ANTHROPOGENIC DISTURBANCES IN THE DEEP SEA

EDITED BY: Ricardo Serrão Santos, Christopher Kim Pham and Jeroen Ingels

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ANTHROPOGENIC DISTURBANCES IN THE DEEP SEA

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The Community Structure of Deep-Sea Macrofauna Associated with Polymetallic Nodules in the Eastern Part of the Clarion-Clipperton Fracture Zone

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Deep-sea areas characterized by the presence of polymetallic nodules are getting increased attention due to their potential commercial and strategic interest for metals such as nickel, copper, and cobalt. The polymetallic nodules occur in areas beyond national jurisdiction, regulated by the International Seabed Authority (ISA). Under exploration contracts, contractors have the obligation to determine the environmental baseline in the exploration areas. Despite a large number of scientific cruises to the central east Pacific Ocean, few published data on the macrofaunal biodiversity and community structure are available for the abyssal fields of the Clarion-Clipperton Fracture Zone (CCFZ). This study focused on the macrofaunal abundance, diversity, and community structure in three physically comparable, mineable sites located in the license area of Global Sea Mineral Resources N.V. (GSR), at ~4,500 m depth. A homogeneous but diverse macrofaunal community associated with the sediment from polymetallic nodule areas was observed at a scale of 10 to 100 s of km. However, slight differences in the abundance and diversity of Polychaeta between sites can be explained by a decline in the estimated flux of particulate organic carbon (POC) along a southeast-northwest gradient, as well as by small differences in sediment characteristics and nodule abundance. The observed homogeneity in the macrofaunal community is an important prerequisite for assigning areas for impact and preservation reference zones. However, a precautionary approach regarding mining activities is recommended, awaiting further research during the exploration phase on environmental factors structuring macrofaunal communities in the CCFZ. For instance, future studies should consider habitat heterogeneity, which was previously shown to structure macrofauna communities at larger spatial scales. Acknowledging the limited sampling in the current study, a large fraction (59–85%; depending on the richness estimator used and the macrofaunal taxon of interest) of the macrofaunal genus/species diversity from the habitat under study was characterized.

Keywords: polymetallic nodules, macrofauna, deep sea, CCFZ, biodiversity, community structure, deep-sea mining

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INTRODUCTION

For a long time, hard substrates were thought to be relatively uncommon in the deep sea, but they can occur in regions with recent volcanic activity (hydrothermal vents, mid-ocean ridges), in seeps with carbonate crust formation, in submarine canyons, on seamounts, or in areas with low sedimentation rates. The latter areas allow the development of polymetallic nodules: black spheroidal to discoïdal bodies composed mainly of manganese, iron, silicates and hydroxides, as well as trace metals such as nickel, copper, cobalt, and molybdenum, and rare earth elements (REE; Halbach et al., 1975; Halbach and Fellerer, 1980). Polymetallic nodules were first discovered in 1873 during the historic voyage of HMS Challenger. Researchers in the second half of the twentieth century, discovered that these nodules cover vast areas of the ocean floor below 4,000 m, with highest abundances in the Central Indian Basin (CIB), the Peru basin and especially the area in the equatorial Pacific Ocean off the west coast of Mexico, known as the Clarion-Clipperton Fracture Zone (CCFZ).

Mero (1965) was the first to recognize the potential commercial and strategic interest of the nodules, and in the near future, polymetallic nodules will likely be mined in order to meet growing demands of metals such as nickel, copper, and cobalt (Clark et al., 2013). The polymetallic nodules occur in areas beyond national jurisdiction in common heritage of mankind and are thus regulated by the International Seabed Authority (ISA; Wedding et al., 2015). Anno 2016, the ISA has allocated license areas for potential mining to 15 national and industrial groups of contractors, which are engaged in the assessment of the resources in the area and the environmental assessments prior to mining activities. On the 14th of March 2013, Global Sea Mineral Resources N.V. (GSR) was granted a license for the exploration of polymetallic nodules for a period of 15 years, for an area encompassing 76,728 km^2 in the eastern part of the CCFZ.

Mining for polymetallic nodules will inevitably impact the fauna in the area, not only by removing the nodules themselves, but also by the resuspension and redeposition of sediments in an otherwise very stable habitat (Jumars, 1981; Ingole et al., 2001; Thiel et al., 2001). Therefore, as recommended by the ISA, prior to (test) mining it is important to determine the environmental baseline in the exploration area, to gain insight into natural processes such as dispersion and settling of particles and benthic faunal succession, and to gather other data that may make it possible to acquire the capability necessary to make accurate environmental impact predictions (ISA-LTC, 2013). Moreover, because the populations of fauna associated with polymetallic nodule areas will be subsets of meta-populations that interact through dispersal and colonization, it is important to know the degree of isolation of populations associated with the areas where nodules will be removed and whether a given population serves as a critical brood stock for other populations (ISA-LTC, 2013).

Previous studies have indicated that the presence of nodules influences the abundance, community composition, and distribution of meiofauna, macrofauna, and megafauna in the CCFZ (e.g., Mullineaux, 1987; Tilot, 2006; Veillette et al., 2007; Smith et al., 2008; Vanreusel et al., 2016). Moreover, at regional scales, polymetallic nodules enhance biodiversity of the deep-sea benthos (Smith et al., 2008). Regions exposed to nodule mining are therefore crucial areas from a biodiversity conservation point of view (Smith et al., 2008). Although some (mostly) negative experimental disturbance effects have been observed (Borowski and Thiel, 1998; Bluhm, 2001; Ingole et al., 2001; Radziejewska et al., 2001; Miljutin et al., 2011), predicting the impact of nodule mining on biodiversity remains difficult because of the poor ecological baseline knowledge of the area. Hence, in order to understand the possible impact of future mining of deep-sea mineral resources, knowledge of the fauna associated with areas of nodules is crucial. Despite the recently large number of scientific cruises to the central east Pacific Ocean, still very little is known of the macrofaunal biodiversity and community structure at the abyssal fields of the CCFZ (Paterson et al., 1998; Glover et al., 2002; Smith et al., 2008; Janssen et al., 2015) and almost no published data are available on the macrofaunal composition in the GSR license area (but see: Hecker and Paul, 1979; Wilson, 2016). The lack of biological data from the CCFZ and the deep sea in general is linked to its remoteness, the consequently high financial cost associated with deep-sea sampling, and difficulties in observing and sampling organisms.

To bridge this knowledge gap, we conducted a base line study collecting quantitative data on macrofaunal abundance, taxonomic composition, and diversity in the GSR license area, focusing on three sites with a high suitability for deep-sea mining. We hypothesize that (1) macrofaunal community composition does not vary over scales of 10 to 100 s of km, and (2) polymetallic nodule abundance, coverage, and volume do not structure the macrofaunal community in the GSR license area. In addition, we estimated the minimum number of samples required to detect the majority of the estimated species richness in nodule rich sediments of the GSR license area.

MATERIALS AND METHODS

Study Area and Sampling Design

The biological and environmental sampling conducted during expedition GSRNOD15A served as a biological baseline study gathering data on the fauna and relevant environmental variables within the Global Sea Mineral Resources N.V. (GSR) license area, centered around 12–17° N, 122–129° W in the CCFZ. The CCFZ is located in the mesotrophic Pacific abyss, positioned between the eutrophic abyssal sediments around the equator and the oligotrophic sediments underlying the North Pacific central gyre. GSRNOD15A samples were collected from September 10th to October 19th 2015 aboard the RV "Mt. Mitchell" at depths varying from 4,470 to 4,569 m. Within the GSR license area, three 10 × 20 km sampling sites (B6S02, B4S03, and B4N01) located between ∼60 and 270 km apart from each other (**Figure 1**) were selected based on the presence of polymetallic nodules and suitability for future deep-sea mining from (1) available ecological literature, (2) bathymetry and slope maps, and (3) backscatter intensity data (giving an indication of the potential presence of polymetallic nodules) collected during a previous expedition to the license area (August–September 2014). Although we lacked a comprehensive evaluation of the

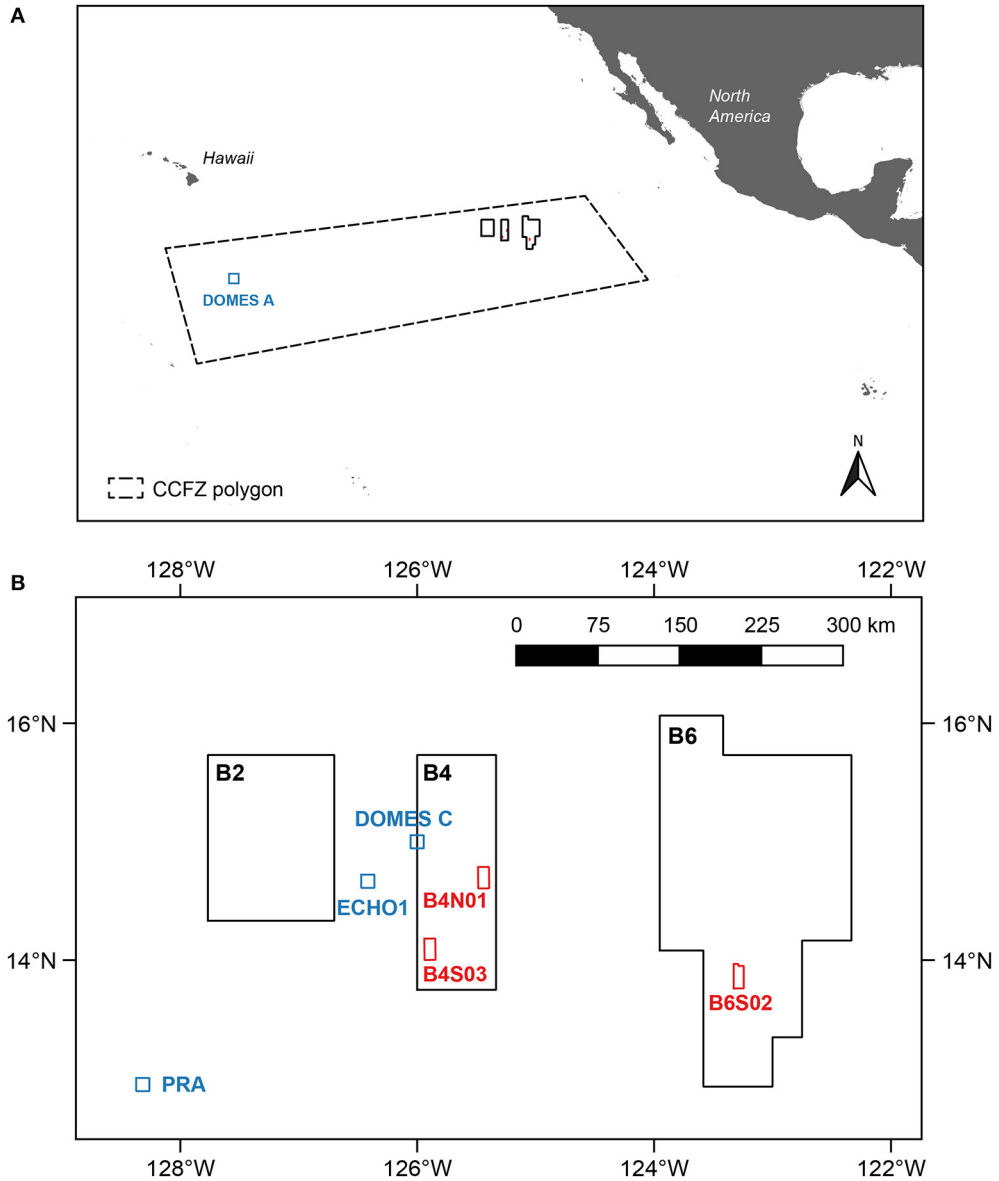


FIGURE 1 | The central Equatorial Pacific indicating (A) the Global Sea Mineral Resources (GSR) license area located within the Clarion-Clipperton Fracture Zone (CCFZ) and **(B)** the three sites (B6S02, B4S03, and B4N01; red) sampled during expedition GSRNOD15A. The coordinates of the CCFZ polygon were based on the working definition of the CCFZ used by Glover et al. (2015). Other stations and sites in the CCFZ examined for macrofauna are indicated in blue.

habitat heterogeneity covered by the selected sites, sites were expected to be as similar as possible with respect to bathymetry and slope.

Sampling Strategy and Sample Analysis

In order to compare sampling sites, sediment environmental parameters (i.e., granulometry, sediment sorting coefficient and porosity, total organic matter, and total organic carbon and nitrogen) were measured from an undisturbed sediment core (internal diameter: 10 cm, depth: 0–10 cm), collected with a MC-800 multicorer (MUC) and replicated ($n = 3$) per site. The cores were processed in a cold lab container (4°C) and, following siphoning the overlying water off the sediment core, they were sliced per cm down to a depth of 10 cm. Subsequently, each slice was stored at −20°C until further analysis. At each site a minimum of three and a maximum of five boxcores were collected using a MK-III spade box corer (0.25 m^2 surface, 0.6 m sediment depth; **Table 1**). As recommended by the ISA (2015), the surface area of the box cores was not subsampled but entirely preserved for macrofauna (metazoan organisms retained on a 300-μm mesh sieve) analysis. Upon recovery of the box corer, the overlying water was siphoned off in order to avoid resuspension of the surficial sediments and the associated fauna. Subsequently, the box core surface was photographed and all nodules were

TABLE 1 | Date, position and depth of the box core (BC) and multicore (MUC) deployments within the Global Sea Mineral Resources (GSR) license area located in the Clarion–Clipperton Fracture Zone (CCFZ).

Site	Deployment	Date	Depth (m)	Latitude	Longitude
B6S02	BC011	21/09/2015	4549	13° 53′ 38.54″ N	123° 17′ 49.34″ W
	BC013	21/09/2015	4560	13° 53′ 17.45″ N	123° 17′ 19.39″ W
	BC015	22/09/2015	4560	13° 52′ 59.66″ N	123° 16′ 55.96″ W
	MUC001	23/09/2015	4526	13° 51′ 13.85″ N	123° 15′ 18.46″ W
	MUC002	23/09/2015	4525	13° 51′ 14.89″ N	123° 14′ 45.73″ W
	MUC003	24/09/2015	4522	13° 50′ 44.12″ N	123° 15′ 9.32″ W
B4S03	BC018	27/09/2015	4501	14° 6′ 44.93″ N	125° 52′ 17.29″ W
	BC019	27/09/2015	4488	14° 7′ 3.65″ N	125° 52′ 46.38″ W
	BC021	28/09/2015	4477	14° 6′ 15.66″ N	125° 52′ 39.76″ W
	BC024	1/10/2015	4569	14° 3′ 20.07″ N	125° 55′ 35.27″ W
	MUC004	29/09/2015	4490	14° 7′ 4.08″ N	125° 52′ 44.76″ W
	MUC005	29/09/2015	4498	14° 6′ 45.14″ N	125° 52′ 17.65″ W
	MUC006	29/09/2015	4470	14° 6′ 15.19″ N	125° 52′ 41.84″ W
B4N01	BC026	4/10/2015	4509	14° 42′ 23.08″ N	125° 27′ 40.25″ W
	BC027	4/10/2015	4501	14° 42′ 22.86″ N	125° 26′ 32.75″ W
	BC029	5/10/2015	4504	14° 42′ 22.90″ N	125° 27′ 5.90″ W
	BC035	9/10/2015	4505	14° 38′ 50.82″ N	125° 24′ 31.82″ W
	BC036	9/10/2015	4514	14° 40′ 17.40″ N	125° 27′ 21.82″ W
	MUC007	6/10/2015	4509	14° 42′ 22.68″ N	125° 27′ 39.13″ W
	MUC008	7/10/2015	4504	14° 42′ 22.79″ N	125° 27′ 4.68″ W
	MUC009	7/10/2015	4501	14° 42′ 23.36″ N	125° 26′ 31.34″ W

removed from the sediment surface, washed, and individually measured and weighed for further analysis. The overlying water and the washed nodule residue was pooled with sediment from the 0–3 cm layer and sieved through a 300-μm mesh size with cold (4°C) filtered sea water. The sieve residue was immediately bulk-fixed in pre-cooled (−20°C) 96% absolute EtOH and stored at −20°C. Every 3 to 5 h, the sample containers were carefully shaken to guarantee penetration of the EtOH through the sediment and prevent the water inside the samples from freezing (Riehl et al., 2014). After 24 h, the EtOH was decanted and replaced by new pre-cooled 96% absolute EtOH, to ensure a high EtOH concentration (Riehl et al., 2014). Subsequently, the samples were kept frozen (−20°C) until further analysis. Sediment from the 3–5 and 5–10 cm layers was sieved through a 300-μm mesh size with non-cooled filtered sea water. The sieve residues were bulk-fixed in 10% seawater-buffered formaldehyde for 48 h and subsequently preserved in 80% denatured EtOH until further processing.

In the laboratory, sediment samples were dried (60°C) and granulometry was analyzed by means of a Malvern Mastersizer hydro 2000 G. Measured granulometric variables were median grain size, sand (>63 μm), and mud (<63 μm) content, sediment sorting (a measure for the spread distance of the various grain sizes, quantified by the sediment sorting coefficient SC; Giere, 2009) and sediment porosity (measured as the difference in sediment weight before and after drying). The total amount of organic matter (TOM) was determined based on weight loss after combustion for 2 h at 500°C. Total organic carbon (TOC) and nitrogen (TN) were measured using a Flash 2000 NC Sediment Analyser of Interscience (Thermo scientific). Prior to analysis, the samples were acidified with 1% HCl to remove inorganic carbon. Nodule surface coverage was estimated from the photographs. The 0–3 cm bulk-fixed sediment samples were rinsed with chilled 99% denatured EtOH. The 3–5 cm and 5–10 cm bulk-fixed sediment samples were stained with 0.01% Rose Bengal and rinsed after 24 h. Sample residues were transferred to (chilled) sorting dishes and absolute EtOH (−20°C) was added. Using a Leica MZ16 stereomicroscope all macrofauna (including Nematoda, Copepoda, Ostracoda, and Kinorhyncha; which are generally not considered macrofauna) was sorted, counted and photographed with a Nikon DS-Fi2 camera. Polychaeta and Isopoda, two of the most abundant macrofaunal taxa in the CCFZ (e.g., Hessler and Jumars, 1974; Paterson et al., 1998; Glover et al., 2001), representing different functional and reproductive strategies, were identified to genus or species level wherever possible using original scientific literature, identification keys and expert knowledge. All other taxa were identified to order, class, or phylum level. Following identification, specimens were preserved separately in 2 ml vials containing cooled (−20°C) absolute EtOH in case of the 0–3 cm layers and 99% denatured EtOH in case of the 3–5 and 5–10 cm layers.

Data and Statistical Analyses

Because the assumptions of parametric statistical approaches were not fulfilled, differences in (1) the set of sediment environmental variables and (2) nodule parameters (i.e., nodule abundance, nodule surface coverage and nodule volume) between sites were investigated by means of a one-way permutational ANOVA (Permanova) in which "site" was a fixed factor with three levels (B6S02, B4S03, and B4N01). The analysis was based on a Euclidean distance resemblance matrix and performed on untransformed, normalized (for the sediment environmental variables) data. Where a significant effect was found, pair-wise tests between the sites were carried out. The Monte Carlo p-value (p_{MC}) was reported instead of the permutation p-value (p_{PERM}) if the number of possible permutations was <100 (Anderson et al., 2008). Although Permanova makes no explicit assumptions regarding the distribution of the data, a test for the homogeneity of multivariate dispersions was ran, using the PERMDISP routine. None of the PERMDISP tests was significant, indicating no differences in dispersion among the sites (Anderson et al., 2008). In addition, sediment environmental data was visualized by a Principal Coordinates Analysis (PCO; Anderson et al., 2008). Based on Spearman correlations, only variables that correlated >50% with one of the first two PCO axes were plotted.

The total macrofaunal community was investigated both at higher taxon and lower taxon level. Additionally, a detailed study of Polychaeta and Isopod (both at higher—i.e., family—, and lower—i.e., genus or species—taxon level) was performed. Prior to further analyses, macrofaunal abundances were standardized to individuals per m^2. Differences in the macrofaunal taxon abundance N and biodiversity indices (taxon richness T, Shannon-Wiener diversity index H', Pielou's evenness index J' and the Hurlbert rarefaction index for 50 individuals ET_{50}) between sites were tested with a one-way PERMANOVA based

on a Euclidean distance resemblance matrix and performed on untransformed data. Similarly, differences in the relative abundance of the most dominant total macrofauna taxa, Polychaeta and Isopoda, were tested between sites. Except for the higher taxon richness T of Polychaeta, the total macrofauna and Hurlbert's rarefaction ET_{50} of higher taxa Polychaeta, and for the relative abundance of the isopod families Haploniscidae and Ischnomesidae, the PERMDISP tests were not significant for the factor "site," indicating no differences in dispersion among the sites (Anderson et al., 2008).

Taxon-accumulation curves (TAC), plotting the cumulative number of taxa recorded as a function of the number of sites/samples studied, were produced by randomly adding sites/samples and repeating this procedure 9,999 times. Additionally, the Chao1, Jacknife2, and Bootstrap estimators were used to extrapolate the TAC's and estimate the total taxon richness in the GSR license area, as suggested by Magurran (2004). The minimum number of additional samples required to detect 95 and 100% of the estimated asymptonic taxon richness was calculated using the non-parametric method proposed by Chao et al. (2009). This statistically rigorous method uses the Chao1 nonparametric estimators of asymptotic taxon richness for abundance data. Differences in the faunal community compositions between sites were tested with a multivariate 1-factor PERMANOVA (and visualized by a PCO), which was based on Bray-Curtis resemblance matrices of fourth-root transformed macrofaunal abundance data. In case a significant effect was found, pair-wise tests among sites were carried out. According to the PERMDISP tests, the factor "site" showed no differences in dispersion (Anderson et al., 2008).

To investigate relationships between nodule parameters (i.e., nodule abundance, nodule surface coverage, and nodule volume) and macrofaunal abundance and diversity indices over all sites, Spearman-rank correlations were calculated. A significance level of $p < 0.05$ was used in all tests. All statistical analysis were conducted in the open source software R (version 3.3.1; R Development Core Team, 2015) and in PRIMER v6 with the PERMANOVA+ add-on software (Clarke and Gorley, 2006; Anderson et al., 2008).

RESULTS

Sediment Environmental Variables

The set of sediment environmental variables differed significantly between sites (1-factor Permanova, pseudo-$F_{(2, 6)} = 5.828$, $p_{PERM} = 0.005$) and pair-wise tests showed that differences were significant between all three sites: B6S02-B4S03 ($p_{MC} = 0.0495$), B6S02-B4N01 ($p_{MC} = 0.0096$), B4S03-B4N01 ($p_{MC} = 0.0441$). The PCO analysis graphically showed that the sediment environmental variables distinguished between the three sites (**Figure 2**). PCO axis 1 explained 57.7% of the variation inherent in the resemblance matrix and separated site B6S02 from sites B4S03 and B4N01. This separation resulted from the elevated median grain size, sand content and sediment porosity and the lower mud content and sorting coefficient in B6S02 compared to the other two sites (**Table 2**, **Figure 2**). PCO axis 2 explained 26.4% of the total variation and distinguished site B4N01 from site B6S02 and B4S03, which was due to the elevated content of TOM, TOC, and TN in B4N01 (**Table 2**, **Figure 2**).

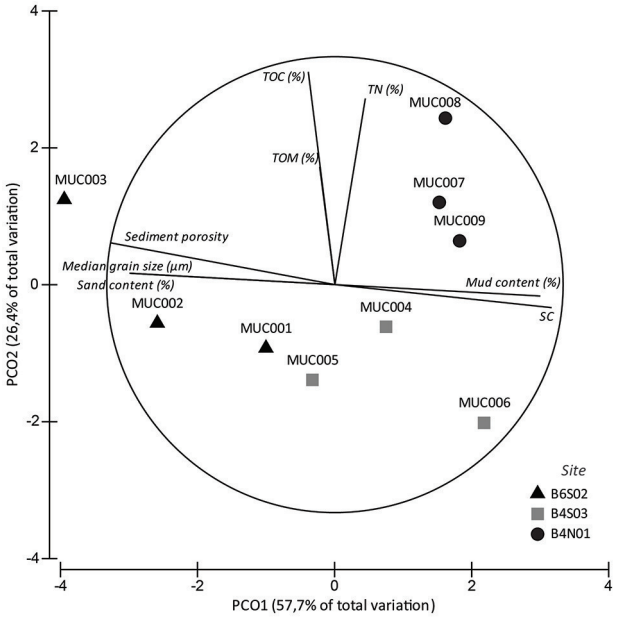


FIGURE 2 | PCO analysis of the sediment environmental variables in sites B6S02, B4S03, and B4N01 based on Euclidean distance similarities of untransformed, normalized data. Vectors represent sediment variables correlating >50% (based on Spearman correlation coefficients) with one of the two PCO axes.

TABLE 2 | Mean (±SE) sediment environmental variables and polymetallic nodules parameters for the three sites within the GSR license area.

	B6S02	B4S03	B4N01
SEDIMENT ENVIRONMENTAL VARIABLES			
Median grain size (µm)	18.5 ± 0.6	16.5 ± 0.5	15.8 ± 0.2
Sand content (%)	11.3 ± 0.6	8.5 ± 0.4	8.0 ± 0.4
Mud content (%)	88.7 ± 0.6	91.5 ± 0.4	92.0 ± 0.4
SC	1.25 ± 0.01	1.29 ± 0.02	1.30 ± 0.01
Sediment porosity	0.874 ± 0.006	0.858 ± 0.004	0.856 ± 0.004
TOM (%)	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
TOC (%)	0.49 ± 0.02	0.41 ± 0.01	0.54 ± 0.02
TN (%)	0.17 ± 0.01	0.15 ± 0.004	0.21 ± 0.01
TOC/TN	3.53 ± 0.07	3.29 ± 0.17	3.03 ± 0.03
POLYMETALLIC NODULE PARAMETERS			
Nodule abundance (kg.m^{-2})	27.33 ± 0.62	22.22 ± 2.41	19.30 ± 2.18
Nodule surface coverage (%)	31.11 ± 8.43	36.19 ± 2.95	39.34 ± 7.02
Nodule volume (cm^3)	69.67 ± 13.68	92.63 ± 6.95	54.75 ± 7.90

Sediment data was derived from an undisturbed sediment core (Ø 10 cm, 0–10 cm deep) collected with a MC–800 multicorer (MUC) and replicated (n = 3) per site. The polymetallic nodule abundance, surface coverage and volume were derived from the box corer (BC) deployments (n = 3, 4, and 5 in sites B6S02, B4S03, and B4N01, respectively). SC, the sediment sorting coefficient (a measure for the spread distance of the various grain sizes); TOM, the total amount of organic matter; TOC, the total amount of organic carbon; TN, the total amount of nitrogen.

Polymetallic Nodules

Of the nodule variables measured, only volume differed significantly between the sites (1-factor Permanova, pseudo-$F_{(2, 9)} = 4.873$, $p_{PERM} = 0.039$) and pair-wise tests showed a significantly higher mean nodule volume (±SE) in site B4S03 (92.63 ± 6.95 cm^3) compared to site B4N01 (54.75 ± 7.90 cm^3, $p_{MC} = 0.009$; **Table 2**). Although not significantly different, mean nodule abundance (±SE) was lowest in site B4N01 (19.30 ± 2.18 $kg.m^{-2}$), intermediate in site B4S03 (22.22 ± 2.41 $kg.m^{-2}$) and highest in site B6S02 (27.33 ± 0.62 $kg.m^{-2}$; **Table 2**). The mean nodule surface coverage (±SE) per site was 39.34 ± 7.02% in B4N01, 36.19 ± 2.95% in B4S03 and 31.11 ± 8.43% in B6N02 (**Table 2**).

Total Macrofauna Abundance, Taxon Diversity, and Community Composition

A total number of 7,028 macrobenthic organisms representing 100 different taxa were sampled inside the GSR license area, at the three sites (Supplementary Material 1). The average total macrofaunal abundance (±SE) in the 0–10 cm sediment layer was 604 ± 56 $ind.m^{-2}$ at site B6S02, 627 ± 50 $ind.m^{-2}$ at site B4S03 and 542 ± 66 $ind.m^{-2}$ at site B4N01 and did not vary significantly among sites (1-factor Permanova, pseudo-$F_{(2, 9)} = 0.5756$, $p_{PERM} = 0.60$). In terms of abundance, the macrofaunal community was dominated by Nematoda (49.6%), Copepoda (15.8%), Polychaeta (12%), Tanaidacea (11.5%), Isopoda (2.8%), Ostracoda (1.4%), Amphipoda (1.3%), and Bivalvia (1.3%). The remaining taxa comprised 4.3% of total macrofaunal abundance. The relative abundances of the dominant taxa did not differ significantly between sites (1-factor Permanova, for all tests $p_{PERM} > 0.05$). After exclusion of Nematoda, Copepoda, Ostracoda and Kinorhyncha (taxa generally construed as typical meiofauna taxa), the average total macrofaunal abundance (±SE) was 199 ± 15 $ind.m^{-2}$ at site B6S02, 202 ± 22 $ind.m^{-2}$ at site B4S03 and 186 ± 30 $ind.m^{-2}$ at site B4N01 (**Table 3**). Similarly, differences in total macrofauna abundance between sites were not statistically significant (1-factor Permanova, pseudo-$F_{(2, 9)} = 0.1104$, $p_{PERM} = 0.89$; **Table 3**). The macrofaunal community excluding the meiofauna taxa was dominated by Polychaeta (36.1%), Tanaidacea (34.6%), Isopoda (8.6%), Amphipoda (3.9%), and Bivalvia (3.9%). The remaining taxa comprised 12.8% of the total macrofaunal abundance (**Figure 3**). Only the average relative abundance of Polychaeta [1-factor Permanova, pseudo-$F_{(2, 9)} = 9.9609$, $p_{PERM} = 0.0089$] and Tanaidacea (1-factor Permanova, pseudo-$F_{(2, 9)} = 4.5042$, $p_{PERM} = 0.046$) differed significantly among sites. Pair-wise comparisons showed a significantly lower relative abundance of Polychaeta (±SE) at B4N01 (29.1 ± 2.6%) compared to B6S02 (41.1 ± 2.1%; $p_{MC} = 0.018$) and B4S03 (40.5 ± 1.5%; $p_{MC} = 0.0092$), and a significantly lower relative abundance of Tanaidacea at B6S02 (22.3 ± 2.2%) compared to B4N01 (44.8 ± 3.1%; $p_{MC} = 0.0018$).

Taking into account all lower macrofaunal taxa (excluding meiofauna), Shannon-Wiener diversity H', Pielou's evenness J', and Hurlbert rarefaction ET_{50} were significantly affected by the factor "site" (**Table 3**). Pair-wise Permanova tests revealed that these three indices were significantly higher at B6S02 compared to B4N01, but not at B4S03 except for ET_{50}. (**Table 3**). The number of macrofaunal taxa T did not differ between sites. However, only 32% of the taxa were shared among all three sites. When excluding meiofauna, 92 macrofaunal taxa (lowest identifiable taxon level) were identified in the GSR license area, of which 29% was shared between all sites. Taxon-accumulation curves suggest that 64 to 85% of the total number of lower macrofaunal taxa from the three sites in the sampling area had been identified (**Figure 4**). Bootstrap estimates of total number of macrofauna taxa were 106 and 108 with increasing number of sites and samples, respectively, whereas Chao1 estimated 135 and 135 taxa and Jacknife2 estimated 134 and 145 taxa, respectively. B6S02 had a higher number of observed taxa (S_{obs}) and a higher estimated total taxon richness (Chao1, Jacknife2, and Bootstrap) than B4S03 and B4N01 (**Figure 5**). The minimum number of additional samples required to encompass 100% of the taxon richness estimated by Chao1 is 123 samples, compared with 36 samples to encompass 95% of the estimated taxon richness.

Multivariate analysis revealed no site effect on macrofaunal community composition (1-factor Permanova, pseudo-$F_{(2, 9)} = 1.25$, $p_{PERM} = 0.11$; Supplementary Material 2). No statistically significant correlations between total macrofaunal abundance or lower taxon diversity indices (excluding meiofauna) and the polymetallic nodule parameters were observed ($p > 0.05$), except for a moderately strong, positive correlation between nodule abundance and H' ($r_S = 0.69$, $p = 0.016$) and ET_{50} ($r_S = 0.69$, $p = 0.017$), respectively.

Polychaeta Abundance, Species Diversity, and Community Composition

Polychaeta were the most abundant macrofauna in the study area. In total, 844 polychaetes, representing 53 taxa, were collected (Supplementary Material 1). The average polychaete abundance (±SE) was highest at B6S02 (83 ± 10 $ind.m^{-2}$) and B4S03 (81 ± 6 $ind.m^{-2}$), and lowest at B4N01 (54 ± 10 $ind.m^{-2}$). The average polychaete abundance did not differ significantly among sites (1-factor Permanova, pseudo-$F_{(2, 9)} = 3.3959$, $p_{PERM} = 0.088$). The polychaete specimens represented 24 families, of which 8 families accounted for 74.4% of polychaete abundance: Spionidae (17.1% of the total abundance), Paraonidae (13.7%), Cirratulidae (12.3%), Goniadidae (9.5%), Capitellidae (7.6%), Lumbrineridae (5.7%), Acrocirridae (4.3%), and Paralacydoniidae (4.3%) (**Figure 3**). The remaining 25.6% of the total polychaete abundance belonged to less dominant families (see Supplementary Material 1). One-way Permanova revealed that the factor "site" affected the average relative abundance of Spionidae (pseudo-$F_{(2, 9)} = 7.8781$, $p_{PERM} = 0.0066$), Cirratulidae (pseudo-$F_{(2, 9)} = 5.4806$, $p_{PERM} = 0.030$), and Lumbrineridae (pseudo-$F_{(2, 9)} = 16.402$, $p_{PERM} = 0.0065$). Pair-wise comparisons showed a significantly higher relative abundance of Spionidae (±SE) in site B4S03 (25.2 ± 3.5%) compared to B6S02 (8 ± 2.9%, $p_{MC} = 0.015$) and B4N01 (15.6 ± 2.3%, $p_{MC} = 0.047$), a significantly higher relative abundance of Cirratulidae in B4S03 (21.4 ± 4.6%) compared to B4N01 (5.5 ± 2.7%, $p_{MC} = 0.016$), and a significantly higher

TABLE 3 | Overview of the calculated community descriptors (mean ± SE) and the 1–factor Permanova main and pair–wise tests for the total macrofauna, the Polychaeta, and the Isopoda communities of the three sites within the Global Sea Mineral Resources (GSR) license area.

Macrofauna group	B6S02 ($n = 3$)	B4S03 ($n = 4$)	B4N01 ($n = 5$)	Main Test			Pair–wise test (p_{MC})		
				MS	Pseudo–$F_{2, 9}$	p_{PERM}	B6S02–B4S03	B6S02–B4N01	B4S03–B4N01
TOTAL MACROFAUNA (EXCL. MEIOFAUNA)									
Taxon abundance *N*	199 ± 15	202 ± 22	186 ± 30	302.40	0.1104	0.889	–	–	–
Taxon richness *T (low)*	27.3 ± 1.8	20.5 ± 2.5	20.2 ± 3.0	55.23	1.7849	0.231	–	–	–
Shannon diversity index *H′ (low)*	2.98 ± 0.08	2.46 ± 0.20	2.24 ± 0.13	0.52	5.6845	**0.030**	0.0853	**0.0066**	0.3604
Pielou's eveness index *J′ (low)*	0.903 ± 0.008	0.819 ± 0.036	0.757 ± 0.024	0.02	6.4891	**0.024**	0.1112	**0.0047**	0.1846
Hurlbert rarefaction index ET_{50} *(low)*	21.2 ± 0.9	16.2 ± 1.5	15.5 ± 1.3	32.79	4.6238	**0.047**	**0.0479**	**0.0196**	0.7218
POLYCHAETA									
Taxon abundance *N*	83 ± 10	81 ± 6	54 ± 10	1090.40	3.3959	0.088	–	–	–
Taxon richness *T (high)*	12 ± 0	8.0 ± 0.3	8.8 ± 1.0	17.93	8.1515	**0.015**	**0.0001**	**0.0475**	0.2904
Taxon richness *T (low)*	14.0 ± 0.6	12.0 ± 1.2	10.4 ± 2.0	12.23	1.0669	0.383	–	–	–
Shannon diversity index *H′ (high)*	2.31 ± 0.04	1.80 ± 0.04	2.06 ± 0.10	0.23	8.9415	**0.012**	**0.0006**	0.1370	0.0578
Shannon diversity index *H′ (low)*	2.50 ± 0.02	2.34 ± 0.10	2.20 ± 0.18	8.74×10^{-2}	0.9914	0.408	–	–	–
Pielou's eveness index *J′ (high)*	0.930 ± 0.018	0.892 ± 0.011	0.961 ± 0.009	5.17×10^{-3}	9.4171	**0.006**	0.1080	0.1320	**0.0019**
Pielou's eveness index *J′ (low)*	0.949 ± 0.016	0.948 ± 0.009	0.966 ± 0.012	4.66×10^{-4}	0.8249	0.454	–	–	–
Hurlbert rarefaction index ET_{50} *(high)*	11.8 ± 0.1	7.4 ± 0.3	8.8 ± 0.9	16.82	8.0310	**0.014**	**0.0001**	0.0502	0.2739
Hurlbert rarefaction index ET_{50} *(low)*	13.7 ± 0.5	11.8 ± 1.2	10.3 ± 2.0	10.96	1.0364	0.398	–	–	–
ISOPODA									
Taxon abundance *N*	17 ± 4	18 ± 5	15 ± 4	9.60	0.1214	0.890	–	–	–
Taxon richness *T (high)*	3.7 ± 0.7	3.0 ± 0.4	3.0 ± 0.6	0.50	0.3553	0.778	–	–	–
Shannon diversity index *H′ (high)*	1.23 ± 0.16	0.98 ± 0.10	0.94 ± 0.26	8.81×10^{-2}	0.4727	0.693	–	–	–
Pielou's eveness index *J′ (high)*	0.972 ± 0.016	0.934 ± 0.054	0.970 ± 0.010	1.78×10^{-3}	0.3798	0.922	–	–	–
Hurlbert rarefaction index ET_{50} *(high)*	3.7 ± 0.7	3.0 ± 0.4	3.0 ± 0.6	0.50	0.3553	0.781	–	–	–

N, taxon abundance (ind.m^{-2}); T, taxon richness; H′, Shannon–Wiener diversity index; J′, Pielou's evenness index; and ET_{50}, the Hurlbert rarefaction index for 50 individuals. Site was a fixed factor with three levels (B6S02, B4S03, and B4N01). Analysis were based on an Euclidian distance resemblance matrix and performed on untransformed data. In case of significant differences ($p < 0.05$) p-values are in bold. High, analysis on higher taxon (family) level; low, analysis on lower taxon (genus/species) level; p_{PERM}, permutation p-value, p_{MC}, Monte Carlo p-value.

relative abundance of Lumbrineridae in B6S02 (15.7 ± 2.6%) compared to B4S03 (1.5 ± 1.5%, $p_{MC} = 0.0039$) and B4N01 (1.7 ± 1.7%, $p_{MC} = 0.0031$).

When calculated based on the genus/species level, none of the polychaete community descriptors were significantly affected by the factor "site," whereas all four descriptors differed significantly between sites at the family level (**Table 3**). Pair-wise tests revealed that *T*, *H′*, and ET_{50} were significantly higher at B6S02 compared to B4S03, but not B4N01, except for *T*. *J′* was significantly higher at B4N01 compared to B4S03 (**Table 3**). A total of 24 polychaete families occured in the GSR license area. According to taxon-accumulation curves, most (84–93%) of the polychaete families in the area were identified during GSRNOD15A. The total number of families with increasing number of samples was estimated at 26, 27, and 29 by Bootstrap, Chao1, and Jacknife2, respectively. At the genus or species level, 53 polychaete taxa were identified, however only 26% of these were shared among the three sites. Moreover, taxon-accumulation curves suggest that only part (62–85%) of the total taxon richness has been characterized (**Figure 4**). Bootstrap estimated that with increasing number of sites and samples there are, respectively, 61 and 63 polychaete taxa in the study area, whereas Chao1 estimated 77 and 77 taxa and Jacknife2 estimated 78 and 85 taxa, respectively. B6S02 had a higher number of observed polychaete taxa (S_{obs}) and a higher estimated total taxon richness (Jacknife2 and Bootstrap) than B4S03 and B4N01. However, based on the Chao1 estimator,

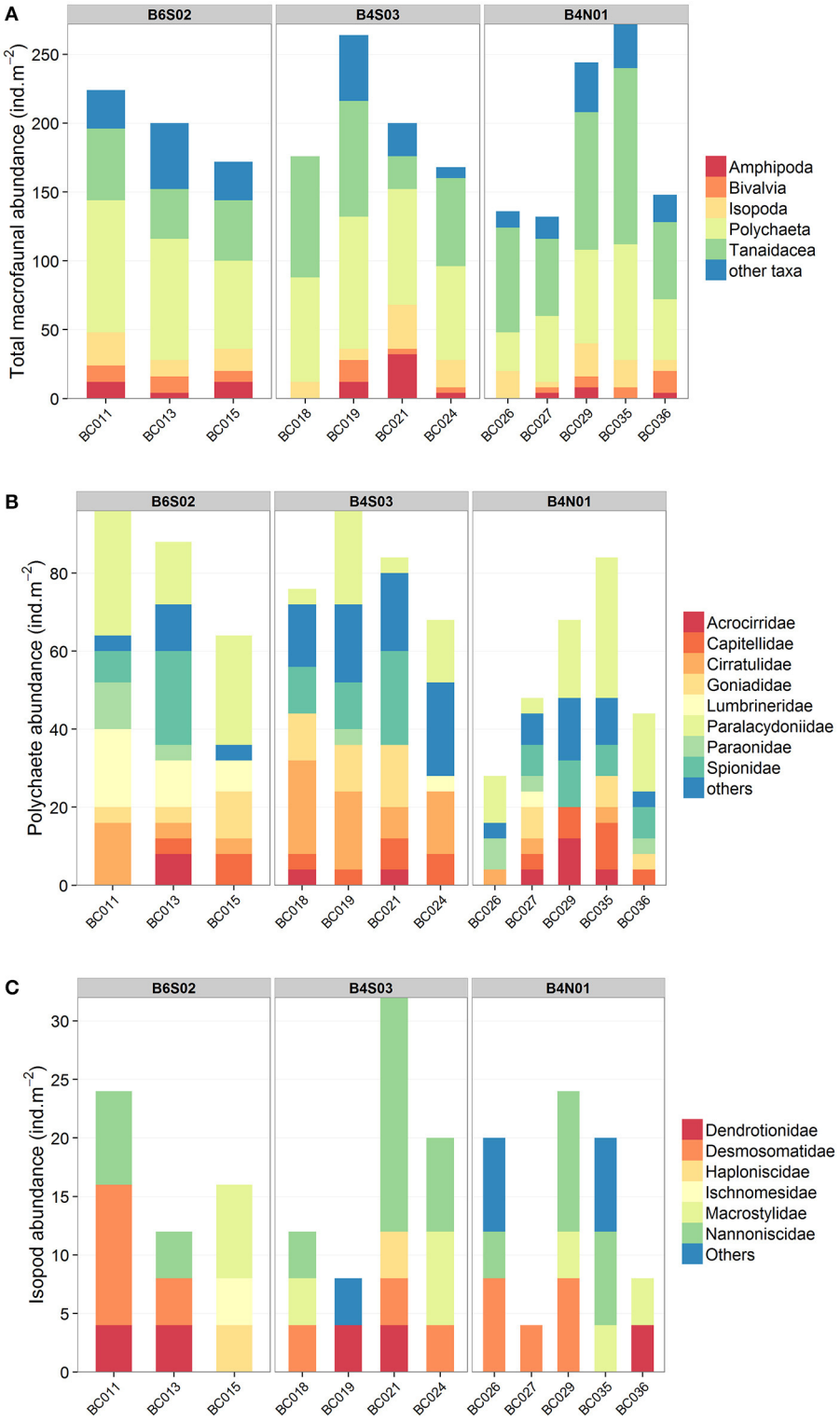


FIGURE 3 | Macrofauna composition of the box cores (0–10 cm) taken at sites B6S02, B4S03, and B4N01 in the GSR license area. (A) Total macrofauna (higher taxon level). Nematoda, Copepoda, Ostracoda, and Kinorhyncha were not included since these taxa are generally construed as typical meiofauna taxa. Other taxa include Acari, Brachiopoda, Decapoda, Chaetognatha, Cumacea, Gastropoda, Mysida, Nemertea, Oligochaeta, Ophiuroidea, Priapulida, Pycnogonida, Scaphopoda, and Sipuncula. **(B)** Polychaeta (family level). Other families include Amphinomidae, Glyceridae, Magelonidae, Maldanidae, Nereididae, Nephtyidae, Opheliidae, Oweniidae, Phyllodocidae, Polynoidae, Sabellidae, Sigalionidae, Syllidae, Terebellidae, Trichobranchidae. **(C)** Isopoda (family level). Others include isopod specimens which could not be identified to the family level.

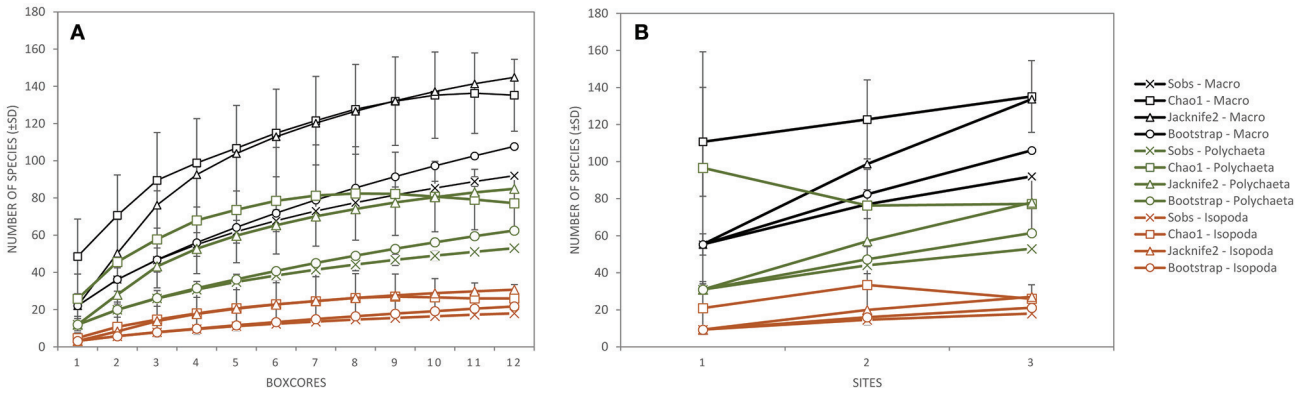


FIGURE 4 | The total number of species observed (±*SD*; S_{obs}) and the total taxon richness estimates (Chao1 ± *SD*, Jacknife2, and Bootstrap) of the total macrofauna (excluding meiofauna; black), Polychaeta (genus/species level; green) and Isopoda (genus/species level; orange) as a function of (A) the number of box cores collected, and **(B)** the number of sites sampled during expedition GSRNOD15A in the GSR license area.

site B4S03 had higher total taxon richness than sites B6S02 and B4N01 (**Figure 5**). The minimum number of additional samples required to encompass 100% of the taxon richness estimated by Chao1 is 76 samples, while 24 samples are required to encompass 95% of the estimated taxon richness.

Multivariate analysis revealed no site effect on polychaete community composition (1-factor Permanova, pseudo-$F_{(2, 9)} = 1.085$, $p_{PERM} = 0.37$; Supplementary Material 3). Polychaete abundance was significantly and positively correlated with nodule abundance ($r_S = 0.61$, $p = 0.036$). No other statistically significant correlations between the lower or higher polychaete taxon diversity indices and the polymetallic nodule parameters were observed ($p > 0.05$).

Isopoda Abundance, Species Diversity and Community Composition

Regarding Isopoda, a total of 200 organisms representing 18 different taxa were sampled in the GSR license area (Supplementary Material 1). All belonged to the superfamily Janiroidea. The average isopod abundance (±SE) was 17 ± 4 ind.m^{-2} at B6S02, 18 ± 5 ind.m^{-2} at B4S03, and 15 ± 4 ind.m^{-2} at B4N01 and did not differ significantly between sites (1-factor Permanova, pseudo-$F_{(2, 9)} = 0.1214$, $p_{PERM} = 0.89$). In terms of abundance, 90% of the Isopoda belonged to six different families: Nannoniscidae (34% of the total isopod abundance), Desmosomatidae (24%), Macrostylidae (16%), Dendrotionidae (10%), Haploniscidae (4%), and Ischnomesidae (2%) (**Figure 3**). Damaged isopod specimens which could not be identified to the family level comprised 10% of the total isopod abundance. Average relative abundance was not significantly affected by the factor "site" (1-factor Permanova, for all tests $p_{PERM} > 0.05$) for any of the isopod families.

None of the isopod community descriptors (whether calculated based on genus/species level or family level) were significantly affected by the factor "site" (**Table 3**). Seven isopod families were identified in the GSR license area. Based on taxon-accumulation curves, Bootstrap estimated a total number of seven families, whereas Chao1 and Jacknife2 estimated eight families. Hence, isopod family diversity was largely captured (88–100%) by our samples. At the genus or species level, only 11% of the taxa were shared among the three sites, while 59–83% of the isopod taxon richness has been characterized: 18 taxa were observed, whereas Bootstrap estimated 21 and 22 taxa with increasing number of sites and samples, respectively, Chao1 estimated 26 and 26 taxa, respectively, and Jacknife2 27 and 31 taxa, respectively (**Figure 4**). In line with Polychaeta, when taking into account three boxcores, the number of observed isopod taxa (S_{obs}) and the estimated total taxon richness (Chao1, Jacknife2, and Bootstrap) was higher at B6S02 compared to B4S03 and B4N01 (**Figure 5**). The minimum number of additional samples required to encompass 100% of the taxon richness estimated by Chao1 is 54 samples, while 22 samples would be sufficient to encompass 95% of the estimated taxon richness.

Multivariate analysis revealed no site effect on isopod community composition (1-factor Permanova, pseudo-$F_{(2, 9)} = 0.9296$, $p_{PERM} = 0.54$; Supplementary Material 4). We found no statistically significant correlations between the isopod abundance or the lower or higher taxon diversity indices and the polymetallic nodule parameters ($p > 0.05$).

DISCUSSION

The Macrofauna Community and Habitat Heterogeneity in the GSR License Area

This study provides one of the first insights in the abundance, diversity, and community composition of macrofauna associated with nodule areas in the GSR license area in the CCFZ (but see: Hecker and Paul, 1979; Wilson, 2016). Although habitat differences between the sampling sites based on sediment environmental data was larger than expected based on bathymetry and slope maps, apparently they had no large impact on total macrofauna and polychaete abundances. However, total macrofaunal genus/species diversity as well as polychaete family diversity was significantly higher in the eastern side of the

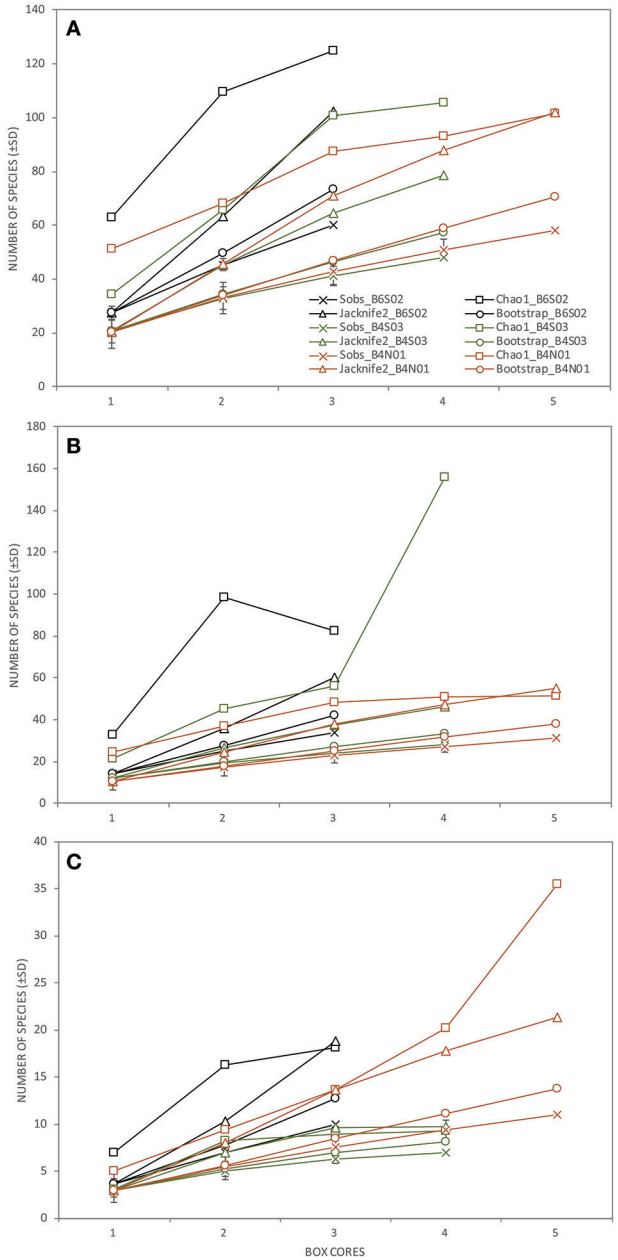


FIGURE 5 | The total number of species observed (±*SD*; S_{obs}) and the total taxon richness estimates (Chao1, Jacknife2, and Bootstrap) of (A) the total macrofauna (excluding meiofauna; lowest taxon level), **(B)** Polychaeta (genus/species level), and **(C)** Isopoda (genus/species level) as a function of the number of box cores collected per station (B6S02: black, B4S03: green, B4N01: orange) sampled during expedition GSRNOD15A in the GSR license area.

GSR license area (B6S02). Since we found no clear between-site difference in nodule parameters (estimated from a limited number of box core deployments), diversity differences might be driven by sediment related habitat differences and the flux of surface-produced particulate organic carbon (POC) to the seafloor. POC generally constitutes the main carbon and energy source in the deep sea and is assumed to be one of the main drivers of the distribution and composition of deep-sea benthic communities (Ruhl and Smith, 2004; Wei et al., 2010). Lutz et al. (2007) modeled seafloor POC flux based on water depth and seasonal variability in remote-sensed net primary productivity between 19 August 1997 and 24 June 2004. Gridded POC flux data from Lutz et al. (2007) was used to calculate the average annual seafloor POC flux for each of the GSR sites in ArcGIS 10.3 using the Zonal statistics tool. Based on the annual average POC flux, a latitudinal and longitudinal gradient characterized the GSR license area, with briefly increasing POC values from B4N01 (1.51 g C_{org} m^{-2} $year^{-1}$) to B4S03 (1.56 g C_{org} m^{-2} $year^{-1}$) to B6S02 (1.61 g C_{org} m^{-2} $year^{-1}$). This increase in POC from the northwest to the southeast, along with changes in sediment environmental parameters, likely contributed to the significantly higher total macrofauna and polychaete taxon diversity at B6S02 compared to B4N01.

Similarly, Smith et al. (2008) reported a substantial decline in macrofauna, and especially polychaete abundance and diversity from the eastern French license area (IFREMER/AFRENOD east zone) and Kaplan site East to the western French license area (IFREMER/AFRENOD west zone) and Kaplan site West in the CCFZ. This decline likely results from a gradual decrease in the flux of POC from east to west (Smith and Demopoulos, 2003). Consequently, Smith et al. (2008) observed a large habitat difference between the western and eastern areas, with the western area characterized by higher substrate heterogeneity (Smith et al., 2008). In line with Smith et al. (2008), Hecker and Paul (1979), Wilson (2016), and Paterson et al. (1998) reported a decline in overall macrofauna and polychaete abundances from higher to lower productivity sites in the eastern equatorial Pacific. The trends observed in these studies are more pronounced than the findings reported here, which is probably due to the large distance between their sampling sites (~1,500, 2,800, 2,800, and 2,500 km, respectively), and hence a potentially high degree of habitat heterogeneity at broader spatial scales in the northeast Pacific abyss.

In general, macrofaunal abundance, diversity, and community composition were similar between sites in the GSR license area, implying that the macrofaunal community associated with polymetallic nodule areas is, at least at scales of 10 to 100 s of km, somewhat homogeneous. This finding is in line with results from a megafauna study suggesting that the deep-sea megafauna in the eastern CCFZ (dominated for 63.5% by sessile morphotypes) may be relatively homogenous on scales of 1 to 100 s of km (Amon et al., 2016). However, small differences in estimated seafloor POC flux, nodule abundance and sediment environmental variables among sites, might contribute to slight differences in macrofaunal abundance and diversity observed in the GSR license area.

Comparison with Other Studies in the Area

Nematoda, Copepoda, Ostracoda, and Kinorhyncha are often considered typical meiofaunal taxa and are therefore generally not included in macrofauna studies. To enable comparison of macrofaunal abundances in our samples from the GSR license area with other nodule-bearing sites in the Pacific (Hessler

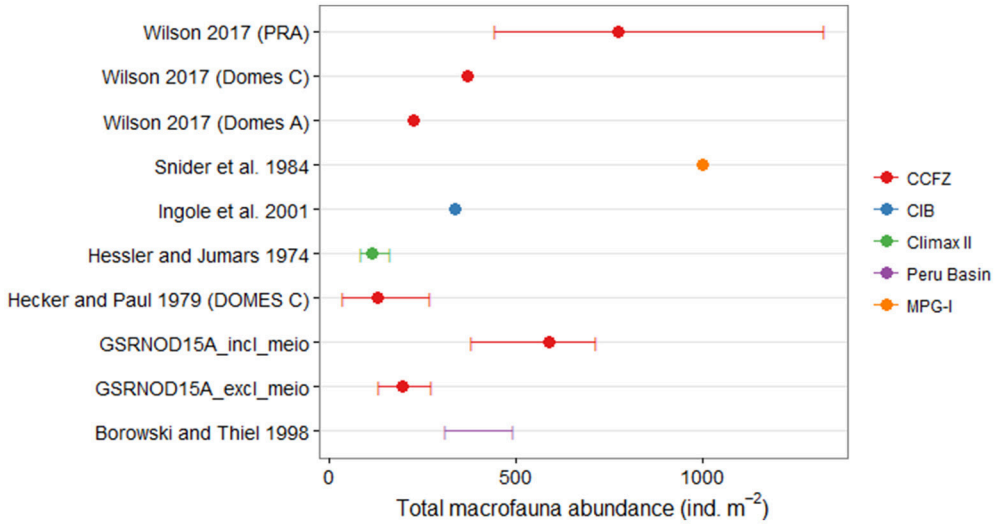


FIGURE 6 | Comparison of the average macrofauna abundances (ind.m^{-2}) and the abundance range (if available) between the current study (GSRNOD15A) and other macrofauna studies conducted at abyssal nodule sites. For the current study, abundances are reported for all macrofauna taxa both including and excluding typical meiofauna taxa (i.e., nematodes, copepods, ostracods, and kinorhynchs). Colors indicate the studied areas. The macrofauna study of Ingole et al. (2001) was the only one, besides this study, that involved typical meiofauna taxa. CCFZ, Clarion-Clipperton Fracture Zone; CIB, Central Indian Ocean Basin. Locations of the CCFZ macrofauna studies mentioned here are show in **Figure 1**.

and Jumars, 1974; Snider et al., 1984; Borowski and Thiel, 1998) and the Indian Ocean (Ingole et al., 2001), macrofauna abundances were determined both including and excluding the typical meiofauna taxa.

Excluding typical meiofaunal taxa, macrofaunal abundances observed in our study (average: 195 ind.m^{-2}, range: 132–272 ind.m^{-2}) fell within the lower to middle range of macrofaunal abundances reported for other nodule-bearing sites (**Figure 6**). The only other macrofaunal study that did not omit typical meiofauna taxa was that of Ingole et al. (2001), and the average macrofauna abundance (336 ind.m^{-2}) these authors reported was lower than the macrofaunal abundance including meiofauna in our study (average: 586 ind.m^{-2}, range: 380–712 ind.m^{-2}; **Figure 6**). The macrofauna at the Pacific MPG-I site (Snider et al., 1984) and at the PRA site (a region set aside and excluded from mining as a stable reference area; Wilson, 2016) exhibited aberrantly high abundance ($\geq$1,000 ind.m^{-2}), relative to other areas (**Figure 6**). Only Polychaeta and Isopoda were identified to the lowest possible taxon level, however, 100 different macrofauna taxa were identified from 12 box cores in the GSR license area. Hessler and Jumars (1974) collected 108 macrofaunal species in 10 box cores at the CLIMAX II site in the oligotrophic central North Pacific, compared to 381 macrofaunal species reported for the more productive DOMES sites in the eastern Equatorial Pacific (Hecker and Paul, 1979). Because different taxonomic resolutions and sampling efforts limit comparisons of taxon richness between this study and other macrofauna studies conducted at abyssal nodule sites, caution is recommended regarding the interpretation of the results.

Polychaetes were the predominant macrofaunal taxon at the nodule-bearing MPG-I (Snider et al., 1984) and CLIMAX II sites (Hessler and Jumars, 1974) in the northeast Pacific, and in nodule fields of the South Pacific (Borowski and Thiel, 1998; predisturbance conditions) and the Central Indian Ocean (Ingole et al., 2001; predisturbance conditions). Similarly, polychaetes comprised a considerable fraction (21–44%) of macrofaunal abundances in the GSR license area. Polychaete abundances in the PRA site (situated within zone A1 of the GSR license area), were elevated compared to the ECHO/DOMES C site (located in zone A3 of the GSR concession area; Paterson et al., 1998; Glover et al., 2002; Wilson, 2016), which likely results from the more productive waters in which PRA is located (Wilson, 2016). The polychaete taxon richness in the GSR license area (53 taxa identified) is higher than in the oligotrophic CLIMAX II site (46 species; Hessler and Jumars, 1974), slightly lower than the 69 species identified during the Kaplan project (Smith et al., 2008), and only half as high as the number of species in the DOMES A (104 species), PRA (100 species), and ECHO (113 species) sites (Paterson et al., 1998). The sampling intensity in the latter studies varied between 10 (CLIMAX II) and 47 (DOMES A) box core samples per site, and thus was much higher than in our study.

Comparison with other nodule-bearing sites revealed somewhat low macrofaunal abundance and taxon diversity in the GSR license area. The polychaetes and isopods sampled from the GSR license area were identified to lower taxonomical levels. However, identification of all macrofauna to a lower taxonomical level, preferably to species, would be required to fully characterize biogeographical patterns in the CCFZ (ISA, 2015). Despite the limited sampling in our study (12 box cores), we characterized a large fraction (59–85%) of the macrofaunal genus/species diversity (depending on the richness estimator used and the macrofauna taxon of interest) belonging to this specific habitat type (i.e., nodule rich sediments). However, a more realistic picture of macrofaunal abundance and diversity

in the habitat type under study requires more extensive sampling (e.g., increasing the number of deployments per site; Grassle, 1991) as indicated by the higher than observed species diversity estimates. Previous studies have shown that most species in the deep-sea macrofauna are rare (Grassle and Maciolek, 1992). Rare species have to be considered in any ecological study or management plan appreciating the overall importance of local-scale ecological interactions (Gage, 2004). Because rare species may represent transient propagules of populations well established elsewhere (Gage, 2004), they represent key elements of the ecosystem when considering colonization of previously disturbed (e.g., mined) habitats. The additional sampling effort necessary to capture all the estimated taxa in this study ranged from 5.47 (Isopoda) to 11.28 (total macrofauna) times the original sampling size (i.e., ranging from 66 to 135 box cores). Chao et al. (2009) suggest using the 95% fraction because it would encompass most taxa, with more realistic sampling objectives. The effort needed to reach this 95% fraction is substantially lower, ranging from 2.82 (Isopoda) to 3.96 (total macrofauna) times the original sample size (i.e., ranging from 34 to 46 box cores). This estimate of the number of replicates applies only to the specific habitat type. A more realistic view on abundance and species richness will have to consider habitat heterogeneity in the GSR license area. However, most abyssal studies never reach this number of quantitative replicates [e.g., in the CCFZ, Hecker and Paul (1979) collected 8 box cores in DOMES C, Paterson et al. (1998) collected 15 box cores from the ECHO site, Wilson (2016) report 16 boxcores from the PRA site and 50 boxcores from DOMES A (of which 24 in November 1977 and 26 in May 1978), and Smith et al. (2008) collected 13, 7, and 4 box cores in sites Kaplan East, Central and West, respectively]. Hence, as pointed out by Wilson (2016), capturing all species is unrealistic because of the logistics of processing large numbers of samples; working with smaller numbers of samples and using estimation techniques will therefore have to suffice.

Implications for Management

Notwithstanding differences in the sediment environmental variables and the POC flux to the seafloor, we observed a homogeneous macrofaunal community associated with polymetallic nodule areas over spatial scales of 10 to 100 s of km. This observed homogeneity in the macrofaunal community might dampen the expected mining impact on macrofaunal diversity at small scales through recovery from adjacent, unimpacted areas, however, a precautionary approach is recommended because of the large uncertainty in the spatial and temporal scale and intensity of deep-sea mining activities (Wedding et al., 2015). Therefore, prior to investigating the impact of (test) mining and the potential selection of protection zones, a better understanding of regional habitat heterogeneity and associated fauna should be achieved. In order to estimate this ecological variability in the area, habitat mapping is required (Cordes et al., 2016). Indeed, the macrofaunal community might be structured by differences in habitat heterogeneity between areas suited for mining and areas that are not mineable, such as differences in seabed slope and nodule parameters, which were not taken into account in our study. If the turnover of species between areas characterized by considerable slope (e.g., $\geq$3%) and relatively flat areas are found to be small, slope areas can be designated as preservation reference zones, preserving portions of diverse habitats and associated macrofaunal biodiversity, and ecosystem functioning.

CONCLUSION

One of the first insights in the abundance, diversity, and community composition of macrofauna associated with nodule areas in the GSR license area in the CCFZ is provided.

A homogeneous but diverse macrofaunal community associated with the sediment from polymetallic nodule areas was observed at a scale of 10 to 100 s of km.

Differences in the abundance and diversity of Polychaeta among sites can be explained by a decline in the estimated flux of POC along a southeast-northwest gradient, as well as by small differences in sediment characteristics and nodule abundance.

Despite the limited sampling in our study (12 box cores), we characterized a large fraction (59–85%) of the macrofaunal genus/species diversity belonging to the habitat type under study (i.e., nodule rich sediments).

A more realistic view on abundance and species richness will have to consider habitat heterogeneity in the GSR license area.

AUTHOR CONTRIBUTIONS

Conceived and designed the sampling design: EP and AV. Performed the sampling: EP and LC. Processed the samples: BD, TR, and PB. Analyzed the data: BD. Wrote the paper: BD, EP, TR, PB, and AV.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00103/full#supplementary-material

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Impaired Short-Term Functioning of a Benthic Community from a Deep Norwegian Fjord Following Deposition of Mine Tailings and Sediments

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The extraction of minerals from land-based mines necessitates the disposal of large amounts of mine tailings. Dumping and storage of tailings into the marine environment, such as fjords, is currently being performed without knowing the potential ecological consequences. This study investigated the effect of short-term exposure to different deposition depths of inert iron ore tailings (0.1, 0.5, and 3 cm) and dead subsurface sediment (0.5 and 3 cm) on a deep water (200 m) fjord benthic assemblage in a microcosm experiment. Biotic and abiotic variables were measured to determine structural and functional changes of the benthic community following an 11 and 16 day exposure with tailings and dead sediment, respectively. Structural changes of macrofauna, meiofauna, and bacteria were measured in terms of biomass, density, community composition and mortality while measures of oxygen penetration depth, sediment community oxygen consumption and ^{13}C-uptake and processing by biota revealed changes in the functioning of the system. Burial with mine tailings and natural sediments modified the structure and functioning of the benthic community albeit in a different way. Mine tailings deposition of 0.1 cm and more resulted in a reduced capacity of the benthic community to remineralize fresh ^{13}C-labeled algal material, as evidenced by the reduced sediment community oxygen consumption and uptake rates in all biological compartments. At 3 cm of tailings deposition, it was evident that nematode mortality was higher inside the tailings layer, likely caused by reduced food availability. In contrast, dead sediment addition led to an increase in oxygen consumption and bacterial carbon uptake comparable to control conditions, thereby leaving deeper sediment layers anoxic and in turn causing nematode mortality at 3 cm deposition. This study clearly shows that even small levels (0.1 cm) of instantaneous burial by mine tailings may significantly reduce benthic ecosystem functioning on the short term. Furthermore, it

reveals the importance of substrate characteristics and origin when studying the effects of substrate addition on marine benthic fauna. Our findings should alert decision makers when considering approval of new deep-sea tailings placement sites as this technique will have major negative impacts on benthic ecosystem functioning over large areas.

Keywords: submarine tailings placement, anthropogenic disturbance, deep sea, benthos, algal carbon, pulse-chase analysis, ^{13}C-labeling

INTRODUCTION

The extraction of mineral resources on land produces large amounts of fine waste material known as mine tailings (Jamieson, 2011). About 60% (iron) to 99.99% (gold) of the ore processed in mines is discarded as non-economic by-product resulting in an annual waste production of 14 billion tons of fine tailings worldwide (Jones and Boger, 2012; Vogt, 2013). While many solutions for the recycling of tailings have been proposed (Bian et al., 2012; Adiansyah et al., 2015), the vast majority is discarded in landfills, lakes, riverine systems, and the marine environment. The environmental and socio-economic consequences of tailings disposal can be devastating so that a proper management and sustainable use of mine tailings requires more attention (Franks et al., 2011; Bian et al., 2012; Adiansyah et al., 2015). For reasons of risk reduction on land as well as economic and esthetical considerations (Kvassnes and Iversen, 2013) the disposal of inert tailings material into streams and the marine environment known as riverine tailings disposal (RTD), submarine tailings placement (STP) and deep-sea tailings placement (DSTP) have been proposed and implemented (Vogt, 2013). But, due to the irreversible environmental impacts resulting from direct tailings discharge into riverine systems the use of this approach has seized and RTD is no longer implemented (Vogt, 2013). Submarine tailings placement, however, is allowed and applied at 14 mining sites worldwide (status in 2013) while new sites are still being targeted (Vogt, 2013). Currently, 0.6% of all industrial-sized mines discharge their tailings into the marine environment with Norway as main contributor (Vogt, 2013). In 2013, Norway, the country with most STP sites worldwide, had 7 operational STP sites, and 2 sites in a planning stage (Kvassnes and Iversen, 2013).

STP occurs mainly at continental margins at depths between 30 and 1,000 m or more including highly productive ecosystems such as fjords and canyons (de Leo et al., 2010; Vogt, 2013; Thurber et al., 2014). Continental margins are significant contributors to biodiversity and productivity and fulfil an important role for the provision of ecosystem services (Walsh, 1991). These include a wide range of regulatory services such as nutrient cycling, natural carbon sequestration and primary and secondary production, but also direct provisioning services such as fisheries and mineral or genetic resources (Armstrong et al., 2012; Thurber et al., 2014). Despite this key role, only few studies have assessed the impacts of mine tailings disposal on the functioning of bathyal benthic communities. The environmental impacts of STP can be manifold, including hyper-sedimentation, changes in grain size, smothering of benthic fauna and toxicity by the release of heavy metals or chemicals (Ramirez-Llodra et al., 2015). To prevent irreversible impacts on the environment it is of particular interest to get an understanding of the risks of STP and the magnitude of its impact on marine biota and ecosystem functioning. A large amount of scientific data is available in the "gray literature" from monitoring programs accompanying STP operations (Ramirez-Llodra et al., 2015). However, many of these monitoring studies only report impacts on one or few aspects of the ecosystem and often good baseline studies are lacking (Ramirez-Llodra et al., 2015). Furthermore, to be able to give recommendations for future environmental management with regard to land-based mining but also regarding possible future marine mining scenarios, it is crucial to investigate threshold values for tailings deposition. And although monitoring studies have incorporated the impact of tailings disposal at various distances from the outfall (Olsgard and Hasle, 1993) it is difficult to infer threshold values for the directly impacted communities as benthic fauna might naturally vary with depth and distance from the outfall due to changes in grain size or food availability. Especially the composition of meiofauna taxa and nematode species in particular and their vertical distribution in the sediment are strongly determined by abiotic factors such as grain size and sediment oxygenation (Jansson, 1967; Higgins and Thiel, 1988; Coull, 1999; Moodley et al., 2000b) making this group particularly vulnerable to sediment burial and disturbance.

Some important ecosystem functions e.g., organic matter remineralization or primary and secondary production are strongly driven by biotic factors such as biomass, diversity, or bioturbation (Lohrer et al., 2004; Danovaro et al., 2008; Braeckman et al., 2010). Moreover, different trophic levels and functional groups can exhibit tight interactions, and changes in the structure of one taxon can have strong repercussions on others which in turn influences ecosystem functioning (Gilbertson et al., 2012; Piot et al., 2013). Therefore, to fully understand the complexity of the processes shaping the structure and functioning of benthic ecosystems under various stressors, well-designed, controlled experiments resembling natural conditions as close as possible, including multiple trophic levels, and community-based approaches, are needed (Hale et al., 2011; Wernberg et al., 2012). Oxygen consumption is a good proxy for the depth-integrated overall remineralization of organic matter by benthic communities through aerobic and anaerobic processes (Moodley et al., 1998; Middelburg et al., 2005; Glud, 2008) and has been proven a very useful indicator in various impact studies (Vanaverbeke et al., 2008; e.g., Sweetman et al., 2010, 2014). Furthermore, the processing of organic matter can be traced through different trophic levels by introducing labile organic material with isotopically enriched carbon signatures (Middelburg et al., 2000; Boschker and Middelburg, 2002; van Oevelen et al., 2006a). In combination with estimates of biomass

and density it is also possible to assess the relative contribution of each biotic component to the observed functional changes (van Oevelen et al., 2006b).

To understand the potential environmental impacts of DSTP on the structure and functioning of soft-sediment communities and to assess threshold levels for tailings deposition, we conducted a microcosm experiment with soft-bottom fauna from a deep Norwegian fjord. For this purpose, natural, undisturbed sediments were incubated in the laboratory under *in-situ* conditions and subjected to three different levels of deposition with mine tailings. Structural changes of the macrofaunal, meiofaunal and bacterial communities were assessed using measures of biomass, densities, and diversity. Furthermore, mortality was assessed in macrofauna by "life-checking" and in meiobenthic nematodes by using a staining technique with Trypan Blue. Next to these structural impacts we investigated ecosystem functioning responses such as oxygen consumption dynamics and phytodetritus processing by the different biotic compartments making use of stable isotope tracing techniques. To isolate the effect of the tailings from deposition with natural sediment an additional experimental treatment (i.e., the deposition of dead sediment) was included in the experimental setup.

We hypothesized that (I) Exposure to burial with mine tailings will alter the benthic community structure on the short term due to mortality and changes in vertical community distribution; (II) Changes in benthic community structure due to tailings disposal will cause a reduced processing of organic matter as assessed from O_2 consumption, ^{13}C-labeled phytodetritus uptake and production of dissolved inorganic carbon and (III) The response of benthic organisms to tailings is different than to a deposition event with natural subsurface sediment.

MATERIALS AND METHODS

Study Site and Sediment Collection

Sediments were collected from 207 m depth in the Norwegian Hadangerfjord (59°43.48′N, 5°24.18′E, SW Norway) on board MS Solvik (October 28, 2014). Three sediment cores (∼14 cm) were subsampled from 14 boxcore deployments with a total of 42 subsampled sediment cores. The cores were transported to the laboratory at the International Research Institute of Stavanger (IRIS, Norway) and maintained in the dark at *in-situ* temperature (8°C) for acclimatization. The cores were continuously supplied with fresh, cooled and sand-filtered seawater (salinity: 33.02) from a nearby fjord by a flow-through system via gravity feed. Twelve cores were randomly assigned to the following treatments ($n = 4$): 0.1, 0.5, and 3 cm of mine tailing (MT) addition. Six cores were assigned to the following treatments ($n = 3$): 0.5 and 3 cm dead sediment (DS) addition. Inert, ground up tailings from a Norwegian iron ore mine were used for the MT treatments. For the DS treatment, subsurface sediment (<20 cm) was taken from the boxcores, temporarily frozen (−80°C for more than 3 days) to kill fauna and thawed prior to application. Four cores served as controls, i.e., no tailings or sediment was added. The remaining cores were used to measure granulometry of the natural sediment, for nematode staining tests and to determine the necessary amount of substrate required to build up to the target deposition thickness. The latter was experimentally determined as follows: Slurries of tailings and sediment were made from a known amount of material with the estimate to build 1 cm and were added to two separate spare cores. After 24 h of settlement the actual sediment height was measured and more tailings/sediment was added until a total build-up of 10 cm. From this information the required amount of tailings/sediment was calculated.

Experiment Set-Up and Incubation Procedure

To start the experiment, the respective amounts of substrate were added to the cores and were left for 24 h to allow settlement of particles. The different treatment thicknesses of the tailings addition could be easily distinguished from the Control (**Figure 1A**) with visual inspection as the mine tailings formed a dense, gray-reddish layer with a separation of fine, light particles on top and coarser, darker particles below (**Figures 1B–D**). This distinction was less clear for the dead sediment addition treatments (**Figures 1E–F**).

The incubation procedure involved a series of manipulations, measurements, and samples obtained at the various time points throughout the incubation duration of 11 and 16 days for the MT and DS treatments, respectively (**Figure 2**).

After substrate addition at T0, particles were allowed to settle for 24 h. One day after tailing addition (T1) the overlying water in the cores was clear indicating that full settlement of particles had taken place and oxygen penetration depth (OPD) in the sediment was determined. At day 8 (T8) and day 13 (T13) for MT and DS, respectively, rates of sediment community oxygen consumption (SCOC) were determined followed by a second OPD measurement. Subsequently, at day 9 (T9) for the MT and day 14 (T14) for the DS treatments, 31 mg (equivalent to 47 mmol C m^{-2}) dried *Skeletonema costatum*, that was enriched in ^{13}C (28% enrichment), was pipetted homogenously on the sediment surface in the cores and SCOC was measured once again. The isotopically enriched algae provided a tool to trace ^{13}C carbon throughout the benthic assemblage and enable quantification of fresh organic carbon processing. At day 10 (T10) for the MT treatments and day 15 (T15) for the DS treatments the experiment was terminated by sampling the sediment for faunal and sediment analyses. The different incubation durations for the two treatments are the result of logistic difficulties.

The Control followed the same time frame and sampling procedures as the MT treatments, thus, with an incubation time of 11 days.

After the experiment, sediments were sampled by inserting a smaller meiofauna core (∼5 cm) into the experimental core and the overlying water in the experimental cores was carefully siphoned off. The sediment surrounding the meiofauna core was sliced in three intervals (0–2, 2–5, and 5–10 cm) measured from the surface of the added substrate. Each layer was homogenized in a bucket and subsamples were taken with 30 mL syringes and immediately stored frozen at −21°C for analysis of sediment characteristics (porosity, total organic carbon, particulate organic

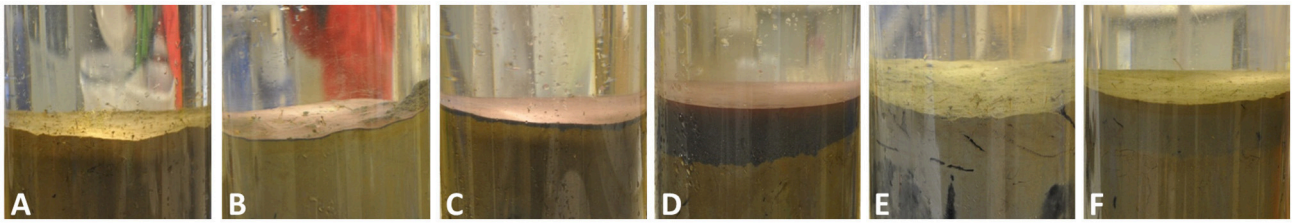


FIGURE 1 | Surface layers of the incubation cores 24 h after deposition. **(A)** Control **(B)** 0.1 cm mine tailings **(C)** 0.5 cm mine tailings **(D)** 3 cm mine tailings **(E)** 0.5 cm dead sediment **(F)** 3 cm dead sediment.

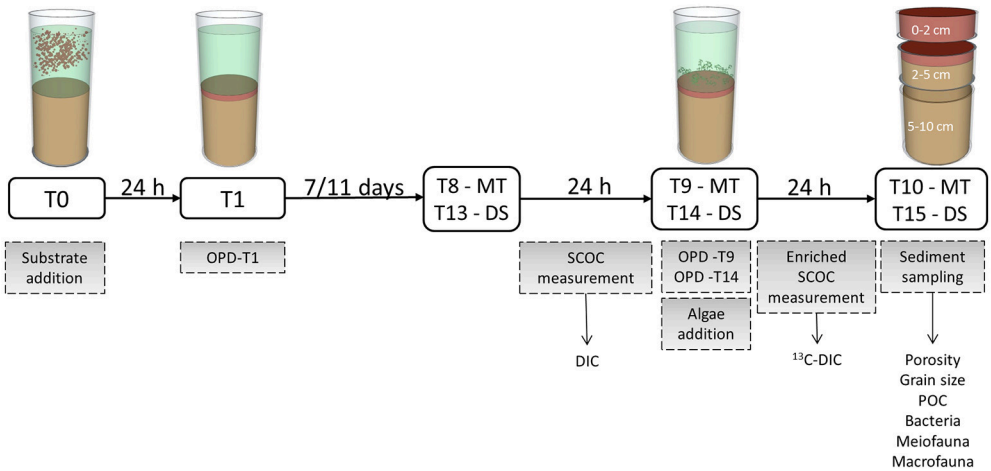


FIGURE 2 | Scheme of manipulations and samplings during the experiment. Treatments included the addition of mine tailings (MT) with 0.1, 0.5, and 3 cm thickness and the addition of dead sediment (DS) with 0.5 and 3 cm thickness. "T" Indicates the days of incubation after substrate addition. Actions and measurements are shown in gray boxes while arrows indicate from which measurements the different variables were obtained. Abbreviations: OPD, Oxygen penetration depth; SCOC, Sediment community oxygen consumption; DIC, Dissolved inorganic carbon; POC, Particulate organic carbon.

carbon) and bacterial specific phospholipid-derived fatty acids (PLFA). Sediment porosity was determined for each treatment and sediment layer by weight loss after freeze-drying. The sediment granulometry of two separate control cores, each sliced in 0.5 cm intervals and of one mine tailings sample were measured by laser diffraction with a Malvern Mastersize 2000 particle analyzer (Malvern Instruments, UK). Grain size classes were determined according to the Wentworth scale (Wentworth, 1922). After acidification, total organic carbon content (TOC) was quantified with a Thermo Flash EA 1112 elemental analyzer (Thermo Fisher Scientific, USA).

Analyses Determining Structural Changes of the Benthic Community

The remaining sediment from the experimental core was sieved over a 500 and 38 μm sieve. The macrofauna fraction (>500 μm) was qualitatively screened under a stereomicroscope ("life-check," see Moodley et al., 1997) before fixation in 4% formaldehyde to avoid abundance overestimates which could result from the lack of decomposition of dead organisms under low temperature conditions in a short-term experiment. In all samples, all encountered macrofauna specimens were found to be alive. The preserved samples were later identified to the lowest taxonomic level.

After siphoning off the overlying water of the meiofauna core, the sediment was sliced in intervals of 0.5 cm starting from the surface of the added substrate down to 2 cm depth of the natural sediment and in 1 cm intervals down to 5 cm depth. After slicing of the meiofauna cores, 5 or 10 mL of 4% Trypan blue solution (see Section Nematode Staining Test) were added to the 0.5 and 1 cm sediment slices, respectively, and samples were shaken vigorously before incubation for 2 h in sampling vials to ensure sufficient exposure with the stain. Subsequently, the samples were washed with filtered (10 μm) seawater on a 32 μm sieve until most of the stain was washed out and fixed on 4% buffered formaldehyde. Meiofauna was extracted from the sediment by washing the samples over two stacked sieves of 1 mm and 32 μm. The 32 μm fraction was subjected to density centrifugation with Ludox HS40 (Dupont) at 3,000 rpm [specific density of 1.18, (Heip et al., 1995)]. Centrifugation was done three times and the supernatant was sieved (32 μm) and fixed in 4% buffered formaldehyde. Meiofauna was identified to higher taxon level with a stereomicroscope (50x magnification) and nematodes were categorized in "stained" and "unstained". Copepods and their nauplii were removed from the analysis because those taxa

were found after sieving (32 μm) the seawater from the flow through system for 30 min. This may point toward the ability of copepods and nauplii to penetrate or inhabit the sand filter. No other meiofauna taxa or larger organisms were found after sieving the water. No meiofauna was found when checking a 30 mL subsample of mine tailings.

Biomass of the three biotic size classes (macrofauna, meiofauna, bacteria) is expressed as organic carbon content per area (mg C m^{-2}) and was directly calculated from the ratio mass spectrometer output (see Section Analyses Determining Functional Changes of the Benthic Community for Full Description).

Nematode Staining Test

Trypan blue is a dye commonly used in cell viability assessments (Louis and Siegel, 2011) that has already been successfully applied to assess soil nematode mortality (Womersley and Ching, 1989). In order to assess nematode mortality, a new staining protocol with Trypan blue was developed and tested prior to the experiment. To test the protocol, the upper 1.5 cm of two spare cores were sliced in 0.5 cm intervals and three sample vials containing slices of one core were submerged in hot water (±80°C) for 10 minutes to kill the meiofauna. After 2 h, all samples (dead and live) were stained with 3 mL of 4% Trypan blue solution (prepared with distilled water) and left to incubate for 2 h. In the live samples 16.42% ± 6.84 of nematodes were stained while in the dead samples this percentage was 77.75% ± 7.97. However, a proportion of 7.05% ± 1.13 and 13.27% ± 4.77 of the nematodes in live and dead samples, respectively, were left "uncategorized" due to an incomplete staining of the bodies.

Analyses Determining Functional Changes of the Benthic Community

Oxygen penetration depth (OPD) was determined by means of a microprofiler equipped with oxygen microsensors (50 μm tip; Unisense A.S., Denmark). After 2-point sensor calibration (0% calibration: Na_2SO_3; 100% calibration: air-bubbled seawater), oxygen concentration in each core (1 profile per core) was measured by penetrating in 100 μm steps into the sediment until oxygen concentration was below detection limit (0.3 μM).

SCOC was measured over a 24 h period in the dark in cores that were sealed off with lids that were fitted with a stirrer and an oxygen optode (PreSens, Germany). During this period the cores were disconnected from the water flow through system. Water samples of 10 mL (filtered through a 0.2 μm filter and conserved by the addition of 10 μL $HgCl_2$) were taken in headspace vials at the beginning and at the end of the incubation for later analysis of the dissolved inorganic carbon (DIC) flux and ^{13}C-DIC measurements. For these analyses a headspace of ∼1.5 ml was created by injecting Helium gas through a septum. The samples were subsequently acidified with 20 μL concentrated H_3PO_4 to transform DIC into gaseous CO_2. A 500 μL sample of the CO_2 was then injected into a HP 61530 gas chromatograph (Hewlett-Packard/Agilent, USA) connected to a DELTA-Plus Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA) to determine DIC concentrations and ^{13}C composition as described by Moodley et al. (2000a). The ^{13}C composition in the freeze-dried, grinded sediment subsamples was quantified with a Thermo Flash EA 1112 elemental analyzer (Thermo Fisher Scientific, USA) coupled with an DELTA V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA).

The >500 μm macrofaunal fraction was grouped in the following taxonomic groups: Echinodermata, Mollusca, Polychaeta, Sipunculida. Within each sample, each taxonomic group was individually freeze-dried to determine total dry weight. After homogenization, a weighted subsample was taken for isotope and biomass analysis (as described above for sediment, e.g., Moodley et al., 2005). In two samples, one individual or pieces of a sea pen (Pennatulacea) were found which were removed from the stable isotope analysis. The meiofauna fraction (>38 μm) of the experimental core sediment was fixed on 4% buffered formaldehyde for stable isotope analysis of nematodes. For this purpose 130 randomly hand-picked nematodes per sample were transferred to a few drops of Milli-Q water in silver capsules (8 × 5 mm) that had been pre-combusted for 4 h at 450°C. The nematode samples were dried overnight at 60°C, acidified with 20 μl 2% HCl and dried on a hot plate at 60°C. After acidification the samples were closed and bulk C and N content as well as δ^{13}C and δ^{15}N were measured as described for the sediment.

Bacterial tracer uptake and biomass was based on concentrations of bacteria specific PLFA (i14:0, i15:0, a15:0, i16:0, and 18:1ω7c; Middelburg et al., 2000). These PLFAs were extracted from freeze-dried, grinded sediment using the Bligh and Dyer method (Bligh and Dyer, 1959) according to the protocol by Boschker (2004). Subsequently the extracted PLFAs were derivatized to fatty acid methyl esters (FAME) that were analyzed by GC/C-IRMS (HP 61530 gas chromatographer, Hewlett-Packard/ Agilent, USA; DELTA-Plus Isotope Ratio Mass Spectrometer, Thermo Fisher Scientific, USA) with a polar analytical column (ZB5-5MS; 60 m length, 0.32 mm diameter, 0.25 μm film thickness; Phenomenex, USA).

The incorporation of ^{13}C into macrofaunal, nematode and bacterial biomass was calculated as described by different papers (Middelburg et al., 2000; Moodley et al., 2002; van Oevelen et al., 2006a; Guilini et al., 2011). Total algal tracer uptake is expressed as the quotient of total ^{13}C uptake (I) and the ^{13}C content in *S. costatum* (28%) (see Moodley et al., 2002).

Statistical Analyses

To include the depth dependency of samples, all data sets containing depth information were analyzed using a Permanova analysis with a nested design allowing statistical comparison between treatments and within depth layers (Primer software version 6.1.11 with the Permanova+ add-on, Clarke and Gorley, 2006; Anderson et al., 2008). **Table 1** shows the Permanova designs for the various variables. When the main test yielded significant differences pairwise tests were performed and, if the number of possible permutations was lower than 100, Monte Carlo tests were applied to estimate *p*-values (p(MC)) with increased accuracy. Graphs were computed using Prism 6 (GraphPad Software, Inc.). Throughout the manuscript, data is reported as mean and standard error, unless mentioned otherwise.

TABLE 1 | Permanova designs for the analysis of various parameters.

Parameter	Factor	Nested in	Fixed/Random
Porosity, TOC	Treatment		Fixed
Faunal densities, diversity and community structure	Depth		Fixed
Biomass of macrofauna, meiofauna and bacteria	Core ID	Treatment	Random
Uptake of macrofauna, nematodes and bacteria			
OPD; SCOC	Treatment		Fixed
	Time		Fixed
	Core ID	Treatment	Random

As grain size was measured in one spare core it was not analyzed statistically. Porosity and TOC were tested with Permanova for differences between treatments and the control within each layer.

Standardized densities in individuals per m^2 and individuals per 10 cm^2 were used for macro- and meiofauna analysis, respectively. Total (whole core) meiofauna and macrofauna densities were analyzed using ANOVA in R (R.Core Team, 2013) to assess differences between treatments after the assumptions of normality and homogeneity of variances were assured. Pairwise comparisons were conducted by means of the TukeyHSD test. For reasons of better comparison, statistical analyses were done on data from the sediment intervals of 0–2, 2–5, and 5–10 cm starting at the added substrate surface for macrofauna and bacteria and on 0–2 and 2–5 cm intervals for meiofauna. Nematode distribution and mortality on a high depth interval resolution was analyzed graphically and descriptively. The contribution of specific macro- and meiofauna taxa to differences in their community composition was determined by a similarity of percentages (SIMPER) analysis. Shannon and Simpson diversity indices and Pielou's evenness index were calculated for meio- and macrofauna densities and tested in terms of whole core diversity (ANOVA) and per depth layer (Permanova).

Due to the repeated measures character of OPD and SCOC data a Permanova analysis was used to test for differences between the factors Treatment and Time (Permanova design, **Table 1**). Univariate data of DIC was analyzed using ANOVA to assess differences between treatments after the assumptions of normality and homogeneity of variances were assured. Total (whole core) tracer uptake of the different biotic compartments was analyzed with Permanova as assumptions for normality and homogeneity of variances were not met. Depth layer information was included with Permanova as described above.

RESULTS

Effect of Substrate Addition on Abiotic Variables

The natural sediment was composed of fine silt characterized by a median grain size of 11.94 ± 0.34 μm in the 0–2 cm layer, 12.26 ± 0.13 μm in the 2–5 cm layer and 12.59 ± 0.56 μm in the 5–10 cm layer. In contrast, the mine tailings were composed of very fine sand with a median grain size of 101.31 μm. The difference in median grain size between the mine tailings and the natural sediment was also reflected in the lower porosity of the added layers of tailings compared to the natural sediment [Permanova, $p(MC) \leq 0.003$ **Figure 3A**]. Additionally, TOC was lower in the layers with added tailings compared to control cores [Permanova, $p(MC) \leq 0.013$, **Figure 3B**].

Structural Changes of the Benthic Community

Total macrofauna densities ranged from 12,971 ± 3,573 ind. m^{-2} in the 3 DS treatment to 20,040 ± 2,780 ind. m^{-2} in the 0.1 MT treatment. Though total densities did not significantly differ between treatments, a decreasing trend with sediment depth was observed. Within each sediment depth layer community composition was similar between treatments and control. Within each mine tailings treatment and the control, the community composition in the 0–2 cm layer was different from the deeper layers [Permanova, $p(MC) \leq 0.0400$]. Main contributors to the dissimilarities between the 0–2 and 2–5 cm and between the 0–2 and 5–10 cm layers were *Kelliella militaris* (bivalve, 6.5%, 7.23%), *Macrochaeta* sp. (polychaete, 4.11%, 4.45%), *Ophelina modesta* (polychaete, 3.39%, 3.84%), *Mendicula pygmaea* (bivalve, 3.27%, 3.54%), *Sipuncula* sp. (2.14%, 4.05%) and *Nucula nitidosa* (bivalve, 2.83%, 3.46%) with much higher densities in the top (0–2 cm) layer and *Paramphinome jeffreysii* (polychaete, 4.26 %, 3.67 %) and *Levinsenia gracilis* (polychaete, 2.27%, 3.67%) showing the opposite trend. This depth effect in community composition was not observed in the DS treatments, thus, all organisms were distributed similarly throughout those cores.

Total meiofauna densities (including stained and unstained nematodes) were significantly lower (712 ± 252 ind. 10 cm^{-2}) in the 3 DS treatment compared to the control (2080 ± 93 ind. 10 cm^{-2}, TukeyHSD, $p = 0.0115$). This difference was attributed to a reduction in nematode densities (TukeyHSD, $p = 0.0105$) which was the most abundant taxon (94.91 ± 0.34 % of meiofauna community). Nematodes were found in high densities in all surface layers, indicating that the animals were able to move into the added MT and DS substrate (**Figure 4**). Interestingly, mortality in the MT treatment was substantially higher as compared to the controls (**Figure 4**), with mortality exceeding 80% in the 0.5 MT and 3 MT treatment. Furthermore, mortality in the 3 DS treatment was high throughout the whole core and not only in the added substrate layer (17.74–63.56%). The addition of mine tailings had no effect on the whole-core meiofauna community composition (faunal mortality not taken into account). In both DS treatments, however, it differed from the control [Permanova, $p(perm) \leq 0.0073$] due to reduced densities of nematodes, oligochaetes, and polychaetes (SIMPER). When taking depth layers into account we observed that all taxa were equally successful in migrating into the mine tailings since community composition remained similar in the Control and MT treatments within the upper 2 cm. Also within the deeper layer (2–5 cm) meiofauna community composition remained similar in all treatments when compared to the

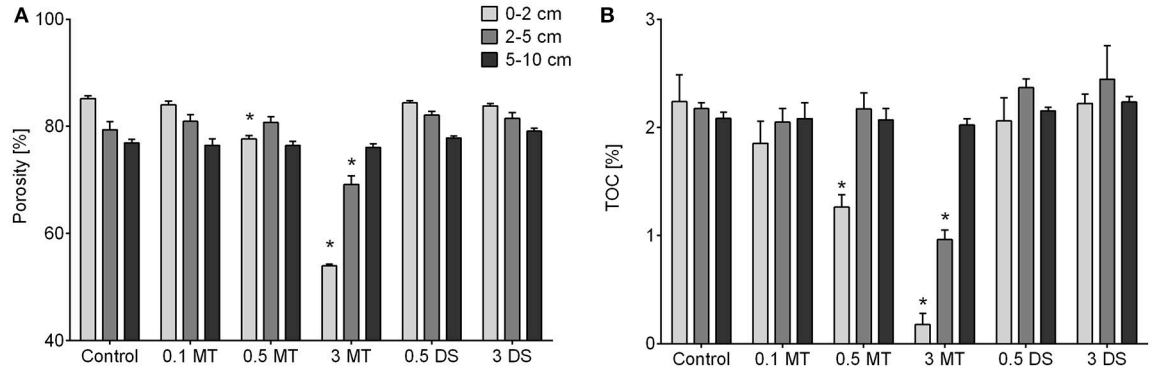


FIGURE 3 | (A) Porosity and **(B)** total organic carbon (TOC) content of all depth layers per treatment. Error bars denote standard error and an asterisk indicates significant ($p < 0.05$) differences with the control. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition.

Control [Permanova, p(perm) $\leq$ 0.0162]. Interestingly, within each treatment, meiofauna community composition differed between depth layers (0–2 and 2–5 cm) for all treatments except the 3 MT and 3 DS treatments [Permanova, p(MC) $\leq$ 0.049]. Differences could be attributed to lower abundances of Kinorhyncha, Ostracoda, Nematoda, and Polychaeta in the 2–5 cm layer (SIMPER). Overall meiofauna higher taxon diversity and evenness were low and did not differ between treatments with the exception that evenness was significantly higher in the upper layer of the 3 DS treatment compared to the control due to reduced nematode densities and, thus, reduced dominance of this taxon. Diversity and evenness did not differ between treatments.

In the control situation, total biomass was highest for bacteria (9824.93 $\pm$ 1503.20 mg C m^{-2}) followed by macrofauna (716.47 $\pm$ 109.45 mg C m^{-2}) and nematodes (413.67 $\pm$ 90.01 mg C m^{-2}; **Figure 5**). The high variability in macrofaunal biomass did not reveal any differences between treatments or depth. For bacteria and nematodes, however, the addition of 3 cm of mine tailings resulted in a reduced biomass in the 0–2 cm layer compared to the control [Permanova, p(MC) $\leq$ 0.0341]. Furthermore, nematode biomass was reduced in the upper layer of the 3 DS treatment and the 2–5 cm layer of the 0.5 MT treatment [Permanova, p(MC) $\leq$ 0.0197].

Functional Changes of the Benthic Community

Under control conditions oxygen penetrated 1.17 $\pm$ 0.11 cm deep into the sediment at the start of the experiment and remained stable throughout the experiment (**Figure 6**). The OPD of the 3 cm mine tailings treatment was 2.98 $\pm$ 0.54 cm, which means that oxygen did not reach beyond the tailings, leaving the natural sediment anoxic. Moreover, the OPD decreased to 1.49 $\pm$ 0.18 cm at the end of the experiment [Permanova, p(perm) = 0.0098]. The deposition of dead sediment resulted in a shallower OPD compared to the Control (0.61 $\pm$ 0.05 cm and 0.57 $\pm$ 0.06 cm for 0.5 DS and 3 DS, respectively) after the deposition event [Permanova, p(MC) $\leq$ 0.036], but gradually deepened and approached control conditions at the end of the experiment.

After 8 (MT) and 13 (DS) days of incubation, the sediment community oxygen consumption (SCOC) was reduced in the 0.5 and 3 MT treatments compared to the Control [Permanova, p(perm) $\leq$ 0.0236] while in the 3 DS treatment SCOC increased [Permanova, p(perm) = 0.0342, **Figure 7**]. The SCOC increased in all treatments after addition of the algal tracer [Permanova, p(perm) = 0.0001, **Figure 7**].

A large fraction of 85.21 $\pm$ 1.40 % of the added algal carbon was not processed and remained in the form of particulate organic carbon (POC) in the sediment, especially in the 0–2 cm layer (**Figure 8A**). Of the added algal carbon 9.70 $\pm$ 1.01 % was respired into dissolved inorganic carbon (DIC; **Figure 8B**). No significant differences in total ^{13}C-POC or algal ^{13}C-DIC were found between treatments.

Total tracer-C uptake was reduced in the 3 MT treatment for nematodes and macrofauna (Permanova, $p \leq 0.0038$) and for bacteria (Permanova, $p = 0.0523$, borderline significant, **Figures 8C–E**). Additionally, uptake by nematodes was lower for the 0.5 MT, 0.5 DS, and 3 DS treatment [Permanova, p(MC) $\leq$ 0.0365]. When taking depth into account, a significant decrease in tracer uptake by nematodes was observed in the top layer (0–2 cm) of the MT and DS treatments. In general, uptake was highest in the upper 2 cm for bacteria and nematodes while macrofaunal uptake was high in the upper 5 cm with significantly lower values in the 5–10 cm layer (p(MC) < 0.01).

DISCUSSION

With the continuing STP and prospect of new STP sites from land based mining facilities in many locations, but also with the perspective of deep-sea mineral extraction (e.g., massive sulfides and polymetallic nodules) and associated disposal of waste sediment, there is an urgent need for quantitative assessments of the environmental impact of tailings deposits in marine environments (Mengerink et al., 2014; Ramirez-Llodra et al., 2015; Levin et al., 2016). Due to the slow growth and long life spans of many deep-sea taxa (Young, 2003) they are particularly vulnerable to the impacts associated with tailings placement

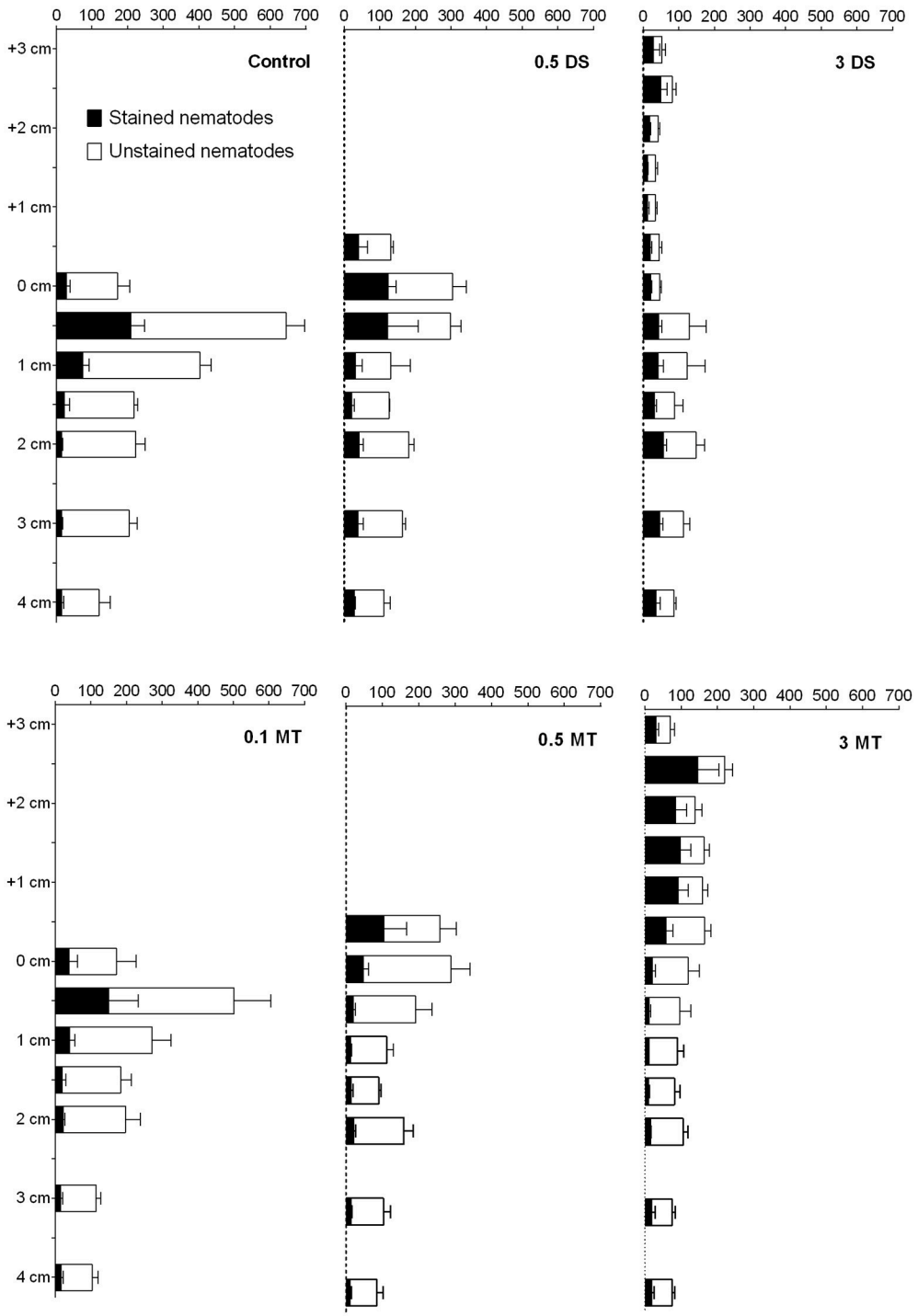


FIGURE 4 | Mean abundances (x-axis) of stained (dead) and unstained (alive) nematodes in the different substrate addition treatments. Error bars indicate standard error. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition.

such as hyper sedimentation, changes in grain size, or toxicity effects (Kvassnes and Iversen, 2013). To our knowledge, this experimental study is the first of its kind to investigate the impacts of different levels of inert mine tailings and sediment deposition on both, structure and functioning, of upper bathyal soft-bottom communities.

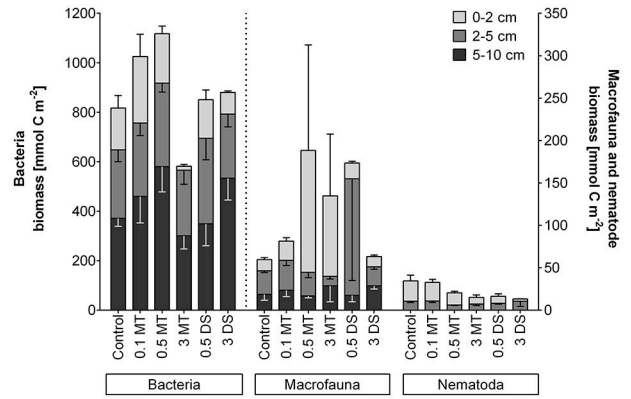


FIGURE 5 | Biomass per depth and treatment in the three biological compartments measured: Bacteria (left y-axis), macrofauna and Nematoda (right y-axis). Error bars depict standard errors and point downwards for the 2–5 cm and 5–10 cm data for better visualization. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition.

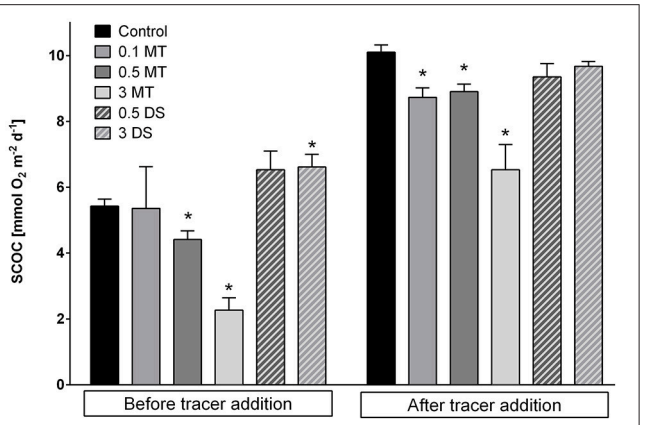


FIGURE 7 | Mean sediment community oxygen consumption (SCOC) in mmol O_2 $m^{-2}d^{-1}$ before and after tracer addition for the different substrate addition treatments. Error bars denote standard error and an asterisk indicates significant ($p < 0.05$) differences with the control. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition.

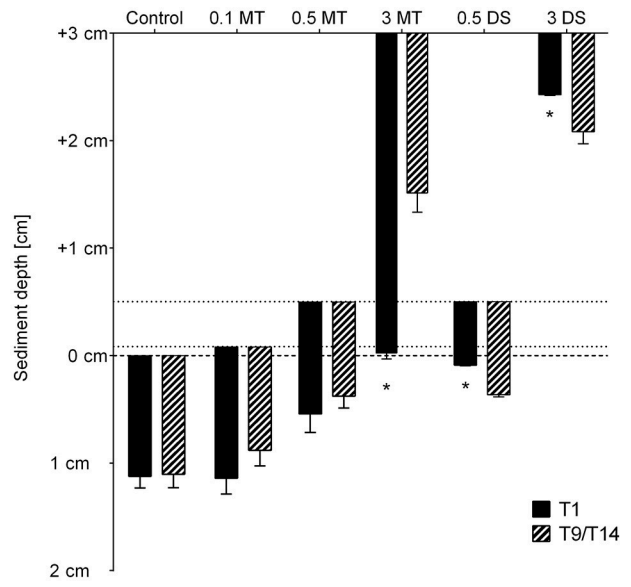


FIGURE 6 | Oxygen penetration depth (OPD) in the sediments of the different treatments one day after settlement of the substrate (T1) and at the end of the experiment (T9/T14). Error bars denote standard error and an asterisk indicates significant ($p < 0.05$) differences with the control. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition. The dashed line at y = 0 represents the natural sediment surface while above this layer the added substrate surface is indicated.

Substrate Addition Induces Structural Changes of the Benthic Community

Sediment characteristics substantially determine the composition of benthic assemblages and while meiofauna communities are strongly influenced by sediment grain size (Jansson, 1967; Coull, 1999), macrofauna community structure is most likely governed by factors of e.g., food availability, sediment oxygenation, and biotic interactions rather than grain size (Snelgrove and Butman, 1994; Seiderer and Newell, 1999). In our experiment, the physical modifications from substrate addition were visible in the different properties of the new, added substrates with much coarser grain size, lower porosity and a low TOC in the mine tailings and relatively high TOC in the dead sediment. These results confirm the successful addition of both substrates and physical modification of the upper sediment layers.

Furthermore, the addition of each of the two substrates immediately changed oxygen conditions in the natural sediment. The two most extreme treatments (3 MT and 3 DS) resulted in anoxic conditions of the natural sediment throughout the entire experiment duration. The first hypoxia related response of benthic invertebrates is migration to more suitable areas in the sediment, although successful migration strongly depends on the mobility of the individual taxa or species and the properties of the added substrate (e.g., grain size, porosity; Jansson, 1967; Maurer et al., 1986; Hendelberg and Jensen, 1993; Diaz and Rosenberg, 1995; Wetzel et al., 2001). Accordingly, we observed migration into the added substrate of most metazoan taxa and an upward shift in meio- and macrofauna community composition in all treatments. Successful migration of benthic organisms into added, non-native substrates is well-known (Maurer et al., 1986; Schratzberger et al., 2000; Bolam, 2011), however, the upward shift in our experiment was accompanied by a high nematode-mortality in both substrate additions. Without the applied nematode staining technique this observation would have been missed and the impacts of substrate addition on nematodes would have been underestimated.

In the mine tailings, nematode mortality was possibly linked to a strongly reduced food availability. Compared to other taxa,

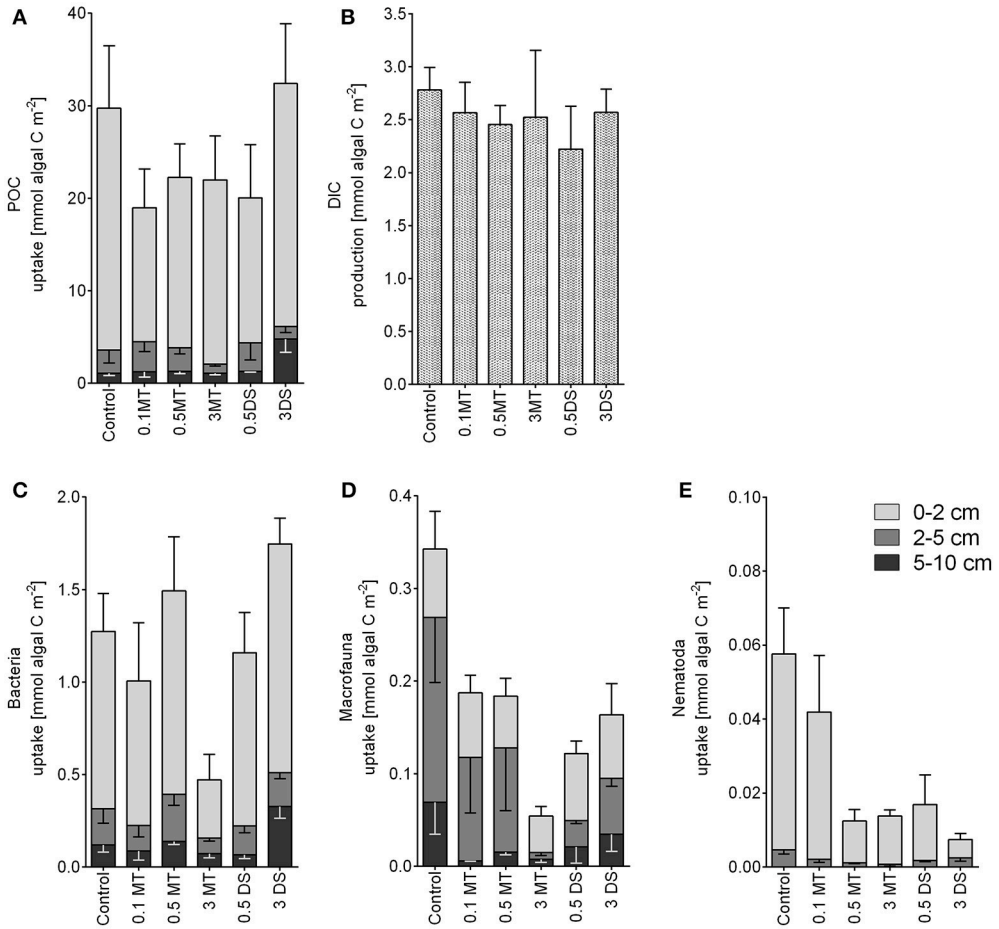


FIGURE 8 | Particulate organic carbon (POC, A) per treatment and depth; **(B)** dissolved inorganic carbon (DIC, B) per treatment; and uptake of algal ^{13}C per depth and treatment of **(C)** Bacteria, **(D)** Macrofauna and **(E)** Nematoda. Error bars depict standard errors and point downwards for the 2–5 cm and 5–10 cm data for better visualization. Abbreviations: 0.1 MT = 0.1 cm mine tailing addition, 0.5 MT = 0.5 cm mine tailing addition, 3 MT = 3 cm mine tailing addition, 0.5 DS = 0.5 cm dead sediment addition, 3 DS = 3 cm dead sediment addition.

nematodes are often reported to be able to survive situations of severe stress, such as temporary hypoxia or seawater acidification (Josefson and Widbom, 1988; Widdicombe et al., 2009; Schade et al., 2016) and the capability to change feeding modes to some extent may be advantageous in those situations (Moens and Vincx, 1997). Bacterial growth strongly depends on the availability of organic matter (Danovaro, 1996; Kirchman and Rich, 1997; Eiler et al., 2003) so despite the fact that the tailings were well oxygenated, the low organic carbon content did not support strong bacterial growth resulting in very low food availability for meio- and macrofauna inside the added layer. The survival of nematodes in low food conditions is very species dependent but generally an incubation time of 10 or more days, as applied here, is sufficient to set the worms at their limit (Ott, 1972; Ott and Schiemer, 1973; Wieser et al., 1974).

In contrast, the mechanism behind the nematode mortality and reduced densities in the DS treatment is likely different from that of the MT treatment. High bacterial biomass and total organic carbon in the added dead sediment, compared to conditions in the control, should have led to higher concentrations of food for the nematodes. Nevertheless, we observed a strong reduction in nematode densities and high proportions of dead nematodes in all layers throughout the core. Here, the cause is likely linked to the anoxic conditions in the sediment produced by the high bacterial activity in terms of carbon uptake and resulting increased oxygen demand. In a laboratory experiment with an intertidal meiofauna community, Steyaert et al. (2007) found that suboxic and anoxic conditions for 14 days led to a decrease by about a third in nematode densities with species specific survival. Similarly, other studies report hypoxia associated mortality of nematode fauna and shifts in meiofauna community composition toward hypoxia adapted species (Hendelberg and Jensen, 1993; Moodley et al., 1997; Van Colen et al., 2009; Wetzel et al., 2001). Low nematode densities in the 3 DS treatment possibly result from a combination of the high mortality and decomposition of dead nematodes, potentially also facilitated by the longer incubation time in the dead sediment treatments.

Macrofaunal responses were less obvious and organisms showed no signs of mortality. Nevertheless, while tailings addition resulted in an upward shift of the entire macrofauna community, dead sediment addition resulted in a less clear distinction between surface and subsurface community composition, compared to the control situation. The stress response of macrofauna is species-specific and depends on their mobility, oxygen requirements, and feeding type (Chou et al., 2004; Hinchey et al., 2006; Smit et al., 2008). In a metanalysis, Smit et al. (2008) predicted from marine species sensitivity distributions that instantaneous burial with 5.4 cm of natural sediment would negatively affect about half of the 32 analyzed macrofauna species. Furthermore, burial with 0.63 cm already affected 5% of the tested macrofauna species. Substrate addition and especially tailings addition may lead to emigration or death of certain macrofaunal organisms on a longer term if requirements of oxygen and food availability are not met. While several studies on shallow water ecosystems report on the effects of burial with sediment following dredging (Bonvicini pagliai et al., 1985; De Grave and Whitaker, 1999; Thrush and Dayton, 2002; e.g., Bolam, 2011) or strong hydrodynamic disturbances (Miller et al., 2002 and citations therein; e.g., Dernie et al., 2003), we need to be cautious in comparing these results with tailings burial. Indeed, as observed in this study, the specific characteristics of tailings in terms of grain size, organic matter content and porosity (disregarding any toxicological properties) compared to the natural sediment led to differing structural responses and community functioning (see Section Community Functioning Changes as a Result of Structural Changes Induced by Substrate Deposition).

The differential response of meiofauna and macrofauna to low oxygen concentrations and starvation contradicts the general perception that the meiofauna are more resistant to different stressors than macrofauna. Many authors studying the effect of hypoxia on benthic communities report a more negative effect of long hypoxic events on macrofauna, including mortality, whereas meiofauna is generally less affected and can withstand long hypoxic events (Weigelt and Rumohr, 1986; Josefson and Widbom, 1988; Diaz and Rosenberg, 1995; Van Colen et al., 2009). However, these studies focused on hypoxia in the water column which was not the case in our experiment where the overlying water was always well oxygenated. Here, the greater capacity of macrofauna to move vertically in the sediment could have enabled organisms to reach oxygenated layers as well as food-rich surface layers ensuring their survival. The loss of distinction between the macrofaunal community composition in different depth layers in the dead sediment treatments could be a first indication to support this hypothesis. Thus, while meiofauna could not compensate the very rapid occurrence of anoxic conditions in the sediment by vertical migration, macrofauna may have been more successful in doing so.

Despite a comparatively low standing stock, the high activity and life cycle turnover rates of meiobenthic organisms make them a particularly important part of the benthic environment when it comes to biomass production and food consumption (Gerlach, 1971; Coull, 1999). Furthermore, they can exert a strong impact on other benthic organisms by enhancing bacterial productivity (Gerlach, 1978) and influencing macrobenthic species interactions which can result in modified ecosystem properties (Piot et al., 2013). Therefore, the high nematode mortality induced by substrate burial may lead to strong repercussions on other benthic organisms on a longer term.

Community Functioning Changes as a Result of Structural Changes Induced by Substrate Deposition

In this study we observed strong, negative effects of substrate addition on the benthic community structure in terms of biomass and composition. These changes led to adverse effects of benthic functioning in terms of respiration rates and organic matter processing. Oxygen is a key element in the aerobic respiration and metabolism of organisms and, thus, tightly linked to the mineralization of organic carbon and the activity of benthic organisms. Therefore, OPD and SCOC can provide a reliable indication of organic matter remineralization rates (Moodley et al., 1998) and, combined with stable isotope carbon uptake data of the biota, has proven to be a good tool to assess ecosystem functioning and responses of the benthos to environmental disturbances (Bratton et al., 2003; Sweetman et al., 2010, 2014, 2016).

Immediately after settlement of the added substrates, a shift in OPD occurred in most treatments. OPD did not decrease at 0.1 and 0.5 cm tailings addition but shifted upwards leaving previously oxygenated deeper sediment layers anoxic. Furthermore, despite an increase in OPD at 3 cm of tailings addition compared to the control, the underlying, previously oxygenated sediment became anoxic because oxygen did not penetrate through the mine tailings layer to the natural sediment anymore. Similarly, Cummings et al. (2009) observed an upward shift of the OPD when marine sediments were exposed to very thin layers of terrestrial sediments. In this study OPD (measured throughout 3 days) penetrated ± 0.7 mm in the control situation, while after the addition of ± 1.1 mm of terrestrial sediment oxygen only penetrated ± 0.3 mm into the underlying sediments. Thus, comparable to our study, organisms inhabiting the surface sediment will become exposed to a deterioration of biogeochemical conditions after the addition of a non-native substrate. In our experiment, however, OPD in the tailings treatments became shallower toward the end of the experiment possibly by a higher biogenic activity inside the tailings due to faunal migration. Sediment deposition resulted in a decreased OPD at the start of the experiment that might be linked to strong microbial respiration due to high organic matter contents of the added sediment. Also in this case, underlying sediment layers were exposed to biogeochemical changes negatively affecting structure and functioning of biota. Toward the end of the experiment OPD deepened to values comparable to the control indicating a possible stabilization of the biogeochemical conditions in the dead sediment treatments to a pre-disturbance state.

Values of SCOC in our control incubations were comparable to those reported in other studies with Norwegian fjord fauna (Ishida et al., 2013; Sweetman et al., 2014, 2016). The SCOC

measurements after 8 and 13 days of incubation (MT and DS, respectively) informed about the effect of substrate addition on the SCOC, while the second measurement informed about the response of the sediment community to input of algal detritus. Mine tailings burial reduced oxygen consumption in the 0.5 and 3 cm treatments, but SCOC increased in the dead sediment treatments when compared to the control. Low organic matter content associated with low bacterial biomass and faunal mortality may explain the reduced SCOC measurements in the mine tailings treatments. On the contrary, high organic matter content and an increase in SCOC in the dead sediments may point toward increased bacterial activity as they play a key role in the carbon turnover in marine sediments (Rowe and Deming, 1985; Deming and Baross, 1993). However, in the dead sediment treatments, addition of fresh organic matter in the form of labeled algae did not lead to a pronounced increase in oxygen consumption as it did in the mine tailings treatments and the control. This is possibly due to the already high rate of organic matter processing and strong bacterial respiration resulting from the organic matter input originating from the dead sediment itself. It is important to note that SCOC increased in all treatments after algae addition but, at the same time, the negative effect of tailings addition on the processing of a new food source became more pronounced when compared to the control situation. This way even a deposition of 0.1 cm sufficed to induce a significant reduction in SCOC, illustrating that the benthic community was hampered to process fresh organic matter by as little as 0.1 cm of tailings.

Bacteria dominate deep-sea ecosystems in terms of abundance and biomass and are the main contributors to organic matter remineralization (Wei et al., 2010; Danovaro et al., 2014). Similarly, in our experiment we observed that bacteria had a higher biomass and took up considerably more added algal carbon compared to macro- and meiofauna. Interestingly, at 0.1 cm tailings addition, bacterial tracer uptake and biomass remained close to control conditions while a decreasing trend of tracer uptake was already visible for macro- and meiofauna. Here, the tailings layer may have posed a physical barrier for those organisms to reach the new source of organic matter present on the sediment surface. At 3 cm tailings addition, low bacterial biomass and possibly reduced faunal activity in terms of bioturbation lead to a lower fraction of the added algae being transported to deeper layers and an overall reduced tracer uptake. Bioturbation by infauna strongly influences ecosystem functioning, especially in sediments where disturbances are low, as it provides structure to the sediments and is responsible for irrigation, transport of nutrients and organic matter to deeper layers and providing various microhabitats for meiofauna and bacterial communities (Mermillod-Blondin et al., 2004; Meysman et al., 2006; Braeckman et al., 2010).

Continental margins are responsible of 10–15% of the global ocean primary production and fulfil an important role in the sequestration of atmospheric carbon and transport to the deep-ocean (Muller-Karger et al., 2005; Fennel, 2010). Furthermore, with high denitrification rates these regions adjacent to land boundaries act as a barrier for nitrogen input from land and atmosphere into the open ocean (Fennel, 2010). This disproportional contribution to the total ocean nutrient cycling is the result of tight biological interactions, biogeochemical transformations facilitated by microorganisms and characteristic hydrodynamics (Renaud et al., 2007; Hofmann et al., 2011). Therefore, disturbing these ecosystems by activities such as mine tailings placement, can have implications on a much larger scale.

The Origin of the Added Substrate Results in Differential Responses

This study clearly illustrated how both substrates used in this experiment resulted in differential responses. Mine tailings addition mainly induced food-limitation for all benthic compartments in the added layers, whereas the high bacterial respiration in the dead sediment layer initially led to oxygen limitation in deeper layers. In the sediment addition treatments, oxygen conditions seemed to return to conditions similar to the control indicating a possible biochemical recovery of the sediments to normal conditions after the 15 day period. With bacterial and macrofaunal biomass and uptake being similar to control conditions it is possible that those taxa might recover relatively fast. Meiofauna, however, suffered most in this scenario with strongly reduced densities, low carbon uptake, and increased mortality. The interconnectedness of the three benthic compartments is widely acknowledged and changes in one taxon can have strong repercussions on the other (Gerlach, 1971, 1978; Alongi and Tenore, 1985; Evrard et al., 2010; Piot et al., 2013), thus we cannot exclude the possibility of adverse effects on the long term. As a naturally occurring phenomenon, marine organisms are to some extent adapted to sedimentation and resuspension, and ecosystems may show increased resilience to sediment disturbance, particularly if they are subjected to a high intensity of natural disturbance (Schratzberger and Warwick, 1998; Leduc and Pilditch, 2013). However, man-made sedimentation events may exceed natural variability in sediment load and frequency and may induce permanent changes in the ecosystem. In the mine tailings treatments no signs of recovery of the benthic community to control conditions were observed within the experiment duration of 11 days. In fact, monitoring studies for STP have shown that after cessation of extensive tailings discharge (up to 4–5 cm tailings addition per year during >20 years) it may take 1 to 4 years before the tailings are fully recolonized while differences in community compositions still prevail (Olsgard and Hasle, 1993; Burd, 2002). However, it remains uncertain if, accompanied with faunal recovery, also ecosystem function will return to normal values. Unfortunately, controlled experiments to determine threshold levels for sediment overburden and tolerated frequencies are largely missing (Miller et al., 2002).

Our study contributes to reducing this knowledge gap since comparing different deposition depths and substrates allows us to gain some information on threshold values and differentiate the effects of substrate characteristics on benthic community structure and functioning. When applying the precautionary principle in a STP scenario, instantaneous depositions with as little as 0.1 cm of tailings over large areas have to be avoided to maintain ecosystem functioning in terms of organic matter

remineralization at normal levels. Structural changes of biota with reduced biomass and shifts in vertical distribution become apparent at 0.5 cm burial with tailings and intensify at 3 cm tailings deposition. It remains unclear how fast biological communities can recover from the short-term effects and how repeated burial with tailings will affect species survival on a longer term. Furthermore, macrofauna is often used to assess and monitor environmental impacts, but was actually the most tolerant group in our experiment while the response of meiofauna was much more pronounced. Therefore, monitoring studies should make use of a more integrated approach covering multiple size groups representing different functional traits and trophic levels.

CONCLUSION

Our research clearly shows that burial with both, mine tailing or dead sediment, has strong negative effects on the biota and the functioning of benthic communities. However, the processes behind the impacts were different between the two substrate additions.

The most severe effects were observed at 3 cm of tailings deposition with a reduction of bacterial and meiofaunal biomass by more than half, reduced algal carbon uptake of all biological compartments and reduced sediment community oxygen consumption. However, already at 0.1 cm tailings deposition, the ability of the benthic community to process organic matter was significantly reduced while the structure of the community remained largely unaffected at this level. This emphasizes the importance of using multiple trophic levels and an ecosystem-based approach in laboratory experiments including measures of ecosystem functioning. The addition of dead sediment, on the other hand, resulted in an increase in bacterial activity causing severe anoxia in the underlying sediment layers entailing decreased meiofaunal biomass and changes in the vertical distribution of macrofauna. While productivity in terms of bacterial biomass and carbon turnover are enhanced on the short term, mortality of nematodes and resulting shifts in benthic community composition might induce unforeseen consequences on the longer term. While possibly less obvious in measurements of abundance, physical disturbance and changes in sediment characteristics may substantially influence infauna community composition, particularly that of meiofauna (Schratzberger et al., 2000; Leduc and Pilditch, 2013). Zeppilli et al. (2016) identified a positive exponential relationship between nematode biodiversity and ecosystem functioning and efficiency in different deep-sea habitats. Hence, reductions in biodiversity and changes in community composition may result in decreased ecosystem functioning and a reduced resilience of the system to different additional stressors (Gessner and Hines, 2012; Steudel et al., 2012; Zeppilli et al., 2016).

We need to be aware of the differential response to burial with different substrates when assessing impacts of mine tailings placement on the benthic environment. This study shows that the particular characteristics of the sediment e.g., organic matter content, porosity or grain size strongly influence the biochemistry inside the sediments and the way the ecosystem responses to substrate burial. Therefore, more research is needed using substrate with similar characteristics of the effectively placed tailings. Furthermore, our study indicates that vast areas impacted by low tailings deposition might experience a reduced carbon mineralization capacity, especially over the short-term. While the thickness of mine tailings in the direct surrounding of the deposition site can reach very high values with deposition rates of several m y^{-1}, the seafloor may still be impacted by deposition rates of 1 mm y^{-1} several km off the deposition site (Olsgard and Hasle, 1993). The wider implications will depend on the scale of tailings discharge and the resilience of the targeted ecosystem. If DSTP is implemented in more regions worldwide environmental managers need to be aware that even small deposition rates might negatively impact very large areas disrupting the functioning of important benthic environments.

AUTHOR CONTRIBUTIONS

AKS, DvO, and AV generated the project funding and conceived the idea of this integrated experiment, that was further developed and implemented by LM, LMe, TS, and SW. KG and SW provided further input to the conceptual design and practical implementation of the study. LM, SW, LMe, and TS collected and analyzed the data. The authors collectively interpreted the data. LMe and TS wrote the manuscript with input from all authors.

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Limited Spatial and Temporal Variability in Meiofauna and Nematode Communities at Distant but Environmentally Similar Sites in an Area of Interest for Deep-Sea Mining

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To be able to adequately assess potential environmental impacts of deep-sea polymetallic nodule mining, the establishment of a proper environmental baseline, incorporating both spatial and temporal variability, is essential. The aim of the present study was to evaluate both spatial and intra-annual variability in meiofauna (higher taxa) and nematode communities (families and genera, and *Halalaimus* species) within the license area of Global Sea mineral Resources (GSR) in the northeastern Clarion Clipperton Fracture Zone (CCFZ), and to determine the efficiency of the current sampling of meiofauna and nematode diversity. In October 2015, three polymetallic nodule-bearing sites, about 60–270 km apart, located at similar depths (*ca.* 4,500 m) were sampled, of which one site was sampled in April in that same year. Despite the relatively large geographical distances and the statistically significant, but small, differences in sedimentary characteristics between sites, meiofauna and nematode communities were largely similar in terms of abundance, composition and diversity. Between-site differences in community composition were mainly driven by a set of rare and less abundant taxa. Moreover, although surface primary productivity in April exceeded that in October, no significant changes were observed in sedimentary characteristics or in meiofauna and nematode communities. At all sites and in both periods, Nematoda were the prevailing meiofaunal phylum, which was in turn dominated by Monhysterid genera and *Acantholaimus*. Our findings support the earlier purported notion of a low degree of endemism for nematode genera and meiofauna taxa in the deep sea, and hint at the possibility of large distribution ranges for at least some *Halalaimus* species. Taxon richness estimators revealed that the current sampling design was able to characterize the majority of the meiofauna and nematode taxa present. To conclude, implications of the present findings for environmental management and future research needs are provided.

Keywords: polymetallic nodules, Nematoda, environmental baseline, biodiversity, *Halalaimus*

INTRODUCTION

The deep-sea bed (>200 m water depth) constitutes the largest benthic ecosystem on Earth (Tyler et al., 2016). Nevertheless, although deep-sea sampling efforts have surged in recent years (Stuart et al., 2008; McClain and Schlacher, 2015), the deep-sea floor, and especially the abyss (>3,000 m; Stuart et al., 2008), remains largely undersampled (Ramirez-Llodra et al., 2010). Moreover, there are relatively few high-resolution temporal (i.e., intra-annual) biological data collected in the deep sea (Galeron et al., 2001; Veit-Köhler et al., 2011; Guilini et al., 2013; Lins et al., 2014), which is linked to its remoteness and the consequently high financial cost associated with deep-sea sampling. As a consequence, our comprehension of the level of biodiversity, as well as of the degree of spatial and temporal variability in benthic community composition and biodiversity in the deep sea, and the responsible drivers, is still incomplete (Rex and Etter, 2010; McClain and Schlacher, 2015). In the last decades, human exploitation activities, including fisheries, and oil and gas exploration, are expanding progressively to offshore, deeper waters in response to an increasing demand for biological and mineral resources, and to technological innovations (Glover and Smith, 2003; Ramirez-Llodra et al., 2011). Unfortunately, our inadequate knowledge about the structure and dynamics of the deep-sea benthic ecosystem, hinders the accurate prediction (prior to exploitation) or evaluation (following exploitation) of the environmental impacts that may arise from these activities.

One exploitation activity that may potentially take place in the near future is the mining of polymetallic nodules (Clark et al., 2013). Polymetallic nodules, potato-shaped accretions rich in commercially interesting metals, occur at abyssal depths underneath low-productive waters. The Clarion Clipperton Fracture Zone (CCFZ) in the northeast Pacific is considered to harbor one of the largest high-grade nodule reservoirs and has therefore gained the most attention from industries and governments (Lodge et al., 2014). The first contracts for the exploration of polymetallic nodules in the CCFZ were granted by the International Seabed Authority (ISA) in 2001, and currently 15 license areas have been assigned (https://www.isa.org.jm/deep-seabed-minerals-contractors). Contractors are required to collect environmental data to establish an environmental baseline, incorporating both spatial and temporal variability, against which to assess the likely effects of their activities on the marine environment (ISA, 2002, 2013).

Meiofauna (>32 μm) is the most abundant and one of the most diverse components of the metazoan benthos in the deep sea (Sinniger et al., 2016) including the CCFZ (Smith and Demopoulos, 2003). Studies investigating temporal and/or spatial trends in meiofaunal communities in the CCFZ are limited to the 9°N and 5°N "EqPac" stations (Lambshead et al., 2003) and four currently assigned license areas, i.e., IOM[1] (Radziejewska et al., 2001), IFREMER[2] (Mahatma, 2009; Miljutina et al., 2010; Miljutin et al., 2015), DORD[3] (Kaneko et al., 1997; Shirayama and Fukushima, 1997) and CIIC[4] (Trueblood and Ozturgut, 1997; study conducted by the National Oceanographic and Atmospheric Administration's Ocean Minerals and Energy Division; see also the meta-analytical papers by Vanreusel et al., 2010; Radziejewska, 2014; Singh et al., 2016 and the paper by Jones et al., 2017). Temporal variability in CCFZ meiofauna was so far only assessed by comparing different years (Trueblood and Ozturgut, 1997; Shirayama, 1999; Radziejewska et al., 2001). Also for the mega-, macro-, and micro-fauna intra-annual variability in the CCFZ remains to be evaluated.

In this study, samples were collected in the license area of Global Sea mineral Resources (GSR) in the northeastern CCFZ, to evaluate both spatial and intra-annual variability in meiofauna and nematode communities. To this end, three nodule-bearing sites at similar depths (*ca.* 4,500 m) were sampled (between roughly 60 and 270 km apart), of which one was sampled during two different cruises in 2015 (April-May: SO239, September-October: GSRNOD15A). As these three sites are of potential interest for deep-sea mining, the results presented here are of direct relevance to environmental (spatial) management which aims, amongst others, at preserving biodiversity. Information on the level of biodiversity, as well as on the distribution ranges and connectivity of taxa, will aid in the delineation of impact reference zones (IRZs, to evaluate impacts of mining) and preservation reference zones (PRZs, to protect certain taxa or to allow for mitigation of mining-induced alterations in biodiversity) within the license area (ISA, 2013). Both types of reference zones need to be similar in faunal abundance, composition and diversity to the targeted mine site, and the PRZs also need to be positioned as such as to allow for recolonization of the targeted mine sites (ISA, 2013). Specifically, the following questions were addressed here:

- Do similarly deep nodule-bearing sediments (i.e., similar macro-habitats) separated by more than 60–270 kms harbor similar meiofauna and nematode communities in terms of abundance, composition and diversity?
- Do meiofauna and nematode communities at 4,500 m water depth display intra-annual variability?
- What portion of meiofauna and nematode diversity is characterized by the current sampling?

MATERIALS AND METHODS

Study Area

The Clarion Clipperton Zone (CCFZ), that part of the Pacific abyss bounded by the Clarion and Clipperton fracture zones (see **Figure 1**), is located in the Eastern Tropical Pacific (ETP). Primary productivity in the ETP is moderate but the region displays considerable spatio-temporal variability (Pennington et al., 2006). Both longitudinal and latitudinal gradients in

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[3]Deep Ocean Resources Development company, Japan.
[4]Cook Islands Investment Corporation, Cook Islands.

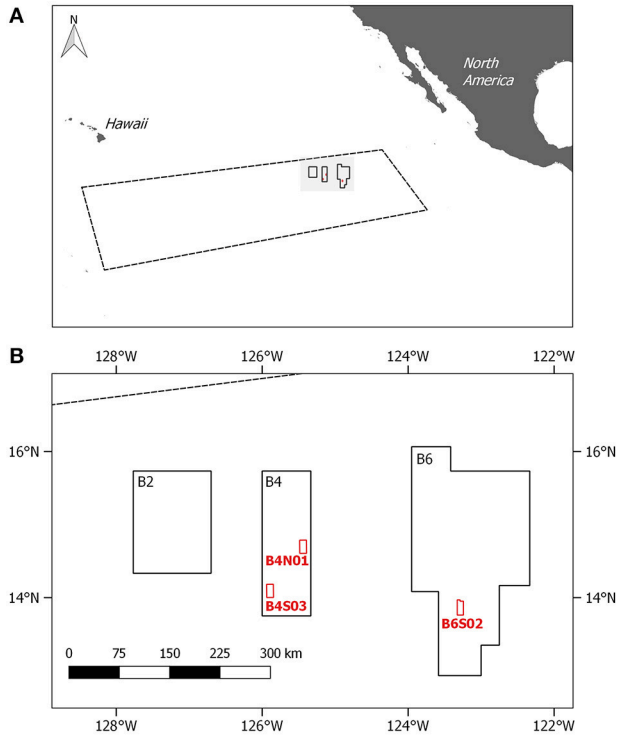


FIGURE 1 | (A) Location of the Clarion Clipperton Fracture Zone (CCFZ); coordinates based on the working definition of Glover et al. (2015) with an indication of the GSR license area. **(B)** Location of the three sites sampled within the GSR license area during GSRNOD15A (in red). Site B6S02 was also sampled for meiofauna and environmental variables during cruise SO239.

surface water production and the resultant flux of particulate organic carbon (POC) to the seafloor have been observed in the CCFZ (Smith et al., 1997; Wedding et al., 2013). Interannual variability in primary productivity is driven by interannual El Niño-Southern Oscillations (ENSO) and multidecadal Pacific Decadal Oscillations (though the effects are strongest near the equator, in eastern coastal boundary regions, and in the central north Pacific; Fiedler, 2002), but also global warming is thought to affect primary productivity in the ETP (Behrenfeld et al., 2006). In contrast, intra-annual variability is considered to be relatively low (Amos and Roels, 1977; Pennington et al., 2006).

In 2013, the Belgian company GSR was granted a license for the exploration for polymetallic nodules in the CCFZ by the International Seabed Authority for an area of 76,728 km^2. The GSR license area, located in the northeast of the CCFZ, is divided in three non-adjacent zones, called B2, B4, and B6. During the exploration cruise in 2015 (termed GRSNOD15A), three sites (each *ca.* 10 × 20 km) were targeted, of which two are located in B4 (B4S03 and B4N01) and one in B6 (B6S02). Samples for meiofauna (including nematodes) and sediment environmental variables (i.e., bacterial biomass and sediment characteristics) were collected in the frame of a biological baseline study to characterize spatial and intra-annual variability.

Sampling Strategy and Onboard Sample Processing

At each site, sediments were sampled in triplicate with a multicorer (MUC) aboard the *RV Mt. Mitchell* during the GSRNOD15A cruise (September-October 2015) to the GSR license area (see **Table 1**). At site B6S02, MUC001–MUC003 were deployed at approximately the same locations as those of MUC deployments 121, 124, and 146, respectively, done during the JPI Oceans aboard the *RV Sonne* which took place in April–May 2015 (i.e., cruise SO239; Haeckel and Arbizu, 2015). This sampling strategy allowed for the evaluation of intra-annual or seasonal variability in sediment characteristics and in meiofauna and nematode communities at this site (SO239 samples were not analyzed for bacterial biomass). Given the sampling dates (see **Table 1**), the SO239 MUC samples collected at site B6S02 will be referred to as the "April 2015" samples, whilst the GSRNOD15A MUC samples from B6S02 will be designated as the "October 2015" samples. Per MUC deployment, 1 core was analyzed for meiofauna and nematode community attributes, whilst another core was studied for sediment characteristics. During GSRNOD15A, two additional cores from each MUC deployment were sampled for the quantification of sediment bacterial biomass and phytopigments. Unfortunately, the high-performance liquid chromatography (HPLC) analysis (following Wright and Jeffrey, 1997; Van Heukelem and Thomas, 2001) of the sediment samples yielded concentrations below the detection limit (<20 ng g^{-1}). The MUC aboard the *RV Sonne* and the *RV Mt. Mitchell* had an internal diameter of 94 and 100 mm, respectively. From MUC124 (obtained during the SO239 cruise), no core was available for the study of sediment characteristics. Hence, to evaluate intra-annual variability in sediment characteristics at site B6S02, we analyzed data from one of the cores from MUC125 (**Table 1**). Sediment cores were processed in a cold lab container which was set at 4°C, close to the *in situ* bottom water temperature. From the cores intended for meiofauna analysis, the 5 cm of water standing on top of the sediment as well as the top 0–5 cm of the sediment were added to one sample container. To each sediment sample a roughly equal amount of 10% seawater-buffered formaldehyde was added. Sediment cores intended for the analysis of bacterial biomass (GSRNOD15A) and sediment characteristics (SO239 and GSRNOD15A) were sliced vertically (GSRNOD15A: per cm to 5 cm depth, SO239: 0–1 cm, 1–5 cm) and stored at −20°C till further analysis.

Sample Analysis

Analysis of Environmental Variables

To determine whether the difference in the timing of sampling (April vs. October 2015) was associated with a difference in surface net primary productivity (NPP), we extracted Vertically Generalized Production Model (VGPM) estimated NPP (Behrenfeld and Falkowski, 1997) based on MODIS data for site B6S02 for January 2015–October 2015. Monthly-averaged NPP values were downloaded in HDF format from the Ocean Productivity website (http://www.science.oregonstate.edu/ocean.productivity/index.php) and converted to geotiff using the

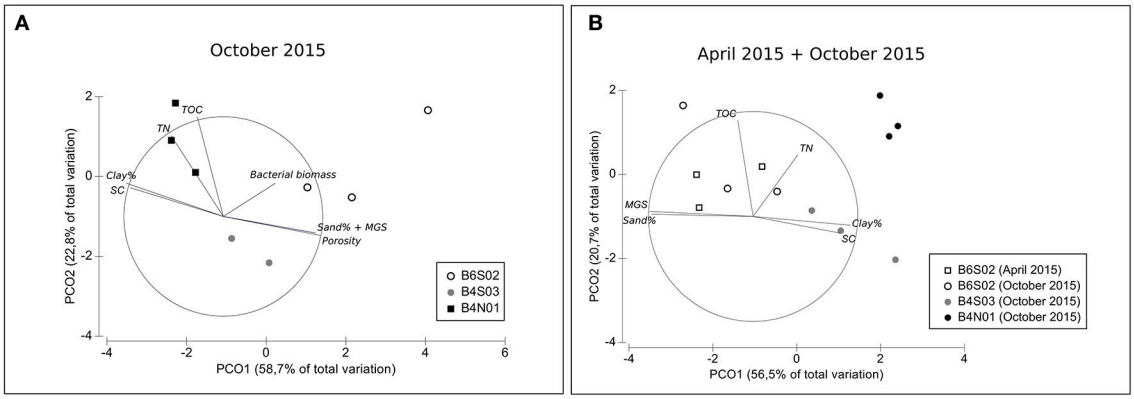


FIGURE 2 | PCO plots of sediment-depth integrated (0–5 cm) environmental variables for all three sites sampled in the GSR license area **(A)** during GSRNOD15A (October 2015, all sites) and **(B)** during GSRNOD15A (October 2015, all sites) and SO239 (April 2015, site B6S02). MGS, median grain size; SC, sediment sorting coefficient; TN, total nitrogen content; TOC, total organic carbon content. Vectors represent sediment characteristics correlating >50% (based on Spearman correlation coefficients) with one of the two PCO axes. Note that in the second PCO analysis **(B)** sediment porosity and bacterial biomass were excluded from the analysis as these variables were not measured on samples collected in April 2015.

TABLE 1 | Details of the multicorer (MUC) deployments during sampling cruises GSRNOD15A (October 2015) and SO239 (April 2015).

Site	Cruise	Deployment	Date	Lat (DD)	Long (DD)	Depth (m)
B6S02	SO239	MUC121	08/04/2015	13.854133	−123.254933	4,526
		MUC124	08/04/2015	13.854133	−123.244833	4,525
		MUC125	*09/04/2015*	*13.851117*	*−123.237000*	*4,524*
		MUC146	12/04/2015	13.845667	−123.251667	4,522
	GSRNOD15A	MUC001	23/09/2015	13.853847	−123.255129	4,526
		MUC002	23/09/2015	13.854136	−123.246030	4,525
		MUC003	24/09/2015	13.845595	−123.252588	4,522
B4S03	GSRNOD15A	MUC004	29/09/2015	14.117804	−125.879102	4,490
		MUC005	29/09/2015	14.112544	−125.871569	4,498
		MUC006	29/09/2015	14.104218	−125.878287	4,470
B4N01	GSRNOD15A	MUC007	06/10/2015	14.706303	−125.460873	4,509
		MUC008	07/10/2015	14.706333	−125.451303	4,504
		MUC009	07/10/2015	14.706489	−125.442040	4,501

DD, decimal degrees. Coordinates are those of the research vessel during landing of the MUC. Water depth is based on multibeam data retrieved during a previous GSR sampling cruise (GSRNOD14A). MUC125 (in italics) was analyzed for abiotic sediment data only, as from deployment MUC124 there was no core available for these analyses.

SeaDAS software. In QGIS, the point sampling tool was used to extract NPP values for all MUC deployment locations. Monthly NPP values for B6S02 were calculated as the average NPP over all deployments.

Sediments were weighed before and after drying (60°C) to determine water content, which was in turn used to estimate porosity, assuming a dry sediment density of 2.55 g cm^{-3}. Grain size analysis of ca. 1 g of sediment was performed with a Malvern Mastersizer hydro 2000 G. The granulometric variables used in this study to describe the physical environment of the meiobenthos were: median grain size (MGS), the percentage of sand (grain size > 63 μm), clay (grain size < 4 μm), and silt (4 μm < grain size < 63 μm; Wentworth, 1922), and the sediment sorting coefficient (SC). SC, which is a measure for the spread distance of the various grain sizes, was calculated following Giere (2009). Total organic carbon (TOC) and total nitrogen (TN) content were measured on 200 mg samples using a Flash 2000 NC Sediment Analyser of Interscience (Thermo scientific) after acidification with 1% HCl to remove inorganic carbon.

Per 1-cm sediment layer (0–5 cm), lipids were extracted from *ca.* 3 g of freeze-dried sediment using a modified Bligh and Dyer (1959) method (Boschker et al., 1999). The polar lipid fraction was obtained by fractionation on silicic acid, and derivatized using mild alkaline methanolysis to yield fatty acid methyl esters (FAMEs). The concentration of these FAMEs was determined with a gas chromatograph-mass spectrometer (GC-MS) from Agilent (GC-6890N + MS-5973N). Samples were run in splitless mode (1 μl injection per run, injector temperature of 250°C)

using a HP-88 column (Agilent J&W, 0.2 mm internal diameter × 60 m) with a He flow rate at constant pressure. The FAME C19:0 (Fluka 74208) was added as an internal standard. Oven temperature was initially set at 50°C for 2 min, followed by a ramp at 25°C min^{-1} to 75°C, and then a second and final ramp at 2°C min^{-1} to 230°C with a final 4 min hold. FAMEs were identified by comparing the retention times and mass spectra with those of two external standards, i.e., the Bacterial Acid Methyl Esters Mix (Sigma-Aldrich, catalog #47080-U) and the Supelco 37 component FAME mix (Sigma-Aldrich, Supelco #47885), and available ion spectra in the WILEY and a self-constructed library. These analyses were done using the software MSD ChemStation by Agilent Technologies. Individual FAMEs were quantified by linearly regressing the chromatographic peak areas against known concentrations of the standards in the Supelco 37 component FAME mix. Bacteria-specific phospholipid-derived fatty acids (PLFAs) that were present in all of our samples were i15:0, a15:0, and i16:0. As these PLFAs make up roughly 13% of all bacterial PLFAs (based on literature sources mentioned by Middelburg et al. (2000), and 5.6% of the total carbon content in bacterial cells represents PLFA carbon (Brinch-Iversen and King, 1990), the concentrations of these three PLFAs enabled the estimation of bacterial biomass. PLFA concentrations were measured per unit weight of sediment but were converted to unit surface area taking into account sediment porosity.

Analysis of Meiofauna and Nematodes

Sediments were washed over a 32 μm sieve (no upper sieve was used), and the sieve residue was subjected to three Ludox centrifugation rounds to extract the meiofauna (Burgess, 2001). After the last round, the supernatant was poured over a 32 μm sieve and the sieve residue was stored in borax-buffered 4% formaldehyde. Next, a drop of a 1% Rose Bengal-formaldehyde solution was added to stain the organic material, facilitating recognition of meiofauna. Metazoan meiofauna (hereafter referred to as "meiofauna") was sorted, enumerated, and identified at higher taxonomic level using a stereomicroscope following Higgins and Thiel (1988). Meiofauna counts were converted to abundances per surface area as ind. 10 cm^{-2}, which is commonly done in ecological meiofauna studies. To account for differences in sediment volume caused by the presence of nodules, we estimated sediment volume of each MUC sample (0–5 cm) by (1) assessing the volume of meiofauna extraction residues (>32 μm) using a measuring cylinder and, (2) calculating the volumetric fraction of the sediment with a grain size <32 μm based on granulometric analyses. Per sample about 120 nematodes were hand-picked at random, mounted on slides, and identified to family and genus level (where possible). Additionally, the life stage/gender was noted if possible (female/male/juvenile). Some genera are hard to distinguish based on morphological characteristics, especially when dealing with juveniles (e.g., *Thalassomonhystera/Monhystrella*, and *Microlaimus/Aponema*), so these were pooled in so-called genus groups. *Halalaimus* was further identified down to species level because of (1) the relatively high relative abundance of this genus at each site (2) the pre-existence of molecular data for *Halalaimus* sampled in the CCFZ, including the GSR license area, which can at a later stage be linked to the morphological data. Specimens that resembled but were not morphologically identical to a certain taxon, were designated as "aff" to assign the morphological affinity with that taxon.

Data Analysis

For each sample, diversity was evaluated for meiofauna higher taxa, nematode families and nematode genera using the following indices: taxon richness (higher meiofauna taxa: T, nematode families: F, nematode genera: G), Pielou's evenness (J'), Shannon–Wiener diversity (H'), expected taxon richness for a sample of 51 individuals [higher taxa: ET(51), nematode families: EF(51), nematode genera: EG(51)]. For *Halalaimus* species, diversity was only assessed in terms of species richness (S) because of the small sample size. Moreover, taxon-accumulation curves (TACs), plotting the cumulative number of taxa observed (T_{obs}) as a function of the number of sites/samples or periods studied, were produced for all taxonomical levels by randomly adding sites/samples or periods and repeating this procedure 9999 times. Additionally, the non-parametric Chao1 (determined by the number of taxa that have only one or two individuals in the complete sample set), Jacknife2 ("jack2;" a function of the number of taxa retrieved in one or two samples) and Bootstrap ("boot;" dependent on the set of proportions of samples that contain each taxon) estimators (see also Magurran, 2004; Gotelli and Colwell, 2010) were used to extrapolate the TACs to obtain an estimate of total taxon richness in the GSR license area. The usage of non-parametric estimators to estimate the (minimal) total number of taxa present was recommended by Gotelli and Colwell (2010). The comparison of T_{obs} and the taxon richness estimators allowed for an estimation of the percentage of total taxon richness characterized by our sampling strategy for all taxonomical levels considering all MUC samples and all sites. In addition, the minimum number of additional samples required to detect 95 and 100% of the estimated asymptotic taxon richness was calculated using the non-parametric method based on taxon counts (Chao1) *sensu* Chao et al. (2009). The average number of individuals identified per sample was used to estimate the additional number of samples needed.

Differences between sites and periods regarding sedimentary environmental variables, total meiofauna abundance, diversity indices, and meiofauna and nematode community composition were examined using a one-way PERMANOVA analysis with "site" (levels: B6S02, B4S03 and B4N01) or "period" (levels: April and October) as a fixed factor. Additionally, for site B6S02 the relative abundance of nematode juveniles (relative to total nematode abundance) was compared between April and October to check for potential recruitment, by means of a one-way PERMANOVA. The significance level was set at 0.05. No statistical tests were conducted for spatial and temporal differences in *Halalaimus* species richness and composition, because of the small number of individuals and samples available. Significant main PERMANOVA tests were followed by pairwise PERMANOVA tests. Permutational *P*-values (P_{PERM}) were interpreted when the number of unique permutations >100; alternatively, Monte Carlo *P*-values (P_{MC}) were considered.

Each PERMANOVA analysis was followed by a test for the homogeneity of multivariate dispersions (PERMDISP); these results were only mentioned in case of a significant *P*-value. Meiofauna abundances were corrected for differences in sediment volume between samples, by including sediment volume as a covariate in the PERMANOVA analyses. For the (untransformed) univariate biological (total meiofauna and relative nematode juvenile abundance, and diversity indices) and (previously normalized) multivariate abiotic sediment data, Euclidean distance was used to construct resemblance matrices. Multivariate biological data (i.e., meiofauna higher taxon, nematode family and genus composition, and *Halalaimus* species composition) were standardized and square-root transformed (to down weigh the importance of the most dominant taxa) prior to the construction of resemblance matrices using Bray-Curtis dissimilarities. In case of significant differences in taxonomic composition between sites, the BIO-ENV BVStep procedure (Spearman Rank correlation) was run to determine which subset of taxa best explained the full multivariate pattern (Clarke and Warwick, 1998). This procedure can be construed as a generalization of the SIMPER (Similarity of Percentages) routine in Primer. Unlike BVStep, however, SIMPER compares two groups of samples at a time, and therefore does not cater for more gradual changes in community composition (Clarke and Gorley, 2006). Because sediment porosity and bacterial biomass were only measured on samples collected in October 2015 (GSRNOD15A), we conducted two separate Principal COordinates (PCO) analyses: one for the October 2015 samples (GSRNOD15A) only (to visualize differences in environmental variables between sites) and another one for the April and October 2015 samples together (to visualize differences in environmental variables between sites and between periods for site B6S02). Environmental variables that correlated strongly (>50%, Spearman Rank correlation) with one of the two PCO axes were plotted as vectors, indicating which variables relate the most to the patterns observed. Except for the PCO pots, which were created in Primer, all plots were made with the R package ggplot2 (Wickham, 2009; R Core Team, 2016). All analyses were run in Primer v 6.1.11 (Clarke and Gorley, 2006) and the PERMANOVA + add-on (Anderson et al., 2008).

RESULTS

Environmental Variables

Spatial Variability

The sediment at all three sites sampled in the GSR license area was dominated by silts (72.2–74.0%), followed by clay (14.8–19.4%) and sand (7.3–12.2%; **Table 2**). Sediments were highly porous (0.85–0.88) and poorly sorted (SC: 1.2–1.3), and total organic carbon (TOC) and total nitrogen (TN) content ranged between 0.4–0.6% and 0.1–0.2%, respectively (**Table 2**). The entire suite of sediment environmental variables (0–5 cm) varied significantly between sites [main PERMANOVA: Pseudo-$F_{(2, 6)} = 5.06$, $P_{PERM} < 0.01$], owing to a significant difference between B6S02 and B4N01 (pairwise PERMANOVA: $t = 2.73$, $P_{MC} = 0.01$). The segregation between these two sites is mainly illustrated by the first PCO axis, which correlates most strongly with granulometric characteristics, and to a lesser extent with bacterial biomass (**Figure 2A**). Compared to B4N01, site B6S02 had slightly coarser, better sorted sediments with a higher percentage of sand, and a lower silt and clay content, and higher sediment bacterial biomass (see **Table 2**).

TABLE 2 | Average (±*SD*) sediment-depth integrated (0-5 cm) environmental variables per site sampled in the GSR license area during GSRNOD15A (October 2015, all sites) and SO239 (April 2015, site B6S02).

	B6S02		B4S03	B4N01
	April 2015	October 2015	October 2015	October 2015
Bacterial biomass (mg C m^{-2})	–	416.6 ± 132.1	237.7 ± 23.5	270.1 ± 20.4
MGS (μm)	18.56 ± 0.87	18.50 ± 1.05	16.52 ± 0.92	15.85 ± 0.30
Sand (%)	11.01 ± 1.06	11.31 ± 1.04	8.53 ± 0.65	7.99 ± 0.64
Silt (%)	72.71 ± 0.57	72.68 ± 0.25	73.18 ± 0.67	73.16 ± 0.76
Clay (%)	16.28 ± 0.52	16.02 ± 1.15	18.29 ± 1.16	18.85 ± 0.19
Porosity (–)	–	0.87 ± 0.01	0.86 ± 0.01	0.86 ± 0.01
SC (–)	1.24 ± 0.04	1.25 ± 0.02	1.29 ± 0.03	1.30 ± 0.02
TOC (%)	0.51 ± 0.03	0.49 ± 0.04	0.41 ± 0.03	0.54 ± 0.03
TN (%)	0.13 ± 0.00	0.17 ± 0.02	0.15 ± 0.01	0.21 ± 0.01

MGS, median grain size; SC, sediment sorting coefficient; TN, total nitrogen content; TOC, total organic carbon content. Porosity and bacterial biomass were not determined for sediment samples collected in April 2015. Bacterial biomass for site B4S03 was based on two replicate MUC samples as one of the lipid extracts from MUC006 was accidentally spilled in the lab.

Intra-Annual Variability

At site B6S02, average daily NPP in April (271.2 g C m^{-2} d^{-1}) exceeded that in October 2015 (214.7 g C m^{-2} d^{-1}; see **Figure 3**). Moreover, the months prior to April 2015 (SO239) displayed the highest NPP values overall, whereas the months preceding GSRNOD15A were characterized by the lowest NPP. This difference in NPP between the two periods was not reflected in a dissimilarity in sediment characteristics [main PERMANOVA: Pseudo-$F_{(1, 4)} = 0.55$, $P_{MC} = 0.64$; see **Table 2** and **Figure 2B**].

Meiofauna and Nematode Communities

Spatial Variability

Total meiofauna abundances in the top 0–5 cm of the sediment did not differ significantly between the three sites sampled during GSRNOD15A [main PERMANOVA: Pseudo-$F_{(2, 5)} = 2.16$, $P_{PERM} = 0.22$]. Nevertheless, on average, abundances declined from B6S02 (151.1 ± 54.2 ind. 10 cm^{-2}) to B4S03 (106.6 ± 29.3 ind. 10 cm^{-2}) and B4N01 (88.1 ± 55.0 ind. 10 cm^{-2}). Meiofauna higher taxon composition differed significantly between sites according to the main PERMANOVA test [Pseudo-$F_{(2, 6)} = 2.15$, $P_{PERM} = 0.03$], but the pairwise tests could not identify pairwise differences (all $P_{MC} > 0.05$; see also Supplementary Figure 1A). Each site was dominated by the same taxa; nematodes prevailed (attaining relative abundances of 85.1–93.3% over all GSRNOD15A samples), followed by copepods (1.5–10.6%) and nauplii (1.1–7.1%; see **Figure 4A**). The other taxa encountered always contributed <1% to total meiofauna abundance. Among-sample compositional

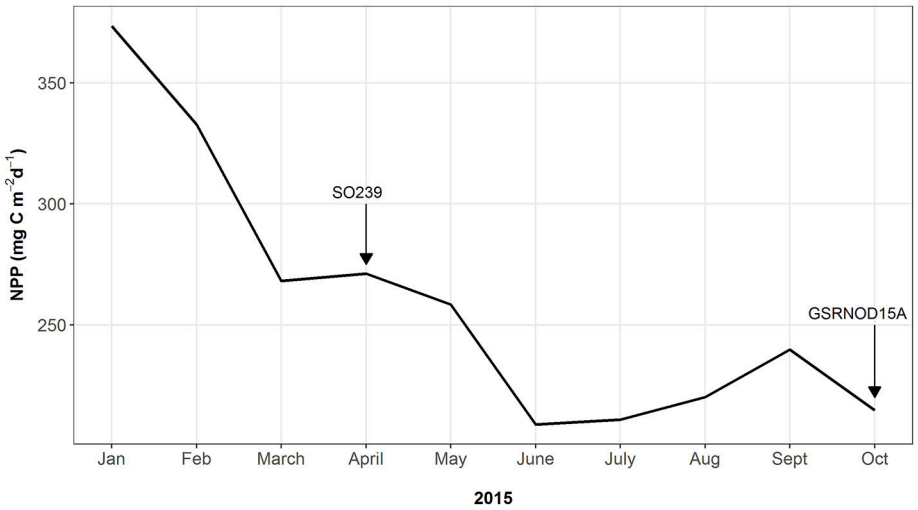


FIGURE 3 | Monthly-averaged daily net primary productivity (NPP) for the surface water at site B6S02 for January–October 2015. Arrows indicate the timing of the SO239 cruise (April 2015) and that of the GSRNOD15A cruise (October 2015).

differences were mainly driven by the less abundant taxa as the BVStep procedure selected Isopoda, Ostracoda, Polychaeta, and Tantulocarida as the subset best explaining the overall multivariate pattern (Rho = 0.69, $P = 0.03$). Moreover, these taxa were either restricted to (Isopoda restricted to B6S02) or (nearly) absent from one of the sites (Ostracoda and Tantulocarida absent from B4N01 and B6S02, respectively; Polychaeta only found in one B4N01 sample). None of the meiofauna taxon diversity indices computed differed significantly between the three sites (for all indices: main PERMANOVA: $P_{PERM} > 0.05$; **Table 3**).

Nematode family composition (**Figure 4B** and Supplementary Figure 1B) showed no marked differences between sites [main PERMANOVA, Pseudo-$F_{(2, 6)} = 1.21$, $P_{PERM} = 0.23$], and more than half of the families were shared between sites (Supplementary Figure 2A). The prevailing families were Monhysteridae (26.9 ± 6.4%), Chromadoridae (21.1 ± 2.6%), and Desmoscolecidae (13.9 ± 4.6%), and together they comprised 47.4–72.6% of total nematode abundance. The PERMDISP test indicated a significantly different multivariate dispersion between sites [$F_{(2, 6)} = 7.13$, $P_{PERM} = 0.03$], caused by the lower dispersion for B4S03 (average dispersion: 11.63 ± 0.56%) relative to the other sites (B6S02: 15.47 ± 0.82%, B4N01: 15.89 ± 1.15%). Except for nematode family richness (F), there were no significant differences in family diversity indices between sites (main PERMANOVA: all $P_{PERM} > 0.05$). Pairwise PERMANOVA tests showed significantly fewer families at site B4N01 (14.00 ± 1.73) compared to B4S03 (17.7 ± 0.58; $t = 3.48$, $P_{MC} = 0.03$; **Table 1**). Although, average family richness at B6S02 (18 ± 1.73) also exceeded that at B4N01, the relatively high amongst-replicate variability for B6S02 resulted in a non-significant difference between B6S02 and B4N01 ($t = 2.83$, $P_{MC} = 0.05$). Although the main PERMANOVA test revealed a significant "site" effect on nematode genus composition [Pseudo-$F_{(2, 6)} = 1.63$, $P_{PERM} = 0.02$], the pairwise tests were unable to differentiate amongst pairs of sites (all $P_{MC} > 0.05$; see also Supplementary Figure 1C). As shown in **Figure 4C**, all three sites were dominated by the same set of nematode genera. *Monhystrella/Thalassomonhystera* (27.0 ± 6.4%) and *Acantholaimus* (15.9 ± 4.0%) dominated the nematofauna in all samples, except for MUC005 (B4S03) where *Prototricoma* was subdominant (14.4%). The subset of genera best explaining the among-sample compositional differences constituted 11 genera (BVStep: Rho = 0.90, $P = 0.02$), of which the majority was only present in few samples and mostly represented by one individual. Amongst these 11 genera were *Diplopeltula*, *Manganonema,* and *Tricoma*, which attained relatively high relative abundances in only a few samples (**Figure 4C**). More than 50% of the genera were restricted to one site (Supplementary Figure 2A). Site B6S02 was characterized by the highest nematode generic evenness (J′) [main PERMANOVA: Pseudo-$F_{(2,6)} = 6.83$, $P_{PERM} = 0.02$; pairwise PERMANOVA: B6S02-B4N01, $t = 3.92$, $P_{MC} = 0.02$, B6S02-B4S03: $t = 3.92$, $P_{MC} = 0.04$]. The values of the other genus diversity indices were similar between the three sites (all $P_{PERM} > 0.05$). **Figure 5A** compares the values of three genus richness estimators (i.e., Bootstrap, Chao 1, and Jackknife2) between the three sites sampled during GSRNOD15A in function of the number of MUC deployments. Although G and expected genus richness [EG(51)] did not vary significantly between sites, the genus richness estimators calculated here were consistently lowest for site B4N01, intermediate for B4S03 and highest for B6S02.

Figure 4D shows a highly variable *Halalaimus* species composition both within and between sites (see also Supplementary Figure 1D). *Halalaimus abyssus* ($n = 6$) and *H.* aff. *delamarei* ($n = 9$) were overall the most abundant; these were also the two only species shared between all sites (see also Supplementary Figure 2A). Half of the species was restricted to one site (see Supplementary Figure 2A). *Halalaimus* species

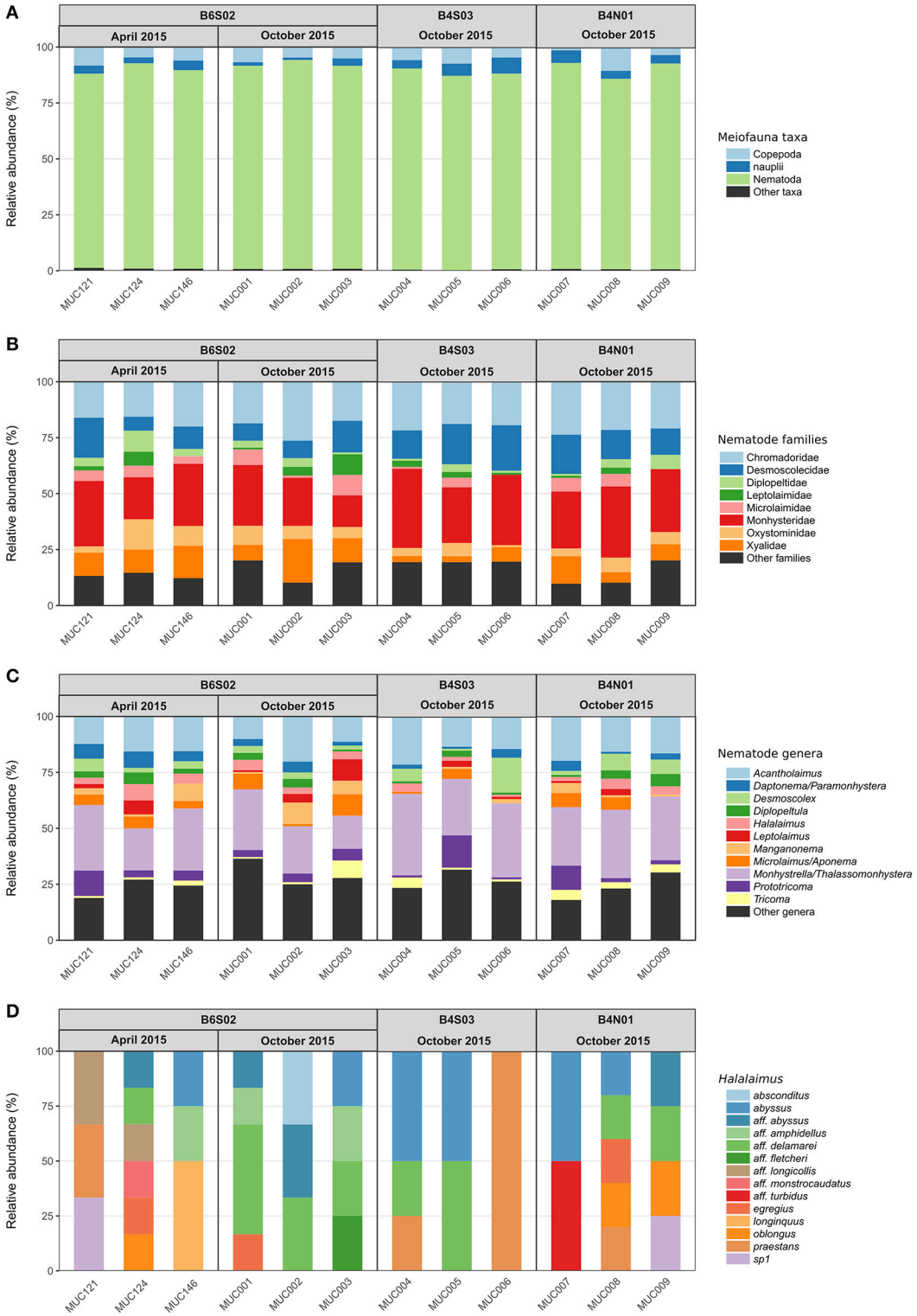


FIGURE 4 | Relative abundances of **(A)** higher meiofauna taxa, **(B)** nematode families, **(C)** nematode genera, and **(D)** *Halalaimus* species per multicorer (MUC) sample for all sites sampled in the GSR license area during GSRNOD15A (October 2015, all sites) and SO239 (April 2015, site B6S02). "Other taxa" are higher meiofauna taxa that contributed <1% to total meiofauna abundance. "Other families" comprised <1% of the total number of nematodes identified per sample, and "other genera" comprised <5% of the total number of nematodes identified per sample.

TABLE 3 | Average (±SD) counts and values of diversity indices for meiofauna (higher) taxa, nematode families, nematode genera, and *Halalaimus* species per site sampled in the GSR license area during GSRNOD15A (October 2015, all sites) and SO239 (April 2015, site B6S02).

	B6S02		B4S03	B4N01
	April 2015	October 2015	October 2015	October 2015
MEIOFAUNA HIGHER TAXA				
N	1684.94 ± 410.69	1187.06 ± 425.82	837.57 ± 230.00	691.71 ± 431.99
T	7.00 ± 0.00	8.00 ± 1.00	5.67 ± 1.15	6.33 ± 0.58
H'	0.46 ± 0.07	0.37 ± 0.05	0.47 ± 0.04	0.42 ± 0.10
J'	0.23 ± 0.04	0.18 ± 0.03	0.27 ± 0.05	0.23 ± 0.06
ET(51)	3.31 ± 0.16	2.99 ± 0.21	3.12 ± 0.11	2.99 ± 0.21
NEMATODE FAMILIES				
N	97.33 ± 8.08	117 ± 12.53	108.67 ± 3.79	110.33 ± 1.15
F	16.33 ± 2.08	18.00 ± 1.73	17.67 ± 0.58	14.00 ± 1.73
H'	2.24 ± 0.17	2.31 ± 0.15	2.12 ± 0.13	2.08 ± 0.14
J'	0.80 ± 0.04	0.80 ± 0.03	0.74 ± 0.04	0.79 ± 0.02
EF(51)	12.91 ± 1.31	13.29 ± 1.08	13.10 ± 0.84	11.23 ± 1.47
NEMATODE GENERA				
N	97.33 ± 8.08	116.00 ± 12.53	107.00 ± 4.00	109.33 ± 1.53
G	26.33 ± 2.31	29.67 ± 5.03	29.00 ± 3.00	26.33 ± 3.51
H'	2.68 ± 0.15	2.83 ± 0.14	2.51 ± 0.24	2.57 ± 0.12
J'	0.82 ± 0.03	0.84 ± 0.02	0.74 ± 0.05	0.79 ± 0.00
EG(51)	19.27 ± 1.60	20.37 ± 1.80	18.64 ± 2.14	18.06 ± 1.92
***HALALAIMUS* SPECIES**				
N	4.33 ± 1.53	4.33 ± 1.53	2.33 ± 1.53	3.67 ± 1.53
S	4.00 ± 1.73	3.67 ± 1.53	2.00 ± 1.00	3.67 ± 0.58

N, number of individuals identified; T, meiofauna (higher) taxon richness; H', Shannon–Wiener diversity; J', Pielou's evenness; ET(51), expected taxon richness for 51 individuals; F, nematode family richness; EF(51), expected nematode family richness for 51 individuals; G, nematode genus richness; EG(51), expected genus richness for 51 individuals; S, number of species.

richness and the values of all three species richness estimators, but also the number of *Halalaimus* individuals identified, were lowest for site B4S03 (see **Table 3** and **Figure 5B**).

Intra-Annual Variability

At site B6S02, differences in total meiofauna abundance (0–5 cm) between April and October 2015 were statistically insignificant [main PERMANOVA, Pseudo-$F_{(1,3)} = 4.00$, $P_{PERM} = 0.11$]. Nevertheless, average meiofauna abundances were higher in April (242.3 ± 59.2 ind $10\,cm^{-2}$) than in October 2015 (151.1 ± 54.2 ind. $10\,cm^{-2}$). The relative abundance of nematode juveniles did not change significantly between April (36.5 ± 8.2%) and October [37.8 ± 5.4%; main PERMANOVA, Pseudo-$F_{(1,4)} = 0.05$, $P_{MC} = 0.84$]. Meiofauna higher taxon composition was comparable between April and October 2015 [main PERMANOVA: Pseudo-$F_{(1,4)} = 1.78$, $P_{MC} = 0.20$], with nematodes being the dominant taxon (90.3 ± 2.3% over all B6S02 samples), and copepods (5.9 ± 1.4%) and nauplii (2.8 ± 1.2%) the second and third, respectively, most abundant groups (**Figure 4A** and Supplementary Figure 1A). Also meiofauna taxon diversity was comparable between April and October (all diversity indices: $P_{MC} > 0.05$). No differences could be discerned in nematode family [main PERMANOVA: Pseudo-$F_{(1,4)} = 0.61$, $P_{MC} = 0.67$; **Figure 4B** and Supplementary Figure 1B] or genus composition [main PERMANOVA: Pseudo-$F_{(1,4)} = 1.11$, $P_{MC} = 0.38$; **Figure 4C** and Supplementary Figure 1C] at site B6S02 between April and October 2015. All nematode family and genus diversity indices showed similar values for the two periods (main PERMANOVA, all $P_{MC} > 0.05$; see **Table 3**). *Halalaimus* species composition varied greatly within and between periods (see **Figure 4D** and Supplementary Figure 1D). Species richness was similar between April and October (**Table 3**). Although PERMANOVA tests failed to uncover an intra-annual change in meiofauna higher taxon, nematode family, and nematode genus composition, a considerable proportion of taxa was found only in samples from April or October (see Supplementary Figure 2). As shown by taxon accumulation curves in function of the number of periods sampled (data not shown), the investigation of site B6S02 during two different periods in 1 year resulted in, on average, 20% more meiofauna taxa, 10% more nematode families, 31% more nematode genera, and 44% more *Halalaimus* species compared to sampling only 1 period. The taxa that were restricted to one period were all rare, whilst the most abundant taxa were present in both periods.

Sampling Efficiency of Meiofauna and Nematode Diversity

Overall, 14 meiofauna taxa, 28 nematode families, 80 nematode genera, and 14 *Halalaimus* species were identified from the April and October 2015 samples collected in the GSR license area. **Table 4** contains the observed number of taxa (T_{obs}) and the values of 3 taxon richness estimators (i.e., Chao1, Jack2, and boot) for meiofauna higher taxa, nematode families, nematode genera and *Halalaimus* species given the nine MUC samples collected during GSRNOD15A (October 2015). For meiofauna higher taxa, Chao1 could not be defined, as doubletons (i.e., taxa found twice) were absent. In October 2015, we encountered 14 higher meiofauna taxa, 28 nematode families, 77 nematode genera and 11 *Halalaimus* species within the GSR license area. Between 75.7 and 89.7% (based on sites), or between 76.5 and 91.5% (based on MUCs) of all meiofauna taxa present were found. For nematode families, the current sampling captured between 84.3 and 94.9% (sites) or between 87.8 and 94.9% (MUCs) of nematode family richness. For *Halalaimus*, it was estimated that 66.7–85.3% (sites) or 70.5–85.9% (MUCs) of the species present was sampled. In agreement, the *Halalaimus* species accumulation curves appeared to start leveling off (**Figures 5D,F**). Nematode genus richness was captured to the least extent by our sampling as between 65.5 and 85.7% (sites) and 60.4 and 85.6% (MUCs) of all genera were detected. The change in genus richness in function of the number of MUC samples (**Figure 5C**) and sites (**Figure 5E**) confirms the incomplete characterization of nematode genus richness, as none of the curves reach an asymptote. Based on Chao1, we need 1 (or 1.1 times the current sampling effort) or 12 additional samples (or 2.3 times the current sampling effort) to characterize 95 or 100%, respectively, of total nematode family richness. To characterize 95 and 100% of total nematode genus richness, an extra 22 (3.4 times the current sampling effort) or 72 (9 times the current sampling effort) MUC samples, respectively,

are required. For *Halalaimus*, 8 (2 times the current sampling effort) or 19 (3 times the current sampling effort) additional samples are needed to capture 95 or 100% of total species richness within this genus.

DISCUSSION

Spatial Variability in Meiofauna and Nematode Communities within the GSR License Area

Despite the large geographical distances (60–270 km) and the statistically significant (albeit small) differences in sediment granulometry between the three nodule-bearing sites sampled in the GSR license area, the abundance, composition and diversity of meiofauna and nematode communities were largely comparable. This resemblance in abyssal meiobenthic communities between sites located around 60 km apart is presumably related to the largely similar environmental setting, with all sites constituting the same macro-habitat (*cfr.* Vanreusel et al., 2010) being equally deep nodule-bearing deep-sea silts. Similarly, geographically disparate, isobathic sites in various nodule-free deep-sea regions were shown to harbor similar meiofauna and nematode communities, at least as long as sediment granulometry and food availability were comparable (e.g., Lambshead et al., 2003; Sebastian et al., 2007; Pape et al., 2013; Lins et al., in press).

Several studies documented an effect of taxonomic resolution on spatial patterns in deep-sea benthic communities (Narayanaswamy et al., 2003; Muthumbi et al., 2011; Lins et al., in press). In the GSR license area, both the meiofauna (this study) and macrofauna (De Smet et al., 2017; this issue) were spatially homogenous irrespective of the taxonomical level considered. However, lower-level taxonomical identifications (family, genus, or species) were in both investigations restricted to particular taxa (this study: Nematoda; De Smet et al., 2017: Isopoda and Polychaeta). To make accurate inferences about the taxonomic resolution required to detect macro-ecological patterns in benthic size groups, more taxa need to be identified down to lower taxonomical level.

Although, overall no large spatial variability was observed in terms of sediment environmental variables and meiobenthic communities within the GSR license area, there were some subtle differences between sites in terms of specific meiofauna and nematode community attributes and environmental variables. First of all, there was a (non-significant) tendency of site B6S02 having higher sedimentary bacterial biomass, meiofauna abundances, and total estimated nematode genus richness relative to the other two sites sampled. This finding may be ascribed to the higher food availability at B6S02, inferred from seafloor POC flux calculated according to Lutz et al. (2007) [i.e., B6S02 (1.61 g C_{org} m^{-2} yr^{-1}) > B4S03 (1.56 g C_{org} m^{-2} yr^{-1}) > B4N01 (1.51 g C_{org} m^{-2} yr^{-1})]. In agreement, higher benthic bacterial biomass (Smith et al., 1997) and meiofauna abundances (Smith et al., 2008) have been linked before to elevated POC flux rates to the seabed in the CCFZ. Both polychaete (Glover et al., 2002; Wilson, 2016) and nematode species (Lambshead et al., 2003) diversity showed a positive relationship with seafloor POC flux for a variety of sites in the Eastern Tropical Pacific, including the CCFZ, though these studies covered a wider range of productivity. Likewise, De Smet et al. (2017), who sampled the same three sites in the GSR license area, recorded the highest polychaete (family) diversity at site B6S02. The rather small POC flux gradient covered here may explain the absence of statistically significant results. Secondly, even though the meiofauna and nematode communities at the three sites were dominated by the same taxa, there were some minor compositional differences owing to the less abundant or rare taxa, of which most had a seemingly limited spatial distribution (although this may simply be an artifact of under-sampling). Spatial distribution patterns of dominant and rare meiofauna taxa did not concur in the few nodule-free deep-sea systems investigated (Bianchelli et al., 2008; Gambi et al., 2010), suggesting these are driven by different processes. Several theories exist to explain patterns in taxon richness and coexistence (Holt, 2003; Leibold et al., 2004), but the mechanisms underlying these in the deep sea are still not fully understood (Gage, 1996; Gray, 2002; McClain and Hardy, 2010).

Intra-Annual Variability in Meiofauna and Nematode Communities

In oligotrophic deep-sea areas, like the CCFZ, biological responses to seasonally fluctuating organic matter input are generally restricted to bacteria and protozoans (Gooday, 2002). This may explain the lack of significant intra-annual variability in metazoan meiofauna (higher taxon level) and nematode (families and genera) abundance, composition and diversity observed at site B6S02 in the GSR license area. Nevertheless, interannual differences in nematode abundance (Miljutin et al., 2015), nematode genus composition (Radziejewska et al., 2001; Miljutin et al., 2015) and nematode genus diversity (Miljutin et al., 2015) observed in the CCFZ, were thought to be driven by interannual differences in primary productivity and the resultant flux of organic matter to the seabed. Possibly, the magnitude of the temporal difference in primary productivity observed here was not large enough to elicit a response by the meio- and nemato-fauna. Miljutin et al. (2015) mentions a difference in surface productivity (in terms of surface water chlorophyll a concentration) of a factor 1.6 between the 2 years the IFREMER area was investigated, which is slightly higher than that observed here (1.3). However, even in the Southern Ocean where the effect of an eight-fold difference in productivity was examined, no significant change in meiofauna standing stock was noted (Veit-Köhler et al., 2011). Alternatively, the meiofauna and nematode community may have reacted to the seasonally varying input of organic matter by changing vertical distribution (Veit-Köhler et al., 2011) or biomass, both of which were not assessed here.

Sampling Efficiency of Meiofauna and Nematode Diversity

The foregoing shows that the present sampling strategy allowed for the detection of macro-ecological patterns, but the question remains whether it adequately captured meiofauna and nematode diversity. Our study potentially characterized more

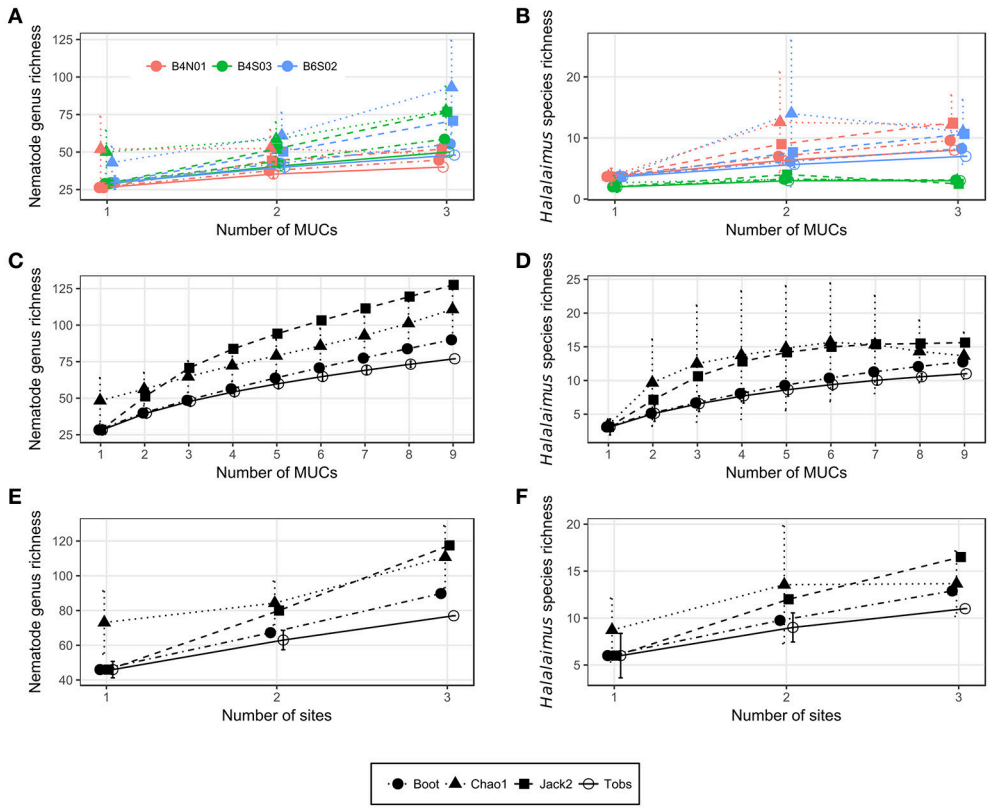


FIGURE 5 | Nematode genus **(A,C,E)** and *Halalaimus* species **(B,D,F)** accumulation curves for **(A,B)** all MUCs taken per site, **(C,D)** all MUCs taken, and **(E,F)** all sites sampled during GSRNOD15A. Error bars denote standard deviations and are only provided for T_{obs} and Chao1 in the PRIMER v6 software. Boot, Bootstrap; Jack2, Jackknife 2; T_{obs}, observed taxon richness.

TABLE 4 | Observed (T_{obs}) and estimated taxon richness for higher meiofauna taxa, nematode families, nematode genera, and *Halalaimus* species based on the accumulation of GSRNOD15A MUC samples ($n = 9$) and sites ($n = 3$).

		T_{obs}	Chao1	Jack2	Boot
Higher meiofauna taxa	MUC sample	14	–	18.3	15.3
	Sites		–	18.5	15.6
Nematode families	MUC samples	28	29.5 ± 2.29	31.9	29.9
	Sites			33.2	30
Nematode genera	MUC samples	77	110.8 ± 18.0	128	90
	Sites			118	89.8
Halalaimus species	MUC samples	11	13.7 ± 3.5	15.6	12.8
	Sites			16.5	12.9

than 90% of meiofauna taxa and nematode families present in the GSR license area, but comparatively more samples are needed to characterize the majority of the nematofauna at a genus level and the nematode genus *Halalaimus* at the species level. To accurately assess the level of biodiversity, it is crucial to work at the highest taxonomic resolution possible, i.e., species level (as recommended by ISA, 2015). The morphological identification of deep-sea nematodes, especially to species level, is time-consuming and difficult owing to the high number of undescribed species and, specifically for abyssal nodule areas, the dominance of speciose genera like *Thalassomonhystera* and *Acantholaimus* (Miljutina et al., 2010; Miljutin and Miljutina, 2016). Moreover, differences in diagnostic characteristics between different genera are not always unambiguous (Miljutina et al., 2010), which is why several genera were grouped here. As a consequence, the only species-level studies conducted in the CCFZ assigned nematodes to morphospecies (Lambshead et al., 2003; Miljutina et al., 2010), leaving these datasets incomparable and therefore hampering a region-wide assessment of biodiversity and connectivity. Furthermore, traditional morphological biodiversity assessment does not allow for the detection of cryptic species (Bhadury et al., 2008), which may be common amongst deep-sea nematodes including those inhabiting the CCFZ (Smith et al., 2008). A promising technique for rapid biodiversity analysis is metabarcoding of environmental samples (Taberlet et al., 2012; Fonseca et al., 2014), although currently it cannot yet serve as

a tool to reliably and accurately estimate (deep-sea) nematode diversity (Porazinska et al., 2009; Dell'Anno et al., 2015). One of the biggest problems with employing this approach for deep-sea nematodes is the lack of an adequate reference database (Dell'Anno et al., 2015; Sinniger et al., 2016). Hence, combined morphological and molecular biodiversity assessment is still warranted (Sinniger et al., 2016).

Based on the Chao 1 estimator, to capture 95 or 100% of the nematode genera present in the GSR license area, sampling effort would need to increase three- to nine-fold, respectively. As the full characterization of nematode genus richness required more samples than that of family richness, it is expected that even more samples are needed to adequately assess total nematode species richness. However, in agreement with Wilson (2016), the collection and processing of this estimated total number of samples required to capture meiobenthic diversity (nearly) fully, is highly impractical. In practice, the level of sampling effort should be a compromise between practical feasibility and the degree of coverage of total biodiversity (e.g., 90% of the total number of species present). The latter can be estimated through the extrapolation of taxon accumulation curves with taxon richness estimators, as was done in the present study. However, the minimal fraction of biodiversity to be covered or the minimal sampling effort in environmental baseline studies (and later on, in monitoring studies) in the CCFZ has so far not been stipulated by the International Seabed Authority (ISA), the regulator for the exploration for deep-sea mining in the Area Beyond National Jurisdiction (ISA, 2012; Durden et al., 2016).

Comparison with Other Abyssal (Nodule-Bearing) Site Studies

There are considerable discrepancies amongst this and previous studies on meiofaunal ecology in abyssal nodule areas in terms of sampling methodology, location, and timing (see Supplementary Table 1 and Supplementary Figure 3). This greatly complicates the comparison of meiofauna and nematode community descriptors between studies. Nevertheless, despite these aforementioned differences in sampling strategies, meiofauna densities documented for the GSR license area were broadly similar to those reported for other nodule-bearing abyssal sediments, including the CCFZ (Supplementary Figure 4).

As generally observed for deep-sea sediments world-wide (e.g., Lampadariou and Tselepides, 2006; Shimanaga et al., 2007; Veit-Köhler et al., 2011) including (CCFZ) nodule-bearing sediments (Snider et al., 1984; Renaud-Mornant and Gourbault, 1990; Shirayama, 1999; Ahnert and Schriever, 2001; Mahatma, 2009), the meiofauna in the GSR license area was dominated by nematodes (>85%). The predominant nematode families (i.e., Monhysteridae, Chromadoridae, and Desmoscolecidae) and genera (i.e., *Monhystrella/Thalassomonhystera* and *Acantholaimus*) in the GSR license area also attain high relative abundances in other nodule-bearing sites in the CCFZ (Renaud-Mornant and Gourbault, 1990; Lambshead et al., 2003; Miljutina et al., 2010) and other basins (Vopel and Thiel, 2001; Singh et al., 2014), as well as in nodule-free CCFZ sediments (Radziejewska et al., 2001). Moreover, these taxa are common in nodule-free abyssal sediments from around the World Ocean too (Miljutina et al., 2010; Vanreusel et al., 2010; Singh et al., 2016). Note, however, that subtle differences exist in the order of dominance of nematode taxa in abyssal nodule-bearing sediments, which may reflect spatial and/or temporal patterns or which may simply be a consequence of the different sediment depth strata sampled (see Supplementary Table 1). Six out of the fourteen *Halalaimus* species identified from the GSR license area were described for the first time from a nodule-bearing site in the Peru Basin (Bussau, 1993). Amongst those six species is *H. egregius* which was also reported for the eastern IFREMER license area (see Supplementary Figure 3) west off the GSR area (Miljutina et al., 2010). Thus, morphological data suggest potential connectivity between the GSR and the IFREMER license area in the CCFZ (100s of kilometers apart), and between these two CCFZ areas and the Peru Basin (1,000s of kilometers apart). However, molecular data are needed to confirm whether these are in fact the same species or whether they constitute cryptic species. Apparently, the degree of cryptic speciation in CCFZ nematodes is considered to be substantial (Smith et al., 2008).

In conclusion, it seems that the same suite of meiofauna and nematode taxa prevail (although in differing orders of dominance) in nodule-bearing (and nodule-free) sediments sampled within license areas (at a spatial scale of 10s–100s km), in different CCFZ license areas (100s–1,000s km) and in different regions (1,000s–10,000s km). These dominant taxa also occur in other deep-sea macro-habitats (though, again, not necessarily with the same proportions), supporting the notion of a low level of endemism for meiofauna taxa and nematode genera in the deep sea (Bik et al., 2010, 2012; Zeppilli et al., 2011). The fact that geographically disparate nodule-bearing CCFZ license areas and abyssal regions share potentially the same *Halalaimus* species, implies that a wide distribution range may not be limited to higher taxonomical levels. The existence of widespread deep-sea nematode species has already been reported based on morphological data (Miljutin et al., 2010). Moreover, identical ribosomal sequences, potentially representing the same species, were retrieved from sites separated by more than 10,000 km for taxa belonging to the Enoplida, an order to which also *Halalaimus* belongs (Bik et al., 2010).

Implications for Environmental Management and Suggestions for Future Research

The present findings indicate that distant, equally deep nodule-bearing sediments within the GSR license area are inhabited by similar meiofauna communities, and that the dominant taxa also occur in remote nodule-bearing and nodule-free deep-sea localities. Thus, based on water depth and nodule presence, different macro-habitats, with each habitat expected to harbor similar meiofaunal communities, can be delineated through habitat mapping (Lamarche et al., 2016). Together with similar data on other benthic size groups (macro- and mega-fauna), the current meiofaunal data will aid the spatial planning of PRZs and IRZs in the GSR license area.

Given that intra-annual variability in meiofaunal composition, standing stock, and diversity might be limited, any temporal changes observed in these community attributes during the monitoring of mined sites will most probably be directly resulting from mining activities. Any potential mining-induced alterations in meiofaunal communities may then be mitigated by recolonization from these PRZs, given that these are within the range of the maximal dispersal distance. Nonetheless, it is important to note that species-level, and preferably molecular, data are needed to confirm these acclaimed broad distribution ranges of meiofauna taxa and high connectivity between different deep-sea sites. Moreover, more research is needed on the influence of habitat heterogeneity, driven by spatial variability in, for instance, topography, nodule abundance and nodule size, on meiofaunal communities to ascertain the suitability of candidate preservation zones. Understanding the mechanisms responsible for taxon distributions, will help to gauge the extinction risks and recovery rates of taxa. Finally, the high proportion and the significant contribution to spatial variability in community composition of rare meiofauna and nematode taxa in the GSR license area, together with their potentially elevated extinction risk, warrant closer scrutiny of their functional importance. In highly diverse systems, like the deep sea, the presence of many rare taxa may act as a buffer against taxon loss following e.g., environmental disturbances (Naeem, 1998). In highly diverse terrestrial and coastal ecosystems, rare taxa have been shown to possess unique functional traits (Mouillot et al., 2013). For (nodule-bearing) deep-sea systems, however, information on the functional importance of rare taxa is lacking.

AUTHOR CONTRIBUTIONS

Conceived and designed the sampling design: EP, AV. Performed the sampling: EP, FH. Processed the samples: EP, TB. Analyzed the data: EP. Wrote the paper: EP, AV. Critically reviewed the paper: FH, TB.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00205/full#supplementary-material

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Characterization of Methane-Seep Communities in a Deep-Sea Area Designated for Oil and Natural Gas Exploitation Off Trinidad and Tobago

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Exploration of the deep ocean (>200 m) is taking on added importance as human development encroaches. Despite increasing oil and natural gas exploration and exploitation, the deep ocean of Trinidad and Tobago is almost entirely unknown. The only scientific team to image the deep seafloor within the Trinidad and Tobago Exclusive Economic Zone was from IFREMER in the 1980s. That exploration led to the discovery of the El Pilar methane seeps and associated chemosynthetic communities on the accretionary prism to the east of Trinidad and Tobago. In 2014, the E/V *Nautilus*, in collaboration with local scientists, visited two previously sampled as well as two unexplored areas of the El Pilar site between 998 and 1,629 m depth using remotely operated vehicles. Eighty-three megafaunal morphospecies from extensive chemosynthetic communities surrounding active methane seepage were observed at four sites. These communities were dominated by megafaunal invertebrates including mussels (*Bathymodiolus childressi*), shrimp (*Alvinocaris* cf. *muricola*), *Lamellibrachia* sp. 2 tubeworms, and *Pachycara caribbaeum*. Adjacent to areas of active seepage was an ecotone of suspension feeders including Haplosclerida sponges, stylasterids and *Neovermilia* serpulids on authigenic carbonates. Beyond this were large *Bathymodiolus* shell middens. Finally there was either a zone of sparse octocorals and other non-chemosynthetic species likely benefiting from the carbonate substratum and enriched production within the seep habitat, or sedimented inactive areas. This paper highlights these ecologically significant areas and increases the knowledge of the biodiversity of the Trinidad and Tobago deep ocean. Because methane seepage and chemosynthetic communities are related to the presence of extractable oil and gas resources, development of best practices for the conservation of biodiversity in Trinidad and Tobago waters within the context of energy extraction is critical. Potential impacts on benthic communities during oil and gas activities will likely be long lasting and include physical disturbance during drilling among others. Recommendations for the stewardship of

these widespread habitats include: (1) seeking international cooperation; (2) holding wider stakeholder discussions; (3) adopting stringent environmental regulations; and (4) increasing deep-sea research to gather crucial baseline data in order to conduct appropriate marine spatial planning with the creation of marine protected areas.

Keywords: Caribbean, stewardship, EBSA, VME, E/V *Nautilus*, Cruise ID NA054, anthropogenic impacts

INTRODUCTION

Trinidad and Tobago, the most southerly complex of islands in the Caribbean chain, is the largest oil and natural gas producer in the Caribbean region (Ministry of Energy and Energy Industries, 2017). Historically, the energy sector has focused on terrestrial and shallow coastal waters (Rajnauth and Boodoo, 2013), but with the improvements in deepwater technology, increasing global demands for energy, and dwindling reserves elsewhere in Trinidad and Tobago, deepwater (>1,000 m) exploration for oil and natural gas is currently underway in several lease blocks, with exploitation to begin in late 2017 (**Figure 1**). Potential impacts on seabed communities can result from activities in the oil and gas exploration phase, but are most likely to occur during the production phase. These include but are not limited to the physical disturbance of the seabed during drilling and associated activities, addition of chemical toxicants and sedimentation from drilling muds, and light and noise pollution (Cordes et al., 2016). Impacts on the water column are also likely from the input of sewage water, cooling water, processed cuttings etc. (Cordes et al., 2016). These impacts may persist for many years to decades (Ramirez-Llodra et al., 2011; Cordes et al., 2016). It is difficult to understand and manage the environmental impacts of these activities in the deep sea without knowing what habitats might be affected and the ecology and biogeography of the fauna that inhabit them.

The Caribbean Sea is one of the most important hotspots of shallow-water marine biodiversity in the world (Miloslavich et al., 2010, 2011; Tittensor et al., 2010). The limited exploration of the deep Caribbean means that there is insufficient information to make similar statements about the deep-sea species richness and diversity. Scientific exploration of the deep ocean off Trinidad and Tobago has been limited to only three major expeditions, resulting in limited knowledge of this ecosystem and its contribution to biodiversity. In 1884, the USS *Albatross* collected one benthic trawl from depths of ∼1,600 m, resulting in the collection of approximately 200 specimens (Townsend, 1901). During the 1980s, scientists from IFREMER discovered extensive chemosynthetic communities associated with mud volcanoes and methane seeps, resulting from the tectonic compression of fluid-rich marine sediments during the subduction of the Atlantic Plate under the Caribbean Plate that creates the extensive Barbados Accretionary Prism (Westbrook and Smith, 1983; Le Pichon et al., 1990; Olu et al., 1996, 1997; Sibuet and Olu, 1998). Similar seep environments were also discovered off Barbados and Venezuela at depths of 1,680–5,000 m (Jollivet et al., 1990; Olu et al., 1996, 1997; Sibuet and Olu, 1998). Methane seeps are patchy areas of the seafloor where methane and other hydrocarbons seep out of underground reservoirs through fractures and faults caused by tectonic activity, resulting in patchy ephemeral chemosynthetic communities, and enhancing deep-sea environmental heterogeneity (Hecker, 1985; Sibuet and Olu, 1998; Levin and Sibuet, 2012). Trinidad and Tobago's Exclusive Economic Zone (EEZ) has likely hosted chemosynthetic fauna since the Cenozoic, as there has been a rich fossil history uncovered in terrestrial deposits with many similarities between modern and past species (Gill et al., 2005).

El Pilar is the only explored area located within the EEZ of Trinidad and Tobago, and was found to be inhabited by at least 12 previously unknown species of chemosynthetic fauna (Jollivet et al., 1990; Olu et al., 1996; Sibuet and Olu, 1998). Two areas within the El Pilar site were explored in the 1980s: "Dome 1" featured small beds of the mussel *Bathymodiolus childressi*, *Neovermilia* serpulids, and thickets of vestimentiferans (Olu et al., 1996). "Dome 2" hosted an enormous *B. childressi* community with mussel densities of 800 ind.m^{-2} comprising a range of sizes (Olu et al., 1996). There were also dense mussel shell aggregations present (Olu et al., 1996). *Bathymodiolus* mussels and vestimentiferans are foundation species, creating habitat for other species and modifying the physical and chemical environment (Govenar, 2010). *Neovermilia* serpulids were abundant around the mussel beds, whereas shrimp (*Alvinocaris muricola*), anomuran crabs (*Lithodes manningi*), large brachyuran crabs, echiuran worms, and chitons (*Leptochiton micropustulus*) were observed in the mussel beds (Olu et al., 1996). Both domes also featured at least three species of gastropods (Olu et al., 1996). Many of the species associated with the mussel and tubeworms assemblages have since been described and are considered to be endemic to seeps (Jollivet et al., 1990; Olu et al., 1996).

With the impending start of deepwater hydrocarbon extraction off Trinidad and Tobago and the close affiliation of methane seeps with extractable oil and gas resources, the urgency for the regulation and management of these and other potential resources (fisheries, polymetallic nodules and other minerals, as well as bioprospecting) within the national jurisdiction has become more significant. Previous studies suggest that many deep-sea ecosystems have low resilience and recovery potential, but more importantly, that there is usually a high degree of uncertainty, so the precautionary approach should prevail (Ramirez-Llodra et al., 2011; Cordes et al., 2016; Huvenne et al., 2016; Van Dover et al., 2017). Environmental legislation should reflect modern environmental conservation practices by defining: (1) clear environmental goals and objectives, (2) the framework for environmental assessment and monitoring, (3) particular habitats or species that should be protected, and (4) the boundaries of areas designated for spatial management. Networks of protected areas (CBD Decision IX/20, Annex II) that capture 30–50% of the area (and representative habitats) within a region (Lodge et al., 2014), that consider cumulative

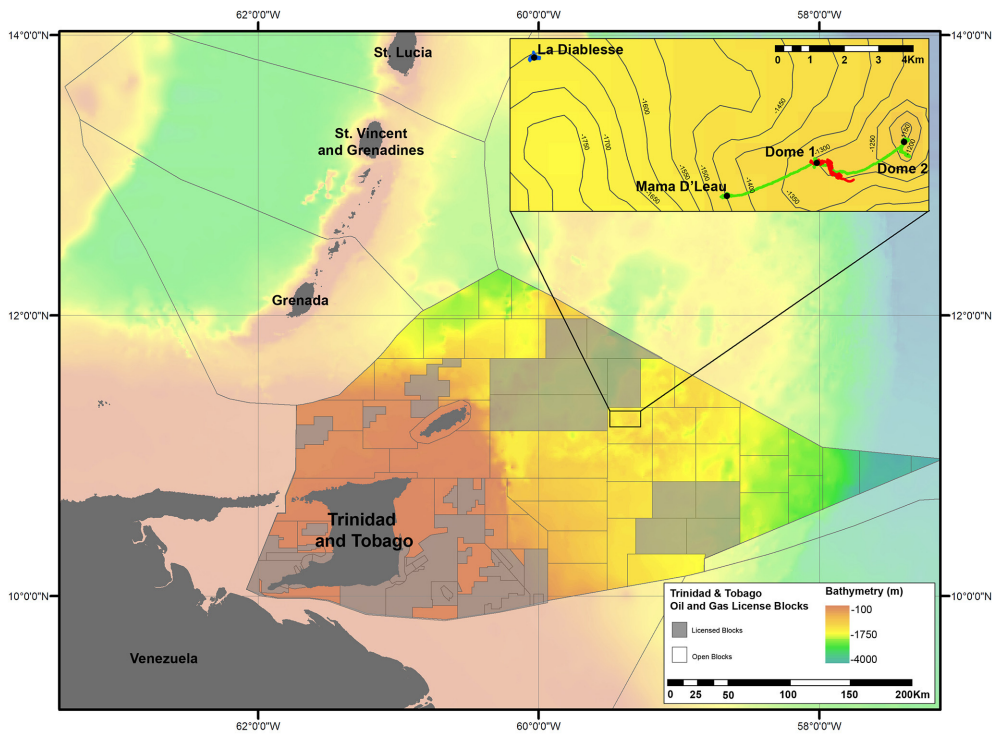


FIGURE 1 | Locations of the four methane seep sites surveyed at El Pilar off Trinidad and Tobago during NA054. Gray lines denote Exclusive Economic Zones (EEZs) in the main map. Within the Trinidad and Tobago EEZ, oil and gas lease blocks can be seen. The blocks that have been leased are colored transparent gray. ROV dives tracks can be seen in the inset map: green = Dive H1392, red = Dive H1393, and blue = Dive H1394. Seep sites are indicated by black circles and the site name. Lease blocks were adapted from a map by the Ministry of Energy and Energy Affairs, Government of the Republic of Trinidad and Tobago. Gridded bathymetric data provided by the General Bathymetric Chart of the Oceans (GEBCO) 30 arc-second grid (accessed via http://www.gebco.net/). Map and associated shapefiles created in ESRI ArcMap (version 10.3.1).

impacts of sectoral activities and climate change in their design, and that ensure ecosystem services are not interrupted, are a key component of regional environmental management plans. In Trinidad and Tobago, there is presently no environmental legislation to accompany the expansion of deepwater exploration and exploitation of oil and gas, leaving known and unknown communities vulnerable. Knowledge of important ecological areas, such as methane seeps, are a key first step in designing protected area networks.

This paper details the findings from the exploration of Trinidad and Tobago's deep ocean by Remotely Operated Vehicles (ROVs). In 2014, E/V *Nautilus*, in collaboration with scientists from Trinidad and Tobago, visited two previously sampled (Jollivet et al., 1990; Olu et al., 1996), and two unexplored areas of the El Pilar site (Carey et al., 2015). Here we describe the chemosynthetic communities and ecotones associated with the two previously visited sites ("Dome 1" and "Dome 2") and the two new sites ("Mama D'Leau" and "La Diablesse"), including species richness, assemblage structure and ecological observations. We also compare these communities with other chemosynthetic communities in the Caribbean, Gulf of Mexico and off Costa Rica. Given the ecological and scientific importance of methane-seep communities, we recommend ways to increase the stewardship of Trinidad and Tobago's deep sea.

MATERIALS AND METHODS

ROV Surveys and Collections

E/V *Nautilus* conducted three exploratory ROV dives at four sites within El Pilar to the east of Trinidad and Tobago from 5 to 8 October 2014 (NA054) using the two-bodied system comprising ROV *Hercules* and towsled-style ROV, *Argus* (**Figure 1** and **Table 1**). ROV *Hercules* was tethered to *Argus* via a neutrally buoyant tether while *Argus* was attached to the ship by 0.68 fiber cable, which allows real-time transmission of video, navigation, and data from the vehicles to operators and scientists aboard the ship. The ROV was equipped with two manipulators, a suction sampling system, push cores, and scaling lasers to measure objects. A TrackLink 5000 ultra-short baseline (USBL) navigation system was used to geolocate both ROVs, a Paroscientific Digiquartz pressure sensor was used to determine vehicle depth, and a IXSEA Octans III fiber-optic gyrocompass was used to determine vehicle attitude. The imaging and lighting suite was comprised of two Insite Pacific Zeus high-definition cameras (one on each vehicle) and CathX Aphos LED lampheads, which supply 28,000 lumens each. Additional lights and utility cameras aided in sampling and operational aspects of the dives.

Exploratory video surveys were undertaken at all four sites (**Figure 1** and **Table 1**): two of these had been visited

TABLE 1 | Sites surveyed during NA054.

Site	Latitude (N)	Longitude (W)	Min. Depth (m)	Max. Depth (m)	Date (DD.MM.YY)	ROV Dive
"Dome 2"	11.235	−59.345	998	1070	05.10.14, 06.10.14	H1392
"Dome 1"	11.231	−59.364	1190	1271	06.10.14, 07.10.14	H1392 H1393
"Mama D'Leau"	11.223	−59.392	1264	1274	06.10.14	H1392
"La Diablesse"	11.260	−59.444	1586	1629	07.10.14	H1394

previously ("Dome 1" and "Dome 2") and two were unexplored ("Mama D'Leau" and "La Diablesse"). "Mama D'Leau" and "La Diablesse" were chosen for exploration after the shipboard EM302 multibeam water-column data detected numerous strong bubble plumes emanating from circular dome-like structures and ridges in the area (Carey et al., 2015). CTD (temperature, salinity, depth) data were collected throughout the dives, as well as a temperature probe used to collect specific temperature measurements. Faunal samples were collected with the ROV *Hercules* manipulators and suction sampler, and used as voucher specimens to aid identifications based on imagery. Once the ROV was on deck, fauna were quickly transferred to containers containing chilled seawater. Each sample was photographed, and a tissue subsample taken. Two types of biological samples were archived at Harvard University's Museum of Comparative Zoology (MCZ) for easy access by researchers worldwide: voucher samples (intact/whole organisms) and tissue samples (subsamples of a population or whole organism) that are used for genetic analysis.

Telepresence Capabilities

E/V *Nautilus* is equipped with a high bandwidth output satellite system. This is used to send video and data to the Inner Space Center at the University of Rhode Island's Graduate School of Oceanography, where it is then distributed via the Inner Space Center and NautilusLive websites directly to Exploration Command Centers for both public and scientific consumption. Through the *Nautilus* Scientist Ashore Program, a network of scientists around the world are connected to the watch-standing scientists on the ship to participate in the exploration in real time. A real-time chat between the watch leader and scientists ashore expands the expertise on board and allows for broader participation by scientists.

Analysis of Imagery and Samples

Video from the high-definition camera on the ROV was reviewed and image frames archived of each identifiable megafaunal morphospecies. Criteria used for the selection of megafaunal morphospecies was that individuals were greater than 1 cm in maximum dimension and that there were sufficient details to identify them to a putative "species-level" morphospecies. Morphospecies that could not be identified to species but appeared morphologically distinct were assigned a unique informal species name (e.g., Polynoidae sp. 1). These were then identified by taxonomic experts (see section Acknowledgements) or using the literature. Fauna collected were also identified to the lowest possible taxonomic level. The presence or absence of each species was documented at each site using all available imagery. This process provided an estimate of the minimum number of morphospecies in the surveyed areas (species richness) and will aid in delimiting species ranges.

Statistical Analyses

Species accumulation curves and richness estimates were made using Primer v.6 (Clarke and Gorley, 2006). Since the species accumulation curve indicated that the species inventory had not yet reached asymptote and was continuing to accumulate, the recommendations of Magurran (2004) were followed and the Chao 1, Jacknife 2, and Bootstrap estimators were used to estimate total species richness. Sorensen's coefficient was used to compare the similarity at morphospecies level between the presence/absence data of the faunal assemblages at the four sites visited.

RESULTS

Site Descriptions within El Pilar

A total of 83 megafaunal morphospecies was identified from four sites (**Figures 2–4** and **Table 2**). There were 58 morphospecies recorded at "Dome 1," 46 morphospecies at "Dome 2," 19 morphospecies at "Mama D'Leau," and 20 morphospecies at "La Diablesse" (**Table 2**). Eight morphospecies were shared across all locations (cf. *Neovermilia* sp., *Lamelllibrachia* sp. 2, cf. Amphipoda spp., *Chaceon* cf. *fenneri, Alvinocaris* cf. *muricola, Aldrovandia* cf. *affinis, Pachycara caribbaeum*, and *Bathymodiolus childressi*; **Table 2**). These are likely underestimates due to poor image resolution, difficulty identifying fauna from images, and the presence of cryptic species. Differences in morphospecies richness among sites is likely due to a higher sampling effort at "Dome 1" and "Dome 2" rather than from distinct species diversity and distributions. Cnidaria was the most species-rich phylum, with a total of 22 morphospecies observed, followed by the Arthropoda (16 morphospecies) (**Figure 4**). Species-accumulation curves suggest that megafaunal morphospecies richness has not been fully characterized in areas surveyed during NA054 (**Figure 4**). Chao-1 and Jacknife 2 estimated 129 (Chao-1 s.d. = 19.5) total morphospecies, whereas Bootstrap estimated 96 morphospecies (**Figure 4**). Communities at "Mama D'Leau" and "La Diablesse" were the most similar with 60% Sorensen's similarity, followed

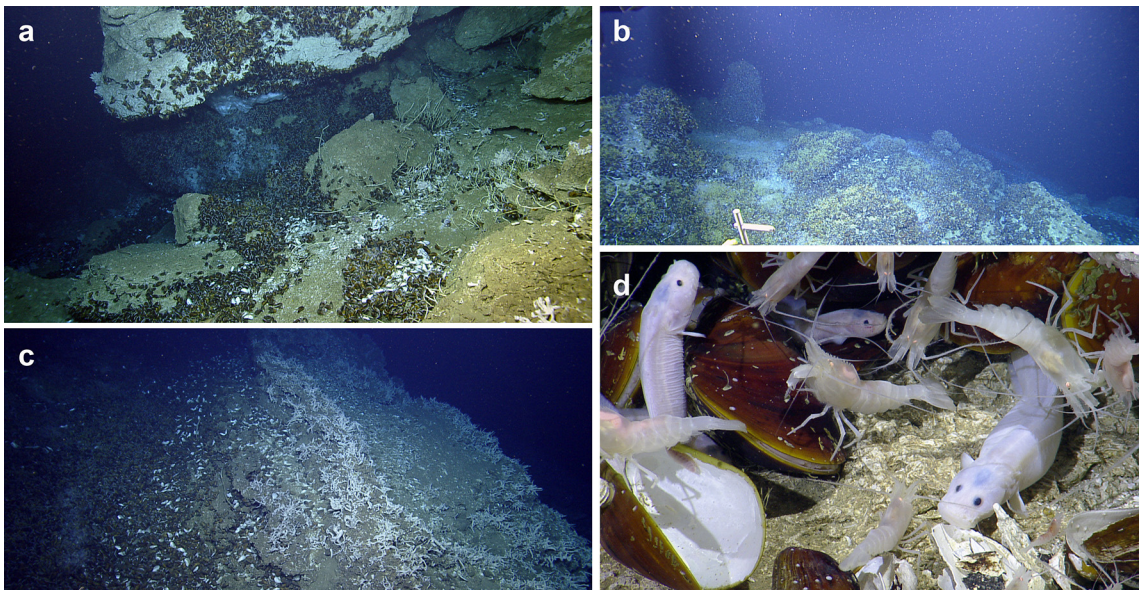


FIGURE 2 | Methane-seep sites at El Pilar off Trinidad and Tobago. **(a)** The methane seep on "Dome 2" containing methane hydrate under an authigenic carbonate ledge surrounded by *Bathymodiolus childressi*, *Lamellibrachia* sp. 2 and other fauna. **(b)** Pinnacle structures covered in high abundances of *B. childressi* and other fauna at a methane seep on "Dome 1." **(c)** Chemosynthetic communities in depressions at "Mama D'Leau," dominated by *B. childressi*, are surrounded by authigenic carbonates hosting Haplosclerida n. sp. and *Lamellibrachia* sp. 2. **(d)** *B. childressi*, *Alvinocaris* cf. *muricola, Pachycara caribbaeum,* and Gastropoda sp. 1 at a methane seep at "La Diablesse."

by communities at Domes 1 and 2 (52% similarity). The communities at "Dome 1" were 47% similar to "Mama D'Leau" and 43% to "La Diablesse," whereas "Dome 2" had 43% similarity with "Mama D'Leau" and 30% with "La Diablesse."

"DOME 1": Three areas of "Dome 1" at the El Pilar site were explored moving from southeast to northwest. There was an extensive chemosynthetic community of adult and juvenile *Bathymodiolus childressi*, *Lamellibrachia* sp. 2, *Alvinocaris* cf. *muricola, Pachycara caribbaeum, Kanoia* cf. *meroglypta* and cf. *Munidopsis* sp. 1 growing on authigenic carbonate pinnacles (**Figure 2**). There were also crevices with thickets of *Lamellibrachia* sp. 2. indicating subsurface seepage. Downslope was bubble venting and reduced (dark) sediment with a different habitat featuring *Stenohelia* cf. *profunda*, Haplosclerida n. sp., sparse *Laubiericoncha* cf. *myriamae* shells, *Neovermilia* sp. and dead and live *Lamellibrachia* sp. 2 in high abundances on the carbonates. Further from visibly active seepage was another habitat comprised of several large octocorals with commensals, the octopus *Graneledone* n. sp., actiniarians and fish (**Table 2**).

Moving northwest to the second site at "Dome 1," there were both sedimented areas and active seeps with methane hydrates, *Bathymodiolus childressi*, *Lamellibrachia* sp. 2, *Alvinocaris* cf. *muricola, Pachycara caribbaeum*, cf. *Neovermilia* sp., cf. *Munidopsis* sp. 1, *Chaceon* cf. *fenneri,* amphipods, *Kanoia* cf. *meroglypta* and *Bathynerita* cf. *naticoidea*. Surrounding the mussel bed were authigenic carbonates covered with beds of cf. *Neovermilia* sp., Haplosclerida n. sp. and *Stenohelia* cf. *profunda*. Several *B. childressi* shell deposits were also observed. There were also sedimented areas with depressions that contained seeps, some of which were well developed, containing bacterial mats, *B. childressi*, *K.* cf. *meroglypta*, *Lamellibrachia* sp. 2, *A.* cf. *muricola,* cf. *Neovermilia* sp., cf. *Munidopsis* sp. 1, *P. caribbaeum* and cf. *Methanoaricia* sp., while others were sparsely populated.

The most northwest site was mostly sedimented and inactive with small carbonates and only four discrete areas of seepage. The first area of activity was dominated by extensive beds of cf. *Neovermilia* sp., with *Lamellibrachia* sp. 2, *Stenohelia* cf. *profunda*, cf. Zoanthidae sp., and several other morphospecies also present. The other seep communities were in depressions and were dominated by dead and live cf. *Neovermilia* sp., as well as bacterial mats, *Bathymodiolus childressi*, *Lamellibrachia* sp. 2, Haplosclerida n. sp., *S.* cf. *profunda* and *Kanoia* cf. *meroglypta.* There were also several mobile crustacea and chordates present as well as many dead *Laubiericoncha* cf. *myriamae* shells. In total, 24 of the 58 morphospecies observed at "Dome 1" were found at no other site (**Table 2**).

"DOME 2": "Dome 2" of the El Pilar site, previously explored by Olu et al. (1996), hosted an area of active seepage with associated chemosynthetic communities within a depression. This was centered on an authigenic carbonate overhang where methane hydrate was accumulating (**Figure 2**). Amphipods were abundant on the methane hydrate, as well as in the surrounding mussel bed, which was dominated by *Bathymodiolus childressi*, *Alvinocaris* cf. *muricola*, and *Pachycara caribbaeum*. There were abundant cf. *Neovermilia* sp., Haplosclerida n. sp., *Stenohelia* cf. *profunda* and *Lamellibrachia* sp. 2 on and in cracks of the carbonates surrounding the mussel bed. Adjoining the chemosynthetic community was a large area of empty *B. childressi*

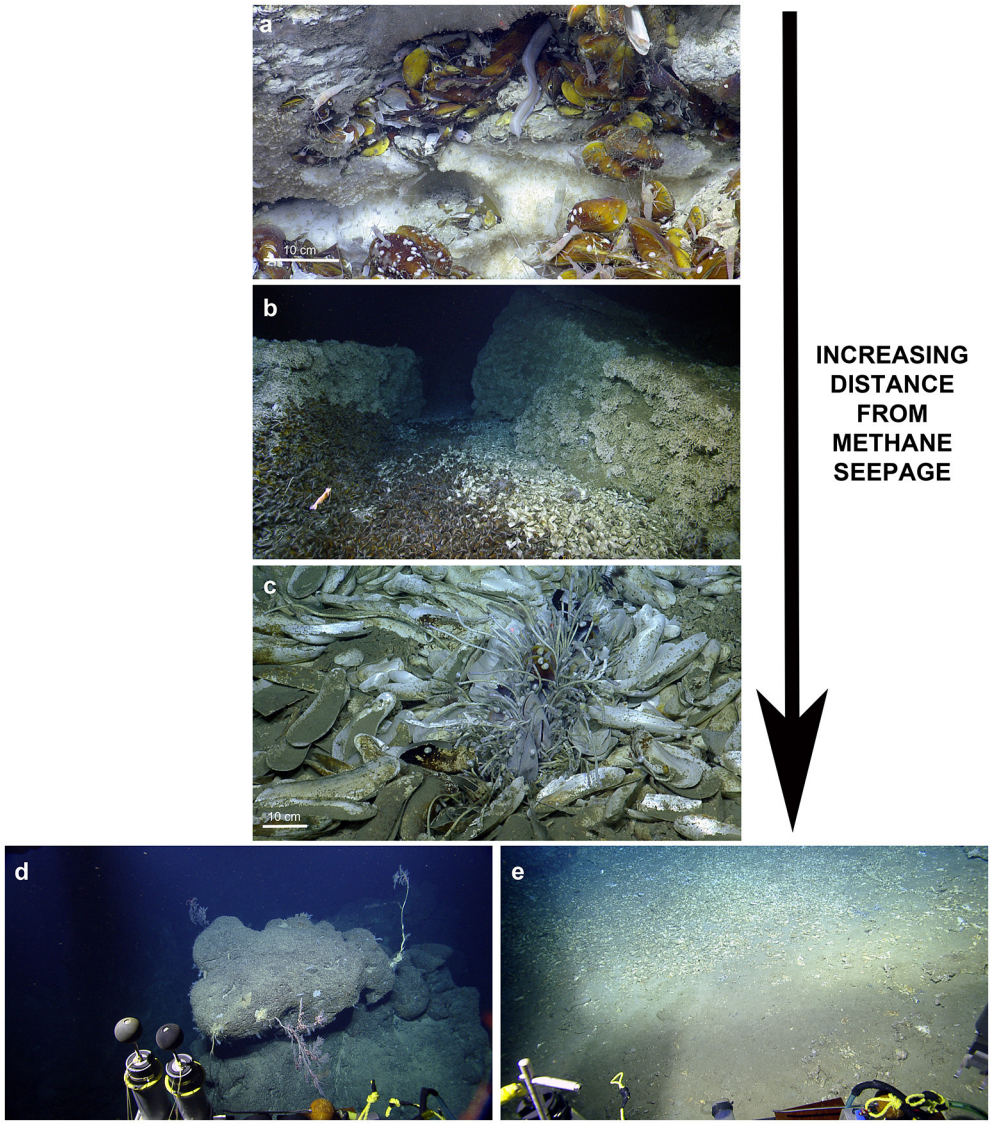


FIGURE 3 | Zonation at El Pilar off Trinidad and Tobago. The five habitats observed at the four El Pilar sites, in order of increasing distance from methane seepage: **(a)** Dense aggregations of amphipods on methane hydrate, which is surrounded by chemosynthetic *Bathymodiolus childressi*, *Alvinocaris* cf. *muricola*, *Pachycara caribbaeum,* and other fauna. **(b)** Abundant filter-feeders (e.g., *Neovermilia* serpulids, *Stenohelia profunda*, Haplosclerida n. sp.) on authigenic carbonate structures adjacent to *Bathymodiolus* mussel beds. **(c)** *Bathymodiolus* shell aggregations, which can sometimes have discrete bushes of *Lamellibrachia* sp. 2, live *B. boomerang*, *A.* cf. *muricola*, *Kanoia* cf. *meroglypta* gastropods, and other small fauna. Furthest from the active methane seeps are **(d)** authigenic carbonates that host large sparse octocorals and their commensals and/or **(e)** inactive sedimented areas.

shells. South of the active seep were large carbonates that hosted at least five species of octocorals (cf. Chrysogorgiidae sp., cf. *Plumarella* sp. 1, cf. *Plumarella* sp. 2, cf. *Thouarella* sp., cf. Keratoisidinae -B1 clade sp.) and their commensals (**Figures 3**, **5**). Of the 46 morphospecies observed at "Dome 2," 18 were observed at no other site (**Table 2**).

"MAMA D'LEAU": "Mama D'Leau" had never been visited previously and was comprised of chemosynthetic habitats on a slope amongst large authigenic carbonates (**Figure 2**). At "Mama D'Leau," the carbonates hosted abundant Haplosclerida n. sp., *Stenohelia* cf. *profunda* and *Lamellibrachia* sp. 2, peripheral to areas of seepage that were inhabited by bacterial mats, *Bathymodiolus childressi* with Gastropoda sp. 1 living on the shells, *Alvinocaris* cf. *muricola, Lamellibrachia* sp. 2, *Pachycara caribbaeum*, cf. *Munidopsis* sp. 1 and *Kanoia* cf. *meroglypta* (**Figure 2**). The chemosynthetic communities tended to be in depressions and were surrounded by large beds of empty *B. childressi* shells. Several other species were observed in this area including cf. *Erenna* sp., *Graneledone* n. sp., and *Plinthaster* cf. *dentatus* (**Figure 5**). All 19 morphospecies observed at "Mama D'Leau" were observed at other sites explored during NA054 (**Table 2**).

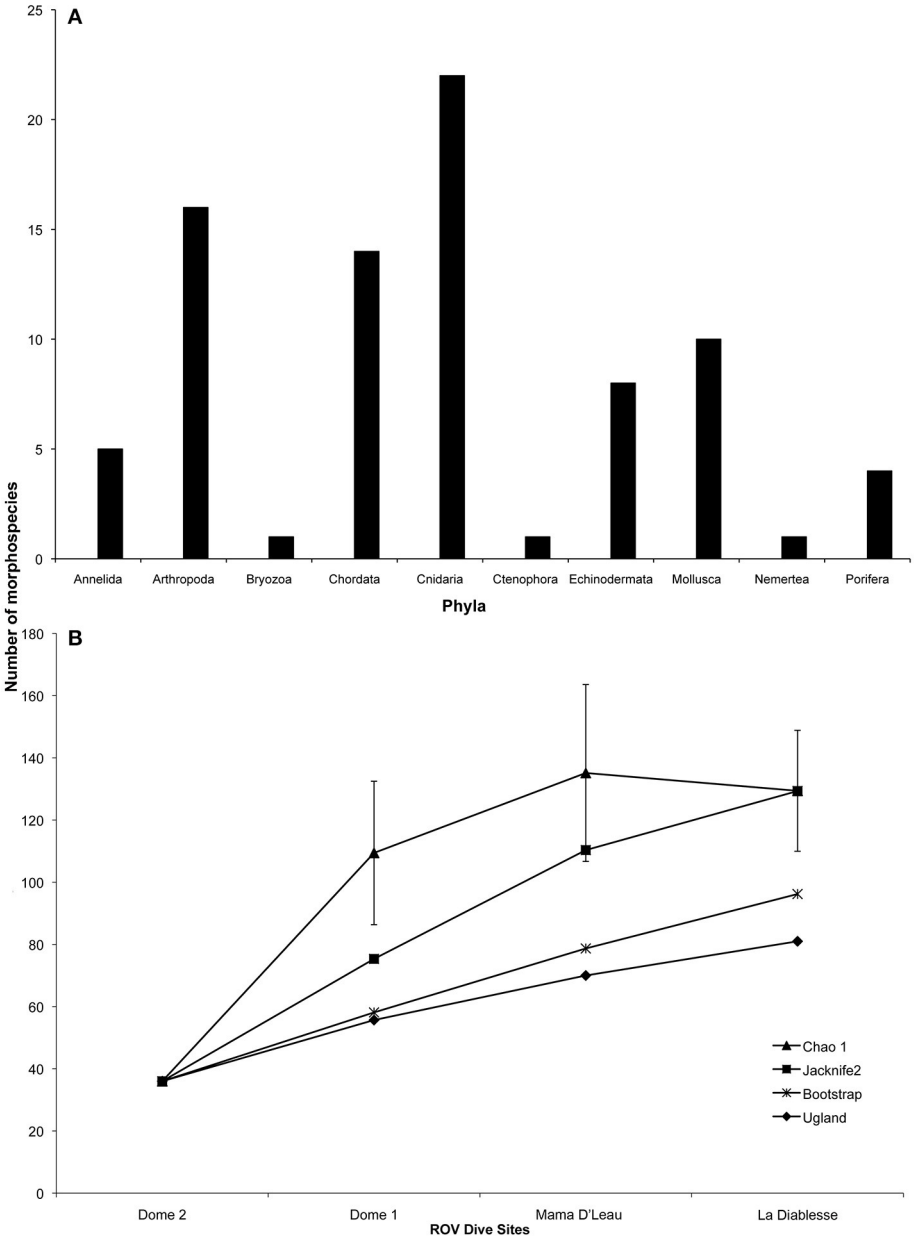


FIGURE 4 | Megafaunal morphospecies richness observed at El Pilar off Trinidad and Tobago. **(A)** Megafaunal morphospecies richness by phylum. **(B)** Chao-1 (±s.d.), Jacknife2, Bootstrap, and Ugland (UGE) estimates of megafaunal morphospecies richness with increasing number of ROV dive sites.

"LA DIABLESSE": The chemosynthetic communities at "La Diablesse" comprised large *Bathymodiolus childressi* beds on authigenic carbonates and had never been imaged before. These beds also housed *Alvinocaris* cf. *muricola, Pachycara caribbaeum*, cf. Amphipoda spp., cf. Gastropoda sp. 1, and *Paralomis* cf. *arethusa*. There were several areas of methane-gas escape and methane hydrate, which were covered in amphipods. Downslope from the live *B. childressi* beds were large beds of empty shells of *B. childressi* and *B. boomerang*, which had sparse thickets of *Lamellibrachia* sp. 2, *Kanoia* cf. *meroglypta*, Demospongiae sp. 2 and bacterial mats interspersed. These shell aggregations adjoined sedimented areas, which hosted a different complement of fauna that included *Paroriza* cf. *pallens* and Ophiuroidea sp. (**Figures 2**, **3** and **Table 2**).

Habitat Zonation within the El Pilar Sites

Five different habitats were observed at all four of the deep-sea sites that were surveyed: (a) active methane seeps dominated by *Bathymodiolus* mussel beds, (b) peripheral abundant filter-feeders (e.g., *Neovermilia* sp., *Stenohelia profunda*, Haplosclerida n. sp.) on authigenic carbonate structures adjacent to mussel beds, (c) mussel shell aggregations, (d) sparse octocorals

TABLE 2 | Megafaunal morphospecies observed from all imagery collected during NA054 off Trinidad and Tobago.

Phylum	Class (Subclass)	Order (Infraorder)	Family	Genus/Morphospecies	"Dome 2"	"Dome 1"	"Mama D'Leau"	"La Diablesse"
Annelida	Polychaeta (Echiura)			cf. Echiura sp.	–	+	–	–
	(Errantia)	Phyllodocida	Polynoidae	cf. Polynoidae sp. 1	–	+	–	–
				cf. Polynoidae sp. 2	–	–	–	+
	(Sedentaria)	Orbiniida	Orbiniidae	cf. *Methanoaricia* sp.	–	+	–	–
		Sabellida	Serpulidae	cf. *Neovermilia* sp.	+	+	+	+
			Siboglinidae	*Lamellibrachia* sp. 2	+	+	+	+
Arthropoda	Malacostraca	Amphipoda		cf. Amphipoda spp.	+	+	+	+
		Decapoda (Anomura)		cf. Decapoda sp.	+	–	–	–
			Chirostylidae	*Gastroptychus* cf. *spinifer*	+	+	–	–
			Lithodidae	*Lithodes* cf. *manningi*	+	+	–	+
				cf. *Paralomis* n. sp.	–	+	–	+
				Paralomis cf. *arethusa*	–	+	+	+
			Munidopsidae	cf. *Munidopsis* sp. 1	+	+	+	–
				cf. *Munidopsis* sp. 2	+	–	–	–
			Munididae	cf. *Munida* sp.	+	+	–	–
			Paguroidea	cf. Paguroidea sp. 1	–	+	–	–
				cf. Paguroidea sp. 2	+	+	–	–
		(Brachyura)	Geryonidae	*Chaceon* cf. *fenneri*	+	+	+	+
		(Caridea)		cf. Caridea sp. 1	+	–	–	–
			Alvinocarididae	*Alvinocaris* cf. *muricola*	+	+	+	+
			Nematocarcinidae	cf. *Nematocarcinus* sp.	+	+	–	–
			Thoridae	cf. *Lebbeus* sp.	+	+	–	–
Bryozoa	Stenolaemata	Cyclostomatida	Horneridae	cf. Horneridae sp.	–	+	–	–
Chordata	Actinopterygii	Alepocephaliformes	Alepocephalidae	*Alepocephalus* cf. *agassizii*	–	+	–	–
		Gadiformes	Macrouridae	*cf. Coryphaenoides* sp.	+	–	–	–
				Nezumia cf. *aequalis*	+	+	–	–
		Notacanthiformes	Halosauridae	*Aldrovandia* cf. *affinis*	+	+	+	+
		Ophidiiformes	Ophidiidae	cf. Ophidiidae sp. 1	+	+	–	–
				cf. Ophidiidae sp. 2	+	–	–	–
				Barathrites cf. *parri*	+	+	–	–
			Bythitidae	cf. Bythitidae sp.	+	–	–	–
		Perciformes	Zoarcidae	*Pachycara caribbaeum*	+	+	+	+
				cf. Zoarcidae sp.	–	+	–	–
		Zeiformes	Oreosomatidae	cf. *Allocyttus* sp.	+	–	–	–
	Elasmobranchii	Squaliformes	Etmopteridae	*Centroscyllium* cf. *fabricii*	–	–	–	–
		Rajiformes	Rajidae	*Fenestraja* cf. *plutonia*	–	+	–	–
	Holocephali	Chimaeriformes	Chimaeridae	*Hydrolagus* cf. *affinis*	–	+	–	+
Cnidaria	Anthozoa (Hexacorallia)	Actiniaria		cf. Actiniaria sp. 1	–	+	–	–
				cf. Actiniaria sp. 2	–	–	–	+
			Amphianthidae	cf. *Amphianthus* sp.	–	+	–	–
			Hormathiidae	cf. *Paraphelliactis* n. sp.	+	+	–	–
		Zoantharia		cf. Zoantharia sp.	–	+	–	–
	(Octocorallia)	Alcyonacea		cf. Alcyonacea sp. 1	+	–	–	–
				cf. Alcyonacea sp. 2	–	+	–	–
			Chrysogorgiidae	cf. Chrysogorgiidae sp.	+	–	–	–

(Continued)

TABLE 2 | Continued

Phylum	Class (Subclass)	Order (Infraorder)	Family	Genus/Morphospecies	"Dome 2"	"Dome 1"	"Mama D'Leau"	"La Diablesse"
			Primnoidae	cf. *Plumarella* sp. 1	−	+	−	−
				cf. *Plumarella* sp. 2	+	+	−	−
				cf. *Thouarella* sp.	+	+	−	−
			Isididae	cf. Keratoisidinae (B1 clade) sp.	+	−	−	−
				cf. *Keratoisis* sp.	−	+	−	−
	Hydrozoa			cf. Hydrozoa sp. 1	+	−	−	−
				cf. Hydrozoa sp. 2	−	+	−	−
			Stylasteridae	*Stenohelia* cf. *profunda*	+	+	+	−
		Siphonophorae	Erennidae	cf. *Erenna* sp.	−	+	+	−
			Pyrostephidae	*Bargmannia* cf. *lata*	−	+	−	−
			Rhodaliidae	cf. Rhodaliidae sp.	+	−	−	−
		Trachymedusae	Halicreatidae	cf. *Halitrephes* n. sp.	−	+	−	−
				cf. Halitrephes sp.	+	−	−	−
		Narcomedusae	Aeginidae	*Aeginura grimaldii*	+	−	−	−
Ctenophora	Tentaculata	Cydippida		cf. Cydippida sp.	+	−	−	−
Echinodermata	Asteroidea			cf. Asteroidea sp.	+	−	−	−
		Spinulosida	Echinasteridae	cf. Echinasteridae sp.	+	−	−	−
		Valvatida	Goniasteridae	cf. Goniasteridae sp.	−	+	−	−
				Plinthaster cf. *dentatus*	+	+	+	−
	Holothuroidea	Aspidochirotida	Synallactidae	*Paroriza* cf. *pallens*	−	−	−	+
		Elasipodida	Pelagothuriidae	*Enypniastes* cf. *eximia*	−	+	−	−
	Ophiuroidea			cf. Ophiuroidea sp.	−	−	−	+
		Euryalida	Asteroschematidae	cf. *Asteroschema* sp.	+	+	−	−
Mollusca	Bivalvia	Mytiloida	Mytilidae	*Bathymodiolus boomerang*	−	−	−	+
				Bathymodiolus childressi	+	+	+	+
		Venerida	Vesicomyidae	*Laubiericoncha* cf. *myriamae* (shells only)	+	+	+	−
	Cephalopoda	Octopoda	Octopodidae	cf. *Graneledone* n. sp.	−	+	+	−
		Vampyromorpha	Vampyroteuthidae	*Vampyroteuthis* cf. *infernalis*	−	−	+	−
	Gastropoda			cf. Gastropoda sp. 1	−	+	+	+
		Vetigastropoda	Cataegidae	*Kanoia* cf. *meroglypta*	−	+	+	+
			Fissurellidae	cf. Fissurellidae sp.	+	+	−	−
		Cycloneritimorpha	Phenacolepadidae	*Bathynerita* cf. *naticoidea*	−	+	−	−
		Neogastropoda	Muricidae	cf. *Trophon* sp.	−	+	−	−
	Polyplacophora	Lepidopleuridae	Leptochitonidae	*Leptochiton* cf. *micropustulosus*	+	−	−	−
Nemertea				cf. Nemertea sp. 1	−	+	−	−
Porifera	Demospongiae			cf. Demospongiae sp. 1	+	−	−	−
				cf. Demospongiae sp. 2	−	+	−	+
		Haplosclerida		Haplosclerida n. sp.	+	+	+	−
		Poecilosclerida	Cladorhizidae	cf. Cladorhizidae sp.	−	+	−	−

Presence at each surveyed site is indicated by +, while absence is indicated by −.

and other non-chemosynthetic fauna on authigenic carbonate structures further from mussel beds, and (e) inactive (no evident methane seepage or indicators of seepage) sedimented areas (**Figures 3a–e**). Areas of methane hydrate in active seeps (1) were covered with dense populations of amphipods (approximately 10,000 ind.m^{-2}, although this is likely an underestimate) (**Figure 3**). Surrounding the methane hydrate were beds of *Bathymodiolus childressi*, the dominant fauna that live in closest proximity to the methane seepage (**Figure 2**). Mean mussel densities were generally consistent between sites apart

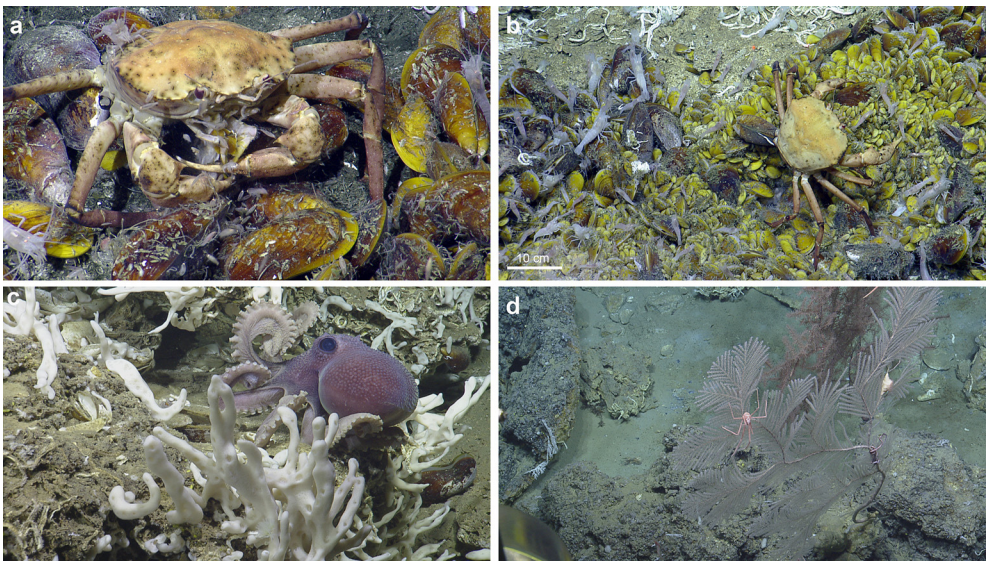


FIGURE 5 | Ecological observations at El Pilar. **(a)** *Chaceon* cf. *fenneri* and amphipods consume a *Bathymodiolus childressi* at "Dome 1." **(b)** Variably sized *Bathymodiolus childressi* and *Alvinocaris* cf. *muricola* indicate ongoing recruitment at "Dome 1." **(c)** A new species of *Graneledone* octopus seen by its *Bathymodiolus* shell midden at "Dome 1." **(d)** A large *Plumarella* sp. 2 with commensal *Amphianthus* actiniaria and Chirostylidae sp. (foreground) with a large *Thouarella* sp. (background) at "Dome 1."

from at "Dome 1" ("Dome 1": 712 ind.m^{-2}, "Dome 2": 277 ind.m^{-2}, "Mama D'Leau": 269 ind.m^{-2}, "La Diablesse": 312 ind.m^{-2}). Maximum densities were much higher when juvenile mussels were present, e.g., at "Dome 1": 1353 ind.m^{-2}. Always inhabiting these mussel beds were *Alvinocaris* cf. *muricola* (mean densities - "Dome 1": 199 ind.m^{-2}, "Dome 2": 29 ind.m^{-2}, "Mama D'Leau": 32 ind.m^{-2}, "La Diablesse": 142 ind.m^{-2}), and *Pachycara caribbaeum* (mean densities - "Dome 1": 20 ind.m^{-2}, "Dome 2" and "Mama D'Leau": 1 ind.m^{-2}, "La Diablesse": 15 ind.m^{-2}). These mean densities are likely underestimates given the likelihood of multiple layers of fauna in the mussel beds. Other fauna were also common within the mussel beds including cf. *Neovermilia* sp. and cf. Gastropoda sp. on *B. childressi* shells, cf. *Munidopsis* sp. 1, *Chaceon* cf. *fenneri*. *Plinthaster* cf. *dentatus, Kanoia* cf. *meroglypta,* and several lithodid species. A number of other polychaete and mollusc species were observed in the beds but more rarely (**Table 2**). Occasionally, there were bushels of *Lamellibrachia* sp. 2 in the live mussel beds but these were mostly seen on carbonates surrounding the *Bathymodiolus* beds, along with dense populations of cf. *Neovermilia* sp., Haplosclerida n. sp., and *Stenohelia* cf. *profunda*. Adjacent to these habitats were large beds of dead *Bathymodiolus* spp. shells on sediment, sometimes with small patches of *Lamellibrachia* sp. 2 or *B. boomerang* (**Figure 3**). Further away from the active seepage and chemosynthetic communities but located on the large carbonates were isolated octocorals and their commensals, as well as actiniarians (**Figure 3**). Sedimented areas surrounded the carbonates and seeps (**Figure 3**). There were no temperature anomalies observed at the four sites investigated (ambient temperature was 4.0–5.7°C).

Trophic Interactions and Recruitment within the El Pilar Sites

At "Dome 1," a *Chaceon* cf. *fenneri* was observed feeding on *Bathymodiolus childressi*, while scavenging amphipods appeared to benefit from sloppy feeding by the crab (**Figure 5**). Other feeding interactions inferred included several *C.* cf. *fenneri* crabs missing legs and an octopus (*Graneledone* n. sp.) dwelling with a *B. childressi* shell midden (**Figure 5**). All four sites showed ongoing recruitment of chemosynthetic species: At "Dome 1," there were high densities of gastropod eggs on adult *Bathymodiolus childressi*, a gravid *C.* cf. *fenneri* crab, and varying sizes of *Alvinocaris* cf. *muricola* shrimp (2.9–11.8 cm total length) and *B. childressi* mussels (0.9–17.1 cm total length). "La Diablesse" also showed varying sizes of *B. childressi*, *A.* cf. *muricola,* and *Pachycara caribbaeum* eelpouts (1.8–14.6, 2.3–11.6 cm and 5.1–24.7 total length respectively) (**Figure 5**). At "Mama D'Leau," there was also ongoing recruitment of *B. childressi* as indicated by variable shell size. "Dome 2" showed little recruitment with few young *B. childressi* observed, mostly in patches amongst beds of adult mussels and clutches of gastropod eggs on adult mussel shells.

DISCUSSION

Ecology of the Communities

We provide new insights into the seep communities at four sites in El Pilar off Trinidad and Tobago. Our results indicate that Trinidad and Tobago's deep sea is poorly characterized with the majority completely unknown, emphasized by at least five of the morphospecies observed appearing to be new to science: cf.

Haplosclerida n. sp., cf. *Graneledone* n. sp., cf. *Paraphelliactis* n. sp., cf. *Paralomis* n. sp. and cf. Halicreatidae n. sp (Voight and Kurth, 2017) (E. Rodriguez, pers. comm.; D. Lindsay, pers. comm., E. Macpherson, pers. comm., S. Pomponi, in prep.). *Pachycara caribbaeum*, a zoarcid present in large numbers at every site at El Pilar, has also been described from these sites as well as an additional Caribbean site recently (Anderson et al., 2016). Morphospecies richness within our surveys appears to be high (83 morphospecies) relative to other chemosynthetic habitats in the region e.g., 32 morphospecies were observed at the Cayman vent fields (Plouviez et al., 2015). Species richness in this area remains undersampled indicating that more data are required to better understand how these diverse assemblages will be impacted by oil and gas exploration and exploitation. There do not appear to have been any major changes at "Dome 1" and "Dome 2" during the last 30 years: both sites continue to have active seepage and thus have similar faunal assemblages dominated by *Bathymodiolus* mussels (Jollivet et al., 1990; Olu et al., 1996).

The five major habitats observed reemphasises that seeps are patchy areas with conspicuous zonation of the dominant taxa and demonstrates that there are expanded footprints beyond the source of seepage (Olu-LeRoy et al., 2004; Levin et al., 2016). The majority of species found closest to the areas of seepage in mussel beds benefit from chemosynthetic nutrition. For example, *Bathymodiolus childressi* are known to have methanotrophic bacteria and thus live in active seep sites where methane is present in the bottom water above the carbonates (Olu et al., 1996). *Bathymodiolus* mussels are foundation species that are responsible for modifying the environment at chemosynthetic habitats and provide hard substrate and shelter for many smaller morphospecies, as was observed during this study (Cordes et al., 2010; Levin et al., 2016). Interestingly, high abundances of amphipods on methane hydrate have not been seen before and suggests that the amphipods may occupy a similar trophic niche to the hesionid *Hesiocaeca methanicola*, which grazes on the abundant free-living chemoautotrophic bacteria on the hydrate (**Figure 3**; Fisher et al., 2000; Becker et al., 2013).

Always inhabiting mussel beds were *Alvinocaris* cf. *muricola* and *Pachycara caribbaeum*. *A*. cf. *muricola* has been suggested to specialize on a variety of food items including free-living bacteria and meiofauna (Becker et al., 2013). *Pachycara caribbaeum* predate on *Rimicaris hybisae* at the Von Damm vent field, so it is plausible that this species may feed on *A*. cf. *muricola* or other small crustaceans within the mussel beds (Anderson et al., 2016). Other scavengers or carnivores include *Chaceon cf. fenneri* and amphipods, cf. *Munidopsis* sp. 1, *Graneledone* n. sp. and several lithodid species (**Figure 5**; Macpherson, 1994; Levin et al., 2016). Molluscs common in the mussel beds and likely grazing on bacteria included cf. Gastropoda sp. on *B. childressi* shells, *Bathynerita naticoides*, and *Kanoia* cf. *meroglypta*. These molluscs, *A*. cf. *muricola* and *P. caribbaeum* are vent/seep endemics as they are absent from the surrounding benthic areas, whereas the other mobile scavengers are likely opportunistic vagrant fauna (Carney, 1994; Olu et al., 1996). Fishes and echinoderms observed in the vicinity of the seep communities are also likely vagrants (Olu et al., 1996).

Lamellibrachia sp. 2, cf. *Neovermilia* sp., Haplosclerida n. sp., and *Stenohelia* cf. *profunda* were the main inhabitants of the ecotone between live *Bathymodiolus* beds and authigenic carbonates hosting non-chemosynthetic fauna. *Lamellibrachia* sp. 2 are associated with endosymbiotic sulfur-oxidizing bacteria that depend on sulfides being produced in sediments that they absorb via their tubes, which are either partially buried in the sediments or slotted into cracks in carbonate concretions (Fisher, 1990; Olu et al., 1996). Thus, they can live in less active locations, such as on authigenic carbonates, because of their ability to mine sulfide (Freytag et al., 2001) and are considered endemic foundation species (Cordes et al., 2010; Levin et al., 2016). The other epifauna in this ecotone are likely seep-endemic filter feeders benefiting from the horizontal advection of particulate organic material from the mussel beds (Olu et al., 1996; Sibuet and Olu, 1998).

Adjacent to and usually downslope from active areas were large beds of dead *Bathymodiolus* spp. shells overlying sediment, sometimes with small patches of *Lamellibrachia* sp. 2 and *B. boomerang* (**Figure 3**). *B. boomerang* hosts both sulfur-oxidizing and methanotrophic bacteria, allowing them to use either methane or hydrogen sulfide and thus be well adapted to survive in a range of potential fluid compositions (Von Cosel and Olu, 1998). Shell aggregations may be as a result of *B. childressi*'s inability to tolerate fluctuations in fluid flow, and likely host diverse macrofaunal communities (Olu et al., 1996; Levin et al., 2016).

Further away from the active seepage and chemosynthetic communities but located on the authigenic carbonates was an ecotone of isolated octocorals and their commensals (actiniarians, ophiuroids, squat lobsters etc.), and other fauna (**Figure 3**). Here the authigenic carbonates are used by benthic background fauna as a source of hard substrate and shelter, and also provide access to food through the position in the current (Olu et al., 1996; Quattrini et al., 2015; Levin et al., 2016). Beyond that ecotone were sedimented areas that had a different faunal assemblage mostly comprised of deposit feeders (**Figure 3**).

Biogeographical Comparisons with in the Region

Many of the fauna observed off Trinidad and Tobago during NA054 have been observed at other chemosynthetic sites within the region despite the ephemeral patchy nature of these habitats. The closest surveyed deep-sea sites to El Pilar are Orenoque A (1,680–1,700 m) and Orenoque B (1,950–2,080 m), which are 115 km apart, and appear to have similar faunal assemblages to El Pilar, suggesting larval exchange between all three areas (Olu et al., 1996). There appear to be at least 15 species in common with Orenoque A (Olu et al., 1996). Orenoque A, like El Pilar, was dominated by *Bathymodiolus childressi* and *B. boomerang* but also featured live and dead vesicomyids, *Alvinocaris* cf. *muricola* and vestimentiferans (although it is unclear which species) (Olu et al., 1996; Von Cosel and Olu, 1998, 2008; Karasevaa et al., 2016). At Orenoque A, maximum *Bathymodiolus* densities appear to be similar (256 ind.m^{-2} for adults rising to 1,670 ind.m^{-2} for juveniles), and there was also evidence of high levels of

recruitment (Olu et al., 1996). There were also amphipods, *Bathynerita naticoidea*, *Neovermilia* sp., *Paralomis arethusa*, and *Munidopsis* sp. 1 and sp. 2 observed in the mussel beds (Jollivet et al., 1990; Macpherson, 1994; Olu et al., 1996; Bellan-Santini, 1997). The presence of octopuses and zoarcids can be inferred to be *Graneledone* n. sp. and *Pachycara caribbaeum* but need to be confirmed. Similar to the El Pilar seeps, surrounding the mussel beds was an ecotone of filter-feeding *Neovermilia* sp. and sponges attached to the carbonates, followed by an outer eoctone of octocorals (Olu et al., 1996). Orenoque B appeared to have a slightly different faunal assemblage to El Pilar and Orenoque A, in that there was only *B. boomerang* present and an absence of filter feeders, which may be as a result of the difference in depths (Olu et al., 1996). Mud volcanoes known from 4,700 to 5,000 m off Barbados are dominated by *Calyptogena*, galatheids, gastropods, and cladorhizids and appear to have no faunal overlap with the communities at El Pilar despite the close proximity (Le Pichon et al., 1990; Vacelet et al., 1996; Olu et al., 1997). This is likely due to the large differences in depth and geology.

Methane seeps located on the deep slopes of Kick'em Jenny submarine volcano off Grenada may have at least seven species in common despite very different geology (Carey et al., 2014, 2015). These include *Alvinocaris* cf. *muricola*, *Bathymodiolus boomerang*, *Laubiericoncha* cf. *myriamae*, *Lamellibrachia* sp. 2, *Neovermilia* sp., Gastropoda sp. 1 and *Kanoia* cf. *meroglypta*. The co-occurrence of several species is not surprising given the close proximity (450 km) (Carey et al., 2014). There is also faunal overlap between El Pilar, Orenoque A and Kick'em Jenny with the Von Damm vent field in the Mid-Cayman Spreading Centre (MCSC) despite an approximate distance of 2,000 km: *Lamellibrachia* sp. 2 (genetically identical – 100%), *B. childressi* and *Pachycara caribbaeum* (Connelly et al., 2012; Plouviez et al., 2015; Anderson et al., 2016). *A.* cf. *muricola* may also co-occur but further investigations need to be undertaken (Plouviez et al., 2015). The overlap with Von Damm vent field but not Beebe vent field, which is also in the MCSC, is likely due to the similar sedimented and shallow nature of the Von Damm vent field when compared with the Beebe vent field (4,960 m). The seep-specific fauna, *Lamellibrachia* sp. 2 (genetically identical−100%), *A.* cf. *muricola*, and *B. childressi*, as well as *Bathynerita naticoidea* and *K.* cf. *meroglypta* also dominate both the El Pilar seeps and several Gulf of Mexico seeps of similar depths (Sibuet and Olu, 1998; Cordes et al., 2007b; Miglietta et al., 2010; Olu et al., 2010).

The predominantly easterly current in the region suggests that there are at least two stepping stones (Kick'em Jenny and Von Damm vent field, as well as others as yet undiscovered) for the westward and northerly migration of fauna from the seeps on the Barbados Accretionary Prism (El Pilar and Orenoque) up through the Caribbean and into the Gulf of Mexico (Bergquist et al., 2003; Cordes et al., 2007b; Arellano et al., 2014; Carey et al., 2014). This number should further increase if organic falls are considered, but still emphasizes the importance of each of these sites for dispersal (Smith et al., 1989, 2015; Dando et al., 1992; Feldman et al., 1998). However, the potential for dispersal among these sites depends on the depth range within which the larvae of various species travel and the duration of the larval life spans, of which very little is known (Tyler and Young, 1999; Cordes et al., 2007b). Arellano et al. (2014) reported that the larvae of *Bathymodiolus childressi* and *Bathynerita naticoidea* migrate into shallower waters (<100 m depth) allowing them to take advantage of faster surface currents that may facilitate long-distance dispersal. It was estimated that *B. naticoidea* could be in the planktonic dispersal phase for 7–12 months, whereas *B. childressi* could migrate for up to 16.5 months. Additionally Young et al. (2012) estimated larvae originating near Barbados had the potential of colonizing virtually the entire Caribbean area and the southeastern Gulf of Mexico, with most individuals being retained in the Caribbean, during a larval life of 13 months.

Further afield, the El Pilar seep communities exhibit affinities (at least eight overlapping genera and many overlapping families) with the hydrothermal seep fauna of Jaco Scar at 1,800–1,900 m off Costa Rica in the Pacific Ocean. Beyond the foundation taxa *Lamellibrachia* and *Bathymodiolus*, common taxa present at El Pilar and Jaco Scar include *Pachycara, Neovermilia, Alvinocaris, Paralomis, Kanoia,* and *Graneledone* (Levin et al., 2012). Notably absent were vesicomyid clam communities common in the Pacific. There are also some affinities with taxa reported from the newly discovered Pescadero Transform Fault at 2,400 m in the Guaymas Basin (Goffredi et al., 2017). Thus, similarities of the Atlantic equatorial belt seep fauna may extend into the Pacific, with depth zonation playing a key role in community structure (Olu et al., 2010).

Stewardship of the Deep Ocean of Trinidad and Tobago

Methane seeps frequently occur in areas of economic interest because of their direct association with oil and gas-rich fluids (Sibuet and Olu, 1998; Bergquist et al., 2003; Cordes et al., 2003, 2016; Bernardino et al., 2012; Jones et al., 2014). Although there has been wide-ranging deep-sea exploration off Trinidad and Tobago, the majority of this work has been undertaken by oil and natural gas companies and the data have not been made publically available. The four study sites described in this paper host methane seeps and associated chemosynthetic communities. Furthermore, as previously concluded by Jollivet et al. (1990) and Carey et al. (2015), the abundance of methane gas plumes (85) detected in the limited EM302 water-column data (approximately 140 km^2) suggests that a large number of as yet undiscovered chemosynthetic communities populate this area east of Trinidad and Tobago (**Figure 6**). However, it cannot be concluded that each of these gas plumes represents a unique site.

Methane seeps are of particular importance given their high primary productivity, relatively high biomass and diversity, and high endemicity. Seeps have also been shown to provide regulating, provisioning and cultural ecological services that are likely irreplaceable (e.g., biogeochemical cycling, enhancing fisheries, and providing inspiration respectively; Levin et al., 2016). A combination of slow growth and long life spans for some seep taxa (Cordes et al., 2007a; Durkin et al., 2017), and variable and/or poorly understood recruitment (Cordes et al., 2003; Arellano and Young, 2009), means that recovery from

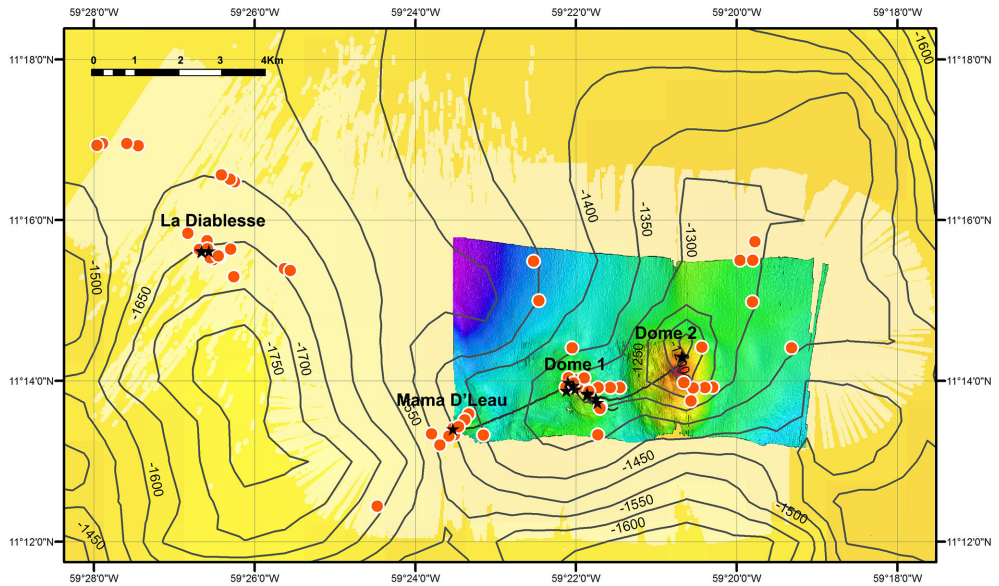


FIGURE 6 | Locations of prospective methane-seep sites. Red dots are the locations of methane bubbles emitted from the seafloor (indicative of seepage) that were identified from water column data using the shipboard EM302 multibeam, while black stars are locations of bubble streams visually confirmed with the ROV *Hercules*. Gridded bathymetric data provided by the General Bathymetric Chart of the Oceans (GEBCO) 30 arc-second grid (accessed via http://www.gebco.net/). Map and associated shapefiles created in ESRI ArcMap (version 10.3.1). The high-resolution central section was constructed from unpublished ship-based bathymetry data from the EV *Nautilus*. The entire light yellow area was surveyed with the ship-based multibeam but due to adverse surveying conditions, only the water column data were useful.

impacts can be prolonged (Cordes et al., 2016). The fauna that live in peripheral ecotones and habitats of these sites are also distinct from the fauna in other areas of the deep sea, so deserve recognition as a distinct biodiversity component and consideration for protection (Demopoulos et al., 2010; Levin et al., 2016). Furthermore, as seeps are relatively small and patchy, they may be more susceptible to anthropogenic impacts as the scale of the disturbance will likely be more concentrated, and island-like habitats are particularly prone to local extinction (Rowden et al., 2016). Given these attributes, methane seeps are considered to have the characteristics of vulnerable marine ecosystems (VME), defined by the FAO as vulnerable to bottom fishing. Methane seeps overlap with the following VME criteria: an ecosystem that hosts endemic taxa, is structurally complex and fragile, and contains difficult-to-recover species (e.g., those with slow growth rates, late maturity and that are long-lived. VME criteria are similar to those of ecologically and biologically significant areas (EBSAs) as defined by the UN Convention on Biological Diversity (CBD). EBSAs are "discrete areas, which through scientific criteria (similar to those listed for VMEs; see Ardron et al., 2014 for comparison of VME and EBSA criteria), have been identified as important for the health and functioning of our oceans and the services that they provide" (UNEP-WCMC, 2014; Cordes et al., 2016). The EBSA concept was developed under the United Nations Convention on Biological Diversity, which Trinidad and Tobago has been a signatory on since 1992 (Dunn et al., 2014; Cordes et al., 2016), but EBSAs have yet to be incorporated into any formal management structures (Dunn et al., 2014).

In order to balance the needs of industry with those of the environment, Trinidad and Tobago must consider marine stewardship (the careful and responsible planning and management of resources) including, but not limited to, conservation (the preservation of resources). Given the current absence of a regional environmental management plan that includes area-based management for both conservation and exploitation needs, and the imminent deep-sea oil and gas exploitation, it is critical that Trinidad and Tobago begin to address the governance of the deep sea and its associated resources with systematic planning and conservation targets. Furthermore, given the increased exploitation of the deep sea for a range of industries worldwide, e.g., mineral extraction, methane hydrate extraction, fisheries etc., there is a dire need for holistic environmental management, including local legislation. To begin to fill this stewardship gap, we recommend the following:

(1) Given the prevalence of uncertainty with regard to the deep sea off Trinidad and Tobago, there is a need for a precautionary approach in order to protect and minimize significant environmental harm to what is still unknown. In current practice, protection of 30–50% of a management area for conservation, protection of 30–50% of each representative habitat, and protection of all important areas (meeting EBSA or VME criteria) through a network of protected areas is a first step in regional environmental management planning in the deep sea (Wedding et al., 2015). With regard to oil and gas and mineral extraction at the contractor level, the first two

steps of the mitigation hierarchy (avoid, minimize) should be emphasized: restoration and offsetting are not deemed practical at this time for deep-sea ecosystems (Bank, 2012; Van Dover et al., 2017). For example, deep coral reefs (also potentially present in Trinidad and Tobago's EEZ) meet EBSA criteria and are thus targets for protection. Huvenne et al. (2016) assessed the effectiveness of a deepwater cold-water coral MPA, following 8 years of fisheries closure. The results suggested that even after 8 years as an MPA, there was low resilience and slow recovery potential of this deep-sea ecosystem. This resilience may be further reduced by warming and acidification acting as cumulative stressors, adding to the uncertainty of system responses (Levin and Le Bris, 2015). This research highlighted the importance of the application of the precautionary principle in deep-sea conservation.

(2) Comprehensive environmental assessments, including but not limited to baseline research to characterize the natural variability, should be mandatory. Ideally, surveys should include high-resolution mapping, seafloor imagery surveys, and physical samples to characterize the faunal community and ensure proper species identifications. This faunal characterization should consist of a combination of morphological and molecular taxonomy of not only the large conspicuous species (megafauna) but also smaller size classes (microbes, meiofauna, and macrofauna) (Zeppilli et al., 2017). Beyond describing the biodiversity, environmental assessments should include measurements needed to evaluate the status and change in ecosystem functions and the services they support, consistent with ecosystem-based management. These measurements should incorporate the dynamics of the physico-chemical environment (including changing climate variables), presence/absence of habitats that meet EBSA and VME criteria, connectivity, benthic-pelagic coupling, and resilience in order to enable sound decision making and environmental management. While some of these costs should be covered by the industries working in the deep ocean, developing research funding independent of industry is also important. Furthermore, scientific research should extend to include Environmental Impact Assessments with monitoring before, during and after the activities as well as at control reference sites (Cordes et al., 2016). However, the reliability of EIAs depends on the quality of existing baseline data (Cordes et al., 2016). For example, following the Deepwater Horizon incident in the Gulf of Mexico, one of the most well-studied areas of the deep ocean, baseline data were found to be lacking in the immediate vicinity of the impacts and for many key components of the ecosystem (Cordes et al., 2016).

(3) Legal framework & regional agreements for all types of deep-sea exploitation need to be drawn up and enforced at a standard that is equal to or better than those internationally and reflects modern practices of environmental conservation. For example, rigorous oil and gas regulations need to be put in place as per the recommendations of Cordes et al. (2016) e.g., buffer zones, and activity, temporal and spatial management. Local and regional spatial planning should be enforced after engaging all relevant stakeholders. This includes industry, government, policymakers, civil society, scientists, economists and local communities, and should aim for binding outcomes from those discussions. For spatial management to be effective, data on the distribution of features of conservation interest is essential (see second recommendation above). One of the goals of the spatial management should be to implement a network of MPAs in order to preserve significant and representative communities (EBSAs) locally and regionally (Dunn et al., 2014), similar to those proposed by de Mello Baez Almada and Bernardino (2016) off Brazil. As a Small Island Developing State, taking advantage of global expertise (e.g., the Deep-Ocean Stewardship Initiative) can aid this process. Given the ecological and scientific value of the El Pilar site and other seep areas within the Trinidad and Tobago EEZ, and the ongoing development of the deep-sea energy sector in the same area, it is timely for Trinidad and Tobago to develop a regional environmental management plan with stakeholders to protect the marine environment in the context of extractive activities.

AUTHOR CONTRIBUTIONS

DA, JG, LM, and NR were integral in ROV video surveys and sample processing while at sea on the E/V *Nautilus*. DA collected and analyzed the data from imagery, while CVD analyzed the samples. DA wrote the main manuscript text with assistance from JG, LL, LM, NR, and CVD. LM prepared **Figures 1**, **6**, while DA prepared **Figures 2**–**5**. All authors reviewed the manuscript.

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Deep-Sea Mega-Epibenthic Assemblages from the SW Portuguese Margin (NE Atlantic) Subjected to Bottom-Trawling Fisheries

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Bottom-trawling fisheries are a common threat to the health of continental margins worldwide. Together with numerous environmental and biological processes, physical disturbance induced by trawlers can largely shape the benthic habitats and their associated assemblages. At the SW Portuguese Margin, crustacean bottom trawlers have exploited deep-sea habitats for a few decades, but its effects on the benthic biodiversity are practically unknown. During the spring-summer of 2013 and 2014, several Remotely Operated Vehicle (ROV) video transects were used to investigate mega-epibenthic abundance, composition, and diversity in soft-sediment areas subjected to varying trawling pressures off Sines and Setúbal (200–800 m). Differences in mega-epibenthic assemblages were linked with environmental changes (depth, grain size, primary productivity) and trawling disturbance. The effect of trawling was assessed between segments with similar habitat characteristics, i.e., muddy-sand bottoms between 300 and 500 m. Areas subjected to intensive trawling pressure showed a generally flattened seabed, with abundant recent trawl marks (up to 3 scars.100 m^{-1}), indicating that the seabed physical integrity was compromised. Significant negative correlations were detected between various mega-epibenthic diversity indices [S, H′, and $ET_{(20)}$] and trawling pressure (h.$cell^{-1}$.y^{-1}). Furthermore, the distinct mega-epibenthic assemblages and absence of several sessile erect morphospecies at both low and highly disturbed locations by trawling off Sines, namely all seapen morphospecies found in non-trawled areas, demonstrates the negative influence of trawling fisheries on the benthic component of the study area. Also, low dissimilarity between assemblages from the main fishing grounds and the adjacent low-disturbance locations, suggests that the potentially negative influence of trawling can extend beyond the targeted areas (e.g., by the plumes of re-suspended sediments). The observed deleterious effects of trawling on

mega-epibenthic fauna together with the intensification of trawling pressure in the study area, stress the need for adequate monitoring programs and regulatory measures to halt the long-term loss of biodiversity and allow the sustainability of fisheries at the SW Portuguese Margin.

Keywords: mega-epifauna, diversity, sediment composition, disturbance, trawling, deep sea, West Iberian Margin

INTRODUCTION

Continental margins are considered productive and diverse regions in the deep sea (Levin and Dayton, 2009). They encompass several unique habitats, such as submarine canyons, seamounts, and even chemosynthesis-based habitats (e.g., pockmarks and mud volcanoes). Hence, benthic faunal biodiversity at margins is complex as it is shaped by the interaction of numerous environmental and biological processes (e.g., substrate sorting, water-mass properties, productivity regimes, predation, competition), but also to some extent, by the increasing pressure from anthropogenic activities (e.g., fisheries, pollution, mineral, and oil extraction; Levin et al., 2001; Levin and Dayton, 2009; Ramírez-Llodra et al., 2011).

Among the several anthropogenic activities occurring in deep waters worldwide, bottom-trawling fisheries are identified as one of the most destructive, affecting primarily the continental shelf and upper slope, seamounts, and submarine canyons (Ramírez-Llodra et al., 2011). General impacts caused by trawling practices are relatively well-established for the shelf areas, although the magnitude and duration of the effects largely depends on the characteristics of the targeted habitats, gears used, and trawl intensity and frequency (Jennings and Kaiser, 1998; NRC, 2002). Moreover, the low selectivity of trawling practices directly causes a high mortality of both target and non-target species and alterations or destruction of seabed habitats (NRC, 2002). Indirectly, trawling also promotes shifts in benthic community composition and diversity, while trophic webs may also be affected, namely by the increase in carrion available from both on-site mortality and discard practices (NRC, 2002). Also, effects of trawling are highly dependent on the faunal size-groups, as a greater vulnerability is linked with the large-sized fauna (macro and megafauna; Jennings et al., 2001; Duplisea et al., 2002). In this context, megafaunal organisms, defined by Grassle et al. (1975) as animals >1 cm either easily detected in photographs/videos or collected by trawl nets, are particularly sensitive to repeated trawling disturbance. Subsequently, changes in megafauna assemblages can result in depletion of several ecosystem functions, since megafauna is known to promote important benthic-pelagic coupling processes (Soltwedel et al., 2009), and function as "ecosystem engineers." For example, mega-epibenthic organisms can promote habitat complexity and induce changes in the sediment biogeochemistry via bioturbation, but also by serving as biogenic habitats for smaller fauna (e.g., corals; Buhl-Mortensen et al., 2010).

It is postulated that trawling practices may have stronger effects on the deep-sea mega-epibenthic fauna in comparison with shallower areas (Clark et al., 2015). This arises from the typical characteristics of deep-sea species, particular life-history traits (k-selected; e.g., slow growth, high longevity), metabolic rates (low productivity) and reproductive strategies (e.g., intermittent spawning events), which make them more vulnerable and less resilient to the effects of trawling practices (Thrush and Dayton, 2002). Heavier trawl gears and more localized practices can also exert a stronger pressure on deep-sea habitats (Clark et al., 2015). Yet, the effects on deep-sea benthic habitats and mega-epibenthic assemblages are still debated and geographically dependent, since in most cases we lack either background knowledge of the biodiversity on the long-term targeted areas, or an obligatory legislation that requires impact assessment and monitoring programmes at recent fishing grounds (Clark et al., 2015). The most obvious effects identified so far include the large-scale changes of the seabed topography and sediment dynamics (e.g., fishing grounds at the upper flank of La Fonera canyon, Catalan margin; Puig et al., 2012). With each trawling haul, the seafloor is flattened and large amounts of sediment are re-suspended, often resulting in alterations of both surface and sub-surface sediment properties, namely organic matter concentrations, grain size composition, and porosity (Martín et al., 2014; Oberle et al., 2016). These high turbidity periods often extend beyond the fishing grounds, indirectly impacting non-target areas by smothering filter-feeding organisms and increasing mortality rates of their faunal assemblages (Greathead et al., 2007; Leys, 2013; Clark et al., 2015). Effects on mega-epibenthic fauna include the decline of both standing stocks (abundance and biomass) and species richness, and changes in community composition (Clark et al., 2015 and references therein). In addition, the damage of long lived habitat-forming organisms (i.e., sponges and corals) in seamounts areas, have shown a very low recoverability and marked community shifts of their associated fauna (Koslow et al., 2001; Clark and Rowden, 2009; Williams et al., 2010; Yesson et al., 2016). In more extreme cases, alterations of the mega-epibenthic faunal distribution patterns at different spatial scales can also occur (Althaus et al., 2009). It is important to stress that current knowledge pertains mostly to rather charismatic and vulnerable hard substrate habitats such as cold-water coral areas and seamounts (Clark et al., 2015 and references therein). Less focus has been directed to study the effects of bottom trawling on fauna inhabiting soft sediments from slopes and canyons along continental margins worldwide (e.g., Atkinson et al., 2011; Buhl-Mortensen et al., 2015; Murillo et al., 2016; Yesson et al., 2016). Yet, some indications arise regarding their potential for a faster recovery after disturbance (Yesson et al., 2016). Hence, it is crucial to increase our knowledge related to trawling effects at these areas that naturally contrast from hard-bottom areas, so we can adequately adjust the current management actions to allow

for a sustainable exploitation of natural resources, and maintain a good environmental status.

The Iberian Margin has been identified as one of the most disturbed regions by bottom-trawling fisheries in Europe. This activity affects 40–90% (depending on the substrate type) of the areas beyond the six nautical miles limit down to ca. 1,000 m water depths and is associated with a large footprint per unit of landing with ca. 13–17 $km^{-2}t^{-1}$ depending on the depth range considered (Eigaard et al., 2016; Bueno-Pardo et al., 2017). Moreover, few studies have attempted to understand the trawling impacts on the benthic assemblages and are limited by the absence of adequate control areas (Morais et al., 2007; Fonseca et al., 2014). The present study aims to address this issue by investigate the upper slope mega-epibenthic assemblages in a southwest Iberian margin area subjected to long-term crustacean bottom trawling. Specifically, we hypothesized that (i) the spatial and temporal environmental heterogeneity in the study region (i.e., water depth, sediment composition, annual productivity) will affect mega-epibenthic composition and community structure; (ii) Changes in the mega-epibenthic abundance, diversity, composition, and community structure are altered by different degrees of bottom-trawling pressure (including no-, low-and high trawling pressure). These hypotheses will be tested using multivariate analyses.

METHODOLOGY

Study Area

The West Iberian Margin (WIM) is characterized by a relatively narrow shelf with a steep and irregular continental slope, incised by several large submarine canyons. It is exposed to complex seasonal hydrodynamic processes, driven by wind forcing, local bathymetry, and prominent topographic features, such as the Setúbal canyon (Fiúza, 1983; Relvas et al., 2007). During spring and summer, northerly winds induce relatively weak upwelling regimes, reaching a maximum off cape of Sines (SW Portugal). The inverse tends to occur during winter, with downwelling regimes and strong storm events, driven by south-westerly winds, although pulse episodes can occur at all seasons (Fiúza, 1983; Relvas et al., 2007). The high surface primary production generated during upwelling extends often for ca. 30–40 km offshore, but in some areas phytoplankton bloom filaments can reach as far as 200 km offshore. The relevant contribution of the surface productivity peaks to total standing stock and primary production have a significant impact on the food webs, supporting productive fisheries along the WIM (Picado et al., 2014).

Fishing activities along the WIM comprise various *métiers*, of which deep-water otter trawling, often designated as "crustacean bottom trawling," as one of the most economically important, accounting for more than 30% of the total landing sale values (Campos et al., 2007). Crustacean trawling fisheries at the WIM are typically restricted to the South and Southwest regions off Portugal, where the most landed and valuable species include several deep-water crustaceans species, such as the Norway lobster (*Nephrops norvegicus*), red and rose shrimps (*Aristeus antennatus* and *Parapenaeus longirostris*, respectively), but also a few demersal fish species such as blue whiting (*Micromesistius poutassou*) and the European hake (*Merluccius merluccius*; Campos et al., 2007; Bueno-Pardo et al., 2017). In 2014, the total declared landings of these species altogether for the SW Portuguese region were relatively low (ca. 50 t y^{-1}, and about 5% of the total landings). Yet, this region yielded ∼30% of the total trawling effort in Portugal (Bueno-Pardo et al., 2017). While not all of these species show the same habitat preferences, their distribution often overlaps at the soft sediment areas (mud and muddy-sand) between 200 and 800 m water depths (Monteiro et al., 2001). Fishing grounds along the Portuguese margin are delimited by legal restrictions defined by the initial official regulation from July in 1987[1], which prohibits trawling within six nautical miles from the coastline.

Based on the vessel monitoring system (VMS) satellite data compiled by DGRM (MAMAOT, 2012), a region of interest in the SW Portuguese margin was delimited at ∼37°40′-38°20′N; 08°50′-09°20′W, along the upper continental slope (200–800 m water depth) off Sines and in the vicinity of Setúbal canyon (**Figure 1A**, **Table 1**). Here, two main seabed types can be identified considering the habitats scheme of the European Union Nature Information System (EUNIS; Davies et al., 2004) and detailed sediment charts from Instituto Hidrográfico (2005a,b): coarser sediments (A6.3: deep-sea sand) at shallower depths (ca. 200–300 m) until the self-break/upper slope transitions areas, while finer sediment types occur at deeper locations (>300 m; A6.4: deep-sea muddy-sand, with variable mud, and carbonate contents). Owing to the occurrence of the Norway lobster habitat (fine sediments near the shelf break) and proximity to Sines harbor, this region is heavily targeted by crustacean trawlers. On the other hand, the 6 nm limit creates a trawling-free area between cape Sines and cape Espichel (Setúbal area), allowing the comparison between heavily fished and non-fished areas at similar depths and sediment types (**Figure 1A**).

ROV Dive Surveys and Sampling Design

A total of six ROV survey transects were performed. The surveys were designed taking into consideration the available information from the VMS satellite data and the known distribution of sediment types. In 2013 (RV Belgica, cruise 2013/17), two transects (6,000 and 11,500 m; D13_1 and D13_3, respectively) were outlined perpendicularly to the coastline from the upper continental slope to shallower areas. These covered several types of sediments (sand to muddy sand) and crossed a gradient of trawling pressures, including heavily fished deeper areas and the transition to less or no fished shallower areas (**Figure 1B**). In 2014 (RV Pelagia, cruise 64PE387), four shorter transects (<4,000 m) were delineated only in areas of similar sediment type (muddy sand). Two transects running parallel to the coastline focused on trawling target and adjacent non-target areas (D14_3 and D14_4; respectively **Figure 1C**) in the main fishing ground off Sines. Additionally, two other transects (D14_1 and D14_2, **Figure 1A**), were initiated near the flanks of

[1]Diário da República, Decreto regulamentar n° 43/87 de 17 de Julho, Ministério da Agricultura, Pescas e Alimentação, 1ª Série - n°162 de 17 de Julho de 1987.

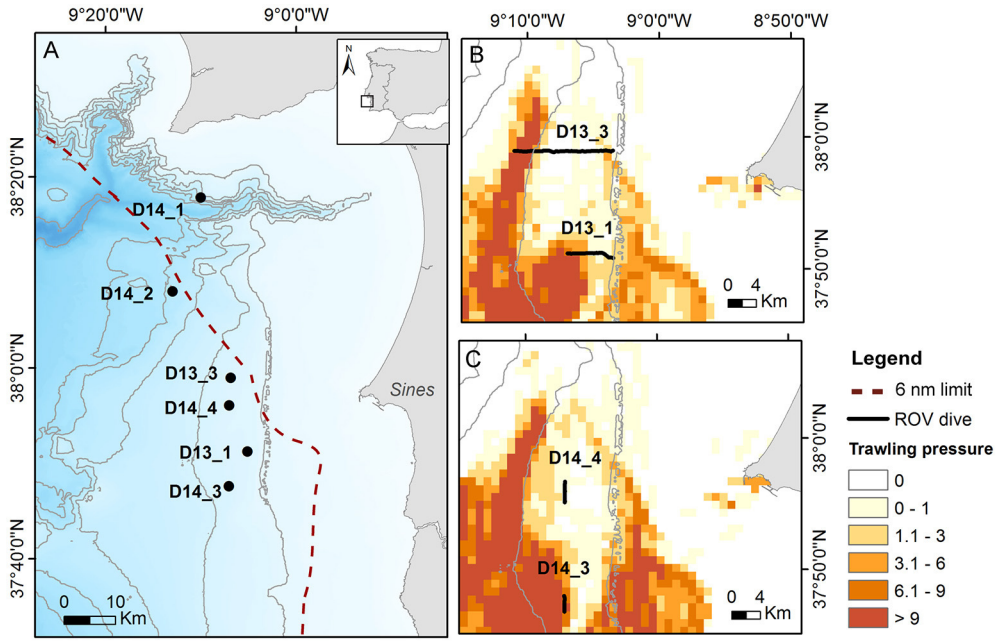


FIGURE 1 | (A) Map of the study area indicating the locations of ROV dive transects in relation to the distribution of the crustacean trawlers annual trawling pressure ($h.cell^{-1}.y^{-1}$) for **(B)** 2013 and **(C)** 2014. Setúbal canyon area is not shown ($0\,h.cell^{-1}.y^{-1}$).

TABLE 1 | Metadata on ROV dive transects.

Cruise	Dive Code	Date	Position coordinates				Depth range (m)	Total length (m)	Segments analyzed (%)	EUNIS Habitat	Trawling pressure
			Start		End						
			Lat (N)	Long (W)	Lat (N)	Long (W)					
RV Belgica 2013/17	D13_1	11/06/13	37.85257	−9.117838	37.847216	−9.049816	208–318	6,002	60.0	A6.3/A6.4	LT/HT
	D13_3	12/06/13	37.98379	−9.187216	37.983141	−9.05709	228–441	11,405	78.9	A6.3/A6.4	LT/HT
RV Pelagia 64PE387	D14_1	03/05/14	38.292665	−9.169028	38.29948	−9.162458	425–720	951	66.7	A6.4	NT
	D14_2	04/05/14	38.133224	−9.219712	38.133139	−9.213610	740–786	534	80.0	A6.4	LT
	D14_3	04/05/14	37.772635	−9.117301	37.799775	−9.117666	343–348	3,020	43.3	A6.4	HT
	D14_4	05/05/14	37.906349	−9.116855	37.946467	−9.116353	287–309	4,400	59.1	A6.4	LT

EUNIS Habitats classification: A6.3: Deep-sea sand; A6.4: Deep-sea muddy-sand; Trawling pressure includes: NT, no trawling pressure, LT, low trawling pressure, and HT, high trawling pressure.

the Setúbal canyon, where trawling pressure is null, and in the case of D14_1, it was located within the 6 nm limit. Both dives were not fully completed as planned (longer transects) owing to safety reasons, due to the risk of entanglement in the numerous fishing traps deployed at depths of ca. 450 m.

The video transects were performed using the ROV *Genesis*, a sub-Atlantic Cherokee-type Remotely Operated Vehicle from VLIZ (Vlaams Instituut voor de Zee). Each video recording was obtained using two forward-looking standard definition black and white (Kongsberg OE15–100a) and color cameras (Kongsberg OE14–366/367) at a speed of ∼0.4 $m.s^{-1}$ and altitude of ∼1 m above the seabed. In addition, digital still images were acquired at ∼30-s intervals using a high definition camera (Canon PowerShot G5). Accurate geo-positioning of both video and stills was obtained though the IXSEA global acoustic positioning system.

Image Analysis and Faunal Characterization

Video recordings were analyzed in segments of 100 m (linear distance sampling unit) calculated from the geo-positioning data. At each segment, all specimens visible in the footage were counted and identified to the lowest taxonomic level possible using additional high-resolution stills taken during the dives. Some of the species captured digitally could be confirmed by the identified specimens collected for macrofauna studies

within the same sampling campaigns. In many cases, it was not possible to accurately assign specimens to species level and they were thus grouped into separate morphospecies, based on distinct morphological characteristics. Taxonomic classification followed the World Register of Marine Species database (WoRMS Editorial Board, 2016; www.marinespecies.org). Typical pelagic organisms (Ctenophora, Scyphozoa, and pelagic fishes) were also identified but not counted, since these organisms sometimes followed the ROV lights for long distances, not allowing their accurate quantification. Note that demersal fish species were included in our analysis, due to their direct interaction with the seabed. Video observations also included the description of seabed characteristics (e.g., bioturbation evidence, topography, ripple marks, phytodetritus patches) and any evidence of disturbance by trawling operations (trawl scars). Trawl scars were classified into "eroded"—scars where evident bioturbation and/or collapsed tracks; and "recent"—scars that were clearly undisturbed by bottom currents or faunal activity.

Due to technical issues, the reference scale normally provided by the laser points was not available and consequently the field view area was not estimated, which hindered biomass estimates and estimates of abundance per area (thus expressed per 100 m). The segments were performed at a relatively constant camera position and altitude, allowing the comparison among dives in both years. When this was not possible (e.g., no visual contact or varying altitude, high sediment resuspension, strong illumination), segments of "poor image quality" were excluded from the analysis to avoid low confidence level observations, resulting in the analysis of ∼65% of the video recordings (**Table 1**).

Environmental Parameters

Geographical information system software ArcGIS v10.3.1 was used to compile environmental data pertaining to each segment obtained from various sources as mentioned below.

Seabed habitats and bathymetric data were acquired from the European Marine Observation and Data Network portal—EMODnet (European Commission, 2016; http://www.emodnet.eu). Seabed habitats were classified following the EUNIS scheme (Davies et al., 2004) and the refined information from the available seabed sediment charts from Instituto Hidrográfico (2005a,b). Deep-sea sand (A6.3) included MdS1 (medium sand, grain size dominant fraction: 500–250 mm with <10% mud and <30% carbonate content) and FiS1 (fine sand, grain size dominant fraction: 250 mm−63 μm with <10% mud and <30% carbonate content). Deep-sea muddy-sand (A6.4) included SM2 (sandy-mud with 25–50% mud and 30–50% carbonate content), MS2 (muddy-sand with 10–25% mud and 30–50% carbonate content), and MS1 (muddy-sand with 10–25% mud and <30% carbonate content). Charts referring to the sediment composition were confirmed by several sediment samples collected for macrofauna studies within the same sampling campaigns. The monthly average surface Net Primary Production (avNPP; $g.C.m^{-2}.month^{-1}$) values were obtained from the Vertically Generalized Productivity Model (VGPM) available on the Ocean productivity database (Behrenfeld and Falkowski, 1997). The VGPM model uses a standard algorithm calculated based on MODIS aqua satellite data for chlorophyll *a* concentrations, photosynthetically active radiation and sea-surface temperature. Temporal variability of the monthly surface Net Primary Production over 1 year prior to each sampling campaign was expressed as the seasonal variation index (SVI), calculated from dividing the standard deviation by the monthly average of the NPP (Lutz et al., 2007):

$$SVI = \frac{\sigma(NPP)}{\overline{NPP}}$$

Trawling Pressure

Annual trawling pressure estimates ($h.cell^{-1}.y^{-1}$; where each cell size corresponds to 0.01 × 0.01 decimal degrees) were used as a proxy for the intensity of disturbance caused by crustacean trawlers to the seabed during the 2 years of this study. Trawling pressure was calculated based on VMS position data of the deep-water otter trawlers in operation along the Portuguese Margin, often designated as "crustacean trawlers." This data was provided by DGRM and processed according to Bueno-Pardo et al. (2017). Trawling pressure data allowed to classify each segment into one of the following classes: no (NT: $0\,h.cell^{-1}.y^{-1}$), low (LT: $0.1–1.5\,h.cell^{-1}.y^{-1}$), and high (HT: $>1.5\,h.cell^{-1}.y^{-1}$) trawling pressure. In fact, both NT and LT locations are assumed to be not directly disturbed. However, NT label was attributed to the segments within the 6 nm limit and with null trawling pressure values, while LT segments were assigned to segments that corresponded to relatively undisturbed areas adjacent to the main fishing ground (HT).

Data Analysis

Mega-epibenthic faunal abundances (ind.100 m^{-1}: individuals per 100 m of linear distance), composition and diversity were investigated using both uni- and multivariate data analyses performed with the software PRIMER v6 and PERMANOVA+ (Clarke and Gorley, 2006; Anderson et al., 2008). Prior to the exploration of the biological dataset in relation to trawling disturbance, the relationship between the mega-epibenthic assemblages and all acquired environmental variables [depth, sediment type (categorical predictor variable based on mud and carbonate content percentage range), avNPP, SVI, and trawling pressure] was computed by means of the distance-based linear model (DISTLM) analysis. The DISTLM routine was run using the adjusted-R^2 as selection criterion and the stepwise selection procedure on normalized environmental data and the distance-based redundancy analysis (dbRDA) plot was computed to illustrate the DISTLM model (Anderson et al., 2008).

In addition to trawling pressure, a strong relation between the other environmental variables and the biological dataset was observed in the DISTLM analysis. Thus, to further investigate the sole influence of trawling on the mega-epibenthic assemblages, only a subset of the dataset with relatively similar habitat characteristics was analyzed: segments characterized by muddy-sand sediments within two narrow bathymetric

ranges (either 300–400 or 400–500 m) for each year. Each bathymetric range was analyzed separately, as follows: a 2-factor layout, with "Year" as fixed factor and "Trawling" as a random factor nested in "Year," was used for the 300–400 m depth range, and a 1-factor layout, with "Trawling" as the fixed factor, was used for the 400–500 m (replicate samples from both years were not available). A Non-metric multidimensional scaling (nMDS) ordination based on the Bray-Curtis similarity matrix after 4th root transformation was performed followed by the permutational multivariate analysis of variance (PERMANOVA) to test for differences in mega-epibenthic assemblages among groups (1-factor and 2-factor nested design for the subset of data). Morphospecies contributions (%) for the observed similarity within and dissimilarity between groups were analyzed through the SIMPER analysis.

Species richness (S), Shannon-Wiener diversity (H′), evenness (J) (Pielou, 1966), and Hurlbert's expected number of taxa [$ET_{(20)}$; Hurlbert, 1971] were used to examine diversity patterns. k-dominance (Lambshead et al., 1983) and Hurlbert's rarefaction curves were plotted to assess for differences in community structure. Lastly, non-parametric Spearman correlations were calculated between trawling pressure and mega-epibenthic faunal abundance, as well as trawling pressure and various diversity values [S, H′, and $ET_{(20)}$], assuming no dependence among variables (Quinn and Keough, 2002). Significant correlation values were adjusted by using the Bonferroni correction (Shaffer, 1995), which was calculated by dividing the significance value of each test by the number of hypothesis tested. Correlation analyses were run using the software GraphPad PRISM v6 (GraphPad Software, www.graphpad.com).

RESULTS

Environmental Variability

General Seabed Characterization

Overall, the distribution of the different sediment types mapped in the geological charts was confirmed by the video observations. Coarser sediments (medium and fine sands included in A6.3, surveyed in 2013) were concentrated at shallower locations (ca. 200–300 m) along the self-break/upper slope transitions and characterized by a little phytodetritus coverage. In opposition, finer sediments (A6.4 deep-sea muddy-sand) were mostly found at depths >300 m. Most segments surveyed in 2013 presented frequent ripple marks and heterogeneous patches of organic detritus material deposited on the seafloor. In 2014, most segments were deprived of evident phytodetritus coverage across all segments, which contrast with the higher annual average surface net primary production (avNPP; $g.C.m^{-2}.month^{-1}$) and smaller monthly fluctuations (lower SVI values) observed for 2014 (**Table 2**).

Segments from the flanks of the Setúbal canyon were characterized by a heterogeneous seabed microtopography, with muddy-sand sediments (A6.4) and little evidence of detrital material.

TABLE 2 | Summary of average surface Net Primary Production (avNPP; $g.C.m^{-2}.month^{-1}$) and seasonal variation index (SVI) values per dive.

Dive	avNPP	SVI
D13_1	345.5 ± 46.25	0.51 ± 0.018
D13_3	339.2 ± 46.14	0.51 ± 0.017
D14_1	438.1 ± 50.30	0.41 ± 0.031
D14_2	356.5 ± 34.40	0.36 ± 0.000
D14_3	410.7 ± 53.54	0.43 ± 0.029
D14_4	410.2 ± 51.35	0.41 ± 0.000

For each dive an average of the values of 100 m segments is shown.

Mega-Epibenthic Assemblages in Relation to Environmental Variables

A total of 27,953 individuals were counted and subsequently assigned to 71 different morphospecies, belonging to at least 50 families and eight phyla. Six pelagic species and eight benthic morphospecies present in the reduced visibility segments could not be quantified and therefore were not included in further analyses. The list of all observed taxa is provided in the Supplementary Table 1. Overall, the most abundant phylum was Annelida (66% of the total abundance), however only represented by four morphospecies. Contrastingly, the phyla Cnidaria (13%; 11 morphospecies) and Chordata (11%; 18 morphospecies) showed intermediate abundances but high taxa richness. The remaining phyla were less abundant, but not necessarily less diverse: Echinodermata (4%; 15 morphospecies), Arthropoda (3%; 11 morphospecies), Mollusca (1%; 9 morphospecies), Porifera (2%; 2 morphospecies), and Nemertea (<0.01%; 1 morphospecies).

The mega-epibenthic assemblages showed a large variation within and among dives, where spatial (depth, sediment composition, trawling disturbance) and temporal (years) factors appeared to, at least partially, determine the observed variability (**Figure 2**). In detail, shallower areas off Sines (c.a. 200–300 m, only surveyed in 2013) yielded the highest abundances of the study, reaching 531 ind.100 m^{-1} at 250 m depth, and the lowest diversity, with $ET_{(20)}$ ranging from 3.0 to 3.4. Here, mega-epibenthic fauna was typified by high numbers of the polychaete *Hyalinoecia tubicola* (83–88% of the total assemblage) regardless of the sediment type (sand or muddy-sand).

Muddy-sand sediments at the upper slope off Sines (ca. 300–500 m, surveyed both in 2013 and 2014) showed much lower abundances, typically under 150 ind.100 m^{-1}, but higher diversity, with $ET_{(20)}$ ranging from 6.2 to 8.5. Faunal composition gradually changed with increasing water depth. Yet, the assemblages were generally dominated by different morphospecies of tube-dwelling anemones (subclass Ceriantharia, Spirularia ind.; 19–57%) and hexacorallian anemones (2–52%), namely epibenthic actiniarians (mostly *Actinauge richardi*) and zoantharians (commensal, attached to hermit crabs). Several benthic fish morphospecies (Actinopterii: 6–21%) and few crustaceans morphospecies (Malacostraca: 3–17%) were also well represented. The 2014 surveys were marked by the presence of higher abundances of Crinoidea (10–17%), but also Porifera (21%) and Ophiuroidea (18%) in D14_4.

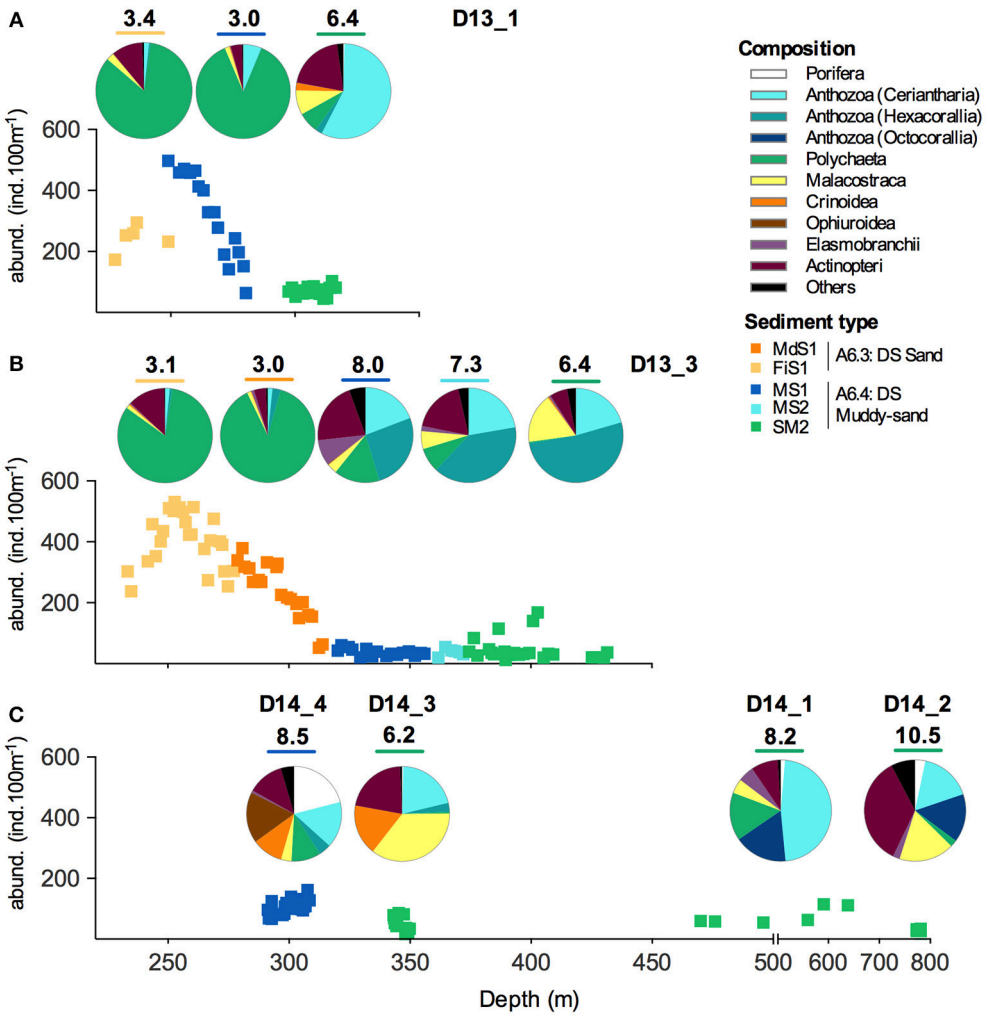


FIGURE 2 | Distribution of the mega-epibenthic fauna abundance (ind.100 m^{-1}) in relation to depth and sediment type (MdS1, medium sand; FiS1, fine sand; MS1, muddy-sand with <30% carbonate content; MS2, muddy-sand with 30–50% carbonate content; SM2, sandy-mud). The pie charts show the taxonomic composition for each sediment type in the different dives: **(A)** D13_1; **(B)** D13_3; and **(C)** all 2014 dives. Hulbert's expected number of taxa [$ET_{(20)}$] for each assemblage is indicated above the respective pie charts. "Others" represent all the taxa that contribute with <1% to the total abundance.

Muddy sediments at the Setúbal region (450–800 m) showed also low abundances, with 22.5 ± 3.75 and 71.8 ± 11.6 ind.100 m^{-1}, but higher evenness leading to $ET_{(20)}$ values of 8.2 and 10.5 for D14_1 and D14_2, respectively. Communities were typically composed by the anthozoan subclass Ceriantharia (16–47%) and Octocorallia (15–17%), but also with relevant contributions of various other taxa such as Actinopterii (9–35%), Malacostraca (5–17%), and Polychaeta (2–15%).

The DISTLM model analysis demonstrated that all six individual environmental variables were significantly correlated with the mega-epibenthic community structure (marginal tests; $p < 0.01$; Supplementary Table 2). The best explanatory model (adjusted $R^2 = 0.42852$) and sequential tests recognized by order of importance, sediment type (18%), SVI (11%), depth (9%), avNPP (4%), and trawling pressure (TP; 2%), explaining a total of 44.8% of the observed variability (Supplementary Figure 1; Supplementary Table 2). Thus, because of the strong separation between the assemblages surveyed in the years 2013 and 2014, driven overall higher avNPP in 2014, but also depth, sediment type, and trawling pressure (Supplementary Figure 1), the putative effect of trawling disturbance on the mega-epibenthic assemblages was further analyzed only within segments pertaining muddy-sand sediments at two major depth ranges: 300–400 and 400–500 m (**Table 3**).

Bottom-Trawl Fisheries Disturbance

Evidence of Trawling Disturbance on the Seabed

In total, 149 trawl scars were detected in the present study, mostly associated with the higher trawling pressure areas (HT; 61.1%) and muddy-sand sediments (73.8%).

Undisturbed locations (NT) near the Setúbal canyon flanks were not associated with trawl marks (**Table 3**) and showed an

overall heterogeneous microtopography and frequent evidence of faunal activity and bioturbation, numerous tracks, and variously sized burrows and mounds; which are often associated with mud-burrowing decapods, such as the Norway lobster, *N. norvegicus* (**Figures 3A,B**). In contrast, both low (LT) and highly disturbed (HT) segments were characterized by the presence of either discontinuous or continuous ripple marks. Particularly in 2013, comparatively considerable less bioturbation evidence (e.g., fewer and smaller burrows and tracks; **Figures 3C–F**) was observed for these areas. LT segments showed consistently low numbers of trawl scars (ca. 0.15 trawl scars.100 m^{-1}). Most scars observed at LT segments in 2013 were classified as "recent," while scars observed in 2014 were mostly characterized as "eroded" (**Table 3**). The number of scars observed in the trawling target areas (HT) was up to 19 times higher than at the LT areas (**Table 3**). Note that this number may be greatly underestimated owing to the repeated operation of trawlers over the same trajectories.

TABLE 3 | Characterization of the trawling scars observed in muddy-sand sediment (A6:4) segments within 300–400 and 400–500 m water depths (selected dataset).

Area	N° of 100 m segments	TP (h.cell^{-1}.y^{-1})	Trawl scars	
			Average ± SE	Eroded scars (%)
300–400 m				
LT (13)	16	0.03 ± 0.027	0.18 ± 0.136	25.0
LT (14)	26	0.39 ± 0.051	0.15 ± 0.072	100.0
HT (13)	15	5.55 ± 0.393	0.53 ± 0.192	50.0
HT (14)	13	8.90 ± 0.191	2.85 ± 0.406	18.9
400–500 m				
NT (14)	3	0.00 ± 0.000	0.00 ± 0.000	0.0
HT (13)	23	11.24 ± 1.622	2.09 ± 0.492	4.3

TP: trawling pressure. NT, no trawling pressure; LT, low trawling pressure, and HT, high trawling pressure.

Mega-Epibenthic Assemblages in Relation to Trawling Disturbance

The nMDS plot (**Figure 4**) shows a segregation of the mega-epibenthic assemblages according to trawling pressure for both years. PERMANOVA results (**Table 4**) confirms significant differences in mega-epibenthic assemblages from different "trawling pressure" groups ($p < 0.001$) within the same depth range, independently of the sampling year ($p = 0.3181$). Morphospecies contributions for these differences analyzed through the SIMPER analysis, showed a maximum dissimilarity of 90.5% between assemblages from NT and HT segments, while dissimilarity between LT and HT segments was 64.3% (Supplementary Tables 3, 4). The comparison between NT and LT was not computed due to depth-range differences. The major contributors to the dissimilarity between NT and HT segments (400–500 m; **Table 5** and Supplementary Table 4) were the dominant morphospecies in these groups: Spirularia ind. 1, *Kophobelemnon* sp., *Galeus melastomus*, and other Pennatulidae at NT segments; anthozoan anemones, such as *A. richardi* and the tube-dwelling Spirularia ind. 2, and high abundances of the motile predator hermit crabs with their commensal anemones

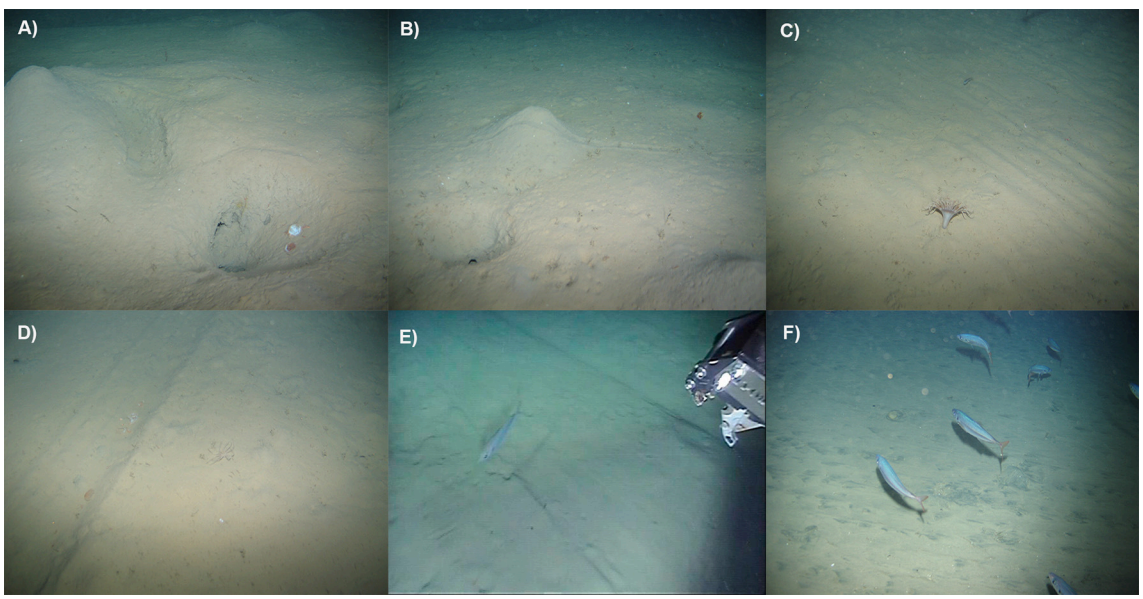


FIGURE 3 | Seabed image samples from the study area within muddy-sand sediments (A6.4). **(A,B)** Undisturbed locations at the Setúbal area (NT) showed heterogeneous topography and large faunal activity (e.g., tracks, burrows, and mounds of various sizes). Older evidence of trawling disturbance was demonstrated by the **(C)** chain/nets scars and **(D)** trawl doors marks with clear signs of bioturbation activity. Recent passages by trawlers were evidenced by **(E)** large door marks and **(F)** adjacent locations with flattened seabed surface with no recent faunal activity evidence. Photo credits: VLIZ and UGent.

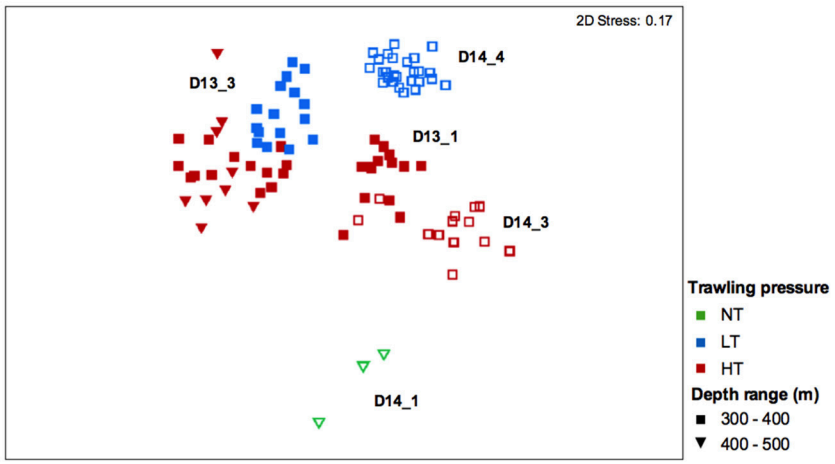


FIGURE 4 | nMDS plot for comparison of mega-epibenthic assemblages from muddy-sand sediments segments between 300–400 and 400–500 m subjected to varying trawling pressure (selected dataset). NT, no trawling pressure; LT, low trawling pressure; HT, high trawling pressure. Closed symbols: 2013 segments; Open symbols: 2014 segments.

TABLE 4 | PERMANOVA main results based on the mega-epibenthic faunal community composition dataset of the 2-factor nested design [Year and Trawl (Year)] for muddy-sand sediments between 300 and 400 m water depths and 1-factor design (Trawl) for depths 400–500 m.

Source of variation	df	SS	MS	Pseudo-F	P	Perm.	P(MC)	Estimate component of variation
300–400 m								
Year	1	31,733	31,733	1.5406	0.1715	6	0.2502	297.4
Trawl (Year)	2	40,863	20,431	22.532	**0.0001**	9,907	0.0001	1051.3
Res	77	69,821	906.77					906.77
Total	80	142,420						
400–500 m								
Trawl	1	18,309	18,309	21.365	**0.0007**	2,546	0.0001	3288.1
Res	24	20,567	856.96					856.96
Total	25	38,876						

For tests with permutations lower than 100, Monte Carlo results were considered; Values in bold represent significant values.

(Zoantharia ind.) in HT segments. Differences between LT and HT segments (300–400 m) were largely explained by the presence of Porifera ind. 2 and Ophiuroidea ind. 1, limited to LT segments in 2014, high abundance of the predator shrimp, *Plesionika* sp., in HT segments, but also by various morphospecies with low individual contributions (e.g., *H. tubicola*, Spirularia ind. 2, *Caryophyllia* sp., small sized Comatulida ind. 1, and Comatulida ind. 2.; **Table 5** and Supplementary Table 3).

Differences in composition between disturbed and undisturbed areas were supported by the consistently higher diversity and evenness values of the mega-epibenthic assemblages at NT [$H' = 2.33$; $J = 0.778$; $ET_{(20)} = 9.1$; $K_1 = 27.7$], and LT [$H' = 2.25$–2.38; $J = 0.646$–0.690; $ET_{(20)} = 8.0$–8.5; $K_1 = 20.1$–20.9], when compared to HT [$H' = 1.84$–2.09, $J = 0.558$–0.603, $ET_{(20)} = 6.2$–7.4; $K_1 = 34.8$–39.2; **Table 5**]. This is further confirmed by the lower rarefaction curves and higher dominance curves displayed by the HT assemblages at both depth ranges (**Figure 5**). All rarefaction curves approximate asymptotic values, apart from the NT segments at the deeper areas (400–500 m, **Figure 5D**), indicating that the survey was insufficient to fully evaluate the biodiversity at the Setúbal sites.

A significant negative correlation (after Bonferroni correction) was detected between trawling pressure and the estimated diversity indices: species richness ($R = -0.5169$, $p < 0.001$), Shannon–Wiener diversity ($R = -0.6347$, $p < 0.001$) and $ET_{(20)}$ ($R = -0.6335$, $p < 0.001$; **Figures 6B–D**). Contrastingly, no significant correlation between trawling pressure and mega-epibenthic faunal abundances was observed (**Figure 6A**). It is noteworthy the record of large aggregations of the hermit crab Paguroidea ind. 1 in two segments under high trawling pressure ($19\ h.cell.^{-1}.y^{-1}$). The high abundances of this species largely contributed to the high variability in faunal abundances observed in the HT areas.

DISCUSSION

The sustainable exploitation and management of deep-sea resources can only be achieved by a good knowledge on the

TABLE 5 | Abundance and biodiversity results from muddy-sand sediment areas at subjected to varying trawling disturbance (selected dataset).

Trawling pressure	n	N	S	$ET_{(20)}$	H′	J′	Dominance			
							Taxa	Morphospecies	FG	%
300–400 m										
LT (13)	16	42.4 ± 2.99	26	8.0	2.25	0.690	HEX	*Actinauge richardi*	Pr/Su	20.1
							CER	Spirularia ind. 2	Pr/Su	19. 0
							ACT	*Gadiculus argenteus*	Pr	18.5
							POL	*Hyalinoecia tubicola*	Sc/Dt	15.5
							ELA	*Galeus melastomus*	Pr	7.2
LT (14)	26	101.7 ± 4.82	40	8.5	2.38	0.646	POR	Porifera ind. 2	Su	20.9
							OPH	Ophiuroidea ind. 1	Dt	18.5
							CER	Spirularia ind. 2	Pr/Su	15.4
							POL	*Hyalinoecia tubicola*	Sc/Dt	10.6
							ACT	*Gadiculus argenteus*	Pr	9.8
HT (13)	15	71.1 ± 4.16	36	7.4	2.09	0.582	CER	Spirularia ind. 2	Pr/Su	35.0
							DEC	*Plesionika* sp.	Pr/Om	18.1
							ACT	*Gadiculus argenteus*	Pr	16.1
							CRI	Comatulida ind. 1	Su	8.2
							CER	Spirularia ind. 3	Pr/Su	4.5
HT (14)	13	66.2 ± 10.59	21	6.2	1.84	0.603	DEC	*Plesionika* sp.	Pr/Om	34.8
							ACT	*Gadiculus argenteus*	Pr	20.0
							CRI	Comatulida ind. 1	Su	17.2
							CER	Spirularia ind. 3	Pr/Su	11.6
							CER	Spirularia ind. 5	Pr/Su	8.6
400–500 m										
NT (14)	3	53.0 ± 4.82	20	9.1	2.33	0.778	CER	Spirularia ind. 1	Pr/Su	27.7
							OCT	*Kophobelemnon* sp.	Su	23.3
							ELA	*Galeus melastomus*	Pr	12.4
							OCT	Pennatulacea ind.1	Su	11.3
							ACT	*Gadiculus argenteus*	Pr	4.4
HT (13)	23	47.3 ± 1.53	29	6.4	1.88	0.558	HEX	Zoantharia ind.	Pr/Su	39.2
							CER	Spirularia ind. 2	Pr/Su	17.2
							DEC	Paguroidea ind. 1	Pr/Su	16.7
							HEX	*Actinauge richardi*	Pr/Su	12.4
							ACT	*Gadiculus argenteus*	Pr	3.9

NT: no trawling pressure; LT: low trawling pressure; HT: high trawling pressure; n: number of the pooled segments; N: average abundance ± SE: standard error; S: morphospecies richness; $ET_{(20)}$: Hulbert's expected number of species per 20 individuals; H': Shannon–Wiener diversity (ln base); J': Pielou's evenness. Taxa include: POR (Porifera), CER (Anthozoa: Ceriantharia – Spirularia), HEX (Anthozoa: Hexacorallia), OCT (Anthozoa: Octocorallia), POL(Polychaeta), DEC (Malacostraca: Decapoda), CRI (Crinoidea), ELA (Elasmobranchii), ACT (Actinopterii). Feeding group (FG) includes: Pr, Predator; Sc, Scavenger; Om, omnivores; Dt, Detritus feeder; Su, Suspension/Filter feeder.

biodiversity and ecosystem functions of the concerned area. This has been proven difficult when, in addition to the environmental and biological processes, anthropogenic activities, particularly fisheries, are also influencing the mega-epibenthic assemblages (Ramírez-Llodra et al., 2011). This work was fundamentally driven by the limited information available on the impacts caused by crustacean bottom-trawling fisheries which have been active along the Portuguese coast since the late 70's. To our knowledge, only few *in-situ* observations were performed aiming to describe the mega-epibenthic faunal biodiversity there, and those were mostly concentrated in submarine canyon areas (Pattenden, 2008; Duffy et al., 2012; Fonseca et al., 2014; Gomes-Pereira et al., 2015). Yet, even less attempt has been made to identify the possible impact of fisheries on the benthic habitat and faunal assemblages (Morais et al., 2007; Fonseca et al., 2014). Hence, we discuss here mega-epibenthic faunal composition and diversity changes from areas subjected to varying trawling pressure using video and photographic methods.

It is important to refer that some limitations are associated within the present study. Specifically, the low taxonomical resolution associated with identification certain taxa (e.g., Porifera and Anthozoa), may have resulted in the underestimation of the overall biodiversity in study region as we only assigned a separate morphospecies when clear morphological characters were identified. This issue is usually associated with photographic/video surveys, in areas where

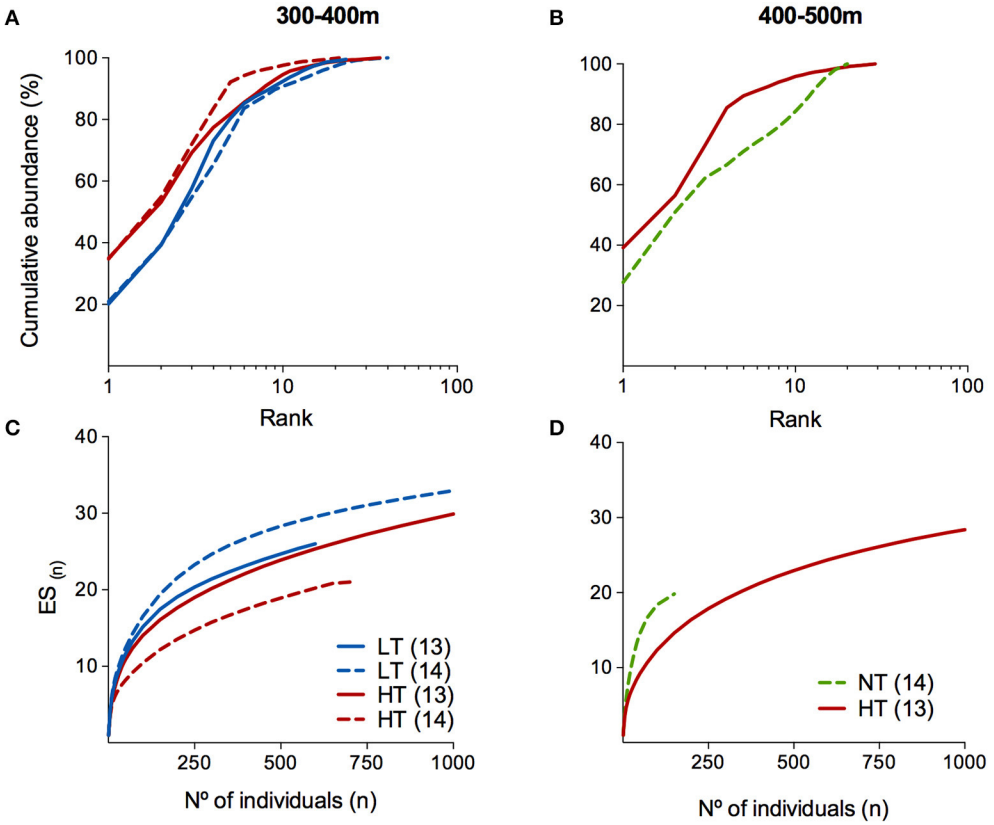


FIGURE 5 | Diversity comparison among the different disturbed areas through **(A,B)** k-dominance curves and **(C,D)** rarefaction curves (Hurlbert's expected number of species) computed based on the selected dataset for mega-epibenthic assemblages at depth of 300–400 m (Left) and 400–500 m (Right) within muddy-sand sediments. NT, no trawling pressure; LT, low trawling pressure; HT, high trawling pressure.

the understanding of biological biodiversity is still limited and is not associated with additional sampling, however it represents currently the best available tool to accurately quantify mega-epibenthic specimens (Bicknell et al., 2016). Furthermore, imagery surveys are essential to describe both faunal distribution and activity (e.g., bioturbation and feeding behavior), but also more importantly, to investigate direct evidence of physical disturbance on the seabed (e.g., presence and condition of trawl marks), otherwise impossible or counterproductive when using destructive methods such as trawl samplers (Bicknell et al., 2016). Secondly, because the laser points were not available due to technical issues, we were not able to estimate biomass differences across areas, even though the influence of bottom-trawling fisheries on this measure has been frequently reported (NRC, 2002).

Mega-Epibenthic Assemblages Associated with Environmental Variability

The effects of trawling fisheries on mega-epibenthic assemblages are fundamentally difficult to isolate from the environmental variability. Here, we observed marked differences in faunal assemblages linked with both spatial and temporal variability of the environmental and trawling disturbance conditions experienced along a relatively narrow depth range (c.a. 200–800 m). Depth-related changes in sediment sorting and fishing disturbance conditions (trawling pressure), together with the expected decrease in food supply (not directly investigated here) were accompanied by changes in mega-epibenthic fauna abundance, composition and diversity.

In the area off Sines, the overall higher abundances that characterized the shelf-break assemblages (c.a. 200–300 m), regardless of the sediment type, contrasts with the sharp abundance decline at depths greater than 300 m both at Sines and Setúbal areas. An abrupt decline in the benthic standing stocks (both abundance and biomass) is usually observed with increasing water depth. These declines in standing stocks are generally linked with a major decline of particulate organic matter supply to the seafloor (Rex et al., 2006). Furthermore, the high abundance and low diversity values at shallower depths resulted from the dominance of a single species, the onuphid polychaete *Hyalinoecia tubicola*, present in large aggregations and often feeding on carrion. This opportunistic scavenger has been reported in several regions of the NW Atlantic, including at the Portuguese margin (Fauchald and Jumars, 1979; Ravara and Moreira, 2013). *Hyalinoecia tubicola* displays a wide bathymetric

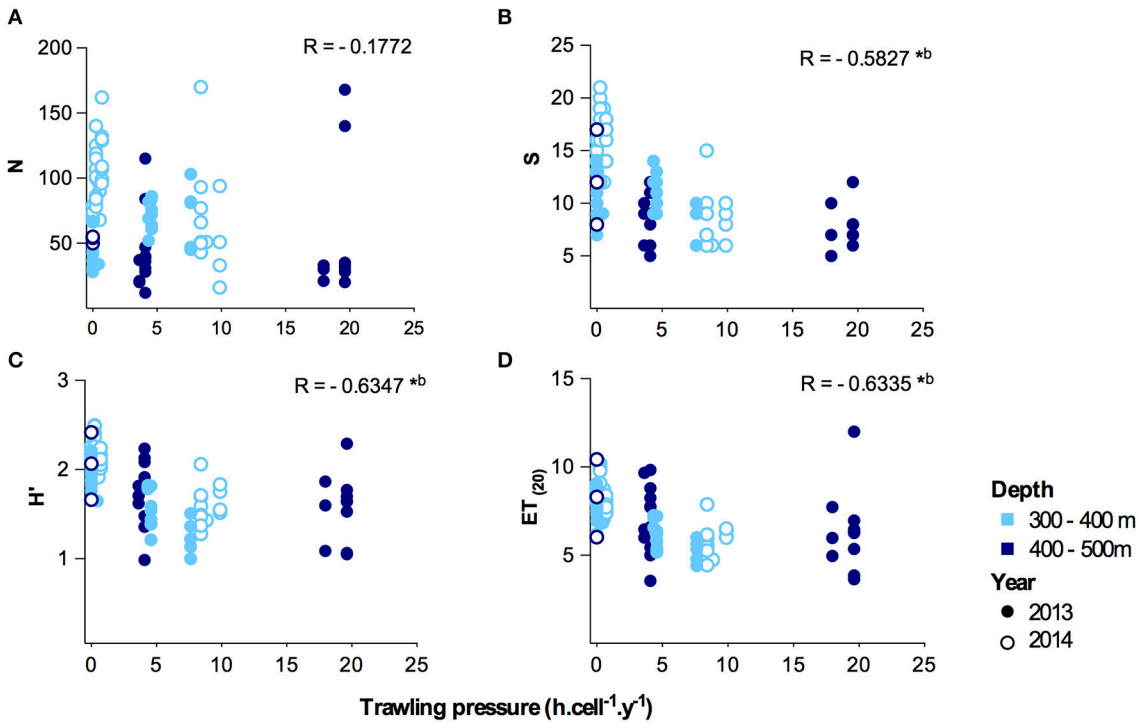


FIGURE 6 | Relationship between annual trawling pressure (h.cell^{-1}.y^{-1}) and **(A)** abundance (N; ind.100m^{-1}) **(B)** morphospecies richness (S), **(C)** Shannon-Wiener diversity index (H′) and **(D)** $ET_{(20)}$ Hulbert's expected number of taxa for 20 individuals per depth range. *Indicates significant correlation; [b]Indicates significant correlations after Bonferroni correction.

distribution, but is only dominant in relatively shallow and hydrodynamic areas (Grassle et al., 1975), thus it is not surprising that here shallower coarser sediment areas seemed to create a suitable habitat for this polychaete species, otherwise mainly absent at deeper locations. Furthermore, remains of dead crabs and other animals were frequently observed during the surveys off Sines. They probably originated from discarding practices which are common along the Portuguese margin (Monteiro et al., 2001), and may allow the maintenance of the abundant *H. tubicola* populations.

The upper slope segments off Sines (>300 m) were characterized by a shift to finer sediments (but also different trawling regimes). This area showed distinct mega-epibenthic assemblages from the ones observed at the shelf-break, typified by the presence of tube-dwelling anemones and other mud-burrowing fauna (e.g., the Norway lobster). Sediment preferences by both epibenthic and infaunal organisms are often reported in other studies and have been linked to life style and feeding habits (e.g., deposit feeders may select certain grain-size classes; Levin et al., 2001; Murillo et al., 2016). The preference of burrowing organisms for finer sediment types has been related with the higher stability of this sediments. A higher sediment stability allows for example the construction of burrows and tunnels (or even large galleries in the case of the Norway lobster), which otherwise would collapse in unstable sandy sediments (Afonso-Dias, 1998).

Differences in both morphospecies composition and diversity were also largely associated with different geographic locations (Setúbal and Sines areas; >300 m) and distinct long-term trawling disturbance regimes (discussed in more detail in sections Crustacean Trawling Fisheries and Seabed Physical Integrity and Mega-Epibenthic Fauna Vulnerability to Physical Disturbance). While we recognize the possible influence of canyon conditions (e.g., high energy bottom currents) at the Setúbal region (reference areas—NT), the naturally high dynamic conditions and productivity regimes of the WIM (Lavaleye et al., 2002), may attenuate the normally observed dissimilarities in community composition between canyon and slopes habitats (e.g., Ramírez-Llodra et al., 2010). In contrast with the typical dominance of deposit-feeders in other European regions (e.g., the Celtic Margin), the upper slope assemblages along the WIM tended to exhibit a naturally high proportion of sessile filter-feeders communities, often described as "canyon indicators" (Lavaleye et al., 2002). These "canyon indicators" were represented here by several morphospecies of the sub-class Octocorallia. The presence of current ripple marks parallel to the isobaths lines confirms the high energy hydrodynamic conditions along the self-break and upper slope off Sines.

Besides spatial variability, the mega-epibenthic assemblages also showed differences between years. As stressed before, these temporal changes must be interpreted with caution because of the differences in the alignment of the dives

(perpendicular or parallel to the coastline) and of sediment types and depths surveyed in 2013 and 2014. Temporal fluctuations in environmental conditions, namely the lower seasonal fluctuations and higher surface productivity in 2014 may explain the observed increase in dominance of detritivores (e.g., ophiuroids). The influence of other stressors that we were not able to directly investigate here (e.g., water masses properties, bottom currents, etc.), likely also contributed to these interannual differences. It is also important to mention that extreme storms occurred during the winter of 2013–2014 (Instituto Português do Mar e da Atmosfera, 2014), and those were not recorded in the winter of 2012–2013. These extreme events resulted in severe beach erosion and transport of large amounts of OM rich sediments from terrestrial origins toward deeper areas (Sanchez-Vidal et al., 2012; Diogo et al., 2014), likely providing additional food sources for detritivores and deposit feeders in the surveyed area.

Crustacean Trawling Fisheries and Seabed Physical Integrity

The initial characterization made by the Portuguese government—Direcção Geral dos Recursos Naturais, Segurança e Serviços Marítimos (DGRM) (MAMAOT, 2012) in the context of the European Union's Marine Strategy Framework Directive highlights trawling fisheries as one of the most pervasive activities along the Portuguese margin. Furthermore, the Portuguese government has issued a ban for bottom-trawling activities in the high seas areas comprising the Azorean EEZ and the claimed extended continental shelf beyond the 200 nautical miles[2]. However, these interdictions do not include continental slope and submarine canyon areas along the Portuguese mainland, which are the principal target habitats of deep-water crustacean trawlers.

Fishing effort distribution patterns in the mainland differ greatly between northern and southern regions (north and south of Cape Espichel, respectively). These differences are primarily related to the distribution of different target species and their preferred habitats. In the north, the most landed species include several cephalopod and demersal fish species that occur in coarse sediments along the continental shelf; in the south region, the most valuable species include several deep-water crustacean species (e.g., the Norway lobster, red and rose shrimps), which typically occur at muddy and muddy-sand habitats between the shelf break and 700 m water depths (Campos et al., 2007; Bueno-Pardo et al., 2017). Our results show the highest evidence of disturbance (trawl scars) in muddy-sand sediment bottoms (300–500 m depth) and an increase of up to 5 times in the observed number of trawl scars from 2013 to 2014, which are consistent with the fishing effort distribution and the increase in trawling pressure off Sines reported by Bueno-Pardo et al. (2017). This recently observed shift in trawling activity toward the Southwest region, mostly toward deeper locations (Bueno-Pardo et al., 2017), is of particular concern because it is likely to exert an unprecedented pressure on the deep-dwelling benthic assemblages and should be followed by an adequate monitoring programme.

While the most direct evidence of trawling pressure on benthic habitats are illustrated by the trawl scars, other seabed features could also help to characterize the effect of trawling in this area. Both the direct evidence of trawl fisheries impact (number and condition of the trawl scars), as well as the microtopography and bioturbation evidence (as proxy of the "ecosystem engineers" faunal activity) could help to infer the physical integrity of the seafloor; which is crucial for benthic biodiversity and ecosystem functioning (Rice et al., 2012; Thurber et al., 2014). The studied areas included in this research suggest that seabed integrity was largely compromised at disturbed locations off Sines. In the most severe cases (several HT segments) the seabed showed a completely flat appearance, and overall both HT and LT areas displayed low structural complexity. These observations contrasted with the area off Setúbal, which has never been trawled, and where the presence of a complex microtopography, represented by numerous tracks from crawling fauna, variously sized burrows and mounds was observed. These mentioned seafloor characteristics are indicative of the presence of "ecosystem engineering" fauna, responsible for performing several fundamental functions in the environment, such as promoting sediment carbon cycling, enhancement of water-sediment flux microhabitat provision and refuge for associate fauna (Thurber et al., 2014).

Mega-Epibenthic Fauna Vulnerability to Physical Disturbance

Among the most evident impacts associated with the low selectivity of bottom-trawling practices are the direct removal of large biomasses of target species, incidental catches of non-target species (by-catch), and overall increased *in-situ* mortality of damaged individuals. The indirect effects on the benthic habitats may include compromised seabed integrity (mentioned above), changes in benthic community trophic structure and size spectrum, and decreased mega-epibenthic fauna diversity (Jennings and Kaiser, 1998; NRC, 2002). However, the results of different studies are often inconsistent. For example, Atkinson et al. (2011) reported a decline in both mega-epibenthic faunal abundance and species richness from low to highly disturbed areas (reference conditions not available). In the Barents Sea, Buhl-Mortensen et al. (2015) investigated a wide range of soft and hard-substrate bottoms, and they have observed significant declines in abundance in sand and hard substrates locations, while muddy bottoms showed no distinct patterns regarding changes in abundance. In the south Portuguese margin, Morais et al. (2007) and Fonseca et al. (2014) identified a depletion of mega-epibenthic organisms abundances and diversity in fine sediment locations that suffered intense exploitation by crustacean trawlers with little evidence of recovery, while rocky and coarse sand substrates (avoided by trawlers to not damage the nets), promoted refuge for several sensitive species that included a large crinoid bed of *Leptometra celtica* (Fonseca et al., 2014). Moreover, most studies on soft sediment faunal assemblages impacted by trawling are flawed by the lack of reference pristine areas of the same habitat type.

[2]Diário da República, Portaria n° 114/2014 de 28 de Maio, 1ª série n°102 de 28 de Maio de 2014.

By comparing mega-epibenthic assemblages subjected to different levels of trawling pressures only in areas with similar sediment types and depth ranges, our study attempts to minimize the effects of other confounding environmental variables. Overall, the mega-epibenthic assemblages under higher levels of trawling pressure showed low diversity (taxa richness and evenness) in agreement with previous reports form the Southern Portugal coast (Morais et al., 2007; Fonseca et al., 2014). Differences in community composition were mostly marked between undisturbed locations (NT) and highly impacted sites (HT). Undisturbed areas were characterized by a more diverse fauna, showing a wider range of feeding modes and life styles. Among the most dominant taxa here were small tube-dwelling Spirularia ind. 1, several filter-feeding seapen species (e.g., *Kophobelemnon* sp., *Pennatula* sp.) anchored to the seabed and small predatory sharks (*G. melastomus*). In contrast, the typical dominant fauna of impacted areas included large and robust anemone species (*A. richardi* and tube-dwelling Spirularia ind. 2) and several highly mobile fish species and decapods with an opportunistic feeding behavior (predatory-scavenging; e.g., the arrow shrimp—*Plesionika* sp. and the hermit crabs—Paguroidea ind. 1). The presence of abundant motile predators or scavengers in HT segments is consistent with previous observations reporting a rapid response after disturbance of such species (e.g., Dannheim et al., 2014; Almeida et al., 2017) but also experimental works performed in the deep sea (Bluhm, 2001; Gerdes et al., 2008). In fact, there is often an increased food availability for these trophic groups in recurrently trawled areas, which results from both the on-site mortality or injured fauna, but also from discarding practices (Ramsay et al., 1996; NRC, 2002; Castro et al., 2005). The low commercial value of many by-catch species (e.g., Henslow's crab) at the WIM often leads to discarding of an average of 40–70% of the fished biomass by crustacean trawlers (Borges et al., 2001; Monteiro et al., 2001).

Differences between LT and HT mega-epibenthic assemblages were less pronounced than between NT and HT. Because LT areas are adjacent to the main fishing grounds (HT areas), they are likely influenced by trawling-induced turbidity. Pervasive high turbidity owing to sediment re-suspension during trawling operations (Puig et al., 2012; Martín et al., 2014) causes smothering of filter feeding fauna and can lead to overall lower abundances (Greathead et al., 2007). Lastly, the lower dissimilarity between HT and LT assemblages off Sines (64%) when compared to NT vs. HT areas (91%), suggests that the long-term influence of physical disturbance led to a significantly altered state of the mega-epibenthic assemblages in areas beyond the ones directly targeted by crustacean trawlers.

CONCLUSIONS

This work showed relevant differences in mega-epibenthic assemblages, linked both with environmental heterogeneity in the study region and trawling disturbance. The marked differences in morphospecies community composition and lower diversity in the disturbed locations, as well evidence of deleterious effects in areas beyond the ones directly targeted by crustacean trawlers, are indicative of strong effects of bottom-trawling activities on the mega-epibenthic assemblages off the SW Portuguese margin. Future recovery assessments would require historical analysis on both trawling pressure and community-based information (not currently available to our knowledge). Nevertheless, the observed deleterious effects of trawling on mega-epibenthic fauna, together with the intensification of trawling pressure in the study area stress the need for adequate monitoring programs and regulatory measures to halt the long-term loss of biodiversity and allow the sustainability of fisheries at the SW Portuguese Margin.

Lastly, it is important to point that trawl disturbance evidence on the seabed, assessed through the number and condition of the trawl scars, supports the Vessel Monitoring Systems (VMS) mapping and trawling pressure estimates performed by Bueno-Pardo et al. (2017), for the Portuguese Margin. While this method shows constraints related with data acquisition and background information of benthic habitat biodiversity, VMS data shows great potential for the identification of areas of interest in the deep sea that may need further monitoring.

AUTHOR CONTRIBUTIONS

MC, AV, and NL generated the study idea and funding. MC, AV, SR, and LL were responsible for the ROV video data acquisition, further analyzed by SR, EC, and JA. JB-P processed VMS data used to obtain trawling pressure estimates. SR wrote the manuscript with significant contribution from all authors.

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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.2017.00350/full#supplementary-material

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frontiers in Marine Science

REVIEW
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Identifying Toxic Impacts of Metals Potentially Released during Deep-Sea Mining—A Synthesis of the Challenges to Quantifying Risk

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In January 2017, the International Seabed Authority released a discussion paper on the development of Environmental Regulations for deep-sea mining (DSM) within the Area Beyond National Jurisdiction (the "Area"). With the release of this paper, the prospect for commercial mining in the Area within the next decade has become very real. Moreover, within nations' Exclusive Economic Zones, the exploitation of deep-sea mineral ore resources could take place on very much shorter time scales and, indeed, may have already started. However, potentially toxic metal mixtures may be released at sea during different stages of the mining process and in different physical phases (dissolved or particulate). As toxicants, metals can disrupt organism physiology and performance, and therefore may impact whole populations, leading to ecosystem scale effects. A challenge to the prediction of toxicity is that deep-sea ore deposits include complex mixtures of minerals, including potentially toxic metals such as copper, cadmium, zinc, and lead, as well as rare earth elements. Whereas the individual toxicity of some of these dissolved metals has been established in laboratory studies, the complex and variable mineral composition of seabed resources makes the *a priori* prediction of the toxic risk of DSM extremely challenging. Furthermore, although extensive data quantify the toxicity of metals in solution in shallow-water organisms, these may not be representative of the toxicity in deep-sea organisms, which may differ biochemically and physiologically and which will experience those toxicants under conditions of low temperature, high hydrostatic pressure, and potentially altered pH. In this synthesis, we present a summation of recent advances in our understanding of the potential toxic impacts of metal exposure to deep-sea meio- to megafauna at low temperature and high pressure, and consider the limitation of deriving lethal limits based on the paradigm

of exposure to single metals in solution. We consider the potential for long-term and far-field impacts to key benthic invertebrates, including the very real prospect of sub-lethal impacts and behavioral perturbation of exposed species. In conclusion, we advocate the adoption of an existing practical framework for characterizing bulk resource toxicity in advance of exploitation.

Keywords: deep-sea mining, toxicology, manganese nodules, hydrothermal vents, adaptive management

INTRODUCTION

Whilst the technological challenges of mineral recovery from deep water are significant (e.g., Collins et al., 2013; Glasby et al., 2015), they are no longer regarded as insurmountable obstacles to the exploitation of seabed resources by deep-sea mining (DSM) (Hannington et al., 2017). Consequently, the start—and subsequent expansion—of DSM will soon become contingent on: (a) a viable economic assessment (see Petersen et al., 2016; Hannington et al., 2017) and, (b) an appropriate legislative framework.

Legislatively, under the UN Convention on the Law of the Sea (UNCLOS), legal responsibility for mineral exploitation in the "Area Beyond National Jurisdiction" (or the "Area") rests with the International Seabed Authority (ISA). Early in 2017 the ISA published draft Environmental Regulations for deep-sea resource exploitation within the Area (ISA, 2017). This draft code advocates the Precautionary Principle in the initial pursuit of deep-sea mineral resources and a framework of Adaptive Management to monitor and regulate exploitation by licensed contractors. However, these two concepts present challenges for the ISA, as have been recently reviewed by Jaeckel (2016) and Le et al. (2017). These reviews have argued that key challenges remain with the identification of environmental risks of DSM, relevant to the application of the Precautionary Principle, and in the development of appropriate monitoring technologies with which to support the Adaptive Management of exploitation.

Recently, great progress has been made in constraining the toxic risks of mineral extraction under the unique conditions of high pressure and low temperature, as well as presenting these risks in an operationally appropriate manner to support decisions on exploitation licensing. At this key time for the development of DSM exploitation legislation, this synthesis paper provides a summation of the scientific community's latest understanding of the challenges in quantifying the absolute toxic risks of metal exposure to benthic organisms (meiofauna to megafauna). In part, this synthesis presents the key conclusions of the "Ecotoxicology" working group of the EC FP7 Project "Managing Impacts of Deep Sea Resource Exploitation" (MIDAS; https://www.eu-midas.net/), placed within the wider context offered by the research developments of other teams.

Deep-sea ore deposits comprise complex mixtures of potentially toxic elements including, for example: copper, cadmium, zinc, and lead, as well as rare earth elements, such as lanthanum and yttrium (Petersen et al., 2016). These ore deposits form in distinct geological settings, as has been extensively and recently reviewed (Petersen et al., 2016). *In situ*, the surface of these ore deposits are weathered and present minimal toxic risk. Indeed, nodules and massive sulfides can provide valuable substrate for a diverse faunal assemblage (e.g., Wang et al., 2013; Amon et al., 2016; Vanreusel et al., 2016). DSM, whether of seafloor massive sulfides (SMS), polymetallic nodules, or cobalt-rich crusts, may break up these ore deposits and may release toxic concentrations of metals into the environment at distinct phases of the mining cycle, affecting organisms within different marine compartments (**Figure 1**). Metals may be released in different physical phases (in solution, as ground particulates of different size and shape, or adsorbed onto the surface of particulates (see: Fallon et al., 2017 and references therein), which will affect their bioavailability to organisms. For example, metals in solution may be taken up across permeable tissues such as gill or gut surfaces, whilst particulate or adsorbed metals might be taken into the gut of deposit feeders. In the longer term, metal accumulation in tissues presents a further toxic risk for predatory species higher in the food chain, as they can consume contaminated prey items.

It is expected that the mining of massive sulfides or cobalt-rich crusts will involve crushed ore being pumped as a slurry from the seafloor mining tool, or via a collector after stockpiling on the sea bed (**Figure 1B**), to the surface mining support vessel via a riser pipe (Hoagland et al., 2010). Whilst polymetallic nodules may be collected intact (**Figure 1A**), nodules in the Clarion-Clipperton Fracture Zone in the Pacific Ocean are friable and readily crumble with handling. Consequently, for all resources, there is a risk that extraction will release metals in dissolved and fine particulate phases into the water column as a plume over potential scales of 100–1,000 s of km^2 (Oebius et al., 2001). Current operator plans include the transfer of bulk ore from the mining support vessel to a barge for transfer to shore and processing (**Figure 1C**) and this slurry will require dewatering before and/or after transfer (Hoagland et al., 2010). Dewatering the ore slurry at the surface may also release metals into the marine environment in the return water (**Figure 1D**). Releasing return water into the ocean at the surface or mid-depth (**Figures 1D,E**, respectively) will disperse potential toxicants widely (100–1,000 s of km) and may impact organisms within the water column. Return water, potentially at above-ambient temperature, that is discharged above the seafloor may disperse as a thermally buoyant plume over a large area, carrying toxicants with it (**Figure 1F**). The impacts of these potential metal releases must be understood for their potential to cause "serious harm" to deep sea marine ecosystems, the avoidance of which is a fundamental legal remit of the ISA (Levin et al., 2016).

There is increasing evidence of the need for incorporation of an Ecosystem Service (ES) approach into the environmental management of the deep sea, and specifically DSM (Danovaro et al., 2017; Le et al., 2017, respectively). Since the publication

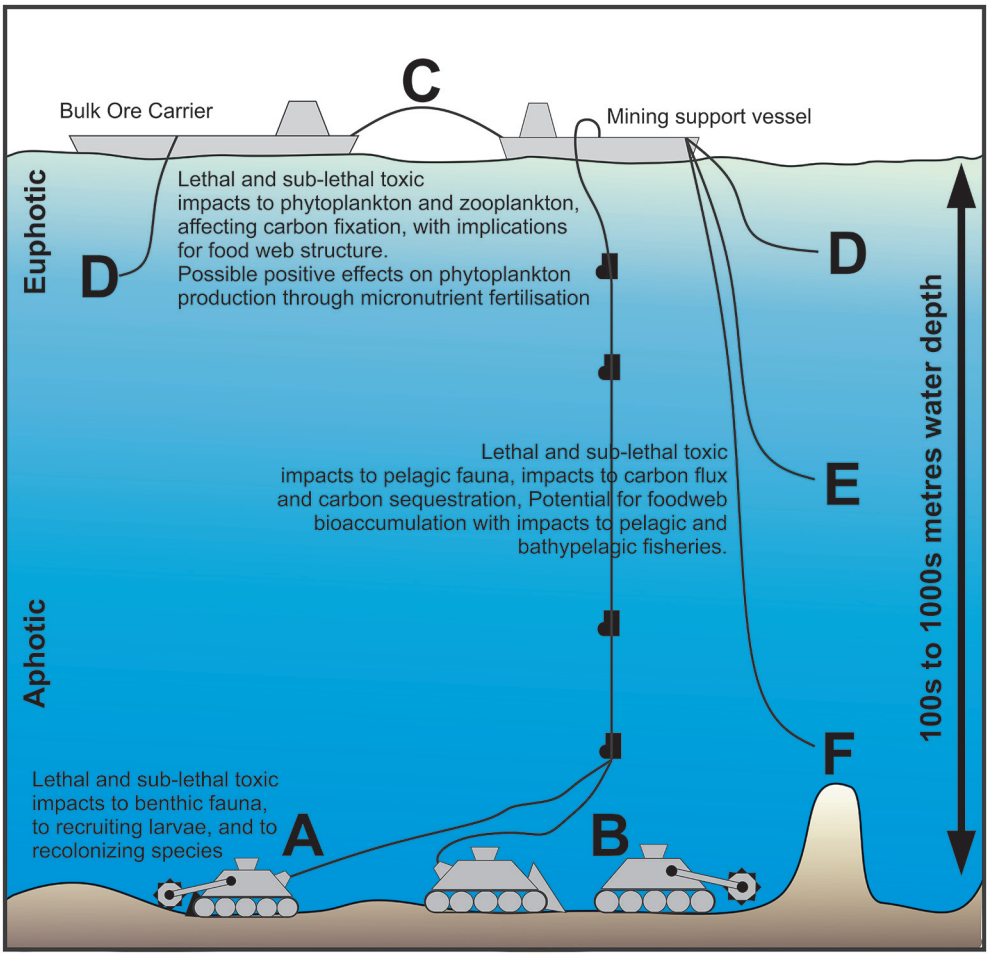


FIGURE 1 | Schematic representation of potential deep-sea mining operations (not to scale), identifying stages at which toxic metals may be released to the environment. Metals may be released in particulate or dissolved phases associated with localized plumes from mining collectors **(A)**, or individually controlled mining tools and collectors **(B)** operating in tandem. Dewatering ore slurry at the mining support vessel or transportation barge **(C)** may introduce toxic concentrations of metals into the euphotic zone, impacting photosynthetic primary producers or zooplankton communities and potentially affecting the sequestration of carbon dioxide in the surface ocean, as well as pelagic food webs driving the flux of carbon to the sea floor. Mid-water discharge **(D,E)**, whilst away from the productive euphotic zone, may impact vertically migrating zooplankton—again altering the vertical flux of carbon to the sea floor. Whilst the discharge of water near the seabed **(F)** will limit mid-water impacts, the release of thermally altered and buoyant water may disperse contaminants over a much larger area than that immediately impacted by the mining tools.

of the Millennium Ecosystem Assessment in 2005, the role of ecosystems in providing resources and services for the benefit of human kind has been recognized, and this is applicable to terrestrial as well as shallow and deep-sea ecosystems (Le et al., 2017). Consideration of ES incorporates a holistic view of species' distributions, abundance and diversity (Ecosystem Structures), as well measures of species' activity (Ecosystem Functions/Supporting Services) (Le et al., 2017; see also **Figure 2**).

Whilst, our knowledge of deep sea ecosystems and the diversity of life that they contain has been rapidly growing in recent years (Ramirez-Llodra et al., 2010), it is clear that we have only just begun to constrain the true diversity of life throughout the deep ocean. Deep sea communities are typified by species with slow growth and delayed maturity; characteristics which make them particularly susceptible to anthropogenic impacts or other environmental perturbation. These joint considerations: a current paucity of available data on deep sea community structure and the life history characteristics of known deep sea species, mean that there is a high probability that, should large scale deep sea mineral exploitation be prosecuted in the near future, we may in fact irreversibly impact unique biological and genetic resources even before they have been identified (Thornburg et al., 2010; Harden-Davies, 2017).

Abyssal benthic environments support biologically diverse species assemblages of meio-, macro- and megafauna (note, microfauna are explicitly not considered in this synthesis). These biologically diverse communities are comprised of populations with low overall abundance as a result of the absent primary production over the vast majority of the abyssal sea floor, and

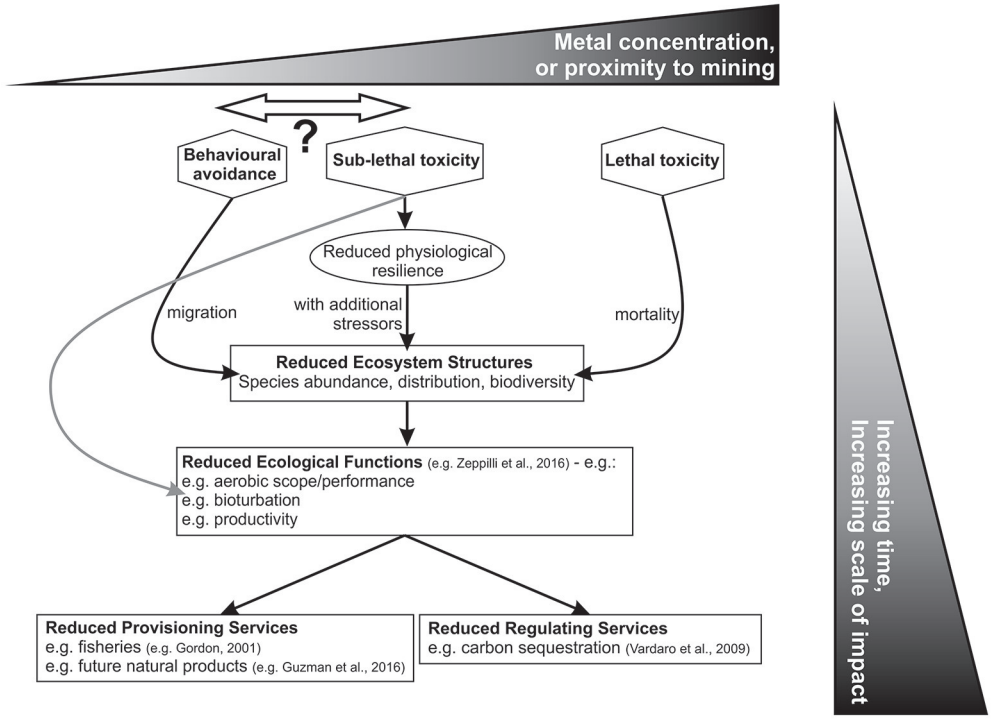


FIGURE 2 | Representation of how behavioral, sub-lethal and lethal impacts of metal exposure on individual organisms can, over time or with increasing magnitude of the metal release, scale to produce impacts on Ecosystem Structures, Ecological Functions and thence to Ecosystem Services (adapted from Le et al., 2017). Shown from left to right is the effect of increasing metal concentration, or increased proximity to the metal release. Behavioral modifications or sub-lethal effects will occur at low metal concentrations or far from a mining site whilst lethal impacts at high metal concentrations or at locations close to metal release will occur. Sub-lethal exposure can result in a reduction in organism performance that cause direct impacts to Ecological Function (gray line) or, in combination with other physiological stressors (e.g., hypoxia, restricted diet, post-spawning) can result in organism mortality that cause direct impacts to Ecosystem Structures. Note that the relative positions and relative order of behavioral and sub-lethal effects is variable.

a complete reliance upon periodic phytodetrital nutrient supply from the surface ocean thousands of meters away. This unique placement renders them energy limited (Smith et al., 2008), therefore restricting overall species abundance.

Nonetheless, recent studies in the Clarion Clipperton Fracture Zone have identified diverse epifaunal communities of filtering feeding organisms, including hexactinellid sponges, crinoids, hydroids, and soft corals, that are reliant on the nodules as substrate for attachment (Amon et al., 2016; Vanreusel et al., 2016). Dominant mobile megafauna associated with abyssal nodule fields include key benthic bioturbating groups, such as holothurians (sea cucumbers) and ophiuroids (brittlestars) (Amon et al., 2016).

In contrast, biological communities that colonize massive sulfides at hydrothermal vents are potentially supplied with a source of energy to support chemosynthetic primary production. These systems can also occur at shallower depths, receiving greater carbon flux from the surface ocean (Van Dover, 2000). As a result, active vent communities are normally comprised of a restricted diversity of fast-growing species that can occur at locally very high abundance. Faunal assemblages typically include crustaceans, molluscs and polychaete worms although with distinct biogeographic provinces (e.g., Van Dover et al., 2002), many of which indirectly rely on the vent fluid as an energy resource (Boschen et al., 2016; and references therein). Hydrothermal vent communities can show high endemism with high rates of speciation recorded; certain taxa being geographically restricted to a single, or small number of active vent sites (e.g., Wolff, 2005). As a consequence, whilst a single vent system might be seen as a productive and robust system, it may represent the only instance of that community within the deep ocean. Moreover, inactive, or relic, vents that might be first targeted for the exploitation of SMS, may support a more diverse faunal assemblage, including organisms that are not tolerant of vent conditions (e.g., relatively warmer waters and higher metal concentrations) or that do not rely on the vent fluids as a source of energy (Marsh et al., 2012; Sarrazin et al., 2015).

Water column, or pelagic communities, that might be impacted by plume release at mid-depths (**Figures 1D,E**; Ellis, 2001) mediate the transfer of energy and organic carbon from primary producers in the euphotic zone to the sea floor (Robison, 2009), but also from hydrothermal plumes on the deep sea floor (Phillips, 2017). Already studies have demonstrated that metals can exert a toxic effect to marine phyto- and zooplankton species (e.g., Hirota, 1981; Hu, 1981; Moraitouapostolopoulou and Verriopoulos, 1982; Caroppo et al., 2006; Fuchida et al., 2017) and can lead to metal accumulation (bioaccumulation) in higher trophic levels of food chains (Amiard Triquet et al., 1993).

In contrast, others have noted that some metals (including for example: copper, zinc, iron) represent essential micronutrients and that long-term exposure to low concentrations might create positive effects in pelagic communities through the improved function of some planktonic species (e.g., Loka Bharathi et al., 2005).

The bathypelagic zone (below ∼1,000 m and above 4,000 m) has been regarded as the largest ecosystem on the planet (Robison, 2009) and represents ∼75% of the volume of the ocean (Ramirez-Llodra et al., 2010). In addition to deep water fish that are exploited commercially (Gordon, 2001), this ecosystem supports a diverse assemblage of gelatinous and other zooplankton, which also play a pivotal role in the sequestration of carbon from the surface ocean to the deep sea floor, in so doing contributing to the global regulation of atmospheric carbon dioxide and thence the Earth's climate (Packard and Gomez, 2013; Teuber et al., 2014).

These different deep water habitats provide considerable Ecosystem Services for humankind; services that need to be afforded protection in the long term as they are both unique and also non-restorable (Le et al., 2017; see **Figure 2**). Indeed, the provision of Ecosystem Services has been argued as one standard for assessing "serious harm" in the context of DSM Environmental Impact Assessments (EIAs) (Le et al., 2017), because these services link environmental health to human well-being. Provisioning Services include supporting fisheries for human nutrition (e.g., Gordon, 2001) and novel natural products (e.g., Guzman et al., 2016), whilst Regulating Services include the absorption of carbon from the atmosphere and its subsequent sequestration with the deep ocean interior and within the seabed (e.g., Vardaro et al., 2009). At the sea floor diverse and stable biological communities represent a biodiversity resource which provides a reservoir of biological and genetic resources that might present the source of future natural products of further benefit to human kind (Harden-Davies, 2017).

The continued provision of Ecosystem Structures and Functions by marine communities in the vicinity of DSM requires the species assemblage to persist into the future (Zeppilli et al., 2016). Aside from the direct removal of settlement surface (or substrate) on which larvae may settle, the introduction of excess metals into the environment may exert a toxic effect on the resident fauna or may lead to organisms avoiding those areas exposed to high metal concentrations (**Figure 2**; see below). In either case, the disappearance of organisms from a system, or a reduction in the performance of a species or organism within a system, may result in a reduction in biodiversity and potentially increase ecosystem instability, which will present a risk to the continued provision of Ecosystem Services into the future.

However, it should be borne in mind that impacts to Ecosystem Services may represent a relatively slowly responding metric, one that integrates complex interactive effects (not just metal toxicity) over large spatial scales, and may therefore not be relied upon to provide an early or sensitive indication of toxic effects from released metals. It can be argued that the identification of any significant adverse impact, impacts that may precede any change in Ecosystem Services, should also constitute "serious harm" to the marine environment, and therefore should be appropriately quantified before large-scale mineral exploitation is licensed.

MINERAL RESOURCE TOXICITY TO INDIVIDUAL ORGANISMS CANNOT BE RELIABLY PREDICTED

For many commonly-studied metals, existing acute toxicological data [lethal concentrations (LC_{50}) and effective concentrations (EC_{50})] are available, but only for shallow-water biological species (e.g., Crompton, 1997). These data identify concentrations of metals which are either lethal, or "effective," for 50% of the exposed population over a designated period, conventionally 72 or 96 h. Alternatively, more recent toxicological studies have adopted a variable exposure duration that matches a desired sub-lethal endpoint (Simpson et al., 2017). The US EPA ECOTOXicology Database (ECOTOX; https://cfpub.epa.gov/ecotox/) is an online resource that summarizes all available metadata included within each ecotoxicology publication, and this database is updated quarterly. However, interrogation of this database at the end of 2013, at the start of the MIDAS project, identified that no data were available for any deep-sea taxa.

Through DSM, the toxic effects of metals will act potentially at *in situ* deep-sea temperatures and pressures (high pressure—up to 60 MPa, low temperature—down to 2°C), which are very different from those of laboratory exposures reported in the ECOTOX database (conventionally set at standard conditions of a temperature of 20°C and a pressure of 0.1 MPa) (Mestre et al., 2014). Brown et al. (2017a) have contrasted the toxic limits of metals in solution at low temperature (10°C) and high hydrostatic pressure (10 MPa) with those recorded under standard conditions of temperature and pressure (20°C and 0.1 MPa). These initial experiments made use of the shallow-water shrimp *Palaemon varians* as an experimental model; a species that has a close phylogenetic relationship to deep-water hydrothermal-vent shrimp species (Tokuda et al., 2006). These initial experiments showed that both copper and cadmium toxicity were significantly reduced at low temperatures at 96 h, but that the effects of high hydrostatic pressure were more complex. Whilst copper toxicity was significantly increased at high hydrostatic pressure, cadmium toxicity was not. Consequently, copper toxicity was lower than cadmium toxicity at 20°C but greater than cadmium toxicity at 10°C, and remained greater than cadmium toxicity at 10.0 MPa (Brown et al., 2017a).

Similar results were found in an acute copper toxicity study with the experimental model organism *Halomonhystera disjuncta* GD1 (Mevenkamp et al., 2017); a nematode that is phylogenetically closely related to the deep-sea species *H. hermesi* (Van Campenhout et al., 2014; Tchesunov et al., 2015) that inhabits cold-seep ecosystems. In *H. disjuncta* GD1, cold temperatures (10°C) reduced copper toxicity, whilst toxicity was increased when nematodes were exposed to high hydrostatic pressure (10 MPa) (Mevenkamp et al., 2017).

Whilst these observations of temperature-mediated toxicity are not new (e.g., Lewis and Horning, 1991; Heugens et al., 2003;

Khan et al., 2006; Prato et al., 2008; Barbieri et al., 2013), to the extent that authors have suggested the use of a correction factor in applying LC_{50} values for different temperature environments (Wang et al., 2014), the moderating effects of temperature may not be consistent within the real world (see Chapman et al., 2006). Moreover data indicate that the determination of any "temperature correction factor" will be different at high hydrostatic pressure for each metal in question and would have to be empirically determined for each case, and potentially for each biological species (see also: Kiffney and Clements, 1996; Gonzalez-Rey et al., 2007; Wang et al., 2014; for example).

A further issue of using existing data to regulate mining activity is that many assessments of metal toxicity are based on a single metal presented at a single oxidation state. Mineral ores represent complex mixtures of metals that are site-specific (e.g., Hering, 1971; Scott, 2001; Glasby et al., 2015; Petersen et al., 2016) and that change with mineral weathering (Koschinsky et al., 2003). It is therefore extremely difficult to predict the exact toxic potential of a mineral resource *in situ* from laboratory studies on single metals, or even metal mixtures (see also the recent discussion of Belzunce-Segarra et al., 2015).

As evidence of this complexity, laboratory studies of the toxicity of copper and cadmium mixtures in the decapod crustacean *Palaemon varians* were assessed by testing deviation from independent addition reference model (IA) predictions (see Jonker et al., 2005; Pan et al., 2015). Brown et al. (2017a) assessed 96-h lethal toxicity in a mixture of copper and cadmium based on the predicted LC_{29} of both metals (derived from the modeled mortality responses to individual metals in each temperature/pressure treatment), and compared with mortality in a control treatment and in individual LC_{29} exposures to copper and cadmium. IA predicted that mortality in a binary mixture with LC_{29} of both constituents would be 50%; however, the interaction of copper and cadmium appeared potentiating. Most importantly, whilst temperature did not significantly affect the interaction of the two metals when exposed in combination, high hydrostatic pressure significantly increased the toxic effect. These data emphasize the potential key role of pressure in mediating the toxic effect of plumes generated through DSM (Brown et al., 2017a).

The complexity of metal mixture toxicity has recently been reviewed (Pan et al., 2015, and references therein) and a detailed discussion is not presented here. Nonetheless, it is clear from our studies and those of other groups, that the scientific community is not in a position to reliably predict the combined toxic effect of metal mixtures *in situ* at mining sites. Ultimately, there are fundamental differences in metal uptake from solution, and the subsequent toxic effect in organisms, that are mediated by low temperature and high pressure. These findings confound the principle of regulating DSM on the basis of exposure thresholds established under standard conditions. We conclude that existing toxicological data of metals in solution established at standard laboratory conditions should not *necessarily* be applied to the context of DSM.

We argue that to continue to establish lethal limits for single metals, or simple combinations of metals, in the laboratory will only produce incremental progress in our understanding of "real world" toxicity of mineral resources. If the scientific community continues with this approach, progress in this field will be too slow to be incorporated usefully into recommendations for contractors (see also, the arguments in Jager et al., 2006). As an alternative, we propose that it will be necessary to assess the "bulk toxicity" of each mineral deposit to identify *a priori* the potential toxic risk of each mineral resource to be mined within a license area (e.g., Harris et al., 2014; Fallon et al., 2017; Simpson et al., 2017; see also discussion of the "Weight Of Evidence" approach below). In practice, it actually may not be necessary to quantify the individual toxicity of each metal within each resource (although assessment of mineral composition is undoubtedly integral to resource classification; e.g., International Council on Mining Metals, 2013). It may only be necessary to determine—under controlled, ecologically relevant, conditions—the bulk lethal toxicity of that resource for a number of different locally-relevant biological proxy organisms. However, assessments of bulk toxicity should be considered for all relevant life cycle stages of the proxy organisms, including larval stages which are known to be more sensitive (e.g., Rainbow, 2007; Simpson and Batley, 2007; Casado-Martinez et al., 2009; Camusso et al., 2012; Hedouin et al., 2016), and should consider all appropriate physical phases for the metal (e.g., in solution/aqueous, as particulates of relevant size and shape, or adsorbed onto the surface of particulates; see below). A similar approach could be adopted to determine the bulk lethal toxicity of any return waters from surface dewatering before any discharge takes place, potentially as part of a contractor's Environmental Impact Assessment (ISA, 2017).

THE PHYSICAL STATE OF THE METAL TOXICANT IS IMPORTANT

Any metals released during the mining cycle will occur in different physical states (Simpson and Spadaro, 2016). Metals may enter solution/aqueous phase and be taken up across the gills, body wall and digestive tracts of exposed animals. Alternatively, crushed mineral particles, and dissolved metals that may adsorb onto sediment particles or flocculates may be ingested; this may particularly be the case for metals released during dewatering of ore slurry (for example Campana et al., 2012, 2013; Camusso et al., 2012). These various exposure routes have implications to predictions of toxicity as well, not least because the vast majority of data listed within databases such as the US EPA ECOTOX database are based on organisms exposed to metals in solution.

For example: Simpson and Spadaro (2016) have identified that metals in solution have greater potential toxicity than in particulate phases. Exposure to particulate copper, in the form of chalcopyrite (100% $CuFeS_2$) or chalcocite (80% Cu_2S), resulted in a reduced absolute mortality in both bivalves *Spisula trigonella* and benthic amphipods *Melita plumulosa* compared to copper in solution. Similarly, Farkas et al. (2017) have recently reported minimal toxicity in the pelagic copepod *Calanus finmarchicus*. exposed to $5\,g\ l^{-1}$ of the fine-grained fraction of marble processing tailings. However, Farkas et al. (2017) do note that this

pollutant did constitute a sub-lethal energetic burden to exposed copepods that took these fine-grained particulates into the gut (energetic implications and sub-lethal impacts are considered further in section Sub-Lethal Impacts of Chronic Exposure Should Be Considered).

These recent studies reinforce the necessity to determine the bulk toxicity of the mined resource in all relevant phases prior to the extraction of metals under an exploitation license.

SUB-LETHAL IMPACTS OF CHRONIC EXPOSURE SHOULD BE CONSIDERED

The holistic view of organisms within their ecosystem providing the basis for Ecosystem Structures, Ecological Functions and Services (see earlier discussion, and Le et al., 2017), is in marked contrast to the paradigm of acute "lethal toxicity." As discussed, acute toxicity is conventionally assessed in terms of the, rather artificial, construct of "96-h LC_{50}." However, DSM—particularly of polymetallic nodules—may continue within a license block for years to decades and the potential anthropogenic impacts may take place at unprecedented temporal and spatial scales (Glover and Smith, 2003; Selck et al., 2017). Key seabed organisms at considerable distances from the mined site may be subject to chronic metal exposures that are orders of magnitude lower than a lethal dose, but for very extended periods (Newman, 2010; Simpson et al., 2017).

A considerable body of research has identified the mechanisms by which metals in the marine environment might exert toxic effects in marine species (e.g., Hollenberg, 2010 and references in that special issue; Wu et al., 2016; **Figure 3**). Briefly, redox-active metals including iron, copper, cobalt, chromium and nickel, can catalyze the formation of reactive oxygen and reactive nitrogen species that can bind with lipids and cause lipid peroxidation and damage to cell membranes (Stohs and Bagchi, 1995; Ayala et al., 2014). Redox-inactive metals, including cadmium and zinc, also exert toxicity by binding with the sulfhydryl groups of proteins (Valko et al., 2016). Excess metals may also lead to the production of reactive species, including superoxide ($O_2^-\bullet$), peroxides (ROOR), singlet oxygen, peroxynitrite ($ONOO^-$), hydroxyl radicals (OH•) and nitric oxide (NO), which may also produce damage in animal tissues (Stocker and Keaney, 2004; Aitken and Roman, 2008; Martinez-Finley and Aschner, 2011). In response, organisms have multiple different mechanisms for the detoxification of reactive oxygen or nitrogen species to minimize damage (**Figure 3**). For example, organisms can produce thiol-rich metal binding proteins (e.g., metallothioneins; e.g., Hardivillier et al., 2004) that can either be sequestered as inclusion bodies or, if forming soluble complexes, can be secreted from the cells. Redox-inactive metals can be bound to glutathione to form non-toxic metal complexes. Reactive species are variously detoxified by enzymes including: superoxide dismutase (SOD), catalase (CAT), and peroxidases (PEROXs) (e.g., Company et al., 2004, 2006a,b, 2007, 2008; Bebianno et al., 2005; Gonzalez-Rey et al., 2008). Recently, Auguste et al. (2016), Martins et al. (2017) and Mestre et al. (2017) have assessed the potential sub-lethal impacts of exposure to dissolved metals specifically in a range of deep sea species, including molluscs and echinoderms that do not inhabit metal-rich environments, and molluscs and crustaceans from hydrothermal-vent habitats; habitats that are naturally metal rich. For example, copper concentrations from the mussel *Bathymodiolus azoricus* habitat on the Mid-Atlantic Ridge (MAR) have been recorded between 0.17 μM at ~850 m water depth at Menez Gwen to 2 μM at ~2,300 m water depth at Rainbow (Kádár et al., 2005) hydrothermal vent sites.

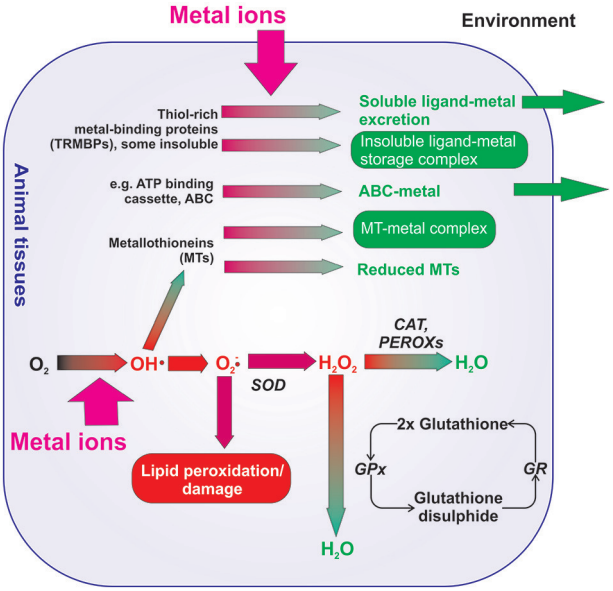


FIGURE 3 | Simplified synthesis of the routes of metal toxicity and detoxification in animal tissues. Metals in the environment that enter animal tissues can be sequestered by various metal binding proteins, including metallothioneins and other thiol-rich peptides, and either stored or excreted from those tissues. Metallothioneins can also detoxify hydroxyl radicals within cells. Redox active metals can drive the formation of superoxide anions ($O_2^-\bullet$) via hydroxyl radicals (OH•) that, if unregulated, can lead to lipid peroxidation and damage to cellular membranes. Superoxides can be enzymatically converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide (H_2O_2) is enzymatically converted to water by catalases and peroxidases (CAT and PEROXs, respectively) or by the action of glutathione peroxidase (GPx) and glutathione disulphide reductase (GR). Synthesis adapted from: Stocker and Keaney (2004), Aitken and Roman (2008), and Martinez-Finley and Aschner (2011).

Auguste et al. (2016) established the effects of copper exposure on the expression of tissue metallothioneins and lipid peroxidation as well as effects of the activity of key antioxidant enzymes in different tissues of the hydrothermal vent shrimp *Rimicaris exoculata* collected from the TAG hydrothermal vent field on the MAR and maintained at 30 MPa and 10°C. Shrimp were held in solutions of either ~25 or ~250 μg l^{-1} copper for 72 h, which were at least two orders of magnitude less than the 72-h LC_{50} concentration of ~35,000 μg l^{-1} for *Palaemon varians* at 0.1 MPa and 10°C reported by Brown et al. (2017a). Auguste et al. (2016) demonstrated that even shrimp that have evolved to live in the metal-rich environment of a hydrothermal vent field (*R. exoculata*) are sensitive to copper exposure in solution. Similarly,

copper exposure in hydrothermal vent mussels *Bathymodioulus azoricus* collected from the "Lucky Strike" hydrothermal vent site on the MAR and maintained at 17.5 MPa at ∼6°C for 96 h caused elevated lipid peroxidation at copper concentrations in excess of 300 $\mu g\ l^{-1}$, indicating lipid membrane damage within these tissues.

In these studies, however, data showed that effects of sub-lethal metal exposure were not consistent across all tissues within each organism. In the mussel *B. azoricus*, although lipid peroxidation was identified in several tissues, gills were more affected whilst mantle and digestive gland were found to be more resilient (Martins et al., 2017). This differential response may be related to the prominent role of the gill in harboring chemosynthetic symbionts and located at the interface between the internal milieu and external vent environment. Similarly, whilst the induction of metallothionein was significant in *Rimicaris exoculata* gill tissue after 72 h, this was not the case in muscle tissue (Auguste et al., 2016). In this latter case the differential response seen in different tissues of the shrimp was considered to result from differences in the route of uptake of the metal over the time course of the experiment.

Clearly, deep-sea species do have the ability to upregulate detoxification pathways in response to metal exposure and this can occur at relatively low concentrations of metals in solution. However, even with the upregulation of detoxification pathways, damage to tissues evidenced as lipid peroxidation can still be observed. Importantly, it is also clear that even those deep-sea species that inhabit metal-rich environments of active hydrothermal vents are physiologically responsive to metals in solution, although the relative sensitivity of vent and "off vent" species, and how this sub-lethal toxic stress might be phenotypically manifest in deep sea species, remains to be fully constrained.

As discussed, cellular protective mechanisms certainly respond to metal exposure, with upregulated metallothioneins, glutathione, molecular chaperones, antioxidants and/or cellular repair pathways increasing basal metabolic demand (**Figure 3**; see also Calow, 1989, 1991; Cherkasov et al., 2006; Ivanina and Sokolova, 2008; Ivanina et al., 2008; Sokolova and Lannig, 2008). Whilst successful detoxification might allow for organisms to survive chronic exposure to metals in solution and associated with sediment plumes, the expression of these pathways consumes energy. Basal metabolic rates will increase in association with detoxification and damage repair, as extensively reviewed by Sokolova et al. (2012). Elevated basal metabolic rate will ultimately reduce the energy available for other processes including growth, reproduction and locomotion (e.g., associated with foraging). These impacts may have significant further implications for the role and persistence of species (see **Figure 2**), even outside of the immediate mining footprint, that should be incorporated into the assessment of exploitation license applications. This is especially the case when the area being considered is adjacent to existing areas of exploitation. For example, in resource/food limited environments such as abyssal plains (Smith et al., 2008), or in environments that are routinely hypoxic (Peña et al., 2010), organisms may not have sufficient energy reserves or aerobic scope to meet the metabolic demand of continued tissue detoxification. Alternatively, at key times of the year—for example periods of reproduction or spawning—benthic organisms may be very susceptible to exposure to metal concentrations that would "normally" be considered to be sub-lethal (e.g., Martins et al., 2011; Levin et al., 2016).

BEHAVIORAL AVOIDANCE BY MOBILE ORGANISMS MAY INDICATE TOXIC IMPACTS IN REAL TIME

As reviewed above, the maintenance of Ecosystem Services within a system impacted by mining is dependent on the species that remain, and in their individual performance and ecological role—itself an integration of physiology and behavior—in that system (Simpson et al., 2017).

Detailed electrophysiological studies have demonstrated that the vent shrimp *Mirocaris exoculata* and the phylogenetically close shallow-water shrimp *Palaemon elegans* have the sensory ability to detect hydrothermal fluid cues like the hydrogen sulfide, as well as food solutions (Machon et al., 2016). They therefore at least possess the potential to detect and respond to changes in their immediate environment, either to remain in close proximity to vent fluids or to move away from high concentrations of metals.

These sensory abilities extend beyond the crustacean taxa. Bivalve molluscs and gastropod snails can withdraw into their shells and either hold the two valves tightly shut or close their opercula opening to avoid detrimental conditions (e.g., Kádár et al., 2001). For example, the fresh water clam *Corbicula fluminea* can close its valves in response to threshold concentrations of 5.6 or 19.5 $\mu g\ l^{-1}$ copper for response times of 300 and 30 min, respectively (Jou et al., 2016). Indeed, Hartmann et al. (2016) have argued that mussel behavior could be used as a biomarker for toxicological applications, and a similar case has been made for gastropod molluscs exhibiting avoidance behaviors when presented with repellent solutions (Hagner et al., 2015).

Of direct relevance to abyssal polymetallic nodule mining, Brown et al. (2017b) have reported consistent avoidance behaviors in echinoderms presented with copper contaminated sediments. In 96-h laboratory exposures at 4°C the shallow-water holothurian *Holothuria forskali* consistently avoided sediments contaminated with copper at concentrations of $5\ mg\ l^{-1}$ by climbing onto the side of the treatment tank (Brown et al., 2017b). These behaviors resulted in no significant induction of antioxidant enzyme activity. These behaviors were mirrored by the abyssal holothurian *Amperima* sp. exposed to copper-contaminated sediments at a depth of 4,167 m in the Peru Basin (Brown et al., 2017b) and were also sufficient to avoid induction of antioxidant enzymes in the bulk tissue extracts.

These data demonstrate that macro- and megafaunal species have the sensory capacity to detect metals in the environment and that, in at least some species, this can result in the expression of avoidance behaviors to help protect the organism from toxic effects. It can be concluded that mobile species exposed to contaminated plumes have the potential to demonstrate

chronic impacts by moving away from areas of contamination during exploitation. These migrations may produce long-lived direct and indirect (through species interactions) changes in the biological community structure at sites far field from the immediate mining site, creating downstream effects on system Ecological Functions and ultimately Ecosystem Services (**Figure 2**).

Moreover, avoidance behaviors do not necessarily constitute a permanent solution to toxicant exposure in marine organisms. For example, valve closure in molluscs represents only a temporary avoidance behavior as bivalve molluscs must open their valves periodically to irrigate their gills with oxygenated water (Byrne et al., 1991). Also, for many benthic species, including those at bathyal and abyssal depths, the speed at which a behavioral response can be elicited may be too slow to represent a viable means to avoid toxicant exposure (Ward et al., 2013). In addition, the potential variability in the "typical" behavioral response to toxicant exposure, which may impede their application as biomarker responses, has been identified by other research teams (e.g., Garcia-March et al., 2008; Melvin et al., 2017). Behavioral avoidance is also not possible for sedentary or attached species. As recently reported (e.g., Amon et al., 2016; Vanreusel et al., 2016), polymetallic nodules support a diverse assemblage of sedentary epibenthic fauna, including sponges and soft corals, which cannot move or otherwise avoid environmental exposure. These sessile fauna create vertical habitat for mobile species (Buhl-Mortensen et al., 2010) and their removal from the abyssal system through mining would create downstream impacts for other mobile fauna, impacting Ecosystem Structures and Functions.

A WAY FORWARD? A HOLISTIC ASSESSMENT OF POTENTIAL TOXICITY USING THE ESTABLISHED WEIGHT OF EVIDENCE (WOE) APPROACH TO QUANTIFY THE TOXIC RISK OF DEEP-SEA MINING TO BIOLOGICAL SPECIES AND COMMUNITIES

Based on current information, including the results of the MIDAS Project Ecotoxicology working group, we argue that it is not possible to predict *a priori* the absolute toxicity, and therefore identify exposure thresholds, of mining different seabed resources at bathyal and abyssal depths. Indeed, to propose absolute exposure limits based on an incomplete understanding of the mineral resource composition, exposure route and duration, and biological species involved, would be scientifically flawed and would run counter to the ethos of the Precautionary Principle recently advocated by the ISA.

However, it is possible to adopt a Weight Of Evidence (WOE) approach to quantify the risk associated with mining a particular resource. WOE approaches have been advocated for quantifying the toxic risk of exposure to metals in sediments in the absence of absolute data on lethal or effective concentrations (Chapman et al., 1998). Since this time the use of the WOE has been refined to address further uncertainties in the methodology and recommendations on their refinement have since been advocated (e.g., Batley et al., 2002). They have since found application in the assessment of risk in terrestrial (e.g., Milton et al., 2003; Semenzin et al., 2008) and aquatic systems (e.g., Schiesari et al., 2007; Martín-Díaz et al., 2008; Kellar et al., 2014).

Using this approach, multiple Lines Of Evidence (LOE), including a characterization of the mineral resource (chemical composition, grain size), the accumulation of metals within organism tissues, the organism tissue biomarker response, and additional bioassays (MICROTOX, or similar), it is possible to quantify the risk associated with mining (Piva et al., 2011; Benedetti et al., 2012, 2014; Regoli et al., 2014; Bebianno et al., 2015; Mestre et al., 2017).

Applying this approach, with multiple LOE to locally relevant "canary" species in the vicinity of the mining site or validated shallow-water ecotoxicological proxy species, would provide a mechanism for regulators and contractors to develop a holistic overview of the toxic risk of mining and resource. Appropriate "canary species" could be identified as either: (a) biomass dominants within the local biological community (e.g., abyssal sponges or holothurians, or hydrothermal vent shrimp and mussels), or (b) key species necessary to maintain Ecosystem Services (e.g., abyssal holothurians, abyssal meiofauna), or (c) species which could be captured and caged for routine monitoring during exploitation (e.g., bivalve molluscs such as *Bathymodiolus azoricus,* which is phylogenetically close to the deeper-dwelling *B. puteoserpentis,* and which naturally inhabits hydrothermal vent environments but which can survive at atmospheric pressure rendering it tractable for laboratory experiments). Ultimately, the precise selection of appropriate canary species will be habitat dependent, and still requires much more comprehensive datasets of the resident biological communities in potential mining locations, particularly in the case of nodule fields at abyssal depths.

Using the WOE approach it will be possible to identify high-risk resources or high-risk communities within a license block during contractor exploration or contractor Environmental Impact Assessments prior to exploitation licenses being issued by the regulating authority—the ISA in the case of the mining within the Area. Exploitation of these high-risk areas could be restricted until appropriate mitigation is in place (through operating procedure, or through mining tool design) or could be used to identify lower risk areas within a license block that could be prioritized for exploitation.

The WOE approach has been applied and validated within the shallow waters of Portmán Bay, Spain during the MIDAS project (Mestre et al., 2017). Nevertheless, further testing and validation of the WOE is required before this approach can be universally recommended to the ISA for use with the Area. The full implications of adopting an *a priori* WOE approach at bathyal and abyssal depths should be tested in the field, potentially by adopting independent scientific advisors/agencies into a flexible and staged implementation of the Adaptive Management of pilot

mining or as part of the first exploitation contracts issued by the ISA (Jaeckel, 2016).

In conclusion, the combined results of the EC MIDAS Ecotoxicology workgroup and other teams reviewed here identify the important, but as yet unpredictable, interacting roles of temperature and pressure in mediating the toxicity of metals to marine invertebrate fauna. As a consequence of these interactions, combined with the variable metal composition of different mineral reserves, it is not possible to predict acute thresholds for marine invertebrates that may be exposed to toxic concentrations of metals during DSM operations. Moreover, we conclude that acute exposure limits represent an artificial measure of possible impact, which do not identify sub-lethal and longer-term chronic responses that may disrupt Ecological Functions and Ecosystem Services within the deep sea. Our data indicate that future regulation of DSM exploitation should consider the potential for the perturbation of normal behaviors of deep-sea species, changes which may also affect the provision of Ecosystem Services in the long term. In order to quantify the toxic risk of mineral exploitation, we advocate the incorporation of existing "Weight of Evidence" approaches into environmental impact assessments. We identify the potential for these approaches to be further developed and validated through continued collaboration of scientific researchers and mining contractors during any future pilot mining operations, according to the principles of Adaptive Management.

AUTHOR CONTRIBUTIONS

CH, ST, MB, IM, MC, BS, AS-V, CG, AD, RD, VG, PW conceived the project and secured funding. All authors contributed to the science described in the manuscript. CH led manuscript production with contributions and comments from all authors.

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REVIEW
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An Overview of Seabed Mining Including the Current State of Development, Environmental Impacts, and Knowledge Gaps

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Rising demand for minerals and metals, including for use in the technology sector, has led to a resurgence of interest in exploration of mineral resources located on the seabed. Such resources, whether seafloor massive (polymetallic) sulfides around hydrothermal vents, cobalt-rich crusts (CRCs) on the flanks of seamounts or fields of manganese (polymetallic) nodules on the abyssal plains, cannot be considered in isolation of the distinctive, in some cases unique, assemblages of marine species associated with the same habitats and structures. In addition to mineral deposits, there is interest in extracting methane from gas hydrates on continental slopes and rises. Many of the regions identified for future seabed mining are already recognized as vulnerable marine ecosystems (VMEs). Since its inception in 1982, the International Seabed Authority (ISA), charged with regulating human activities on the deep-sea floor beyond the continental shelf, has issued 27 contracts for mineral exploration, encompassing a combined area of more than 1.4 million km^2, and continues to develop rules for commercial mining. At the same time, some seabed mining operations are already taking place within continental shelf areas of nation states, generally at relatively shallow depths, and with others at advanced stages of planning. The first commercial enterprise, expected to target mineral-rich sulfides in deeper waters, at depths between 1,500 and 2,000 m on the continental shelf of Papua New Guinea, is scheduled to begin early in 2019. In this review, we explore three broad aspects relating to the exploration and exploitation of seabed mineral resources: (1) the current state of development of such activities in areas both within and beyond national jurisdictions, (2) possible environmental impacts both close to and more distant from mining activities and (3) the uncertainties and gaps in scientific knowledge and understanding which render baseline and impact assessments particularly difficult for the deep sea. We also consider whether there are alternative approaches to the management of existing mineral reserves and resources, which may reduce incentives for seabed mining.

Keywords: deep sea mining, biodiversity loss, seabed disturbance, regulations, manganese nodule, seamount, hydrothermal vent, International Seabed Authority

INTRODUCTION

Rising demand for minerals and metals, in tandem with the depletion of land-based resources, has led to a surge of interest in marine mineral resources. Although no commercial scale deep-sea mining has taken place, a range of mining operations are active in the shallow seabed. However, exploration contracts for deep-sea resources have been awarded to companies from countries including China, the United Kingdom, Belgium, Germany, France and Japan for three different mineral resources: seafloor massive sulfides (SMS), ferromanganese crusts and polymetallic nodules. Mining the seabed carries significant environmental concerns, some of which have been highlighted over the past 5 years in relation to applications for mining in continental shelf regions (for example, ironsands and phosphorite mining in New Zealand waters; New Zealand Environmental Protection Authority, 2016). Given the nature, scale and location of proposed seabed mining activities, serious and widespread negative impacts on biodiversity are inevitable and likely to be irreversible (Van Dover et al., 2017). Other impacts include conflict with other users of the sea, such as the fishing industry and pharmaceutical firms looking to exploit marine genetic resources (Armstrong et al., 2012).

The deep sea (areas covered with >200 m depth of seawater) covers around 360 million km^2 of the Earth's surface (∼50%) and represents 95% of the global biosphere in terms of inhabitable volume (Thistle, 2003; Smith et al., 2009; Danovaro et al., 2014). Topographically, much of the deep ocean floor is abyssal plain at depths exceeding 3,000 m with features that include submarine canyons, oceanic trenches and ridges, hydrothermal vents and seamounts. Yet the vast majority of the deep-sea environment is unexplored and much remains to be discovered about the distinctive biodiversity associated with the deep seabed. Only a fraction of the deep sea has been scientifically studied and there are many valid concerns relating to seabed mining, one of which is the disturbance to as-yet-undescribed biota. In the past 20 years, for example, newly reported species range from invertebrates, such as a yeti crab, to large marine vertebrates including elusive beaked whales (Ramirez-Llodra et al., 2011; Thompson et al., 2012; Dalebout et al., 2014; Thatje et al., 2015; Araya, 2016; Vanreusel et al., 2016). Some deep-sea species with long life spans are vulnerable to physical disturbance because of their slow growth rates. For example, the Greenland shark (*Somniosus microcephalus*) dives to around 1,200 m and is described as the longest living vertebrate, reaching maturity at 156 ± 22 years with a lifespan of at least 392 ± 120 years (Neilsen et al., 2016). The black coral (*Leiopathese* spp.), a deep ocean species found off the Azores, is known to have a colony lifespan of up to 2,320 ± 90 years, arguably one of the longest living organisms on Earth (Carreiro-Silva et al., 2013).

In this review, we respond to the growing interest in exploitation of deep-sea mineral resources by drawing together information from peer-reviewed and other technical literature on developments within this industry. Here, we explore three broad aspects relating to the exploration and exploitation of seabed mineral resources: (1) the current state of development of such activities in areas both within and beyond national jurisdictions, (2) possible environmental impacts both close to and more distant from mining activities and (3) the uncertainties and gaps in scientific knowledge and understanding which render baseline and impact assessments particularly difficult for the deep sea. We also consider whether there are alternative approaches to the management of existing mineral reserves and resources, which may reduce incentives for seabed mining.

THE LOCATIONS OF MARINE MINERAL RESOURCES AND GAS HYDRATES

Mineral extraction from sediments and structures across the deep sea has been proposed at several habitat types—the abyssal plains, hydrothermal vents and seamounts along the mid-ocean ridges. Three main resources are of commercial interest: manganese nodules (MN) on the abyssal plains, particularly in the Pacific Ocean; SMS at hydrothermal vents, including off the coast of Papua New Guinea; and cobalt-rich crusts (CRC), which are found at seamounts worldwide with the largest deposits in the Pacific Ocean (**Figure 1**). In addition to metal-rich deposits, there is interest in extracting methane from gas hydrates associated with marine sediment on continental slopes and rises (in addition to beneath terrestrial permafrost). Other continental shelf resources of commercial interest include diamonds, ironsands (rich in titanomagnetite and lime-soda feldspars for steel production), and phosphorites. Shallow seabed mining for diamonds has been taking place off the coast of Namibia since 2001 by Diamond Fields International Ltd.

Manganese (Polymetallic) Nodules of the Abyss

Manganese nodules form on vast deep-water abyssal plains and comprise primarily of manganese and iron, though significant amounts of other metals are also found in these structures (**Figures 2A,B**). Nodules are potato-like in shape, 4–10 cm in diameter, and are thought to form in a process that takes millions of years in which manganese in seawater adsorbs to a nodule substance that is oxidized by bacteria and becomes nodule matrix (Blöthe et al., 2015; Vanreusel et al., 2016). The main constituents of interest in addition to manganese (28%) are nickel (1.3%), copper (1.1%), cobalt (0.2%), molybdenum (0.059%), and rare earth metals (0.081%) (Hein et al., 2013). Nodules also contain traces of other commercially relevant elements including platinum and tellurium, which are important constituents of technological products such as photovoltaic cells and catalytic technology (**Table 1**) (Hein et al., 2013; Antoni et al., 2017; Ojo and Dharmadasa, 2017).

Nodule accumulations of economic interest have been found in four geographical locations: the Clarion-Clipperton Fracture Zone (CCZ) in the north-central Pacific Ocean; the Penrhyn Basin in the south-central Pacific Ocean; the Peru Basin in the south-east Pacific; and the center of the north Indian Ocean. In the Pacific Islands region, the manganese nodule deposits with the greatest abundance and concentration of metals are found in the EEZ of Rarotonga (the Cook Islands). Other areas with high abundance are the two eastern island groups of the Republic of

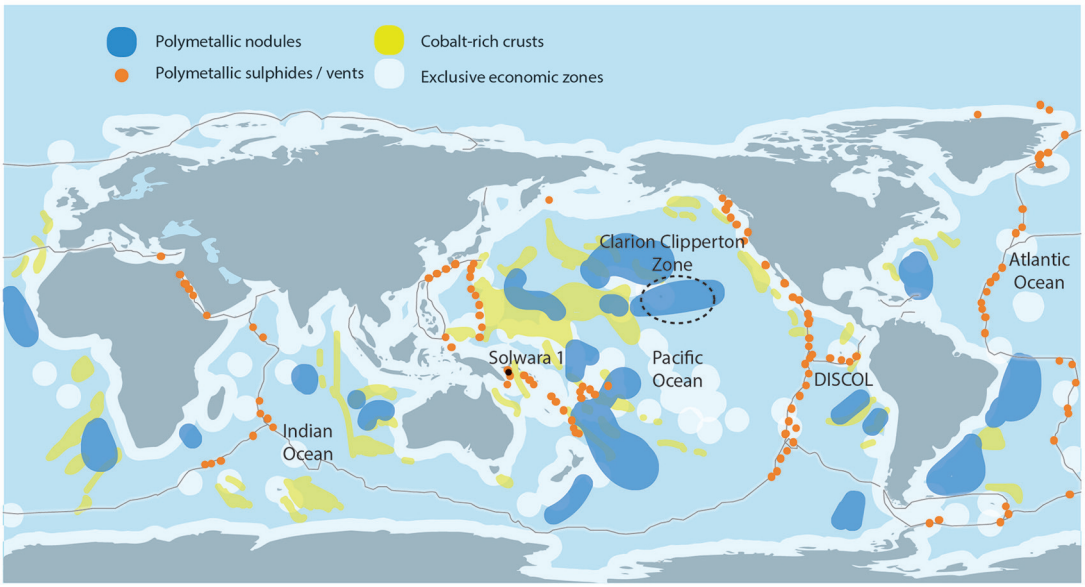


FIGURE 1 | A world map showing the location of the three main marine mineral deposits: polymetallic nodules (blue); polymetallic or seafloor massive sulfides (orange); and cobalt-rich ferromanganese crusts (yellow). Redrawn from a number of sources including Hein et al. (2013).

Kiribati (Phoenix and Line Islands), to a lesser extent the western Kiribati group (Gilbert Islands) and within the EEZ of the island nations Tuvalu and Niue (Secretariat of the Pacific Community, 2011).

Polymetallic nodules grow extremely slowly, at a rate of only rate of several mm to several cm per million years (Halbach et al., 1980; Gollner et al., 2017). Very few studies have investigated nodule fauna because of their inaccessibility on the abyssal plains, but they have been reported to provide some of the only hard substrate for marine species at those locations and therefore removal may result in significant habitat loss (Glover and Smith, 2003; Veillette et al., 2007; Vanreusel et al., 2016). Certain sponges and molluscs are unique to the surfaces of nodules, and nematode worms and crustacean larvae have been found within crevices (Thiel et al., 1993). Vanreusel et al. (2016) report higher densities of both sessile and mobile fauna living on or near manganese nodules than in nodule-free areas of the abyssal plains—in nodule-rich areas, a recorded 14–30 sessile individuals per 100 m^2, and 4–15 mobile individuals per 100 m^2; while in nodule-free areas there were up to 8 sessile individuals per 100 m^2 and 1–3 mobile individuals per 100 m^2.

Seafloor Massive Sulfides at Hydrothermal Vents

Seafloor massive sulfides (SMS), which are associated with both active and inactive hydrothermal vents along oceanic ridges, have a high sulfide content but are also rich in copper, gold, zinc, lead, barium, and silver (**Figure 2C**; Hein et al., 2013). More than 200 sites of hydrothermal mineralization occur on the seafloor and, based on previous exploration and resource assessment, around 10 of these deposits may have sufficient tonnage and grade to be considered for commercial mining. The technological viability to explore and extract marine mineral deposits is determined by the depth at which the minerals are found (Boschen et al., 2013, 2016).

Deep-sea vents are primarily concentrated along Earth's mid-oceanic ridge systems in the Pacific, Atlantic, Arctic, and Indian oceans (Van Dover et al., 2002). Ongoing exploration and resource evaluation indicate that polymetallic seafloor massive sulfide deposits in the Pacific have high copper concentrations with significant enrichment in zinc, gold and silver and are located in comparatively shallow water (<2,000 m; Secretariat of the Pacific Community, 2011; Hein et al., 2013).

Hydrothermal vent communities were first described in 1977, but since then only ∼10% of the deep ridge habitat has been explored (Ramirez-Llodra et al., 2010). In the past decade, ridge habitats have attracted attention from research teams trying to understand ecosystem dynamics and also by companies interested in exploiting minerals for commercial purposes (Beaulieu et al., 2015). Hydrothermal vents are found at 1,000–4,000 m depth and are characterized by temperatures up to 400°C and high acidity (pH 2–3), yet they support vast communities of organisms (Ramirez-Llodra et al., 2007). Chemosynthetic bacteria form the basis of vent ecosystems, and in turn support a large biomass of invertebrates that include molluscs, annelid tube-dwelling worms, and crustaceans (Van Dover et al., 2002). Some researchers think that life of Earth originated at hydrothermal vents (Martin et al., 2008). Around 85% of vent species are considered to be endemic (Ramirez-Llodra et al., 2007) with an average of two new vent species described every month in the 25-year period following their discovery (Van Dover et al., 2002). Within

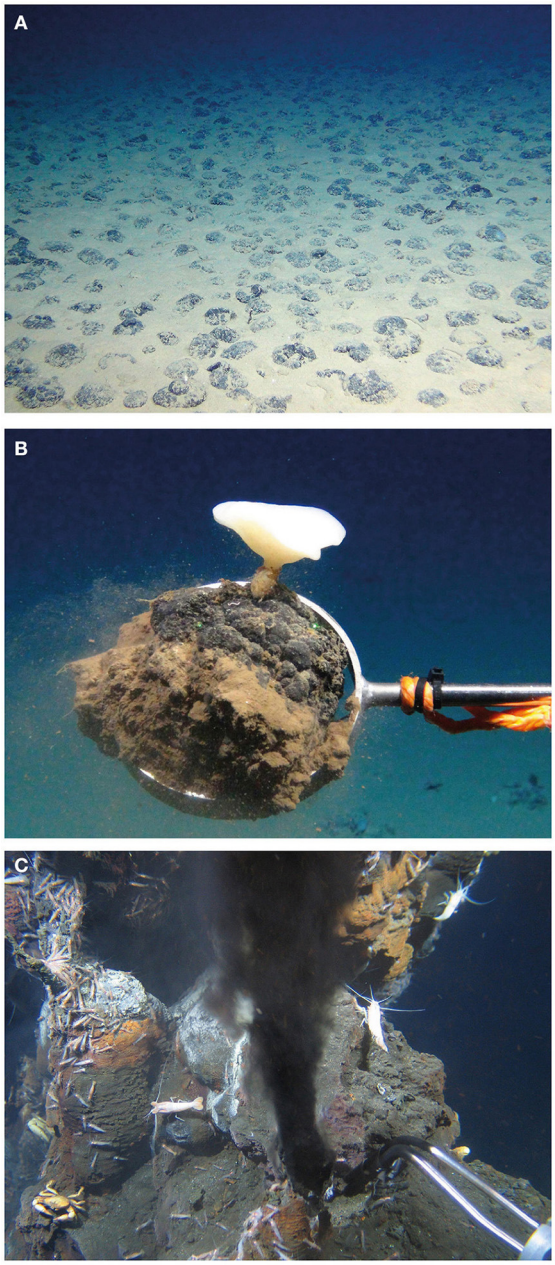


FIGURE 2 | Types of mineral deposit. **(A)** Manganese nodules in the Clarion Clipperton Zone, Pacific Ocean, **(B)** ROV KIEL 6000 holding a manganese nodule with associated fauna, **(C)** Black smoker (vent) on the mid-Atlantic ridge with deep sea shrimps and temperature measuring device. Photo taken by ROV KIEL 6000 during cruise M78/2. Copyright ROV Team; GEOMAR Helmholtz Centre for Ocean Research, Kiel.

the past decade, newly described species include a yeti crab (*Kiwa* spp.) that lives 2,600 m deep near hydrothermal vents in the Antarctic Ocean and farms chemosynthetic bacteria on its claws (Thurber et al., 2011; Thatje et al., 2015), and four species of deep-sea worm that live near vents in the Pacific Ocean (Rouse et al., 2016). Goffredi et al. (2017) found a high diversity of fauna inhabiting vents in the southern Gulf of California, reporting that only three of 116 macrofaunal species that they observed or collected were found on all four of the vent fields they studied. The research team's findings have implications for seafloor massive sulfide mining because destroying a discrete community at one vent could have connectivity implications for communities at nearby vents. Conservation issues could arise if recolonization of a mined vent is by species from a neighboring vent rather than by species that had colonized the vent before mining had taken place.

Cobalt-Rich Crusts at Seamounts

Cobalt-rich crusts, also referred to as ferromanganese crusts, form on the slopes and summits of seamounts and contain manganese, iron and a wide array of trace metals (cobalt, copper, nickel, and platinum; Hein et al., 2013). Based on grade, tonnage and oceanographic conditions, the central equatorial Pacific offers the best potential for crust mining, particularly within the EEZ of Johnston Island (USA), the Marshall Islands and international waters in the mid-Pacific seamounts. The EEZs of French Polynesia, Republic of Kiribati and the Federated States of Micronesia are also considered as potential locations to exploit cobalt crusts; smaller reserves have been recorded in Tuvalu, Samoa and Niue. Cobalt-rich crust mining is more technologically challenging than harvesting manganese nodules from abyssal plains because crusts are attached to rock substrates. Cobalt is of economic interest because the metal has wide-ranging uses that include those in superalloys, such as in jet aircraft engines, and in battery technology (Hein et al., 2013).

Globally, there are estimated to be more than 33,400 seamounts rising 1,000 m or more from the seafloor, and more than 138,000 smaller features known as knolls (rising 500–1,000 m) and hills (rising $<$ 500 m; Pitcher, 2007). Yesson et al. (2011) estimated that seamounts occupy an area of ∼17.2 million km^2 or 4.7% of the global ocean floor. The physics of currents associated with seamounts create oceanographic upwelling that delivers nutrients to surface waters that promote the growth of animals including corals, anemones, featherstars, and sponges (Koslow et al., 2001; Yesson et al., 2011).

Rowden et al. (2010) describe seamounts as oases on the abyssal plains because they often support higher epibenthic species diversity and biomass than nearby slopes. The oases hypothesis appears to depend on the geophysical context in which the seamount exists. Seamounts, in tandem with persistent hydrographic features such as oceanic fronts, have been shown to support high levels of primary productivity and provide a habitat for pelagic species. In such circumstances, seamounts can connect benthic and pelagic ecosystems. For example, fish and marine mammals are known to aggregate over seamounts, using them either for foraging or resting (Garrigue et al., 2015; Morato et al., 2016). Reisinger et al. (2015) tagged and tracked killer whales (Orcinus orca) and found that they spent time hunting over certain seamounts, which suggests that these oceanic features are a source of prey for these mammals. As well as supporting marine fauna including cetaceans, pinnipeds, and turtles for feeding, seamounts are thought to be navigational features during migrations and as breeding grounds (Yesson et al., 2011).

TABLE 1 | A summary of the uses of major resources found on the seabed.

Resource	Symbol	Uses	References
Copper	Cu	Electricity production/distribution – building wires and telecommunication cables/circuit boards. Transport sector—vehicle brakes, radiators and wiring, copper-nickel alloys are non-corrosive and provide material for the hulls of ships. Ecorys (2012) classes mid-ocean ridge copper deposits as areas of "high" economic interest.	British Geological Survey, 2007 Goonan, 2009 Ecorys, 2012 United States Geological Survey, 2012b
Silver	Ag	Mobile phones, PCs, laptops and batteries currently use the largest volumes of silver, many of the newer uses of silver focus on its antibacterial properties. Silver used domestically in mirrors, jewelery and cutlery. Ecorys (2012) classes mid—ocean ridge silver deposits as areas of "high" economic interest.	Ecorys, 2012
Gold	Au	Predominantly jewelery, although has also been used in electrical products. However, the total amount of material used for electricity is decreasing as base metal-gold alloys are increasingly providing a cheaper alternative to pure gold in electrical products. Ecorys (2012) classes mid—ocean ridge gold deposits as areas of "high" economic interest.	Ecorys, 2012 British Geological Survey, 2007 United States Geological Survey, 2012a
Zinc	Zn	Galvanizing steel or iron to prevent rusting, also commonly used as an alloy in the production of brass and bronze. Zinc is also used in the production of paint, as well as pharmaceutical products as a dietary supplement. Ecorys (2012) classes mid—ocean ridge zinc deposits as areas of "high" economic interest.	British Geological Survey, 2004 Ecorys, 2012
Manganese	Mn	Mainly used in construction industry due to its sulfur fixing, deoxidizing, and alloying properties. It is preferred over other more expensive alternatives. Ecorys (2012) classes manganese crusts and nodules at intraplate seamounts as areas of "low" economic interest.	Ecorys, 2012 Geoscience Australia, 2012 Blöthe et al., 2015
Cobalt	Co	Primarily used in production of super alloys with exceptional resistance to high temperatures, for example those used to make aircraft gas turbo engines. Also used in rechargeable batteries—notably lithium-ion batteries used in hybrid electric vehicles. These batteries contain high proportions of cobalt as 60% of the cathode in lithium-ion batteries is composed of lithium-cobalt oxide. Ecorys (2012) classes deep sea and intra plate seamount deposits of cobalt as areas of "moderate" and "low" economic interest, respectively. Cobalt is also found in manganese nodules.	British Geological Survey, 2009 Ecorys, 2012 United States Geological Survey., 2012c
Rare Earth Elements	REEs	Set of 17 elements including the 15 in the lanthanide series, plus scandium and yttrium. Used in the widest group of consumer products of any group of elements and have electronic, optical, magnetic and catalytic applications. Trends suggest that "green"—carbon reducing—technologies such as hybrid and fully electric cars, catalytic convertors, wind turbines and energy efficient lighting are key growth areas for REEs in the future. Demand for rare earth elements is increasing by 5–10% annually. Ecorys (2012) classes intraplate seamount deposits of REEs and yttrium as areas of "low" and "moderate" interest, respectively.	British Geological Survey, 2011 Ernst Young., 2011 Ecorys, 2012MIDAS, 2016
Tin	Sn	Used in the high-tech industry for manufacture of items such as smartphones and laptops in which the metal is used in solder. Also found in tinplate and in compounds that are used to make plastics, ceramics and fire retardants.	Geoscience Australia, 2016
Gas Hydrates		Gas hydrate is a solid ice-like form of water that contains mainly methane gas molecules in its molecular cavities. Methane from gas hydrates may constitute a future source of natural gas. Note that the high methane content of these hydrates and their potential adoption as a fuel resource could make them key sources of Carbon emission. According to the United States Geological Survey, the world's gas hydrates may contain more organic carbon than the world's coal, oil, and other forms of natural gas combined. Estimates of the naturally occurring gas hydrate resource vary from 10,000 trillion cubic feet to more than 100,000 trillion cubic feet of natural gas.	Sloan, 2003 Kretschmer et al., 2015

Some specific information on economic interest in these resources in European waters has been provided where available.

Gas Hydrates

Gas hydrates are ice-like solid crystalline structures that form when water molecules create a cage-like structure around a gas molecule at low temperature and high pressure. Although gas hydrates are not seabed mineral deposits, they have been included in this review because their exploitation is also close

to becoming a reality, and could in itself result in substantial impacts on surrounding deep-sea ecosystems. Gas hydrates may contain methane, ethane, propane or butane, though methane hydrate is the most common that occurs naturally. Potentially large quantities of gas hydrates are available—for example, 1 m^3 methane hydrate can yield 164 m^3 methane gas (Makogon et al., 2007; Duan et al., 2011) but commercial extraction of natural gas has not yet taken place because the process is technologically complex and costly. Estimates of the global mass of marine methane hydrates vary: Piñero et al. (2013) estimate in the region of 550 Gt C, but Kretschmer et al. (2015) suggests the higher figure of 1,146 Gt C. Reserves of gas hydrates are widely distributed and ∼220 deposits have been identified across the globe in the sediment of marine continental slopes and rises and on land beneath polar permafrost; continental shelf margins contain 95% of all methane hydrate deposits in the world (Demirbas, 2010; Chong et al., 2016).

Formation of gas hydrates depends upon a number of factors including accumulation of particulate organic carbon at the seafloor, the microbial degradation of organic matter and its related generation of methane, and composition of the gas (Makogon et al., 2007; Piñero et al., 2013). Methane hydrates are most commonly found at seawater depths of 1,000–3,000 m; they do not usually form in water <600 m deep because the water is too warm, but in the Arctic hydrates may form in shallow waters of around 250 m, where water temperature at the seabed is as low as −1.5°C (Buffett and Archer, 2004).

REGULATION AND MANAGEMENT

The legal framework governing anthropogenic activity on the ocean depends upon distance from land. A coastal state's territorial sea, in accordance with the 1982 United Nations Convention on the Law of the Sea (UNCLOS), extends to 12 nautical miles (22 km) from its coastline and includes the air space, the water body to the seabed and the subsoil (United Nations Convention on the Law of the Sea, 1982). Coastal states have exclusive rights and jurisdiction over the resources within their 200-nautical mile (370 km) exclusive economic zone (EEZ). Some states have an extended continental shelf beyond the EEZ within which they have sovereign rights over the seabed and any mineral resources, though not over the water column (**Figure 3**). Further out to sea is the area beyond national jurisdiction (ABNJ), which is a term used to describe both the seabed "Area" and the high seas water column above. UNCLOS designates the "Area" as the common heritage of mankind. The legal framework for the Area is provided by UNCLOS. The responsibility for the regulation and control of mineral-related activities in the Area is with the International Seabed Authority (ISA), comprised of the States Parties to UNCLOS.

Three sections in UNCLOS are particularly relevant to deep-sea mining: Article 136, Article 137.2, and Article 145, which respectively cover the common heritage of mankind, resources and the protection of the marine environment.

UNCLOS Article 136—Common Heritage of Mankind

The Article states that:

> "The Area and its resources are the common heritage of mankind."

Jaeckel et al. (2017) note that the principle of the common heritage of mankind in relation to the marine environment needs to be developed by the ISA. As well as sharing the benefits of marine resources for current and future generations, the common heritage of mankind principle also includes environmental conservation and preservation of the Area. As interest in commercial seabed minerals mining escalates, exploitation regulations will need to be refined and agreed upon by all interested parties. Setting conservation targets that incorporate research will help to determine the necessary measures to provide effective environmental protection.

UNCLOS Article 137.2—Legal Status of the Area and Its resources

The Article states that:

> "All rights in the resources of the Area are vested in mankind as a whole, on whose behalf the Authority shall act. These resources are not subject to alienation. The minerals recovered from the Area, however, may only be alienated in accordance with this Part and the rules, regulations and procedures of the Authority."

UNCLOS Article 145—Protection of the Marine Environment

The Article states that:

> "Necessary measures shall be taken in accordance with this Convention with respect to activities in the Area to ensure effective protection for the marine environment from harmful effects which may arise from such activities.
>
> To this end the Authority shall adopt appropriate rules, regulations and procedures for *inter alia*:
>
> (a) The prevention, reduction, and control of pollution and other hazards to the marine environment, including the coastline, and of interference with the ecological balance of the marine environment, particular attention being paid to the need for protection from harmful effects of such activities as drilling, dredging, excavation, disposal of waste, construction and operation or maintenance of installations, pipelines and other devices related to such activities;
>
> (b) The protection and conservation of the natural resources of the Area and the prevention of damage to the flora and fauna of the marine environment."

With reference to part (a) of Article 145, control of pollution and other hazards in the marine environment also includes protection of the coastline, indicating that the regulations are not limited to protecting only the Area.

In relation to (b), there is currently discussion among the scientific community, specialists in maritime law and other interested parties to define the measurement or threshold of

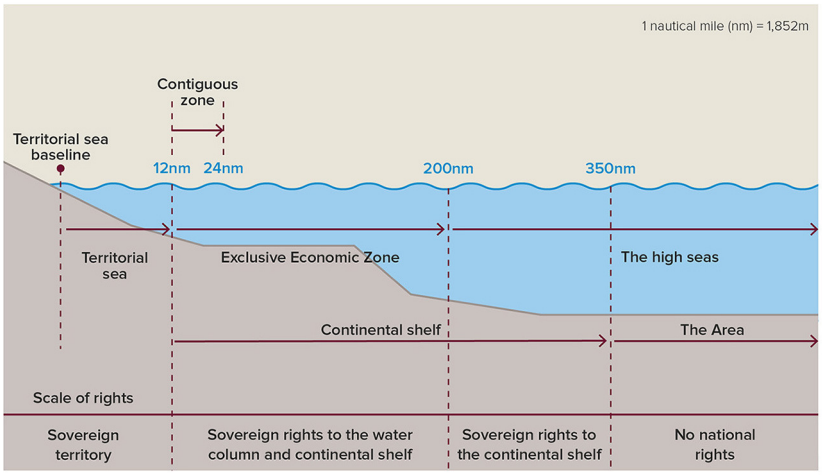


FIGURE 3 | A schematic showing jurisdictional zones from a nation's coast. Courtesy: The Royal Society, London.

harmful effects and what might constitute "acceptable harm" to an ecosystem from seabed mining. Levin et al. (2016) stress that it is crucial to understand marine biodiversity and define what effects would be harmful to the deep-sea environment to enable effective regulation of mining activities.

The International Seabed Authority

The ISA was established in 1982 by UNCLOS and is an autonomous intergovernmental body with 167 members. The ISA is responsible for the mineral resources and the marine environment in the Area. The ISA considers applications for exploration and exploitation of deep-sea resources from contractors, assesses environmental impact assessments and supervises mining activities in the Area.

To date, the ISA has approved 27 exploration contracts (www.isa.org.jm/deep-seabed-minerals-contractors). To compare with past years—17 contracts were active in 2013 and 8 were active in 2010 (**Table 2**). Contractors who have been granted exploration contracts are entitled to explore for minerals over a designated area of the seabed. Contracts are valid for 15 years, after which the contract can be extended for a further 5 years. Exploration contracts for polymetallic nodules cover up to 75,000 km^2, for SMS cover up to 10,000 km^2, and for cobalt-rich ferromanganese crusts cover a maximum 20 km^2. Seventeen contracts for exploration of seafloor massive sulfide deposits have been awarded to 16 contractors, 7 of which are national government bodies and 8 of which are companies with a sponsoring state. All six contracts for exploration relating to SMS and all four contracts to explore CRCs have been awarded to national governments. Exploitation regulations are currently under development but the ISA expects them to be finalized in the next 2 years. Accordingly, exploitation regulations were discussed at the authority's 23rd session, with calls from non-governmental observer organizations for greater transparency and for establishment of a separate environment committee. The session also included discussions on contractor compliance or non-compliance, on the need for the ISA to increase efforts to ensure environmental protection and on the establishment of a measure of "acceptable harm" to the environment from mining activity. Also discussed was the need to consider vulnerable marine ecosystems (VMEs) and ecologically or biologically significant marine areas (EBSAs) when issuing contracts, and how the ISA would justify biodiversity loss when its remit is to manage the Area on behalf of mankind (ENB, 2017).

Minerals exploration is also taking place within national waters and licenses to exploit the seabed for minerals in the exclusive economic zones (EEZ) have been issued by Papua New Guinea and Sudan/Saudi Arabia (**Table 3**). The most advanced project, which is closest to commercial exploitation, is in Papua New Guinea by Canadian registered Nautilus Minerals Inc. (hereinafter Nautilus Minerals).

An environmental permit and mining lease have been granted by the government of Papua New Guinea (Nautilus Minerals, 2011) though environmental concerns have been raised by indigenous communities, suggesting that mining will cause irreversible damage, disrupt their cultural practices and affect food sources (FSRN, 2017).

Regional Seabed Mining Guidelines

In addition to the ISA, regional guidelines that focus on the management of seabed mining are currently under development. One example is the MIN-Guide initiative in the European Union (http://www.min-guide.eu/mineral-policy). The MIN-Guide initiative is an online repository for information on minerals and related policies for Member States. In the Pacific, the Deep Sea Minerals Project was a collaboration between the Pacific Community and the European Union initiated in 2011. The Deep Sea Minerals Project aimed to

TABLE 2 | A summary of mineral exploration contracts in the Area approved by the ISA as of June 2017 including the start and end dates for these contracts.

Exploration contract holder	Sponsor	Location	Resource	Contract start date	Contract end date
China Minmetals Corporation	Government of China	CCZ	Polymetallic nodules	May 12, 2017	May 11, 2032
Cook Islands Investment Corporation	Government of Cook Islands	CCZ	Polymetallic nodules	July 15, 2016	July 14, 2031
UK Seabed Resources Ltd.	Government of United Kingdom of Great Britain and Northern Ireland	CCZ II	Polymetallic nodules	March 29, 2016	March 28, 2021
		CCZ I	Polymetallic nodules	February 8, 2013	February 7, 2028
Ocean Mineral Singapore Pte Ltd.	Singapore company majority owned by Keppel Corporation. Minority shareholders: Seabed Resources Ltd. (Lockheed Martin UK Holdings Ltd.); and Singapore-based Lion City Capital Partners Pte. Ltd.	CCZ	Polymetallic nodules	January 22, 2015	January 21, 2030
G-Tec Sea Minerals Resources NV	Government of Belgium	CCZ	Polymetallic nodules	January 14, 2013	January 13, 2028
Marawa Research and Exploration Ltd.	State enterprise of the Republic of Kiribati	CCZ	Polymetallic nodules	January 19, 2015	January 18, 2030
Tonga Offshore Mining Limited	Government of Tonga. Subsidiary of Nautilus Minerals Inc.	CCZ	Polymetallic nodules	January 11, 2012	January 10, 2027
Nauru Ocean Resources Inc.	Government of Nauru	CCZ	Polymetallic nodules	July 22, 2011	July 21, 2026
Federal Institute for Geosciences and Natural Resources of Germany	Government of Germany	CCZ	Polymetallic nodules	July 19, 2006	July 18, 2021
Government of India	n/a	Indian Ocean	Polymetallic nodules	March 25, 2002	March 24, 2017
Institut français de recherche pour l'exploitation de la mer (IFREMER)	Government of France	CCZ	Polymetallic nodules	June 20, 2001	June 19, 2016
China Ocean Mineral Resources Research and Development Association	Government of China	CCZ	Polymetallic nodules	May 22, 2001	May 21, 2016
Government of the Republic of Korea	n/a	CCZ	Polymetallic nodules	April 27, 2001	April 26, 2016
Yuzhmorgeologiya	Russian Federation	CCZ	Polymetallic nodules	March 29, 2001	March 28, 2016
Interoceanmetal Joint Organization	Governments of Bulgaria, Cuba, Czech Republic, Poland, Russian Federation and Slovakia	CCZ	Polymetallic nodules	March 29, 2001	March 28, 2016
Government of India		Central Indian Ocean	SMS	September 26, 2016	September 5, 2031
Institut français de recherche pour l'exploitation de la mer	Government of France	Mid-Atlantic Ridge	SMS	November 18, 2014	November 17, 2029
Government of the Republic of Korea		Central Indian Ridge	SMS	June 24, 2014	June 24, 2029
Government of the Russian Federation		Mid-Atlantic Ridge	SMS	October 29, 2012	October 28, 2027
China Ocean Mineral Resources Research and Development Association		Southwest Indian Ridge	SMS	Nov 18, 2011	Nov 17, 2026
Companhia De Pesquisa de Recursos Minerais (the Geological Survey of Brazil)	Government of Brazil	Rio Grande Rise, South Atlantic Ocean	CRC	November 9, 2015	November 8, 2030
Ministry of Natural Resources and Environment of the Russian Federation	Russian Federation	Magellan Mountains, Pacific Ocean	CRC	March 10, 2015	March 9, 2030
Japan Oil, Gas, and Metals National Corporation (JOGMEC)	Government of Japan	Western Pacific Ocean	CRC	January 27, 2014	January 26, 2029
China Ocean Mineral Resources Research and Development Association (COMRA)	Government of China	Western Pacific Ocean	CRC	April 29, 2014	April 28, 2029

All contract holders must either be owned by a government or sponsored by a government. CCZ, Clarion Clipperton Zones of the Pacific Ocean; SMS, seafloor massive sulfide deposits; CRC, cobalt rich crusts. Source: International Seabed Authority.

TABLE 3 | A summary of some seabed mining operations on continental shelves.

Contract holder (country of registration)	Description of contract holder	Location	Type of mineral	Project status (year awarded if known)
Nautilus Minerals Inc. (Canada)	Publically listed company	Bismarck Sea, PNG (Solwara 1 Project)	SMS	*Mining contract, active (2011)*
Diamond Fields International (Canada)	Limited company	Atlantis II Basin, Red Sea	SMS	*Mining contract, active (2010). Project currently on hold because of contractual issues with partnership company*
Diamond Fields (Namibia) a subsidiary of Diamond Fields International	Limited company	Namibia	Diamonds	*Mining contracts x4, active (2009, 2007 & 2007; 2000 is pending renewal; expected contract renewal as of November 2017)*
Diamond Fields (South Africa),	Limited company	Western Cape, South Africa	Phosphorites	Prospecting contract, active (2014)
Trans-Tasman Resources (New Zealand)	Limited company	South Taranaki Bight, west coast of North Island	Iron ore sands	*Three projects with an exploration permit, a mining permit and a prospecting permit.*
Trans-Tasman Resources (New Zealand)	Limited company	Westland sands, Ross to Karamea, west coast of South Island	Iron ore sands	Prospecting contract, active (2016)
Chatham Rock Phosphate (New Zealand)	Limited company	Chatham Rise, east side, South Island	Phosphorites	*Mining contract, active (2013). Company refused environmental mining consent approval (2015).*
Bluewater Minerals (Solomon Islands) Ltd. (Solomon Islands)	Limited company	Temotu and Western provinces, Solomon Islands	SMS	Prospecting contract, active (2007)
Green Flash Trading 251 (South Africa)	Limited company	Groen River to Cape Town, South Africa	Phosphorites	Prospecting contract, active (2014)
Green Flash Trading 257 (South Africa)	Limited company	Cape Town to Cape Infanta, South Africa	Phosphorites	Prospecting contract, active (2014)
Namibian Marine Phosphate (Pty) Ltd. (Namibia)	Limited company	Sandpiper Marine Phosphate Project, Walvis Bay, Namibia	Phosphorites	*Mining contract, active (2011). Project pending EIA approval (June 2017).*

This is not an exhaustive list and text in italics indicates exploitation (active mining) contracts, all other contracts refer to exploration. SMS, seafloor massive sulfide deposits.

improve governance and management of deep-sea mineral resources across the region in accordance with international law. The project had 15 member Pacific Island countries: Rarotonga (the Cook Islands), Federated States of Micronesia, Fiji, Republic of Kiribati, Marshall Islands, Nauru, Niue, Palau, Papua New Guinea, Samoa, Solomon Islands, Timor Leste, Kingdom of Tonga, Tuvalu, and Republic of Vanuatu. In August 2012, a Regional Legislative and Regulatory Framework was launched that aimed to improve management of marine mineral resources, with particular attention paid to the protection of the marine environment and ensuring that Pacific Island countries receive appropriate financial compensation (http://dsm.gsd.spc.int/public/files/2014/RLRF2014.pdf). Mining within EEZ areas is under the jurisdictions of national governments.

The possibility of prospecting for and extraction of gas hydrates in future decades has initiated discussion concerning regulations and management policy. No coordinated international regulations are in place to cover gas hydrate extraction, but national policies have been developed by coastal states including Japan, China, the United States, India, and Malaysia (Zhao et al., 2017).

MINING TECHNOLOGY AND PROCESSES

All proposed seabed minerals mining operations are based on a similar concept of using a seabed resource collector, a lifting system and support vessels involved in offshore processing and transporting ore. Most proposed seabed collection systems envisage the use of remotely operated vehicles, which would extract deposits from the seabed using mechanical or pressurized water drills (**Figure 4**). Development of deep-sea minerals mining technology is underway, though the greater depths involved present additional challenges. Mining for SMS at hydrothermal vents would involve mechanical removal of the ore and transportation to a support vessel to extract the necessary materials. Harvesting nodules would mean retrieving the potato-sized deposits from the seafloor then pumping the collected material to a surface vessel through a vertical riser pipe (Blue Nodules, 2016; Jones et al., 2017).

Natural gas would be extracted from reservoirs of gas hydrate in marine sediment or beneath terrestrial permafrost using one of three main methods: depressurization of the reservoir; increasing the temperature; or injecting chemical inhibitors (Makogon et al., 2007; Chong et al., 2016).

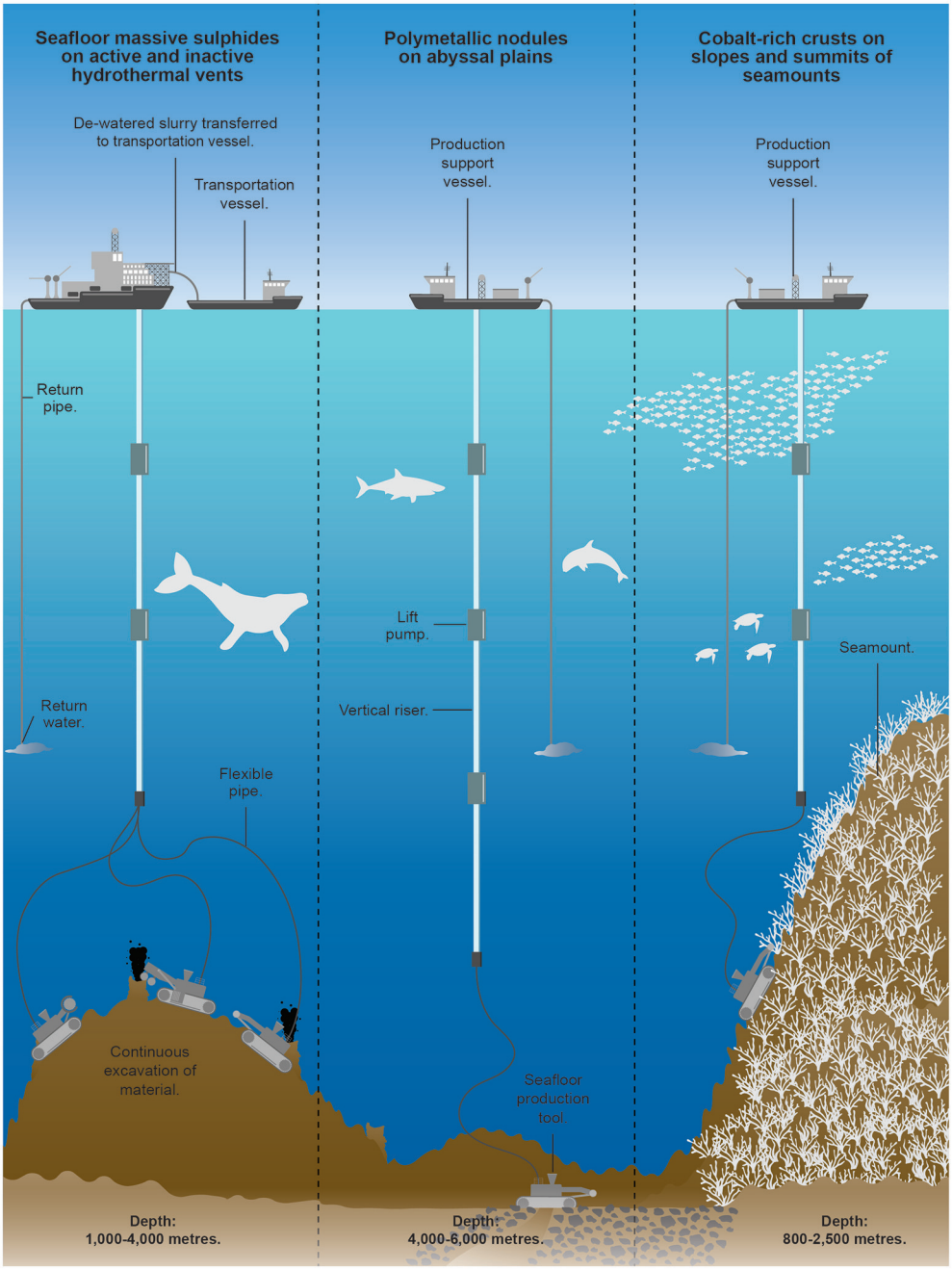


FIGURE 4 | A schematic showing the processes involved in deep-sea mining for the three main types of mineral deposit. Schematic not to scale.

Quantity and Quality of Marine Reserves of Marine Minerals

Polymetallic nodules form at a rate of several mm to several cm per million years (Halbach et al., 1980). Densely covered nodule fields (areas with >10 kg per m^2) that contain at least 1% copper and nickel are found in areas of the North and South Pacific Ocean at depths of 3,000–6,000 m in regions where there is no sedimentation from seamounts or accumulation of carbonate. Nodules are found in many areas of the Pacific Ocean, though for technical and economic reasons only a small percentage of nodules will be suitable for commercial exploitation. The composition of nodules is not uniform. Research has shown that deposits found just several 100 m apart can vary appreciably in composition—the concentration of minerals in nodules found in the North Pacific belt is greater than the South Pacific; percentage values from the former region are reported as: Mn 22–27; Ni

1.2–1.4; Cu 0.9–1.1; Co 0.15–0.25; Fe 5–9 (Halbach et al., 1980). Polymetallic nodules are also found in the Indian Ocean.

Seafloor massive sulfides are most likely to yield copper and zinc, though some also contain commercially significant grades (metal content) of gold (0–20 ppm) and silver (0–1,200 ppm; Hoagland et al., 2010). Data obtained from sampling suggest that the grades of some marine sulfide deposits, especially copper content, are higher than their terrestrial counterparts. Research suggests that seafloor sediment may also be a valuable source of rare earth elements (the lanthanides, plus scandium, and yttrium), with estimated reserves of more than 100 million metric tons (Kato et al., 2011). Most marine seafloor massive sulfide deposits are in the range 1 to 5 million (Hoagland et al., 2010). Nautilus's Solwara 1 site has an indicated seafloor massive sulfide resource of 0.87 million tons, with 1.3 million tons of inferred resource (Hoagland et al., 2010). At 90 million tons, the metalliferous muds of the Atlantis II Deep site in the central Red Sea may form the only seafloor massive sulfide deposit similar in scale to terrestrial sources (Hoagland et al., 2010; Thiel et al., 2015). Most companies focus on exploration of non-active hydrothermal vents.

Cobalt-rich crusts on seamounts can potentially yield multiple metals—manganese, cobalt, nickel, rare earth elements, tellurium, and platinum. The technology and methodologies to assess resources on seamounts is being developed but mining CRCs is not yet technologically feasible (Du et al., 2017).

The potential amount of natural gas in global reserves of gas hydrates are estimated to be around 1.5×10^{16} m^3 (at sea level) but more precise estimates are difficult because field data are scarce (Makogon et al., 2007). Countries including Japan, China, India, and the United States are investigating the resource potential of gas hydrates and in 2012, JOGMEC, the US Department of Energy and US oil firm ConocoPhillips began testing a method to extract methane by using CO_2 (Jones, 2012). Japan claimed to be the first country to successfully extract gas from methane hydrate in 2013 from an offshore location in its EEZ (BBC, 2013). In July 2016, a partnership comprising the US Geological Survey, the Government of India and the Government of Japan found a deposit of natural gas hydrate in the Bay of Bengal, India (United States Geological Service, 2016). Japan and China reportedly extracted methane hydrates in mid-2017 (Arstechnica, 2017). The German Submarine Gas Hydrate Reservoirs (SUGAR) project, launched in 2008 and financed by two federal ministries and German industries, is co-ordinated by GEOMAR and is investigating the potential of obtaining natural gas from gas hydrate reserves (GEOMAR, 2017, see http://www.geomar.de/en/research/fb2/fb2-mg/projects/sugar-i/).

Permission to Exploit Minerals

Projects in international and national waters are focusing on how feasibly to locate and extract minerals from the ocean for commercial gain. Two companies have been awarded permission for exploitation, though neither has begun commercial operations—they are Nautilus Minerals and Diamond Fields International. Both companies plan to operate in the EEZ, in the Bismarck Sea and the Red Sea, respectively. Legal terminology varies depending on the country; some countries award licenses, some award permits and others award contracts to exploit mineral resources.

The main commercial focus of Nautilus Minerals is the Solwara 1 project to extract high-grade copper and gold from seafloor massive sulfide (SMS) deposits located at depths around 1,500–2,000 m in the Bismarck Sea. In 2009, Papua New Guinea granted Nautilus Minerals an environmental permit for the development of Solwara 1 in the Bismarck Sea for a term of 25 years and then, in 2011, awarded the company its first mining lease, which covers an area of ∼59 km^2 (Nautilus Minerals, 2017).

Solwara 1 covers an area of 0.112 km^2, 30 km off the coast of Papua New Guinea at a depth of 1,600 m. The project is projected to have a lifespan of 2.5 years and will focus on the extraction of copper, which has a grade of ∼7%, and gold, with an average grade of 6 g per ton. During its lifetime, an estimated 1.3 million tons of material per year would be extracted from the site (Nautilus Minerals, 2008). Although commercial mining is yet to begin, in April 2012 Nautilus signed its first customer, China-based Tongling Nonferrous Metals Group Co. Ltd. (Nautilus Minerals, 2011). Nautilus Minerals's three seafloor production tools—built in the UK by Newcastle-based Soil Machine Dynamics—arrived in Papua New Guinea in early 2017. All three tools are undergoing 4-month-long submerged trials in an enclosed excavation on Motukea Island—the tools will not be deployed into the ocean and there will be no discharge of cut material into the environment. Nautilus expects delivery of its production support vessel at the end of 2018 (Nautilus Minerals, personal communication) and initial deployment and testing at Solwara 1 to begin in the first quarter of 2019, subject to financing (Nautilus Minerals, 2016a).

Diamond Fields International (DFI), together with its joint venture partner Manafa, acquired a 30-year exclusive deep-sea metal mining license, also for seafloor massive sulfide deposits, in June 2010 for activities within the Atlantis II basin in the Red Sea, ∼115 km west of Jeddah. The Atlantis basin is comprised of four interlinked sub-basins lying ∼2,000 m below sea level and is widely acknowledged as the largest known polymetallic marine "sedex" (sedimentary exhalative) deposit in the world. There is evidence of extensive and continuous mineralization of zinc, copper, silver, gold, lead, and other metals (Ransome, 2010). The Atlantis II project is currently on hold because of a legal dispute (Diamond Fields International, 2016).

ENVIRONMENTAL IMPACTS OF MINING AND THE POTENTIAL FOR SEABED RECOVERY

When commercial exploitation of marine resources was first suggested in the 1960s, scant regard was given to environmental consequences. Several decades on, an increasing number of commercial operations are in the pipeline and companies have been prospecting in international and national waters. In parallel with commercial interest in seabed minerals, there has been a deepening scientific understanding of marine ecosystem services

and biodiversity. Increased knowledge has, in turn, highlighted the potential consequences of mining in the deep sea (**Figure 5**). In the past decade, for example, the implications of the rapid loss of marine species are becoming apparent. Biodiversity loss has led to discussions about ways to help marine ecosystems to develop resilience to climate and physical change, for example by establishing marine reserves, and studies have attempted to assess the environmental impacts of mining (McCauley et al., 2015; O'Leary et al., 2016; Roberts et al., 2017). Seabed disturbance experiments include the German project DISCOL (disturbance and recolonization experiment) and follow-up study, MIDAS (managing impacts of deep sea resource exploitation). MIDAS was a multidisciplinary programme across 11 countries partly funded by the European Commission. Its 3-year investigation was completed in October 2016 (MIDAS, 2016). Conclusions included the potential for release of toxic elements during the mining process and the difficulty of predicting the impact of release using data from laboratory experiments involving only one element. Data on deep-sea biodiversity are scarce, and investigating the genetic connectivity and ascertaining the impacts to biota will require long-term studies. With regard to the longevity of impacts following the cessation of mining, MIDAS found that seafloor habitats did not recover for decades following disturbance and concluded that it was likely that the effects of commercial mining would be evident for longer timeframes. In summary, small-scale trials cannot accurately predict the full consequences of commercial-scale mining. MIDAS worked alongside industry partners to investigate best practices, and in its conclusion to that work proposed an environmental management strategy that adopted a precautionary approach that would incorporate adaptive management.

Environmental management of the Area is by the ISA, which to date has only needed to implement regulations relating to exploration. As interest in commercial exploitation advances, the ISA is now in the process of developing a regulatory framework for exploitation. A working draft titled "Regulations and Standard Contract Terms on Exploitation for Mineral Resources in the Area" was issued in July 2016, and a discussion paper, "Regulations on Exploitation for Mineral Resources in the Area (Environmental Matters)" was made public in January 2017 (https://www.isa.org.jm/files/documents/EN/Newsletter/2017/Mar.pdf). A report from a workshop held in Berlin, Germany, in March 2017 to develop a long-term environmental strategy for the Area is available on the ISA website (https://www.isa.org.jm/document/towards-isa-environmental-management-strategy-area).

Following on from the Berlin workshop, the ISA released revised draft regulations in August 2017 titled, "Draft Regulations on Exploitation of Mineral Resources in the Area" with a short list of questions to be addressed (https://www.isa.org.jm/files/documents/EN/Regs/DraftExpl/ISBA23-LTC-CRP3-Rev.pdf).

Environmental management of exploitation in the Area will ideally involve different levels of assessment, some of which will be carried out by the ISA and some by contractors. At the time of writing, the most recent draft of the exploitation regulations does not address environmental management in detail and specific protocols have yet to be developed. Ideally, the process will involve a strategic environmental assessment and plan, overseen by the ISA, that covers activity across the entire Area. Regional environmental assessments and plans will be prepared by the ISA for smaller zones within the Area and mining contractors would commission environmental impact assessments and statements for the specific area of their contracts. However, full details of how the ISA will manage the environmental aspects relevant to exploitation have not been finalized, there is a dearth of published baseline environmental data and questions remain, including who or what body will manage and monitor areas of particular environmental interest.

Nodule Removal from the Abyss

The physical recovery of manganese nodules will take millions of years because deposition rate of new nodules is slow (Halbach et al., 1980; Gollner et al., 2017). After the removal of nodules, it is unknown whether associated biota will recover. A number of experiments have investigated the impact of nodule removal on the benthic environment in the Clarion-Clipperton Zone have shown highly variable results. For example, an experimental extraction of nodules from the CCZ was conducted in 1978 (the so-called OMCO experiment), and the area revisited in 2004 to assess the recovery of the benthic habitat. Despite the ensuing 26 years, tracks made by the mining vehicles were still clearly visible and there was a reduced diversity and biomass of nematode worms within the disturbed tracks when compared to surrounding undisturbed areas (Miljutin et al., 2011).

Experiments carried out to date have been conducted on much smaller scales than proposed commercial operations, and some tests did not involve recovery of nodules. Vanreusel et al. (2016) examined a track that had been experimentally mined 37 years ago and found that the once nodule-rich area was devoid of fauna, indicating that mining can permanently damage nodule habitat and lead to significant biodiversity loss (**Figure 6**). Tilot (2006) analyzed 200,000 photographs and 55 h of video footage (taken since 1975) to investigate the biodiversity and distribution of benthic megafauna associated with polymetallic nodules in the CCZ. The study found the polymetallic nodule ecosystem to be a unique habitat for suprabenthic megafauna.

Seafloor Massive Sulfides from Hydrothermal Vents

Remotely operated machines will inevitably cause direct physical impact to the seabed, changing its topography through suction or drilling, removal of substrate and by machinery movements. Mining that targets hydrothermal vent chimneys will remove those features entirely, leaving a flatter topography with a more uniform surface and compressed sediment in many areas that could be unsuitable for habitat recovery and recolonization. Van Dover (2010) suggests that mining will alter the distribution of vents but the mineral component of chimneys could reform over time if the vents remain active. Hekinian et al. (1983) reported physical chimney growth of 40 cm over 5 days at some locations in the East Pacific Rise. However, it is unknown how long it would take for the recovery of vent-associated species. Van Dover (2014) assessed the impact of anthropogenic activity (scientific research and commercial exploration) on

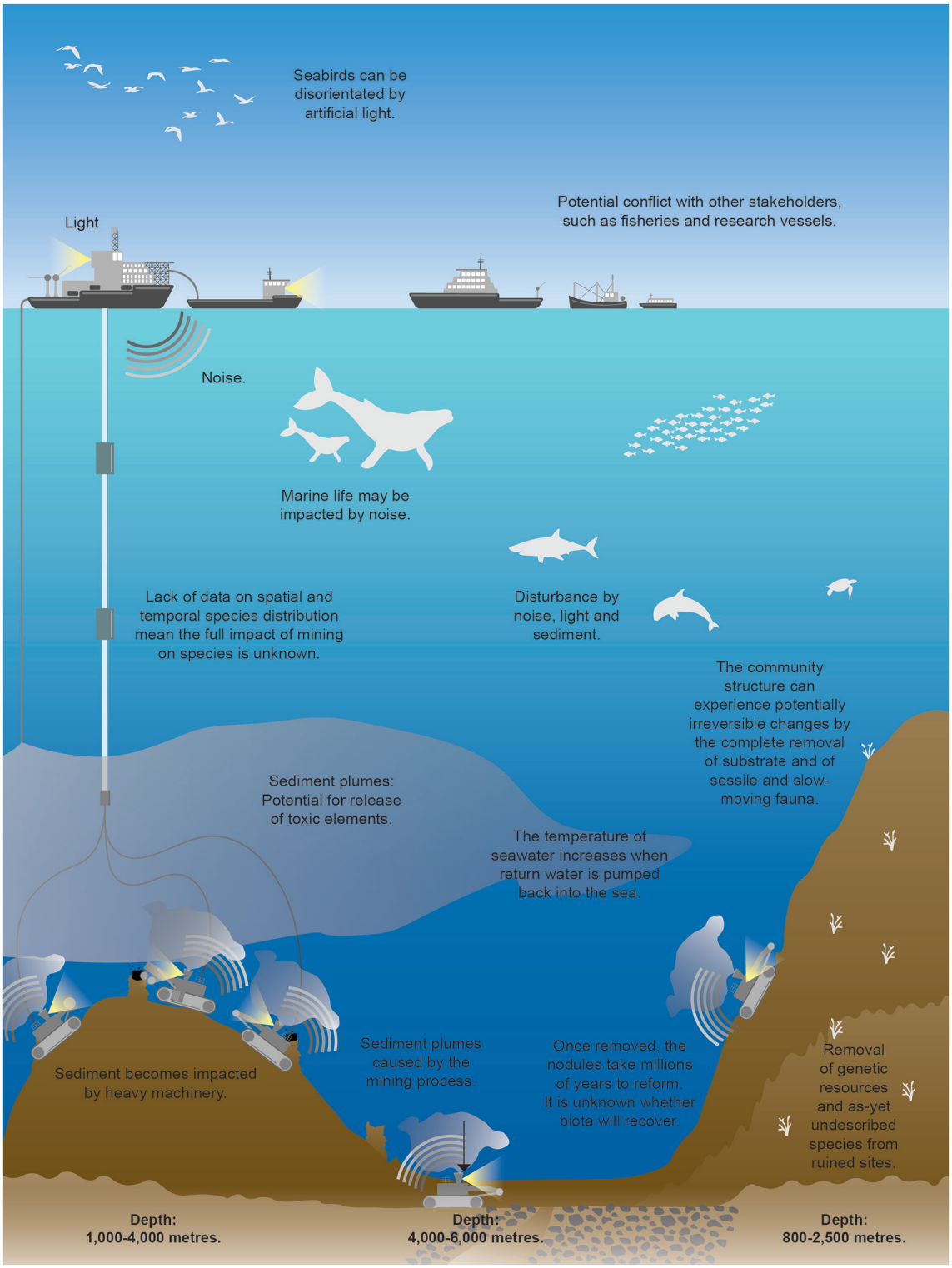


FIGURE 5 | A schematic showing the potential impacts of deep-sea mining on marine ecosystems. Schematic not to scale.

ecosystems surrounding hydrothermal vents and suggest that factors likely to impact vent communities include light and noise pollution, discarded materials, crushing seabed organisms and heavy vehicles compacting the seabed. In addition, intentional or unintentional transport of species (in ballast water, on equipment or relocation of fauna prior to mining activity) to a different

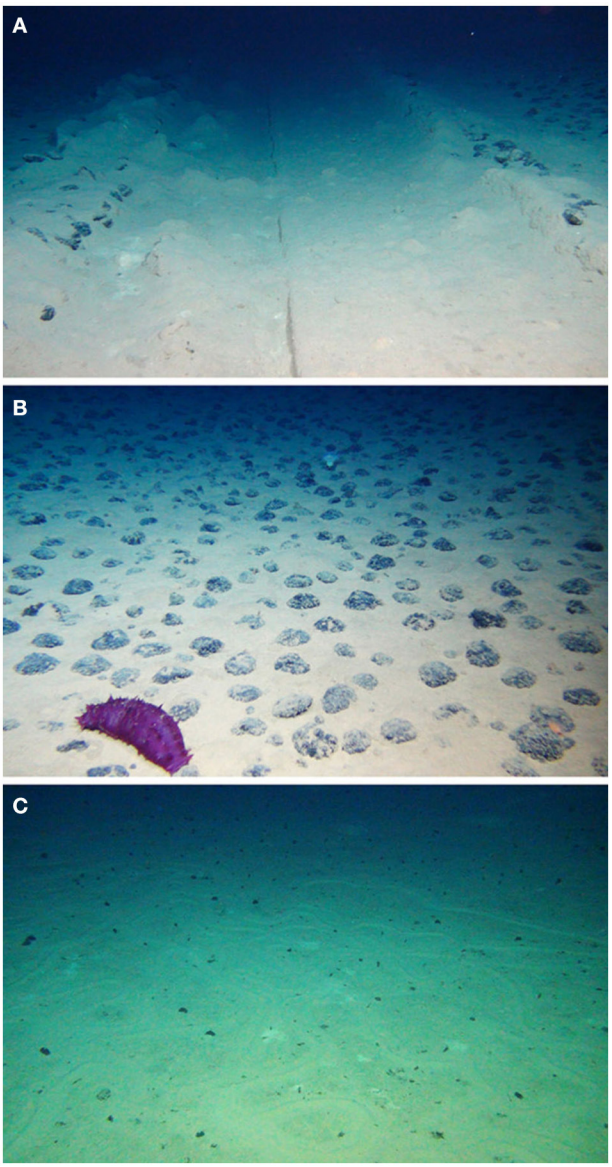


FIGURE 6 | Examples of seafloor morphology and disturbance. **(A)** Thirty-seven year old OMCO track (IFREMER license area); **(B)** Nodule landscape (IFREMER license area); **(C)** Nodule-free landscape (IOM area). Copyright: ROV Kiel 6000 Team/GEOMAR Kiel, EcoResponse cruise with RV Sonne, April–March 2015.

location can introduce potentially invasive species to habitats (Van Dover, 2014). Van Dover (2010) also refer to potentially unique communities associated with inactive vent sites and mining at these locations could permanently change community structures.

Nautilus Minerals expects its mining operations to take place 24 h a day, 365 days per year at Solwara 1. The operation will use three large robotic machines: an auxiliary vehicle will prepare and flatten the seabed by leveling chimneys and destroying habitats, a bulk cutter will leave cut material on the seabed, then a collecting machine will gather the material as slurry and it will be pumped up a rigid pipe to the production support vessel on the sea surface. Approximately 130,000 m^3 of unconsolidated sediment will be moved by Nautilus Minerals over a mining period of 30 months (Nautilus Minerals, 2008, 2016b).

Removal of Cobalt-Rich Crusts from Seamounts

Mining CRC deposits on seamounts will cause direct mortality to sessile organisms. Levin et al. (2016) suggest that such mining may also cause benthic, mesopelagic (200–1,000 m) and bathypelagic (1,000–4,000 m) fish mortality. The extent of mining on seamounts will dictate the level of impact, but it is likely that intensive mining could disrupt pelagic species aggregations due to the removal of benthic fauna, the presence of machinery and disruption as a result of noise, light and suspended sediments in the water column.

Gollner et al. (2017) discuss the potential impacts of mining in the context of what is known from activities such as fisheries, in particular trawling, that remove substrate and associated organisms from seamounts. Though there are few data on recovery of species after intensive periods of trawling, the negative impact of deep-sea fisheries on seamounts is well-documented with noted declines in faunal biodiversity, cover, and abundance (Clark et al., 2016). Many seamount species, such as the sessile corals, are thought to be slow growing (from a few micrometers to ∼1 mm per year), long-lived (up to millennia), and susceptible to physical disturbance and for these reasons it has been suggested that seamounts be globally managed as VMEs (Clark and Tittensor, 2010; Fallon et al., 2014; Watling and Auster, 2017). The impact of marine mining may be more intensive than trawling because the removal of substrata will be complete. Such removal on a commercial scale accompanied by slow species recovery rates will likely lead to irreversible changes in benthic (and possibly pelagic) community structure on and around seamounts (Gollner et al., 2017).

Extraction of Gas Hydrates

Gas hydrates have attracted attention commercially as a potential future energy resource (Lee and Holder, 2001) but prospecting and any subsequent extraction of gas hydrates from seabed (or permafrost) reserves carries potentially considerable environmental risk. The greatest impact would be accidental leakage of methane during the dissociation process. Methane is a greenhouse gas that is 28 times more potent than carbon dioxide according to the assigned global warming potential over 100 years (IPCC, 2014). Other possible impacts of methane hydrate extraction include subsidence of the seafloor and submarine landslides, which could cause even greater instability in remaining hydrate deposits. Anthropogenic activity that leads to increased water temperature at seabed level could also destabilize and melt the hydrates. Dissociation of methane hydrates to form free methane could release large quantities of methane gas into the sea or atmosphere, adding to ocean acidification and/or global warming (Kretschmer et al., 2015). If increasing quantities of methane hydrate is destabilized and released, atmospheric temperature may rise leading to a positive carbon-climate feedback (Archer,

2007; Zhao et al., 2017). Concerns are also that mining could affect biota—chemosynthetic life and higher order organisms have been found on seafloor hydrate mounds. Fisher et al. (2000) noted a previously undescribed species of polycheate worm or "ice worm" (*Hesiocaeca methanicola*) that was able to burrow into sediment to reach the hydrate deposits.

Sediment Plumes

Commercial mining activity will have widespread environmental consequences. Deep-sea sediment plumes will be created by seafloor production vehicles—specifically the cutter and the collector—as well as by risers and processed material that is discharged as waste-water by the surface support vessel (Boschen et al., 2013; Van Dover, 2014; Gollner et al., 2017). Dewatering waste, side cast sediment and sediment released during the mining process are thought to be the main wastes released during mineral recovery. Dewatering waste may contain fine sediment and heavy metals that would be resuspended when discharged into the water column (Nautilus Minerals, 2008). The side-casting of sediment waste on the seafloor minimizes the need for transport to the surface or land-based storage, but would nonetheless lead to major physical alterations and would smother the benthic habitat. In relation to the proposed mining at Solwara 1, Nautilus Minerals state that their waste sediment and rock, an estimated 245,000 tons (Nautilus Minerals, 2008), will be side cast at the edge of the mining zone. Discharged return water will be returned at 25–50 m above the seabed. The returning slurry may contain suspended particles (smaller than 8 μm), be warmer than sea temperature at that depth and contain a high concentration of metals if leaching occurs from ore during mining.

The release of potentially toxic plumes is likely to impact habitats well beyond the area of mining, though details such as the volume and direction of plume travel are not yet fully understood. Some models suggest that sediment released close to the seabed may, in some circumstances, be confined to deep water and not move into the upper water column because of differences in water density (Bashir et al., 2012). However, suspended particulate matter and settlement of sediment could cover a wide area depending on discharge volume, vertical stratification and ocean currents.

According to Nautilus Minerals (2008) and Boschen et al. (2013), the release of plumes from a sulfide test-mining site at Solwara 1 resulted in sedimentation of up to 500 mm within 1 km of the discharge site and some material dispersing up to 10 km away. Records of natural sedimentation rates at hydrothermal vents range from <2 mm in some sites (Atkins et al., 2000) to <0.03 mm in others (Costa et al., 2016). Natural sedimentation rates are thought to be only few millimeters per 1,000 years in both abyssal and seamount habitats.

Making predictions of potential plume movements using models is a complex task in the absence of extensive data on plumes, upwelling, and oceanographic currents (Luick, 2012). Commercial seabed mining has not begun and therefore it is difficult to predict the impacts, but some terrestrial mining operations can help to predict potential consequences of mining operations. For example, tailings disposed of at sea from the terrestrial Lihir Gold mine in Papua New Guinea are estimated to have spread over an area of 60 km^2 from the point of discharge because of subsurface currents (Shimmield et al., 2010). Even when plumes are restricted to deep waters, impact to benthic communities cannot be avoided considering that the overall topography of the seabed could be altered and organisms will endure some extent of smothering. Such smothering will impede gas exchange and feeding structures in sessile organisms and could cause a number of other as yet unquantified impacts as a result of exposure to heavy metals and acidic wastes (Van Dover, 2010). The presence of sediment plumes could delay or prevent recolonization of mined areas through altered larval dispersal, mortality of larvae and success of larval settlement (Gollner et al., 2017).

Suggestions of technological modifications that could be employed to lessen the effect of plumes include reducing the size of the plumes and the toxicity of sediment particles, and by minimizing the accidental escape of suspended sediment during the cutting process (Boschen et al., 2013). The discharge of wastewater at the sea surface could impact marine ecosystems by causing turbidity clouds and affecting commercial fish species, as well as, in some cases, causing algal blooms (Namibian Marine Phosphates, 2012).

Increased Noise

Submerged remotely operated vehicles will increase underwater ambient noise, as will support vessels on the sea surface. Most deep-sea species generally only experience low-levels of noise, such that anthropogenic noise, particularly if occurring on a non-stop basis, will substantially increase ambient sound levels (Bashir et al., 2012). Studies on deep sea fish reveal that some species communicate using low sound frequencies (<1.2 kHz; Rountree et al., 2011) and it is thought that other benthic species may use sensitive acoustic systems to detect food falls up to 100 m away (Stocker, 2002). Anthropogenic noise is known to impact a number of fish species and marine mammals by inducing behavior changes, masking communication, and causing temporary threshold-shifts in hearing or permanent damage depending on the species, type of noise and received level (Gomez et al., 2016; Nedelec et al., 2017).

Nautilus Minerals plans to operate its seafloor production tools and offshore vessels 24 h per day, with operations on the surface and seafloor using artificial lighting. The company expects that the noise from its seafloor production tools and mining support vessels will add to ambient noise levels but precise noise characteristics of the equipment are unknown. In its environmental impact statement (EIS), Nautilus Minerals did not measure ambient noise or assess sound attenuation in relation to proposed commercial operations at Solwara 1 but referred to an older published study from the Beaufort Sea, Canada, for suggested ambient noise levels (Richardson et al., 1990). Mitigation strategies were not suggested by the company in its EIS.

Anthropogenic Light

Sunlight readily penetrates the euphotic zone (approximately the uppermost 100 m of the ocean, depending upon conditions), enabling photosynthesis, but relatively little sunlight penetrates the dysphotic zone (200–1,000 m). The aphotic zone is below the penetration of sunlight but is not completely dark: low light in the deep sea has been shown to originate from bioluminescence (Craig et al., 2011) and geothermal radiation (Beatty et al., 2005) and organisms have adapted to the conditions. Continuous mining activity that employs floodlighting on surface support vessels and seafloor mining tools would vastly increase light levels on a long-term basis and this would be a change from current conditions at proposed mining sites. For example, most of the light detected at two hydrothermal vents (one on the East Pacific Ridge, the other in the Mid-Atlantic Ridge) was near-infrared (Van Dover et al., 1996). Herring et al. (1999) found that vent-inhabiting deep-sea shrimps [*Rimicaris exoculata* and *Mirocaris* (*Chorocaris*) *fortunate*] suffered permanent retinal damage by the use of floodlights on manned submersibles surveying vent chimneys on the Mid-Atlantic Ridge.

Nocturnal artificial lighting on vessels has been shown to disorientate seabirds, particularly fledglings, leading to "fallout," in which the birds fly toward the light source and become exhausted or collide with man-made objects (Troy et al., 2013). Research is needed to determine the extent to which Beck's petrel (*Pseudobulweria becki*)—a species listed as critically endangered on the International Union for the Conservation of Nature Red List and which is native to Papua New Guinea and the Solomon Islands—would be attracted by artificial light used in proposed mining operations. If increased light levels were to persist, other mobile organisms might migrate away from the mine site. To date, there is no evidence that Nautilus has investigated ambient light levels at the Solwara 1 site or considered the likely significance of such impacts in any detail (Nautilus Minerals, 2008).

Increased Temperature

Drilling and vehicle operation during mining will release heat, as will dewatering waste that is returned to the deep sea. Steiner (2009) suggests that waste materials may be as much as 11°C warmer than the surrounding seawater, which is in line with estimates by Nautilus Minerals, which states that processed return water may result in an increase in temperature of 5.8–11.4°C (Nautilus Minerals, 2008). Very little is known about the impact of such temperature increases on deep-sea organisms, though it is thought that the deep sea has a relatively stable temperature and changes could affect growth, metabolism, reproductive success and survival of some deep-sea species (Bashir et al., 2012).

Biodiversity Loss and the Potential for Habitat Recovery

Deep-sea mining will inevitably cause loss of biodiversity on a local scale. Depending on factors such as the type of impact (for example, sediment plumes or noise), the type of mining and the ecosystem, biodiversity across a much wider area could be affected. The geographic and temporal scale of mining activities will affect the level and type of impact. For instance, extraction of SMS may target several hectares per year, whereas the area of cobalt-crust mining may range from tens to hundreds of square kilometers (Hein et al., 2009) and that of manganese nodule mining from hundreds to thousands of square kilometers per operation per year (Wedding et al., 2015). Mining activities will result in the direct mortality of organisms, removal and fragmentation of substrate habitat and degradation of the water column and seabed by sediment plumes (Van Dover et al., 2017).

The extent of habitat fragmentation because of mining is difficult to predict, given that there have been no large-scale trials. Mining large, continuous fields of manganese nodules will create a mosaic of smaller-sized fields, and mining SMS will lead to further fragmentation of an ecosystem that is, naturally, unevenly spaced but heavily dependent on association with specific and localized seabed features. The extent of resource extraction and plume dispersal will influence the size of the remaining fragments. Vanreusel et al. (2016) analyzed videos taken during test mining for nodules in the CCZ and suggest that mining removes almost all epifauna. Benthic organisms span a range of sizes with different ecological characteristics that dictate the nature and extent of their dispersal, mobility, and feeding strategies. The response of benthic organisms to the likely habitat fragmentation induced by mining will vary widely and will be challenging to predict because little is known about the life history or patterns of genetic diversity of many deep-sea species (Boschen et al., 2013).

Habitat modification may extend from the vicinity of mining operations to far-field effects, which are defined as those that are detectable more than 20 km away from the mining site. Reasons for degradation of the marine environment include drifting sediment plumes and low frequency noise propagation, which could alter species distributions, ecosystem functioning or even seemingly unconnected processes such as carbon cycling (Nath et al., 2012; Le et al., 2017).

The potential for benthic communities to recover is likely to vary substantially between locations and will be influenced by the duration of mining operations (Van Dover, 2011). Slow-growing deep-sea organisms typically have correspondingly low resilience to change (Rodrigues et al., 2001; Gollner et al., 2017). Recovery of benthic communities is difficult to estimate because colonization rates are not known for most species and there are few data on population size, reproductive biology, dispersal and, therefore, connectivity (Hilário et al., 2015). In the absence of commercial operations, recovery studies rely on study of the aftermath of natural extinction events such as volcanic eruptions or on deliberate disturbance experiments, but the spatial and temporal scales differ from commercial mining and so extrapolating results to determine ecological responses to seabed mining has limited application (Jones et al., 2017).

The extraction of manganese nodules removes the habitat for nodule dwelling organisms, making recovery of these communities almost impossible given the long time periods required for nodule formation. The first long-term disturbance and recolonization experiment (DISCOL) was established in the Peru Basin in 1989 in the southeast Pacific Ocean at a depth of 4,140–4,160 m. The experiment replicated on a small scale

the disturbance that would be caused by commercially mining manganese nodules by plow harrowing a circular area of the seabed measuring 10.8 km^2. The aim of the project was to monitor recolonization of benthic biota. The experimental area was sampled five times: before, immediately after the disturbance, then after 6 months, 3 and 7 years. After 7 years, the tracks made by the plow were still visible. Mobile animals began to repopulate the disturbed area soon after the damage was caused, but even after 7 years the total number of taxa was still low when compared to pre-disturbance data (Bluhm, 2001). A recent project conducted under the JPI Oceans initiative, Ecological Aspects of Deep-Sea Mining, revisited the DISCOL experiment area in 2015 after a 20-year hiatus. Preliminary results and observations note that the original plow marks are still visible and there has been only a low level of recolonization, suggesting that disturbing nodules for commercial mining will cause long-term damage to the benthic ecosystem (JPI, 2016). A meta-analysis of 11 such test studies (including DISCOL) carried out by Jones et al. (2017) reports that the effects of nodule mining are immediate and severe, and note that although signs of recovery were observed within 1 year following disturbance, at most sites, there was a significant reduction in the number of recolonizing species. Few species groups recovered to pre-mining baseline conditions even after two decades and Jones et al. (2017) suggest that, even after smaller scale test mining experiments, the community-level effects of nodule mining are likely to be severe.

After mining seafloor massive sulfide deposits, vent community recovery will rely on the continuation of the hydrothermal energy source and presence of all species to enable repopulation. Community composition changes are likely due to recolonization of substrates by early successional species and the loss of species sensitive to change (Bashir et al., 2012). Mullineaux et al. (2010) reported recolonization of a vent following a natural volcanic eruption, but with a change in species composition and the presence of immigrant species from distant vent sites, possibly up to 300 km away. Shank et al. (1998) monitored a hydrothermal vent eruption and its recovery, reporting that large increases in faunal assemblages were only noted 3–5 years post-eruption and predicted that it could take up to 10 years for dominant megafauna to return. Sustained mining activity will have very different impacts to one-off natural events and the likelihood and extent of recovery of mined vent sites is highly uncertain (Van Dover, 2011). In an attempt to mitigate disturbance caused by mining, Nautilus Minerals proposes to temporarily transplant large organisms and clumps of substrate to a refuge area before mining and return them to their original position when mining ceases (Nautilus Minerals, 2008). The proposals have not yet been field-tested.

Data indicating the recovery of biota on seamounts following physical disturbance are scarce. Studies looking at seamounts that have been overexploited by trawler fishing indicate uncertainty as to whether recovery of deep-sea fish populations is possible because species are slow growing and bottom trawling (in common with mining operations) causes severe physical disturbance to the seabed. Additional challenges arise when predicting seamount recovery because seamounts vary widely in size, location and environmental conditions (Clark et al., 2010, 2012; Gollner et al., 2017).

Mining extinct vents only is anticipated to minimize impacts to vent species, because extinct sites are considered to host fewer species than active sites. Extinct vents are largely unstudied because they are difficult to locate without a hydrothermal plume (Van Dover, 2010). However, it may be challenging to determine whether a particular vent is extinct or temporally inactive; some reports suggest vent systems can be inactive for several years before reactivating (Birney, 2006). For example, vent activity was highly variable over a 3-year period of investigation at Solwara 1 (Nautilus Minerals, 2008). Suzuki et al. (2004) reported that inactive vents still supported the growth of chemolithotrophic microorganisms because there can still be sufficient hydrothermal energy. Van Dover (2010) noted that extinct vents with no detectable emissions nevertheless still hosted suspension feeding and grazing invertebrates. Such "extinct" or inactive vent systems may therefore prove to be far from extinct in relation to marine life.

Possible Conflicts

Seabed mining impacts have the potential to conflict with subsistence and commercial fishing, and shipping activities. The analysis of deep-sea biota for novel chemical compounds that could be used in medicines is another area of growing commercial interest. Legal cases could be brought if, for example, a sediment plume crosses a boundary and causes harm to the marine environment of a coastal state or to the area outside a contractor's allocated site. Disputes could arise if surface exclusion zones around seabed mining operations reduce access to fishing areas and/or change shipping or navigational routes, whether in EEZs or in the Area. For example, an exclusion zone of 23 × 9 km has been proposed in Namibian waters in relation to exploitation of seabed phosphate deposits, which would impact on key commercial fishing grounds for hake, horse mackerel and monkfish (Namibian Marine Phosphates, 2012). It is reported that fishing activities will cease in the immediate mining area and the exclusion zone due to habitat removal and increased levels of maritime traffic (Namibian Marine Phosphates, 2012). In another example, fishing companies were active opponents to a proposal for ironsand mining off New Zealand's west coast (New Zealand Environmental Protection Authority, 2017).

Armstrong et al. (2012) refers to the deep sea as the largest reservoir of genetic resources and many companies already hold patents for pharmaceuticals discovered there. For example, enzymes from deep-sea bacteria have been used in the development of commercial skin protection products by the French company Sederma for many years (Arico and Salpin, 2005). Hydrothermal vent species are of particular interest because they have unusual symbiotic relationships, are resistant to heavy metals and yield thermotolerant enzymes with a number of commercial uses (Ruth, 2006; Harden-Davies, 2017). The market for marine genetic resources is large and reached many billion US dollars by 2010 (Leary et al., 2010). Despite the significant economic value of deep-sea discoveries, there are concerns that mineral mining could destroy genetic resources before they have been fully understood or even discovered. There are also uncertainties surrounding the legal framework

underpinning discoveries made in the Area (Ruth, 2006; Harden-Davies, 2017).

DISCUSSION

Interest in obtaining minerals and resources from the deep sea has gained momentum over the past decade but so too has the desire to survey, monitor, explore and understand deep-sea ecosystems. The oceans are the least explored ecosystems on Earth even though they cover 71% of Earth's surface (an area of 362 million km^2), of which 90% is considered the deep sea. Although only around 0.0001% of the deep seafloor has been investigated, it is evident that the deep ocean has particularly rich biodiversity (Tyler et al., 2003; Ramirez-Llodra et al., 2010). Advances in technology have made it possible to explore some of the deepest reaches of the ocean, leading to the discovery of hundreds of previously undescribed species but also making commercial exploitation of seabed minerals a real possibility. To date, no deep-sea commercial mining has taken place, nor have there been pilot operations to enable accurate assessment of impacts (Van Dover, 2014).

The resource closest to large-scale extraction is SMS by Nautilus Minerals at the Solwara 1 site in the national waters of Papua New Guinea, where exploitation is scheduled to begin in early 2019. The project has required significant financial investment and the company is under pressure to commence operations that will yield economic returns.

In this paper, we have outlined some of the very significant questions that surround plans for large-scale commercial minerals mining, whether within continental shelf boundaries or in the Area. When the mining of deep-sea minerals was first proposed several decades ago, knowledge of the deep-sea environment was relatively poor, as was our understanding of the potential impacts of seabed mining. Though our understanding of deep-sea biodiversity remains limited, it is evident that many species have specific life-history adaptations (for example, slow growing and delayed maturity; Ramirez-Llodra et al., 2010). Recovery from human-mediated disturbance could take decades, centuries or even millennia, if these ecosystems recover at all. Myriad impacts relate to seabed mining including the potential for conflicts with the interests of other users of the sea. At the time of writing, the ISA was in the process of developing a regulatory framework for managing mining in the Area. The details of the environmental management framework the ISA will adopt is still unclear. Key issues that need to be defined before commercial mining operations begin, including how states can meet their duty, as stipulated in UNCLOS Article 145, to effectively protect the marine environment. As understanding deepens with respect to ecosystem services and the role of the oceans in mitigating climate change, it seems wise to ensure that all necessary precautions are taken before any decision to allow deliberate disturbance that could have long-lasting and possibly unforeseen consequences.

Current activity in the Area is subject to exploration regulations by the ISA, but exploitation will have a far greater environmental impact than exploration, and because of this, biodiversity loss as a consequence of commercial operations is the topic of current debate. For example, questions are being asked such as: "What threshold of loss of biota would equate to significant adverse changes in the marine environment?" with regard to protecting the marine environment during prospecting and exploration activities, the current ISA regulations state that contractors must avoid causing serious harm to the marine environment, which the ISA defines as:

> "any effect from activities in the Area on the marine environment which represents a significant adverse change in the marine environment determined according to the rules, regulations and procedures adopted by the Authority on the basis of internationally recognized standards and practices."

The phrase "significant adverse change" has come under particular scrutiny from scientists and specialists in marine law, who are calling for clarification with empirical data thresholds to determine what constitutes "harm" (see for example, Levin et al., 2016).

Mitigation

Mitigation techniques that have been proposed to monitor the potential impacts to biodiversity and aid recovery of mined areas are untested so far. Indeed, Van Dover (2011, 2014) and Van Dover et al. (2017) stressed that we do not know how to mitigate impacts or restore deep-sea habitats successfully. Van Dover (2014) outlines a hierarchy of possible mitigation methods, including: (1) avoidance (such as by establishing protected reserves within which no anthropogenic activity takes place), (2) minimization (such as by establishing un-mined biological corridors, relocating animals from the site of activity to a site with no activity, minimizing machine noise or sediment plumes) and (3) restoration (as a last resort, because avoidance would be preferable). A fourth mitigation method is offset (the contractor would pay for the establishment of a dedicated reserve or for research), although Van Dover states that there is no such framework in place for hydrothermal systems and suggests initiating discussions on the topic among stakeholders with an interest in deep sea mining. For hydrothermal vent ecosystems, a deeper understanding of the ways in which these ecosystems are likely to be impacted and respond to commercial mineral extraction activities would help to determine the likelihood of natural recovery. An advanced understanding of hydrothermal vent ecology is necessary but that will require funding for research, long-term monitoring and thorough environmental impact assessments prior to authorizing any commercial activity (Van Dover, 2014).

In its mining code (currently only applicable to prospecting and exploration not exploitation), the ISA discusses establishing preservation reference zones (PRZ; areas in which no mining takes place) and impact reference zones (IRZ; areas set aside for monitoring the impact of mining activity). Under the current system, the ISA code requires mining companies to propose the locations and sizes of PRZs and IRZs (International Seabed Authority, 2012, 2013) but, as discussed by Vanreusel et al. (2016) with specific reference to polymetallic nodules, this

situation could mean contractors apply PRZs to economically unimportant areas rather than those that are environmentally important. One point to note is that IRZs and PRZs are distinct from marine protected areas because they are intended to be tools for environmental monitoring, not for the conservation of biodiversity. Lallier and Maes (2016) recommend that the ISA mining code be developed to prioritize environmental protection through the application of the precautionary approach, but it is unclear how this would work on a practical basis, or whether protective measures would be effective. A number of countries, including Canada, the United States, Mexico and Portugal, have established marine protected areas to protect hydrothermal vents and other deep-sea features (Van Dover, 2014), but it is unclear how beneficial these will be.

Other strategies that have been suggested to mitigate the impact of deep-sea mining during the exploitation phase include reducing the area impacted by plumes; de-compacting sediment under the seafloor production tools; and leaving a proportion of nodules on the seabed (such as the largest and the smallest). However, to date there has been no large-scale deep-sea mining test and no assessment of whether any one strategy or combination of strategies would lessen any impact on biodiversity and ecosystem processes.

Some opponents of deep-sea mining imply that any mitigation measures seem futile. An article published in *Science* in 2015 called for the ISA to suspend approval for new exploration contracts and not approve any exploitation contracts until marine protected areas are designed and implemented for the high seas (Wedding et al., 2015). These authors also suggested that protected areas are designated *before* new exploration contracts are awarded.

Uncertainties, Knowledge Gaps, and Areas for Future Research

Many questions and uncertainties surround deep-sea mining, including those stemming from the complexity and scale of the proposed operations, and those arising from legal uncertainties relating to proposed exploitation in the Area and the fact that no large-scale impact trials have yet taken place. In this review, we have presented some of the key issues, but very substantial and significant knowledge gaps remain.

Data indicating the recovery of deep-sea biota following physical disturbance are scarce and thus this is an area warranting additional research. There is an absence of baseline data from potential mining sites because only a fraction of the ocean has been studied in depth due to the logistical complexity and financial constraints of accessing the deep sea. Future studies could focus on understanding deep-sea ecology (for example, local endemism, demographic and genetic connectivity relating to dispersal modes) in the proposed mining zones.

Discussions are underway to develop the legal framework to regulate exploitation, including issues of environmental protection, accountability, interactions across international and national boundaries, and also between claims, with input from marine scientists, legal specialists, and non-governmental organizations. Uncertainties surrounding deep-sea ecology and ecological responses to mining-related activities mean that environmental management strategies would need to be tailored to incorporate natural temporal and spatial variability of deep-sea ecosystems (Clark et al., 2010). The impact of noise on deep-sea organisms is not well-studied, which represents another significant knowledge gap in the management of commercial activities.

Alternatives Approaches

It is widely accepted that demand for metals for use in clean energy and emerging technologies will increase in the next decades, raising the likelihood of supply risk. In response, retrieving metal resources from seabed mining has been identified as one of five sectors with a high potential for development within the European Commission's blue growth strategy (European Commission, 2017a). The strategy aims to provide support to long-term sustainable growth in the marine and maritime sectors within the region, and the European Commission optimistically estimates that by 2020, 5% of the world's minerals could be sourced from the ocean floor (Ehlers, 2016). If technological challenges are overcome, the annual turnover of marine minerals mining within Europe could grow from zero to 10 billion Euros by 2030 (Ehlers, 2016).

However, there are alternatives to exploiting virgin stocks of ore from the seabed. Such approaches include: substituting metals in short supply, such as rare earths, for more abundant minerals with similar properties (United States Department of Energy, 2010; Department for Environment, Food and Rural Affairs, 2012); landfill mining (Wagner and Raymond, 2015); and collection and recycling of components from products at the end of their life-cycle. Other novel options include the potential to recover lithium and other rare metals from seawater (Hoshino, 2015).

A European Commission initiative, adopted in 2015, supports the transition toward a circular economy that promotes recycling and reuse of materials—from production to consumption—so that raw materials are fed back into the economy (European Commission, 2017b), though the strategy will depend on developing the necessary technology as well as changing consumer behavior. Recycling, though crucial, is unlikely to provide sufficient quantities of metals to satisfy requirements in future years which has prompted suggestions that reducing use of metals in products will be a necessary part of product design (United Nations Environment Programme, 2013a).

Increasing the longevity of technological devices and promoting responsible e-waste recycling could be achieved through manufacturer take-back schemes, in which component materials can be safely and effectively recovered for reuse. Recycling metals carries its own challenges, which include potential release of toxic substances during processing and limitations during metals recovery that mean not all components can be isolated (United Nations Environment Programme, 2013a). A shift in focus to reducing consumption and, in addition, better product design (United Nations Environment Programme, 2013b). Closing the loop on metals use is possible because in theory all metals are recyclable, though we are some years away from achieving such a system (Reck and Graedel, 2012). Improving consumer access to recycling and streamlining manufacturing processes can be a more efficient

and economically viable method of sourcing metals than mining virgin ore and could greatly reduce or even negate the need for exploitation of seabed mineral resources.

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Scientific Considerations for the Assessment and Management of Mine Tailings Disposal in the Deep Sea

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Deep-sea tailings disposal (DSTD) and its shallow water counterpart, submarine tailings disposal (STD), are practiced in many areas of the world, whereby mining industries discharge processed mud- and rock-waste slurries (tailings) directly into the marine environment. Pipeline discharges and other land-based sources of marine pollution fall beyond the regulatory scope of the London Convention and the London Protocols (LC/LP). However, guidelines have been developed in Papua New Guinea (PNG) to improve tailings waste management frameworks in which mining companies can operate. DSTD can impact ocean ecosystems in addition to other sources of stress, such as from fishing, pollution, energy extraction, tourism, eutrophication, climate change and, potentially in the future, from deep-seabed mining. Environmental management of DSTD may be most effective when placed in a broader context, drawing expertise, data and lessons from multiple sectors (academia, government, society, industry, and regulators) and engaging with international deep-ocean observing programs, databases and stewardship consortia. Here, the challenges associated with DSTD are identified, along with possible solutions, based on the results of a number of robust scientific studies. Also highlighted are the key issues, trends of improved practice and techniques that could be used if considering DSTD (such as increased precaution if considering submarine canyon locations), likely cumulative impacts, and research needed to address current knowledge gaps.

Keywords: deep-sea tailings disposal (DSTD), improved practice, challenges, environmental management, stakeholders

MINE TAILINGS DISPOSAL: AN ISSUE OF GROWING CONCERN

A rise in world population together with increased rates of economic growth in low and middle income countries over the last century has dramatically increased the demand for metals and minerals (Dold, 2014). In addition, over the span of the twentieth century, the demand for metals and minerals in high income countries has grown exponentially. For example, demand in the USA

grew from a little over 160 million tons to ∼3.3 billion tons (Morse and Glover, 2000). According to the United Nations Environment Programme (UNEP), the amount of minerals, ores, fossil fuels, and biomass consumed globally per year could triple between current day and 2050 (NCIR, 2013).

Mining is defined as the acquisition of non-renewable resources from the environment, mostly accomplished by either open-pit surface mines or underground mines on land. Mining, as with other extractive industries, will always have a social and environmental impact. A major challenge for the industry is how to minimize these impacts during all stages of mining from exploration, through operations and on to mine closure. As demand for metals and minerals grows, environmental sustainability is a growing concern. The subject explored here is the disposal of mine tailings into the marine environment. Tailings are the fine-particle waste produced after extracting the desired metal from the ore. A typical copper ore is less than 1% copper and, therefore, 99% is produced as tailings (Vogt, 2012). Similarly, in gold production a typical ore contains less than 0.1% gold with 99.9% of the processed material classified as mine tailings. As high-grade ores become rarer, and technology and consumer need demands the exploitation of low-grade ores, the issue of mine waste disposal is set to increase.

Historically a relatively small number of mines have discharged tailings and mining waste into the marine environment. Originally this waste was discharged into surface waters, for example at Chañaral, Chile from 1938 to 1975 (Dold, 2014) and Jordan River Copper Mine, Canada from 1972 to 1974 (Shimmield et al., 2010). Over time, this approach has evolved to more sophisticated methods such as piped discharges, with the final depth of discharge varying from a few tens of meters to several hundred meters under the sea surface. This process is referred to as either submarine tailings disposal (STD) in shallow/intermediate waters (shallow, 0–200 m, intermediate depths 200–1,000 m) and deep-sea tailings placement (DSTP) at depths below 1000 m (GESAMP WG42). Here we advocate for a change to the current term DSTP, to a more precise terminology Deep-Sea Tailings Disposal (DSTD). The use of the term "disposal" is considered to be more accurate, as tailings are discarded at depth (where a density/turbidity current transports the tailings to a resting place on the deep seafloor) rather than the tailings being placed in a specific contained area at the outfall or pipe mouth.

Shallow STD has been utilized in a number of coastal mining sites around the world to date. However, whilst most early operations were poorly regulated, and tailings were discharged into the sea as a matter of convenience, modern STD operations now commonly follow an environmental impact assessment (EIA), with baseline studies undertaken to improve understanding of risks. However, shallow STD often leads to severe environmental damage and adverse effects on local biota (Castilla and Nealler, 1978; Loring and Asmund, 1989).

Since the early 1970s, detailed engineering has been incorporated in the practice of most marine tailings disposal operations. At the same time, both the tailings outfall and the final tailings deposition area have been progressively designed to be located deeper (below sea level) in an attempt to minimize environmental impacts (Shimmield et al., 2010; Ramirez-Llodra et al., 2015), the process termed DSTD. The general features of DSTD have been outlined by Apte and Kwong (2004), Shimmield et al. (2010), Reichelt-Brushett (2012), Vogt (2012), Ramirez-Llodra et al. (2015), and Morello et al. (2016).

The concept of DSTD is based on discharge at the edge (usually 100–300 m depth) of an extended drop-off, to a final deposition depth of 1,000 m or more, and at a depth below the euphotic surface mixing zone (Ellis and Ellis, 1994). To achieve deeper deposition, the discharge must be at a location where the tailings slurry from the pipeline will form a density/turbidity current flowing coherently with minimal plume dispersal until it reaches the base of the drop-off. Frequently submarine canyons have been considered suitable by DSTD practitioners, including those beyond fringing reefs in tropical sites (Reichelt-Brushett, 2012). DSTD systems should be designed to prevent tailings reaching surface waters and approval of the EIA will be dependent on the DSTD design achieving this constraint. Any subsurface plumes or upwelling of tailings back into shallow waters will expose coastal environments to increased physical (e.g., suspended solids) or chemical stressors (e.g., metals/metalloids), where toxic components may enter the food chain and have detrimental effects on a wide range of marine organisms. It is important that tailings do not enter the mixed layer as coral species in tropical regions have been found to be particularly sensitive to increased concentrations of suspended solids (Flores et al., 2012; Jones et al., 2015).

In 2015, only 16 of the current 2,500 large industrialized mines worldwide utilize STD/DSTD (GESAMP, 2016). These are restricted to a few countries, namely Norway, Papua New Guinea (PNG), Philippines, Indonesia, France, Turkey, and Chile (Dold, 2014). However, an additional 15–20 mines are already considering STD/DSTD as a future disposal option (GESAMP, 2016). **Figure 1** displays DSTD operations (PNG, Greece, Indonesia, France and Turkey), both active and closed, reporting the average annual tailing disposal in million tons. A number of case studies from DSTD operations can be found in the Supplementary Material.

This paper builds on previous reviews and case studies to consider (1) tailings disposal options (2) impacts and recovery potential from tailings disposal in the deep sea, and (3) the role of scientific information in analysis of case studies. With a focus on DSTD the following aspects are discussed; societal and environmental impacts, approaches to reducing environmental impact, future prospects, legal controls and constraints, science gaps and DSTD relevance to broader environmental management in the deep ocean.

ASSESSING MINE TAILINGS DISPOSAL OPTIONS

Mine tailings management and/or disposal options are important considerations for government regulators during the EIA approval process associated with new mining activities. Each site will have a different set of constraints, which will influence the decision on the tailings disposal options. The main mine

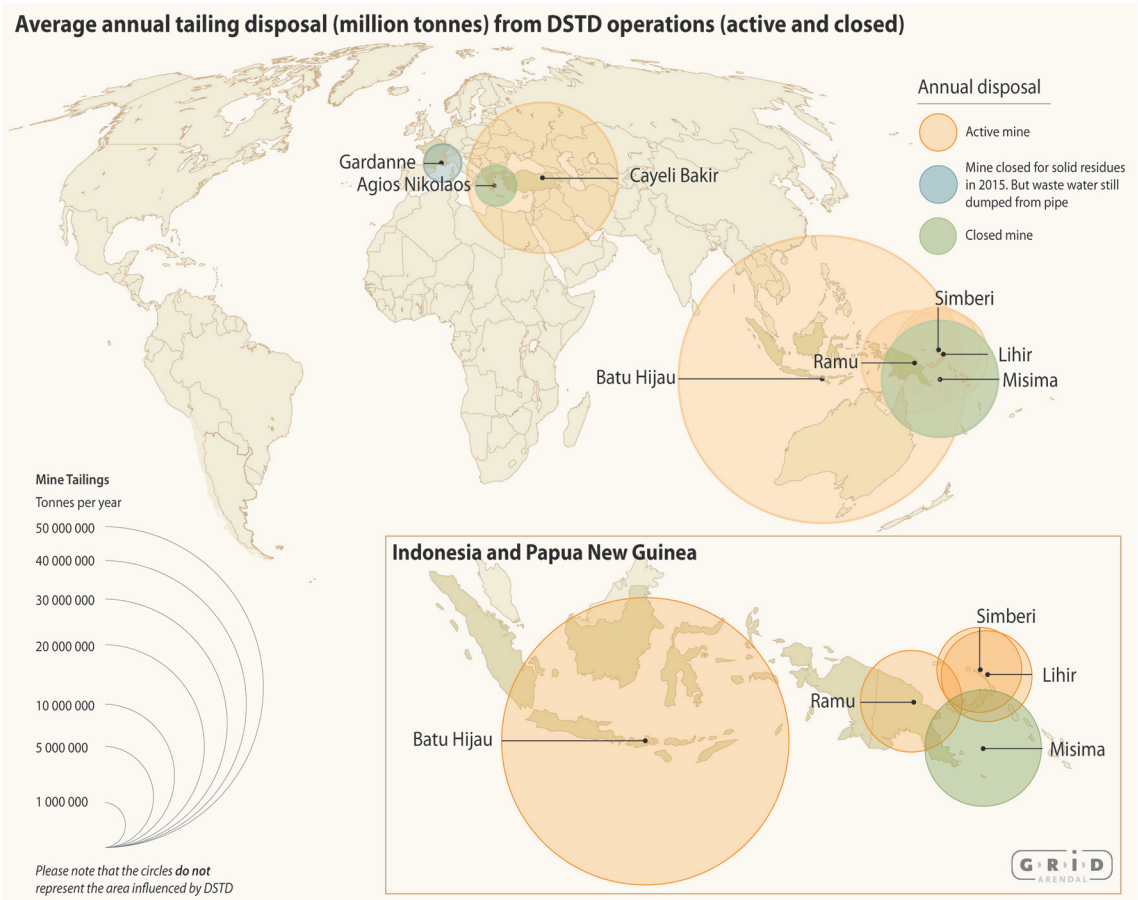


FIGURE 1 | Average annual tailing disposal (million tons) from DSTD operations (active and closed).

tailings disposal options include: tailing storage facilities (TSFs) in the form of dams or ponds (holding wet tailings or partially dewatered pastes) and underground tailings disposal on land, riverine tailings disposal and marine tailings disposal (STD/DSTD); however, some of these are not permitted in some countries. The disposal of mine tailings both on-land or in the marine environment presents unique challenges. The choice of disposal option is dependent on the physical and chemical nature of the tailings, the mine topography, climatic conditions, along with socio-economic considerations; each of the different disposal options has advantages and disadvantages.

In a large number of cases land-based storage in terrestrial impoundments or tailings dams is the norm for the constraint of mine tailings. At least 3,500 mine tailings dams/impoundments exist worldwide, but they are not without environment and public safety issues. The main issues include: the size of the footprint and loss of land that could be used for other activities, potential contamination of surface waters and groundwater, and the short- and long- term safety and integrity of the engineered facilities. There have been 138 significant recorded failures of mine tailings storage dams (Vogt, 2012).

On land, management options frequently include a combination of disposal and storage techniques and include reuse and backfilling as priorities. Geochemical developments that aim to change the character of tailings are making substantial headway in the industry, and processes to better manage acid generation and development of tailings pastes (Palkovits, 2007) are proving potential risk reduction tools. Further to this, appropriate storage of mine tailings may enable reprocessing of old tailings wastes when new technologies become available to further extract the target metal or when minerals other than those originally sought become valuable. Hence many mine tailings may have a series of potential uses immediately or in the future, but only if they are stored in a manner that enables reprocessing.

The success of land disposal is often dependent on climatic conditions and seismic activity. In areas of frequent tectonic activity and high rainfall, there is an increased risk of dam failure and concerns over contaminated mine water that may influence the water quality in local waters bodies. In some countries such as Indonesia and PNG, on-land storage facilities are considered difficult and potentially unstable due to the mountainous terrain, the high risk of earthquake events and rainfall up to 3 m per year. Combined with social demands on the customary lands, this has led to mines in these countries choosing marine disposal. Similarly, in Norway, suitable land for disposal of mine tailings

near the fjords is not readily available. However, it is also important to realize that underwater earthquakes, tsunamis, currents and upwelling are also risk factors associated with STD/DSTD in many countries.

The tailings disposal option is considered one of the most important decisions during the mining feasibility assessment, each disposal method having environmental implications that will require management. Therefore, mining waste should be managed with respect to what is an acceptable environmental impact which may be determined by the various processes involved with EIA and include community engagement in the process, as well as considering the technical and economic feasibility of the disposal method. When considering disposal options, historically, short-term profit was of higher interest than long-term solutions to tailings containment and management (e.g., Palkovits, 2007). Economic, environmental and social considerations (Vanclay, 2004) are now part of the reporting process for many mining operations. However, in countries where environmental legislative frameworks and enforcement are poor, considerations of longer-term environmental and social impacts/threats may not be fully recognized and their level of importance may be lower in the decision-making process.

Regulators currently rely on information provided by EIAs, which undergo an independent review process, to inform decisions and, like many industries, it is recognized that there is a lack of information on potential long-term impacts of DSTD. Work to fill knowledge gaps is often limited by finances, along with sampling capabilities on temporal and spatial scales, and a lack of background understanding of ecosystems likely to be impacted. In this context the understanding of the consequences of DSTD on ecosystems and ecosystem processes is currently limited. Robust scientific knowledge is even scarcer for bathyal and abyssal ecosystems, including sedimentary slopes, submarine canyons, seamounts, or habitat-building fauna such as cold-water corals that may be impacted by DSTD (Ramirez-Llodra et al., 2011). In many cases, the physical, geochemical and biological information available from areas considered for DSTDs is lacking, and knowledge is based only on baseline studies commissioned in the framework of the mining activity. The main limitation to such studies is the remoteness of deep-sea ecosystems, the study of which comes with high financial and technological costs; hence, conducting the necessary research for a robust EIA and monitoring of DSTDs and their impacts, before, during and post-operation is challenging.

There has been some development of numerical assessment frameworks for selecting the most suitable tailings waste management options (e.g., Kizil and Muller, 2011). Some frameworks enable details on other options such as TSF in assessments that can potentially feed into a more complex options analysis that considers environmental and social values.

Comparisons of disposal options require a supporting framework that acknowledges/assesses constraints, impacts, and risks across terrestrial and oceanic boundaries and which go some way to applying ecosystem values to these systems. Here, an outline framework for considering disposal options has been developed, highlighting the complexity that exists at the site level and the need to incorporate social and environmental linkages (**Figure 2**). Such a framework may help resolve conflicting views on how the receiving environment is valued. It has been developed as a decision tree approach to risk assessment (e.g., ANZECC/ARMCANZ, 2000; Reichelt-Brushett, 2012), and incorporates social values that are informed by stakeholder engagement activities identifying risk assessment tools and approaches for deep-sea mining and STD/DSTD (e.g., Reichelt-Brushett et al., 2016). Consider for example, what may be deemed "acceptable" in terms of the movement and distribution of tailings in a terrestrial setting as compared to an oceanic setting.

There is increased pressure and a growing trend in leading mining companies to improve the sustainability of tailings disposal methods (ESMAP/World Bank/ICMM., 2005). The concept of mine tailings "management" is a longer-term construct that may extend for several decades after mine closure. Such a process is becoming more common practice at mine sites throughout the world and some countries require substantial bonds to be paid to ensure that longer-term management processes can be afforded in what is a relatively volatile industry. This approach is partially in response to abandoned mine sites and processing operations that have resulted in serious health risks to communities and are requiring multimillion dollar clean-up operations with complex liabilities (e.g., Barth and McNichols, 1994; Hanrahan et al., 2016).

DSTD IMPACTS AND ECOSYSTEM RECOVERY POTENTIAL

Historically, the deep seabed has been regarded as an almost unlimited repository for waste (liquid and solid) (Ramirez-Llodra et al., 2011). This misconception has changed in the latter half of the twentieth century, in part as a function of stakeholder scrutiny of the increasing interest in deep-sea living and non-living resources (Mengerink et al., 2014). Increasingly, the deep seabed is being targeted as a provider of mineral resources. Both the extraction of minerals from the seabed and disposal of mining waste products into the deep sea from either land-based or seabed mining are of growing concern due to the disturbances that can be caused to both littoral and deep-sea benthic ecosystems (e.g., Lee and Correa, 2005; Hughes et al., 2015; Levin et al., 2016).

The waste materials from DSTD consist of a slurry of predominantly finely-crushed rock materials, formed after the mineralized material has been processed. This material contains mud, silt and sand, water, low concentrations of targeted minerals (e.g., gold, copper, and silver), and measureable concentrations of other metals such as arsenic, cobalt, nickel, mercury, lead, zinc, as well as processing wastes such as sodium cyanide, lime and other acids (Ramirez-Llodra et al., 2015). DSTD EIAs require rigorous studies of hydrology and local topography to determine feasibility of site selection, together with comprehensive studies aimed to create baselines of abiotic (environmental) and biotic (ecology and function) properties of the actual seabed and overlying ecosystems in order to assess potential environmental impacts. However, although these discharged tailings are expected to be permanently deposited in a deep-water environment, the potential for plume dispersal and tailing resuspension, and the

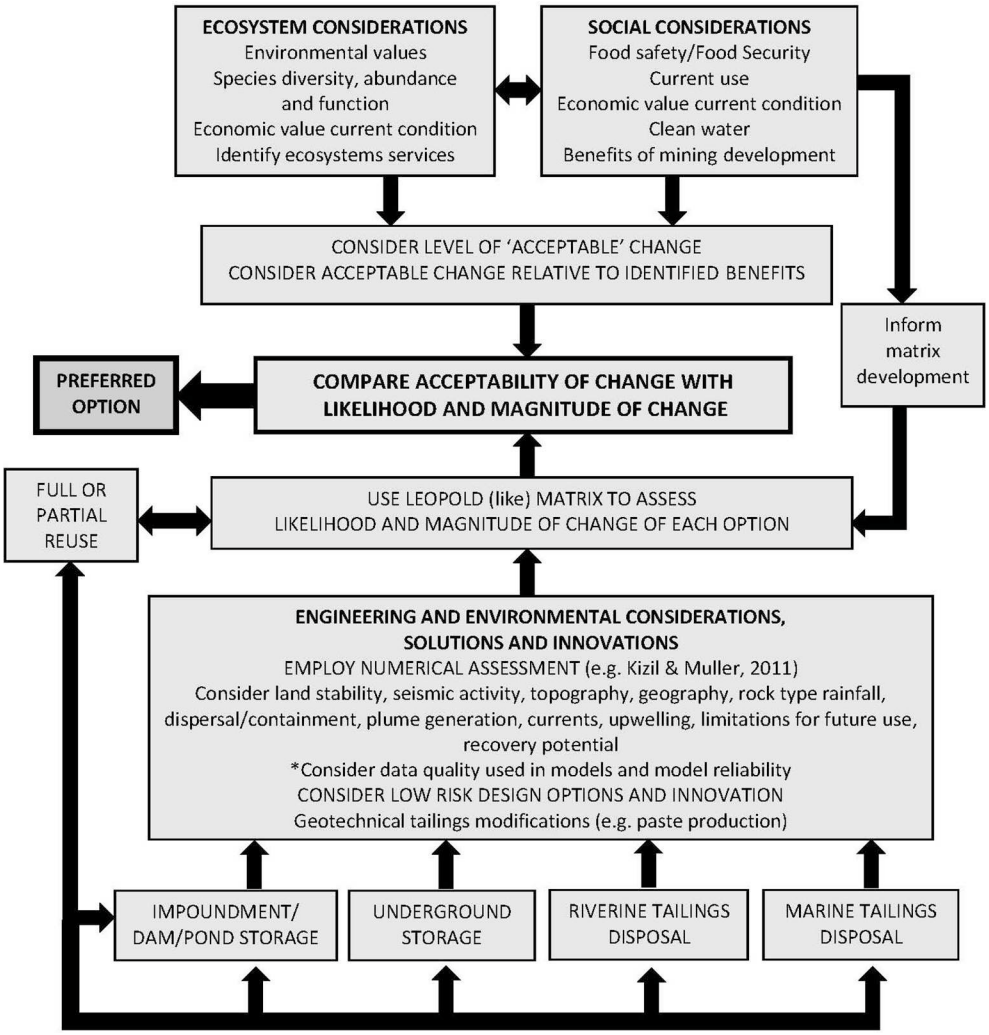


FIGURE 2 | Possible framework for considering options for tailings disposal.

consequences for the marine ecosystem in the water column and on the seafloor are uncertain over long time scales.

The post-depositional fate and behavior of tailings disposed in the deep sea will have major effects on the sedimentary and geochemical environment, and on the integrity and recovery of faunal communities. Submerged tailings constitute both a potential source of remobilized dissolved metals and metalloids, as well as processing chemicals (e.g., acids, flocculants and floatation agents) to pore water and overlying water (Perner et al., 2010; Shimmield et al., 2010; Angel et al., 2013; Simpson and Spadaro, 2016) by bacterially-mediated, sediment diagenetic processes associated with the remineralization of organic matter (Middelburg and Levin, 2009; Bourgeois et al., 2017). For instance, increased copper concentrations can affect microbial biomass and metabolic activity leading to reduction of their assimilative capacity, and hence impaired crucial ecosystem services such as carbon and nutrient cycling (Jonas, 1989; Almeida et al., 2007). Under high rates of sedimentation associated with tailings disposal, crucial reactions involved in the oxidation of organic matter may be altered, disrupting biogeochemical zonation of electron acceptor sequencing. For instance, O_2 and NO_3 could be depleted more rapidly than they can be restored (Pedersen, 1984); this would be exacerbated if bioturbating fauna were reduced. In shallow waters, limed tailings have been observed to diminish benthic phosphorous regeneration relative to natural sediments, with potential depletion of productivity (Pedersen and Losher, 1988).

The degree of physical and possible toxic impact of a mine tailings discharge will depend on several abiotic and biotic factors. These factors include the location of the outfall pipe, the volume disposed and the physical, chemical and hydrodynamic conditions of the targeted area, together with the degree of tolerance/sensitivity of the organisms in the local site and adjacent areas that may be affected by increased water turbidity

and concentrations of metals or metalloids due to tailing plumes (Mineral Policy Institute, 1999; McKinnon, 2002). Most of the known effects of tailings on marine environments that are described in the literature have been based on tailings disposal in littoral, nearshore shallow waters and coastal fjords (e.g., Kathman et al., 1983; Burd, 2002; Lee and Correa, 2005; Kvassnes and Iversen, 2013; Mevenkamp et al., 2017). Studies published in the open literature specific to the impacts of DSTD are fewer (e.g., Hughes et al., 2015), and those in the gray literature are often not readily accessible (e.g., Shimmield et al., 2010; LIPI, 2014; Simpson and Angel, 2015).

Deep-sea organisms may differ from those in shallow water in having slower growth rates, greater longevity, and less exposure to disturbance or variable environmental conditions, increasing their vulnerability to the changes related to tailings disposal. The benthic marine fauna can be impacted in a number of different ways by discharged tailings. The deposited material can kill the organisms directly through smothering and asphyxiation, through contact or poisoning via ingestion or exposure to water-dissolved substances. Mortality can also occur through the destruction of sensitive juveniles and through the killing of prey organisms (Brewer et al., 2007; Shimmield et al., 2010; Reichelt-Brushett, 2012). Benthic fauna at the disposal site and in the vicinity of the plume may bioaccumulate metals from tailings porewater and ingestion of sediment (Rainbow, 2007; Casado-Martinez et al., 2010; Campana et al., 2012). These impacts can lead to ecosystem-level changes. In shallow water situations, such as marinas, elevated sediment copper levels alter faunal composition and reduce macrofaunal biodiversity, total biomass and individual body size, as compared to sites with lower sediment copper concentrations (Neira et al., 2011, 2014).

Where mine tailings have been disposed, shifts in meiofaunal and macrofaunal community structure have been observed, with reduced biodiversity (e.g., Lee and Correa, 2005; Shimmield et al., 2010; Hughes et al., 2015); these effects on the environment can last several years after tailings disposal has ceased. Monitoring around the Island Copper Mine located on Vancouver Island (British Columbia) showed lower benthos diversity and abundance in tailings depositional areas, but no consistent reduction in crab catch associated with tailings discharge. Extensive benthic repopulation was observed in areas that had not undergone tailings deposition for 12 months, although assemblages of recolonizing organisms differed from reference sites (Poling et al., 1993). This shift in composition after recovery seems to be a common pattern and could result in shifting ecosystem functions (Gollner et al., 2017).

A large disturbance such as tailings disposal may result in changes in the flux, species composition of settling larvae and colonists, which could be facilitated by the disappearance of former residents (Mullineaux et al., 2010). Characterization of regional water masses and their seasonality is of great relevance, as these waters can transport different assemblages of colonizing larvae at different times of the year (e.g., Calderon-Aguilera et al., 2003; Adams et al., 2011). Benthic animals tend to recolonize disturbed areas at a slower rate with increasing water depth (Smith and Hessler, 1987). Tailings deposition, with increased metal mobilization and altered biogeochemistry, could create "no settlement zones" for larvae (Marinelli and Woodin, 2002). Shallow-water field experiments using defaunated sediments, with contrasting natural metal loading, spiked sediments, translocation and replacement showed changes in recolonizing infaunal and hard-substrate fauna composition with reduced biodiversity and lower structural complexity (Hill et al., 2013; Neira et al., 2015). As the same high-level taxa are present and the same principles govern sediment assemblages in shallow and deep waters, analogous responses could be expected for mine tailings disposed in the deep sea, but at a much larger spatial scale, covering a wider range of habitats and ecosystems. In deep continental margin settings, sediment recolonizes are facilitated by strong currents (Levin and DiBacco, 1995), and in chemosynthetic systems, by hydrogen sulfide (Levin et al., 2006). Thus, the order of species arrival, and the rate at which the faunal community will regenerate after tailings disposal has ceased will be site-specific, and also species-specific (Hughes et al., 2015). However, even higher-taxon level identification is sufficient to detect large-scale tailings impact in shallow water environments (Lee and Correa, 2005) and deep-sea sediments (Montagna et al., 2013; Hughes et al., 2015; Mevenkamp et al., 2017).

In most places where DSTD are conducted or proposed, knowledge of the deep-sea ecosystem, including the faunal composition, the functions, and the services provided are poorly known. Such knowledge becomes particularly important at the regional scale beyond the impact area, as larger areas may be impacted by resuspension, slope failure, plume sheering, etc., as well as by changes in connectivity affecting source populations for recovery.

SOCIETAL IMPACT AND MITIGATION

Reducing the Risk of Environmental Impacts

General "good practice" for all proposed mining operations commences with background and feasibility studies, including an EIA. These practices are intended to engage and inform communities, government and the industry proponents of the benefits and risks posed for triple bottom line outcomes (social, environmental and economic). All stakeholders are then provided a period of time to consider the studies, and comment, before potential revisions are made and approvals are sought for the options providing the best outcomes.

The EIA should clearly articulate the pertinent issues and uncertainties surrounding the short- and long-term impacts, including cumulative impacts. Within the EIA, tailings management will also consider options for on-land tailing impoundments (TSF, dams, ponds). The attributes of DSTD that are reported as favorable by mining companies over TSFs frequently include:

- Less impact to land (e.g., clearing of forest, loss of agricultural land)
- Less impact to communities that may be displaced or have land-based livelihoods altered
- Lower risks associated with natural events (high rainfall or earthquakes; floods)

- Avoidance of many of the problems of acid rock drainage, and its long term management

Within the vicinity of the DSTD outfall, significant adverse impacts on the marine ecosystem are predicted in the form of (i) reduction in seawater quality due to elevated turbidity and dissolved metal/metalloid concentrations (ii) burial of benthic organisms, and (iii) potential reduced habitat for demersal fish. When the DSTD ceases operation at completion of ore processing, the intent would be that the seawater quality improves within days to weeks and the benthic ecosystem recovers during a period of years (e.g., within 2–10 years), but the timescales for the recovery of ecosystems are poorly understood. Other frequently-cited positive environmental attributes of DSTD which are uncertain include:

- The ability to engineer the DSTD such that the tailings remain below the surface mixed layer and photic zone (beneath the pycnocline), flow rapidly to great depths (slopes $>12^{\circ}$), and deposit within predictable regions of the sea floor.
- The depositional area can be chosen to be a zone of relatively low biological productivity (i.e., not impacting a vulnerable ecosystem), and maintain low chemical reactivity and low physical disturbance of the tailings in the future.
- The availability of locations that have an absence of upwelling events or seasonal mixing, or that are truly low productivity environments.

Considerable evidence exists that tailing plumes cannot be avoided, and the fate of plumes of fine tailings particles within the water column has been difficult to predict (Boschen et al., 2013; Hughes et al., 2015). Tailing plumes contribute to sometimes large amounts of tailings depositing outside predicted areas (e.g., 15–80% outside), requiring the need to develop better 3D modeling techniques that are capable of more accurately predicting tailings deposition areas (footprints). Demonstrating that no tailings resurface and disperse within the euphotic zone as a dissolved or particulate plume requires extensive monitoring which will be required as part of the Operational Environmental Management Plan (OEMP) of the mine, without which the mine cannot operate. The ecological risk assessment should include evaluating the risk of adverse effects to aquatic organisms both within the water column (pelagic organisms) and sediment environment (benthic organisms).

The first requirement for good practice concerns the completion of comprehensive, high-quality baseline studies that provide information on the receiving environment: detailed bathymetry and physical oceanography (e.g., local and seasonal information on frequency and intensity of currents and current-shearing, upwelling and downwelling, storms), sedimentology, and ecosystem (e.g., coastal and deep-sea community structure, function, connectivity, and resilience). To achieve suitable levels of background information on the dynamics of the abiotic and biotic systems, studies will generally need to be conducted over many years. A limited number of studies have addressed interactions and connections among abiotic and biotic systems, and new methods and approaches are needed. There are many knowledge gaps, for example, how the daily vertical migrators and benthopelagic coupling of living organisms are influenced by tailing plumes (Morello et al., 2016).

The second stage of good practice involves the engineering and modeling. Engineering aspects include: design, quality, and operational management for the life of the DSTD. These elements will include the de-aeration of the tailings and other conditioning to achieve the desired density and rheology, the tailing pipe network, materials, maintenance, stability of the optimal outfall depth, and quality controls for detection of leakage. A suitable level of understanding and monitoring of residual process chemicals (e.g., xanthates for floatation, lime for pH control, or specialized flocculants) is also needed. The engineering may need frequent adaptions to cope with changes in ore processing that may influence the environment downstream of the DSTD. The behavior of the tailings is modeled and takes into account oceanographic conditions, tailings volume and composition to predict the behavior of the discharge (direction, rates of transport and deposition) in relation to the bathymetry to estimate the tailings footprint. Based on this, ecological models could be developed outlining the estimated main and potential areas of impact to the water column and benthos. Tailings depositions outside initial model-predicted areas of DSTDs are estimated as ∼15% at Batu Hijau, Indonesia (LIPI, 2014; Simpson and Angel, 2015).

Due to the uncertainty surrounding predicted environmental risks of DSTDs, there is a need to undertake extensive monitoring and ongoing review of operations (stage three of good practice). The monitoring program should be designed to provide transparent evidence that the environmental management objectives are being met, e.g., demonstration of minimal impacts to the biologically productive surface waters (e.g., the surface mixed layer and photic zone), of no tailings deposition in near-shore coastal environments, and for impacts from the deep-sea deposition to be occurring in the predicted area. The tailings management systems and monitoring programs should span the processes from the mine to the sea, starting with the upstream management of ores and mine water on site and in the processing plant, then proceeding to tailings management via controls relating to engineering (e.g., tailings rheology, integrity of land seabed pipes) and tailing quality (e.g., quantities of oxidized forms), possible treatment options (e.g., re-sulfidization), and finally to monitoring of tailings disposal impacts within the marine environment. The management and monitoring programs should provide information that enables issues to be rapidly identified (e.g., extremes such as pipe breakages or surfacing tailings), and allows continuous improvement to the management and monitoring programs. This is detailed in the OEMP and is a requirement of the environment permit.

Routine monitoring should include a network of stations both within and beyond the predicted DSTD impact zone (encompassing the full water depth range of the receiving environment). The monitoring should include: the volume, physical and chemical characteristics of the tailings prior to discharge (e.g., crucial parameters monitored daily, and other parameters weekly to monthly); the coastal environment

surrounding the DSTD (e.g., CTD profiles, seawater quality, total suspended solids, sediment quality, and various components of the marine ecosystem (Shimmield et al., 2010; Simpson and Angel, 2015).

Further technical studies may be necessary to support, validate or investigate aspects of the operations or potential impacts that cannot be adequately evaluated from routine monitoring data (LIPI, 2014), or to modify and optimize closure plans (stage four of good practice). Specialist studies may be undertaken upstream of the DSTD (e.g., relating to changes in processing techniques that influence tailings properties) or downstream (e.g., hydrodynamics, plume behavior, chemistry or ecological impacts). These studies should take advantage of new technologies to optimize data acquisition in remote deep-sea habitats for example, higher resolution bathymetry mapping, advanced autonomous underwater vehicles (AUVs) and advances in eco-genomics-based methods to provide more holistic information on impacts to ecological community structure, functions connectivity and resilience. These studies will often be vital to improving monitoring programs, and adapting management practices for positive outcomes during the mine life and post-closure (ongoing good practice).

For all assessments, there is a need to consider multiple lines of evidence (LOE) in order to adequately evaluate risks posed by mine tailings to the environment (Simpson and Batley, 2016; Mestre et al., 2017). For many deep-sea assessments, the desired LOE may not be readily assessed using existing tools, resulting in greater uncertainty. Prime examples include the lack of species that are representative of deep-sea environments that can be utilized for aquatic toxicity testing (Mestre et al., 2014; Brown et al., 2017), and the inadequate knowledge of deep-sea ecosystem structures, functions and connectivity to enable informed ecological assessments. Consequently, there will be a need to develop new tools to provide new LOE and to validate these for the specific assessment purposes. The assessments, approvals and monitoring will continue to improve as new science-based tools are developed to cover all aspects of chemistry, ecotoxicology, ecology, physical oceanography, and topography. This development is necessary to enable the most informed and robust management decisions associated with DSTD.

Although some of these aspects of reducing the risk of environmental impacts from DSTD outlined above are considered and incorporated within the approval and permitting processes, it is important that all are fully addressed. Owing to the numerous unavoidable uncertainties, it is recommended that permits are not issued for the entire mine life, but instead are for limited terms (e.g., 3–5 years) with thorough scrutiny of compliance and all operating procedures that may influence the environmental management objectives. This review process should enable clarification or improvements to be made to the objectives and permit requirements.

THE FUTURE OF DSTD

Potential Locations for Future DSTDs

For future DSTDs on the continental margin, a major consideration is that the slope is steeper than 12 degrees, to facilitate the formation of a turbidity current that will transport the tailings to a deep (below 1,000 m), and stable soft-sediment seafloor area (Shimmield et al., 2010; Ramirez-Llodra et al., 2015). Physically, it is essential that the selected site is not affected by upwelling events and is located in a low-energy environment (Shimmield et al., 2010). Ecologically, the transport and deposition areas should have low-productivity ecosystems, and avoid biodiversity hotspots and vulnerable communities (such as cold-water coral and sponge reefs, seamounts or cold seeps, amongst others). Submarine canyons are geomorphological structures that form deep incisions in most shelves and slopes around the world (Fernandez-Arcaya et al., 2017). Due to their particular geomorphological features, canyons modify the local hydrography and act as enhanced transport pathways of material from the productive coastal and shelf zones to the deep basins (Puig et al., 2014; Fernandez-Arcaya et al., 2017) influencing coastal upwelling process (Sobarzo and Djurfeldt, 2004). This enhanced downslope transport of particles has been used to justify certain canyons as preferential target sites for DSTD initiatives. Submarine canyons have been used for DSTDs in PNG (Basamuk canyon; ongoing), Indonesia (Senunu canyon, Sumbawa; ongoing) and France (Cassidaigne canyon; ongoing). The increased amount of scientific data available from canyons suggests that these complex geomorphological features can serve as essential habitats to marine communities, supporting an enhanced productivity and biodiversity, and are used as hatching, nursery or refuge areas. Canyons with steep rocky walls often support communities of sessile filter feeders such as corals (Roberts et al., 2009) or sponges (Schlacher et al., 2007, 2010), and the soft-sediment axis provides habitat to a variety of mobile fauna, including commercial species (Company et al., 2012). Furthermore, many canyons support complex marine food webs that include benthic species, but also pelagic decapods, fish, sharks and mammals attracted by the increased productivity and habitat heterogeneity (Vetter et al., 2010; van Oevelen et al., 2011; Moors-Murphy, 2014). The vulnerability of some of these biological communities, together with the limited knowledge of the processes and their temporal variability that drive canyon ecosystems, call for precaution and thorough long-term studies when considering a canyon as a DSTD site. In addition, the composition, diversity and ecosystem functions supported by canyons differ amongst regions, and thus it is essential to conduct local studies where canyons are proposed sites for DSTD activities.

Cumulative Impacts

Current trends for the disposing of mine tailings are increasingly considering deep-sea areas as final waste-deposit sites. At the same time, an increasing number of industries are targeting deep-sea resources, both mineral and biological (Ramirez-Llodra et al., 2011), contaminants are spreading to the deepest parts of the ocean (Jamieson et al., 2017), and ocean warming, acidification and deoxygenation affect ocean ecosystems globally, with impacts reaching the deep ocean (Levin and Le Bris, 2015). The synergies of different direct and indirect stressors on an ecosystem may result in a magnified effect with often poorly understood consequences, particularly in the deep sea where empirical studies are lacking (Ramirez-Llodra et al., 2011).

Below we describe key impacts that may coincide in space and time with DSTD operations, highlighting the need for strategic environmental assessments that will take into account multiple activities within a region.

Fishing

The slopes of many continental margins, including some canyon habitats, are fished intensively, with depth of operations gradually increasing as technologies develop (Morato et al., 2006). Those DSTD impacts that affect the water column and sediments (plumes, contaminants) could affect the diel vertical migrators and resident mesopelagic species as well as the benthic and demersal fish in ways that ultimately influence fisheries. Some studies, although not all, report an increase of metal content in fish tissues (Mol et al., 2001; Powell and Powell, 2001; Scroggins et al., 2002) and suggest that tailing plumes can disrupt migration routes and displace populations of commercially-important species (Sheaves, 2001; Brewer et al., 2007).

Confrontation between the fishing and mining industries have taken place in New Zealand and Namibia, where seabed phosphate mining licenses were applied for in regions that were closed to fishing (New Zealand) or that support a strong fishing industry (Namibia) (Levin et al., 2016). In Chile, mining is concentrated in the northern part of the country, and environmental impacts associated with this activity have a long history, among them, deposition of hundreds of millions of tons of tailings from copper mining into coastal beaches over the years (e.g., Vásquez et al., 1999; Lancellotti and Stotz, 2004). In 1990 disposal of untreated tailings was banned and further regulations and environmental requirements have been put in place (Advanced Conservation Strategies, 2011) in part because of concerns about fisheries. Potential conflicts of interest are possible if mining activity and future DSTD installations are near Benthic Resources Fisheries and Management Areas, and other types of Marine Protected Areas. In this regard, and in order to investigate the gaps related to the use of DSTP a number of mining companies formed an independent Consortium to conduct an impartial evaluation of DSTD (The DSTP Initiative, 2014). Mining projects have been rejected by the Chilean government arguing that mining close to MPAs could affect marine resources such as fisheries, as well as biodiversity, and other environmental services. An example is the case of Dominga Mining Company (Andes Iron) project, which was finally rejected in August 2017 by the Environmental Assessment Commission (http://www.bbc.com/news/world-latin-america-41007462?SThisFB).

Deep-Seabed Mining

Although the exploitation of deep-sea minerals has yet to begin, the interest in deep-seabed mining and the number of exploration licenses continue to grow internationally (Van Dover, 2011; Mengerink et al., 2014). The first *in situ* test mining for seafloor massive sulfides (SMS) took place in Japanese waters in 2017. It is likely to be several years before any commercial exploitation begins. In addition to potential cumulative risk and impacts, DSTDs and deep-sea mining activities have several processes in common (e.g., dispersal of sediment plumes; smothering of benthic fauna) and thus there is great benefit to sharing new knowledge, new technologies, methodologies and experience for the development of good practices and impact minimization.

Marine Genetic Resources

Among the ecosystem services provided by deep ocean ecosystems, there is increasing recognition of the value of genetic resources, including genes, proteins and natural products (Harden-Davies, 2017). New pharmaceuticals, industrial agents and biomaterials are originating from the deep sea, however, because so little of the deep ocean has been explored, most genetic resources have yet to be discovered. Disturbance to the sea floor from DSTD as well as extractive industries runs the risk of causing loss of these resources before they known.

Climate Change, Ocean Acidification, and Ocean Deoxygenation

The ocean environment below 200 m is changing rapidly as a result of heat uptake from the atmosphere (ocean warming), CO_2 uptake from the atmosphere (ocean acidification), and oxygen loss from thermal effects on oxygen solubility, stratification and ventilation (ocean deoxygenation) as well as from enhanced nutrient input (Levin and Le Bris, 2015; Sweetman et al., 2017). Deep continental margin ecosystems are highly vulnerable to these climate-related stressors, which act cumulatively with direct human disturbance from bottom trawling, oil and gas extraction and spills, pollution, and potentially seabed mining (Levin and Le Bris, 2015). The inorganic and organic particles including contaminants within the plumes from DSTD have the potential to further alter biogeochemistry at the seafloor and in the water column, compounding climate-related temperature, oxygen and pH stress. These cumulative effects are likely to be widespread, altering habitat properties, many ecological functions, (e.g., biodiversity, calcification by habitat-forming species like cold water corals) and ecosystem services (e.g., carbon sequestration, nutrient cycling, and fisheries production).

LEGISLATION AND RESEARCH GAPS

Current Legislation

When considering DSTD, it is important to consider the international legislation framework. The "Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter 1972," the "London Convention" is one of the first global conventions to protect the marine environment from human activities and has been in force since 1975. Its objective is to promote the effective control of all sources of marine pollution and to take all practicable steps to prevent pollution of the sea by dumping of wastes and other matter. Currently, 87 States are Parties to this Convention.

In 1996, the "London Protocol" was agreed to further modernize the Convention and, eventually, replace it. The Protocol entered into force on 24 March 2006 and there are currently 45 Parties to the Protocol. Under the Protocol all dumping is prohibited, except for wastes on the "reverse list." Rather than state which materials may not be dumped, the

1996 Protocol restricts all dumping except from a permitted list of eight major categories, including "Inert, inorganic geological material," under which tailings may fall.

Although the national waters of a State are excluded from both the Convention and Protocol, Parties to the Protocol have the option to apply its rules to their waters if they wish (Article 7).

It is important to note that the London Convention and Protocol (LC/LP) do not cover discharges from land-based sources such as pipes and outfalls, wastes generated incidental to normal operation of vessels, or placement of materials for the purposes other than mere disposal, providing such disposal is not contrary to aims of the Convention. Therefore, the LC/LP do not directly apply to DSTD. In addition the LC/LP allows the dumping of "inert, geological material", and mining organizations argue that as mine tailings are geological in origin, they are also "inert," and therefore do not contravene the LC/LP.

In October 2008 (under increasing pressure from non-government organizations, particularly Greenpeace) the governing bodies under the LC/LP agreed for a more detailed assessment of mine tailings, in order that effective control of sub-sea tailings discharges may be considered and communicated to relevant bodies, including the United Nations Environment Program (UNEP) Global Programme of Action (GPA) for Protection of the Marine Environment from Land-Based Activities. The GPA is unique in that it directly addresses the connectivity between terrestrial, freshwater, coastal and marine ecosystems. GPA targets major threats to the health, productivity, and biodiversity of marine and coastal environments resulting from human activities on land. Importantly the GPA is not binding, but provides a framework for governments in close partnership with all stakeholders to address some of the most significant threats to marine ecosystems.

In 2009, the International Maritime Organization (IMO) submitted a paper entitled "Initial proposals for co-operation between the London Convention and Protocol and the UNEP Global Programme of Action for Protection of the Marine Environment from Land-based Activities (GPA)," (Annex 4), which considers coastal management issues and investigates options for co-operation between the LC/LP and the UNEP-GPA and the UNEP Regional Seas programme to deal with coastal management issues. This collaborative policy response is still being considered.

Although pipeline discharges and other land-based sources of marine pollution fall beyond the regulatory scope of the LC/LP, it is noted by LC/LP Scientific Groups that the discharge of such tailings frequently falls beyond the scope of any effective international regulatory control. The LC/LP has been interested in riverine and submarine disposal of tailings and associated wastes, including cooperation of the LC/LP Secretariat at the IMO with UNEP cooperation of the LC/LP GPA, in gathering information on the issue. The LC/LP Secretariat commissioned a report on the issue, which was submitted to the LC/LP Scientific Groups and discussed at the LC/LP meetings in November 2013. LC/LP Scientific Groups agreed there is a need for international guidance and/or codes of conduct to be developed but, as GESAMP noted, there is a governance gap and it is not clear which international body should take the lead. The LC/LP agreed to establish an intersessional correspondence group.

Further international concern led to an international workshop held in June 2015 in Peru led by IMO-GESAMP, and co-organized by the MITE-DEEP project (funded by the Norwegian Research Council and INDEEP) and the Chilean DSTD initiative. The final report of the Workshop has been published by the GESAMP Office and the LC/LP Secretariat (GESAMP, 2016). In addition, there is a working group in the process of developing guidelines; the group is led by Perú. Most recently in 2017 a new working group (WG42) has been established by GESAMP (sponsored by IMO and UNEP) on the impacts of wastes and other matter in the marine environment from mining operations, including marine minerals mining. Finally, as reported in their Annual Report, OSPAR are also considering the need for Guidance on the deep-sea disposal of mine tailings (OSPAR Commission, 2017).

In 2014, the European Commission started a process to review and adapt the first (2009) Reference Document on Best Available Techniques for Management of Tailings and Waste-Rock in Mining Activities, to include all new knowledge and available techniques. The release of this reviewed "Best Available Techniques Reference Document for the Management of Waste from Extractive Industries" is expected at the end of 2017. However, the draft version of this document focuses mostly on land-based processes of extractive waste and discusses only briefly issues related to submarine tailing disposal.

Research Priorities to Address Current Knowledge Gaps

A thorough review of the pros and cons of DSTDs against a land-based dam for the management of mine tailings under different scenarios (e.g., existing and potential DSTDs) would be a useful exercise. The main limitation to conducting this essential exercise before making the decision to dispose tailings in the marine or land systems is the limited knowledge of many marine community structures and processes, including the value of the services provided. Here, we highlight some of the major gaps in knowledge that need to be addressed, before a rigorous science-based evaluation of the advantages and disadvantages of marine vs. land tailings management can take place. The priority topics for future research have been selected based on discussions during the GESAMP/MITE-DEEP/INDEEP workshop and post-workshop deliberations and include expert comments from a variety of scientific disciplines, industry and policy makers from broad geographical regions. Detailed consideration should be given to the following issues:

- *Tailings dispersal* in the water column: there is a need for better spatio-temporal physical oceanographic data to feed into more accurate models (e.g., data on shearing currents and plume generation) and ground-truthing of models.
- Engineering developments to change *tailings behavior* (turbidity currents, plume generation) to reduce impacts.
- *Post-deposition behavior* of tailings: what are the potential physical (resuspension, slope failures) and chemical (reactions

in water column and sediments) processes affecting deposited tailings and their components?
- Inventories of the *materials being deposited*: what is the nature of processing chemicals and what is their behavior in different environments?
- Detailed *faunal community* studies: what is the composition and structure of benthic and pelagic fauna (using morphological and molecular tools)?
- *Ecosystem function*: what are the trophic relationships in the ecosystem? What bentho-pelagic coupling processes are found? What are the reproductive patterns of the key species and are they affected by tailings deposition? What microbial processes take place in the sediment? What ecosystem services derive from these functions and how can they be impacted by DSTDs?
- Effects on *pelagic early life-history* stages: what is the effect of plumes (particles and toxicity) on eggs, larvae and juveniles?
- Effect on *recolonization and settlement*: what are the different effects of varying grain size, organic matter content and grain shape/sharpness? What are the sensitivities of early life stages to mine-derived chemical contaminants?
- *Ecotoxicity*: although most EIAs include ecotoxicity tests, these are conducted on standard, shallow-water species, and the results may not be relevant to deep-sea species. There is an urgent need to develop similar analyses for deep-sea species, but the difficulty of collecting and maintaining deep-sea fauna alive in laboratory conditions continues to limit these studies.
- *Evaluate cumulative impacts* from different direct and indirect stressors.
- *Identify thresholds* to evaluate serious harm.
- *Assess long-term fate* of tailings in deep-sea ecosystems.
- *Value deep-sea ecosystem services* to provide the necessary information to managers assessing the cost-benefit of DSTD applications and deciding upon required compensation for lost services.
- *Circular economy:* further research is necessary in the reprocessing of tailings to extract value and minimize waste volume.
- *Legislation and standard rules/guidelines for Good Practice*: further rollout of guidelines for the use of DSTD.

DSTD IN A BROADER CONTEXT

Although DSTD is a very local practice associated with terrestrial mining, the activities and impacts associated with disposal of terrestrial tailings in the deep sea are likely to affect a broader spectrum of stakeholders. While most of these human activities occur within a national jurisdiction, they may impact transboundary organisms that exhibit ontogenetic or migratory movements into international waters, or the national waters of other countries.

As suggested above, the monitoring and research conducted for DSTD may also inform management of other activities. For example, the substrate modifications, sediment plumes, sediment deposition, toxic compounds/heavy metals and, to a lesser extent, noise are also features of deep-seabed minerals mining. Long term studies of plume behavior, benthic impacts, and faunal recovery rates as described for the Batu Hijau project in Indonesia (LIPI, 2014; Simpson and Angel, 2015) or the Lihir gold mine in PNG could inform regulation or even decision making about seabed mining, which has been proposed within Exclusive Economic Zones (EEZs) of many island nations in the Western Pacific Ocean (SPC, 2013), as well as in international waters (Levin et al., 2016). Although the seabed mineral resources differ (massive sulfides, polymetallic nodules, and cobalt crusts), their extraction will resuspend sediments and yield many similar physical impacts to DSTD. The emerging regulations and science of deep-seabed mining have much to gain by utilizing the scientific studies carried out on DSTD, and by learning from the past regulatory successes and failures of DSTD.

At the international level, there are a number of deep-focused oceanographic data networking programs that may be able to help inform plume modeling, risk assessment and post-disposal recovery. Among these are the nascent Deep Ocean Observing Strategy (www.deepoceanobserving.org), a GOOS project which will help coordinate accessibility of deep-sea biological and hydrographic data, and the Ocean Biogeographic Data System (OBIS), which is initiating a deep-sea node for biological/biodiversity data. The Deep Ocean Stewardship Initiative (DOSI, http://dosi-project.org/) and the International Network for Scientific Investigations of Deep-Sea Ecosystems (INDEEP, http://www.indeep-project.org/) both facilitate capacity building and can offer scientific and policy expertise on various aspects of anthropogenic impact in the deep ocean, including DSTD. Engagement with these entities, and with other international activities such as the UN Sustainable Developmental Goal (SDG) 14, which advocates for sustainable oceans, may be helpful to regulators in developing countries where much of the current DSTD occurs, as these often do not have their own long-term, deep-ocean monitoring programs, or scientists and policy experts familiar with the deep ocean.

There are now numerous organizations associated with UNCLOS that regulate or are responsible for various aspects of biodiversity in the ocean (Ardron and Warner, 2015): the Convention on Biological Diversity (CBD) in national waters, the International Seabed Authority for the Area (international seafloor), the Food and Agriculture Organization (FAO) for fish in international waters, the International Maritime Organization (IMO) for contaminants in international waters. Each of these organizations has identified some form of protected area—Ecologically or Biologically Significant Marine Areas (EBSAs) by the CBD, Vulnerable Marine Ecosystems (VMEs) by the FAO, Areas of Particular Environmental Interest (APEIs) by the International Seabed Authority (ISA), and Particularly Sensitive Sea Areas (PSSAs) by the IMO (Diz et al., 2017). There is an understanding that we need to identify, monitor, and minimize DSTD sediment plumes, contaminants or other ecosystem alterations that are transported from the margins into national Exclusive Economic Zones (EEZs) and international waters, especially those that may affect protected areas. The new UN treaty being negotiated

to protect biodiversity in international waters is addressing impact assessment, marine protected areas, genetic resources and capacity building (Blasiak et al., 2016), all issues relevant to DSTD.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

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The Benthic Megafaunal Assemblages of the CCZ (Eastern Pacific) and an Approach to their Management in the Face of Threatened Anthropogenic Impacts

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We present here the results of a UNESCO/IOC baseline study of the megafaunal assemblages of the polymetallic nodule ecosystem of 5 areas within the Clarion Clipperton Zone (CCZ) of the eastern Pacific Ocean. The work was undertaken with a view to investigating the structure of the epifaunal populations associated with the benthic biotopes being targeted for nodule mining and developing an appropriate set of management tools and options. The general characteristics of nodule ecosystem and assemblages and their sensitivity to deep-sea mining are discussed in relation to water masses, surface to seabed water circulation, the nepheloid layer and processes taking place at the sediment interface. Management tools considered include species diversity and vulnerability indexes, GIS systems, zoning, and 3D rapid environmental assessment (REA). These strategies are proposed for trial during pilot mining operations within the CCZ.

Keywords: deep sea mining, polymetallic nodule ecosystem, epibenthic megafauna, vulnerable marine ecosystems, ecosystem-based marine spatial planning

INTRODUCTION

Knowledge of the structure of its megafaunal assemblages is essential to understanding the functioning of any deep-sea ecosystem (Rex and Etter, 2010). Typically this faunal component includes a significant fraction (17–50%) of benthic abyssal biomass (Haedrich and Rowe, 1977). Since the megafauna is also one of the principal agents of bioturbation at the depositional interface of the deep-sea benthos (Mauviel and Sibuet, 1985; Levin et al., 1986), it can influence many other biological and geochemical components of the deep ocean, in particular concerning the nodule ecosystem (Sharma and Rao, 1992) (**Figure 1**). In particular, the benthic fauna plays an important role in carbon cycling and mineralization within the epibenthos. Interestingly it also contributes to the genesis of polymetallic nodules, bioturbation along with bottom currents playing a role in allowing nodules to remain on the seafloor (Dugolinsky et al., 1977; Du Castel, 1985; Mullineaux, 1987; Thiel et al., 1993; Veillette et al., 2007a,b).

Megafaunal assemblages also serve as a good indicator of the status of a habitat in the face of natural and anthropogenic impacts (Bluhm et al., 1995), and may be used to measure the variation in flux of particulate organic carbon (Smith et al., 1997), to identify critical environmental parameters, to characterize selected habitats and associated nodule facies (Tilot, 2006a), and to measure rates of recolonization under natural or impacted conditions (Tilot, 1988, 1989, 1990, 1991; Bluhm, 1997, 2001).

The large equatorial polymetallic nodule belt of the Clarion Clipperton Zone (CCZ) (118°–157° W/9°–16° N), wherein lie the most economically important deposits (Morgan, 2000; Petersen et al., 2016) (**Figure 2**), covers about 2 million km^2 of abyssal hills and escarpments in the eastern Pacific (Halbach et al., 1988; Kotlinski, 1998). Considerable regional-scale variation in the geological environment (topography, erosion by deep ocean currents and regional deposition of sediments) has led to a classification of nodule deposits and the recognition of a series of distinct "*nodule-facies*" (Hoffert and Saget, 2004; Hoffert, 2008), differentiation which has been based on a combination of photographic study and of collection of samples for morphological and geochemical assessment (**Table 1**).

We had the opportunity to participate in a comprehensive study of the biodiversity and distribution of epibenthic megafauna of the CCZ, originally with IFREMER (L'Institut Français de Recherche pour l'Exploitation de la Mer), France, and funding from the EU and the Institut océanographique, France (Tilot, 1988, 1989, 1991, 1992, 2006c; ESCO CNRS IFREMER, 2014), following which the work was updated and expanded (Tilot, 2006a), with particular emphasis on the echinoderm fauna (Tilot, 2006b), with the support of the Intergovernmental Oceanographic Commission (IOC) of UNESCO (published in 3 vol. see: http://unesdoc.unesco.org/images/0014/001495/149556e.pdf#223), in order to establish a UNESCO/IOC baseline. Because of unavoidable conditions, the findings could not be published at the time, other than is restricted circulation reports.

More recently, however, this research has led to the development of options for the management and conservation of the nodule ecosystem, taking in to account relevant scientific, legal and institutional issues (Tilot, 2010a). Similar options have been proposed for other deep-sea habitats and discussed at a UNESCO meeting of experts (Académie des Sciences d'Outre-Mer (ASOM), 2010) which provided an international platform for consideration of these issues (Tilot, 2011) and initiated the development of a number of management and conservation tools (Tilot, 2013, 2014). In particular, the application of a strategic approach to marine spatial planning and of the concept of marine sanctuaries to the high seas was considered (Tilot, 2004; Ardron et al., 2008). The present study has taken this approach further to propose the application of multilayer Rapid Ecological Assessment (REA) (Price et al., 1998; Price, 2004), of management indexes (Tilot et al., 2008a,b; Tilot, 2013, 2016) and of ecohydrodynamics (Moreno Navas et al., 2014), to one or more of the UNESCO/IOC baseline sites.

The present support for international scientific collaboration in multidisciplinary research investigating the environmental impacts of extracting energy resources and minerals from the deep sea provides an opportunity to present our findings. In particular, our areas of study are in line with the objectives of the research programme funded under the European Commission's Framework 7 initiative (e.g. Midas) and the present joint action on deep seabed mining impact initiated under the Strategic Research and Innovation Agenda of the JPI Healthy and Productive Seas and Oceans programme funded by EU's Horizon 2020. The results we present here contribute toward the urgently required full description of the referential state of the areas to be mined (Gollner et al., 2017).

METHODS AND STUDY AREA

The study areas, data from which were used during the UNESCO/IOC baseline study (Tilot, 2006a,b, 2010a), were the NORIA region, within which lie the NIXO 45 and NIXO 41 sites, the American site ECHO 1, and the consortium site IOM BIE (Russia, Bulgaria, Cuba, Poland, Czech Republic, Slovakia) (**Figure 2**); the most detailed and recent work focussed on NIXO 45, a particularly well explored and sampled site within Ifremer's marine geosciences programmes. **The NORIA (AFERNOD)** area covers about 450,000 km^2 delimited between 125°W/135°W and 11°N/16°N, and was surveyed from 1974 on a grid pattern with a station every 2.4 km, in order to determine the structural, bathymetric and sedimentary environment of the nodules. The **NIXO 45** site lies within 130°00′W/130°10′W, 13°56′N/14°08′N, at a mean depth of 4,950 m. The **NIXO 41** site lies within 127°W/130°W, 12°10′N/13°35′W at an depth of 4,700–5,000 m, and extends eastwards to NIXO 45. The **ECHO I** site is located at 14°40′N-125°25′W at a depth of 4,500 m. And the **IOM BIE** site has a midpoint at 11°04′N; 119°40′W and an average depth of 4,300 m.

The methods used for data collection were those standard at the time. The platforms used for video and still photography and physical sampling within the UNESCO/IOC baseline study areas were: the French towed device "Remorquage Abyssal d'Instruments pour l'Exploration" (RAIE), the suprabenthic camera sleigh "Troika," the French camera coupled free sampler "ED1," the autonomous unmanned "Epaulard," the French manned submersible "the Nautile" for the French sites, the American "Deep Tow Instrumentation System" for ECHO I site and for the IOM BIE site, the Russian towed cameras MIR-1 and NEPTUN, and also box cores with photo cameras. Details of these platforms and the photographic and other sampling gear and methods are provided in the UNESCO/IOC report (Tilot, 2006a) (**Table 2**; **Figure 3**).

The assessment of the megafaunal communities presented here is based on the analysis of more than 200,000 photos of the seafloor and more than 55 h of videos. To permit effective interpretation, a photographic atlas was assembled for all the observed megafaunal morphotypes, identified to the highest practicable taxonomic level in collaboration with an international network of taxonomists. The atlas was annotated with observations on the morphology, ethology and feeding behavior of each taxon thus further facilitating identification of the species observed on the imagery. Comparisons were also made with the fauna recorded from the Peruvian basin analyzed during the DISCOL cruise (Tilot, 1989). Information on



FIGURE 1 | Photos of megafauna in the nodule ecosystem taken by the manned submersible "Nautile." From top left to bottom right starting from top: (1) Holothurian *Psychropotes longicauda* being collected over facies C 15% ©IFREMER; (2) Swimming Holothurian *Peniangone leander* ©IFREMER; (3) Holothurian *Psychropotes longicauda* on facies C 40%©IFREMER; (4) Fish *Ophidiid Barathrites* sp., over facies C15%©IFREMER; (5) Holothurian *Psychronaetes hanseni* on facies C20% ©IFREMER; (6) Crustacean Decapoda *Plesiopenaeus* sp. or *Aristaeomorpha* sp. ©IFREMER; Source: Tilot (2010b).

the geographic and bathymetric distribution of each taxon was compiled from data from abyssal regions, mainly in the Pacific and Atlantic, outside the CCZ. Hypotheses of identification for each taxa were collated on the advice of international specialists listed in Appendix I of UNESCO/IOC baseline volume 1 (Tilot, 2006a) (Supplementary Material Document 1).

Data was analyzed using a programme developed by Ifremer for studying the spatial distribution of megafauna photographed *in situ* (Sibuet, 1987). This computer program adds in successive increments the surface area of each photograph calculated from the camera altitude. A multivariate analysis of relationships was then performed with a CYBER 992-31 calculator using SPAD N software.

The bottom topography and distribution of the main facies in NIXO 45 were mapped with facies type being superimposed on plots of several seabed terrain variables using QGIS. These variables included depth, slope (degrees of inclination), aspect (the orientation of the seafloor measured in degrees), and rugosity (a non-metric measure of topographic unevenness).

Value and Sensitivity Indices for NIXO 45

Based on data gathered during the UNESCO/IOC baseline survey of NIXO 45 we developed two provisional indices for management use. The first is a Value Index which incorporates the perceived significances of a range of physico-chemical and biological features described in the Results section. The second is a Sensitivity Index which is based on the likely impacts of exploitation on the observed communities as judged by the available literature. We then assess, based on the available data, the component values for each of the facies recorded in the study and so calculate first the Value Index and then the Sensitivity Index for each facies. The significance and derivation of this approach is explained further in the Discussion section.

RESULTS

Since space limits the extent to which full data can be included, we present here only the main findings. Examples of the megafauna detected through use of video and still imagery are shown in **Figure 2**.

NORIA

Within the wider NORIA area a total of 159 taxa were recorded representing 13 phyla. These were mainly cnidarians (59 taxa), echinoderms (50 taxa including 32 holothurians), chordates (23 taxa including 17 fish) and sponges (21 taxa). Suspension feeders (68 sessile and 10 mobile taxa), that were mainly cnidarians and sponges, displayed a higher taxonomic richness than deposit feeders, that were mainly echinoderms (60 taxa, among which 50 taxa are mobile) and carnivores/scavengers (45 taxa).

NIXO 45

The NIXO 45 site was studied in greater detail that the others. Within it, facies BP and A occurred on slopes and close to escarpments, facies B on the hills rising to about 4,750 m, facies C on the valleys, facies O ancient sediments on the whole area

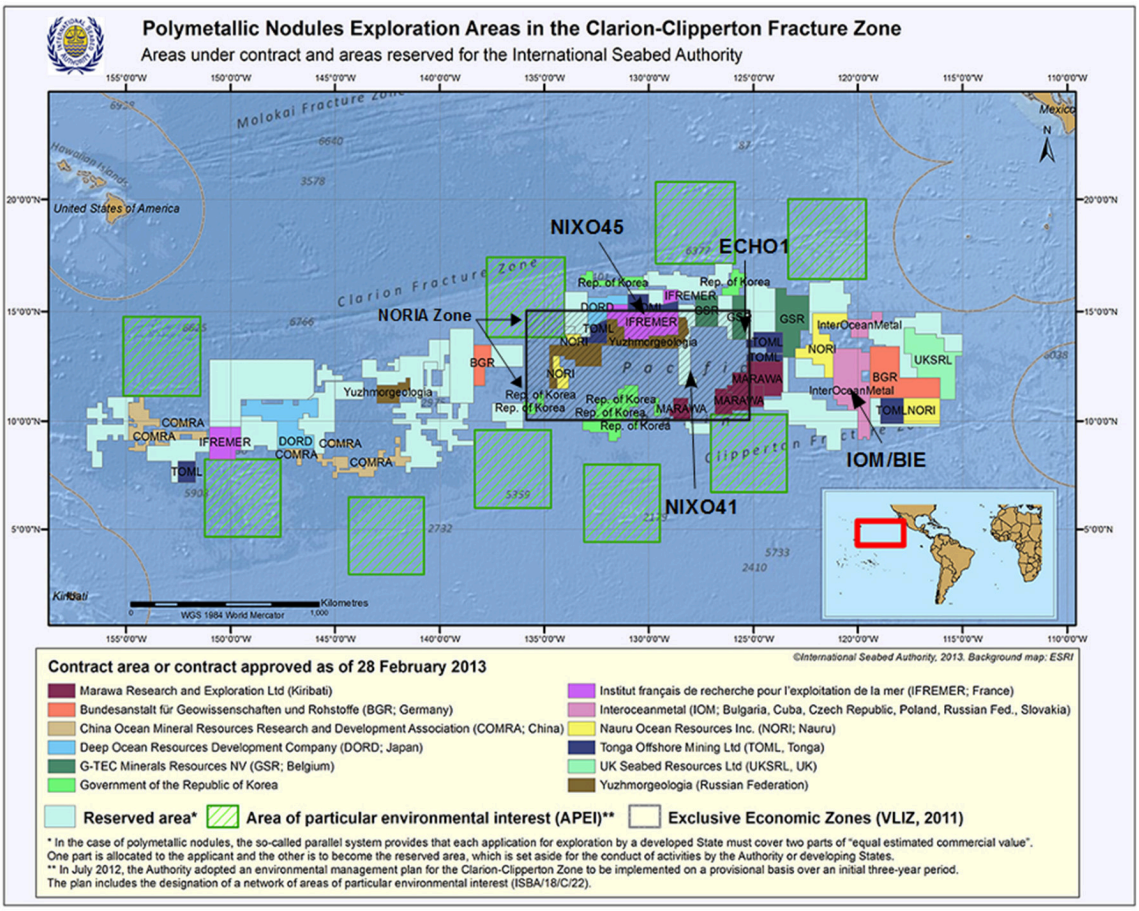


FIGURE 2 | Updated map adapted from https://www.isa.org.jm of the CCZ showing the areas under contract and the areas reserved for the International Seabed Authority, as well as the Areas of Particular Environmental Interest (APEI) and the UNESCO/IOC Baseline Study areas (NORIA, NIXO 45, NIXO 41, Echo 1, IOM BIE).

deeper than 5,000 m and on the accumulation areas, and facies O recent sediments in some transition areas on the valleys when facies C is absent (Du Castel, 1985; Tilot, 2006a). Part of NIXO 45 containing minor structures such as plateaux, hills, secondary valleys and a central depression, with a maximum depth of 5,150 m, is illustrated in **Figure 4**.

Within Nixo 45, 134 different taxa were identified from the analysis of 70,000 m^2 of photographed surface; cnidarians outnumbered echinoderms (as in NORIA as a whole) and sponges. Nodule coverage and abiotic factors such as slope, topography and bathymetry appeared to largely determine the abundance and composition of the faunal assemblage. Overall a higher taxonomic richness was recorded on all nodule facies, with 48 taxa recorded on facies C 2–15%. However, 36 taxa were found on Facies O with recent sediments, more than on facies B and BP (34 taxa), likely because this facies is located on the same valleys as facies C and represents a transition facies between patches of facies C with low nodule coverage. This facies may have nodules under the surface which is not the case with facies O on ancient sediments located in deeper basins. More specifically the habitats in decreasing order of species richness were: facies C 10, facies O with recent sediments, facies C15%, facies C20%, facies BP 35%, facies C2–5%, facies B 50%, facies BP 50%, facies O with old sediments, facies C40%, facies C30%, and facies B40%.

The overall faunal abundance of 553 ind/ha encompassed mainly, in decreasing order, 258 ind/ha cnidarians (mainly actinians and octocorallians), 180 ind/ha echinoderms (holothurians and crinoids) and 56 ind/ha sponges. There were overall more suspension feeders than deposit feeders and carnivores/scavengers, irrespective of nodule-facies. More specifically, there were more sessile suspension feeders than mobile deposit feeders, mobile carnivores/scavengers, motile suspension feeders or sessile deposit feeders. Preferential habitats for suspension feeders were facies O with recent sediments and nodule-facies C 10% on slopes. Among motile suspension feeders, a predominance of actinians was observed, in decreasing order, on facies BP 35%, facies C 30%, facies B 50%, facies C 15%, and on facies O. The preferential habitat for deposit feeders, mainly echinoids and holothurians, was facies C 10%.

Overall, taxa are more abundant on, in decreasing order, the following facies,: facies C 10% on slopes $>15°$, facies O in recent sediments, facies C 15%, facies C 20%, facies BP 35%, facies C

TABLE 1 | Nodules-facies classification based on visual identification from samples and imagery (Voisset and Hein, 1978; Hoffert and Saget, 2004).

Nodule-facies	A	BP	B	C	C+
CRITERIA					
Degree of burial (%)	0	0–30	30–60	60–100	30–60
Est. average diameter in cm	<2–10	2.5–4.5	2.5–10	2.5–>15	2.5–7.5
Morphology	Spherical, polylobate	Heterogen fragments	Disk, ellipsoid polylobate, fragments	Spherical, ellipsoid	Spherical, ellipsoid fragments
Aspect of upper surface	Very smooth	Smooth	Smooth, some granular	Very hummocky equatorial thickening	Hummocky Granular some smooth
Sediment on upper surface	No	Some	Some	A lot	Yes
Aggregated nodules	Very frequent	Very frequent	Never	Never	Never
Density on the seabed	High (30–50%)	High (35–50%)	High (40–50%)	Low (5–20%)	Medium (20–40%)

2–5%, facies B 50%, facies BP 50%, facies O in old sediments, facies C 40%, facies C 30%, and facies B 40% (**Figure 5**).

Some phyla prevail on specific facies, e.g., cnidarians, echinoderms and sponges, on facies C 10% and facies C 30% on slopes. The Echiurian bonnelid worms, *Jacobia birsteini*, have been identified in association to large mounds (more than 2 m long, 80 cm wide and 50 cm high) (Tilot, 1988, 1995, 2006a) which evidence suggests to increase turbulence, induce an exchange with the interstitial water in galleries and influence the depth of nodule cover. They are present, at densities of up to 16ind/ha, mainly in facies B40%, facies B50%, facies BP35%, and facies BP35%. On videos from the Nautile their density was much higher (44 ind/ha), due probably to better viewing conditions (lenses, distance and angle) and the patchiness of populations. There are 13 preferential habitats which can be ranked according to nodule coverage and slope >15° as shown in **Figure 5**. Some facies in NIXO 45 have exclusive taxa such as facies O with ancient sediments (Oligocene to Miocene) and facies C 15%. Habitat heterogeneity was found to be the main factor structuring the distribution of megafaunal assemblages at different scales. In addition there is a marked patchiness in the distribution of specific megafaunal taxa, e.g., of the actinid *Sincyonis tuberculata* which aggregates in great densities in sampling areas above 1,600 m^2 on facies C and above 800 m^2 on facies O according to the Levis, David and Moore indexes and Fisher's coefficient. When comparing data collected with the autonomous unmanned Epaulard and the manned submersible Nautile, the same overall mean abundances (508 ind/ha) and abundances of individual phyla were observed, e.g., cnidarians at 228 ind/ha and echinoderms at 132 ind/ha.

Given the more detailed work undertaken within NIXO 45 it was possible to distinguish the different megafaunal assemblages characteristic of the main facies within this area (see also Tilot, 1992, 2006a). These were elucidated using multi-dimensional scaling (**Figures 6**, **7**) as follows:

Facies O on Ancient Sediments

This facies is characterized by a majority of suspension feeders, notably sponges *Pheronema* sp., ringed hexactinellids *Cladorhiza* sp. (which were most abundant on this facies), octocorallians of the families Primnoidae and Isididae, fixed crinoids of the family Hyocrinidae, and actinians from the families Hormathiidae, Actinoscyphiidae and Amphianthidae (with *Amphianthus bathybium* prevailing). Detritus feeders present are mainly asteroids from the family Pterasteridae, and the holothurians *Synallactes aenigma* and *Benthodytes lingua*. Taxa unique to this facies are sponges of the family Cladorhizidae, sedentary polychaetes with characteristic rounded mounds, asteroids. *Hymenaster violaceus*, holothurians *Benthodytes lingua*, holothurians, gastropods Pterotracheidae and Liparid fish.

Facies O on Recent Sediments

This facies is characterized among suspension feeders mainly by octocorallians, among detritus feeders by isopods of the family Munnopsidae and asteroids of the family Porcellenasteridae, and among carnivores by Ophidioid and Ipnopid fishes. A particular form of bioturbation, the "witch ring" (Heezen and Hollister, 1971), associated with polychaete worms, is typically abundant on this facies. Unique to this facies are the holothurians *Psychropotes longicauda*, the siphonophores *Physonectes* and sedentary polychaetes of the family Cirratulidae.

Facies C 5–10%

This facies is characterized by a high abundance of suspension feeders including octocoralliarids of the families Isididae, Primnoidae and Umbellulidae, corallimorpharids of the family Sideractiidae (*Nectatis singularis* has a high abundance only on this facies) and hexactinellid sponges of the family Hyalonematidae among which *Hyalonema* sp., *Poecillastra* sp., *Phakellia* sp., and *Esperiopsis* sp. are the most abundant. Detritus feeders are mainly the holothurians *Peniagone gracilis*, *Mesothuria murrayi, Paelopatides* sp., *Pannychia moseleyi* and *Enypiastes eximia*, the echinoids *Plesiodiadema globulosum*, isopods of the family Munnopsidae and the sipunculids *Nephasoma elisae*. Carnivores are represented by an Ophidioid fish, a jellyfish of the family Trachynemidae and the decapod *Plesiopenaeus* sp. Unique to this facies are alveolate hexactinellid sponges, the demosponges *Phakellia* sp., bivalves of the family Vesicomyidae and the decapods *Plesiopenaeus* sp.

Facies C 15–25% Including Slopes >15°

This facies is characterized by a high diversity; it is dominated by the suspension feeding actinids *Liponema* spp., *Actinernus verrill, Bolocera* sp., *Actinoscyphia* sp., the sponges *Caulophacus* sp. (which are most abundant on this facies) and *Poecillastra* sp., ring- or dish-shaped hexactinellids of the family Rosselidae, Euretidae, and Cladorhizidae, *Cornucopia* sp. (on slopes), crinoids of the family Antedonidae and polychaetes of the family Sabellidae. Detritus feeders are mostly echinoids of the family Aeropsidae, the holothurians *Peniagone vitrea*, *Meseres macdonaldi*, *Benthodytes* sp., and an asterid of the

TABLE 2 | Details of the different observation platforms and methodologies used on the study sites, together with information on the lengths of transects and numbers of images captured.

Equipment	Photo and video sampling
The free sampler "ED1," a camera coupled to a sampling device, was conceived by the EGMO company. The camera allows a slightly oblique angle of shot in relation to the vertical, photographing an area of about 1 m^2.	A total of 3,213 stations (or photographs) were taken in the CCZ during different campaigns from 1975 onwards and these photographs were examined for this study.
The **"troika"** is a towed suprabenthic camera sleigh, 2.5 × 1 × 1.6 m, developed by Commander J.Y. Cousteau, equipped with 2 Benthos "372" cameras, a Hydroproducts SDA video camera, an Edgerton 382 flash, and a 200 W spotlight. The area covered by photographs is from 1 to 2.5 m^2 and the definition allows identification of objects > than 5 cm	The troika was used in the NORIA area, at sites A and B of the COPANO cruise in 1979 in the CCZ (site A: 132°44′W−16°34′N; site B: 133°20′W-14°30′N). The interval between shots was 6 seconds. A combined total of 2,400 photographs taken at these 2 sites were analyzed in this study.
The **"R.A.I.E" (Remorquage Abyssal d'Instruments pour l'Exploration)** (**Figure 3**) was developed for Ifremer to investigate polymetallic deposits on the seabed. It is equipped with two cameras, one Benthos 377 cameras (28 mm and 16 mm) able to take 3,800 shots and one Benthos 387. The angle of view is subvertical, inclined at 10°, to avoid recording the ballast in the photographic field. For an altitude of 3 m, for example, the surface that is photographed is 7 m^2. The interval between shots in the version of "R.A.I.E." used in the nodule zones is of 16 s.	Close to 76,200 photographs, relating to a total distance of 1,447 km and covering about 144 ha, were taken by the "R.A.I.E." in the NORIA zone. In the NIXO 45 site, a random selection of 48,100 photographs, covering an area of about 91ha, collected by the "Epaulard" and the RAIE was analyzed in sets of 200 photographs each set covering about 3,800 m^2. In the NIXO 41 site, 18,000 photographs were collected during the SEANOD 2 cruise in Dec-Jan 1980–1981, along 274 km of transect, resulting in analysis of 34.2 ha of the seafloor.
The **"Épaulard"** (**Figure 3**) is an autonomous unmanned submersible developed by Ifremer. This submersible is 4 × 2 × 1.1 m in size and is piloted from the surface by acoustic controls to a depth of 6,000 m. During survey work the "Épaulard" is maintained at a height about 5 m from the ocean floor, so that the photographs obtained each cover about 25 m^2. It is equipped with a Benthos 377 camera capable of taking 5,000 photographs at a rate of one every 5 s.	The present study has involved detailed examination of 84,937 photographs, taken since 1975 along 227 km of transect and covering about 160 ha of the NORIA area. In the NIXO 45 site, a random selection of 48,100 photographs, covering an area of about 91 ha, were collected by the "Epaulard" and the "R.A.I.E." and have been analyzed in sets of 200 photographs, each covering about 3,800 m^2.
The **"Nautile"** (**Figure 3**) is an autonomous manned submersible, 8 × 2.7 × 3.45 m, developed by Ifremer and the D.T.C.N. It is capable of descending to 6,000 m and moving at a maximum speed of 2.5 knots during a dive of up to 13 h. It is equipped with a video camera, two photographic cameras and six searchlights (3,000 W). The width of the field covered is about 2 m. Wakefield and Genin (1987) enabled to adapt a Canadian Perspective grid. The cameras can be oriented, generally one oblique and one vertical.	During the NIXONAUT cruise in 1988, the "Nautile" was able to make 16 dives over the nodule zones, in particular NIXO 45, resulting in a total of 55 h of video footage and 2,090 photographs of the seabed in the CCZ that were examined in this study.
The **"Deep Tow Instrumentation System,"** developed for the Scripps Institute of oceanography in the USA, is a metal chassis towed near the seabed. A 35 mm video camera and two Benthos cameras with 50 mm (wide angle) and 70 mm (telephoto) objective lenses. During the ECHO I cruise in June 1983, the height of the camera above the floor was 10 m. As this was often too high for the recognition of the fauna, the 70 mm telephoto objective was used to reveal greater detail.	During the ECHO I expedition (Spiess et al., 1987), the Deep Tow made 11 photographic transects and collected about 5,500 photographs over about 12,400 m^2 of ocean floor, analysis of which is included in this present study.
The Russian **towed cameras MIR-1 and NEPTUN,** as well as **a modified ORETECH (Ocean Research Equipment Technology Inc., USA) towed deep-sea camera system,** were used during a benthic impact assessment experiment, conducted by the Interoceanmetal Joint Organization (IOM) in the CCZ in 1994-1997, the impact being generated by the Deep Sea Sediment Resuspension System (DSSRS).	A total of 14 DSSRS tows were carried out within a site of 1.5 × 2.0 km, and the impact observed from deep-sea camera tows and sediment samples. In October 1994, five transects (7.13–10.13 km long) were surveyed with the MIR-1, resulting in a total of 3,523 technically acceptable frames; in July 1995, five transects (1.91–2.94 km) were surveyed with the NEPTUN yielding a total of 1,038 frames; and in April 1997, four transects (3.0–10.3 km) were surveyed with the modified ORETECH, producing a total of 3,299 frames (Radziejewska and Stoyanova, 2000).

family Brisingidae with 10 arms and the peracarid Cumaceans. The carnivores present are mostly decapods *Nematocarcinus* sp., archaeogastropods and siphonophores of the families Rhodaliidae and Bithitidae, such as *Typhlonus* sp. Unique to this facies are sponges *Hyalonema* sp., members of the family Caulophacidae, the siphonophores *Physonectes*, Chiroteuthid cephalopods, Galatheids with a rounded rostrum, neogastropods of the family Turridae, polychaetes of the family Polynoidae or Aphroditidae and the fish *Coryphaenoides yaquinae*.

Facies C 30–40%

This facies is characterized mainly by the suspension feeding sponges, *Euplectella* sp. and the dark ophiuroids, *Ophiomusium* sp. Common detritus feeders are the holthurians *Peniagone intermedia*, and, in particular, the swimming holothurians *Enypniastes eximia*. Carnivores are represented by polychaetes of the family Hesionidae and Aphroditidae. Unique to this facies are the cephalopods *Benthesicymus* sp., jellyfish of the family Nausithoidae, the holothurians *Orphnurgus* sp. and *Amperima*

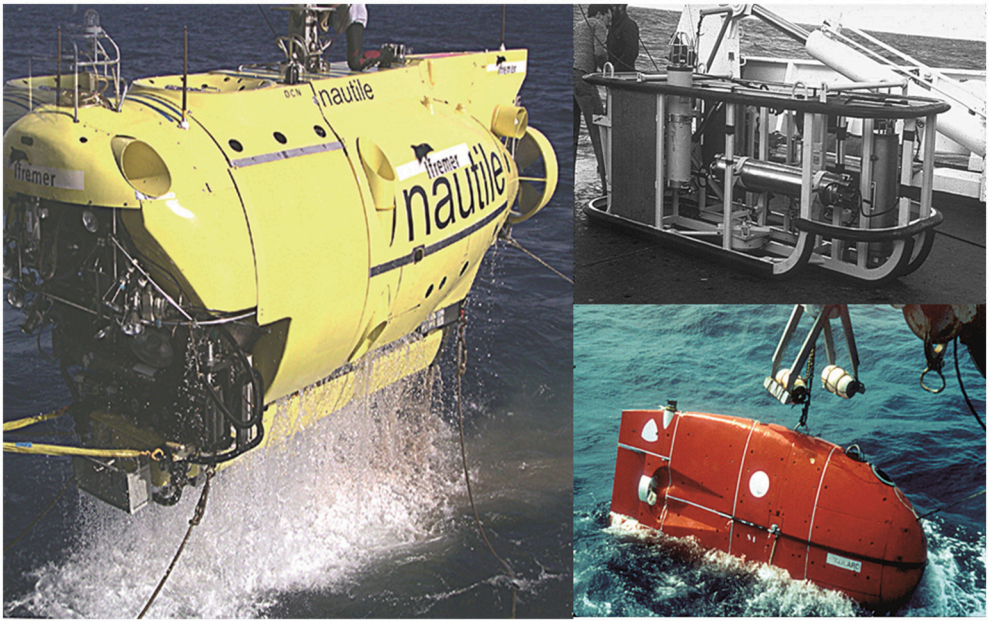


FIGURE 3 | Principle platforms deployed during exploration and sampling of the French NORIA area. **(Left)** Manned submersible "Nautile" **(Top right)** Towed "R.A.I.E"; **(Bottom right)** Remote controlled submersible "Epaulard" (© all figures IFREMER) http://unesdoc.unesco.org/images/0014/001495/149556f.pdf, volume 1, Figures 20, 28, 30 (Tilot, 2006a), http://unesdoc.unesco.org/images/0014/001495/149556e.pdf#223, volume 3, Appendix I, Figures 10, 11 (Tilot, 2010b).

naresi and an unknown, about 30 cm diameter, apparently free-living ascidian.

Facies B (40–50%)

This facies is characterized by a dominance of suspension feeders over detritus feeders. The suspension feeders are mostly the antipatharids *Bathypates patula* and *Bathypates lyra* and the brisingids *Freyella* sp. The detritus feeders are mostly the holothurians *Psychronaetes hanseni* and *Benthodytes typica* and the asteroids *Hymenaster* sp. Also found are aggregations of the actinids *Sincyonis tuberculata* and of swimming aphroditid polychaetes. Unique to this facies are octocoralliarids of the family Umbellulidae, the antipatharids *Schizpathes crassa* and holothurians of the family Deimatidae, *Deima validum*.

Facies BP (35–50%)

This facies is characterized by the prevalence of fixed suspension feeders including vase-shaped sponges, *Poecillastra* sp., which are most abundant on this facies, the actinids *Bolocera* sp., *Sincyonis tuberculata* and *Actinoscyphia*, and the ophiuroids, *Ophiomusium armatum*. Detritus feeders are mainly the holothurians *Synallactes aenigma, Synallactes profundi, Peniagone leander*, and *Benthodytes* sp. (e.g., *B. incerta)*, while the carnivores are mostly polynoid polychaetes. Unique to this facies is a two-horned shaped Hexactinellid sponge.

Facies A (30%)

This facies, which in fact is mainly present in NIXO 41, is characterized by a dominance of suspension feeders over detritus feeders, not due to an abundance of cnidarians as on other facies, but to the high abundance of the ophiuroids, *Ophiomusium armatum*. Other echinoderms present include the holuthurians *Synallactes profundi, Deima validum, Benthodytes* sp., *Mesothuria murrayi, Peniagone leander*, and *Psychronaetes hanseni,* the echinoids *Plesiodiadema globulosum*, and the crinoids *Fariometra parvula*. Cnidarians are mainly represented by actinians (*Actinernus verrill*, *Sincyonis tuberculata),* and by the sponges P*heronema* sp. and other hexactinellids. The bonellid worms *Jacobia birsteini* are particularly abundant on this facies. Unique to the facies are the holothurians *Peniagone vitrea* and *Amperima rosea*.

Facies RCVO (Rocky, with Carbonate Bars and Volcanic Outcrops)

This facies has a much lower diversity of megafauna, consisting mostly of suspension feeding octocorallians, with, in decreasing order of abundance, sponges (*Cornucopia* sp.), actinians and crinoids. Very few detritus feeders or carnivores are evident.

NIXO 41

NIXO 41 appeared to be very similar to NIXO 45 in terms of taxonomic richness and faunal abundance, despite the different methods and different platforms employed (different towed cameras and the manned submersible Nautile). The two areas showed the same orders of abundance and the same dominance of suspension feeders over deposit feeders and carnivores, whatever the facies, save that NIXO 41 tended to display a prevalence of ophiuroids instead of cnidarians.

Overall, the order of abundance of trophic groups within NIXO 41 may be characterized as sessile deposit feeders > mobile deposit feeders > sessile suspension feeders > mobile carnivores/scavengers > motile suspension feeders. The

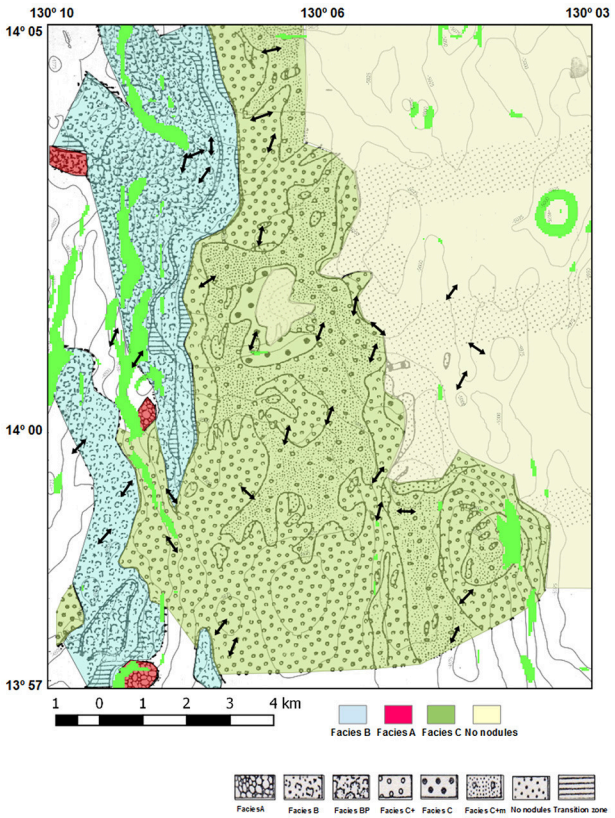


FIGURE 4 | Modified from Du Castel (1985); NIXO 45 GIS Bathymetry, currents and the 4 main Facies represented. The areas with slope greater than 15° are highlighted in green. Bottom current directions derived from the analysis of imagery of the seabed, in particular of the direction of suspension feeders.

relative abundances of megafauna were, in decreasing order of abundance: sipunculids, echinoderms (mainly echinoids and holothurians), echiurians, sponges, and cnidarians (mainly actinians). No taxon was found to be exclusive to NIXO 41, all the taxa recorded having already been identified in NIXO 45. The overall relative taxonomic richness of the facies was, in decreasing order, facies A 30% > C 30% > B 35%. The taxonomic richness, faunal composition, and levels of abundance of individual taxa on facies B 35% were very similar to those recorded on the same facies in NIXO 45. However Facies C+ 30% in NIXO 41 had a greater faunal abundance than the same facies in NIXO 45. The preferred habitat of suspension and deposit feeders was found to be facies C+ 30% with recent sediments. Facies A 30% was found to resemble facies B 40% in NIXO 45 in its population of cnidarians, and sloping facies C 20–40% in NIXO 45 in having a majority of echinoderms and a relatively high density of sessile deposit feeders, with the bonnelid echiurian worms also present.

ECHO I

In ECHO I, we recorded 61 taxa over around 25,200 m^2 sampled over three nodule-facies, with a higher faunal richness (36 taxa) being recorded on facies C 40% than on facies B 45% (23 taxa). Interestingly dredge tracks, produced by pilot-scale mining tests (OMA) in 1978, were still undisturbed and had been partly recolonized by only eight taxa (on facies O). There was an overall dominance of deposit feeders over suspension feeders and carnivores/scavengers, and more specifically an overall relative dominance of sessile deposit feeders over mobile deposit feeders, over sessile suspension feeders, over mobile carnivores/scavengers and over motile suspension feeders.

Findings that differed from NIXO 45 and NIXO 41 were that suspension feeders prevailed on facies nodule B 45% and C 40% on old sediments, with the most actinians (167 ind/ha) occurring on facies C40%. Deposit feeders, mostly echinoderms (mainly echinoids and holothurians) were observed to prevail on facies O. Overall the order of abundance of the main taxa was: sipunculids (50 ind/ha), echinoderms (mainly echinoids and holothurians), echiurians, sponges and cnidarians (mainly actinians). The overall predominance of echinoderms, mainly holothurians and echinoids, observed on photographs from ECHO I (taken with a 50 mm lens) differed from that recorded in NIXO 45, but resembled that recorded in NIXO 41 for facies A 30% and facies C 30%, except that here echinoderms were mainly represented by ophiuroids instead of holothurians, as in NIXO 45. Deposit feeding sipunculids, echinoids and holothurians, were particularly abundant (332 ind/ha) on facies O with old sediments.

IOM BIE

Noticeable at the IOM BIE site was a greater faunal abundance and richness on facies B 45% and facies C 40% than on facies O. Overall there were similar numbers of deposit feeders, of suspension feeders and of carnivores/scavengers. However suspension feeders prevailed on the nodule bearing facies, while, as in ECHO I, deposit feeders were slightly more abundant on facies O. On all facies the predominance of echinoderms (mainly holothurians and ophiuroids) over sponges and cnidarians (hydrozoans) was less marked than at the other sites. As in ECHO I, sponges showed a marked preference for nodule facies C 40%, where echiurians were also particularly abundant. The highest diversities were recorded on gently undulating plains and horst slopes, intermediate diversities on horst tops and trough axles, lower diversities on trough slopes, and the lowest diversity on volcanic slopes. That apart, megafauna appeared more abundant on nodules that were diagenically grown i.e. D-Type and D1 subtype (Cu>Ni>1.2%) than elsewhere.

Value and Sensitivity Indices for NIXO 45

Based on data gathered during the survey work in NIXO 45 we assessed the typical (modal) values of each indicator within both Value and Sensitivity Indexes, and hence the value of each indice for each facies. The resulting values are shown in **Tables 3A,B**. The values support the view that overall type C facies may be considered the most vulnerable, with type C facies 15–20% on a slope of >15° obtaining very similar scores.

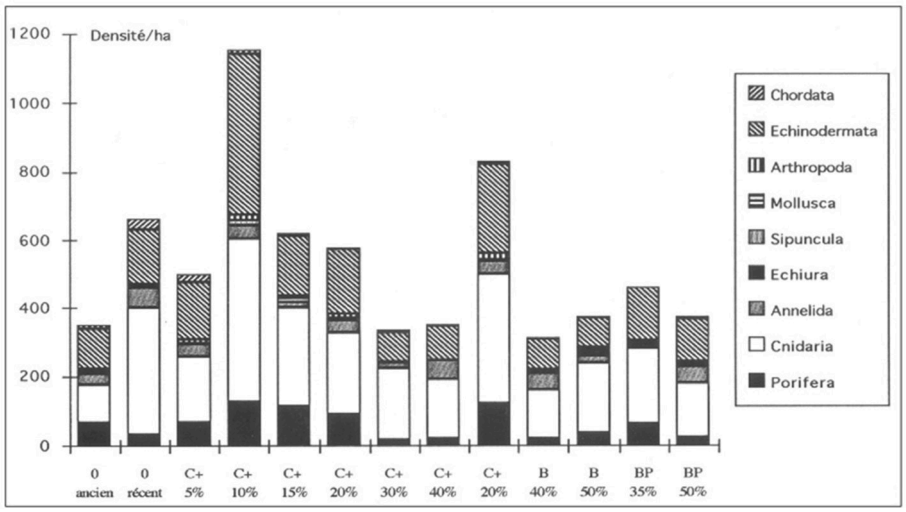


FIGURE 5 | Histogram of the partitioned faunal abundance for each nodule-facies on NIXO 45 (http://unesdoc.unesco.org/images/0014/001495/149556f.pdf, volume 1 Figure 41).

DISCUSSION

Comparison of Different Observation Platforms

Unavoidably, different platforms and cameras with different characteristics were used at different sites. Whereas "Epaulard" could provide only a downward looking vertical view, with the manned submersible "Nautile," organisms were observed from an oblique viewpoint and their behavior captured on video with high quality imagery facilitating reliable taxa identification, in particular when coupled with sampling. In this way it was possible to film the complex swimming movements of some holothurians at several meters above the seafloor (Tilot, 1990). Nevertheless, with "Nautile" it was not practical to undertake numerous replicate quantitative surveys as with the others, in particular the Epaulard. Now the ROVs (such as IFREMER's VICTOR 6000) would produce the best results.

In most cases the abundance estimates obtained from the different platforms appeared very similar. However a comparison at NIXO 45 of data obtained using "Nautile" with that obtained using "Epaulard" found that, using "Nautile," between 1.4 and 1.7 times as many suspension feeders and detritus feeders were recorded as with "Épaulard"; it was unclear whether this was because of differences in effective sampling area or observer error. In contrast the overall abundances of carnivores recorded from the two platforms were quite similar, although in part only because greater abundances of errant polychaetes in photographs taken by "Épaulard" were balanced out by "Nautile" recording greater numbers of fish, some of which seemed to be attracted to the submersible. The largest difference observed was the four times as many echiurian burrows observed from "Nautile," since these were much more easily detected given "Nautile's" oblique, wider field of view.

"Épaulard" and "R.A.I.E." were equipped with the same camera and consequently took similar photographs. However "R.A.I.E." was more difficult to maneuver; being towed it was subject to oscillations but better able to explore broken terrain than "Épaulard." The American "Deep Tow Instrumentation System" and the Russian towed cameras MIR-1 and NEPTUN were even more susceptible to oscillation. Deep Tow 50 mm lens images were often off target, although at other times the higher magnification significantly assisted species identification. But it was not possible to make quantitative comparisons between platforms because they were not available at the same sites, and modest differences in abundance on the same facies were as likely due to differences between sites as between gear. Nevertheless, our experience confirmed conclusions in the literature that the quality of the images does vary with the optical characteristics of the cameras and the construction of the observation system.

Seafloor Topography, Polymetallic Nodule Genesis and Distribution

The patterns of occurrence of the faunal communities described above needs to be viewed against the topography of the seabed and the processes involved in the formation and growth of the nodules themselves. The CCZ has an average depth of 5,500 m and is mainly composed of abyssal hills, covered by nodule fields interspersed with some nodule-free sediment areas (Hoffert, 2008). The hills, elongated in a north-south direction, run parallel to each other, and have irregular, elliptically domed summits and steep, sometimes escarpment-like sides, although in places rocky substrates and seamounts occur (Menard, 1964; Halbach et al., 1988; Kotlinski, 1998). In the Eastern Equatorial Pacific Ocean, sediment coverage is about 20% thicker within morphological depressions than on the ridges and, under the influence of sedimentary processes, the contours tend to soften (Shor, 1959).

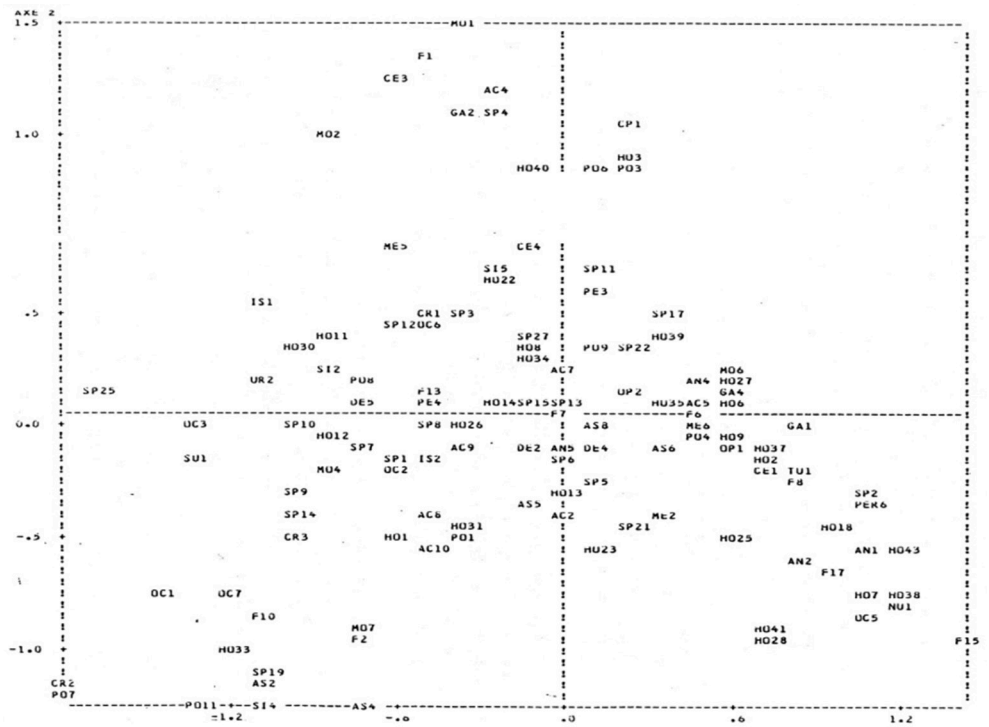


FIGURE 6 | Multi-Dimensional Scaling (MDS) Plot to show the degrees of similarity and difference in distribution patterns of the principal taxa recorded in NIXO 45. (http://unesdoc.unesco.org/images/0014/001495/149556f.pdf, volume 1 Figure 46).

Locally at some sites, such as NIXO 45, seabed morphology comprises horsts and grabens (linear ridges and furrows) that tend to generate stronger currents on slopes that are favored by sessile suspension feeders.

Polymetallic nodules generally lie on the sediment, forming a more or less well developed superficial cover, but can also be buried to several meters within the sediments (Hoffert, 2008). The distribution of nodules seems to be more influenced by the degree of slope than by factors such as longitude, latitude and depth (Friedrich and Plüger, 1974; Wang et al., 2001). The growth of nodules is linked to the migration of manganese across the sediments, and its precipitation and accumulation in the form of concentric layers by oxidation close to the water-sediment interface (Hartmann, 1979). There appear to be two conditions that determine the growth of nodules: the proximity of the CCD and the degree of exposure to currents (von Stackelberg and Beiersdorf, 1991; Hoffert, 2008). The rate of growth of nodules has been estimated by radiochronological methods at several millimeters (4–9 mm) per million years (Harada and Nishida, 1979; Heye, 1988), and the time for reconstitution of nodules at 1 million years for 1–2 mm size nodules (McMurtry, 2001). The role of bioturbation, in particular by deposit feeders, and the role of currents, which are sufficient to transport fine particles, alter chemical properties and reduce the real rate of sedimentation. Both factors have been proposed as important in maintaining the position of the nodules at the sediment surface (Hartmann, 1979; Schneider, 1981; Hoffert, 2008).

The Structure of Faunal Communities in the CCZ

Taken together, the UNESCO/IOC and ISBA/Kaplan/Nodinaut studies (ISBA, 2008) provide evidence of both intra-regional and within-area variability of the structure of benthic faunal assemblages in the CCZ in relation to variation in biotic and abiotic parameters at different scales of space and time. The data revealed a relatively high taxonomic diversity within all faunal categories, with many species new to science. These findings have been confirmed in the more recent literature (Peterson et al., 1998; Glover et al., 2002; Wang et al., 2010; Janssen et al., 2015; Paterson et al., 2015). Key faunal groups within the CCZ are the cnidarians, echinoderms and sponges among the megafauna, and polychaete worms, nematode worms and protozoan foraminifera among the macrofauna and meiofauna; these taxa represent >50% of faunal abundance and species richness in abyssal sediments and display a broad range of ecological and life history types. The faunal assemblages encompass true abyssal species, notable among them Isopoda, Nematoda and Foraminifera and Echinodermata. These conclusions have also been confirmed by more recent studies (Ramirez-Llodra et al., 2011).

Other comparable studies have also supported the view that habitat heterogeneity ("nodule facies," micro-heterogeneity at the nodule level, patches of detritus, biogenic structures and bioturbation), in combination with other factors (bottom currents, sediment chemistry, varying trophic input and

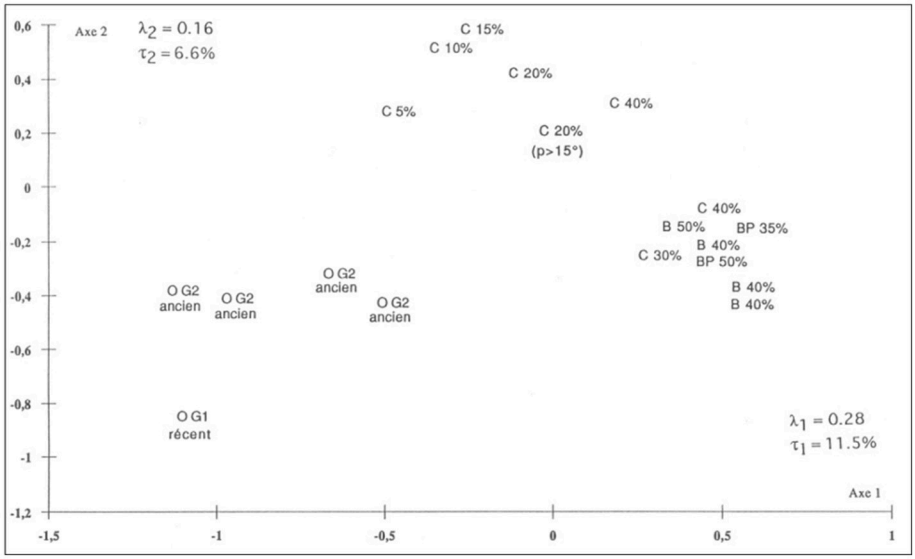


FIGURE 7 | Multi-Dimensional Scaling (MDS) Plot to show the degrees of similarity and difference in the edaphic parameters recorded at each of 20 survey stations within NIXO 45 together with the fascies recorded at each station. (http://unesdoc.unesco.org/images/0014/001495/149556f.pdf, volume 1 Figure 47).

sedimentation rate), are responsible for driving the structure of the epibenthic faunal assemblages. There is clearly a markedly greater abundance and taxonomic richness of megafauna and macrofauna in nodule bearing areas, while meiofauna prefers nodule free areas. This general pattern has been observed not only in the CCZ (Mullineaux, 1987; Morgan, 1991; Radziejewska, 1997; Radziejewska and Stoyanova, 2000; Galeron et al., 2006; ISBA, 2008; Stoyanova, 2008; Gooday et al., 2015; Amon et al., 2016; Vanreusel et al., 2016) but also within the eastern Pacific in the Peru basin, where the nodule crevice fauna has been found to be distinctly different from that living in the sediment in the proximity of the nodules (Tilot, 1989; Thiel et al., 1993).

Nodules clearly provide a distinct habitat for both infaunal communities and for the sessile suspensivore fauna e.g. sponges, actinids, stalked crinoids, octocorallians, sedentary polychaetes, antipatharids, and tunicates, that settle on the nodules. This key conclusion has been supported by Thiel et al. (1993) and Veillette et al. (2007b). In addition, it appears that the nature of the sediments may differ, with softer sediments being located outside of the nodule fields compared to within them. The ISBA/Kaplan/Nodinaut project (ISBA, 2008) also found that megafaunal suspension feeders tend to prevail on nodule-covered areas, while sponges and deposit feeders, particularly holothurians, dominate in the nodule free areas.

That nodule abundance and abiotic factors such as slope, topography and bathymetry, largely determine the abundance and composition of faunal assemblages has been supported in UNESCO/IOC baseline study in which different megafaunal assemblages were found associated with 13 distinctive habitats. However, at a regional scale, the work also provided evidence that (a) there tend to be more suspension feeders at western sites and more deposit feeders at eastern sites, mirroring a gradient in oceanic primary productivity from west to east, and (b) there is an increase in taxonomic richness of megafaunal and macrofaunal assemblages moving from east to west, reflecting a trend of greater substrate heterogeneity at both nodule and facies levels.

As evidenced in Heezen and Hollister (1971) and Kennett (1982), fixed organisms are good indicators of bottom currents. The analysis of the imagery from NIXO 45 found that most suspension feeding assemblages occurred on facies B and C in the eastern and western hills (Tilot, 2006a). Their distribution indicated the general direction of N40-N50 bottom currents. In addition to the main current, water moving in other directions may be observed due to local topography, with secondary currents in contrasting directions resulting within ridge or furrow formations (Flood, 1983). These furrows may be ancient, resulting from the presence of stronger currents in past geological time. The varying near-bed velocity field plays a role in the supply of edible particles to benthic suspension feeders since the horizontal flux of organic seston is a function of the interaction of particles of varying densities with the near-bed velocity field (Muschenheim, 1987). As evidenced in Tilot (2006a), the preferred habitats for suspension feeders are nodule facies vs. facies O, except for facies O on recent sediments. Suspension feeders are particularly abundant on facies C 5–10%, C 15–20% with slope $>15^{\circ}$.

The community structure of the faunal assemblages varies substantially, with a significant turnover in species being evident over a latitudinal range. For foraminifera and polychaetes, the turnover occurs at scales of 1,000–3,000 km across the CCZ (ISBA/Kaplan/Nodinaut) (Levin et al., 2001; ISBA, 2008). For megafauna, the turnover occurs at a larger scale. In the present study (Tilot, 2006a), the high values of the standard deviations of overall abundances of taxa indicates a high within-site variability, likely the result of the non-random dispersion of fauna widely

TABLE 3A | "Value Indexes" for NIXO 45 based on results of UNESCO/COI baseline survey (Tilot, 2006a).

VALUE INDEX Facies/criteria (top number highest value)	O Old sed	O Recent sed	A 30–50%	B 40–50%	C 5–10%	C 15–20% With slope >15°	C 25–40%	BP 35–50%	RCVO rock-outcrop
% nod cover (1–6)	–	–	3	6	1	2	4	5	–
Interest for miners (1–3)	–	–	1	3	2	3	3	3	–
Slope/escarpments (0–3)	0	0	1	2	0	3	0	1	3
Hills (1–3)	1	3	2	3	1	2	2	1	1
Plateaux, valleys (0–3)	0	2	1	3	3	3	3	2	1
Deeper basins (0–1)	1	0	0	0	0	0	0	0	0
Taxon abundance (1–9)	3	7	5	2	9	8	4	6	1
Taxon diversity (1–9)	5	8	4	2	9	7	3	6	1
Susp feeders (1–9)	2	7	3	5	9	8	4	6	1
Detr feeders (1–9)	5	6	4	3	9	8	7	2	1
Carnivores (1–9)	7	5	1	9	6	3	4	8	2
Echinoderms (1–9)	3	6	4	2	9	8	7	5	1
Cnidarians (1–9)	2	6	2	3	9	8	7	4	1
Sponges (1–9)	5	3	2	4	7	9	8	6	1
Echiurian (1–3)	2	3	3	3	2	2	3	3	–
Bioturbation (1–3) superficial to deep	3	3	2	3	(1),3	(1),3	1	2	–
Total	38	59	38	53	80	77	60	60	14
Value index/10	4	6	4	5	8	8	6	6	2

Bioturbation (1–3) was scored as: 1, Surface tracks: (between nodules, circular furrows); 2, Intermediary bioturbation (furrows, lines, imprints); 3, Deep bioturbation (several cm to ms): tumuli, burrows, rosette. The simplified Value Index Score obtained by summing all values and dividing by 10 to give values on a 10-point scale.

reported in the literature (Schneider et al., 1987; Kritzer and Sale, 2010). This phenomenon is particularly evident at the taxon level with e.g., the actinid *Sincyonis tuberculata* aggregating at greater densities in sampling areas above 1,600 m^2 on facies C and above 800 m^2 on facies O according to the Levis, David and Moore indexes and Fisher's coefficient (Tilot, 1992, 2006a).

Primary Productivity, Particulate Organic Carbon Fluxes and Trophic Input

Primary productivity (NECC) in the CCZ is rather low, ranging from 210 to 327 mg C m^3 d^{-1} (Smith and Demopoulos, 2003; Pennington et al., 2006) and in consequence particulate organic carbon fluxes (POCF) range from 0.5 to 1.6 g C $m^{-2}y^{-1}$, placing the CCZ within the broadly defined mesotrophic abyss, as defined by Hannides and Smith (2003). However, considerable variation in the vertical flow of particles has been described (Karl et al., 1996; Baldwin et al., 1998; Karl, 2002; Smith and Rabouille, 2002) and seems related to seasonal patterns, upwelling and El Niño events (Dymond and Collier, 1988; Ruhl and Smith, 2004). The openness of the ocean system leads to benthic-pelagic coupling that may be not only dynamic but highly variable (Rowe, 1971; Pfannkuche and Lochte, 1993).

This variation in flux results in variable deposition of phytodetritus, often in patches, on the sediment surface (Smith et al., 1997, 2008b; Beaulieu and Smith, 1998; Radziejewska and Stoyanova, 2000; Radziejewska, 2002; Stoyanova, 2008), causing in turn significant changes in the structure of the epibenthic megafaunal assemblages and measurable effects on ecosystem function (Smith et al., 1993; Lauerman et al., 1997; Drazen et al., 1998; Thurston et al., 1998; Danovaro, 2008; Loreau, 2008).

There is evidence however that factors other than the varying vertical deposition of phytodetritus can contribute to trophic input and so influence the structure of the epibenthic assemblages. There is a horizontal contribution to nutritive sediment particles in suspension as a result of currents originating to the west in zones of high primary production. In addition, particular edaphic and hydrological conditions above the bottom layer, perhaps related to the topography of the ocean floor, can favor the collection of nutritive sediment particles. In addition, of course the carcasses of larger pelagic organisms provide a significant input of carbon to benthic food webs (Dayton and Hessler, 1972; Haedrich and Rowe, 1977; Smith and Baco, 2003; Tyler, 2003).

Community Dynamics and Diversity

Deep sea benthic megafauna are generally small in body size (with some exceptions e.g. bonnelid worms), show low biomass (less than 2 g m^{-2}) and are extremely delicate, feeding on a thin layer of organic matter at the water-sediment interface. The deep sea megafauna is also characterized by slow metabolic growth, slow maturation, low reproductive potential and low rates of colonization, although compensated for to some extent by greater longevity (Gray, 1977; Gage and Tyler, 1991; Smith and Demopoulos, 2003), all adaptations to extreme environmental

TABLE 3B | "Sensitivity Index" values for NIXO 45 based on results of UNESCO/COI baseline survey (Tilot, 2006a).

Facies/criteria (top number highest value)	O Old sed	O Recent sed	A 30–50%	B 40–50%	C 5–10%	C 15–20% slope >15°	C 25–40%	BP 35–50%	RCVO rock-outcrop
% nod cover (1–6) Weight 1	–	–	3	6	1	2	4	5	–
Interest for miners (1–3) Weight 0	–	–	0	0	0	0	0	0	–
Slope/escarpments (0–3) Weight 2	0	0	2	4	0	6	0	2	6
Hills (1–3) Weight 2	2	6	4	6	2	2	4	2	2
Plateaux, valleys (0–3) Weight 1	0	2	1	3	3	3	3	2	1
Deeper basins (0–1) Weight 0	0	0	0	0	0	0	0	0	0
Taxon abundance (1–9) Weight 1	3	7	5	2	9	8	4	6	1
Taxon diversity (1–9) Weight 2	10	16	8	4	18	14	6	12	2
Susp feeders (1–9) Weight 2	4	14	6	10	18	16	8	12	2
Detr feeders (1–9) Weight 0	0	0	0	0	0	0	0	0	0
Carnivores (1–9) Weight 1	7	5	1	9	6	3	4	8	2
Echinoderms (1–9) Weight 1	3	6	4	2	9	8	7	5	1
Cnidarians (1–9) Weight 2	4	12	4	6	18	16	14	8	2
Sponges (1–9) Weight 2	10	6	4	8	14	18	16	12	2
Echiurian (1–3) Weight 2	4	6	6	6	4	4	6	6	0
Bioturbation (1–3) superficial to deep Weight 2	6	6	4	6	4	4	2	4	0
Total	53	86	52	72	106	104	78	84	21
Sensitivity index/12	5	9	5	7	11	10	8	8	2

*The values in the table were obtained by weighting the values from **Table 3A** (by a factor 0–2) according to their likely sensitivity to environmental impacts such as nodule mining, as judged from knowledge of the ecosystem and information contained in the literature. The Simplified Sensitivity Index Score was obtained by summing all values and dividing by 10 to give values on a 12-point scale.*

conditions (Rex, 1983). As a result the deep-sea fauna may be viewed as a non-equilibrium community in which, following a patch mosaic model, there develops a dynamic mosaic of microhabitats on which species might specialize (Grassle and Sanders, 1973). The patch mosaic phenomenon enables larvae and juveniles specializing on patches of different sorts to grow until they can compete effectively with other species and so coexist and reproduce (Jumars et al., 1990). Thus, temporal and spatial environmental variability are driving factors within the deep-sea ecosystem (Smith et al., 2001). This variability induces fluctuations in biological processes (Tyler, 1988; Gage and Tyler, 1991; Smith and Kaufmann, 1999; Gooday, 2002) including opportunistic feeding (Billett et al., 1988; Jumars et al., 1990) and rapid community responses to variation at the deep-sea floor (Smith et al., 1986). Thus, contrary to possible expectation, the deep-sea benthos is surprisingly species-rich (Snelgrove and Smith, 2002).

Water Masses, Ocean Circulation, Bottom Currents and Climate Variation

Water Masses and Major Currents

The Pacific Ocean is the largest yet freshest of the three major ocean basins. As a result of its lower salinity, the northern North Pacific shows no deep water formation and only a weakened intermediate water formation (Talley et al., 2011). This is an important factor to be born in mind in relation to deep sea mining, especially given the associated operations (including sediment dispersal) anticipated to take place in mid-water. Otherwise the CCZ is dominated by the North Equatorial Current (NEC), which is the broad flow of the subtropical gyre driven westwards by trade winds between 8 and 20°N (Fiedler and Talley, 2006). This ocean region, associated with the highly productive equatorial upwelling to the east, is identified as the North Pacific Tropical Gyre province (NPTG) (Longhurst, 2007). Within it, based on the analysis of samples collected by the 2010 Spanish Malaspina Expedition within the area 7°N−20°N and 99°W−167°W, seven separate water masses can be identified at depths between 200 and 4,000 m (Catalá et al., 2015a,b) (**Figure 8**).

The thermohaline and intermediate waters (500–1,500 m) in the CCZ present unique features as they undergo excessive aging (Catalá et al., 2015b). This anomaly is associated with (1) the high biological productivity of the area, which exports organic material from the surface to the deep ocean (Feely et al., 2004), and (2) the sluggish circulation of the North Pacific that facilitates organic matter sinking. The high productivity of this area together with the weak sub-thermocline currents results in a pronounced oxygen minimum layer (OML) (Kessler, 2006).

A potentially significant management issue is the desirability of preserving the oldest water masses, located in the North East Pacific between 1,800 and 3,500 m according to Talley et al. (2011), because of their unique properties, which stem from their perpetual reworking in the deep ocean. They are characterized by a distinct biogeochemistry, and greater molecular diversity, heterogeneity and complexity of dissolved

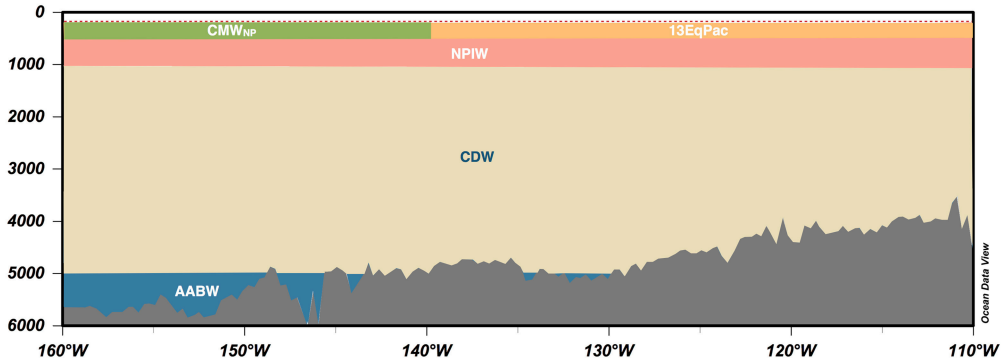


FIGURE 8 | Water masses in the CCZ region. The results are based on the water mass analysis performed in the Malaspina 2010 Circumnavigation (Catalá et al., 2015a,b). Only the most abundant water mass for each site was plotted. The dashed red line represents the upper limit of the water mass analysis (200 m). The lower limit of the Malaspina water mass analysis was at 4,000 m. Below this depth, the information has been extracted from Talley et al. (2011). AABW, Antarctic Bottom Water; CDW, Circumpolar Deep Water; NPIW, North Pacific Intermediate Water; CMWNP, North Pacific Central Mode Water; 13EpPac, Equatorial Pacific Central Water (13°C) (Tsuchiya, 1981).

organic matter (Dittmar, 2015). Given the water masses age, its microbial communities are expected to be adapted to these hypoxic and inhospitable conditions, resulting in a different deep-sea microbial biodiversity. This singular biogeochemistry and microbial flora is presumed to have an impact on the nodule ecosystem via the water column food chain and pelagic-benthic transfer to the sediments; it may also influence nodule genesis. These oldest water masses are thus very vulnerable but the impact of deep-sea mining on them remains difficult to quantify.

Eddy Kinetic Energy and Bottom Currents

The CCZ shows sea-surface elevation variability of 4 cm or more, the maximum sea-height variability denoting excess eddy kinetic energy at the surface that is then transmitted to the sea floor in water depths exceeding 3,000 m (Hollister and Nowell, 1991; Kontar and Sokov, 1994). Many regions of the world ocean show evidence of benthic storm activity in which cold currents in the deep ocean accelerate into powerful sediment-transporting events (e.g., Gardner and Sullivan, 1981; Nowell et al., 1982; Hollister and McCave, 1984; Klein, 1987; Quirchmayr, 2015). Bottom currents in the northeastern tropical Pacific are predominantly weak (Shor, 1959), between 2 and 25 cm/s (Amos et al., 1977), but the flow is characterized by alternation of periods of stronger, quasi-unidirectional currents and periods of slower water movement (McCartney, 1982; Kontar and Sokov, 1994, 1997). Nevertheless, several hydrographic disturbances, such as changes in deep ocean current or periodic "benthic storms," have been recorded during long-term *in situ* monitoring (Richardson et al., 1993; Kontar and Sokov, 1994; Aller, 1997); such changes will in turn impinge on the sediment surface itself (Tkatchenko and Radziejewska, 1998).

Climate Variation and Global Warming

Long-term datasets from the Pacific Ocean basin show that deep-sea communities are strongly affected by climate variation (Smith et al., 2009), as are those of the upper-ocean (Levitus et al., 2000; Barnett et al., 2005). Global warming is anticipated to increase stratification while reducing vertical mixing and nutrient exchange from deeper depths (Sarmiento et al., 2004; Behrenfeld et al., 2006); this in turn is expected to reduce primary production and carbon export to the deep-sea (Huisman et al., 2006), thus impacting the deep ocean ecosystem (Glover et al., 2010), including in particular that of the northeast Pacific (McGowan et al., 1998).

Sedimentation Rates and Mixing, Sediment Plumes

The sediment cover in the CCZ is mainly biogenic, but has been subjected to diagenetic processes to varying extent (Hannides and Smith, 2003; Hoffert, 2008). The surface layer is highly hydrated and can be several 100 m thick, depending on the age of the crust and prevailing sedimentation rates (Menzies et al., 1973; Kotlinski, 1999). Sedimentation rates in the CCZ are currently assessed to be 0.1–10 cm per thousand years (Gage and Tyler, 1991; Smith and Demopoulos, 2003). Although the currents are weak (2–25 cm/s), they are sufficient to transport fine particles and thus, in places, to reduce the rates of sedimentation. Yet they are too slow to cause the erosion of even lightly consolidated sediments (Hoffert, 2008). This explains why in ECHO I dredge tracks produced in 1978 are still visible over 30 years later (Tilot, 1991, 2006a; ISBA, 2008).

The environmental impact studies that have been conducted on nodule ecosystems in the CCZ, the Indian Ocean and the Peru basin (e.g., Amos et al., 1977; Ozturgut et al., 1980; Tilot, 1989; Foell et al., 1990; Thiel et al., 1991, 2001; Bluhm et al., 1995; Fukushima, 1995; Tkatchenko et al., 1996; Radziejewska, 1997; Schriever et al., 1997; Trueblood et al., 1997; Shirayama, 1999; Fukushima et al., 2000; Chung et al., 2001; Oebius et al., 2001; Sharma et al., 2001) mostly focused on alterations to the sediment-nodule interface and impacts to epibenthic faunal communities over very localized areas, compared to the size and the number of areas to be mined. Several reviews (e.g., Smith, 1999; Sharma, 2015; Jones et al., 2017) concluded that there is a lack of information on the potential impact of sediment burial

at the scale of the CCZ and that there would be considerable long-term negative effects on the ecosystem.

These arise because, according to Sharma (2015), for every ton of manganese nodule mined, 2.5–0.5 tons of sediment will be resuspended. Adjacent areas will obviously experience the highest sedimentation rates, but sediment plumes will remain in suspension over long periods and also travel laterally. It is considered that significant sediment loads will clog the filter feeding apparatus of most benthic fauna. Hannides and Smith (2003) estimate that the mining proposed would severely affect benthic communities over 20,000–45,000 km^2 of seabed, with resettlement of up to 95% of the sediment particles released within a period of 3–14 years, depending on the depth of release (Rolinski et al., 2001). That this is so can be appreciated if one considers that even with a current as low as 1 cm per second, water-borne sediment would travel more than 3,000 km within a period of 10 years. Even while still suspended in the water column, the sediment plume is anticipated to have various effects depending on the particular water masses involved.

Ecological Characteristics and Sensitivity Impacts

Given the foregoing it appears that the ecological characteristics of the nodule ecosystem within the CCZ, and their sensitivity to natural and anthropogenic impacts may be summarized as in **Table 4**. This indicates the great sensitivity of much of the fauna to the impacts to be expected from nodule mining. This conclusion in turn highlights the need to manage operations so as to minimize disturbance, an issue we consider in the next sections.

Options for Management of the Impacts of Deep Sea Mining

The Need for Reference Areas and Tridimensional Management

The study of environmental and edaphic conditions expands our understanding of the role of suprabenthic faunal communities in the nodule ecosystem in the CCZ, beyond that developed previously during the UNESCO/IOC and ISBA/Kaplan/Nodinaut studies and elsewhere (Amos et al., 1977; Aller, 1997; Glover and Smith, 2003). The associations between different functional assemblages and particular biotopes, especially within the different nodule facies, reflect the critical factors that drive the species population dynamics; these will likely include environmental heterogeneity, sediment loads and current variability and strength, as well as biotic factors. This dependence of the biological community on precise physical environment needs to be borne in mind when considering the management of such physically driven biotopes, as does the fact that these environments are one of the world's few that remain relatively pristine (Tilot, 2010a). The changes to the seabed and overlying water-column brought about by the mining activity will inevitably impact the different benthic communities present. Further both natural impacts (natural climate variation, benthic storms, El Niño events...) and anthropogenic disturbances (pollution, fishing, seabed mining, oil and gas extraction, disposal of wastes...) generally result in degradation and homogenization of habitats across broad areas (Glover and Smith, 2003; Thiel, 2003; Smith et al., 2008a).

Unfortunately, unlike fisheries for example, polymetallic nodule mining cannot be managed as a sustainable activity, since the reconstitution of nodules following removal, and the restoration of the nodule ecosystem, if ever possible, will take several million years, based on measured rates of nodule formation and growth (Ghosh and Mukhopadhyay, 2000; McMurtry, 2001). Further, commercial nodule mining is envisioned to be the largest-scale human activity ever to affect the deep sea, most probably taking place at a regional scale over an area of more than 3 million km^2 of the deep sea floor. The processes will impact not only the benthos, but also the water column and the surface of the ocean (and even the layer of air over the ocean), at a global regional scale. Thus, faunal communities particular to the nodule ecosystem may even be threatened with extinction.

The temporal and spatial scales of disturbances will naturally determine whether these habitats are able to sustain themselves or change to the extent that the original pristine state can never be recovered (Gundersen and Pritchard, 2002; Berkes et al., 2003; Gollner et al., 2017). The extent of impacts will have crucial consequences for management, since it is easier to repair a damaged ecosystem than restore, it once destroyed. During impact and post-impact phases, changes in species composition of the benthos almost invariably occur, often favoring short-lived species that can quickly colonize after the disturbances (Hughes et al., 2003). Subsequently alternate ecosystem states may be maintained through density-dependent mortality effects (e.g., owing to altered predator-prey ratios) or as a result of populations failing to achieve density thresholds required for reproductive success (Cury and Shannon, 2004). In the face of such threats to ecosystem viability, the critical need to safeguard all aspects of marine as well terrestrial biodiversity has been widely recognized by both scientists and decision takers, and enshrined within a suite of international agreements, in particular, the Convention on Biological Diversity and the United Nations Convention on the Law of the Sea. An emerging multidisciplinary consensus stresses the importance of assessing and actively promoting ecosystem resilience (Peterson et al., 1998; Folke et al., 2004), to achieve which it will be key to establish deep-sea reference areas in the proximity of the deep-sea mining areas.

In addition, it is now widely accepted that for the effective protection of marine biodiversity and marine resources the establishment of marine protected areas (MPAs) is essential (Tundi Agardy, 1994; Margules and Pressey, 2000; Roberts et al., 2001; Edgar et al., 2007). The numbers and extent of MPAs has increased rapidly over recent decades, with a target of protecting 10% of ocean areas incorporated in the Convention on Biological Diversity and a target of 30% recommended by the 2014 Sydney World Parks Congress. While almost all earlier MPAs were designated in coastal waters and within the territorial waters of individual nations or territories, increasingly a move toward declaring large open ocean and high seas protected areas is evident (Corrigan and Kershaw, 2008; Game et al., 2009; O'Leary et al., 2012; Gianni et al., 2016).

TABLE 4 | Table summarizing the potential effects of nodule exploitation on all aspects of the environment and ecology of the CCZ.

General ecological characteristics	Sensitivity to natural and anthropogenic impacts
Primary productivity, particulate organic carbon flux (POCF), trophic input to the seabed	More sensitive. There will be a cumulative impact of natural and anthropogenic activities at the surface, in the water column and on the seabed. Recovery rates need to be assessed for each stage and when cumulated.
Biological features: biomass, respiration, reproduction, growth, recolonization	More sensitive. These processes will show only a slow recovery even if there is some survival. Recent research evidences rapid adaptation to variable trophic input as a result of opportunistic behavior of some taxa, but this represents a change in ecology. It is desirable to test their response to DSM impact and identify thresholds for survivorship and reorganization of food chains.
Biodiversity: taxonomic richness, taxon abundance and distribution at different temporal and spatial scales	Highly sensitive. Although true species diversity is poorly known since populations are undersampled, the high within-site taxonomic diversity observed here implies serious consequences for attempts to conserve the biodiversity of the CCZ. Many species are new to science and therefore need to be protected from extinction until their conservation status is determined. Further various biotic and abiotic parameters influence the species distribution patterns at both regional and intra-area scales. In particular biodiversity and abundance of megafauna is greater on nodule areas vs. nodule free areas, in particular in facies C 5–20% nodules + slopes >15%. Also, information is lacking on sensitivity to impacts of recolonization across different spatial-scale dependence.
Ambient parameters: temperature, light, pressure	Temperature is 1–4°C, pressure increases with depth to 500 bar at 5,000 m; there is no light and very low nutrient levels, although many organisms produce bioluminescence. Organisms may therefore be functioning at the limits of their physiology and have limited ability to cope with additional impacts such as light, vibration and sediment plumes.
Deep Ocean circulation, water masses, bottom currents	Effects of mining unknown, but most probably long lasting and complex in the water column. The low oxygen values of the thermohaline and intermediate waters will likely hinder organic material decomposition, thus increasing sinking rates to the ocean floor. The abyssal domain in the CCZ contains the oldest waters anywhere in the oceans and contains a reservoir of unique and great molecular diversity.
Sedimentation rates and sediment mixing	There is a lack of information on the sensitivity to sediment burial on the scale of the CCZ. There would inevitably be considerable long-term negative biological impact on the majority of the fauna, especially suspension feeders.
Deep seafloor: topography and global geochemical cycles	The ecosystem would be sensitive to topographic modification since the heterogeneity of the substrate and topography play an important role in structuring the faunal assemblages.
Polymetallic nodules: nodule growth and processes maintaining nodules on the seabed	Polymetallic nodule mining cannot be considered as a sustainable activity since the reconstitution of the initial status, even if possible, would take at least 1 million years, based of known rates of nodule growth. Most probably there would be an impact at a regional scale, over an area of more than 3 million km^2 of the deep sea floor.
The structure of faunal communities: distribution at different temporal and spatial scales	Highly sensitive. There are a range of biotic and abiotic parameters that determine the structure of the megafaunal communities at both regional and intra-site scales. As mentioned above, biodiversity and abundance of megafauna are enhanced on nodule areas, compared to nodule-free areas. As also mentioned, some taxa evidence highly non-random distributions, making protection a greater challenge.

However, an issue resulting from the mining planned in the CCZ is that even the earliest mining activity will have an environmental impact at a regional scale, both disturbing ecosystems and even covering nodule fields that are the mining target in surrounding areas. Consequently, the recognition of marine protected areas "where sea-floor mining and other deleterious activities are not allowed" is considered impracticable. Instead the International Seabed Authority (ISBA) has referred to "Areas of Particular Environmental Interest (APEI)," and also to "Impact Reference Zones" and "Preservation Reference Zones" (Smith et al., 2008b; Wedding et al., 2017), the purposes of which would be to assist the ISBA in taking decisions concerning the modalities of nodule exploitation and its impact both on the ecosystems concerned and on the future of mining activities.

To serve both as foci for natural recolonization and as valid reference sites, nodule ecosystem conservation areas need to remain effectively unimpacted, to secure which extensive buffer zones, as designated around many large protected areas, are also essential. They should also be fully representative of the areas to be exploited in terms of depth, physical and chemical nature of the substratum, overlying chemistry and oceanography of the water column, and broader climatology, all of which

ideally require monitoring at different temporal and spatial scales (Freiwald and Roberts, 2006; Académie des Sciences d'Outre-Mer (ASOM), 2010; Tilot, 2010a).

In conformity with the principle of ecosystem-based management and the commitments enshrined in the Convention on Biological Diversity, marine spatial planning in the high seas/deep sea needs to pursue an integrated approach that both considers all stakeholders and sectors of activity and fully appreciates the tri-dimensional status of the oceans (Tilot, 2004; Ardron et al., 2008). The necessary marine spatial planning will require cumulative impact studies that monitor the extent of impact at all scales (Ardron et al., 2008; Foley et al., 2010). In the pelagic domain, numerous species are using the CCZ as feeding grounds near the surface (0–200 m for Bluefin tuna according to Block et al., 2002, see map). Some species extend their range to a depth of 1,000 m, such as for yellowfin tuna, bigeye tuna and swordfishes, to 1,500 m for Bottlenose whale or even approximately 3,000 m for sperm whale (FAO Fisheries and Aquaculture, 2000; Block et al., 2011; Karleskint et al., 2012; Schor et al., 2014, see maps).

The need must also be emphasized for all international instruments and institutions concerned with the High Seas to coordinate in securing the necessary strategic perspective of marine spatial planning (Ardron et al., 2008; Maes, 2008; Académie des Sciences d'Outre-Mer (ASOM), 2010; Tilot, 2010a). In addition, given the complexity of the system and how little knowledge of its function is available, it is strongly advised that the management system incorporates strong elements of adaptive or reactive management, so that preventative action can be taken so soon as new information becomes available.

Measuring Biodiversity

It is broadly accepted that a key objective of environmental management is to protect, if not examples of all habitats and ecosystem in a pristine state, then at least as much of a region's biodiversity as is possible. The well-established arguments for preserving both marine and terrestrial species have been well documented and widely accepted and will not be rehearsed here. But, since complex species record matrices such as those summarized here cannot be easily interpreted, it is relevant to consider how best to use them to compare the biodiversity and ecological value of contrasting nodule field habitats, given that a major problem in any study of this type of environment is that only a limited area can be sampled, and that only for conspicuous megafauna.

Historically several indices of species richness, including simple species number, have been used, both to assess the diversity of an area or habitat and to monitor environmental impacts which almost invariably result in a loss of diversity. However a well-known problem with most traditional indices, including a simple count of the number of species present (species richness), is that the values obtained are highly sensitive to the amount of sampling completed (sample size), making comparisons between studies or even different habitats problematic. Some indices such as Simpson's D attempt to compensate for varied sample size, and also to include a measure of the distribution of species between different abundance categories (evenness). Of the widely used diversity indices, we are most inclined to use Fisher's log alpha, since it is generally considered to have good discriminant ability as well as low sample size sensitivity, while being relatively insensitive to the pattern of relative abundances across species (Kempton and Taylor, 1976; Hayek and Buzas, 1997).

However there are two aspects of diversity that traditional diversity indices do not consider: taxonomic diversity—whether the species present are closely or distantly related; and functional diversity—whether the species present perform similar or contrasting functional roles within the ecosystem (Williams et al., 1996; Price, 2002). It has been considered a priority to develop new metrics that are process oriented and that account for ecosystem dynamics across temporal and spatial scales (Steneck, 2001; Price et al., 2007), highlighting so far as possible the importance of key functional groups, ecological roles and species interactions (Hughes et al., 2005). More recently, a number of such indices have been developed that seem relatively intuitive and comprehensive. These include *average taxonomic distinctiveness* (which is a measure of the mean taxonomic distance between species within a sample or study area), and *variation in taxonomic distinctiveness* which is a measure of the variance in taxonomic distance between species; both are relatively insensitive to sample size (Warwick and Clarke, 2001). In addition, complementing the idea of taxonomic distinctiveness, the concept of *taxonomic similarity*, the mean taxonomic distance between species from different samples or sites, has been introduced, allowing comparison across areas at different scales; this measure is likewise relatively insensitive to sampling intensity (Izsak and Price, 2001; Price, 2002; Price and Izsak, 2005). It is increasingly considered that such t*axonomic diversity* measures of biodiversity at both within-habitat and within-region scales, including across and between large ocean provinces, are invaluable for comparing and monitoring environments where full species lists are impracticable to attain (Warwick and Clarke, 1995, 2001; Gray, 1997; Price, 1999; Izsak and Price, 2001), and we propose their wider use for future assessing and monitoring in the CCZ.

Monitoring and Rapid Environmental Assessment

Long-term monitoring involving the collect of time-series data has been routinely used in coastal and shallow-water ecosystems, such as coral reefs and mangrove forest, for research and management purposes (http://www.gcrmn.org). A wide variety of different methodologies are used, many incorporating the use of still or video imagery (see for example Tilot, 2003; Tilot et al., 2008a,b). However, as with baseline assessments, a major issue is the limited time and resources that can be allocated to sampling at any one site, to which may be added the extremely large areas over which competent monitoring is often desired. Such issues are especially acute when the need to monitor the benthic environment of the CCZ is considered. Deployment of platforms onto the seabed is extremely expensive, only a tiny fraction of the benthic area can be sampled even during an extended voyage, and only a small proportion of the biota can be detected. In response to similar problems workers in shallow and coastal environments have developed protocols for Rapid

Environmental (or Ecological) Assessment (REA), typically for use in situations where large areas, hundreds of km across, need to be assessed, but where no more than a day at most can be spent in sampling at any one site (Price, 1999). In such a situation it is more useful for management purposes to obtain a less precise estimate of abundances across a large sample area, than a very precise estimate over a small sampling area, such as a limited number of quadrats, as is typically attempted in more academic ecological studies.

REAs have in common that they accept semi-quantitative abundance estimates of biological taxa and of environmental factors, often assessed on a pre-defined (often 5- or 6-, or 10-point) scale (Van der Maabel, 1979; Lévesque, 1996; Tait and Dipper, 1998), and that in order to assess diversity or monitor the presence of keystone trophic groups they restrict species identification to pre-selected orders or families, for example birds or echinoderms or fish, such as butterflyfish (Hourigan et al., 1988; Roberts et al., 1988; Montevecchi, 1993; Whitfield and Elliott, 2002; Price, 2004). Such systems have been widely developed and used on land by nature conservation agencies, for example in the widely adopted *phase I* habitat and vegetation classification surveys used by the UK agencies, and have been extended for use in intertidal and subtidal environments, with different habitats and sub-habitats being distinguished on the basis of semi-quantitative but standardized visual estimates of the abundance of key taxa (Nature Conservancy Council, 1990; Rodwell, 1991; Davies et al., 2001). While individual estimates may lack precision, provided methods are applied consistently, statistical methods can be applied to the data, enabling quantitative conclusions to be drawn with equal confidence. Monitoring of deep-sea benthos using the methods described is comparable in that only reasonably conspicuous megafauna can be recorded and that key environmental factors such as sediment type, nodule density and seabed slope, must be assessed visually on a predefined scale. Recording of megafauna via still or video imagery appears to resemble traditional quadrat or transect methodology, but study of such large quantities of imagery can be hugely time-consuming if it is desired to identify every specimen even to genus level, besides which, it must be stressed, many of the megafauna visible in surveys have yet to be identified or even described. Hence there is a strong case for, at least in routine monitoring, subsampling of images, and identification only to order or family level, or trophic or functional group, while restricting determination of species number to a few selected taxa. Where subsequently there is concern about a particular area or taxa, then the archived imagery can be analyzed in full (i.e., without sub-sampling), at a later date, in order to gain greater statistical precision.

In addition, individual taxa may be used as indicator species. For example the Echiurian worm *Jacobia birsteini* (family Bonellidae) appears to be a strong indicator of specific conditions of current, nodule abundance and size, lithographic sediment features, and trophic conditions favoring the presence of such deposit feeders. By the mounds they build, they generate habitat heterogeneity and also influence the dynamics of epifaunal assemblages; they also have a salient role in sediment irrigation and mixing, and so an action on nodule diagenesis (Tilot, 1995, 2006a,b, 2010b). A second good indicator would be the presence of swimming deep sea holuthurians (Elasipods), such as *Enypniastes eximia* and *Peniagone leander*, that seem to be benthopelagical, since they can sometimes be observed at great distance above the seabed (Rogacheva et al., 2012).

Adoption of REA principles does not however exclude the necessity for monitoring of key water column parameters, such as turbidity, current speed, dissolved oxygen concentration, production (chlorophyll), alkalinity etc. It is essential to establish a permanent or semi-permanent tri-dimensional monitoring system recording key environmental variables related to the different mining activities that will take place not only on the seabed, but also in the water column and at the surface. Most of these variables can be measured with sensors, for example current velocity using Lowered Acoustic Doppler Current Profilers (LADCP). Judging by the recent results of Catalá et al. (2015a,b), and the recent research on hydrodynamics close to the seabed (Moreno Navas et al., 2014), and on nepheloid layers (Gardner et al., 1984) in particular for nodule fields (Hoffert, 2008), attention will need to be paid to the selected environmental parameters within five particular depth zones: (i) from the surface to 200 m, (ii) in the central domain (200–500 m), (iii) within the intermediate domain (500–1,500 m), with a pronounced high productivity and pronounced oxygen minimum layer, (iv) in the abyssal domain (>1,500–200 m above the seabed) where are located the oldest water masses of the oceans (between 1,800 and 3,500 m), and v) close to the seabed (200 m above to the seabed including the bottom nepheloid layer which may extend >200 m above the seabed into the abyssal domain), a most interesting layer with direct implications for the functioning of the nodule ecosystem and its genesis. The data should enable the management body to set up guidelines that can be adapted over time and space to reflect expected impacts (Tilot, 2004, 2010a, 2014).

Ecohydrodynamics and Predictive Modeling

Generally, the study of ecological communities has been dominated by a single-scale taxonomically narrow approach, which cannot describe the interaction of environmental and stochastic processes across contrasting functional guilds or spatial scales (Weiher et al., 2011). A novel approach to the understanding of deep-sea benthos is the use of mathematical and geospatial tools, such as 3D hydrodynamic models and particle dispersion models, species distribution models, combined with Geographic Information Systems (GIS), to understand how topography, hydrography and species ecology influence the structure of faunal assemblages (Nihoul, 1981; Nihoul and Djenidi, 1991; Harkema and Weatherly, 1996; Moreno Navas et al., 2014). Such ecohydrodynamic modeling can provide a better understanding of the interaction of such variables as food supply, larval transport, community composition, sediment dynamics, and pollutant dispersion, in response to hydrological factors such as currents, internal waves, upwelling, downwelling and bottom topography (Henry et al., 2013). It has been concluded that often lateral and vertical advection of particles plays an important role in the functioning of deep-sea ecosystems.

Such an approach may also be used to predict the effects of anthropogenic activities. Ecohydrodynamics have for example been applied to a cold-water coral reef ecosystem to predict the effects of bottom fishing and renewable energy installations on reef connectivity and ecosystem function (Watson and Morato, 2004; Henry et al., 2013; Moreno Navas et al., 2014; Roberts and Cairns, 2014). In relation to manganese nodule mining from the deep sea, several studies have modeled sediment transport (Jankowski and Zielke, 2001; GESAMP, 2016), but a broader ecohydrodynamic approach could greatly assist in predicting the nature and scale of the ecological changes likely to be triggered by nodule mining in different sea-floor areas. Similar modeling would also facilitate adoption of a metapopulation persistence approach, since knowledge of dispersal patterns is needed to estimate the patterns of recruitment between different habitat areas and types, so providing key information for marine environmental managers (Botsford and Hastings, 2006).

Use of Management Indices

The interpretation of even the most intuitive diversity indices represents a challenge to non-specialists. With this in mind, several simpler types of index have been developed for presentation to managers and government. Two increasingly common types are Environmental Sensitivity or Vulnerability Indices, used to indicate the priority that should be accorded to different areas or habitats in protecting them from impact, and Management Alert systems, that are used to assist managers determine when action is required to control developing environmental stress, such as fishing pressure or pollution discharge.

Habitat mapping often based on ground-truthed multispectral imagery imported in to a GIS base map has become one of the most important tools in ecology, finding wide use in resource assessment, environmental impact assessment and biodiversity conservation (e.g., Brown and Collier, 2008; Cogan et al., 2009). Environmental Sensitivity Index (ESI) mapping takes this approach a stage further by, within the GIS, weighting the layers representative of different habitats and communities to indicate both their sensitivity to different classes of impact and the likelihood of those different impacts occurring (Buckley, 1982; Tortell, 1992). Such ESI maps, when complemented by maps of socioeconomic data, can be an invaluable tool for managers in working with stakeholders to identify vulnerable locations, establish protection priorities, and identify strategies for minimizing undesirable environmental consequences and promoting desirable ones. These tools have been widely used in terrestrial environments for many years, although there remains a need to incorporate the value of habitats as surrogates for ecosystem services, because there can be important co-benefits or opportunity costs associated with their conservation and or loss during development (Fraschetti, 2012). ESIs have also been widely applied to the marine environment, where their first application was to risk management of spills in the oil industry (Jensen et al., 1993).

Based on data gathered during the survey of NIXO 45 we assessed the typical (modal) values of each component indicator, and so the values of both Value and Sensitivity Indexes for each facies (**Tables 3A,B**). The values support the view that overall type C facies may be considered the most vulnerable, with type C facies 15–20% on a slope of $>15^{\circ}$ obtaining very similar scores. These facies are significant because, based on the data described here, they host the most diverse and abundant megafauna. After facies C, facies O with recent sediments have the next highest richness and abundance values. Among other topographic variables, aspect and changes in current speed appear to be important factors the principal factor influencing cold-water coral reef biodiversity, in particular that of suspension feeders, through locally increasing current strength (Henry et al., 2013; Moreno Navas et al., 2014). It is an issue that miners are most interested on facies C and B with dense nodule coverage.

Alert Systems

Alert Systems, in contrast, are still innovative in their application to marine areas, but have for example been proposed in relation to coastal areas and MPAs in the Red Sea, Gulf of Oman and Arabian Gulf (Tilot, 2003, 2016; IUCN/ROWA, 2016). Levels of key biological (e.g., live coral cover) or physical (e.g., sediment load) or chemical (e.g., oil hydrocarbon) parameters are set to trigger management action to halt the damaging activity (such as an oil leak) and implement restitution activities (e.g., oil spill clear up) as previously determined. Often Alert Systems discriminate more than one threshold, often using a traffic lights system (Halliday et al., 2001; Ceriola et al., 2007; Barange et al., 2010), with for example blue indicating pristine, green indicating minimal impact, orange indicating that one or more critical levels of impact are being approached, and red that immediate management intervention is required. There is a need to develop both sensitivity indexes and alert systems for application to the deep sea, particularly in response to the anticipated huge extent of future nodule mining operations. Both maps and alert system should incorporate increasing knowledge of the CCZ nodule ecosystem, so as to inform the implementation of strict environmental guidelines and secure the desirable conservation-based management responses. The effectiveness of both sensitivity maps and alert systems will be greatly enhanced if ecohydrodymanic models can be incorporated in them, enabling managers to visualize more directly the way in which short- or long-term changes in hydrodynamic environment will impact the biota.

AUTHOR CONTRIBUTIONS

VT discusses the results of a unique comprehensive study she performed on imagery taken on 5 sites of the CCFZ and proposes options for conservation and management using a marine spatial planning approach on the basis of her experience in other marine domains. She proposes with the team to apply these tools to one of the sites she studied. RO worked with VT on the interpretation of the results and the development of 3D environmental assessments, vulnerability indexes and other tools adapted from experience in shallower waters in the world. JMN worked on the GIS and Ecohydrodynamics modeling adapted to the selected study sites on the basis of his experience in cold-water coral communities and coastal zone management. TC

contributed with the analysis of water mass samples of the CCZ taken during the Malaspina circumnavigation expedition in 2010. Her results were fed into the discussions, in particular for the 3D monitoring of the water column.

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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.2018.00007/full#supplementary-material

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CORRECTION
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Corrigendum: The Benthic Megafaunal Assemblages of the CCZ (Eastern Pacific) and an Approach to their Management in the Face of Threatened Anthropogenic Impacts

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A corrigendum on

The Benthic Megafaunal Assemblages of the CCZ (Eastern Pacific) and an Approach to their Management in the Face of Threatened Anthropogenic Impacts
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In the original article, there was an error, in that the role of studies to which text refers was unclear. Corrections have been made to three paragraphs and to the acknowledgements as follows:

Introduction, paragraph 4:

We had the opportunity to participate in a comprehensive study of the biodiversity and distribution of epibenthic megafauna of the CCZ, originally with IFREMER (L'Institut Français de Recherche pour l'Exploitation de la Mer), France, and funding from the EU and the Institut océanographique, France (Tilot, 1988, 1989, 1991, 1992, 2006c; ESCO CNRS IFREMER, 2014), following which the work was updated and expanded (Tilot, 2006a), with particular emphasis on the echinoderm fauna (Tilot, 2006b), with the support of the Intergovernmental Oceanographic Commission (IOC) of UNESCO (published in 3 vol. see: http://unesdoc.unesco.org/images/0014/001495/149556e.pdf# 223), in order to establish a UNESCO/IOC baseline. The third volume published in 2010 expanded the interpretation of a referential state with additional information from other surveys in the region in a collaborative scientific effort. Because of unavoidable conditions, the findings could not be published at the time, other than in restricted circulation reports.

Methods and study area, paragraph 1, last two sentences:

The IOM BIE site (Radziejewska, 1997, 2002; Kotlinski, 1998; Tkatchenko and Radziejewska, 1998; Radziejewska and Stoyanova, 2000; Stoyanova, 2008) serves as complementary source of information to complete the interpretation of the referential state of the CCFZ on a regional and latitudinal scale. This site has a midpoint at 11°04′N; 119°40′W and an average depth of 4,300 m.

Results, IOM BIE, first sentence:
The analysis of the faunal communities recorded on IOM BIE site imagery (Radziejewska and Stoyanova, 2000; Stoyanova, 2008) revealed a greater faunal abundance and richness on facies B 45% and facies C 40% than on facies O.

Acknowledgements, third and fourth sentences:
Later support was provided by the "Intergovernmental Oceanographic Commission of UNESCO (IOC-UNESCO) and the Government of Flanders to update discussions, comparing results with additional information from other surveys in the region. Concerning the IOM BIE site, this would not have been possible without the scientific collaboration of Dr Ryszard Kotlinski (formerly Director-General of IOM), Dr Valcana Stoyanova (IOM) and Prof Teresa Radziejewska (Palaeoceanology Unit, Faculty of Geosciences, University of Szczecin) during the second phase of UNESCO/IOC project (2008–2010) as reflected in vol 3 of IOC Technical Series 69.

The authors apologize for this error and state that these does not change the scientific conclusions of the article in any way.

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Has Phytodetritus Processing by an Abyssal Soft-Sediment Community Recovered 26 Years after an Experimental Disturbance?

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The potential harvest of polymetallic nodules will heavily impact the abyssal, soft sediment ecosystem by removing sediment, hard substrate, and associated fauna inside mined areas. It is therefore important to know whether the ecosystem can recover from this disturbance and if so at which rate. The first objective of this study was to measure recovery of phytodetritus processing by the benthic food web from a sediment disturbance experiment in 1989. The second objective was to determine the role of holothurians in the uptake of fresh phytodetritus by the benthic food web. To meet both objectives, large benthic incubation chambers (CUBEs; 50 × 50 × 50 cm) were deployed inside plow tracks (with and without holothurian presence) and at a reference site (holothurian presence, only) at 4100 m water depth. Shortly after deployment, ^{13}C- and ^{15}N-labeled phytodetritus was injected in the incubation chambers and during the subsequent 3-day incubation period, water samples were taken five times to measure the production of ^{13}C-dissolved inorganic carbon over time. At the end of the incubation, holothurians and sediment samples were taken to determine biomass, densities and incorporation of ^{13}C and ^{15}N into bacteria, nematodes, macrofauna, and holothurians. For the first objective, the results showed that biomass of bacteria, nematodes and macrofauna did not differ between reference sites and plow track sites when holothurians were present. Additionally, meiofauna and macrofauna taxonomic composition was not significantly different between the sites. In contrast, total ^{13}C uptake by bacteria, nematodes and holothurians was significantly lower at plow track sites compared to reference sites, though the number of replicates was low. This result suggests that important ecosystem functions such as organic matter processing have not fully recovered from the disturbance that occurred 26 years prior to our study. For the second objective, the analysis indicated that holothurians incorporated 2.16 × 10^{-3} mmol labile phytodetritus C m^{-2} d^{-1} into their biomass, which is one order of

magnitude less as compared to bacteria, but 1.3 times higher than macrofauna and one order of magnitude higher than nematodes. Additionally, holothurians incorporated more phytodetritus carbon per unit biomass than macrofauna and meiofauna, suggesting a size-dependence in phytodetritus carbon uptake.

Keywords: stable isotopes, Pacific Ocean, DISCOL, C/N-ratio, stoichiometry, carbon limitation, Holothuroidea, deep-sea mining

INTRODUCTION

Abyssal plains, i.e., the ocean floor between 3,000 and 6,000 m water depth, cover more than 50% of the Earth's surface and form the largest ecosystem on earth (Smith et al., 2008). Although the remote abyssal plains seem to be far away from anthropogenic influences, they experience increased pressure from oil and gas extraction activities and disposal of litter and waste (Ramirez-Llodra et al., 2011). In the near future, they may also be affected by climate change (Sweetman et al., 2017), as well as by potential deep-sea mineral extraction of polymetallic nodules (Ramirez-Llodra et al., 2011; Petersen et al., 2016). Polymetallic nodules are potato-like deposits that grow extremely slowly at a rate of millimeters per million years (Guichard et al., 1978). They are typically found at the sediment surface at an average density of 15 kg m^{-2} in the Clarion-Clipperton Zone (CCZ, NE Pacific), 10 kg m^{-2} in the Peru Basin (SE Pacific) and 4.5 kg m^{-2} in the Central Indian Ocean Basin (Kuhn et al., 2017). Nodules provide hard substrate that is essential for some sessile epifauna and associated megafauna (Purser et al., 2016; Vanreusel et al., 2016).The extraction of polymetallic nodules will not only eliminate this hard substrate, but will also disturb and resuspend the surface sediment (Thiel and Tiefsee-Umweltschutz, 2001), which is critical for detritus feeding mobile epifauna and the biota that inhabit the sediment (Borowski and Thiel, 1998; Bluhm, 2001; Borowski, 2001).

We presently lack sufficient knowledge to assess if, and at what rate, the ecosystem will recover from these disturbances (Mengerink et al., 2014). The first insights on ecosystem recovery and resilience were provided by small-scale disturbance experiments that have been carried out at several sites in the Pacific and Indian Ocean (Jones et al., 2017). These studies focused on recovery of density and diversity of meiofauna, macrofauna, and megafauna (Ingole et al., 2000, 2005; Ahnert and Schriever, 2001; Bluhm, 2001; Borowski, 2001). For the DISCOL ("DISturbance and reCOLonization experiment;" Bluhm, 2001) area in the Peru Basin (tropical SE Pacific), in particular, it was found that recovery of megafauna densities after 26 years was between 11% (Anthozoa) and 167% (Holothuroidea) (Gollner et al., 2017). Macrofauna recovered to 85% within seven years (Borowski, 2001) and meiofauna densities after 26 years had recovered to 90% (Gollner et al., 2017). However, the recovery of key ecosystem functions such as nutrient cycling, organic matter processing, and secondary production (Thurber et al., 2014) has not been assessed in detail yet.

Pulse-chase experiments using stable isotopically labeled substrates have been used to study ecosystem functioning and food web dynamics in the deep sea (Middelburg, 2014). These studies are performed either *in-situ* with benthic landers (Moodley et al., 2002; Sweetman and Witte, 2008) or *ex-situ* with sediment cores onboard research vessels (Guilini et al., 2010). In both approaches, phytodetritus, e.g., diatoms or coccolithophores (Jeffreys et al., 2013), or zooplankton fecal pellets (Mayor et al., 2012) that have been enriched in ^{13}C and/ or ^{15}N are added to the benthic ecosystem (*pulse*) to track the uptake and processing of this material by microorganisms, meiofauna, metazoan macrofauna, and foraminifera (*chase*). Despite the high importance of deep-sea megafauna, dominated by holothurians, as e.g., grazers of labile phytodetritus (Miller et al., 2000; Gallucci et al., 2008; Amaro et al., 2010), logistic challenges of deep-sea research have so far hampered the inclusion of megafauna in these stable isotope studies. This represents a major gap in our understanding of abyssal food webs (van Oevelen et al., 2012). Due to slow recovery rates and high vulnerability of deep-sea megafauna to mechanical disturbance (Bluhm, 2001; Vanreusel et al., 2016; Stratmann et al., in review), insight on the contribution of megafauna to important ecosystem functions is particularly relevant in the context of deep-sea mining impact assessments.

In this study, we conducted pulse-chase experiments at the DISCOL experimental area (DEA) to quantify ecosystem recovery of an abyssal ecosystem following a sediment disturbance event that occurred 26 years prior to our study. The DEA is a 10.8 km^2 large circular area (Bluhm, 2001) that was plowed diametrically 78 times in 1989 (Foell et al., 1990, 1992) to induce a disturbance that would mimic small-scale mining of polymetallic nodules. We deployed newly designed benthic incubation chambers that enabled us to include larger megafauna in pulse-chase studies. A recent visit to the DEA allowed us to assess (1) the recovery of ecosystem functioning in the form of carbon uptake and partitioning 26 years after a disturbance event and (2) the contribution of holothurians to the total uptake of fresh phytodetritus.

MATERIALS AND METHODS

Design of the Benthic Incubation Chambers

Benthic incubation chambers (henceforth called CUBEs in reference to their cubical design) were designed by the Royal Netherlands Institute for Sea Research with a large Poly(methyl methacrylate) (PMMA) chamber (50 × 50 × 50 cm, **Figure 1A**, **Supplementary Figure 1**). The CUBEs have an open bottom to enclose a megafaunal specimen on the seafloor. Each CUBE is further equipped with a stirring plate to ensure homogenously mixed water and an injection port with a 30 mL syringe to add a tracer (e.g., phytodetritus or bromide) at a preset time.

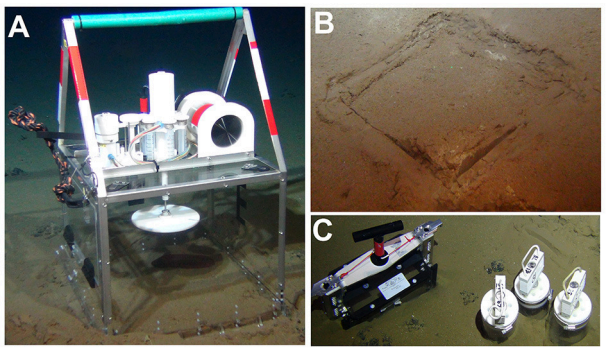


FIGURE 1 | *In-situ* pulse-chase tracer experiment with holothurian at 4,100 m depth in the Peru Basin. **(A)** Deployment of the benthic incubator (CUBE) over *Benthodytes* sp. in a plow track in the DEA west. **(B)** Imprint of CUBE in the sediment after its removal by ROV at the end of the incubation. **(C)** Sampling of sediment inside the CUBE imprint for macrofauna (bladecorer), meiofauna, bacteria, and sediment characteristics (pushcores). Photos by ROV Kiel 6000 (GEOMAR, Kiel, Germany).

A rosette with six 35 mL sampling syringes is fitted on the CUBE for repeated water sampling and a 6,000 m-rated oxygen optode (Contros HydroFlash® O_2; Kongsberg Maritime Contros GmbH, Germany) is inserted in the chamber top for continuous oxygen measurements. A titanium housing holds the battery pack, controller board and temperature sensor. The chamber has a PMMA door (22 × 42 cm) on one side that can be opened and closed by the ROV to allow sampling of enclosed specimens. We also noticed that a deployment with "the door open" significantly minimized sediment disturbance. Four ventilation valves in the top of the CUBE ensure that all air escapes during the descent, but that the CUBE remains sealed at the seafloor. All edges and corners are fortified with a stainless steel 316 frame that ends in a stainless-steel grip bar (total CUBE height: 100 cm). The stirring plate has a diameter of 20 cm, is adjustable in height and has an adjustable stirring capacity from 0 to 100% (0–18 rpm). Stirring tests using uranine as passive tracer for seven different stirring capacities (10, 20, 30, 40, 50, 75, 100%) showed that the uranine concentration reached an equilibrium in the CUBE between 1.5 min (100% stirring capacity) and 6.3 min (10% stirring capacity). This mixing speed is fast enough to consider the water column inside to be homogenously mixed during the incubation. The battery pack consists of 12 D-sized alkaline batteries connected in series (∼18 V power if new batteries are used) and supplies power to the motors of the sampling rosette, stirring plate, injection device and O_2 optode. The CUBEs run fully autonomously following a script that is saved on a micro SD or SDHC card and the program is activated when the ROV triggers a start flap, i.e., a flexible plastic flap at the side of the CUBE (**Figure 1A**).

Experimental Set-Up and Sampling Procedures

The study was conducted during RV Sonne cruise SO242-2 to the DEA (−88.45°E, −7.07°N; **Figure 2A**) in September 2015 and included three different treatments. Two CUBE incubations were performed at the southern reference station (**Figures 2A,C**) with an enclosed holothurian (one identified later as *Benthodytes* sp. and one as *Amperima* sp.) and are henceforth referred to as "Ref+hol." Three incubations were conducted inside the 6-m wide plow tracks (**Figures 2A,B**) each enclosing a holothurian (identified later as twice *Amperima* sp. and *Peniagone* sp. and will be referred to as "PT+hol") and two incubations inside the plow tracks without a holothurian, referred to as "PT-hol." We note that this (unbalanced) experimental design was a compromise following from practical and logistical limitations, while allowing us to focus on ecosystem recovery ("Ref+hol" vs. "PT+hol") and the impact of holothurians ("PT+hol" vs. "PT-hol").

The CUBEs were deployed on a dedicated lander (i.e., the elevator; Linke, 2010) that was placed at a specific location (reference site, disturbed site within a plow track) using a video-guided release system. The ROV KIEL 6000 lifted a CUBE from the elevator and placed it gently over a holothurian to trap it (**Figure 1A**, treatments "PT+hol" and "Ref+hol") or directly on the seafloor (treatment "PT-hol"). The incubation was subsequently started by moving the start flap. Each CUBE deployment lasted 3 days. The first water sample was taken after 45 min, followed by the injection of 0.5 g dry weight (DW) (equivalent to 40 mmol C m^{-2} and 5 mmol N m^{-2}) freeze-dried *Skeletonema costatum* (29 at% ^{13}C and 37 at% ^{15}N). Stirring capacity was increased from 50 to 100% for 10 min and then stopped for 45 min to allow for a homogeneous settling of the freeze-dried diatoms. Afterwards, stirring was set to 25% and subsequently water samples were taken at 0.07, 1, 2, and 3 days after the start of the incubation. Unfortunately, oxygen concentrations could not be measured due to the sensor malfunctioning. At the end of the experiment, the side door of the CUBE was opened and the holothurian was carefully sampled with the suction sampler into the suction container of the ROV. The CUBE was lifted and put aside to leave a clear imprint in the sediment (**Figure 1B**). A bladecorer (20.5 × 9 × 30 cm; designed by Max Planck Institute for Marine Microbiology, Germany) for macrofauna sampling was pressed into the sediment at a random place within the imprint square and released. The bladecorer was left in place until three push cores were taken from within the experimental area to minimize sediment disturbance (**Figure 1C**).

Aboard RV Sonne, the water samples were filtered through a 0.2 µm syringe filter in 20 mL headspace vials for DIC and ^{13}C-DIC concentration analysis and preserved by the addition of 20 µL 0.24 mol L^{-1} $HgCl_2$. The overlying water from the push cores was carefully siphoned off and sieved (32 µm). Subsequently, the cores were sliced in 0–2 and 2–5 cm intervals in a climate room at *in-situ* temperature (2.9°C) and meiofauna from the overlying water was added to the 0–2 cm layer. The different sediment layers of the three push cores from a single CUBE deployment were pooled and homogenized to reduce spatial variability and ensure sufficient amounts of sediment for the planned analyses. Subsequently, two 35 mL subsamples were taken and stored frozen at −21°C for bacterial specific phospholipid-derived fatty acid (PLFA) analysis, sediment porosity, bulk N/^{15}N and organic C/^{13}C

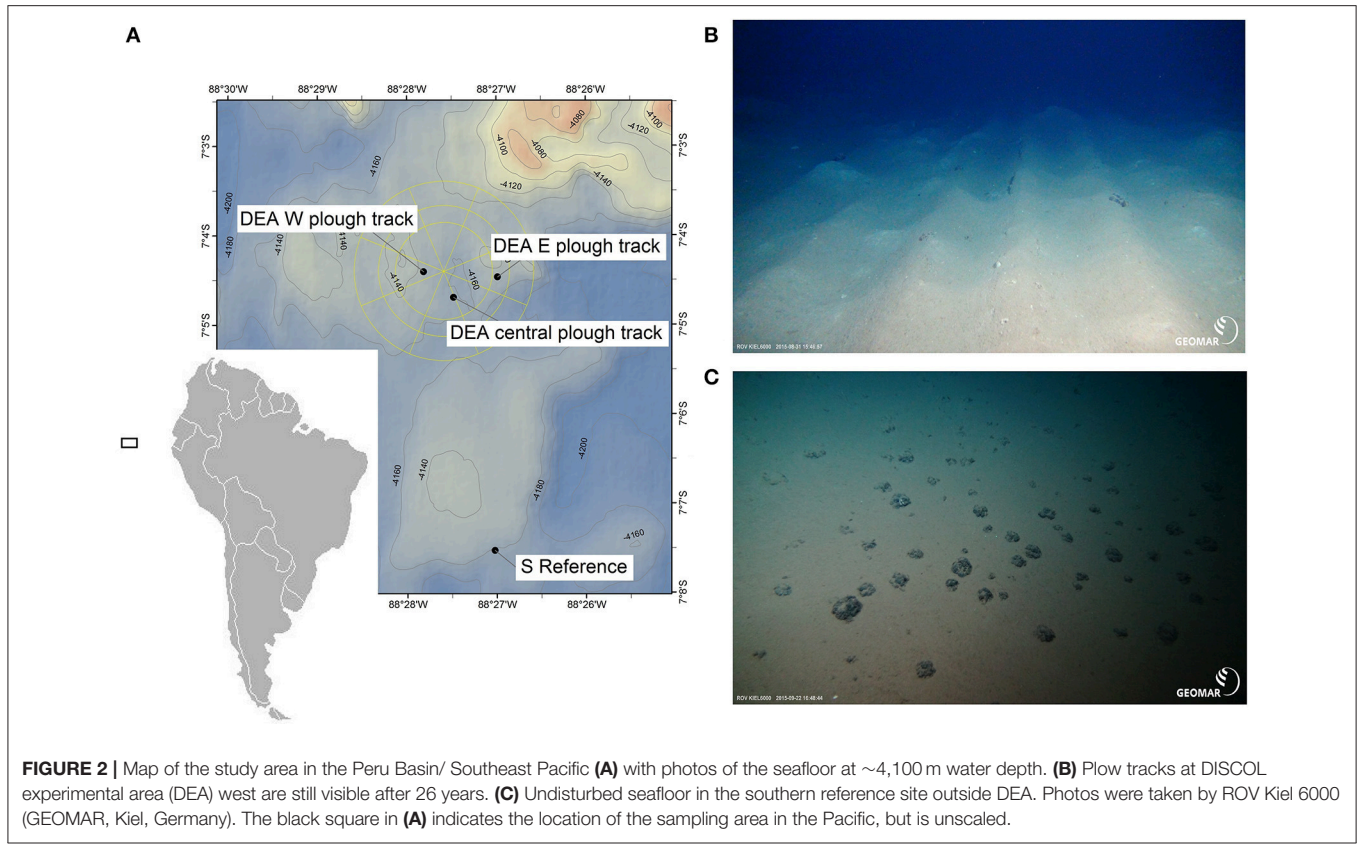


FIGURE 2 | Map of the study area in the Peru Basin/ Southeast Pacific **(A)** with photos of the seafloor at ∼4,100 m water depth. **(B)** Plow tracks at DISCOL experimental area (DEA) west are still visible after 26 years. **(C)** Undisturbed seafloor in the southern reference site outside DEA. Photos were taken by ROV Kiel 6000 (GEOMAR, Kiel, Germany). The black square in **(A)** indicates the location of the sampling area in the Pacific, but is unscaled.

determination. The remaining sediment was fixed in 4% borax-buffered formaldehyde for meiofauna analysis (abundance and isotope enrichment). The upper 5 cm of the sediment in the bladecorer was sieved over a 500 μm sieve with filtered seawater (0.7 μm) at *in-situ* temperature. All macrofauna were fixed in 4% borax-buffered formaldehyde.

Sediment Analysis

Sediment porosity was determined by the weight difference between wet sediment and freeze-dried sediment assuming a sediment density of 2.55 g cm^{-3} (Haeckel et al., 2001). The organic C/^{13}C and N/^{15}N of ∼20 mg freeze-dried, acidified sediment was measured with at Thermo Flash EA 1112 elemental analyzer (EA; Thermo Fisher Scientific, USA) coupled to a DELTA V Advantage Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher Scientific, USA).

Bacteria Analysis

Bacterial biomass and incorporation of phytodetritus ^{13}C into bacteria were determined through the analysis of bacterial-specific PLFAs. Lipids were extracted from ∼2.5 g freeze-dried, finely ground sediment with a modified Bligh and Dyer extraction method (Boschker et al., 1999; Moodley et al., 2002). The lipid extract was fractioned into different lipid classes on a silicic-acid column by sequentially eluting with chloroform (neutral lipids), acetone (glycolipids), and methanol (polar lipids). The polar lipid fraction was derivatized to fatty acid methyl esters (FAME) and measured on a HP 61530 gas chromatograph (Hewlet Packard/ Agilent, USA) coupled with a DELTA-Plus Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA) with a polar analytical column (ZB5-5MS; 60 m length, 0.32 mm diameter, 0.25 μm film thickness; Phenomenex, USA).

The bacterial biomass was calculated based on the concentration of the bacterial specific PLFAs i14:0, i15:0, a15:0, i16:0, and 18:1ω7c, assuming that these represent 28% of the C in all bacterial PLFA and that PLFAs represent 6% of the total C in bacterial cells (Middelburg et al., 2000).

Meiofauna Analysis

Meiofauna was extracted from sediment by washing the samples over a 32 μm sieve and subsequent density centrifugation of the fraction retained on the 32 μm sieve with Ludox HS40 (Dupont) at 3,000 rpm (specific density of 1.18; Heip et al., 1985). The centrifugation was repeated three times, the supernatant was sieved (32 μm) and fixed again in 4% borax-buffered formaldehyde. Meiofauna were organisms counted for density estimates and identified to higher taxon level with a stereomicroscope (50x magnification).

Due to the small body-size of meiofauna, several hundreds of organisms needed to be pooled to have sufficient biomass for the analysis of organic C, ^{13}C, N, and ^{15}N. Therefore only the most abundant taxon, i.e., nematodes, was analyzed. A total of 500 nematodes were randomly handpicked per sample (or less when

not enough nematodes were present in a sample) and transferred to a few drops of Milli-Q water in 8 × 5 mm silver capsules (pre-combusted for 4 h at 450°C). The samples were dried overnight at 60°C, acidified with 20 µL 2% HCl and dried again at 60°C on a hot plate. Capsules were closed and organic C and N content was analyzed following the procedure described above for sediment.

Macrofauna Analysis

Macrofauna was counted for density estimates and identified to lowest taxonomic level and when possible to family level in case of polychaetes using a stereomicroscope. For the analysis of organic C, ^{13}C, N, and ^{15}N, dried, whole organisms were packed in pre-combusted 8 × 5 mm silver capsules, measured following the procedure for meiofauna. Unfortunately, 39% of the macrofauna samples were compromised during sample preparation for the elemental analyzer after density determination and had to be discarded.

Megafauna Analysis

Individual holothurian specimen were retrieved from the ROV suction containers, measured for length, height and width and dissected to separate gut and gut contents from the other somatic tissue. All tissue samples were shock frozen in liquid nitrogen and stored at −21°C. After freeze-drying the somatic tissue excluding the gut tissue was manually ground to fine powder and organic C, ^{13}C, N, and ^{15}N were measured in ∼2 mg holothurian powder as described above for macrofauna.

For the holothurian biomass determination, wet weight (WW) of each specimen was calculated based on the length (L) of the organism using the length-wet weight relationship $WW = 0.859 \times L^{1.813}$ ($n = 13$, $R^2 = 0.68$) identified for intact holothurians from Brown et al. (in review). Holothurian WW was converted to DW using a conversion determined for the holothurians in the Peru Basin in Brown et al. (in review) ($DW = 0.04 \times WW + 0.13$; $n = 13$, $R^2 = 0.98$) and then converted into organic C using the organic C content measured for dried tissue sample of each individual (C content in holothurians ranged from 1.96 to 9.69%).

DIC Analysis

For the analysis of DIC and ^{13}C-DIC, He-gas was injected through the septum into the head-space vials to create a headspace of ∼1.5 mL. The sample was acidified with 10 µL concentrated H_3PO_4 per 1 mL water to transform all inorganic C into gaseous CO_2 in the headspace. From the headspace, a 500 µL sample was taken and injected into a Flash 1112 Series elemental analyzer (EA) coupled via a Conflo III to a Thermo Delta V continuous flow isotope ratio mass spectrometer (IRMS) for the analysis of DIC concentration and isotopic composition (Gillikin and Bouillon, 2007).

Calculations

The incorporation (I) of phytodetritus C and N into particulate organic carbon (POC) and particulate nitrogen (PN), i.e., phytodetritus C and N recovered from the sediment, and bacteria, meiofauna, several macrofauna specimen and megafauna was calculated as follows. The R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the samples and is calculated based on the $^{13}C/^{15}N$ output of the IRMS:

$$R_{sample} = (\delta^{13}C/1000 + 1) \times R_{standard} \text{ or}$$
$$R_{sample} = (\delta^{15}N/1000 + 1) \times R_{standard} \quad (1)$$

where $R_{standard}$ for C is 0.0111802 and for N it is 0.0036782. The fraction (F) of the heavy isotopes ^{13}C and ^{15}N in the sample (F_{sample}) and background ($F_{background}$) material is calculated as:

$$F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R+1) \quad (2)$$

The incorporation of phytodetrital C and N (I) is:

$$I = (F_{sample} - F_{background}) \times \textit{total C} \text{ or } \textit{N pool/phytodetritus enrichment} \quad (3)$$

Macrofauna incorporation rates could not be estimated for all incubations due to unfortunate sample loss (see above). Therefore, the total phytodetritus C and N incorporation rate is defined as the sum of bacteria and nematode phytodetritus C and N incorporation in the top (0–2 cm) and bottom sediment layer (2–5 cm) plus the phytodetritus C and N incorporation into holothurians.

The bulk carbon:nitrogen-ratio (C_{bulk}:N_{bulk}-ratio) of organisms (meiofauna and macrofauna) or somatic tissue (holothurians) and phytodetritus was calculated as the molar ratio. The C_{uptake}:N_{uptake}-ratio was calculated as incorporation of phytodetritus C in the organism divided by the incorporation of phytodetritus N in the organism.

The total biomass of nematodes was calculated as the biomass of an individual nematode (C content of individual nematode = C content of number of measured nematodes divided by number of measured nematodes) times the density of nematodes in the sediment that was determined during counting of meiofauna. The biomass of macrofauna was calculated as the taxon-specific (polychaetes, arthropods, nematodes) macrofauna density times the average tax on specific individual biomass.

Statistical Analysis

Due to the low number of replicates ($n = 2$ for Ref+hol, $n = 3$ for PT+hol, $n = 2$ for PT-hol), differences in density, biomass and ecosystem functioning, i.e., phytodetritus C and N uptake and phytodetritus C-DIC production, between treatments were assessed by comparing the 83.4% confidence intervals (CI). These 83.4% CI were calculated by bootstrapping 10,000 boots-trap replicates using the "boot" package in R (Canty and Ripley, 2017) and represent a type 1 error probability (α) of 0.05 (Knol et al., 2011). This means that the difference in means between treatments is statistically not significant for $\alpha = 0.05$ when the 83.4% CI overlap. Data are presented as mean with lower 83.4% CI—upper 83.4% CI, except for contributions to total density or uptake which are presented as mean ± standard deviation. The C_{uptake}:N_{uptake}-ratios and C_{uptake}:N_{uptake}-ratio were presented as median with 1st quantile and 3rd quantile.

For the analysis of differences in benthic community composition between treatments, the density data of meiofauna

and macrofauna of the upper 5 cm were combined and square-foot transformed before applying the "Analysis of Similarities" (ANOSIM) routine for Bray-Curtis similarity in PRIMER 6 (Clarke and Gorley, 2006).

A one-sample Wilcoxon signed-rank test on log_{10}-transformed data was conducted to determine whether the difference between the median C_{uptake}:N_{uptake}-ratio of nematodes, macrofauna or holothurians combined for all sites and the C_{uptake}:N_{uptake}-ratio of the added phytodetritus were significant.

RESULTS

Visual Inspection of the Sites

Plow marks from the 26-year old disturbance were clearly visible as several centimeter-high ripples and valleys (**Figure 2B**). The sediment surface inside the plow tracks was a mosaic of original brownish surface sediment and white patches originating from sediment that was turned upside down during plowing. The plowing effectively buried the polymetallic nodules into the sediment leaving the plow tracks cleared of the typical hard substrate-providing surface nodules. In contrast, the reference site (**Figure 2C**) had a very smooth brown sediment surface with a homogenous distribution of surface polymetallic nodules.

Benthic Biomass, Density, and Community Composition

Based on the 83.4% CIs, mean biomass of bacteria and macrofauna (**Table 1**) in the upper 5 cm of sediment did not differ between reference site, plow track site or presence of holothurians. In contrast, the mean nematode biomass in the lower (2–5 cm) sediment layer differed significantly between treatments PT-hol [0.22 (0.21–0.22) mmol C m^{-2}] and Ref+hol [0.19 (0.19–0.20) mmol C m^{-2}], but not between PT+hol and PT-hol or Ref+hol in the same sediment layer. Also, the mean nematode biomass in the upper sediment layer (0–2 cm) was not different between the reference sites, plow track site or presence of holothurians.

Total mean meiofauna density was not significantly different between the treatments based on the 83.4% CI (**Table 2**). Nematodes contributed most to the total meiofauna density in the upper 5 cm of sediment (Ref+hol: 88.43 ± 0.19%, PT+hol: 90.62 ± 1.36%, PT-hol: 88.33 ± 0.52%), followed by harpacticoid copepods (Ref+hol: 5.74 ± 0.70%, PT+hol: 5.01 ± 1.09%, PT-hol: 6.98 ± 0.11%) and nauplii (Ref+hol: 4.01 ± 0.77%, PT+hol: 2.92 ± 0.67%, PT-hol: 3.13 ± 0.58%).

Based on the 83.4% CI the total macrofauna densities of the different treatments were significantly different from each other (**Table 2**). About 25% of the macrofauna consisted of polychaetes at Ref+hol (26.14 ± 1.61%) and PT+hol (25.40 ± 9.91%). At PT-hol, polychaetes contributed only 8.33 ± 0.00% to the total macrofauna density and the macrofauna assemblage was dominated by harpacticoids (33.33 ± 0.00%), followed by isopods, tanaids, and nematodes that contributed each 12.50 ± 5.89% to the total macrofauna density. Large nematodes were the second largest contributor to macrofauna density in the Ref+hol treatment (21.59 ± 4.82%), whereas this role was taken by ostracods in the PT+hol treatment (15.08 ± 14.35%). When the combined meiofauna and macrofauna species composition in the upper 5 cm was compared, no significant difference was detected between the three treatments (ANOSIM: $p = 0.87$).

TABLE 1 | Mean biomass and (lower 84.3% CI – upper 84.3% CI) (mmol C m^{-2}) of bacteria, nematodes, and macrofauna in the three different treatments Ref+hol (incubation at reference station outside DEA including holothurians), PT+hol (incubation inside plow tracks inside DEA including holothurians), and PT-hol (incubation inside plow tracks inside DEA without holothurians).

		Bacteria biomass	Nematodes biomass	Macrofauna biomass
PT+hol	0–2 cm	13.81 (8.21–19.45)	0.27 (0.19–0.34)	
	2–5 cm	10.90 (2.47–19.20)	0.24 (0.15–0.33)	
	0–5 cm			0.94 (0.58–1.29)
PT-hol	0–2 cm	13.98 (11.44–16.52)	0.31 (0.15–0.46)	
	2–5 cm	17.82 (8.67–26.89)	0.22 (0.21–0.22)	
	0–5 cm			1.08 (0.37–1.79)
Ref+hol	0–2 cm	11.77 (11.73–11.81)	0.20 (0.16–0.24)	
	2–5 cm	12.73(9.32–16.12)	0.19 (0.19–0.20)	
	0–5 cm			1.90 (0.94–2.85)

TABLE 2 | Total mean density and (lower 84.3% CI—upper 84.3% CI) of meiofauna (ind. 10 cm^{-1}) and macrofauna (ind. m^{-2}) in the three different treatments Ref+hol (incubation at reference station outside DEA including holothurians), PT+hol (incubation inside plow tracks inside DEA including holothurians), and PT-hol (incubation inside plow tracks inside DEA without holothurians).

		Meiofauna density	Macrofauna density
PT+hol	0–2 cm	122 (108–136)	
	2–5 cm	81 (52–111)	
	0–5 cm		363 (343–383)
PT-hol	0–2 cm	118 (82–155)	
	2–5 cm	52 (43–61)	
	0–5 cm		654 (654–654)
Ref+hol	0–2 cm	108 (101–114)	
	2–5 cm	62 (56–69)	
	0–5 cm		518 (439–599)

Phytodetritus Processing

The total mean amount of phytodetritus C (mmol C m^{-2}) that was incorporated into bacteria, nematodes and holothurians (if present) was 0.39 (0.56–1.42) in the PT-hol treatment, 0.99 (0.57–1.43) in the PT+hol treatment and 2.36 (1.80–2.91) in the Ref+hol treatment (**Figures 3A–C**). Based on the 83.4% CI, phytodetritus C uptake by bacteria (mmol C m^{-2}) in the upper sediment layer was significantly different between Ref+hol (0.271–0.447) and PT-hol (0.131–0.269). In the lower sediment layer phytodetritus C uptake by bacteria (mmol C m^{-2}) differed significantly between Ref+hol (0.461–0.509) and PT+hol (0.013–0.040) and between Ref+hol (0.271–0.447) and PT-hol (0.035–0.338). Nematodes in the 0–2 cm

sediment layer from the PT-hol treatment (1.10×10^{-3} – 1.90×10^{-3} mmol C m^{-2}) incorporated significantly more phytodetritus C than nematodes from the same sediment layer of the Ref+hol treatment (6.00×10^{-4} – 9.00×10^{-4} mmol C m^{-2}). In the 2–5 cm sediment layer, the uptake of phytodetritus C (mmol C m^{-2}) by nematodes was in the same range at Ref+hol [4.50×10^{-4} (3.00×10^{-4} – 6.00×10^{-4})] and PT+hol [4.33×10^{-4} (3.00×10^{-4} – 6.00×10^{-4})], but less than at PT-hol [8.50×10^{-4} (6.00×10^{-4} – 1.10×10^{-3})]. Holothurians incorporated (not significantly) more phytodetritus C in treatment Ref+hol [1.52 (1.03–2.00) mmol C m^{-2}] than in treatment PT+hol [0.71 (0.29–1.11) mmol C m^{-2}], but had also a smaller biomass (mmol C ind^{-1}) in the later [Ref+hol: 122.04 (114.60–129.40); PT+hol: 36.77 (28.60–44.85)]. Most of the phytodetritus C (mmol C m^{-2}) that was added to the CUBEs was recovered in the sediment (POC) [Ref+hol: 5.02 (5.01–5.03), PT+hol: 7.29 (2.76–11.80), PT-hol: 6.47 (4.91–8.01); **Figure 3D**] and in the gut content of holothurians [Ref+hol: 0.84 (0.02–1.64), PT+hol: 0.43 (0.03–0.82)]. Respiration of phytodetritus C-DIC (mmol C m^{-2}) occurred linearly over time (**Figure 4**) and after 3 days of incubation it was 3.19 (2.97–3.42) at PT-hol, 2.22 (1.59–2.86) at PT+hol and 3.07 (2.65–3.49) at Ref+hol (**Figure 3E**). Hence, based on the 83.4% CI, phytodetritus C-DIC respiration was significantly lower in treatment PT+hol compared to treatment PT-hol.

Nematodes incorporated between 8.52×10^{-7} (5.83×10^{-7} – 1.12×10^{-6}) mmol N m^{-2} phytodetritus N (PT+hol) and 1.51×10^{-6} (1.23×10^{-6} – 1.78×10^{-6}) mmol N m^{-2} phytodetritus N (PT-hol; **Figure 5**) and therefore significantly less phytodetritus N at PT+hol than at PT-hol. Holothurians took up 0.15 (0.04 – 0.26) mmol N m^{-2} phytodetritus N in treatment PT+hol and 0.31 (0.20–0.41) mmol N m^{-2} phytodetritus N in treatment Ref+hol (**Figure 5**). Between 0.88 (0.36–1.40) mmol N m^{-2} phytodetritus N (PT+hol) and 1.06 (0.73–1.38) mmol N m^{-2} phytodetritus N (Ref+hol) was traced back in the sediment (PN; **Figure 5**).

C:N-Ratio

The phytodetritus that was added in the pulse-chase experiment had a C_{uptake}:N_{uptake}-ratio of 6.54 and C_{bulk}:N_{bulk}-ratio of 7.73. The (median) C_{uptake}:N_{uptake}-ratio of nematodes from both sediment layers combined ($n = 7$), macrofauna ($n = 20$) and holothurians ($n = 5$) was 16.56 (1st quantile: 14.34, 3rd quantile: 7.75), 11.44 (1st quantile: 9.38, 3rd quantile: 18.74), and 4.88 (1st quantile: 4.88, 3rd quantile: 5.06), respectively (**Figure 6**). The C_{bulk}:N_{bulk}-ratios were 7.12 (1st quantile: 6.57, 3rd quantile: 7.68), 5.57 (1st quantile: 3.62, 3rd quantile: 6.27), and 4.48 (1st quantile: 4.46, 3rd quantile: 4.63) for nematodes, macrofauna and holothurians, respectively. The difference between the median of the $\log_{10}$-transformed C_{uptake}:N_{uptake}-ratios of nematodes, macrofauna, somatic tissue of holothurians and the $\log_{10}$-transformed C_{uptake}:N_{uptake}-ratio of the added phytodetritus (0.82) was significant for nematodes ($Z = 2.48$, $p = 0.01$) and macrofauna ($Z = 3.92$, $p \leq 0.001$), but not for holothurians ($Z = -0.67$, $p = 0.63$).

DISCUSSION

This study shows that the ecosystem function of a previously disturbed seafloor had not completely recovered 26 years post-disturbance. Here we relate these results to other studies on small-scale deep-sea disturbances. We also compare the processing of labile phytodetritus by the benthos with similar pulse-chase studies and discuss the role of holothurians in deep-sea ecosystem functioning.

Recovery of Ecosystem Functioning from Deep-Sea Mining

As ecosystem functions in the deep sea are often interrelated to ecosystem services, such as nutrient regeneration and fisheries (Thurber et al., 2014), it is of major concern to decipher which processes of ecosystem functioning are able to recover from deep-sea mining and over what timescales. However, only few studies on ecosystem functions in deep-sea disturbance experiments have been conducted so far, hence a direct comparison between studies is cumbersome.

Our study showed that the bacteria in the 2–5 cm sediment layer did not incorporate phytodetritus C in equal amounts at reference sites compared to plow track sites. This is intriguing, because bacteria can incorporate up to 32% of the total processed label at the Pakistan margin (Woulds et al., 2009) and contribute 74% to total sediment community oxygen consumption at the lower continental slope and in abyssal plains (Heip et al., 2001). Reasons for this difference could be a varying level of sediment reworking by the holothurians inside the CUBEs that made more or less phytodetritus accessible to the subsurface bacteria or a different bacteria composition in the 2–5 cm sediment layer. During the plowing of the seafloor in 1989 parts of the surface sediment were removed and sediment was relocated inside the plow tracks. Hence, the sediment of the 2–5 cm layer in the reference area may be different from the 2–5 cm layer inside the plow tracks. This sediment difference may also cause a concurrent difference in the bacterial composition.

When comparing the total amount of phytodetritus C incorporated in bacteria and nematodes in the upper 5 cm of sediment and taken up by holothurians, the uptake was significantly lower at plow track sites compared to reference sites. This difference might be caused by lower, though not significantly, uptake of phytodetritus carbon by holothurians at the plow track site compared to the reference site. However, it might also indicate that ecosystem function in the form of phytodetritus C processing takes more than 26 years in the DEA to recover from the small-scale disturbance experiment in 1989. In contrast, ecosystem function in the form of respiration of large megafauna, i.e., holothurians, in the DISCOL area recovers faster. Stratmann et al. (in review) compared the respiration rates of holothurians in plow tracks inside DEA ("disturbed") with the respiration rates of holothurians outside plow tracks inside DEA ("undisturbed") and with rates from holothurians at reference sites ("reference") ∼4 km away from DEA. Measured rates of oxygen consumption were not significantly different at the disturbed, undisturbed and reference sites,

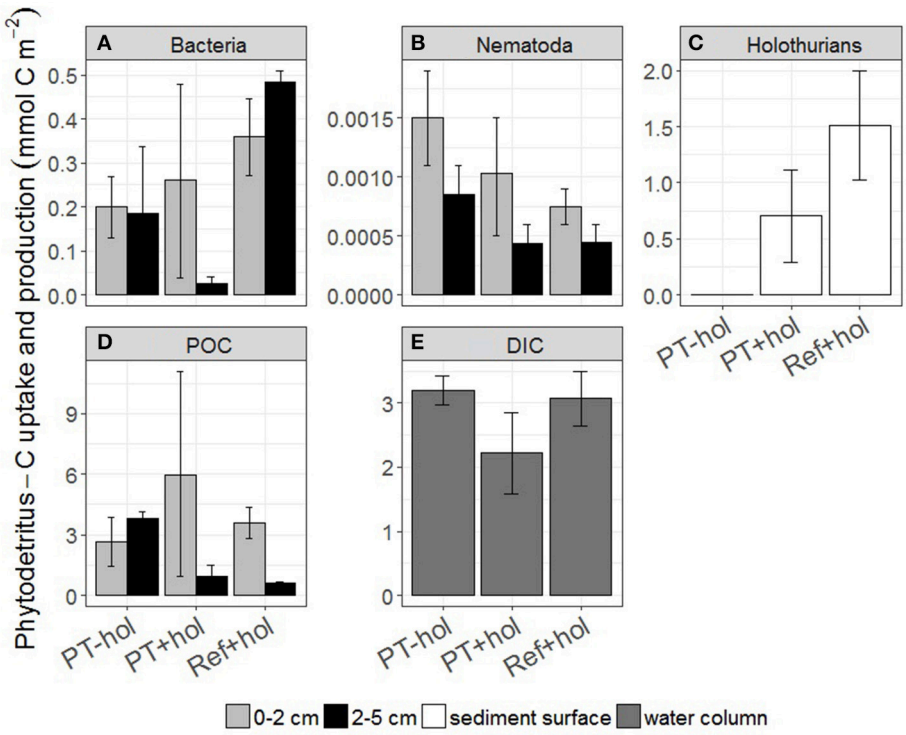


FIGURE 3 | Uptake and production of phytodetritus carbon (in mmol C m^{-2}) **(A)** bacteria, **(B)** Nematoda, **(C)** holothurians, **(D)** POC, i.e., phytodetritus C recovered from the sediment, and **(E)** DIC per sediment depth interval (in cm) and treatment. Error bars show the 83.4% confidence intervals. PT-hol, incubation inside plow tracks inside DEA without holothurian; PT+hol, incubation inside plow tracks inside DEA including holothurians; Ref+hol, incubation at the reference station outside DEA including holothurians.

indicating holothurian community respiration recovery within 26 years.

While future deep-sea mining is unlikely in the Peru Basin, exploration licenses have been issued in the CCZ. Here, ecosystem functioning in the form of nutrient cycling and sediment community oxygen consumption was reported for the French claim of the CCZ (Khripounoff et al., 2006). The authors deployed a benthic lander at a reference station and inside a 2.5 m-wide mining track, which was created in 1978 by dredging away the upper 4.5 cm of the sediment during a small-scale deep-sea mining experiment (Khripounoff et al., 2006). Sediment community oxygen consumption and nutrient fluxes of silicate, nitrate and phosphate were not different between the reference and disturbed location 26 years after the disturbance (Khripounoff et al., 2006), leading the authors to conclude that ecosystem functioning in terms of nutrient cycling had recovered.

Although these studies indicate that ecosystem function recovery takes at least 26 years, time scales for recovery from industrial-size deep-sea mining scenarios will likely be much larger. The DISCOL experiment was conducted in a relatively lightly disturbed area of 10.8 km^2, of which only about 22% were plowed within a month (Thiel and Schriever, 1989). In contrast, a single mining operation will likely remove polymetallic nodules over an area of 300–800 km^2 per year (Smith et al., 2008) and last for 15–30 years (Levin et al., 2016). Moreover, we did not take cumulative effects of deep-sea mining into account like the overlap of sediment plumes from close-by mining operations, changes in POC export fluxes due to climate-change or biodiversity loss due to deep-sea mining (Levin et al., 2016; Sweetman et al., 2017; Van Dover et al., 2017; Yool et al., 2017).

Role of Holothurians in Labile Phytodetritus Uptake

Another main objective of our study was to quantify the role of holothurians in phytodetritus processing. Due to an unfortunate loss of macrofauna samples, we have insufficient data to determine the total phytodetritus uptake by macrofauna except for one deployment. In this deployment (PT+hol), the macrofauna incorporated a total of 1.66×10^{-3} mmol phytodetritus C m^{-2} d^{-1} (0.4% of total uptake), suggesting that macrofauna play a comparatively limited role in the phytodetritus uptake in the Peru Basin. This is also supported by a comparison with *in-situ* studies on short-term phytodetritus processing by Woulds et al. (2009). At sites between 1,200 and 1,850 m at the Pakistan margin metazoan macrofauna took up between (mean ± std) 1 ± 0.5 and 4 ± 1% of the processed label (Woulds et al., 2009). Therefore, the role of holothurians in the processing of phytodetritus at the DISCOL

site can be estimated by neglecting the missing macrofauna contribution. However, this will overestimate the importance of holothurians, because the megafauna density in the CUBEs (1 holothurian specimen 0.25 m^{-2}, extrapolated to 4 holothurian specimen 1 m^{-2}) is two orders of magnitudes higher than under natural conditions (0.02 holothurian specimen m^{-2}; Stratmann et al., in review). To correct for the scale-effect of the incubation chamber and allow translation of the results to natural conditions, we multiplied the average holothurian uptake (0.09 mmol phytodetritus C ind.$^{-1}$ d^{-1}) with the holothurian density in the Peru Basin (240 ind. ha^{-1}; Stratmann et al., in review) to find a daily uptake of phytodetritus C by holothurians of 2.16×10^{-3} mmol phytodetritus C m^{-2} d^{-1}. Hence, based on data from the PT+hol treatment, the contribution of holothurian incorporation of labile phytodetritus to the total uptake of this carbon pool in the Peru Basin is one order of magnitude lower than the contribution of bacteria (7.30×10^{-2} mmol phytodetritus C m^{-2} d^{-1}), but one order of magnitude higher than the contribution of nematodes (4.80×10^{-4} mmol phytodetritus C m^{-2} d^{-1}). It is also 1.3 times larger than the contribution of macrofauna (1.66×10^{-3} mmol phytodetritus C m^{-2} d^{-1}) assuming that the uptake measured for this specific incubation is representative for the whole experiment. Even though bacterial uptake is one order of magnitude higher than the megafauna uptake, the uptake of megafauna is the highest of the metazoans suggesting that their activity should be considered in the structure of deep benthic food webs.

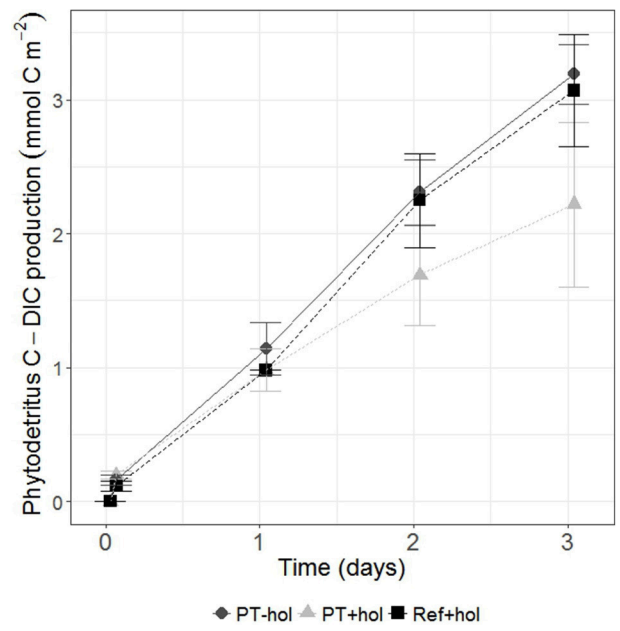


FIGURE 4 | Production of phytodetritus C-DIC (in mmol C m^{-2}) over time (in days) for all treatments. Error bars show the 83.4% confidence intervals. PT-hol, incubation inside plow tracks inside DEA without holothurians; PT+hol, incubation inside plow tracks inside DEA including holothurians; Ref+hol, incubation at the reference station outside DEA including holothurians.

Size-Class Dependent Uptake of Phytodetritus

Because half of the labile detritus that reached the seafloor on the Porcupine Abyssal Plain (PAP) in the NE Atlantic was modeled to be available for deposit feeders (Durden et al., 2017), it is interesting to investigate which size class of metazoan deposit-feeding benthos will most likely benefit from it. Levin et al.

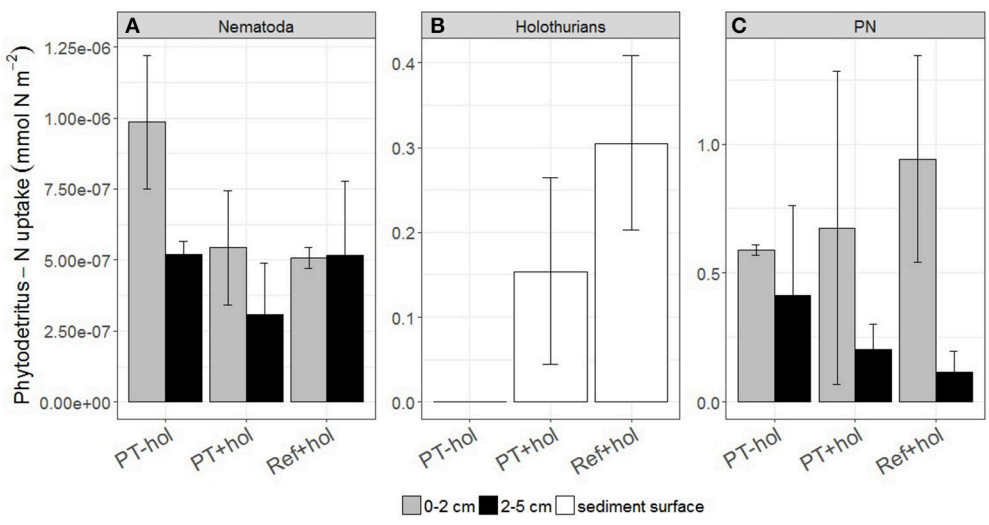


FIGURE 5 | Uptake of phytodetritus nitrogen (in mmol N m^{-2}) by **(A)** Nematoda, **(B)** holothurians, and **(C)** particulate nitrogen (PN), i.e., phytodetritus N recovered from the sediment, per sediment depth interval (in cm) and treatment. Error bars show the 83.4% confidence intervals. PT-hol, incubation inside plow tracks inside DEA without holothurians; PT+hol, incubation inside plow tracks inside DEA including holothurians; Ref+hol, incubation at the reference station outside DEA including holothurians.

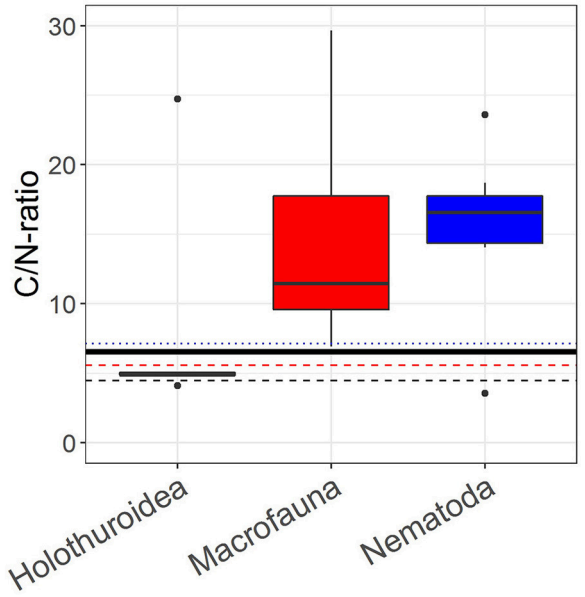


FIGURE 6 | Boxplots of the C_{uptake}:N_{uptake}-ratio, i.e., the incorporation of phytodetritus C in the organism divided by the incorporation of phytodetritus N in the organism, of holothurians ($n = 5$), macrofauna ($n = 20$), and nematodes ($n = 14$). The black solid line represents the C:N-ratio of the added phytodetritus (*Skeletonema costatum*), the black dashed line shows the median C:N-ratio, i.e. the molecular C:N-ratio, of somatic tissue of holothurians, the red dashed line shows the median C:N-ratio of macrofauna and the blue dotted line the median C:N-ratio of nematodes.

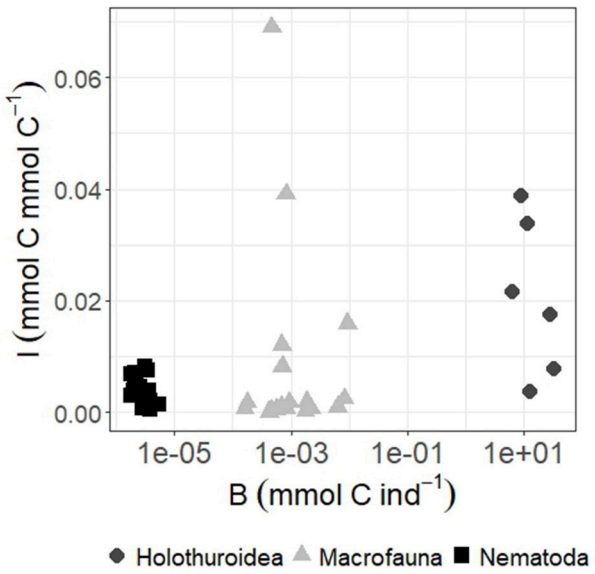


FIGURE 7 | Biomass-specific phytodetritus C incorporation I (in mmol phytodetritus C mmol C−1) per individual biomass B (mmol C ind−1) of holothurians, macrofauna, and Nematoda.

(1999) hypothesized that larger macrofauna organisms have better access to freshly deposited organic matter than smaller macrofauna organisms and therefore would be first in taking it up. These authors tested this hypothesis in one of the first *in-situ* tracer studies at the North Carolina slope (NE Atlantic). The regression of dry weight of macrofauna against diatom tracer uptake across taxa showed no relation with macrofaunal body size (Levin et al., 1999). However, the tested size was limited to macrofauna (>300 μm), whereas in the present study, we studied a substantially broader size spectrum ranging from nematodes/meiofauna (32 μm to 1 mm; 10^{-6} to 10^{-5} mmol C ind^{-1}), macrofauna (>500 μm, 10^{-4} to 10^{-2} mmol C ind^{-1}) to holothurians (>1 cm; 10^{0} to 10^{2} mmol C ind^{-1}) (**Figure 7**). A non-linear regression analysis of these data shows a highly significant positive relationship between individual size B and biomass-specific phytodetritus carbon uptake I [$I = 0.09B^{0.4}$; $n = 43$, $R^2 = 0.78$; $F_{(1,42)} = 145.9$, $p \leq 0.0001$]. Hence, our results suggest that larger organisms in the deep-sea may be more important in exploiting labile phytodetritus than smaller ones and therefore the link between surface productivity and organism productivity might be stronger for larger fauna as opposed to smaller size classes. However, the holothurians we included are selective feeding species, i.e., *Peniagone* sp. and *Amperima* sp. (Wigham et al., 2003; FitzGeorge-Balfour et al., 2010). It will be interesting to find out how strong the correlation between size class and fresh phytodetritus uptake is when other feeding types, such as the fermenters *Psychropotes longicauda* and *Pseudostichopus villosus* (Roberts et al., 2001), conveyor belt feeders or funnel feeders (Massin, 1982), are included.

C:N Stoichiometry

The results of the faunal uptake data were also explored for a potential size-dependent difference in uptake of phytodetritus carbon compared to nitrogen. A comparison of C_{uptake}:N_{uptake}-ratio showed that nematodes and macrofauna took up more phytodetritus carbon relative to phytodetritus nitrogen, whereas holothurians incorporated slightly more phytodetritus nitrogen relative to phytodetritus carbon when comparing it to the food source *S. costatum* (**Figure 6**). The C_{uptake}:N_{uptake}-ratio of the incorporated phytodetritus by nematodes and macrofauna was also higher than the bulk chemical composition of their body tissue, suggesting that they retained preferentially more carbon as compared to nitrogen. Such a dissimilar incorporation has been used to infer carbon vs. nitrogen limitation of benthic organisms. For example, a higher assimilation efficiency or proteinaceous N (mean ± std: 24.6 ± 10.5%) was found for the shallow-water mussel *Mytilus edulis* as compared to carbohydrates (16.7 ± 7.1%) or proteinaceous C (9.5 ± 3.1%) (Kreeger et al., 1996). This led the authors to conclude that *M. edulis* was more nitrogen limited than energy or protein limited and the higher assimilation efficiency of nitrogen would be a strategy to compensate for the low nitrogen content in their food. Although based on a comparatively short incubation time, the higher carbon retention as compared to nitrogen by nematodes and macrofauna may indicate that the nematodes and macrofauna at this specific site are more carbon or energy limited as opposed to nitrogen limited.

CONCLUSION

This is one of the few studies that investigate ecosystem function in an abyssal plain that was previously disturbed by a mimicked deep-sea mining experiment. Despite the low number of replications, the results indicate that the processing of fresh phytodetritus has not fully recovered after 26 years as the uptake of fresh phytodetritus by bacteria, nematodes and holothurians is significantly lower in plow tracks compared to reference sites.

Furthermore, the deployment of large (0.25 m^2) benthic incubation chambers allowed to determine the role of holothurians in the uptake of phytodetritus and showed that their uptake is highest compared to the other metazoans (meiofauna, macrofauna). The analysis of size-class dependent uptake of the phytodetritus resulted in a higher biomass-specific uptake of phytodetritus for holothurians than for nematodes implying that for the metabolism of holothurians phytodetritus is relatively more important than for the metabolism of smaller size classes. Additionally, the elevated C:N-ratios of incorporated phytodetritus in nematodes and macrofauna relative to their tissue C:N ratio let us speculate that these benthic organisms are likely more carbon than nitrogen limited at this particular study site.

AUTHORS CONTRIBUTIONS

AS, DvO, and AV: generated the project funding and conceived the idea of this experiment; AS, DvO, TS, and LM: performed the experiment, collected and analyzed the data; TS, LM, and DvO interpreted the data; TS and DvO: wrote the manuscript with input from all authors.

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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.2018.00059/full#supplementary-material

Supplementary Figure 1 | Technical drawing of the benthic incubation chamber CUBE with indicated dimensions given in mm. 1, oxygen optode; 2, injection device with the start flap; 3, stirring plate; 4, sampling rosette six 35 mL sampling syringes. The arrow points toward the start flap.

Data Availability

The raw data to this article can be found at: https://doi.pangaea.de/10.1594/PANGAEA.885565

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Impacts of Bottom Trawling and Litter on the Seabed in Norwegian Waters

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Bottom trawling and seabed littering are two serious threats to seabed integrity. We present an overview of the distribution of seabed litter and bottom trawling in Norwegian waters (the Norwegian Sea and the southern Barents Sea). Vessel Monitoring System (VMS) records and trawl marks (TM) on the seabed were used as indicators of pressure and impact of bottom trawling, respectively. Estimates of TM density and litter abundance were based on analyses of seabed videos from 1,778 locations, surveyed during 23 cruises, part of the Norwegian seabed mapping programme MAREANO. The abundance and composition of litter and the density of TM varied with depth, and type of sediments and marine landscapes. Lost or discarded fishing gear (especially lines and nets), and plastics (soft and hard plastic and rubber) were the dominant types of litter. The distribution of litter reflected the distribution of fishing intensity (density of VMS records) and density of TM at a regional scale, with highest abundance close to the coast and in areas with high fishing intensity, indicated from the VMS data. However, at a local scale patterns were less clear. An explanation to this could be that litter is transported with currents and accumulates in troughs, canyons, and local depressions, rather than reflecting the fisheries footprints directly. Also, deliberate dumping of discarded fishing gear is likely to occur away from good fishing grounds. Extreme abundance of litter, observed close to the coast is probably caused by such discarded fishing gear, but the contribution from aggregated populations on land is also indicated from the types of litter observed. The density of trawl marks is a good indicator of physical impact in soft sediments where the trawl gear leaves clear traces, whereas on harder substrates the impacts on organisms is probably greater than indicated by the hardly visible marks. The effects of litter on benthic communities is poorly known, but large litter items, such as lost fishing gear may add to the direct negative effects of bottom trawling.

Keywords: marine litter, bottom trawling, deep-sea, anthropogenic impact, vessel monitoring system

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INTRODUCTION

The Norwegian exclusive economic zone (EEZ) is large (2.1 Mkm^2), and most of it is deep sea (>200 m). It includes a long coastline with numerous fjords, a wide shelf with banks, inserted canyons, and troughs, and an abyssal plain with depths down to 3,970 m. The size of the Norwegian population and industry sectors that may impact the marine ecosystems is small, with the fisheries, petroleum industry, and shipping as major activities. Bottom trawling and seabed

littering are probably the two most serious threats to seabed integrity [Descriptor 6 in the European Union's Marine Strategy Framework Directive (MSFD); Galgani et al., 2015; Eigaard et al., 2016]. Knowledge about the distribution and intensity of these pressures are crucial for designing relevant regional management plans with the aims to achieve good environmental status (GES) (EC, 2008).

Fishing with bottom trawl has long traditions on the continental shelf in Norwegian waters. The target species in this area are mainly cod and haddock, but also deep sea prawns. These commercial stocks have for periods been fished extensively. Bottom trawling is mainly confined to areas where the target species aggregate. While the prawn fishery has been conducted both far north, including areas around Svalbard, and far south in the Skagerrak branch of the North Sea, the cod fishing areas has been gradually expanding northwards in recent years. It is known that bottom trawling has negative effects on benthic communities and habitats (Løkkeborg and Fosså, 2011; Lyubin et al., 2011; Puig et al., 2012; Buhl-Mortensen et al., 2013, 2016; Jørgensen et al., 2015). Especially sessile megafauna is negatively affected by breakage and dislodgement. Long-lived sessile megafauna, such as corals and sponges may need decades to fully recover from serious impact (Kaiser et al, 2006), whereas the recovery is quicker after moderate impact (Buhl-Mortensen, 2017).

Litter is present in all marine ecosystems, and may accumulate in the deep sea, especially at high latitudes (Bergmann and Klages, 2012; Galgani et al., 2015; Tekman et al., 2017). It has been documented by numerous studies that litter, especially plastics are harmful to marine birds and mammals. Less is known about how marine litter affects the benthic invertebrates and habitats (Mordecai et al., 2011). The effects of marine litter are various with clear, direct physical impacts like strangulation, tissue damage, and intestinal blockage in vertebrates. Generation of microplastic, leakage of environmental poisonous chemicals and introduction of alien substrate habitats are less visible, but not less serious. Lost fishing gear is a common type of litter in areas with fishing activities, and can result in so-called ghost fishing (Baeta et al., 2009).

The physical impact of petroleum related activities is much more restricted than the impact of bottom trawling. The petroleum industry also represents local sources of litter. The chemical pollution and risks of accidental spills of oil or chemicals is not assessed in this paper.

The main aim of this study is to present an overview of the distribution of fishing intensities (indicated from VMS records), seabed litter, and signs of bottom trawling. Knowledge about the spatial overlap of these pressure indicators is useful when assessing the risk for combined negative effects.

MATERIALS AND METHODS

Study Area

The Norwegian Sea is the northeastern flank of the North Atlantic Ocean. It covers about 1.5 million square kilometers and has an average depth of 1,600 m. The marine landscapes are variable with shallow banks, canyons, and deep-sea basins where the depth reaches 3,000–4,000 m. It borders the Barents Sea off the northern coast of Norway (**Figure 1**), and with the waters of the North Sea to the southeast of the Faroe Islands. The Barents Sea is a high latitude shelf ecosystem located between about 70 and 80° N on the north-western corner of the European continental margin. It is a shelf area (about 1.6 million km^2, mean depth 230 m) bounded in west and north by the deep basins of the Norwegian Sea and the Nansen Basin of the Arctic Ocean. The Norwegian Current, a branch of the Gulf Stream, transports warm water to the north past the United Kingdom (UK), through the Norwegian Sea and on into the Barents Sea.

The bottom topography guides the currents and controls the distribution of water masses in the Barents Sea (Loeng, 1991). The Norwegian Current splits into two main branches, one flowing into and through the Barents Sea from southwest to northeast, the other flowing around the western and northern flanks of the Barents Sea as the West Spitsbergen Current (Skagseth, 2008; Ingvaldsen and Loeng, 2009; Ozhigin et al., 2011).

MAREANOs mapping has so far covered 10 types of landscapes in Norwegian waters (Thorsnes et al., 2009; www.MAREANO.no/en). Landscape is defined as "large geographical areas with a visually homogeneous character." Nine types of marine landscapes have been identified within the MAREANO mapping area: (1) Strandflat, (2) Fjord, (3) Continental shelf plain, (4) Marine valley, (5) Shallow marine valley, (6) Smooth continental slope, (7) Continental slope plain, (8) Marine canyon, (9) Deep sea plain.

Observations of Sediments, Trawl Marks, and Litter

The results of this study are based on analyses of seabed videos from 1,778 locations surveyed during 23 cruises between 2006 and 2017, part of the Norwegian seabed mapping programme MAREANO (Buhl-Mortensen et al., 2015). The study area (the extent of the MAREANO mapping area to date) covers around 170,000 km^2 of the Norwegian Sea and the southern Barents Sea, and 3.735.900 m^2 of the seabed has been directly observed with seabed video. Most of the observed locations (1,358) are shallower than 700 m depth, whereas 420 locations were deeper.

During the cruises, video transects were annotated in the field with respect to occurrence of sediment types, fauna, trawl marks and litter, using the annotation software CampodLogger vs. 0.39 (developed at Institute of Marine Research). Sediment observations were recorded following a modified Folk scale (Folk, 1954), and litter types were described as detailed as possible in the field, and later assigned to 10 classes (ceramics, glass, metal, wood, paper, hard plastic, soft plastic, rubber, fishing gear, and unspecified). This dataset has previously been used to describe the distribution and content of litter in Norwegian waters (Buhl-Mortensen and Buhl-Mortensen, 2017), but the results on observed sediment types and trawl marks have not been published in full extent. Dominating sediment type for each location was estimated as the most frequent recorded sediment type. Additional data on seabed litter from other studies were used for comparison. For studies only providing weight of litter, numbers were converted to number of items using the same assumed weight per item as in Buhl-Mortensen and Buhl-Mortensen (2017).

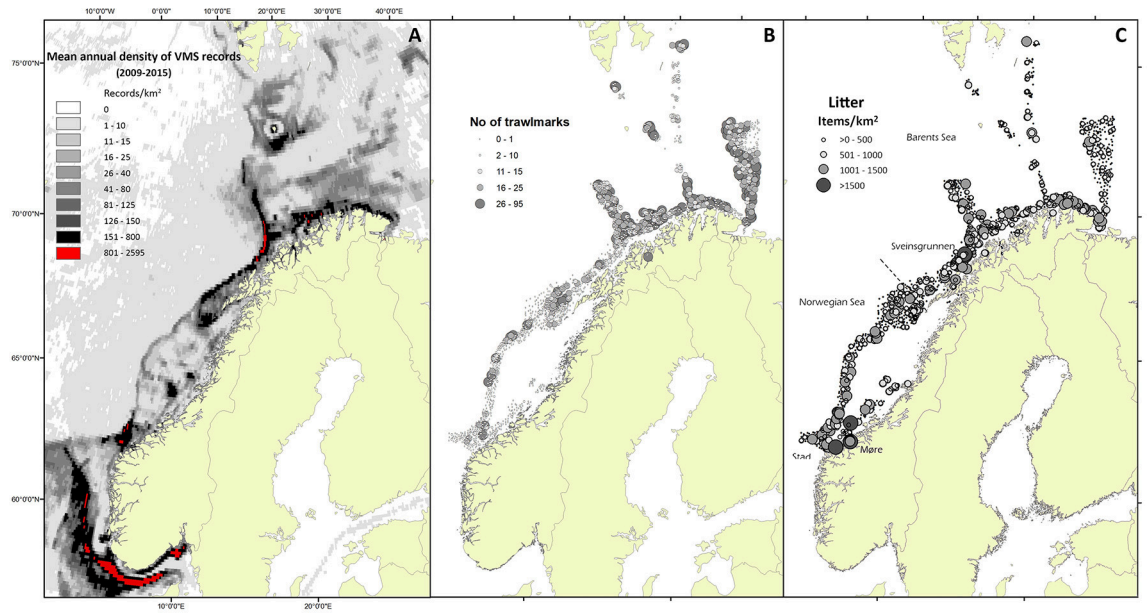


FIGURE 1 | Overview of indicators of human pressure **(A)** and impact **(B,C)** on the seabed. **(A)** Annual mean density of VMS records (pings) based on aggregated data for the period 2009–2015. **(B)** Number of trawlmarks observed during visual inspections part of the Mareano mapping programme. **(C)** Density of seabed litter observed during visual inspections (Mareano).

Vessel Monitoring System (VMS) Data from Norwegian Waters

In Norway, VMS was introduced on all Norwegian fishing vessels >24 m long in July 2000. Since then, the Norwegian Directorate of Fisheries has received information about time (minute resolution), vessel position, permit number, heading, and speed approximately every 60 min. In this study, we did not include data from before 2003, because earlier data had a higher frequency of missed records due to a less stable tracking system (Salthaug, 2006). VMS data was filtered based on speed, assuming that trawling is normally performed at a speed between 2 and 5 knots. We used the number of VMS point records as an indicator of fishing intensity (FI). For a general representation of the mean FI we estimated the average number of pings in a course gridnet (4 × 12 km; **Figure 1A**). For correlations between density of VMS records, trawl marks and seabed litter VMS records were counted within a circle (2 km radius) around the center of the Mareano video transects.

We used the VMS records from a 3 years period before the video surveys to estimate relevant FI for each location (e.g., results from surveys conducted in 2006 were related to FI estimates based on 2003–2005 records). Pearson product-moment correlation was used to assess the correlation between abundance of litter, density of TM, and FI.

RESULTS

Distribution of Fishing Intensity (VMS Data)

Figure 1A shows the distribution of mean annual FI indicated by the aggregated VMS data. The areas indicated in red had a mean annual FI >800 VMS records/km^2. The largest continuous areas with high FI were found in the North Sea, outside the range of the MAREANO mapping area. Relative large areas along the shelf break, along the coast of northern Norway, and at six areas on the continental shelf had an FI between 150 and 800 records/km^2 (**Figure 1A**). Four landscapes were more intensely fished than others (**Table 1**): Highest FI was found for locations near the coast, within the strandflat landscape (mean of 27.8 records/km^2). Locations on the continental shelf plain, in fjords, and in marine valleys had between 6.2 and 9.1 records/km^2. The remaining five landscapes had less than six records/km^2. The deep sea plain had the lowest FI.

Distribution of Trawl Marks and Sediment Types

TM may appear as trenches or furrows on the seabed (**Figure 2**). Their shape reflects the part of the trawl gear that has made the impact. The doors leave the deepest tracks with up to ca 50 cm deep and wide v-shaped trenches. Other parts of the trawl gear may leave more rounded marks or finer striations. Within the MAREANO mapping area, the highest densities of TM were found on the continental shelf plain, with an average density of 11.1 TM per video transect (**Table 1**). Locations in the northeastern part of the mapping area had the highest densities (**Figure 1B**). The density of TM, south of 69°N, was generally lower than further north, and were confined to areas around the shelf break (**Figure 1B**).

It was highest between 200 and 400 m depth (**Figure 3**). However, there was also a less pronounced peak at depths between 600 and 700 m. These two peaks correspond to the

TABLE 1 | Overview of density of litter and trawlmarks (TM) observed during MAREANO cruises and VMS data from the Norwegian Fisheries directorate (from 2003 to 2015).

Marine landscapes	No of st	Depth	Std	Observed litter				VMS records		Observed trawlmarks	
				Weight		No of items		Pings/location	Std	TM/location	Std
				Kg/km^2	Std	Items/km^2	Std				
Deep sea plain	14	2,204	449	51	138	102	276	0.3	0.6	0.0	0.0
Fjord	78	219	105	400	978	781	1,891	6.6	14.9	0.5	3.5
Shallow marine valley	291	259	79	109	222	213	435	5.2	8.0	6.6	12.7
Smooth continental slope	356	788	454	108	224	203	408	3.9	11.8	4.5	8.3
Continental slope plain	40	551	77	36	115	71	230	0.7	1.1	6.0	12.5
Continental shelf plain	619	204	72	85	187	168	367	9.1	24.6	11.1	14.8
Marine valley	290	312	81	125	246	241	476	6.2	15.7	4.7	11.4
Canyon	50	1,095	493	230	464	467	884	2.4	2.4	0.6	1.3
Strandflat	40	122	83	142	245	286	480	27.8	45.8	0.1	0.3
Mean		639		143		281		6.9		3.8	

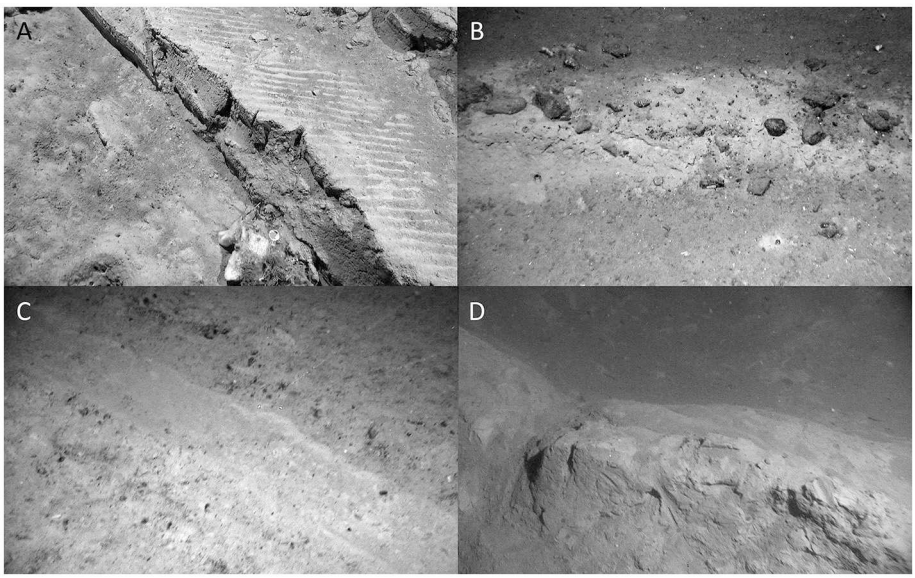


FIGURE 2 | Examples of trawl marks observed on soft **(A,D)** and mixed bottoms **(B,C)** during MAREANO seabed video surveys.

relatively shallow fisheries for white fish on the continental shelf and close to the shelf break, and the deeper fisheries for Greenland halibut on the continental slope (Buhl-Mortensen et al., 2013).

Most of the 1,778 locations were dominated by mixed mud and sand (572 st), and mixed gravelly muds (477 st) (**Table 2**). The density of TM was generally weakly correlated with FI for most sediment types, except for locations dominated by mixed gravelly muds ($R = 0.48$, $p < 0.005$). A strong correlation between TM and FI for bedrock was not significant ($p > 0.05$). Cold-water coral reefs (*Lophelia pertusa*) were observed at 151 locations, and were the dominating bottom type at 41 (**Table 2**). TM were observed at 27% of the locations with coral reefs.

Distribution of Seabed Litter

Litter were observed at 27.4% of the locations. Most observations of litter with densities >1,500 items/km^2 were from depths between 100 and 500 m (**Figure 3**). There was a general, and relatively similar pattern with depth for all parameters (litter abundance, TM density, and FI), with peak values between 100 and 400 m. However, none of these parameters were significantly ($p < 0.05$) correlated with each other.

Highest abundance of litter were found close to the coast and in areas with high fishing intensity, indicated from the VMS data. Different types of fishing gear were the most common (17% of locations) type of litter. Unspecified litter was observed at 8% of the locations, and plastics (soft plastic, hard plastic, and rubber) were observed at 4%. Other types of litter (metal,

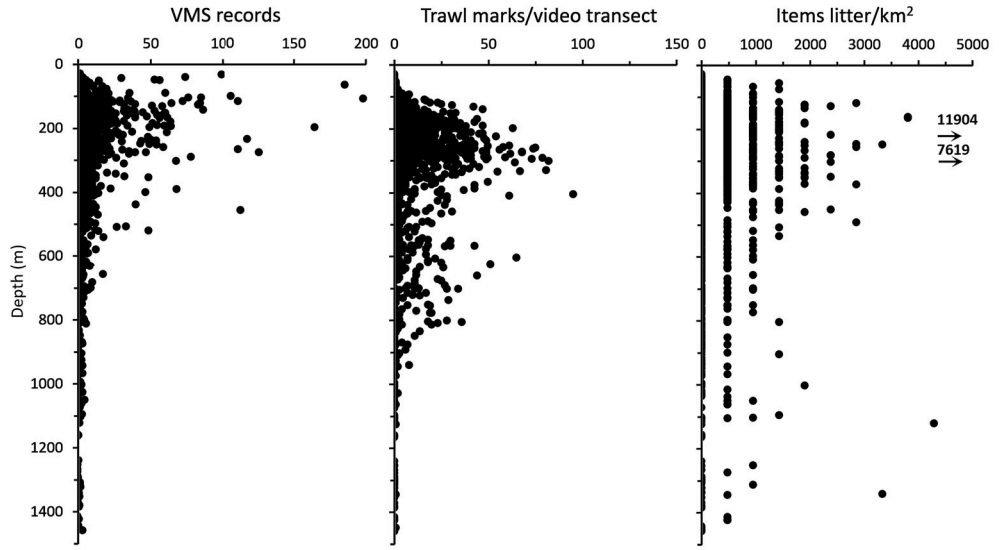


FIGURE 3 | Bathymetric distribution of VMS records (mean values of records per year for 3 years prior to video surveys). Observed trawl marks, and density of observed litter items.

TABLE 2 | Number of locations with dominance of different sediment types (*n*), and correlation (Pearson product-moment correlation) values (*r*) between trawl marks (TM), density of vessel monitoring system records (VMS), and density (no of items per km^2) of observed litter for different substrates.

Sediment types	*n*	TM vs. VMS	Litter vs. VMS	Litter vs. TM
Bedrock	25	0.80	0.05	0.26
Coral reefs	41	−0.07	**0.50***	−0.15
Mixed hard	303	0.00	0.10	0.02
Mixed gravelly muds	69	**0.48****	−0.01	0.03
Mixed gravelly sands	477	0.13	0.08	0.06
Mud and sand	572	0.17	0.26	−0.04
Mud	112	0.02	0.03	−0.07
Sand	171	0.07	0.18	−0.04
Compacted sediments	8	−0.15	−0.15	1.00
All substrates	1,778	0.03	0.12	−0.03

*$p < 0.05$, **$p < 0.005$. Significant correlations are indicated as bold numbers.*

glass, ceramics, fabric, wood, and paper) occurred at <2% of the locations. Plastics were observed at 71 locations, most often (63) only as one item. The highest number of plastic items (three) were observed at one location outside Møre (in the Norwegian Sea; **Figure 1**). This was the same location as contained the highest number of lost/discarded fishing gear.

Extreme densities of litter, observed close to the coast were of dumped or lost fishing gear. Here, wires occurred in curles or bundles, indicating that they were not lost during normal fishing activities.

The abundance of litter reflected FI and densities of TM at a regional scale. However, at a local scale patterns were not so clear. No strong correlation was found for the relation between abundance of litter and TM (**Table 2**). Abundance of litter was strongest correlated with VMS records for locations with coral reefs ($R = 0.50$, $p < 0.005$).

Figure 4 show the average values for number of litter items/km^2 for 100 m depth intervals above 1,000 m and for 400 m intervals below 1,000 m. Litter seems to aggregate in deep water, and the high values around 1,200 m was related to concentrations in canyons, whereas the peak at around 2,400 m represents the foot of the continental slope, where the steep slope meets the level abyssal plain. Lost or discarded fishing gear was the most abundant observed litter, and comprised 70–80% of all observations at shallow (<100 m) locations and at around 600 and 700 m depth. The contribution of plastics to the total amount of litter was greatest at around 600 m depth (21%), and 900 m depth (43%). Plastics were not observed deeper than 1,200 m (**Figure 4**).

DISCUSSION

Methods

The use of VMS records as an indicator of FI has several weaknesses. Using vessel speed as a criterion to identify fishing activities may include vessels in slow transit or other activities than fishing (Skaar et al., 2011). This is probably the reason large areas in deep (>1,000 m) waters were indicated as being fished (although at a low intensity with 1–10 records/year). This is not realistic as these areas are not known to be used for bottom fishing. Detailed spatial patterns of FI cannot be reveled from VMS records since detailed track lines of the trawled paths are not provided because of too low rate of position recording (pings), and lack of direct information about time of fishing. This could explain the generally weak correlations between density of observed trawl marks (TM) and VMS records. Even so, the general patterns (aggregated

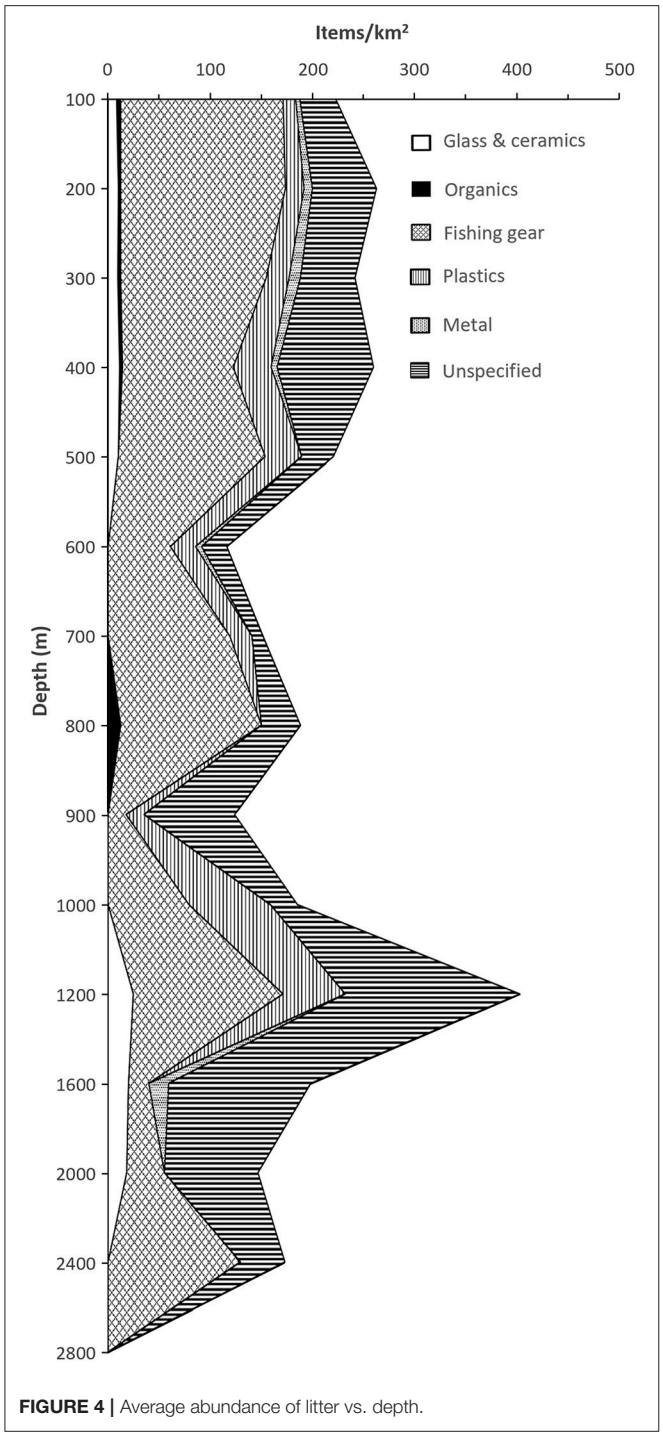


FIGURE 4 | Average abundance of litter vs. depth.

at a broader scale) provide a good indication of areas that could be at risk for compromising the seafloor integrity. The high concentration of trawl marks in the northeastern part of the mapping MAREANO area is not reflected in the VMS records (**Figures 1A,B**). This is probably due to lack of data from Russian vessels, and the high density of observed trawl marks indicate a much higher FI than indicated from the VMS records.

Impacts of Bottom Trawling

Figure 1A shows areas with FI > 800 VMS records/km^2, indicated in red color. According to Buhl-Mortensen et al. (2016) this FI could lead to ca 20% reduction of species richness.

The density of observed TM is a good indicator of trawling impact in soft sediments where the trawl gear leaves clear traces, whereas on harder substrates trawls leave less traces. Thus, the impacts on organisms on hard substrates is probably greater than indicated by TM.

Similar to the findings in this study, Buhl-Mortensen et al. (2016) in general found no strong correlation between density of TM and FI. They found that number of TMs was highest on mud, although FI was larger in sandy mixed bottoms. Also, the longevity of TMs (how long they are visible on the seabed) depends on the type of sediment; its softness and stability (Buhl-Mortensen et al., 2016). Buhl-Mortensen et al. (2016) reported a significant, negative relationship between FI and density and diversity of megabenthos for all substrates, except for mud. They found that most megabenthos taxa decreased in density with increased FI, with some few exceptions for scavengers. Vulnerability may be defined as a response to stressors based on increased mortality, and reduced growth and reproduction. For benthic, deep water species there are few results demonstrating the direct effect of stressors such as physical disturbance, dislodgement, and resuspension of sediments. The results are more indirect, by comparing abundances between areas of different degree of pressure (Buhl-Mortensen et al., 2013, 2016; Jørgensen et al., 2015). Commonly, fragility of taxa is assessed based on organism's size and flexibility (Parker et al., 2009; Parker and Bowden, 2010). Sessile marine megafauna with a size larger than 10 cm, often serve as habitats for other species, and are regarded as local providers of biodiversity hot-spots (Buhl-Mortensen et al., 2010). Many different taxa provide such habitats, but most common in some areas, and particularly on the shelf and shelf break off western and northern Norway, are the sponges and corals (octocorals and scleractinians). Four of the sixteen habitats listed by OSPAR (OSPAR Convention for the protection of the marine environment of the North-East Atlantic) as threatened and/or declining (OSPAR Commission, 2008a,b) occur in deep-water (>200 m) and are characterized by megafaunal invertebrates. The characterizing species for these habitats are regarded as sensitive to anthropogenic stressors such as destructive fishing activities (Buhl-Mortensen et al., 2010), sediment exposure or pollution. **Table 3** summarizes the effects of fishing and litter on benthic organisms and habitats. Negative effects from bottom trawling have been clearest demonstrated for large megabenthos (corals and sponges; Fosså et al., 2002; Mortensen et al., 2005; Buhl-Mortensen et al., 2016; Buhl-Mortensen, 2017). Buhl-Mortensen (2017) studied the health status of *Lophelia* reefs off northern Norway in relation to bottom trawling, and found minimal damage on coastal reefs where little bottom trawling occurs, whereas extensive damage was documented on offshore reefs, exposed to greater FI. The damage observed on the reefs probably occurred more than 10 years before the investigation, when

TABLE 3 | Overview of impact of litter and bottom fishing on physical environment and organisms on hard and soft bottom.

	Litter		Bottom fishing	
	Hard bottom	**Soft bottom**	**Hard bottom**	**Soft bottom**
Impacts on physical environment	Introduction of unstable substrates	Introduction of hard substrates	Displacement of substrates	Resuspension of sediments Changes in seabed morphology Exposure of sediments
Impacts on organisms	Entanglement Tissue damage Abrasion Abrasion	 Introduction of hard bottom organisms	Physical damage Mortality Removal Dislodgement	

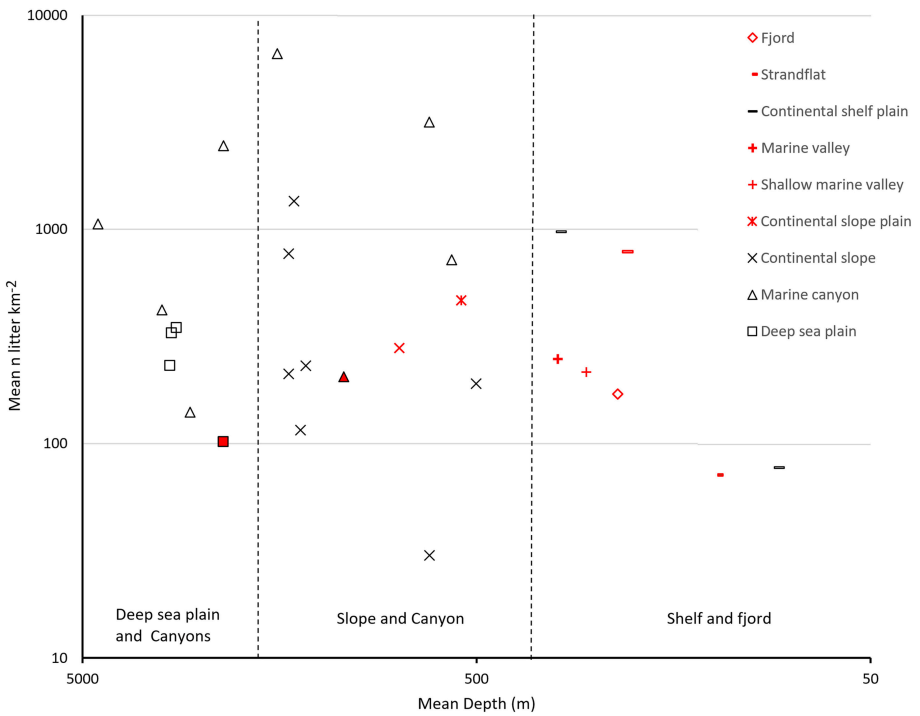


FIGURE 5 | Mean abundance of litter in different marine landscapes at different depth. Data from Pham et al. (2014) and (Buhl-Mortensen and Buhl-Mortensen, 2017).

a ban against trawling on known coral reefs had been implemented.

The petroleum industry represents activities that cause physical impact at more restricted scale than bottom trawling, but also represents local sources of litter. The chemical pollution and risks of accidental spills of oil or chemicals is not assessed in this paper. In a study of effects of oil drilling on *L. pertusa*, Mortensen and Lepland (2007) found increased coral mortality locally (<500 m from the drilling), caused by discharge of drilling mud. Resuspended particles from bottom trawling could lead to similar effects on a greater scale, and studies have indicated great impact on sediment distribution and even changes bottom topography (Palanques et al., 2006; Puig et al., 2012).

Distribution and Sources of Litter

On average, the litter density in Norwegian waters is in the lower range compared to what has been reported in other studies (Pham et al., 2014; Galgani et al., 2015; **Figure 5**).

In our study we found that fishing gear dominates the litter, indicating that local activities are a more common source than long transport of drifting litter. However, the distribution of litter does not reflect the fishing intensity at a local scale (<1 km). This is partly explained by transport with currents of litter

with light buoyancy during sinking, accumulation in troughs, canyons, and local depressions, but also deliberate dumping of waste or old fishing gear outside fishing grounds. Dumping of discarded fishing gear would probably more common outside the best fishing grounds rather than within, to avoid encounters that could damage the gear. "Piles" of wire observed in this study indicate this, and loss of gear due to attachment in the bottom would result in gear being suspended on the seabed.

Much of the fishing gear may also be classified as plastic, but in many cases, it was not possible to identify the material of the lines, nets and ropes. Other studies have found that plastic is the greatest contributor to seabed litter (Watters et al., 2010; Ramirez-Llodra et al., 2013; Tekman et al., 2017). The general pattern with highest densities in canyons reported in several studies (Watters et al., 2010; Mordecai et al., 2011; Ramirez-Llodra et al., 2013) is also clearly reflected in the data presented here. Bergmann and Klages (2012) and Tekman et al. (2017) have recorded increasing densities of litter at the slope west of Svalbard. Their results reports an increase from <4,000 items/km^2 in 2002 to more than 6,000 items/km^2 in 2014. This leads to the question whether the deep sea is a sink hole for marine debris that can be transported with the currents. Tekman et al. (2017) indicate that increased tourism and fishing activity is an important factor to this increase. At a local scale, the FI was not correlated with abundance of litter at any bottom type, except for coral reef ($R = 0.50$, $p < 0.005$). The coral reefs are rugged structures where any type of litter, including fishing gear may get stuck. At a landscape scale, fjords and canyons are units that due to topographical properties act as retention sites for litter.

The effects of litter on benthic communities is poorly known. In addition to direct effects on organisms' biological processes (e.g., respiration and food uptake) certain types of large litter items, such as lost fishing gear may add to the direct negative effects of bottom trawling when caught and dragged along the seabed. Clean-up of lost fishing gear and other litter with dredges could also cause negative physical impact, especially in fragile habitats such as coral reefs and biogenic habitats. It is therefore important to provide relevant maps based on direct information before starting sublittoral and deeper litter removal campaigns.

AUTHOR CONTRIBUTIONS

PB-M and LB-M have contributed to this manuscript by providing data, analyzing, and writing.

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Deep-Sea Mining With No Net Loss of Biodiversity—An Impossible Aim

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Deep-sea mining is likely to result in biodiversity loss, and the significance of this to ecosystem function is not known. "Out of kind" biodiversity offsets substituting one ecosystem type (e.g., coral reefs) for another (e.g., abyssal nodule fields) have been proposed to compensate for such loss. Here we consider a goal of no net loss (NNL) of biodiversity and explore the challenges of applying this aim to deep seabed mining, based on the associated mitigation hierarchy (avoid, minimize, remediate). We conclude that the industry cannot at present deliver an outcome of NNL. This results from the vulnerable nature of deep-sea environments to mining impacts, currently limited technological capacity to minimize harm, significant gaps in ecological knowledge, and uncertainties of recovery potential of deep-sea ecosystems. Avoidance and minimization of impacts are therefore the only presently viable means of reducing biodiversity losses from seabed mining. Because of these constraints, when and if deep-sea mining proceeds, it must be approached in a precautionary and step-wise manner to integrate new and developing knowledge. Each step should be subject to explicit environmental management goals, monitoring protocols, and binding standards to avoid serious environmental harm and minimize loss of biodiversity. "Out of kind" measures, an option for compensation currently proposed, cannot replicate biodiversity and ecosystem services lost through mining of the deep seabed and thus cannot be considered true offsets. The ecosystem functions provided by deep-sea biodiversity contribute to a wide range of provisioning services (e.g., the exploitation of fish, energy, pharmaceuticals, and cosmetics), play an essential role in regulatory services (e.g., carbon sequestration) and are important culturally. The level of "acceptable" biodiversity loss in the deep sea requires public, transparent, and well-informed consideration, as well as wide agreement. If accepted, further agreement on how to assess residual losses remaining after the robust implementation of the mitigation hierarchy is also imperative. To ameliorate some of the inter-generational

inequity caused by mining-associated biodiversity losses, and only after all NNL measures have been used to the fullest extent, potential compensatory actions would need to be focused on measures to improve the knowledge and protection of the deep sea and to demonstrate benefits that will endure for future generations.

Keywords: no net loss, biodiversity offsetting, compensation, mitigation hierarchy, deep-sea mining, Environmental Impact Assessment (EIA)

INTRODUCTION

The Potential Impact of Deep-Sea Mining

There is increasing interest worldwide in the potential for deep-sea mining to serve as an engine for "Blue Growth" and to drive sustainable economic development (European Commission, 2012; Wedding et al., 2015). Most deep-sea ecosystems targeted for mining have some combination of ecological characteristics that make them particularly sensitive to anthropogenic disturbance, such as being largely pristine, highly structured, very diverse, dominated by rare species and (extremely) slow to recover. Accordingly, there is increasing concern that the direct and indirect impacts of mineral extraction in the deep sea will result in a significant loss of biological diversity (herein referred to as biodiversity) (CBD, 1992; Wedding et al., 2015). Direct impacts occur through the removal of target material and associated organisms within the mine site and include the destruction of biota as well as habitat loss, fragmentation, and modification through altered mineral and sediment composition, geomorphology, and biogeochemical processes (Ellis, 2001; Van Dover, 2014; Jones et al., 2017). Potential indirect impacts on the seabed and water column both within and outside of the directly mined area include the smothering of habitat and biota, interference with feeding activities, and the release and spread of nutrient-rich and toxin-laden water from the generation of plumes (Ellis, 2001; Boschen et al., 2013). Additional potentially harmful diffuse effects include those from light, noise and electromagnetic disturbance (Van Dover, 2014; MIDAS, 2016). The scale over which these indirect impacts are likely to occur is largely unknown and most of the effects remain unstudied. The three mineral resource types commonly considered for deep-sea mining each have their own specific environmental contexts, which have each been the subject of targeted scientific study and some proposed management measures—polymetallic nodules (nodules), cobalt crusts (crusts), and seafloor massive sulfides (SMS) associated with hydrothermal vents (vents). However, despite the considerable differences among these resources and the types of ecosystems within which they are located, the scales implicated by deep-sea mining suggest that exploitation of all three resource classes will yield significant biodiversity loss, indicating that a precautionary approach is warranted (Levin et al., 2016).

The Importance of Deep-Sea Biodiversity

While biodiversity loss is recognized as a major global environmental problem (Weikard, 2002), the importance of biodiversity in the deep ocean merits clarification, particularly given that most species (both prokaryotes and eukaryotes) remain undiscovered or unidentified (Higgs and Attrill, 2015; Sinniger et al., 2016; Shulse et al., 2017). Deep-sea biodiversity is valued both for the ecosystem services it provides and for underpinning the health of the oceans by enabling a range of ecological and evolutionary functions that are viewed as necessary to productive, sustainable ecosystems (Thurber et al., 2014). There is increasing reliance on the provisioning services of the deep sea through fisheries, energy and mineral extraction, pharmaceutical prospecting, and the search for industrial agents and bioinspired materials that all derive from deep-sea biodiversity (Mengerink et al., 2014). Deep-sea communities and organisms also play important roles within climate regulation (through the burial of carbon and mitigation of climate change and ocean acidification) and the production of oxygen (through the recycling of nutrients required by phytoplankton). Biodiversity facilitates the provision of food, refuge, habitat, and nursery grounds for species responsible for the services described above. These services, in addition to the intrinsic values ascribed to biodiversity, are often called natural capital and describe the benefits that humans derive from the effective functioning and existence of biodiversity (Soulé, 1985; Hungate and Cardinale, 2017). In the deep ocean, where there is great uncertainty about natural processes, vulnerabilities and the consequences of human impacts, biodiversity serves as a form of evolutionary insurance, acting as a living library that facilitates adaptation and ecosystem resilience to changing environmental conditions (Mace et al., 2014). With the current absence of a detailed understanding of ecological relationships, the precautionary approach advocated by several international commitments and legal obligations including that of the Convention on Biological Diversity (CBD, 1992) suggests that we assume that healthy, functioning deep-sea biodiversity is, as in other realms, highly desirable and provides services beneficial to humankind.

Managing Impacts on Deep-Sea Biodiversity

In recognizing the importance of the marine environment and its living resources, the United Nations Convention on the Law of the Sea (UNCLOS, 1982) sets forth a prescriptive regime for the seabed "Area" beyond national jurisdiction and its mineral resources, designed to achieve international control, sharing of benefits (monetary and non-monetary), and environmental protection. UNCLOS designates the international deep seabed and its mineral resources as the "Common Heritage of Mankind," to be managed on behalf of humankind as a whole, including future generations (Bourrel et al., 2016; Jaeckel et al., 2017;

UNCLOS, Art. 136). The International Seabed Authority (ISA), established under UNCLOS, regulates mining activities (to date limited to exploration) on the international seabed and is required to take measures necessary to ensure the "effective protection of the marine environment from harmful effects", "the prevention, reduction and control of pollution and other hazards to the marine environment" and "the prevention of damage to the flora and fauna of the marine environment" that might arise from mining (Levin et al., 2016; UNCLOS, Arts. 137, 145, 153). Moreover, the international community has recognized the importance of biodiversity conservation more broadly (CBD, 1992; UNGA, 1995, 2006), which should inform the management of deep-sea mining.

With the prospect of commercial mining approaching, the ISA is drafting exploitation regulations that also address environmental considerations. One aspect that the ISA will need to consider is whether the current best practice of other extractive industries, specifically the application of the mitigation hierarchy (IFC, 2012; Ekstrom et al., 2015), can be effectively used in the vent, seamount, and abyssal deep-sea ecosystems likely to be affected by mining.

Here we critically address the challenges of applying the different phases of the mitigation hierarchy to deep-sea mining with the specific aim of no net loss (NNL) of biodiversity. We also consider the recent suggestion that biodiversity offsetting could be employed in the context of deep-seabed mining (ISA, 2016). We first explore the application of NNL to the deep ocean and examine possible avenues for avoiding, minimizing, and remediating adverse effects. We next appraise the potential of biodiversity offsetting, the last resort in the tiered mitigation hierarchy, to address residual and unavoidable harm. Recognizing that the primary obligation of the ISA is to prevent harm to the marine environment, if deep-sea mining is allowed to proceed without adhering to an aim of NNL the benefits arising from any compensatory actions should be considered to outweigh the losses of deep-sea ecosystems and services to humankind as a whole. Here we also explore the difficult question of what considerations ought to be required of any agreed compensatory action should such a scenario arise.

DEEP-SEA MINING AND NO NET LOSS (NNL)

Introduction to NNL

A key tool in environmental management is the mitigation hierarchy (IFC, 2012), which is applied in environmental impact assessment (EIA) and management planning, and is also commonly required by financial institutions and regulatory frameworks (**Figure 1**). Based on an assessment of the potential environmental impacts of an activity, measures to avoid and then minimize the impacts as far as possible are undertaken. After this, opportunities to remediate (i.e., reverse the residual impacts) should be considered before exploring the last resort of biodiversity offsets to address any unavoidable impacts (Ekstrom et al., 2015). Offsetting most commonly involves restoration to assist in the recovery of a quantum of degraded, damaged or destroyed ecosystem equivalent to that lost; and can be active (e.g., replanting, construction of artificial habitats) or passive (e.g., removing threats to promote natural recovery) in nature (Perrow and Davy, 2002; SER, 2004). Sustainable management of resource extraction is of increasing importance to governments and industry alike. In response, aims of NNL or even net gain of biodiversity have been adopted within some public and private policies as part of the mitigation hierarchy (Bull et al., 2013; Le et al., 2017; Niner et al., 2017a). This uptake appears to be increasing despite limited evidence as to the success of the mitigation hierarchy and offsets to realize these aims (Maron et al., 2015b; Gibbons et al., 2017; Lindenmayer et al., 2017). While not yet applied in deep-sea management, NNL is becoming increasingly accepted by established extractive industries such as terrestrial mining and oil and gas operations including those operating offshore in shallower waters (ICMM, 2005; Rio Tinto, 2008; Bayon and Jenkins, 2010; BHP Billiton, 2012; Benabou, 2014; Rainey et al., 2014).

Prospects for NNL in the Deep Sea

Defining what is meant by NNL is critical to its implementation and assessment; however, this detail is seldom provided in offsetting policy (Maron et al., 2015a). NNL suggests that the total "amount" of biodiversity should not be altered by an activity. However, the term "biodiversity" itself also requires definition as interpretation can vary across a range of spatial scales and metrics including those describing genetic diversity, species richness, evenness, species turnover, habitat heterogeneity, ecosystem function, and community or taxonomic distinctness (Sarkar and Margules, 2002; Magurran, 2004). The data requirements for each of these descriptors may be different and the acceptability of potential remediation or offsetting options will vary with the metrics used (Bruggeman et al., 2005, 2009). For example, NNL of ecosystem function or functional diversity, as opposed to species richness, allows for an alternate definition of remediation to include rehabilitation where the full suite of functions are restored across the region even in the absence of the return of all original species (Van Andel et al., 2012). In the case of deep-sea mining, even if a purely functional definition of NNL were to be adopted, defining an ecologically relevant measure of extent and functionality based on current scientific understanding would be extremely challenging. Furthermore, such definitions could mask the risk of local and regional species extinctions and the diminished resilience of ecosystem services (Donohue et al., 2016). Whatever surrogates and methods are employed to measure biodiversity changes in the deep sea, they should be appropriate for assessing management measures and whether adequate protection is being acheived (Magurran, 1988; Bull et al., 2016).

Recent correspondence by Van Dover et al. (2017) and others have questioned the feasibility of an aim of NNL in marine environments and for the deep-sea mining industry. Specifically, OECD and IUCN policy guidelines suggest that offsets are not appropriate where there is uncertainty about the severity, vulnerability, and irreplaceability of biodiversity components lost

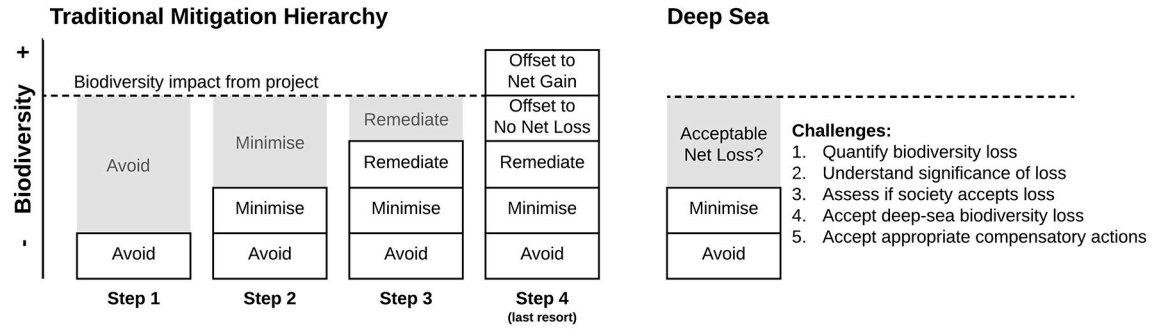


FIGURE 1 | The mitigation hierarchy as applied in its intended sequence and the challenges posed by deep sea application.

and gained (IUCN, 2016; OECD, 2016). Particular challenges in the deep ocean relate to the availability of baseline data (including the large number of unsampled and undescribed species), current abilities to measure and monitor biodiversity losses and gains resulting from human activities such as mining, and a complete lack of proven success in assisted ecological restoration. Studies of biodiversity in the deep sea are challenging owing to cost, remoteness, immense spatial scales, processes that unfold over long time scales (e.g., centuries to millennia for expected recovery from some deep-sea mining impacts), and limited expertise. These factors result in a high degree of uncertainty in (i) local and regional assessments of biodiversity (active hydrothermal vents on local scales are best known but still not fully understood); (ii) the effects of human activities in space and time on biodiversity; and, (iii) the extent to which components of deep-sea biodiversity are vulnerable and irreplaceable. Further complicating impact quantification is the need to account for future scenarios that would unfold in the absence of the impact or offsetting action; these need to integrate current and future effects from other ecosystems stressors such as climate-driven changes (Smith et al., 2008a; Levin et al., 2016). As a result, there is substantial uncertainty associated with EIA and consequent implementation of the mitigation hierarchy for deep-sea projects owing to a lack of ecological and biogeographic knowledge (Smith et al., 2008a; Amon et al., 2016; Levin et al., 2016; Jones et al., 2017). These challenges are compounded by the frequent absence of defined property rights or effective laws that provide for the control of access by others to an area. Without the authority to restrict access to an area, protection for restoration from other damaging uses such as fishing is not possible. The combination of these issues complicates each step of the mitigation hierarchy in deep-sea environments. Indeed, the Environmental Protection Authority of New Zealand referred to the inadequate application of the mitigation hierarchy as a reason for its refusal of the application of Chatham Rock Phosphate Ltd to mine phosphate off New Zealand (NZ EPA, 2015). The decision specified the absence of (1) impact quantification, (2) planned interventions to minimize impacts, and (3) evidence of the effectiveness of measures to support the reversal of impacts as contributing to their decision (NZ EPA, 2015; UNEP-WCMC, 2016). In the next section, we explore these challenges and conditions for applying the mitigation hierarchy appropriately.

Exploring the Mitigation Hierarchy in the Deep Sea

Avoid

The first step in the mitigation hierarchy is avoidance, whereby a measure, once designed into a project, does not require continued effort to remove impacts (Bull et al., 2016). Deep-sea mining will involve direct removal of targeted habitat, yielding loss, fragmentation, and modification of that habitat as well as mortality of associated fauna (Ellis, 2001; Van Dover, 2014; Jones et al., 2017). Given the inevitably destructive nature of the activity, the avoidance of significant biodiversity losses is unlikely to be achievable for some or even most projects (Van Dover et al., 2017). Some impacts might be avoided at a project-level by reducing the footprint of mining within a contracted area and/or by leaving some minerals with associated fauna in place and undisturbed, e.g., through reticulated extraction patterns that leave large, contiguous areas undisturbed by direct mining. However, given that many effects of mining will involve three-dimensional, diffuse, poorly understood, and wide-ranging impacts from sediment plumes, toxicity and noise, the identification of refuge areas free from damaging impacts will not be straight-forward (Ellis, 2001; Thiel et al., 2001; Van Dover, 2014). This is evidenced by the inability to identify control sites unaffected by the resettlement of material in the *Dis*turbance and re*col*onoization (DISCOL) experiment conducted to help evaluate potential deep-sea mining impacts (Thiel et al., 2001). Identification of such refugia will require both modeling and in-situ studies at multiple levels and over various time frames (e.g., the expected decadal duration of nodule mining), to include project, regional, and cumulative impacts. The regulatory framework will need to be both precautionary and adaptive to allow responses such as the cessation/relocation of mining or the modification of impact-free refugia as new data become available and prior to reaching the point of serious harm (Levin et al., 2016). Nonetheless, even with plume management and impact-free refugia, negative impacts, and biodiversity loss will be unavoidable.

Minimize

The second step in the mitigation hierarchy is to minimize losses of biodiversity and other ecosystem damage to the greatest extent possible. Minimization measures include activities that require on-going activity to reduce the significance of impacts (Bull et al., 2016). In the case of deep-sea mining, technologies and practices might be developed and applied to reduce these risks. For example, sediment plumes generated during mineral extraction are considered to be a source of major risk to deep-sea ecosystems resulting in, among other effects, the burial and clogging of animals' feeding apparatus. Investing in engineering design and *in situ* testing of mining tools, including installation and testing of shrouds on cutters, and minimizing the creation of pulverized fine material, might reduce sediment plume dispersion and the spatial extent and temporal persistence of some plume impacts. Other technical innovations might include the design of vehicles to reduce compaction of the seabed and turbulence, or a change in waste disposal techniques to reduce ecotoxicological effects. However, because the industry is in its infancy, the effectiveness of such measures in reducing losses of biodiversity remains wholly untested. Again, regulatory mechanisms, including environmental objectives with precautionary indicators, will be necessary to stimulate the innovation and uptake of improved technologies. Despite the potential to reduce impacts in the future, the destructive nature of mining indicates that current technologies will be unable to avert significant local biodiversity losses through avoidance and minimization. Species extinction rates typically increase exponentially with the percentage of essential habitat lost (Ney-Nifle and Mangel, 2000); thus, biodiversity loss from mining is likely to scale to the ratio of habitat area impacted to the total area of that habitat within the biogeographical province. Biodiversity loss will also be affected by levels of abundance and endemism within a habitat, and rates of population connectivity (Ney-Nifle and Mangel, 2000). To account for these varying impact pathways, minimization efforts should incorporate spatial planning that considers species ranges, habitat distributions, and patterns of connectivity to determine where and how much mining will be allowed to occur (e.g., to avoid massive habitat loss within a single biogeographic province leading to extinction of species requiring that habitat).

Remediate

The third step of the mitigation hierarchy, remediation, seeks to address losses of biodiversity associated with mining after the first two steps of the mitigation hierarchy have been considered and implemented to the greatest extent possible. Remediation under an aim of NNL implies that a reversal of damages incurred by the associated activity is required and feasible (Bull et al., 2016). Requirements for post-mining site remediation are now commonplace for terrestrial projects (Lamb et al., 2015) but a similar approach for the deep sea will face numerous challenges. These challenges include the slow recruitment and growth of the native species that occur in target habitats (manganese nodule fields, seamounts, and sulfide deposits), the potentially vast scales of mining impacts, the limited understanding of the requirements for proper ecosystem function, and the likely high cost of deployment of assisted regeneration strategies and monitoring in the deep sea (Van Dover et al., 2014). Effectiveness and practicability of any remediation technologies or methods at the scale required to address deep-sea mining impacts and achieve NNL has not been demonstrated. Remediation for nodule mining is especially problematic considering the temporal and spatial scales involved. For example, one 30-year nodule mining operation may involve a contract area of 75,000 km^2 roughly the size of Austria or Tasmania, with direct impacts potentially affecting 20–30% of this area (Smith et al., 2008b). There is little evidence of recovery of biodiversity in nodule beds following relatively small-scale, low-intensity disturbances, even after several decades (Miljutin et al., 2011; Vanreusel et al., 2016; Jones et al., 2017). While a nascent academic field of deep-sea benthic assisted restoration science exists (Strömberg et al., 2010; MERCES, 2017[1]), that work is decades away from contributing reliably and responsibly to industrial-scale reduction of biodiversity loss, and it is still unclear whether deep-sea restoration is feasible at all. Although the technology for remediation may appear relatively simple in some respects, for example deploying simulated nodules to the seafloor in mined areas, the remediation scales (of order 10,000 km^2) are daunting, the efficacy of remediation approaches is unknown and will require decades to evaluate (due to the very slow recovery rates of abyssal communities) and run a high risk of failure which could implicate further biodiversity loss.

Even if benthic remediation were technically feasible, it would be further complicated by the extended timespans over which financial commitment would be required. The long recovery periods of most deep-sea environments (excluding some active vent fields), likely to be in the order of decades to centuries, would extend beyond the duration of typical mining contracts. The question then arises as to where responsibility for remediation of a former mine site lies once a mining contract has expired but commitments remain outstanding. An increasing concern with schemes for ecological remediation of terrestrial mine sites, where development consent conditions often require a return to pre-impact ecological condition post activity, relates to the "selling off" of concessions and accompanying environmental liabilities to smaller, less financially stable companies. Smaller companies may be less likely to possess the required expertise, experience, and financial resources to manage the long-term commitment posed by remediation (Lamb et al., 2015). In the case of deep-sea mining in the Area it would be the responsibility of the ISA, together with the relevant sponsoring state, to ensure all long-term environmental commitments by the contractor are sustained, even beyond the contractual period. This may require the use of environmental bonds or similar financial instruments (ISA, 2017) to ensure the contractor is not able to shift responsibility to another entity. Given these ecological, practical and legal challenges, it is likely that residual significant impacts will remain after the first three steps of mitigation hierarchy have been implemented, leading to a potential reliance on biodiversity offsets to meet an aim of NNL, but these too may prove problematic.

[1] Available online at: http://www.merces-project.eu/?q=content/about-project

BIODIVERSITY OFFSETTING IN THE DEEP SEA

Biodiversity offsets are, the last resort of the mitigation hierarchy and are employed to manage residual impacts after the full and robust implementation of avoidance and minimization strategies. They are considered to be "measurable conservation outcomes resulting from actions designed to compensate for residual biodiversity impacts arising from project development after appropriate prevention and mitigation actions have been taken" (BBOP, 2012), with "demonstrably quantifiable equivalence between what is lost and gained" (Bull et al., 2016). The three criteria central to an aim of NNL include the prediction and measurement of biodiversity changes (losses and gains), the provision of evidence to support claims of additionality, and ecological equivalence. These criteria, particularly that of additionality, are designed to ensure that the net balance of biodiversity is only ever measured against the scenario most likely to have occurred in the absence of impact or compensatory intervention (Ferraro and Pattanayak, 2006). The strict application of these criteria, essential to realize an aim of NNL and to avoid offset misuse, have been found to be challenging in marine contexts (UNEP-WCMC, 2016; Niner et al., 2017b).

Possible Biodiversity Offsets for the Deep Sea

The gaps in current ecological knowledge and restoration abilities in the deep sea are also inconsistent with use of assisted biodiversity recovery as an appropriate offset mechanism. As such, offsetting through averting loss might present the only option by which the condition of equivalence could currently be addressed. This averted loss offsetting mechanism is meant to create biodiversity benefit by protecting biodiversity that in the absence of the action, would have been lost. The effect is thus to improve current negative trends in biodiversity of a type similar to that lost. Averted loss benefits arise by removing ecosystem threats, preventing harm from other human activities not associated with the project in question, and in some cases by allowing for natural remediation (Ekstrom et al., 2015). For habitats where seabed mining is likely to be the only major threat, averted loss offsets would not be possible. Such habitats include nodule fields or SMS deposits.

However, averted loss offsets might, in theory, be plausible for crust mining as some seamounts are threatened by mining *and* fishing. Therefore, biodiversity loss attributed to mining could conceivably be offset by protecting equivalent unmined areas from the impacts of bottom-contact fishing and future exploitation through mining. However, the additionality of these efforts would be difficult to establish in light of the United Nations General Assembly resolutions which already commit states to "prevent significant adverse impacts" on vulnerable marine ecosystems, including seamounts, from the impacts of deep-sea fisheries (UNGA, 2006). These commitments have been translated into binding regulations by most of the regional fisheries management organizations (RFMOs) with the legal competence to manage bottom fisheries on the high seas (Gianni et al., 2016). Further, unlike on land where property rights commonly exist, the ability of one international agency (e.g., the ISA) to restrict activities regulated by another competent organization (e.g., RFMOs) in the Area is limited. Accordingly, "buying out" industry players with access to an area would provide little guarantee of protection in areas beyond national jurisdiction (ABNJ) if others unaffected by the financial transaction could move into the protected area (**Table 1**). Given that the ISA has limited jurisdiction applying only to activities associated with seafloor minerals, protecting an area through preventing access would only be possible through international agreement (such as through measures adopted by the relevant RFMO) and only if effective enforcement mechanisms existed. The developing treaty on biodiversity beyond national jurisdiction (BBNJ) may provide a more coordinated approach to the establishment of area-based measures for the protection of marine biodiversity, but at present, such coordination would have to proceed on a very slow and labor intensive case-by-case basis (Freestone et al., 2014).

Offsetting through averted loss could also theoretically apply if an area to be mined is converted to a no-mining area. However, as the seafloor is presently under exploration only and there is no exploitation contract in place in the Area, proving that likely exploitation has been averted will be difficult. Protection of important or significant areas inside mining areas may also be a required component of a contractor's environmental management plan (EMP) to preserve rare or fragile ecosystems and for long-term scientific research and monitoring. A further challenge results from the requirement of contractors to setup preservation reference zones in their claim areas. These zones will need to be ecologically similar to mined areas, but unperturbed by mining, in order to monitor mining impacts (ISA, 2010, 2011, 2012, 2013). However, because unimpacted reference zones are already required by ISA guidelines, they do not provide the additionality required to be considered as an offset.

Extending or increasing these Preservation Reference Zones beyond what will be contractually required within mining concessions could, however, present an option for offsetting biodiversity losses if it could be shown that there was a real (direct or indirect) risk of their future degradation from mining impacts. Without ascertaining this additionality these measures might be used to effectively mask biodiversity losses through "protecting" areas that weren't economically attractive to industry and therefore unlikely to be threatened (**Table 1**). This approach has been adopted by Nautilus Minerals Niugini Ltd, where predicted biodiversity losses associated with the Solwara I project have been "mitigated" to provide colonists to assist with post-mining regeneration (there was no requirement to meet an aim of NNL) by leaving a nearby site, South Su, unmined (Coffey Natural Systems Pty Ltd, 2008). Notwithstanding the fact that Solwara I and South Su feature different assemblages and so would unlikely meet an aim of NNL, in order for this to be considered as an offset, evidence to prove that South Su would otherwise be targeted for mining is required.

Offset Misuse

The term "biodiversity offset" is frequently misapplied and misused (Bull et al., 2016). True offsets require the provision

TABLE 1 | Options for the final step of the mitigation hierarchy and barriers to their effective application in the deep sea.

	Compensatory mechanism type	Barriers to application in the deep sea
Offset	***"Like for like" averted loss*** of biodiversity through the protection of a similar type and equivalent amount of habitat under threat from other existing or planned activities. For example, the removal of bottom trawling pressures to offset mining impacts on seamounts.	Lack of data and agreed surrogates to establish ecological equivalence between biodiversity lost and that restored. Difficulty in proving that in the absence of action, under the most likely future scenario, this biodiversity is likely to be lost and as such equals a bona fide gain in biodiversity. Absence of legal mechanism to limit access. Need for complex and long-term monitoring and compliance regime. Need for long-term significant financial commitment.
Offset	***"Like for like" habitat restoration*** to create/restore new, additional and equivalent biodiversity of a similar type in a different location to that lost to ensure no net loss.	Lack of data and agreed surrogates to establish ecological equivalence between biodiversity lost and that restored. Lack of spatial data to locate and identify degraded similar habitat to that damaged by mining project for restoration. Restoration of deep-sea biodiversity within appropriate space and time scales currently considered unfeasible in most cases. Restoration techniques are unproven and the efficacy of any efforts are likely to be uncertain for many decades. Need for long-term significant financial commitment. Limited legal mechanisms to limit disturbance by other human activities. Need for long-term significant financial commitment.
Compensation	***"Out of kind" habitat restoration*** to create new, additional and equivalent biodiversity of a different type and/or in a different location such as in shallow or coastal environments.	Lack of data and agreed surrogates to establish ecological equivalence between biodiversity lost and that restored. Inconsistencies with the UNCLOS obligation to "ensure effective protection of the marine environment from harmful effects" of mining-related activities in the Area. Low level of proven success of marine ecological restoration techniques for any habitat or species, particularly at large scales; any efforts are likely to be associated with high uncertainty. Need for long-term significant financial commitment. Limited legal mechanisms to limit disturbance by other human activities. Need for long-term significant financial commitment. Lack of competence of the ISA to mandate habitat restoration in areas within national jurisdiction. Restoring habitat within national jurisdiction in order to compensate for habitat loss in the area is unlikely to fulfill expectations under the principle of common heritage of mankind (i.e., it is effectively a transfer of wealth from the international community to recipient coastal states). No societal mandate, or scientific rationale, to support trading up or across habitats, ecosystems, or biogeographic provinces and to accept residual losses of deep-sea biodiversity. Shift in ethical basis of conservation whereby the presence of "easy" alternatives degrades barriers previously based on moral objections to the biodiversity loss.
Compensation	***Additional actions*** that do not seek equivalence or provide biodiversity gains ecologically linked to biodiversity losses, such as capacity building.	Lack of data to establish financial equivalent (such as through an ecosystem services evaluation). Inconsistencies with the UNCLOS obligation to "ensure effective protection of the marine environment from harmful effects" from mining-related activities in the Area. Issues of additionality whereby any such compensation would have to be above any monetary and non-monetary benefit shared by contractors based on royalties, profits etc. from mineral extraction in the Area, and any compensation would have to be reinvested in a way that benefits mankind as a whole. No societal mandate to support a net biodiversity loss. Shift in ethical basis of conservation whereby the presence of "easy" alternatives degrades barriers previously based on moral objections to the biodiversity loss.

of new and additional biodiversity benefits and "measurable and commensurate gains" (Bull et al., 2016). "In kind" or "like for like" offsets refer to conservation actions designed to benefit a similar habitat or the same species/communities, allowing for an assessment of ecological equivalence (**Table 1**). Where equivalence is not demonstrated, such activities cannot be referred to as offsets and should be more accurately termed compensatory measures (Bull et al., 2016). Determining whether a gain in one type of biodiversity compensates for the loss of another type is probably not scientifically or ethically justifiable in the deep sea, especially given the uncertainties that pervade such environments (**Table 1**). This issue becomes increasingly scientifically challenging as the ecological and evolutionary distance between a mining impact area and an "out of kind" compensation action increases, especially if the actions ultimately promote ecosystem functions and services that fundamentally differ from those that were lost. "Out of kind" measures should not be called biodiversity offsets as these involve a trade across dissimilar biodiversity, where losses of one biodiversity type are accepted in place of benefits for different habitats, species and/or communities. Accepting and clearly communicating that these "out of kind" actions do not offset biodiversity loss is important to avoid misinterpretation of the trade-offs and losses implicated in decisions to progress with deep-sea mining projects.

"Trading up," a controversial and "out of kind" conservation action to yield gains in biodiversity in areas considered to be of higher conservation value (BBOP, 2012), has been proposed as a compensatory action, incorrectly labeled as a possible offset, for impacts associated with deep-sea mining. For example, it has been suggested that damage in the deep sea from mining (which will inevitably involve biodiversity loss) might be compensated or offset through an "International Marine Mitigation Bank" (ECO, 2016; ISA, 2016; Fish Reef Project, 2017), which deploys "reef balls"—concrete substrata—to promote coral-reef habitat and biodiversity in shallow-water ecosystems. This and other "trading-up" practices assume that loss of largely unknown species and ecosystems in the deep sea can be exchanged for protecting biodiversity elsewhere. From a scientific perspective, such an assumption is highly questionable. In our view, the relationship between any gain in biodiversity in a shallow-water coral-reef setting and loss of biodiversity in the deep sea is too ambiguous to be scientifically meaningful and cannot be considered to be offsetting deep-sea biodiversity loss (**Table 1**). Moreover, compensating biodiversity loss in the Area with environmental restoration in coastal waters is legally problematic because the ISA's jurisdiction is limited to the Area. Additionally, under the principle of the Common Heritage of Mankind, measurable benefits of mining activities on the international seabed, which include those relating to biodiversity, must accrue to the international community as a whole and particularly to developing states (Jaeckel et al., 2016; UNCLOS, Art. 150). Compensating a few coastal states for a loss of biodiversity that occurs in areas beyond their national jurisdictions would in effect constitute a transfer of natural wealth. Such a scheme appears contrary to the historic intentions of UNCLOS (Nandan et al., 2002) and the Common Heritage of Mankind principle wherein the ISA acts as a trustee for mankind in perpetuity with benefits to be shared equitably (Wolfrum, 1983). These legal concerns may not apply where both mining and a "trading-up" conservation action take place in national waters, although the scientific and ethical concerns remain.

Similarly, no-mining areas, referred to by the ISA as "Areas of Particular Environmental Interest" (APEIs) (ISA, 2011; Wedding et al., 2015) have been incorrectly characterized by some as "strategic offsets" in international waters (Johnson and Ferreira, 2015; IUCN, 2016). Notably, they do not provide new and additional biodiversity benefits and thus do not actually offset residual losses of biodiversity that might be incurred by a mining project. Therefore, these are more accurately seen, not as offsets, but as part of a conservation plan to avoid irreversible harm from the extirpation of species or loss of ecosystem function in the mining region.

NET LOSS AND ENVIRONMENTAL RESPONSIBILITY

Industrial-scale remediation is not demonstrated and likely not feasible, and offsets are impossible, as such it can be reasonably expected that deep-sea mining will result in a net loss of biodiversity in the direct mining footprint and for some distance around it (Levin et al., 2016; Van Dover et al., 2017). These losses may well be irreversible on timescales relevant to management and possibly for many human generations (Amon et al., 2016; Vanreusel et al., 2016; Jones et al., 2017). If neighboring areas are protected from mining and its effects (such as through APEIs), these can be expected to mitigate the ecological damage on a regional scale, and may assist in some local re-colonization and regeneration. Such no-mining areas can be expected to feature as an important mechanism to avoid and minimize some biodiversity loss. However, despite their advantages, protected areas cannot be expected *prima facie* to avert all biodiversity losses associated with mining. Furthermore, protected areas cannot be construed as being offsets because no new gains in biodiversity will have been created.

Owing to our current limited knowledge of deep-sea ecosystem structure and function, we cannot determine the significance and therefore acceptability of the likely biodiversity losses associated with deep-seabed mining. In the face of our ignorance, the precautionary principle requires that we consider the potentially irreversible consequences of any decisions made and ensure that sufficient procedural, substantive, and institutional measures are in place to avoid serious harm (Jaeckel et al., 2017). This would include a transparent and consultative approach to the development of deep-sea mineral exploitation regulations with experts and stakeholders to specify how biodiversity will be measured with sufficient statistical power, what level of net loss of biodiversity and accompanying ecosystem function might be deemed acceptable, and what form of compensation will be provided for the harm that is caused.

Potential Compensatory Actions

While we consider the risks to biodiversity loss from deep-sea mining to be high, the push within the ISA to develop

exploitation regulations indicates that the industry may be progressed prior to an open dialogue on whether such biodiversity losses are ecologically or economically justifiable in terms of its benefit to humankind as a whole (Kim, 2017).

Should deep-sea biodiversity losses and the associated risks of degraded ecosystem services through mining be found to be both justifiable and acceptable, then it is possible, despite scientific concerns, that "out of kind" compensation will be stipulated at the fourth and final stage of the mitigation hierarchy. Given the difficulty in quantifying equivalence across different ecosystems and types of biodiversity, the outcomes of potential compensatory actions may be reduced to financial rather than ecological benefits. The quantification of financial sums would need to be linked to the value of biodiversity loss, calculated based in part on the total value (both instrumental and intrinsic) of the natural capital lost. Based on the Common Heritage of Mankind, the interests of society as a whole, including lost opportunities to future generations, must also be included in such assessment. Given the importance of deep-sea ecosystem services, the levels of evolutionary novelty, and the challenges of restoration as outlined, calculations would be complex and the sums could conceivably be immense.

Ethically, potential compensatory actions beg the difficult question: is one form of life, or evolutionary pathway, equivalent to another? Can the living elements of biodiversity be treated, and traded, as though part of a single currency, even though each species, habitat and evolutionary pathway is unique? And if so, how will the exchange values be set? Can the preservation and recovery of one form of life legitimize the destruction of others (Soulé, 1985)? A further concern is that the acceptance of loss during a biodiversity trade engenders a shift in the ethics of biodiversity protection previously based on moral objection (Ives and Bekessy, 2015; Spash, 2015) and reduces societal pressures to reject proposals with associated environmental damage (**Table 1**). Finally, establishing a marketplace on nature assumes that when components from one ecosystem are exchanged for others, the outcomes are manageable (i.e., the currency allows for easy and equal exchange between units). Unpleasant, possibly irreversible, "surprises" that characterize complex systems built upon unique components, such as found in deep-sea environments, are not taken into account to allow for trading to take place.

If potential compensatory actions are required, these should be limited to actions that benefit the understanding and conservation of deep-sea biodiversity. Capacity building for deep-sea biodiversity research and conservation is one such measure. Again, any capacity-building action on the part of a contractor would have to be *in addition* to the capacity building already required by the sponsoring State or ISA (ISA, 2010, 2012, 2013). Investment in research on biodiversity in the deep sea, including advancing understanding of minimization and remediation of mining disturbances, might serve as a potential compensatory action. However, to qualify as a compensatory action, these research activities would need to supplement those required for baseline studies, and for the development of EIAs and monitoring, which are already contractor responsibilities. For example, potential compensatory actions might include biodiversity research at regional scales (i.e., outside claim areas and within APEIs) to provide a broader biogeographic context for interpreting the consequences of mining on biodiversity, test the efficacy of protected-area networks, or to assess the risks of species extinctions (e.g., through quantification of connectivity, and plume contaminant and sound dispersal). Various fund schemes that would charge entities inflicting damage on the seabed to support relevant research on biodiversity in the deep sea have been proposed as possible conservation actions (Barbier et al., 2014; Mengerink et al., 2014; Johnson and Ferreira, 2015). The need for funding of new research, rather than that already required under national laws, the Law of the Sea, and the ISA's Mining Code, will need to be addressed through clear criteria in guidelines and regulations to avoid the displacement of existing commitments. We suggest that this research should have the goal of developing new knowledge and capacity to protect the marine environment. However, it should always be recognized that this additional capacity has been built on the premise of accepted deep-sea biodiversity loss.

CONCLUSIONS

As exploitation regulations are developed, and contracts for commercial mining in the Area are considered, the inability to achieve and verify a goal of NNL through the mitigation hierarchy should be broadly recognized and debated. If mining is permitted and losses accepted, national governments, the ISA, and deep-sea mining contractors will need to focus even greater attention on the preventive steps of the mitigation hierarchy (avoidance and minimization) using a precautionary and adaptive approach. This should be accompanied by research inside and outside mining areas that add to our knowledge and capacity to better understand and protect deep-sea biodiversity, and that is additional to pre-existing legal requirements (Rainey et al., 2014). Such an approach could involve a staged approach to permitting the development of the industry with a number of small sites of perceived lower risk being exploited to develop mitigatory technologies and to monitor and test predicted impacts (Tinch and van den Hove, 2016). Improved knowledge obtained from a staged strategy should inform the progression of the industry, with future stages of exploitation being contingent on the successful ability to predict and take action to minimize impacts and associated biodiversity loss.

Given the very slow natural rates of recovery in most deep-sea ecosystems targeted for mining, loss of biodiversity in the deep sea is inevitable and may be considered to be "forever" on human time scales. In effect, the actions of one generation will affect the common heritage of humankind for many generations to come. To avoid, or at least ameliorate this inter-generational inequity, deep-sea mining should yield demonstrable economic benefits, as well as benefits from compensatory measures for current and future generations. The ecological consequences of a net loss of biodiversity in the deep sea are poorly understood and approaches for avoidance and minimization of losses remain limited and unproven. As the ISA develops regulations for seabed mining, it is essential that the potential significance and consequences of this loss (including for future generations) are clearly communicated, understood and taken into account. Given the potential scale of harm, this matter is deserving of wider inclusive debate, both within the ISA and internationally. The

scale of biodiversity loss that may be associated with some types of deep-seabed mining and the very limited ability to understand, remediate and offset these losses in "like for like" settings may well preclude their scientific or social acceptance.

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Assessment of Marine Litter in the Barents Sea, a Part of the Joint Norwegian–Russian Ecosystem Survey

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This study presents a large-scale monitoring of marine litter performed in the joint Norwegian–Russian ecosystem monitoring surveys in the period from 2010 to 2016 and contribute to documentation of the extent of marine litter in the Barents Sea. The distribution and abundance of marine litter were calculated by recordings of bycatch from the pelagic trawling in upper 60 m, from bottom trawling close to the sea floor, and floating marine debris at surface by visual observations. The study is comprehensive regarding coverage and number with registrations from 2,265 pelagic trawls and 1,860 bottom trawls, in addition to surface registration between the stations. Marine litter has been recorded from 301 pelagic and 624 of the bottom trawl catches. In total, 784 visual observations of floating marine debris were recorded during the period. Marine litter has been categorized according to volume or weight of the material types plastic, wood, metal, rubber, glass, paper, and textile. Marine litter is observed in the entire Barents Sea and distribution vary with material densities, ocean currents and depth. Plastic dominated number of observations with marine litter, as 72% of surface observations, 94% of pelagic trawls, and 86% of bottom trawls contained plastic. Observations of wood constituted 19% of surface observations, 1% of pelagic trawls, and 17% of bottom trawls with marine litter. Materials from other categories such as metal, rubber, paper, textile, and glass were observed sporadically. Recordings of wood dominated surface observations (61.9 ± 21.6% by volume) and on seafloor (59.4 ± 35.0% by weight), while plastic dominated marine litter observations in upper 60 m depth (86.4 ± 16.5% by weight) over these 7 years. Based on recordings and volume or area covered, mean levels of plastic in the upper 60 m of the Barents Sea were found to 0.011 mg m^{-3} (pelagic) and 2.9 kg km^{-2} at sea floor over the study period. Average levels of marine litter (all material types) at the sea floor were found to be 26 kg km^{-2}.

Keywords: marine litter, plastic, surface, pelagic, sea floor, Barents Sea

INTRODUCTION

During the last years marine litter has received more attention as an important threat to animal life and ecosystem health. Marine litter is defined as any persistent, manufactured, or processed solid material discarded, disposed of, or abandoned in the marine environment (UNEP, 2009). In 2015, 322 million tons of plastic were produced globally, in addition to 61 million metric tons synthetic fibers (Lusher et al., 2017). It has been estimated that in 2010, between 4.8 and 12.7 million metric tons of plastic waste entered directly into the oceans (Jambeck et al., 2015). Population densities and the effectiveness of waste management systems largely determined which country contributed most to uncaptured waste becoming marine debris (Jambeck et al., 2015).

As part of the objectives in the Marine Strategy Framework to achieve good environmental status in all EU marine waters by 2020, marine litter is listed as descriptor ten of eleven descriptors important for this goal. The aim for marine litter is to ascertain that properties and quantities of marine litter do not cause harm to the coastal and marine environment (EU-MSDF, 2008).

Marine debris and microplastics have been reported everywhere people have investigated its occurrence in the marine environment; at surface, in the water column, in biota, at sea floor and in sediments (GESAMP, 2016; UNEP, 2016). It is a classic transboundary issue, in many cases unseen, but end up in the environment, probably to a large degree on the sea floor. Large variation in occurrence and densities of marine litter at the sea floor have been reported from different areas, dependent on distance to coastline, population densities, distance to shipping routes, rivers, topography, water currents, and circulations. Material densities, fouling processes, size and shape are important for transport distance and sedimentation rate. Highest densities of marine litter have been found in submarine canyons, while continental shelves and ocean ridges have the lowest densities (Galgani et al., 2000; Ramirez-Llodra et al., 2011; Pham et al., 2014; Woodall et al., 2015; Buhl-Mortensen and Buhl-Mortensen, 2017). In the Mediterranean, densities of marine litter collected by trawling from deep water areas (mean depth 1,400–3,000 m) ranged from 400 kg km^{-2} at the continental slope south of Palma de Mallorca to densities ranging between 70 and 180 kg km^{-2} at the other sites (Galgani et al., 2000; Pham et al., 2014). In four gulfs in Greece, densities ranged from 7 to 47 kg km^{-2} (Koutsodendris et al., 2008). Densities of litter in the Ryukuy Trench and in the basin of Okinawa through in the Northwest Pacific ranged from 8 to 121 kg km^{-2}, while shallower continental slopes or abyssal plains ranged from 0.03 to 9 kg km^{-2} (Shimanaga and Yanagi, 2016). In the European part of the Atlantic Ocean, 43–74 kg km^{-2} have been recorded in the Bay of Biscay (Lopez-Lopez et al., 2017), while a mean of 123 kg km^{-2} have been estimated offshore at the Norwegian shelf and slope in the Norwegian Sea, and 154 kg km^{-2} have been estimated offshore in the Barents Sea (Buhl-Mortensen and Buhl-Mortensen, 2017). For coastal areas, higher levels were recorded; A mean of 2,510 kg km^{-2} was recorded along the Norwegian coast from Ålesund to Lofoten and 227 kg km^{-2} from Lofoten to the Russian border (ibid).

The main sources of marine litter in Nordic Seas are sea based from maritime activities, such as fisheries, shipping, oil and gas exploration, and tourism. Discharges of human consumables can origin from both sea- and land based activities (UNEP, 2009). Population densities and intensity of the marine activities are important for how large input of marine litter may be (Pham et al., 2014; Jambeck et al., 2015). Abandoned fish gear lead to ghost fishing and entanglement (Gilardi et al., 2010). A vast number of species has been documented to be entangled in, or having ingested marine litter and die from of damage related to marine litter every year (UNEP, 2009; Gall and Thompson, 2015; Kühn et al., 2015). Species like sea birds, sea mammals, turtles, and fish are susceptible to such effects (ibid.).

The Barents Sea is a transition zone between Atlantic and Arctic conditions and is influenced by strong inflow of Atlantic waters with an annual mean inflow of 2 Sverdrup (Sv) and weaker inflow of coastal (∼1 Sv) and Arctic waters (∼1 Sv). Since the 1980s the Barents Sea has gone from a situation with high fishing pressure, cold conditions and low demersal fish stock levels, to the current situation with high levels of demersal fish stocks, reduced fishing pressure, and warmer conditions (ICES, 2017). As boreal species like cod, migrate northwards for feeding, cod fisheries also moves in to the northern areas. The Barents Sea is classified as a clean and rich ocean with low levels of environmental pollution (ICES, 2017). Time-series recorded for the last two decades show that the levels of persistent organic pollutants have been stable or decreasing (ICES, 2017). With regards to marine litter, increased concern is directed to this area as oceanographic modeling indicate the Barents Sea to be a potential gyre for marine litter accumulation (van Sebille et al., 2012). Plastic, as other floating marine debris, could be transported by currents along the coast and in to the open sea (Barnes et al., 2009). Recent studies with microplastic measurements at the surface in the Arctic ocean and the Barents Sea added support for this possibility (Cózar et al., 2017).

This study presents a large-scale recording of marine litter performed in the joint Norwegian–Russian ecosystem monitoring surveys in the period from 2010 to 2016. This comprehensive monitoring program (spatial coverage of 350 thousand square nautical miles and sampling effort of >4,000 stations) of marine litter is unique and contribute to documentation of the extent of marine litter in the ecosystem. Marine litter has been categorized according to volume or weight of the material types plastic, wood, metal, rubber, glass, paper, and textile. We investigated spatial pattern of marine litter taken from the two types of trawls (pelagic trawl: covering 0–60 m and bottom trawl: covering bottom and ca. 5 m above) and floating marine debris at surface observed from the ship.

MATERIALS AND METHODS

Study Area

The Barents Sea is a large shelf area (about 1.6 million km^2) located at high latitudes between 70 and 80°N to the north of Norway and Russia. The mean depth is about 230 m and the maximum depth in the western Barents Sea is about 500 m. Two archipelagos (Spitsbergen and Franz Josef Land) are located in

the northern Barents Sea. The bottom topography is complex with several larger (Central bank) and smaller (North Cape Bank, Spitsbergen Bank, Thor Iversen Bank and Tidley Bank) banks and deeper trenches (Bear Island Channel, St. Anna Trough, Central Bank Basin and Murman Rise in between. In the western part the Bear Island Trough provides a deeper connection with the Norwegian Sea, and in the northeast the St. Anna Trough provides a deeper connection with the Arctic Ocean via the northern Kara Sea (**Figure 1**). The bottom topography with banks and basins steers the currents and governs the distribution of water masses in the Barents Sea (Loeng, 1991). Warm and saline Atlantic waters flow into the southwestern Barents Sea from the Norwegian Sea. The North Atlantic Drift splits into two main branches, one flowing into and through the Barents Sea from southwest to northeast, the other flowing around the western and northern flanks of the Barents Sea as the West Spitsbergen Current (**Figure 1**, Skagseth et al., 2008; Ingvaldsen and Loeng, 2009; Ozhigin et al., 2011). Cold fresh Arctic waters arrive from the Arctic Ocean, entering the Barents Sea between Nordaustlandet and Franz Josef Land and between Franz Josef Land and Novaya Zemlya. The Norwegian Coastal Current flows eastwards following the coastline and bring fresh water from the northern Norwegian and Russian coasts in to the Barents Sea.

The Monitoring Activities and Sampling

The Barents Sea Ecosystem Survey covered the entire Barents Sea with 35 nautical miles between stations (**Figure 2**, Survey activities 2010–2016; Eriksen et al., 2017). Two to three Norwegian vessels cover the Norwegian economic zone and

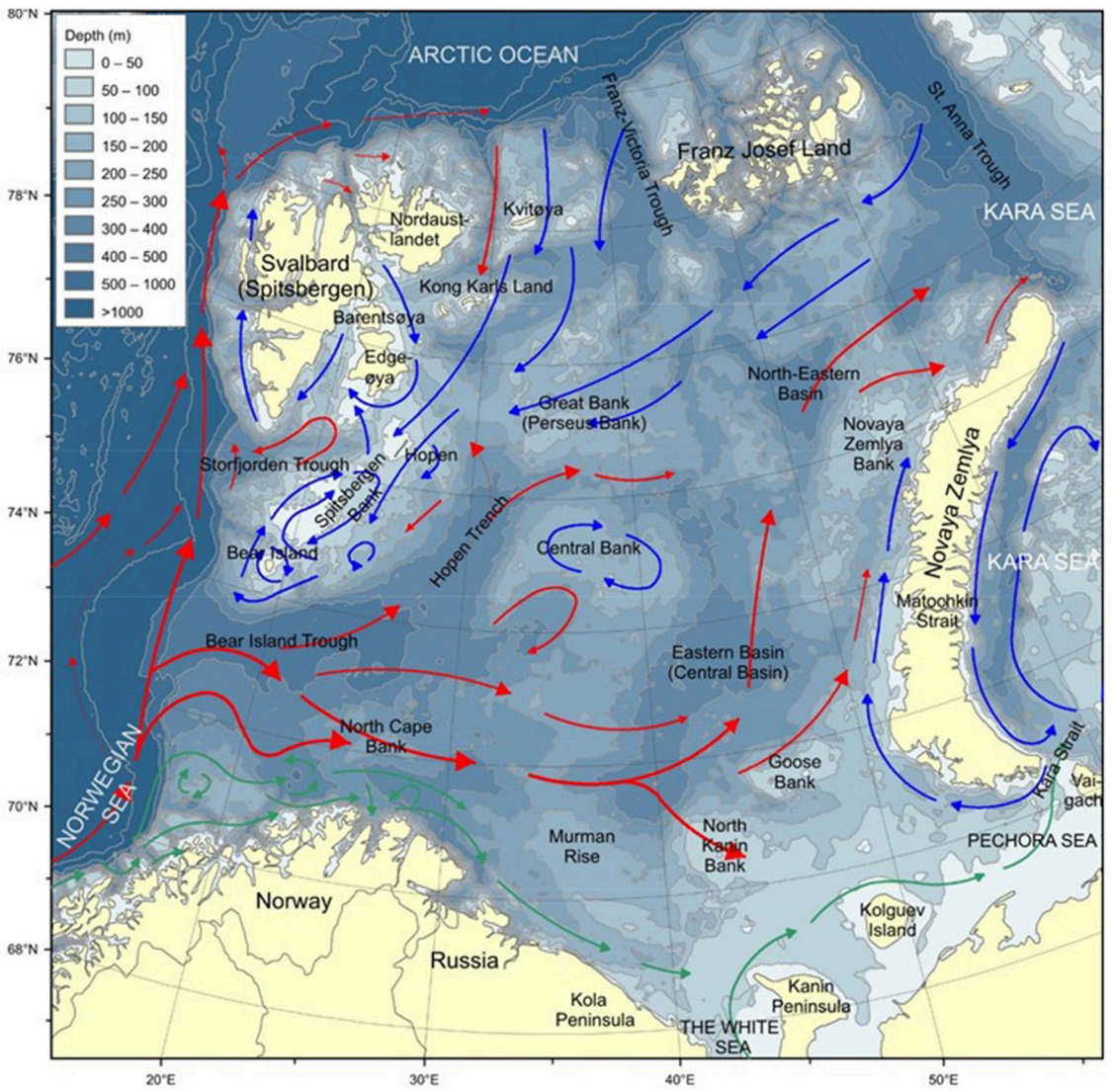


FIGURE 1 | The Barents Sea. Red arrows show Atlantic water currents, blue arrows Arctic currents and green arrows currents of coastal waters. Published with courtesy of Institute of Marine Research, Norway.

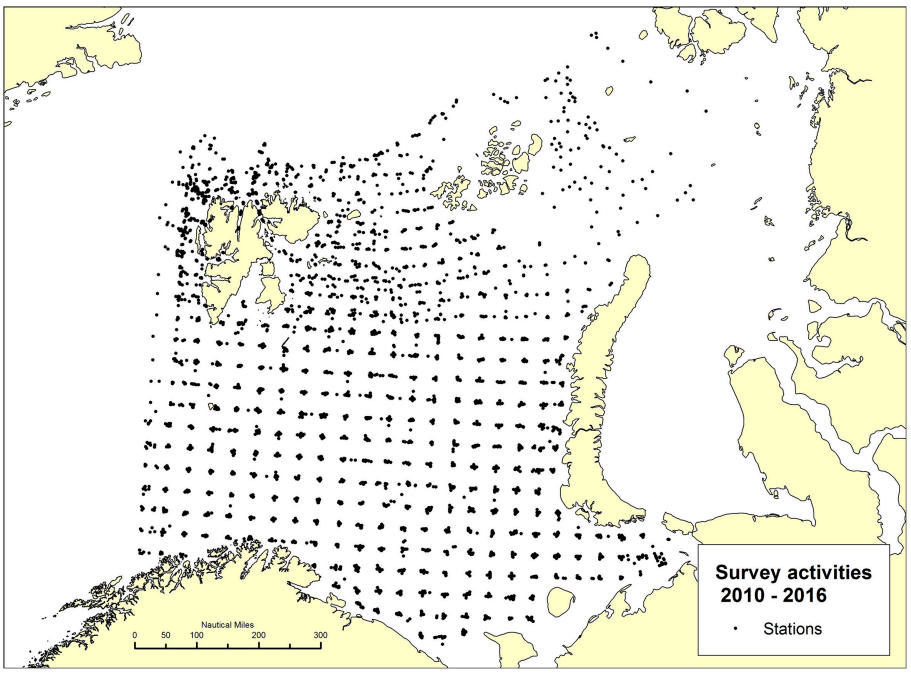


FIGURE 2 | Survey activities 2010–2016. The BESS covers the entire Barents Sea with 35 nautical miles between ecosystem stations. Dots indicate stations, where pelagic and bottom trawl hauls were taken. Location of station varied slightly between years.

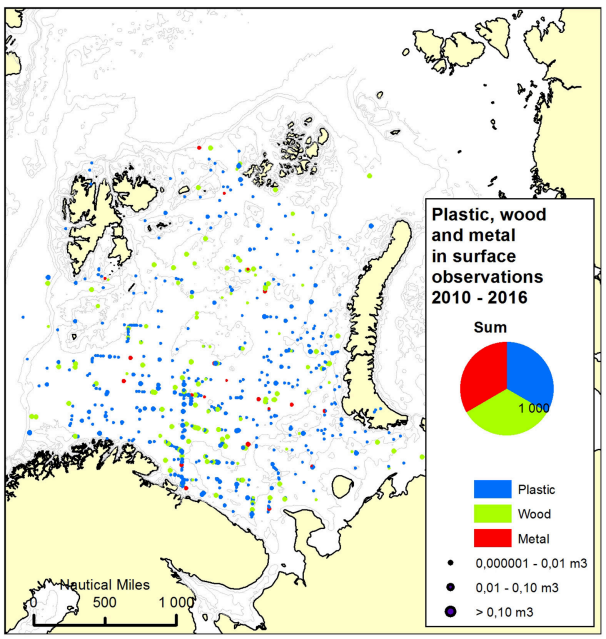


FIGURE 3 | Marine litter from surface observations 2010 to 2016. Observations of plastics (blue), wood (green) and metal (red).

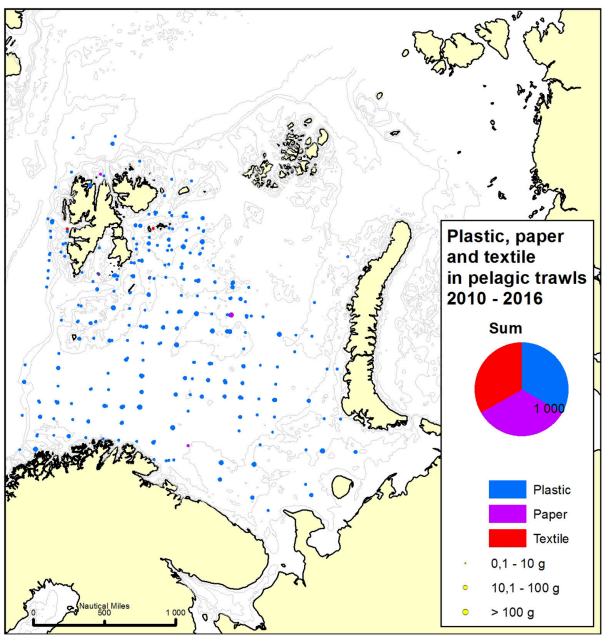


FIGURE 4 | Marine litter from pelagic trawl from 2010 to 2016. Observations of plastics (blue), paper (purple) and textile (red).

the Fisheries protection zone around Svalbard, and one to two Russian vessels cover the Russian economic zone. The indicated stations include a bottom trawl haul, a pelagic trawl haul (0–60 m) and other equipment (more detail in Michalsen et al., 2011). All vessels use standard trawls and trawling procedures and data are comparable between vessels and years.

Trawling

The distribution and abundance of marine litter in upper 60 m are based on pelagic trawling with a small meshed pelagic trawl "Harstad trawl" with a mouth opening of 20 × 20 m, with seven panels and a cod end. The panels have mesh sizes varying from 100 mm in the first part to 30 mm in the end. Pelagic trawling was carried out at three depths, each over a distance of 0.5 nautical mile, with the headline of the trawl located at 0, 20, and 40 m, respectively, and with trawling speed of three knots.

The distribution and abundance of marine litter near the bottom are based on trawling with the standard research bottom trawl "Campelen 1800 shrimp trawl" with 80 mm (stretched) mesh size in the front, cod-ends of 22 mm mesh size and a cover net of 116 mm meshes. The trawl was equipped with a rockhopper ground gear and sweep wire length of 40 m, plus 12 m wire for connection to the doors. Standard tow duration was 15 min at three knots. Trawl performance was constantly monitored by Scanmar trawl sensors, i.e., distance between the doors, vertical opening of the trawl and bottom contact control. From trawl catches, marine litter were sorted and classified according to material type and weight. When starting up registration of marine litter as bycatch from the Barents Sea ecosystem surveys IMR and PINRO decided to use a simple classification of marine litter: plastic, wood, metal, rubber, glass, paper, and textile (some years). The data were recorded (category and numbers) in standard data base on board and later transferred to the IMR/PINRO data base. The data of marine litter do not include information about sources of (e.g., from fisheries, human consumables, or other).

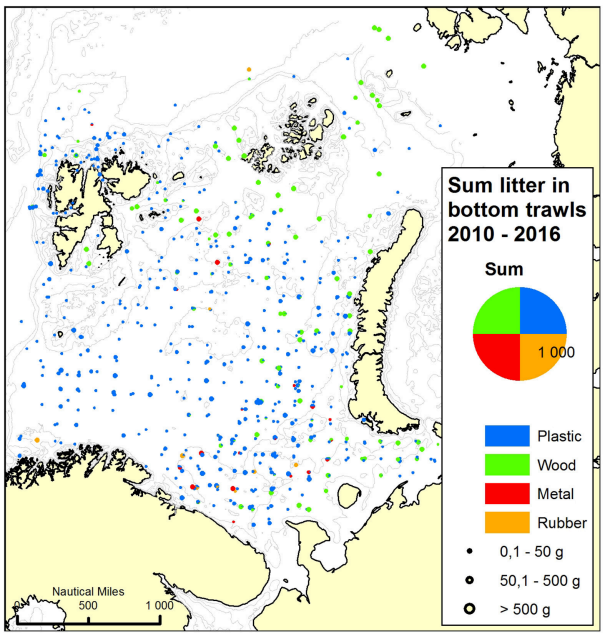


FIGURE 5 | Marine litter from bottom trawl from 2010 to 2016. Observations of plastics (blue), wood (green), metal (red) and rubber (orange).

TABLE 1 | Number of observations of floating marine debris and number of trawls, where any kind of marine litter observed given for each years and sum for the studied period 2010–2016.

Year	Observations of floating marine debris	Pelagic trawls with marine litter	Bottom trawls with marine litter
2010	145	25	63
2011	242	28	94
2012	69	57	144
2013	83	39	127
2014	40	40	53
2015	107	67	106
2016	98	45	37
Sum observations	784	301	624

Visual Observations

During transit between stations (35 nm miles), observations of floating marine debris at surface were recorded by whale observers, and material types and volumes were noted. Visual observations were taken only during day time and when weather and visibility was suitable. Observers recorded approximate volume of the same categories of the floating marine debris. For this reason, data from visual observation have some uncertainties due to several limitations described above and should therefore be interpreted with care, but still contain valuable information. For more information see annual survey reports from the BESS, which are available on https://www.hi.no/tokt/okosystemtokt_i_barentshavet/survey_reports/nb-no.

Data Treatment: Mapping, Composition, and Analyzing

Recordings of marine litter from surface, pelagic trawl and bottom trawl from 2010 to 2016 have been used to prepare GIS maps to map the distribution in the Barents Sea. **Figures 2–5** were made with land maps from ESRI and bathymetric contours

TABLE 2 | Percentage composition of marine litter floating at surface (by volume), or as bycatch in pelagic and bottom trawls (by weight) for the period 2010–2016, presented as mean per year ± SD.

Material	Plastic (%)	Paper (%)	Textile (%)	Wood (%)	Metal (%)	Rubber (%)	Glass (%)	Other (%)
Surface	34.6 ± 22.3	0.3 ± 0.4	0	61.9 ± 21.6	2.3 ± 2.3	0.8 ± 1.8	0	0.1 ± 0.1
Pelagic	86.4 ± 16.5	2.1 ± 5.4	7.4 ± 18.2	0.0 ± 0.1	0.5 ± 0.9	1.7 ± 3.5	0	1.8 ± 3.6
Bottom	18.4 ± 20.4	1.5 ± 3.8	5.2 ± 12.8	59.4 ± 35.0	5.1 ± 9.7	9.8 ± 17.1	0.0 ± 0.1	0.5 ± 1.2

from GEBCO with ArcGIS 10.5.1. The three or four most abundant material types were plotted to indicate area of occurrence and distribution.

Pelagic catches were standardized by filtered volume of water defined by towing distance for each trawling 0.5 nautical miles (926 m), trawl opening (20 × 20 m) and covered depth (0–60 m). Furthermore, the pelagic marine litter (weight per m^3) were calculated by mean weight per haul, frequencies of stations with marine litter and filtered volume. Densities of marine litter at sea floor (weight per km^2) were estimated by mean weight and frequencies of stations with marine litter and covered area (distance * trawl width). Towing time was 15 min for three knots.

Visual estimated volume of the floating marine debris was used for mapping and correlations analyses only. Pearson correlation were used to study relation between annual and total occurrence of categories (plastic and wood only), and environmental parameters (latitude and longitude) (for surface, pelagic, and bottom observations) and depth (for bottom observations).

RESULTS

During the joint Norwegian–Russian Barents Sea ecosystem surveys in the period from 2010 to 2016, large scale recordings of marine litter from surface and as bycatch in pelagic and bottom trawls have been performed. Weight of marine litter of different material types have been recorded from 2,265 pelagic trawls and 1,860 bottom trawls in addition to surface observations between the stations by whale observers. Marine litter has been recorded from 301 pelagic and 624 of the bottom trawl catches. In total, 784 visual observations of floating marine debris were recorded during the period. Annual records of marine litter are summarized in **Table 1**. Plastic dominated number of observations, as 72% of surface observations, 94% of pelagic trawls, and 86% of bottom trawls with marine litter contained plastic. Observations of wood constituted 19% of surface observations, 1% of pelagic trawls with marine litter and 17% of bottom trawls with marine litter. Materials from other categories such as metal, rubber, paper and textile, and glass were observed sporadically.

Surface Observations

Floating marine debris were widely distributed in the Barents Sea, while highest volume of marine litter was observed in the central, eastern and northern areas (**Figure 3**). Wood dominated the floating marine debris observations (61.9 ± 21.6% by volume), while plastic constituted 34.6 ± 22.3% by volume. Metal, rubber and paper were recorded sporadically (**Table 2**).

Larger volume of floating wood was observed in 2010, 2011, and 2015, with estimated volumes of 11.9, 17.0, and 8.7 m^3, respectively. Floating wood significantly correlated with latitude and longitude some years, and indicated northward distribution in 2011 and 2013, north-eastern in 2014 and 2016, and eastward in 2012 (**Table 3**). Larger volume of floating plastic was observed in 2011 and 2012 (11.7 and 5.0 m^3, respectively). Floating plastic were significantly correlated with latitude and longitude some

TABLE 3 | Pearson correlations between marine litter and environmental parameters [latitude and longitude (for surface observations, pelagic, and bottom catches) and depth (for bottom catches)].

Pearson correlation	Floating litter				Pelagic litter			Bottom litter				
	Lat→Plastic	Long→Plastic	Lat→Wood	Long→Wood	Lat→Plastic	Long→Plastic	Lat→Plastic	Long→Plastic	Depth→Plastic	Lat→Wood	Long→Wood	Depth→Wood
2010	−0.12	0.02	−0.07	−0.18	0.22	0.20	−0.27	−0.15	0.23	−0.29	0.42	−0.37
2011	−0.08	0.03	0.28	−0.03	0.09	0.23	−0.09	−0.09	0.09	−0.31	0.13	0.01
2012	−0.12	0.05	−0.12	0.23	0.03	−0.03	−0.21	−0.11	0.07	0.92	−0.58	−0.87
2013	0.31	0.10	0.28	−0.31	−0.17	−0.03	−0.12	0.03	−0.09	0.32	0.09	0.30
2014	−0.05	−0.27	0.39	0.74	0.02	0.20	−0.15	0.03	−0.05	0.14	−0.10	−0.22
2015	−0.16	0.00	−0.05	0.13	−0.12	0.04	−0.18	0.07	−0.12	−0.09	−0.24	−0.11
2016	0.21	−0.07	0.27	0.26	0.01	0.16	−0.19	0.12	−0.16	0.19	0.30	0.15
2010–2016	−0.05	0.00	0.02	−0.04	−0.08	0.03	−0.05	−0.01	−0.04	0.12	0.01	0.03

Red indicates significant ($p < 0.01$) correlations.

TABLE 4 | Marine litter observations, frequencies, and mean amount in the Barents Sea.

Type of trawl	Total no trawls	Total no trawl with marine litter	Mean weight of marine litter, g	% trawls with marine litter	Mean weight plastic, g	% trawls with plastics	Mean marine litter	Mean plastic
Pelagic	2,265	301	57.9	13	7.31	13	0.082 mg m^{-3}	0.011 mg m^{-3}
Bottom	1,860	624	722	33.5	80.3	28.9	26 kg km^{-2}	2.9 kg km^{-2}

years, and indicated northward distribution in 2013 and 2016, while westward in 2014 (**Table 3**).

Pelagic Marine Litter in Upper 60 m

Pelagic marine litter were observed in 13% of all pelagic trawls with a mean of 58 gram per trawl catch (**Table 4**). Marine litter from pelagic trawls distributed wider in the Barents Sea, while highest catches were distributed in the south western and north central areas, and close to the Norwegian and Svalbard coast (**Figure 4**).

Plastic was the bulk (85.1%) of pelagic marine litter observations (**Table 2**) with mean 0.011 mg m^{-3} (**Table 4**). Paper (9.4%) and textile (3.9%) were observed more seldom, while other materials only sporadically (**Table 2**). Pelagic plastic was significantly correlated with latitude and longitude some years, and indicated north-eastern distribution in 2010, and northern distribution in 2011 and 2014 (**Table 3**).

Marine Litter From Bottom Trawl

Marine litter as bycatch from bottom trawling were observed in 33.5% of all bottom trawl hauls with a mean of 772 g per haul (**Table 4**). Marine litter from bottom trawls distributed wider in the Barents Sea, while the highest catches were taken in the western, south eastern, north eastern, and around Svalbard (**Figure 5**). Plastic were observed from the entire Barents Sea, processed wood in the eastern and northern parts, and metal and rubber in the south east (**Figures 5**, **6**). Processed wood dominated the amount of marine litter from bottom trawls with a mean of 66% of the weight of all catches with any type of marine litter. Plastic constituted 11.4% of the weight, but dominated the number of observations. Metal and rubber consisted ∼10% of the weight but from few numbers of observations. On average, 26 kg km^{-2} of marine litter was found in the Barents Sea, with an average of 2.9 kg km^{-2} of plastics-only (**Table 4**).

In 2010 and 2012, plastic from bottom trawling significantly correlated with latitude and indicated southern distribution (**Table 3** and **Figure 6A**). Wood indicated variations in distribution between years (**Table 3**), but were mainly distributed in the eastern and northern parts of the Barents Sea (**Figure 6B**).

Plastic from bottom trawls were widely distributed in the Barents Sea (**Figures 5**, **6A**). High number of plastic observations from bottom trawls were found in the areas of 100 to 300 m depth (**Figure 7**). Large amounts were also found in deeper areas (>400 m, **Figure 7**), along the north and west part off Svalbard (**Figures 5**, **6A**).

DISCUSSION

Occurrence of marine litter, especially plastic and wood, were observed more frequently and over larger areas, while other types (glass, paper, rubber, and textile) were observed seldom and over restricted areas. Wood dominated by weight at surface and on the sea floor, and were most likely transported by rivers, ocean currents, and winds into the open sea. Our results indicate the distribution of marine litter to vary with material densities, ocean currents, and depth. This is in accordance with observation from other areas (Galgani et al., 2000; Barnes et al., 2009; Ramirez-Llodra et al., 2013).

Occurrence of plastic in trawl catches and visual observations increased from the seafloor (11%) to floating at surface (35%) and were highest in the pelagic layer (upper 60 m), with 85% of the recordings. The pelagic layer is an important feeding area during summer-fall, where plankton, juvenile fish, and large pelagic fish stocks occurs, and the accumulation of different type of food sources attract the predators such as larger fish, marine mammals, and sea birds. Plastic particles may be ingested by fish, sea mammals, and sea birds. This could be due to sun reflection resembling reflections in plankton, fish eyes or fish scales, or color (Kühn et al., 2015). Plastic could also resemble the shape of jellyfish, which are a food source for fish and sea birds (ibid). Marine-seasoned microplastics can produce a dimethyl sulfide signature that is also an odorant/smell attractive for living organism to prey (Savoca et al., 2016). Too high levels of ingested marine litter may clog the digestion system and be fatal for the organisms, as has been reported for many organisms like birds, turtles, fish, and sea mammals (Gall and Thompson, 2015; Kühn et al., 2015).

Levels of marine litter at sea floor had mean value of 26 kg km^{-2} in the Barents Sea, while the mean value of plastic only was 2.9 kg km^{-2}. Levels of marine litter by trawl catch reported in this study is lower compared to estimated average levels of marine litter at sea bottom offshore of the Barents Sea of 154 kg km^{-2} observed from video recordings over restricted area as reported by Buhl-Mortensen and Buhl-Mortensen (2017). Different approaches for estimations of marine litter at the sea floor demonstrate the challenge in calculating densities for large areas as both methods contain uncertainties, for example mesh size with trawling and visual observations with video recordings.

Marine litter in the Barents Sea can origin from various marine activities such as discharges from fisheries, ship traffic, oil and gas exploration, and tourism in addition to land based discharges. The Barents Sea is a rich and productive area, with high fishing activities during the whole year (**Figure 8A**). Other marine activities include transportation of goods, oil and gas,

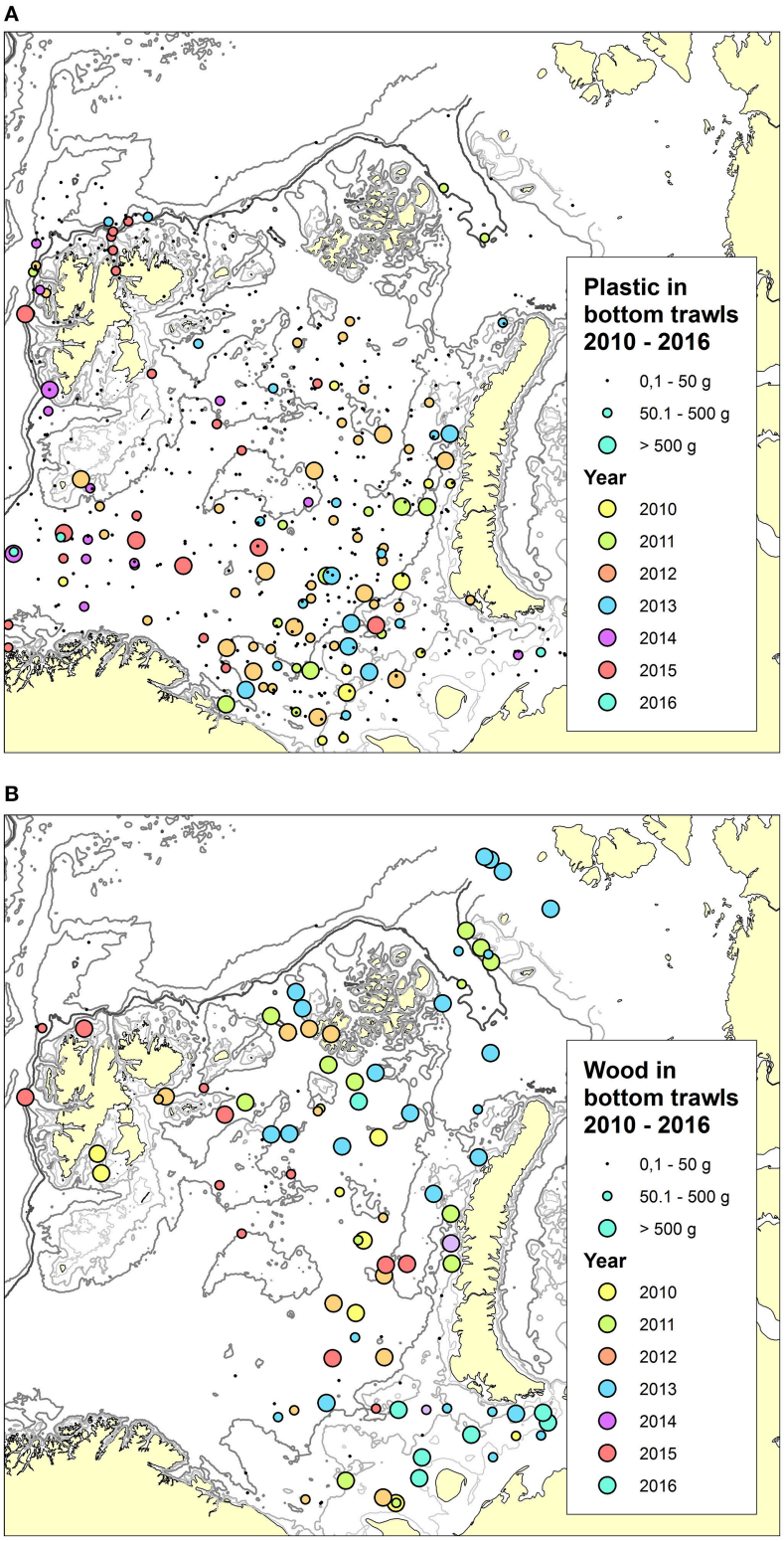


FIGURE 6 | Plastic and wood in bottom trawls from 2010 to 2016, **(A)** Plastic only. **(B)** Wood only. Different colors indicated per year.

and tourism. The main sailing routes are shown in **Figure 8B**. In addition, there are locations for salmon farming along the coast of Norway. Less ice cover and increased oil and gas activities over the recent years have increased ship traffic in the area (Norwegian Ministry of the Environment, 2011; King et al., 2017). Fisheries and other marine activities are the most likely sources of marine debris in the Barents Sea, as also reported from marine litter registrations at beaches at Svalbard (Bergmann et al., 2017) and from sea bottom recordings (Buhl-Mortensen and Buhl-Mortensen, 2017). Our observations indicated larger occurrence of plastic in areas with high intensity of fisheries and ship traffic, which also are retention areas due to ocean currents and depth.

Population densities are low in the adjacent land areas to the Barents Sea both in Norway and in Russia. In the two most northern counties of Norway, Troms and Finnmark, the population densities are 6.4 and 1.6 persons km^{-2}, respectively (www.ssb.no). For the Murmansk county, covering the Kola peninsula the population density is 5.2 persons km^{-2} (https://gov-murman.ru/region/index.php). This is low compared to more densely populated regions as the North Sea and the Mediterranean, and is also reflected in lower discharges from land based activities.

The Barents Sea monitoring documented a wide occurrence of marine debris floating at surface, in the upper 60 m and on the seafloor, and is a comprehensive data set with regards to coverage and number of observations. Even though there are limitations to the catchability and observations, the large number of observation and repeated monitoring strengthens the reliability of the data. Unfortunately, we are at present not able to draw conclusions on whether there are time trends in marine litter during this period. This shows the challenge with interpreting time trends on data from marine litter registrations from a large geographic area, and supports the need for observatory stations to improve the possibility to record changes in deep water environment with time in a defined area (Ruhl et al., 2011). For example, an increase in litter densities at sea floor has been reported from the Arctic deep-sea observatory HAUSGARTEN between 2002 and 2011 (Bergmann and Klages, 2012). On the other hand, studies of microplastics in the surface and the water column in the Baltic Sea (Beer et al., 2017) and at the surface in the North Atlantic subtropical gyre (Law et al., 2010) and East

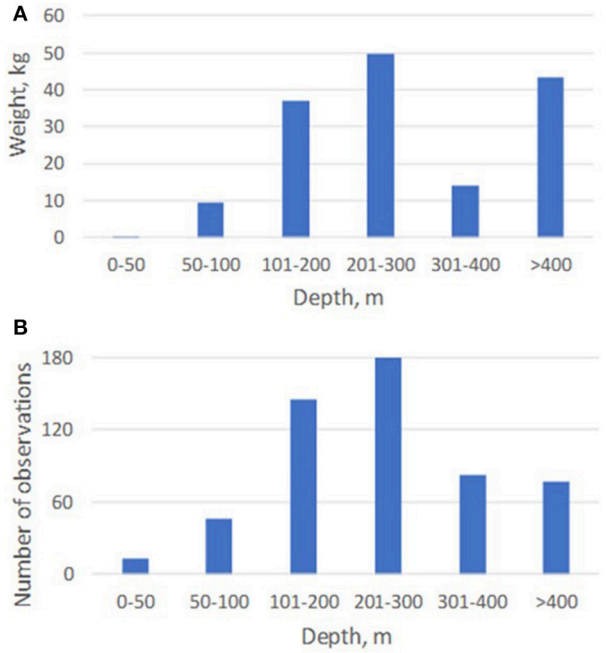


FIGURE 7 | Weight **(A)** and number of observation **(B)** of plastic from bottom trawls separated by depth.

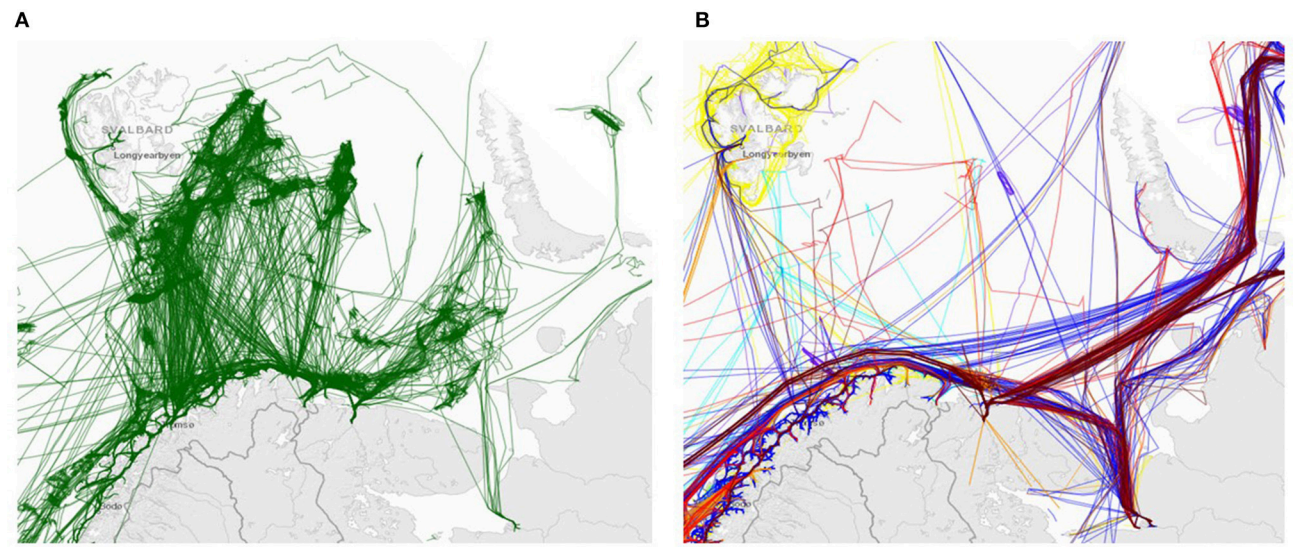


FIGURE 8 | (A) Sailing routes for fisheries (green lines), **(B)** sailing routes for other than fisheries: oil tankers (brown), chemicals/product tankers (red), bulk ship (orange), general cargo ship (blue), cooling/freezing (magenta), passenger (yellow), other activities (peach). Taken from the website havbase.no, period: August 2016.

Pacific gyre (Law et al., 2014) has not revealed a trend over the last decades. This warrants further studies on transport of marine litter and microplastics from surface and water column to the sea floor. We need more knowledge of fate of the continuous discharges of marine litter and microplastics to the Oceans and how it may impact ecosystem health.

AUTHOR CONTRIBUTIONS

BG and EE was responsible for writing the manuscript, BG, TP, EE, PK, PH, and DP contributed substantially with planning, analyzing data, preparing maps and discussions.

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Observations of Litter Deposited in the Deep Waters of Isla del Coco National Park, Eastern Tropical Pacific

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Marine debris is recognized as a major threat to biodiversity and can be found ubiquitously even in remote regions, including deep-sea environments. Isla del Coco National Park is known as one of the best protected areas around the world, a preferred diving destination, and is also renowned for large aggregations of pelagic species that attract illegal commercial fisheries. Despite its pristine reputation, this study demonstrates that anthropogenic debris can affect this remote and well protected area. Images of marine debris were taken using the *DeepSee* submersible, mainly plastic litter from fishing gear were found between 200 and 350 m depth. A total of 40 items of debris were found in 5.48% of 365 dives, 60% of the items were plastics, and local boat and fishing activities provided most of litter. Our aim with this research is to raise awareness about the potential problem of solid wastes and fishing gear deposited in the deep environments of Isla del Coco National Park and to explore the potential implications for the ecological integrity of this protected area, including ghost fishing and the possible ingestion of pollutants. These results provide useful management information for the area, especially if one considers the constant pressure of illegal fishing and tourism at Isla del Coco National Park.

Keywords: submersible research, Costa Rica, conservation, ghost fishing, plastics, marine protected areas, marine debris

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INTRODUCTION

Marine debris, which is defined as persistent, manufactured or processed solids that have been disposed of or abandoned in the marine and coastal environments (Coe and Rogers, 1997), is now recognized as a global problem and one of the major threats to biodiversity (Gall and Thompson, 2015; Browne et al., 2016). There is field evidence for impacts on marine species assemblages due to anthropogenic debris (Browne et al., 2016). Threats to the marine ecosystem arise due to (see revision in Browne et al., 2016): (i) entanglement or ghost-fishing, where animals can be caught and killed by lost fishing gear such as nets or traps (McFadyen et al., 2009); (ii) alteration or destruction of habitat modifying the original community structure (Katsanevakis et al., 2007; Widmer and Hennemann, 2010); (iii) ingestion of debris (Jacobsen et al., 2010; van Franeker et al., 2011), and (iv) rafting, which is a well-known mechanism for dispersal of species, but anthropogenic material can potentially transport alien species across the oceans (Barnes and Fraser, 2003; Barnes and Milner, 2005; Lewis et al., 2005; Molnar et al., 2008). Also, marine debris can be harmful to human health

(Vethaak and Leslie, 2016), has the potential to increase the transport of contaminants (Mato et al., 2001), and it is aesthetically detrimental and hard to remove and control, having in general negative socioeconomic consequences (McIlgorm et al., 2011; Butterworth et al., 2012).

Litter can now be found ubiquitously even in remote regions, as some studies have demonstrated (Bergmann and Klages, 2012; van Cauwenbergue et al., 2013; Woodall et al., 2014), and deep seas are not exempt from its presence (Schlining et al., 2013; Pham et al., 2014). Marine debris can now be found in remote places as the Artic deep waters, where Galgani and Lecornu (2004) found 0.2–0.9 pieces of plastic per linear kilometer at 2,500 m depth, and according to Bergmann and Klages (2012) the amount of litter deposited in the same area has been increasing; the same trend is expected to happen around the world. The deep sea is now recognized as a major sink for microplastic debris (van Cauwenbergue et al., 2013; Woodall et al., 2014). However, anthropogenic debris in deep-sea environments has been less studied since deep-sea surveys are logistically difficult and economically more expensive compared to those conducted in surface waters and along beaches (Ryan et al., 2009).

Isla del Coco is an oceanic island located 500 km off the Pacific continental coast of Costa Rica and is the summit of a seamount on the Coco Submarine Volcanic Range, that extends from the Galápagos Islands to the southern part of Costa Rica (Lizano, 2012; Rojas and Alvarado, 2012). Isla del Coco National Park is known as one of the best protected areas around the world (Edgar et al., 2014), and is recognized for its high diversity, biomass and endemism, features that have made the island a preferred diving destination across the world (Cortés, 2012, 2016). Also, this protected area is renowned for large aggregations of pelagic species, especially sharks (Carcharhinidae and Sphyrnidae), billfish (Istiophoridae) and tuna (Scombridae) (Friedlander et al., 2012; López-Garro et al., 2016). The high abundances of these species attract commercial fishing vessels that enter the protected waters, and illegal fisheries represent one of the major threats of this World Heritage Site (López-Garro et al., 2016).

Since 2006, the submersible *DeepSee* has been used to study deep waters down to 450 m at Isla del Coco (Cortés and Blum, 2008). During submersible dives images of marine debris, such as garbage and fishing gear, have been taken around the island. Estimates of baseline abundance and composition of litter are crucial to implement litter reduction policies (Page et al., 2004; Ryan et al., 2009). Here we present a percentage of immersions where marine debris was observed, and information about the places where it was found. However, our images were obtained from non-systematic samplings, since the submersible was used with touristic purposes. Despite that, these images represent a great opportunity to analyze the problem of solid wastes and fishing gear deposited in the deep waters of Isla del Coco National Park. With the aim to explore the potential implications for the ecological integrity of this protected area, future research should focus on improving our understanding of the faunal composition and the effects that marine debris may have on the local communities. Fishing gear was the most common type of litter found. These results provide useful management information for the area, especially if one considers the constant pressure of illegal fishing.

MATERIALS AND METHODS

Isla del Coco National Park is found in the Pacific Ocean (5°30′-5°34′ N and 87°01′-87°06′ W). This site covers 24 km^2 of terrestrial area, and a protected marine area extending 22.22 km around the island (Cortés, 2008). The Isla del Coco National Park is part of the Isla del Coco Marine Conservation Area (ACMIC: Área de Conservación Marina Isla del Coco), that also protects around 9,640 km^2 of marine ecosystems with seamounts (Área Marina de Manejo Montes Submarinos).

Images of marine debris were captured from videos taken with the *DeepSee* submersible (Cortés and Blum, 2008) around Isla del Coco National Park (**Figure 1**) between 2006 and 2015. *DeepSee* is a three-person submersible with capability to dive to 450 m. When debris was observed the camera recorded the objects for small periods (less than a minute). Videos were recorded with an AVI format on mini-DV tapes by a high definition Sony HDX7 camera (frame size: 1920 × 1080 pixels) and then transcoded with a 4:2:2 low codec to a.mov format. Image grabs were made in the laboratory using iMovie and FinalCut software (Apple Inc.). Observations of debris were not made systematically because the *DeepSee* is used for tourism, and random recordings are focused mostly on large, conspicuous animals.

Marine debris was quantified from 365 dives at 17 different locations (**Figure 1**). Debris images were classified according to material composition and possible origins (boats/coastal origin, fishing debris or diving tourism). Associations of organisms with debris were noted and described, according to our video observations. The possible ecological threats that marine debris may represent were discussed according to the composition of litter and its interaction with the marine fauna.

RESULTS

Marine debris was observed in 20 (5.48%) of the immersions ($n = 365$), a total of 40 images were captured from the videos (**Table 1**). Litter was observed in four diving sites: The Wall 0475 (in ten dives), Piedra 165 (four dives), Piedra Drop (four dives) and Kili (two dives) (**Figure 1**), between 200 and 350 m depth. The most common items of litter deposited in the deep waters were: fishing gear ($n = 15$), beverage cans ($n = 13$), scuba gear ($n = 3$), and other debris (which includes plastic bags, clothes, brooms, chair remains, and product wrappings; $n = 9$). Debris was observed in contact with megafauna in 27.5% of the cases (**Table 1**).

Fishing gear comprised lost lines (**Figure 2**), between 200 and 350 m. One line was attached to a recently deceased silky shark, *Carcharhinus falciformis*, of approximately 250 cm total length at 200 m depth (**Figure 2A**); this shark was not a case of ghost fishing, many sharks are target species of illegal fishing in the vicinity of the National Park. Almost half of the fishing debris was observed in contact with fishes or crabs (**Table 1**). Since some organisms grow in the lines, such as algae and corals, lost fishing lines may represent a suitable feeding place and refuge for some

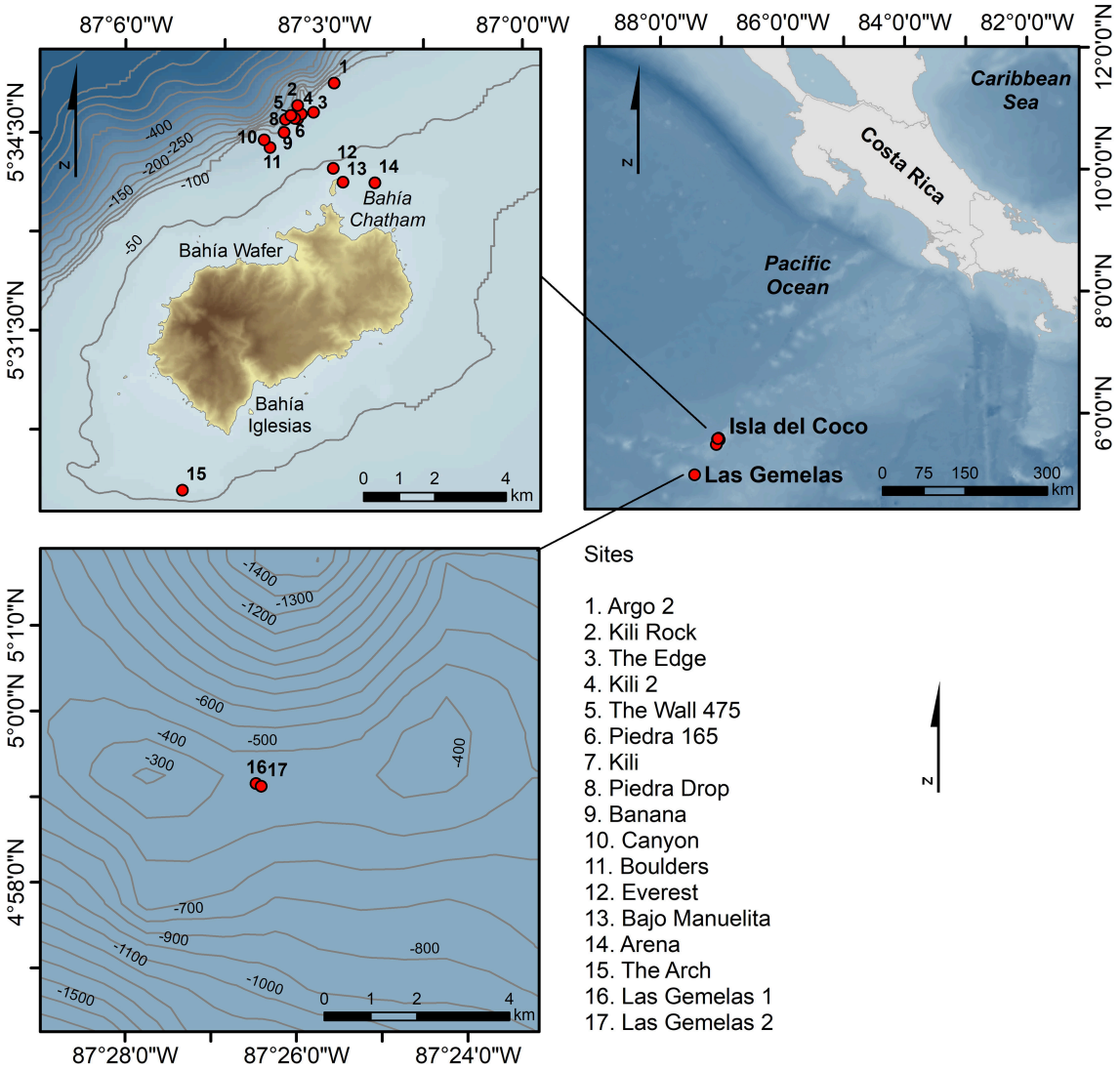


FIGURE 1 | Dive sites (50–450 m depth) at Isla del Coco National Park where the submersible *DeepSee* has acquired images of marine debris (found between 200 and 350 m depth).

species. Crabs were observed feeding next to the lines (**Figure 2B**) or were entangled in the lines (**Figure 2C**). Fishes were observed feeding or looking for refuge between the bundled lines, like the Anthiinae (Serranidae) shown in **Figure 2D**. Scuba gear, which included tanks and fins, and other debris are shown in **Figure 3**.

Most of the litter observed was plastic debris (60%), mainly fishing gear (**Table 1**). Other small litter items made from plastic or containing large amounts of plastic included plastic bags, brooms, product wrappings and scuba gear. Cans represent the second most common item in terms of abundance. Overall, metal represented 35% of the litter composition, whereas 5% of the litter was made with materials other than plastic or metal, such as clothes and unidentified materials.

Considering possible sources of debris, the majority likely came from boats or resulted from coastal activities. However, many debris items were in relatively good condition with little signs of degradation, suggesting that they had not traveled long distances and are the product of activities within or near the conservation area. Fishing activities contributed to 45% of the debris deposited at the bottom. The remaining 5% was from diving activities directly.

DISCUSSION

The images presented here demonstrate that the deep-sea environments of Isla del Coco National Park are exposed to human waste, despite its remoteness and despite being considered one of the best protected areas in the world (Cortés, 2012). Some studies have shown the accumulation of debris items in the deep sea (Galgani et al., 1995, 1996; van Cauwenbergue

et al., 2013; Woodall et al., 2014), and this is the situation at Isla del Coco National Park. However, the frequency in which litter was found in the present study (40 items in total, in 5.48% of the 365 dives) is much lower than that found in other studies; for example, at a similar depth (300 m) Schlining et al. (2013) found 22 items in just three visits to one of the areas with more debris accumulation in Monterey Canyon, California.

TABLE 1 | Characterization of marine debris observed during *DeepSee* human-occupied vehicle immersions at Isla del Coco National Park (2006–2015).

Material	Possible source	Description	Number of items	Depth range (m)	Items in contact with	
					Crabs	Fishes
Metal	Boats/coast	Cans	13	208–310		
Metal/plastic	Diving	Tank	1	300		
Other	Boats/coast	Clothes	1	326		
		Unknown equipment	1	160		1
Plastic	Boats/coast	Brooms	2	200		1
		Plastic bags	3	165–326		
		Product wrappings	1	210		
		Unknown	1	307		1
	Diving	Fin	1	320	1	
	Fishing debris	Fishing line	16	200–350	4	3
	Total		40	200–350	11	

The four sites from which we have debris images are deep and down-current from the island and the anchoring sites of the tourist vessels. The bottom around the island is relatively flat and sandy with a few rocks and seamounts. At a depth of around 180–200 m there is a steep drop to 2,500–3,000 m (Lizano, 2001; Cortés, 2016). We also observed organic detritus, leaves and sticks, from the island at all depths on the insular platform. As Schlining et al. (2013) pointed out, marine debris as a global problem is going to have lasting effects because the rate of debris input is not decreasing and debris accumulation is occurring in certain areas depending on hydrographic and geomorphological characteristics. A long-term systematic monitoring of debris at Isla del Coco National Park is necessary to better understand the distribution and density patterns of litter. Special attention should be given to submarine canyons, which have complex features that facilitate sediment transport and accumulation, and which can act as an effective conduit for the transport of debris into deep sea (Mordecai et al., 2011).

Like other studies, most of the litter items found in Isla del Coco are made of plastic. This is a common situation given around 8 million tons of plastic enter the oceans every year (Jambeck et al., 2015). It has been calculated that between 60 and 80% of all marine debris is plastic (Derraik, 2002; Butterworth et al., 2012), which is similar to the percentage of plastic found in this study. Miyake et al. (2011) found accumulation of litter in deep-sea trenches and depressions, and most items were composed of plastic. Plastics are extremely durable synthetic polymers, and the associated throw-away culture has led to a plastic waste management problem and widespread accumulation of plastic debris (Thompson et al.,

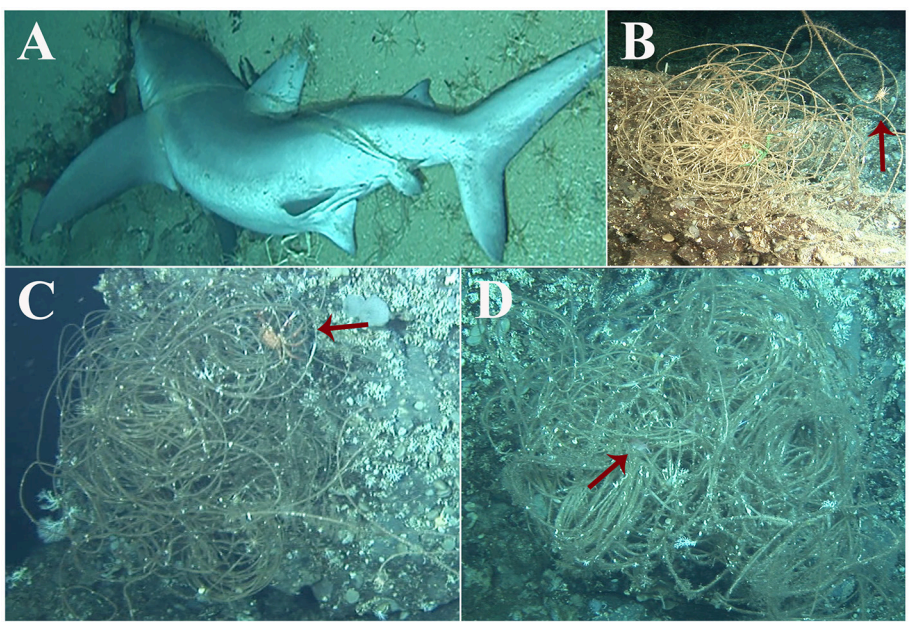


FIGURE 2 | Abandoned, lost or discarded fishing gear deposited in deep environments of Isla del Coco National Park. **(A)** Silky shark (*Carcharhinus falciformis*) attached to a line at 200 m depth. **(B)** A crab presumably scraping food from the fishing line, 302 m depth. **(C)** A tangled crab (red arrow) in a fishing line, 320 m depth. **(D)** A serranid (Anthiinae; red arrow) looking for food or refuge inside a fishing line, 350 m depth.

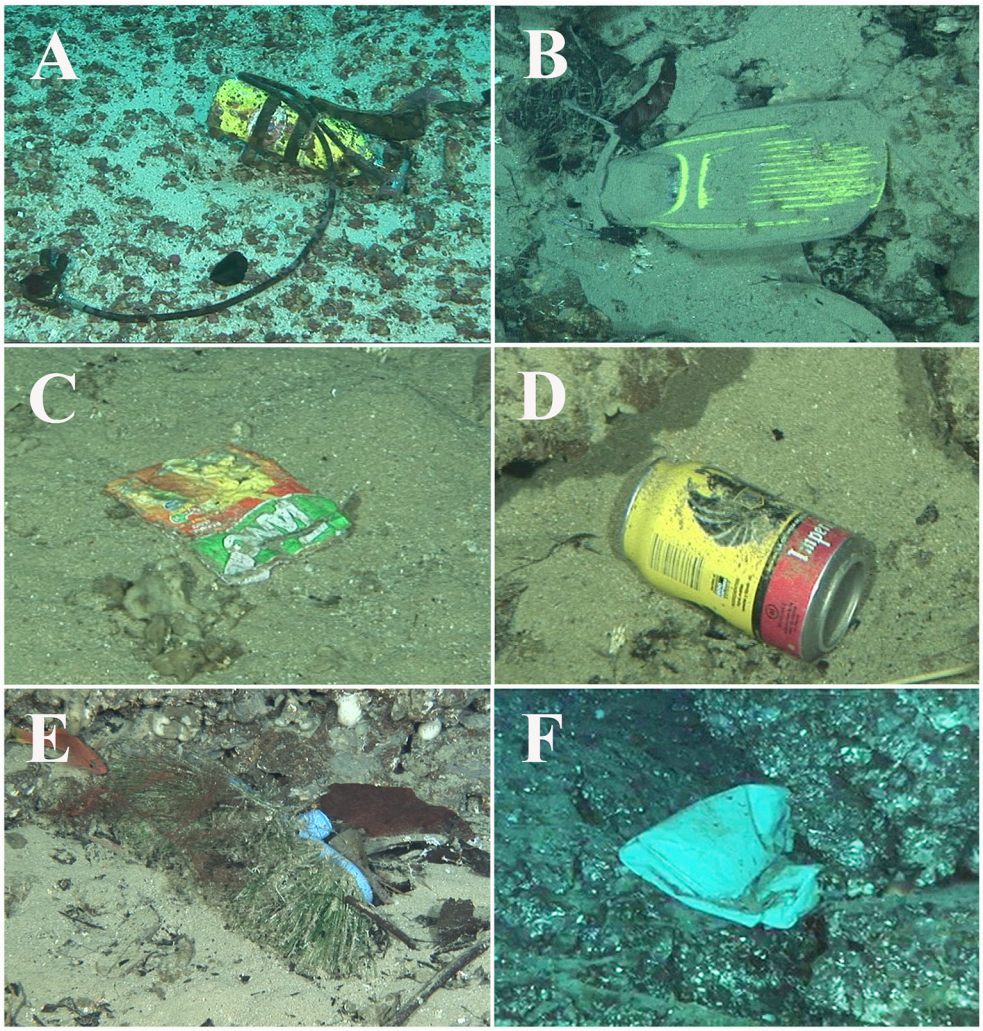


FIGURE 3 | Scuba gear and other debris deposited in deep sediments of Isla del Coco National Park. **(A)** Scuba tank, 300 m depth. **(B)** Fin, 320 m depth. **(C)** Product wrapping, 210 m depth. **(D)** Beer can, 220 m depth. **(E)** Broom, 200 m depth. **(F)** Plastic bag, 240 m depth.

2009; Butterworth et al., 2012). Also, plastics are buoyant, they can be dispersed over long distances and when they settle in sediments they may persist for centuries (Goldberg, 1997). Plastics can be denser or lighter than seawater, those that are buoyant float when first entering the sea, so historically attention has focused on the accumulation on shorelines and at the sea surface (Ryan et al., 2009). However, because of fouling by organisms and adherence of particles, positively buoyant plastics can, over a timescale of weeks to months, become negatively buoyant and sink (Lobelle and Cunliffe, 2011).

In the waters surrounding Isla del Coco, special attention should be paid to the seamounts since they are preferred fishing sites (López-Garro et al., 2016). Starr et al. (2012) observed lost fishing gear while comparing fish assemblages between Isla del Coco National Park and Las Gemelas Seamount, which is located 50 km southwest of Isla del Coco. Overall, they observed lost fishing lines on 33 occasions, but 30 of those were observed in Las Gemelas Seamount in just four dives. At Isla del Coco National Park, Starr et al. (2012) observed 3 lost fishing lines in two of the 12 dives made. The fishing activities produce large amounts of marine plastic debris, and ships in general are an important source of this pollutant (Horsman, 1982; Derraik, 2002). Globally, abandoned, lost and discarded fishing gear (known as ALDFG) compose less than 10% of total marine debris by volume (McFadyen et al., 2009; Pham et al., 2014). In the studied area, illegal fishing is considered one of the major threats against conservation efforts (López-Garro et al., 2016). During 2012 to 2014, surveillance patrols within the Isla del Coco National Park found 108 fishing lines and seized more than 500 km of fishing line (López-Garro et al., 2016). Just in 2007, 1516 illegal boats were observed in the waters of Isla del Coco and 600 km of fishing lines were seized (Castro et al., 2008). This is probably an underestimation of the real situation, since many fishermen are attracted by the high abundance of

pelagic species, mainly tunas and sharks (Arias et al., 2014). The potential effects of ALDFG have been raising awareness over the last years, mainly due to the expansion of fishing efforts, and the use of synthetic and more durable and buoyant materials (Derraik, 2002; McFadyen et al., 2009). Synthetic fishing gear could eventually accumulate in marine ecosystems, having long-term effects on marine biota (Moore, 2008). van Cauwenbergue et al. (2013) suggested that the tracing of fishing effort and gear type would be an important step to elucidate hotspots of litter abundance on seamounts, ridges and banks, which points to the need of better surveillance and fishing records around Isla del Coco.

Many marine species had been harmed or killed by plastic debris (Gall and Thompson, 2015). As some of our images show, crustaceans and fishes are probably looking for food near the lines, since marine debris may acquire encrusting organisms such as bacteria, algae, diatoms, and subsequently other organisms. The entanglement, mainly of crustaceans, is of interest for future research at Isla del Coco. Another source of concern is the possible ingestion of plastic litter by organisms. Ingestion of plastic can block the digestive tract, damage stomach lining and reduce feeding rates, resulting in starvation (Taylor et al., 2016). Also, some chemicals contained in the plastic can have detrimental effects even at very low quantities. For example, polychlorinated biphenyls (PCBs) can produce reproductive disorders, increase risk of diseases, alter hormone levels, and even cause death (Lee et al., 2001). In addition, plastics can fragment to microplastics (less than 5 mm), with potential physical and biochemical impacts on food webs (Andrady, 2011; Law and Thompson, 2014). Since studies have suggested that concentrations of microplastics found in deep-sea environments (and remote places) can be similar to those found in shallow sub-tidal sediments (Woodall et al., 2014), microplastics need to be assessed in future studies in Isla del Coco National Park.

Marine debris can generate other effects that are more difficult to identify. For example, litter on soft-sediments could alter the gas exchange and local biogeochemistry (Goldberg, 1997). Mordecai et al. (2011) reported anoxic sediments underneath a plastic bag. Cans and other metal debris are also persistent and can change the original structure of the benthic community. A detailed analysis of the litter items at Isla del Coco National Park is needed to identify the organisms attached to the litter. This can provide information about possible invasive species, since long distance "rafting" litter could introduce non-native organisms. For example, 7% of litter at beaches in a Norway location was colonized with exotic invasive barnacles and bryozoans. Biological invasions pose an issue of concern in Isla del Coco since this site is known for a high level of endemism (Cortés, 2012), and the presence of litter can increase the risk of alien invasion.

Since marine debris can travel long distances, is difficult to know from where it comes from. However, some of the cans and scuba gear are likely from tourism activity. Tourism is the major economic activity that generates entries to the Isla del Coco National Park conservation efforts. Adequate waste management and tourist awareness programs are necessary to avoid the accumulation of marine debris, allowing Isla del Coco National Park to remain as one of the best protected places of the world and an example of conservation. Since one of the more isolated and best protected places around the world is not exempt of debris, the images shown in this work have important implications for marine protected areas globally because they can serve to promote the reduction of disposable single-use products, adequate waste management, and raise awareness to avoid illegal fisheries especially in a marine area with high abundance of top predators such as Isla del Coco National Park.

AUTHOR CONTRIBUTIONS

BN-E: conceptualization of paper, processed the images, and wrote the paper; JC: conceptualization of paper, organized the project to obtain the images, review in detail all drafts of the paper, procured all the funding.

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frontiers in Marine Science

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Biogeochemical Regeneration of a Nodule Mining Disturbance Site: Trace Metals, DOC and Amino Acids in Deep-Sea Sediments and Pore Waters

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Increasing interest in deep-sea mineral resources, such as polymetallic nodules, calls for environmental research about possible impacts of mineral exploitation on the deep-sea ecosystem. So far, little geochemical comparisons of deep-sea sediments before and after mining induced disturbances have been made, and thus long-term environmental effects of deep-sea mining are unknown. Here we present geochemical data from sediment cores from an experimental disturbance area at 4,100 m water depth in the Peru Basin. The site was revisited in 2015, 26 years after a disturbance experiment mimicking nodule mining was carried out and compared to sites outside the experimental zone which served as a pre-disturbance reference. We investigated if signs of the disturbance are still visible in the solid phase and the pore water after 26 years or if pre-disturbance conditions have been re-established. Additionally, a new disturbance was created during the cruise and sampled 5 weeks later to compare short- and longer-term impacts. The particulate fraction and pore water were analyzed for major and trace elements to study element distribution and processes in the surface sediment. Pore water and bottom water samples were also analyzed for oxygen, nitrate, dissolved organic carbon, and dissolved amino acids, to examine organic matter degradation processes. The study area of about 11 km^2 was found to be naturally more heterogeneous than expected, requiring an analysis of spatial variability before the disturbed and undisturbed sites can be compared. The disturbed sites exhibit various disturbance features: some surface sediments were mixed through, others had the top layer removed and some had additional material deposited on top. Pore water constituents have largely regained pre-disturbance gradients after 26 years. The solid phase, however, shows clear differences between disturbed and undisturbed sites in the top 20 cm so that the impact is still visible in the plowed tracks after 26 years. Especially the upper layer, usually rich in manganese-oxide

and associated metals, such as Mo, Ni, Co, and Cu, shows substantial differences in metal distribution. Hence, it can be expected that disturbances from polymetallic nodule mining will have manifold and long-lasting impacts on the geochemistry of the underlying sediment.

Keywords: heavy metals, deep-sea mining, ecosystem disturbance, long-term changes, DISCOL

INTRODUCTION

Deep-sea mining has been featured prominently on the political and scientific agenda for the past years and experiences a new phase of interest after first major exploration activities in the 1970s and 1980s. Recent advances in deep-sea mining technology, such as the building of collector prototypes, (Gollner et al., 2017) and an increasing number of exploration contracts issued by the International Seabed Authority (ISA): seven of 16 in the last 5 years (International Seabed Authority, 2014) show the need for environmental baseline data and knowledge about the response of deep-sea sediments to impacts by polymetallic nodule mining. The number of articles discussing the need for mining regulation to protect the deep-sea ecosystem and its fauna has skyrocketed in recent years (Glover and Smith, 2003; Davies et al., 2007; Ramirez-Llodra et al., 2011; van Dover, 2011; Barbier et al., 2014; Mengerink et al., 2014; van Dover et al., 2014). Yet, most research on trace metals in deep-sea sediments is approximately 30 years old (Klinkhammer, 1980; Klinkhammer et al., 1982; Sawlan and Murray, 1983; Heggie and Lewis, 1984; Heggie et al., 1986; Müller et al., 1988; Shaw et al., 1990) and recent trace metal research mostly does not focus on the deep-sea (Morford and Emerson, 1999; Morford et al., 2005; Beck et al., 2008; Kowalski et al., 2009). Therefore, more state-of-the-art deep-sea sediment studies focusing on the current issue of mining impacts are needed.

Exploration for polymetallic nodules has been carried out in many different areas of the oceans, with a focus on the Clarion-Clipperton Fractures Zone (CCZ) in the central Pacific (Hein et al., 2013). There, nodules have a high percentage of economically interesting metals—Ni, Cu, and Co (Hein et al., 2013). All ISA exploration contract areas except one are located in the CCZ (International Seabed Authority, 2014); however, some long-term research projects on the impacts of nodule mining on the deep-sea ecosystem have also been carried out in the Peru Basin. Polymetallic nodules in the Peru Basin are characterized by higher growth rates and larger size in comparison to the CCZ nodules (Marchig et al., 2001). Cu contents are generally lower than in CCZ nodules (Wegorzewski and Kuhn, 2014). The overlying waters are more productive than in the CCZ and hence, sediments are characterized by higher organic carbon concentrations of around 0.5 wt% up to rarely 1 wt%, low sedimentation rates (0.4–2.0 cm/ka) and an oxygen penetration depth around 10–15 cm (Haeckel et al., 2001).

In 1989, a DISturbance and reCOLonization (DISCOL) experiment mimicking polymetallic nodule mining was carried out in the Peru Basin. The deep-sea floor was plowed in an area of approximately 11 km^2 (Thiel, 2001). Environmental assessments were carried out 0.5, 3, and 7 years after the disturbance (Thiel, 2001). The assessments, however, mainly focused on fauna (Thiel and Schriever, 1990) and the first geochemical studies in the wider DISCOL area were conducted in 1996 (cruise SO106, ATESEPP project), unfortunately only after the disturbance so that no baseline data from prior to the experiment exists. The six sampling sites of SO106 were spread out across the Peru Basin and only one site was located in the DISCOL experimental area (DEA) (Schriever et al., 1996). It is not known, however, if the 1996 DISCOL sample is from within or outside a plow track because the multi-corer (MUC) sampling then was not TV-guided and the geochemical data (Koschinsky, 2001) does not give a clear picture to draw conclusions about a disturbance.

Mining operations to recover nodules will likely remove or disturb the upper 10–50 cm of sediment and create a sediment plume (Thiel and Schriever, 1990; Oebius et al., 2001; Cronan et al., 2010; Gollner et al., 2017). *Ex-situ* experiments with sediment cores from the Peru Basin showed that pore water metals, dissolved organic carbon (DOC) and nutrients are released when the sediment is stirred up (Koschinsky et al., 2001b). It has been assumed that such a disturbance would also be caused by polymetallic nodule mining (Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001). Depending on the redox zonation of the area and depth of sediment removal, a change in redox zonation can occur. The redox zonation develops as a result of organic matter degradation, following a roughly set sequence in which oxidants are used according to their potential to produce energy: oxygen, nitrate, Mn-oxide, Fe-oxide, and sulfate (Froelich et al., 1979). The redox zonation in marine sediments determines how metals are distributed between the solid phase and pore water: metals are either bound in the solid phase or dissolved in the pore water (König et al., 2001; Koschinsky, 2001). Elements soluble in oxic water (Mo, U, possibly V, As) are released from oxic pore water, but they have similar concentrations in the oxic bottom water (Koschinsky, 2001). Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb have higher concentrations in the sediment pore water than in the bottom water, especially in suboxic pore water, which are up to two orders of magnitude higher than in the bottom water at the sediment-water interface (Koschinsky, 2001). If the oxic layer is thick enough (few cm), most pore water metals diffusing upwards from the suboxic layer will be scavenged and bound to e.g. Mn-oxides because Mn-oxides are effective scavengers and positively charged metal species such as Co, Ni, Cu, Zn, Pb, and Cd are associated with Mn (Koschinsky, 2001). Similarly, if the disturbance is limited to the oxic layer, Mn-oxides would bind most of the released heavy metals in a relatively short period of time

(Koschinsky et al., 2001b) which makes them immobile and they do not diffuse into the bottom water (Koschinsky, 2001; Morford et al., 2005). The depth of the oxic layer hence is a decisive factor for the heavy metal budget (Koschinsky, 2001). Besides the Mn-oxide rich surface layer, polymetallic nodules also act as metal scavengers (Koschinsky et al., 2003). If these nodules are mined, this option of metal scavenging is removed. If, however, the oxic layer is removed or contracted, metals dissolved in the pore water from the suboxic layer can discharge into the oxygenated bottom water, causing the release of dissolved heavy metals and an increase of seawater heavy metal concentrations (König et al., 2001; Koschinsky et al., 2003). Since some heavy metals could potentially reach toxic concentrations with detrimental effects for the fauna, sediment disturbance and potential metal release is a serious issue to be considered with respect to deep-sea nodule mining. Ecotoxicological experiments showed that LC_{50} values for animals subjected to colder temperatures and higher pressures, to simulate deep-sea environmental conditions, for dissolved Cu ranged between 8.85 and 29.4 μmol/L for nematodes (Mevenkamp et al., 2017) and 380–420 μmol/L for shrimp (Brown et al., 2017a). LC_{50} values for Cd ranged between 521 and 548 μmol/L for shrimp (Brown et al., 2017a). Experiments are usually carried out with spiked Cu and Cd concentrations in the μmol/L range (Auguste et al., 2016; Martins et al., 2017; Mevenkamp et al., 2017). Trace metals are an important part of the biogeochemical cycle of the surface sediment and should be well understood before mining commences.

These preliminary experiments from the past thus have shed some light on the geochemical behavior of heavy metals in deep-sea sediments. Yet, no detailed in-situ studies or long-term monitoring of the mining impacts have been carried out in the DISCOL area (as mentioned above, only one station was sampled there in 1996) to determine: (1) degrees of disturbance at different sites to obtain a comprehensive picture of the geographic extent and degree of the disturbance and (2) the processes in the sediment and new equilibrium establishment after a disturbance. This is essential as most geochemical processes in the deep-sea are slow and therefore environmental recovery rates can also be expected to be slow.

As part of the European JPI Oceans MiningImpact project ("Ecological Aspects of Deep-Sea Mining") (GEOMAR, 2017), we revisited the DISCOL area in 2015 during the SO242 cruise with RV SONNE, to study the geochemical long-term development of the site. We aim to answer the following research questions: (1) Are there differences between the reference sites demonstrating natural variability in the particulate fraction and pore water? After 5 weeks and 26 years, (2) are signs of the disturbance still visible in the solid phase and pore water or has a new equilibrium been reached? (3) Are there differences between the disturbed sites across the DEA and between the microhabitats within a disturbed track? Answering these questions will help to understand natural variability and how deep-sea mining could affect the deep-sea floor geochemistry. Since little is still known about sediment recovery after a disturbance, these background studies are extremely valuable—especially if carried out on relatively long time-scales. Decision-makers can also draw from our results as a basis for defining baseline data and threshold values because if the disturbance sites vary considerably, this needs to be taken into account for monitoring in future mining scenarios.

From a research perspective, the DISCOL area provides a good comparison to the well-researched and industrially more pertinent CCZ, where similar studies have been and are being carried out, but the geochemistry is quite different. The DISCOL area has an oxic layer of approximately 10–15 cm (Haeckel et al., 2001), whereas the CCZ sediment is oxic down to ca. 200 cm and deeper (Mewes et al., 2014, 2016; Kuhn et al., 2017). A comparison of these sites will help to assess the possible range of changes in the trace metal cycle during deep-sea mining in relation to the different environmental conditions.

MATERIALS AND METHODS

Site Description

The Peru Basin is located in the south eastern tropical Pacific (Marchig et al., 2001). Predominantly, siliceous and calcareous muds and oozes make up the sediments in this region (Weber et al., 1995). For detailed site description of the DISCOL area and the disturbance experiment carried out in 1989 please refer to Thiel and Schriever (1990); Boetius (2015); Greinert (2015).

During RV SONNE cruise legs SO242/1 and 2 in 2015, the reference sites outside the DEA (**Figure 2A**), as well as undisturbed and disturbed sites inside the DEA were sampled (**Figure 1**). The 26-year old plowed tracks exhibit various disturbance features. Due to the plow harrow, grooves traverse the sediment and form ripples and valleys. Throughout the DEA "white patches" of lighter sediment occur in the disturbed sites. These three features are microhabitats of the disturbed sites, which were sampled to study the disturbance variety (**Figure 2C**).

During leg SO242/1 (Greinert, 2015), a new disturbance in addition to the plow tracks from 1989 was created using an epibenthic sled (EBS), and the affected sites were sampled approximately 5 weeks later during leg SO242/2. The sediment surface layer was visibly removed so that the lighter sediment below the Mn-oxide rich layer became exposed (**Figure 2B**). The EBS track sediment disturbance was created to simulate nodule mining and to be able to take samples for geochemical analyses shortly after the disturbance. This had not been done in the frame of the original DISCOL project and geochemical data from shortly after the impact is missing. Therefore, the EBS track samples add a point in time between undisturbed samples and samples from the 26-year old disturbance. It is important to note, however, that the tracks created 26 years ago were created using a plow harrow, which affects the sediment in a different way than the EBS. The general disturbance impact is comparable, but variations due to the gear used are probable.

Sediment and Pore Water Sampling

Sediment was collected using MUC and ROV push cores (ROV-PUC). TV-guided MUCs allowed for exact sampling of the tracks, while the precision with the ROV was even higher and

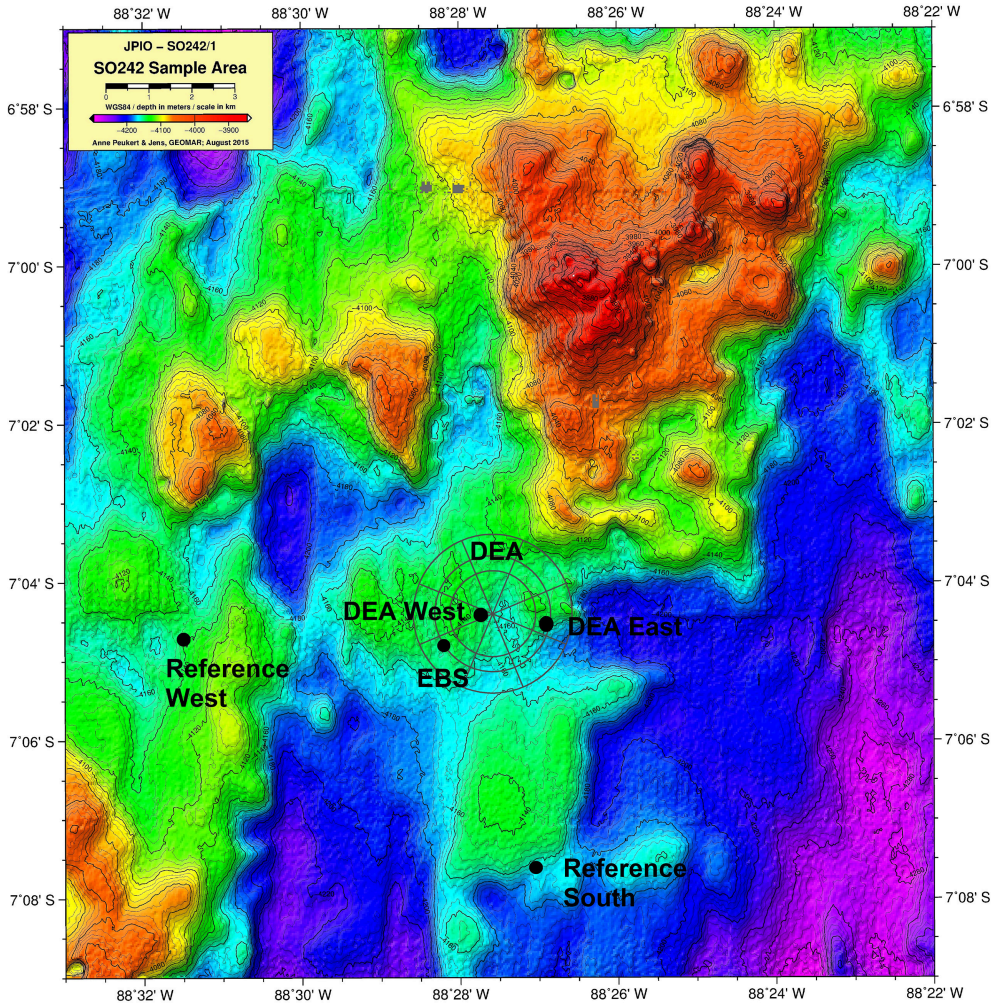


FIGURE 1 | Sampling sites of sediment cores in the DISCOL area (adapted from a map by Anne Peukert, GEOMAR, working group of Jens Greinert). The circle indicates the DISCOL experimental area (DEA) in which the disturbance experiment had been carried out in 1989.

microhabitats within the plow tracks could be sampled. The cores were immediately brought into the 4°C cold room of RV SONNE and for trace element and DOC analyses, sliced into 2 cm layers in a glove bag filled with argon. The sediment slices were transferred into 50 mL acid-cleaned centrifuge tubes in the glove bag and centrifuged at 3,200 rpm for 40 min. The supernatant was filtered through 0.2 μm cellulose acetate syringe filters, pre-cleaned with 0.1 M hydrochloric (HCl) acid and deionized water, again using a glove box. The water overlying the particulate fraction within the MUC liner was sampled as well to get bottom water values for each core. The pore water samples were acidified to pH 2 with concentrated, suprapure HCl and stored at 4°C. Pore water samples for amino acid analyses were taken with rhizons according to the procedure described by Seeberg-Elverfeldt et al. (2005) and frozen. Pore water samples for nitrate analyses were extracted with a low pressure (argon at 3–5 bar) squeezer using 0.2 μm cellulose acetate filters. An overview of the cores taken at different locations in the working area is given in **Table 1**.

Chemical Analyses

All acids used were of suprapure quality (HCl and HF by Merck, $HClO_4$ and HNO_3 by Roth). All PE containers were acid cleaned prior to use to avoid any trace element contamination.

Solid Phase

Major and trace elements

For bulk chemical analyses, centrifuged sediment samples were crushed and dried at 105°C to remove moisture. 100 mg of sediment were then digested with a PicoTrace DAS acid digestion system using 3 mL perchloric acid ($HClO_4$ 70%) and 3 mL hydrofluoric acid (HF 38–40%) at 220°C for 12 h. Samples were evaporated, taken up in 5 mL HCl and evaporated again. This step was repeated before the samples were taken up in a mix of 0.5 M nitric (HNO_3) acid and 0.5% HCl (v/v). Samples were analyzed with ICP-OES (SpectroCiros SOP instrument) for major elements and ICP-MS (Perkin Elmer Nexion 350x) for trace elements. For ICP-OES measurements, the certified reference

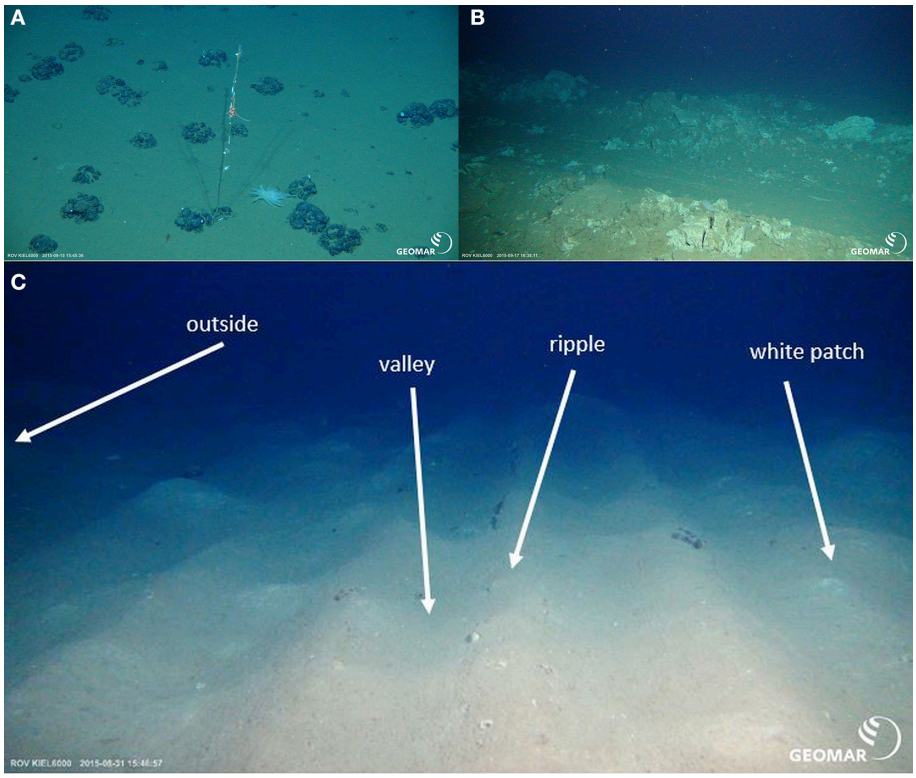


FIGURE 2 | (A) Example of seafloor at a reference site, **(B)** example of an EBS track, **(C)** example of a 26-year old plow track, indicating the four microhabitats outside track, track valley, ripple, and white patch. Pictures copyright ROV KIEL 6000 Team, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany.

TABLE 1 | Overview of cores taken for sediment and pore water trace metal analyses.

Sample ID	Area	Location	Water depth (m)
SO242/1_34MUC	Reference South	7°07.5244′S 88°27.031′W	4162
SO242/1_56MUC	DEA West plow track	7°04.414′S 88°27.760′W	4149
SO242/1_80MUC	Reference West	7°04.542′S 88°31.581′W	4130
SO242/1_108MUC	DEA East plow track	7°04.483′S 88°26.919′W	4169
SO242/2_163ROV-PUC38	DEA East ripple	7°04.493′S 88°26.933′W	4143
SO242/2_166ROV-PUC63	DEA East valley	7°04.478′S 88°26.918′W	4143
SO242/2_166ROV-PUC64	DEA East outside track	7°04.459′S 88°26.924′W	4143
SO242/2_169ROV-PUC10	DEA East white patch	7°04.481′S 88°26.913′W	4144
SO242/2_202ROV-PUC63	DEA EBS track	7°04.953′S 88°28.198′W	4150
SO242/2_211ROV-PUC57	DEA EBS outside track	7°04.967′S 88°28.193′W	4150

material (CRM) MESS-3 was within 5% accuracy of certified values for Cu, K, Mg, and Mn, and within 10% for Ca, Fe, P, and Zn. Accuracy for Al was −13% but too low values for Al in MESS-3 have been reported before (Roje, 2010). Method precision was within 8% for all elements except P (10%), Mg (13%), and Al (21%). Accuracy of BHVO-2 reference material was within 5% for all elements except P and Zn (12%) and method precision was within 4% for all elements except P (13%). Accuracy for MESS-3 and BHVO-2 for ICP-MS measurements were within 3% except for Pb (−8% MESS-3) and Ni (6% BHVO-2). Method precision was within 3% except for Pb (26%). Cd could not be measured reliably in the reference material due to high discrepancies of the two measured isotopes. For detailed information on limit of quantification[1] (LOQ), accuracy, and method precision refer to Supplementary Material 1.

POC and PON

Particulate organic carbon (POC) and particulate organic nitrogen (PON) of the sediment were determined through gas-chromatography of CO_2 and N_2, produced by flash combustion using a Carlo Erba Element Analyzer (NA 1500). Samples were treated with HCl to release carbon bound to carbonates prior to analysis.

Porosity

Porosity was calculated from the weight difference of wet and freeze-dried sediment. For further details see Haeckel et al. (2001).

Pore Water

Major and trace elements

Pore water major elements were measured with ICP-OES (SpectroCiros SOP instrument). Overall accuracy for IAPSO seawater reference material was within 5% for all measured elements except Mg (+11%). Method precision was 2–3% except

[1] 10 * standard deviation of acid blanks for each run.

for Si (17%). For trace elements (As, Cd, Co, Cu, Mn, Mo, U, V), a ICP-MS (Perkin Elmer Nexion 350x) coupled with an apex Q (ESI) introduction system to increase sensitivity and decrease background was used. As, Co, Cu, Mn, and V were measured in a reaction cell in collision cell mode (KED mode) with helium gas, to eliminate interferences. Cd and Co values of all CRMs are below the LOQ and could not be verified. Mo and U were verified with IAPSO and NASS-6 seawater reference material, V, Mn, Cu, and As were checked in NASS-6, SLEW-3, and SLRS-6. Accuracy and method precision vary for each CRM, possibly due to varying salt contents but generally agree with the reference materials. Only Mo values are slightly too high. For detailed information on LOQ, accuracy, and method precision refer to Supplementary Material 1. Ni, Zn, Cr, and Pb concentrations in the pore water could not be reliably quantified because of poor accuracy (Ni and Zn) or too low concentrations (Pb LOQ = 0.01–2.29 μg/kg; Cr LOQ = 0.32–1.42 μg/kg).

For ICP-OES measurements, 10 ppm Y were used as internal standard and for ICP-MS, a mixed internal standard containing Ru, Re, and Bi was used: 2 ppb without APEX and in KED mode and 1 ppb with APEX.

DOC

DOC concentrations [mg/L] were determined via a high temperature combustion method (TOC-V_{CSH} Analyzer, Shimadzu). Inorganic carbon was removed by 2 M HCl prior to injection into the combustion tube where organic carbon is oxidized to CO_2 at 680°C with a platinum catalyst. A 5-point calibration from 0.5 to 5 mg DOC/L was used. The error of measurement is less than 2% (for further analytical details see Brockmeyer and Spitzy, 2013).

Amino Acids

Total hydrolysable dissolved amino acids (DAA) and hexosamines (HA) of selected samples were analyzed with a Biochrom 30 Amino Acid Analyzer after hydrolysis of ca. 3 ml of filtrate with 6 N HCl for 22 h at 110°C under a pure argon atmosphere. A particle free aliquot was evaporated three times to dryness in order to remove the unreacted HCl; the residue was taken up in an acidic buffer (pH 2.2). After injection and subsequent separation with a cation exchange resin, the individual AA monomers were post-column derivatized with o-phthaldialdehyde in the presence of 2-mercaptoethanol and detected with a Merck Hitachi L-2480 fluorescence detector. Duplicate analysis of a standard solution according to this method results in a relative error of 0.1 to 1.3% for the concentrations of individual AA monomers and 0.2 to 3.0% for individual AA monomers of water samples. Due to acid hydrolysis, aspartic acid and asparagine are both measured as Asp and glutamic acid and glutamine are both measured as Glu.

Oxygen

Oxygen was measured *ex-situ* using fiber-optic microsensors (FireStingO2 optodes from Pyroscience GmbH, Aachen, Germany) which were lowered into the MUC sediment with a motorized micromanipulator (MU1, Pyroscience GmbH, Aachen, Germany). Measurements were taken in 500 μm steps, with two optodes at the same time. In total, four to six concentration profiles were completed for each MUC core. Method precision was 1% and the detection limit 1 μmol/L. For further details see Haeckel et al. in Greinert (2015).

Nitrate

Nitrate was analyzed on-board RV SONNE directly after sampling using a Hitachi UV/VIS spectrophotometer. The analysis followed standard analytical procedures, measuring nitrate as sulphanile-naphthylamide after reduction with Cd (Grasshoff et al., 1999). The detection limit was 2 μmol/L and analytical precision was 3%.

Detailed tables with results for trace elements, DOC and DAA can be found online at PANGAEA: https://doi.pangaea.de/10.1594/PANGAEA.880596.

RESULTS

Solid Phase

Two reference sites outside the DEA and two sites inside the DEA—next to an old plow mark (DEA East) and the freshly disturbed EBS track—not directly impacted by plowing, were analyzed for a range of background values and to determine natural spatial variability. Based on the natural background conditions we will compare the 26-year old and 5-week old disturbed sites.

The major elements in the sediments of the undisturbed and disturbed sites are Al, Fe, and Ca (**Figures 3–5**). The latter increases with depth, usually more strongly below 10 cm or displays pronounced peaks between 15 and 20 cm. The major element concentrations in general, as well as the Ca variability, are comparable in the disturbed sites and do not vary substantially from the undisturbed sites (**Figure 3** and Supplementary Material 2). Si could not be measured due to the acid digestion procedure using HF. Porosity decreases with depth from approximately 0.93 at the surface to 0.86 at around 15 cm and is slightly higher in the surface sediments of undisturbed sites compared to disturbed sites. Especially the EBS track has a lower porosity at the surface. The slope of decreasing porosity is steeper in the disturbed sites than in the undisturbed sites (**Figure 3**).

The surface sediments of the undisturbed sites in the DEA have lower Mn concentrations than those of the reference sites but display the same curved profile shape as the reference sites (**Figure 4**). Additionally, the Mn-oxide rich layer is thicker in the reference sites. POC content is higher within the DEA and lower in Reference South and West. Mo, Ni, Co, Cu, and Cd have similarly curved patterns as Mn. We were only able to get reliable results for Cd in the undisturbed site next to the EBS track. Similar to Mn, the above mentioned Mn-associated metals show slightly higher concentrations at the reference sites compared to the undisturbed sites within the DEA, outside DEA East plow track being the undisturbed site with overall lowest concentrations.

All disturbed cores have Mn solid phase concentrations below 1.5 wt.%, with DEA West plow track having the highest concentration (up to 1.26 wt%) and the EBS track having by

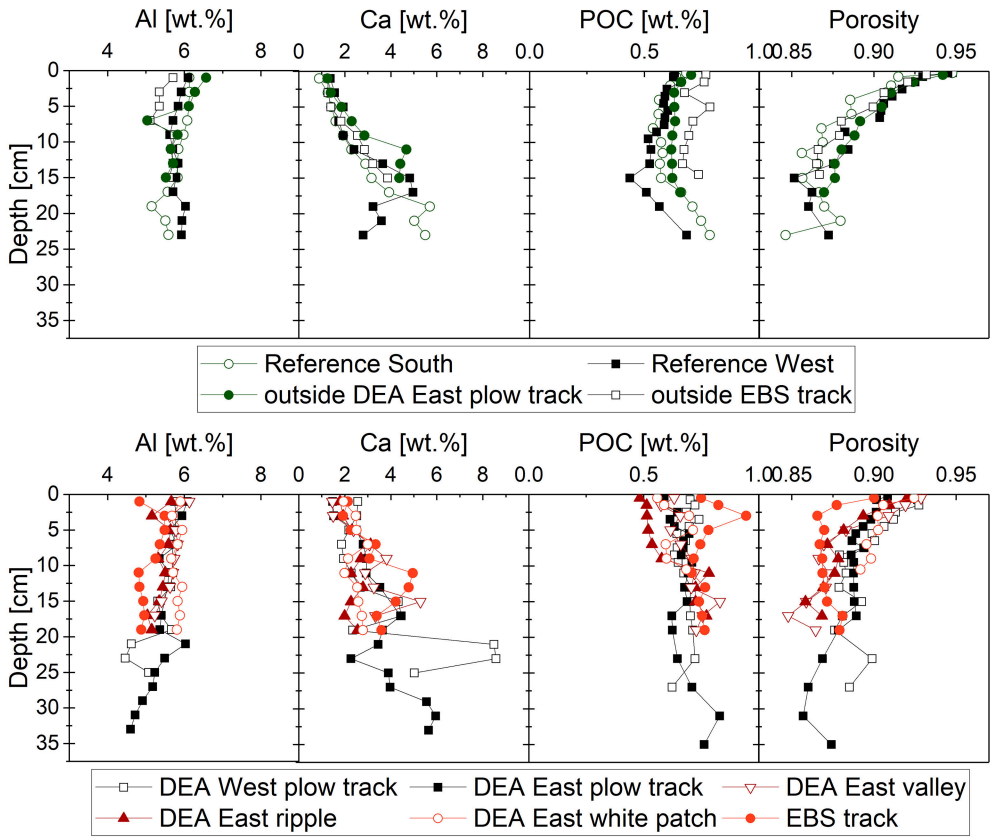


FIGURE 3 | Sediment major element profiles and properties of the four undisturbed and six disturbed sites.

far the lowest concentration (<0.5 wt%) (**Figure 5**). Mo, Ni, Co, and Cu have similar concentrations at the disturbed sites as at undisturbed sites, only the white patch and the EBS track have considerably lower concentrations of Mo, Ni, and Co. These concentrations are in the same range as the concentrations at depth below the Mn-oxide rich layer in the other cores. The profiles of Mn and associated metals do not show the typical curves as it was the case for the undisturbed sites (**Figure 4**). They are rather straight (DEA East ripple and valley) or have peaks at depth (DEA East plow track at 23 cm).

Pore Water

Pore water *ex-situ* oxygen profiles (**Figure 6**) show that the oxygen penetration depth is between 12 and more than 20 cm, decreasing from west to east. It is lower in the DEA plow tracks but this is partly due to the natural gradient. Nevertheless, oxygen profiles from plow track cores are more linear than in undisturbed sites. Oxygen measurements from Vonnahme et al. (in prep.) show that oxygen penetration in the microhabitats is between 11 and 14 cm deep. Nitrate is relatively stable throughout the upper 25 cm of sediment with concentrations between 40 and 60 μmol/L. Below, concentrations slightly decrease as visible in the DEA East plow track profile (**Figure 6**).

The measured pore water major element concentrations are in the same range for each element across all 10 sites—undisturbed and disturbed—and are mostly in the same range as bottom water concentrations (Supplementary Material 3). Only Si shows the typical increase with depth, which is steepest in the EBS track. Trace element concentrations in the pore waters are in the same range for each element across all undisturbed sites as well (**Figure 7**). Mn, Mo, U, and As concentrations are generally in the range of bottom water values. Co, Cu, V, Cd, and DOC pore water concentrations are usually twice as high as bottom water concentrations, at least in the upper centimeters. Co is rarely above the LOQ (0.08–0.22 μg/kg) in pore waters of the undisturbed sites. Based on selected best data, we assume the background concentration to be approximately 0.5 nmol/L. Overall, bottom water and pore water trace element concentrations and profiles at the 26-year old plow tracks (**Figure 8**) are similar to those at undisturbed sites. The surface layer DOC peaks are less pronounced in the disturbed cores but some cores have peaks at greater depths.

In some cores—undisturbed and disturbed—local peaks in certain elements occur which could be sampling artifacts from filtration or nanoparticles. Another explanation would be local redox signals, where Mn and associated metals are released into the pore water while Mo and U get removed due to a reducing

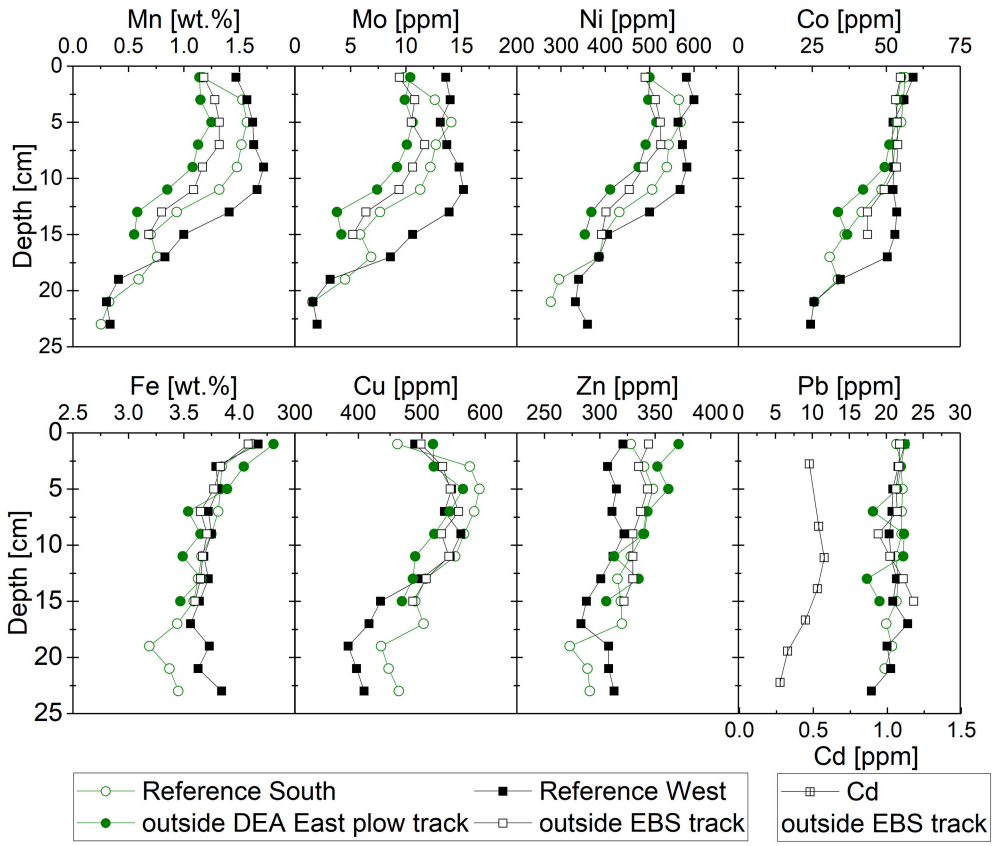


FIGURE 4 | Sediment element profiles of the four undisturbed sites. Reliable Cd results only for outside EBS track.

environment. Diagenetically, however, these peaks cannot be sustained long. These peaks were already found in prior pore water studies in the Peru Basin (see Koschinsky, 2001). Here, they will not be considered further.

Pore water DAA usually have their concentration maxima in the upper 10 cm, best visible for Reference South (**Figure 9**). At Reference South, the DAA peak roughly coincides with the DOC peak. The pore water DAA concentrations in the 26-year old plow tracks are generally in the same range as in the undisturbed cores with DAA concentrations between 2.2 and 11.1 μmol/L. Peaks in the upper 10 cm are not pronounced (**Figure 9**). Especially in the DEA East plow track the pore water DAA spectra differ from the undisturbed samples with relatively higher contents of non-protein amino acids β-Ala, γ-Aba as well as Lys, Val, and Met (Supplementary Material 4).

DISCUSSION

Solid Phase: The Mn-Oxide Rich Layer in the Undisturbed Sites

Mn-oxides are the main host phase for heavy metals in the upper 20 cm in the DISCOL area (Koschinsky et al., 2001a; Marchig et al., 2001). The solid phase Mn content decreases steeply around the oxygen penetration depth due to Mn-oxide utilization in organic matter degradation (**Figure 4**). Oxygen is utilized first because it is energetically the most favorable pathway (Froelich et al., 1979). Once oxygen is consumed, NO_3^- and MnO_2 function as electron acceptors. The processes can run in parallel, even though nitrate is the energetically favorable option. Additionally, it has been suggested that the Mn and N cycles are linked and that MnO_2 can provide O_2 to oxidize N, leading to nitrate formation and Mn-oxide reduction (Mogollón et al., 2016). Since the MnO_2 concentration is declining while NO_3^- is still present in the pore water (**Figure 6**), this might be a relevant process here. The low Mn content (∼0.5 wt%) below the Mn reduction zone is likely bound in detrital minerals or Mn-carbonates and represents the constant level of solid phase Mn for sediment below the Mn-oxide rich layer (Gingele and Kasten, 1994; Koschinsky, 2001). The sediments change color from dark brown in the oxic zone to light brown in the suboxic zone, the tan color is due to Fe(III) in the clay minerals (König et al., 1997).

The natural variability of Mn in the oxic layer is 1.1 to 1.7 wt%. There is an increase in Mn content with an increase in oxygen penetration depth, from east to west. Additionally, the reference sites have higher Mn concentrations than the undisturbed sites within the DEA. It remains unclear, if solid phase element concentrations of the undisturbed sites within the DEA are lower solely due to natural variability or because they were impacted

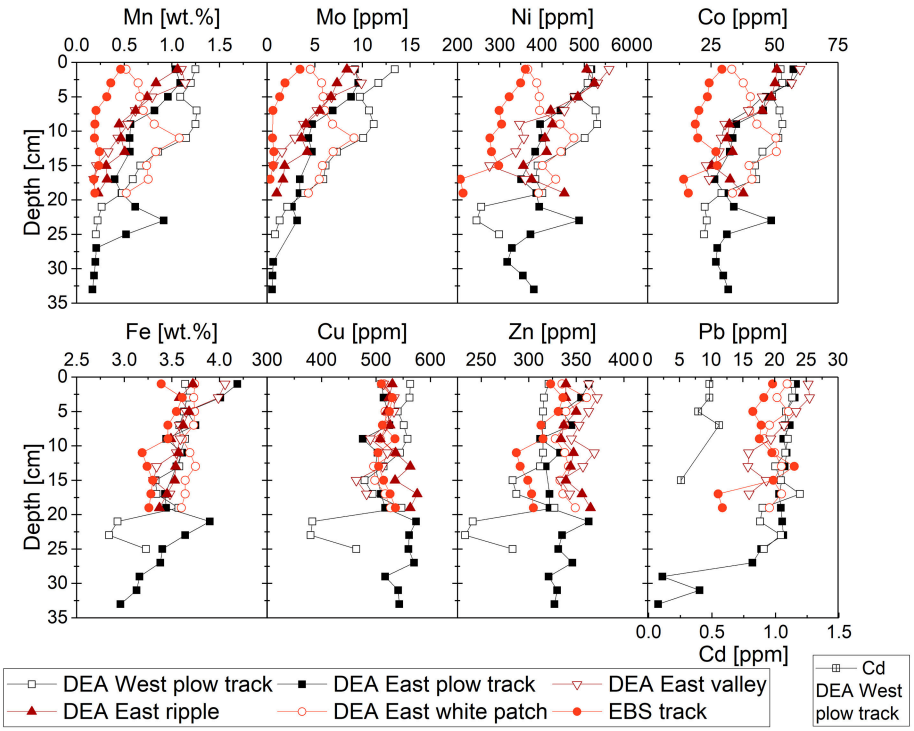


FIGURE 5 | Sediment element profiles of five 26-year old disturbed sites and the 5-week old EBS track. Reliable Cd results only for DEA West plow track.

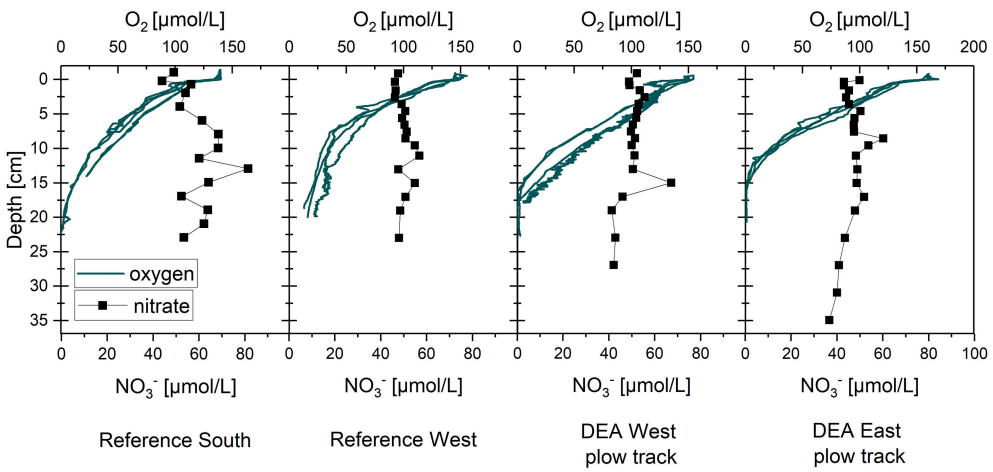


FIGURE 6 | Bottom water and pore water ex-situ oxygen and nitrate profiles of two undisturbed and two disturbed sites (MUCs). In each core, four to six oxygen profiles were measured.

by the disturbance as well. Since the undisturbed sites within the DEA are adjacent to plow tracks, they have likely been impacted by resettling suspended sediment from the plowing. Mo, Ni, Co, and Cu are associated with Mn-oxides which is well known from other studies (Klinkhammer et al., 1982; Heggie and Lewis, 1984; Shaw et al., 1990; Koschinsky, 2001; Morford et al., 2005). Mo and Ni show a similar increase from east to west and from the DEA to the reference sites. Co and Cu do not show such variability, though; the concentrations are in the same range for the four undisturbed sites. Correlation coefficients show that of the four metals Cu is least associated with Mn (**Table 2**). Shaw et al. (1990) only name Mo, Ni, and Co as being associated with Mn-oxides. Cu seems to be neither controlled by the Mn-oxide phase nor by the Fe-oxyhydroxide phase, as indicated by only weak correlation with Mn and Fe (**Table 2**). Since Cu is generally known to show a high affinity to organic matter, we assume that binding to organic

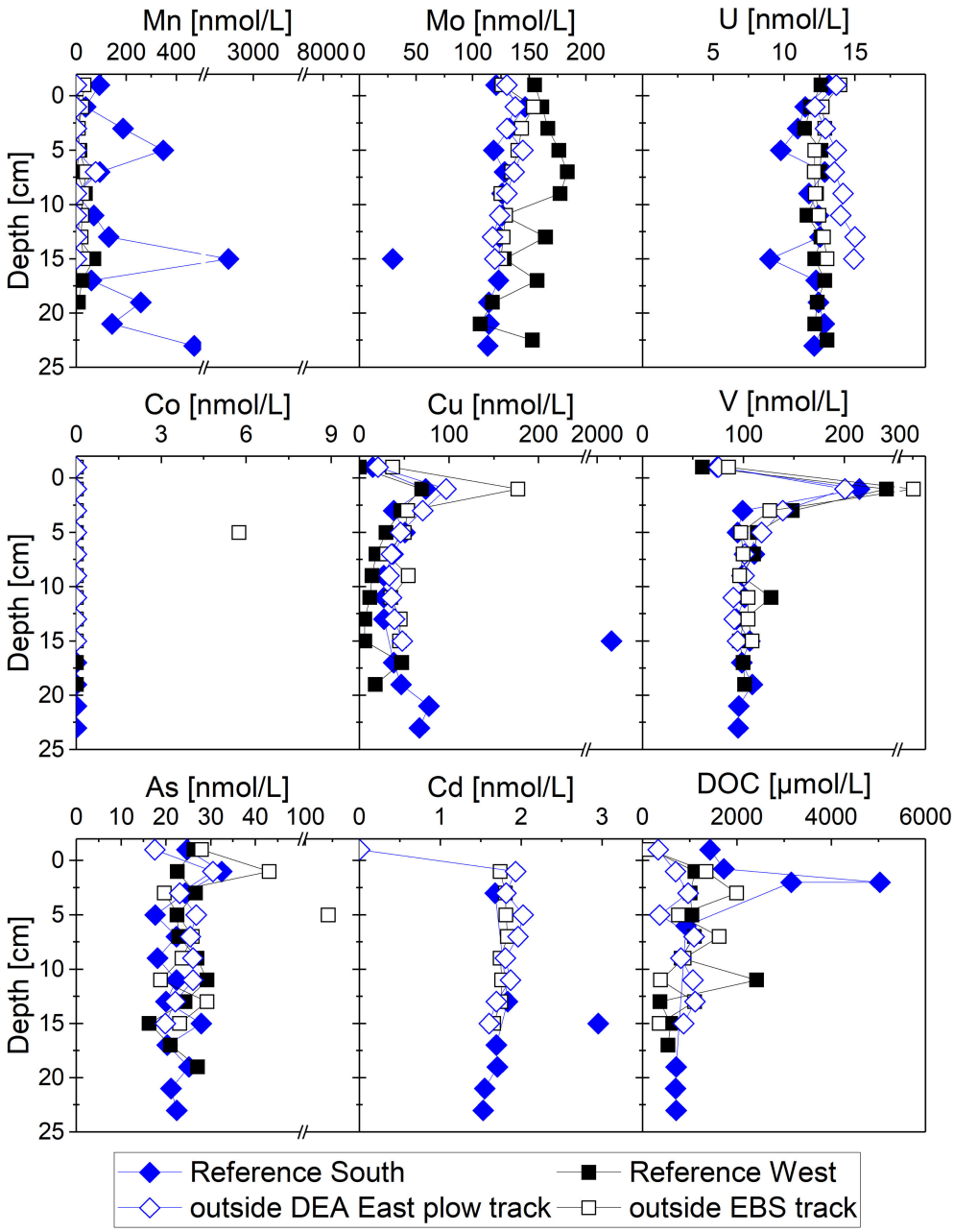


FIGURE 7 | Bottom water and pore water element profiles of the undisturbed sites. The uppermost values refer to bottom water concentrations measured in the supernatant retrieved above the sediment surface in the MUC liner.

functional groups may play a role in controlling Cu distribution in the surface sediment.

Disturbance Impacts on the Solid Phase: Sediment Removal, Redeposition, and Inversion

Disturbed sediments have lower solid phase Mn concentrations than the undisturbed sediments in the upper 15 cm, suggesting that the top Mn-oxide rich layer has been removed or mixed. This is explicit in the 1 month old disturbance—the EBS track—but also still visible in the profiles of the 26-year old plow tracks (compare Mn in **Figures 4**, **5**). Even though the highest concentrations of the disturbed sites (DEA West plow track) are in the range of the Mn concentrations of the undisturbed sites within the DEA, a comparison of the overall ranges shows that the disturbed sites on average show lower Mn concentrations. Comparing average Mn concentrations of undisturbed and disturbed sites in the individually sampled layers, the 26-year

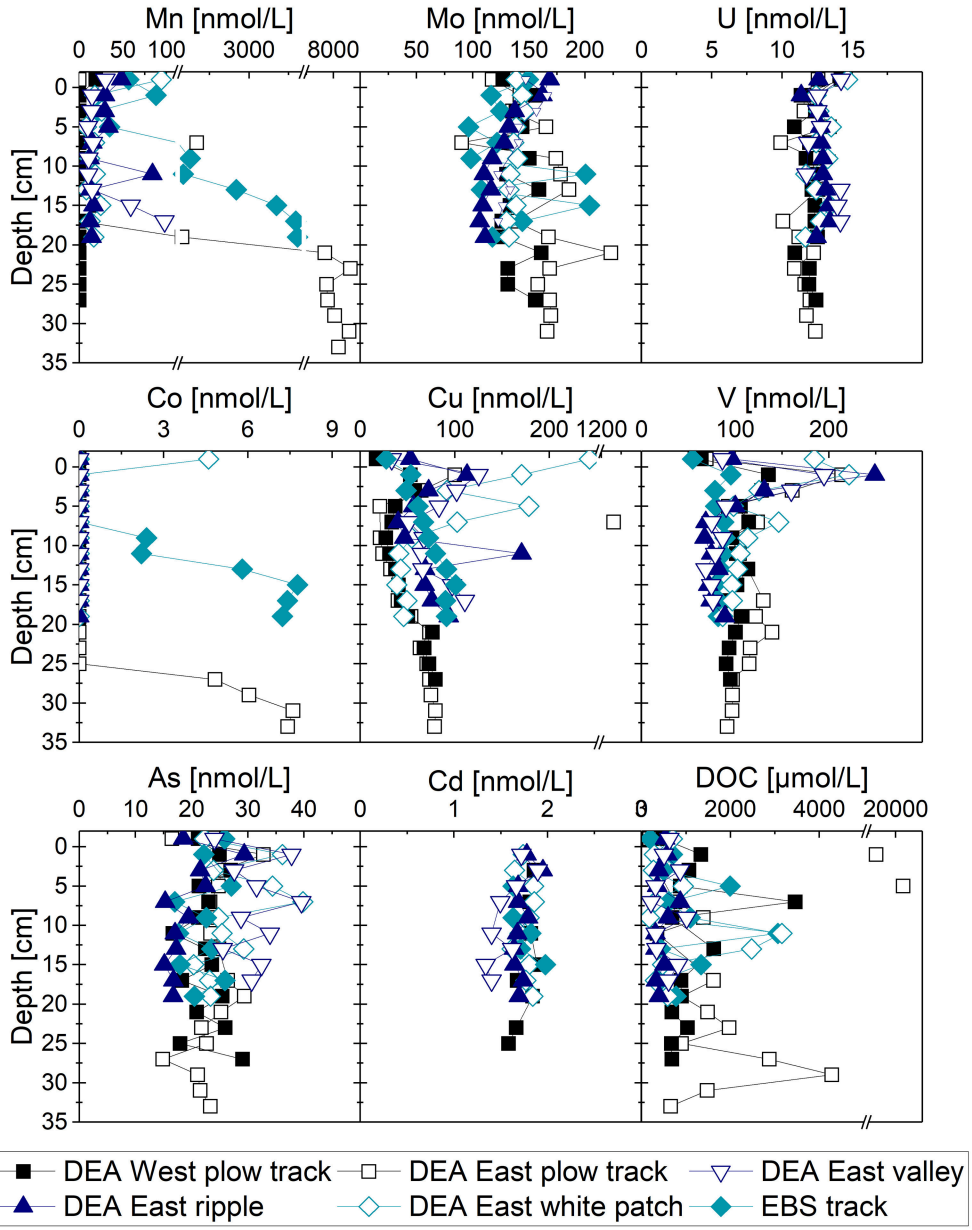


FIGURE 8 | Bottom and pore water element profiles of five 26-year old disturbed sites and the 5-week old EBS track. The uppermost values refer to bottom water concentrations measured in the supernatant retrieved above the sediment surface in the MUC liner. Mn and Co below the LOQ for DEA West plow track.

old disturbed sites have 20 to 47% less Mn in the upper 16 cm. Similarly, there is 17 to 48% less Mo in disturbed surface sediments, while Co, Ni and Cu contents are successively less impacted: Co 7 to 25%, Ni 5 to 18% and only Cu −7 to 7%.

The plowing removed most of the Mn-oxide rich layer in the plow tracks and disturbed microhabitats. Only a thin layer is left, the thickness varying between the disturbed sites but being clearly reduced compared to the undisturbed sites. Specifically the white patch does not have a Mn-oxide rich layer left at the sediment surface. The same is true for the EBS track and the tan sediment layer that is usually beneath the dark brown Mn-oxide layer lies at the surface. Since the EBS track sediment is now exposed to the bottom water, trace metals diffuse from the suboxic pore water until a sufficiently thick Mn-oxide layer has formed that scavenges the trace metals. The higher the Mn-oxide content in the surface layer, the lower the diffusive flux of heavy metals into the bottom water and the higher the sorption capacity (Fritsche et al., 2001). Mn-oxides should form with time and the Mn, Mo, Ni, Co, and Cu concentrations in the particulate fraction should slowly increase to establish the typical layering but the time scale of these processes is unknown. The particulate fraction has not yet recovered in these parts but at DEA West and East plow tracks

the Mn-oxide rich layer is building up again and it is thicker than in the microhabitats. In conclusion, both, Mn layer thickness and Mn content, are lower in the disturbed sites.

In addition to sediment removal, suspended sediment was deposited on the plow tracks. The sedimentation of suspended sediment was most measurable in the track valley as indicated by increased concentrations of Mn, Mo, Ni, Co, and Fe in the DEA East valley in the upper 2 to 4 cm (**Figure 5**). The sediment seems to be composed of different material than the sediment further downcore, possibly resettled particles from the Mn-oxide rich layer that were suspended during the plowing. Moreover, the porosity of the valley's surface layer is higher compared to other disturbed sites, proving that loose material was deposited on top. Both impacts have also been found in other biogeochemical investigations (Vonnahme et al., in prep.).

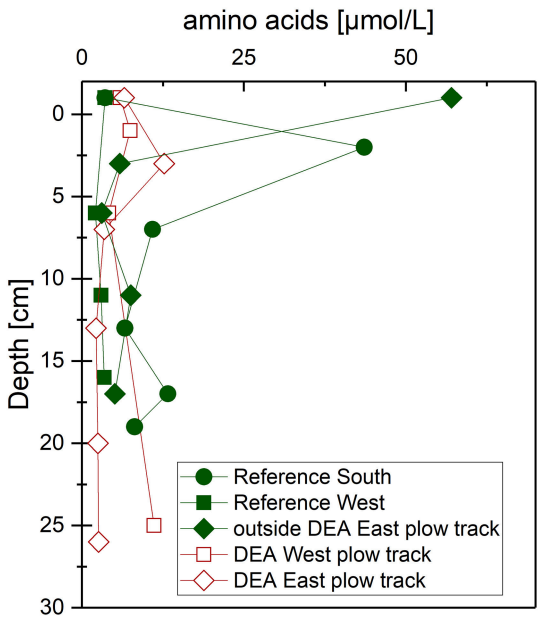


FIGURE 9 | Sum of dissolved amino acid (DAA) concentration profiles of three undisturbed and two disturbed sites. The uppermost values refer to bottom water concentrations measured in the supernatant retrieved above the sediment surface in the MUC liner.

A third impact is sediment inversion, where Mn-oxide rich surface sediment got plowed to greater depth. This is visible in the white patch and the DEA East plow track profiles. At the white patch, the porosity also increases again below this depth, supporting the assumption that surface sediment got turned. At both sites, Mn, Mo, Ni, Co, and Cu show elevated concentrations at 11 and 23 cm, respectively. In other areas of the Peru Basin, a Mn peak at the redox boundary was discovered (Koschinsky, 2001). Internal redox cycling of Mn around the redox boundary can lead to such pronounced solid phase Mn peaks (Burdige, 1993). This is comparable to the marked Mn peak at DEA East plow track (**Figure 5**) and could be an explanation for the peak because at 25 cm depth the dissolved Mn concentration increases drastically, a clear sign that Mn-oxides are reduced. Since the upper part of the core shows disturbance impacts, we assume that the peak at 23 cm is another sign of the disturbance.

Pore Water Natural State and Impacts Visible 5 Weeks Post-disturbance

The degradation of organic matter during early diagenesis at the sediment-water interface releases various elements to the pore water—e.g., V, Cu, Mo, and DOC (Sawlan and Murray, 1983; Heggie et al., 1986; Shaw et al., 1990; Koschinsky, 2001; Kowalski

TABLE 2 | Correlation coefficients of Mn and Fe with Cu, Co, Ni, and Mo, calculated in Excel.

	Mn	Mn	Mn	Mn	Mn	Mn	Mn	Mn	Mn	Mn
Mn	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cu	0.88	0.96	0.90	0.78	0.84	−0.18	−0.51	0.71	0.01	−0.12
Co	0.94	0.87	0.97	0.95	0.97	0.92	0.84	0.98	0.92	0.71
Ni	0.98	0.96	0.99	0.99	0.97	0.94	0.78	0.94	0.93	0.78
Mo	0.99	0.98	0.99	0.98	0.98	0.90	0.98	1.00	0.97	0.97
	Fe	**Fe**	**Fe**	**Fe**	**Fe**	**Fe**	**Fe**	**Fe**	**Fe**	**Fe**
Fe	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mn	0.76	0.26	0.57	0.44	0.85	0.88	0.88	0.94	0.00	0.73
Cu	0.55	0.23	0.39	−0.18	0.99	−0.11	−0.57	0.58	−0.03	0.46
Co	0.88	0.23	0.67	0.61	0.84	0.78	0.64	0.94	0.21	0.21
Ni	0.84	0.43	0.63	0.44	0.94	0.78	0.48	0.92	−0.19	0.73
Mo	0.75	0.21	0.57	0.32	0.83	0.81	0.88	0.96	0.15	0.83
	Reference South	Reference West	Outside DEA East plow track	Outside EBS track	DEA West plow track	DEA East plow track	DEA East ripple	DEA East valley	DEA East white patch	EBS track

The entire profiles were correlated.

et al., 2009). In this study, we clearly see this release in form of a marked peak in the top 2 cm of the concentration profiles for V, Cu, and DOC, and to a lesser extent for As (**Figures 7**, **8**). Extensive V peaks at the sediment-water interface can be sustained due to complexation by DOC (Emerson and Huested, 1991; Morford et al., 2005) and because it is not significantly adsorbed to Mn-oxides (Koschinsky, 2001). There are no major differences in the pore water profiles of undisturbed and 26-year post-disturbance sites; the pore water is recovered. Only at DEA West plow track the usual trace metal peak in the top 2 cm is less pronounced, which is interesting considering that DEA West plow track has the least impacted solid phase. The typical peaks of V, Cu, and As in the top 2 cm are not clearly developed yet in the EBS track profile (**Figure 8**; teal filled diamonds). Even though this feature is already visible in the V profile, the extent of the peak is less than half the concentration of that in the undisturbed and 26-year old disturbed sites from DEA East. This could also be a sign of a lower microbial activity and hence lower rates of organic matter degradation, which usually releases metals to the pore water at the sediment-water interface (Sawlan and Murray, 1983; Heggie et al., 1986; Shaw et al., 1990).

Typically, pore water concentrations of Mn and some associated metals (e.g., Mo, Ni, Co, Cu) increase in the Mn reduction zone as the solid phase concentrations decrease (Froelich et al., 1979; Heggie and Lewis, 1984; Koschinsky, 2001; Morford et al., 2005), approximately below 20 cm in the Peru Basin. This phenomenon is rarely visible in our data except for Cu (**Figures 7**, **8**), as the cores are usually too short to cover the entire Mn reduction zone or the Mn and Co pore water concentrations are below the detection limit when they first increase. The increase is only visible at DEA East plow track (**Figure 8**) because it is a 35 cm long MUC and we were not able to retrieve those sediment depths with any other core. The Mn and Co concentration increase is natural since it occurs below the redox boundary where such increase is expected, even though the profile is from a disturbed site. Samples taken at the DISCOL area in 1996 also show increasing pore water Mn below 25 cm (Koschinsky, 2001). Correlating with the Mn and Co release, DOC, Mo, and V concentrations increase at the depth of Mn release in the DEA East plow track core. The EBS example indicates, however, that 5 weeks post-disturbance the pore water shows signals of the impact and Mn and Co are already detectable in the pore water at ca. 8 cm depth. This is markedly closer to the sediment-water interface than for the undisturbed and 26-year old disturbed sites. Also, Mo is more variable below 8 cm compared to the largely conservative profiles in the undisturbed sites. Therefore, this difference can be clearly attributed to the disturbance. The sediment profiles show that the entire Mn-oxide rich layer was removed with the EBS so that suboxic pore water with dissolved Mn and Co must have been at the sediment-water interface. After 5 weeks, Mn and Co have already been removed from the pore water due to diffusion of oxygen into the sediment and concentrations in the top 8 cm are within the natural background range. According to Einstein-Smoluchowski calculations the diffusional length for 5 weeks is 7–8 cm. König et al. (2001) predicted diffusion of oxygen into the sediment after a disturbance at short time-scales; the establishment of the original redox zonation might, however, well take a few centuries. The natural redox zonation has, however, not been established yet and the pore water, as well as the particulate fraction, is in the process of approaching a new equilibrium.

Trace Metal Fluxes to the Ocean

The increased concentration of V and Cu in the surface pore water suggests diffusion to the bottom water to some degree (**Table 3**) (also see Koschinsky, 2001). Fluxes of the trace metals which have concentrations in the range of bottom water (Mo) are negligible or metals diffuse from the bottom water into the pore water (Mn) (**Table 3**). Trace metal input would, however, be considerably enhanced when metals would diffuse from the suboxic pore water after removal of the Mn-oxide rich layer due to deep-sea mining (**Table 3**). Nevertheless, metals do not reach concentrations potentially toxic to animals, e.g., Cu release 0.3 $\mu mol^{*}m^{-2}{*}month^{-1}$ compared to lowest LC_{50} values of 8.85 μmol/L (Mevenkamp et al., 2017). The trace metal concentrations and fluxes in the upper cm are already reduced after 5 weeks, as the data from the EBS track shows (**Figure 8**). The diffusion of oxygen into the sediment leads to oxidation of the dissolved metal ions and removal from the pore water. Similarly, the metal ions released into the bottom water will be quickly scavenged by particles in the oxic bottom water and are not expected to greatly impact the trace metal budget of the ocean. Therefore, the numbers for diffusive fluxes after the disturbance presented in **Table 3** are a worst-case scenario and will probably be lower, even within the first month after the disturbance, because they decline every day. Further non-steady state modeling would be needed to portray a realistic post-disturbance scenario.

DOC and DAA as Indicators of Organic Matter Degradation

DOC and DAA can be intermediates of sedimentary organic matter degradation. DOC and DAA concentrations are elevated in pore waters compared to bottom water so that the sediments are sources of DOC and DAA to the water column (Lahajnar et al., 2005; Burdige and Komada, 2015). In the pore water, DOC and especially the more reactive DAA, may be further degraded to inorganic nutrients or reintegrated into the sediment by bacterial uptake or sorption processes (Burdige and Martens, 1990; Ding and Henrichs, 2002). DAA concentrations in near-shore pore water are elevated in the upper 25 cm and drop to values of 2-5 μmol/L at depth (Burdige and Martens, 1990), which also fits well with our deep-sea pore water data (**Figure 9**). DAA bottom water and pore water spectra are dominated by Ser>Gly>Ala>His>Orn>Asp (Supplementary Material 4), irrespective of disturbance and redox-zonation, which is quite different from sediment and suspended matter spectra that are dominated by Gly, Asp, Glu, and Ala or, respectively, by Gly, Glu, Asp, and Ser (Gaye et al., 2013) but similar to DAA spectra from the water column (Ittekkot and Degens, 1984). Pore waters tend to accumulate those amino acids which are preferably removed from the particulate phase, including Ser, Gly, and Glu (Seifert et al., 1990) as well as degradable amino acids (e.g., Met) and basic amino acids (e.g., Lys) preferentially sorbed to

TABLE 3 | Diffusive fluxes of selected dissolved metal ions across the sediment-water interface and potential fluxes across the sediment-water interface when the Mn-oxide rich layer is removed; based on gradients across the redox-boundary in cores from this study.

	Diffusive flux across the sediment-water interface				Potential diffusive flux across the sediment-water interface when oxic layer removed		
	[μmol*m^{-2}*month^{-1}]				[μmol*m^{-2}*month^{-1}]		
Site	**Mn**	**Cu**	**V**	**Mo**	**Mn**	**Cu**	**Co**
Reference South	3	−4	−15	−3			
Reference West	−1	−5	−20	−1			
Outside DEA East plow track		−5	−13	−1			
Outside EBS track	1	−9	−28	−3			
Average undisturbed	**1**	**−6**	**−19**	**−2**			
DEA West plow track		−2	−7	−3			
DEA East plow track		−6	−14	−2	−79	−0.3	−0.07
DEA East ripple	1	−4	−15	1			
DEA East valley	1	−6	−11	−2			
DEA East white patch	5	5	−4	−1			
Average disturbed 26 years old	**2**	**−3**	**−10**	**−1**			
EBS track	−2	−2	−4	3	−21	−0.2	−0.03

Negative fluxes indicate diffusion from the pore water to the bottom water and positive fluxes indicate diffusion from bottom water to the pore water. The assumptions are that the system is steady state and diffusion the only process. $F = -\phi D_{sed} \frac{\delta C}{\delta x}$ *(ø = average porosity of the sediment in each core for the used depth;* $D_{sed} = \frac{D_{SW}}{\theta 2}$*;* D_{SW} *Mn = 3.02E-10* $m^2 s^{-1}$*;* D_{SW} *Cu = 3.22E-10* $m^2 s^{-1}$*;* D_{SW} *Co = 3.15E-10* $m^2 s^{-1}$*;* D_{SW} *V = 5E-10* $m^2 s^{-1}$*;* D_{SW} *Mo = 5E-10* $m^2 s^{-1}$*);* D_{SW} *at temperature 0°C from Schulz (2006) after Boudreau (1997), closest diffusion coefficient values considering deep-sea temperatures of 1.85°C (Brown et al., 2017b), except for V and Mo, where only general diffusion coefficients published in Emerson and Huested (1991) and Scholz et al. (2011) were used. For a more detailed table including the pore water and bottom water metal concentrations see Supplementary Material 5.*

mineral surfaces (Ding and Henrichs, 2002). The latter indicate the degradation or desorption of amino acids of the particulate pool. In addition, pore waters also accumulate the non-protein amino acids β-Ala, γ-Aba, and Orn which are either degradation products of proteinaceous amino acids or are not taken up by bacteria (Seifert et al., 1990; Davis et al., 2009). It has been shown experimentally with cores from the Peru Basin that a few hours after a disturbance, particulate AA concentrations in the sediment and DOC in pore waters sharply increased. The increase in particulate AA was attributed to enhanced bacterial activity which could be related to spreading of fresh organic matter from deeper layers (Koschinsky et al., 2001b) and augmented by the oxygen availability in the upper sediments which may reinforce organic matter degradation (Lee, 1992). After 26 years, however, the concentration differences between disturbed and undisturbed sites are not so visible anymore so that degradation possibly slowed down due to decreasing quality of organic matter (Vonnahme et al., in prep.). The high DOC concentration in the DEA East plow track surface layer should therefore not be a remnant of the 1989 disturbance but rather due to a recent incident, such as a local input of organic material or bioturbation.

CONCLUSION

The solid phase results of the undisturbed sites show natural variability with respect to element concentrations, yet the profile shapes agree. The pore water profiles do not show major differences between the undisturbed and the 26-year old disturbed sites. Five weeks post-disturbance, the impacts were still visible in the pore water profiles but signs of regeneration in the upper centimeters were already visible and an elevated metal flux to the ocean seems to prevail on even shorter time scales. Differences in DOC and DAA concentrations as well as spectra are not visible or cannot be attributed to the disturbance after 26 years. In general, the re-establishment of a new steady-state in the solid phase takes longer than in the pore water. The differences between undisturbed and 26-year old disturbed sites, especially the loss or redistribution of Mn-oxide rich sediment, are clearly visible in the profiles. An important finding of our study is that degrees and types of disturbance differ strongly among the disturbed sites. The EBS track is quite distinct due to its recency but even the other five 26-year old disturbed sites vary with respect to concentrations of the metals—especially Mn, Mo, Ni, and Co—as well as profile shapes in the solid phase. As discussed above, these can be results of different disturbance impacts such as removal, mixing, redeposition of suspended sediment, and inversion or most often a unique combination of several impacts.

The geochemical variability which was discovered in the undisturbed as well as disturbed sites elucidates that the deep-sea is a highly complex system that is still poorly understood as has also been recently shown for the CCZ (Mewes et al., 2014, 2016; Mogollón et al., 2016; Kuhn et al., 2017; Volz et al., in review). With respect to polymetallic nodule mining, it will be necessary to carry out baseline studies on the geochemistry of the potentially impacted sites and reference sites for quite a high number of locations to assess the heterogeneity of both, the natural area and the types of impact. The difficulty of gaining representative baselines and ranges of disturbance impacts is a

general challenge for deep-sea mining related research and has been discussed elsewhere, too (see for example Jones et al., 2017).

Metal concentrations in pore water are not suitable for monitoring purposes because their concentrations quickly reach a new steady-state after a disturbance, probably on time scales of months. Therefore, they could imply that the system has recovered which truly is not the case for other components. In the DISCOL area, the disturbance impact was most pronounced in the Mn-oxide rich top layer. Since it was shown in this study and previous work (Shaw et al., 1990; Koschinsky, 2001; Morford et al., 2005) that many other metals – such as Mo, Ni, Co, and Cu, are associated with Mn-oxides in this layer, Mn is a key parameter for monitoring, if not all parameters can be measured due to financial, technical, and time constraints in an industrial mining scenario. In addition to the oxygen penetration depth, knowing the Mn concentration in the solid phase and pore water gives a lot of insights into the geochemical system at this site, including potential release of other trace metals, and would be a useful parameter to measure pre-mining and post-mining for monitoring purposes. Mn is a good indicator for disturbance in sediments with a relatively shallow oxic layer, such as the Peru Basin. Areas with a different redox-zonation might have different key parameters because a largely oxic system (such as the CCZ where deep-sea polymetallic nodule mining is most likely to start) will be differently impacted (also see Cronan et al., 2010; Rühlemann et al., 2011; Mewes et al., 2014, 2016; Mogollón et al., 2016; Kuhn et al., 2017; Volz et al., in review). We are only able to draw this conclusion about Mn as a key parameter, however, because this small site has been extensively studied over a long period of time. Baseline studies are vital to (1) understand the system, (2) select key parameters, and (3) define thresholds. More extensive research in different geochemical seafloor systems and on a larger scale needs to be carried out before it can be determined what a negative impact on the environment may be and which thresholds should therefore not be exceeded.

AUTHOR CONTRIBUTIONS

SP: research design, data collection, trace metal analyses and data interpretation, article drafting and revision. MH, AK, and SK: research design. MH: sampling, oxygen, POC, PON, porosity data collection, analyses and interpretation. BG: DOC and DAA analyses and data interpretation. AK, MH, BG, and SK: article revision. SP, MH, BG, SK, and AK: final approval of the version to be published.

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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.2018.00117/full#supplementary-material

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Out of Sight, But Within Reach: A Global History of Bottom-Trawled Deep-Sea Fisheries From >400 m Depth

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Deep-sea fish species are targeted globally by bottom trawling. The species captured are often characterized by longevity, low fecundity and slow growth making them vulnerable to overfishing. In addition, bottom trawling is known to remove vast amounts of non-target species, including habitat forming deep-sea corals and sponges. Therefore, bottom trawling poses a serious risk to deep-sea ecosystems, but the true extent of deep-sea fishery catches through history remains unknown. Here, we present catches for global bottom trawling fisheries between years 1950–2015. This study gives new insight into the history of bottom trawled deep-sea fisheries through its use of FAO capture data combined with reconstructed catch data provided by the *Sea Around Us* - project, which are the only records containing bycatches, discards and unreported landings for deep-sea species. We illustrate the trends and shifts of the fishing nations and discuss the life-history and catch patterns of the most prominent target species over this time period. Our results show that the landings from deep-sea fisheries are miniscule, contributing less than 0.5% to global fisheries landings. The fisheries were found to be overall under-reported by as much as 42%, leading to the removal of an estimated 25 million tons of deep-sea fish. The highest catches were of Greenland halibut in the NE Atlantic, Longfin codling from the NW Pacific and Grenadiers and Orange roughy from the SW Pacific. The results also show a diversification through the years in the species caught and reported. This historical perspective reveals that the extent and amount of deep-sea fish removed from the deep ocean exceeds previous estimates. This has significant implications for management, conservation and policy, as the economic importance of global bottom trawling is trivial, but the environmental damage imposed by this practice, is not.

Keywords: deep-sea fisheries, deep-sea, fisheries management, global fisheries, bottom-trawling, habitat destruction, environmental impact

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INTRODUCTION

The history of global fisheries is one of full- or over-exploitation, with a few exceptions (Pauly and Zeller, 2016). One of the most controversial fishing practices known to date is bottom trawling, which can be dated back to as early as 1376, when concerns and complaints were raised by fellow fishermen about a new destructive and wasteful fishing habit (Roberts, 2007). The extension of

bottom trawling to the deep-sea occurred in the second half of the twentieth century, prompted by technological advances and a decline in shallow water fisheries (Koslow et al., 2000). The fisheries industry, particularly in Europe, North America, and the former Soviet Union, pushed into ever deeper water in search of more fish. In many cases, these fisheries were promulgated on the high seas where there were few, if any, regulations (Bensch et al., 2009a). Many of those fisheries, especially those targeting seamounts have been shown to be "boom and bust" fisheries, and lasting from less than a decade to a couple of decades (Clark et al., 2007) before they are no longer economically viable.

The deep sea is an ecosystem different from that of shallower water. Here organisms, including fish, generally live for long times, have low fecundity, mature at older ages, and have lowered metabolism and slow growth. Drazen and Haedrich (2012) found that for 41 shallow and deep dwelling fish species with sufficient life history data, there was a consistent trend of increasing longevity, decreasing fecundity, and decreasing potential rate of population increase with depth. In addition, deep-sea fish may be more vulnerable to the fishery by aggregating on seamounts for mating or taking advantage of trapping of vertically migrating nekton by seamount topography (Morato and Clark, 2007). Such is the case of the Orange roughy, where fisheries, in order to be economically viable, have targeted spawning and feeding aggregations (Clark, 1999; Roberts, 2002). It is, therefore, likely that deep-sea fisheries could easily and rapidly, over-exploit fish species living on seamounts and ridges, or along the continental slopes of the world.

Fishing in the deep is difficult, and requires large vessels with very heavy gear in order to reach species living at depths of as much as 2,000 m. Deep-sea fishing vessels are often of 80–100 m length, weighing in at 2,000 gross tons or more. In some distant water fisheries, the vessel may be much larger and house crew and capabilities for processing and freezing the catch while at sea. Deep trawl gear is usually in the form of an otter trawl which uses metal "doors" that can weigh up to 5,000 kg in order to get the net to the bottom and keep the net mouth open while being pulled across the seafloor. The trawl can be very wide, with total distance including the sweeps, bridles and ground gear amounting to 80–200 m. The ground gear of a deep-sea trawl is equipped with steel bobbins and/or stiff rubber discs that are designed to allow the net to move over rough bottom without getting "hung up" (Clark and Koslow, 2007). This equipment guarantees that bottom trawling is the most efficient fishing method in the deep-sea, but also the most destructive as it permanently removes the benthic habitats, typically comprising long-lived habitat-forming species, such as deep-sea corals and sponges (Clark et al., 2016). To date, no recovery over decadal time scales have been observed on seamounts targeted by bottom trawling (Williams et al., 2010).

Deep-sea fisheries have been summarized in general terms (Priede, 2017), for specific species (Shotton, 2016) and parts of the ocean, such as seamounts (Clark, 2009), FAO fishing regions, or for relatively short periods of time (Bensch et al., 2009a). However, a comprehensive historical analysis of bottom trawled fisheries describing the major trends with data beyond that provided by FAO, is currently missing. The purpose of this study is to produce a more complete global history of those fisheries promulgated primarily with bottom trawl gear below 400 m. This is achieved by complementing the data set produced by the FAO for the period of 1950–2015 (FAO, 2017), with unreported landings, bycatch and discards data for the period of 1950–2014 from the *Sea Around Us* research initiative. We document the history of the species targeted, the countries involved, and the parts of the global ocean that have supported these fisheries and highlight shifts in their patterns through the years covered by the data. In addition, we pinpoint discrepancies between the two data sets to show that the fishing pressure encountered by certain species has not been accounted for in current conservation efforts, which is potentially leading to the mismanagement of deep-sea trawl fisheries.

METHODS

When we started this study, there was no comprehensive compilation of fish species caught as part of the deep-sea fisheries of the world (Bensch et al., 2009a). Therefore, we developed a list of fish species caught primarily by deep-sea bottom trawling either as target species or bycatch, using records from EU Annex 1 and 2, FishBase (Fishbase.org), the fisheries literature, and from the compilation by Priede (2017) (**Table 1**). This list of demersal species was reviewed by independent experts to be sure we had not overlooked any species known to be taken, at least in moderate to large numbers, primarily by bottom trawling. A small number of species within this list are also caught with a longline, along with bottom trawling and there is no differentiation between catches by gear types. Global capture data (in tonnage) for species was extracted from the Food and Agriculture Organization of the United Nations (FAO) capture data set using FishstatJ (v. 3.01) for the years 1950–2015. As the FAO data only includes the capture landings reported by national governments, a set of estimated unreported capture data for the same species for the years 1950–2014 was provided by the *Sea Around Us* (www.seaaroundus.org) at the University of British Columbia. The reconstructed catch data includes species for which FAO did not require reports in the past, as well as unreported landings and discards generated by deep-sea fisheries (Zeller et al., 2017). Both data series were also used to calculate the number of species caught per year.

The *Sea Around Us* reconstructed capture data is estimated using a seven-step methodology developed by Zeller et al. (2007) and recently modified and detailed in Zeller and Pauly (2016). The method includes sourcing data from reporting entities, in particular FAO, but also local agencies, identification of sectors not covered in the official reports, finding additional sources of information searching literature and archives, developing data anchor points in the time series, and consulting local experts to fill in the data gaps, then interpolating between anchor points for missing data components. Lastly, the uncertainty associated with each reconstruction is quantified.

Deep-sea fish species exhibit a continuously changing range of life-history traits with depth (Drazen and Haedrich, 2012). In order to make catch levels more comparable, we divided our list of deep-sea species into two groups. The shallower group

TABLE 1 | List of species taken primarily by bottom trawls at depths greater than 400 m and for which there is data in the FAO FishStat J landings database (a) and also included are species whose trawl fisheries are primarily between 200–400 m (b).

Common name	Scientific name
(a) >400 m depth	
Antarctic toothfish	*Dissostichus mawsoni*
Arctic skate	*Amblyraja hyperborea*
Baird's slickhead	*Alepocephalus bairdii*
Beaked redfish	*Sebastes mentella*
Bigeye grenadier	*Macrourus holotrachys*
Bigspined boarfish	*Pentaceros decacanthus*
Birdbeak dogfish	*Deania calcea*
Black cardinal fish	*Epigonus telescopus*
Black dogfish	*Centroscyllium fabricii*
Black oreo	*Allocyttus niger*
Black scabbardfish	*Aphanopus carbo*
Blackbelly rosefish	*Helicolenus dactylopterus*
Blackmouth catshark	*Galeus melastomus*
Blue antimora	*Antimora rostrata*
Blue ling	*Molva dypterigia*
Bluntnose sixgill shark	*Hexanchus griseus*
Chimaeras, etc. *nei*	Chimaeriformes
Common Atlantic grenadier	*Nezumia aequalis*
Common mora	*Mora moro*
Deep-sea smelt	*Glossanodon semifasciatus*
Dogtooth grenadier	*Cynomacrurus piriei*
Great lanternshark	*Etmopterus princeps*
Greater argentine	*Argentina silus*
Greenland halibut	*Rheinhardtius hippoglossoides*
Greenland shark	*Somniosus microcephalus*
Grenadier cod	*Tripterophycis gilchristi*
Grenadiers *nei*	Macrouridae
Gulper shark	*Centrophorus granulosus*
Kamchatka flounder	*Atheresthes evermanni*
King dory	*Cyttus traversi*
Kitefin shark	*Dalatias licha*
Knifetooth dogfish	*Scymnodon ringens*
Leafscale gulper shark	*Centrophorus squamosus*
Little sleeper shark	*Somniosus rostratus*
Longfin codling	*Laemonema longipes*
Longnose velvet dogfish	*Centroscymnus crepidater*
Lowfin gulper shark	*Centrophorus lusitanicus*
Mediterranean slimehead	*Hoplostethus mediterraneus*
Mouse catshark	*Galeus murinus*
Northern wolffish	*Anarhichas denticulatus*
Norway redfish	*Sebastes viviparus*
Norwegian skate	*Dipturus nidarosiensis*
Orange roughy	*Hoplostethus atlanticus*
Pacific sleeper shark	*Somniosus pacificus*
Patagonian toothfish	*Dissostichus eleginoides*
Pelagic armourhead	*Pseudopentaceros richardsoni*
Portuguese dogfish	*Centroscymnus coelolepis*
Rabbit fish	*Chimaera monstrosa*
Ridge scaled rattail	*Macrourus carinatus*
Roughhead grenadier	*Marcrourus berglax*
Roughsnout grenadier	*Trachyrincus scabrus*
Round ray	*Rajella fyllae*
Roundnose grenadier	*Coryphaenoides rupestris*
Sailfin roughshark	*Oxynotus paradoxus*
Shortspine African angler	*Lophius vaillanti*
Silvery John dory	*Zenopsis conchifer*
Slender armorhead	*Pseudopentaceros wheeleri*
Slickheads *nei*	Alepcephalidae
Slimeheads *nei*	Trachichthyidae
Smooth oreo dory	*Pseudocyttus maculatus*
Spiky oreo	*Neocyttus rhomboidalis*
Spiny scorpionfish	*Trachyscorpia cristulata*
Splendid alfonsino	*Beryx splendens*
Straightnose rabbitfish	*Rhinochimaera atlantica*
Thorntooth grenadier	*Lepidorhynchus denticulatus*
Velvet belly	*Etmopterus spinax*
Whitson's grenadier	*Macrourus whitsoni*
(b) 200–400 m depth	
Alfonsino	*Beryx decadactylus*
Alfonsinos *nei*	Berycidae
Angler	*Lophius piscatorius*
Anglerfishes *nei*	Lophiidae
Benguela hake	*Merluccius polli*
Blackbellied angler	*Lophius budegassa*
Blackspot(=red) seabream	*Pagellus bogaraveo*
Blue grenadier	*Macruronus novaezelandiae*
Bluenose warehou	*Hyperoglyphe antarctica*
Boarfish	*Capros aper*
Boarfishes *nei*	Caproidae
Cape bonnetmouth	*Emmelichthys nitidus*
Capro dory	*Capromimus abbreviatus*
Catsharks, nursehounds *nei*	*Scyliorhinus* spp.
Chilean grenadier	*Coelorinchus chilensis*
Deep-water cape hake	*Merluccius paradoxus*
Dories *nei*	Zeidae
Eaton's skate	*Bathyraja eatonii*
Golden redfish	*Sebastes norvegicus*
Greater forkbeard	*Phycis blennoides*
Hapuku wreckfish	*Polyprion oxygeneios*
Ling	*Molva molva*
Longnose spurdog	*Squalus blainville*
Megrim	*Lepidorhombus whiffiagonis*
Monkfishes *nei*	Lophiidae
Offshore silver hake	*Merluccius albidus*
Patagonian grenadier	*Macruronus magellanicus*

(Continued)

TABLE 1 | Continued

Common name	Scientific name
Pink cusk-eel	*Genypterus blacodes*
Rubyfish	*Plagiogeneion rubiginosum*
Sandpaper fish	*Paratrachichthys trailli*
Shallow-water cape hake	*Merluccius capensis*
Shortspine thornyhead	*Sebastolobus alascanus*
Skilfish	*Erilepis zonifer*
Splitnose rockfish	*Sebastes diploproa*
Spotted wolffish	*Anarhichas minor*
Thorntooth grenadier	*Lepidorhynchus denticulatus*
Wreckfish	*Polyprion americanus*

Not included are species taken largely or exclusively by long lines.

comprises slope species that inhabit depths of ∼200–400 m. These species typically mature faster and have higher fecundity resulting in life history characteristics that are more closely allied with species of the continental shelf. As such they provide more productive fisheries with higher magnitude of catches and will be considered in a future paper. The second group, which is the main focus of this study, comprises species living and being caught mostly by bottom trawls below 400 m. These species exhibit more typical deep-sea traits such as slow growth, late maturation and low fecundity, and by comparison have lower weights of catches. Data handling and analysis was conducted in the software "R" (R Core Team, 2016) or with Microsoft Excel®.

Long-term patterns of catch by country was illustrated using the Density Equalizing Cartogram routine implemented in the "Cartogram geoprocessing tool" in ArcGIS (v. 10.2.2). This routine uses a diffusion-based method (Gastner and Newman, 2004) and changes the shape of each polygon (in this case, country) in a manner that reflects the amount of an attribute (catch) associated with each polygon. The final product is a world-map of countries altered according to their deep-sea fishery catch in relation to that of all other countries. We constructed a cartogram for each year from 1950 to 2015.

We added a color gradient to the cartograms to give an indication of the magnitude of catches for each year. In the country analysis, we treated the data for Russian Federation and that of USSR as a single country whereas those formerly in the Soviet Union have their own entries in the FAO database after they became independent. Cartograms were generated separately for the two depth groups using both the reported data from FAO and the reconstructed total estimated catch. The cartograms for the 200–400 m depth group will be reported in a future paper.

RESULTS

Global, Regional and Country Specific Trends

The total amount of deep-sea fish caught was ∼14 million (FAO) and ∼25 million tons (FAO + SAU) through the historical period of 1950–2015 (**Table 2a**). Unreported landings and discards contributed almost equally to the discrepancy between the two data sets (Table S1). Deep-sea bottom-trawled fisheries account for less than 0.5% of the global estimated catch of all fisheries. The first large-scale deep-sea fishery catches are from 1956 by the Soviets who, based on reconstructions, discarded an estimated ∼84,000 t of Greenland halibut in the Northeast Atlantic (Tables S1). The discards were potentially bycatch of the more valuable cod and herring fisheries, which were the predominant target species at that time (Garfield, 1959). From here onwards, both reported landings and unreported catch steadily increased through the 1960s, after which there were three major periods of peak catches, reflected more in the total estimated catch than in the reported FAO data (**Figure 1**). These peaks represent sudden increases in catch of one or two species, such as Greenland halibut and Longfin codling, whose populations were newly discovered and rapidly exploited (**Figure 2**, and Supplementary Material, cartogram animation). While the early catches were dominated by just a few species from one or two areas, the deep-sea fisheries history becomes more complex adding more species and regions, with large estimated unreported catches between 1985 and 2010 and an estimated ∼600,000 t of fish being caught in the mid-1970s late 1980s and early 2000s.

TABLE 2 | Total reported (FAO data) and total estimated (FAO + SAU) catch (tons) for the period of 1950–2015 showing (a) the contribution of deep-sea bottom trawl fisheries to global landings, (b) a breakdown of total deep-sea (>400 m) fish landings by FAO fishing region.

(a)	Reported	Total estimated
Global fisheries catch	3,916,784,240	5,183,437,078
Deep-sea bottom trawl catch	14,397,146	24,905,883
Portion of global catch	<0.4%	<0.5%
(b) Fishing region	**FAO**	**FAO + SAU**
Atlantic, Northeast	5,714,858	8,862,807
Pacific, Northwest	1,810,983	5,639,395
Pacific, Southwest	1,575,128	4,281,410
Atlantic, Northwest	3,365,650	3,749,962
Atlantic, Southwest	558,596	586,350
Indian Ocean, Antarctic	261,666	359,503
Pacific, Northeast	263,759	308,787
Pacific, Southeast	264,929	287,715
Mediterranean and Black Sea	103,857	256,111
Atlantic, Southeast	138,663	140,928
Atlantic, Eastern Central	101,561	134,600
Indian Ocean, Eastern	54,740	99,149
Atlantic, Antarctic	96,483	95,223
Pacific, Antarctic	45,792	42,857
Indian Ocean, Western	25,563	36,109
Arctic Sea	12,617	12,617
Pacific, Western Central	0	7,817
Atlantic, Western Central	68	2,368
Pacific, Eastern Central	2,233	2,175

The total reported and estimated catch of each region is presented in **Table 2b**, from which it can be seen that four FAO

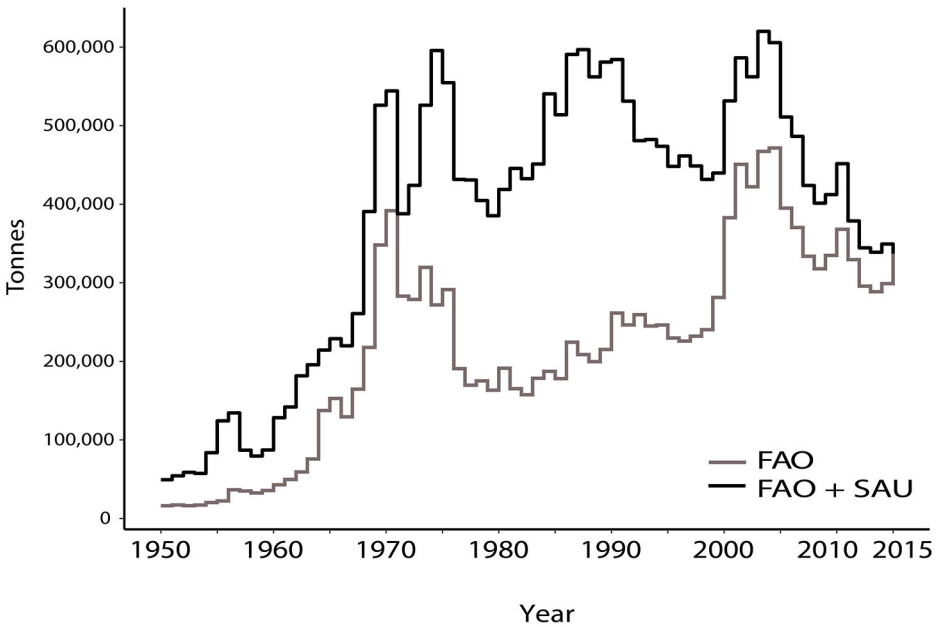


FIGURE 1 | Total catches of deep-sea (>400 m) bottom trawled species by the FAO and *Sea Around Us* not data series for the period of 1950–2015.

regions are responsible for the majority of landings: the Northeast and Northwest Atlantic, and the Northwest and Southwest Pacific. These areas are more productive and have high amounts of organic matter falling to the deep sea floor (Lutz et al., 2002; Watling et al., 2013) and as such the deep-sea fish populations in those areas are also very productive. The time period between 1975–2000 includes a notable decrease in landings from the NE Atlantic, which is reflected in the FAO data; in this same period there was an increase in the estimated total catch from the SW and NW Pacific fisheries (**Figure 2**). Catches in the NW Pacific, however, showed an overall sudden peak followed by a trend to reduction in landings until recently, which is linked to the collapse of the once extremely abundant Longfin codling stocks (**Figure 2**). The SW Pacific fisheries rely on aggregating species, such as Orange roughy and Smooth oreo dories found on and around topographic features, such as offshore banks and mid-ocean ridges that provide a great amount of area at fishable depth within the region. However, as fishing grounds become depleted, there is an overall trend of reduction in catches (**Figure 2**) with productivity of the fisheries questioned leading to stricter management (Schlacher et al., 2014). For example, catch quotas for the Orange roughy were lowered in the 1990s once scientific evidence emerged showing slow growth, high longevity (Mace et al., 1990) and rapidly declining biomass for some of the stocks (Clark, 2001).

We have illustrated the changing patterns of catch for individual countries in a series of cartograms, one for each year for both reported landings and estimated total catches. The cartograms are presented in **Figure 3**, and the remainder have been made into an animation and are included as Supplementary Material. The FAO cartograms show the pattern of change of landings, first from the North Atlantic, where Greenland halibut, Longnose velvet dogfish, and Blue ling were the mainstay of the fishery. Initially these species were caught in relatively low numbers, with reported landings for the countries being 20,000 t or less. As of 1950 the countries landing any deep-sea fish were Ireland, Norway, and a few other European nations. Through the 1950s and 60s, catches steadily increased, mostly in the North Atlantic as those three species continued to be exploited.

During this period the Soviet Union expanded into deep-sea fisheries. In 1969 Slender armorhead was discovered on the Hawaiian—Emperor Seamounts and in 1970 an estimated 149,820 t were caught (**Figure 3**). The Soviet Union was the primary country targeting Slender armorhead, and as a result it is the first country to report total deep-sea fishery landings in the range of 100,000–240,000 t. However, there are also records of the Japanese fleet targeting the Slender armorhead fishery during this time with catches of ∼30,000 t (Clark et al., 2007). Unfortunately, the FAO and the reconstructed data sets used in this study have no record of this and overall provide poor records for the Japanese deep-sea fisheries.

From the late 1970s and through the 1980s other deep-sea fish species were targeted, in particular Orange roughy, Longnose grenadier and other miscellaneous grenadiers (listed by FAO as "Grenadiers *nei*") and by 1990, the Patagonian toothfish. The 1980s and 90s also represent a period where the disparity between reported landings and total catch estimates was the largest. As will be noted below, these two decades saw very large landings of Longfin codling by Russia, and up to 50% under-reporting of Orange roughy by New Zealand, and systematic under-reporting of Greenland halibut being caught in the North Atlantic.

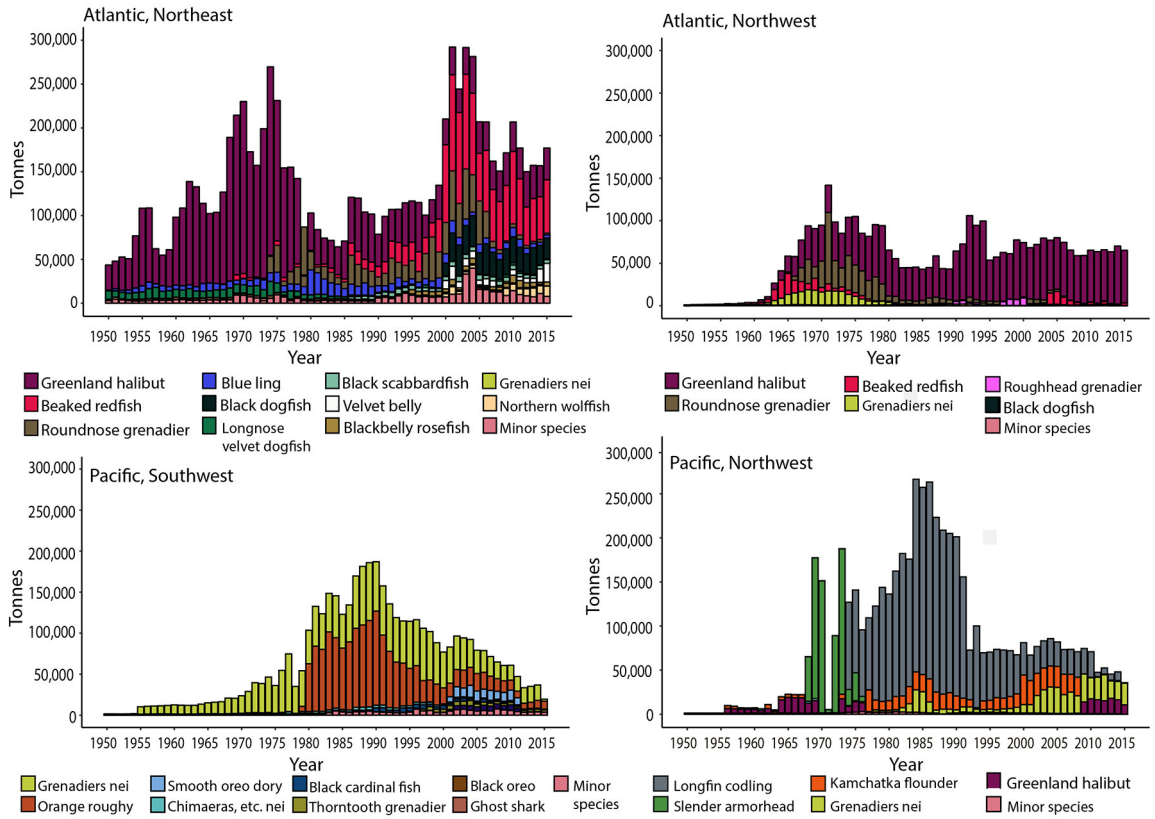


FIGURE 2 | Estimated total catches (*Sea Around Us* data) of deep-sea fish caught primarily at depths >400 m for the major fishing regions showing species targeted. **(A)** Northeast Atlantic, **(B)** Northwest Atlantic, **(C)** Southwest Pacific, and **(D)** Northwest Pacific. Species under the Minor species category include those contributing less than 1% to the total landings of a region.

The diversification of countries fishing in the deep sea and of species landings being reported increase, while the trend for under-reporting decreases during the 1990s and into the 2000s. At the same time the global catch numbers decrease to about half of peak values seen in the 1980s. This pattern is correlated with a decrease in landings from the SW Pacific, which consisted mostly of Grenadiers and Orange roughy, and an increase in catches from the NE Atlantic where the diversity as well as the tonnage of the catch increased (**Figure 2**). In the NW Pacific the large reduction in catch, which buoyed the values in the 1980s and 90s, was due to the heavy exploitation of Longfin codling. This species was replaced by catches of Grenadiers (mostly Popeye and Giant, although not specifically reported) and Greenland halibut from the Bering Sea.

Comparisons between the FAO records and the reconstructed catches show a steady increase in both data series for the number of species being caught, or at least recorded (**Figure 4**). This can be expected in the FAO data series, as a large number of species were not reported in the past, because of different regulation or the species were often grouped under a category of "*nei*." However, the reconstructions also show an increase in the diversity of species being caught, with an increase of up to 30 species from the 1950s to the modern era, where the data sets converge. This implies that a true diversification in the species targeted is likely and it is not simply an artifact caused by regulations leading to ungrouping of species.

Individual Fisheries

The 72 species or species groups being caught primarily with bottom trawls mostly at depths greater than 400 m, their reported (FAO) catch data, and the total estimated catch (FAO+SAU) are presented in **Table 3**. In the following section we give more detail about the most fished and vulnerable deep-sea fishery species, the nations who fish them, and catch trends since 1950. Biological information on individual species not specifically cited has been taken from FishBase.org. and all the catch values described here can be found in the Supplementary Data Tables (Table S1).

Greenland Halibut

Greenland halibut (*Reinhardtius hippoglossoides* Walbaum, 1792), also known as Turbot. The species is found in both the North Atlantic and North Pacific Oceans usually at depths of 500–1,000 m. This is a very old fishery in West Greenland, NW Atlantic, being fished commercially using line and hooks since the mid-1800s (Bowering and Brodie, 1995). For many years gill nets were used, but after 1966 large trawlers from Poland, German Democratic Republic and the USSR began taking Greenland halibut as trawl bycatch in the Redfish and Roundnose

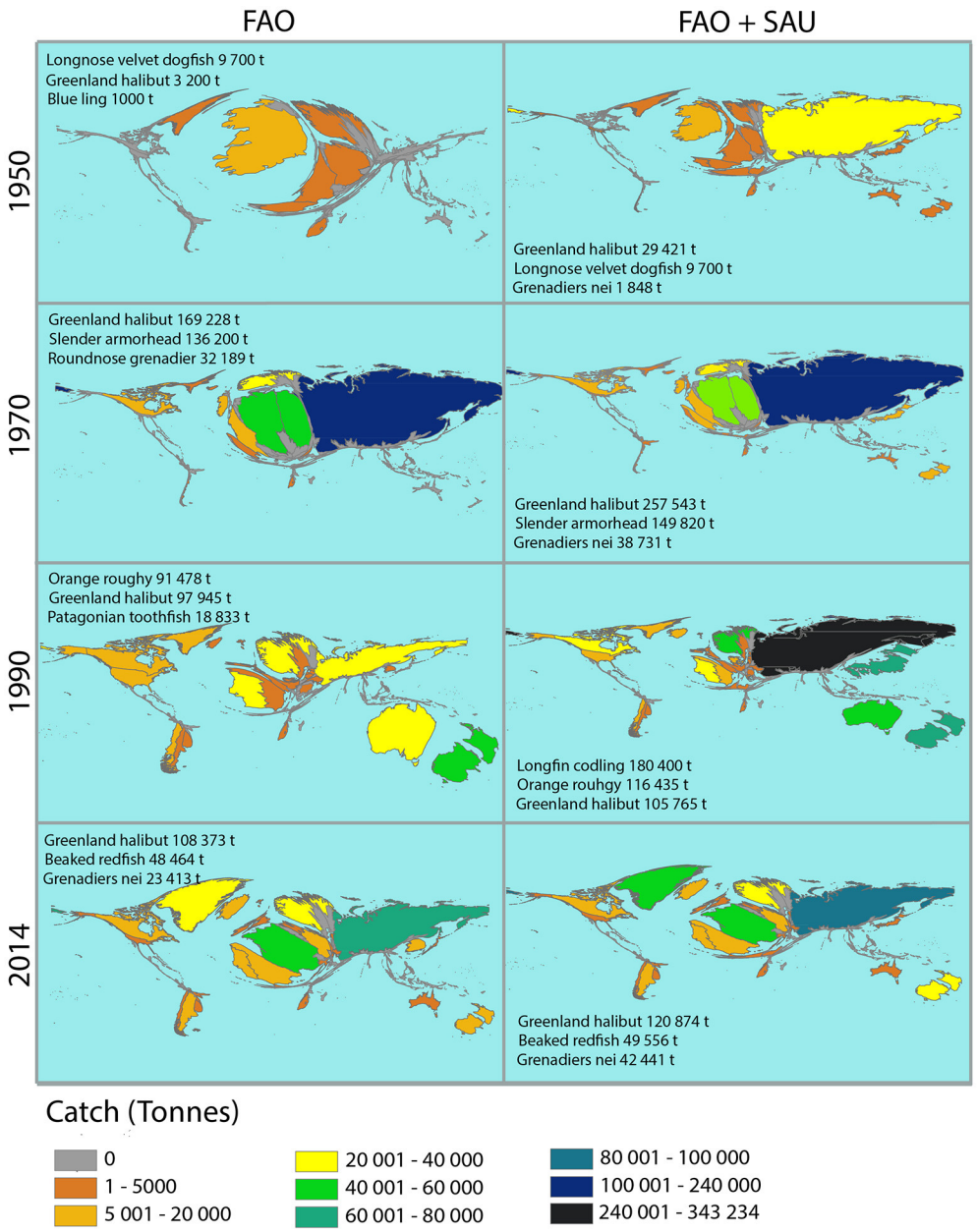


FIGURE 3 | Selected cartograms illustrating the changing patterns of deep-sea (primarily >400 m) trawled fish catch by country for the years 1950, 1970, 1990, and 2014. Maps are based on FAO and FAO + SAU (*Sea Around Us*) catch data. The size of the country reflects the reported catch (tons) of deep-sea bottom-trawled species. Each country is also color-coded according to range of catch data. The species within the figure represent the top three species with catches in tons. A full set of cartograms for the period 1950–2015 is available as an animation in the Supplementary Material.

grenadier fisheries. Indeed, in 1966, the reconstructed data series reveals the Soviets discarding up to 47,000 t of Greenland halibut. Canada entered the directed fishery for this species in the mid-1960s (summarized in Bowering and Brodie, 1995), reporting landings of ~80,000 t over the decade. Portugal entered in the 1980s with minor catches and Spain in the 1990s reported landings of ~170,000 t. Spain's dominance in this fishery likely led to the Canada – Spain "turbot war" of 1993 (Haedrich et al., 2001). On the Newfoundland slope, most of the fish caught between 1991 and 1996 were below the size at 50% maturity (Haedrich et al., 2001). This species had, by far, the highest reported landings through the history of the FAO data set (~4.89 million tons), and also the highest total catches when unreported landings and discards are included (~7.64 million tons) (**Table 3**). The greater part of the catch was from the North Atlantic, first from the northeast, and later from the northwest, with smaller numbers from the North Pacific. Only about 60% of the total estimated catches were reported to FAO. In fact, the

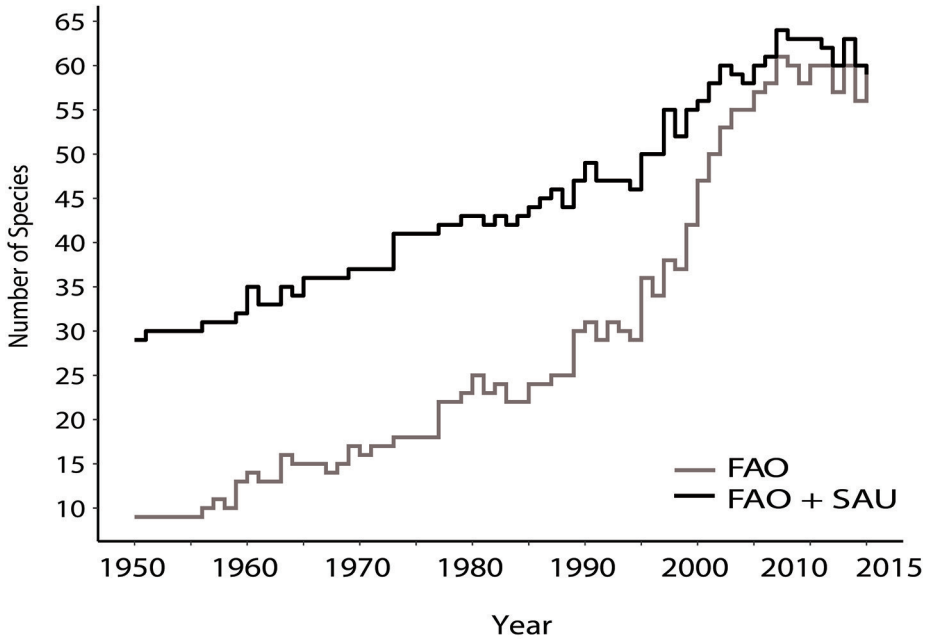


FIGURE 4 | Number of deep-sea species primarily caught at depths >400 m by bottom trawling each year in the FAO and *Sea Around Us* data series for the period 1950–2015.

catches were likely under-reported by more than 75% for 39 of the 66 years considered here (**Figure 5**, Table S1). A major difference between the two data sets arises from the reconstructed discard estimates, which amount to a total of 2.5 million tons with ∼1.8 million tons assigned to the Soviet/Russian fleet operating within the Barents Sea (Table S1). In the 1990s discards amounted to 350,000 t, of which 200,000 t were by the Russian fleet in the Barents Sea and 80,000 t in the Norwegian Sea. In comparison, the reported landings for Russia only amount to 47,000 t over the whole decade. The large quantities of discards within this period were likely caused by lowered quotas as the Greenland halibut stocks suffered a decline and became regulated in 1992 (Honneland and Nilssen, 2001; Nedreaas and Smirnov, 2004). The discard estimates made by Jovanović et al. (2008) use discard data between 1996 and 2006 and assume a similar discard policy for the species throughout the fishery's history potentially leading to an overestimation of the discard values.

Longfin Codling

Longfin codling (*Laemonema longipes* Schmidt, 1938), also known as Forked hake in Japan, is a deep-water member of the Gadiformes, in the Family Moridae, primarily inhabiting the NW Pacific continental slope from Kamchatka to Hokkaido and into the Okhotsk Sea. Its depth distribution is 80–1,830 m, but the most abundant catches are at 800 m depth (Yokota and Kawasaki, 1990). The fishery is promulgated both by Japanese and Russian vessels, the latter sometimes in contract to Japan. Savin (2013) noted that the biomass of the species in the area where the fishery occurs can reach values as high as 360,000 t. The lowest stock values were recorded in 1984 (116,000 t) and 2006 (66,800 t). Savin (2013) noted that the fishery by Russian vessels started in 1974, followed by Japan in 1976. There seems to be no evidence of a fishery for Longfin codling before that. Even so, by the third year, the landings (unreported to FAO) in Russia were approximately 100,000 t. After the establishment of the EEZ by Japan, some Russian vessels were allowed to fish under contract in Japanese waters. For some reason, except for 3 years (1978–1980), neither country reports catches of Longfin codling under its own name and there are hardly any records of this fishery existing in the FAO database. Instead, the landings are most likely included under the category "marine fishes *nei*" (Alexei Orlov, personal communication). Thus, estimating the actual catches of this species has been difficult and rely heavily on reconstructed records from the *Sea Around Us* (**Figure 5**) and data recorded in Russian fishery reports (Savin, 2013). In the end, this species is estimated to have produced about 3.5 million tons of fish, making it the second most productive deep-sea fish caught by trawls and causing one of the biggest discrepancies between the reported landings and total catch estimates.

Orange Roughy

Orange roughy (*Hoplostethus atlanticus* Collett, 1889) has a very wide distribution, from NW and NE Atlantic, throughout much of the eastern Atlantic, south Central Indian, and SW and SE Pacific. It is one of the oldest commercially exploited fish species, reaching more than 100 years of age (Andrews et al., 2009). Orange roughy matures at the age of 20–35 (Horn et al., 1998) and spawning occurs in dense aggregations around topographic features such as seamounts, and rises as well as along the continental slope (Branch, 2001). These aggregations are fished and provide high catch rates, leading to rapid overexploitation. The long, slow growth and late maturity of this species, coupled

TABLE 3 | A list of deep-sea bottom trawled species, including target and bycatch species caught primarily below 400 m depth, and their reported (from FAO database) and estimated total [FAO data with unreported estimates from the *Sea Around Us* (SAU)] in tons for the period of 1950–2015.

Species	FAO	FAO + SAU
Greenland halibut	4,887,801	7,639,088
Longfin codling	8,663	3,509,542
Grenadiers	571,055	2,993,797
Orange roughy	1,317,328	1,815,861
Beaked redfish	1,399,585	1,679,056
Roundnose grenadier	999,020	1,354,031
Patagonian toothfish	901,591	1,034,946
Kamchatka flounder	547,459	716,491
Slender armorhead	610,331	671,361
Blue ling	538,905	549,388
Black dogfish	444,243	492,497
Blackbelly rosefish	363,261	408,816
Longnose velvet dogfish	328,619	328,667
Black scabbardfish	223,544	224,984
Antarctic toothfish	158,671	163,280
Velvet belly	128,951	157,963
Chimaeras, etc. *nei*	1,876	132,195
Smooth oreo dory	123,130	123,130
Common mora	90,164	97,586
Northern wolffish	85,850	92,161
Ridge scaled rattail	79,750	84,724
Splendid alfonsino	2,052	83,866
Black cardinal fish	59,970	78,113
Portuguese dogfish	42,655	75,975
Thorntooth grenadier	71,523	71,523
Roughhead grenadier	63,673	64,136
Deep-sea smelt	1,339	56,271
Greater argentine	49,677	54,905
Black oreo	54,315	54,315
Blackmouth catshark	7,552	53,750
Slimeheads *nei*	22,466	47,131
Ghost shark	NA	44,363
Little sleeper shark	42,500	42,500
Leafscale gulper shark	33,686	34,367
Baird's slickhead	29,160	29,773
Kitefin shark	15,534	20,992
Gulper shark	16,275	20,348
Rabbit fish	4,244	18,599
Mediterranean slimehead	158	17,672
Greenland shark	3,289	13,513
Bluntnose sixgill shark	452	10,867
King dory	7,591	9,362
Longnose spurdog	7,612	8,632
Norway redfish	6,113	7,226
Knifetooth dogfish	6,298	6,372
Pelagic armourhead	5,812	5,941
Silvery John dory	5,243	5,243
Spiny scorpionfish	5,143	5,143
Common Atlantic grenadier	38	4,937
Mouse catshark	4,700	4,700
Birdbeak dogfish	3,271	4,536
Blue antimora	2,163	4,498
Lowfin gulper shark	2,995	3,526
Spiky oreo	2,732	2,929
Blunt scalyhead	1,690	1,690
Roughsnout grenadier	1,487	1,517
Whitson's grenadier	1,445	1,445
Round ray	463	463
Shortspine African angler	454	454
Giant boarfish	356	356
Slickheads *nei*	334	334
Arctic skate	223	223
Grenadier cod	143	168
Splitnose rockfish	149	149
Norwegian skate	57	143
Sailfin roughshark	139	139
Bigeye grenadier	73	73
Bigspined boarfish	45	52
Pacific sleeper shark	36	36
Great lanternshark	20	20
Straightnose rabbitfish	3	3
Dogtooth grenadier	1	1

with its low reproductive output, makes recovery slow. The first reported landings of Orange roughy occurred in 1977, by foreign trawlers off New Zealand, but the start of the fishery occurred in New Zealand in 1979 (Branch, 2001). In 1980, in the Chatham Rise area, which is the largest and oldest Orange roughy fishery, virgin catches were about 9–10 t/tow (Clark, 2001), but by the late 1990s catches had decreased to 2–3 t/tow. Also, in the 1990s the fishery moved out of New Zealand waters to the Louisville Ridge seamount chain where catches varied from 1.4 to 2.7 t/tow. Other Orange roughy fisheries include those off Australia, NE Atlantic in the mid-1980s and 1990s, off Namibia in the 1990s, and off Chile and in the Southern Indian Ocean in the late 1990s (**Figures 4**, **5**). Virtually all these fisheries are operating on stocks that are less than 30% of virgin biomass, and several have lasted only a few years (Branch, 2001; Foley et al., 2011). The reconstructed data suggests that for this fishery, landings were under-reported by as much as 50% between the years of 1981–89. Overall, half a million tons of unreported Orange roughy have been caught in the period of 1950–2015, mainly by Japan (∼225,000 t), New Zealand (∼162,000 t) and South-Korea (∼35,000 t). The FAO admits its records underestimate Orange roughy catches with trade analysis confirming at least a 30% underestimation in the year 2001 (Lack et al., 2003). Simmons et al. (2016) note that discrepancies in tray weights, conversion factors, and false reporting in order to avoid income tax liability have all contributed to under reporting of Orange roughy from New Zealand waters. Since

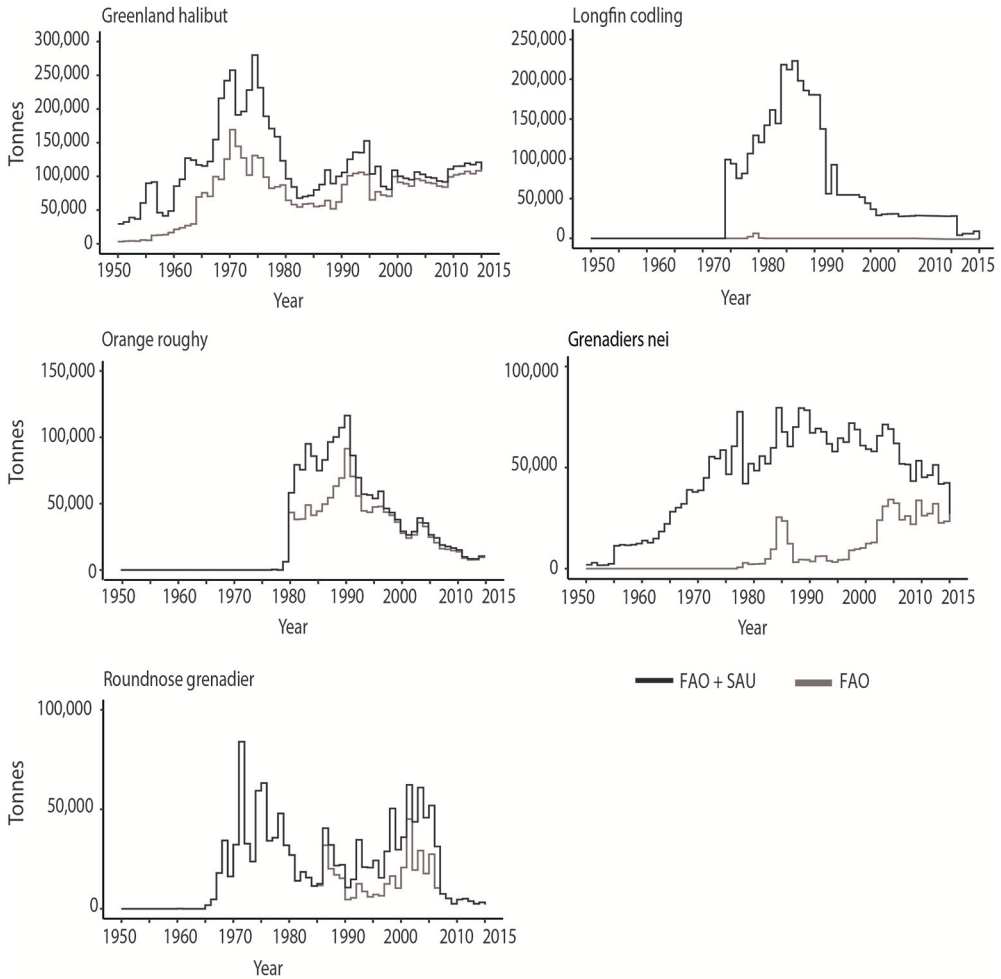


FIGURE 5 | Catch data for some of the most abundantly deep-sea (>400 m) bottom-trawled species between 1950–2015, which show strong discrepancies between reported (FAO) and estimated total (FAO + *Sea Around Us*) values.

1986, the establishment of the Quota Management System, also gave incentive to under report due to quota restrictions and in order to avoid penalties. In the Chatham Rise fishery, after years of reduced TACs (total allowable catches), the stocks have shown signs of recovery (Doonan et al., 2015) and currently, the fishery is certified as sustainable by the Marine Stewardship Council (MSC) with TACs set at ∼7,000 t/year. However, to date, the Orange roughy fishery remains contentious, with scientists and non-governmental organizations (NGOs) raising concerns about its sustainability (Watling, personal observation).

Grenadiers

Grenadiers (Macrouridae) *nei* (not elsewhere included) is a category consisting of grenadiers, whiptails and rattails, which are not recorded separately, or species that were not recorded separately until relatively recently. There are about 360 species in this group extending over a wide depth range of 110–7,000 m, making them widespread in the deep sea. Most species are caught as bycatch and are small or not edible so are either discarded or processed as fishmeal. A few of the larger species, with better quality meat, are targeted and their landings are recorded separately by FAO. FAO did not show any landings for Grenadiers *nei*, until 1977, with total landings now reaching 571,000 t, of which ∼400,000 t has been reported by Russia from the NW Pacific. These commercially targeted fisheries, include species, such as the Giant, Popeye and Pacific grenadier (Tuponogov et al., 2008), whose landings are not yet recorded separately by the FAO. The *Sea Around Us* estimates the amount of caught but discarded grenadiers to be close to 2.5 million tons since 1950 (**Figure 5**). These numbers include some species that were discarded in the early years, but became a targeted fishery later, such as Roundnose grenadier. Two million tons of discards are assigned within the New Zealand EEZ (economic exclusive zone) mostly from the fishing entities of New Zealand (1 million tons) and Japan (0.5 million tons). Rattails are a common bycatch product of the Orange roughy, oreo, hoki, hake, ling and arrow squid fisheries and of the scampi trawling (Anderson,

2012; Ballara and O'Driscoll, 2015; Anderson et al., 2017). The bycatch estimates (% of total catch) made by observers on New Zealand vessels are 0.7% for Orange roughy, 6% for hoki and 30% for scampi fisheries of the total catch, with the majority discarded (Anderson, 2012; Anderson et al., 2017; Ballara and O'Driscoll, 2015). The contrasting discard rates between the official records and the reconstructions are potentially caused by the "observer" effect, in which fishing behavior is modified for the better due to the presence of observers (Simmons et al., 2016). The reconstructions also suggest that the discarding of Grenadiers remains relatively high, when taking into account the fact that the catches for target species have notably decreased (**Figure 5**).

Roundnose Grenadier

Roundnose grenadier (*Coryphenoides rupestris* Gunnerus, 1765), is a benthic to benthopelagic species found along the continental slopes and the Mid-Atlantic Ridge of the North Atlantic Ocean typically at 400–1,200 m depth. Russian trawlers first caught Roundnose grenadiers as bycatch in their cod and redfish fisheries on the Canadian eastern slope (Atkinson, 1995). It was soon suggested, due to the high numbers and high quality of the fish, that a targeted fishery could be developed in the North Atlantic, with the Danish, developing a fishery in the 1980s in the Skagerrak. The highest reported landings peaked in 1971 at 84,000 t, but declined steadily after 1975 (**Figure 5**). Haedrich et al. (2001) noted that important biological information for this species was not known until after 1975, by which time the fishery had begun to decline. Both the FAO reported landings and *Sea Around Us* estimated total catches show fluctuations in the catch, and from 1986 to 2006 the reported catch was from 78 to 26% of the estimated unreported catch (**Figure 5**). The discrepancy between the data sets arise from reconstructed discards from Denmark between early 1990s to 2006, which are estimated to 10,000–20,000 t per year amounting overall to 350,000 t. This species is a common bycatch and discard in the demersal mixed trawl fisheries in Skagerrak, Kattegat and North Sea (Gibson et al., 2010) with discard rates of 28% in weight in the NE Atlantic. As the juveniles and adults co-exist within the same area, trawls catch small, non-marketable fish, which are discarded at sea (Pawlowski and Lorance, 2009). The decline in stocks led to an agreement between the EU and Norway, setting the TAC to zero within the Norwegian waters since 2006 (ICES, 2016). Devine et al. (2006) noted that the steep drop in abundance would qualify *C. rupestris* as endangered under IUCN criteria.

Beaked Redfish

Beaked redfish (*Sebastes mentella* Travin, 1951) is an oceanic migratory fish inhabiting the waters of the northern North Atlantic at 300–1,400 m depth. It is worth noting that this fishery has been mixed, especially in early years, with the Golden redfish (*Sebastes marinus*) fishery. This was the second-most landed fish over the 66 years of the FAO database, even though the fishery started in the 1960s, and had landings that were quite modest through the 1960s to 1990s (**Figure 6**). Starting in 2000, however, landings increase, reaching 1.4 million t reported to FAO. Iceland and Russia caught the majority of this fish since 2000. The discrepancies between the reported and estimated total catches arise from 275,000 t of reconstructed discards starting from the late 1980s (**Figure 6**). The discards are mainly assigned to Iceland (197,000 t) and Norway (76,500 t), despite both countries having discard bans since 1989 and 1987, respectively (Condie et al., 2014). Nakken (1998) reported that high amounts of undersized redfish were discarded prior to the introduction of sorting grids in shrimp trawlers in 1995. Redfish is also a common bycatch of the cod and haddock fisheries in the Barents Sea (Little et al., 2015). The regulations for this fishery include over quota catches being withdrawn from the following year's quota and size limitations, in addition to bycatch limits with undersized fish landed being counted at 50% of the fish's weight against the annual quota (Moffat et al., 2009). Furthermore, temporary and permanent closures of fishing areas can occur when juvenile fish are caught in excess (Little et al., 2015) thus indirectly providing some incentive to discard undersized redfish.

Slender Armorhead

Slender armorhead (*Pentaceros wheeleri* Hardy, 1983), also sometimes known as Pelagic armorhead or Longfin armorhead, is a benthopelagic species of the North Pacific, typically at depths of 400–600 m. It forms spawning aggregations on the southern Emperor and northern Hawaiian Ridge seamounts (Boehlert and Sasaki, 1988). The fishery for Slender armorhead followed a classic "boom and bust" pattern, starting with its discovery by Soviet trawlers in 1967 (Humphreys et al., 1984). According to the FAO landings data, the Soviet fleet fished the area until the stock was exhausted in 1977 (**Figures 2**, **6**). In the second year of the fishery, 145,000 t of fish were reported as landed, with a subsequent peak of 150,000 t in 1973. The catch steadily declined to 200 t in 1977. After years of no landings, fewer than 5 tons per year have been landed from these seamounts over the last decade (Table S1). Humphreys et al. (1984) also cite a series of Japanese works that show Japan having caught between 25,000 and 35,000 t of armorhead from the southern Emperor seamounts from 1970 to 1976. None of these catches are recorded in the FAO database (version 2016) or in the catch estimates reconstructed by the *Sea Around Us*.

Patagonian Toothfish

Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898), also known as Chilean seabass, is a benthopelagic species. It is widely distributed around the Southern Ocean, mostly outside the Antarctic Convergence, while its congener, Antarctic toothfish (*D. mawsoni* Norman, 1937) lives mostly on the Antarctic shelf and slope within the Convergence. The Patagonian toothfish is typically found between 50 and 1,500 m water depth. The toothfish fishery is both a longline and bottom trawl fishery, with the latter method becoming more common outside Antarctic waters where trawl usage is allowed. The bulk of the catch is from the FAO area in the SW Atlantic, that is, the Argentine shelf, Falkland Islands, and South Georgia, with Pacific, Southeast (Chile slope) and Indian Ocean, Antarctic (Kerguelen Plateau) not far behind. Chile, France, and Argentina, in that order,

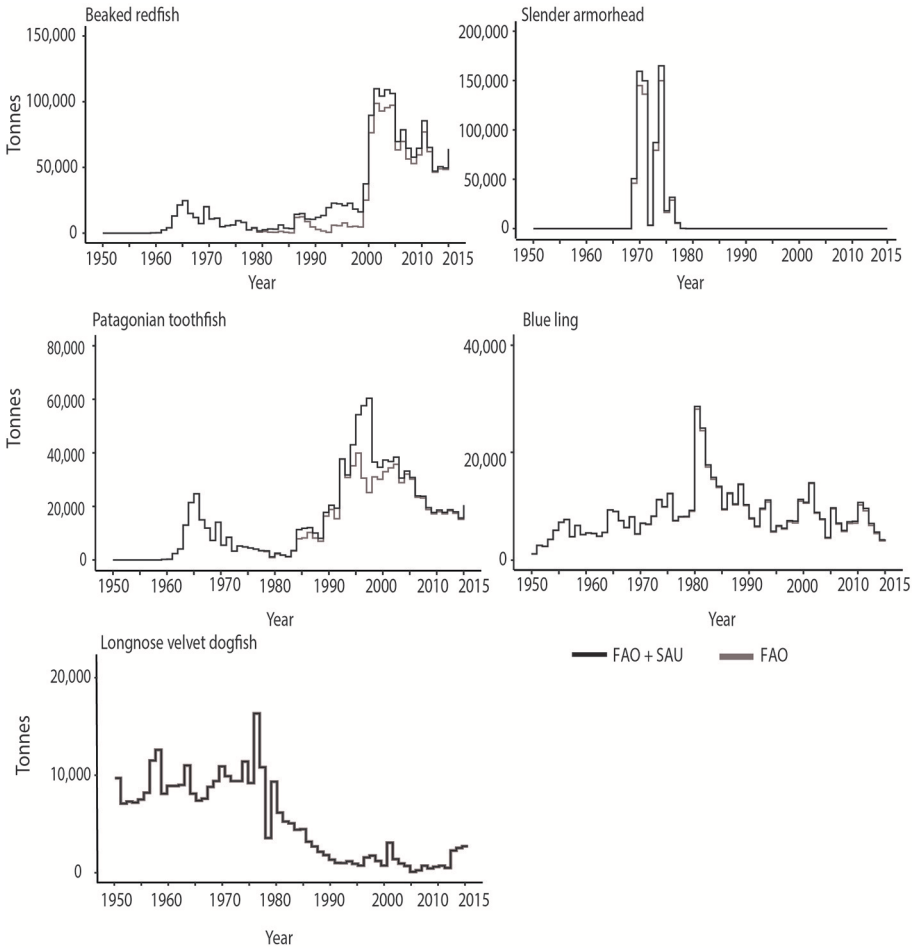


FIGURE 6 | Catch data for some of the most captured deep-sea (>400 m) bottom-trawled species between 1950–2015, which have nearly similar reported (FAO) and estimated total (FAO + *Sea Around Us*) values.

landed most of the catch. There was a ∼10% difference in the 1990s between the reported and unreported estimate of total catches (**Figure 6**), although Collins et al. (2010) suggest that illegal, unreported, and unregulated (IUU) catches might be under-estimated by as much as 50% in some areas. The unreported landings amount to a total of 125,609 t, half of which were from the Prince Edward Islands in South-Africa. The reconstructions suggest that there were half a dozen nations under-reporting their landings. Some of these, such as Panama, are known for providing flags of convenience for other nations (Bialek, 2003). Since 1999, in response to high IUU fishing, the fishery has been managed using a Catch Documentation Scheme (CDS) by the CCAMLR, in which the fish are tracked from the point of landing and throughout the trade cycle (Bialek, 2003).

Blue Ling

Blue ling (*Molva dypterygia* Pennant, 1784) is a benthic, non-migratory species distributed within the NE and NW Atlantic and the western Mediterranean, typically between depths of 350–500 m. It aggregates for mating along the continental slope and on offshore banks and seamounts, which makes it vulnerable for serial depletion (ICES, 2017). Blue ling fisheries have been recorded in FAO landings data since 1950 with landings from Norway and Germany in the NE Atlantic. Faroe Islands entered the fishery in the early 1970s. Peak catches occurred in the 1980s (**Figure 6**), after which catches were strongly reduced, partly due to restricting catches to periods when mating aggregations were not occurring, and to management measures reducing total allowable catches in the NE Atlantic. In the NE Atlantic, two of the depleted spawning areas have remained closed since 1993 and since 2003 ICES has advised for no direct fishery and a reduction in bycatch (ICES, 2017).

Longnose Velvet Dogfish

Longnose velvet dogfish (*Centroscymnus crepidater* Barbosa du Bocage and de Brito Capello, 1864), also known under many other common names, such as Black shark and Deepwater dogfish in Australia, Pailona à long nez in France, and Sapata

preta in Azores. This shark is benthic and widespread globally, being found on bathyal ridges and continental slopes at depths between 230 and 1,500 m, in all oceans, except the western Atlantic, central Pacific and polar waters. In the FAO database, Ireland was the only country reporting landings of this species until 2002 (**Figure 2**). Subsequently, France and United Kingdom entered the fishery, with France landing relatively large amounts, as high as 2,460 t in 2010. Estimated unreported landings exceed those reported to FAO by only minor amounts (**Figure 6**) due to some discarding of this species.

DISCUSSION

Comparing Reported and Estimated Total Landings

This study complemented the FAO records with reconstructed unreported landings and discards from the *Sea Around Us* to estimate more accurate catch levels for deep-sea fisheries. The analysis reveals that overall deep-sea fisheries are likely to have captured 42% more fish than what was reported to FAO. Specifically, the period between years 1975 and 2000 was characterized by the highest catches, much of which were not reported to FAO (**Figure 1**). Catch data from FAO has often come under attack for being inaccurate at best, and unreliable at worst (Lobo and Jacques, 2017; Pauly and Zeller, 2017; Ye et al., 2017). It should be kept in mind, however, that FAO reports in its database only what is reported to it. For some areas of the world, at various time periods, the "official" landings reported to FAO closely parallel the best in-country records. An example of this is the NE Atlantic since the establishment of the European Union's Common Fishery Policy in 1983. Fish catches are managed through a variety of steps that begin with scientists from member countries contributing information to the ICES advisory working groups, after which total allowable catches (TACs) are designated for each species. The data ICES receives is based on each country's monitoring procedures and can reflect haul data and ship logs monitored by observers. Many countries use shipboard observers to verify catch numbers, but observer coverage is highly variable and spotty between nation, representing sometimes only 5% of the vessel trips (Auster et al., 1996; Lorance et al., 2010). The presence of the observers is known to lead to modified behavior by the fishermen, leading to better, more careful fishing and reporting (Simmons et al., 2016).

When observers are not present it is possible that catch data only reflects what was kept and landed at the dock, with fish that were discarded not being included. The unwanted fish were either not of high enough quality, not large enough, or not of interest or marketability or could not be landed due to restrictions in quotas (Zeller et al., 2017). It is improbable that deep-sea fish, once caught along with tens of thousands of other fish in a trawl net from the cold ocean, then deposited into the hold where the catch is sorted, would survive once returned to the ocean. Therefore, those dead fish should have been part of the catch levels reported, but they were not, although there are efforts now in some areas to record the discarded fish numbers (Pawlowski and Lorance, 2009). Indeed, our results indicate that one of the major discrepancies with FAO records and total estimates, arises from the high amount of discards involved in the deep-sea fisheries, totaling 6 million tons over the study period. The high amounts of discards is not unexpected, as bottom trawling is known to generate the highest discard rates in comparison to other fishing gear (Zeller et al., 2017). It is important to record this discard data, because ignoring it and using only landings data to model population dynamics, results in poor stock assessments and biased fishing patterns leading to mismanagement of deep-sea fisheries (Pawlowski and Lorance, 2009).

Another notable discrepancy between the data sets is the number of different species being caught. This difference might be expected as the FAO did not require the reporting of many of these species in the earlier time periods, but the increasing trend is also apparent in the FAO + SAU data set. This increase in species being caught, especially since the mid-1990s (**Figure 4**) suggests that fishing vessels no longer concentrate their efforts on only a few economically viable species. Instead, it is likely that as the most valuable fish stocks are depleted and are more heavily regulated, a broader range of species become targeted and markets developed for them.

It is evident that catch data from both FAO and the *Sea Around Us*, contains uncertainties. The *Sea Around Us* has engaged multiple teams of people in most fishing countries to try to estimate the unreported catches (Pauly and Zeller, 2016). The method of estimating can vary from country to country, so it is possible that the accuracy of the estimates will also vary according to country. In some areas, for example, the Russian Far East and Japan, estimates for the landings of some fish species were corrected as, we found, during the study, additional literature that indicated more precisely when certain fisheries began. One of the most extreme cases of unreported fish landings concerns the Longfin codling fishery, which as previously described had only minor landings in the FAO database, despite producing 100,000 t in the third year of the fisheries existence (Savin, 2013). Another case illustrating the uncertainty with both the FAO and *Sea Around Us* data sets, is the absence of landings for Japan's Slender armorhead fishery within the Hawaiian-Emperor seamount chain, despite records stating catches of 30,000 t (Clark et al., 2007). Despite these limitations, the FAO landings data complemented with the *Sea Around Us* reconstructions is the only data compilation available for estimating reported and unreported landings and discards for deep-sea fisheries.

Ecological Consequences of Deep-Sea Trawling

There are two long term consequences of the deep-sea bottom trawl fishery. Bottom trawling enables rapid exploitation of fish species and indiscriminately catches whatever fish are in the path of the trawl. It is also known to physically alter the benthic habitat (Haedrich et al., 2001) by removing or crushing habitat-forming species (Koslow et al., 2001; Williams et al., 2010; Clark et al., 2016). Merrett and Haedrich (1997) make two observations about the management of deep-sea fisheries that are still important

today. First, they make the analogy of the distant-water trawler as a "roving predator" (p. 227) seeking prey throughout the world's ocean. Unfortunately, the predator has evolved far faster than the prey. The first distant-water trawler, the *Fairtry*, was built in the 1950s in England. It was a fully-developed floating fish factory, capable of both catching and processing fish. It became the model for vessels fishing far from ports where normally fish stored on ice would be landed. The capability of the predator to consume large numbers of fish was born, and then kept from extinction by the provision of financial subsidies, at least in some areas, to help allay the enormous costs of operating such large vessels at sea for long periods of time. Of the world's 13 biggest high seas bottom trawling nations, there is only one (New Zealand), which does not provide subsidies, suggesting that many deep-sea trawl fisheries would have ended much sooner in the absence of subsidies (Sumaila et al., 2010). In view of these developments, Merrett and Haedrich (1997) conclude that "the deep-sea fishery should not be considered a fishery at all. There is a much stronger analogy to a mining operation wherein an ore body is exploited to depletion and then new sources (mines, virgin stocks) are sought" (p. 228).

For many of the target species, recruitment and restoration of populations is a possibility if the level of exploitation is strongly reduced or eliminated, or the exploitation strategy is altered for a number of years, as was the case for Blue ling (Large et al., 2010). On the other hand, several other species have been fished to very low numbers, often in a decade or two. Patterns of depletion are apparent in the Slender armorhead fishery, where the population was reduced to a fraction of its virgin biomass in 8 years. Similarly Longfin codling estimated landings were as high as 200,000 t in 1986, and 55,000 t in 1994, and Roundnose grenadier estimated catches were greater than 60,000 t in 2001 but a rapid decline in stocks lead to a moratorium in 2006. We show that considerable fish biomass has been removed from the deep-sea, particularly in certain areas and while we do not understand the consequences of that removal yet, it is likely that the deep-sea ecosystem is being changed. For example, many of these fish species, such as the Greenland halibut, are top-predators within their habitats and removing them could cause trophic cascading as previously seen in cod fisheries (Frank et al., 2005). Others are mid-level predators and their removal may have more subtle consequences related to the removal of biomass that would otherwise recycle in the benthic ecosystem as these fish grow, reproduce and die.

The impact of trawling goes beyond the capture of fish populations since the benthic fauna gets removed from the seabed, thus comprising a large fraction of the bycatch (Probert et al., 1997; Anderson and Clark, 2003). The routine use of trawls in these fisheries results in considerable environmental modification, loss of habitat structure, and reduction of biodiversity, especially on seamounts (Clark et al., 2016). In addition, where these fisheries occur along the continental slope, re-suspended sediments can flow down-slope into deeper waters, covering organisms that would otherwise have been out of the way of the trawl (Pusceddu et al., 2014). In order to obtain 42% higher catches of deep-sea fish, it is likely that trawling has covered larger areas (even when considering that trawl fisheries often cover the same ground repetitively), resulting in unknown amounts of additional bycatch of benthic species.

Many of the largest bottom-trawled fisheries, such as Orange roughy and Slender armourhead fisheries occur also on topographic highs, such as seamounts and ridges. Their irregular topography offers a mosaic of habitats while influencing local current velocities and often delivering food particles at a slightly higher rate to fauna. These habitats are often heavily populated by suspensions feeders, such as habitat forming deep-sea corals and sponges that have been shown to be hundreds of years old, along with a variety of other species (Koslow et al., 2000; Duineveld et al., 2004; O'Hara et al., 2008; Watling et al., 2011). The deep-sea fish also take advantage of seamounts, where they often aggregate to feed, spawn and live (Clark et al., 2007). These aggregations make ideal candidates for deep-sea fisheries, resulting in deep-sea trawl fisheries targeting the summits and occasionally the sides of seamounts at depths shallower than 2,000 m (Clark et al., 2007).

All seamounts where bottom trawling occurred and that have been investigated with remotely operated vehicles (ROVs) or towed cameras show large cleared areas where communities of suspension feeders once lived (Koslow et al., 2001; Waller et al., 2007; Williams et al., 2010; Clark et al., 2016). Despite knowing that these species live for centuries, we do not know what their rate of reproduction and pattern of recruitment is, so we have no certain way of determining how long it will take for the community to recover.

Non-governmental organizations (NGOs) such as the Deep-Sea Conservation Coalition (www.savethehighseas.org/) and Bloom Association (www.bloomassociation.org/) have also argued against deep-sea bottom trawl fisheries because of the damage such fishing does to benthic communities. In Europe, these and other NGOs initiated a campaign to ban bottom trawling in deep water, culminating in legislation in 2016. The legislation is complex, but essentially bans bottom trawling in waters deeper than 800 m (European Parliament and the Council of the European Union, 2016) thus providing some protection to both bottom habitat and non-targeted deep-sea fish species.

Within this context, it is worth considering the economic importance of deep-sea fisheries. Our analysis reveals that deep-sea fisheries focusing on species caught primarily below 400 m contribute a mere ∼0.5% to the total global capture fisheries (**Table 2**). While locally, deep-sea fisheries can be of economic importance, as for example in New Zealand, where in 2009 the Orange roughy fishery was estimated to be worth $282 million, globally their economic importance is trivial.

The Impact of Management on Deep-Sea Trawling Fisheries

Much of the reductions in catches reported, especially after 2000, are due to management measures that have been taken to reduce the possibility of species being over-fished. In some cases, such as with Blue ling, the problem of fishing on spawning aggregations was recognized and protection areas were introduced (Large et al., 2010). However, some of the NE Atlantic spawning

aggregations have yet to recover and remain closed to date (ICES, 2017). In other cases, landings have been restricted as catch numbers declined e.g., for the NE Atlantic, see ICES-WGDEEP (2017) (see also Villasante et al., 2012). Sometimes these management measures might have come too late. In the NE Atlantic, for example, Roundnose grenadier landings were always much below the TAC set for them (ICES-WGDEEP, 2017, p. 374). Similarly, the allowable catch numbers for Orange roughy in the NE Atlantic have been set at zero for several years and the MSY at roughly 30% of virgin biomass in the SW Pacific (Francis and Clark, 2005). For most deep-sea fisheries, the lack of biological information combined with assumptions from shallow fisheries prevents them from being suitably regulated from the start. Combining this, with the much higher catches documented here and hence not officially accounted for, has led to regulations that might not have been strict enough to allow recovery, particularly in mixed-trawl fisheries. For fisheries, such as the Greenland halibut, concerns of depletion led to a reduction of quota which in turn caused high discards from 1992 onwards. Some nations have enforced discard bans, but Iceland among others has a record of discarding Beaked redfish, potentially because vessels are not able to acquire quota within the transferable quota system or the fish are undersized. The diversification of target species through time reveals that the indiscriminate nature of deep-sea trawling creates a market for new target species as the stocks of the previous species decline and/or become more tightly regulated.

Finally, there is the problem, of managing trawling in the high seas, that is, in areas of the ocean beyond any national jurisdiction. In 2006 an estimated 285 vessels were involved in high seas trawling, with the fisheries often occurring on isolated topographic structures, such as seamounts and ridges (Bensch et al., 2009b). Management of fisheries and habitats in these waters has been proposed through United Nations General Assembly (UNGA) Resolutions. These call for the formation of Regional Fisheries Management Organizations (RFMOs) who are responsible for setting allowable catches of species under their jurisdiction and for limiting damage to Vulnerable Marine Ecosystems (VME). The latter are defined on the basis of "indicator species" agreed to by all nations and listed in various FAO documents such as Thompson et al. (2016). As seamounts have extensive coverage of VME indicator species and are often trawled leading to high degree of environmental damage, Watling and Auster (2017) have proposed that seamounts as a whole should be considered as Vulnerable Marine Ecosystems. This would warrant seamounts a stronger set of protections as laid out by UNGA Resolutions (Bensch et al., 2009a; Rogers and Gianni, 2010) and potentially also limit or eliminate catches of seamount-associated fish species, thus limiting the associated environmental damage.

CONCLUSION

This study describes historical patterns in catches of deep-sea trawling fisheries since 1950–2015 by comparing and complementing the FAO landings data with reconstructed unreported landings and discards. The catches were shown to be underestimated by 42% with both unreported landings and discarding contributing equally to the discrepancy between the data sets. The major fisheries for this period include the Greenland halibut fishery in the North Atlantic, the Longfin codling in the NW Pacific and the Orange roughy in the SW Pacific. The reconstructed catches also suggest high discarding of Greenland halibut, Beaked redfish, Roundnose grenadier and the grouping Grenadiers *nei*. The new catch estimates suggest that much more biomass, encompassing both fish and habitat-forming species, has been removed from the deep-sea, altering this ecosystem in ways that have yet to be understood.

AUTHOR CONTRIBUTIONS

LW and CN conceived the study. LV, LW, and MD analyzed and interpreted the data. LV prepared the figures. LV and LW prepared the animation. LV and LW drafted the text and all authors revised and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00098/full#supplementary-material

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Bacterial Community Response in Deep Faroe-Shetland Channel Sediments Following Hydrocarbon Entrainment With and Without Dispersant Addition

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Deep sea oil exploration is increasing and presents environmental challenges for deep ocean ecosystems. Marine oil spills often result in contamination of sediments with oil; following the Deepwater Horizon (DwH) disaster up to 31% of the released oil entrained in the water column was deposited as oily residues on the seabed. Although the aftermath of DwH was studied intensely, lessons learned may not be directly transferable to other deep-sea hydrocarbon exploration areas, such as the Faroe-Shetland Channel (FSC) which comprises cold temperatures and a unique hydrodynamic regime. Here, transport of hydrocarbons into deep FSC sediments, subsequent responses in benthic microbial populations and effects of dispersant application on hydrocarbon fate and microbial communities were investigated. Sediments from 1,000 m in the FSC were incubated at 0°C for 71 days after addition of a 20-hydrocarbon component oil-sediment aggregate. Dispersant was added periodically from day 4. An additional set of cores using sterilized and homogenized sediment was analyzed to evaluate the effects of sediment matrix modification on hydrocarbon entrainment. Sediment layers were independently analyzed for hydrocarbon content by gas chromatography with flame ionization detection and modeled with linear mixed effects models. Oil was entrained over 4 cm deep into FSC sediments after 42 days and dispersant effectiveness on hydrocarbon removal from sediment to the water column decreased with time. Sterilizing and homogenizing sediment resulted in hydrocarbon transport over 4 cm into sediments after 7 days. Significant shifts in bacterial populations were observed (DGGE profiling) in response to hydrocarbon exposure after 42 days and below 2 cm deep. Dispersant application resulted in an accelerated and modified shift in bacterial communities. Bacterial 16S rRNA gene sequencing of oiled sediments revealed dominance of *Colwellia* and of *Fusibacter* when dispersant was applied over oiled sediments. The increased relative abundance

of anaerobic hydrocarbon degraders through time suggests creation of anoxic niches due to smothering. The study showed that hydrocarbons can entrain deep sediments to over 4 cm in a short time and that FSC indigenous bacteria are able to respond to a contamination event, even at a low temperature, reflecting the *in situ* conditions.

Keywords: oil spill, deep-sea sediment, hydrocarbon degradation, hydrocarbon entrainment, bacteria, dispersant, pollution, Faroe-Shetland Channel

INTRODUCTION

Oil and gas exploration in the deep sea is increasing as shallow and more accessible sources become depleted (Leffler et al., 2011; Ramirez-Llodra et al., 2011). The Faroe-Shetland Channel (FSC) is an area of deep-water hydrocarbon exploration (Smallwood and Kirk, 2005; Gallego et al., 2018) where hydrocarbon prospecting is occurring at depths of up to 1,500 m (Lagavulin well: 1,567 m depth, 62°39′N, 1°7′W). There are numerous concerns over environmental implications stemming from oil drilling activity, production and potential spillages/release to areas such as the FSC which require further investigation (Cordes et al., 2016).

The exposure of the marine environment to hydrocarbons can cause serious detriment to localized and wide-scale regional ecosystems (Mearns et al., 2010). Oil can be released to the water column directly, as witnessed following the *Deepwater Horizon* (DwH) well blowout in the Gulf of Mexico (GoM) in 2010 (Atlas and Hazen, 2011; Schrope, 2011; Montagna et al., 2013; Joye et al., 2016). More commonly oil is released to surface waters in shipping accidents such as the *Exxon Valdez* spill in 1989 (Atlas and Hazen, 2011) and *Prestige* spill in 2002 (Acosta-González et al., 2015). Following an oil spill in the water column or sea surface hydrocarbons are transported to the seabed, often in large quantities and across vast areas (Valentine et al., 2014). The quantity of oil transported to the seabed during the DwH was estimated to range between 1.8 and 31% of the water column-entrained oil (Lubchenco et al., 2010; Chanton et al., 2014; Valentine et al., 2014). There have been several oil-to-seabed transportation mechanisms proposed (Romero et al., 2015): (1) a combination of advective transport and oceanic currents carried the oil entrained plume into the continental slope leaving a "bathtub" ring of oily residue (Valentine et al., 2014); (2) the formation of oil-mineral aggregates and marine oil snow were deposited to the seafloor (Passow et al., 2012; Ziervogel et al., 2012); (3) a loss of buoyancy of oil droplets and oil-mineral aggregates in the water column resulted in deposition (Gong et al., 2014a) and (4) the ingestion of oil or oil-mineral aggregates by zooplankton and excretion as fecal pellets that settled on the seabed (Muschenheim and Lee, 2002).

The sedimentation pulses that took place following the DwH spill (Chanton et al., 2014; Valentine et al., 2014; Romero et al., 2015) led to the formation of flocculent oily material on the seabed which covered corals (White et al., 2012), reduced natural bioturbation (Brooks et al., 2015) and altered the microbial community (Yang et al., 2016). Although it was determined that the indigenous microflora and fauna were capable of responding to and degrading the influx of hydrocarbons (Kimes et al., 2014), major shifts within sediment microbial populations in the months following the spill had varying effects on geochemical cycling and redox conditions (Kimes et al., 2013; Scott et al., 2014; Hastings et al., 2016). Anaerobic conditions often prevailed in normally aerated sediments due to smothering by floc and rapid oxygen consumption (Yang et al., 2016), yet Mason et al. (2014a) and Kimes et al. (2013) both detected aerobic and anaerobic hydrocarbon degraders in sediments surrounding the Macondo well following DwH. The shift in redox state to anaerobic conditions meant that deposited hydrocarbons would undergo slower degradation compared to aerobic processes (Head et al., 2006; Widdel et al., 2010), leading to persistence of harmful and toxic oil components in the environment (Hylland, 2006; Marini and Frapiccini, 2013). Seabed-bound hydrocarbons are subjected to physical and biological processes which can translocate them (Konovalov et al., 2010; Zuijdgeest and Huettel, 2012). Solutes can be transported into sediments by diffusive and advective pore water fluxes (Huettel et al., 2014) and hydrocarbons may desorb from sediment, dissolve in the water column and be transported to remote locations (Zhao et al., 2015) where they may be degraded within the water column. The environmental conditions in the deep sea vary with location and a greater understanding of how hydrocarbons entrain and are removed from deep sea sediments is required to assess environmental risks in the event of an oil spill similar to DwH in a different location such as the FSC.

Deepwater hydrocarbon exploration in the FSC has been underway for over 20 years (Austin et al., 2014; Gallego et al., 2018) with fields such as Schiehallion (350–450 m) in 1993 and more recently the North Uist prospect (∼1300 m) in 2012. Increased exploration in this region at great depths presents a potential risk of oil spills in the FSC and an outcome analogous to the deep sea intrusion layers and sedimentation pulses observed following DwH is certainly possible. Direct application of lessons learned from DwH may, however, be misguided as the environmental conditions differ to those in the GoM (The Energy and Climate Change Committee, 2011). Temperatures at depths > 1000 m in the FSC are ∼0°C, thus colder than temperatures in the GoM (∼5°C), and are accompanied by an extreme hydrodynamic regime comprising complex multidirectional water masses (Berx et al., 2013). The transport processes of hydrocarbons in the FSC are likely to be more complex than those observed in the GoM and oil would inevitably be dispersed over a vast area (Main et al., 2016). The FSC is an important region hosting diverse benthic habitats and

parts of it have been designated as marine protected areas (e.g., the Faroe-Shetland Sponge Belt Nature Conservation Marine Protected Area; Joint Nature Conservation Committee, 2014). The FSC is known to have intense benthic ecological activity and bioturbation (Jones et al., 2007; Gontikaki et al., 2011), potentially enhancing hydrocarbon transport into sediments. Unlike GoM sediments, FSC microflora and bacterial communities have not been pre-exposed to hydrocarbons, a factor which contributed to the efficiency of microbial oil degradation following DwH (Hazen et al., 2010; Joye et al., 2014). Hydrocarbon degradation in the FSC may be slower compared to that in the GoM due to ∼0°C temperatures prevailing below 600 m (Ferguson et al., 2017). The FSC is of high environmental interest as a proxy for potential oil spills in deep arctic ecosystems since North Atlantic Deep Water formed in the Arctic flows southwards at depths over 600 m in the channel (Berx et al., 2013). In particular, ∼50% of North Atlantic Deep Water flows through the FSC. It follows that an oil spill in this location would entail the risk of contaminating the North Atlantic's deep water supply.

In response to the DwH blowout, 7 million liters of chemical dispersant (mainly Corexit 9500 and 9527A) were applied to both the surface slick and deep sea plume. Dispersant reduces the surface tension of the oil-water interface, transforming large globules of oil into smaller droplets enhancing solubilization and dissolution; this is proposed to enhance biodegradation rates as hydrocarbons become more bioavailable (Kleindienst et al., 2015b). However, there are concerns over the toxicity and degradability of dispersants (Scarlett et al., 2005) and their efficacy is inconclusive with studies reporting both suppression (Kleindienst et al., 2015b) and stimulation of microbial oil degradation (Baelum et al., 2012). The effect of dispersants on oil-sediment interactions is currently under investigation and contrasting effects on hydrocarbon sorption to marine sediments have been observed (Zhao et al., 2015). Oil dispersants selectively enhance sorption of aliphatic and aromatic hydrocarbons to marine sediments at different dispersant concentrations (Zhao et al., 2016). These effects varied with type of oil dispersant used, highlighting that an understanding of the effects of commercial dispersants on oil-sediment-microbe interactions requires further investigation.

The aims of this study were to evaluate the propensity of hydrocarbons to be transported into and out of FSC sediments following oil-sediment particle deposition and the subsequent bacterial community response over time. An additional aim was to determine the effects of a commercial oil dispersant (Superdispersant-25, SD25 hereafter) on these processes, and identify bacteria responding to oil in the presence or absence of SD25 treatments in deep sea sediments. To achieve this, the following hypotheses were developed: (1) post-depositional transport of hydrocarbons in surficial sediments at *in situ* temperatures triggers a stratified bacterial community response, (2) chemical dispersant increases hydrocarbon mobility and accessibility to microbial communities enhancing shifts to a hydrocarbon-degrading population and (3) sedimentary matrix modification results in enhanced mobility of hydrocarbons through surficial sediments. To our knowledge, this is the first study to evaluate hydrocarbon transport and subsequent bacterial community response in naturally stratified deep sea sediments of subarctic origin at *in situ* temperatures.

MATERIALS AND METHODS

Sediment and Seawater Collection, Transportation, and Preparation

Sediment and seawater samples were collected on May 2014 and May 2015 on-board *MRV Scotia* (cruise numbers: 0514S and 0515S, respectively) in the FSC from two stations (Supplementary Figure 1). Sediments from station A (1000 m deep; 61°35.02′N, 4°14.64′W), collected in 2015 cruise using a maxi-corer (OSIL, UK) fitted with acrylic cores (internal diameter = 10 cm, length = 60 cm), were used in "undisturbed sediments" experiments. Four sub-cores (acrylic, internal diameter = 3.6 cm, length = 30 cm) of ∼10 cm sediment depth were collected from each Maxi-corer core on-board. The sub-cores were stored fully submerged in seawater baths inside a temperature-controlled unit at 0°C that allowed water circulation and were individually aerated via air stones attached to an air pump to prevent anoxia. Following transport to the laboratory, the sub-cores were allowed to acclimatize for 10 days at 0°C until initiation of the experiments. Seawater was collected from station A using a rosette equipped with Niskin bottles during the 2015 cruise to be used in the undisturbed experiment reservoir system. Sediments were collected using a van Veen grab from station B at 180 m depth (69° 49.08′N, 5° 21.03′W) during the 2015 cruise and used in the production of oil-sediment pellets (see below).

A second set of sediments, collected using a van Veen grab during the 2014 cruise from station A, were sterilized and homogenized, and used in the transport experiment ("modified sediments," hereafter). Once transported to the lab modified sediments were mechanically homogenized and autoclaved at 120°C and 100 kPa for 21 min. Thereafter, sub-cores were made up to 10 cm with the modified sediment and filled up with UV-filtered seawater (0.5 μm filter) collected from the Ythan estuary. Sediment and seawater characterization was performed using methods described in (Supplementary Methods 1).

Model Oil and Artificial Oil-Sediment Pellets

A model oil was prepared with 20 hydrocarbons with a resulting density of 880 kg m^{-3}, similar to a medium crude oil (Ferguson et al., 2017). The model oil was composed of 64.9% aliphatic hydrocarbons, 30.0% combined monoaromatic and PAHs and 5.1% resin fractions. The hydrocarbons used to make the model oil, and their characteristics are listed in Supplementary Table 1. Model oil was filter-sterilized through a 0.22 μm PTFE filter (VWR).

Oil-sediment pellets (OSPs) were made using the 300–350 μm fraction of station B sediments after removal of organic carbon (450°C, 12 h). This ensured that the sediment used was homogenous in terms of organic matter and particle size. To make the 3-cm OSPs, 4 g of treated sediment were placed in tin foil and 1 ml of model oil and 1 ml of MilliQ water (18.2 MΩ cm, 25°C) were added. The OSPs were stored at −20°C

until use. To evaluate the exact amount of model oil components retained in individual pellets, three replicate OSPs were extracted by Soxhlet. On average, 0.41 ± 0.09 g (error = standard deviation, n = 3) of the oil was retained in the OSPs (Supplementary Table 1).

Incubation of Oiled Seawater-Sediment Systems

Undisturbed sediment sub-cores were removed from the water baths and lined around a rosette fitted with magnets. The cores were subject to three treatments; no oil (control), oil only (O-treatment) and oil and dispersant (OD-treatment). O- and OD-treatments were inoculated with artificial OSPs. All sub-cores were sealed with modified rubber stoppers on the upper end, leaving no headspace (Supplementary Figure 2). The modified stoppers were fitted with magnetic stirrers and two hollow steel pipes ∼8 cm above the sediment surface to enable water exchanges to take place throughout the experiment. The rosette rotated to move the magnetic stirrers inside the sub-cores and simulated advection of supernatant water (20 rpm). The system was kept in darkness and held at 0°C for the duration of the experiment. Supernatant water was periodically replaced to emulate replenishment of seawater in the water column and prevent anoxia in surficial sediments. Each sub-core was connected to a water reservoir (225 ml) and water was exchanged between sub-cores and reservoirs using a 520S peristaltic pump (Watson Marlow) at a rate of 25 ml min^{-1} for 20 min twice per week. SD25 (Oil Technics, UK) was added to OD-treatment reservoirs (33 µl, 1:30 SD25:oil ratio, based on manufacturer's recommendation) when water exchanges were performed to evaluate the effect of SD25 on transport and solubilization of hydrocarbons. Triplicate sub-cores were analyzed per time point, treatment and sediment type.

Hydrocarbon Extraction and Analysis

At specified time intervals (7, 21, 42, and 71 days) hydrocarbons were quantified from each of the 4 depth layers (0–1, 1–2, 2–4, and 4–10 cm). Approximately 10 g of sediment from each section was stored in glass vials with PTFE caps at −20°C prior to hydrocarbon extraction. Supernatant seawater was collected and stored in amber vials with PTFE caps and maintained at −20°C until extraction. Hydrocarbons were extracted from sediment by Soxhlet extraction for ∼40 cycles (5 h in total) using 100 ml dichloromethane. Hydrocarbons were liquid-liquid extracted from the total volume of seawater of each core (∼150 ml) with 3 × 10 ml dichloromethane and the resulting extractions combined. Extraction recovery rates for all components were performed in triplicate and can be found in Supplementary Table 1. Hydrocarbon extractions were analyzed by gas chromatography fitted with a flame ionization detector (GC-FID) using a previously described system and method (Ferguson et al., 2017). Calibration curves (6-point) were determined for each compound of the model oil. Laboratory control samples were analyzed to establish the effect of the sediment matrix and extraction procedure on the recovery of model oil compounds. Toluene was added as an internal standard to account for injection error (1 µl ml^{-1}). The limits of detection and quantification were defined as chromatographic signal to noise ratios of 3 and 10, respectively.

Bacterial Community Analysis

DNA Extractions

Total genomic DNA was extracted from 0.4 g sediment using the FastDNA™ SPIN Kit for Soil and FastPrep®-24 instrument (both MP Biomedicals, Cambridge, UK), according to manufacturer's instructions. Purified DNA was stored at −20°C until further analysis.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed on all sediment layers and shows differences in bacterial profile through depth. The conserved V3-V5 region of bacterial 16S rRNA gene was amplified using the primer pair 341F (with GC clamp) (Muyzer et al., 1993) and 907R (Muyzer and Smalla, 1998). Each PCR reaction contained 1 µl of target DNA extract (diluted to < 20 ng μl^{-1}), 2 µl PCR water, 45 µl Red Taq DNA Polymerase Master Mix with 1.5 mM $MgCl_2$ and 1 µl of each primer (10 µM). Amplification was carried out on a Techne thermal cycler (Techne, UK) using a step-down PCR programme as follows: initial denaturation at 95°C for 4 min, followed by 10 cycles of 94°C for 30 s, 62°C for 45 s, and 72°C for 1 min, followed by 25 cycles of 94°C for 30 s, 57°C for 40 s, and 72°C for 1 min, plus final extension for 10 min at 72°C. The PCR products were checked on a 1.2% (wt/v) agarose gel with gel electrophoresis for 40 min at 90 V. Gels were stained with GelRed™ and visualized on a UV transilluminator (UGenius 3, Syngene, UK).

DGGE using a TV-400 DGGE system (Scie-Plas, UK) was performed on the PCR amplified products. PCR product was loaded onto a 6% (wt/vol) polyacrylamide gel with a denaturant gradient of 30–70% (100% corresponds to 7 M urea and 40% vol/vol formamide). Electrophoresis was performed in 1 × TAE buffer at 60°C for 16 h at 100 V. The gel was stained with GelRed™ for 60 min and visualized on a UV transilluminator. All three incubation replicates for each time point were analyzed by DGGE to ensure reproducibility but a single replicate for each treatment was loaded on a single DGGE gel to evaluate shifts over time between all treatments. DGGE bands that potentially represented different strains were excised with a sterile scalpel and transferred to sterile 1.5 ml micro-centrifuge tubes containing 50 µl sterile molecular water. DNA was eluted from bands over 48 h at 4°C. Eluted DNA was re-amplified using the same primer pair (without GC clamp) and PCR programme. PCR products were then purified (E.Z.N.A cycle purification kit according to manufacturer's instructions; Omega) and quantified using a Genova Nano spectrophotometer (Jenway). Purified DNA was then diluted to a concentration of 6 ng μl^{-1} using sterile PCR water and sequenced by paired-end Sanger sequencing (Source Bioscience, Bellshill, Scotland). Sequences were quality checked using SeqTrace (Stucky, 2012) and run through the Basic Local Alignment Search Tool (BLASTn) for nucleotide closest match.

Next-Generation Sequencing

Paired-end (2 × 300 bp) Illumina MiSeq sequencing of the 16S rRNA gene V3–V4 variable region was performed on the upper sediment layer as previously described (Ferguson et al., 2017). Average read depth was 39,838 ± 2,206 (SEM) per sample for 39 samples, except for 3 samples (<10,000) which were omitted from downstream analysis. Bioinformatics analysis was carried out on the Maxwell High Performance Computing Cluster at the University of Aberdeen, using Mothur v 1.39.0. Chimera detection was performed using UCHIME (Edgar et al., 2011). OTU clustering was performed at 97% similarity and taxonomic assignment obtained with SILVA (Quast et al., 2012). The raw sequencing data is available in the European Nucleotide Archive (ENA) under the accession number PRJEB25813.

Statistical Analysis and Calculations

Statistical analysis was carried out using the package *nlme* (Pinheiro et al., 2017) within R environment (R Core Team, 2017). Preliminary data exploration was undertaken to establish the best approach to analyse the sediment profile data. To model the entrainment of hydrocarbons in the sediments, linear mixed effects models were used due to the correlation of hydrocarbon concentrations at each depth interval within individual cores. [Hydrocarbon] was used as the response variable ($x^{0.4}$-transformed to ensure normality of residuals), core identity was specified as a random effect and hydrocarbon identity (HCID) was nested within core identity to allow for variation in total [hydrocarbon] content between cores and variation in average [hydrocarbon] between hydrocarbons within cores. An autoregressive residual correlation term (AR1) was included in the model, such that the concentration of a hydrocarbon was allowed to depend on the concentration of the same hydrocarbon directly above it in each core. Treatment, HCID, depth and time (both as factors) were included as fixed effects in the model and all possible interactions between them. Model selection was performed by stepwise elimination of non-significant terms (from higher to lower order terms) using the likelihood ratio test and maximum likelihood estimation. Once a minimal adequate fixed effect model structure was determined, it was refitted using restricted maximum likelihood estimation (Zuur et al., 2009). Separate models were fitted for undisturbed and modified sediment incubations.

To evaluate the partitioning of hydrocarbons between sediment and water phases, a distribution coefficient was developed that describes the ratio of total hydrocarbon mass in supernatant water to total hydrocarbon mass in sediment. This is not a true partition coefficient as this typically refers to distribution between two immiscible liquids. It is a distribution coefficient due to the presence of dissolved hydrocarbons in interstitial water but in this work, the sediment "phase" encapsulates the hydrocarbons dissolved and dispersed in the interstitial water as well as those adsorbed to and absorbed into sediment particles, while the supernatant "phase" encapsulates the hydrocarbons dissolved and dispersed in the water column. The distribution coefficient was defined as:

$$K_{ws} = \log \frac{m_w}{m_s}$$

Where m_w is the total hydrocarbon mass in supernatant phase and m_s the total hydrocarbon mass in the sediment phase. Where no hydrocarbon was detected in either phase, the minimum detected concentration of that hydrocarbon in that phase was used instead of zero. K_{ws} values below and above zero indicate preferential partitioning to the sediment and the supernatant water phase, respectively. The effect of SD25 application and time was evaluated using a linear mixed effects model following the same procedure as for the sediment transport models above with the exception that no autoregressive correlation was implemented into the model because [hydrocarbon] in one core did not depend on [hydrocarbon] in other cores. Separate models were developed for undisturbed and modified sediment incubations. Underlying model assumptions were validated graphically for hydrocarbon transport and K_{ws} models (Supplementary Figures 3–6).

Effective diffusivities (D_{eff}) for model oil compounds were calculated following Thibodeau and Mackay (2011) assuming the sediments were porous and saturated. Briefly, D_{eff} is calculated as a ratio of aqueous diffusivity divided by physical and chemical resistance terms which take into account sediment properties and organic matter content.

Based on the analysis of DGGE images using Phoretix 1D analysis software (version 4.0; TotalLab Ltd), bacterial community analysis was represented by the relative band intensities within lanes as previously performed (McCaig et al., 2001). The statistical analysis of microbial communities was performed using the package *vegan* (Oksanen et al., 2017) in R. A distance matrix was generated using the Bray-Curtis method from the relative band intensity data (function vegdist) and the treatment effects on the community structure were visualized by non-parametric multi-dimensional scaling (nMDS) (function metaMDS). Hierarchical clustering (function hclust) was performed using the unweighted pair group method with arithmetic mean (UPGMA). Statistical differences between bacterial communities between treatments and times were analyzed using permutational multivariate analysis of variance (PerMANOVA) (function adonis; 999 restarts).

The operational taxonomic unit (OTU) table resulting from the Illumina sequencing analysis was imported into R with the package *phyloseq* (function import_biom) (Mcmurdie et al., 2013). Singletons from the whole database were removed and samples rarefied to the smallest sample read depth (function rarefy_even_depth). Plots representing the bacterial structure were visualized using the *ggplot2* package (Wickham and Chang, 2009). Alpha diversity analysis was performed in package *phyloseq* (function estimate_richness). To assess differences in alpha diversity over time and between treatments, analysis of variance was performed where diversity estimates was the response variable and the interaction between treatments and day were the explanatory variables after log transformation of the data. For beta diversity analysis, a distance matrix was produced (function vegdist) with the Jaccard index and community structure differences between samples were visualized by nMDS as described above. PerMANOVA was performed to detect significant differences in community composition. In order to identify the taxa

whose changes of abundance between treatments are more significant, differential abundance testing (function deseq) was carried out on non-subsampled data as recommended by package developers in package *DESeq2* (Love et al., 2014).

RESULTS

Sediment Properties and Hydrocarbon Effective Diffusivities

The sediment from station A was silt dominated (81–82%) and contained relatively low quantities of carbon (1.7–1.9% TOC and 0.9% TIC) (Supplementary Table 2). The BTEX components and naphthalene had the highest effective diffusivities ($D_{eff;} > 29$ cm^2 $year^{-1}$) (Supplementary Table 1). For aliphatics, D_{eff} decreased with increasing carbon chain length. Similarly, for PAHs, as the number of rings increased, D_{eff} decreased (Supplementary Table 1).

Transport Into Sediments

In undisturbed sediments, hydrocarbon entrainment varied with HCID over time (Supplementary Figures 7–9, Supplementary Table 4, Supplementary Data 1), interaction: HCID × Depth (factor) × Time (factor): $LR_{df=271} = 240.74$, $P = 0.0001$). SD25 application significantly affected hydrocarbon entrainment over time (**Figures 1**, **2**, Supplementary Figures 7–9, Supplementary Table 4, Supplementary Data 1, Treatment × Depth (factor) × Time (factor), $LR_{df=9} = 162.9$, $P < 0.0001$). Hydrocarbons were detected over 4 cm deep on day 42 for both treatments (**Figures 1**, **2**, Supplementary Figures 7–9, Supplementary Data 1, $[TPH]_{O-Treatment} = 73.1 \pm 96.0$ $\mu g\ g^{-1}$, $[TPH]_{OD-Treatment} = 124.0 \pm 161.9$ $\mu g\ g^{-1}$, error = standard deviation) but not in the OD-treatment on day 71 ($[TPH]_{O-Treatment} = 22.3 \pm 22.2$ $\mu g\ g^{-1}$, error = standard deviation).

In modified sediments, hydrocarbons from all fractions were detected over 4 cm depth in the sediment on day 7 (Supplementary Figures 10–13). As in undisturbed sediments, SD25 application significantly affected [hydrocarbon] by time and depth in modified sediments (Supplementary Figures 10–14, Supplementary Table 3, Supplementary Data 2, Interaction Treatment × Time (factor) × Depth (factor), $LR_{df=9} = 167.21$, $P < 0.0001$). However, during model simplification, [hydrocarbon] across hydrocarbons over time was found to be non-significant (Interaction HCID × Treatment × Time (factor): $LR_{df=57} = 74.31$, $P = 0.0615$). In contrast to undisturbed sediments, there was no evidence that hydrocarbon entrainment varied across hydrocarbons over time (Interaction HCID × Time (factor) × Depth (factor): $LR_{df=171} = 92.59$, $P = 1$) or for the effect of SD25 on hydrocarbon entrainment to vary with hydrocarbon (Interaction HCID × Treatment × Depth (factor): $LR_{df=57} = 39.73$, $P = 0.9603$). The two-way interactions of HCID with treatment and time were also found to be not significant in modified sediments (HCID × Treatment: $LR_{df=57} = 25.68$, $P = 0.1392$ and HCID × Time (factor): $LR_{df=57}$: 70.25, $P = 0.1117$).

Water-Sediment Distribution Coefficients (K_{ws})

In undisturbed sediments, there was no evidence of K_{ws} values changing over time ($LR = 4.06$, $d.f._3$, $P = 0.2555$). However, SD25 application significantly affected K_{ws} values differently between hydrocarbons (**Figure 3**, Supplementary Table 5, Supplementary Data 3, interaction HCID × Treatment: $LR_{df=19} = 80.12$, $P < 0.0001$). SD25 application increased K_{ws} values of all hydrocarbons except BTEX components and naphthalene. In modified sediments, the effect of SD25 application varied with hydrocarbon and time (Supplementary Figure 15, Supplementary Table 6, Supplementary Data 4, Interaction HCID × Time × Treatment, $LR_{df=19} = 101.63$, $P = 0.0001$). For the O-treatment, K_{ws} increased in day 21 and decreased thereafter. For the OD-treatment, K_{ws} was lower than in the O-treatment for most components for day 7 but increased in day 21, remained higher than for the O-treatment in day 42 and decreased in day 71 (Supplementary Figure 15).

Depth Profile of Bacterial Community Structure in Hydrocarbon and Superdispersant-25 Treated Sediments

According to DGGE analysis the bacterial community structure in the upper layer (0–1 cm) of the controls remained relatively unchanged over time (Supplementary Figures 16A,B). Bacterial communities from layer 1 controls days 0–21 and day 0 O-treatment were grouped together by cluster analysis (Supplementary Figure 16C). Ordination analysis of DGGE patterns of the top layer revealed differences in bacterial community structure between C- and both O- and OD-treatments (Supplementary Figure 16B). The presence of oil (O- and OD-treatments) significantly changed community composition compared to controls (PerMANOVA; $R^2 = 0.46$, $P = 0.0009$). There were successional shifts in band patterns of O- and OD-treatments from day 7 to 71. The bacterial structure on day 42 in the OD-treatment was similar to O-treatment on day 71. However, this structure had changed in the parallel OD-treatment, indicating similar trajectories but at significantly different rates (PerMANOVA; $R^2 = 0.93$, $P = 0.0009$).

Bacterial community structure of the control in the upper layer was similar to both C- and O-treatments in the deeper layers (Supplementary Figure 17A). The composition in these treatments was dominated by the presence of a core group of organisms (hereinafter referred to as "core group"). The core group was consistently present regardless of time or treatment. However, within the top layer of O-treatment incubations where [hydrocarbon] was highest, bands decreased in density and abundance in days 21–71. Moreover, there was a shift in bacterial structure at 1–2 cm deep in the O-treatment at days 42–71 (when hydrocarbons had migrated into the sediment) that clustered together with 0–1 cm deep O-treatment days 21–71 (Supplementary Figures 17B,C). Below 2 cm, there was dominance of the core group with no noticeable shifts. There was a significant difference between bacterial communities as a function of depth across both treatments (PerMANOVA; $R^2 = 0.48$, $P = 0.0009$).

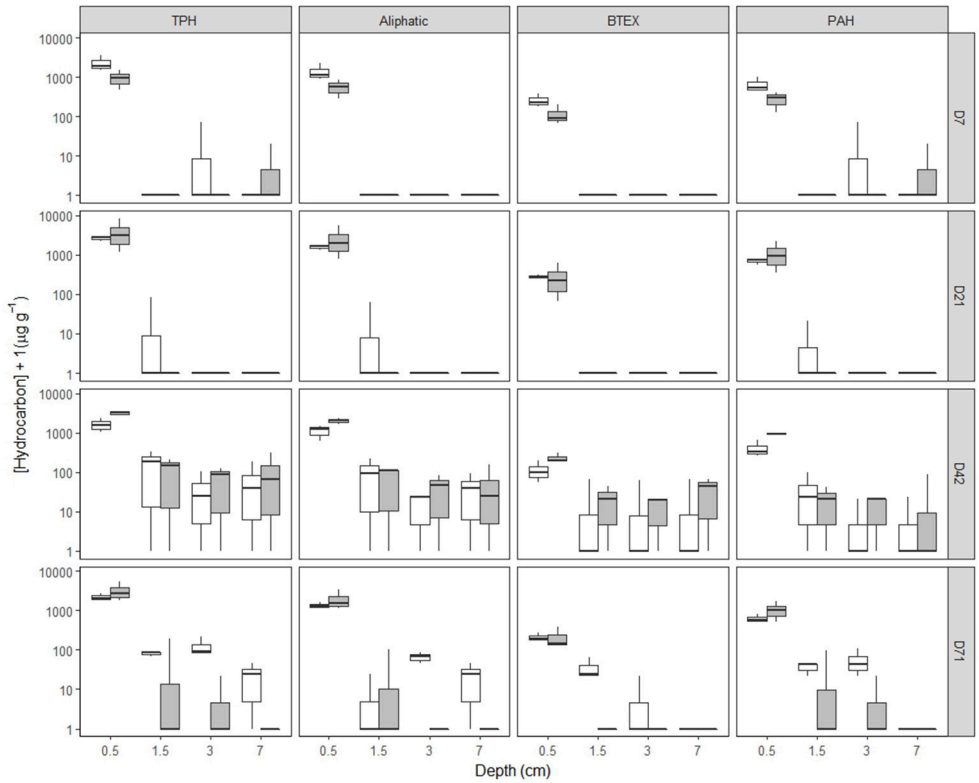


FIGURE 1 | [Hydrocarbon] of hydrocarbon fractions by time, depth and treatment in Faroe-Shetland Channel undisturbed sediments. From top to bottom, days 7, 21, 42, and 71 and left to right total petroleum hydrocarbons (TPH), aliphatic, BTEX and polyaromatic hydrocarbons (PAH) fractions. Box plots represent [hydrocarbon] for oil (white) and oil and dispersant (gray) treatments ($n = 3$). Depths shown are the average depth of the sections analysis (i.e. 0–1, 1–2, 2–4, and 4–10 cm correspond to 0.5, 1.5, 3, and 7 cm deep, respectively). Note [hydrocarbon] has been log(x+1) scaled.

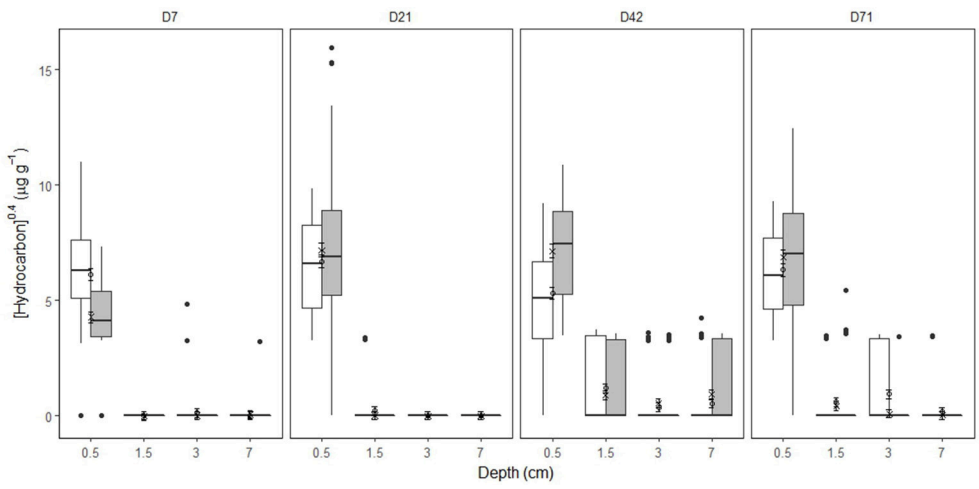


FIGURE 2 | [Hydrocarbon] by time, depth and treatment in Faroe-Shetland Channel undisturbed sediments. From left to right, days 7, 21, 42, and 71. Box plots represent [hydrocarbon] for oil (white) and oil and dispersant (gray) treatments ($n = 60$). Linear mixed effects predictions are shown for oil (circles) and oil and dispersant (crosses) treatments. Error bars represent standard error. Depths shown are the average depth of the sections analysis (i.e. 0–1, 1–2, 2–4, and 4–10 cm correspond to 0.5, 1.5, 3, and 7 cm deep, respectively). Note [hydrocarbon] has been $x^{0.4}$- transformed.

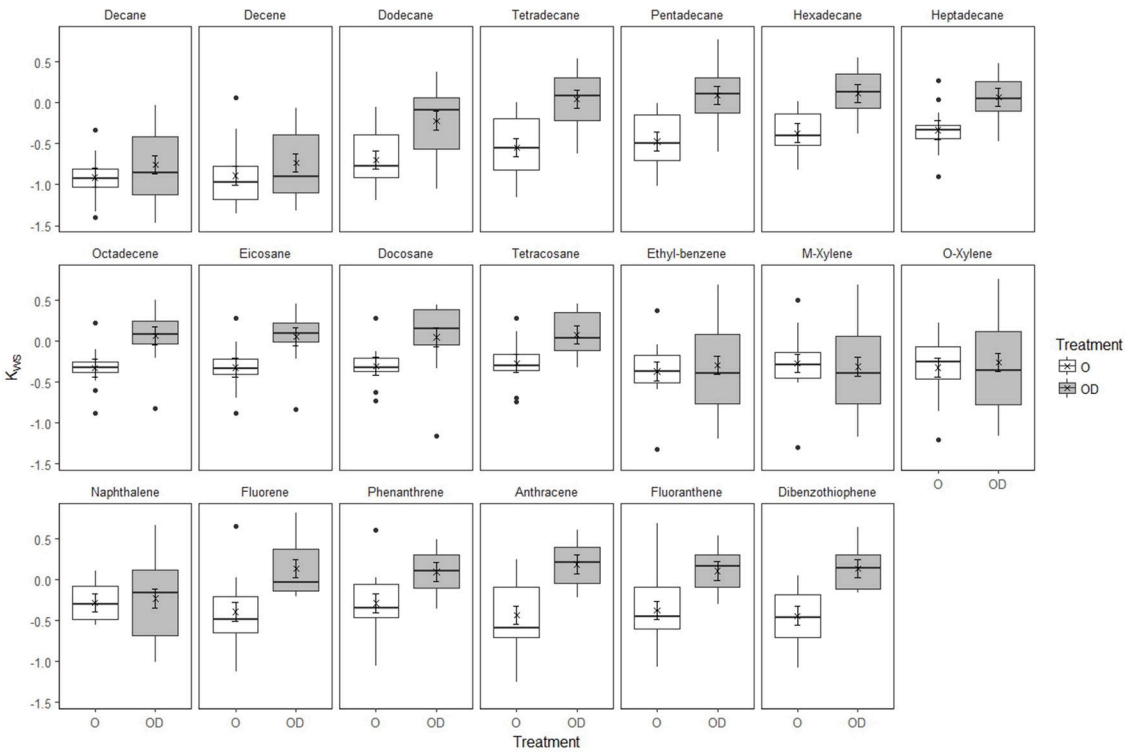


FIGURE 3 | Effect of Superdispersant-25 on K_{WS} by hydrocarbon in undisturbed Faroe-Shetland Channel sediments. Box plots represent the raw data for oil (white) and oil and dispersant (gray) treatments ($n = 12$). Linear mixed effects model predictions are shown for the oil (circles) and oil and dispersant (crosses) treatments. Error bars represent standard error.

Differences between C- and O-treatment communities were only identified within 0–1 cm deep (PerMANOVA; $R^2 = 0.59$, $P = 0.0009$).

To identify the bacterial members of communities DGGE bands were excised and sequenced. All bands were classified as members of Proteobacteria, except band A, which was part of the Firmicutes phylum (Supplementary Table 7). Bands classified as Proteobacteria were characterized as γ-Proteobacteria, except bands K and L which were categorized as δ-Proteobacteria. Bands B and C showed similar homology to *Colwellia rossensis* strain ANT9279 (100% identity) and *Colwellia psychrerythraea* strain 34H (99%), respectively. *Colwellia* related strains were most prominent in layer 1 in O-treatment incubations but also present in other treatments and depths at a lower density. Bands H and J showed high sequence homology to *Pseudoalteromonas* sp. K8 (99%) and *Pseudoalteromonas translucida* KMM 520 (99%), respectively. *Pseudoalteromonas* related strains appeared to be stimulated by oil exposure within 0–1 cm, particularly band J which was only present on day 71. The core group was mainly represented by bands D to I. Band D diminished in the presence of oil whereas band G was more prominent in the O-treatment; both matched to uncultured γ-Proteobacteria. Band F initially increased in density from days 0–21 but then decreased from days 42–71 showing potential succession to *Pseudoalteromonas* spp.

Bacterial Community Structure at the Sediment/Water Interface

Illumina sequencing of the top centimeter of sediment was performed to examine the O- and OD-treatments induced bacterial shifts in more detail. The relative abundance of taxa within the bacterial communities was consistent among replicates early in the study but became increasingly divergent over time (**Figures 4**, **5**). Bacterial communities from all treatments were dominated by Proteobacteria across all time points (46–66%) except for OD-treatment day 71 where composition was shared between Proteobacteria (41%) and Firmicutes (28%). Firmicutes was represented in the other two treatments but only with maximum contribution of 9% within the controls and 17% in the O-treatment. The phylum Parcubacteria represented <1% at all time points and treatments except O-treatment day 71 where it averaged 5%. The phylum Actinobacteria was found at consistent levels throughout all time points within controls. Within Proteobacteria, the majority of organisms were assigned to the orders Alteromonadales and Xanthomonadales.

The presence of oil selected for Alteromonadales, which averaged 14% in day 0 of both C- and O-treatments. By day 71 this reduced to 10% in controls but increased to 39% by day 71 in the O-treatment. In contrast, in the OD-treatment it peaked at 32% on day 21 but decreased to 8% by day 71

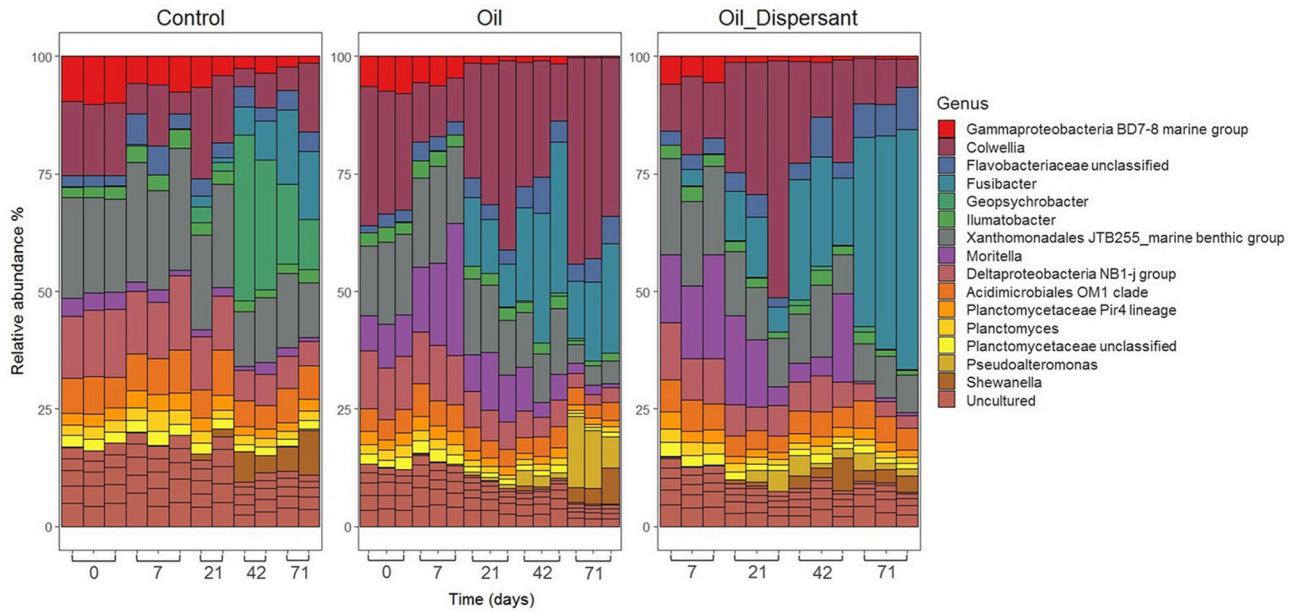


FIGURE 4 | Relative abundance plots visualizing bacterial community composition and dynamics within the top centimeter of Faroe-Shetland Channel sediment over time. Time in days is displayed left to right along the x axis. Treatment is grouped as control (left box), oil (middle) and oil and dispersant (right). Bars represent the top 20 OTUs for each sample grouped at the genus level (clustered at 97%).

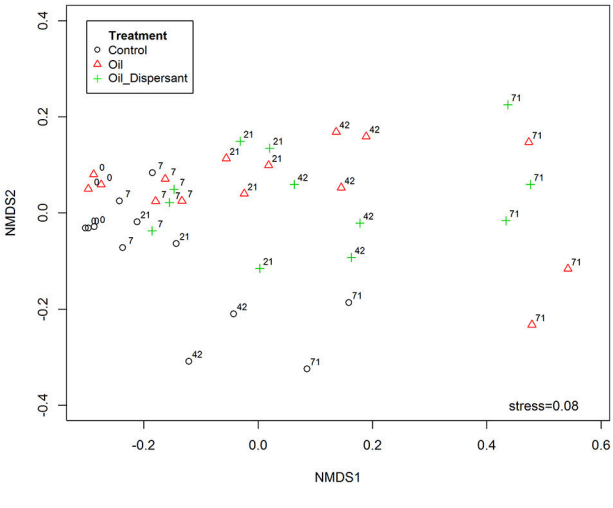


FIGURE 5 | Effect of treatment and time on bacterial community composition within the top centimeter of Faroe-Shetland Channel sediment, assessed using non-metric multidimensional scaling (nMDS), based on Jaccard index following square root transformation of sample data. Treatments are indicated as control (black circles), oil (red triangles) and oil and dispersant (green crosses). Numbers placed next to treatments indicate time points in days.

indicating succession to other organisms. At the genus level, *Colwellia* represented the most prominent OTU in both O- and OD-treatments on day 21 (**Figure 4**). It then decreased in the OD-treatment but increased to 27% in O-treatment. *Moritella* was present at 3% on day 0 in all treatments. However, by day 7 it had risen to 10% in both O and OD-treatments before decreasing at similar rates down to 1% by day 71. *Pseudoalteromonas*, *Pseudomonas* and *Oleispira* were present in oiled treatments at a lower abundance (1–10%). Oil selected for Candidatus Campbellbacteria, the predominant order within Parcubacteria, which was not present above 0.05% in any treatment other than O-treatment day 71 where it had increased to 6% in two of the three replicates. Application of dispersant resulted in increased abundance of the order Clostridiales, with relative abundance of 1% at day 7 before increasing to 29% at day 71 in the OD-treatment (Supplementary Figure 18). The most prominent member in the OD-treatment by day 71 was *Fusibacter* (27%), which was also present in O-treatment (11%).

Oil exposure had negative effects on Xanthomonadales which was consistently present in controls but decreased in relative abundance from 10% in both oiled treatments at day 7, to 5% by day 71 (Supplementary Figure 18). Genera which markedly increased in relative abundance in controls include *Geopsychrobacter* which was <0.5% at day 0 yet increased to 18% by day 42 and Desulfuromonadales which increased in relative abundance from day 42 onwards in controls. A group of taxa were seen to withstand oil contamination such as Acidimicrobiales OM1_clade and Planctomycetaceae Pir4_lineage and may represent the aforementioned core group (**Figure 4**).

Statistical Analysis of Illumina Sequenced Bacterial Communities

Ordination analysis revealed dissimilarity of oil treated (both O- and OD-) communities with controls (**Figure 5**). The effect

of treatment significantly explained variation in community structure (PerMANOVA; $R^2 = 0.16$, $P = 0.001$). There was clustering of day 71 incubations of O- and OD-treatments away from earlier time points. Furthermore, there appeared to be gradual community divergence over time within replicates across all treatments. The overall effect of time was significant in community composition (PerMANOVA; $R^2 = 0.38$, $P = 0.001$). The richness (observed OTUs) and diversity (Shannon index, Supplementary Figure 19) of the microbial communities across all treatments significantly decreased with time (ANOVA; $F = 196.053$, $P = 0.001$; $F = 60.586$, $P = 0.001$, respectively). When considering the interacting effects of treatment and time there were significant variations in community richness and diversity (ANOVA; $F = 3.776$, $P = 0.006$; $F = 5.124$, $P = 0.001$ respectively).

Differential abundance testing using *DESeq2* determined which taxa were significantly more abundant between treatments (**Figure 6**). *Pseudoaltermonas*, *Oleispira*, *Moritella*, Candidatus Campbellbacteria, *Pseudomonas*, *Colwellia* and *Fusibacter* were more significantly abundant (all adjusted $p < 0.001$) in O- and OD-treatments. Members of the genera *Geopsychrobacter* and *Desulfuromonas* were more significantly (adjusted $p < 0.001$) abundant in controls.

DISCUSSION

The experimental design was unique in the way that it was developed to replicate a scenario following an oil spill in the deep sea where oil residues would be deposited to the seabed. Natural sediment structure was carefully maintained within cores and kept under *in situ* temperatures, an important factor in understanding and comparing contaminated sediments as was recently stressed (Acosta-González and Marqués, 2016). The use of OSPs enabled retention of oil within the top centimeter of sediment and aimed at replicating heavy oiling of sediments. This likely resulted in the formation of anoxic micro-niches as witnessed at previous spill sites (Yang et al., 2016) and represents a realistic microcosm. SD25 use aimed to simulate continuous application of dispersant at the wellhead as may be performed in a future oil spill response. To replicate appropriate field conditions, whole-core incubations were run and subjected to seawater flushing to ensure an aerobic water column and sediment-water interface, and replenishment of natural seawater nutrients. Flushing would have resulted in mobilization of oil from the sediment and removal of oil from the system, effectively diluting the oil concentration within the water column over time as expected *in situ*.

Hydrocarbon Transport Into Sediments

Hydrocarbons from all fractions were detected below 4 cm deep from day 7 to day 71 suggesting that the hydrocarbons in the model oil have the capability of entraining undisturbed FSC sediments relatively rapidly (**Figures 1**, **2**, Supplementary Figures 7–9). Limited transport of C20–24 aliphatics and higher ring-number PAHs can be attributed to two key factors: (1) interactions with the minerals and organic matter in the upper centimeters of sediment (Stoffyn-Egli and Lee, 2002) and (2) continuous water replacement of supernatant water promoting the partition to the water column over intra-sediment transport. However, on day 71 C10 aliphatics were not detected below 2 cm in either treatment and C12+ aliphatics were not detected over 3 cm in the OD-treatment suggesting that these hydrocarbons may have been biodegraded in deep FSC sediments within 71 days. Metagenomic profiling of sub-surface sediments at a depth of 1.5–3 cm within 3 km of the well following the DwH spill revealed the dominance of known hydrocarbon degraders from the class δ-Proteobacteria (Kimes et al., 2013). The effect of SD25 was not found to be significant for individual hydrocarbons, it was significant for the Treatment × Depth × Time interaction (**Figures 1**, **2**, Supplementary Figures 7–9). Therefore, the influence of SD25 on post-depositional transport of hydrocarbons in this work was unclear. C22–24 aliphatics were not detected below 2 cm deep at any time point suggesting limited transport of longer chained aliphatics. This is in agreement with the corresponding calculated D_{eff}, which are the lowest of the model oil components (12.8–12.9 cm year^{-1}). As found for aliphatics, BTEX and PAHs were also more abundant in day 42 than in day 71 over 1 cm deep (Supplementary Figures 7–9) and may have been degraded during the experiment. There is evidence for local 1000 m deep sediment microbial communities degrading these hydrocarbons (Ferguson et al., 2017). This is further supported by findings of microbial shifts at 1–2 cm from day 42 (Supplementary Figure 17). The entrainment of hydrocarbons over 4 cm deep into FSC sediments is of significance because it will encourage the consumption of oxygen by aerobic microbes and deplete available oxygen in the sediment rendering it anoxic. This suggests that hydrocarbon biodegradation beyond this will be limited by oxygen availability and dominated by anaerobic microbial communities (Widdel et al., 2010). There was no detected shift in the microbial community structure below 2 cm deep suggesting slower rates of metabolism than in surficial sediments. The half-life of PAHs in the GoM has been shown to be twice as long in sediments over 1000 m deep than at 100–150 m deep (Tansel et al., 2011). Due to the cold deep water temperatures in the FSC, PAH residence times are expected to be higher than in the GoM. Discussion on hydrocarbon transport in modified sediments can be found in the Supplementary discussion.

In undisturbed sediments, the application of SD25 increased K_{ws} values of most hydrocarbons with the exception of BTEX and naphthalene, the most water-soluble components of the model oil, but the interaction was not significant over time (**Figure 3**, Supplementary 10–12). In contrast, the effect of SD25 application in modified sediments changed significantly over time and followed a similar trend for most hydrocarbons revealing a time lag in K_{ws} which was consistent across hydrocarbons (Supplementary Figure 15). K_{ws} values increased from day 7 to day 21 to equilibrate with the supernatant water phase in both treatments. In 42-day incubations, K_{ws} values decreased for the O- but not for the OD-treatment. The decrease in K_{ws} in the O-treatment can be explained by desorption hysteresis, whereby hydrocarbons adsorbed onto fine particles and organic matter can remain adsorbed despite experiencing conditions

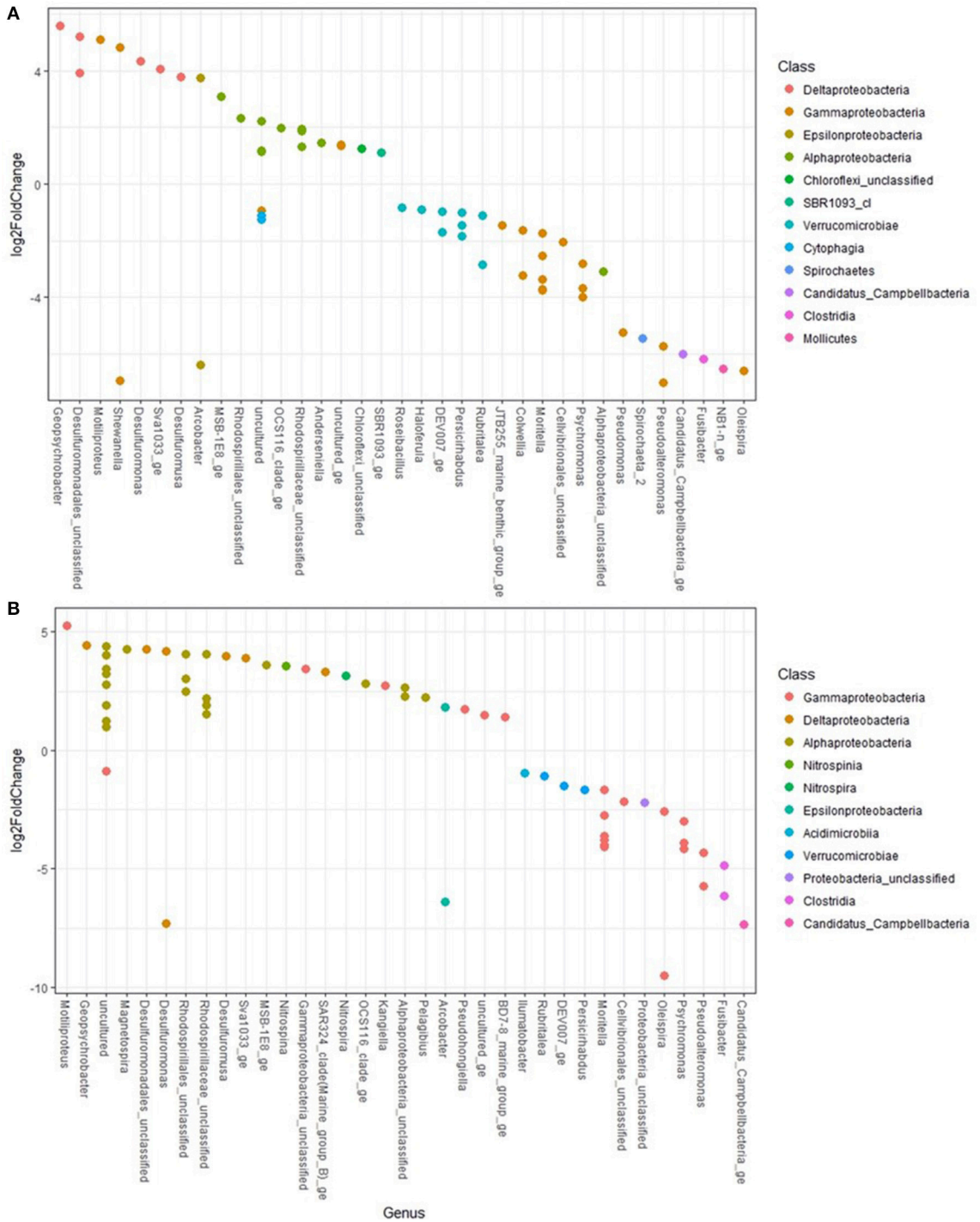


FIGURE 6 | Differential abundance testing of taxa between treatments using *DESeq2* found in **(A)** control (+log2FoldChange) and oil treatments (-log2FoldChange) and **(B)** control (+) and oil and dispersant treatments (–).

which would favor partition to the water column (Gong et al., 2014b). The sustained K_{ws} values in the OD-treatment suggest that SD25 application facilitates the partitioning of hydrocarbons to the water column and reduces the impact of hysteresis. This contradicts the findings of Zhao et al. (2015) where they show that PAH uptake increases with SD25 application. In their experimental setup the [hydrocarbon] used are much lower than those used here (0–6000 mg l^{-1} for naphthalene and 1-methylnaphthalene, 0–280 mg l^{-1} for pyrene) suggesting that this effect is only prevalent at low [hydrocarbon].

Bacterial Community Response Following Hydrocarbon Entrainment

Diverse microbial communities inhabit deep sea sediments and their structure and diversity is dependent upon environmental conditions such as depth and organic carbon content (Biddle et al., 2011). These microbes are believed to make up a "seed bank" of taxa which are niche-dependent (Gibbons et al., 2013). Certain bacterial taxa capable of utilizing hydrocarbons are present within the seed bank in very small numbers and bloom once provided with their preferred carbon source (Syutsubo et al., 2001; Head et al., 2006). Differential abundance testing identified certain taxa that were undetectable in controls but were able to respond to oil exposure. Shifts in relative abundance resulted in a modification to the richness, evenness and subsequently diversity of microbial communities. A significant reduction in diversity of oil contaminated samples compared to control samples over time in this study agrees with previous work (Hazen et al., 2010; Baelum et al., 2012; Dubinsky et al., 2013). Yet, the continued presence of a "core" group revealed by both molecular analyses suggests tolerance of selected sediment bacterial communities. This could be caused by the incubation design, whereby the environment/sediment provided a buffering effect, mitigating the impact of oil as opposed to intrusive incubation methods that more readily expose communities to toxic fractions of oil such as slurries and liquid incubations.

Following the sedimentation pulse during DwH, metagenomic analysis of surficial sediments revealed OTU dominance of uncultured γ-Proteobacteria and *Colwellia* spp. (Mason et al., 2014b). *Colwellia* was identified here as most responsive to hydrocarbon exposure in oiled treatments. *Colwellia* is believed to play an active role in hydrocarbon degradation throughout the oceans (Valentine et al., 2010; Redmond and Valentine, 2012; Mason et al., 2014a) and within sediments (Mason et al., 2014b). Strains identified in this study had similarity of 100% to *C. rossensis* strain ANT9279 isolated from Arctic sea ice (Brinkmeyer et al., 2003) and 99% to *C. psychrerythraea* isolated from Arctic sea sediment, confirming its ability to function in cold environments. Both of these strains were matched to clones from a library constructed from cold DwH plume waters and linked to short chain alkane degradation (Valentine et al., 2010) and PAH degradation in surface slick and plume samples (Gutierrez et al., 2013). Single cell genomic analysis of a *Colwellia* strain from the DwH hydrocarbon plume (matching 84% to *C. psychrerythraea* 34H) revealed the organism has genes for denitrification, adaptations to cold environments, nutrient acquisition and hydrocarbon degradation (Mason et al., 2014a). The presence of denitrification genes suggest anaerobic respiration capability, which would allow growth in areas of oxygen depletion as witnessed within the DwH plume (Joye et al., 2011; Kessler et al., 2011) and in anoxic sediments (Yang et al., 2016). It is plausible to find both aerobic and anaerobic organisms in the same location; Yang et al. (2016) identified both aerobic α-Proteobacteria and anaerobic δ-Proteobacteria within the same site surrounding the Macondo well. This is concurrent with evidence in this study where from days 21 in both O- and OD-treatments over 50% of dominant taxa were a combination of *Colwellia* and *Fusibacter* genera which are capable of anaerobic respiration. Yang et al. (2016) showed this may be the result of heterogeneous redox conditions forming within the sediment due to smothering by oil residues and the development of anoxic micro-niches. *Fusibacter* are generally halotolerant fermentative anaerobes capable of reducing a range of sulfur species and often isolated at mesophilic temperatures (Serrano et al., 2017). Several strains have been isolated from hydrocarbon related environments including the type strain *Fusibacter paucivorans* isolated from an oil-producing well (Ravot et al., 1999) and *Fusibacter bizertensis* isolated from a corroded kerosene storage tank (Smii et al., 2015). However, this genus is yet to be directly linked to PAH degradation (Kappell et al., 2014) and the ability of Firmicutes to form endospores may infer an ability to survive when other organisms cannot.

Numerous known hydrocarbon degraders were detected in this study including *Pseudoalteromonas*, *Oleispira*, *Pseudomonas* and *Moritella*. *Pseudoalteromonas* is regularly found in oil polluted environments such as surface oil slicks (Yang et al., 2014), the water column (Chakraborty et al., 2012; Gutierrez et al., 2013; Chronopoulou et al., 2014), deep sea sediments (Yang et al., 2016) and coastal ecosystems (Kostka et al., 2011; Kappell et al., 2014). *Pseudoalteromonas* is commonly linked to PAH degradation and can thrive once aliphatic resources are low or depleted (McKew et al., 2007; Dubinsky et al., 2013) and increased in relative abundance by day 71 in O-treated incubations. *Oleispira* was significantly more abundant in O-treatment incubations in this study and is active in cold environments (Yakimov et al., 2003; Kube et al., 2013; Gentile et al., 2016). *Pseudomonas* can degrade various structured hydrocarbons from cold environments (Bacosa et al., 2010; Viggor et al., 2013). Relative abundance data suggests *Pseudomonas* phylotypes were most prominent toward the end of the incubation period. However, in a simulation study of the DwH spill *Pseudomonas* were most abundant at 6 days of incubation with crude oil (Hu et al., 2017). *Moritella* is also capable of hydrocarbon degradation (Bagi et al., 2014) and was dominant by day 7 but then decreased in relative abundance over time. In addition, several *Moritella* species have been classified as piezophiles (Yanagibayashi et al., 1999; Xu, 2003) as has *Colwellia* (Oger and Jebbar, 2010) and these physiological traits reflect their sampling environment. Most interesting was the enrichment of Candidatus Campbellbacteria which was undetectable (<0.05% relative abundance) in controls but rose to 6% by day 71 in the O-treatment. Candidatus Campbellbacteria belongs to Parcubacteria and has been detected in marine sediments and anoxic environments including the Mariana Trench (León-Zayas et al., 2017). Although never cultured, it has been detected in hydrocarbon polluted environments (Salam et al., 2017).

Effects of Dispersant Application on Bacterial Community Structure

Commercial dispersants have long been used in oil spill response efforts and there are a wide variety of US EPA approved dispersants on the market (Kleindienst et al., 2015a) most notably Corexit 9500 and 9527, both used in the DwH clean-up strategy (Seidel et al., 2015). In the UK, one of the approved and most readily available dispersants is SD25 which was found to be less toxic than Corexit varieties (Scarlett et al., 2005). Therefore, it was

used in this study as an oil spill remediation treatment to assess the effect on hydrocarbon transport in sediments and bacterial community composition.

The application of SD25 had a significant effect on microbial community composition and selected for a different range of taxa, although there were similarities between O- and OD-treatments. *Fusibacter* was strongly selected for by SD25 application and this trend has never been witnessed before. The presence of an additional carbon-rich substrate may have allowed *Fusibacter* to thrive, outcompeting other taxa. Additionally, the increased solubilization of oil in seawater by SD25 may have allowed *Fusibacter* to utilize hydrocarbons faster than other taxa. Community shifts within the OD-treatment followed a similar pattern to the O-treatment; however, it appeared to occur faster. *Moritella* was initially selected for, followed by *Colwellia,* as was apparent in both O- and OD-treatments. Yet, in OD-treatment the decline of *Colwellia* occurred in day 42 and was succeeded by *Fusibacter*.

Dispersant has been reported to significantly increase hydrocarbon degradation and enrich the presence of hydrocarbon degrading bacteria (Baelum et al., 2012). SD25 was found to significantly enhance oil degradation rates by communities obtained from 1,000 m in the FSC however, there were no changes in community structure (Ferguson et al., 2017). Conversely, addition of dispersant has been shown to have limited, if any, impact on biodegradation (Foght and Westlake, 1982; Macías-Zamora et al., 2014) and in one case dispersant suppressed the activity of natural oil-degrading microorganisms (Kleindienst et al., 2015b). Thus, the notion that SD25 may have modified the bacterial response compared to oil only treatment may not necessarily mean that it enhanced biodegradation. Dispersants dissolve and disperse oil in water, leading to an increased oil droplet surface area and potential biodegradation stimulation (Kleindienst et al., 2015a). However, sediment particles present an increased surface area for oil adsorption and may reduce dispersant effectiveness (Macías-Zamora et al., 2014). It is often not possible to discern whether the microbial community shift is due to increased oil biodegradation or other influencing factors. Lindstrom and Braddock (2002) observed quicker mineralization of Corexit EC9500A than that of crude oil and dispersed oil implying that Corexit dispersant offered an alternative carbon source and was more selectively degraded. Whether this detracts from oil removal or positively "conditions" the microbial community for oil degradation remains to be answered.

CONCLUSION

This study assessed the transport of hydrocarbons and subsequent microbial response in FSC sediments following OSP deposition, in the presence and absence of SD25. This is the first study to replicate heavy oiling of naturally stratified deep sea sediments. Conditions were kept representative by maintaining *in situ* temperatures, replenishing seawater and continuously applying dispersant. A deep water oil spill in the FSC would likely have profound impacts on benthic ecosystems due to its unique oceanographic conditions (Bett, 2012). Biodegradation rates are reduced at low temperatures (Sharma and Schiewer, 2016; Ferguson et al., 2017). Additionally, low temperatures will enhance the extent of adsorption of hydrocarbons to sediments (Delle Site, 2001; Zhao et al., 2015). Adsorption of oil by sediment has been shown to promote biodegradation by capturing hydrocarbons (Yang et al., 2008). However, sequestration of hydrocarbons by sediments may also reduce their bioavailability in the long term through entrainment into organic matter and particle micropores that are inaccessible to microbes (Haritash and Kaushik, 2009).

The results of this study provide realistic data regarding hydrocarbon transport, mobilization and the ability of natural bacterial populations to respond and will aid in oil spill response decision-making in the UK. Key findings were:

(1) Deposited oil was transported to over 4 cm deep into FSC sediments following OSP deposition within the timeframe of the experiment, indicating that hydrocarbons can entrain deep into FSC sediments. Microbial shifts below 2 cm in the sediment were witnessed from 42 days onwards.
(2) The use of modified sediment matrix resulted in enhanced oil transport into FSC sediments with respect to undisturbed sediments. This highlights the importance of considering sediment handling and pre-treatment which may influence patterns of hydrocarbon transport.
(3) Deposition of hydrocarbons on surficial sediments forced bacterial communities to undergo significant shifts. The bacterial community response to oil exposure was related to members of the genera *Colwellia, Fusibacter* and *Pseudoalteromonas*. Microbial profiling demonstrated that shifts were primarily taking place within the top centimeter of sediment from day 7.
(4) SD25 application in undisturbed sediments increased the K_{ws} values of all hydrocarbons except C10 aliphatics, BTEX components and naphthalene indicating that SD25 selectively enhances the water-solubility of oil components. In modified sediments, SD25 application had a similar effect for all hydrocarbons.
(5) SD25 application resulted in a significant change in the bacterial community structure with strong dominance of *Fusibacter*. Shifts at parallel time points suggest an accelerated and modified response at the presence of dispersant.

The results of this study provide insight into hydrocarbon transport and subsequent microbial community shifts in FSC deep sediments following OSP deposition and the role of SD25 on these processes.

AUTHOR CONTRIBUTIONS

LJP, LDP, EG, UW, and JA: conceived the study; LJP, LDP, and AG: collected the samples; LJP and LDP: conducted the experiments; LJP, LDP, and TC: designed the statistical analysis for hydrocarbon transport and LJP, LDP, and CG-R designed the microbial community analyses. LJP and LDP: wrote the manuscript with input from all authors.

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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.2018.00159/full#supplementary-material

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