The contribution of microbial communities in polymetallic nodules to the diversity of the deep-sea microbiome of the Peru Basin (4130 − 4198 meter depth)

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Abstract. Industrial-scale mining of deep-sea polymetallic nodules will remove nodules in large areas of the seafloor. The regrowth of the nodules by metal precipitation is estimated to take millions of years. Thus, for future mining impact studies, it is crucial to understand the role of nodules in shaping microbial diversity and function in deep-sea environments. Here we investigated microbial community composition based on 16S rRNA gene sequences retrieved from sediments and nodules of the Peru Basin (4130 − 4198 m water depth). The nodule field of the Peru Basin showed a typical deep-sea microbiome, with dominance of the classes Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, and Acidimicrobiia. Nodules and sediments host distinct bacterial and archaeal communities, with nodules showing lower diversity and a higher proportion of sequences related to potential metal-cycling bacteria (i.e. Magnetospiraceae, Hyphomicrobiaceae), bacterial and archaeal nitrifiers (i.e. *AqS1*, unclassified Nitrosomonadaceae, *Nitrosopumilus*, *Nitrospina*, *Nitrospira*), and bacterial sequences found in ocean crust, nodules, hydrothermal deposits and sessile fauna. Sediment and nodule communities overall shared a low proportion of Operational Taxonomic Units (OTU; 21 % for Bacteria and 19 % for Archaea). Our results show that nodules represent a specific ecological niche (i.e. hard substrate, high metal concentrations, and sessile fauna), with a potentially relevant role in organic carbon degradation. Differences in nodule community composition (e.g. Mn-cycling bacteria, nitrifiers) between the Clarion-Clipperton Fracture Zone (CCZ) and the Peru Basin suggest that changes in environmental setting (e.g. sedimentation rates) play also a significant role in structuring the nodule microbiome.

1 Introduction

Polymetallic nodules (or manganese nodules) occur in abyssal plains (4000−6000 m water depth) and consist primarily of manganese and iron, as well as many other metals and rare earth elements (Crerar and Barnes, 1974; Kuhn et al. 2017). Nodules are potato- or cauliflower-shaped structures with typical diameters of 4−20 cm and are typically found at the sediment surface or occasionally buried in the uppermost 10 cm sediment horizon. The mechanisms of nodule formation are not completely elucidated. The current understanding is that they are formed via mineral precipitations from bottom waters (*Hydrogenetic* growth) or pore waters (*Diagenetic* growth) involving both abiotic and microbiological processes (Crerar and Barnes, 1974; Riemann, 1983; Halbach et al., 1988; Wang et al., 2009). The formation of nodules is a slow process that is estimated to range between thousands and millions of years per millimetre growth (Kerr, 1984; Boltenkov, 2012).

Rising global demand for metals has renewed interests in commercial mining of deep-sea nodule deposits. Mining operations would remove nodules, disturb or erode the top decimeters of sediment, and create near bottom sediment plumes that will resettle and cover the seafloor (Miller et al., 2018). Although the first nodules have been discovered in the 1870's (Murray, 1891), only little is known about the biodiversity, biological processes and ecological functions of the nodules and their surrounding sediments as specific deep-see habitat. Major questions remain, for example as to spatial turnover on local and global scales, the role of the microbial community in and around nodules, and the role of nodules as substrate for endemic species. Hence, there is the need to thoroughly characterize baseline conditions as a requirement for any mining operations as these will require assessments of impacts associated with mining.

Extensive and dense nodule fields are found in different areas of the Pacific and Indian Oceans. Nodule accumulations of economic interest have been found in four geographical locations: the Clarion-Clipperton Fracture Zone (CCZ) and the Penrhyn Basin in the central north and south Pacific Ocean, respectively; the Peru Basin in the south-east Pacific; and in the center of the northern Indian Ocean (Miller et al., 2018). To our knowledge the Peru basin is the only region that does not have exploration activities and plans for mining so far. Previous work on the structure of microbial communities of nodule fields by 16S rRNA gene sequencing focused on the CCZ and the central South Pacific Ocean (Xu et al., 2007; Wu et al., 2013; Tully and Heidelberg, 2013; Blöthe et al., 2015; Shulse et al., 2016; Lindh et al., 2017). All studies showed that polymetallic nodules harbour microorganisms that are distinct from the surrounding sediments and overlying water. They indicate that nodule communities show a pronounced spatial variability, but these results are so far not conclusive. Similar microbial communities were observed in nodules collected at distances of 6000 km and 30 km (Wu et al., 2013; Shulse et al. 2016), while Tully and Heidelberg (2013) found that nodule communities varied among sampling sites (<50 km). Besides, potential Mn-oxidizers and -reducers such as *Alteromonas*, *Pseudoalteromonas*, *Shewanella* and *Colwellia* were proposed as a core of the nodule microbiome involved in the formation of nodules (Wu et al. 2013; Blöthe et al., 2015), but they were not found in all nodules sampled so far (Tully and Heidelberg, 2013; Shulse et al. 2016). The lack of knowledge on the diversity and composition of microbial assemblages of other nodule provinces makes it difficult to

assess whether observed differences within the CCZ may reflect regional differences in environmental conditions (e.g. input of organic matter, bathymetry, topography, sediment type), or in abundance and morphology of nodules, or in the colonization of the nodules by epifauna and protozoans.

In this study we investigated the diversity and composition of bacterial and archaeal communities associated with manganese nodule fields of the Peru Basin. The Peru Basin is located about 3000 km off the coast of Peru and covers about half of the size of the CCZ, which is 5000–9000 km away. The present-day organic carbon flux in this area is approximately two times higher than in the CCZ, resulting in higher content of organic carbon in the surface sediments (>1 % *vs* 0.2−0.6 % in the CCZ), and a shallower oxic-suboxic front (10 cm *vs* tens of meters sediments depth in the CCZ; Müller et al., 1988; Heackel et al., 2001; Volz et al., 2018). As a consequence of differences in environmental conditions (e.g. organic carbon flux, carbonate compensation depth, sediment type, topography and near-bottom currents), the Peru basin and the CCZ host manganese nodules with different geological features (Kuhn et al. 2017). This includes: i) nodules from the Peru Basin are often larger, with a typical cauliflower shape, compared to those in CCZ that have a discoidal shape and a size of 2-8 cm (Kuhn et al. 2017); ii) average nodule abundance in the Peru Basin is lower (10 kg m^{-2}) than in CCZ (15 kg m^{-2}; Kuhn et al. 2017); iii) Mn nodules from the Peru Basin are thought to be mainly formed by suboxic diagenesis, whereas CCZ nodules apparently exhibit a mixture of diagenetic and hydrogenetic origin (von Stackelberg 1997; Chester and Jickells 2012); iv) while Peru Basin and CCZ nodules consist of the same type of mineral (disordered phyllomanganates), they have a different metal content (Wegorzewski and Kuhn 2014; Wegorzewski et al. 2015).

An increasing number of studies and policy discussions address the scientific basis of ecological monitoring in deep-sea mining, highlighting the need to identify appropriate indicators and standards for environmental impact assessments and ecological management. A key aspect is avoiding harmful effects to the marine environment, which will have to include loss of species and ecosystem functions. The primary aims of this study were to assess the structure and similarity of benthic microbial communities of nodules and sediments of the Peru Basin nodule province, and to compare them with those of other global deep-sea sediments and nodules in the CCZ. The focus was on similarity comparisons in order to investigate endemism and potential functional taxa that could be lost due to the removal of manganese nodules by mining activities. To achieve this, the hypervariable 16S rRNA gene regions V3-V4 for Bacteria and, V3-V5 for Archaea were amplified from DNA extracted from nodules and surrounding sediments and sequenced using the Illumina paired-end MiSeq platform. The hypotheses tested were i) nodules shape deep-sea microbial diversity, ii) nodules host a specific microbial community compared to the surrounding sediments. Secondary aim of this study was to investigate the nodule features that may play a major role in shaping microbial community composition and microbially-mediated functions.

2 Methods

2.1 Sample collection

Sediment samples and polymetallic nodules were collected as a part of the MiningImpact project of the Joint Programming Initiative JPI Healthy and Productive Seas and Oceans (JPI Ocean) on board of R/V Sonne (expedition SO242/2; 28th of August - 1st of October 2015) in the Peru Basin around 7° S and 88.5° W. Samples were collected at three sites outside the seafloor area selected in 1989 for a long-term disturbance and recolonization experiment (DISCOL; Thiel et al., 2001), for this reason they were called "Reference Sites": Reference East, Reference West, and Reference South. Sediment samples were collected using TV-guided MUltiple Corer (TV-MUC) at three stations per site (Table 1). The cores were sliced on board in a temperature-controlled room (set at *in situ* temperature), and aliquots of sediment were stored at −20 °C for DNA extraction. Manganese nodules where sampled, using a TV-MUC, or a Remotely Operated Vehicle (ROV KIEL6000, GEOMAR, Germany): one nodule at Reference West and four nodules at Reference South. The nodules were partly located at the surface or buried down to 3 cm below the seafloor (bsf) with diameters of a few cm. Nodules were gently rinsed with 0.22-µm filtered cold bottom seawater to remove adhering sediment, stored in sterile plastic bags at −20 °C and crushed before DNA extraction in the home lab. From the nodules collected with the ROV, only the surface layer was scraped off using a sterile spoon, and subsequently crushed and frozen (−20 °C). Sedimentary metadata (e.g. cell counts, pigments and organic carbon content, porewater profiles, and porosity) and a map of the study area are available in Vonnahme at al. (in press). Focusing entirely on sediments, that publication also includes a discussion of the variability of environmental settings and microbial communities.

2.2 DNA extraction and sequencing

The DNA was extracted from 1 g of wet sediment (0-1 cm layer) and from 1 g of wet nodule's fragments using the FastDNA™ SPIN Kit for Soil (Q-BIOgene, Heidelberg, Germany) following the protocol provided by the manufacturer. An isopropanol precipitation was performed on the extracted DNA, and DNA samples were stored at −20 °C. As control for DNA contamination (negative control), DNA extraction was carried out on purified water after being in contact with sterile scalpel and plastic bag.

Amplicon sequencing was done at the CeBiTec laboratory (Centrum für Biotechnologie, Universität Bielefeld) on an Illumina MiSeq machine. For the 16S rRNA gene amplicon library preparation we used the bacterial primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATC TAATCC-3′), and the archaeal primers Arch349F (5′-GYGCASCAGKCGMGAAW-3′) and Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Wang and Qian, 2009; Klindworth et al., 2013), which amplify the 16S rRNA gene hypervariable region V3-V4 in Bacteria (400−425 bp fragment length) and the V3-V5 region in Archaea (510 bp fragment length). The amplicon library was sequenced with the MiSeq v3 chemistry, in a 2x300 bp paired run with >50,000 reads per sample, following the standard instructions of the 16S rRNA gene Metagenomic Sequencing Library Preparation protocol (Illumina, Inc., San Diego, CA, USA).

149 The quality cleaning of the sequences was performed with several software tools. CUTADAPT
150 (Martin, 2011) was used for primer clipping. Subsequently the TRIMMOMATIC software (Bolger et
151 al., 2014) was used to remove low-quality sequences starting with the following settings:
152 SLIDINGWINDOW:4:10 MINLEN:300 (for Bacteria); SLIDINGWINDOW:6:13 MINLEN:450 (for
153 Archaea). In case of bacteria data this step was performed before the merging of reverse and forward
154 reads with PEAR (Zhang et al., 2014). Low-quality archaeal sequences were removed after merging
155 the reads in order to enhance the number of retained reads due to the increase in archaeal 16S rRNA
156 gene fragment length. All sequences were quality controlled with FastQC (Andrews, 2010). Where
157 necessary, more sequences were removed with TRIMMOMATIC with larger sliding window scores
158 until the FastQC quality control was passed (average quality score per sample >34 for Bacteria and >22
159 for Archaea). Clustering of sequences into OTUs (operational taxonomic units) was done using the
160 SWARM algorithm (Mahé et al., 2014). The taxonomic classification was based on the SILVA rRNA
161 reference database (release 132), at a minimum alignment similarity of 0.9, and a last common ancestor
162 consensus of 0.7 (Pruesse et al., 2012). Workflow and scripts applied in this study can be found in
163 Hassenrück et al. (2016). Raw sequences with removed primer sequences were deposited at the
164 European Nucleotide Archive (ENA) under accession number PRJEB30517 and PRJEB32680; the
165 sequences were archived using the service of the German Federation for Biological Data (GFBio;
166 Diepenbroek et al., 2014).
167 The total number of sequences obtained in this study is reported in Table S1. Absolute singletons
168 (SSOabs), i.e. OTUs consisting of sequences occurring only once in the full dataset (Gobet et al., 2013)
169 were removed (Table S1). Similarly, contaminant sequences (as observed in the negative control) and
170 unspecific sequences (i.e., bacterial sequences in the archaeal amplicon dataset, and archaeal,
171 chloroplast, and mitochondrial sequences in the bacterial dataset) were removed from amplicon data
172 sets before the analysis (Table S1). The dominant OTU sequences and OTU sequences highly abundant
173 in the nodules were subjected to BLAST search (BLASTn; GeneBank nucleotide database 12/06/2019;
174 Altschul et al., 1990) in order to identify in which others habitats the closest related (i.e. >99 %)
175 sequences have been previously reported.

176 **2.3 Data analysis**

177 The first three Hill Numbers, or the effective number of species, were used to describe alpha-diversity:
178 species richness (H_0), the exponential of Shannon entropy (H_1), and the inverse Simpson index (H_2;
179 Chao et al., 2014). Coverage-based and sample-size-based rarefaction (based on actual number of
180 sequences) and extrapolation (based on double number of sequences) curves were calculated for the
181 Hill's numbers using the R package iNEXT (Hsieh et al., 2018). Calculation of the estimated richness
182 (Chao1) and the identification of unique OTUs (present exclusively in one sample) were based on
183 repeated (n = 100) random subsampling of the amplicon data sets. Significant differences in alpha-
184 diversity indices between substrates (i.e. manganese nodules and sediments) were determined by
185 analysis of variance (ANOVA), or by non-parametric Kruskal-Wallis test (KW) when ANOVA's
186 assumptions were not satisfied.

Beta-diversity in samples from different substrates and from different sites was quantified by calculating an Euclidean distance matrix based on centred log-ratio (CLR) transformed OTU abundances (function *clr* in R package compositions) and Jaccard dissimilarity based on a presence/absence OTU table. The latter was calculated with 100 sequence re-samplings per sample on the smallest dataset (40613 sequences for Bacteria and 1835 sequences for Archaea). Euclidean distance was used to produce non-metric multidimensional scaling (NMDS) plots. The Jaccard dissimilarity coefficient was used to perform hierarchical clustering (function *hclust* in R package vegan, using the complete linkage method), and the dissimilarity values for cluster nodes were used to calculate the number of shared OTUs between/within groups. The permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used to test difference in community structure and composition.

Differentially abundant OTUs and genera were detected using the R package ALDEx2 (Fernandes et al. 2014) at a significance threshold of 0.01 and 0.05 for Benjamini–Hochberg (BH) adjusted parametric and non-parametric (KW) P-values, respectively. We only discuss the taxa that were at least two times more abundant in nodules than in sediments (i.e. (Log2(Nodule/sediment) ≥1) and with a sequences contribution of total number of sequences ≥1 % (for genera) or ≥0.1 % (for OTUs).

All statistical analyses were conducted in R using the core distribution with the additional packages vegan (Oksanen et al. 2015), compositions (Van den Boogaart et al., 2014), iNEXT (Hsieh et al., 2018), and ALDEx2 (Fernandes et al. 2014).

3 Results

3.1 Microbial alpha-diversity

Bacterial and archaeal communities in 5 nodules and 9 sediment samples (Table 1) were investigated using specific sets of primers for Bacteria and Archaea on the same extracted pool of DNA per station. The number of bacterial sequences retrieved from DNA extracted from sediments and nodules was on average 5±5 and 25±14 times higher, respectively, than those obtained for archaea (t-test: $p<0.001$, df=11, t=4.5).

Table S1 shows the statistics of sequence abundance and proportion of singletons and cosmopolitan types. Sequence abundances of bacteria were comparable between sediments and nodules. Cosmopolitan OTU, i.e. those present in 80 % of the sediments and nodule samples, were only 9 % of all taxa (77 % of all sequences), whereas rare OTUs occurring only in <20 % of all samples represented 50 % the taxa (4 % of all sequences). Sediments *vs* nodules contained only 4 and 2 %, respectively, of endemic taxa, defined as those were abundant in either substrate but rare in the other. Thus the contribution of unique OTUs to the total number of OTUs was lower in manganese nodules than in sediments samples (Table 2, Figure 1a). Bacterial and archaeal diversity was investigated calculating the total number of OTUs (Hill number q=0; H_0) and the estimated richness (Chao1), and the unique OTUs (present exclusively in one station). For this analysis, the latter were calculated with sequence re-sampling, to overcome differences in sequencing depth. Abundance-based coverage estimators, exponential Shannon (Hill number q=1; H_1) and inverse Simpson (Hill number q=2; H_2),

were also calculated. The rarefaction curve indicates that the richness (H_0) of the less abundant and rare OTUs was somewhat underestimated both in nodules and in sediments (Figure S1 a-b). However, the bacterial and archaeal diversity was well described for the abundant OTUs (H_1 and H_2; Figure S1 a-b); with more than 90 % of the estimated diversity covered (Figure S1 c-d). Both in sediments and nodules the alpha-diversity indices were higher for Bacteria than for Archaea (t-test: $p<0.0001$, df=12, t=8.0−16.0), while the contribution of unique OTUs to the total number of OTUs was comparable (Table 2). Bacterial communities in manganese nodules have lower Hill numbers and Chao1 indices compared to those associated to sediments (Table 2, Figure 1a). Archaeal communities showed the same patterns for diversity indices and unique OTUs, with exception for the H_2 index that did not show significant difference between nodules and sediments (Table 2, Figure 1b).

3.2 Patterns in microbial community composition

The changes in microbial community structure at OTU level (beta-diversity) between substrates and samples were quantified by calculating Euclidean distances from CLR transformed OTU abundance. Shared OTUs were estimated by calculating Jaccard dissimilarity from OTU presence/absence based on repeated random subsampling of the amplicon data sets. Microbial communities associated with manganese nodules differed significantly from those found in the sediments (Figure 2, Table S2). Also, significant differences were detected in sediment microbial community structure among the different sites (PERMANOVA; Bacteria: $R^2 = 0.384$; $p = 0.003$; $F_{2,8} = 1.87$; Archaea: $R^2 = 0.480$; $p = 0.013$; $F_{2,8} = 2.31$; Table S2), and between communities associated with nodules and sediment at Reference South site (PERMANOVA; Bacteria: $R^2 = 0.341$; $p = 0.023$; $F_{1,6} = 2.59$; Archaea: $R^2 = 0.601$; $p = 0.029$; $F_{1,6} = 7.53$; Table S2), which was the only site where the number of samples allowed for the test. “Site” defined by geographic location, and “Substrate”, i.e. origin from sediment or nodule, explained a similar proportion of variation in bacterial community structure (27 % and 23 %, respectively). “Substrate” had a more important role in shaping archaeal communities than “Site” (explained variance 35 % and 19 %, respectively; Table S2). The number of shared OTUs between nodules and sediments (Bacteria: 21 %; Archaea: 19 %) was lower than those shared within nodules (Bacteria: 30 %; Archaea: 30 %) and within sediments (Bacteria: 31 %; Archaea: 32 %) (Figure S2).

Bacterial communities in manganese nodules and sediments were dominated by the classes Gammaproteobacteria (26 %), Alphaproteobacteria (19 %), Deltaproteobacteria (9 %), Bacteroidia (5 %), Acidimicrobiia (4 %), Dehalococcoidia (4 %), Planctomycetacia (4 %), Nitrospinia (3 %), and Phycisphaerae (3 %), which accounted for more than 75 % of the total sequences (Figure 3). All archaeal communities were dominated by Thaumarchaeota (*Nitrosopumilales*), which represented more than 95% of all sequences. The remaining small proportion of sequences was taxonomically assigned to *Woesearchaeia* (Figure S2b). Nodule and sediment samples showed similar compositions of most abundant bacterial genera (contribution to total number of sequence ≥1 %; Figure S2a). 69 bacterial genera (9 % of all genera) were differentially abundant in the nodules and in the sediment, accounting for 36 % and 21 % of total sequences retrieved from nodules and sediments, respectively (ALDEx2: ANOVA adjusted $p<0.01$ and KW adjusted $p<0.05$; Figure 4 and Table S3). Of those only one unclassified genus within the family of Sphyngomonadaceae and the genus *Filomicrobium* were

exclusively found in nodules and not in the sediment samples, and their contribution to the total number of sequences was less than 0.06 %. Genera that were more abundant in the nodules than in the sediments included: unclassified Alphaproteobacteria (7 %), *Nitrospina* (4 %), unclassified SAR324 clade (Marine group B; 3 %), unclassified Hyphomicrobiaceae (3 %), Pirellulaceae Pir4 lineage (2 %), unclassified Methyloligellaceae (1 %), unclassified Pirellulaceae (1 %), *Acidobacteria*, unclassified Subgroup 9 (1 %) and Subgroup 17 (1 %), Nitrosococcaceae *AqS1* (1 %), Calditrichaceae *JdFR-76* (1 %), and *Cohaesibacter* (1 %) (Figure 4 and Table S3). In the sediment we identified 21 genera that were more abundant than in the nodules, but all together they represented only 3 % of total sequences recovered from sediments. 128 OTUs were highly abundant in nodules (ALDEx2: ANOVA adjusted $p<0.01$ and KW adjusted $p<0.05$), which accounts for 24 % of total sequences retrieved from nodules (Table 3a). The closest related sequences (≥99 % similarity) were retrieved from ocean crusts (30 %), from nodule fields (26 %), from hydrothermal/seep sediments and deposits (21 %), from worldwide deep-sea sediments (16 %), and associated to invertebrates (7 %; Table 3b and Figure 5).

4 Discussion

Industrial-scale mining of deep-sea polymetallic nodules may remove nodules and the active surface seafloor layer at a spatial scale ranging from ca. 50000−75000 km^2 per claim to ca. 1 million km^2 including all current exploration licences (Miller et al., 2018). The regrowth of nodules will take millions of years, thus it is unknown if the associated biota could recover at all (Simon-Lledo et al., 2019). The response of microbial communities to the loss of nodules and seafloor integrity is largely unknown. It may play an important role in the ecological state of the seafloor habitat due to the many functions bacteria and archaea hold in the food-web, element recycling, and biotic interactions, beyond representing the largest biomass in deep-sea sediments (Joergensen and Boetius 2007). It is thus crucial to understand the role of nodules in shaping microbial diversity and in hosting microbes with important ecological functions. So far, only few studies were carried out to investigate the microbiota of nodule fields, and most of them were focused on identifying microbes involved in metal cycling. Here, we investigated similarity of microbial community structures in sediments and nodules retrieved from the Peru Basin. The objectives of this study were: i) compares the microbes of nodules fields with microbiota of deep-sea sediments, in order to identify specific features of microbial diversity of nodule fields; ii) elucidates differences in diversity and in microbial community structure between sediments and nodules; iii) investigates potential microbially-mediated functions and the major drivers in shaping microbial communities associated to the nodules.

4.1 Microbial diversity of nodule fields is distinct from other deep-sea areas

Benthic bacterial assemblages in sediments and nodules of the Peru basin showed the typical dominance of the classes Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, and Acidimicrobiia, as reported for deep-sea sediments worldwide (Bienhold et al., 2016; Figure 3) and in the Pacific Nodule Province (Wang et al., 2010; Wu et al., 2013; Shulse et al., 2016; Lindh et al., 2017). However at higher taxonomic resolution we detected substantial differences to the microbial

community composition of other deep-sea regions. Sediments of the Peru Basin bacteria classes were depleted in sequence abundances of Flavobacteria, Gemmatimonadetes and Bacilli, whereas sequence abundances of the Chloroflexi (i.e. Dehalococcoidia), Planctomycetes (i.e. Pirellulaceceae, Phycisphaeraceae) and the genus *Nitrospina* were higher compared to other deep-sea regions (Bienhold et al., 2016, Varliero et al., 2019). Dehalococcoidia and Planctomycetes were previously reported as important component of benthic microbial assemblages in the Pacific Ocean (Wang et al., 2010; Wu et al., 2013; Blöthe et al., 2015; Walsh et al., 2016; Lindh et al., 2017). Their contribution to the total community was found to increase in organic matter depleted subsurface sediments (Durbin and Teske, 2011; Walsh et al., 2016).

Dominant OTUs (>1 %) belonged to unclassified Actinomarinales, Gammaproteobacteria, Subgroup 21 (phylum Acidobacteria), and to genus *Woesia* (family Woeseiaceae). Members of Actinomarinales and Woeseiaceae are cosmopolitan types in deep-sea sediments (Bienhold et al., 2016). For Actinomarinales there are no cultured relatives, and the function of this group remains unknown. In the case of Woeseiaceae, one representative is in culture (*Woeseia ocaeni*). *W. ocaeni* is an obligate chemoorganoheterotroph (Du et al., 2016), suggesting a role in organic carbon remineralization for members of that family, as recently confirmed by analysis of deep-sea assembled genomes (Hoffmann et al., 2020). Closest related sequences of Subgroup 21 have been reported in deep-sea sediments (Schauer et al. 2010) and across Pacific nodule fields (Wu et al., 2013), but also in association with deep-sea benthic giant foraminifera (Xenophyophores) and in surrounding sediments (Hori et al., 2013). The subgroup 21-like OTU was also one of the 10 most abundant OTUs retrieved from nodules (0.9 %). Xenophyophores have agglutinated tests and can grow to decimetre size, suggesting that members of Subgroup 21 may be colonists of biological and/or hard substrates.

Within the class Alphaproteobacteria the most abundant OTUs (>0.5 %) belonged to unclassified genera of the families Magnetospiraceae (order Rhodospirillales), Hyphomicrobiaceae (order Rhizobiales), and Kiloniellaceae (order Rhodovibrionales). Magnetospiraceae and Hyphomicrobiaceae are the most abundant families in nodules with >2 % of OTUs. Closely related sequences have been reported previously across Pacific Nodule Provinces (Xu et al., 2007; Shulse et al., 2016). The family of Magnetospiraceae includes microaerophilic heterotrophs, able of magnetotaxis and iron reduction (i.e. genus Magnetospirillum; Matsunaga et al. 1991; Schuler and Frankel, 1999), and thus the members of this family could play a role in Fe(III) mobilization, affecting its bioavailability. Hyphomicrobiaceae-like sequences found in this study are related to genera *Hyphomicrobium* and *Pedomicrobium* (sequence identity 97 %), which have been reported to be involved in manganese cycling (Tyler, 1970; Larsen et al., 1999; Stein et al., 2001). A potential contribution of these groups in metal cycling in manganese nodules is also suggested by the presence of closest related sequences in ocean crust (Santelli et al., 2008; Lee et al., 2015), which typically hosts epilithic and endolithic microbial communities of chemolithotrophic metals-oxidizers (Staudigel et al., 2008). Similarly, Kiloniellaceae related OTUs might be involved in metal-cycling as closely related sequences were found in marine basalts (Mason et al., 2007; Santelli et al., 2008) and inside other manganese nodules

(Blöthe et al., 2015). Most of the marine cultivates in the family Kiloniellaceae belong to genus *Kiloniella*, that have been isolated from marine macroalga (Wiese et al., 2009), the guts of Pacific white shrimp (Wang et al., 2015), marine sponge (Yang et al., 2015), spider crab and clam (Gerpe et al., 2017), and from the surface water of a polynia in the Western Antarctic Sea (Si et al., 2017). Besides, Kiloniellaceae-like sequences were found in sponges (Cleary et al., 2013), sea star larvae (Galac et al., 2016) and in seamount's iron mats (Scott et al., 2017). The presence of rich sessile and mobile metazoan communities associated to nodules offers various potential hosts for members of Kiloniellaceae. *Kiloniella* is a chemoheterotrophic aerobe, and the draft genome of an isolate from the gut of a Pacific white shrimp shows potential for denitrification and iron acquisition and metabolism (Wang et al., 2015). Thus, either as free-living or host-associated life, the potential contribution of Kiloniellaceae in metal cycling requires further investigation.

Archaea were also present in sediments of the Peru Basin, with Nitrosopumilaceae (phylum Thaumarchaeota) dominating the archaeal communities (Figure S2b). Archaeal sequences comprised a lower portion of total sequences retrieved from sediments (6 – 45 %) and nodules (<1 – 7 %) of Peru basin, and they were lower in nodules compared to the sediments. Our data differed from what was reported by Shulse et al. (2016) for CCZ, especially for nodules (ca. 20%). We cannot rule out that the observed differences in microbial community structure partly reflect the different sets of primers used in our study and by Shulse et al. (2016). As both primer sets amplified the same hypervariable region of 16S rRNA gene (V4) we assume that biases are small enough to justify the comparison. The majority of member of Nitrosopumilaceae are believed to be capable of oxidation of ammonia to nitrite, the first step of nitrification (Offre et al., 2013). Archaeal ammonia oxidizers have a higher affinity for ammonia than bacterial ammonia oxidizers, and they are favoured in environments with low ammonia concentrations (Martens-Habbena et al., 2009). The Peru Basin has higher particulate organic-carbon fluxes as compared to central Pacific Ocean (Haeckel et al., 2001; Mewes et al., 2014), which results in higher remineralisation rates and higher ammonia fluxes. These limit the thickness of oxygenated sediments to 10 cm in the Peru Basin while they can reach up to 2-3 m depth in the CCZ (Haeckel et al., 2001; Mewes et al., 2014; Volz et al., 2018). Hence differences observed between CCZ and Peru nodule fields in the contribution of archaeal sequences to microbial assemblages are likely due to ammonia availability, which is controlled by organic matter fluxes.

4.2 Microbial community structure differs between sediments and nodules

Beta-diversity of microbial community structure in the Peru Basin sediments showed remarkable spatial OTU turnover already on a local scale (<60 km; Figure S2), which is at the higher end of previous microbial beta-diversity estimates for bathyal and abyssal seafloor assemblages (Jacob et al., 2013; Ruff et al., 2015; Bienhold et al., 2016; Walsh et al., 2016; Varliero et al., 2019). Here we focused specifically on the contribution of nodules to diversity, which could be a critical parameter in the ecological assessment of nodule removal. Analysis of community composition at OTU level shows that nodules and sediments host distinct bacterial and archaeal communities (Figure 2), as previously reported also for CCZ (Wu et al., 2013; Tully and Heidelberg, 2013; Shulse et al., 2016; Lindh et al.

2017). Albeit the microbial communities in the sediment showed significant differences between sites, the low number of shared OTUs between sediments and nodules <20 % supports the presence of specific bacterial and archaeal communities associated with polymetallic nodule habitats. However, the proportion of truly endemic, unique nodule OTUs was also low (Figure 1a, Table S1), nonetheless it is relevant to highlight that nodule removal would lead to a loss of specific types of microbes in a mined deep-sea region (Blöthe et al., 2015).

Microbial communities associated with nodules are significantly less diverse than those in the sediments, and the decrease in diversity was observed both in rare and abundant bacterial types (Figure 1 and Figure S1). This seems to be a common feature of polymetallic nodules (Wu et al., 2013; Tully and Heidelberg, 2013; Zhang et al., 2014; Shulse et al., 2016; Lindh et al. 2017). However, a recent meta-analysis of 16S rRNA gene diversity reports no significant differences in microbial biodiversity between nodules and sediments within the studied habitats in the CCZ (Church et al., 2019). Church and colleagues also pointed out that the findings are so far not conclusive due to the limited number of studies and differences in methods (e.g. PCR primers, sequencing approaches), which may also be a reason for the differences between the meta-analysis and the results of this study. Tully and Heidelberg (2013) suggested that lower microbial diversity in the nodules might be due to less availability of potential energy sources (e.g. organic matter) compared to sediments. Despite that the sedimentation rate exceeds the growth rate of nodules, the nodules are typically exposed to bottom water and not covered by sediments (Peukert et al., 2018). Although, it is unknown whether physical mechanisms (e.g. current regime or seasonal events) or biological processes (e.g. grazing, active cleaning) are responsible for lack of sediments accumulation on nodules, the decrease of microbial diversity with the decrease of organic matter availability is in accordance with positive energy-diversity relationship reported for deep-sea sediments (Bienhold et al., 2012). However, the presence of foraminiferal assemblages (Gooday et al., 2015) and specific sessile metazoan communities (Vanreusel et al., 2016) on the surface of nodules may represent a potential source of transformed organic matter (e.g. dissolved organic matter) and catabolic products, which may represent a much more valuable energy source for microbes than refractory particulate organic matter sinking from the water column. Furthermore, higher microbial diversity in the sediments than in the nodules could be the result of the accumulation of allochthonous microbes, as suggested by the higher proportion of rare and unique OTUs in the sediments. Lastly, the nodules offer hard substrate and presence of metals, which can select for specific Bacteria and Archaea. Similarly, hydrothermal deposits have typically lower bacterial diversity than deep-sea sediments despite chemical energy sources being highly available (Ruff et al., 2015; Wang et al., 2018). We propose that the decreased diversity of abundant OTUs in nodules, observed especially for Bacteria, suggests selection for colonists adapted to specific ecological niches associated with nodules (e.g. high metals concentration, hard substrate, presence of sessile fauna).

4.3 Potential functions of microbial communities associated to nodules

The presence of a large proportion of bacterial community with low abundance in the sediments, but enriched in the nodules both at the level of genera (35 %) and OTUs (24 %) (Figure 4, Table S3 and

Table 3a) indicates niche specialization. The most abundant OTUs (13 % of the bacterial community) in nodules include unclassified Hyphomicrobiaceae, Magnetospiraceae, Alphaproteobacteria, Arenicellaceae and SAR324, *Nitrospina*, *AqS1*, Methyloligellaceae, Subgroup 9, Subgroup 17, Kiloniellaceae, *Cohaesibacter* and JdFR-76, which closest related sequences have been retrieved from Pacific nodules (e.g. Wu et al. 2013; Blöthe et al., 2015), basaltic rocks (e.g. Mason et al 2007; Santelli et al 2008; Mason et al., 2009; Lee et al., 2015), sulfide and carbonate hydrothermal deposits (e.g. Sylvan et al., 2012; Kato et al., 2015), and giant foraminifera (Hori et al., 2013; Table 3b and Figure 5). There are currently no cultivated representatives and metabolic information for these members of the Bacteria, and it is not known whether they have metal tolerance mechanisms or they are actively involved in metal cycling. The high abundance of potential metal reductive (i.e. Magnetospiraceae) and oxidizers (i.e. Hyphomicrobiaceae), and presence of encrusting protozoans (Gooday et al., 2015), microbial eukaryotes (Shulse et al., 2016) and metazoans (Vanreusel et al., 2016) create specific ecological niches, which may be at least partially responsible for the observed selection of microbial taxa in nodules. Overall, these findings suggest that bacterial groups adapted to lithic or biological substrates preferentially colonize nodules, likely favoured by manganese and iron availability, formation of biofilms and presence of sessile fauna communities.

The reduction and dissolution of Mn oxides by dissolved organic matter (e.g. humic compounds) occurs typically in photic or reducing aquatic environments (Sunda et al., 1983; Stone and Morgan, 1984; Stone, 1987; Sunda and Huntsman, 1994). However reductive dissolution of Mn oxides by dissolved organic substrates has been observed also in dark oxygenated seawater (Sunda et al., 1983; Sunda and Huntsman, 1994), suggesting that it could be a relevant abiotic process in manganese nodules. Indeed, this reaction yields manganese(II) and low-molecular-weight organic compounds (Sunda and Kieber, 1994), which potentially may favour Mn-oxidizing Bacteria and microbial exploitation of refractory dissolved organic matter. Intense extracellular enzymatic activities have been reported for seafloor-exposed basalts (Meyers et al., 2014), raising the question of whether the closely related microbes associated with nodules might have comparable degradation rates. Furthermore, nodules host diversified communities of suspension feeders such as serpulid tubeworms, sponges, corals and crinoids (Vanreusel et al., 2016), which filter microbes and POC from the bottom water and release DOM and catabolic metabolites (e.g. ammonia). Thus, nodules may act as hot spots of organic carbon degradation. Albeit metabolic activity has never been quantified on nodules and sequence abundances are lower, the increased abundance of nitrifiers in nodules compared to the sediments reported for Pacific Nodule Province (Tully and Heidelberg, 2013; Shulse et al., 2016) and in this study could indicate a high metabolic activity. Nitrifiers catalyse the oxidation of ammonia, a catabolic product of heterotrophic metabolism, to nitrite and eventually to nitrate. In the CCZ the nitrifier community was composed of archaeal ammonia-oxidizing *Nitrosopumilus*, which represented a large portion of the microbial assemblages (up to 20 %), and a minor contribution of bacterial nitrite-oxidizing *Nitrospira* (Tully and Heidelberg, 2013; Shulse et al., 2016). Peru Basin sediments and nodules showed more diversified nitrifier communities, which are enriched by ammonia oxidizing *AqS1* (1 %) and unclassified Nitrosomonadaceae (1 %) and by nitrite-oxidizing *Nitrospina* (4 %) and *Nitrospira* (1 %; Figure 4, Table S3 and Table 3a). *Nitrospina* are not commonly reported for deep-sea

sediments, but they are the dominant nitrite oxidizers in the oceans (Luecker et al., 2013). They have recently been reported as symbiont of deep-sea glass sponges (Tian et al., 2016), which also commonly colonize FeMn nodules (Vanreusel et al., 2016). The Nitrospina-related OTUs detected in the nodules showed only low similarity with pelagic *Nitrospina gracilis* and *Nitrospina*-like sequences found in deep-sea glass sponge (sequence identity of 93 %), but were closely related with sequences recovered from marine basalts (Mason et al., 2007; Santelli et al. 2008; Mason et al. 2009), suggesting nodules as a native habitat.

5 Conclusions

The sediments of nodule fields in the Peru Basin host a specific microbial community of bacterial taxa reported for organic carbon poor environments (i.e. Chloroflexi, Planctomycetes) and potentially involved in metal-cycling (i.e. Magnetospiraceae, Hyphomicrobiaceae). Nodule communities were distinct from sediments and showed a higher proportion of sequences from potential Mn-cycling bacteria including bacterial taxa found in ocean crust, nodules and hydrothermal deposits. Our results are in general agreement with previous studies in the CCZ, confirming that nodules provide a specific ecological niche. However remarkable differences in microbial community composition (e.g. Mn-cycling bacteria, nitrifiers) between the CCZ and the Peru Basin also show that environmental settings (e.g. POC flux) and features of FeMn nodules (e.g. metal content, nodule attached fauna) may play a significant role in structuring the nodule microbiome. Due to limitations in the available datasets and methodological differences in the studies existing to date, findings are not yet conclusive and cannot be generalized. However, they indicate that microbial community structure and function would be impacted by nodule removal. Future studies need to look at these impacts in more detail and should address regional differences, to determine the spatial turnover and its environmental drivers, and the consequences regarding endemic types.

Furthermore, our results suggest that the removal of nodules, and potentially also the blanketing of nodules with plume sediments resuspended during the mining operations may affect the cycling of metal and other elements. Future work is needed to characterize metabolic activities on and in nodules, and to understand factors and processes controlling nodule colonization. Specifically, restoration experiments should take place to test whether artificial substrates favour the recovery of microbial and fauna communities, and their related ecological functions.

Data availability

Raw sequences with removed primer sequences were deposited at the European Nucleotide Archive (ENA) under accession number PRJEB30517 and PRJEB32680.

Author contributions

A.B, **F.J**, **F.W.** and **M.M** conceived the study. **A.B**, **F.J**, **F.W.** and **T.R.W.** performed sampling activities. **M.M.** compiled and analysed the data. **M.M.** wrote the paper with the contribution from all Authors.

Competing interest

The authors declare that they have no conflict of interest.

Special issue statement

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Figure captions

768 Figure 1. Comparison of diversity indices and unique OTUs between manganese nodules and
769 sediments for (a) bacterial and (b) archaeal communities. H_0: number of OTUs (q=0); H_1: exponential
770 Shannon (q=1); H_2: inverse Simpson (q=2); Unique: OTUs present exclusively in each station
771 (percentage relative to total OTUs of whole dataset). Chao1 and Unique OTUs were calculated with
772 100 sequence re-samplings per sample to the smallest dataset (40613 sequences for Bacteria and 1835
773 sequences for Archaea). Red line shows the median. F: statistic *F-ratio*, with subscript numbers
774 reporting the degrees of freedom between groups and within groups, respectively; p: probability level;
775 KW-test: Kruskal-Wallis test; χ^2: Chi square test value, with subscript numbers reporting the degrees
776 of freedom between groups and sample size, respectively.

777 Figure 2**.** Non-metric multidimensional scaling (NMDS) plot based on Euclidean distance similarity
778 matrix of bacterial (a) and archaeal (b) community structure at OTU level. Sequence abundances of
779 OTUs were centre log-ratio transformed. Permutational multivariate analysis of variance
780 (PERMANOVA) showed significant differences between nodule and sediment associated microbial
781 communities (for details see Table S1). Each sample (dot) is connected to the weighted averaged mean
782 of the within group distances. Ellipses represent one SD of the weighted averaged mean.

783 Figure 3. Bacterial community structure at dominant class level (cut-off ≥1 %). MN: manganese
784 nodules; MUC: sediments.

785 Figure 4. Genera highly abundant in nodules (ALDEx2: glm adjusted p<0.01; KW adjusted p<0.05).
786 Base 2 logarithm of the ratios between geometric mean centred sequences number of nodule (Nod) and
787 sediment (Sed), and average of the sequences contribution of total number of sequences (%) retrieved
788 in nodules and in sediments are shown. For details see Table S3.

789 Figure 5. Habitats coverage for the closest related sequences (≥99 % similarity) to OTUs highly
790 abundant in the nodules. For details see Table 4a-b.

791 **Table captions**

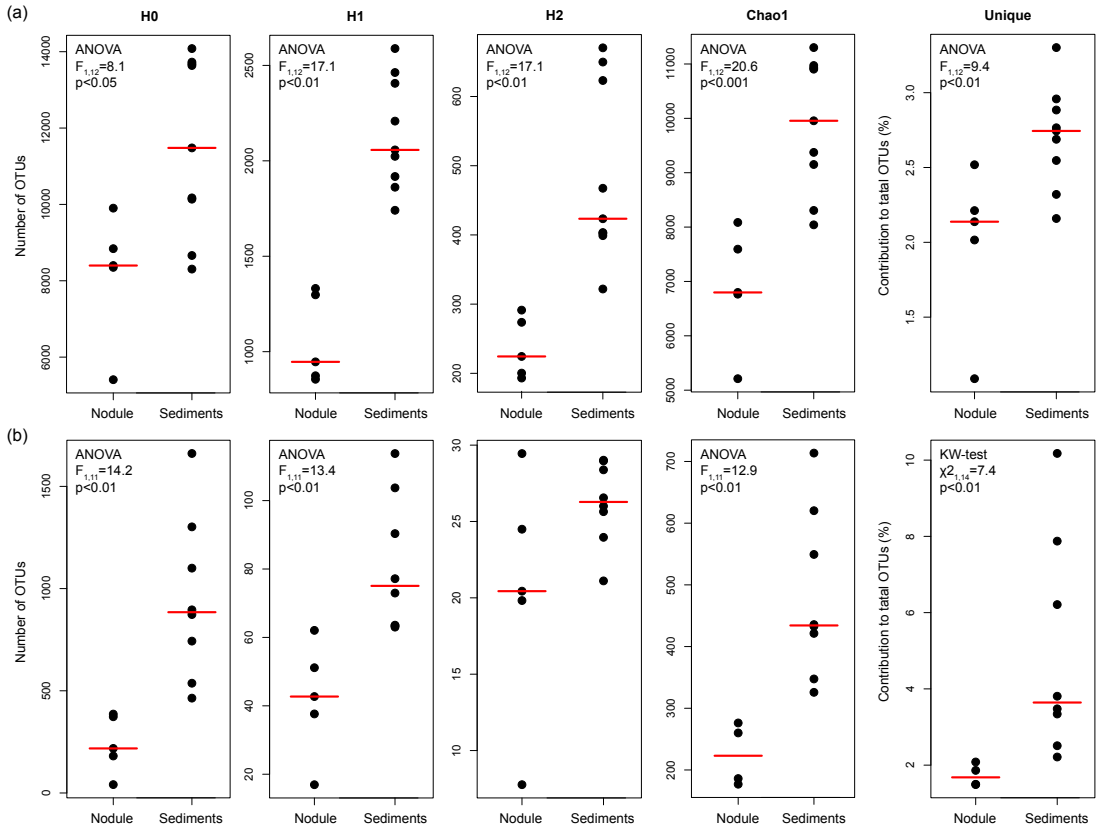
792 Table 1. Stations list and description of investigated sites/substrates.

793 Table 2. Bacterial and archaeal diversity indices and unique OTUs for all nodules and sediment
794 samples. Indices and unique OTUs were calculated without singletons.

795 Table 3. (a) OTUs highly abundant in nodules (ALDEx2: glm adjusted p<0.01; KW adjusted p<0.05).
796 Only OTUs ≥0.1 % are reported. Base 2 logarithm of the ratios between geometric mean centred
797 sequences number of nodule (Nod) and sediment (Sed), and average of the sequences contribution of
798 total number of sequences (%) retrieved in nodules and in sediments are shown. (b) Closest related
799 sequences as indemnified with BLASTn (NCBI nucleotide database 12/06/2019).

800

801 **Figure 1.**



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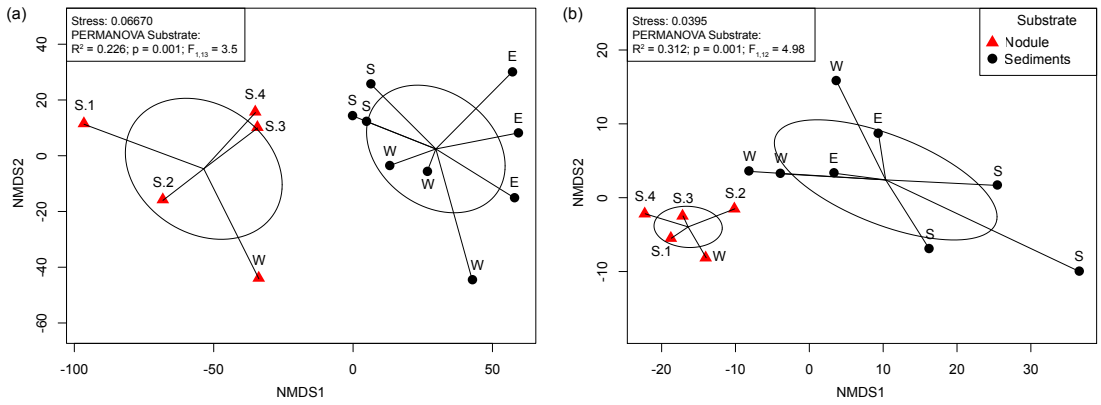
804 **Figure 2.**



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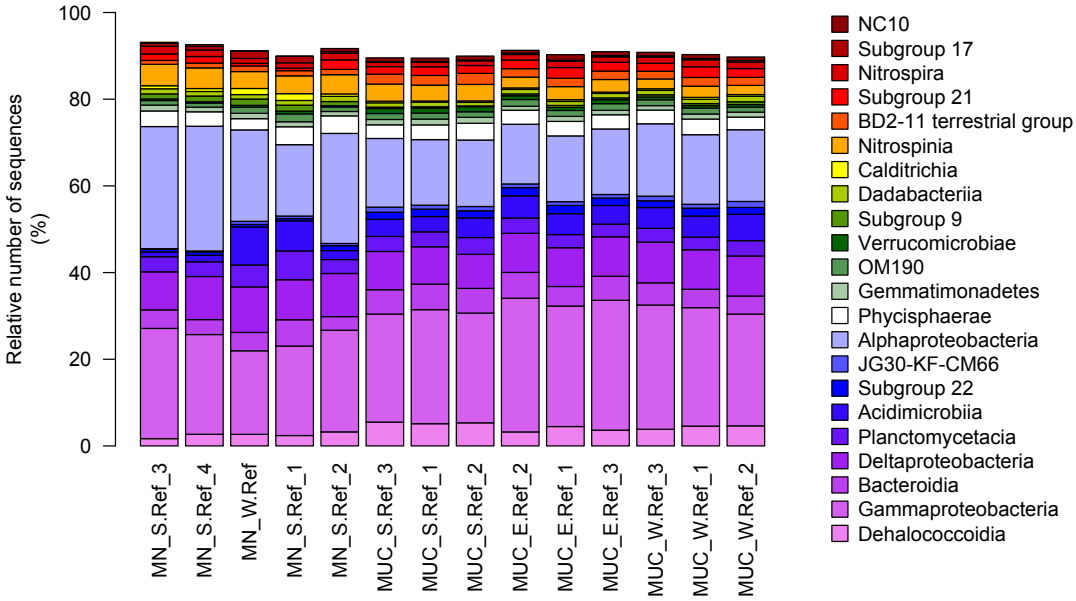
807 **Figure 3.**



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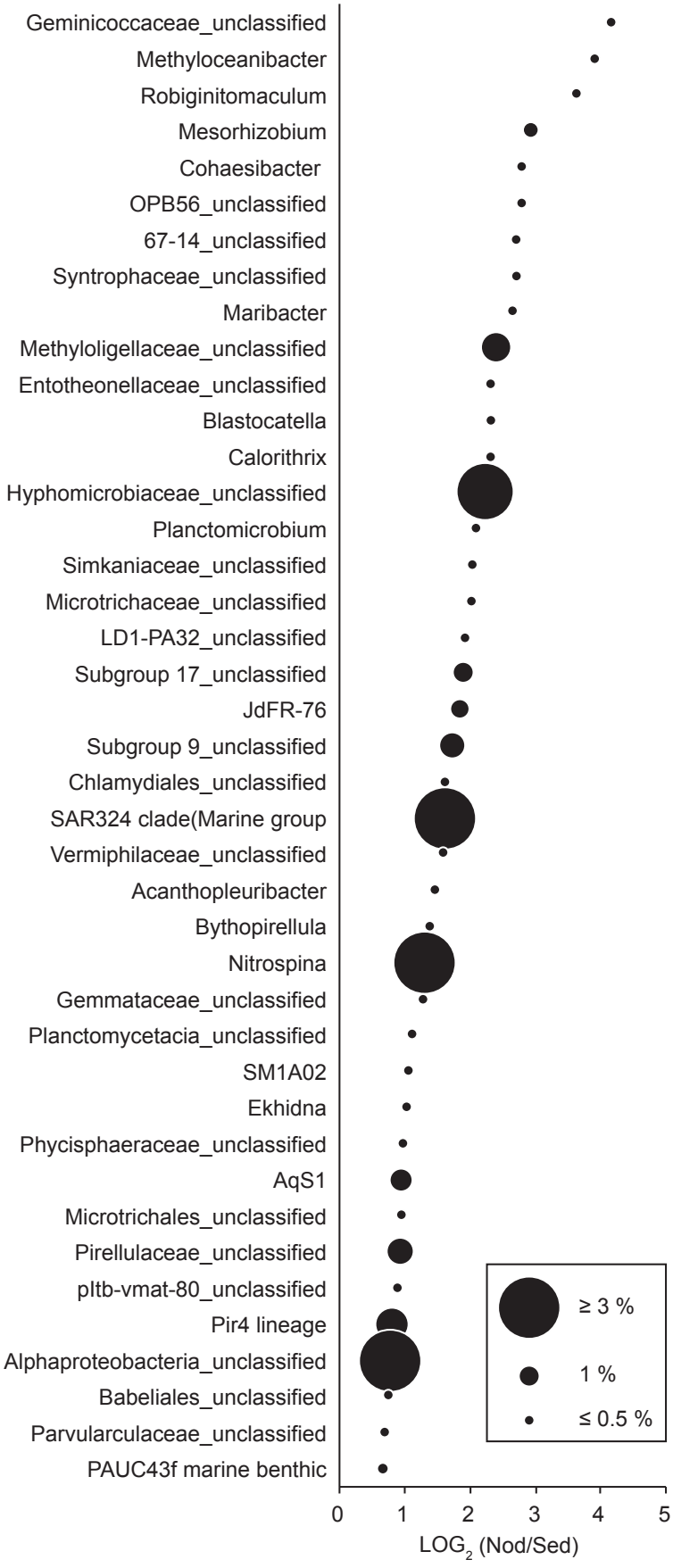
809 **Figure 4.**



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811 **Figure 5.**

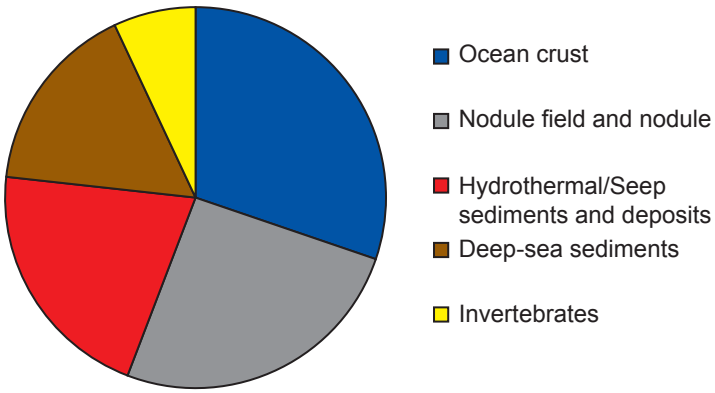

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Table 1.

Station	Sample ID	Sampling Time	Latitude (N)	Longitude (E)	Depth (m)	Device	Site	Sediment layer (cm bsf)	Substrate
SO242/2_147	MUC_E.Ref_1	02.09.15	−7.1007	−88.414	4198.2	MUC	Reference East	0-1	sediments
SO242/2_148	MUC_E.Ref_2	02.09.15	−7.1006	−88.414	4195.8	MUC	Reference East	0-1	sediments
SO242/2_151	MUC_E.Ref_3	03.09.15	−7.1006	−88.414	4197.8	MUC	Reference East	0-1	sediments
SO242/2_194	MN_W.Ref	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference West	surface	nodule
SO242/2_194	MUC_W.Ref_1	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference West	0-1	sediments
SO242/2_194	MUC_W.Ref_2	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference West	0-1	sediments
SO242/2_194	MUC_W.Ref_3	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference West	0-1	sediments
SO242/2_198	MN_S.Ref_1	16.09.15	−7.1262	−88.450	4145.6	ROV	Reference South	surface	nodule
SO242/2_198	MN_S.Ref_2	16.09.15	−7.1262	−88.450	4145.6	ROV	Reference South	surface	nodule
SO242-2_208	MN_S.Ref_3	19.09.15	−7.1256	−88.450	4150.7	MUC	Reference South	surface	nodule
SO242-2_208	MN_S.Ref_4	19.09.15	−7.1256	−88.450	4150.7	MUC	Reference South	surface	nodule
SO242/2_208	MUC_S.Ref_1	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference South	0-1	sediments
SO242/2_208	MUC_S.Ref_2	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference South	0-1	sediments
SO242/2_208	MUC_S.Ref_3	15.09.15	−7.0761	−88.526	4129.5	MUC	Reference South	0-1	sediments

MUC: TV-guided MUltiple Corer; ROV: Remote Operated Vehicle (Kiel 6000); bsf: below seafloor.

Table 2.

Bacteria	Sequences n. [a]	Sequences n. [b]	H_0	H_1	H_2	Chao1 [c]	sd	Unique (%) [c]	sd
MUC_E.Ref_2	226078	161443	13638	2024	402	10930	201.6	2.7	0.1
MUC_E.Ref_3	218324	166847	13680	2057	423	10972	200.1	2.9	0.1
MUC_E.Ref_1	222924	164985	14082	2208	467	11302	166	3.0	0.1
MN_W.Ref	209563	159724	9902	1296	290	8085	143.6	2.2	0.1
MUC_W.Ref_1	137990	104301	11480	1918	403	9955	164	2.8	0.1
MUC_W.Ref_2	112259	81103	10171	1862	399	9151	148.3	2.3	0.1
MUC_W.Ref_3	236896	178985	13727	1741	322	10905	198.9	3.3	0.1
MN_S.Ref_1	313418	236498	8841	853	192	6798	138.3	2.5	0.1
MN_S.Ref_2	220364	172668	8399	872	199	6766	132.8	2.1	0.1
MN1_S.Ref_3	114074	43932	5409	945	223	5211	73.28	1.1	0.1
MN2_S.Ref_4	64218	76729	8351	1329	272	7594	124.2	2.0	0.1
MUC_S.Ref_1	77424	65890	10137	2588	670	9374	110.5	2.7	0.1
MUC_S.Ref_2	58575	45832	8662	2406	623	8306	93.85	2.2	0.1
MUC_S.Ref_3	59503	40613	8306	2463	650	8041	84.73	2.5	0.1

Archaea	Sequences n. [d]	Sequences n. [b]	H_0	H_1	H_2	Chao1 [c]	sd	Unique (%) [c]	sd
MUC_E.Ref_2	40952	34494	896	63	21	433	52	3.5	0.5
MUC_E.Ref_3	25090	20215	743	73	26	421	50	3.3	0.6
MUC_E.Ref_1	na	na	na	na	na	na	na	na	na
MN_W.Ref	11737	12623	373	51	24	260	33	2.1	0.4
MUC_W.Ref_1	18097	14878	537	63	24	348	36	2.5	0.5
MUC_W.Ref_2	37656	31192	873	77	28	436	53	3.8	0.6
MUC_W.Ref_3	13031	10444	464	64	26	326	31	2.2	0.4
MN_S.Ref_1	7423	5384	218	38	20	186	26	1.5	0.3
MN_S.Ref_2	15314	9472	386	62	29	276	29	1.5	0.4
MN1_S.Ref_3	6099	1835	181	43	20	177	15	1.9	0.3
MN2_S.Ref_4 [e]	722	182	41	17	8	na	na	na	na
MUC_S.Ref_1	34166	29221	1100	90	27	549	66	6.2	0.6
MUC_S.Ref_2	41633	36378	1302	104	29	620	71	7.9	0.8
MUC_S.Ref_3	75344	64433	1661	114	29	714	75	10.2	0.9

H_0: number of OTUs; H_1: exponential Shannon; H_2: inverse Simpson; Unique: OTUs present exclusively in one station (percentage relative to total OTUs of whole dataset); na: not available.

[a] after the merging of forward and reverse reads;

[b] after removal of un-specific and contaminants sequences (see Methods for details);

[c] calculated with 100 sequence re-samplings per sample to the smallest dataset (40613 sequences for Bacteria and 1835 sequences for Archaea), average data and standard deviation (sd) are given;

[d] after quality trimming of merged forward and reverse reads;

[e] due to extremely low number of sequences, this sample was not included in analyses requiring sequences re-sampling.

Table 3.

(a)

Phylum	Class	Order	Family	Genus	OTU	LOG2 (Nod/Sed)	Nodule (%)	Sediment (%)
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobiaceae_unclassified	otu29	2	2.5	0.7
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Magnetospiraceae	Magnetospiraceae_unclassified	otu11	2	2.2	0.7
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu31	8	1.5	0.0
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu83	8	0.9	0.0
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu160	6	0.3	0.0
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu249	7	0.2	0.0
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu66	8	0.7	0.0
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu78	2	0.6	0.1
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu202	3	0.4	0.0
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu317	1	0.2	0.1
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu947	4	0.2	0.0
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu588	2	0.1	0.0
Proteobacteria	Deltaproteobacteria	SAR324 clade(Marine group B)	SAR324 clade(Marine group B)_unclassified	SAR324 clade(Marine group B)_unclassified	otu425	2	0.1	0.0
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu68	3	1.4	0.1
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu227	6	0.2	0.0
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu215	3	0.2	0.0
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	otu636	6	0.2	0.0
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu434	3	0.1	0.0
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicellaceae_unclassified	otu36	2	1.4	0.4
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicellaceae_unclassified	otu162	5	0.4	0.0
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu97	4	0.5	0.0
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu266	2	0.2	0.1
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu521	6	0.2	0.0
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu346	4	0.2	0.0
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu991	5	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu113	2	0.5	0.1
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu184	7	0.3	0.0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu234	3	0.2	0.0
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu255	6	0.5	0.0
Proteobacteria	Gammaproteobacteria	Nitrosococcales	Nitrosococcaceae	AqS1	otu122	2	0.8	0.3
Acidobacteria	Subgroup 17	Subgroup 17_unclassified	Subgroup 17_unclassified	Subgroup 17_unclassified	otu326	5	0.7	0.0
Acidobacteria	Subgroup 17	Subgroup 17_unclassified	Subgroup 17_unclassified	Subgroup 17_unclassified	otu865	1	0.1	0.1
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	JdFR-76	otu171	1	0.6	0.2
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	JdFR-76	otu541	4	0.2	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu357	3	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu435	4	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu370	3	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu467	6	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu450	2	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu519	3	0.1	0.0
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Cohaesibacter	otu71	4	0.7	0.1
Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinales_unclassified	Actinomarinales_unclassified	otu163	2	0.3	0.1
Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinales_unclassified	Actinomarinales_unclassified	otu532	6	0.2	0.0
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu342	3	0.3	0.0
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu674	3	0.1	0.0
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonadaceae_unclassified	otu203	1	0.4	0.2
Proteobacteria	Alphaproteobacteria	Kordiimonadales	Kordiimonadaceae	Kordiimonas	otu86	2	0.4	0.1
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Cyclobacteriaceae_unclassified	otu233	3	0.4	0.0
Dadabacteria	Dadabacteriia	Dadabacteriales	Dadabacteriales_unclassified	Dadabacteriales_unclassified	otu347	3	0.2	0.0
Dadabacteria	Dadabacteriia	Dadabacteriales	Dadabacteriales_unclassified	Dadabacteriales_unclassified	otu1016	3	0.1	0.0
Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	67-14_unclassified	otu324	3	0.3	0.0
Proteobacteria	Deltaproteobacteria	NB1-j	NB1-j_unclassified	NB1-j_unclassified	otu344	1	0.1	0.0
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellulaceae_unclassified	otu538	2	0.1	0.0
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	Microtrichaceae_unclassified	otu669	2	0.1	0.0
Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	Blastocatella	otu489	3	0.1	0.0
Entotheonellaeota	Entotheonellia	Entotheonellales	Entotheonellaceae	Entotheonellaceae_unclassified	otu788	4	0.1	0.0
Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 10	otu711	3	0.1	0.0
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Kangiellaceae	Kangiellaceae_unclassified	otu744	5	0.1	0.0
Proteobacteria	Gammaproteobacteria	Thiohalorhabdales	Thiohalorhabdaceae	Thiohalorhabdaceae_unclassified	otu571	6	0.1	0.0
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Ekhidna	otu651	5	0.1	0.0
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	otu579	6	0.1	0.0
Gemmatimonadetes	BD2-11 terrestrial group	BD2-11 terrestrial group_unclassified	BD2-11 terrestrial group_unclassified	BD2-11 terrestrial group_unclassified	otu1439	3	0.1	0.0

(b)

OTU	NCBI ID ≥ 99% similarity	Habitat(s)
otu29	KT748605.1; JX227334.1; EU491654.1	basaltic crust; nodule fields
otu11	JX227511.1; JQ013353.1; FJ938664.1	nodule fields; deep-sea sediments; cobalt-rich crust
otu31	MG580220.1; KF268757.1	Mariana subduction zone sediments; heavy metal contaminated marine sediments
otu83	MG580220.1; JN621543.1	Mariana subduction zone sediments; manganese oxide-rich marine sediments
otu160	MG580740.1; JX227257.1	Mariana subduction zone sediments; nodule fields
otu249	JQ287236.1; KM051824.1	inactive hydrothermal sulfides; basaltic crust
otu66	JX226721.1 [a]	nodule fields
otu78	JN860354.1; HQ721444.1	hydrothermal vents; deep-sea sediments;
otu202	MG580143.1; JX227690.1; JN860358.1	Mariana subduction zone sediments; nodule fields; hydrothermal vents
otu317	JX227432.1; AY627518.1	nodule fields; deep-sea sediments;
otu947	JX226721.1 [a]	nodule fields
otu588	LC081043.1	nodule
otu425	JX227680.1; FJ938661.1	nodule fields; cobalt-rich crust
otu68	JN886931.1; FJ752931.1; KJ590663.1	hydrothermal carbonate sediments; polychaete burrow environment; biofilm
otu227	MG580382.1; AM997732.1	Mariana subduction zone sediments; deep-sea sediments
otu215	KC901562.1; AB015560.1	basaltic glasses; deep-sea sediments
otu636	HM101002.1; EU491612.1; KC682687.1	Marine Sponge Halichondria; ocean crust;
otu434	EU287401.1; JN977323.1	Subsurface sediments; marine sediments
otu36	JX227383.1; KY977840.1; AM997938.1	nodule fields; Mariana subduction zone sediments; deep-sea sediments
otu162	FN553503.1; AM997671.1	hydrothermal vents; deep-sea sediments
otu97	JX227693.1; FJ024322.1; EU491736.1	nodule fields; ocean crust
otu266	AB694157.1; JX227083.1	deep-sea benthic foraminifera; nodule fields
otu521	KY977757.1; KT336088.1; JX227223.1	Mariana subduction zone sediments; nodules; nodule fields
otu346	KY977757.1; JX227223.1	Mariana subduction zone sediments; nodule fields
otu991	JX227363.1; AM997733.1	nodule fields; deep-sea sediments
otu113	JX226757.1; EU491557.1	nodule fields; ocean crust
otu184	EU491404.1	ocean crust
otu234	EU491604.1	ocean crust
otu255	JX227709.1; FJ437705.1; KM110219.1	nodule fields; hydrothermal deposits
otu122	MG580277.1; AM997814.1; AJ966605.1	Mariana subduction zone sediments; deep-sea sediments; nodule fields
otu326	JN886905.1; KT748584.1	hydrothermal carbonate sediments; basalt crust
otu865	JX227375.1; FJ938651.1; AY225640.1	nodule fields; cobalt-rich crust; hydrothermal sediments
otu171	AM997407.1; FJ205352.1; EU491267.1	deep-sea sediments; hydrothermal vents; ocean crust
otu541	AB694393.1	deep-sea benthic foraminifera
otu357	EU236317.1; GU302472.1	marine sponge; hydrocarbon seep
otu435	KY609381.1; KM051717.1; JX226899.1	Fe-rich hydrothermal deposits; basaltic crust; nodule fields
otu370	EU491648.1 [a]	ocean crust
otu467	FN553612.1; AB858542.1; KM051770.1	hydrothermal vents; sulfide deposits; basaltic crust
otu450	AM997745.1; KM051762.1; EU491108.1	deep-sea sediments; basaltic crust; ocean crust
otu519	GU220747.1; MG580729.1	Fe-rich hydrothermal deposits; Mariana subduction zone sediments
otu71	FJ205181.1; JX226787.1	hydrothermal vents; noduel fields
otu163	JX227427.1; JN886907.1; EU491661.1	nodule fields; hydrothermal carbonate sediments; ocean crust
otu532	EU491402.1; JX227188.1; EU374100.1	ocean crust; nodule fields; deep-sea sediments
otu342	JX227410.1; FJ205219.1; KT336055.1	nodule fields; hydrothermal vents; nodules
otu674	JX227662.1; KT336085.1; FJ938601.1	nodule fields; nodules; cobalt-rich crust
otu203	KP305065.1; FJ938598.1	corals; cobalt-rich crust
otu86	AM997620.1; FJ938474.1	deep-sea sediments; cobalt-rich crust
otu233	JX227464.1; AM997441.1	nodule fields; deep-sea sediments
otu347	JX227062.1; EU491655.1	nodule fields; ocean crust
otu1016	KF616695.1; KM396663.1; EU491261.1	carbonate methane seep; brine seep; ocean crust
otu324	JX226791.1; JN886912.1	nodule fields; hydrothermal carbonate sediments
otu344	EU438185.1; KY977824.1	deep-sea sediments and hydrohtermal vents; ocean crust
otu538	KM356353.1; JX226930.1; DQ996924.1	carbonate methane seep; nodule fields; deep-sea sediments
otu669	EU491619.1; MG580068.1; KT748607.1	ocean crust
otu489	EU491660.1; MG580531.1; AM998023.1	ocean crust; deep-sea sediments
otu788	JN886890.1; MG580099.1	hydrothermal carbonate sediments; ocean crust
otu711	JX193423.1; GU302449.1; AY225643.1	mariculture sediments; hydrocarbon seep; ocean crust
otu744	AB831375.1; EU290406.1; KM454306.1	deep-sea methane-seep sediments; marine sponge; marine sediments
otu571	JQ287033.1; AM911385.1; EU236424.1	hydrothermal sulfides; cold-water corals; sponges
otu651	KT972875.1 [a]	outcrops
otu579	EU491573.1; KT336070.1	ocean crust; nodules
otu1439	JN886922.1; KC747092.1; JN884864.1	hydrothermal carbonate sediments; deep-sea sediments; methane seep

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834 [a] ≥ 98% similarity.