

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

Massimiliano Molari<sup>1</sup>, Felix Janssen<sup>1,2</sup>, Tobias R. Vonnahme<sup>1,a</sup>, Frank Wenzhöfer<sup>1,2</sup>, and Antje Boetius<sup>1,2</sup>

<sup>1</sup>Max Planck Institute for Marine Microbiology, Bremen, Germany

<sup>2</sup>HGF MPG Joint Research Group for Deep-Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

<sup>a</sup>present address: UiT the Arctic University of Tromsø, Tromsø, Norway

Correspondence: Massimiliano Molari (mamolari@mpi-bremen.de)

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Abstract. Industrial-scale mining of deep-sea polymetallic nodules will remove nodules in large areas of the sea floor. 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 microbialcommunity 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 the oceanic crust, nodules, hydrothermal deposits, and sessile fauna. Sediment and nodule communities overall shared a low proportion of operational taxonomic units (OTUs; 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) also play 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 of the sediment horizon. The mechanisms of nodule formation are not completely elucidated. The current understanding is that they are formed via mineral precipitation 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 of 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 decimetres of sediment, and create near-bottom sediment plumes that would resettle and cover the sea floor (Miller et al., 2018). Although the first nodules were discovered in the 1870s (Murray and Renard, 1891), only little is known about the biodiversity, biological processes, and ecological functions of the nodules and their surrounding sediments as a 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 characterise 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 north-central and south-central Pacific Ocean, respectively; the Peru Basin in the south-eastern Pacific; and in the centre 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 has focused on the CCZ and the south-central Pacific Ocean (Xu et al., 2007; Wu et al., 2013; Tully and Heidelberg, 2013; Blöthe et al., 2015; Shulse et al., 2017; 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 and 30 km (Wu et al., 2013; Shulse et al., 2017), while Tully and Heidelberg (2013) found that nodule communities varied among sampling sites ( < 50 km). Besides, potential Mn oxidisers 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., 2017). 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), in the abundance and morphology of nodules, or in the colonisation 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 2 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 oxicsub-oxic front (10 cm vs. tens of metres of sediment depth in the CCZ; Müller et al., 1988; Haeckel 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): (i) nodules from the Peru Basin are often larger, with a typical cauliflower shape, compared to those in the CCZ, which have a discoidal shape and a size of 2-8 cm (Kuhn et al., 2017); (ii) the average nodule abundance in the Peru Basin is lower  $(10 \text{ kg m}^{-2})$  than in the CCZ  $(15 \text{ kg m}^{-2})$ ; Kuhn et al., 2017); (iii) Mn nodules from the Peru Basin are thought to be mainly formed by sub-oxic 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 deepsea 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 that (i) nodules shape deep-sea microbial diversity and (ii) nodules host a specific microbial community compared to the surrounding sediments. The 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 Healthy and Productive Seas and Oceans (JPI Oceans) on board the R/V Sonne (expedition SO242/2; 28 August-1 October 2015) in the Peru Basin around 7° S and 88.5° W. Samples were collected at three sites outside the sea floor area selected in 1989 for a long-term disturbance and recolonisation 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 a 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 were sampled using a TV MUC or a remotely operated vehicle (ROV; KIEL 6000, 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 sea floor (b.s.f.) and had diameters of a few centimetres. 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  $(-20 \,^{\circ}\text{C})$ . 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. (2020). 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 fragments using the FastDNA<sup>TM</sup> 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. To control for DNA contamination (negative control), DNA extraction was carried out on purified water after being in contact with a sterile scalpel and a plastic bag.

Amplicon sequencing was done at the CeBiTec laboratory (Center for Biotechnology, Bielefeld University) 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') as well as the archaeal primers Arch349F (5'-GYGCASCAGKCGMGAAW- 3') and Arch915R (5'-GTGCTCCCCGCCAATTCCT-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 MiSeq v3 chemistry in a 2bp × 300 bp paired run with > 50 000 reads per sample, following the standard instructions of the 16S Metagenomic Sequencing Library Preparation protocol (Illumina, Inc., San Diego, CA, USA).

The quality cleaning of the sequences was performed with several software tools. CUTADAPT (Martin, 2011) was used for primer clipping. Subsequently the TRIMMO-MATIC software (Bolger et al., 2014) was used to remove low-quality sequences starting with the following settings: SLIDINGWINDOW:4:10 MINLEN:300 (for Bacteria) and SLIDINGWINDOW:6:13 MINLEN:450 (for Archaea). In the case of Bacteria data, this step was performed before the merging of reverse and forward reads with a paired-end read merger (PEAR; J. Zhang et al., 2014). Low-quality archaeal sequences were removed after merging the reads in order to enhance the number of retained reads due to the increase in archaeal 16S rRNA gene fragment length. All sequences were quality-controlled with FastQC (Andrews, 2010). Where necessary, more sequences were removed with TRIMMOMATIC using larger sliding window scores until the FastQC quality control was passed (average quality score per sample: > 34 for Bacteria and > 22 for Archaea). Clustering of sequences into OTUs (operational taxonomic units) was done using the SWARM algorithm (Mahé et al., 2014). The taxonomic classification was based on the SILVA rRNA reference database (release 132) at a minimum alignment similarity of 0.9 and a last-common-ancestor consensus of 0.7 (Pruesse et al., 2012). The workflow and scripts applied in this study can be found in Hassenrück et al. (2016). Raw sequences with removed primer sequences were deposited at the European Nucleotide Archive (ENA) under accession number PRJEB30517 and PRJEB32680; the sequences were archived using the service of the German Federation for Biological Data (GFBio; Diepenbroek et al., 2014).

The total number of sequences obtained in this study is reported in Table S1 in the Supplement. Absolute singletons (SSO<sub>abs</sub>), i.e. OTUs consisting of sequences occurring only once in the full dataset (Gobet et al., 2014), were removed (Table S1). Similarly, contaminant sequences (as observed in the negative control) and unspecific sequences (i.e. bacterial sequences in the archaeal amplicon dataset as well as and archaeal, chloroplast, and mitochondrial sequences in the bacterial dataset) were removed from amplicon datasets before the analysis (Table S1). The dominant OTU sequences and OTU sequences highly abundant in the nodules were searched using the Basic Local Alignment Search Tool (BLASTn; GeneBank nucleotide database, 12 June 2019; Altschul et al., 1990) in order to identify the

Station	Sample ID	Sampling time (dd.mm.yy)	Latitude	Longitude	Depth (m)	Device	Site	Sediment layer (cm b.s.f.)	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

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

MUC: TV-guided multiple corer; ROV: remote-operated vehicle (Kiel 6000); b.s.f.: below sea floor.

other habitats in which the most closely related (i.e. > 99%) sequences have been previously reported.

#### 2.3 Data analysis

The first three Hill numbers, or the effective number of species, were used to describe alpha diversity: species richness  $(H_0)$ , the exponential of Shannon entropy  $(H_1)$ , and the inverse Simpson index (H2; Chao et al., 2014). Coveragebased and sample-size-based rarefaction (based on the actual number of sequences) and extrapolation (based on double the number of sequences) curves were calculated for the Hill numbers using the R package iNEXT (Hsieh et al., 2018). Calculation of the estimated richness (Chao1) and the identification of unique OTUs (present exclusively in one sample) were based on repeated (n = 100) random subsampling of the amplicon datasets. Significant differences in alpha diversity indices between substrates (i.e. manganese nodules and sediments) were determined by analysis of variance (ANOVA) or by the non-parametric Kruskal–Wallis (KW) test when the ANOVA's assumptions were not satisfied.

Beta diversity in samples from different substrates and from different sites was quantified by calculating a Euclidean distance matrix based on OTU abundances that have underwent centred log-ratio (CLR) transformation (*clr* function in the R package compositions) and Jaccard dissimilarity based on a presence–absence OTU table. The latter was calculated with 100 sequence resamplings per sample on the smallest dataset (40 613 sequences for Bacteria and 1835 sequences for Archaea). Euclidean distance was used to produce nonmetric multidimensional scaling (NMDS) plots. The Jaccard dissimilarity coefficient was used to perform hierarchical clustering (*hclust* function in the 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 or 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 2 times more abundant in nodules than in sediments, i.e. Log2(Nodule/sediment)  $\geq$  1, and with a sequence contribution of total number of sequences of  $\geq$  1 % (for genera) or  $\geq$  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), iN-EXT (Hsieh et al., 2018), and ALDEx2 (Fernandes et al., 2014).

#### 3 Results

#### 3.1 Microbial alpha diversity

Bacterial and archaeal communities in five nodules and nine 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  $5 \pm 5$  and  $25 \pm 14$  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

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abundances of Bacteria were comparable between sediments and nodules. Cosmopolitan OTUs, i.e. those present in 80 % of the sediments and nodule samples, made up only 9% of all taxa (77 % of all sequences), whereas rare OTUs occurring in only < 20% of all samples represented 50\% of the taxa (4 % of all sequences). Sediments and nodules contained only 4 % and 2 %, respectively, of endemic taxa, defined as those that were abundant in one 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, Fig. 1a). Bacterial and archaeal diversity was investigated calculating the total number of OTUs (Hill number q = 0;  $H_0$ ), the estimated richness (Chao1), and the unique OTUs (present exclusively at one station). For this analysis, the latter were calculated with sequence resampling to overcome differences in sequencing depth. Abundancebased 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 (Fig. S1a-b in the Supplement). However, the bacterial and archaeal diversity was well described for the abundant OTUs ( $H_1$  and  $H_2$ ; Fig. S1a–b), with more than 90% of the estimated diversity covered (Fig. S1c-d). In both 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 with sediments (Table 2, Fig. 1a). Archaeal communities showed the same patterns for diversity indices and unique OTUs with the exception of the  $H_2$  index that did not show significant difference between nodules and sediments (Table 2, Fig. 1b).

#### 3.2 Patterns in microbial-community composition

The changes in microbial-community structure at the OTU level (beta diversity) between substrates and samples were quantified by calculating Euclidean distances from CLRtransformed OTU abundance. Shared OTUs were estimated by calculating Jaccard dissimilarity from OTU presence or absence based on repeated random subsampling of the amplicon datasets. Microbial communities associated with manganese nodules differed significantly from those found in the sediments (Fig. 2, Table S2). Furthermore, 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 the 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. originating from sediments or nodules) 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%; Fig. 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 (Fig. 3). All archaeal communities were dominated by Thaumarchaeota (Nitrosopumilales), which represented more than 95% of all sequences. The remaining small proportion of sequences were taxonomically assigned to Woesearchaeia (Fig. S2b). Nodule and sediment samples showed similar compositions of most abundant bacterial genera (contribution to total number of sequence:  $\geq 1\%$ ; Fig. S2a). A total of 69 bacterial genera (9% of all genera) were differentially abundant in the nodules and in the sediment, accounting for 36% and 21% of the total sequences retrieved from nodules and sediments, respectively (ALDEx2: ANOVA-adjusted p of < 0.01 and KW-adjusted p of < 0.05; Fig. 4 and Table S3). Of those only one unclassified genus within the family Sphingomonadaceae 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%; Fig. 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 the total sequences recovered from sediments. A total of 128 OTUs were highly abundant in nodules (ALDEx2: ANOVAadjusted p of < 0.01 and KW-adjusted p of < 0.05), which accounts for 24 % of the total sequences retrieved from nodules (Table 3a). The most closely related sequences ( $\geq$  99 % similarity) were retrieved from the oceanic crust (30%), from nodule fields (26%), from hydrothermal or seep sediments and deposits (21%), and from worldwide deep-sea sediments (16%), and 7% were found in association with invertebrates (Table 3b and Fig. 5).



**Figure 1.** Comparison of diversity indices and unique OTUs between manganese nodules and sediments for (**a**) bacterial and (**b**) archaeal communities.  $H_0$ : number of OTUs (q = 0);  $H_1$ : exponential Shannon (q = 1);  $H_2$ : inverse Simpson (q = 2); unique: OTUs present exclusively at each station (percentage relative to total OTUs of whole dataset). Chao1 and Unique OTUs were calculated with 100 sequence resamplings per sample for the smallest dataset (40 613 sequences for Bacteria and 1835 sequences for Archaea). The red line shows the median. *F*: statistic *F* ratio, with subscript numbers reporting the degrees of freedom between groups and within groups, respectively; *p*: probability level; KW test: Kruskal–Wallis test;  $\chi^2$ : chi-square test value, with subscript numbers reporting the degrees of freedom between groups and sample size, respectively.

#### 4 Discussion

Industrial-scale mining of deep-sea polymetallic nodules may remove nodules and the active surface sea floor layer at a spatial scale ranging from ca.  $50\,000-75\,000\,\mathrm{km}^2$  per claim to ca.  $1 \times 10^6 \,\mathrm{km}^2$ , including all current exploration licences (Miller et al., 2018). The regrowth of nodules will take millions of years; thus it is unknown whether the associated biota could recover at all (Simon-Lledo et al., 2019). The response of microbial communities to the loss of nodules and sea floor integrity is largely unknown. It may play an important role in the ecological state of the sea floor 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 have been 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 the similarity of microbial-community structures in sediments and nodules retrieved from the Peru Basin. The objectives of this study were (i) to compare the microbes of nodules fields with microbiota of deep-sea sediments in order to identify specific features of microbial diversity of nodule fields, (ii) to elucidate differences in diversity and in microbial-community structure between sediments and nodules, and (iii) to investigate potential microbially mediated functions and the major drivers in shaping microbial communities associated with the nodules.

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

Bacteria	Sequences n <sup>a</sup>	Sequences n <sup>b</sup>	$H_0$	$H_1$	$H_2$	Chao1 <sup>c</sup>	SD <mark>TS1</mark>	Unique (%) <sup>c</sup>	SD
MUC_E.Ref_2	226 078	161 443	13 638	2024	402	10930	201.6468247	2.7	0.1
MUC_E.Ref_3	218 324	166 847	13 680	2057	423	10972	200.1030792	2.9	0.1
MUC_E.Ref_1	222 924	164 985	14 082	2208	467	11 302	165.975377	3.0	0.1
MN_W.Ref	209 563	159 724	9902	1296	290	8085	143.6020775	2.2	0.1
MUC_W.Ref_1	137 990	104 301	11 480	1918	403	9955	164.0064362	2.8	0.1
MUC_W.Ref_2	112 259	81 103	10171	1862	399	9151	148.2778837	2.3	0.1
MUC_W.Ref_3	236 896	178 985	13 727	1741	322	10905	198.9227666	3.3	0.1
MN_S.Ref_1	313 418	236 498	8841	853	192	6798	138.3369372	2.5	0.1
MN_S.Ref_2	220 364	172 668	8399	872	199	6766	132.8050941	2.1	0.1
MN1_S.Ref_3	114074	43 932	5409	945	223	5211	73.28194894	1.1	0.1
MN2_S.Ref_4	76729	64 218	8351	1329	272	7594	124.1994144	2.0	0.1
MUC_S.Ref_1	77 424	65 890	10137	2588	670	9374	110.4517313	2.7	0.1
MUC_S.Ref_2	58 575	45 832	8662	2406	623	8306	93.85000713	2.2	0.1
MUC_S.Ref_3	59 503	40 6 1 3	8306	2463	650	8041	84.73235998	2.5	0.1
Archaea	Sequences n.d	Sequences n. <sup>b</sup>	$H_0$	$H_1$	$H_2$	Chao1 <sup>c</sup>	SD	Unique (%) <sup>c</sup>	SD
Archaea MUC_E.Ref_2	Sequences n. <sup>d</sup> 40 952	Sequences n. <sup>b</sup> 34 494	<i>H</i> <sub>0</sub> 896	<i>H</i> <sub>1</sub> 63	H <sub>2</sub> 21	Chao1 <sup>c</sup> 433	SD 52	Unique (%) <sup>c</sup> 3.5	SD 0.5
Archaea MUC_E.Ref_2 MUC_E.Ref_3	Sequences n. <sup>d</sup> 40 952 25 090	Sequences n. <sup>b</sup> 34 494 20 215	<i>H</i> <sub>0</sub> 896 743	H <sub>1</sub> 63 73	H <sub>2</sub> 21 26	Chao1 <sup>c</sup> 433 421	SD 52 50	Unique (%) <sup>c</sup> 3.5 3.3	SD 0.5 0.6
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1	Sequences n. <sup>d</sup> 40 952 25 090 NA	Sequences n. <sup>b</sup> 34 494 20 215 NA	H <sub>0</sub> 896 743 NA	H <sub>1</sub> 63 73 NA	H <sub>2</sub> 21 26 NA	Chao1 <sup>c</sup> 433 421 NA	SD 52 50 NA	Unique (%) <sup>c</sup> 3.5 3.3 NA	SD 0.5 0.6 NA
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623	<i>H</i> <sub>0</sub> 896 743 NA 373	H <sub>1</sub> 63 73 NA 51	H <sub>2</sub> 21 26 NA 24	Chao1 <sup>c</sup> 433 421 NA 260	SD 52 50 NA 33	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1	SD 0.5 0.6 NA 0.4
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878	<i>H</i> <sub>0</sub> 896 743 NA 373 537	<i>H</i> <sub>1</sub> 63 73 NA 51 63	H <sub>2</sub> 21 26 NA 24 24	Chao1 <sup>c</sup> 433 421 NA 260 348	SD 52 50 NA 33 36	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5	SD 0.5 0.6 NA 0.4 0.5
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192	H <sub>0</sub> 896 743 NA 373 537 873	<i>H</i> <sub>1</sub> 63 73 NA 51 63 77	H <sub>2</sub> 21 26 NA 24 24 28	Chao1 <sup>c</sup> 433 421 NA 260 348 436	SD 52 50 NA 33 36 53	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8	SD 0.5 0.6 NA 0.4 0.5 0.6
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_3	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444	H <sub>0</sub> 896 743 NA 373 537 873 464	H <sub>1</sub> 63 73 NA 51 63 77 64	H <sub>2</sub> 21 26 NA 24 24 28 26	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326	SD 52 50 NA 33 36 53 31	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_1	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384	H <sub>0</sub> 896 743 NA 373 537 873 464 218	H <sub>1</sub> 63 73 NA 51 63 77 64 38	H <sub>2</sub> 21 26 NA 24 24 24 28 26 20	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186	SD 52 50 NA 33 36 53 31 26	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_1 MN_S.Ref_2	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423 15 314	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384 9472	H <sub>0</sub> 896 743 NA 373 537 873 464 218 386	H <sub>1</sub> 63 73 NA 51 63 77 64 38 62	H <sub>2</sub> 21 26 NA 24 24 24 28 26 20 29	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186 276	SD 52 50 NA 33 36 53 31 26 29	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5 1.5	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3 0.4
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_1 MN_S.Ref_2 MN1_S.Ref_3	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423 15 314 6099	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384 9472 1835	H <sub>0</sub> 896 743 NA 373 537 873 464 218 386 181	H <sub>1</sub> 63 73 NA 51 63 77 64 38 62 43	H <sub>2</sub> 21 26 NA 24 24 28 26 20 29 20	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186 276 177	SD 52 50 NA 33 36 53 31 26 29 15	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5 1.5 1.5 1.9	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3 0.4 0.3
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MUC_W.Ref MUC_W.Ref_2 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_1 MN_S.Ref_2 MN1_S.Ref_3 MN2_S.Ref_4 <sup>e</sup>	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423 15 314 6099 722	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384 9472 1835 182	$\begin{array}{c} H_0 \\ 896 \\ 743 \\ NA \\ 373 \\ 537 \\ 873 \\ 464 \\ 218 \\ 386 \\ 181 \\ 41 \end{array}$	$ \begin{array}{r} H_1 \\ 63 \\ 73 \\ NA \\ 51 \\ 63 \\ 77 \\ 64 \\ 38 \\ 62 \\ 43 \\ 17 \\ \end{array} $	$\begin{array}{c} H_2 \\ 21 \\ 26 \\ NA \\ 24 \\ 24 \\ 28 \\ 26 \\ 20 \\ 29 \\ 20 \\ 8 \end{array}$	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186 276 177 NA	SD 52 50 NA 33 36 53 31 26 29 15 NA	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5 1.5 1.5 1.9 NA	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3 0.4 0.3 NA
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_1 MN_S.Ref_2 MN1_S.Ref_3 MN2_S.Ref_4 <sup>e</sup> MUC_S.Ref_1	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423 15 314 6099 722 34 166	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384 9472 1835 182 29 221	$\begin{array}{c} H_0 \\ 896 \\ 743 \\ NA \\ 373 \\ 537 \\ 873 \\ 464 \\ 218 \\ 386 \\ 181 \\ 41 \\ 1100 \\ \end{array}$	$\begin{array}{c} H_1 \\ 63 \\ 73 \\ NA \\ 51 \\ 63 \\ 77 \\ 64 \\ 38 \\ 62 \\ 43 \\ 17 \\ 90 \end{array}$	H <sub>2</sub> 21 26 NA 24 24 28 26 20 29 20 8 27	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186 276 177 NA 549	SD 52 50 NA 33 36 53 31 26 29 15 NA 66	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5 1.5 1.5 1.9 NA 6.2	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3 0.4 0.3 NA 0.6
Archaea MUC_E.Ref_2 MUC_E.Ref_3 MUC_E.Ref_1 MN_W.Ref MUC_W.Ref_1 MUC_W.Ref_2 MUC_W.Ref_3 MN_S.Ref_3 MN_S.Ref_2 MN1_S.Ref_3 MN2_S.Ref_4 <sup>e</sup> MUC_S.Ref_1 MUC_S.Ref_1 MUC_S.Ref_2	Sequences n. <sup>d</sup> 40 952 25 090 NA 11 737 18 097 37 656 13 031 7423 15 314 6099 722 34 166 41 633	Sequences n. <sup>b</sup> 34 494 20 215 NA 12 623 14 878 31 192 10 444 5384 9472 1835 182 29 221 36 378	$\begin{array}{c} H_0 \\ 896 \\ 743 \\ NA \\ 373 \\ 537 \\ 873 \\ 464 \\ 218 \\ 386 \\ 181 \\ 41 \\ 1100 \\ 1302 \end{array}$	$\begin{array}{c} H_1 \\ 63 \\ 73 \\ NA \\ 51 \\ 63 \\ 77 \\ 64 \\ 38 \\ 62 \\ 43 \\ 17 \\ 90 \\ 104 \end{array}$	$\begin{array}{c} H_2 \\ 21 \\ 26 \\ NA \\ 24 \\ 28 \\ 26 \\ 20 \\ 29 \\ 20 \\ 8 \\ 27 \\ 29 \end{array}$	Chao1 <sup>c</sup> 433 421 NA 260 348 436 326 186 276 177 NA 549 620	SD 52 50 NA 33 36 53 31 26 29 15 NA 66 71	Unique (%) <sup>c</sup> 3.5 3.3 NA 2.1 2.5 3.8 2.2 1.5 1.5 1.5 1.9 NA 6.2 7.9	SD 0.5 0.6 NA 0.4 0.5 0.6 0.4 0.3 0.4 0.3 NA 0.6 0.8

 $H_0$ : number of OTUs;  $H_1$ : exponential Shannon;  $H_2$ : inverse Simpson; unique: OTUs present exclusively at one station (percentage relative to total OTUs of whole dataset); NA: not available. <sup>a</sup> After the merging of forward and reverse reads. <sup>b</sup> After removal of unspecific and contaminant sequences (see Methods for details). <sup>c</sup> Calculated with 100 sequence resamplings per sample for the smallest dataset (40613 sequences for Bacteria and 1835 sequences for Archaea; average data and standard deviation, SD, are given). <sup>d</sup> After quality trimming of merged forward and reverse reads. <sup>e</sup> Due to an extremely low number of sequences this sample was not included in analyses requiring sequence resampling.

## 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; Fig. 3) and in the Pacific Nodule Province (Wang et al., 2010; Wu et al., 2013; Shulse et al., 2017; Lindh et al., 2017). However at higher taxonomic resolution we detected substantial differences in 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) and Planctomycetes (i.e. Pirellulaceceae, Phycisphaeraceae) as well as 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 components 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 the genus *Woeseia* (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

nodules and in sedir	nents is shown. (b) The m	nost closely related sequences with	I definity (ID) $\geq 33\%$ ; as identified	I WIUI BLASTII (Veilebalik liuciec		ase, 12 June .	2019).
(a) Phylum	Class	Order	Family	Genus	OTU	LOG2 (Nod/Sed)	Nodule (%)
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobiaceae_unclassified	otu29	2	2.48
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Magnetospiraceae	Magnetospiraceae_unclassified	otul 1	2	2.19
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu31	8	1.50
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu83	8	0.90
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu160	6	0.33
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	otu249	7	0.20
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu66	8	0.65
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu78	2	0.62
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu202	3	0.37
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu317	1	0.24
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu947	4	0.17
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu588	2	0.14
Proteobacteria	Deltaproteobacteria	SAR324 clade (marine group B)	SAR324 clade (marine group B)_unclassified	SAR324 clade (marine group B)_unclassified	otu425	2	0.13
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu68	3	1.41
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu227	6	0.25
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu215	3	0.24
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	otu636	6	0.19
Nitrospinae	Nitrospinia	Nitrospinales	Nitrospinaceae	Nitrospina	otu434	3	0.14
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicellaceae_unclassified	otu36	2	1.35
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicellaceae_unclassified	otu162	5	0.38

(a) Phylum	Class	Order	Family	Genus	OTU	LOG2 (Nod/Sed)	Nodule (%)
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu97	4	0.51
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu266	2	0.23
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu521	9	0.16
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu346	4	0.15
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Woeseiaceae	Woeseia	otu991	5	0.10
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu113	5	0.51
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu184	7	0.30
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	Methyloligellaceae_unclassified	otu234	3	0.19
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu255	9	0.48
Proteobacteria	Gammaproteobacteria	Nitrosococcales	Nitrosococcaceae	AqS1	otu122	5	0.85
Acidobacteria	Subgroup 17	Subgroup 17_unclassified	Subgroup 17_unclassified	Subgroup 17_unclassified	otu326	5	0.66
Acidobacteria	Subgroup 17	Subgroup 17_unclassified	Subgroup 17_unclassified	Subgroup 17_unclassified	otu865	1	0.15
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	JdFR-76	otu171	-	0.61
Calditrichaeota	Calditrichia	Calditrichales	Calditrichaceae	JdFR-76	otu541	4	0.19
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu357	ε	0.15
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu435	4	0.14
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu370	ю	0.14
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu467	9	0.12
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu450	2	0.12
Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Kiloniellaceae	Kiloniellaceae_unclassified	otu519	ε	0.11
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Cohaesibacter	otu71	4	0.74
Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinales_unclassified	Actinomarinales_unclassified	otu163	2	0.33
Actinobacteria	Acidimicrobiia	Actinomarinales	Actinomarinales_unclassified	Actinomarinales_unclassified	otu532	9	0.19
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu342	3	0.28

Table 3. Continued.

Table 3. Continued.							
(a) Phylum	Class	Order	Family	Genus	OTU	LOG2 (Nod/Sed)	Nodule (%)
Acidobacteria	Subgroup 9	Subgroup 9_unclassified	Subgroup 9_unclassified	Subgroup 9_unclassified	otu674	3	0.12
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonadaceae_unclassified	1 otu203	1	0.45
Proteobacteria	Alphaproteobacteria	Kordiimonadales	Kordiimonadaceae	Kordiimonas	otu86	2	0.38
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Cyclobacteriaceae_unclassified	otu233	3	0.36
Dadabacteria	Dadabacteriia	Dadabacteriales	Dadabacteriales_unclassified	Dadabacteriales_unclassified	otu347	3	0.22
Dadabacteria	Dadabacteriia	Dadabacteriales	Dadabacteriales_unclassified	Dadabacteriales_unclassified	otu1016	3	0.11
Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	67-14_unclassified	otu324	3	0.28
Proteobacteria	Deltaproteobacteria	NB1-j	NB1-j_unclassified	NB1-j_unclassified	otu344	1	0.15
Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellulaceae_unclassified	otu538	2	0.12
Actinobacteria	Acidimicrobiia	Microtrichales	Microtrichaceae	Microtrichaceae_unclassified	otu669	2	0.12
Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales	Blastocatellaceae	Blastocatella	otu489	3	0.12
Entotheonellaeota	Entotheonellia	Entotheonellales	Entotheonellaceae	Entotheonellaceae_unclassified	otu788	4	0.11
Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Subgroup 10	otu711	3	0.11
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Kangiellaceae	Kangiellaceae_unclassified	otu744	5	0.10
Proteobacteria	Gammaproteobacteria	Thiohalorhabdales	Thiohalorhabdaceae	Thiohalorhabdaceae_unclassified	otu571	6	0.10
Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Ekhidna	otu651	5	0.10
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacteriaceae_unclassified	otu579	6	0.10
Gemmatimonadetes	BD2-11 terrestrial group	BD2-11 terrestrial group_unclassified	BD2-11 terrestrial group_unclassified	BD2-11 terrestrial group_unclassified	otu1439	ы С	0.08
(b) OTU $ID \ge 99$	9 % similarity	Habitat(s)					
otu29 KT748	605.1; JX227334.1; EU4	91654.1 Basaltic crust, nod	ule fields				
otul1 JX2275	511.1; JQ013353.1; FJ938	3664.1 Nodule fields, deep	o-sea sediments, cobalt-rich crust				
otu31 MG58(	0220.1; KF268757.1	Mariana subductio	n zone sediments, heavy-metal conta	minated marine sediments			
otu83 MG580	0220.1; JN621543.1	Mariana subductio	n zone sediments, manganese oxide-	rich marine sediments			
otu160 MG58(	0740.1; JX227257.1	Mariana subductio	n zone sediments, nodule fields				
otu249 JQ2872	236.1; KM051824.1	Inactive hydrother	mal sulfides, basaltic crust				

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wide         JX226721.1*         Nodul           ouu202         MG580143.1; JY227690.1; JN860358.1         Maria           ouu317         JX226721.1*         Nodul           ouu317         JX227690.1; JN860358.1         Maria           ouu317         JX226721.1*         Nodul           ouu317         JX226721.1*         Nodul           ouu317         JX226731.1; FJ752931.1; KJ590663.1         Hydro           ouu425         JX227680.1; FJ938661.1         Nodul           ouu215         MC901562.1; AB015560.1         Maria           ouu215         MC90102.1; EU491612.1; KJ590663.1         Hydro           ouu216         HM101002.1; EU491612.1; KJ590663.1         Hydro           ouu316         HM101002.1; EU491636.1         Maria           ouu316         HM101002.1; EU491636.1         Nodul           ouu316         HM101002.1; EU491636.1         Nodul           ouu316         HX97757.1; X733208.1; JX227233.1         Nodul <t< th=""><th>Nodule fields Hvdrothermal vents. deep-sea sediments</th></t<>	Nodule fields Hvdrothermal vents. deep-sea sediments
oun78         JN860334.1; HQ721444.1         Hydro           ouu202         MG580143.1; JX227690.1; JN860358.1         Maria           ouu317         JX227432.1; AY627518.1         Nodul           ouu947         JX226721.1*         Nodul           ouu947         JX226721.1*         Nodul           ouu947         JX226721.1*         Nodul           ouu947         JX226721.1*         Nodul           ouu425         JX227680.1; FJ752931.1; KJ590663.1         Hydro           ouu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           ouu215         KC901562.1; AB015560.1         Maria           ouu636         HM101002.1; EU491612.1; KC682687.1         Hadici           ouu36         JX227383.1; KY977840.1; AM097938.1         Nodul           ouu36         JX227333.1; KY977840.1; AM097938.1         Nodul           ouu36         JX227333.1; KT936088.1; JX227223.1         Maria           ouu36         JX2277693.1; FJ024322.1; EU491736.1         Nodul           ouu36         JX2277693.1; FJ024322.1; EU491736.1         Nodul           ouu36         JX2277693.1; FJ024322.1; EU491736.1         Nodul           ouu36         JX227763.1; JY227223.1         Maria           ouu27         M2694157.1; JY227223.	Hvdrothermal vents, deep-sea sediments
otu317         MGS0143.1; JX227690.1; JN860358.1         Maria           otu317         JX227432.1; AY627518.1         Nodul           otu347         JX226721.1*         Nodul           otu347         JX226721.1*         Nodul           otu588         LC081043.1         Nodul           otu588         LC081043.1         Nodul           otu588         LC081043.1         Nodul           otu588         LC081043.1; FJ752931.1; KJ590663.1         Hydro           otu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           otu215         KC901562.1; AB015560.1         Maria           otu215         KC901562.1; AB015560.1         Basatt           otu215         KC901562.1; AB015560.1         Maria           otu215         KC901562.1; AB015560.1         Maria           otu216         HM101002.1; EU491556.1         Nodul           otu434         EU287401.1; JN977323.1         Nodul           otu36         JX2277333.1; KY97732.1; AM997938.1         Nodul           otu316         FN553503.1; AM997671.1         Nodul           otu316         FN553503.1; KT93688.1; JX227223.1         Nodul           otu316         KY977757.1; JX227233.1         Nodul           otu326 <td></td>	
otu317         JX227432.1; AY627518.1         Nodul           otu947         JX226721.1*         Nodul           otu947         JX226721.1*         Nodul           otu425         LC081043.1         Nodul           otu425         JX227680.1; FJ938661.1         Nodul           otu425         JX227680.1; FJ752931.1; KJ590663.1         Hydro           otu217         MG580382.1; AM997732.1         Maria           otu215         KC901562.1; AB015560.1         Basatt           otu215         KC901562.1; AM997732.1         Maria           otu215         KC901562.1; AM997732.1         Maria           otu215         KV901732.1         Maria           otu434         EU287401.1; JN977323.1         Nodul           otu36         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu36         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu36         JX227693.1; JX227083.1         Nodul           otu36         JX227693.1; SU99733.1         Nodul           otu36         JX227693.1; JX22723.1         Maria           otu36         JX227763.1; KY97787.1         Nodul           otu37         KY977757.1; JX227083.1         Nodul           otu162         KY97	860358.1 Mariana subduction zone sediments, nodule fields, hydrothermal vents
otu947         JX226721.1*         Nodul           otu588         LC081043.1         Nodul           otu588         LC081043.1         Nodul           otu6425         JX227680.1; FJ938661.1         Nodul           otu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           otu636         JM01002.1; EU491612.1; KJ590663.1         Hydro           otu215         KC901562.1; AB015560.1         Basatt           otu216         KC901562.1; JM0732.1         Maria           otu215         KC901562.1; AB015560.1         Basatt           otu216         HM101002.1; EU491612.1; KC682687.1         Hydro           otu36         JX227583.1; KY977840.1; AM997938.1         Nodul           otu36         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu36         JX227693.1; FJ024322.1; EU491736.1         Maria           otu36         JX227693.1; JX227083.1         Nodul           otu374         KY977757.1; JX227083.1         Nodul           otu316         JX227363.1; AM997733.1         Nodul           otu162         FN557363.1; JN227723.1         Maria           otu346         KY977757.1; JX227723.1         Nodul           otu341         JX2277353.1; SU491557.1         Nodul <td>Nodule fields, deep-sea sediments</td>	Nodule fields, deep-sea sediments
otu388         LC081043.1         Nodul           otu425         JX227680.1; FJ938661.1         Nodul           otu425         JX227680.1; FJ752931.1; KJ590663.1         Hydro           otu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           otu227         MG580382.1; AB015560.1         Basalt           otu215         KC901562.1; AB015560.1         Basalt           otu215         KC901562.1; BU491612.1; KC682687.1         Halicl           otu434         EU287401.1; JN977323.1         Subsu           otu434         EU287401.1; JN977323.1         Nodul           otu36         JX227383.1; KY977840.1; AM9979733.1         Nodul           otu36         JX2277693.1; FJ024322.1; EU491736.1         Nodul           otu36         JX2277693.1; KT336088.1; JX227223.1         Maria           otu36         JX2277693.1; M997733.1         Nodul           otu36         JX227757.1; KT336088.1; JX227223.1         Maria           otu316         KY977757.1; JY227223.1         Maria           otu36         JX227763.1; JX27723.1         Nodul           otu36         JX227763.1; KT336088.1; JX227223.1         Nodul           otu36         JX227763.1; KT336088.1; JX227223.1         Nodul           otu310 <td< td=""><td>Nodule fields</td></td<>	Nodule fields
otu425         JX227680.1; FJ938661.1         Nodul           otu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           otu277         MG580382.1; AM997732.1         Maria           otu215         KC901562.1; AB015560.1         Basalt           otu215         KC901562.1; AB015560.1         Basalt           otu215         KC901562.1; AB015560.1         Basalt           otu216         HM101002.1; EU491612.1; KC682687.1         Halic/           otu434         EU287401.1; JN977323.1         Subsu           otu434         EU287401.1; JN977323.1         Nodul           otu36         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu36         AB694157.1; JX227083.1         Nodul           otu210         KY97757.1; JX227083.1         Nodul           otu266         AB694157.1; JX22703.1         Maria           otu31         JX227693.1; AM997733.1         Nodul           otu264         KY97757.1; JX227083.1         Nodul           otu27         AB694157.1; JX22703.1         Maria           otu266         AB694157.1; JX22703.1         Nodul           otu264         KY97757.1; JX22703.1         Nodul           otu264         KY97757.1; KT336088.1; JX22723.1         Nodul	Nodule
otu68         JN886931.1; FJ752931.1; KJ590663.1         Hydro           otu227         MG580382.1; AM997732.1         Maria           otu215         KC901562.1; AB015560.1         Basalt           otu216         HM101002.1; EU491612.1; KC682687.1         Halicl           otu434         EU287401.1; JN977323.1         Subsu           otu434         EU287401.1; JN977323.1         Nodul           otu434         EU287401.1; JN977323.1         Nodul           otu434         EU287401.1; JN977323.1         Nodul           otu36         JX2277693.1; FJ024322.1; EU491736.1         Nodul           otu36         AB694157.1; JX227083.1         Nodul           otu521         KY97757.1; KT336088.1; JX227223.1         Maria           otu346         KY97757.1; JX227083.1         Nodul           otu313         JX227363.1; AM997733.1         Nodul           otu346         KY97757.1; KT336088.1; JX227223.1         Maria           otu324         EU491404.1         Occan         Occan           otu133         JX2277363.1; AM997733.1         Nodul         Occan           otu346         KY977757.1; KT336088.1; JX227223.1         Nodul         Occan           otu326         JX227709.1; AM997733.1         Nodul         Occan </td <td>Nodule fields, cobalt-rich crust</td>	Nodule fields, cobalt-rich crust
otu227         MG580382.1; AM997732.1         Maria           otu215         KC901562.1; AM915560.1         Basalt           otu636         HM101002.1; EU491612.1; KC682687.1         Halic/           otu636         HM101002.1; EU491612.1; KC682687.1         Basalt           otu36         JX227383.1; KY977840.1; AM997938.1         Nodul           otu36         JX2277383.1; F1024322.1; EU491736.1         Nodul           otu97         JX227693.1; F1024322.1; EU491736.1         Nodul           otu97         JX227693.1; JX227083.1         Nodul           otu97         JX227693.1; JX227083.1         Nodul           otu366         AB694157.1; JX227083.1         Nodul           otu261         KY977757.1; JX227083.1         Nodul           otu346         KY977757.1; JX227083.1         Nodul           otu131         JX2277363.1; AM997733.1         Nodul           otu134         EU491604.1         Nodul           otu134         EU491604.1         Ocean           otu255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu234         EU491604.1         Ocean           otu255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu255         JX227709.1; F1437705.1; KM110219.1	0663.1 Hydrothermal carbonate sediments, polychaete burrow environment, biofilm
otu215         KC901562.1; AB015560.1         Basalt           otu636         HM101002.1; EU491612.1; KC682687.1         Halici           otu434         EU287401.1; JN977323.1         Subsu           otu36         JX227383.1; KY977840.1; AM997938.1         Nodul           otu162         FN553503.1; FJ024322.1; EU491736.1         Hydro           otu36         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu266         AB694157.1; JX227083.1         Nodul           otu346         KY97757.1; KT336088.1; JX227223.1         Maria           otu346         KY97757.1; KT336088.1; JX227223.1         Maria           otu346         KY97757.1; JX227703.1         Maria           otu346         KY97757.1; EU491557.1         Nodul           otu346         KY97757.1; EU491557.1         Nodul           otu113         JX227703.1; EU491557.1         Nodul           otu113         JX227705.1; EU491557.1         Nodul           otu113         JX227705.1; EU491557.1         Nodul           otu113         JX227705.1; EU491557.1         Nodul           otu347         EU491604.1         Ocean           otu112         MG580277.1; EU491557.1         Nodul           otu255         JX227705.1; FJ437705.1; KM110219.1 </td <td>Mariana subduction zone sediments, deep-sea sediments</td>	Mariana subduction zone sediments, deep-sea sediments
otu636         HMI01002.1; EU491612.1; KC682687.1         Halici           otu434         EU287401.1.1, JN977323.1         Subsu           otu36         JX227383.1; KY977840.1; AM997938.1         Nodul           otu36         JX227693.1; F1024322.1; EU491736.1         Nodul           otu97         JX227693.1; F1024322.1; EU491736.1         Nodul           otu97         JX227693.1; F1024322.1; EU491736.1         Nodul           otu366         AB694157.1; JX227083.1         Nodul           otu521         KY977757.1; JX227083.1; JX227223.1         Maria           otu346         KY97757.1; JX227033.1         Nodul           otu341         JX227363.1; JX227723.1         Maria           otu13         JX2277363.1; JX227723.1         Nodul           otu134         EU491404.1         Nodul           otu133         JX2277363.1; FU491557.1         Nodul           otu113         JX227709.1; F1437705.1; KM110219.1         Nodul           otu1234         EU491604.1         Ocean           otu255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AY225640.1         Nodul           otu326	Basaltic glasses, deep-sea sediments
otu434         EU287401.1; JN977323.1         Subsu           otu36         JX227383.1; KY977840.1; AM997938.1         Nodul           otu162         FN553503.1; AM997671.1         Hydro           otu162         FN553503.1; FJ024322.1; EU491736.1         Nodul           otu266         AB694157.1; JX227083.1         Nodul           otu261         KY977757.1; KT336088.1; JX22723.1         Maria           otu346         KY977757.1; JX227083.1         Maria           otu346         KY977757.1; JX227233.1         Maria           otu346         KY977757.1; JX227223.1         Maria           otu346         KY97757.1; JX227233.1         Nodul           otu346         KY91404.1         Nodul           otu113         JX226757.1; EU491557.1         Nodul           otu113         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu122         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         JX227709.1; FJ43705.1; M09786005.1         Motul           otu1326         JN886905.1; KT748584.1         Nodul           otu865         JX227375.1	C682687.1 Halichondria marine sponge, oceanic crust
otu 36         JX227383.1; KY977840.1; AM997938.1         Nodul           otu 162         FN553503.1; F1024322.1; EU491736.1         Hydro           otu 97         JX227693.1; F1024322.1; EU491736.1         Nodul           otu 266         AB694157.1; JX227083.1         Nodul           otu 521         KY977757.1; JX227083.1; JX22723.1         Maria           otu 521         KY977757.1; JX227088.1; JX22723.1         Maria           otu 346         KY977757.1; JX22723.1         Maria           otu 341         JX227363.1; JX22723.1         Nodul           otu 91         JX227363.1; JX227723.1         Nodul           otu 13         JX227767.1; EU491557.1         Nodul           otu 113         JX2277363.1; AM997733.1         Nodul           otu 113         JX2277363.1; FU491557.1         Nodul           otu 113         JX2277361.1; EU491557.1         Nodul           otu 124         EU491604.1         Ocean           otu 123         JX227709.1; F1437705.1; KM110219.1         Nodul           otu 255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu 122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu 326         JN886905.1; KT748584.1         Nodul           otu 326	Subsurface sediments, marine sediments
otu 162         FN553503.1; AM997671.1         Hydro           otu 97         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu 266         AB694157.1; JX227083.1         Nodul           otu 261         KY977757.1; KT336088.1; JX22723.1         Deep-           otu 346         KY977757.1; JX227083.1         Maria           otu 346         KY97757.1; JX22723.1         Maria           otu 341         JX226757.1; EU491557.1         Nodul           otu 991         JX226757.1; EU491557.1         Nodul           otu 113         JX226757.1; EU491557.1         Nodul           otu 123         BU491604.1         Ocean           otu 234         EU491604.1         Ocean           otu 235         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu 235         JX227709.1; KT748584.1         Maria           otu 122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu 326         JN886905.1; KT748584.1         Nodul           otu 865         JX227375.1; FJ938651.1; AY225640.1	1997938.1 Nodule fields, Mariana subduction zone sediments, deep-sea sediments
otu97         JX227693.1; FJ024322.1; EU491736.1         Nodul           otu266         AB694157.1; JX227083.1         Deep-           otu521         KY977757.1; KT336088.1; JX22723.1         Maria           otu346         KY977757.1; JX2272088.1; JX227223.1         Maria           otu341         JX227363.1; AM997733.1         Nodul           otu991         JX227363.1; AM997733.1         Nodul           otu133         JX226757.1; EU491557.1         Nodul           otu113         JX226757.1; EU491557.1         Nodul           otu113         JX226757.1; EU491557.1         Nodul           otu113         JX226767.1; EU491557.1         Nodul           otu123         JX2267709.1; FJ437705.1; KM110219.1         Nodul           otu254         EU491604.1         Ocean           otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu326         JN886905.1; KT748584.1         Hydrc           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul	Hydrothermal vents, deep-sea sediments
otu 266         AB694157.1; JX227083.1         Deep-           otu 321         KY977757.1; KT336088.1; JX22723.1         Maria           otu 346         KY977757.1; JX22723.1         Maria           otu 346         KY97757.1; JX22723.1         Maria           otu 346         KY97757.1; JX22723.1         Maria           otu 346         KY97757.1; JX22723.1         Maria           otu 13         JX226757.1; EU491557.1         Nodul           otu 113         JX226757.1; EU491557.1         Nodul           otu 184         EU491404.1         Ocean           otu 184         EU491604.1         Ocean           otu 234         EU491604.1         Ocean           otu 234         EU491606.1         Nodul           otu 234         EU491606.1         Nodul           otu 255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu 256         JN886905.1; KT748584.1         Nodul           otu 122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu 326         JN886905.1; KT748584.1         Nodul           otu 865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu 865         JX2277375.1; FJ938651.1; AY225640.1         Nodul	91736.1 Nodule fields, oceanic crust
otu521         KY977757.1; KT336088.1; JX227223.1         Maria           otu346         KY977757.1; JX227223.1         Maria           otu91         JX227363.1; AM997733.1         Maria           otu91         JX226757.1; EU491557.1         Nodul           otu13         JX226757.1; EU491557.1         Nodul           otu113         JX226757.1; EU491557.1         Nodul           otu184         EU491404.1         Ocean           otu184         EU491604.1         Ocean           otu234         EU491604.1         Ocean           otu235         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu326         JN886905.1; KT748584.1         Nodul           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul	Deep-sea benthic foraminifera, nodule fields
otu346         KY97757.1; JX22723.1         Maria           otu991         JX227363.1; AM997733.1         Nodul           otu113         JX226757.1; EU491557.1         Nodul           otu113         JX2266757.1; EU491557.1         Nodul           otu113         JX2266757.1; EU491557.1         Nodul           otu134         EU491604.1         Ocean           otu254         EU491604.1         Ocean           otu255         JX227709.1; F1437705.1; KM110219.1         Nodul           otu255         JX227709.1; F437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu326         JN886905.1; KT748584.1         Hydrc           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu171         AM997407.1; FJ205352.1; EU491267.1         Deep-	227223.1 Mariana subduction zone sediments, nodules, nodule fields
otu991       JX227363.1; AM997733.1       Nodul         otu113       JX226757.1; EU491557.1       Nodul         otu113       JX226757.1; EU491557.1       Nodul         otu184       EU491404.1       Ocean         otu234       EU491604.1       Ocean         otu234       EU491604.1       Ocean         otu234       EU491604.1       Ocean         otu235       JX227709.1; FJ437705.1; KM110219.1       Nodul         otu255       JX227709.1; FJ437705.1; M110219.1       Nodul         otu122       MG580277.1; AM997814.1; AJ966605.1       Maria         otu122       JN886905.1; KT748584.1       Hydrc         otu326       JN886905.1; KT748584.1       Hydrc         otu865       JX227375.1; FJ938651.1; AY225640.1       Nodul         otu171       AM997407.1; FJ205352.1; EU491267.1       Deep-	Mariana subduction zone sediments, nodule fields
otu113       JX226757.1; EU491557.1       Nodul         otu184       EU491404.1       Ocean         otu234       EU491604.1       Ocean         otu255       JX227709.1; FJ437705.1; KM110219.1       Nodul         otu122       MG580277.1; AM997814.1; AJ966605.1       Maria         otu122       MG580277.1; FJ938651.1; AY225640.1       Hydro         otu865       JX227375.1; FJ938651.1; AY225640.1       Nodul         otu171       AM997407.1; FJ205352.1; EU491267.1       Deep-	Nodule fields, deep-sea sediments
otu 184         EU491404.1         Ocean           otu 234         EU491604.1         Ocean           otu 255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu 255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu 256         JX88690277.1; AM997814.1; AJ966605.1         Maria           otu 122         MG580277.1; FJ938651.1; AY225640.1         Hydro           otu 865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu 171         AM997407.1; FJ205352.1; EU491267.1         Deep-	Nodule fields, oceanic crust
otu234         EU491604.1         Occan           otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu326         JN886905.1; KT748584.1         Hydro           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu171         AM997407.1; FJ205352.1; EU491267.1         Deep-	Oceanic crust
otu255         JX227709.1; FJ437705.1; KM110219.1         Nodul           otu122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu326         JN886905.1; KT748584.1         Hydro           otu326         JN886905.1; KT748584.1         Hydro           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu171         AM997407.1; FJ205352.1; EU491267.1         Deep-	Oceanic crust
otu 122         MG580277.1; AM997814.1; AJ966605.1         Maria           otu 326         JN886905.1; KT748584.1         Hydro           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu171         AM997407.1; FJ205352.1; EU491267.1         Deep-	10219.1 Nodule fields, hydrothermal deposits
otu326         JN886905.1; KT748584.1         Hydro           otu865         JX227375.1; FJ938651.1; AY225640.1         Nodul           otu171         AM997407.1; FJ205352.1; EU491267.1         Deep-	J966605.1 Mariana subduction zone sediments, deep-sea sediments, nodule fields
otu865 JX227375.1; FJ938651.1; AY225640.1 Nodul otu171 AM997407.1; FJ205352.1; EU491267.1 Deep-	Hydrothermal carbonate sediments, basalt crust
otu 171 AM997407.1; FJ205352.1; EU491267.1 Deep-	25640.1 Nodule fields, cobalt-rich crust, hydrothermal sediments
	491267.1 Deep-sea sediments, hydrothermal vents, oceanic crust
otu541 AB694393.1 Deep-	Deep-sea benthic foraminifera

 $^* \geq 98~\%$  similarity.

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Table 3. Cor	ntinued.	
(b) OTU	$ID \ge 99\%$ similarity	Habitat(s)
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*	Oceanic 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, oceanic crust
otu519	GU220747.1; MG580729.1	Fe-rich hydrothermal deposits, Mariana subduction zone sediments
otu71	FJ205181.1; JX226787.1	Hydrothermal vents, nodule fields
otu163	JX227427.1; JN886907.1; EU491661.1	Nodule fields, hydrothermal carbonate sediments, oceanic crust
otu532	EU491402.1; JX227188.1; EU374100.1	Oceanic 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, oceanic crust
otu1016	KF616695.1; KM396663.1; EU491261.1	Carbonate methane seep, brine seep, oceanic crust
otu324	JX226791.1; JN886912.1	Nodule fields, hydrothermal carbonate sediments
otu344	EU438185.1; KY977824.1	Deep-sea sediments and hydrothermal vents, oceanic crust
otu538	KM356353.1; JX226930.1; DQ996924.1	Carbonate methane seep, nodule fields, deep-sea sediments
otu669	EU491619.1; MG580068.1; KT748607.1	Oceanic crust
otu489	EU491660.1; MG580531.1; AM998023.1	Oceanic crust, deep-sea sediments
otu788	JN886890.1; MG580099.1	Hydrothermal carbonate sediments, oceanic crust
otu711	JX193423.1; GU302449.1; AY225643.1	Mariculture sediments, hydrocarbon seep, oceanic 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*	Outcrops
otu579	EU491573.1; KT336070.1	Oceanic crust, nodules
otu1439	JN886922.1; KC747092.1; JN884864.1	Hydrothermal carbonate sediments, deep-sea sediments, methane seep



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



Figure 3. Bacterial-community structure at the dominant class level (cut-off of  $\geq 1\%$ ). MN: manganese nodules; MUC: sediments.

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 remineralisation for members of that family, as recently confirmed by analysis of deep-sea assembled genomes (Hoffmann et al., 2020). The most closely 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 Sub-

group 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., 2017). The family Magnetospiraceae includes microaerophilic heterotrophs, which are capable of magnetotaxis and iron reduction (i.e. genus *Magnetospirillum*; Matsunaga, 1991; Schüler and Frankel, 1999), and thus the

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**Figure 4.** Taxa highly abundant in nodules (ALDEx2: glm-adjusted p of < 0.01; KW-adjusted p of < 0.05). The base 2 logarithm of the ratios between the sequence number of nodules (Nod) and sediments (Sed) centred by geometric mean as well as the average contribution of total number of sequences (%) retrieved in nodules and in sediments is shown. For details see Table S3.

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members of this family could play a role in Fe(III) mobilisation, affecting its bioavailability. Hyphomicrobiaceaelike sequences found in this study are related to the 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 cy-



**Figure 5.** Habitat coverage for the most closely related sequences  $(\geq 99\%$  similarity) to OTUs highly abundant in the nodules. For details see Table 3a–b.

cling in manganese nodules is also suggested by the presence of the most closely related sequences in the oceanic crust (Santelli et al., 2008; Lee et al., 2015), which typically hosts epilithic and endolithic microbial communities of chemolithotrophic metal oxidisers (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 the genus Kiloniella and have been isolated from marine macroalgae (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 polynya in the Amundsen Sea in the West Antarctic Ice Sheet (Si et al., 2017). Furthermore, Kiloniellaceae-like sequences were found in sponges (Cleary et al., 2013), sea star larvae (Galac et al., 2016), and in the iron mats of seamounts (Scott et al., 2017). The presence of rich sessile and mobile metazoan communities associated with 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 (Fig. S2b). Archaeal sequences comprised a lower portion of total sequences retrieved from sediments (6%–45%) and nodules (< 1%–7%) of the Peru Basin, and they were lower in nodules compared to sediments. Our data differed from what was reported by Shulse et al. (2017) for the 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. (2017). As both primer sets amplified the same hypervariable region of the 16S rRNA gene (V4) we assume that biases are small enough to justify the comparison. The majority of members of Nitrosopumilaceae are believed to be capable of oxidation of ammonia to nitrite, the first step of nitrification (Offre et al., 2013). Archaeal ammonia oxidisers have a higher affinity for ammonia than bacterial ammonia oxidisers, 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 the 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 Basin 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; Fig. S2), which is at the higher end of previous microbial beta diversity estimates for bathyal and abyssal sea floor 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 (Fig. 2), as previously reported for the CCZ as well (Wu et al., 2013; Tully and Heidelberg, 2013; Shulse et al., 2017; Lindh et al., 2017). Although 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 (Fig. 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 (Figs. 1 and S1). This seems to be a common feature of polymetallic nodules (Wu et al., 2013; Tully and Heidelberg, 2013; G. Zhang et al., 2014; Shulse et al., 2017; 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 the fact 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 the lack of sediment accumulation on nodules, the decrease in microbial diversity with the decrease in organic matter availability is in accordance with the positive energydiversity 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 the 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 metal concentration, hard substrate, presence of sessile fauna).

## 4.3 Potential functions of microbial communities associated with 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%; Fig. 4, Tables S3 and 3a) indicates niche specialisation. 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, of which the most closely related sequences have been retrieved from Pacific nodules (e.g. Wu et al., 2013; Blöthe et al., 2005; 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 Fig. 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-reducing Bacteria (i.e. Magnetospiraceae) and oxidisers (i.e. Hyphomicrobiaceae) and the presence of encrusting protozoans (Gooday et al., 2015), microbial eukaryotes (Shulse et al., 2017) 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 colonise nodules, likely favoured by manganese and iron availability, formation of biofilms, and the presence of sessile fauna communities.

The reduction and dissolution of Mn oxides by dissolved organic matter (e.g. humic compounds) typically occur 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 also been observed 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 may potentially favour Mn-oxidising Bacteria and microbial exploitation of refractory dissolved organic matter (DOM). Intense extracellular enzymatic activities have been reported for sea-floorexposed basalts (Jacobson 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 particulate organic carbon (POC) from the bottom water and release DOM and catabolic metabolites (e.g. ammonia). Thus, nodules may act as hotspots of organic-carbon degradation. Although 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 the Pacific Nodule Province (Tully and Heidelberg, 2013; Shulse et al., 2017) and in this study could indicate 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 ammoniaoxidising Nitrosopumilus, which represented a large portion of the microbial assemblages (up to 20%) and a minor contribution of bacterial nitrite-oxidising Nitrospira (Tully and Heidelberg, 2013; Shulse et al., 2017). Peru Basin sediments and nodules showed more diversified nitrifier communities, which are enriched by ammonia-oxidising AqS1 (1%) and unclassified Nitrosomonadaceae (1%) and by nitriteoxidising *Nitrospina* (4%) and *Nitrospira* (1%; Fig. 4, Tables S3 and 3a). *Nitrospina* are not commonly reported for deep-sea sediments, but they are the dominant nitrite oxidisers in the oceans (Luecker et al., 2013). They have recently been reported as symbionts of deep-sea glass sponges (Tian et al., 2016), which also commonly colonise 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 sponges (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 the oceanic 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. Mncycling 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 generalised. 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 as well as 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 characterise metabolic activities on and in nodules and to understand factors and processes controlling nodule colonisation. 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 numbers PRJEB30517 (https://www.ebi.ac.uk/ena/data/view/PRJEB30517; Centre for Polar and Marine Research, 2020) and PRJEB32680 (https://www.ebi.ac.uk/ena/data/view/PRJEB32680; Max Planck Institute for Marine Microbiology, 2020).

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*Author contributions.* AB, FJ, FW, and MM conceived the study. AB, FJ, FW, and TRV performed sampling activities. MM compiled and analysed the data. MM wrote the paper with contributions from all authors.

*Competing interests.* The authors declare that they have no conflict of interest.

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