Contrasting archaeal and bacterial community assembly processes and the importance of rare taxa along a depth gradient in shallow coastal sediments

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Running Title: Microbial communities in marine sediments

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Abstract

Marine microbial communities assemble along a sediment depth gradient and are responsible for processing organic matter. The high-resolution mechanisms of the vertical assembly processes in marine sediments remain poorly described. We analyzed 31 depth layers of 3 sediment cores from the shallow sediment zone at 3 stations in the Bohai Sea, and obtained high-resolution vertical profiles (2 cm per sample) of microbial communities. We analyzed 78 archaeal and 76 bacterial communities based on 16S rRNA gene amplicon sequencing, together with 14 selected metagenomes. We grouped these samples into three layers (Top, 0-18 cm; Middle, 18-38 cm; and Bottom, below 38 cm) to analyze trends in diversity and assembly processes along the depth gradient. We found that alpha diversity increased for the *Thaumarchaeota*-dominated archaeal community but decreased for the *Proteobacteria*-dominated bacterial community as depth increased. The mechanisms determining archaeal community assembly were mostly deterministic, while bacterial community assembly was mostly stochastic. Co-occurrence networks among different taxa and key functional genes revealed a tight community with low modularity in the bottom sediment, and disproportionately more interactions among low abundance ASVs. This suggests a significant contribution to community stabilization by rare taxa, and suggests that the bottom layer, rather than surface sediments may represent a hotspot for benthic microbial interactions.

Keywords: Marine sediment; microbial assembly; stochasticity; determinism; rare taxa
Importance: Marine sediments harbors a giant microbial diversity. To date, our understanding of the mechanisms determines their distribution remain limited. We identified different mechanism dominated the distribution of archaeal and bacterial assemblies mostly may due to the metabolic plasticity in bacteria. We demonstrated an increased microbial co-occurrence network with depth. We further stressed the importance of rare taxa in the microbial community based on the co-occurrence network of the relative abundance of different taxa and different key genes in marine biogeochemical cycling. This work reveals the complex mechanisms determines the distribution of microbial assemblies in coastal marine sediments.

Introduction

Over 70% of the Earth’s surface is covered by the ocean, and microbes in marine sediments account for up to one-third of living biomass on the planet (1). The settled organic matter from the overlying water column provides sufficient carbon resources for microbes in marine sediments. Sediment-inhabiting microorganisms are involved in the processing of this carbon while cycling other nutrients, e.g., nitrogen, sulfur, and iron (2, 3). Single-gene (e.g., 16S rRNA) or metagenome-based surveys of marine sediments have revealed a diversity of deeply-branched, uncultured bacterial and archaeal phyla, including the Candidate Phyla Radiation in the bacterial domain and the Asgard Superphylum in the archaeal domain (4–6). Knowledge about the composition, interaction, and dynamics of microbial communities in marine sediments is crucial for understanding microbial ecology.
The Bohai Sea is a semi-enclosed marginal sea of the Northwest Pacific. The biogeochemistry in the Bohai Sea is complex. First, it is jointly influenced by terrestrial inputs, e.g., the discharge from rivers (7), and the neighboring North Yellow Sea (8). Second, the Bohai Sea has a high rate of sedimentation (over 10 mm•yr\(^{-1}\)) (9). Third, the Bohai Sea is characterized by clear seasonal variations in environmental conditions, e.g., temperature, chlorophyll-a, and primary production (10). Moreover, recently observed seasonal hypoxia events in the bottom waters of the Bohai Sea in summer (11, 12) may increase buried organic matter due to insufficient degradation. Furthermore, the redox gradients in the sediment change rapidly along the depths.

Extensive studies of microbial communities in the Bohai Sea have commonly revealed a strong effect of anthropogenic activities on microbial distribution (13–16). The microbial composition in marine sediments is largely affected by both the abiotic or environmental factors (17–19), such as the nutrient availability and redox gradients, and the biotic factors, e.g., competition and predation. However, due to the complexity of the biogeochemical conditions, the mechanism of prokaryotic distribution has not been well studied in the Bohai Sea. Energy-limitation is usually a key factor in determining the distribution of prokaryotes in marine sediments (20), but it may have a different role in the shallow zone of coastal sediments. The high sedimentation rate and buried organic matter during seasonal hypoxia in the Bohai Sea may affect the distribution of the archaeal and bacterial communities differently, due to their metabolic versatility and differences along depth in the shallow zone. For example, a study on the geographic and seasonal distribution of archaea in the coastal surface sediments showed
that the distribution of archaeal communities was mainly impacted by stochasticity rather than determinism (21). Thus, it remains unclear what the patterns in vertical distributions of bacterial and archaeal communities are and what determines the patterns in the coastal sediments. The Bohai Sea sediment environments are ideal targets to study the patterns of vertical distributions of prokaryotic communities and the processes that determine their distribution.

We hypothesized that the vertical distributions of benthic prokaryotic communities, which include both bacteria and archaea, are primarily affected by the biogeochemical conditions rather than stochastic processes. In this study, we examined the spatial distribution (from the surface to 62 cm below the surface maximum) of both the bacterial and archaeal communities with high spatial resolution (2 cm for each sample), together with samples selected for metagenome sequencing at three different stations in the shallow sediment zones of the Bohai Sea. We assessed different ecological processes governing the microbial community assembly. The co-occurrence of different taxa and key functional genes also stressed the importance of rare taxa in the microbial interactions driving element cycling in marine sediments.

**Results**

We collected 3 sediment cores from 3 stations (M3, M8, and BHB10) in the Bohai Sea. Each core was sub-sampled every 2 cm with 78 sediment samples in total. We analyzed the biogeochemistry in the porewater and sediment samples. The archaeal and bacterial
communities were analyzed based on 16S rRNA gene amplicon sequencing. We further selected 14 samples for metagenomic sequencing.

**Geochemistry in sediments**

To classify the sediment cores into three layers (top, middle, and bottom), we characterized the vertical change of the biogeochemical conditions in sediments. The concentrations of measured geochemical characteristics showed different patterns along the sediment depth gradients at different stations (Fig. S1, https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). PCA revealed different biogeochemical conditions among samples from different depths at three stations (Fig. S1), which we classified as three layers: top, middle, and bottom. The biogeochemical conditions at three stations (BHB10, M3, and M8) and three different layers were significantly different ($P < 0.01$) based on three non-parametric multivariate statistical tests (MRPP, ANOSIM, and Adonis), and the effect of sampling sites was more profound than depth (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). The biogeochemical conditions in the bottom sediments at three stations were more similar to each other than those in the surface sediments at three stations (Fig. S1).

**Alpha and beta diversity of bacterial and archaeal communities**

Generally, the species richness of the archaeal community was lower than that of the bacterial community (Fig. 1). Archaeal alpha diversity increased from top to bottom sediments (Fig. 1a and S2), especially for the top layer, while bacterial alpha diversity
decreased (Fig. 1b and S2). To estimate the separation pattern of archaeal and bacterial composition, PCoA at the ASV level was performed. Based on Bray-Curtis dissimilarity, the composition showed a clear separation along the primary principal coordinate for both archaeal (29% and 18%, respectively) and bacterial community (29% and 14%, respectively) (Fig. 1e and 1f). NMDS ordination further revealed that the archaeal (Stress = 0.10) and bacterial (Stress = 0.10) communities were grouped by three sampling sites and layers (Fig. S3). Three non-parametric multivariate statistical tests (MRPP, ANOSIM, and Adonis), together with the WPGMA analysis, further confirmed the significant differences in archaeal and bacterial communities in different layers at different locations ($P < 0.01$) (Fig. S3, https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). It is noteworthy that samples from station BHB10 were more different than samples from the other two stations, especially for the bacterial community. Overall, the results revealed that both the archaeal and bacterial communities were variable at different depths in the three study sites.

**Taxonomic assignment in sediments**

An average of 89 ± 7 % and 83 ± 7 % of sequences in each sample could be assigned to archaeal and bacterial phylum levels, respectively. The relative abundance of archaea and bacteria in the Bohai Sea sediments showed taxon-specific distribution patterns according to the depths and sampling sites. The sediment samples were dominated by *Thaumarchaeota* (29 ± 24 %), followed by *Euryarchaeota* (23 ± 12 %), *Crenarchaeota*
(20 ± 16 %), and Woesearchaeota (13 ± 12 %) for the archaeal community (Fig. 2a, https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). More than half of the bacterial communities were Proteobacteria (50 ± 14 %), which mainly consisted of Deltaproteobacteria (26 ± 6 %), Gammaproteobacteria (18 ± 10 %), and Alphaproteobacteria (4 ± 1 %) (Fig. 2b).

**ASV occurrence pattern and spatial distribution of microbial community**

Approximately 1% (110 out of 8,809 and 299 out of 32,893 for archaeal and bacterial ASVs, respectively) of ASVs occurred in ≥ 50% of all samples. Among these 110 ASVs, only 9 Proteobacteria ASVs, 2 Chloroflexi ASVs, and other two unclassified bacterial ASVs were present in all samples. There were strong positive correlations between the samples that included these ASVs and the relative abundance of both archaeal and bacterial communities, regardless of the station and sampling depth (Fig. S4). This suggests that rare taxa have limited habitats or their abundances are below the limit of detection. This pattern was more obvious for archaea than bacteria, as evidenced by the higher rho value for the archaeal community than the bacterial community (Fig. 3a and 3b). The occurrence patterns of ASVs assigned to each taxon showed that Woesearchaeota and Proteobacteria had 3,740 and 12,709 ASVs, and were the most diverse phyla in the archaeal and bacterial communities. However, the most abundant taxon in the archaeal community, Thaumarchaeota, only had 250 ASVs in total and many of them were widely distributed in the samples. More than half of ASVs in each taxon, which contributed a total relative abundance over 1% in each domain, were only
present in one sample, except for Crenarchaeota and Pacearchaeota (Fig. 3c). However, ASVs that occurred in only one sample had low relative abundance.

The dissimilarity of microbial communities between samples increased with depth for both archaeal and bacterial assemblages in all three sites (Fig. 3d and 3e), and this trend was stronger in the archaeal community than in the bacterial community. To reveal the effect of phylogeny on the change of dissimilarity, the dissimilarity of each major lineage (i.e., relative abundance > 1%) was examined. The dominant lineages, e.g., Thaumarchaeota, Euryarchaeota, and Crenarchaeota for the archaeal community, Deltaproteobacteria, Gammaproteobacteria, Chloroflexi, and Alphaproteobacteria for the bacterial community, generally exhibited less dissimilarity than the rare lineages (Fig. S5). The correlation between dissimilarity and depth decreased gradually with the relative abundance of each clade (Fig. S5).

Determinism vs stochasticity influencing the structure of the microbial community

A tb-RDA revealed that all chemical variables (nitrate, nitrite, ammonium, DIP, DIC, CO, methane, and sulfate) and depth collectively affected the archaeal and bacterial composition. However, the first two axes explained the variance for only 13% and 11% in the archaeal community, and 10% and 8% in the bacterial community (Fig. S6). A Mantel test further confirmed the insignificant Spearman’s correlations between the collective environmental conditions and the microbial communities (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). The changes in archaeal and bacterial communities were
significantly associated with nitrite, ammonium, sulfate, and CO concentrations in the sediments \( (P < 0.05) \) (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples).

To further reveal the mechanisms behind the observed patterns of archaeal and bacterial distributions, we used infer Community Assembly Mechanisms by Phylogenetic-bin-based null model analysis (iCAMP) (22). Based on the null model analysis, in the archaeal community deterministic processes accounted for 67\%, 53\%, and 51\% of the assembly in top, middle, and bottom sediments, respectively. This means that heterogeneous selection and homogeneous selection were responsible for over half of the archaeal community assembly. In contrast, in the bacterial community, stochastic processes accounted for 57\%, 60\%, and 59\% of the assembly in top, middle, and bottom sediments, respectively. This means that homogenizing dispersal, dispersal limitation, drift and others were dominant for the bacterial community assembly (Fig. 4a). The homogeneous selection was the most important process among the five processes (22), with a minor contribution from heterogeneous selection and homogenizing dispersal to explain both the archaeal and bacterial communities variation in all three layers of sediments (Fig. 4a).

**Microbial co-occurrence networks**

We retained 547, 470, and 620 archaeal and bacterial ASVs for the co-occurrence network analysis from top, middle, and bottom layers, respectively. They occurred in more than half of the samples in each layer. After filtration \( (P < 0.01 \text{ and } \rho > 0.7) \),
there were 22,028 edges among 281 archaeal and 563 bacterial nodes (edge/node ratio $= 26$) for the overall co-occurrence network. 3,828 edges among 83 archaeal and 283 bacterial nodes (edge/node ration $= 10$) composed the co-occurrence network in the top layer. 5,061 edges among 128 archaeal and 210 bacterial nodes (edge/node ration $= 15$) composed the co-occurrence network in the middle layer. 17,611 edges among 200 archaeal and 328 bacterial nodes (edge/node ration $= 33$) composed the co-occurrence network in the bottom layer (Fig. 5 and S7). These ASVs compromised 3% archaeal diversity, 2% bacterial diversity, 58% archaeal abundance, and 51% bacterial abundance.

The clustering coefficient of 1000 random networks was much lower than the corresponding network (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). This suggests the observed network structure was non-random and unlikely to be due to chance. Based on the Kolmogorov-Smirnov test, each observed network was distinct ($P < 0.001$) (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). As a whole, the overall network was the most robust, and the robustness of the network increased from the top to the bottom (Fig. S7). The increasing average degree, network density, degree centralization, and clustering coefficient; and the decreasing average path length, network diameter, betweenness centralization, and modularity provided additional evidence that network complexity increased from the top to the bottom of the sediment gradient (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples).
Module 1, the largest module, was mainly composed of ASVs from *Proteobacteria* and *Woesearchaeota*. The most abundant archaeal and bacterial ASVs did not have high connectivity with other ASVs in the community. The ASVs that were widely connected formed module 2, and were generally in low abundance and from different phyla, including *Proteobacteria*, *Euryarchaeota*, *Chloroflexi*, and some unclassified groups (Fig. 5). This suggests an important role of rare taxa in maintaining the stability of microbial networks in marine sediments. There were more ASVs included in the bottom sediment networks, albeit with a low relative abundance of each ASV. The number of network modules decreased from the top to the bottom sediments, but the connectivity increased (Fig. 5). It is of note that the relative abundance of archaeal ASVs only represented the abundance within the archaeal community, thus their relative abundance is not directly comparable with the bacterial ASVs. Keystone taxa maintain the structure of the network, and maintain community function. These were identified based on the topological roles of each node (23).

**Microbial functional diversity in marine sediments**

To further explore connections between microbial diversity and functional genes in marine sediments, 14 samples were deeply shotgun sequenced (752,062,594 to 975,955,532 paired reads for each sample) and assembled to contigs (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). Based on annotations from the IMG/M pipeline, a total of 7,764 unique KOs (KEGG Orthology) were identified. We selected a set of genes encoding...
for the catalytic or key subunit of enzymes (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples) involved in the redox reaction of oxygen, nitrogen, and sulfur. These are important for element cycling in marine sediments and energy conservation for microbes, especially in the shallow zone of coastal sediments where we took samples. The counts and the normalized abundance of the identified KOs in the assembly represent the microbial diversity and abundance of that specific KO, respectively. The ratios of the diversity of a specific gene in the three layers were similar to that for the abundance. Several archaeal and bacterial phyla had strong correlations with the diversity or abundance of those key genes (Fig. 6 and S7). However, the relative abundance of a phylum was not associated with the intensity of correlations with genes, regardless of the phylum’s abundance or diversity. The high-abundance archaeal phyla, i.e., *Thaumarchaeota* (29%) and *Euryarchaeota* (23%), were associated with nitrogen and sulfur cycling, but the correlations were less strong than other low-abundance phyla. There were no correlations between the key genes and the other abundant archaeal phyla, e.g., *Crenarchaeota* (20%) and *Woesearchaeota* (13%). The dominant bacterial phylum, *Proteobacteria* (50%), only had correlations with the abundance of a few genes (*nirA*, *nrfA*, and *hmp*) (Fig. 6). The phyla that were strongly correlated with the diversity and abundance of key genes, i.e., *Aenigmarchaeota* (0.18%), *Gemmatimonadetes* (0.46%), *Kiritimatiellaeota* (0.23%), *Lentisphaerae* (0.02%), and WPS-2 (0.02%), were mainly rare taxa in the marine sediments (Fig. 6 and S7). Notably, *Gemmatimonadetes* had the strongest correlation with key processing genes (*ccoN, nrfA, nirS, narG, nirD, phsA*,
asrA, and fccB), suggesting this phylum was actively involved in carbon, nitrogen, and sulfur cycling in the shallow zones of coastal marine sediments.

Discussion

Spatial heterogeneity in benthic prokaryotes affected by sediment biogeochemistry

Bohai Sea represents a typical coastal sea largely affected by anthropogenic activities and freshwater inputs. In the current study, we performed extensive analyses of sediment samples at different depths ranging from surface to 62 cm with a high resolution of 2 cm per sample at three stations in the Bohai Sea. Consistent with a study on the vertical variation of bacterial communities in the Chinese coastal sea, the alpha diversity of bacterial communities decreased from the top to bottom, but with a locational difference (24). The abundance of the microbial communities decreased by one order of magnitude from the top to the bottom sediment within our sampling depth in this study, similar to the previous study (24) on the dominance of the bacterial community (25). Generally, microbial biomass and diversity decrease with increasing sediment depth. However, the alpha diversity of the archaeal community in this study increased from the top to bottom sediment, mainly contributed by station BHB10, which could be due to tolerance of low energy availability (26) or to preferential availability of the buried organic matter after degradation to archaea. The increasing archaeal diversity with increasing sediment depth contradicts prior results in these shallow zones (27, 28). This could be explained by the higher resolution sampling
depths employed by our study. Heterogeneous biogeochemical conditions in the sediments supported diverse microbial communities.

Both archaeal and bacterial communities in the sediments were influenced by different environmental factors, such as nitrite, ammonium, sulfate, and CO (Fig. S6). However, there was no significant correlation between depth and the entire archaeal or bacterial communities. The relative abundance of most archaeal or bacterial phylum was significantly correlated with the concentration of nitrite and sulfate, suggesting that the role of element cycling was more important to the microbial communities than depth. Although the abundances of both archaeal and bacterial phyla changed in different layers, only the abundances of most archaeal phyla were statistically correlated with the depth. This indicates that the bacterial community was more adapted to this shallow zone of sediments despite the fact that archaeal taxa are more tolerant to the low energy conditions. The distance-decay patterns based on the abundance in different geographic locations revealed that prokaryotic dispersal was strongly governed by the high environmental heterogeneity or spatial isolation, further explaining marine microbial spatial dynamics (21, 29). We applied the geographic definition at fine-scale to further reveal that bacterial taxa had a lower decay rate and had more chances to disperse nearby than the archaeal taxa, and this pattern was evident for the highly abundant taxa (30).

Consistent with previous studies on the microbial communities in the north Chinese marginal seas, the bacterial community was dominated by *Deltaproteobacteria*
and *Gammaproteobacteria* (25, 31) representing the key players for sulfate reduction and sulfur oxidation (32), respectively, in this area. This co-abundant pattern of sulfate reducers and sulfur oxidizers has been reported in the coastal Yamada Bay (33), which further supports the active sulfur cycling from the top to bottom in the shallow zone of sediments in the Bohai Sea. Unlike the relatively stable abundance of each bacterial taxa in different samples, the archaeal phyla showed a dramatic shift in geographic and spatial scale. *Thaumarchaeota* (mainly *Nitrosopumilales* class) was the overall dominant archaeal taxa, however, they were most dominant in the top layer of the sediments, mainly attributed to their well-known role as ammonia oxidizers (34). The abundance of the other two dominant archaeal classes, *Thermoprotei* and *Thermoplasmata*, belonging to *Crenarchaeota* and *Euryarchaeota*, and was significantly correlated with different biogeochemical conditions and increased from top to bottom (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples). The abundance of *Thermoprotei* is negatively correlated with salinity in different habitats, e.g., the Pearl River estuary (35), the Baltic Sea (36), and the Chilika Lagoon (37). Thermoplasmata is metabolically versatile (38) and able to adapt to both marine and freshwater environments with the preference for high organic matter sediments (39). The increased abundance of *Thermoprotei* and *Thermoplasmata* from the top to the bottom suggests that the type, rather than the total quantity of organic matter, may determine the distribution of these two archaeal classes in the shallow coastal zones of sediment where the overall organic matter was rich with diverse
sources (40, 41). The similar shift of these two archaeal classes further indicates that they may share the same preferred organic matter.

Different roles in determining the archaeal and bacterial communities

The low relative importance of homogenizing dispersal (4%) suggests habitat heterogeneity and barriers to connectivity in the shallow zone of coastal sediments. The higher contribution of homogenizing dispersal in the bacterial assembly (3%) than in the archaeal assembly (2%) further supports the metabolic versatility of bacteria which may more easily adapt to changing environments than archaea. The effects of drift are most significant when selection is weak and alpha diversity (the local species diversity) is low. However, there was no method available to assess the relative importance of diversification in shaping microbial community structure (42).

At different geographic scales, the dominant mechanism affecting the distribution of benthic communities varied. For example, the benthic marine microeukaryotes were mainly affected by determinism in marine sandy beaches at large geographic scale (~ 12,000 km) (43) and stochasticity in intertidal sediments at small geographic scale (~ 20 km) (44). Besides the geographic sampling scale, the type of organism may also affect the mechanism of their distribution. Of note, determinism and stochasticity, respectively, mainly determined the distribution of the bacterial assembly in North China plain soil (45) and the archaeal assembly in eastern Chinese marginal sea surface sediment (21) at a similar geographic scale (1000 ~ 1500 km). Due to the redox gradients in the sediment, the respiratory electron acceptors shift with decreasing
energy production efficiency from the top to bottom in marine sediments (46). Thus, the effects of selection are considered to surpass other ecological processes, e.g., diversification, dispersal, and drift, as the major force driving the vertical distribution of microbial communities in marine sediments (20, 47, 48). Interestingly, our present study revealed that the archaean assembly was mainly governed by determinism, while the bacterial assembly was mainly dominated by stochasticity in the shallow zone of coastal marine sediments. This strong adaptability and metabolic plasticity to the change in environments in the bacterial community is consistent with previous findings in a river-bay system (49). The distribution of archaea, which were generally smaller than bacteria, was more determined by the environmental condition. This also challenges the size-plasticity hypothesis, i.e., the metabolism of smaller organisms is more plastic and tolerant to the change of environments than those of larger organisms (50). The decreased relative importance of determinism from the top to the bottom for both the archaean and bacterial assemblies may correlate with the decreased bioavailability of organic matter, which requires more effort to utilize. Previous studies revealed the importance of a geographic sampling scale in determining the mechanism of microbial distribution and the difference between archaean and bacterial communities (43, 44). Here, our current study further stresses the significant role of depth in the ecological processes. However, the separate analysis of ecological processes in archaean and bacterial assemblies may not represent the real scenario in situ due to the frequent interactions between archaea and bacteria. For example, there is a syntrophic association between the sulfate-reducing bacteria and methane-oxidizing archaea (51),
and there may be possible competition for simple sugars between archaea and bacteria due to the redundancy of central metabolism (19) in marine sediments. Thus, the effects of interactions between the archaeal and bacterial communities on the ecological processes are overlooked when we analyze the ecological processes in different domains.

The co-occurrence network indicated increased microbial interactions in the bottom layers of sediments

Co-occurrence networks have been applied in various habitats to infer the complex interactions that occur within microbial communities (21, 49, 52). We constructed a co-occurrence network at the ASV level for the integrated archaeal and bacterial communities to explore the interactions in the whole community. Of note, the abundance of the archaeal community was lower than the bacterial community, however, the archaeal ASVs still significantly contributed to the network. The disproportion of the number of connections and the relative abundance of each node revealed the importance of rare taxa to support the function and stability of the community (53). Our results revealed increased microbial interactions from the top to the bottom sediment in the shallow zone, which contradicts a previous study where it was reported to decrease in the deep-sea sediments (53). The organic-rich sediments in the coastal shallow zone supported diverse microbial communities, however, the bioavailability of labile organic matters still decreased from the top to the bottom. The metabolic hand-off (54) may be needed for the microbial community to utilize the
nutrients resulting in a tighter network in the bottom layer than in the top layer in this shallow zone.

The co-occurrence network between the taxa and functional genes involved in oxygen, sulfur, and nitrogen redox reactions revealed that different phyla were correlated with the abundance or diversity of the functional genes. This may be explained by the functional redundancy in the microbial community (55). Thus, one taxon could be replaced by another one due to its same function. Moreover, the correlation between low-abundance phyla and functional genes further stressed the key role of rare taxa in the microbial community involved in various processes of biogeochemical cycling.

Materials and Methods

Study site and sample collection

Samples were collected during a cruise by research vessel (R/V) Chuangxin Yi (18-26, August 2018). Sediments from three stations (BHB10, M3, and M8; Fig. S1 and https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples), with the water depth ranging from 17.0 to 30.5 m, in three different mud zones in the Bohai Sea, were collected using a stainless-steel box-sampler. Sediment samples were processed immediately on board for biogeochemical analysis, including methane, CO, nitrate, nitrite, ammonium, dissolved inorganic phosphate (DIP), and dissolved inorganic carbon (DIC) (See detailed procedures in Supplementary material).
Sample processing

Sediment samples were taken every 2 cm. They were classified as top (0-18 cm), middle (18-38 cm), and bottom (below 38 cm) layers based on the changes in biogeochemical conditions at three stations. DNA was extracted from sediment samples, and sequenced on an Illumina HiSeq 2500 platform. The sequences were processed using QIIME2 (56). Statistical analyses and co-occurrence network analysis were performed with R. infer Community Assembly Mechanisms by Phylogenetic-bin-based null model analysis (iCAMP) (22) (http://ieg3.rccc.ou.edu:8080/) was used to quantify the ecological processes governing the archaeal and bacterial communities in the sediments. 14 samples were selected for metagenome sequencing. Metagenomic data were assembled using IDBA-UD v1.0.9 (57). Scaffolds ≥ 2,000 bp were annotated by the Integrated Microbial Genomes & Microbiomes (IMG/M) using DOE-JGI Metagenome Annotation Pipeline (MAP v.4) (58). Detailed methods for sampling, sequence data processing and statistical analysis were presented in supplementary material.

Data deposition

Amplicon sequence data were deposited into the National Center for Biotechnology Information (NCBI). All data were registered under BioProject PRJNA743900.

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Supplementary material includes:

- Figures S1-S7
- Supplementary Materials and Methods
- Supplementary Results and Discussion

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Figures

Figure 1. The archaeal and bacterial diversity in the Bohai Sea sediments at three stations (BHB10, M3, and M8) at different depths. (a) Shannon index of each sample for the archaeal community, (b) Shannon index of each sample for the bacterial community, (c) Shannon index for the archaeal community in different layers (top: 0-18 cm; middle: 18-38 cm; and bottom: below 38 cm), (d) Shannon index for the bacterial community in different layers. Significant differences between layers are marked by stars (**P < 0.001; *P < 0.05). Principal coordinate analysis (PCoA) of amplicon sequence variants (ASVs) abundance based on the Bray–Curtis dissimilarity for the archaeal (e) and bacterial (f) communities colored by depth in three stations (BHB10, M3, and M8) with 95% confidence.

Figure 2. Stacked bar chart showing the relative abundance of the archaeal taxa (a) and bacterial taxa (b) at different depths in the three stations in the Bohai Sea sediments.

Figure 3. The occurrence pattern of ASVs in different samples and the pattern of dissimilarity along with depth. The relationship, indicated by Spearman’s rank correlation, between the relative abundance of each archaeal (a) and bacterial (b) ASVs and the occurrence across all the samples. (c) Occurrence patterns of ASVs belonging to the taxa in phylum level with relative abundance over 1% for archaeal or bacterial community. Numbers in the parentheses above the phylum name indicate the number of ASVs shown in the figure for each phylum. Numbers within the figure represent the total number of ASVs for each phylum, the number of ASVs occurring only in one
sample (not included in the figure for better visualization), and their corresponding relative abundance (percentage in parentheses). Relationships between the Bray-Curtis dissimilarities and the depth between each sample for archaeal (d) and bacterial (e) communities.

**Figure 4.** Ecological processes determining the archaeal and bacterial assemblies based on null model analysis with iCAMP in marine sediments. (a) The relative importance of different mechanisms governing the community in different layers. (b) Relative abundance of the top 15 abundant phylogenetic groups (bins), which account for a total relative abundance of 63.99% among the total 162 archaeal bins and 28.45% among the 599 bacterial bins. (c) Relative abundance of the classified genus with the greatest relative abundance in each bin. The relative importance of ecological processes across different phylogenetic groups in the top (d), middle (e), and bottom (f) layers.

**Figure 5.** Co-occurrence networks and Zi-Pi plot of the archaeal and bacterial ASVs in three different layers: (a, b, and c) top layer, (d, e, and f) middle layer, and (g, h, and i) bottom layer. The cutoff of shown connections were $P < 0.01$ and $|\rho| > 0.7$. The size of each node is proportional to the relative abundance of each ASV (a, d, and g), and the number of connections (b, e, and h). The nodes were classified into four categories: (I) peripherals with $Z_i \leq 2.5$ and $P_i \leq 0.62$; (II) connectors with $Z_i \leq 2.5$ and $P_i > 0.62$; (III) module hubs with $Z_i > 2.5$ and $P_i \leq 0.62$; and (IV) network hubs with $Z_i > 2.5$ and $P_i > 0.62$. Numbers denote the ASV counts in each category (c, f, and i).
Figure 6. Correlation of the relative abundance of each archaeal and bacterial phylum and the relative abundance of functional genes involved in oxygen, nitrogen, and sulfur redox reactions. The cutoff of shown connections were $P < 0.01$ and $|\rho| > 0.7$. 
Figure 1

The archaeal and bacterial diversity in the Bohai Sea sediments at three stations (BHB10, M3, and M8) at different depths. (a) Shannon index of each sample for the archaeal community, (b) Shannon index of each sample for the bacterial community, (c) Shannon index for the archaeal community in different
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Stacked bar chart showing the relative abundance of the archaeal taxa (a) and bacterial taxa (b) at different depths in the three stations in the Bohai Sea sediments.

Figure 3

The occurrence pattern of ASVs in different samples and the pattern of dissimilarity along with depth. The relationship, indicated by Spearman's rank correlation, between the relative abundance of each
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**Figure 4**

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Figure 5

Co-occurrence networks and Zi-Pi plot of the archaeal and bacterial ASVs in three different layers: (a, b, and c) top layer, (d, e, and f) middle layer, and (g, h, and i) bottom layer. The cutoff of shown connections...
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**Figure 6**

Correlation of the relative abundance of each archaeal and bacterial phylum and the relative abundance of functional genes involved in oxygen, nitrogen, and sulfur redox reactions. The cutoff of shown connections were $P < 0.01$ and $|\rho| > 0.7$.

**Supplementary Files**
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