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

Xianzhe Gong[1,2*], Zhiyi Chen[3], Ye Deng[1,4], Duo Zhao[1], Ping Gao[5], Liang Zhang[6], Qichao Tu[1], Lingyun Qu[5], Liwen Zheng[1], Yong Zhang[1], Chao Song[7], Jihua Liu[1 *]

1, Institute of Marine Science and Technology, Shandong University, Qingdao, Shandong, 266237, China

2, Marine Science Institute, University of Texas at Austin, Port Aransas, TX, 78373, USA

3, State Key Laboratory of Microbial Technology, Shandong University, Qingdao, Shandong, 266237, China

4, CAS Key Laboratory for Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (CAS), Beijing, 100085, China

5, First Institute of Oceanography, Ministry of Natural Resources, Qingdao, 266237, China

6, Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, Singapore 637141, Singapore

7, Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Qingdao, Shandong, 266237, China

*Correspondence:

Dr. Xianzhe Gong

xianzhe.gong@gmail.com

Dr. Jihua Liu

liujihua1982@foxmail.com

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Figure S1. Background of Bohai Sea. **(a)** Sampling map in the Bohai Sea and the sampling schematic diagram. Three sampling sites (station BHB10, M3, and M8) were marked by a circle with the water depth (numbers in parenthesis) and the depth of sediment cores at each station. **(b)** Biogeochemical characters, including carbon monoxide (CO), dissolved inorganic carbon (DIC), dissolved inorganic phosphate (DIP), methane (CH_4), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and sulfate (SO_4^{2-}), at three sampling sites. **(c)** Principal component analysis (PCA) of biogeochemical characters in different samples with 95% confidence at three sampling sites.

Figure S2. The archaeal and bacterial alpha diversity in the Bohai Sea sediments at three stations (BHB10, M3, and M8) at different depths. **(a)** Chao 1 index of each sample for the archaeal and bacterial community, and in different layers (top: 0-18 cm; middle: 18-38 cm; and bottom: below 38 cm) (From left to right). **(b)** Shannon index of archaeal and bacterial communities in the Bohai Sea sediments at three stations (BHB10, M3, and M8) at different depths. **(c)** Chao 1 index of archaeal and bacterial communities in the Bohai Sea sediments at three stations (BHB10, M3, and M8) at different depths. Significant differences between layers are marked by stars (***P < 0.001, **P < 0.01, and *P < 0.05).

Figure S3. Difference of archaeal and Bacterial communities based on Bray–Curtis dissimilarity in different samples. **(a)** Non-metric multi-dimensional scaling (NMDS) analysis of amplicon sequence variants (ASVs) abundance in archaeal community colored by depth in three stations with 95% confidence. **(b)** NMDS of bacterial community colored by depth in three stations with 95% confidence. The heatmap of the distribution pattern of the archaeal **(c)** and bacterial **(d)** community in different samples

with the indication of sampling site, depth, and the classified layers of each sample clustered by the weighted pair group method with averaging (WPGMA).

Figure S4. The occurrence pattern of archaeal (a, e, i, c, g, and k) and bacterial (b, f, j, d, h, and l) ASVs in different samples at three stations: BHB10, M3, and M8, and in three layers: top, middle, and bottom. The relationship is indicated by Spearman's rank correlation between the relative abundance of each ASV and each category.

Figure S5. Bray-Curtis dissimilarity patterns of each archaeal and bacterial phylum. Numbers in the parenthesis after the phylum name denote the percentage of relative abundance of that phylum.

Figure S6. Transformation-based redundancy analysis (tb-RDA) illustrating the relationship between the microbial community and environmental variables at different stations with 95% confidence and depths with different colors. (a) archaeal community; (b) bacterial community.

Figure S7. Co-occurrence networks and characters. Network of the overall archaeal and bacterial communities based on the pairwise Spearman's correlation between ASVs. The size of each node is proportional to the relative abundance of each ASV **(a)**, and the number of connections **(b)**. The robustness of the microbial network in the entire and three layers of sediments measured by the average degree **(c)** and natural connectivity **(d)**. **(e)** Correlation of the relative abundance of each archaeal and bacterial phylum and the counts of functional genes involved in oxygen, nitrogen, and sulfur redox reactions in each assembly. The cutoff of shown connections were $P < 0.01$ and $|rho| > 0.7$.

Supplementary 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 Table S1), 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. At each station, a polyvinyl chloride (PVC) tube with an 11 cm internal diameter was inserted into the box-sampler after carefully removing topwater to take sediment core samples. The PVC tube was drilled with three side-holes at each layer, and the vertical sampling intervals were 2 cm from the top. The holes were covered with tape before being inserted into the sediment. The sediment core was processed on board immediately depending on the type of analysis, as described below. All types of sub-sampling were processed from the surface sediment (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples).

Methane and CO measurement

A 2 mL sediment sample was collected using a cut plastic syringe through the hole. All samples were transferred immediately to a 10 mL glass serum vial with 4 mL 3% NaOH solution and 1.5 g NaCl preloaded, and the vial was crimp-capped with a butyl rubber septum. The serum vial containing samples was shaken and stored upside down at -20 °C until analysis. The concentrations of CH_4 and CO in the headspace were measured as previously described (1). The porosity of the sample is defined as the difference between 1 and the ratio of dry weight (weight of samples after being completely dried at 65 °C) and wet weight. Calibrated Henry's law and porosity were

used to calculate the whole volume of CH_4 or CO in the glass serum vial, and finally, transformed to the concentration of CH_4 or CO in the porewater (C_p). C_p was calculated as:

$$C_p = \frac{C_g * V_g + C_l * (V_l + V_p)}{V_p} \qquad (1)$$

where C_g is the measured concentration of gas in the glass serum vial, C_l is the concentration of gas in the liquid phase calculated by C_g and Henry's Law constants (2), V_g is the volume of gas-phase (4 mL), V_l is the volume of liquid phase (4 mL), and V_p is the value of porosity multiply the volume of sediment (2 mL).

Porewater analysis

The porewater sample was extracted using Rhizon samplers (pore size of 0.15 μm) (3). The first 1 mL of the extracted porewater was discarded, and then ~10 mL was collected before the samples were aliquoted for the analysis of individual pore-water parameters. Four mL of porewater samples for nitrate, nitrite, ammonium, and dissolved inorganic phosphate (DIP) analysis were stored at -20 ℃ until analysis as described previously (4, 5). For sulfate analysis, 4 mL of the extracted porewater was flushed with a stream of moist CO_2 for 2 minutes to remove the hydrogen sulfide (H_2S) from the sample. Sulfate samples were stored at -20 ℃ until analysis. Sulfate was measured by an ICS-900 Ion Chromatography system (Dionex Corporation, CA, U.S.A.). Dissolved inorganic carbon (DIC) samples were stored in 2-mL brown glass vials without headspace with the addition of 0.02% to 0.05% $HgCl_2$ at 4 ℃ until analysis as described before (4).

Sediment sub-sampling, DNA extraction, and sequencing

Three 2-3 mL sediment samples were collected from each layer using a cut plastic syringe through the previously drilled holes and preserved at -80 °C. DNA was extracted by DNeasy PowerSoil Kit (Qiagen, Germany), quantified using Qubit DNA BR Assay Kit (Thermo Fisher Scientific, MA, U.S.A.), and stored at -80 °C until further analysis. Three DNA extracts from the same layer were mixed before sequencing. 16S rRNA fragments in the DNA extraction were amplified by using the primer pair 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (6, 7), and the primer pair 349F (5′-GYGCASCAGKCGMGAAW-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (8, 9) targeting for bacterial and archaeal communities, respectively. The PCR products were sequenced on an Illumina HiSeq 2500 platform.

16S rRNA gene amplicon sequence denoising and variants clustering

The sequencing data were processed using QIIME2 (10). In brief, the raw paired-end sequences were quality-filtered, denoised, merged, chimera-checked, and clustered into amplicon sequence variants (ASVs) using dada2 (11). Taxonomy was assigned using the ribosomal database project (RDP) pipeline with a bootstrap cutoff of 80%, and any assignment with a confidence lower than 50% was considered unclassified. Extrinsic domain ASVs, singletons, doubletons, and the ASVs which are 50 bp shorter than the target sequences, i.e., 421 and 430 bp for archaeal and bacterial communities, respectively, were removed before downstream analysis. Samples were rarefied at the depth of 8,651 and 11,200 reads for 78 archaeal and 76 bacterial samples, respectively (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-Bohai-Sea-sediment-samples).

Statistical analyses on biogeochemical characteristics and microbial diversity

To quantify the effect of depth on the microbial communities, we classified the sediments into three layers, top (0-18 cm), middle (18-38 cm), and bottom (below 38 cm), based on the changes in biogeochemical conditions at three stations. We used principal component analysis (PCA) to investigate the correlation of biogeochemical variables and differences in porewater chemistry at three stations. Chao 1 (richness estimator) and Shannon index were calculated in QIIME2 for alpha diversity analysis. For beta diversity, a distance matrix based on Bray-Curtis dissimilarities was calculated for principal coordinate analysis (PCoA) and non-metric multi-dimensional scaling (NMDS) analysis using the Vegan package v2.5-7 (12) in R, and for cluster analysis of weighted pair group method with averaging (WPGMA) using pheatmap package v1.0.12 in R. Three different complementary non-parametric multivariate statistical tests: analysis of similarity (ANOSIM) (13), non-parametric multivariate analysis of variance test (Adonis) (14), and multiple response permutation procedure (MRPP) were used based on the Bray-Curtis dissimilarities to test the differences in microbial diversity among layers and sites. The statistical tests of biogeochemistry were based on the Euclidean distance. All tests were performed using the Vegan package v2.5-7 (12) in R. The correlations between the microbial communities and environmental characters were calculated with Mantel test, and also through transformation-based redundancy analysis (tb-RDA) using the Vegan package v2.5-7 (12) in R.

Metagenomic sequencing assembly and annotation

Among all the sediment samples, 14 representatives covering the top, middle, and bottom layers at three stations were selected for metagenomic sequencing. After quality control, the number of clean paired-end reads in each sample ranged from 752,069,594 to 975,955,532 (https://github.com/Xianzhe-Gong/16SrRNA-gene-analysis-from-

Bohai-Sea-sediment-samples). Metagenomic reads from each sub-sample were individually assembled using IDBA-UD v1.0.9 (the constant kMaxShortSequence was modified to 250 bp) with the following parameters: -pre_correction -mink 65 -maxk 115 -step 10 -seed_kmer 55 (15). Scaffolds ≥ 2,000 bp were submitted to the Integrated Microbial Genomes & Microbiomes (IMG/M) using DOE-JGI Metagenome Annotation Pipeline (MAP v.4) for gene prediction and functional annotation (16). The occurrence frequency of KOs (KEGG Orthology) was counted considering the microbial diversity containing that specific KO in the assembly. The abundance of each scaffold was calculated by mapping raw reads to the scaffold normalized by the length of the scaffold and the number of sequencing reads in that sample. The abundance of each KO was the sum of the abundance of each scaffold containing that KO in the assembly.

Distribution pattern of archaeal and bacterial communities

To reveal the contribution of different ecological processes to the community, infer Community Assembly Mechanisms by Phylogenetic-bin-based null model analysis (iCAMP) (17) (http://ieg3.rccc.ou.edu:8080/) was used to quantify the ecological processes governing the archaeal and bacterial communities in the sediments. The rooted trees of the archaeal and bacterial ASVs were separately generated in QIIME2 (10) with all representative sequences. Pairwise vertical distances and pairwise Bray-Curtis dissimilarities within communities in the same site were calculated to reveal relationships that are similar to distance-decay relationships. ASVs belonging to the same phylum were also examined in the same way.

Network analysis

ASVs representing over 0.1% across all samples and occurring in more than 50% of samples were retained for co-occurrence network analysis. Co-occurrence networks were constructed, and the topology features of the network were analyzed using the Igraph and Psych package in R based on the pairwise Spearman's correlation with the cutoff of $P < 0.01$ and |rho| > 0.7 being considered as a valid relationship. Node degree and natural connectivity of the network were used to evaluate the sparsity (18) and robustness (19) of the network, respectively. Meanwhile, 1000 Erdös-Réyni random networks in an equal size were constructed to compare the topology features of observed and random networks (20). We further performed the two-sample Kolmogorov-Smirnov test to assess the difference between networks by bootstrapping node attributes with 10,000 iterations (21). The network was visualized in Gephi v0.9.2 (https://gephi.org). The random matrix theory (RMT)-based molecular ecological networks (MENs) was used to analyze the network interactions in microbial communities (22, 23).

The Spearman's correlation matrix between the microbial taxa and the selected KOs was calculated using the Hmisc package in R, and filter with the cutoff of $P < 0.01$ and |rho| > 0.7 being considered as a valid relationship. The network was analyzed using the Igraph package in R, and visualized in Cytoscape v3.9.1 (24).

Supplementary Results and Discussion

The concentrations of CO were more than 12.37 μM in surface sediments, decreased to less than 7.80 μM at 20-22 cm, and remained relatively stable below 22 cm at three stations. The concentration of DIC decreased from 3.03 in 0-6 cm sediments to ~2.60 mM in sediments below 26 cm at station M3, while it reached the highest concentration of 4.16 mM and 4.75 mM at 12-14 cm and 10-12 cm at stations M8 and BHB10,

217 respectively, and decreased to below 3.50 mM in the bottom sediments. The
218 concentrations of DIP were relatively stable from top to bottom at stations BHB10 and
219 M3 (between 0.63 and 1.84 μM). At station M8, DIP reached the highest concentration
220 (7.47 μM) at 12-14 cm and dropped to 0.91 μM at the bottom. Despite the highest
221 concentration of CH_4 being 0.73 μM at 20-22 cm at station M8, all concentrations of
222 CH_4 were below 0.73 μM at all sampling sites. The concentrations of NH_4^+ were high,
223 mostly more than 50 μM in all samples. The concentrations of NO_3^- and NO_2^- were
224 below 1 μM, except for the concentration of NO_3^- (1.43 μM) at the 42-44 cm sample at
225 station BHB10. The concentration of SO_4^{2-} was constant (17.79-27.61 μM) in all
226 samples.

227 The Bohai Sea represents a typical coastal sea largely affected by anthropogenic
228 activities and freshwater inputs. In the current study, we performed extensive analyses
229 of sediment samples at different depths ranging from surface to 62 cm with a high
230 resolution of 2 cm per sample at three stations in the Bohai Sea. Station BHB10 is
231 located in the Bohai Bay, the second-largest bay in the southwest of the Bohai Sea,
232 receiving industrial and domestic wastewater discharges from the vicinity of Tianjin
233 (with over 9 million population) and its adjacent megacity, Beijing (25). Station M3 is
234 located in the middle of the Bohai Sea, and station M8 is located in the south end of
235 Liaodong Bay. Geographically, station M3 is located at the same distance as station
236 BHB10 and M8 (Fig. S1). However, the hydrology at station BHB10 was different from
237 the other two stations since station BHB10 is located closer to the coast, especially to
238 the large city. There was strong heterogeneity and stratification of inorganic nutrients
239 in the Bohai Sea water in different locations and seasons (4, 26), with the recently
240 reported seasonal hypoxia in the bottom water during the summer (27, 28). Based on
241 the previous study, the sedimentation rate was around 4.8 mm•yr^{-1} at a sampling site

close to station M3 (29). In addition, we observed low nitrate and nitrite concentrations and high ammonium concentration in the porewater in this study, suggesting the active aerobic respiration and denitrification processes in the surface sediments. Compared to other coastal areas, the concentration of organic matter in the sediments in the Bohai Sea was higher than in its adjacent Yellow Sea (30), which would form a deep layer of sediments containing high concentrations of organic matter due to the high sedimentation rate. We further observed heterogeneous biogeochemical conditions in the sediments. The significant difference of biogeochemical characters between different stations and layers (Table S4), especially the differences in the concentrations and composition of organic matter, supported the diverse microbial communities in the sediments in this area (31), as well as the other regions, such as Western Antarctica (32), the Eastern Mediterranean Sea (33), and East China Sea (34).

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 in the sediments, but with the locational difference (31). The abundance of the microbial communities decreased for one order of magnitude from the top to the bottom sediment within our sampling depth in this study based on the report from the previous study (31) with the dominance of the bacterial community (35). Generally, microbial biomass and diversity decrease with the 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, could be due to the capability of tolerant to low energy availability (36) or the buried organic matters after degradation were more favored by different taxa of archaea than bacteria. The increased archaeal diversity with the increasing sediment depth in these shallow zones based on the high resolution of sampling depth was contradicted with the results based on the archaeal

diversity in marine sediments by the low resolution of sampling depth in previous studies (37, 38). The heterogeneous biogeochemical conditions in the sediments supported diverse microbial communities. Beta-diversity analysis revealed distinct archaeal and bacterial communities in different layers and stations, with the tendency that the similarity of community at station M3 and M8 were closer to each other than station BHB10, which may be due to the strong influence of terrestrial input from the coastal area at station BHB10. Beta-diversity analysis revealed distinct archaeal and bacterial communities in different layers and stations, with stations M3 and M8 having more similar communities than station BHB10. This may be due to the strong influence of terrestrial input from the coastal area at station BHB10.

Vertically, the relative abundance of *Thaumarchaeota* (*Nitrosopumilus* and *Nitrososphaera* clade) and *Woesearchaeota* decreased with depth, while the relative abundance of *Crenarchaeota* (mostly belong to *Thermoprotei* clade) and *Euryarchaeota* (*Methanomassiliicoccus* and *Methanosphaera*) increased with depth for the archaeal community in all three sampling sites, with the most marked trend at station M3 (Fig. 2a). In general, the relative abundances of *Woesearchaeota* and *Pacearchaeota* were much higher at station BHB10 than those at the other two stations. *Crenarchaeota* were rare in the top sediments at stations M8 and BHB10. Comparatively, the top sediment samples at station BHB10 were mainly populated by the *Nitrosopumilus* clade of *Thaumarchaeota*, which comprised 69 ± 22 % of the top sediment samples at station BHB10. The relative abundance of *Proteobacteria* decreased from 74 ± 1 % to 31 %, 52 ± 3 % to 34 ± 3 %, and 52 ± 3 % to 39 ± 4 % from the top sediments to the bottom sediments at station BHB10, M3, and M8, respectively, and this decrease was mainly attributed to *Gammaproteobacteria*. The second most abundant bacterial taxon was *Chloroflexi*, comprising 11 ± 8 % of the bacterial

community across all samples, with a clear trend of increased relative abundance from the top sediments to the bottom sediment in all three sampling sites. The relative abundance of *Bacteroidetes*, *Acidobacteria*, *Planctomycetes*, and *Actinobacteria* were over 2% across all samples (Fig. 2b) and decreased from top to bottom with the exception that *Planctomycetes* was the most abundant in the bottom sediment sample at station M8.

To explore phylogenetic consistency of assembly processes, ASVs were divided into 161 archaeal and 599 bacterial groups ('bins' hereafter) using iCAMP (17). The top 15 most abundant archaeal bins contributed 64% relative abundance of the archaeal community, while the 15 most abundant bacterial bins only represented 28% of the bacterial community (Fig. 4b). Many of the bins could not be classified to the class level (Fig. 4b). We further quantified the relative importance of different ecological processes in each bin. The most dominant archaeal bin (bin 15, > 28% relative abundance) was the unclassified *Nitrososphaera* family, which was governed by homogeneous selection in all layers (Fig. 4). The most classified archaeal and bacterial classes were *Methanomassiliicoccus* and *Woeseia*, respectively, among the top 15 abundant bins. Archaeal bin 41, bin 43, bin 53, and bin 52 all belong to *Mehanomassiliicoccus*, but only bin 43 was dominated by deterministic processes (Fig. 4 d-f). *Woeseia* class, distributed in bin 436, bin 431, bin 430, bin 432, and bin 348, also showed different mechanisms for the community assembly of different constituent bins. Among the top abundant 30 bins (15 each for the archaeal and bacterial bins), only bin 10 (unclassified archaea), bin 436 (*Woeseia* class), and bin 377 (*Pelobacter* class) shifted from the deterministic-dominated to stochastic-dominated processes from the top to the bottom layers (Fig. 4 d-f). The plot of within-module connectivity (Zi) and among-module connectivity (Pi) showed that there were more module hubs, defined as

highly connected nodes within the module, and connectors, defined as nodes that highly linked modules within the sediment layer, in the top sediments (Fig. 5). The overall large number of connectors in the network suggests well-connected microbial communities in marine sediments.

This study provides a better understanding of spatial patterns and mechanisms underlying the microbial community in coastal marine sediments. We demonstrated that deterministic processes govern the archaeal community, while the bacterial community is governed by stochastic processes from the top to the bottom in the shallow zone of the coastal marine sediments. This indicates stronger metabolic plasticity and adaptation to the change in environments in the bacterial assembly than in the archaeal assembly. A co-occurrence network highlighted the key role of rare taxa. Moreover, increased connection in the community from the top to the bottom suggested the community in the bottom sediment may be more vulnerable if the tightly connected nodes are destroyed. Our study also emphasized the importance of finely resolved sampling intervals of the sediment core to properly interpret the ecological progress and the patterns of co-occurrence networks in the marine sediments. Consequently, further studies at different resolutions are needed to answer the question of what processes are involved in shaping the microbial communities in marine sediments.

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