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[Article]

Bacterial Communities from the Water Column and the Surface Sediments along a Transect in the East Sea

Jeong-Kyu Lee, Keun-Hyung Choi*

Department of Marine Environmental Science, Chungnam National University, Daejeon 34134, Korea

Corresponding Author
Keun-Hyung Choi
Department of Marine Environmental Science, Chungnam National University, Daejeon 34134, Korea
E-mail : keunhchoi@cnu.ac.kr

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We determined the composition of water and sediment bacterial assemblages from the East Sea using 16S rRNA gene sequencing. Total bacterial reads were greater in surface waters (<100 m) than in deep seawaters (>500 m) and sediments. However, total OTUs, bacterial diversity, and evenness were greater in deep seawaters than in surface waters with those in the sediment comparable to the deep sea waters. Proteobacteria was the most dominant bacterial phylum comprising 67.3% of the total sequence reads followed by Bacteriodetes (15.8%). Planctomycetes, Verrucomicrobia, and Actinobacteria followed all together consisting of only 8.1% of the total sequence. *Candidatus* Pelagibacter ubique considered oligotrophic bacteria, and Planctomycetes copiotrophic bacteria showed an opposite distribution in the surface waters, suggesting a potentially direct competition for available resources by these bacteria with different traits. The bacterial community in the warm surface waters were well separated from the other deep cold seawater and sediment samples. The bacteria exclusively associated with deep sea waters was Actinobacteriacea, known to be prevalent in the deep photic zone. The bacterial group Chromatiales and Lutibacter were those exclusively associated with the sediment samples. The overall bacterial community showed similarities in the horizontal rather than vertical direction in the East Sea.

Keywords: Bacterial community, Metagenomics, the East Sea, Massive parallel sequencing

Introduction

Bacteria are recognized as important agents in biogeochemical processes in all aquatic ecosystems, including sediments. They are involved not only in the re-mineralization of organic matter both in water and sediments, but they also play major roles in food web structure and dynamics (Azam, 1998; Azam et al., 1983; Guilini et al., 2010). Studies show not only short-term but also long-term bacterial responses to environmental changes (Giovannoni and Vergin, 2012). Therefore, their composition reflects the environmental characters in which they are living and their changes over time (Walsh et al., 2015).

The extent of the diversity of marine prokaryotes is not well known, primarily because of poor cultivability. Techniques permit the characterization of such organisms without culturing using 16S rRNA sequences as templates for the polymerase chain reaction (PCR) obtained directly from biomass (Britschgi and Giovannoni, 1991; Fuhrman et al., 1993b). For more than a decade, Sanger sequencing and fluorescence-based electrophoresis technologies have dominated the DNA sequencing field. The potential of terminal-restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) have been extensively used to separate and detect OTUs and thus to characterize marine bacterioplankton communities (Moeseneder et al., 1999).

Recent progress concerning the genomics method, especially development of massive parallel genomic sequencing (Rothberg and Rothberg, 2015) in high-throughput DNA sequencing and high-performance computing and bioinformatics, has caused a substantial expansion in genomic discovery (Cottrell et al., 2005; Rogers and Venter, 2005). Surveys using this extensive parallel sequencing have been performed in many marine environments, including estuarine and coastal waters (Feng et al., 2009; Pommier et al., 2010), oligotrophic open ocean (Pommier et al., 2010; Agogué et al., 2011), and marine sediments (Agogué et al., 2011; Bolhuis and Stal, 2011; Sogin et al., 2006). These studies could contribute to improve the overall understanding of the global

patterns of marine bacterial diversity (Ladau et al., 2013) and to comprehend local and global biogeochemical processes (DeLong, 2009).

However, even with improved molecular tools, our understanding of microbial diversity in aquatic environments is still limited due not only to the highly variable physical and biogeochemical conditions but also to the sometimes difficult access in sampling (such high sea and deep-sea environments). Spatial variations in the bacterial community along environmental gradients or across environments could provide a better picture of bacterial distribution and community structure (Walsh et al., 2015).

The East Sea is a deep, semi-enclosed marginal sea located in the northwest Pacific. It is also called a "miniature ocean" due to the similarity of its dynamic characteristics with that of a global ocean (Ichiye, 1984). The Tsushima Warm Current (TWC) supplies heat, water, and materials horizontally through the Tsushima/Korea Straits (TKS) to the East Sea with double peaks of volume transport in May and October. Nitrogen fluxes through the western channel of the Tsushima/Korea Straits (TKS) sustain high primary productivity in the southwestern Japan Sea (Onitsuka et al., 2007), affecting ecosystem dynamics in the East Sea.

In this study we used 16S rRNA sequencing to determine the composition of water and sediment bacterial assemblages in the East Sea. The study area sits on various geomorphic features from the coastal to continental basin encompassing continental shelf and slope and partly covering the area the Tsushima Warm Current (TWC)'s influence. Our primary focus was to find spatial patterns of bacterial communities and to gain an overall understanding of bacterial diversity in this marine system affected by the TWC. We also tried to compare bacterial communities in the warm surface water, cold deep-sea environments, and sediments in the East Sea.

Materials and Methods

1. Site description, sample collection and characterization

Seawater samples were collected at several depths from the surface to the bottom at six stations along a transect from the coastal to the open sea deep basin in the East Sea in May 2017 (Fig. 1). The sampling stations were selected to capture the influence of the Tsushima Warm Current (TWC) flowing north along the transect during the cruise. Water sampling was done at several depths, mostly on the surface (generally <100 m) for all stations.

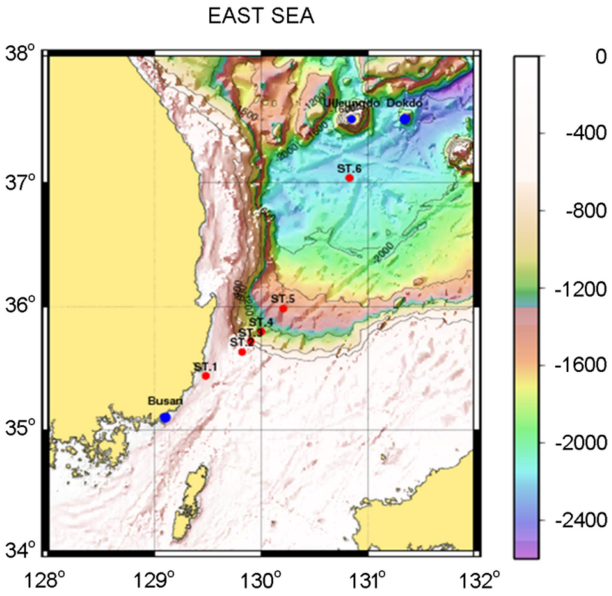

Fig. 1. Sampling stations for bacterial communities along a transect in the East Sea. The contours and vertical bars show water depths (m) on a negative scale.

Deep water samples were also collected, and sampling often reached down to the bottom (600 m at ES4, 1,400 m at ES5, and 1,900 and 2,100 m at ES6).

Standard oceanographic properties, including water temperature, salinity, fluorescence, and dissolved oxygen were measured. Temperature and salinity profiles were obtained for the downward casts of conductivity, temperature, and depth (CTD) rosette profiler (Seabird-911, Sea-Bird Scientific Ltd., USA) only to 200 m. Dissolved oxygen (DO) and fluorescence were also measured during the casts with a multi-sonde attached to the CTD profiler. The water samples were collected using Niskin bottles attached to the rosette multi-sampler. Phosphate samples were frozen on board following filtration through 47 mm GFF filters, and its concentration was quantified spectrophotometrically in the laboratory (spectrophotomer: Cary 100 UV-VIS, Varian Inc., Palo Alto, USA) following Grasshoff et al. (Grasshoff et al., 2009). For bacterial analysis, water samples of 1 ℓ at each sampling depth were filtered onto 47 mm 0.2 μm cellulose ester membrane filters (Advantec Nissei Kaisha, Ltd., Japan), and the filters were immediately placed in 15 ml tubes in liquid nitrogen. Surface sediment samples were collected using a box corer (BX-610 Box Corer, Ocean Instruments, San Diego, USA). All apparatus for filtering waters and sediment sampling were sterilized with 80% ethanol and air-dried prior to sampling to reduce bacterial contamination.

2. Nucleic acid extractions and high-throughput sequencing of 16S rRNA

Each sequenced sample was prepared according to the Illumina 16S Metagenomic Sequencing Library protocols. Both DNA quantification and quality were measured by PicoGreen® (Thermo Fisher Scientific Inc., Waltham, MA, USA) and Nanodrop™ (Thermo Fisher Scientific Inc., Waltham, MA, USA). The 16S rRNA genes were amplified using 16S V3-V4 primers: 16S Amplicon PCR Forward Primer (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG), and 16S Amplicon PCR Reverse Primer (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). Input gDNA (12.5 ng) was amplified with 16S V3-V4 primers, and a subsequent limited-cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. The final products were normalized and pooled using PicoGreen®, and the size of libraries were verified using the LabChip GX HT DNA High Sensitivity Kit (PerkinElmer Inc., Waltham, MA, USA). The amplicons were finally sequenced using the MiSeq™ platform (Illumina Inc., San Diego, CA, USA).

3. Sequencing data processing

After completion of the sequencing run, we performed quality-based trimming and filtering using Trimmomatic v.0.33 (Bolger et al., 2014). Read joining and taxonomical classification were processed with mothur v1.39.5 (Schloss et al., 2009). The selected paired end reads were joined into contigs, in which contigs of incorrect length (>465 bp, 2.5% of all reads used in this study) and those that contained ambiguous bases were excluded. Unique contigs that were identified exactly one time from all samples were removed for further analysis of taxonomic identification. SILVA reference database version 132 (Quast et al., 2013) was used for 16s rRNA V3-V4 amplicon reads alignment. Sequences were pre-clustered, which allowed for up to 2nt difference between the sequences. Chimeras were identified and removed using UCHIME (Edgar et al., 2011) in mothur. Sequences were classified using the RDP v.16 database (Cole et al., 2014), and only sequences that were classified as bacteria-derived were kept. Subsampled sequences were clustered into Operational Taxonomic Units (OTUs) at a 97% similarity cutoff, and reference sequences for each OTU were determined.

4. Statistical analyses

Community analysis was performed to compare differences among the water and sediment samples. All statistical analyses were performed in R (v3.5.1, (R Core Team, 2018). Community diversity (Shannon index) and evenness (Shannon's evenness index) were calculated and compared between groups. Bray-Curtis dissimilarity indices were used to generate community distance matrices. These dissimilarity matrices were then used to create a dendrogram, using complete linkage in cluster analysis. Bacterial community structure from the water and sediment samples were visualized in non-metric multidimensional scaling (NMDS) plots generated from the Bray-Curtis dissimilarity index matrices of log-transformed OTU data by using the 'metaMDS' function in Vegan Package within R environment (Oksanen et al., 2013).

Results

1. Water mass characteristics

Water temperature shows the presence of cold deep waters (2℃) sitting below a 150~200 m depth with maximum surface temperature reaching 16℃ during the study period (Fig. 2a). Surface temperatures in the open water (such as ES4, 5, and 6) were slightly warmer than in the coastal region (ES1 and 2).

Salinity showed the presence of less saline water mass from the coast up to locations between ES4 and ES5 in the water column down to about 40 m depth (Fig. 2b). The vertical salinity structure for stations from ES1 to ES4 shows a subsurface maximum, which sits on top of less saline deep waters. Meanwhile, salinity declined with depth at the ES5 and 6 open water stations.

A temperature-salinity (T-S) diagram was constructed from water temperature data and salinity with the resulting isopycnal lines drawn on top of the two parameters. The T-S plot shows that water density is primarily regulated by temperature, and the intrusion of the water mass of low salinity and higher water temperature in the surface layer indicates the transport of TWC into the region (Fig. 2c).

Phosphate concentration showed a strong very similar vertical stratification of water temperature distribution (Fig. 2d), indicating that the surface water was nutrient-depleted. The surface concentration was as low as <0.2 μM, while it was up to 1.5 μM at around 200 m depth. Fluorescence data showed overall strong subsurface maxima in all stations, except around ES4, at which point its values were high throughout the water column (Fig. 2e).

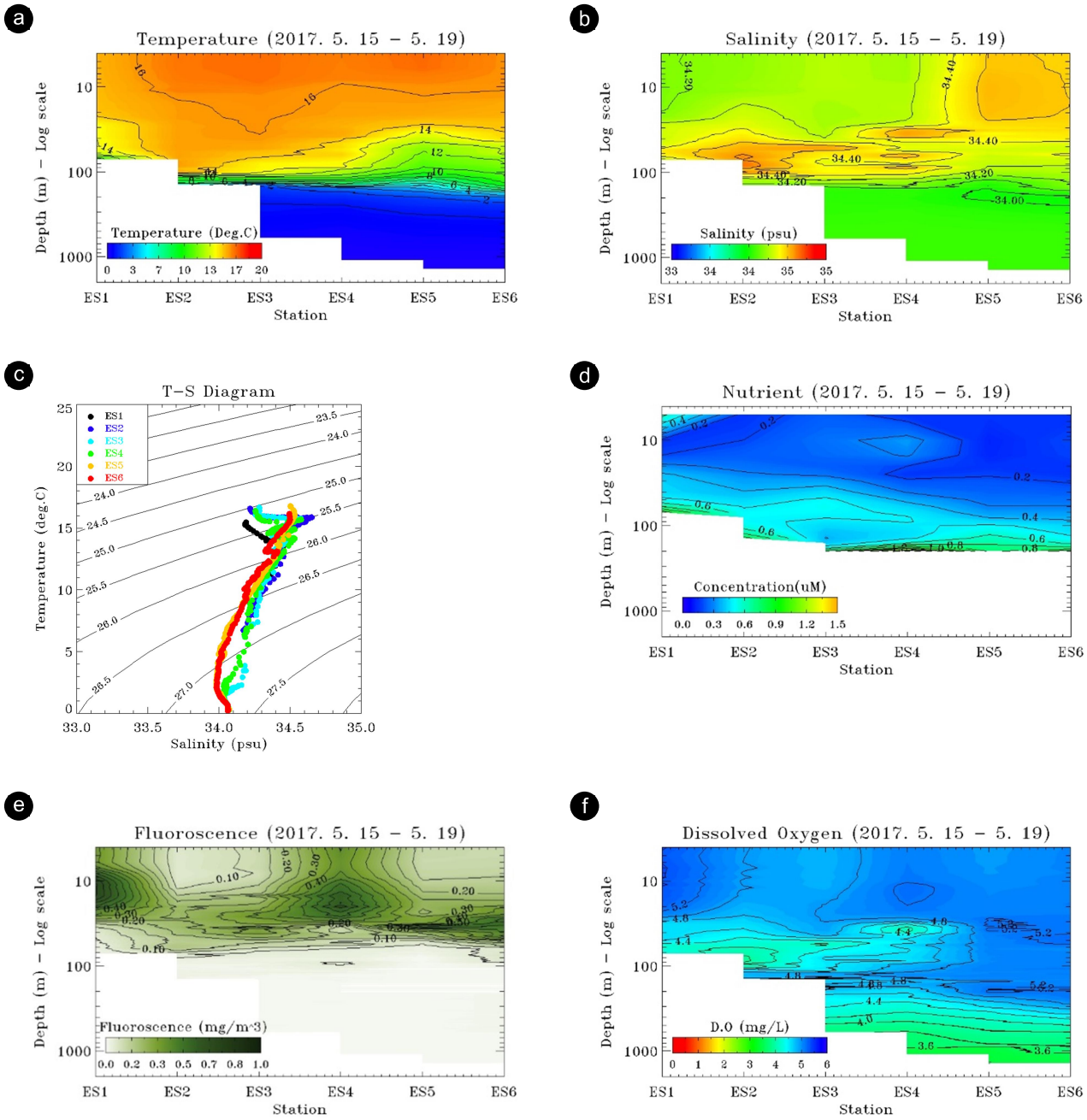

Fig. 2. Water column characteristics and physicochemical properties: (a) water temperature; (b) salinity; (c) temperature-salinity profile; (d) phosphate concentration; (e) fluorescence; and (f) DO. Please note that the vertical scale is logarithmic. The stations along the x-axis are equally spaced to better show various parameters' distributions.

DO showed rather a complex pattern (Fig. 2f). It decreased with depth, then increased again at around 100~200 m depth, then decreased again with depth for the ES2 to ES4 stations. The elevation in water between the two layers of lower oxygen was presumably due to the intrusion of northern seawater. At stations ES5 and 6, deep mixing of DO down to 200~300 m was observed.

2. Overview of sequence reads

Using 16S rRNA gene Illumina sequencing, a total 6,637,818 high

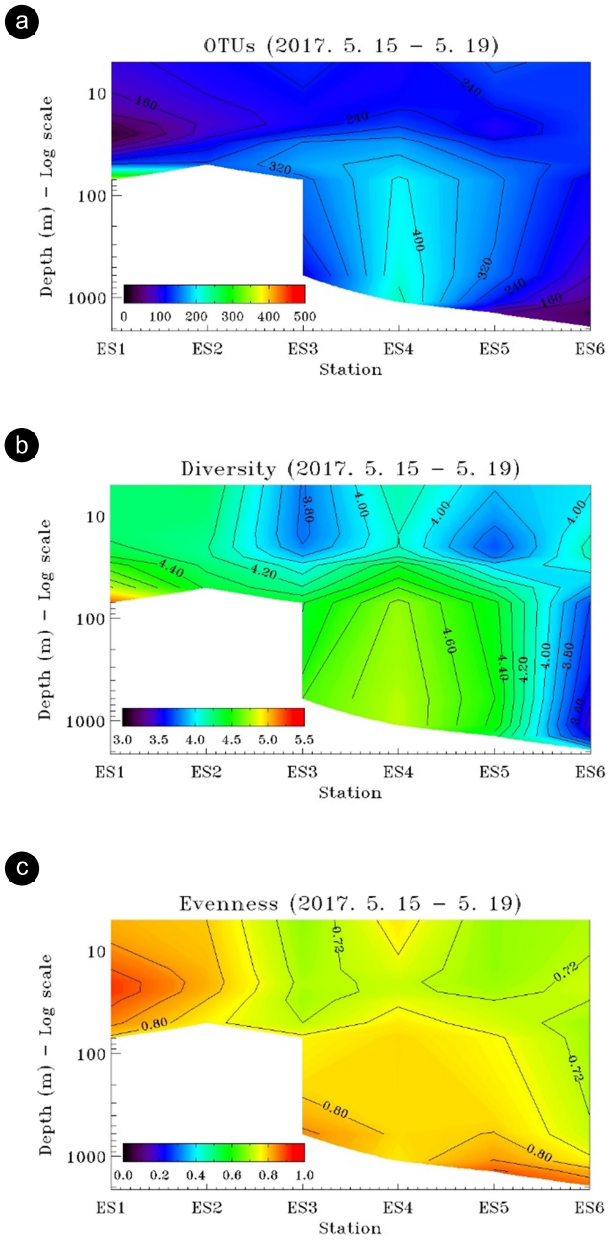

Fig. 3. Distribution of total operating taxonomic units (OTUs), bacterial diversity, and evenness in the study area's water samples.

quality reads were generated from 30 samples with an average of 214,123 reads/sample (minimum reads/sample = 106,638; maximum read/sample = 320,370). Because of the uneven sequencing depth, 100,000 randomly selected paired end reads per sample from the initial clean reads were retained for further analysis (supplementary Table S1). After quality control filtering and chimera removal, the reads were clustered into 3,225 OTUs. After classification using the SILVA database classifier at a confidence threshold of 97%, 64% (19,860 sequences) of all qualified reads were assigned to the Bacteria domain. One particular OTU, identified as a Burkholderia, comprised >70% of the total reads and even made up >90% of the total reads from sediment. Such overwhelming dominance of one particular OTU might have been an artifact, and as such, this OTU was removed for further analysis.

3. Total OTUs and bacterial diversity

Total OTUs ranged from 99 to 714 (mean 268±125) for the water column and from 57 to 238 (mean 133±77) in the sediments (Fig. 3a, 4). The variation was similar with a coefficient of variation (CV) of 46% for the water column and 58% for the sediments. For OTUs in the water column, their numbers generally were higher in the deeper parts of the stations and were lower in the coastal surface layer and the deepest part of station ES6 (Fig. 3a). Although lower OTUs were found in the sediments than in the water column, it appeared that total OTUs in the sediment environment generally followed the pattern in the overlying water column with the highest OTU observed at station ES4 (Fig. 4).

Bacterial diversity ranged from 3.79 to 5.22 (mean 4.28±0.36) in the water column and from 3.52 to 5.33 (mean 4.50±0.67) in the sediment (Fig. 3b and 4). The variation was larger in the sediment a CV of 15% as compared to 8% in the water column. Bacterial diversity generally followed the pattern of the OTU distribution. Diversity in the coastal station was higher compared to deep stations and appeared to rise at depth (Fig. 3b). At deep water stations ES3-5, diversity in the surface layer was low but high at mid-depth and deep waters.

Bacterial evenness ranged from 0.69 to 0.92 (mean 0.78±0.07) in the water column and from 0.87 to 0.97 (mean 0.95±0.05) in the sediment (Fig. 3c and 4). The variation, however, was larger in the water column with a CV of 9% as compared to 5% in the sediment. Evenness generally followed a pattern similar to that of diversity, high in coastal water and at deep waters of open water stations.

4. Major bacterial taxa and their distribution

A total of 22 bacterial phyla, including an unclassified one, were detected in this study (Fig. 5). Proteobacteria was the most dominant bacterial phylum in the water samples and comprised 67.3% of the total sequence reads followed by Bacteriodetes (15.8%). Planctomycetes, Verrucomicrobia, and Actinobacteria fol-

lowed next, all together consisting of only 8.1% of the total sequence reads (Fig. 5). Deep sea water samples contained lower reads compared to surface water samples. Relatively lower reads were detected in the sediment than in the overlying water columns for the major Proteobateria and Bacteriodetes phyla (Fig. 5).

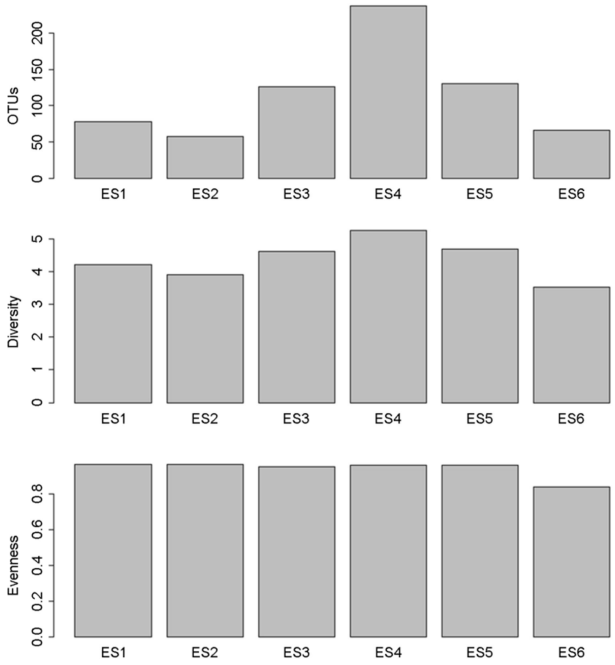

Fig. 4. Distribution of total operating taxonomic unit (OTUs), bacterial diversity, and evenness in the study area's sediment samples.

The main bacterial OTUs sharply increased moving through station ES4 and to the north (Fig. 5). *Candidatus* Pelagicbacter, Gammaproteobacteria, and Planctomycetaceae bacteria were distributed extensively through the water column except in coastal waters in which their abundance was very low (<200 reads) (Fig. 6). Flavobacteriaceae, Amylibacteria, and Rhodopirulle rhodobacteria Rhodopirellula were largely concentrated in the surface layer, but they also exhibited low abundance in coastal waters (Fig. 6). Variovax was more abundant in the subsurface waters although their presence was small. Many of these bacteria were highly concentrated at station ES4's water column with an opposing *Candidatus* Pelagicbacter distribution pattern, which showed the highest abundance away from ES4.

Non-metric dimensional scaling (NMDS) was performed with the data of the top 10 most abundant OTUs for each sampling site, which resulted in a total of 77 OTUs for analysis. NMDS results showed differences between the bacterial communities of the upper water columns and those of the deep-sea seawater and the sediment samples (Fig. 7). Cluster analysis displayed similar results to those obtained from NMDS analysis. The analysis of similarities based on the dissimilarity matrix of the bacterial OTUs among the samples showed that separation into three groups

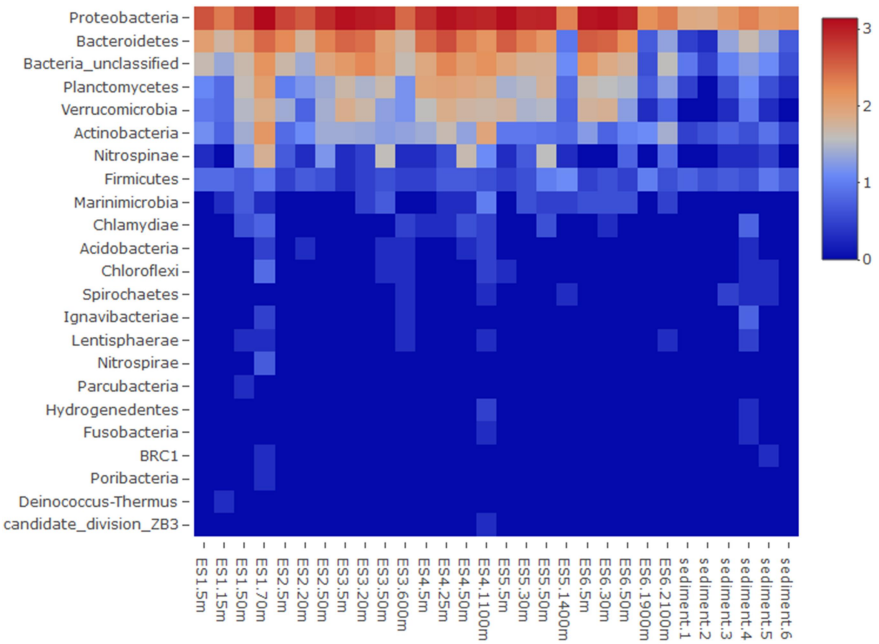

Fig. 5. Total sequence reads' distribution by bacterial phylum at different stations of different depths and sediments. Note that the scales of the reads are on log10.

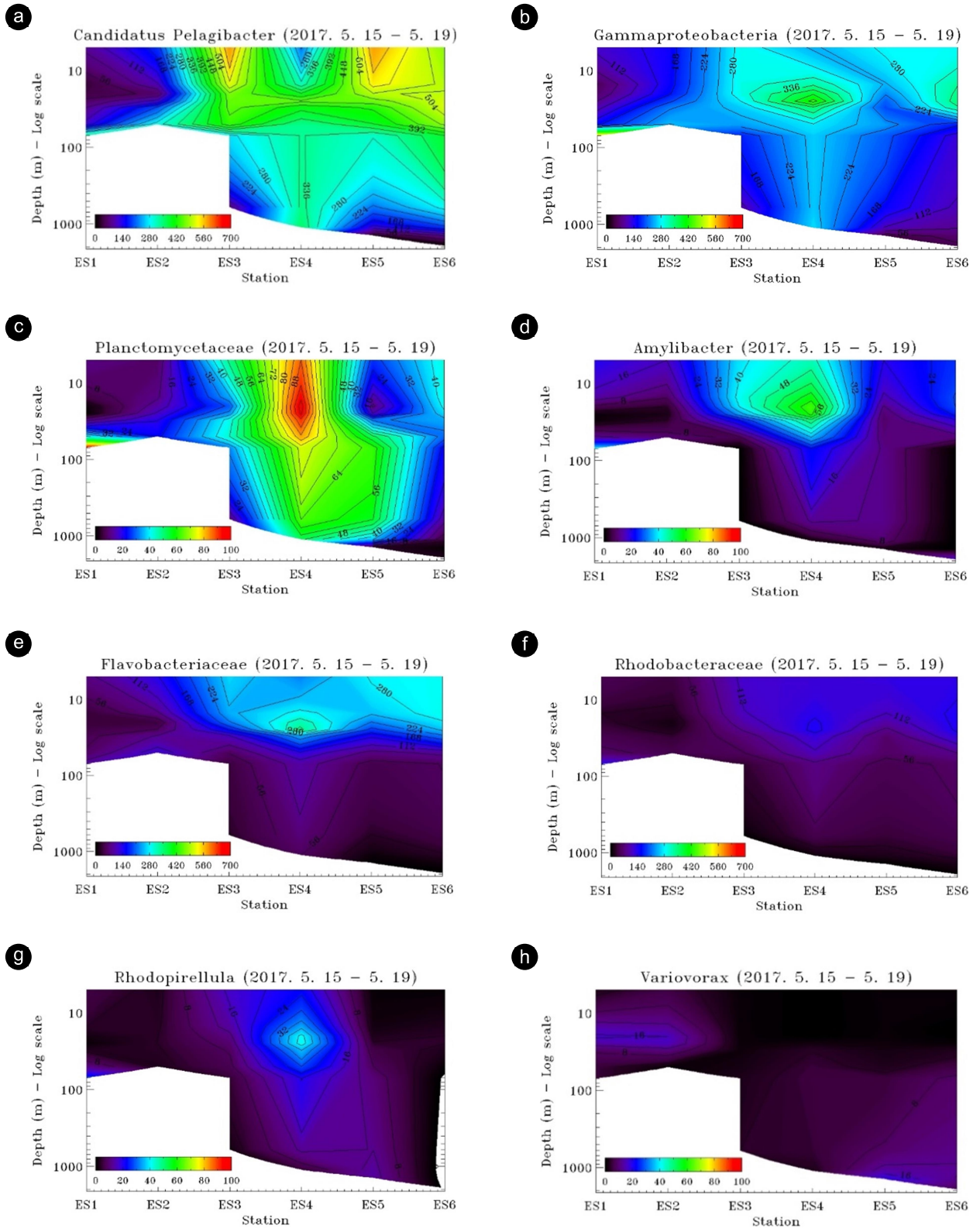

Fig. 6. Distribution of major bacterial taxa in the water column of the study area. The stations on the x-axis are equally spaced to better show the distributions.

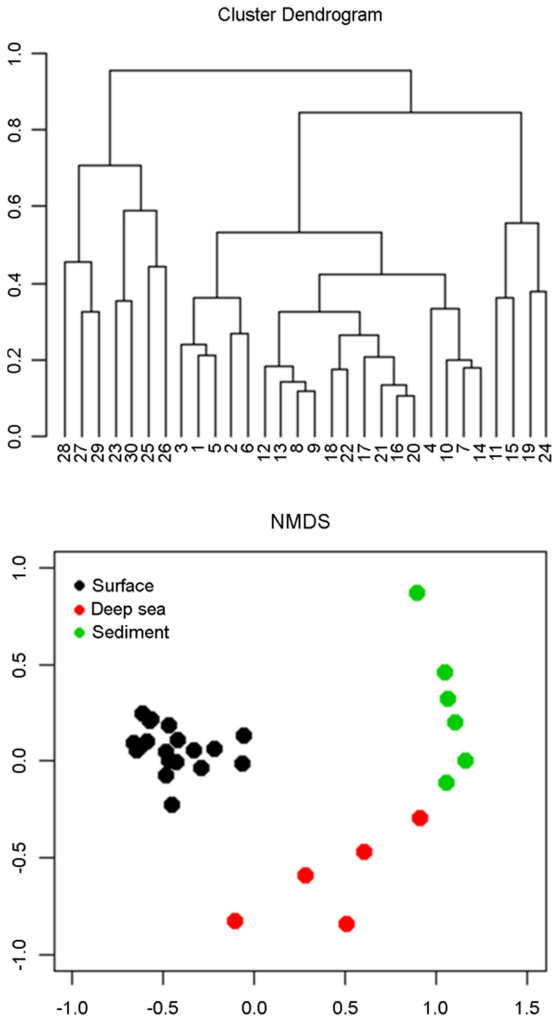

Fig. 7. Multivariate analysis of bacterial community of the top 10 OTUs for each sampling site. Cluster analysis results on the left panel and non-metric multidimensional scaling results on the right panel.

based on the cluster analysis and NMDS were statistically well-supported (r = 0.97, p < 0.001).

Indicator species analysis was further performed (Cáceres and Legendre, 2009) to determine indicators of site groups using the OTU data (Table 1). We used the R Package 'indicspecies' by the same authors, which allows indicators to consist of species combinations in addition to single species. For surface water samples, 22 bacterial OTUs were associated (such as group 1, p <0.05), whereas eight bacterial OTUs were in Group 2 (deep-sea seawater samples), and just four OTUs in Group 3 (sediment samples).

Table 1. Indicator species analysis on the seawater and sediment samples showing the representative OTUs for each group (p <0.05) that was obtained from cluster analysis and non-metric dimensional analysis

Group	Otu ID	Associated taxa
Group 1	Otu0004	Gammaproteobacteria_unclassified
	Otu0005	*Candidatus*_Pelagibacter_unclassified
	Otu0007	Gammaproteobacteria_unclassified
	Otu0008	Flavobacteriaceae_unclassified
	Otu0009	Amylibacter
	Otu0010	Alphaproteobacteria_unclassified
	Otu0011	Rhodobacteraceae_unclassified
	Otu0012	Flavobacteriaceae_unclassified
	Otu0014	Flavobacteriaceae_unclassified
	Otu0015	Rhodospirillaceae_unclassified
	Otu0016	Alphaproteobacteria_unclassified
	Otu0022	Planctomycetaceae_unclassified
	Otu0024	Flavobacteriaceae_unclassified
	Otu0026	Nitrospina
	Otu0027	Gammaproteobacteria_unclassified
	Otu0029	Flavobacteriaceae_unclassified
	Otu0032	Rhodobacteraceae_unclassified
	Otu0037	Rhodobacteraceae_unclassified
	Otu0046	Flavobacteriaceae_unclassified
	Otu0051	Alphaproteobacteria_unclassified
	Otu0128	Actinobacteria_unclassified
	Otu0013	Gammaproteobacteria_incertae_sedis_unclassified
Group 2	Otu0036	Proteobacteria_unclassified
	Otu0048	Acidimicrobiales_unclassified
	Otu0068	Gammaproteobacteria_unclassified
	Otu0074	*Candidatus*_Pelagibacter_unclassified
	Otu0098	Flavobacteriaceae_unclassified
	Otu0104	Gammaproteobacteria_unclassified
	Otu0170	Nitrospina
Group 3	Otu0182	Gammaproteobacteria_unclassified
	Otu0267	Gammaproteobacteria_unclassified

Table 1. Indicator species analysis on the seawater and sediment samples showing the representative OTUs for each group (p <0.05) that was obtained from cluster analysis and non-metric dimensional analysis (Continued)

Group	Otu ID	Associated taxa
Group 3	Otu0302	Chromatiales_unclassified
	Otu0360	Gammaproteobacteria_unclassified
	Otu0435	Lutibacter

Discussion

1. Major bacterial taxa

In the current study, proteobacteria (Alphaproteobacteria and Gammaproteobacteria) were predominant in water and sediment samples (Fig. 5). This finding is consistent with previous studies showing that these bacteria are dominant in marine environments (Feng et al., 2009; Sekiguchi et al., 2002; Seo et al., 2017). In the South Sea of Korea located southwest of the current study area, the majority of the 19,860 sequences were affiliated with Alphaproteobacteria (58.2%), Gammaproteobacteria (7.9%), and Bacteroidetes (13.9%). Alphaproteobacteria was the most abundant bacterial class. In the Changjiang Estuary and the East China Sea, Proteobacteria (72.9%) was the most abundant phylum, followed by Firmicutes (6.4%), Bacteroidetes (4.6%), and Actinobacteria (4.1%) (Feng et al., 2009). Actinobacteria were abundant in the Changjiang Estuary in addition to the South Sea and the East China Sea surface waters (Seo et al., 2017). Rhodobacteraceae were also abundant in the South Sea of Korea.

Gammaproteriobacteria also dominated in the sediments as well (Fig. 6). In Changjiang Estuary, Proteobacteria were the most abundant phyla in the sediment samples (73%). Previous studies also showed that Proteobacteria were the dominant bacterial phylogenetic lineage in most surface marine sediments, often comprising >50% of the microbial biomass (Ravenschlag et al., 2001). Furthermore, it seems that Gammaproteobacteria was the most significant clade present in most marine sediments (Dyksma et al., 2016; Franco et al., 2017; Ravenschlag et al., 2001). For example, in the upper 2-cm layer of sediment, Gammaproteobacteria accounted for up to 10.5% of the total cell counts and 20% of prokaryotic rRNA in the Smeerenburgfjorden sediments (Ravenschlag et al., 2001).

Some of the dominant bacteria at family or genus level (Fig. 6) are known to be important in in the ocean surface water carbon cycle. *Pelagibacter* is an abundant member of the SAR11 clade in the phylum Alphaproteobacteria. SAR11 members and its relatives may be the most abundant organisms in the ocean (Henson et al., 2018), and it can comprise about 25% of all microbial plankton cells. During the summer, they may account for approximately half the cells present in temperate ocean surface waters (Morris et al., 2002). The family Flavobacteriaceae comprises more than 100 genera (http://www.bacterio.cict.fr), constituting a major phylogenetic group within the phylum Bacteroidetes (Bernardet and Nakagawa, 2006). Many members of the family Flavobacteriaceae originated from marine environments. The Cytophaga-Flavobacterium cluster within the phylum Bacteroidetes often accounts for >10% of the total microbial community in coastal and offshore waters (Barbeyron et al., 2008). Moreover, flavobacteria have been found in high abundance during natural and induced phytoplankton blooms, suggesting utilization of polymeric organic matter in marine systems (Kirchman, 2002).

Planctomycetes include free-living in addition to attached organisms. These highly diverse bacteria have been proposed to contribute to the global carbon cycle via turnover of complex carbohydrates in marine sediments and marine snow (Glöckner et al., 2003; Žure et al., 2015a). They possess phenotypic characteristics unusual for the domain Bacteria, including reproduction by budding, and an intracellular membrane-bounded compartmentalization (Jenkins et al., 2002). Planctomycetes *Rhodobacteraceae* are deeply involved in sulfur and carbon biogeochemical cycling and symbiosis with aquatic micro- and macro-organisms (Pujalte et al., 2014). Rhodopirellula is an abundant marine member of the bacterial phylum Planctomycetes. Cultivation studies revealed the presence of several closely related Rhodopirellula species in coastal sediments of the North Sea (Žure et al., 2015b). The genus Rhodopirellula also belongs to the widespread bacterial phylum Planctomycetes (Wagner and Horn, 2006).

Relatively little information is available for Amylibacter, a genus of the bacterial family, Rhodobacteraceae. A few species have been isolated from marine hosts, and *Amylibacter marinus* appears to be the only bacteria that has been isolated from surface seawater (Teramoto and Nishijima, 2014). As members of the family Comamonadaceae (Betaproteobacteria), the genus Variovorax has been found to inhabit diverse environments, including the ice surfaces of glaciers (Ciok et al., 2016), soils (Yoon et al., 2006) and deep marine sediments (Wang and Gu, 2006).

2. Community structure among different habitats

Although bacterial reads were higher in the surface water, its diversity and evenness were lower than that seen in coastal and deep seawaters. This is consistent with open ocean survey in which taxonomic richness was generally highest in the water-column O_2 minimum zone than the surface waters (Walsh et al., 2015). Bacterial communities in the warm surface waters were well separated from the other deep cold seawater and sediment samples (Fig. 7). However, the cluster analysis does not entirely support the distinction of the surface bacterial community influenced by the TWC from the East Sea water as it appears the association within the surface layer may have been more closely related to bacterial depth distribution. Bacteria can have different niche preferences in marine environments, which include not only physical properties (such as temperature, salinity, DO, and pH) but also chemical properties (such as nutrient concentrations). For instance, phosphate and DO concentrations have a significant influence on the bacterial community composition in the South Sea of Korea (Seo et al., 2017). Water temperature is a major factor deriving ocean bacterial activity with the highest activity normally observed during the summer (Price and Sowers, 2004). Several studies are available for bacterial community spatial succession along environmental gradients such as salinity (Campbell and Kirchman, 2013; Sekiguchi et al., 2002), but a study dealing with temperature gradients is rare.

The bacteria exclusively associated with Group 2 (deep seawaters) was Actinobacteriacea (Table 1). These organisms appear to be prevalent in the deep photic zone at or around the deep chlorophyll maximum. Marine actinobacterial groups may be also important players for nutrient cycling in the marine environment (Mizuno et al., 2015). Our results indicate that they can extend their habitat to deep cold seawaters. The bacterial group Chromatiales (class Gammaproteobacteria) and Lutibacter (family Flavobacteriaceae) were those exclusively associated with the sediment samples (Table 1). Chromatiales are generally known as sulfur-oxidizing Gammaproteobacterium (A Bazylinski et al., 2016), and their importance in the sulfur cycle has only recently gained attention (Lavy et al., 2018). Lutibacter bacteria are common to tidal flat sediments and are non-spore forming, facultative anaerobic (Choi et al., 2013; Choi and Cho, 2006) and carotenoid-containing chemoheterotrophs (Sundararaman and Lee, 2017). Thus, the presence of these bacteria from the coastal sediment to the deep ocean basin suggests a role for sulfur and organic carbon cycling in the East Sea sediment. Such clustering of the sediment bacterial community despite wide environmental gradients was also observed in the sediment samples collected from the Changjiang Estuary and the East China Sea (Feng et al., 2009). Clustering of bacteria collected from the sediment supports the general feature that bacterial types are widely dispersed in similar habitat types (Feng et al., 2009; Fuhrman et al., 1993a; Mullins et al., 1995).

3. Influence of The Tsushima Warm Current

The TWC flows into the East Sea through the Korea/Tsushima Strait and then continues along the Japanese and Korean coasts to higher latitudes in the East Sea. It shows that water density is primarily regulated by temperature, and the intrusion of the water mass of low salinity and high seawater temperature into the surface layer indicates transport of TWC into the region, most likely between stations ES1/ES2 and ES4 (Fig. 2). The influence of the TWC seems to be limited to ES4 at the study transect, the point at which the continental slope ends (Fig. 1) as TWC may flow further north and is not involved the current study area. However, TWC's downward effects are clearly limited in the surface layers from 30 to 70 m (Fig. 2) as indicated in downward heat supplied by the current (Onitsuka et al., 2007). To some extent, TWC intrusion in the surface water in the region appears to affect both bacterial communities and abundance. Major bacterial OTUs sharply increase between stations ES2 and ES4 (Fig. 5). Flavobacteriaceae, Amylibacteria, Rhodopirulle rhodobacteria, and Rhodopirellula were largely concentrated on the surface layer, but they also exhibited low abundance in coastal waters. However, the cluster analysis does not entirely support the differences in surface bacterial community influenced by the TWC from the East Sea water (Fig. 7) as it appears the association within the surface layer may be more closely related to bacterial depth distribution.

The confluence of two water masses of TWC and East Sea water mass may lead to such increased biological activity in association with the geomorphic feature along this transect at which point the continental shelf ends and the continental slope begins. These potential increased biological activities may account for the reduced level of DO at the subsurface layer compared to ambient environments (Fig. 3). Such enhanced biological activities may have occurred in the sediment environment as well (Fig. 4). The ES4 station had the highest diversity of sedimentary bacterial genotypes among all sampling stations in the study area, and ES4 also showed the highest number of Proteobacteria and Bateriodetes among the sediment samples (Fig. 5). The continental slope serves as a focus for the deposition of organic matter produced on

continental shelf (Moriarty et al., 1991).

Many marine bacteria have evolved to grow optimally at either high (copiotrophic) or low (oligotrophic) nutrient concentrations, enabling different species to colonize distinct trophic habitats in the oceans. In general, oligotrophs, and not the more readily isolatable copiotrophs, dominate the ocean's free-living microbial populations (Lauro et al., 2009). *Candidatus* Pelagibacter ubique is considered as oligotrophy (Giovannoni et al., 2005), whereas Planctomycetes, a group known to become dominant during phytoplankton blooms, have trophic signatures that can be described as copiotrophic with some traits of oligotrophy (Lauro et al., 2009; Morris et al., 2006). Their distribution can be seen as a direct competition over the resources and *Candidatus* Pelagibacter dominates where biological activity is high.

In summary, in the East Sea, bacterial community in the warm surface waters were well separated from the other deep cold seawater and sediment samples. Overall bacterial community showed more of similarity in horizontal than in vertical direction in the East Sea. The confluence of the warm Tsushima Currents and the more saline East Sea water appears to create an environment for enhanced biological activity as does the continental slope compared to other benthic environments. Distribution of oligotrophic and copiotrophic bacteria in the surface waters suggest a potential direct competition of these bacteria of different traits over resources.

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Supplementary Table S1

Sample name	Number of total reads (w/o trim)	Number of total read (with Trimming)
Eatsea-sediment-1	190,840	187,432
Eatsea-sediment-2	160,108	156,954
Eatsea-sediment-3	218,866	215,002
Eatsea-sediment-4	306,324	301,210
Eatsea-sediment-5	183,150	179,888
Eatsea-sediment-6	293,556	288,238
ES1-1-5m	237,754	233,634
ES1-2-15m	239,956	235,784
ES1-3-50m	296,650	291,618
ES1-4-70m	267,886	263,770
ES2-1-5m	200,806	196,698
ES2-2-20m	175,342	171,720
ES2-3-50m	236,960	232,574
ES3-1-5m	235,582	231,448
ES3-2-20m	326,658	320,370
ES3-3-50m	201,116	197,132
ES3-4-600m	216,036	212,092
ES4-1-5m	296,080	290,790
ES4-2-25m	245,298	241,706
ES4-3-50m	108,460	106,638
ES4-4-1100m	145,758	143,642
ES5-1-5m	181,686	178,944
ES5-2-30m	160,034	157,460
ES5-3-50m	128,956	126,958
ES5-4-1400m	149,888	147,614
ES6-1-5m	288,866	284,774
ES6-2-30m	219,678	215,648
ES6-3-50m	185,748	182,380
ES6-4-1900m	251,898	247,428
ES6-5-2100m	225,150	221,438