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Characteristics of long-term changes in microbial communities from contaminated sediments along the west coast of South Korea: Ecological assessment with eDNA and physicochemical analyses

Aslan Hwanhwi Lee[a,1], Junghyun Lee[a,1], Junsung Noh[a], Changkeun Lee[a], Seongjin Hong[b], Bong-Oh Kwon[c], Jae-Jin Kim[d], Jong Seong Khim[a,*]

[a] School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Ocean Environmental Sciences, Chungnam National University, Daejeon 34134, Republic of Korea
[c] Department of Marine Biotechnology, Kunsan National University, Kunsan 54150, Republic of Korea
[d] Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University, Seoul 02841, Republic of Korea

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A B S T R A C T

The environmental DNA (eDNA) metabarcoding was applied to assess benthic ecological health in the west coast of South Korea by investigating a long-term microbial community change (2015–17). The ecological interaction among microorganisms, from phylum to family level, and their associations to environmental variables across the five regions were highlighted. As part of the study, the available chemistry and toxicological data in the regions during the monitoring periods were incorporated into an integrated sediment triad assessment. The bacterial communities were dominated by Proteobacteria (34.2%), Bacteroidetes (13.8%), and Firmicutes (10.8%). Proteobacteria and Bacteroidetes dominated consistently across regions and years, while Firmicutes and Cyanobacteria significantly varied by region and years ($p < 0.05$). The abundance of this phylum declined over time with the increasing abundance of Cyanobacteria, indicating their independent interactions to certain environmental changes. Planctomycetes and Gemmatimonadetes linked to some contaminants (ΣPAHs and Cu), implying indicator taxa. Overall, eDNA-based microbial community analysis combined with exposures of contaminants and responses of microorganisms is a promising strategy for the assessment of benthic ecological health in contaminated sediments from coastal waters.

1. Introduction

The west coast of Korea meets the Yellow Sea, in which diverse marine biological organisms, including bacterial communities, inhabited (Park et al., 2014). Several major rivers, including Han River, Geum River, and Yeongsan River of South Korea, are discharged into the west coast. These inputs provide resources promoting civilization and anthropogenic activities, which have both positive and negative effects on the coastal ecosystems including microbial communities (Marti et al., 2017; Xie et al., 2017a; Lee et al., 2020a).

Since the late 2000s, with more rapid urbanization, sediment contamination along the Korean coast has been monitored (Jeon et al., 2017; Kim et al., 2020) and assessed with marine ecological apparatus via the measurement of sediment quality, such as with a triad approach involving the examination of benthic community health, toxicity, and chemical analyses (Khim and Hong, 2014; Lee et al., 2018a; Lee et al., 2020c). In the previous study, long-term perspectives with the measurement of persistent toxic substances (PTSs), including polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and metals and its toxicity were assessed (Kim et al., 2020). More recently, we successfully employed environmental DNA (eDNA) analyses (Xie et al., 2017a; Lee et al., 2020a), which are now increasingly used to assess the structures of aquatic and benthic communities in coastal waters and elsewhere (Zhang, 2019).

The sediment microbiome is one aspect of the sensitive taxa representing benthic community health. The eDNA metabarcoding of this microbiome is now considered to be a sensitive and powerful method for the characterization of benthic community health status and trends, which can be linked to the assessment of contaminated sediments (Xie et al., 2017a, 2018; Yang et al., 2018). It has increased the amount of

* Corresponding author.
E-mail address: jskocean@snu.ac.kr (J.S. Khim).
[1] These authors contributed equally to this work.

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taxonomic and eco-genomic information available for freshwater and marine taxa, rendering it more reliable and effective (Sunagawa et al., 2015; Lee et al., 2019). Overall, metagenomic technology using eDNA bioinformatics shows promise for characterization of the complexity of sediment microbial ecosystems composed of enumerable individual taxa (Amann et al., 1995; Sharmin et al., 2013; Gibbons et al., 2014).

The present study was conducted in five regions along the west coast of South Korea in 2015–17, as part of our continuing long-term ecological monitoring in the west coast of South Korea since the late 2000s (Jeon et al., 2017; Kim et al., 2020; Lee et al., 2020a). We used a high-throughput sequencing (HTS) approach to examine microbial communities for the characterization of sediment quality and benthic community health, as applied in this region in 2010–14 (Lee et al., 2020a). In benthic microbial communities from habitats where anthropogenic pollutants have been discharged and have accumulated in freshwater and seawater sediments, specific phyla demonstrated interaction with environmental pollutants and correlation matrices. Benthic microbial community analysis permits the characterization of sediment health and quality via the assessment of microbiota exposure to PTSs. The specific objectives of this study were: 1) to determine the spatio-temporal distribution of sediment microbial composition along the west coast of South Korea, 2) to compare bacterial community data with previously reported long-term monitoring data, and 3) to explore interactions between bacterial taxa and specific environmental variables of concern.

2. Materials and methods

2.1. Study area and samples

Surface sediment samples were collected once a year between 2015 and 2017 from 15 locations (11 seawater sites and 4 freshwater sites) in five regions along the west coast of South Korea: Sihwa Lake (region A), Asan and Sapgyo Lakes (region B), the Taean Coast (region C), Geum River estuaries (region D), and Yeongsan River (region E) (Fig. 1; Table S1). All samples were transported immediately at 4 °C to the laboratory, where they were stored at −20 °C until freeze-dried and ground with a mortar prior to analysis.

As indicators of microbial community growth, data on long-term changes in chemical oxygen demand (COD) and total nitrogen (T-N) concentrations were collected in 2000–2017, as part of monitoring for the establishment of marine environmental standards in South Korea (Fig. 1). These concentrations indicate water quality under efficient primary treatment (Silva-Bedoya et al., 2016); according to the South Korean marine environmental standards.

2.2. eDNA metabarcoding and bioinformatics analysis

Total DNA was extracted from sediment samples (0.5 g dry mass) using the DNeasy Powersoil kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Sequencing libraries of bacterial communities were constructed using amplicon next-generation sequencing of 16S ribosomal RNA (rRNA) genes (V3–V4 region) with forward primer, 5′–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3′; reverse primer 5′–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–3′ (Klindworth et al., 2013; Lee et al., 2020b). The libraries were sequenced on the Illumina MiSeq platform (Macrogen, Korea) according to the manufacturer's instructions.

Sequences were obtained by DNA amplification and processed using QIIME v1.9.1 (Caporaso et al., 2010). All reads were checked and trimmed for quality (maximum homopolymers, $n > 6$) after the removal of chimeras (Edgar, 2013). Low-quality and short sequences, as well as those with polymerase chain reaction bias, lack of annotated references, and lineage filtering, were discarded (Bragg et al., 2013; Torres-Oliva et al., 2016). After noise removal and checking of raw read quality, the microbial diversity of the remaining sequences was analyzed. The sequences were clustered into operational taxonomic units (OTUs) using a ≥97% similarity cut-off and the average linkage method with VSEARCH (Rognes et al., 2016). Of note, the variation in OTU readings across the sampling regions was relatively great compared to that between sampling years (Fig. S1). A taxonomic

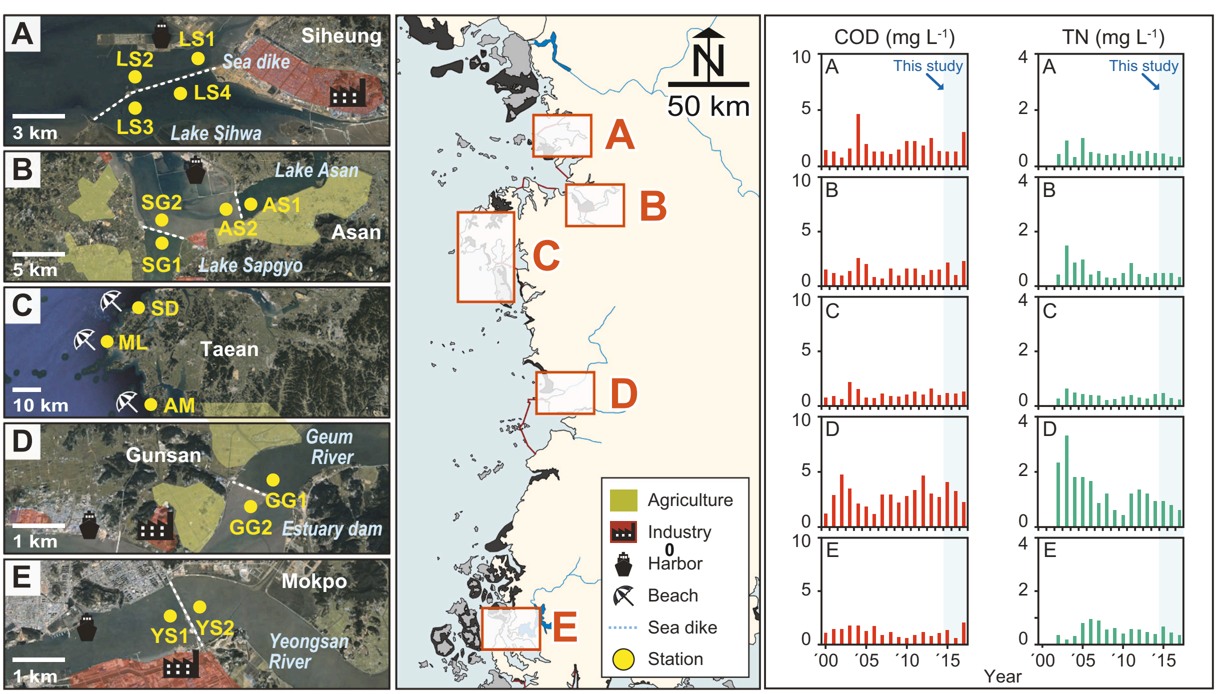


Fig. 1. Map showing the five regions of the study areas, such as Lake Sihwa (A), Lakes Sapgyo and Asan (B), the Taean coast (C), Geum River Estuary (D), and Yeongsan River Estuary (E) including 15 locations, and long-term changes in COD and T-N of the environments along the five regions.

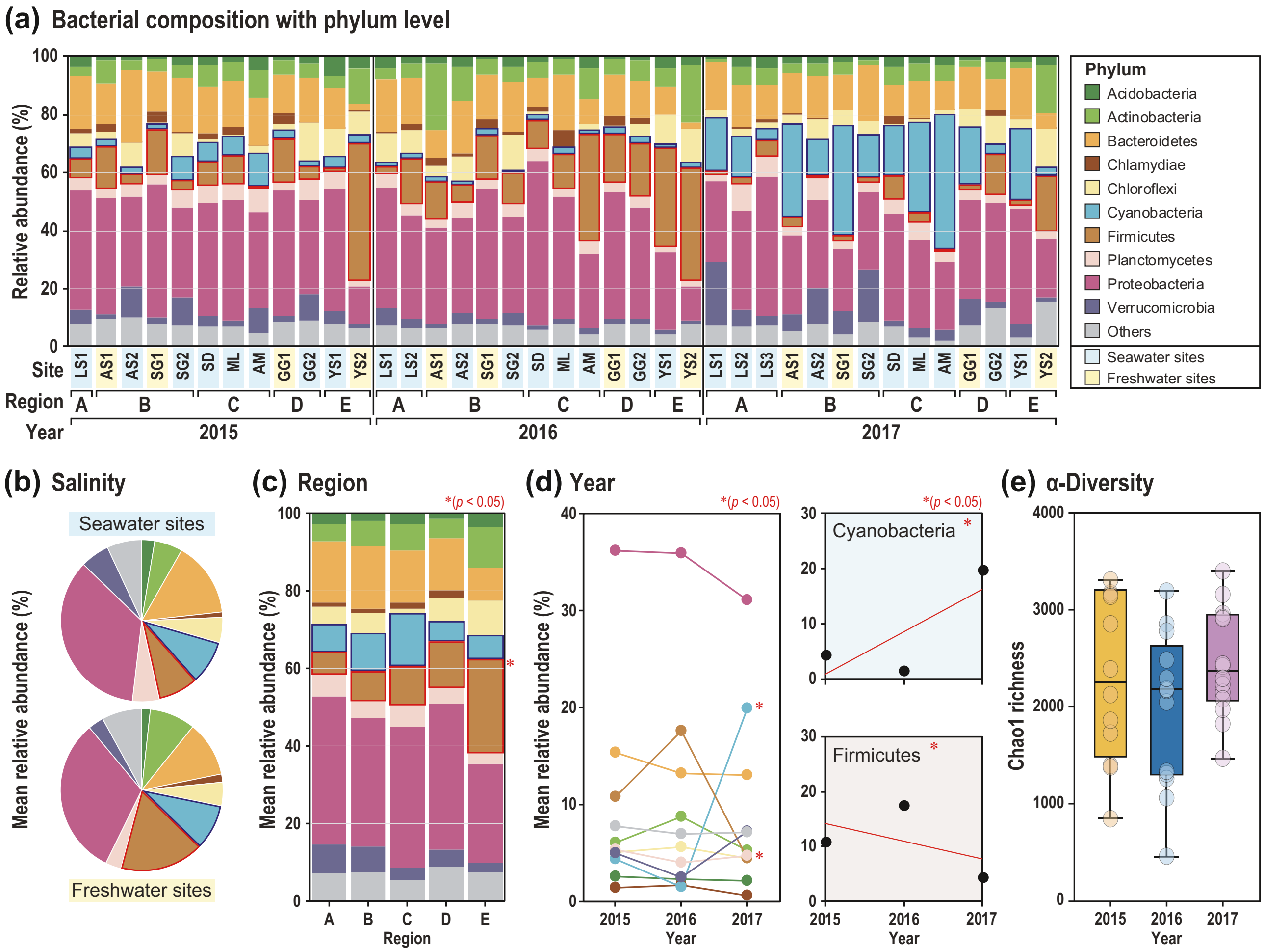


Fig. 2. The structures of the bacterial communities at phylum level in sediments along the west coast of South Korea. Panels: (a) relative abundance of the dominant bacterial at phyla across all samples, statistical comparisons for mean relative abundances at the phylum level between (b) salinity, (c) sampling region, and (d) sampling years, and (e) alpha-diversity estimated with Chao1 richness along with year. With the exception of the top 10 phyla group, "Others" were grouped in panel (a). An asterisk indicates a significant difference at p values < 0.05 (*).

assignment was performed for the representative sequence that was most abundant in each OTU using the Greengenes database (http://greengenes.secondgenome.com). Alpha diversity (Chao1 richness) indices were calculated using QIIME (Chao, 1984). After taxonomic profiling, we investigated differences in the relative abundance of the microbial taxa among study areas. Beta diversity was analyzed because microbial taxa differed in relative abundance across the years and regions.

2.3. Statistical analyses

To analyze beta diversity, principal coordinate analysis with permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis similarity was performed using PRIMER-e 6 (PRIMER-E Ltd., Plymouth, UK). Changes in microbial composition were examined using a two-way analysis of variance with Prism GraphPad 6 (GraphPad Software), and PERMANOVA with PRIMER-e 6 (Miranda et al., 2018). Dendrograms for the sediment samples were constructed based on the similarity of bacterial diversity over the years and regions, by similarity percentage analysis with PRIMER-e 6.

The relative contributions of environmental pollutants with the given data of PAHs, APs, styrene oligomers (SOs), and metals (Kim et al., 2020), to differences in community structure, were determined by distance-based redundancy analysis (dbRDA) based on the Bray-Curtis similarity of all data, using PRIMER-e 6 (Glasl et al., 2019). Spearman rank correlation coefficients were calculated to support the dbRDA-based plotting of fitted variation. Correlation matrices were generated using the R software (version 3.6.0, http://www.R-project.org/). The R packages "igraph", "ggraph", "corrplot", and "network" were used for microbial network analysis (Layeghifard et al., 2018). Network analysis reveals symbiotic relationships with chemical variables (Corel et al., 2016; Tipton et al., 2019).

2.4. Integrated assessment

The integrated assessment used four characteristics of sediments, including concentrations of PTSs (15 PAHs, 6 APs, and 10 SOs), metals [as hazard quotients (ΣHQ_{metal})], toxic potencies [H4IIE-*luc* bioassays for determining AhR-mediated potencies (presented as $\%BaP_{max}$) and MVLN bioassays for determining ER-mediated potencies (presented as $\%E2_{max}$)], and sediment-microbiome, and ratio to mean (RTM) values were calculated (Cesar et al., 2009; Kim et al., 2020). The RTMV method is used to convert values for each variable of interest in lines of evidence (LOE) to non-dimensional values by dividing the value obtained by the arithmetic mean obtained for all stations and all years (Cesar et al., 2009; Khim et al., 2018). RTMVs were combined through the calculation of a mean, thus producing a single new value for each LOE. These single new values were plotted in three-axis graphs to producing a triangular pyramid reflecting each class at each survey time point.

3. Results and discussion

3.1. Characteristics of HTS data

In total, 1,123,200 bacterial sequences from the V3 and V4 regions of 16S rRNA were obtained from the sediment samples. The sequences remaining after quality filtering contained 8764 bacterial OTUs representing 65 phyla, 211 classes, 418 orders, 648 families, 1019 genera, and 1080 species. Sample YS1, collected in 2015, contained the most OTUs ($n = 2768$), and sample SG1, collected in 2015, contained the fewest ($n = 370$).

3.2. Bacterial communities and their spatial distribution

At the phylum level, bacterial community composition varied among sediments and dominated by Proteobacteria (mean 34.2%), Bacteroidetes (13.8%), Firmicutes (10.8%), Cyanobacteria (9.0%), Actinobacteria (6.7%), Chloroflexi (5.0%), Verrucomicrobia (5.0%), Planctomycetes (4.7%), Acidobacteria (2.3%), and Chlamydiae (1.2%) (Fig. 2a). The relative abundance of other individual phyla was < 1%. Proteobacteria were distributed widely among samples, and the Proteobacteria composition was similar to that observed in samples collected from the same locations in 2010–14 (Lee et al., 2018b; Lee et al., 2020a). In contrast to previous findings, however, Cyanobacteria and Chlamydiae were among the 10 dominant phyla, and Gemmatimonadetes was not. Although determining the cause of cyanobacteria blooms can be elusive, contaminated sediments with nutrient enrichments are often critical for bloom initiation, development, and species succession (Glibert et al., 2011). Previous study reported that marine sediment Chlamydiae have pathways for metal resistance and encoded a multicopper oxidase. Also, single lineage of Chlamydiae could represent sediment contamination (Dharamshi et al., 2020). In general, the dominant phyla in the sediment samples were similar to those observed in other regions of the world (Nemergut et al., 2013; King et al., 2015; Xie et al., 2018).

The mean relative abundance of the bacterial phyla varied with salinity and sampling region (Fig. 2b and c). Salinity and spatial variability are regarded as major contributors to spatiotemporal changes of microbial assemblages (Xie et al., 2017b; Lee et al., 2020a), but the microbial communities did not differ significantly with differences at phylum level in aspect of salinity (Fig. 2b). Of note, irregular discharges of freshwater through sea dikes may cause temporary dilution affecting salinity gradients (Yim et al., 2018). For example, the mean relative abundance of Firmicutes differed significantly among study regions ($p < 0.05$; Fig. 2c), while not significantly different between salinity. Firmicutes was dominant and abundant in region E due to the abundance of Bacilli at freshwater sites in this region (Fig. 3a). Meantime, the abundance of Proteobacteria in region E decreased with increasing Firmicutes abundance (Fig. 2a and c). Differences in the relative abundance of these two phyla in the microbiome have been reported to reflect ecosystem changes (Wang et al., 2012; Zhang et al., 2019). Higher levels of Firmicutes and lower levels of Proteobacteria in region E may reflect changes in physicochemical parameters, such as pH, temperature, and the presence of contaminants (Zhang et al., 2019). Actinobacteria were very abundant at freshwater sites. Actinobacteria have been reported to dominate in freshwater sediments, and their growth can be enriched at freshwater sites (Tamaki et al., 2005; Wang et al., 2012). Bacterial diversity was absolutely lesser at SG1 and GG1 than those in other locations (Fig. 2e), but the number of species changed greatly in locations with shifts in the density of dominant phyla (Fig. 3b).

To scrutinize the spatial similarity in microbial communities, cluster analysis was performed using all the data obtained from 2015 to 17 (Fig. 4a). Clusters for regions C and E contained mixed data from all sampling years. However, region E showed the statistical difference with region B and C (Table S2a). Spatial dissimilarity has resulted in region E, and seawater and freshwater sites showed no significant grouping (Fig. 4). Region E clustering was caused by the dominance of Firmicutes communities; seemingly due to the great relative abundances of Firmicutes (23.9%) and Actinobacteria (10.4%) as well as the lower relative abundances of other dominant phyla in the very region (Proteobacteria, 25.5%; Bacteroidetes, 8.5%). The dissimilarity of region C from other regions was due mainly to the great relative abundance of Cyanobacteria (13.8%). COD and T-N concentrations were consistently lowest in region C, and showed similar decreasing tendencies from region A to region B (Fig. 1).

3.3. Temporal variation in bacterial communities

The mean relative abundance of Cyanobacteria and Firmicutes differed significantly across sampling years ($p < 0.05$; Fig. 2d). The abundance of Cyanobacteria increased to 19.9%, and that of Firmicutes decreased to 4.5% in 2017. Increases in the abundance of Actinobacteria and Firmicutes were observed in 2016. Cyanobacteria and Verrucomicrobia showed similar tendencies in decreasing and increasing relative abundance between 2016 and 2017 (Fig. 3a). Although Chao1 richness values varied greatly cross the locations each year but did not show significant differences among years from 2015 to 2017, on average (Fig. 2e). By region, however, regions A, B, and D exhibited temporal dissimilarity between 2015 and 2017 (Fig. 4a).

For the three sampling years, the top 10 families per site, in terms of relative abundance, together comprised 67 of 648 families found to be present. The top 20 of these 67 families belong to 8 phyla (Fig. 4a). The top 10 families in individual sediment samples accounted for 51.7% of the mean relative abundance. The greatest frequencies of the top 10 families were found in samples from SG1, GG1, and ML collected in 2015–16 (59.1–58.5%, 56.8–58.2%, and 56.5–57.1%, respectively), and in samples from AM, YS1, and ML collected in 2017 (68.3%, 61.5%, and 60.9%, respectively). The three Firmicutes families (Alicyclobacillaceae, Bacillaceae, and Paenibacillaceae) were dominant in sediments from mangrove wetlands, where organic detritus mineralization and the recycling of essential nutrients in soluble matter occur (Liu et al., 2017).

The dbRDA scatter diagram, based on principal component plotting for phylum abundance, showed temporal dissimilarity between 2015–17 and 2010–14 (Fig. 4b), suggesting the occurrence of shifts in microbial community structure and function. Several dominant phyla (Actinobacteria, Firmicutes, and Proteobacteria) demonstrated variation by sampling region and year (Fig. S2); these groups have been found to dominate in other marine and estuarine sediments (Gibson et al., 2014; Xie et al., 2017b). Among yearly differences in relative abundance, that for Cyanobacteria was particularly pronounced, driving the difference between samples collected in 2017 and those collected in previous years (Fig. 2d). Firmicutes was more abundant in region E than in other regions in 2010–14, and Firmicutes and Verrucomicrobia showed compositional shifts during this period (Lee et al., 2020a). The regional variation in benthic microbial communities was thus likely influenced by the increase in Firmicutes abundance over time in region E. The consistent abundance of Firmicutes in region E encompasses a shift in phylum density from seawater to freshwater sites between the present and previous measurement periods (Lee et al., 2020a).

A major shift in the relative abundance of the top 10 families from 2015 to 2017 was observed; Lee et al., 2020a reported that the previous data also showed such a shift. In 2015 and 2016, Chitinophagaceae (Bacteroidetes), Cryomorphaceae (Bacteroidetes), Alicyclobacillaceae (Firmicutes), Bacillaceae (Firmicutes), Paenibacillaceae (Firmicutes), Caulobacteraceae (Proteobacteria), Sphingomonadaceae (Proteobacteria), Oxalobacteraceae (Proteobacteria), and TM7 families were abundant; *cf.* the corresponding abundances all decreased in 2017. The

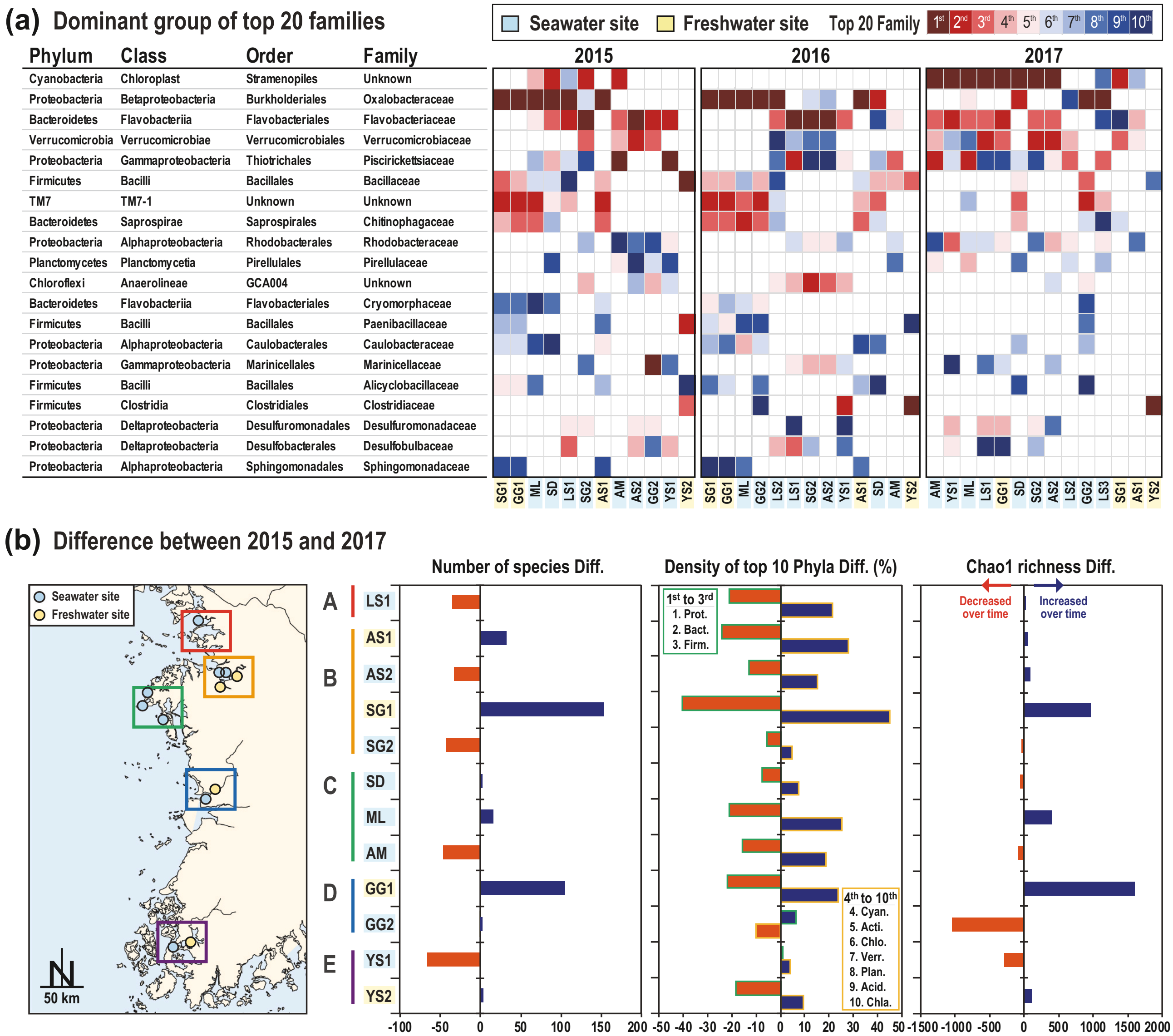


Fig. 3. Structures of bacterial communities (at Family level) in sediments along the west coast of South Korea. Panels: (a) the twenty most abundant bacterial families at each site were marked for all sampled years, (b) differential analyses between the years of 2015 and 2017 in the sampling locations composed of number of species, density of the top 10 phyla, and Chao1 richness.

dbRDA plot showed slight dissimilarity of bacterial communities over time (Fig. 4b), due to this temporal variability in the top 10 families. The general declines in COD and T-N concentrations over time may have resulted in the decrease in Firmicutes family abundance (Sattley et al., 2008).

In 2017, the structure of the benthic bacterial communities was drastically reversed (Fig. 3a); the main components were Flavobacteriaceae (Bacteroidetes), stramenopiles (Cyanobacteria), Rhodobacteraceae (Proteobacteria), Piscirickettsiaceae (Proteobacteria), and Verrucomicrobiaceae (Verrucomicrobia). Piscirickettsiaceae (Proteobacteria), Rhodobacteraceae (Proteobacteria), and Flavobacteriaceae (Bacteroidetes) are known to be effective producers of extracellular enzymes and extracellular polymeric substances for the mineralization of petroleum hydrocarbons (Kamalanathan et al., 2018; Ribicic et al., 2018). Our findings may reflect the interaction of photosynthetic bacterial assemblage abundance with the occurrence of algal blooms in 2017 (Kang et al., 2018; Zerrifi et al., 2018). Of note, such spatio-temporal distribution of small-sized microorganisms could be explained by physical factors including tide and wave; e.g., the West Coast Korean current from the South to West Sea (Hwang et al., 2014; Jeong et al., 2017).

Differential analyses provided further information about the shift in bacterial communities between 2015 and 2017 (Fig. 3b). Microbial diversity increased over this period in samples from SG1 and GG1. Consistent differences in Chao1 richness and the number of OTUs were observed. Specifically, the density distribution of the top 10 phyla shifted. In many sampling sites such as SG1, GG1, and ML, the density of the top three phyla decreased and that of the phyla ranked fourth to tenth increased markedly between 2015 and 2017 (Fig. 3b). These shifts may reflect changes in aquatic salinity gradients (Campbell and Kirchman, 2013; Kirchman et al., 2005). The T-N concentration in region D increased from 2010, exceeding 600 μg L^{-1} (Fig. 1). In region E, this concentration tended to decrease from 2005, but a peak exceeding 2000 μg L^{-1} was observed in 2015. The number of species increased between 2015 and 2017 at all freshwater sites (Fig. 3b).

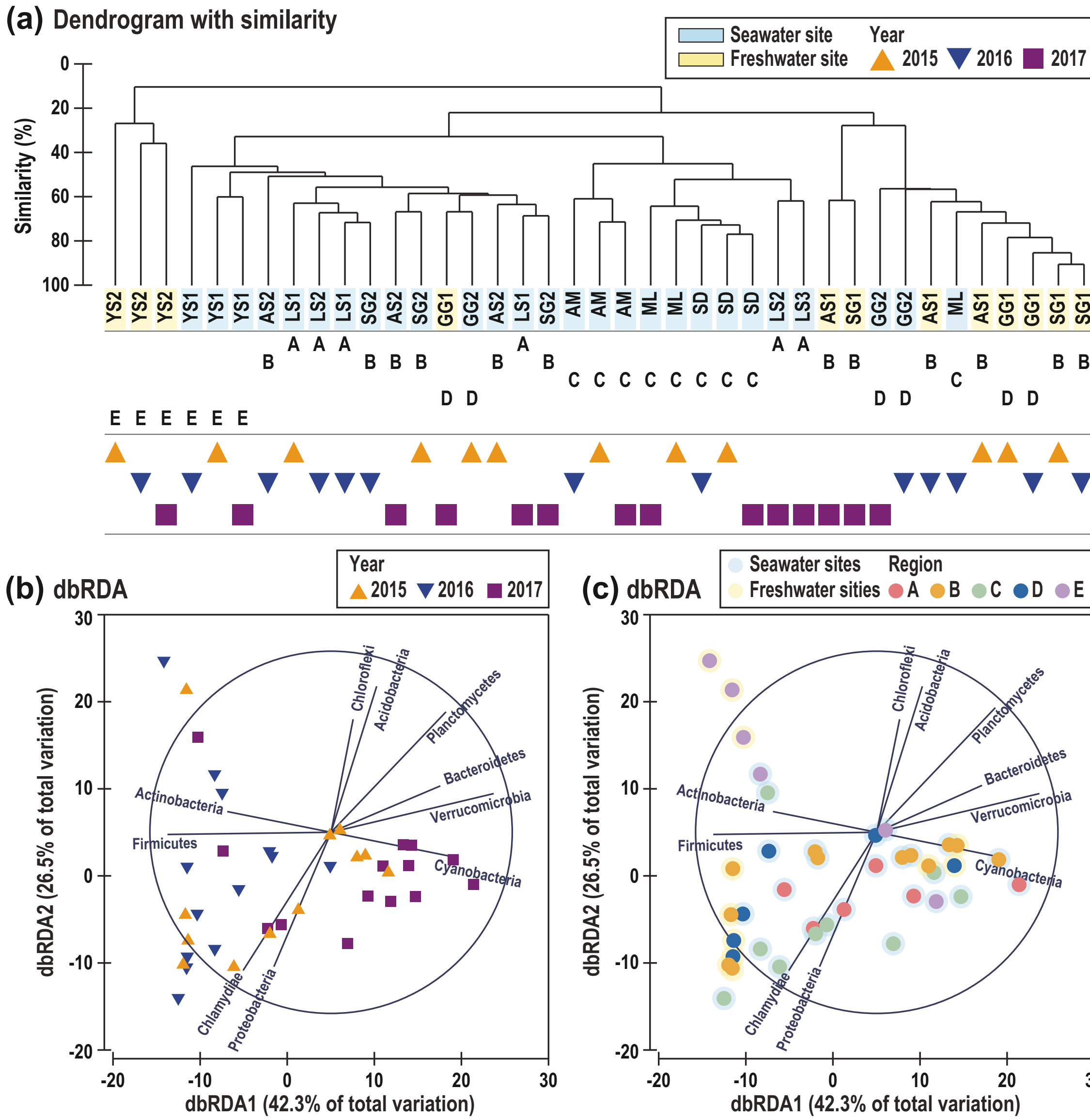


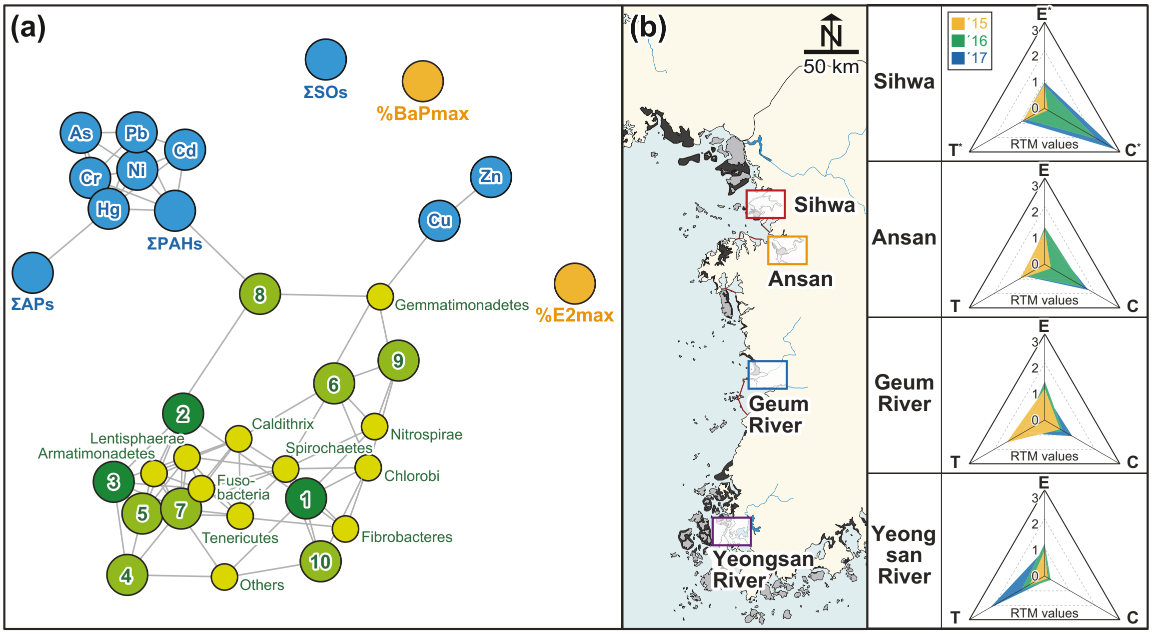
Fig. 4. Classifications of sampled sites based on environmental conditions and biotic composition. Panels: (a) dendrogram based on a Bray–Curtis similarity of bacterial 16S rDNA sequences clustered into OTUs at 97% cut-off, (b) scatter diagram of distance-based redundancy analyses (dbRDA) along the year using Bray-Curtis similarity index, and (c) plotting dbRDA along the region from 2015 to 17. Blue vectors (Spearman correlation test) point in the direction of the increased values for any given variable. Sediments with similar environmental profiles or bacterial compositions for any given variable (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

3.4. Integrated assessment

Planctomycetes and Gemmatimonadetes showed strong correlations with chemical variables (ΣPAHs, Cu, and Zn) (Fig. 5a). Cu and Zn were more closely related to Gemmatimonadetes than were other metal variables in the network analysis. In our previous study, Cd showed a stronger influence on bacterial community structure at the phylum level in 2010–14 (Yao et al., 2017; Lee et al., 2020a). The microbial structure has changed since that time, and the predominant group of bacterial phyla mostly showed correlations with ΣPAHs, Cd, and Pb among the 13 variables, possibly serving as pollution indicator taxa (Lee et al., 2020a). The decrease in the abundance of Firmicutes from 15.2% in 2015 to 1.8% in 2017 might have been caused by a decrease in the Cd concentration in same period (Kim et al., 2020).

To further understand the overall status of the benthic community health of the region and common trends in sediment quality over 3-year period, RTMVs were calculated in each LOE. Direct numerical comparisons between different LOEs could lead to bias in assessing sediment contamination. For example, the chemical contamination RTMVs seemed to overestimate the degree of pollution due to relatively low contamination levels for target compounds. For this reason, we focused on the interpretation of the change in RTMVs by year and region in the present analysis (Fig. 5b).

Comparing RTMVs calculated for samples with respect to time (2015 to 2017) and region (A to E, except C) revealed only benthic community index RTMVs decreased from 1.04 in 2015 to 0.95 in 2017. The results indicated that benthic environments were improved in ecological aspect from 2015 to 2017. However, the toxic effect RTMVs increased from 0.99 in 2015 to 1.18 in 2017. The discrepancy between benthic community and toxicology data indicated that their casual relationships might not be additive and rather complex under independent mechanisms between dose and responses. Meanwhile, during the study period, the chemical contamination (region A and B) and benthic community index RTMVs (region D) showed the greatest LOE, while toxic effect RTMVs exhibited the greatest LOE in region E (Fig. 5b). Thus, bacterial communities can be shifted in response to the



*E: benthic community structure, T: biological effect, C: chemical contamination

Fig. 5. Microbial responses with (a) network analysis presenting distances among bacterial communities and physicochemical data (ΣPAHs, ΣAPs, ΣSOs, and metals), biological effect (%BaP_{max} and %$E2_{max}$). Microbial benthic community by relative abundance for network analysis and by H′ index and Chao 1 data for integrating by use of the ratio to mean (RTM) values method to identify how conditions within the sampled sites changed from 2015 to 2017.

change of marine environments, particularly in the region being influenced by anthropogenic activities. Presumably, because the geographical features of each region would vary, it might be challenging to address an accurate relationship between triad components in the dynamic coastal environments of Korea. Overall, the present work demonstrated reaffirming the utility of "the multiple LOEs approach" with microbial community structure in sediment quality assessment.

4. Conclusions

The eDNA analysis for sediments collected from the coastal regions of South Korea generally well explained the spatiotemporal changes linked to the sediment pollution. Benthic bacterial communities showed smaller spatial variations compared to the temporal variations, particularly with a large difference between 2015 and 2017, indicated year-round change in the surrounding environment. Microbial diversity also shifted over time in 2010–14 and showed somehow different patterns compared to those in 2015–17. At the phylum level, Proteobacteria remained dominant, but region shifts were observed in the dominant phyla of Bacteroidetes, Cyanobacteria, and Firmicutes from 2015 to 2017. Differential analyses clearly demonstrated a reversal of phylum dominance over time. Microbial community structure influenced by selected environmental variables over time was also evidenced. Major shifts in Cyanobacteria and Firmicutes families, attributable in part to environmental contaminants and shifts in other microbial communities, were characteristic. Microbial network analysis indicated the (in)direct influence of 13 environmental variables. Integrated physiochemical and biological assessment enables the characterization of intricate relationships within microbial communities. Overall, benthic microbial communities provide key information about structural and functional variability in marine ecological systems under anthropogenic influence.

CRediT authorship contribution statement

Aslan Hwanhwi Lee: Conceptualization, Data curation, Visualization, Writing - original draft
Junghyun Lee: Investigation, Formal analysis, Data curation, Visualization, Writing - review
Junsung Noh: Investigation, Formal analysis, Visualization
Changkeun Lee: Investigation, Formal analysis, Visualization
Seongjin Hong: Conceptualization, Writing - review & editing
Bong-Oh Kwon: Project administration, Writing - review & editing
Jae-Jin Kim: Formal analysis, Writing - review & editing
Jong Seong Khim: Conceptualization, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111592.

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<Supplementary Materials>

Characteristics of long-term changes in microbial communities from contaminated sediments along the west coast of South Korea: Ecological assessment with eDNA and physicochemical analyses

Aslan Hwanhwi Lee[1], Junghyun Lee[1], Junsung Noh, Changkeun Lee,
Seongjin Hong, Jae-Jin Kim, Bong-Oh Kwon, Jong Seong Khim*

Supplementary tables

Supplementary figures

[1] These authors contributed equally to this work.

* **Corresponding author.** *E-mail address*: jskocean@snu.ac.kr (J.S. Khim).

Supplementary tables

Table S1. Sediment sampling site characteristics

Province	Region	Sites	Latitude	Longitude	Geographical description	Salinity
Gyeonggi	(A) Lake Shihwa	LS1	N37° 20.093’	E126° 41.370’	Coastal area, outside of sea dike	Seawater
		LS2	N37° 19.543’	E126° 39.427’	Coastal area, outside of sea dike	Seawater
		LS3	N37° 18.657’	E126° 36.618’	Inside of sea dike	Seawater
Chungnam	(B) Asan	AS1	N36° 53.600’	E126° 54.742’	Inside of sea dike	Freshwater
		AS2	N36° 54.929’	E126° 54.317’	Coastal area, outside of sea dike	Seawater
	(B) Sapgyo	SG1	N36° 52.728’	E126° 49.633’	Inside of sea dike	Freshwater
		SG2	N36° 53.704’	E126° 49.148’	Coastal area, outside of sea dike	Seawater
	(C) Taean	SD	N36° 50.312’	E126° 11.004’	Coastal area (beach; Sinduri)	Seawater
		ML	N36° 47.027’	E126° 08.185’	Coastal area (beach; Manlipo)	Seawater
		AM	N36° 32.403’	E126° 19.588’	Coastal area (beach; Anmyundo)	Seawater
Jeonbuk	(D) Geum River	GG1	N36° 02.347’	E126° 74.223’	River, inside of dam	Freshwater
		GG2	N36° 00.510’	E126° 73.537’	Coastal area, outside of dam	Seawater
Jeonnam	(E) Yeongsan River	YS1	N34° 78.930’	E126° 44.417’	Coastal area, outside of dam	Seawater
		YS2	N34° 78.198’	E126° 46.271’	River, inside of dam	Freshwater

Table S2. Average similarity (%) of sediment bacterial communities between (a) regions and (b) sampling years, determined by permutational multivariate analysis of variance

(a)

	A	**B**	**C**	**D**	**E**
A					
B	75.140				
C	74.948	74.550			
D	74.985	75.033	74.584		
E	70.024	**70.261****	**70.059***	70.659	

(b)

	2010	**2011**	**2013**	**2014**	**2015**	**2016**	**2017**
2010							
2011	76.775						
2013	**73.278****	72.898					
2014	**74.523***	73.416	76.156				
2015	**71.145****	**68.700****	**70.545****	**71.846****			
2016	**70.131****	**68.639****	**73.292****	**72.941****	83.240		
2017	**67.499****	**64.722****	**64.816****	**67.162****	**81.024****	**77.627****	

*$p < 0.05$, **$p < 0.01$.

Supplementary figures

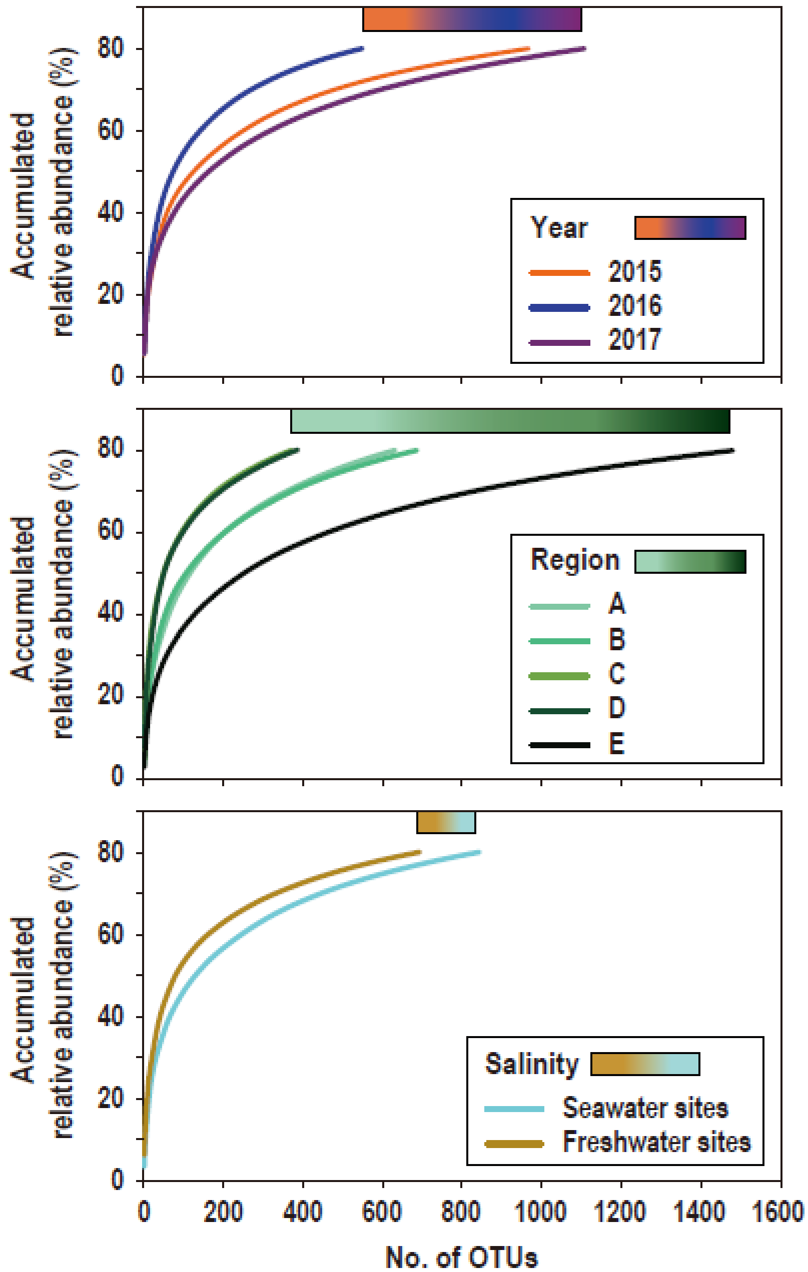

Fig. S1. Number of OTUs occupying 80% of the mean relative abundance along year, region, and salinity.

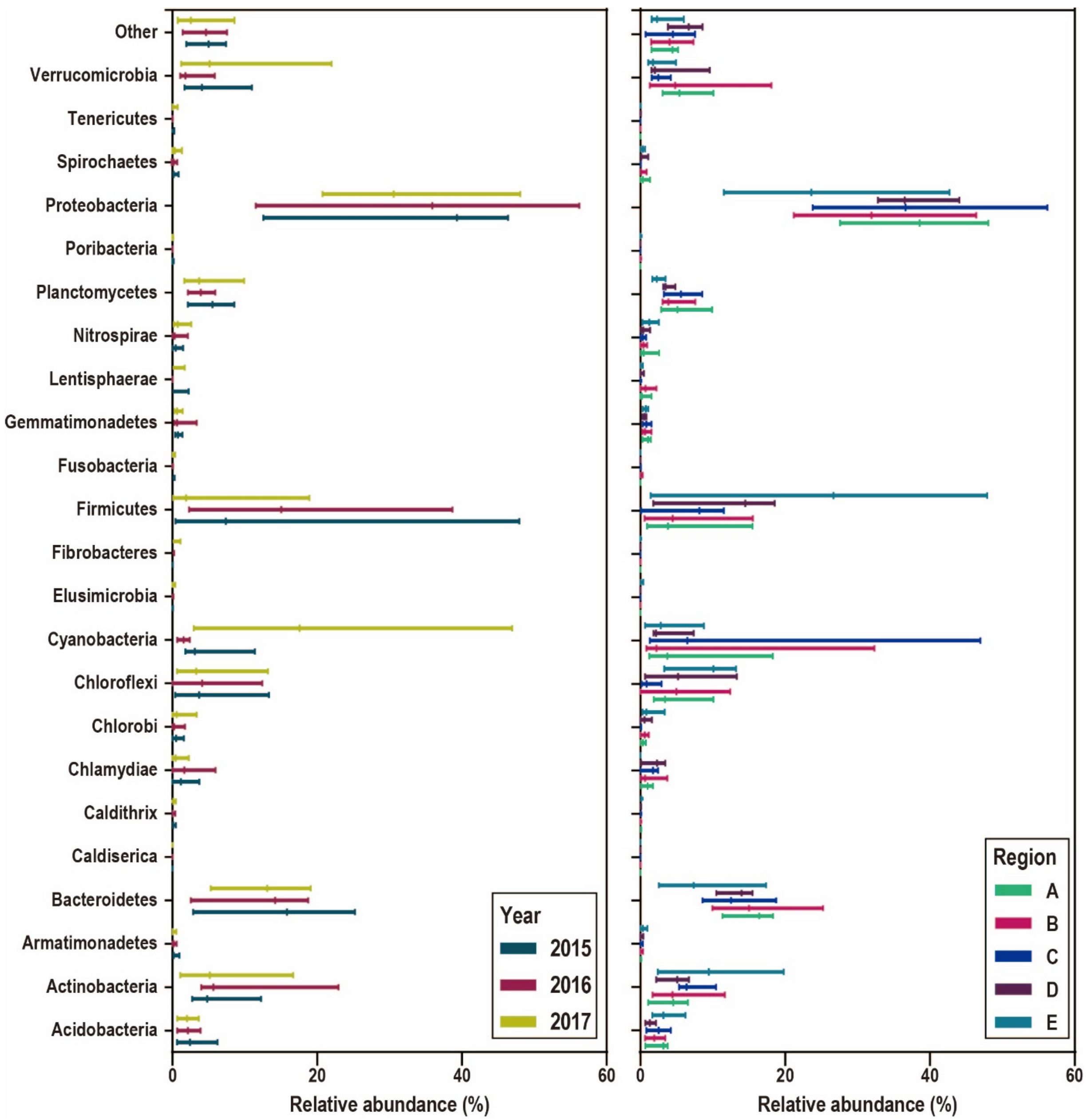

Fig. S2. Relative abundances of dominant bacterial phyla across sampling years and regions.