Environment International 137 (2020) 105569

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Integrated assessment of west coast of South Korea by use of benthic bacterial community structure as determined by eDNA, concentrations of contaminants, and in vitro bioassays

Aslan Hwanhwi Lee[a,1], Junghyun Lee[a,1], Seongjin Hong[b], Bong-Oh Kwon[a], Yuwei Xie[c,d], John P. Giesy[c,d,e,f], Xiaowei Zhang[c,*], 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] State Key Laboratory of Pollution Control & Resource Reuse, School of the Environment, Nanjing University, Nanjing 210003, China
[d] Department of Veterinary Biomedical Sciences & Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[e] Department of Environmental Sciences, Baylor University, Waco, TX, USA
[f] Department of Zoology and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA

A R T I C L E I N F O

Handling Editor: Tieyu Wang

Keywords:
eDNA
In situ microbial community
Next generation sequencing
Persistent toxic substances
In vitro bioassay
Sediment pollution

A B S T R A C T

During the past few decades, contamination of sediments by persistent toxic substances (PTSs) has been observed in estuarine and coastal areas on the west coast of South Korea. The contaminants are suspected to cause toxicities in aquatic biota, but little is known about their ecological effects, particularly on benthic microbial communities. In this study, an eDNA-based assessment was applied along with classic assessments of exposure, such as chemistry and in vitro bioassays, to evaluate condition of benthic bacterial communities subjected to PTSs. Two strategies were adopted for the study. One was to conduct a comprehensive assessment in space (by comparing seawater and freshwater sites at five coastal regions) and in time (by following change over a 5-y period). Although we found that bacterial composition varied among and within years, some phyla, such as Proteobacteria (28.7%), Actinobacteria (13.1%), Firmicutes (12.7%), and Chloroflexi (12.5%) were consistently dominated across the study regions. Certain bacterial groups, such as Firmicutes and Verrucomicrobia have been linked to contamination at some sites in the study area and at specific points in time. Bacterial communities were not significantly correlated with salinity or AhR- and ER-mediated potencies, whereas concentrations of PAHs, APs, and certain metals (Cd and Hg) exhibited significant associations to the structure of bacterial communities at the phylum level. In fact, the relative abundance of microbes in the phylum Planctomycetes was significantly and negatively correlated with concentrations of PAHs and metals. Thus, the relative abundance of Planctomycetes could be used as an indicator of sedimentary contamination by PAHs and/or metals. Based on our correlation analyses, Cd and ER-mediated potencies were associated more with bacterial abundances at the class taxonomic level than were other PTSs and metals. Overall, the eDNA-based assessment was useful by augmenting more traditional measures of exposure and responses in a sediment triad approach and has potential as a more rapid screening tool.

1. Introduction

The west coast of South Korea, along with nearby rivers/estuaries, is part of the Yellow Sea region, a region that has become highly urbanized and industrialized (Jeon et al., 2017). Various types of anthropogenic contaminants from agricultural, industrial, and domestic activities have been discharged into coastal sediment, which is the final sink for those contaminants (Khim and Hong, 2014). Based on results of previous toxicological studies, concentrations of persistent toxic substances (PTSs) in sediments off the west coast of South Korea, were deemed to pose risks to biota (Hong et al., 2012; Jeon et al., 2017; Khim et al., 1999). PTSs, such as polycyclic aromatic hydrocarbons (PAHs),

---

[*] Corresponding authors.

E-mail addresses: aslan.lee@snu.ac.kr (A.H. Lee), jhl092688@snu.ac.kr (J. Lee), hongseongjin@cnu.ac.kr (S. Hong), bongkwon@snu.ac.kr (B.-O. Kwon), yuwei.xie@usask.ca (Y. Xie), jgiesy@aol.com (J.P. Giesy), zhangxw@nju.edu.cn (X. Zhang), jskocean@snu.ac.kr (J.S. Khim).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envint.2020.105569
Received 14 October 2019; Received in revised form 24 January 2020; Accepted 9 February 2020

alkylphenols (APs), metals, and metalloids, accumulating in coastal sediments can adversely affect aquatic and/or benthic ecosystems at all levels of biological organization from molecules to communities (Fent, 2001).

To evaluate of quality of sediments contaminated by PTSs, conventional sediment assessments use chemical analyses combined with determination of toxicological effects. However, this approach focuses on a limited suite of ecotoxicological effects and often lacks ecological relevance. In addition, the conventional approach cannot capture effects on populations and communities in natural ecosystems (de Castro-Català et al., 2016). Therefore, to comprehensively evaluate sediment quality, a triad approach was applied, wherein physicochemical data, such as salinity, pH, and concentrations of contaminants, toxicological data, and benthic community structure data are concurrently examined (Lee et al., 2018). However, few studies have addressed ecological association between concentrations of PTSs and benthic communities (Lee et al., 2018; Yoon et al., 2017).

Within benthic communities, microbial communities play an important role in biogeochemical cycling (Fischer and Pusch, 2001), decomposition of contaminants, and in providing other functions necessary for sustaining aquatic ecosystems (Reed and Martiny, 2013; Xie et al., 2017). Microbial communities, including bacteria, are extremely sensitive to changes in physicochemical conditions, such as temperature, pH, salinity, and concentrations of contaminants (Gibbons et al., 2014; Herlemann et al., 2011; Xie et al., 2016, 2017). Bacteria respond rapidly to changing environmental conditions and adapt their degree of activity, diversity, or community structure (Xie et al., 2016, 2017). This means that the composition of in situ bacterial communities can potentially be used to monitor key elements of sediment quality. However, despite their potential importance in indicating sediment quality, characteristics of in situ bacterial communities have been neglected in conventional sediment quality assessment approaches (Torsvik et al., 2002).

More than 99% of microorganisms that have been observed in nature cannot generally be cultivated or phenotypically identified with standard culture techniques. However, with environmental DNA (eDNA) metabarcoding, most microbial communities can be identified (Amann et al., 1995). Thus, approaches that use metagenomic level analysis to characterize the complexity of microbial ecosystems in sediments could provide a rapid and efficient way to identify and monitor benthic microbial communities and enumerate individual taxa (Gibbons et al., 2014; Sharmin et al., 2013). Improvements in methods for metabarcoding eDNA, in concert with advances in bioinformatics analysis, could provide a promising approach for improving ecological risk assessments (Zhang, 2019).

From the 1970s to the early 1990s, many sea dikes (estuary dams) and landfill projects occurred along the west coast of South Korea (Fig. 1). As a result, most major rivers and estuaries along the west coast have been disconnected from the open sea, leading to different salinity regimes on the two sides of the dams. Salinity is thought to be the major factor affecting bacterial community composition (Campbell and Kirchman, 2013; Wu et al., 2006), more important than the influence of temperature or pH (Lozupone and Knight, 2007). Several studies on shifts in bacterial community composition along aquatic salinity gradients have substantiated the influence of salinity on bacterial composition (Campbell and Kirchman, 2013; Kan et al., 2008; Kirchman et al., 2005). However, most of these gradient studies were conducted in one region and/or over a short duration. To bridge the multiple environmental variables including salinity and chemical contaminants, in vitro toxicological tests, and bacterial communities in the assessment of sediment quality, a comprehensive five-year field study on the west coast of South Korea had been conducted. We investigated effects of salinity and concentrations of toxic chemicals on bacterial composition in sediments. The toxic chemicals included dioxin-like and estrogenic chemical pollutants, measured as aryl hydrocarbon receptor (AhR)-mediated potencies and estrogen receptor (ER)-mediated potencies, because those endpoints represent a large spectrum of dioxin-like and estrogenic chemicals that might exist in contaminated sediments (Hong et al., 2016; Lee et al., 2017).

Specific objectives of the present study were to: (1) characterize spatio-temporal distributions of benthic bacterial communities in sediments along the west coast of South Korea; (2) compare in situ bacterial communities and endpoints of chemical analyses and in vitro bioassays; and (3) identify bacterial taxa indicative of specific environmental variables.

2. Materials and methods

2.1. Study area and sediment collection

Surface sediments were collected during May, annually between 2010 and 2014 from 15 sites (11 seawater sites and 4 freshwater sites) in five coastal regions from rivers, estuaries, and open sea along the west coast of South Korea. The five regions include Lakes Sihwa (Region A), Asan & Sapgyo (Region B), as well as the Taean Coast (Region C), and estuaries of the Geum River (Region D) and Yeongsan River (Region E) (Fig. 1 and Table S1).

The inside of the estuary dams or sea dikes (i.e., landward) is composed of freshwater except for Lake Sihwa, which has a tidal power plant that has allowed tides to pass through since its construction in 2011 (Fig. 1 and Table S1). In contrast, seaward sides of dikes are saline. Sediments were immediately transported, at 4 °C, to the laboratory and stored at –20 °C until analyses. Samples were freeze-dried and ground with a mortar and pestle prior to analyses.

2.2. Next generation sequencing and bioinformatics analyses

Total DNA was extracted from 0.25 g aliquots of each homogenized surface sediment by use of a Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA). Detailed descriptions for amplifying bacterial 16s rRNA genes (V3 fragment) have been previously published (Xie et al., 2016). Triplicate PCR reactions were performed for each sample to minimize potential PCR bias. Products of PCR were checked, purified and quantified. All purified products of PCR were pooled equally for subsequent sequencing. Sequencing adapters were linked to purified DNA fragments with the ION proton sequencer (Life Technologies, CA, USA) following the manufacturer's instructions.

Low quality raw reads (mean quality score < 20, scanning window = 50) and sequences which contained ambiguous 'N' and were shorter than 100 bp but longer than 180 bp in length were discarded by using the Quantitative Insights into Microbial Ecology toolkit (Caporaso et al., 2010). Chimeras were removed and clustered operational taxonomic units (OTUs) with a similarity cutoff of 97% following the UPARSE pipeline method (Edgar, 2013). Taxonomy of bacterial OTUs was assigned to representative sequences by use of the Ribosomal Database Project classifier against the Greengenes database (DeSantis et al., 2006; Wang et al., 2007). Observed OTUs were rarefied at equal sequencing depth to reduce biases resulting from differences in sequencing depth. Alpha-diversity (Shannon indices) was calculated on all twenty equal-depth rarefactions and then averaged them.

2.3. Analyses of chemicals and toxicological tests

Concentrations of 15 PAHs and six APs and in vitro ecotoxicological assays [H4IIE-*luc* bioassay for determining AhR-mediated potencies (presented as $\%BaP_{max}$) and MVLN bioassay for determining ER-mediated potencies (presented as $\%E2_{max}$)] were obtained for sediments collected from the same locations during 2010 to 2014, Jeon et al. (2017). Concentrations of eight metals (Cd, Cr, Cu, Hg, Li, Ni, Pb, and Zn) and one metalloid (As) were also reported for the sediments in a previous study by Kim (2020). Metals and metalloids were expressed in sum of hazard quotients (ΣHQ_{metal}) by Ryu et al. (2016). All prior

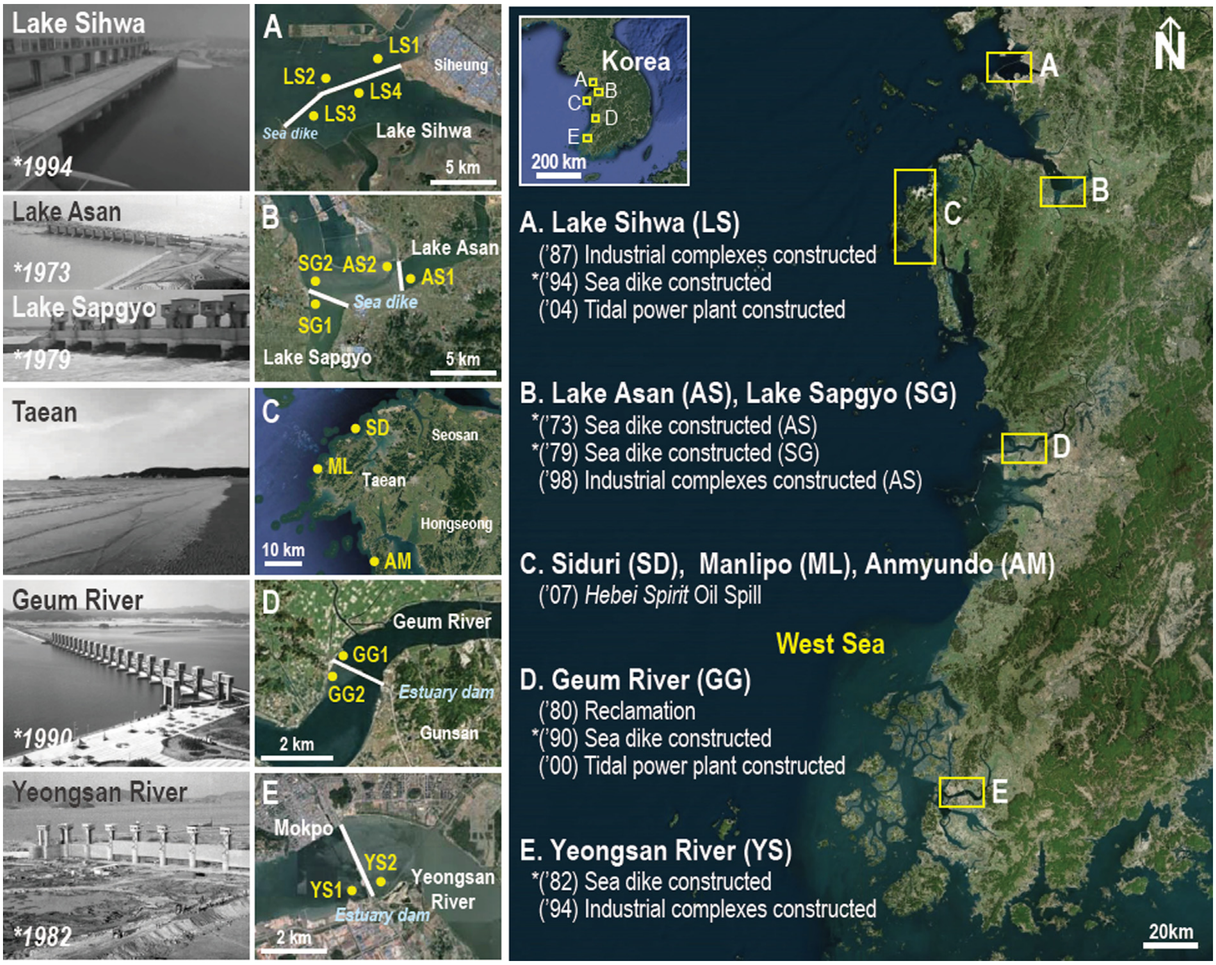

Fig. 1. Pictures and satellite images of sampled sites from five regions of the west coast of South Korea and brief summary of activities related to sampling each region. Panels: (A) Lake Sihwa (LS), (B) Lakes Sapgyo (SG) and Asan (AS), (C) Taean coast, including Sinduri (SD), Manlipo (ML), Anmyundo (AM), (D) Geum River (GG) Estuary, and (E) Youngsan River (YS) Estuary.

chemical measurements and toxicological results are summarized in Fig. S1 of Supplementary Materials.

2.4. Statistics analyses

SPSS 23.0 (SPSS INC., Chicago, IL) and PRIMER 6 statistical software (PRIMER-E Ltd, Plymouth, UK) with the PERMANOVA + add-on package (Clarke and Gorley, 2006; Lozupone and Knight, 2005) were used to perform statistical analyses. We set statistical significance at $p < 0.05$. The bacterial community with phyla data was reduced by eliminating species that contributed < 1% of total abundance. Abundance was log-transformed [ln (x + 1)] and normalized to balance it across the recorded taxa in the measure of similarity (Clarke and Warwick, 2001). The nonparametric Mann-Whitney U (M–W) and Kruskal-Wallis (K-W) with Bonferroni correction were used to detect significant differential features between the salinity, sampled regions, and years. Subsequently, to perform categorize of bacterial assemblage composition, cluster analysis (CA) was performed using a Bray–Curtis similarity matrix (Legendre and Legendre, 2012).

All environmental variables including ΣPAHs, ΣAPs, metals and metalloid (including ΣHQs), and toxicological results were log-transformed and normalized following procedures by Xie et al. (2017). Discriminating chemical and toxicological results by sampled year was confirmed by canonical analysis of principal coordinates (CAP) with Euclidean distance matrix. Next, principal coordinate analysis (PCoA) applied to the distance matrix to enable comparisons of biota among sedimentary environments. This allowed assessment of potential interactions between environmental variables and compositions of bacterial communities, at the phylum taxonomic level, based on the Bray-Curtis similarity matrix and Spearman's rank correlation coefficients (Lozupone and Knight, 2005). PCoA is useful for reducing and representing patterns present in distance matrices by displaying dissimilarities among objects (Gower, 1966). Finally, to identify specific bacterial taxa that may have responded to the various variables measured, Spearman's rank correlation coefficients were developed for associations between results of chemical and toxicological data and the abundance of bacteria with phylum/class data.

3. Results and discussion

3.1. Description of next generation sequencing data

A total of 4,634,661 bacterial 16s, V3 sequences were obtained for sediments along the west coast of South Korea. Sequences with low quality, short lengths, PCR bias, lack of annotated references or lineage filtering were discarded (Bragg et al., 2013). Due to low sequencing depth, samples collected in 2012 were discarded. After our quality check of raw reads, there remained a total of 2,464,770 reads for further analyses. From those remaining filtered raw reads, the number of 5,001 bacterial OTUs which represented 49 phyla, were discovered. According to rarefaction curves for bacterial communities, most of the abundant bacterial OTUs were saturated from more than 80,000 sequences per sample (Fig. S2).

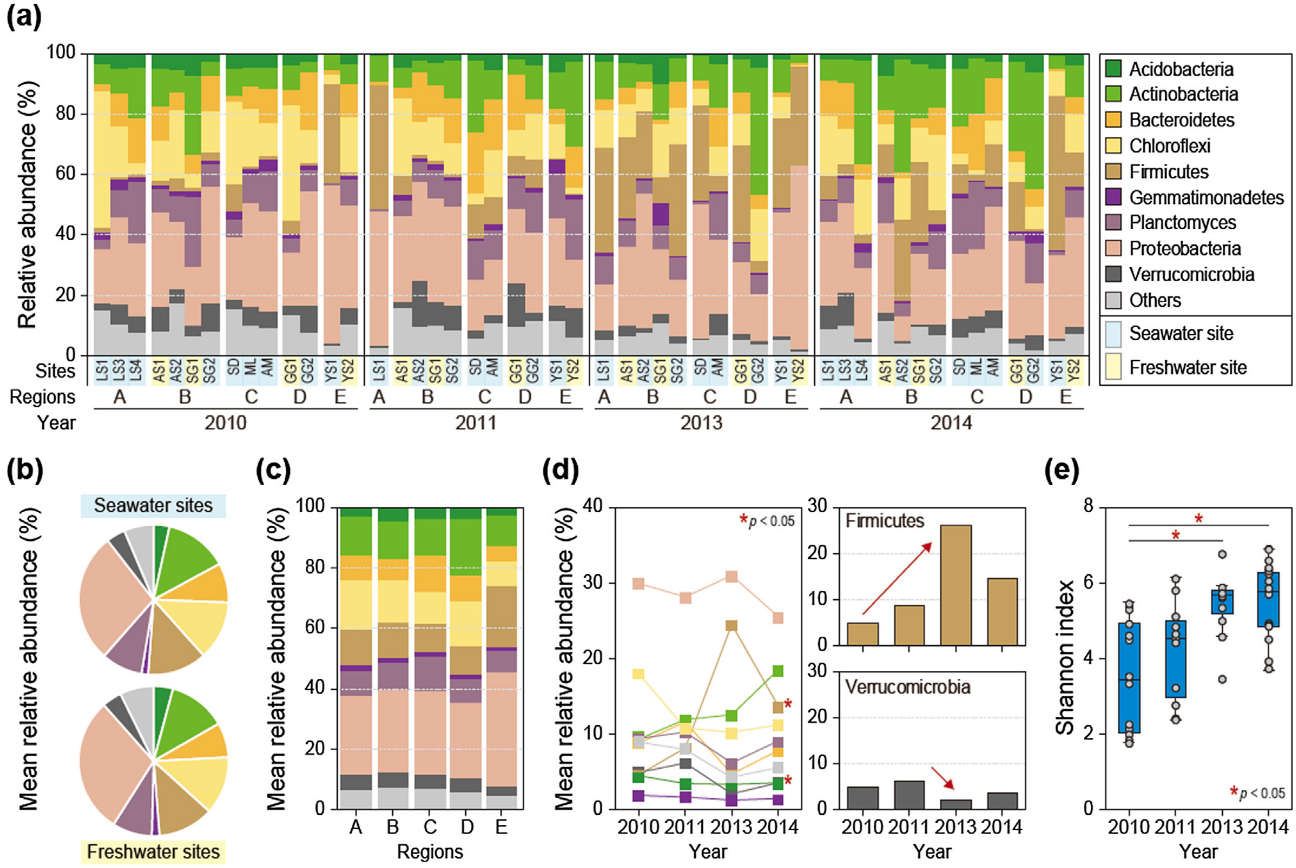

Fig. 2. The structures of the bacterial communities at phylum taxonomic level in sediments along the west coast of South Korea. Panels: (a) relative abundance of the dominant bacteria at the phylum level across all samples, statistical comparisons for mean relative abundances at the phylum taxonomic level between (b) salinity, (c) sampling region, and (d) sampling years, and (e) alpha-diversity estimated with Shannon indices of all sediment samples. Low (< 1%) abundant phyla and unresolved taxa were indicated as "Others" in panel (a). Mann-Whitney *U* test for panel (b) and Kruskal-Wallis test for panels (c–e) were obtained with a Bonferroni corrections. Significance was determined at *p* values < 0.05 (*).

3.2. Spatiotemporal distributions of bacterial communities with phylum taxonomic level

At the level of phyla, compositions of bacterial communities varied among sediments and were dominated by Proteobacteria (mean 28.7%), Actinobacteria (13.1%), Firmicutes (12.7%), Chloroflexi (12.5%), Planctomycetes (8.7%), Bacteroidetes (8.2%), Verrucomicrobia (4.2%), Acidobacteria (3.7%), and Gemmatimonadetes (1.5%) (Fig. 2a). As expected, Proteobacteria were the most abundant bacterial phylum from all sediments collected from along the west coast of South Korea between 2010 and 2014. A similar pattern of compositions for bacterial communities in sediments has been observed by several previous studies conducted around the world (Xie et al., 2016; King et al., 2015; Gibbons et al., 2014). These dominant phyla are free-living and also include a number of nitrogen-fixing bacteria (Sharmin et al., 2013; Li et al., 2009).

Relationships between mean, relative abundance salinity, sampled region, and year were observed (Fig. 2b–e). Although salinity and/or spatial variability are known to be major contributors to microbial community structure and function (Xie et al., 2017; Campbell and Kirchman, 2013; Lozupone and Knight, 2007), in this study mean compositions of bacteria, as determined at the level of phyla, exhibited no statistically significant, correlation with salinity (M–W test) or sampled region (K-W test) (Bonferroni-corrected $p < 0.05$) (Fig. 2b and c). This result, which was unexpected, might be due to the fact that salinity gradients are sometimes temporarily diluted in response to an input of freshwater from temporally irregular discharges of freshwater through the sea dikes in our study regions (Noh et al., 2019). Therefore, a better understanding of patterns in spatial proximity and shared environment characteristics will require intensive sediment sampling from along transects that reflect a longer gradient in physicochemical parameters, such as salinity, pH, and contaminant composition.

Among the dominant phyla, Firmicutes and Verrucomicrobia showed significant differences in mean relative abundance across the sampling years (K-W test, Bonferroni-corrected $p < 0.05$) (Fig. 2d). In 2010, Firmicutes and Verrucomicrobia were distributed at similar level, but Firmicutes abundance gradually increased over time, whereas Verrucomicrobia abundance declined over time, with the Firmicutes eventually becoming 20 times more abundant than Verrucomicrobia by 2013. Firmicutes is quite rare phylum in natural sediments, but is dominant taxa in sugar cane processing sites (Sharmin et al., 2013). However, none of the sites we sampled were near sugar cane processing areas. Verrucomicrobia was related to Planctomycetes and was known to exist in majority of which are eutrophic or even heavily polluted (Spring et al., 2016), and were particularly abundant occurrence in marine environments (Cardman et al., 2014). Several reports of Verrucomicrobia occurring in extreme environments, such as in sulfide-rich water and sediments, have been published (Freitas et al., 2012; Spring et al., 2016).

Mean values of Shannon indices from all sites bacterial communities were significantly greater in 2013 and 2014 than in the 2010 (K-W test, Bonferroni-corrected $p < 0.05$) (Fig. 2e). Our results indicate that although only one bacterial community showed a significant increase in diversity over time at the phylum taxonomic level, population change could become even more significant if lower level taxa are examined. Hierarchical clustering of our surface sediment samples also showed a

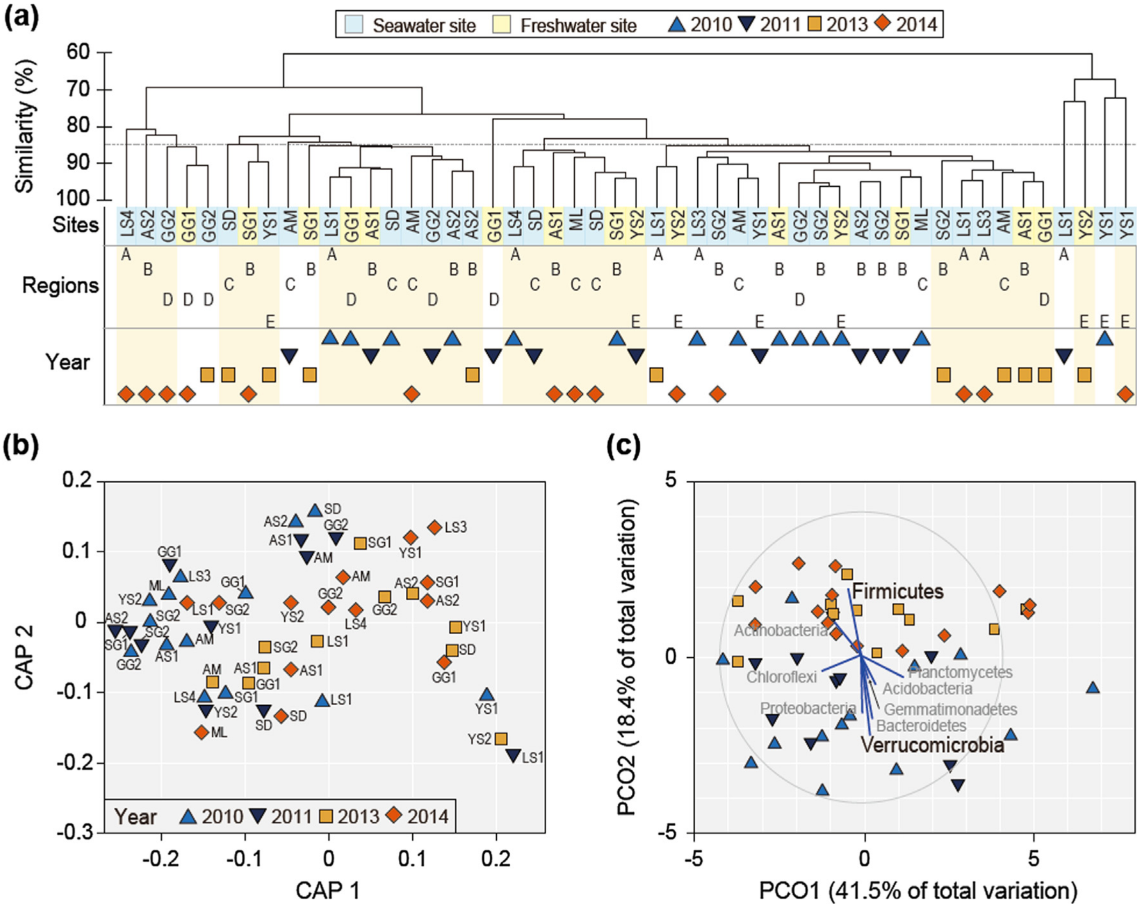

Fig. 3. Classifications of sampled sites based on environmental conditions and biotic composition. Panels: (a) bacterial composition along the west coast of South Korea based on a Bray–Curtis dendrogram of 16S rDNA sequences clustered into OTUs at 97% similarity, followed by orange colored background bars indicating the designated group, (b) scatter diagram of canonical analysis of principal coordinates (CAP) with Euclidean distance matrix, including results of 15 PAHs, 6 APs, and 9 metals and metalloid, and AhR- and ER-mediated potencies grouped by year, (c) Principal coordinates analysis (PCoA) ordinations (first two principal coordinates are displayed) based on Bray-Curtis dissimilarity, showing similarity in community composition between samples. Data are Phylum taxonomic level and have been log-transformed [ln(x + 1)]. 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 are located near each other on the diagram.

separation of bacterial communities by sampling years (2010–11 and 2013–14). High bootstrap values indicated changing environmental influences on bacterial community over time (Fig. 3a). Changes in patterns of diversity could have any number of explanations. However, assuming that sediment contamination favors pollution-tolerant species over non-tolerant species, then complex communities will respond to chemical contamination in a magnitude-dependent effect with respect to toxic pollutants, so that over time microbial communities will increase in diversity as pollution increases (Gillan et al., 2005).

The scatter diagram of CAP of environmental variables (including chemical contaminants and toxicological parameters) show a pattern similar to that observed for bacterial communities (Fig. 3b). Our CAP analysis could partition effects of 'sampling year' for the various environmental variables. We determined temporal trends (which increased, decreased, or did not change) of PTSs and potential toxicity in sediments along the west coast of South Korea by examining linear correlations between concentrations (or toxicities) and sampling years (Fig. S1) (Jeon et al., 2017; Kim et al., 2020). From 2010 to 2014, temporal distributions of PAHs and APs declined from over time, whereas concentrations of metals Cd, Cr, and Hg increased. According to a previous study, PAHs can contribute to reductions in freshwater and marine biotic diversity (Malaj et al., 2014); thus, variations in chemical concentrations might indicate variations in the composition of bacterial communities. In addition, the adverse effects on biota of high concentrations of some metals are due to the metals' abilities to block and inactivate sulfhydryl groups of proteins (Valls and De Lorenzo, 2002). In this study, we found that AhR-mediated potency in sediments declined over time, whereas ER-mediated potency showed a slightly increase from 2010 to 2014 (Jeon et al., 2017). Overall, the concentrations of metals (Cd, Cr, and Hg) and ER-mediated potencies were relatively higher during the 2013–14 period than in the 2010–11 period (Kim et al., 2020).

Despite the spatial differences, the temporal variability of contaminants and toxicological results showed the dominant role contaminants have in shaping the structures of bacterial communities in sediments on the west coast of South Korea (Fig. 3). When we plotted integrated results from chemical, toxicological, and microbiological data with PCoA (Fig. 3c), we found that 59.9% of the variability in the composition of the bacterial assemblages in sediments could be explained by the first two principal component axes (PCoA1 and PCoA2) (Fig. 3c). Bacterial phyla were divided into two groups in the distribution by the PCoA diagram (year 2010–11 and year 2013–14). Distributions of Firmicutes were correlated with profiles of contaminants/toxicological measured during 2013–14, whereas Verrucomicrobia were greatly correlated with 2010–11 samples, which corresponds to the above results. Observed correlations cannot be constructed to indicate causation of either the effects of factors or of dissimilarities between individual sample points, but they do provide insight into where additional work can be focused to determine causation (Clarke and Gorley, 2006).

Due to heterogeneity of estuarine and coastal environments, sediment characteristics and rate of discharge of the river, which correlated most strongly with bacterial communities (Crump and Hobbie, 2005), can vary slightly from year to year, even if the same sites are monitored in the same season each year. However, according to previous study, TOC and TN did not show a large difference over the years (Kim et al., 2020), the effect of changes of PTSs is more effective than that from the differences in sediment properties in terms of spatiotemporal heterogeneity of samples. Due to the inherent heterogeneity of sediments in estuaries and coastal environments, sediment characteristics and river-flow rates, which correlated most strongly with bacterial communities compositions (Crump and Hobbie, 2005), can vary slightly from year to year, even at the same in the same season each year. However, according to a recent study by Kim et al. (2020), the values of TOC and TN do not show significant differences among years and changes over time in coastal sediments are due more to differences in spatiotemporal heterogeneity of PTSs than to differences in the heterogeneity of sediment properties.

3.3. *Temporal variation in the bacterial communities at the class & family taxonomic levels*

Analysis of data presented here at the class and family taxonomic levels provided deeper understanding of factors related to the composition of the dominant fraction of bacterial communities in sediments. For all sampled years, the relative abundances of the top five classes at each site were presented in Fig. 4a. Although the four most frequently occurring classes were Gammaproteobacteria, followed by Anaerolineae, Alphaproteobacteria, and Deltaproteobacteria, the highest relative abundances for all sampled years were Bacilli (24.1%), followed by Gammaproteobacteria (17.5%), Acidimicrobiia (13.6%), and Anaerolineae (13.3%) (Fig. 4a). This result is consistent with several previous studies that characterized microbial communities in sediments (Hullar et al., 2006; Crump and Hobbie, 2005). Although the relative abundance of Gammaproteobacteia was highest in 2013, we found no significant difference among sampled years and steadily dominant. However, at the family taxonomic level, *OM60*, *Halomonadaceae*, *Piscirickettsiaceae*, and *Thiotrichaceae* were significantly different relative to abundance among sampled years. Among these four families, only Halomonadaceae abundance increased over time, while *OM60*, *Piscirickettsiaceae*, and *Thiotrichaceae* decreased over time. A study by Feris et al. (2003) suggested that the presence of Gammaproteobacteria in sediments is positively associated with metal concentrations, whereas Gaboyer et al. (2014) suggested that *Halomonadaceae* may be tolerant to metals such as Cd, Cr, Cu, and Ag. Among the environmental factors, the concentrations of metals (Cd, Cr, and Hg) increased in sediments over time, especially Cd (significantly increased) and *Halomonadaceae*, which is tolerant of Cd have become more abundant.

Classes Bacilli and Clostridia in the Phylum Firmicutes exhibited significantly different abundances by year (Fig. 3b). Within the Phylum Verrucomicrobia, the dominant classes were Verrucomicrobiae and Verruco-5, followed by Spartobacteria and Pedosphaerae (Fig. 3b). Opitutae and Methylacidiphilae were present, but occurred at relatively lower abundances than the other classes (Fig. 3b). According to

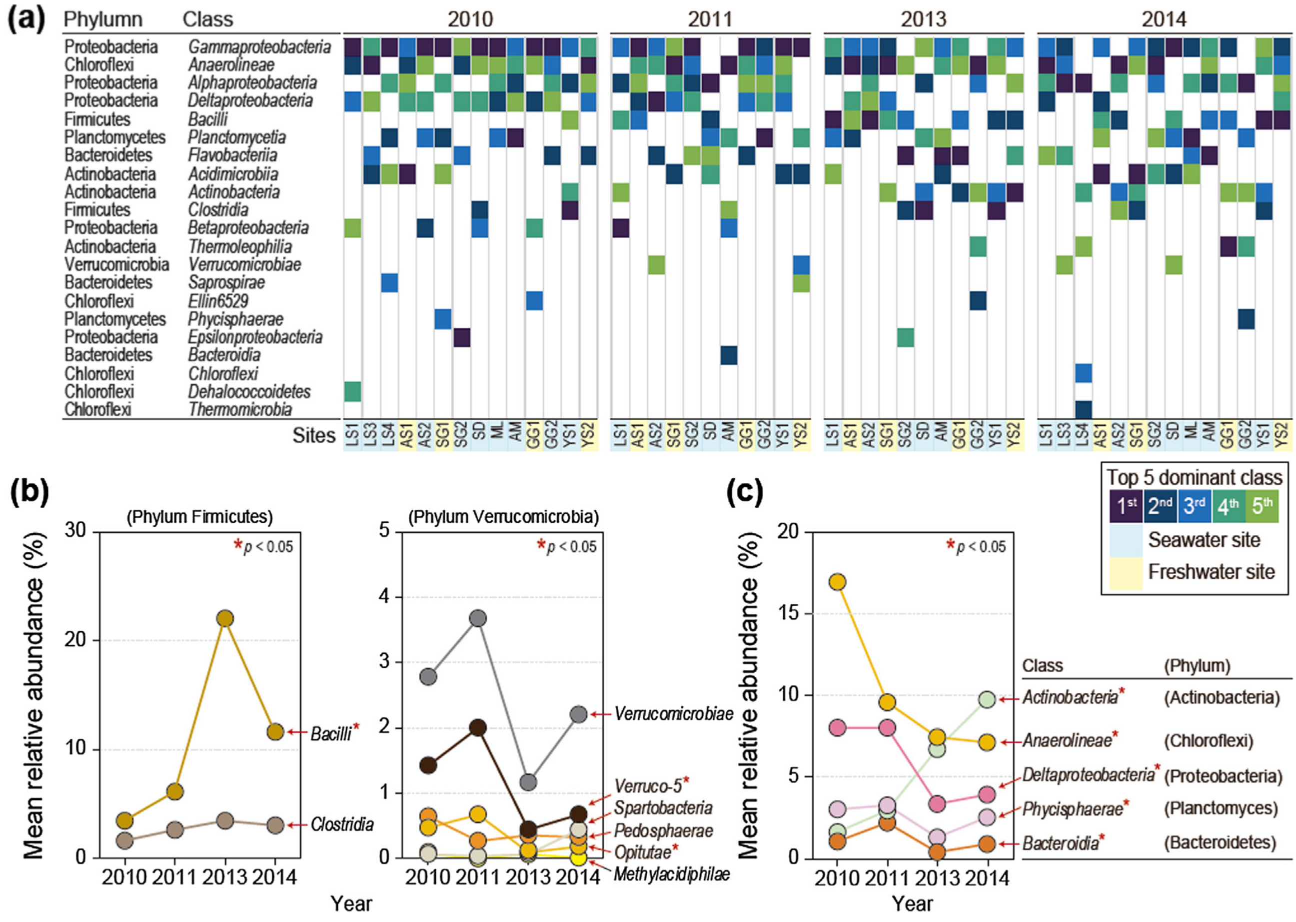

Fig. 4. Structures of bacterial communities (at class taxonomic level) in sediments along the west coast of South Korea. Panels: (a) the five most abundant bacteria at each site were marked for all sampled years, (b) mean relative abundance of bacteria (at the class taxonomic level) that showed differences at phylum taxonomic level, (c) mean relative abundance of bacteria (at the class taxonomic level) that showed no difference at the phylum taxonomic level. The Kruskal-Wallis test, followed by a Bonferroni correction, was performed on data in by panels (b)–(c). Significance was determined at p values < 0.05 (*).

previous studies and consistent with our results, Opitutae is significantly more frequent in the water column, whereas Verrucomicrobiae is more common in marine sediments and to some extent in lakes (Freitas et al., 2012; Arnds et al., 2010; Allgaier and Grossart, 2006). Among the classes of bacteria that exhibited a significant difference in abundance by year, the relative abundances of Bacilli and Actinobacteria showed relatively increasing pattern over time, while abundances of Anaerolineae, Deltaproteobacteria, Phycisphaera, Verruco-5, and Bacterodia showed relatively decreasing pattern over time (Fig. 4b and c).

Desulfobacteraceae and *Syntrophobacteraceae*, which include Deltaproteobacteia, were significantly different in abundance between year 2010 and 2014 ($p < 0.05$). The relative abundance of these two bacterial communities is shown to decrease, which was consistent with the temporal pattern in PAHs concentrations. In previous studies, *Deltaproteobacteia* has been mostly observed in sites highly contaminated by PAHs and has been shown to be crucial in the anaerobic degradation of organic contaminants and the cycling of sulfur compounds (Quero et al., 2015; Sun et al., 2013). Overall, we found that four families of bacteria (*Anaerolinaceae*, *Desulfobacteraceae*, *Piscirickettsiaceae*, and *Spirochaetaceae*) were more concentrated in sediments during the 2010–11 period that in the 2013–14 period (Table S2). Anaerolinaceae, *Desulfobacteraceae*, and *Piscirickettsiaceae* have been found to be positively associated with residual oils in sediments, which indicates relatively great concentrations of PAHs being associated (Xie et al., 2018). Furthermore, presence of *Anaerolinaceae* and *Desulfobacteraceae* indicates the biodegradation of petroleum hydrocarbons (Xie et al., 2018). In the presence of PTSs, these two bacterial families might metabolize PAHs, APs, and metals, resulting in the possible removal of these chemical species from sediments (Chariton et al., 2010; Genderjahn et al., 2018; Lee et al., 2019; Zhang et al., 2017).

3.4. Correlation and response between microbial communities and environmental variables

Significant Spearman's rank correlation between the relative abundance of bacterial individual taxa and the concentration/value of environmental variables facilitated our detection of potential bioindicators of environmental contaminants. Among the measured environmental variables, each bacterial abundance based on phyla were more significantly related to concentrations of ΣPAHs, ΣAPs, and metals (i.e., Cd and Hg) than were AhR- and ER-mediated potencies (Fig. 5a). The concentrations of ΣPAHs negatively correlated with the abundance of Planctomycetes. Abundance of Firmicutes was positively correlated with concentrations of Cd, whereas abundances of Planctomycetes and Verrucomicrobia were negatively correlated with concentrations of Cd. In addition, abundances of Chloroflexi were positively correlated with concentrations of Hg, but negatively correlated with abundances of Planctomycetes. Overall, we speculate that metals have greater influences on shaping structures of microbial communities in sediments than do other variables measured in this study. Responses to changes in concentrations of metals can reduce abundances of less metal-tolerant species in sediments and thus allow other, more-tolerant species to dominate, which in turn reduces biodiversity. In particular, adverse effects on biota of high concentrations of some metals are due to their abilities to block and inactivate sulfhydryl groups of proteins (Valls and De Lorenzo, 2002). Otherwise, non-linear correlations showed between abundant phyla Actinobacteria and Proteobacteria and environmental variables. Because these two phyla were the most abundant in the sediments, the variance at lower bacterial levels are offset, making it difficult to determine relationships between bacterial abundances and environmental variables.

The effect of Cd on bacteria was statistically discernible at both the phylum and class taxonomic levels, whereas concentrations of APs and %E2$_{max}$, which indicate the presence of estrogenic compounds, were only discernible at the phylum and class taxonomic levels, respectively. The abundances of various bacterial taxa were associated with ER-mediated potencies, whereas associations were not observed relative to AhR-mediated potencies. These results are consistent with those of previous studies (Xie et al., 2017). The phyla Acidobacteria and Planctomycetes both showed a positive correlation with ΣPAHs concentrations, whereas at the class taxonomic level, Holophagae (Phylum Acidobacteria) and Brocadiae (Phylum Planctomycetes) exhibited a negative association with ΣPAHs concentrations. Like metals, ΣPAHs can shape the structure of bacterial communities by increasing the relative abundance of Holophagae in a bacterial community. Thus, the relative abundances of Holophagae and Brocadiae could be used as potential indicators of the contaminations of PAHs in sediments.

Patterns of response to contaminants varied among bacterial classes. Bacilli with their ability to reduce soluble and amorphous ferric iron and other oxidized metal species, were resistant to Cd. However, relationships between abundances of bacteria in communities and concentrations of contaminants observed in this study might have been influenced by confounding factors, such as water depth, redox potential, pH, organic carbon contents, and grain sizes of sediments, but such effects can be difficult to differentiate from the toxic effects of the

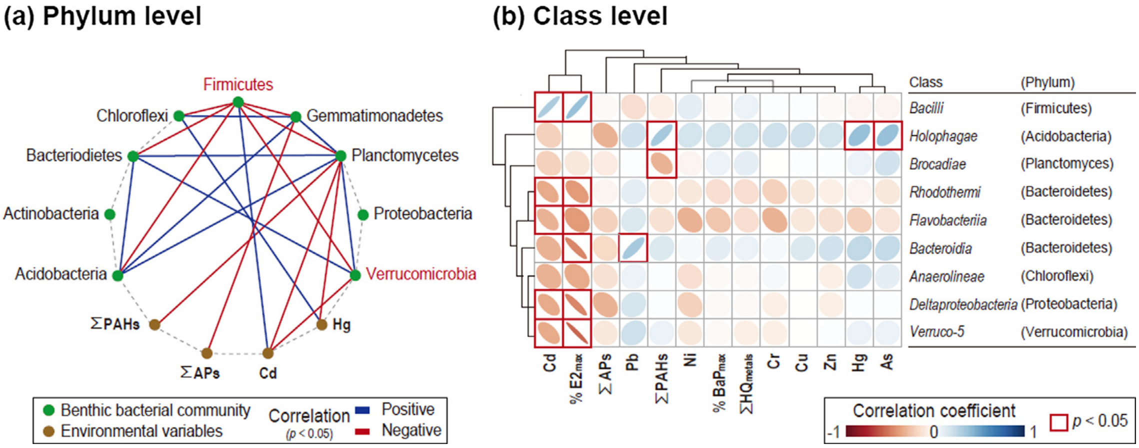

Fig. 5. Correlations and classifications of bacterial assemblages in sediments along the west coast of South Korea at phyla and class taxonomic levels relative to tested environmental variables. Panels: (a) correlation results between environmental variables and bacterial communities of phyla taxonomic levels within the depicted phyla (two phyla that were significantly different among years are depicted in red), (b) pairwise comparisons of environmental variables with a color gradient denoting Spearman's rank correlation coefficients with bacterial community structure based on taxonomic classes. The network of panel (a) was filtered to include only a 'two-tailed' p-value < 0.05.

contaminants (Olson et al., 1990). Further research is needed to investigate other chemicals that might cause changes in structure of microbiota communities in sediments. However, our results highlight the limitations of conventional chemical measurements and the value of the global molecular ecological indicators for monitoring sediment quality.

4. Conclusions

The present study characterized the diversity and structure of bacterial communities and assessed their associations with environmental variables in sediment samples collected from freshwater and seawater sites over five years on the west coast of South Korea. At the phylum taxonomic level, bacterial communities of freshwater sediments did not significantly differ from communities measured in seawater sediments. The structures of bacterial assemblages in sediments appear to be influenced by anthropogenic contaminants and toxicological endpoints. Cd had a stronger influence in shaping the structure of bacterial communities at the phylum taxonomic level, whereas ER-mediated potencies had stronger influence at the class taxonomic level. Thus, to use a bacterial assemblage to assess sediment contamination, one should choose the taxonomic rank at which to analyze the assemblage- (e.g., phylum or class) based on the contaminant of interest. However, there are other environmental variables that we did not measure (e.g., nutrients, grain size, etc.) could also affect the composition of benthic bacterial communities. Because our analysis reveals insights in that how to identify potential contaminants (biotic stressors) from a community perspective, future studies should employ other diagnostic approaches to confirm the identity of stressors that are causing chronic toxicity in sediment biota. Alternatively, mesocosm studies could be conducted to identify the contaminants responsible for influencing biota. Overall, we show that the characterization of in situ bacterial communities can be useful for monitoring and assessing sediment quality in an integrated manner.

CRediT authorship contribution statement

Aslan Hwanhwi Lee: Conceptualization, Investigation, Formal analysis, Visualization, Writing - original draft. **Junghyun Lee:** Conceptualization, Investigation, Formal analysis, Visualization, Writing - review & editing. **Seongjin Hong:** Conceptualization, Formal analysis, Resources, Writing - review & editing. **Bong-Oh Kwon:** Investigation, Project administration, Resources. **Yuwei Xie:** Formal analysis, Writing - review & editing. **John P. Giesy:** Writing - review & editing. **Xiaowei Zhang:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision. **Jong Seong Khim:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision.

Acknowledgments

This work was supported by National Research Foundation of Korea (NRF) grants funded by the South Korean government [grant number NRF-2017R1E1A1A01075067], and also was supported by the project entitled "Marine Ecosystem-Based Analysis and Decision-Making Support System Development for Marine Spatial Planning [grant number 20170325]", funded by the Ministry of Oceans and Fisheries of Korea (MOF), South Korea granted to J.S.K. The research published in this paper is part of the project titled "Next generation solutions to ensure healthy water resources for future generations" funded by the Global Water Futures program, Canada First Research Excellence Fund. Additional information is available at www.globalwaterfutures.ca. Prof. Giesy was supported by the Canada Research Chairs Program of the Natural Science and Engineering Research Council (NSERC) of Canada and a Distinguished, Visiting Professorship from Baylor University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105569.

References

Allgaier, M., Grossart, H.-P., 2006. Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. Aquat. Microb. Ecol. 45, 115–128.

Amann, R.I., Ludwig, W., Schleifer, K.-H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Mol. Biol. Rev. 59, 143–169.

Arnds, J., Knittel, K., Buck, U., Winkel, M., Amann, R., 2010. Development of a 16S rRNA-targeted probe set for Verrucomicrobia and its application for fluorescence in situ hybridization in a humic lake. Syst. Appl. Microbiol. 33, 139–148.

Bragg, L.M., Stone, G., Butler, M.K., Hugenholtz, P., Tyson, G.W., 2013. Shining a light on dark sequencing: characterising errors in Ion Torrent PGM data. PLoS Comp. Biol. 9, e1003031.

Campbell, B.J., Kirchman, D.L., 2013. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. The ISME J. 7, 210–220.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A., Teske, A., 2014. Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of Svalbard. Appl. Environ. Microbiol. 80, 3749–3756.

Chariton, A.A., Court, L.N., Hartley, D.M., Colloff, M.J., Hardy, C.M., 2010. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Front. Ecol. Environ. 8, 233–238.

Clarke, K., Gorley, R., 2006. Primer. PRIMER-e, Plymouth.

Clarke, K., Warwick, R., 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Mar. Ecol. Prog. Ser. 216, 265–278.

Crump, B.C., Hobbie, J.E., 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol. Oceanogr. 50, 1718–1729.

de Castro-Català, N., Kuzmanovic, M., Roig, N., Sierra, J., Ginebreda, A., Barceló, D., Pérez, S., Petrovic, M., Picó, Y., Schuhmacher, M., Muñoz, I., 2016. Ecotoxicity of sediments in rivers: invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools. Sci. Total Environ. 540, 297–306.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.

Fent, K., 2001. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. Toxicol. In Vitro 15, 477–488.

Feris, K., Ramsey, P., Frazar, C., Moore, J.N., Gannon, J.E., Holben, W.E., 2003. Differences in hyporheic-zone microbial community structure along a heavy-metal contamination gradient. Appl. Environ. Microbiol. 69, 5563–5573.

Fischer, H., Pusch, M., 2001. Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. Freshwat. Biol. 46, 1335–1348.

Freitas, S., Hatosy, S., Fuhrman, J.A., Huse, S.M., Mark Welch, D.B., Sogin, M.L., Martiny, A.C., 2012. Global distribution and diversity of marine Verrucomicrobia. ISME J. 6, 1499–1505.

Gaboyer, F., Vandenabeele-Trambouze, O., Cao, J., Ciobanu, M.-C., Jebbar, M., Le Romancer, M., Alain, K., 2014. Physiological features of Halomonas lionensis sp. nov., a novel bacterium isolated from a Mediterranean Sea sediment. Res. Microbiol. 165, 490–500.

Genderjahn, S., Alawi, M., Mangelsdorf, K., Horn, F., Wagner, D., 2018. Desiccation-and saline-tolerant bacteria and archaea in Kalahari pan sediments. Front. Microbiol. 9, 2082.

Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M.L., Gilbert, J.A., 2014. Human and environmental impacts on river sediment microbial communities. PloS one 9, e97435.

Gillan, D.C., Danis, B., Pernet, P., Joly, G., Dubois, P., 2005. Structure of sediment-associated microbial communities along a heavy-metal contamination gradient in the marine environment. Appl. Environ. Microbiol. 71, 679–690.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53, 325–338.

Herlemann, D.P., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579.

Hong, S., Khim, J.S., Naile, J.E., Park, J., Kwon, B.-O., Wang, T., Lu, Y., Shim, W.J., Jones, P.D., Giesy, J.P., 2012. AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: a comparison between Korea and China. Environ. Pollut. 171, 216–225.

Hong, S., Yim, U.H., Ha, S.Y., Shim, W.J., Jeon, S., Lee, S., Kim, C., Choi, K., Jung, J., Giesy, J.P., 2016. Bioaccessibility of AhR-active PAHs in sediments contaminated by the Hebei Spirit oil spill: application of Tenax extraction in effect-directed analysis.

Chemosphere 144, 706–712.

Hullar, M.A.J., Kaplan, L.A., Stahl, D.A., 2006. Recurring seasonal dynamics of microbial communities in stream habitats. Appl. Environ. Microbiol. 72, 713.

Jeon, S., Hong, S., Kwon, B.-O., Park, J., Song, S.J., Giesy, J.P., Khim, J.S., 2017. Assessment of potential biological activities and distributions of endocrine-disrupting chemicals in sediments of the west coast of South Korea. Chemosphere 168, 441–449.

Kan, J., Evans, S.E., Chen, F., Suzuki, M.T., 2008. Novel estuarine bacterioplankton in rRNA operon libraries from the Chesapeake Bay. Aquat. Microb. Ecol. 51, 55–66.

Khim, J.S., Hong, S., 2014. Assessment of trace pollutants in Korean coastal sediments using the triad approach: a review. Sci. Total Environ. 470, 1450–1462.

Khim, J.S., Kannan, K., Villeneuve, D.L., Koh, C.H., Giesy, J.P., 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1 Instrumental analysis. Environ. Sci. Technol. 33, 4199–4205.

Kim, S., Hong, S., Lee, J., Kim, T., Yoon, S.J., Lee, J., Choi, K., Kwon, B.O., Giesy, J.P., Khim, J.S., 2020. Long-term trends of persistent toxic substances and potential toxicities in sediments along the west coast of South Korea. Mar. Pollut. Bull. 151, 110821.

King, G., Kostka, J., Hazen, T., Sobecky, P., 2015. Microbial responses to the Deepwater Horizon oil spill: from coastal wetlands to the deep sea. Annu. Rev. Mar. Sci. 7, 377–401.

Kirchman, D.L., Dittel, A.I., Malmstrom, R.R., Cottrell, M.T., 2005. Biogeography of major bacterial groups in the Delaware Estuary. Limnol. Oceanogr. 50, 1697–1706.

Lee, J., Hong, S., Kwon, B.-O., Cha, S.A., Jeong, H.-D., Chang, W.K., Ryu, J., Giesy, J.P., Khim, J.S., 2018. Integrated assessment of persistent toxic substances in sediments from Masan Bay, South Korea: comparison between 1998 and 2014. Environ. Pollut. 238, 317–325.

Lee, J., Hong, S., Yoon, S.J., Kwon, B.-O., Ryu, J., Giesy, J.P., Allam, A.A., Al-khedhairy, A.A., Khim, J.S., 2017. Long-term changes in distributions of dioxin-like and estrogenic compounds in sediments of Lake Sihwa, Korea: revisited mass balance. Chemosphere 181, 767–777.

Lee, J., Kim, T., Yoon, S.J., Kim, S., Lee, A.H., Kwon, B.-O., Allam, A.A., Al-khedhairy, A.A., Lee, H., Kim, J.-J., Hong, S., Khim, J.S., 2019. Multiple evaluation of the potential toxic effects of sediments and biota collected from an oil-polluted area around Abu Ali Island, Saudi Arabia, Arabian Gulf. Ecotoxicol. Environ. Saf. 183, 109547.

Legendre, P., Legendre, L.F., 2012. Numerical Ecology. Elsevier.

Li, H., Yu, Y., Luo, W., Zeng, Y., Chen, B., 2009. Bacterial diversity in surface sediments from the Pacific Arctic Ocean. Extremophiles 13, 233–246.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Lozupone, C.A., Knight, R., 2007. Global patterns in bacterial diversity. Proc. Natl. Acad. Sci. 104, 11436–11440.

Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. 111, 9549–9554.

Noh, J., Yoon, S.J., Kim, H., Lee, C., Kwon, B.-O., Lee, Y., Hong, S., Kim, J., Ryu, J., Khim, J.S., 2019. Anthropogenic influences on benthic food web dynamics by interrupted freshwater discharge in a closed Geum River estuary, Korea. Environ. Int. 131, 104981.

Olson, R.J., Chisholm, S.W., Zettler, E.R., Armbrust, E.V., 1990. Pigments, size, and distributions of Synechococcus in the North Atlantic and Pacific Oceans. Limnol. Oceanogr. 35, 45–58.

Quero, G.M., Cassin, D., Botter, M., Perini, L., Luna, G.M., 2015. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Front. Microbiol. 6, 1053.

Reed, H.E., Martiny, J.B.H., 2013. Microbial composition affects the functioning of estuarine sediments. ISME J. 7, 868–879.

Ryu, J., Hong, S., Chang, W.K., Khim, J.S., 2016. Performance evaluation and validation of ecological indices toward site-specific application for varying benthic conditions in Korean coasts. Sci. Total Environ. 541, 1161–1171.

Sharmin, F., Wakelin, S., Huygens, F., Hargreaves, M., 2013. Firmicutes dominate the bacterial taxa within sugar-cane processing plants. Sci. Rep. 3, 3107.

Spring, S., Bunk, B., Spröer, C., Schumann, P., Rohde, M., Tindall, B.J., Klenk, H.-P., 2016. Characterization of the first cultured representative of Verrucomicrobia subdivision 5 indicates the proposal of a novel phylum. ISME J. 10, 2801–2816.

Sun, M.Y., Dafforn, K.A., Johnston, E.L., Brown, M.V., 2013. Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. Environ. Microbiol. 15, 2517–2531.

Torsvik, V., Øvreås, L., Thingstad, T.F., 2002. Prokaryotic diversity–magnitude, dynamics, and controlling factors. Science 296, 1064–1066.

Valls, M., De Lorenzo, V., 2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol. Rev. 26, 327–338.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Wu, Q.L., Zwart, G., Schauer, M., Kamst-van Agterveld, M.P., Hahn, M.W., 2006. Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. Appl. Environ. Microbiol. 72, 5478–5485.

Xie, Y., Wang, J., Wu, Y., Ren, C., Song, C., Yang, J., Yu, H., Giesy, J.P., Zhang, X., 2016. Using in situ bacterial communities to monitor contaminants in river sediments. Environ. Pollut. 212, 348–357.

Xie, Y., Wang, J., Yang, J., Giesy, J.P., Yu, H., Zhang, X., 2017. Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. Chemosphere 172, 201–209.

Xie, Y., Zhang, X., Yang, J., Kim, S., Hong, S., Giesy, J.P., Yim, U.H., Shim, W.J., Yu, H., Khim, J.S., 2018. eDNA-based bioassessment of coastal sediments impacted by an oil spill. Environ. Pollut. 238, 739–748.

Yoon, S.J., Hong, S., Kwon, B.-O., Ryu, J., Lee, C.-H., Nam, J., Khim, J.S., 2017. Distributions of persistent organic contaminants in sediments and their potential impact on macrobenthic faunal community of the Geum River Estuary and Saemangeum Coast, Korea. Chemosphere 173, 216–226.

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., Liu, M., Li, D., Li, Y.-F., Zhang, D., 2017. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. Sci. Rep. 7, 12165.

Zhang, X., 2019. Environmental DNA shaping a new era of ecotoxicological research. Environ. Sci. Technol. 53, 5605–5612.

<Supplementary Materials>

Integrated assessment of west coast of South Korea by use of benthic bacterial community structure as determined by eDNA, concentrations of contaminants, and in vitro bioassay

Aslan Hwanhwi Lee [1], Junghyun Lee [1], Seongjin Hong, Bong-Oh Kwon, Yuwei Xie,
John P. Giesy, Xiaowei Zhang [*], and Jong Seong Khim [*]

Supplementary Tables

Supplementary Figures

---

[1] These authors contributed equally to this work.

[*] **Corresponding authors**.

E-mail addresses: zhangxw@nju.edu.cn (X. Zhang); jskocean@snu.ac.kr (J.S. Khim).

Supplementary Tables

Table S1. Latitude/Longitude and region information for each sampling site.

Province	**Region**	**Sites**	**Latitude**	**Longitude**	**Year**					**Remark**	**Salinity on May**
					2010	**2011**	**2012**	**2013**	**2014**	**Geographical description**	
Gyeonggi	Lake Shihwa (A)	LS1	N37° 20.093’	E126° 41.370’	√	√	√	√	√	Coastal area, outside of sea dike	30.9 ± 1.8
		LS2	N37° 19.543’	E126° 39.427’			√			Coastal area, outside of sea dike	32.0 ± 2.0
		LS3	N37° 18.657’	E126° 36.618’	√				√	Inside of sea dike	32.4 ± 2.2
		LS4	N37° 19.494’	E126° 39.340’	√				√	Inside of sea dike	31.9 ±2.5
Chungnam	Asan (B)	AS1	N36° 53.600’	E126° 54.742’	√		√		√	Inside of sea dike	0.5 ± 0.1
		AS2	N36° 54.929’	E126° 54.317’	√	√	√	√	√	Coastal area, outside of sea dike	29.0 ± 4.3
	Sapgyo (B)	SG1	N36° 52.728’	E126° 49.633’	√	√	√	√	√	Inside of sea dike	0.5 ± 0.0
		SG2	N36° 53.704’	E126° 49.148’	√	√	√	√	√	Coastal area, outside of sea dike	29.1 ± 1.7
	Taean (C)	SD	N36° 50.312’	E126° 11.004’	√	√	√	√	√	Coastal area (beach; Sinduri)	32.3 ± 0.1
		ML	N36° 47.027’	E126° 08.185’	√		√		√	Coastal area (beach; Manlipo)	32.0 ± 0.3
		AM	N36° 32.403’	E126° 19.588’	√	√	√	√	√	Coastal area (beach; Anmyundo)	32.4 ± 0.7
Jeonbuk	Geum River (D)	GG1	N36° 01.347’	E126° 44.532’	√	√	√	√	√	River, inside of dam	0.2 ± 0.0
		GG2	N36° 00.510’	E126° 44.117’	√	√	√	√	√	Coastal area, outside of dam	29.8 ± 4.1
Jeonnam	Yeongsan River (E)	YS1	N36° 46.930’	E126° 26.648’	√	√	√	√	√	Coastal area, outside of dam	27.9 ± 2.3
		YS2	N36° 47.198’	E126° 27.767’	√	√	√	√	√	River, inside of dam	0.6 ± 0.3

Table S2. The mean relative abundances of the bacterial communities at family level which showed significantly difference by sampling years in sediments along the west coast of South Korea.

				Relative abundance (%)				Bonferroni-corrected *p* value					
Phylum	**Class**	**Order**	**Family**	**2010**	**2011**	**2013**	**2014**	**10-11**	**10-13**	**10-14**	**11-13**	**11-14**	**13-14**
Actinobacteria	Actinobacteria	Actinomycetales	*Corynebacteriaceae*	0.08	0.58	0.28	0.20		0.00	0.02	0.02	0.05	
			Frankiaceae	0.07	0.08	0.14	0.50						
			Geodermatophilaceae	0.02	0.02	0.06	0.46		0.03				
			Microbacteriaceae	0.12	0.09	0.48	0.84				0.02	0.01	
			Micrococcaceae	0.03	0.08	0.91	0.24		0.01		0.01		
			Micromonosporaceae	0.06	0.07	0.23	1.59					0.04	
			Mycobacteriaceae	0.36	0.82	1.05	0.80				0.02		
			Nocardioidaceae	0.05	0.10	0.30	0.22						
			Sporichthyaceae	0.01	0.03	0.04	0.62						
			Streptomycetaceae	0.03	0.04	0.19	0.22	0.02	0.00	0.01	0.01		
			Streptosporangiaceae	0.00	0.00	0.03	0.55			0.01		0.00	
Chloroflexi	Anaerolineae	Anaerolineales	*Anaerolinaceae*	1.03	0.68	0.44	0.30			0.03			
Firmicutes	Bacilli	Bacillales	*Alicyclobacillaceae*	0.04	0.03	0.10	0.43			0.05		0.04	
			Bacillaceae	0.83	1.41	3.95	4.62		0.01	0.00	0.04	0.01	
			Exiguobacteraceae	0.02	0.03	1.02	0.07		0.00	0.01	0.00		
			Paenibacillaceae	0.05	0.02	0.14	0.55			0.05		0.01	
			Planococcaceae	0.95	1.79	7.53	2.68		0.00	0.02	0.00		
		Lactobacillales	*Carnobacteriaceae*	0.01	0.02	2.95	0.09		0.00		0.00		
Planctomycetes	Planctomycetia	Gemmatales	*Gemmataceae*	0.06	0.15	0.37	0.22		0.05		0.04		
			Isosphaeraceae	0.03	0.08	0.12	0.55			0.02			
Proteobacteria	Alphaproteobacteria	Rhizobiales	*Methylobacteriaceae*	0.03	0.13	0.15	0.34		0.02	0.00			
	Deltaproteobacteria	Desulfobacterales	*Desulfobacteraceae*	2.11	1.74	0.59	0.84			0.04			
		Syntrophobacterales	*Syntrophobacteraceae*	1.04	0.70	0.31	0.31			0.03			
	Gammaproteobacteria	Alteromonadales	*OM60*	0.69	0.54	0.17	0.16			0.01			
		Oceanospirillales	*Halomonadaceae*	3.88	4.66	15.88	8.67		0.01		0.02		
		Thiotrichales	*Piscirickettsiaceae*	2.27	2.38	0.62	0.86			0.05			
			Thiotrichaceae	0.41	0.08	0.12	0.07			0.03			
Spirochaetes	Spirochaetes	Spirochaetales	*Spirochaetaceae*	0.71	0.62	0.16	0.26		0.05	0.04			

Supplementary Figures

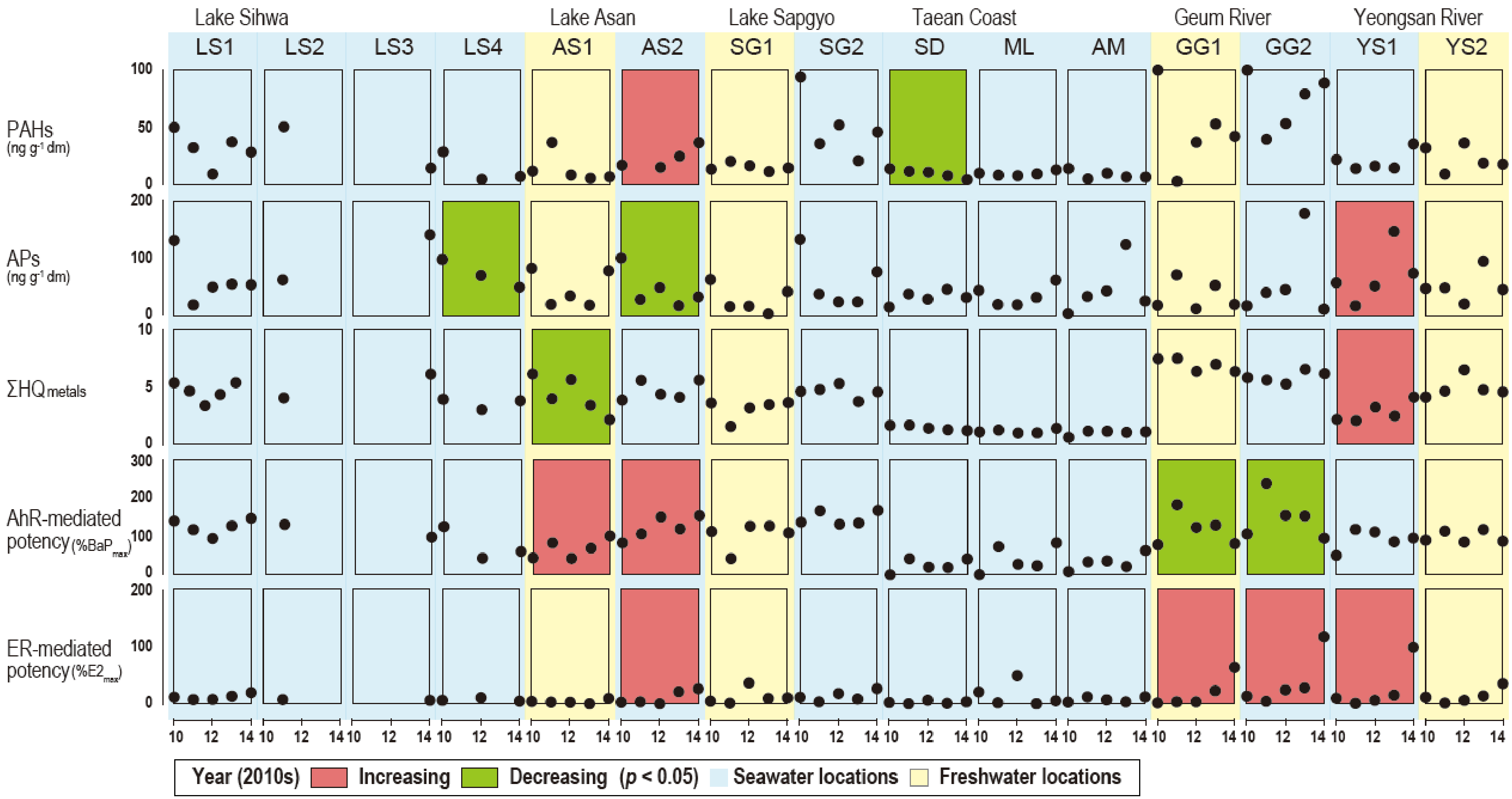

Fig. S1. Spatial and temporal distributions of persistent toxic substances (PTSs) and potential toxicities in sediments along the west coast of South Korea from 2010 to 2014. PTSs include organic pollutants, metals, and the metalloid As. Potential toxicities include AhR- and ER-mediated potencies.

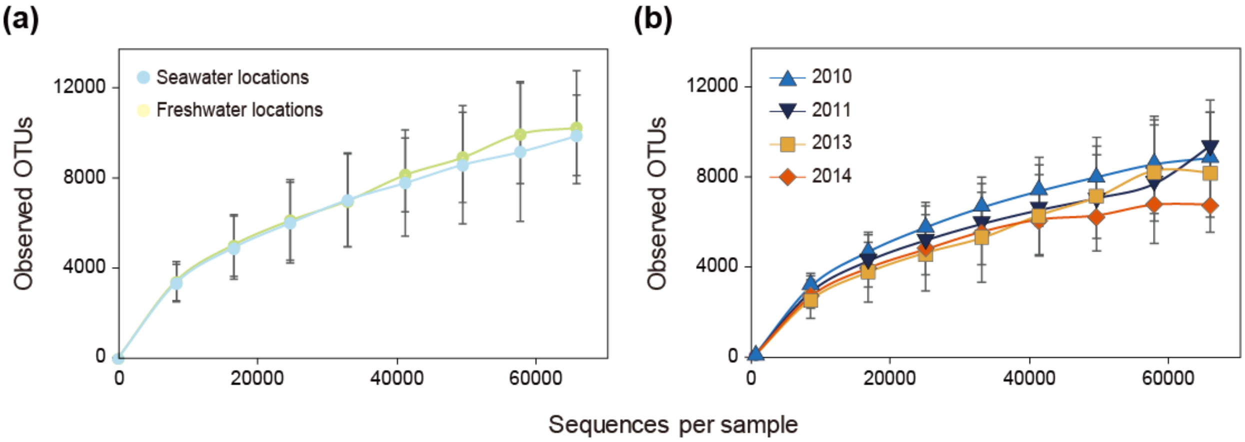

Fig. S2. Rarefaction curves of the observed OTU numbers of bacterial communities (a) by salinity and (b) sampling years in sediments.