African Journal of Biotechnology Vol. 11(9), pp. 2256-2266, 31 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.568
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Spatial and vertical distribution of bacteria in the Pearl River estuary sediment

Fu-Lin Sun[1,2], You-Shao Wang[1,2]*, Mei-LinWu[1], Yu-Tu Wang[1,2] and Qian P. Li[3]

[1]State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
[2]Marine Biology Research Station at Daya Bay, Chinese Academy of Sciences, Shenzhen 518121, China.
[3]Scripps Institution of Oceanography, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.

Accepted 25 May, 2011

In order to investigate the spatial and vertical change of bacteria community structure in the Pearl River estuary sediment, denaturing gradient gel electrophoresis (DGGE) and multivariate statistical analyses were carried out in this study. Results of multidimensional scaling analyses (MDS) were in good agreement with the DGGE band patterns suggesting that vertical depth had a significant impact on sediment bacterial community structure except sample A2. Canonical correspondence analysis (CCA) was also conducted to infer the relationship between environmental variables and bacterial community structure. Bacterial phylotypes in different stations were closely related uniquely to the overlying water environment. Salinity, pH, ammonium, phosphate and silicate were considered to be the key factors driving the changes in bacterial community composition. The neighbour-joining analysis divided bacteria sequences into eight groups, Proteobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, Firmicutes, Planctomycetales and Cyanobacteria. Sequencing analysis results suggested that Proteobacteria and Bacteroidetes were the dominant bacterial groups in the four sediment samples.

Key words: Bacterial community structure, the Pearl River estuary, denaturing gradient gel electrophoresis (DGGE), multivariate statistical analysis.

INTRODUCTION

Particles from runoff and suspended sediments are transported, stored, and modified in estuaries, a buffer zone where freshwater meets saltwater (Jay and Musiak, 1994; Arzayus and Canuel, 2005). Estuary ecosystems often include different habitat types and are subject to physical and meteorological influences. Microbial communities play important roles on the rates of a number of processes including particulate carbon flux, nutrient regeneration and biochemical cycling in estuaries (Binnerup et al., 1992; Sinsabaugh and Findlay, 1995; Pinckney et al., 2001; Yokokawa and Nagata, 2010). On the other hand, coastal microbial communities can change rapidly in response to the change of environmental variables such as nutrients (Hollander and Smith, 2001; Pimenov et al., 2010).

In past decade, distributional patterns of bacterial communities in coastal environments have been well documented (Crump et al., 1999; Scala and Kerkhof, 2000; King et al., 2000; Pernthaler et al., 2002; Bernhard et al., 2005; Freitag et al., 2006). However, studies for understanding the mechanisms of environmental influence on diversity and biogeography of bacterial communities in coastal ecosystems are not equally progressed. Apparently, new field studies are needed to focus on understanding the relationship between microorganisms and the change of ambient environmental properties (Boström et al., 1988; Rauch and Denis, 2008; Hollander and Smith, 2001; Pimenov et al., 2010). It is generally known that microorganisms are important for cycling of nutrients in sediments and overlying water (Clavero et al.,

*Corresponding author. E-mail: yswang@scsio.ac.cn. Tel/Fax: 8620-89023102(O).

Abbreviations: DGGE, Denaturing gradient gel electrophresis; **MDS,** multidimensional scaling; **CCA,** canonical correspondence analysis.

1999; Spears et al., 2007). Since both microbial communities and their living environments are very diverse, statistical analyses of complex linkages between them is thus essential for understanding the field observations. Statistical approaches commonly used to assess the interaction of microorganisms with environmental factors are multidimensional scaling analysis (MDS) and canonical correspondence analysis (CCA). Application of these multivariate statistical techniques in coastal sediment studies suggested that the activities and structures of bacterial communities are strongly influenced by environ- mental change (Rooney-Varga et al., 2005; Henriques et al., 2006; Mosier and Francis, 2008).

The Pearl River estuary, which is located offshore from the mouth of the Pearl River, is an area where freshwater is mixed with seawater. As an interface of land, freshwater and marine environments, estuarine system is extremely complicated and dynamic. In recent decades, the rapid increase of human activities along the Pearl River due to the explosive economic development has become a serious challenge on local environments. For example, water quality of the Pearl River estuary has been significantly influenced by increase anthropogenic discharge of organic nutrients and pollutants into the estuary (Mai et al., 2002; Zhang et al., 2002, 2009; Fu et al., 2003). The high organic matter deposition in coastal systems also promotes rapid digenetic processes in the sediment–water interface, which affects the biogeochemical cycling of carbon, nutrients and many other chemical elements in coastal oceans (Hedges and Keil, 1995). To the best our knowledge, statistical understanding of the relationship between sediment bacteria and the overlying water biogeochemistry however, has not yet been conducted in these regions.

Phylogenetic analysis of 16S rRNA genes using denaturing gradient gel electrophoresis (DGGE) is one of the most valuable tools for studying bacterial community structures in complex systems such as estuarine sediments (Muyzer and Smalla, 1998). One common bias of this technique is the separation of DNA fragment of the same size with different base-pair sequences (Muyzer et al., 1993). The sensitivity of PCR-denaturing gradient gel electrophoresis (PCR-DGGE)technique can however be impacted by low DNA concentrations or by the presence of high concentrations of competing DNA (Ogier et al., 2002). Despite these problems, PCR-DGGE method remains a powerful technique and has been routinely employed for monitoring microbial diversity under the changing environments (Davies et al., 2004).

In this study, we apply PCR-DGGE method to track the vertical diversity of the bacterial community in the Pearl River estuary sediment. Our major goals were to: (1) Understand the geographic pattern of sediment bacterial community in different layers along the Pearl River estuary; (2) Identify the major bacterial phylogenetic groups and their diversity based on culture independent techniques; and (3) Assess the impacts of environmental changes such as nutrient variations on the microbial community structure using multivariate analysis.

MATERIALS AND METHODS

Field samples collection

Sediment samples were collected during August 2009, and geographic locations of sampling sites are shown in Figure 1. In each site, sediment samples collected three times with core sampler was firstly separated into six sections with 2 cm interval from top surface down to a depth of approximately 24 cm. The sediment core was separated into four zones: 0 to 2, 4 to 6, 8 to 12 and 18 to 22 cm. Then sixteen (16) sediment samples were preserved at -20°C freezer. Overlying water samples for physicochemical parameters analysis were also collected using 10 cm syringe for four different sites. These samples were filtered online through 0.45 µm membrane and preserved at -20°C.

Overlying water physicochemical parameters

Dissolved oxygen (DO), salinity and pH were determined *in situ* using the YSI 6600 V2 Sonde water quality monitoring system (YSI Incorporated, USA). Concentrations of ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), silicate (SiO_3-Si) and phosphate (PO_4-P) were determined in standard analytical methods described in "The specialties for marine monitoring" (GB17378.4-1998, China) and Wang et al. (2008).

Total community DNA extraction

DNA samples for total community were extracted from 1.0 g of replication wet samples using the Soil DNA Kit (Biomiga, Inc.). The extracted DNA was re-dissolved in 30 µl of TE buffer (10 mM Tris–HCl, 1 mM Na_2EDTA, pH 8.0), and then three replications DNA extracted in same station were equally mixed together. The quantity and quality of DNA extracted were estimated by electrophoresis of 2.5 µl aliquots on a 0.8% agarose gel and compared to a molecular weight standard (stained using ethidium bromide). The extracted DNA samples were stored at -20°C prior to analysis.

Analysis of the bacteria community structure in the sediment

PCR was used for 16S rRNA gene amplification using a PTC-200 thermal cycler (Bio-Rad laboratories, USA). PCR was performed using primers 341F and 907R to amplify bacterial 16S rRNA genes (Muyzer and Ramsing, 1995). A 40 bp GC-clamp (cgc ccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg ggg g) was added to primer 341F to increase the separation of DNA bands in DGGE gel (Muyzer et al., 1993). The thermal PCR profile was as follows: Initial denaturation at 95°C for 5 min followed by 30 cycles of primer annealing at 55°C for 30 s, chain extension for 30 s at 72°C, denaturation for 30 s at 94°C and a final extension at 72°C for 10 min. PCR products was confirmed by analyzing 2.5 µl of the PCR product on 1.5% (w/v) agarose gel, stained with ethidium bromide (EB) and visualized using the Alpha Imager (Alpha innotech, Japan).

Denaturing gradient gel electrophoresis (DGGE)

A INGENYphorU-2 system (Ingeny International BV,Goes, NL) was used to perform DGGE analysis. 50 µl of the PCR products were

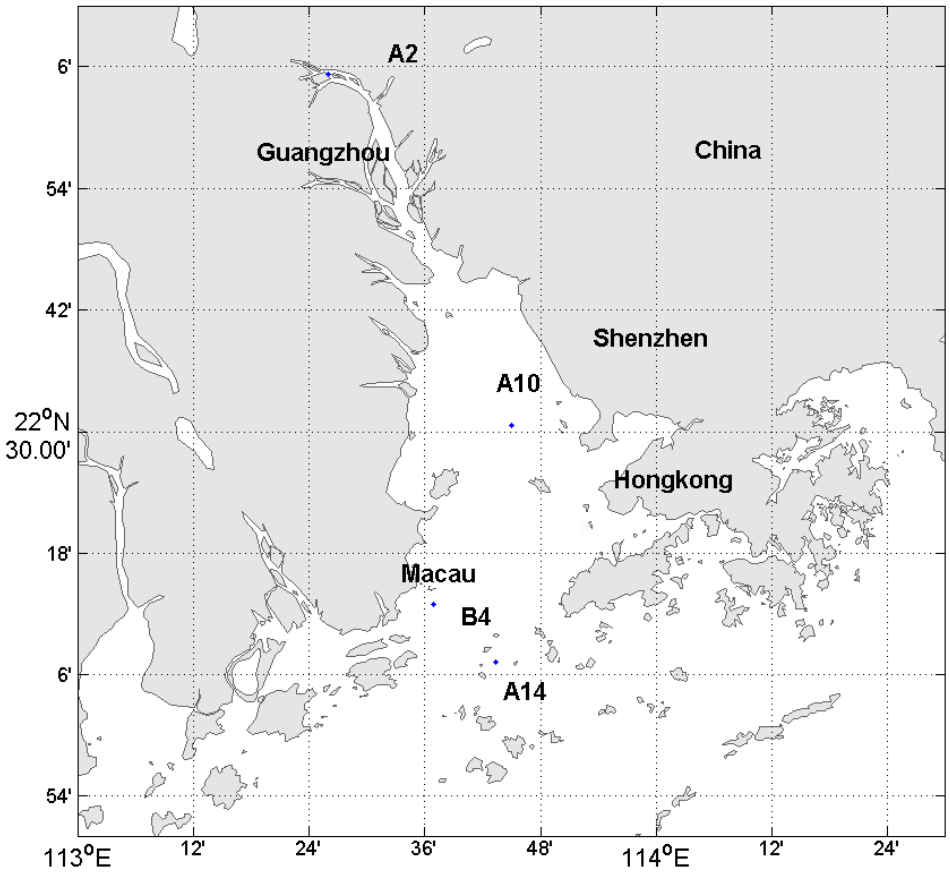


Figure 1. Sampling stations in the Pearl River estuary.

loaded into 8% wt/vol acrylamide gels in 1×TAE buffer (40 mM Tris base, 20 mM acetic acid, and 1 mM disodium EDTA, pH 8.0) containing a linear chemical gradient of 40 to 70% denaturant (100% denaturant is defined as a mixture of 7 M urea and 40% deionized formamide). Gels were pre-run at 80 V in 1x TAE for 30 min at 60°C before the samples were loaded, and then run at 100 V for 18 h at 60°C. Subsequently, gels were stained with ethidium bromide solution and rinsed with distilled water prior to viewing under UV light in an AlphaImager imaging system.

Analysis of DGGE patterns

DGGE banding patterns were digitized and processed using the BandScan software 5.0 and manually corrected for further analysis. Bands were visually identified and distinguished by the distance migrated and intensity in the gel. Base on these, each band was numbered and scored in each of the sediment samples. To assess changes in the genetic diversity of bacterial communities in different sediment samples, DGGE banding patterns were analyzed by multi-dimensional scaling (MDS) analysis (Muckian et al., 2007). For this purpose, the dissimilarity indices were recorded in a binary matrix, which was then analyzed with the program SPSS 18.0 for Windows. The resulting graphical representation, MDS map, showed every band pattern as one plot, and relative changes in community structure were visualized and interpreted as the distance among the plots. The closer the plots were to each other, the more similar were the DGGE banding patterns.

Canonical correspondence analysis (CCA) (CANOCO 4.5; Biometris,Wageningen, the Netherlands) was performed on a statistical analysis of the DGGE profiles versus the environmental variables. The variables included in the analysis (salinity, temperature, dissolved oxygen, ammonium, nitrate, nitrite, silicate, phosphate) were transformed when necessary to correct for deviations from normality. Ordination biplots including the environmental variables and DGGE samples (bands) were used to explain our data. The resulting ordination biplot approximated the weighted average of each species with respect to environmental variables, which were represented as arrows. The length of these arrows indicated the relative importance of that environmental factor in explaining variation in bacterial profiles, while the angle between the arrows indicated the degree to which they were correlated. The bacterial phylotypes was represented as triangles. A Monte-Carlo permutation test based on 499 random permutations was used to test the null hypothesis that bacterial profiles were unrelated to environmental variables.

Cloning of 16S rRNA fragments after DGGE analysis

Dominant bands in the DGGE were excised using a scalpel blade and incubated at 4°C overnight in 20 µl of TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) before they were re-amplified. The positions of the excised bands in the DGGE gel were confirmed with repeated DGGE. Bands showing the expected melting position were amplified with the secondary primer without GC-clamp. The PCR products were purified with the PCR Purification kit (Takara Co. Ltd) and subsequently cloned into pMD18-T plasmid Vector and *E. coli* DH5a cells using pMD18-T cloning vector kit according to manufacturer's instructions (Takara Co. Ltd). Positive clones were identified by PCR

amplification with pMD-18T Vector primer pairs T7 (5V-TAA TAC GAC TCA CTA TAG GG-3V) and M13 (5V-CAG GAA ACA GCT ATG ACC-3V), using the same program as 16S rRNA amplification.

DNA sequencing and phylogenetic tree

Positive recombinants were then submitted for sequencing using an ABI3730 DNA Sequencer (USA) with M13 primer (Shanghai major Biotech Co. Ltd). Nucleotide sequences were compared with those in the GenBank database by BLAST algorithm for tentative identifications. All the sequences obtained in this study have been assigned to the GenBank nucleic acid sequence database with accession numbers HQ010545-HQ010596. Phylogenetic trees of 16S rRNA gene partial sequences were generated using the neighbour-joining algorithms in Mega 4.1 software. The level of support for the phylogenies derived from neighbour-joining analysis was gauged by 1000 bootstrap replicates.

RESULTS

DGGE fingerprint profiles

Analysis of DGGE gel (Figure 2) resulted in a total of 186 detectable bands in 66 different positions (Table 1). The number of bands per sample varied between 4 and 16, indicating a diverse bacterial assemblage in estuary sediments. A total of 52 bands was excised and successfully sequenced (Figure 2). The indices of H, reflecting the structural diversity of the bacterial community, were calculated on the basis of the number and relative intensities of bands on the gel track. Based on Table 1, the results suggested that sites A2 had a slightly higher Shannon index and more microbial species than other sites. A2 DGGE type at different depths was similar to each other compared with other stations. A10 and A14 DGGE types are particularly dramatic changes at all depths.

MDS analysis of DGGE banding pattern

Two-dimensional plots of MDS scores for sediment samples are depicted in Figure 3. The results revealed that the vertical had a significant impact on sediment bacterial community structure. The profiles from cluster A2 site were differentiated from other sites, with an MDS stress value of 0.18 (stress values of below 0.2 indicate that an MDS ordination plot is a good spatial representation of differences between data). The four banding pattern of samples A10 (a, b, c, d), were scattered from each other, it indicated that bacteria in different depths had low similarity.

Bacterial community composition in relation to environmental variables

Table 2 shows the physicochemical parameters of overlying water samples. The CCA biplot is shown in Figure 4. The nitrogen nutrient-associated and DO factors were placed in the same quadrant, showing strong correlation among them. Phylotype-envrionment correlations were especially high for axes 1 and 2 (0.969 and 0.970), indicating a strong relationship between phylotype and environment factors. Each environmental variable is represented by an arrow, which determines an axis. The projection of a taxon (indicated as triangles) on this axis shows the level of the variable where the taxon is most abundant. It is clear that most bacterial phylotypes have a positive correlation with NH_4-N, PO_4-P, S and SiO_3-Si, which was distributed on the both sides of the biplot, including the most sequenced bacterial phylotypes (Figure 2). Bacterial phylotypes of cluster I (most bands belonged to A10 and A14 stations) were positively correlated with S and pH. Bacterial phylotypes of cluster II (most bands belonged to A10 station) showed a significant positive correlation with SiO_3-Si. Bacterial phylotypes of cluster III (bands belonged to A2 station) showed a significant positive correlation with NH_4-N.

Identification and phylogenetic analysis of the predominant bacterial phylotypes based on 16S rRNA gene sequences

Most of the sequences were similar to 16S rRNA sequences reported from uncultured organisms present in environmental samples from sources such as marine sediment, soil, sludge and water.

For phylogenetic analysis, the 16s rRNA gene sequences of the 52 clones in this study were comparable to those from GenBank database. The neighbour-joining analysis divided these bacteria sequences into eleven groups, Proteobacteria (γ, β, α and δ subdivisions), Acidobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, Firmicutes, Planctomycetales and Cyanobacterium (Figure 5). Bands sequences (19, 22, 25, 29, 35, 43 and 52) are identical to cultivable bacteria.

In the γ-Proteobacteria cluster, band 51 had 99% similarity with uncultured Acidobacteriaceae bacterium at the DNA level. In the α-Proteobacteria cluster, Bands 46 and 20 were 99 and 97% identical to 16S rRNA gene sequences of *Hyphomicrobium vulgare* and Rhodobacteraceae bacterium. Bands 19, 29 and 43 had 100% similarity with *Brevundimonas* sp. *Methylobacterium radiotolerans* and Sulfitobacter sp., respectively. Bands 44 and 25 had 99 to 100% similarities with *Limnobacter thioxidans*, which fallen into β-Proteobacteria cluster. In Firmicutes cluster, Bands 38 and 52 was 99 to 100% identical to 16S rRNA gene sequences of *Planomicrobium* sp. and *Bacillus okhensis* strain. Bands 11, 17, 23, 50 and 55 were clustered in the Actinobacteria, and had 95 to99% similarity with Actinobacteria bacterium. Bands (27, 30, 39, 40, 41, 47, 49 and 56) with 94 to 99% similarity to uncultured Bacteroidetes bacterium were belonging into Bacteroidetes cluster. Sequences 21, 26, 28 and 53 had

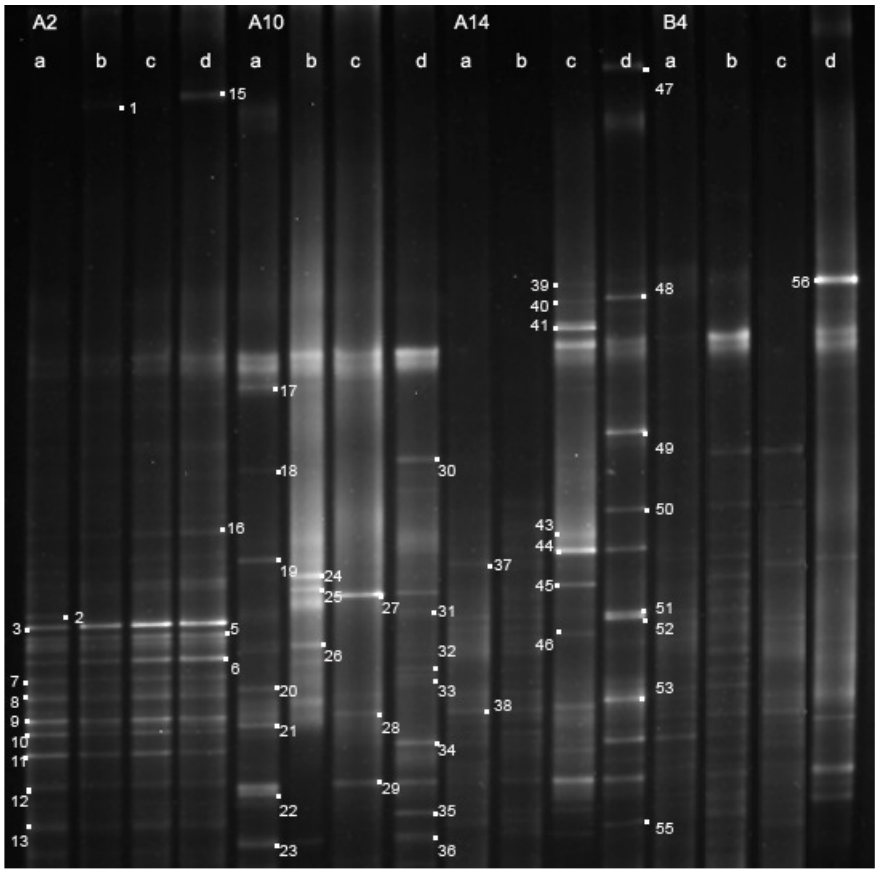


Figure 2. DGGE analyses of bacteria in the Pearl River Estuary sediment. Labels of A2 to B4 at the top of the lanes represent corresponding sampling sites. Predominant bands have been highlighted. The letters of a, b, c and d after station names represent 0 to 2, 4 to 6, 8 to 12 and 18 to 22 cm, respectively.

Table 1. Comparison of the microbial diversity in sediment samples (the H was calculated on the basis of the number and relative intensities of bands on the gel track).

Sample (cm)	Shannon-weaver index (H)	DGGE band number
A2 (0-2)	2.427533	13
A2 (4-6)	2.043275	10
A2 (8-12)	2.373919	15
A2 (18-22)	2.427235	16
A10 (0-2)	2.260979	12
A10 (4-6)	1.529079	8
A10 (8-12)	1.140823	4
A10 (18-22)	1.612296	12
A14 (0-2)	2.208765	10
A14 (4-6)	2.363362	11
A14 (8-12)	2.089545	14
A14 (18-22)	2.372631	14
B4 (0-2)	2.296491	11
B4 (4-6)	1.696156	14
B4 (8-12)	1.310013	6
B4 (18-22)	2.261902	14

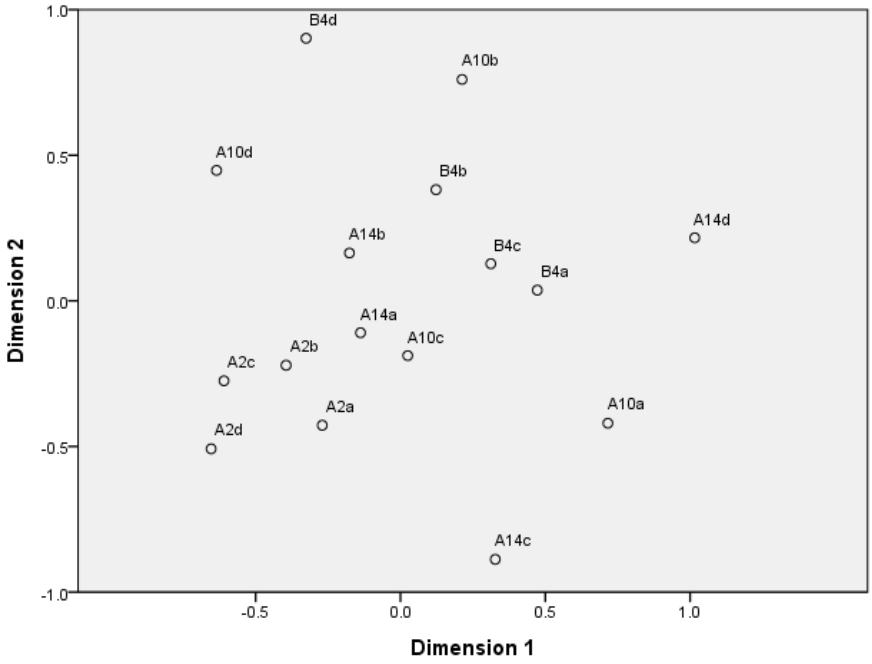


Figure 3. Two-dimensional plots of MDS analyses from DGGE patterns to compare broad-scale differences in bacterial communities.

Table 2. Overlying water characteristics of the Pearl River estuary.

Station	Location		NH_4-N (mg/L)	SiO_3-Si (mg/L)	PO_4-P (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	pH	S (ppt)	DO (mg/L)
A2	113.4333E	23.0867N	2.41	0.835	0.052	1.528	0.169	7.21	0.17	12.56
A10	113.75E	22.51N	0.17	1.665	0.009	1.603	0.073	7.8	16.94	11.73
A14	113.722E	22.12N	0.30	1.005	0.020	1.037	0.071	7.96	30.34	5.04
B4	113.615E	22.215N	0.18	1.155	0.047	1.199	0.005	7.59	14.07	4.77

Locations were determined using global positioning system (GPS); SiO_3-Si, silicate; PO_4-P, phosphate; NH_4-N, ammonium; NO_3-N, nitrate; NO_2-N, nitrite; DO, dissolved oxygen; S, salinity.

98 to 100% similarity with uncultured cyanobacterium.

The bacterial community structure comparison based on DGGE pattern

The result of bacterial community structure from DGGE is summarized in Figure 6a, and the structure comparison among the four vertical stations is displayed in Figure 6b, in percentage of individual group over total community based on the density of their correlated DGGE bands. For all the samples, α-proteobacteria (29.8%), β-proteobacteria (18.6%), γ-proteobacteria (14.76%) and Bacteroidetes (10.59%) generally dominated the DGGE profile.

The DGGE profile of the four layers A2 produced 13, 10, 15 and 16 bands. α-proteobacteria, β-proteobacteria and γ-aproteobacteria were the predominant groups, which accounted for on average 18.47, 41.63 and 26% of the total A2 DGGE intensity. The DGGE profile of the four A10 layers produced 12, 8, 4 and 12 bands, representing nine bacterial groups in this study. The predominant groups were α-proteobacteria (49.4%), Firmicutes (21.6%), Bacteroidetes (55.6%), and α-proteobacteria (37.6%) in each layer, respectively. There were 49 bands in the DGGE profile of A14 layers covering ten bacterial groups. The percentage of α-proteobacteria over the total DGGE intensity dramatically increased to 34.5% in this station. There were 45 bands in the DGGE profile of B4 layers covering seven bacterial groups. The predominant shared groups were α-proteobacteria (31.3%) and Bacteroidetes (19.9%) in this station.

DISCUSSION

DGGE analyses of the whole microbial communities in

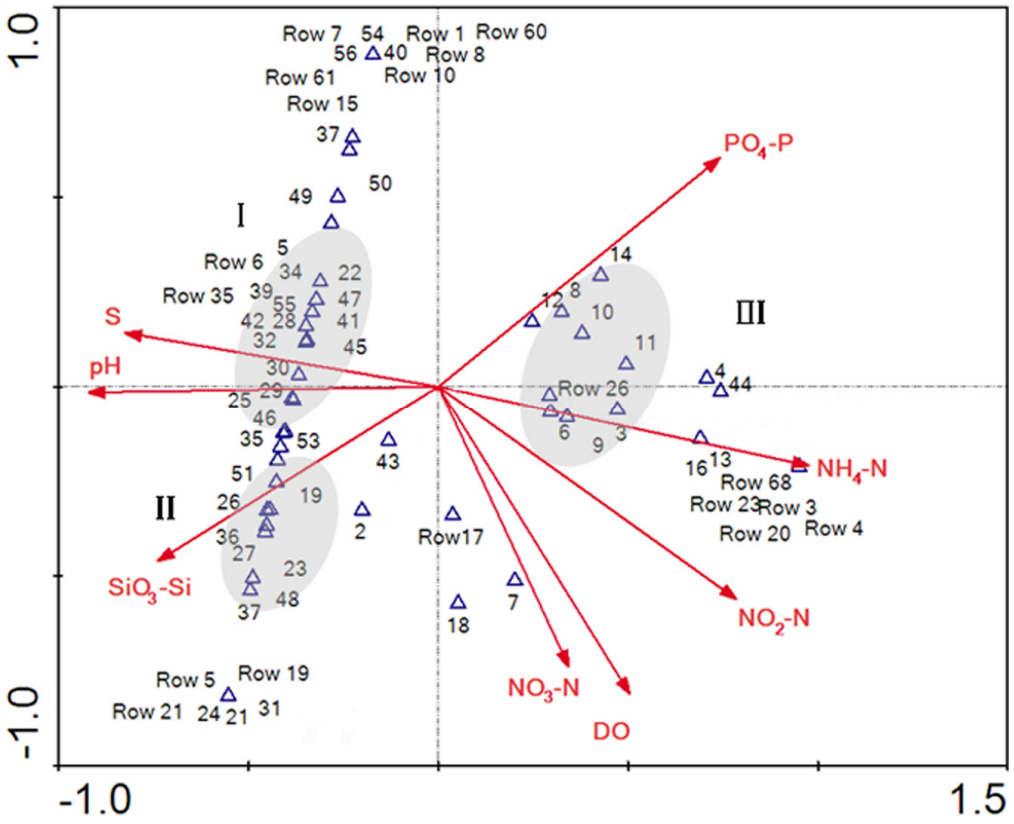


Figure 4. Ordination diagram of DGGE data from canonical correspondence analyses (CCA), with environmental factors as arrows, and individual abundant bacterial phylotypes as triangles. Silicate (SiO_3-Si), phosphate (PO_4-Pi), ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), dissolved oxygen (DO) and salinity (S).

A2 had a slightly higher Shannon index and more microbial species than other sites. Salinity was an important parameter in grouping bacterial communities (Jr and Ducklow, 2000; del Giorgio and Bouvier, 2002). Some freshwater bacteria were inhibited by variations of salinity (Hart et al., 1991; Bernhard et al., 2005). MDS analysis suggested the plots A2 (a, b, c and d) were closer to each other, which indicated that they had similar bacterial community structure. One possible explanation for the observed similarity between surface and deeper layers of the sediment in term of community structure may be the bounding and subsequent deposition of micro-organisms on sediment particles (Novitsky, 1990). B4 stations are all coastal stations showing similar DGGE band types and bacterial groups in each layer. Various layers of other sites, such as A10, were far away from each other, suggesting that they had different bacterial community structure. However, A10 station, located in a zone of mixing freshwater and seawater, had a unique bacterial community distribution in each layer significantly different from other stations. Our analyses also showed that difference in microbial distributional patterns between stations and layers was caused by the difference of their ambient water environments.

CCA analyses of bacterial communities and environmental conditions suggested that compositions of bacterial communities varied with the spatial positions of each sites and their depths, and were strongly correlated with the environmental variables. Our results suggested that the contents of ammonium and phosphate significantly determined the microbial community structures in the sediments of the Pearl River estuary, which are not surprising because nitrogen limitation of low-trophic ecosystem are a common feature in coastal oceans as driven by the overload of phosphorus-contained pollutions (Howarth, 1988; Howarth and Marino, 2006). Heterotrophic bacteria are able to take up dissolved inorganic nitrogen in the form of ammonium and nitrate, although, with a lower preference for the latter (Kirchman et al., 1992). Furthermore, it was suggested that ammonium could sometimes be the dominant N source for marine bacteria (Jorgensen et al., 1994) in certain areas where nitrogen was limited (Zweifel et al., 1993). Fresh water in the inner section of the Pearl River estuary carries a large number of inorganic and organic nutrients, which could have a large impact on the distribution of bacteria. Interestingly, our data indicated that pH and salinity are important factors controlling the bacterial community

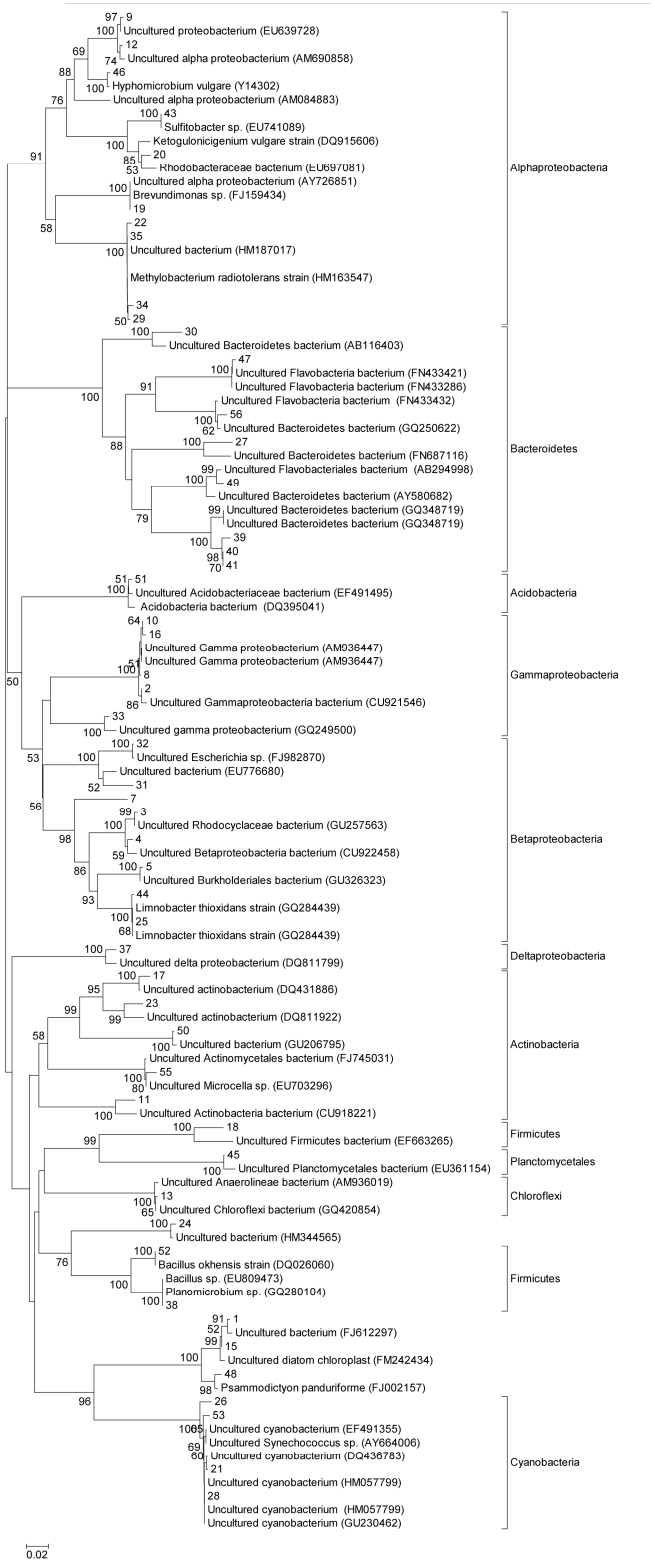
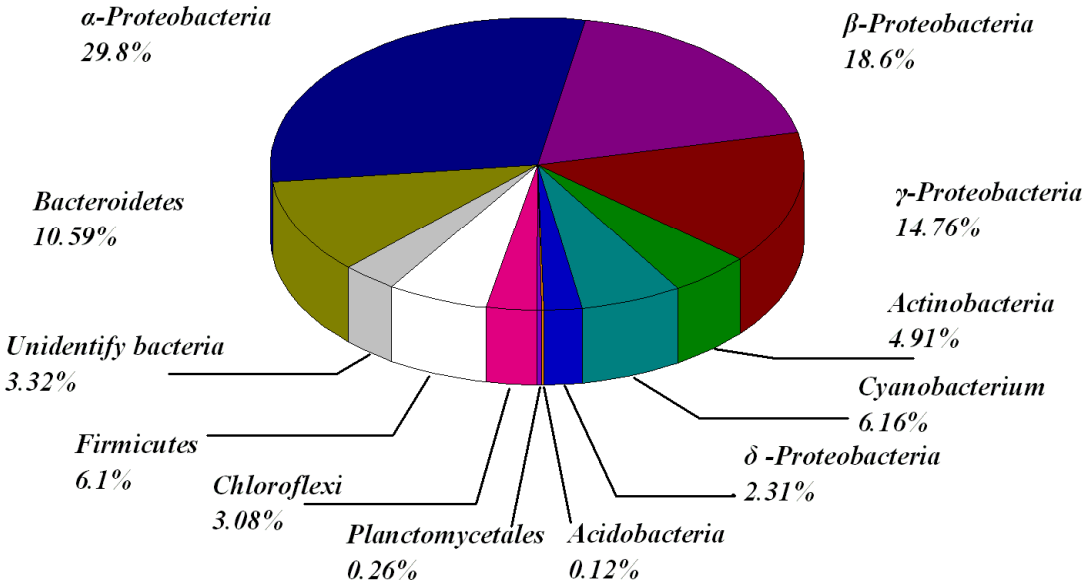


Figure 5. Unrooted phylogenetic tree based on 16S rRNA sequences representing the respective DGGE bands in Figure 2. Bootstrap analysis was based on 1,000 replicates. Bootstrap values from distance analyses are depicted. Bootstrap values less than 50% are not shown.

a



b

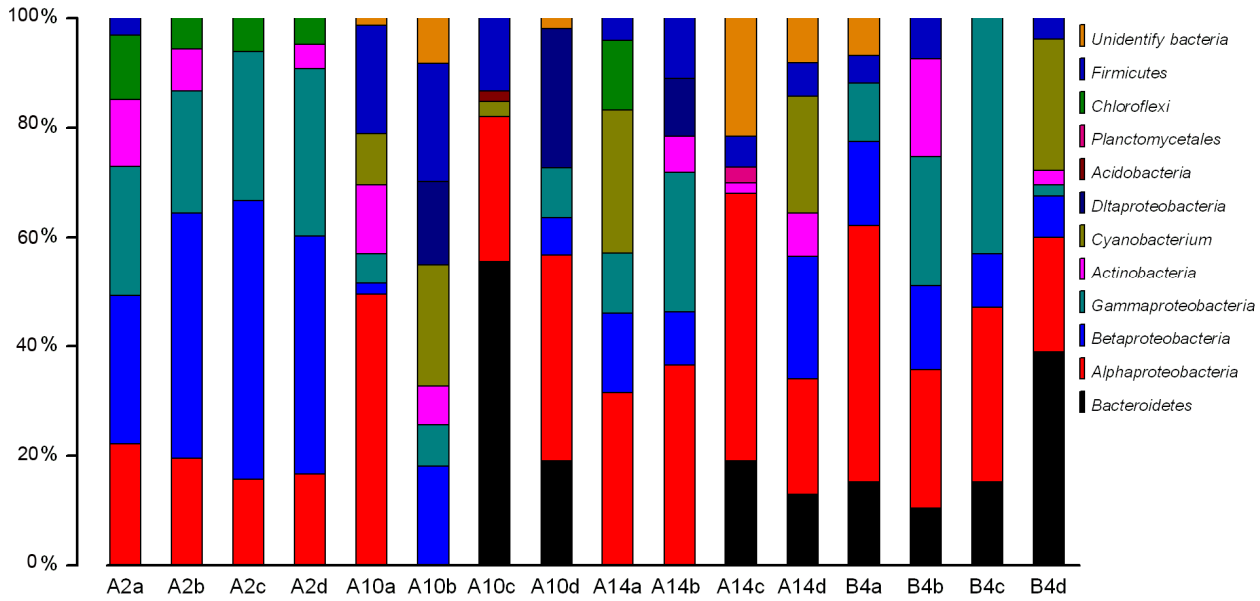


Figure 6. Community structure comparison based on DGGE pattern: a: Analyses of the total DGGE detected bacterial groups; b: Spatial and vertical patterns of the DGGE detected bacterial community structure along the four stations. Similar to Figure 2, the letters of a, b, c and d represent 0 to 2, 4 to 6, 8 to 12 and 18 to 22 cm, respectively. Percentage of each bacterial group (shown by different colors in b section) over total community is based on the density of the correlated DGGE bands.

patterns in estuarine sediments. CCA analysis showed that most bacterial phylotype had positive correlations with pH and S, which suggested that pH and S were the most important factor influencing bacterial distributions and diversities. It is generally believed that pH and S plays an important role on the activities of benthic microbial communities through changing the bio-availability of the major nutrient elements and trace metals as well as their biogeochemical transformation in sediments (Yannarell and Triplett, 2005). Our finding of significant correlation of pH with microbial phylotype supports the recent argument that fluctuation in pH could lead to substantial change of microbial communities by direct microbiological mechanisms (Stepanauskas et al.,

2003; Yannarell and Triplett, 2005). It is also clear that estuaries are subject to the dual impacts from both freshwater and seawater. Therefore, water quality plays an important role on structuring the bacterial communities in sediments. The results here agreed with previous findings that substances required for bacterial growth in coastal and estuarine ecosystems could come from various processes, such as terrestrial runoff (Shiah and Ducklow, 1995).

Phylogenetic analyses based on excising and sequencing the DGGE bands indicated that the fifty-two 16S rRNA gene sequences from this field investigation could be clustered into different taxonomic groups, including Proteobacteria (γ, β, α and δ subdivisions), Acidobacteria, Bacteroidetes, Chloroflexi, Actinobacteria , Firmicutes, Planctomycetales and Cyanobacteria (Figure 5). These results revealed a high diversity of predominant bacteria in the sediments of the Pearl River estuary, which are similar to that found in the sediment of changjiang estuary (Feng et al., 2009). Sequence derived from α-, β-, γ-and δ- Proteobacteria was commonly found in our field sites. Proteobacteria are typically abundant in aquatic systems (Rappé et al., 1997). Three subclasses of proteobacteria (α, β, and γ subdivisions) were equally distributed in coastal samples, while a-proteobacteria was more abundant in estuarine samples. These findings are consistent with those found in the Delaware Bay (Cottrell and Kirchman, 2000). Dominance of Proteobacteria (α-, β-, γ-) in the sediments of the Pearl River estuary is expected as this group of bacteria plays a critical role in biogeochemical cycling of nitrogen, phosphorous, and sulfur in marine environments. We also found Bacteroidetes (band 27, 30, 39, 40, 41, 47, 49 and 56) in our studied sites. As a phylogenetically diverse group, Bacteroidetes is known as one of the hydrolytically fermentative degraders for complex polymers in anaerobic habitats, such as freshwater sediments (Rossello-Mora et al., 1999).

In conclusion, our results have clearly showed the spatial and vertical heterogeneity of the bacteria community structure in the sediments of the Pearl River estuary. In particular, we found significant correlations between the individual bacterial phylotypes with the concentrations of NH_4-N, PO_4-P, S and pH of the overlying water. These findings suggested that water quality is an important player on regulating bacterial community structures in sediments. Moreover, our results suggested that a phylogenetical diversisty of bacteria ranging from *Proteobacteria* to Cyanobacteria was a common feature of the sediments in the Pearl River estuary.

ACKNOWLEDGEMENTS

This research was supported by the Knowledge Innovation Programs of the Chinese Academy of Sciences (No. KZCX2-YW-Q07-02, No. KSCX2-EW-G-12C, No. KSCX2-YW-Z-1024 and No. KSCX2-SW-132), the National Natural Science Foundation of China (No. 41076070), the key projects in the National Science and Technology Pillar Program in the Eleventh Five-year Plan Period (No. 2009BADB2B0606) and the National 908 project (No. 908-02-04-04).

REFERENCES

Arzayus K, Canuel E (2005). Organic matter degradation in sediments of the york river estuary: Effects of biological vs. Physical mixing. Geochim. Cosmochim. Acta, 69: 455-464.

Bernhard AE, Donn T, Giblin AE, Stahl DA (2005). Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. Environ. Microbiol. 7(9): 1289-1297.

Binnerup SJ, Jensen K, Revsbech NP, Jensen MH, Sorensen J (1992). Denitrification, dissimilatory reduction of nitrate to ammonium, and nitrification in a bioturbated estuarine sediment as measured with 15n and microsensor techniques. Appl. Environ. Microbiol. 58: 303-313.

Boström B, Andersen JM, Fleischer S, Jansson M (1988). Exchange of phosphorus across the sediment-water interface. Hydrobiologia, 170: 229-244.

Clavero V, Izquierdo JJ, Fernandez JA, Niell FX (1999). Influence of bacterial density on the exchange of phosphate between sediment and overlying water. Hydrobiologia, 392: 55-63.

Cottrell M, Kirchman D (2000). Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low-and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66 (4): p. 1692.

Crump BC, Armbrust E, Baross JA (1999). Phylogenetic analysis of particle-attached and free-living bacterial communities in the columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. 65: p. 3192.

Davies CE, Hill KE, Wilson MJ, Stephens P, Hill CM, Harding KG, Thomas DW (2004). Use of 16s ribosomal DNA pcr and denaturing gradient gel electrophoresis for analysis of the microfloras of healing and nonhealing chronic venous leg ulcers. J. Clin. Microbiol. 42: p. 3549.

del Giorgio PA, Bouvier TC (2002). Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnol. Oceanogr. 47: 471-486.

Feng BW, Li XR, Wang JH, Hu ZY, Meng H, Xiang LY, Quan ZX (2009). Bacterial diversity of water and sediment in the changjiang estuary and coastal area of the east china sea. FEMS Microbiol. Ecol. 70: 236-248.

Freitag TE, Chang L, Prosser JI (2006). Changes in the community stucture and activity of betaproteobacterial ammonia-oxidizing sediment bacteria along a freshwater-marine gradient. Environ. Microbiol. 8(4): 684-696.

Fu J, Mai B, Sheng G, Zhang G, Wang X, Peng P, Xiao X, Ran R, Cheng F, Peng X (2003). Persistent organic pollutants in environment of the Pearl River delta, china: An overview. Chemosphere, 52: 1411-1422.

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K (1991). A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia, 210: 105-144.

Hedges JI, Keil RG (1995). Sedimentary organic matter preservation: An assessment and speculative synthesis. Mar. Chem. 49: 81-115.

Henriques IS, Alves A, Taco M, Almeida A, Cunha Â, Correia A (2006). Seasonal and spatial variability of free-living bacterial community composition along an estuarine gradient (ria de aveiro, portugal). Estuar. Coast. Shelf Sci. 68: 139-148.

Hollander DJ, Smith MA (2001). Microbially mediated carbon cycling as a control on the [delta] 13c of sedimentary carbon in eutrophic lake mendota (USA): New models for interpreting isotopic excursions in the sedimentary record. Geochim. Cosmochim. Acta, 65: 4321-4337.

Howarth RW (1988). Nutrient limitation of net primary production in marine ecosystems. Annu. Rev. Ecol. Evol. Syst. 19: 89-110.

Howarth RW, Marino R (2006). Nitrogen as the limiting nutrient for

eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnol. Oceanogr. 51: 364-376.
Jay DA, Musiak JD (1994). Particle trapping in estuarine tidal flows. J. Geophys. Res. 99: 20445-20461.
Jorgensen NOG, Kroer N, Coffin RB (1994). Utilization of dissolved nitrogen by heterotrophic bacterioplankton: Effect of substrate c/n ratio. Appl. Environ. Microbiol. 60: p. 4124.
Jr GES, Ducklow H (2000). Changes in bacterioplankton metabolic capabilities along a salinity gradient in the york river estuary, virginia, USA. Aquat. Microb. Ecol. 22: 163-174.
King JK, Kostka JE, Frischer ME, Saunders FM (2000). Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. Appl. Environ. Microbiol. 66(6): 2430-2437.
Kirchman D, Moss J, Keil R (1992). Nitrate uptake by heterotrophic bacteria: Does it change the f-ratio. Arch. Hydrobiol. 37: 129-138.
Mai BX, Fu JM, Sheng GY, Kang YH, Lin Z, Zhang G, Min YS, Zeng EY (2002). Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River delta, China. Environ. Pollut. 117: 457-474.
Mosier AC, Francis CA (2008). Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the san francisco bay estuary. Environ. Microbiol. 10: 3002-3016.
Muckian L, Grant R, Doyle E, Clipson N (2007). Bacterial community structure in soils contaminated by polycyclic aromatic hydrocarbons. Chemosphere, 68(8): 1535-1541.
Muyzer G, de Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59(3): 695-700.
Muyzer G, Ramsing N (1995). Molecular methods to study the organization of microbial communities. Water Sci. Technol. 32: 1-9.
Muyzer G, Smalla K (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Leeuwenhoek. 73(1): 127-141.
Novitsky JA (1990). Evidence for sedimenting particles as the origin of the microbial community in a coastal marine sediment. Mar. Ecol.-Prog. Ser. 60: 161-167.
Ogier J, Son O, Gruss A, Tailliez P, Delacroix-Buchet A (2002). Identification of the bacterial microflora in dairy products by temporal temperature gradient gel electrophoresis. Appl. Environ. Microbiol. 68(8): p. 3691.
Pernthaler A, Pernthaler J, Amann R (2002). Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 68: p. 3094.
Pimenov N, Ulyanova M, Kanapatsky T, Veslopolova E, Sigalevich P, Sivkov V (2010). Microbially mediated methane and sulfur cycling in pockmark sediments of the gdansk basin, baltic sea. Geo-Mar. Lett. 30: 439-448
Pinckney JL, Paerl HW, Tester P, Richardson TL (2001). The role of nutrient loading and eutrophication in estuarine ecology. Environ. Health Perspect. 109: p. 699
Rappé M, Kemp P, Giovannoni S (1997). Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol. Oceanogr. 42(5): 811-826
Rauch M, Denis L (2008). Spatio-temporal variability in benthic mineralization processes in the eastern english channel. Biogeochemistry, 89: 163-180.
Rossello-Mora R, Thamdrup B, Sch fer H, Weller R, Amann R (1999). The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions. Syst. Appl. Microbiol. 22(2): p. 237.
Rooney-Varga J, Giewat M, Savin M, Sood S, LeGresley M, Martin J (2005). Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb. Ecol. 49: 163-175.
Scala DJ, Kerkhof LJ (2000). Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 66: p. 1980.
Shiah FK, Ducklow HW (1995). Regulation of bacterial abundance and production by substrate supply and bacterivory: A mesocosm study. Microb. Ecol. 30(3): 239-255.
Sinsabaugh R, Findlay S (1995). Microbial production, enzyme activity, and carbon turnover in surface sediments of the hudson river estuary. Microb. Ecol. 30: 127-141.
Spears BM, Carvalho L, Perkins R, Kirika A, Paterson DM (2007). Sediment phosphorus cycling in a large shallow lake: Spatio-temporal variation in phosphorus pools and release. In: Gulati RD, Lammens E, Pauw N, Donk E (eds). Shallow lakes in a changing world, Vol. 196. Springer Netherlands, pp. 37-48.
Stepanauskas R, Moran M, Bergamaschi B, Hollibaugh J (2003). Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquat. Microb. Ecol. 31(1): 85-98.
Wang Y, Lou Z, Sun C, Sun S (2008). Ecological environment changes in Daya Bay, China, from 1982 to 2004. Mar. Pollut. Bull. 56 (11): 1871-1879
Yannarell A, Triplett E (2005). Geographic and environmental sources of variation in lake bacterial community composition. Appl. Environ. Microbiol. 71(1): p. 227.
Yokokawa T, Nagata T (2010). Linking bacterial community structure to carbon fluxes in marine environments. J. Oceanogr. 66: 1-12.
Zhang G, Parker A, House A, Mai B, Li X, Kang Y, Wang Z (2002). Sedimentary records of ddt and hch in the pearl river delta, south china. Environ. Sci. Technol. 36: p. 3671.
Zhang L, Yin KD, Wang L, Chen FR, Zhang DR, Yang YQ (2009). The sources and accumulation rate of sedimentary organic matter in the pearl river estuary and adjacent coastal area, southern china. Estuar. Coast. Shelf Sci. 85: 190-196.
Zweifel UL, Norrman B, Hagstrom A (1993). Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar. Ecol. Prog. Ser. 101: 23-23.