Characteristics of Sediment Bacterial Community in Response to Environmental Impacts in a Sewage Polluted River

Authors: Li, Jialin, Lin, Shaoying, and Qin, Song

Source: Journal of Coastal Research, 74(sp1) : 196-206

Published By: Coastal Education and Research Foundation

URL: https://doi.org/10.2112/SI74-017.1

Journal of Coastal Research	SI	74	196–206	Coconut Creek, Florida	2016

Characteristics of Sediment Bacterial Community in Response to Environmental Impacts in a Sewage Polluted River

Jialin Li[†], **Shaoying Lin**[‡], **and Song Qin**[†‡*]

[†] Key Laboratory of Coastal Biology and Bioresource Utilization
Yantai Institute of Coastal Zone Research
Chinese Academy of Sciences
Yantai, Shandong 264003, P. R. China

[‡] College of Environment and Materials Engineering
Yantai University
Yantai, Shandong 264005, P. R. China

www. cerf-jcr. org

www. JCRonline. org

ABSTRACT

Li, J. L.; Lin, S. Y., and Song, Q., 2016. Characteristics of sediment bacterial community in response to environmental impacts in a sewage polluted river. *In*: Harff, J. and Zhang, H. (eds.), *Environmental Processes and the Natural and Anthropogenic Forcing in the Bohai Sea, Eastern Asia. Journal of Coastal Research*, Special Issue, No. 74, pp. 196–206. Coconut Creek (Florida), ISSN 0749–0208.

The Jiaolai River is the main source of industrial and irrigation water for its catchment of 3900 km^2. Anthropogenic activities have caused heavy pollution of this river, but their impacts on biota have never been evaluated. In this study, molecular techniques were applied to investigate the impacts of environmental pollution on the river. Quantitative PCR revealed that total bacterial abundance ranged from 2.90×10^7 to 2.12×10^8 copies/g, with no significant differences among sampling sites or seasons. Bacterial abundance and pore water ammonium concentration were negatively correlated. Cluster analysis revealed that bacterial communities were mainly distributed into groups corresponding to nitrate concentration. Two clone libraries were constructed to compare the bacterial composition of samples with high (J308) and moderate (J304) nitrate impact. Sample J308 was characterized by more members in Clostridia and disappearance of Betaproteobacteria members, which are the primary contributors to nitrogen biogeochemical cycling. Bacterial communities in the sediment were clearly differentiated by environmental nitrogen pollution, suggesting that nitrogen eutrophication was the main environmental problem influencing the Jiaolai River.

ADDITIONAL INDEX WORDS: *Bacterial composition, river sediments, environmental contaminants, river eutrophication.*

INTRODUCTION

Rivers link terrestrial landscapes and oceanic ecosystems because they receive terrestrial inputs via water and transport them to coastal zones (Neu, Neill, and Krusche, 2011). Rivers also serve as the main sources of municipal, agricultural and industrial water for residents living in the basin. However, river pollution by increasing untreated or partly treated wastewater is threatening human health and ecological systems worldwide (Pimpunchat *et al.*, 2009). This situation is more serious in developing countries owing to rapid industrialization and urbanization (Su *et al.*, 2011). In China, 45% of river water was unsuitable for drinking, and 21% didn't meet agricultural standards by monitoring data of more than 400 river sections in 2008 (Jiang, Jin, and Lin, 2011). Accordingly, it is essential to protect and improve river systems, which necessitates understanding the role of contaminants and a greater focus on how groups of organisms and ecological functions respond to pollution stress (Jennerjahn, 2012).

Since sediment is more stable and less variable than surface and interstitial water and serves as a medium for active biogeochemical processes, it is considered to be a reliable recorder of ecological responses to pollution (Liu *et al.*, 2011). Sediment microorganisms play key roles in nutrient cycling, heavy metals immobilization and degradation of organic compounds. In turn, their composition and activity are commonly sensitive to environmental pollutants. Many studies of sediment bacterial ecology have been conducted to assess pollution stresses, clarify biological responses and monitor ecological functions. The results of such studies have revealed that major phyla of the sediment bacterial community such as Proteobacteria, Bacteroidetes and Planctomycetes showed spatiotemporal patterns correlated with depth and seasonal physicochemical parameters within the river system (Xia *et al.*, 2014). Nutrients and heavy metals contamination have also been shown to lead to changes in bacterial biomass, diversity and function (Freese, Karsten, and Schumann, 2006; Sandaa *et al.*, 1999). These effects may arise from sensitive species being replaced by more tolerant species or pollutant degrading microorganisms (Blanck, 2002). Accordingly, application of improved indigenous bacterial communities is thought to be a potential bioremediation strategy to overcome pollution problems associated with local river (Xu *et al.*, 2014). Mahmoudi *et al.* (2013) reported that spilled oil can be rapidly degraded by the indigenous bacterial community of saltmarsh sediment, suggesting that these of microorganisms have the potential for *in situ* bioremediation. However, the bacterial communities in river sediment remain

DOI: 10. 2112/SI74 – 017. 1 received (11 June 2014); accepted in revision (24 September 2015).

*Corresponding author: sqin@ yic. ac. cn

largely unexplored owing to complicated environmental stresses and regional bacterial differentiation.

The Jiaolai River, which is locates in Shandong Province of northeast China, flows into the semi-enclosed Bohai Sea. This river is of great importance owing to its vast flux into the sea and because it provides irrigation and industrial water over a catchment of 3900 km^2. The Bohai Sea is one of the most polluted areas in China owing river discharge (Gao, Zhou, and Chen, 2014), and the Jiaolai River has been heavily contaminated. Specifically, industrial effluent is released into the river from various sources, and its headstream is the most contaminated segment. Peak flows usually occur from June to September, which could lead to increasing contaminants in water. In response to public health and environment sustainability implications, the government has attempted to protect and improve the Jiaolai River by closing heavily polluting factories, and encouraging research into environmental mechanisms and technology to reduce river pollution. Recent studies of bacterial communities involved in N cycling have characterized the activity and taxonomic composition of nitrifiers and denitrifiers in the Jiaolai River estuary (Yang *et al.*, 2014; Zhang *et al.*, 2013). However, there is still a paucity of information regarding the total sediment bacterial community in the Jiaolai River, particularly its distribution characteristics along the river. Moreover, a combined investigation of the bacterial community and environmental variables is needed to determine which factors cause the most stress in the river systems.

To begin addressing the bacterial ecology in the Jiaolai River sediments, we attempted to describe the spatiotemporal distribution of bacterial communities across seasons. We used molecular detection to illustrate the patterns of the total bacterial communities and multivariate statistics to correlate the communities with measured physicochemical variables. Based on the potential pollutants discharged into the river, we considered salinization, eutrophication and heavy metals contamination during the analyses to reveal the environmental impacts. The results revealed distinct bacterial taxa under different pollution levels. This study is the first comprehensive assessment of the bacterial distribution in Jiaolai River sediments. Nutrients contents, especially the nitrate concentration, were found to be associated with considerable changes in bacterial community composition. Overall, the results provide background biodiversity and biogeochemical regarding the primary environmental problems in the Jiaolai River, and with a focus on nutrient removal by environmental technologies.

METHODS

Study sites and sampling procedure

Samples were collected in April, August and October 2012 to cover seasonal variations at four sites along the Jiaolai River from the estuary mouth to the upper steam (Figure 1). The sampling sites were also across the saltwater instrument area with characterized by fresh (J4), brackish (J3) and saline (J1 and J2) groundwater (Han *et al.*, 2014). All 12 samples were labeled with site names following by the month of collection. Sampling could not be conducted during winter because the river was frozen.

Triplicate surface sediment samples (5 cm) were collected from each site using sterile tubes, stored in liquid nitrogen during transport to the laboratory, then preserved at −80° C until analysis. Another sediment sample was collected for measuring environmental parameters and stored in a cooler at 4°C before analysis.

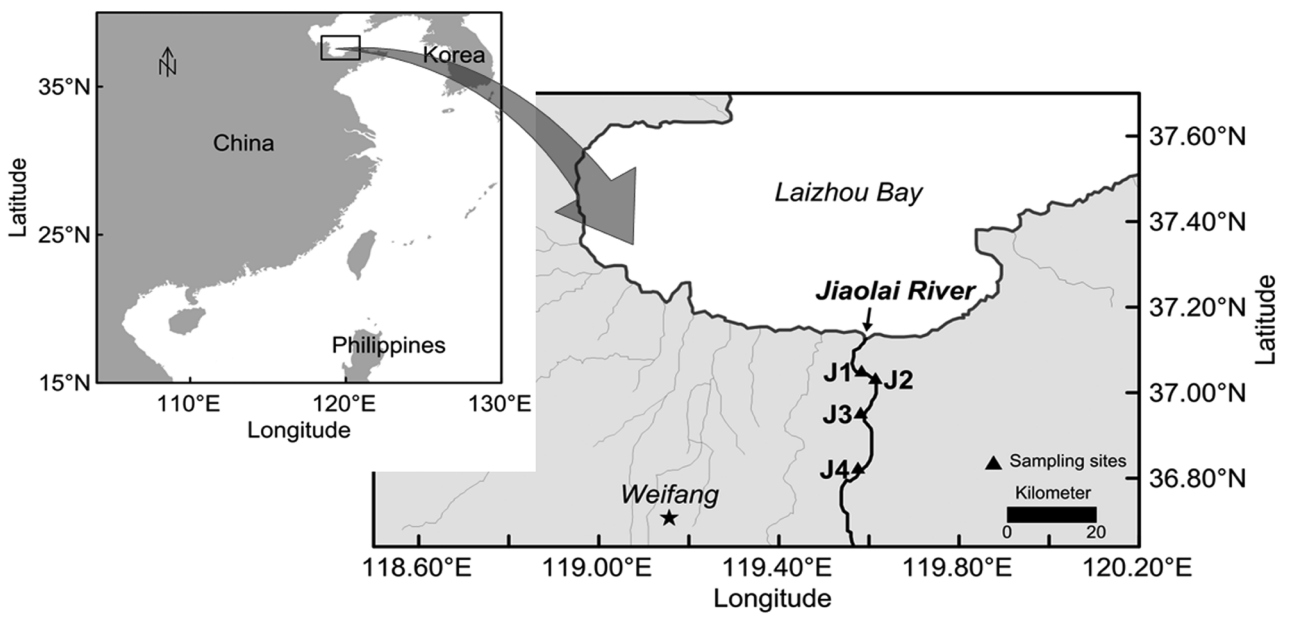

Figure 1. Location of the sampling sites along the Jiaolai River.

Environmental factor analyses

Temperature, salinity and pH of overlying water were measured *in situ* using YSI model 556MPS. Water content was determined based on the weight loss after 48 h of freeze drying. Sediment medium grain size was measured using a Matersizer 2000F laser particle size analyzer (Malvern, England). To determine the NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-} concentrations, the pore water were obtained by collecting supernatants after centrifugated at 5,000g for 10 min. All analyses were measured colorimetrically using standard methods of the AAA3 segmented flow analyzer (Seal Analytical GmbH, German). An Elemental Analyser (Elementar, Vario Micro) was used to measure the total organic carbon (TOC) and nitrogen (TN) contents. Elan DRC II plasma-MS (ICP-MS, PerkinElmer) was used to measure the Pb, Cr, Mn, Ni, Cu, and Zn concentrations after the metals were extracted with 1.0 mol/L HCl(Snape *et al.*, 2004).

Genomic DNA extraction

Total microbial community DNA was extracted from 0.50 g wet weight sediment using a PowerSoil DNA Isolation Kit (MobioLaboratories, USA), then further purified by ethanol precipitation (Adair and Schwartz, 2008). For each sample, triplicate DNA extractions from separate subsamples were pooled to better represent the site by reducing variability of the subsamples (Manter, Weir, and Vivanco, 2010). Final DNA extracts were re-suspended in 50 μl sterile H_2O, then diluted ×10 as PCR templates.

Quantification of sediment 16S rRNAGenes

Real-time quantitative PCR was used to evaluate the bacterial abundance of different samples. Briefly, 16S rRNA gene copies were amplified using the 331F and 797R primers to target a 466 bp fragment (Nadkarni *et al.*, 2002). Plasmids carrying the correct 16S rRNA gene fragment were extracted from *E. coli* prior to quantification using a Nanodrop ND-1000 spectrophotometer. The target abundance for the standard was calculated assuming a molecular mass of 660 Da for double stranded DNA using the following formula:

Gene abundance (copies/ml) = 6.023×10^{23} (copies/mol) × standard concentration (g/ml) / Mw(g/mol), where Mw is the molecular weight of the base pairs of the standard target (284.341 g/mol in this study).

A standard curve was created using a serial dilution of DNA ranging from 10^5 to 10^{11} genes/ml. Genomic DNA was diluted to 20 ng/μl as a template for PCR based on the results of Nanodrop ND-1000 spectrophotometry.

Standard, samples and no-template controls were amplified in triplicate with an ABI 7500 Fast Real-time PCR System(Applied Biosystems, USA) using the SYBR green qPCR method. The 25 μl qPCR mixtures contained the following: 12.5 μl of 2 × SYBR green Premix II (Takara), 0.5 μmol/L final concentration of each primer, 0.8 μg/μl bovine serum albumin (BSA), 0.5 μl ROX Reference Dye II, and 1.0 μl template DNA. Reactions consisted of initial denaturation at 95℃ for 2 min, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 55°C for 30 s, and elongation at 72°C for 45 s. A dissociation curve was run at the end of each reaction to verify amplification of a single PCR product. Data were analyzed against the standard curve using the 7500 software v2.0.6 (Applied Biosystems).

Bacterial communities comparison

Sediment bacterial community compositions were determined by DGGE (denaturant gradient gel electrophoresis) of PCR products using the 341F-GC and 534R primers as described by Muyzer, de Waal and Uitterlinden (1993). PCR products were purified with a Takara MiniBEST DNA Fragment Purification Kit v4.0, prior to running on a 6% polyacrylamide gel in 1 × TAE buffer with loading buffer over a chemical denaturing gradient of urea and formamide equivalent to 40−60% denaturant. Electrophoresis was performed using the D-Code system (Bio-RadLaboratories, UK) at 60V constant current and 60°C for 16 h. Gel images were recorded using the Gel Doc™ XR+ System (Bio-Rad Laboratories, UK) after staining with 1× SYBR green (1∶10,000 dilution, Molecular Probes, USA). DGGE profiles were acquired and identified with the Image Lab™ Software (Bio-Rad Laboratories, UK). DGGE bands were scored based on their relative intensities, which expressed as the ratio between the absolute intensity of that band and the total intensity of all bands in that lane. Each band was defined as operational taxonomic units (OTUs) for analysis. Clustering analysis and diversity indices estimation were performed depending on the DGGE intensity matrix using the Primer v6.1 (Primer-E Ltd, UK). Selected DGGE bands were excised and re-amplified for sequencing by Life Technologies (Beijing, CN).

Two samples were selected based on the cluster results to construct bacterial clone libraries for phylogenetic analyses. Bacterial 16S rDNA was amplified from the genomic DNA with the universal primers 8F and 1492R using the reaction mixture and conditions described by Zhang, Ki and Qian (2008). Cloning of PCR product followed previous detailed procedures as Li *et al.* (2013). Cloned gene fragments were checked by agarose gel electrophoresis. Aliquots (20μl) of individual PCR products were digested with restriction enzymes of *Msp*I and *Hae*III (Takara), then subjected to electrophoresis in 3% agarose to generate restriction fragment length polymorphism (RFLP) profiles. Next, band patterns were used to classify clones into OTUs. Good's coverage was calculated as [1−(n/N)] to illustrate the degrees of bacterial diversity captured, where n is the number of OTUs that had been observed once and N is the total number of OTUs in the sample. One or more clones from each OTU were randomly selected and sequenced by Life Technologies (Beijing, CN). The resulting sequences were checked for chimeras using Uchime v4.2.40 and the silva.gold.bacteria database, then deposited in the GenBank database under accession numbers KM823665 to KM823776. Classification was conducted by Mothur v1.29 using the RDP training set (Schloss *et al.*, 2009).

Statistical analyses

The relationships between bacterial composition(matrix of DGGE profiles) and environmental variables were determined by standard constrained analysis using the Canoco v5.0 software (Biometris, Netherlands). RDA(redundancy analysis) was performed with the linear method since DCA (detrended correspondence analysis) on OUTs variables revealed the length of the first axis gradient was long (>2). Prior to DCA and RDA, OUTs values were square root transformed and environmental variables values were normalized by *z*-score. The significance of the canonical axes was assessed by permutation tests with 999 unrestricted Monte Carlo

permutations ($P<0.05$).

One-way analysis of variance (ANOVA) was processed by SPSS 13.0 to determine whether there were significant differences among the estimated parameters. A P value<0.05 was considered to indicate significance.

RESULTS

Environmental characteristics

A wide variety of parameters were measured to characterize the Jiaolai River sediments. The physical characteristics of the sediments are summarized in Table 1, and the chemical characteristics of the sediments are given in Table 2 and 3. Peak flows in summer corresponded to a sharp decrease of salinity at J1 and J2 in August, which could be explained by the increase of fresh water runoff. The effect of river flow on chemical contaminants was obvious in that the heavy metals concentrations were highest from August to October. Hypernutrification is the main environmental problem in Jiaolai River owing to infiltration by fertilizer, livestock, and point source wastewater. The ammonium concentration in pore water reached more than 500 μmol/L in some samples, while it ranged from 35 to 500 μmol/L in typical eutrophic water samples (Corbett, 2010).

Differences in mean values of environmental variables between sampling sites and seasons were identified by ANOVA. The results revealed significant differences in density, sediment contents, and median grain size (MGS) between sites, as well as significant differences in phosphate concentration and temperature between seasons. Moreover, heavy metals were positively correlated with each other ($P<0.05$).

Table 1. *Physical characteristics of the sediment samples*

Sample	Temperature(℃)	Salinity	pH	Water ratio(%)	MGS(ϕ)	Clay(V %)	Silt(V %)	Sand(V %)
J104	18.31	26.49	8.11	23.74	3.606	1.238	29.136	69.626
J204	15.43	13.02	8.09	28.54	3.664	1.291	26.435	72.274
J304	19.78	2.50	9.09	33.42	3.950	2.835	45.399	51.766
J404	16.50	1.47	8.86	21.70	4.328	3.514	59.607	36.878
J108	24.28	2.65	8.29	23.08	3.573	1.501	16.761	81.738
J208	22.51	3.21	8.06	24.28	3.797	2.703	37.581	59.716
J308	25.60	1.21	7.56	29.42	3.898	4.285	43.199	52.517
J408	26.14	1.06	8.15	27.50	4.563	4.588	59.376	36.035
J110	15.98	25.97	7.72	24.68	3.571	1.416	20.030	78.554
J210	14.17	12.07	7.77	25.74	4.064	3.357	49.047	47.596
J310	15.73	3.68	8.59	20.60	3.824	3.902	41.274	54.824
J410	17.85	2.16	9.10	18.39	5.539	7.030	66.391	26.580

Table 2. *Nutrients, TOC and TN concentrations of the Jiaolai River sediment samples*

Sample	NH_4^+(μmol/L)	NO_2^-(μmol/L)	NO_3^-(μmol/L)	PO_4^{3-}(μmol/L)	TOC(%)	TN(‰)
J104	524.22	0.62	1.05	2.16	0.34	0.56
J204	647.02	0.76	1.53	2.41	0.45	0.57
J304	39.97	1.03	3.58	2.78	0.23	0.72
J404	82.12	0.26	0.37	0.86	0.82	1.03
J108	76.67	5.05	1.96	0.27	0.52	0.71
J208	22.78	2.45	3.55	0.77	0.08	0.15
J308	695.56	8.57	10.69	1.42	0.10	0.35
J408	57.78	0.35	1.90	1.61	1.67	1.54
J110	532.47	0.69	1.73	6.18	0.39	0.27
J210	697.65	1.09	1.35	5.26	1.06	0.64
J310	15.05	2.83	4.61	3.16	0.78	0.43
J410	1.53	0.48	1.24	1.30	0.61	0.22

Table 3. *Heavy metal contents of the sediments*

Sample	Pb(ppm)	Cr(ppm)	Mn(ppm)	Ni(ppm)	Cu(ppm)	Zn(ppm)
J104	0.416	0.267	2.707	0.378	0.347	2.101
J204	0.367	0.365	2.325	0.402	0.289	1.830
J304	0.572	0.423	3.404	0.572	0.456	2.944
J404	0.677	0.318	0.897	0.390	0.376	2.449
J108	0.503	0.274	1.649	0.347	0.238	1.363
J208	0.514	0.240	1.067	0.305	0.249	1.257
J308	0.433	0.170	1.858	0.297	0.300	1.424
J408	1.392	1.166	8.582	1.431	0.888	30.184
J110	0.540	0.438	1.712	0.326	0.312	0.866
J210	0.517	0.449	2.127	0.419	0.383	3.087
J310	0.481	0.527	3.755	0.632	0.568	6.843
J410	1.275	1.187	8.086	0.879	0.871	4.270

Bacterial abundance

Standard curves generated using plasmids containing cloned 16S rRNA genes to relate the threshold cycle(C_T) to gene copies number revealed linearity ($R^2 = 0.98$, $slope = -3.05$). The bacterial abundance and standard deviation (<10%) of each sample was shown in Figure 2. The results revealed a heterogeneous distribution of the sediment bacterial 16S rDNA gene abundance among sampling sites of the Jiaolai River, with J304 containing the highest number of gene copies (2.12×10^8 copies/g dry sediment) and J210 the lowest (2.90×10^7 copies/g dry sediment). No significant differences in bacterial abundance between sites or seasons were observed.

Bacterial abundance was significantly negatively correlated with ammonium content ($R^2 = -0.58$, $P = 0.049$), indicating ammonium overloading in the Jiaolai River system.

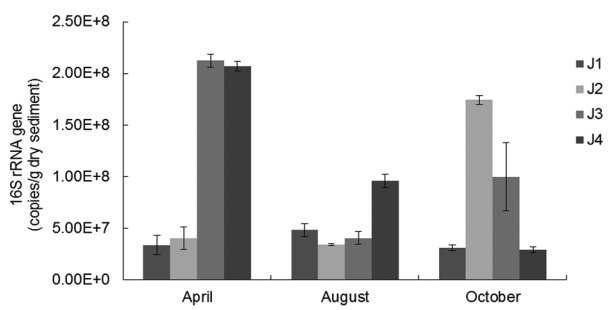

Figure 2. Copy numbers of total bacterial 16S rDNA in sediment samples of Jiaolai River

Bacterial community diversity

To assess differences and dynamics of bacterial diversity in the Jiaolai River sediment, 16S rDNA fragments were analyzed by DGGE and hierarchical cluster analysis was applied based on the DGGE profiles (Figure 3). A total of 43 bacterial OTUs were detected. Bands at the same position in different lanes had identical sequences, which were confirmed by the sequencing results. Fourteen bands were sequenced and blasted with NCBI database (Figure 3A and Table 4). All sequences showed similarities of more than 94% to uncultured bacterium clones from dust, soil, freshwater and seawater. Additionally, they were affiliated to Proteobacteria, Bacteroidetes, Cyanobacteria and uncultured bacterial clones, which are considered the main bacterial phyla in sediment.

As shown in Table 5, the bacterial diversity and richness were calculated from the relative intensity data of the DGGE bands. The most diverse site was J104, for which 29 different bacterial bands were dentified. In contrast, the least diverse site was J304, for which 20 bands were observed. The Shannon-Weaver diversity index and Pielou's evenness varied between 1.98–2.77 and 0.65–0.85 respectively. The mean diversity of J3 was 20.7, which was significantly lower than that of other sites ($P = 0.024$), whereas Shannon-Weaver and Pielou's indicesdid not differ significantly between sites or seasons, although they were slightly higher in samples collected in April.

DGGE banding patterns were used to construct a hierarchical dendrogram (Figure 5B). J308 was more dissimilar from other samples, forming a cluster at 33.6%. Additionally, sample J404 was separated into a sub-cluster at 48.1%. The most similar DGGE band patterns were J204 and J108, which had a similarity of 73.2%. The results showed a heterogeneous distribution of the bacterial composition in Jiaolai River sediment, where no samples were obviously grouped together with sites or seasons.

Correlation of bacterial principal components with environmental variables

RDA of the bacterial composition represented by DGGE profiles was used to reveal their relationship with the environmental variables which passed the forward selection (Figure 4). Covarying variables as heavy metals were checked to minimize collinearity in the RDA analyses. The sum of all eigenvalues indicated that total variation explained by environmental variation of 100.0%. The first two RDA axes explained 45.3% of the cumulative variance in the bacterial composition. RDA1 represented a positive gradient due to nitrate and nitrite, which was indicated by a correlation coefficient of 0.59. RDA2 showed a positive salinity gradient with a correlation coefficient of 0.56.

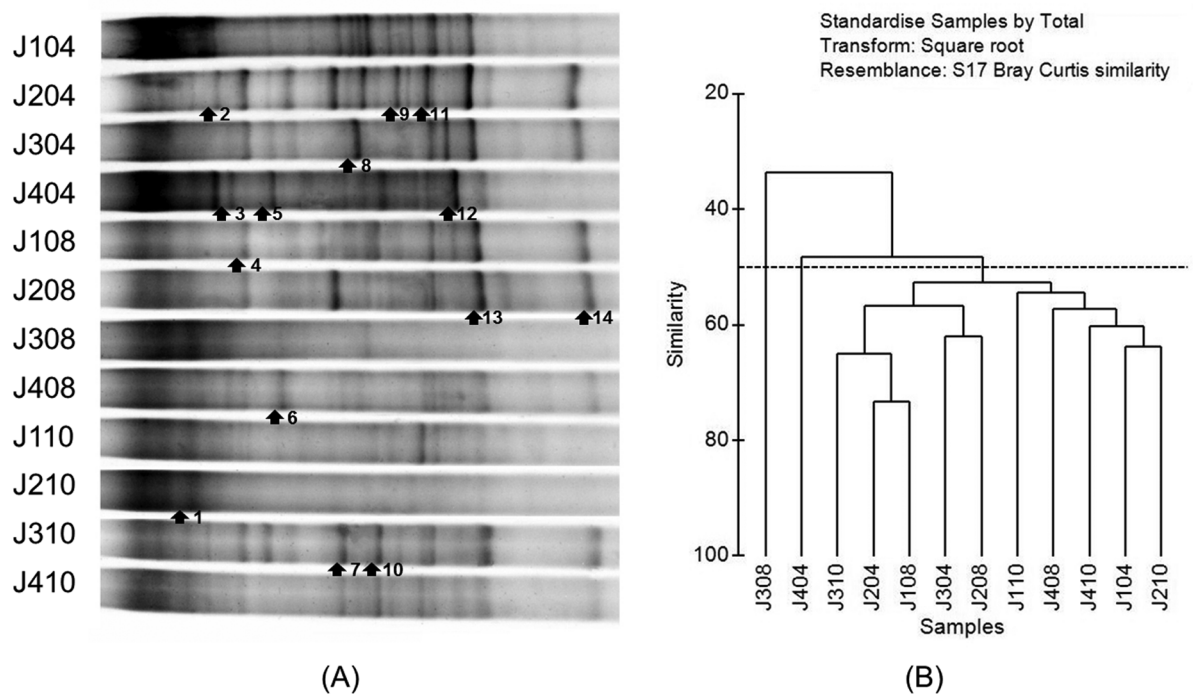

Figure 3. DGGE-profile of bacterial 16S rDNA recovered from the sediments of Jiaolai River with 14 bands (indicated by numbers) recovered for sequencing (A) and cluster result of DGGE patterns (B).

Table 4. *Bacterial phylotype detected by PCR-DGGE*

Band	Classification	Closest match by BLAST (accession No.)	Identity
1	*Actinobacteria*	Soil *Streptomyces* sp. clone (EU873547)	96%
2	*Gammaproteobacteria*	Mangrove soil bacterium clone (GU583977)	97%
3	*Betaproteobactaria*	Humic lake beta proteobacterium clone (AY792245)	96%
4	*Alphaproteobacteria*	Green Bay ferromanganous micronodule bacterium (AF293000)	98%
5	*Flavobacteriia*	Lake sediment bacterium clone (GQ472394)	99%
6	*Flavobacteriia*	Reservoir sediment bacterium clone (DQ833502)	99%
7	*Betaproteobactaria*	Aerosols bacterium clone (KF010682)	94%
8	*Alphaproteobacteria*	Eutrophic bay bacterium clone (KC836063)	98%
9	*Flavobacteriia*	Antarctic lake bacterium strain (AJ441000)	97%
10	*Chloroplast*	Grassland soil bacterium clone (EF516681)	95%
11	*Betaproteobactaria*	Red soil bacterium (FR687424)	94%
12	*Alphaproteobacteria*	RDX-contaminated ground water bacterium clone (EU907883)	98%
13	*Betaproteobactaria*	Water bacterium clone (KM182886)	99%
14	*Betaproteobactaria*	Soil bacterium clone (KF189012)	100%

Table 5. *Diversity indices of different samples based on DGGE profiles*

Index	J104	J204	J304	J404	J108	J208	J308	J408	J110	J210	J310	J410
S^a	29	26	20	27	25	23	21	26	26	24	21	22
H'^b	2.59	2.62	2.39	2.52	2.44	2.47	1.98	2.49	2.77	2.06	2.54	2.21
J'^c	0.77	0.81	0.80	0.76	0.76	0.79	0.65	0.76	0.85	0.65	0.83	0.72

[a] S: Total OTUs;
[b] H': Shannon-Wiener, $H' = -\sum(P_i \ln P_i)$, where P is the percentage of individual OTUs.
[c] J': Pielou's evenness, $E = H/H_{max}$, where $H_{max} = \ln S$.

Based on the partial Monte Carlo permutation test, the forward selection of environmental variables in RDA indicated that the bacterial OTUs were significantly affected by nitrate concentration, and the variance explained by this environmental variable accounted for 16.4% of the total variance in OTUs data ($P<0.05$, $F=2.0$). Ammonium and salinity also showed significant effects, explaining 12.8% and 10.4% of the total variance. Moreover, as shown in Figure 4, nitrate was clearly associated with bacterial communities in four clusters: J308 with high concentration (>10μmol/L), samples in the purple oval with slightly elevated concentrations (3–5 μmol/L), samples in the green oval with medium concentrations (1–2 μmol/L), and J408 with low concentration (<1 μmol/L).

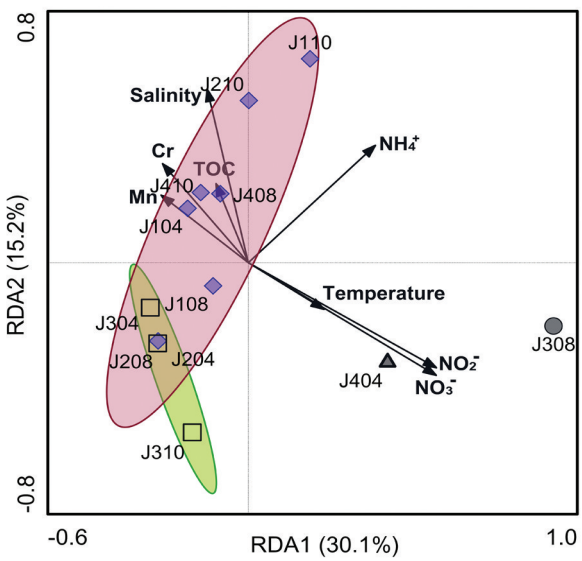

Figure 4. RDA ordination plots for the first two principal dimensions of the relationship between the bacterial communities and environmental variables in Jiaolai River sediments. Correlations between environmental factors and RDA axes were represented by the length and angle of arrows with a cutoff of $r^2 = 0.2$

Bacterial community structure of two representative samples

J304 and J308 were selected for clone library analysis based on the clustering results of DGGE profiles. RFLP screening resulted in 254 clones classified into 112 OTUs (63 from J304 and 49 from J308). Good's coverage was 78.7% at J304 and 83.5% at J308, indicating that bacterial composition could be reflected by the library, although more OTUs should exist *in situ*.

Phylogenic analysis revealed that 112 full-length 16S rDNA sequences were mainly grouped within six phyla as follows: Proteobacteria (47.3%), Firmicutes (27.7%), Chloroflexi (10.7%), Planctomycetes (4.5%), Bacteroidetes (3.6%) and Acidobacteria (3.5%). The Proteobacteria were highly dominant in the library and composed of four subclasses: Gammaproteobacteria (27.7%), Alphaproteobacteria (11.6%), Betaproteobacteria (5.4%), and Deltaproteobacteria (2.7%). There were several clones related to anaerobes, such as the Firmicutes, *Clostridium* sp. and *Bacillus* sp., the Betaproteobacteria, *Denitratisoma* sp., and the Deltaproteobacteria, *Desulfatimicrobium* sp.

The differences between the two libraries based on sequence data were analyzed by phylotype composition and taxonomic features (Figure 5). When compared with J304, remarkable characteristics of the bacterial community of J408 were the disappearance of Betaproteobacteria and the occurrence of Clostridia.

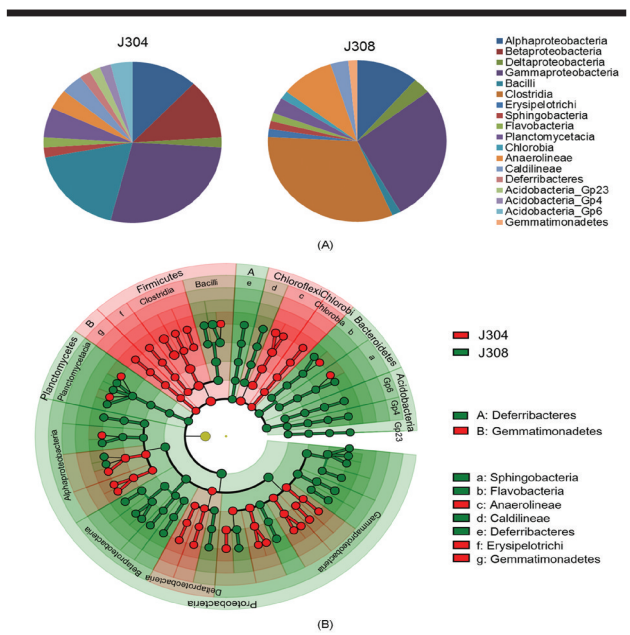

Figure 5. Comparison of the phylotype distribution (A) and detailed different species (B) in J304 and J308 clone libraries

DISCUSSION

DNA-based methods improve our understanding of environmental microbes, especially their vast uncultured components. Construction of 16S rDNA sequences is widely used as a standard tool for identifying structure and function within complex microbial communities because of the ease of amplification, presence of universal primer sites, and alignability across species (Tedersoo *et al.*, 2010). The next generation of high throughput techniques has revealed that environmental microorganisms have much greater diversity than was revealed by Sanger sequencing. Owing to the need for signal amplification of target genes, it is not currently possible to remove the error of PCR biases associated with the two aforementioned techniques. Traditional Sanger sequencing can read full-length 16S rRNA genes, which provides accurate assessment of the taxonomic distribution. Conversely, the increased number of reads enables high throughput sequencing to recover more species and detect rarer individuals (Hamady and Knight, 2009). However, it is likely that these two sequencing method would yield similar results based on the roughly similar phylogenetic information in microbial communities (Schulze-Schweifing, Banerjee, and Wade, 2014; Tedersoo *et al.*, 2010; Wang *et al.*, 2014). Considering the focus of our study, traditional Sanger clone library approaches were used to compare the occurrence and composition of bacterial communities in sediments collected along the Jiaolai River.

Bacterial communities in Jiaolai River sediments

Although the Jiaolai River is an important source of irrigation and industrial water for local populations, no studies have focused on bacterial community dynamics as a result of environmental stresses. In the present study, bacterial community abundance and structure among four sites sampled during different seasons of the same year were explored by molecular and statistical analyses. The bacterial abundance was concurrent with that observed for other freshwater sediments (Dorador *et al.*, 2010; Jiang *et al.*, 2007), but lower than the typical range reported for sediment environments (Bouskill *et al.*, 2010; Eiler and Bertilsson, 2007). The DGGE patterns suggested large numbers of bacterial species and high levels of bacterial diversity in river sediments, which was also confirmed by the Shannon-Weaver diversity index (Bushaw-Newton *et al.*, 2012; Cetecioğlu *et al.*, 2009). Proteobacteria, Bacteroidetes, Firmicutes, Planctomycetes, and Actinobacteria were all recorded in the bacterial communities and were considered to be the most abundant bacterial phyla in the river bacterial database (Newton *et al.*, 2011). The absence of common phyla such as Cyanobacteria did not necessarily mean it was inexistent *in situ*, and may have simply reflected the limitations of DGGE and the library capacity.

DGGE has been shown to be a powerful tool for evaluating differences in bacterial communities based on comparison of DNA fingerprint patterns, although it may not accurately reflect the exact species present in a given mixture owing to its bias and limitations inherent in any electrophoresis technique (Vaz-Moreira *et al.*, 2013). To identify the bands, three were selected from the same position on the DGGE gel of different lanes and sequenced to confirm that they all had the same nucleotide composition. Temporal dynamics and spatial variation of bacterial communities were revealed by cluster analyses of the DGGE profile with dissimilarities between 26.8% and 78.1%. Although variations in bacterial communities were observed with dissimilarities of 40.0% to 78.1% within each site, analysis of 16S rDNA genes of sediment did not reveal changes in the distribution of bacterial communities with respect to sampling month. Seasonality of river bacterial communities has been observed in several river systems, and such changes are mainly driven by temperature and flow rate (Crump and Hobbie, 2005; Feris *et al.*, 2003). A significant relationship between differences in bacterial composition and spatial distance was also previously found in the study of two Central European streams and a South Carolina stream (Beier, Witzel, and Marxsen, 2008). However, neither clustering analyses nor the Mantel test ($P=0.57$) could detect the continuous bacterial distribution in Jiaolai River sediments. These results indicate that heavy pollution has replaced temperature and spatial distance as the greatest influencing factor of community changes.

Bacteria–environmental interaction

Nonpoint source pollution from fertilizer and point source pollution from sewage have had adverse impacts on environmental quality of sediments in the Jiaolai River. Physical and chemical analyses conducted in the present study verified that the sediments were heavily polluted and subjected to excessive nutrification and heavy metals contamination. Moreover, this river has been subject to saline water intrusion due to over-exploitation of groundwater since the 1960s (Xue *et al.*, 1993). It is difficult to evaluate the environmental impacts of these combined anthropogenic pollutions exclusively based on physical and chemical analysis of the interactive effects of contaminants. Bacterial communities were compared to evaluate the integrated effects of pollutants on the biota.

Among the considered environmental variables, it was likely that nutrient concentrations of nitrogen played a significant role in determining bacterial communities in sediments. The relationship between nitrogen eutrophication and bacteria has previously been revealed based on the bacterial abundance being positively coincident with concentrations of nitrogen (Fazi *et al.*, 2013). Here, the ammonium concentration was negatively correlated with bacterial abundance, which supports the idea that ammonium inhibited rather than triggered bacterial growth within the Jiaolai River sediments. A similar relationship between bacterial biomass and nitrogen nutrients was also found in a study of Chesapeake Bay, which receives a large amount of anthropogenic nitrogen every year (Hong *et al.*, 2014). It is expected that other environmental factors including salinity, pH and temperature will influence bacterial distribution as reported in previous studies (Beier, Witzel, and Marxsen, 2008). However, the experimental sites of the current study showed limited differences in bacterial composition in response to the aforementioned variables.

The responses of bacterial communities to the environment through biogeochemical processes were also investigated based on the clone library data. The mainly composition differences of two libraries were Betaproteobacteria and Clostridia. Betaproteobacteria was associated with degradation processes and played a key role in nitrogen cycling, especially for nitrogen fixation and ammonium oxidizing. Ammonium oxidation is traditionally considered as the first and rate-limiting step of nitrification, in which ammonia is oxidized to nitrite and further to nitrate. The extremely high content of nitrate in J408 might restrain

the reaction, then cause the loss of Betaproteobacteria. Anaerobic circumstances might have been responsible for the occurrence of Clostridia as they were obligate anaerobes. However, the dissolved oxygen in the sediment was not considered in this study. Distinct amounts of Betaproteobacteria and Clostridia in the constructed libraries and their consequences could be assumed based on our knowledge of nitrogen cycling. Specifically, (i) excess nitrite and nitrate might inhibit the ammonia-oxidizing steps of nitrification as reaction product and thus reduse the abundance of Betaproteobacteria, which are known to catalyze this oxidization step (McCaig *et al.*, 1999). Additionally, (ii) impeding nitrification pathway may promote the competitive reaction of anammox by the oxidation of ammonium coupled to nitrite with the production of nitrogen, stimulating growth of anaerobic ammonium-oxidizing bacteria. (iii) Denitrification may proceed, impeding nitrification under the high concentration of nitrite and nitrate. These assumptions will be verified by future studies focusing on nitrogen removal processes and bacterial participation, as well as their responses to the environmental variables, especially for eutrophication in the Jiaolai River.

CONCLUSIONS

Bacterial abundance ranged from 2.90×10^7 to 2.12×10^8 copies/g and the main phyla included Proteobacteria, Bacteroidetes, Firmicutes, Planctomycetes, and Actinobacteria. Sediment environmental parameters indicated heavy pollution in the river system, with spatial variations in physical characteristics and seasonal variations in temperature and phosphate concentration. Differences in bacterial abundance and composition were also observed among samples; however, no significant differences between seasons or sites were observed, except that diversity was obviously lower at site J3. Variations in bacterial abundance revealed a significantly negative correlation with ammonium within the sediment. Nitrate concentration has been shown to be a significant contributor of stress that leads to clustering of bacterial communities into groups according to their compositions. Nitrogen eutrophication was shown to be the most serious problem for biota in the Jiaolai River. Research conducted to improve the health of the Jiaolai river system should explore the ecological mechanisms controlling nitrogen removal and develop efficient strategies for remediation of excess nitrogen.

Differences in bacterial composition between two sites characterized by high and medium nitrate levels revealed trends suggested to be related to nitrogen biochemical cycling, which indicated that shifts in bacteria would in turn affect the surrounding environment. Although environmental stresses could drive the functional instability of bacteria, little is known about the consequence of current changes to the ecosystem or if these changes lead to improvement or deterioration (Dong *et al.*, 2009; Zhang *et al.*, 2010). We demonstrated a potential relationship between nitrogen stresses and bacteria corresponding to their cycling process in this study; therefore, future studies should be conducted to determine how bacterial function is regulated by environmental stresses, as well as its linkage to biogeochemical cycling.

ACKNOWLEDGMENTS

The authors would like to thank Shuxian Yu, Jia Yue and Zhengyi Liu for their technical assistance. We acknowledged the Analysis and Test centering of Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences for measurement of environmental parameters. This research work was financially supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA11020305) and the National Natural Science Foundation of China (No. 41106100, No. 41176144).

LITERATURE CITED

Adair, K. L. and Schwartz, E., 2008. Evidence that ammonia-oxidizing archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. *Microbial Ecology*, 56(3), 420-426.

Beier, S.; Witzel, K.-P., and Marxsen, J., 2008. Bacterial community composition in Central European running waters examined by temperature gradient gel electrophoresis and sequence analysis of 16S rRNA genes. *Applied and Environmental Microbiology*, 74(1), 188-199.

Blanck, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. *Human and Ecological Risk Assessment*, 8(5), 1003-1034.

Bouskill, N. J.; Barker-Finkel, J.; Galloway, T. S.; Handy, R. D., and Ford, T. E., 2010. Temporal bacterial diversity associated with metal-contaminated river sediments. *Ecotoxicology*, 19(2), 317-328.

Bushaw-Newton, K. L.; Ewers, E. C.; Velinsky, D. J.; Ashley, J. T., and MacAvoy, S. E., 2012. Bacterial community profiles from sediments of the Anacostia River using metabolic and molecular analyses. *Environmental Science and Pollution Research*, 19(4), 1271-1279.

Cetecioğlu, Z.; Ince, B. K.; Kolukirik, M., and Ince, O., 2009. Biogeographical distribution and diversity of bacterial and archaeal communities within highly polluted anoxic marine sediments from the marmara sea. *Marine Pollution Bulletin*, 58(3), 384-395.

Corbett, D. R., 2010. Resuspension and estuarine nutrient cycling: insights from the Neuse River Estuary. *Biogeosciences*, 7(10), 3289-3300.

Crump, B. C. and Hobbie, J. E., 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnology and Oceanography*, 50(6), 1718-1729.

Dong, L. F.; Smith, C. J.; Papaspyrou, S.; Stott, A.; Osborn, A. M., and Nedwell, D. B., 2009. Changes in benthic denitrification, nitrate ammonification, and anammox process rates and nitrate and nitrite reductase gene abundances along an estuarine nutrient gradient (the Colne Estuary, United Kingdom). *Applied and Environmental Microbiology*, 75(10), 3171-3179.

Dorador, C.; Vila, I.; Remonsellez, F.; Imhoff, J. F., and Witzel, K. P., 2010. Unique clusters of Archaea in Salar de Huasco, an athalassohaline evaporitic basin of the Chilean Altiplano. *FEMS Microbiology Ecology*, 73(2), 291-302.

Eiler, A. and Bertilsson, S., 2007. Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. *Applied and Environmental Microbiology*, 73(11), 3511-3518.

Fazi, S.; Vázquez, E.; Casamayor, E. O.; Amalfitano, S., and Butturini, A., 2013. Stream hydrological fragmentation drives

bacterioplankton community composition. *PLoS ONE*, 8 (5), e64109.

Feris, K.; Ramsey, P.; Frazar, C.; Rillig, M.; Gannon, J., and Holben, W., 2003. Structure and seasonal dynamics of hyporheic zone microbial communities in free-stone rivers of the estern United States. *Microbial Ecology*, 46(2), 200–215.

Freese, H.; Karsten, U., and Schumann, R., 2006. Bacterial abundance, activity, and viability in the eutrophic river Warnow, Northeast Germany. *Microbial Ecology*, 51(1), 117–127.

Gao, X.; Zhou, F., and Chen, C.-T. A., 2014. Pollution status of the Bohai Sea: An overview of the environmental quality assessment related trace metals. *Environment International*, 62, 12–30.

Hamady, M. and Knight, R., 2009. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research*, 19(7), 1141–1152.

Han, D.; Song, X.; Currell, M. J.; Yang, J., and Xiao, G., 2014. Chemical and isotopic constraints on evolution of groundwater salinization in the coastal plain aquifer of Laizhou Bay, China. *Journal of Hydrology*, 508, 12–27.

Hong, Y.; Xu, X.; Kan, J., and Chen, F., 2014. Linking seasonal inorganic nitrogen shift to the dynamics of microbial communities in the Chesapeake Bay. *Applied Microbiology and Biotechnology*, 98(7), 3219–3229.

Jennerjahn, T. C., 2012. Biogeochemical response of tropical coastal systems to present and past environmental change. *Earth-Science Reviews*, 114(1–2), 19–41.

Jiang, H.; Dong, H.; Yu, B.; Liu, X.; Li, Y.; Ji, S., and Zhang, C. L., 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environmental Microbiology*, 9(10), 2603–2621.

Jiang, Y.; Jin, L., and Lin, T., 2011. Higher water tariffs for less river pollution—Evidence from the Min River and Fuzhou City in China. *China Economic Review*, 22(2), 183–195.

Li, J.; Li, F.; Yu, S.; Qin, S., and Wang, G., 2013. Impacts of mariculture on the diversity of bacterial communities within intertidal sediments in the Northeast of China. *Microbial Ecology*, 66(4), 861–870.

Liu, G.-H.; Rajendran, N.; Amemiya, T., and Itoh, K., 2011. Bacterial community structure analysis of sediment in the Sagami River, Japan using a rapid approach based on two-dimensional DNA gel electrophoresis mapping with selective primer pairs. *Environmental Monitoring and Assessment*, 182 (1–4), 187–195.

Mahmoudi, N.; Porter, T. M.; Zimmerman, A. R.; Fulthorpe, R. R.; Kasozi, G. N.; Silliman, B. R., and Slater, G. F., 2013. Rapid degradation of deepwater horizon spilled oil by indigenous microbial communities in Louisiana saltmarsh sediments. *Environmental Science & Technology*, 47(23), 13303–13312.

Manter, D. K.; Weir, T. L., and Vivanco, J. M., 2010. Negative effects of sample pooling on PCR-based estimates of soil microbial richness and community structure. *Applied and Environmental Microbiology*, 76(7), 2086–2090.

McCaig, A. E.; Phillips, C. J.; Stephen, J. R.; Kowalchuk, G. A.; Harvey, S. M.; Herbert, R. A.; Embley, T. M., and Prosser, J. I., 1999. Nitrogen cycling and community structure of proteobacterial β-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments. *Applied and Environmental Microbiology*, 65(1), 213–220.

Muyzer, G.; de Waal, E., and Uitterlinden, A., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59(3), 695–700.

Nadkarni, M. A.; Martin, F. E.; Jacques, N. A., and Hunter, N., 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, 148(1), 257–266.

Neu, V.; Neill, C., and Krusche, A. V., 2011. Gaseous and fluvial carbon export from an Amazon forest watershed. *Biogeochemistry*, 105(1–3), 133–147.

Newton, R. J.; Jones, S. E.; Eiler, A.; McMahon, K. D., and Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews*, 75(1), 14–49.

Pimpunchat, B.; Sweatman, W. L.; Wake, G. C.; Triampo, W., and Parshotam, A., 2009. A mathematical model for pollution in a river and its remediation by aeration. *Applied Mathematics Letters*, 22(3), 304–308.

Sandaa, R. A.; Torsvik, V.; Enger, Ø.; Daae, F. L.; Castberg, T., and Hahn, D., 1999. Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. *FEMS Microbiology Ecology*, 30(3), 237–251.

Schloss, P. D.; Westcott, S. L.; Ryabin, T.; Hall, J. R.; Hartmann, M.; Hollister, E. B.; Lesniewski, R. A.; Oakley, B. B.; Parks, D. H., and Robinson, C. J., 2009. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–7541.

Schulze-Schweifing, K.; Banerjee, A., and Wade, W. G., 2014. Comparison of bacterial culture and 16S rRNA community profiling by clonal analysis and and pyrosequencing for the characterisation of the caries-associated microbiome. *Frontiers in Cellular and Infection Microbiology*, 4, 164–178.

Snape, I.; Scouller, R. C.; Stark, S. C.; Stark, J.; Riddle, M. J., and Gore, D. B., 2004. Characterisation of the dilute HCl extraction method for the identification of metal contamination in Antarctic marine sediments. *Chemosphere*, 57(6), 491–504.

Su, S.; Zhi, J.; Lou, L.; Huang, F.; Chen, X., and Wu, J., 2011. Spatio-temporal patterns and source apportionment of pollution in Qiantang River (China) using neural-based modeling and multivariate statistical techniques. *Physics and Chemistry of the Earth, Parts A/B/C*, 36(9–11), 379–386.

Tedersoo, L.; Nilsson, R. H.; Abarenkov, K.; Jairus, T.; Sadam, A.; Saar, I.; Bahram, M.; Bechem, E.; Chuyong, G., and Kõljalg, U., 2010. 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist*, 188(1), 291–301.

Vaz-Moreira, I.; Egas, C.; Nunes, O. C., and Manaia, C. M., 2013. Bacterial diversity from the source to the tap: a comparative study based on 16S rRNA gene-DGGE and culture-dependent methods. *FEMS Microbiology Ecology*, 83(2), 361–374.

Wang, L.-Y.; Ke, W.-J.; Sun, X.-B.; Liu, J.-F.; Gu, J.-D., and Mu, B.-Z., 2014. Comparison of bacterial community in aqueous and oil phases of water-flooded petroleum reservoirs using pyrosequencing and clone library approaches. *Applied Microbiology and Biotechnology*, 98(9), 4209–4221.

Xia, N.; Xia, X.; Liu, T.; Hu, L.; Zhu, B.; Zhang, X., and Dong, J., 2014. Characteristics of bacterial community in the water and surface sediment of the Yellow River, China, the largest turbid river in the world. *Journal of Soils and Sediments*, 14(11), 1894–1904.

Xu, M.; Zhang, Q.; Xia, C.; Zhong, Y.; Sun, G.; Guo, J.; Yuan, T.; Zhou, J., and He, Z., 2014. Elevated nitrate enriches microbial functional genes for potential bioremediation of complexly contaminated sediments. *The ISME journal*, 8, 1932–1944.

Xue, Y.; Wu, J.; Liu, P.; Wang, J.; Jiang, Q., and Shi, H., 1993. Sea-water intrusion in the coastal area of Laizhou Bay, China: 1. distribution of sea-water intrusion and its hydrochemical characteristics. *Groundwater*, 31(4), 532–537.

Yang, A.; Zhang, X.; Agogué, H.; Dupuy, C., and Gong, J., 2014. Contrasting spatiotemporal patterns and environmental drivers of diversity and community structure of ammonia oxidizers, denitrifiers, and anammox bacteria in sediments of estuarine tidal flats. *Annals of Microbiology*, 1–12.

Zhang, B.; Deng, H.; Wang, H.-l.; Yin, R.; Hallett, P. D.; Griffiths, B. S., and Daniell, T. J., 2010. Does microbial habitat or community structure drive the functional stability of microbes to stresses following re-vegetation of a severely degraded soil? *Soil Biology and Biochemistry*, 42(5), 850–859.

Zhang, W.; Ki, J.-S.; and Qian, P.-Y., 2008. Microbial diversity in polluted harbor sediments I: Bacterial community assessment based on four clone libraries of 16S rDNA. *Estuarine, Coastal and Shelf Science*, 76(3), 668–681.

Zhang, X.; Agogué, H.; Dupuy, C.; and Gong, J., 2014. Relative abundance of Ammonia Oxidizers, Denitrifiers, and Anammox Bacteria in sediments of hyper-nutrified estuarine tidal flats and in relation to environmental conditions. *CLEAN–Soil, Air, Water*, 42(6), 815–823.