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Bacterial diversity in the surface layer of sediments from the East China Sea

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ABSTRACT

Question: What is the bacterial diversity in the surface layer of sediments from the East China Sea?

Methods: We obtained 12 sediment samples at depths of 0–3 cm, 3–5 cm, 5–8 cm, and 8–10 cm at three sediment stations in a coastal area of the East China Sea. We used 16S rRNA gene analyses to characterize the bacteria in the samples. We determined sediment total carbon (TC) and nitrogen (TN) concentrations from dried subsamples. We also measured total organic carbon (TOC) in wet subsamples. We calculated total inorganic carbon (TIC) as TC – TOC. We determined total phosphorus (TP) concentrations colorimetrically from the filtrate remaining after TOC analysis. Redundancy analysis allowed us to describe the relationship between the bacterial communities and the environmental parameters in our samples.

Results: We identified 262 clones and divided them into 15 phylotypes. Proteobacteria (33%), Chloroflexi (9%), and Planctomycetacia (9%) were dominant in the sediments. γ-Proteobacteria comprised a large proportion (15%) of all the clones found. Most bacteria were present in the 3–5 cm or 5–8 cm sediment layers. Sediment total carbon, total nitrogen, total inorganic carbon, and total phosphorus concentrations affected the γ-Proteobacteria, δ-Proteobacteria, ε-Proteobacteria, Chloroflexi, Planctomycetacia, and Bacilli. Total phosphorus and total nitrogen had particularly large impacts on the presence of Chloroflexi and ε-Proteobacteria.

Keywords: bacterial diversity, marine sediment, nutrient concentrations, PCA, RDA, 16S rRNA.

INTRODUCTION

Bacteria are ubiquitous throughout the world's oceans, from coastal to open waters, and in both water and sediments. Community surveys have been performed in many marine environments, including in the oligotrophic open ocean (Fuhrman *et al.*, 1993), coastal temperate waters (Kelly and Chistoserdov, 2001; Acinas *et al.*, 2004), and in marine sediments (Li *et al.*, 1999; Bowman *et al.*,

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2003; Heijs *et al.*, 2008). As important agents for biogeochemical processes in all aquatic environments, benthic bacteria play relevant roles in the structure and dynamics of trophic web networks, and in the remineralization of organic matter (Azam *et al.*, 1983; Azam and Long, 2001). Studies have shown that bacterial fractions in the deep sub-seafloor biosphere may make up 10–33% of the Earth's total biomass, and about 70% of the global prokaryotic biomass (Parkes *et al.*, 1994; Whitman *et al.*, 1998). These studies on microbial ecology and evolution have focused on defining the diversity and distribution of natural microbial communities in the marine environment.

The wide distribution of bacteria is closely related to the diversity of ecosystems. The East China Sea (ECS) is a marginal sea in the northwest of the Pacific Ocean and is influenced by several water masses, including the Kuroshio Current, Tsushima Warm Currents, cold Yellow Sea water, and fresh water from the Yangtze River (Beardsley *et al.*, 1985). Because it is at the interface of land, freshwater, and marine environments, the ECS is extremely complicated and dynamic (Zhang *et al.*, 1999; Jiao *et al.*, 2007). The ECS has a variety of hydrological conditions as well as significant physical and chemical gradients and seasonal differences. Together, all this abiotic diversity promotes a remarkably high biological diversity. The different water masses supply lots of nutrients to the ECS, which play a key role in primary productivity within the marine ecosystem. Deposits of marine creatures to the sea floor also provide high levels of nutrients, in the form of particulate organic material, to the sediment bacteria (Chen and Wang, 2008). In addition, the ECS shelf is relatively shallow, with an average water depth of 60 m, which creates an ever-changing environment and has a considerable impact on the biological conditions for bacterial growth. All of these conditions lead to a high bacterial diversity in the sediments of the ECS.

The economic development and increase in human activities around the coastal areas of the ECS have increased terrigenous inputs into the marine environment; these, in turn, have increased eutrophication and environmental pollution. As a result, the structure and diversity of bacterial communities in the ECS are changing. High organic matter deposition in coastal systems promotes rapid diagenetic processes at the sediment–water interface, which affects the biogeochemical cycling of carbon, nutrients, and many other chemical elements in coastal oceans (Hedges *et al.*, 1999). Knowledge of the sediment microbial communities in the ECS would increase understanding of the ecological effects on biogeochemical cycles, the basis of regulation mechanisms, and the influence of pollutants in ecological degradation.

Although increasing scientific efforts have concentrated on the distribution of bacteria in various oceanic sediments, few data exist for the western part of the Pacific Ocean; this is especially true of analyses that combine bacterial diversity with environmental data. Thus in the present study, we investigated the molecular phylogenic relationships between environmental disturbances and patterns of microbial diversity and activity, and considered their ultimate effects on ecosystem function.

MATERIALS AND METHODS

Sampling effort

We selected three sampling sites (DH-9: 30°45.650′N, 122°06.617′E; DH-13: 31°30.103′N, 123°29.844′E; and DH-18: 30°00.049′N, 122°52.467′E), one within Hangzhou Bay and the other two in the adjacent coastal area of the ECS (Fig. 1). Sediment samples were collected

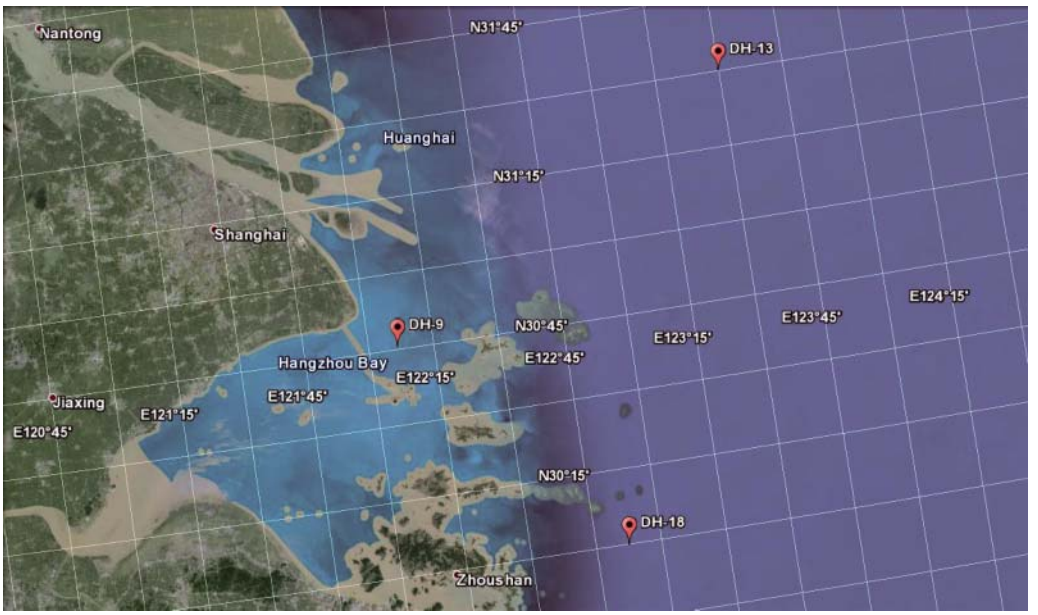


Fig. 1. Map showing the sampling sites in the East China Sea.

using a 0.1-m^2 stainless steel Gray-O'Hara box corer in April 2007. The surface layer (0–10 cm) of sediment from each sectioned core was immediately divided into four layers (0–3, 3–5, 5–8, and 8–10 cm). In total, we obtained 12 marine sediment samples. The samples were labelled according to their geographic location (9, 13, or 18) and layer (1, 2, 3, or 4); for instance, the sample from DH-13 at 3–5 cm depth was labelled sample 132. On collection, we packaged the samples into sterile sample bags and then stored these within further airtight, sterile plastic bags in a freezer at −20°C during our time at sea. The samples were stored at –80°C after returning to the laboratory.

Chemical characteristics

Before analysis, each sediment sample was thawed and manually mixed. Sediment total carbon (TC) and nitrogen (TN) concentrations were determined from dried subsamples using an elemental analyser (Carlo Erba NA1500, Milan, Italy). We also took subsamples (5 g wet weight) and mixed them with 200 mL distilled water in a 500-mL sterile quartz conical flask before rotating for 30 minutes. Following rotation, each subsample was left to settle for 10 minutes and the supernatant was vacuum filtered with a 0.45-μm membrane filter. The level of total organic carbon (TOC) in the filtrate was determined with a TOC analyser (Shimadzu TOC-5050A, Shimadzu Benelux, Den Bosch, the Netherlands) and assumed to represent pore water. Surplus filtrate was stored at −20°C until analysis for total phosphorus (TP). Aliquots of the filtrate were treated with 10 μL ultrapure 1 M $HCl \cdot mL^{-1}$ and stored at 4°C. We determined the TP concentrations colorimetrically on a Nutrient Autoanalyzer-3 (Bran and Luebbe, De Meern, the Netherlands).

DNA isolation, amplification, cloning and sequencing of 16S rRNA gene regions

Each sample was thawed on ice and ~0.5 g (wet weight) was transferred into a Lysing Matrix E tube from a FastDNA spin kit for soil, for DNA extraction with a FastPrep-24 Instrument (MP Biomedicals, Solon, OH, USA). Before PCR amplification, we used a protein nucleic acid detector (Bio-Rad) to measure the concentration and purity of DNA. The bacterial 16S rRNA genes in each sample were amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACG ACTT-3') (Bosshard *et al.*, 2000), with PCR cycling as follows: 95°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, 57.5°C for 30 seconds, and 72°C for 4 minutes, with a final elongation step of 72°C for 10 minutes. The samples were then held at 4°C. The PCR amplifications were performed in a 25-μL volume containing 1 μL template DNA, 2.5 μL $MgCl_2$ buffer, 2 μL of each dNTP, 1 μL of each primer, 0.5 U of Taq DNA polymerase, and 14.5 μL ddH_2O. The PCR products were purified using a TIANgel Midi Purification Kit (TIANGEN), following the manufacturer's instructions. They were quantified with a NanoDrop 3000 (Thermo Fisher Scientific Inc.).

The purified PCR products were cloned into the pMD18-T vector system (TaKaRa, Japan) using *Escherichia coli* DH5α as the host. Positive clones were selected using the blue–white selection method. Libraries were screened for the 1.5-kb 16S rDNA insert by PCR, with M13 primers. The 16S rRNA gene sequences were determined using an ABI 3730XL Automated Sequencer (Applied Biosystems).

Sequence analysis

We submitted the 16S rRNA sequences to the Ribosomal Database Project (http:rdp.cme.msu.edu/) to detect chimeric sequences with the 'Check Chimera tool' and omitted these from the data set (Wang *et al.*, 2007). Similar sequences were identified from the filtered sequences using BLAST (www.ncbi.nlm.nih.gov/BLAST/) to allow phylogenetic analysis. Sequences that were not similar to a known bacterial phylogenetic group in the NCBI taxonomy database were listed as unclassified. Representative sequences of dominant taxa were aligned with CLUSTAL X (Thompson *et al.*, 1994).

Statistical analysis

We calculated Shannon-Wiener indices of diversity, and CHAO1 and ACE estimators of number of species, for all of the samples on the basis of their phylotype distribution (97% similarity). Then, the OTUs were clustered with an overlap identity cut-off of 97% and 99% using DOTUR (Schloss and Handelsman, 2005). Sequences with >97% similarity were considered to be the same phylotype; hence, the phylotype here is defined as a level of grouping that encompasses all sequences that are at least 97% similar. The evolutionary distances of full-length sequences were calculated according to the Kimura two-parameter correction method (Kimura, 1980), and neighbour-joining trees were constructed with 1000 bootstrap replicates using MEGA 4.

Using the vegan package in R (Oksanen *et al.*, 2010), principal components analysis (PCA) was used to test the relationship between taxa and samples, and redundancy analysis (RDA) was employed to further examine the correlation between variation and environmental parameters.

RESULTS

Environmental parameters

At the three sampling locations there were similar chemical characteristics in the pore waters, but the particulate concentrations varied slightly among the different samples (Table 1). The particulate total nitrogen (TN) levels in each sample were significantly different. The average nitrogen contents were low and the TC contents high (19–20 $mg \cdot L^{-1}$), which led to the relatively high TC/TN ratios observed. This indicates that nitrogen might have been limiting throughout. The TOC contents were the highest concentrations of any parameter measured; this may be because of biological waste and human activities. Compared with DH-13 and DH-18, site DH-9 had the lowest concentrations of TN. This may be attributed to the Changjiang and Qiantangjiang rivers, which would have supplied large amounts of fresh water to the area around DH-9. Sampling site DH-18 had the highest TN concentrations, leading to the relatively lower TC/TN ratio observed.

Taxonomic identification and phylogenetic analysis

A total of 262 bacterial 16S rRNA gene sequences were identified (DH-9: 59 clones; DH-13: 101 clones; DH-18: 102 clones). Analysis of the sequences in each sample showed that the ECS sediments had a rich microbial diversity (Fig. 2); Proteobacteria (33%), Chloroflexi

Table 1. Pore water environmental parameters of each sample in the East China Sea

Site	TIC ($mg \cdot L^{-1}$)	TC ($mg \cdot L^{-1}$)	TOC ($mg \cdot L^{-1}$)	TN ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	TC/TN ratio
DH- 9	7.808	19.698	11.890	0.063	0.139	312.67
DH-13	6.576	24.476	17.909	0.120	0.107	203.97
DH-18	6.070	19.701	13.631	0.230	0.141	85.66

Note: TIC = total inorganic carbon, TC = total carbon, TOC = total organic carbon, TN = total nitrogen, TP = total phosphorus.

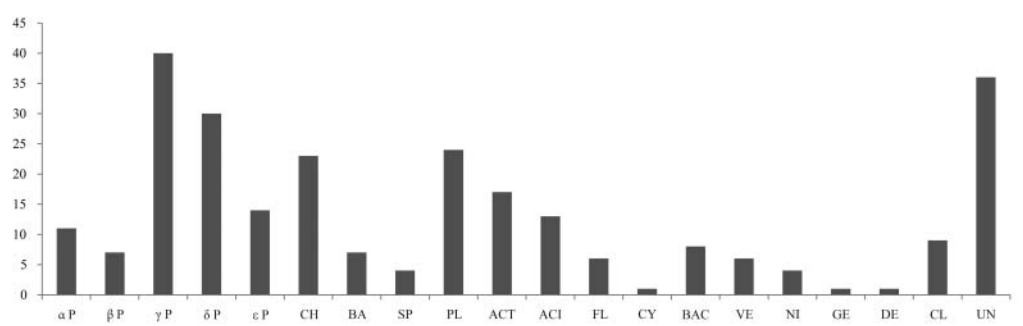


Fig. 2. Proportion of each phenotype in total bacterial sequence. αP, α-Proteobacteria; βP, β-Proteobacteria; γP, γ-Proteobacteria; δP, δ-Proteobacteria; εP, ε-Proteobacteria; CH, Chloroflexi; BA, Bacilli; SP, Spirochaetes; PL, Planctomycetes; ACT, Actinobacteria; ACI, Acidobacteria; FL, Flavobacteria; CY, Cyanobacteria; BAC, Bacteroidetes; VE, Verrucomicrobia; NI, Nitrospira; GE, Gemmatimonadetes; DE, Deinococci; CL, Clostridia; UN, Unclassified.

(9%), and Planctomycetacia (9%) were dominant at these three sample sites. Proteobacteria were represented by the classes α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, and ε-Proteobacteria. Specifically, γ-Proteobacteria comprised 15% of all the clones identified. Other represented groups were Actinobacteria (6%), Acidobacteria (5%), and Bacteroidetes (5%). There were also small numbers of of Spirochaetes, Cyanobacteria, Verrucomicrobia, Nitrospira, Gemmatimonadetes, Deinococci, and Clostridia. Some sequences (14%) were unclassified; these sequences showed very low similarity with known 16S rDNA sequences in the DNA databases, indicating that they were from novel or unculturable bacteria.

Compared with DH-13 and DH-18, the bacterial diversity at site DH-9 appeared low (Fig. 3). The sequences clustered into six major lineages of bacteria: the α, β, γ, δ, and

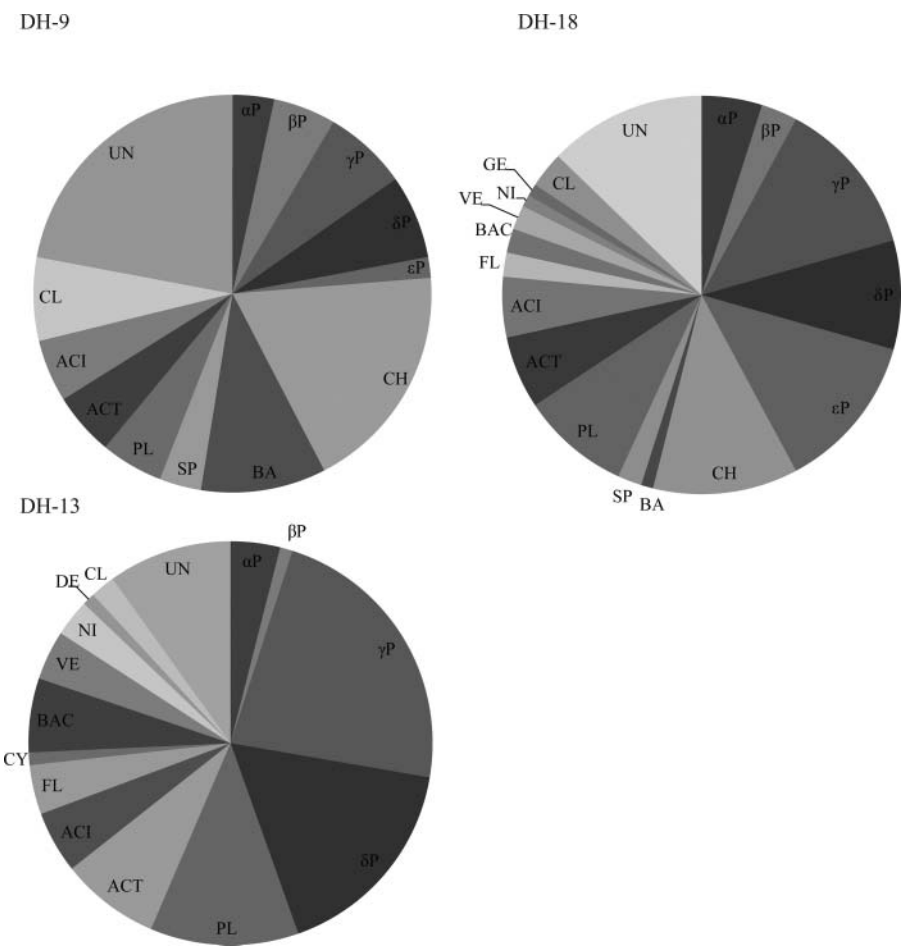


Fig. 3. Composition of clone libraries at class level in each sample. αP, α-Proteobacteria; βP, β-Proteobacteria; γP, γ-Proteobacteria; δP, δ-Proteobacteria; εP, ε-Proteobacteria; CH, Chloroflexi; BA, Bacilli; SP, Spirochaetes; PL, Planctomycetes; ACT, Actinobacteria; ACI, Acidobacteria; FL, Flavobacteria; CY, Cyanobacteria; BAC, Bacteroidetes; VE, Verrucomicrobia; NI, Nitrospira; GE, Gemmatimonadetes; DE, Deinococci; CL, Clostridia; UN, Unclassified.

ε subdivisions of Proteobacteria, as well as Planctomycetacia, Chloroflexi, Actinobacteria, Acidobacteria, and Bacteroidetes. At DH-13 and DH-18, γ-Proteobacteria were the most diverse subphyla, while Chloroflexi were most diverse at DH-9 (accounting for 19%), although Proteobacteria were also dominant there. Bacilli (of Firmicutes) were detected mainly at DH-9. Hence, the presence of Firmicutes may suggest pollution in the nearshore sampling sites (Vieira *et al.*, 2008). ε-Proteobacteria was mainly distributed in the sediments of DH-18. Actinobacteria, Acidobacteria, and Planctomycetacia were widely distributed in all of the ECS sediments sampled. Also, we observed a large proportion of unclassified bacteria.

Clear differences existed between the samples in terms of sediment bacterial diversity and number of species, according to the Chao-1, ACE, Shannon-Wiener, and Simpson's diversity index (Table 2). DH-18 generally appeared to have the highest diversity and number of species, followed by DH-13, then DH-9. Fewer clones were identified at DH-9 than at the other two sampling sites. In fact, the number of clones in each sample was fairly limited. Overall, the indices indicate that some of the sampling sites might have higher bacterial genotype diversity than that uncovered in our current study. More clones from further samples could help us to understand why.

The phylogenetic trees in Figs. 4 and 5 show the taxonomic assignment of the dominant taxa among the 12 samples, taken at different depths. In the deepest sediment layer, there was little bacterial diversity. Most bacteria were concentrated in the sediments at 3–5 cm (second layer) and 5–8 cm (third layer). Within the Proteobacteria (Fig. 4), only β-Proteobacteria were found in the surface layer (0–3 cm), although Chloroflexi and Lentisphaerae were also prominent at this depth. Bacteroidetes, Verrucomicrobia, and Planctomycetes were also detected in the deeper sediment layers. Firmicutes and Planctomycetes were primarily observed at 3–5 cm depth across the three sample sites. Except for Bacteroidetes, almost all the other groups were detected at 3–5 cm. At 5–8 cm, Proteobacteria were abundant and Bacteroidetes, Planctomycetes, and Actinobacteria were also found. In the surface and deepest sediment layers, we found Verrucomicrobia, Lentisphaerae, and 'Candidate division', although not many of these were detected. Our results show that sediment depth had a clear effect on bacterial diversity. The depth-related variations in bacterial community structure could be attributed to vertical changes in the chemical parameters of the sediment, such as NO_3^-, PO_4^-, NH_3^+, and TC.

Distribution with environmental parameters

The PCA analysis showed similar clustering of the bacterial assemblages and their distribution in the three samples (Fig. 6a). For example, all Proteobacteria tended to be close together, reflecting the close relationships among the taxa, whereas the distributions of

Table 2. Measures of bacterial diversity from the surface layer of sediments from the East China Sea

	No. of clones	Bias-corrected Chao-1	Abundance-base coverage estimator (ACE)	Simpson index, *D* (10^{-2})
DH-9	60	287.67	365.40	2.56
DH-13	101	1027.25	1051.96	1.86
DH-18	102	2050.00	2763.59	5.44

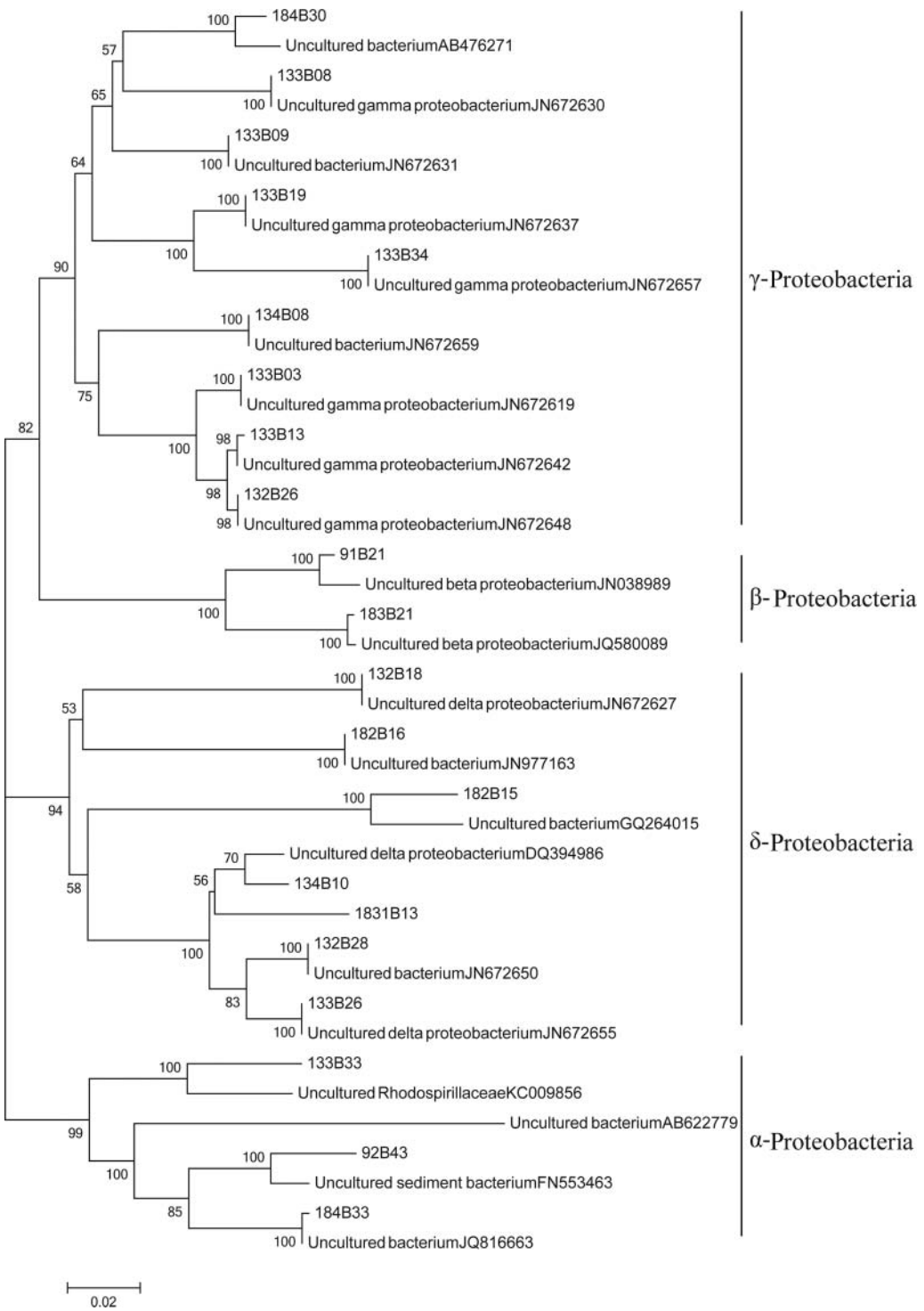


Fig. 4. Phylogenetic tree of Proteobacteria based on 16S rRNA gene sequences.

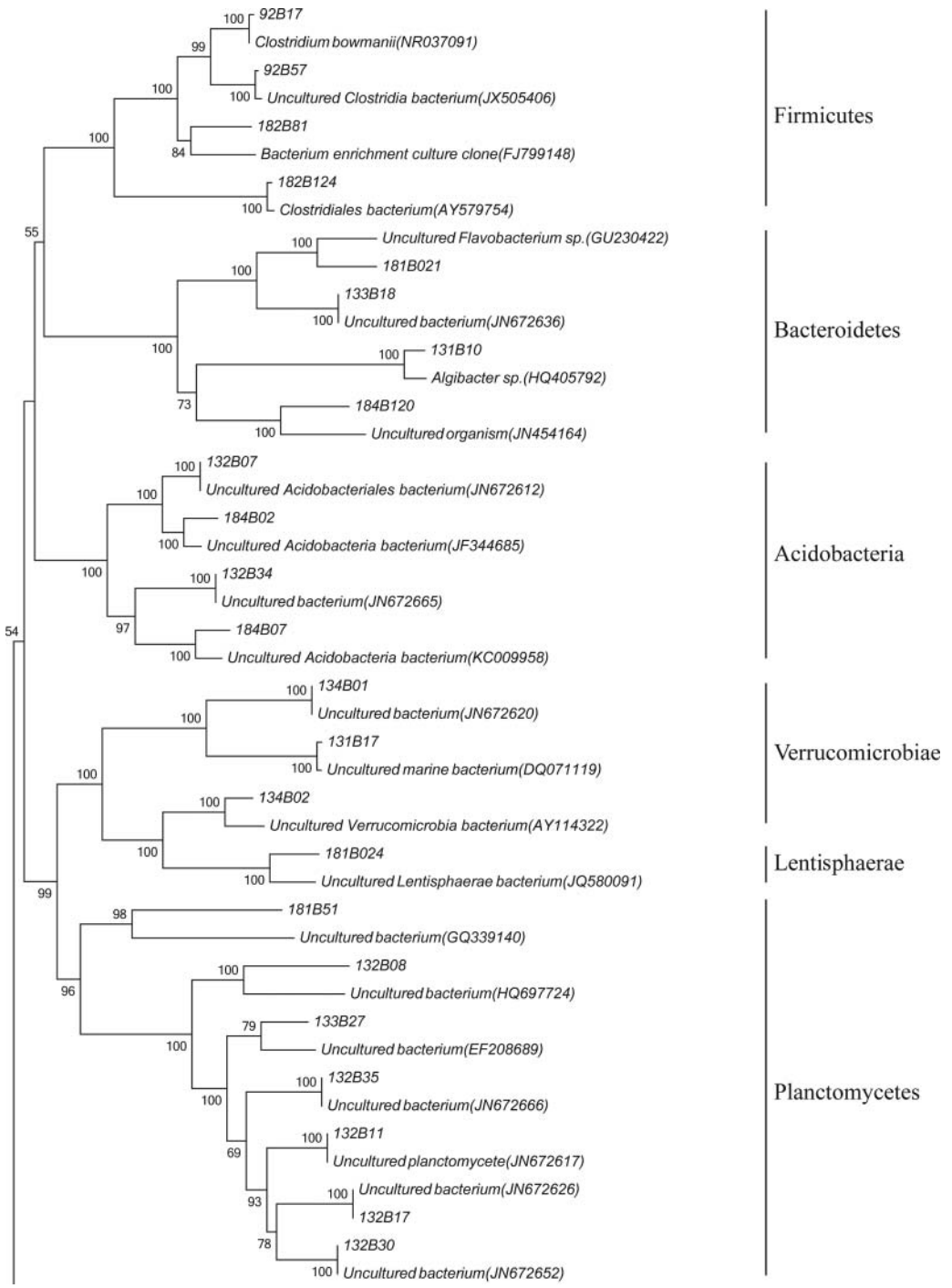
Chloroflexi and Acidobacteria were not close together. Compared with other groups, Clostridia was more similar to Bacilli than other taxa and their distribution trends in the three samples were DH-9 > DH-18 > DH-13. Meanwhile, the number of Clostridia and Bacilli in DH-18 and DH-13 was less than the average. However, α-Proteobacteria and ε-Proteobacteria were mainly at DH-18. Planctomycetes, Actinobacteria, and γ-Proteobacteria were present at DH-13 and DH-18, while Chloroflexi and β-Proteobacteria were most abundant at DH-9 and DH-18. Cyanobacteria were found only at DH-13; this may be due to the geographic location of DH-13, which is some distance from the coast and so likely has relatively poor nutrient levels. This result is consistent with previous studies (Vieira *et al.*, 2008), which found that Cyanobacteria tend to be more abundant in oligotrophic than in eutrophic waters, indicating a relatively pristine environment.

All chemical parameters affected the distribution of bacteria in the ECS sediments. TP and TN had the most influence (Fig. 6b). The TC content in the sediment samples had a positive effect on Planctomycetacia, γ-Proteobacteria, and δ-Proteobacteria, in particular, but a negative effect on ε-Proteobacteria and Chloroflexi (Fig. 6b). Although TIC had a positive effect on Bacilli, it had a negative effect on Planctomycetacia, γ-Proteobacteria, and δ-Proteobacteria. The two elements had a negative effect on the distribution of ε-Proteobacteria; this taxon was strongly negatively correlated with TN content. Chloroflexi were most strongly negatively correlated with TP content. The other taxa that appear in the plot may have only varied on a small scale, or the chemical parameters tested only had a small effect on them. The strongest treatment effect illustrated by the PCA plots was the consistent close association of the composition (Fig. 2).

DISCUSSION

Our study has identified the dominant members of the microbial communities in East China Sea sediments. A sister paper (Jiang *et al.*, 2016) examines the micro-eukaryotes from these same samples. We identified a total of 262 bacterial 16S rDNA clones from three different sampling sites. The sediment cores from each site were divided into four layers: 0–3 cm, 3–5 cm, 5–8 cm, and 8–10 cm. Many common marine phylotypes were identified, with identical sequences to those previously detected in other marine surface sediments (Sekiguchi *et al.*, 2002), deep-sea cold seeps (Li *et al.*, 1999), and cold saline spring sediments (Perreault *et al.*, 2007). All of the sequences derived from the 12 sediment samples fell into 19 bacterial phylogenetic lineages, including the α-, β-, γ-, δ-, and ε-Proteobacteria, as well as Acidobacteria, Bacteroidetes, Chloroflexi, Bacilli, Planctomycetacia, Flavobacteria, Cyanobacteria, Nitrospira, Gemmatimonadetes, Deinococci, Clostridia, Actinobacteria, Spirochaetes, and Verrucomicrobia. Proteobacteria (38%) were the most abundant phylum among all of the sediment samples and γ-Proteobacteria accounted for 15% of the total sequences. Our results agree with those of previous studies that showed that Proteobacteria were the dominant bacterial phylogenetic lineage in most surface marine sediments, comprising >50% of the microbial biomass (Ravenschlag *et al.*, 2001; Bowman and McCuaig, 2003).

Our data indicate that all three sampling sites have a rich microbial diversity, but the diversity differed among the samples according to the index used. For example, five major bacterial lineages (γ-Proteobacteria, δ-Proteobacteria, Planctomycetacia, Actinobacteria and Acidobacteria) were detected in all sediments. γ-Proteobacteria, δ-Proteobacteria, and ε-Proteobacteria were the most abundant Proteobacteria in all of the sediment samples. Chloroflexi was found only at DH-9 and DH-18. Based on the geographic locations of these


92B17
Clostridium bowmanii(NR037091)
92B57
Uncultured Clostridia bacterium(JX505406)
182B81
Bacterium enrichment culture clone(FJ799148)
182B124
Clostridiales bacterium(AY579754)
Firmicutes
Uncultured Flavobacterium sp.(GU230422)
181B021
133B18
Uncultured bacterium(JN672636)
131B10
Algibacter sp.(HQ405792)
184B120
Uncultured organism(JN454164)
Bacteroidetes
132B07
Uncultured Acidobacteriales bacterium(JN672612)
184B02
Uncultured Acidobacteria bacterium(JF344685)
132B34
Uncultured bacterium(JN672665)
184B07
Uncultured Acidobacteria bacterium(KC009958)
Acidobacteria
134B01
Uncultured bacterium(JN672620)
131B17
Uncultured marine bacterium(DQ071119)
134B02
Uncultured Verrucomicrobia bacterium(AY114322)
Verrucomicrobiae
181B024
Uncultured Lentisphaerae bacterium(JQ580091)
Lentisphaerae
181B51
Uncultured bacterium(GQ339140)
132B08
Uncultured bacterium(HQ697724)
133B27
Uncultured bacterium(EF208689)
132B35
Uncultured bacterium(JN672666)
132B11
Uncultured planctomycete(JN672617)
Uncultured bacterium(JN672626)
132B17
132B30
Uncultured bacterium(JN672652)
Planctomycetes

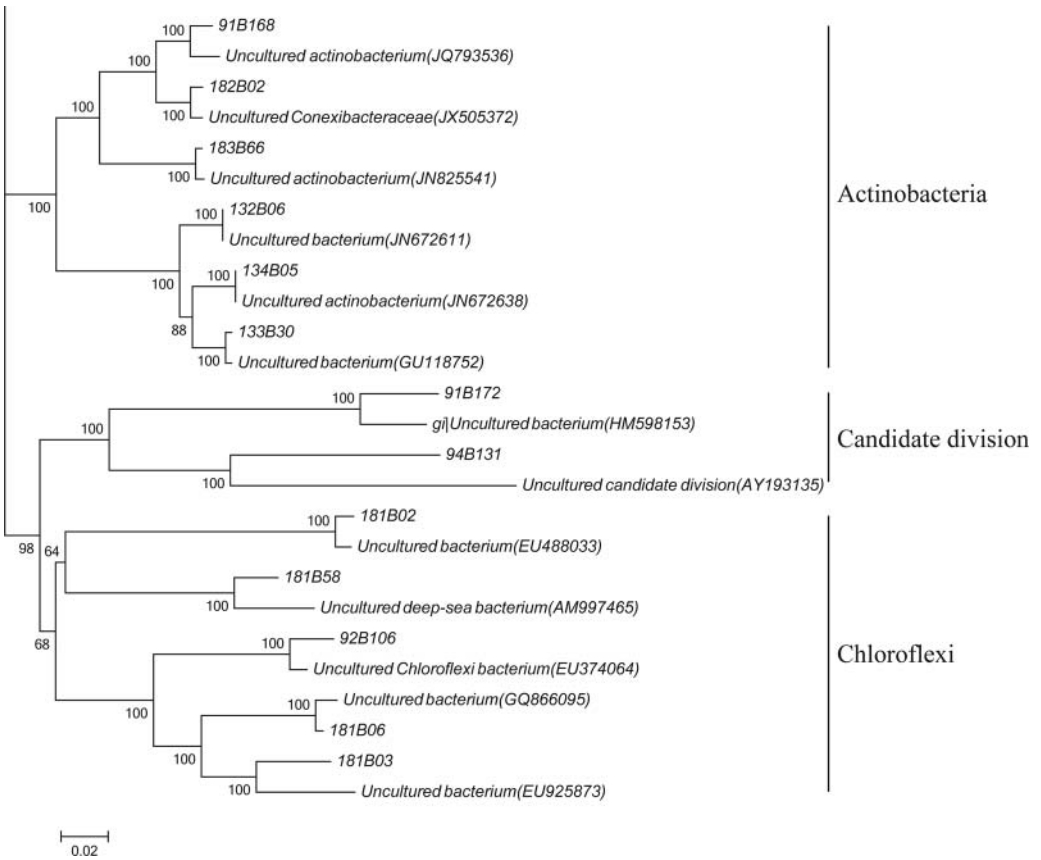


Fig. 5. Phylogenetic tree of other bacteria based on 16S rRNA gene sequences.

two sampling sites, we believe that Chloroflexi can grow in eutrophic environments. As terrestrially impacted seawater has a higher concentration of particles than oligotrophic seawater, which receives relatively low inputs of organic carbon and the associated particles, freely suspended marine bacteria are known to differ between these two environments (Giovannoni and Rappé, 2000). In relation to this, terrestrial environments had less of an impact on the sediments at DH-13 than at DH-9 and DH-18.

However, compared with DH-9, sites DH-18 and DH-13 had higher diversity indices, which may be because DH-9 received high levels of freshwater discharges from the Changjiang and Qiangtang rivers. β-Proteobacteria are commonly detected, as the most numerically dominant cell and clone type, in freshwater lakes (Zwart *et al.*, 2002; Mueller-Spitz *et al.*, 2009), and were mainly observed at DH-9. This provides evidence that DH-9 was affected by freshwater discharges and indicates that freshwater bacteria may have directly affected the bacterial community structure in the area around DH-9. In other words, the deposition of freshwater microorganisms from the water column could explain the phenomenon observed at DH-9.

The total input of particulate organic nitrogen (PON) to deep-sea sediments has been estimated as 24–80 $\mu mol \cdot m^{-2} \cdot day^{-1}$ in different locations and over time (Ni *et al.*, 2001). We

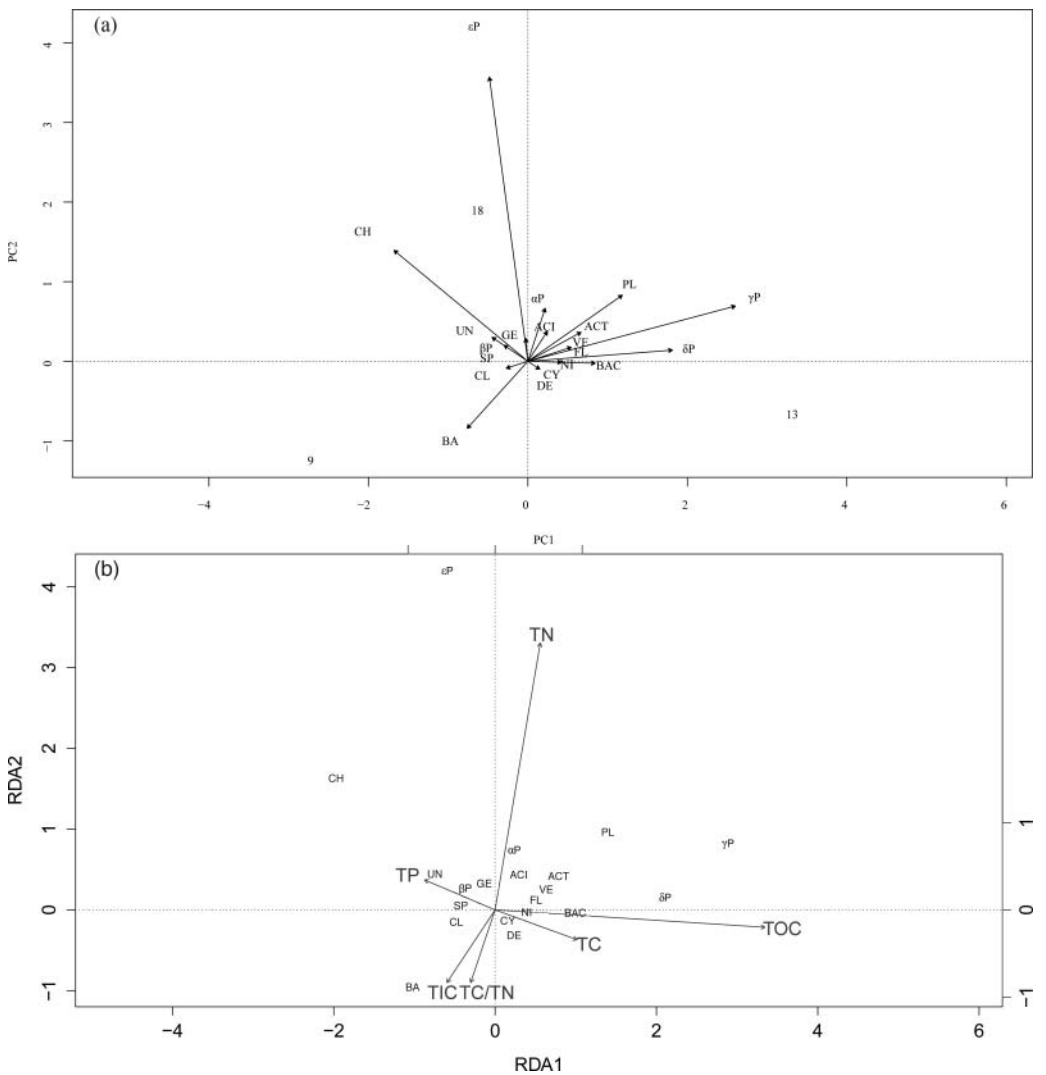


Fig. 6. (a) PCA analysis of the relationship between bacterial distribution and sampling site, and (b) RDA analysis of the relationship between bacterial diversity and environmental factors.

found Flavobacteria, Bacteroidetes, Verrucomicrobia, and Nitrospira only at DH-13 and DH-18; Cyanobacteria and Deinococci only at DH-13; and Gemmatimonadetes only at DH-18. These variations in presence may have been caused by the fact DH-13 was further from the coast, so was less impacted by nutrients.

Different distributions of bacteria among the three sampling sites were not only found in relation to the geographic environment, but also changed with the depth of sediment (to 10 cm). Zeng *et al.* (2005) studied the bacterial community in sediments from the Western Pacific 'Warm Pool' and found the same phenomenon. In this study, different kinds of δ-Proteobacteria, ε-Proteobacteria, and CFB group bacteria existed at different depths. This demonstrates that, to describe comprehensively the status of microorganisms and

reveal the phylogenetic diversity at a specific location, it is necessary to section sample cores and analyse microbial diversity at different depths. Different metabolic processes likely dominate the different sediment layers studied, resulting in the different bacterial communities observed. Böer *et al.* (2009) showed that NO_3^-, NO_2^-, and PO_4^- concentrations in the upper layer of a sediment were greater than at depth, similar to the present study. However, in our study, the 0–3 cm (shallowest) and 8–10 cm (deepest) sediments had the lowest bacterial diversity (Fig. 4); the shallowest sediments had high levels of C, N, and P, so they should have had a high bacterial diversity. The ECS is relatively shallow, so the sediments are more susceptible to the effects of ocean currents, creating a relatively unstable environment. This could explain why we observed low bacterial diversity in the shallowest layer. In the intermediate-depth layer (3–8 cm), the environment would have been relatively stable and, combined with high rates of particle deposition, could have led to the higher number of bacterial clones recovered. In the deepest sediment layer of our study, the low bacterial diversity could be attributed to the low concentrations of C, N, and P observed.

γ-Proteobacteria is an important class, frequently encountered during the analysis of sediment bacterial diversity (Polymenakou *et al.*, 2005; Hunter *et al.*, 2006; Xu *et al.*, 2008). γ-Proteobacteria sulphur oxidizers were dominant in Hornsund fjord marine sediments (in the Arctic) (Ravenschlag *et al.*, 1999). We found that γ-Proteobacteria existed mostly where the C/N ratio was moderate (DH-13); all of the sequences were related to uncultured clones from Genbank. The RDA analysis revealed that TN played a significant role in the distribution of γ-Proteobacteria and Planctomycetacia. Planctomycetes were identified as major players in the global nitrogen and carbon cycles, and perform reactions such as the anaerobic oxidation of ammonium, with the aid of subcellular organelles known as anammoxosomes (Lindsay *et al.*, 2001). TN and TP both had an obviously positive effect on ε-Proteobacteria and Chloroflexi. ε-Proteobacteria were previously found to utilize a wide spectrum of electron donors and acceptors (i.e. hydrogen, sulphur compounds, nitrate, and oxygen), and many of the isolates shared the ability to use the same electron donors and acceptors. Based on the RDA, it seems that most of the ε-Proteobacteria utilized nitrate as an electron acceptor, consistent with the results of Nakagawa *et al.* (2005). The results obtained here also provide evidence that ε-Proteobacteria utilize phosphate as an electron acceptor. Chloroflexi was previously found to have the capability to gain energy from nitrite oxidation, in addition to Proteobacteria and Nitrospirae (Sorokin *et al.*, 2012).

Total carbon had a positive effect on δ-Proteobacteria in this study. Previously, nitrite-oxidizing bacteria were classified as δ-Proteobacteria (Teske *et al.*, 1994). Sulphate-reducing bacteria (SRB) related to Desulfobulbaceae and Desulfosarcina have also been found to be abundant and were affiliated with δ-Proteobacteria (Mußmann *et al.*, 2005), but this result was not observed here. TIC had a positive effect on Bacilli; we consider that these Bacilli have learned to use inorganic carbon as a carbon resource. Salinity has also been shown to be a fundamental indicator in an estuary, because many processes are closely connected with salinity (Sun *et al.*, 2009). However, salinity was not considered in the present study; it may be important in the estuary environment of some of the sample locations.

In summary, this study has revealed the diversity and distribution of bacteria in marine sediments, collected at four different sediment depths from three sampling sites in the East China Sea. There was high bacterial diversity in the sediment environment. We analysed the characteristics of the bacterial diversity and their distribution using C, N, and P parameters. TIC, TC, TP, and TN all had a significant effect on the bacteria. The deposition of marine creatures on the sea floor leads to abundant nutrients, as particulate organic material. This

in turn, and importantly, influences the chemical composition of the sediments and affects global biogeochemical cycles. Season, location, water column production, and plankton all have an influence on the organic material in sediments. To improve our understanding further, more sequences from more samples should be analysed in future studies.

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