



Spatial Variations of Prokaryotic Communities in Surface Water from India Ocean to Chinese Marginal Seas and their Underlining Environmental Determinants

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To illustrate the biogeographic patterns of prokaryotic communities in surface sea water, 24 samples were systematically collected across a large distance from Indian Ocean to Chinese marginal seas, with an average distance of 453 km between two adjacent stations. A total of 841,364 quality reads was produced by the high throughput DNA sequencing of the 16S rRNA genes. Phylogenetic analysis showed that Proteobacteria were predominant in all samples, with Alphaproteobacteria and Gammaproteobacteria being the two most abundant components. Cyanobacteria represented the second largest fraction of the total quality reads, and mainly included *Prochlorococcus* and *Synechococcus*. The semi-closed marginal seas, including South China Sea (SCS) and nearby regions, exhibited a transition in community composition between oceanic and coastal seas, based on the distribution patterns of *Prochlorococcus* and *Synechococcus* as well as a non-metric multidimensional scaling (NMDS) analysis. Distinct clusters of prokaryotes from coastal and open seas, and from different water masses in Indian Ocean were obtained by Bray-Curtis dissimilarity analysis at the OTU level, revealing a clear spatial heterogeneity. The major environmental factors correlated with the community variation in this broad scale were identified as salinity, temperature, and geographic distance. Community comparison among regions shows that anthropogenic contamination is another dominant factor in shaping the biogeographic patterns of the microorganisms. These results suggest that environmental factors involved in complex interactions between land and sea act synergistically in driving spatial variations in coastal areas.

Keywords: prokaryotic diversity, biogeography, surface seawater, high throughput sequencing, 16S rRNA gene, environmental factors

INTRODUCTION

Microbes, estimated to be in the orders of $10^4 \sim 10^6$ cells per milliliter in seawater (Azam and Malfatti, 2007), are believed to play essential roles in food webs (DeLong and Karl, 2005), biogeochemical cycles (Weber and Deutsch, 2010), and global climate changes (Sarmiento et al., 2010; Coelho et al., 2013). However, the diversity and activities of microbes in many regions of

the vast oceans have not been studied yet (Sunagawa et al., 2015), in part because of the sampling difficulties and the fact that large majority of marine microbes have not been obtained in pure culture, which used to be the only available way to describe marine microbes (Giovannoni and Stingl, 2007). In the past few decades, rapid technological advances (Metzker, 2010; Fadrosch et al., 2014) in cultivation-independent molecular surveys, which are based on the analysis of conserved molecular markers (chiefly small subunit ribosomal RNA genes) or PCR-independent metagenomics, has made it possible to detect marine microbiomes in unprecedented detail.

Prokaryotes in surface seawater (<200 m deep) represent a key player in primary production and in feeding of organic matter into biological pump in the ocean (Ducklow et al., 2001; Herndl and Reinthaler, 2013). Understanding of the ecological role of prokaryotes in surface seawater depends on knowledge of the community structure of these organisms. In recent years, efforts have been made to determine the community structures of prokaryotes in surface seawater in several marine regions, such as Mediterranean Sea (Feingersch et al., 2010; Techtmann et al., 2015), Baltic Sea (Herlemann et al., 2011), Red Sea (Qian et al., 2011), South Pacific Gyre (Yin et al., 2013), and North Atlantic Ocean (Hahnke et al., 2013). These studies have provided valuable insights into the microbial community in specific marine regions. Therefore, most of these studies are geographically restricted. More recently, several major programs have been initiated to collect samples from different oceans and to provide insights into the ocean microbiome in a global scale. These include the Sorcerer II Global Ocean Sampling Expedition (Rusch et al., 2007; Yooseph et al., 2007), the Tara Oceans Expedition (Armbrust and Palumbi, 2015), and the International Census of Marine Microbes (ICOMM, <http://icomm.mbl.edu/>). Despite these tremendous efforts, the vast majority of the ocean regions remain unexplored (Sunagawa et al., 2015) and few sampling surveys were made to compare the microbial communities of different marine region. Therefore, the biogeographic patterns of marine microorganisms and the environmental factors determining these patterns in surface water in a broad scale remain to be determined.

In the present study, we systematically collected samples of surface seawater from open seas, coastal regions, and semi-enclosed marginal seas during the DY125-30 cruise with R/V Dayang No.1 from Southwest Indian Ocean to Qingdao, China. The prokaryotic diversity in these samples were assessed and compared. Our results reveal the biogeographic patterns of prokaryotes in previously unexplored marine regions as well as the underlining environmental determinants of these patterns.

MATERIALS AND METHODS

Sample Collection

Twenty four surface seawater samples were collected from a depth of ~4.0 m by an onboard surface seawater sampling system, from May 3 to 26, 2014, at 9:00 a.m. (local time), during the DY125-30 cruise with R/V Dayang No.1 as it traveled from Southwest Indian Ocean to Qingdao, China

(**Figure 1**). Samples (~4.5 L) were filtered through polycarbonate filters with a 0.22- μ m pore size and 47 mm diameter (Merck Millipore) under vacuum. The filters were then sealed in sterile plastic tubes and stored at -80°C until DNA extraction. GPS coordinates and environment characteristics were listed in Table S1 (Supplementary Materials). The temperature, salinity and pH of the water samples were kindly provided by Qunhui Yang and Huaiyang Zhou at Tongji University, Shanghai, China. Average distance between two adjacent sampling sites is about 453 km.

No specific permissions were required for sampling at the above locations as the activities did not involve endangered or protected species and did not occur within a private area.

DNA Extraction, Amplification, and Sequencing

Total DNAs were extracted from samples collected on the membrane filters using the phenol-chloroform extraction method described by Urakawa et al. (2010). The concentration and quality of extracted DNA were determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). The archaeal and bacterial hypervariable regions V4 of the 16S rRNA genes were amplified using the primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), with a unique 8-bp error-correcting Golay barcode to each sample (Caporaso et al., 2011). PCR for each sample was carried out in triplicate in a 20- μ l reaction volume using a GeneAmp 9700 Thermal Cycler (Applied Biosystems, USA) with the following cycling parameters, initial pre-denaturation at 95°C for 3 min, followed by 28 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s, and a final extension at 72°C for 10 min. The PCR mixture contained 0.4 μ l of High Fidelity FastPfu polymerase (TransGen Biotech, Beijing), 4 μ l of 5 \times FastPfu buffer, 2 μ l of 2.5 mM dNTPs, 0.8 μ l of each primer (5 μ M), 10 ng of template DNA, and de-ionized ultrapure water. Negative controls without template were included for each barcoded primer pair to test for reagent contamination. PCR products were visualized on 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) and quantified by the QuantiFluor-ST system (Promega, USA). A mixture of the purified amplicons were pooled in an equimolar ratio and paired-end sequenced (2 \times 300) on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China. The raw sequencing data from this study have been submitted to NCBI Sequence Read Archive database (<http://www.ncbi.nlm.nih.gov/sra/>) with accession no. SRP065252.

Quality-Filtering and Sequence Analysis

To obtain effective reads, the pair-end reads from MiSeq were de-multiplexed and filtered strictly using QIIME v1.7.0 (Quantitative Insights Into Microbial Ecology, <http://qiime.org/index.html>) software package (Caporaso et al., 2010), with the following criteria, as described previously (Bokulich et al., 2013; Hong et al., 2015): (i) the 300-bp reads were truncated at any site that obtained an average quality score of <20 over a 50-bp sliding window, and the truncated reads shorter than 50

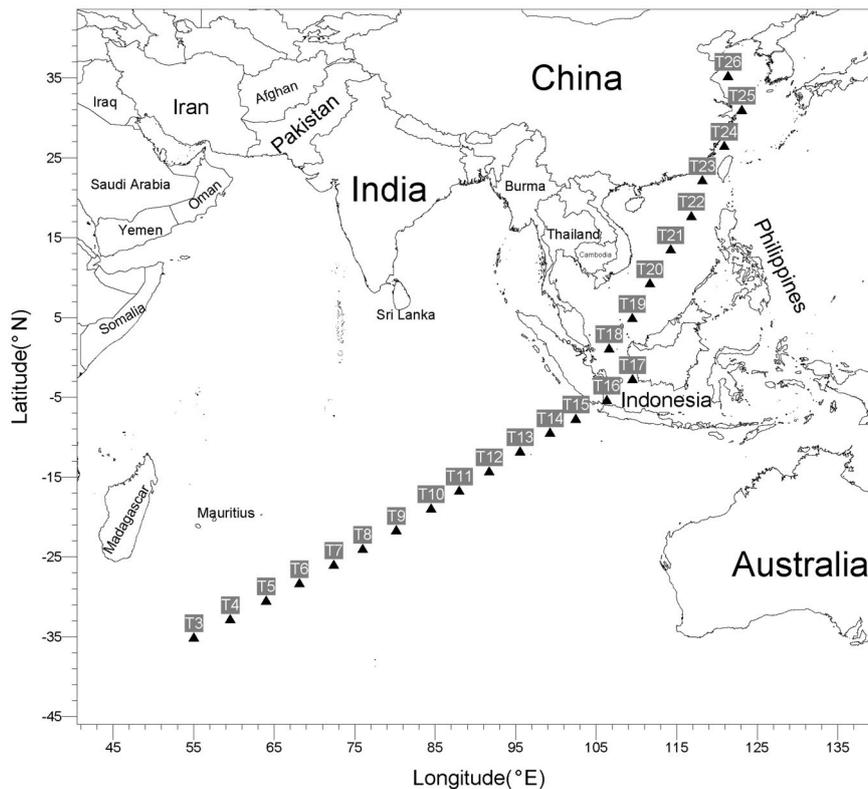


FIGURE 1 | Location of the sampling sites during the DY125-30 cruise.

bp were discarded; (ii) reads with any mismatch in barcode, more than two nucleotide mismatch in primer or containing ambiguous characters were removed; and (iii) overlapping sequences, shorter than 10 bp or with a mismatch ratio of more than 0.2, were eliminated. The chimeric sequences were checked and filtered out by UCHIME (Edgar et al., 2011), and operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE (version 7.1, <http://drive5.com/uparse/>) pipeline (Edgar, 2013). The taxonomic assignment of OTUs was performed by the mother Bayesian classifier (70% confidence) with the MOTHR formatted version of the Ribosomal Database Project (RDP, version 11.1, <http://rdp.cme.msu.edu/>) (Maidak et al., 2001). To avoid the effects of different sequencing depths, all samples were normalized to 25,000 high quality sequences for subsequent analyses. To prevent artificial diversity inflation, the singletons (sequences or OTUs determined at a similarity level of 97% only occurred once in the entire datasets) were removed as putative sequencing errors or PCR amplification artifacts (Kunin et al., 2010).

Diversity Comparison and Statistical Analyses

For each sample, the diversity of microbial community (α -diversity) was assessed by calculating diversity indices, including Chao1 and ACE (estimating the species richness), and Shannon (estimating the species diversity) using MOTHR program

(Schloss et al., 2009). The Good's coverage (C) was calculated as $[1 - (n/N)]$ where n is the number of OTUs that had been observed once and N is the total number of OTUs in the sample. The community comparison of microbial assemblages (β -diversity) was mainly performed with R (v.3.2.2, <http://www.r-project.org/>) packages. Taxa with significant difference in abundance between groups were evaluated with a Welch's t -test in the STAMP program (Parks et al., 2014), the results were filtered based on $p < 0.05$ and an effect size (difference between proportions) of >1 . Geographical distance matrix was calculated from the latitude and longitude coordinates obtained by Global Positioning System using Palaeoecological and Palaeogeographical Analysis Tools package (Vavrek, 2012). Non-metric multidimensional scaling (NMDS) analysis was conducted with fitting of the environmental parameters using the *envfit* function from the Vegan package, to illustrate the relationships between environmental variables and microbial community compositions. Heat map analysis of the 60 most abundant genera in each station was performed by Pretty Heatmaps package (Kolde, 2015).

RESULTS

Environmental Characteristics

A total of 24 surface seawater samples were collected in this study (Figure 1 and Table S1). These include nine samples (T3–T11)

from a subtropical gyre province in Indian Ocean, four samples (T12–T15) from a monsoon gyre province in Indian Ocean, five samples (T18–T22) from semi-enclosed marginal seas, two samples (T16 and T17) from the coastal regions of Indonesia and four samples (T23–T26) from the coastal regions of China. All samples were taken during a short span of 24 days to minimize the seasonal effect. As shown in Table S1, while there was only a small variation in pH among the samples, the level of salinity decreased along the sampling path from Indian Ocean to Chinese marginal seas. The temperature of the surface seawater ranged significantly from 18.3 to 31.4°C, being higher for samples taken closer to the equatorial regions. Surface seawater in Indian Subtropical Gyre Province (IS, 24.8°C on average) was colder than that in Indian Monsoon Gyre Province (IM, 29.7°C on average).

Prokaryotic Diversity and Community Dissimilarity

MiSeq high throughput sequencing of the 16S rRNA genes from the 24 samples yielded a total of 875,520 raw reads. After deleting singletons, 841,364 high quality reads, with 28,863–43,190 reads for each sample, were taxonomically assignable with reference database (Table 1). All samples were rarefied to 25,000 high quality reads for a consistent sequencing depth. In all, 2,419 different OTUs from all samples were assigned at a 97% threshold for sequence similarity and used for further analysis. The Good's coverage ranged from 83.33 to 90.64%, with an average of 85.81%, indicating that the majority of indigenous species were captured in most of the samples. Chao1, ACE, and Shannon indices were calculated to allow the comparison of the richness and diversity of the prokaryotic communities in the samples (Table 1).

The community assemblages prepared from the 24 samples were compared at the OTU level by the Bray-Curtis dissimilarity analysis. As shown in Figure 2, most of the coastal samples are separated from the open sea samples. The samples from the Chinese coastal regions (T25 and T26) cluster together and are separated from the other coastal samples. T20, which is located in the southwest of South China Sea (SCS), a semi-enclosed marginal sea, is grouped with the coastal samples and not with the other open sea samples, pointing to a possible terrestrial anthropogenic contamination. Interestingly, the open seawater samples from IM are clustered with those from the semi-closed marginal seas, but not with those from IS (Figure 2). Similar clustering of the prokaryotic assemblages of our surface seawater samples is also obtained by the NMDS analysis (Figure 3).

To test whether the observed similarities and differences between samples taken from various marine regions in microbial community composition are significantly ($p < 0.05$) correlated with the environmental parameters detected in this study (i.e., latitude, longitude, geographic distance, salinity, temperature, and pH in the sampling regions), the environmental vectors were fitted with the NMDS plot using the *envfit* function from the *vegan* package (Oksanen et al., 2015), with 999 permutations (Figure 3). The highest correlations exist for latitude ($r^2 = 0.7744$, $p = 0.001$), longitude ($r^2 = 0.7111$, $p = 0.001$), and, hence, geographic distance (calculated from longitude and latitude, $r^2 = 0.7553$, $p = 0.001$). Salinity ($r^2 = 0.7694$, $p =$

TABLE 1 | Diversity indices and richness estimators of the surface marine water.

Station	Quality Reads	OTUs	ACE	Chao1	Shannon	Good's Coverage (%)
T3	43,190	571	1,035	894	4.22	87.23
T4	40,325	676	1,199	1,054	4.64	85.09
T5	29,114	725	1,094	1,051	4.27	85.54
T6	30,626	870	1,117	1,115	4.76	88.31
T7	36,001	887	1,221	1,156	4.29	86.09
T8	41,426	843	1,190	1,150	4.32	84.11
T9	28,863	1,090	1,375	1,361	5.2	88.04
T10	31,613	678	989	965	3.27	85.02
T11	41,152	909	1,576	1,376	4.41	84.69
T12	41,390	931	1,276	1,264	4.46	83.95
T13	30,442	921	1,203	1,136	4.44	86.44
T14	36,446	962	1,382	1,366	4.42	83.36
T15	41,458	979	1,293	1,300	4.89	86.32
T16	30,418	624	1,585	1,153	3.85	84.50
T17	29,108	779	1,144	1,113	4.01	84.60
T18	29,368	880	1,588	1,273	4.76	83.33
T19	34,040	795	1,097	1,085	4.23	85.60
T20	33,095	657	1,234	1,015	3.42	85.34
T21	36,765	800	1,408	1,245	4.47	84.94
T22	43,108	747	1,339	1,046	4.16	85.13
T23	36,178	712	1,291	1,089	3.36	84.93
T24	36,986	481	955	747	3.46	87.85
T25	30,195	420	700	584	4.02	90.64
T26	30,057	515	944	754	3.77	89.92
Total	841,364	2,419 ^a	–	–	–	

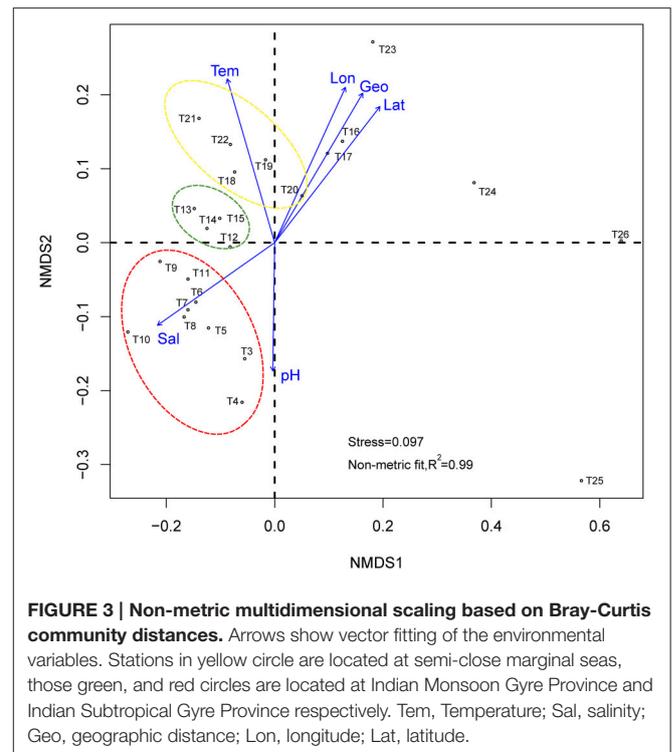
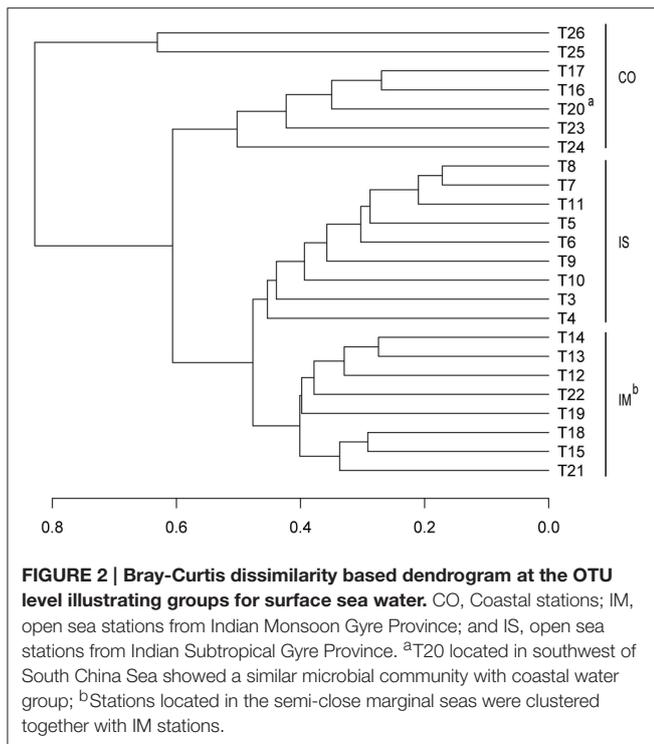
^aA total of 2,419 different OTUs were obtained after all samples being normalized to 25,000 high quality reads.

0.001) and temperature ($r^2 = 0.7015$, $p = 0.001$) are also highly correlated, whereas pH ($r^2 = 0.3412$, $p = 0.013$) appears to be a slightly weak correlation with a relatively high p -value. Briefly, all of the tested environmental parameters are statistically significant in explaining the similarity and dissimilarity of the samples.

Community Structures

A total of 13 phyla were identified from the 24 samples, and the five most abundant phyla contributed up to 93.9% of the total quality reads (Figure 4 and Table S2). We found that most of the quality reads were affiliated with various members of Proteobacteria (46.1% of the total), among which Alphaproteobacteria (25.3%) and Gammaproteobacteria (17.5%) were the most abundant classes and were found in all sampled locations (Figures 4, 5A). Cyanobacteria accounted for the second largest fraction of the total quality reads (21.8%) (Figure 4), and were mainly represented by the widely distributed genera in the ocean, *Prochlorococcus* and *Synechococcus* (Figure S1).

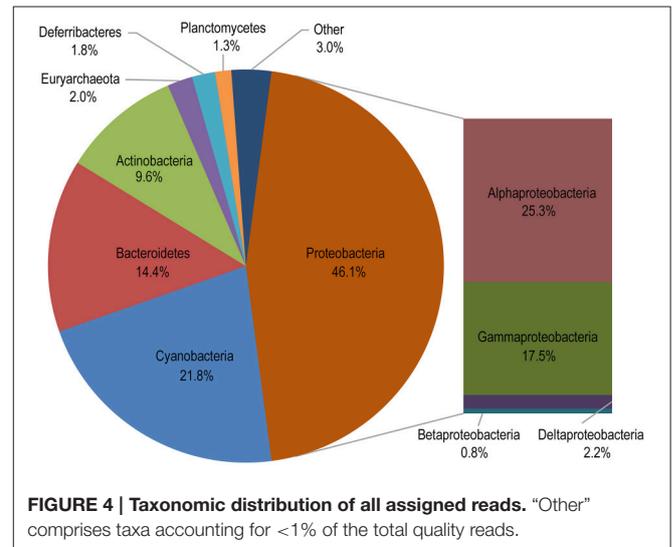
When sequences were assigned to the taxonomic rank of “order,” 10 taxonomic groups were found to make up 81.3% of the total quality reads (Figure 5B). Cyanobacteria (20.3%)



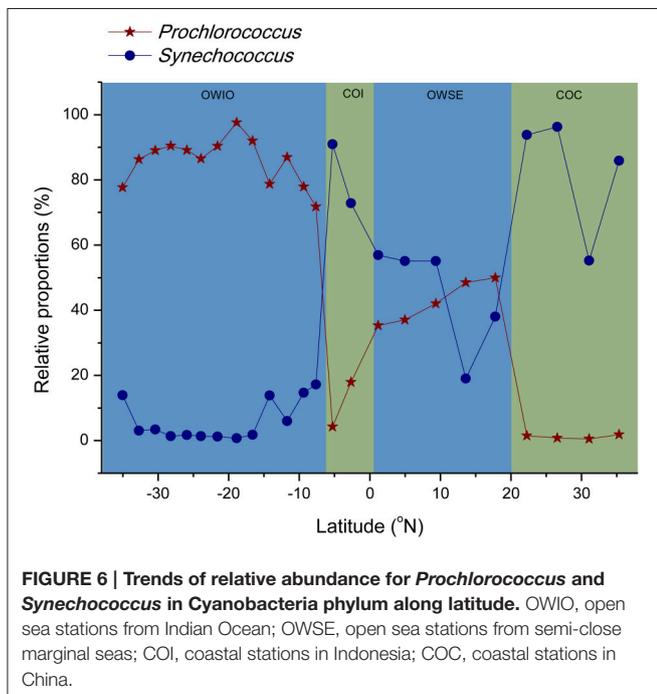
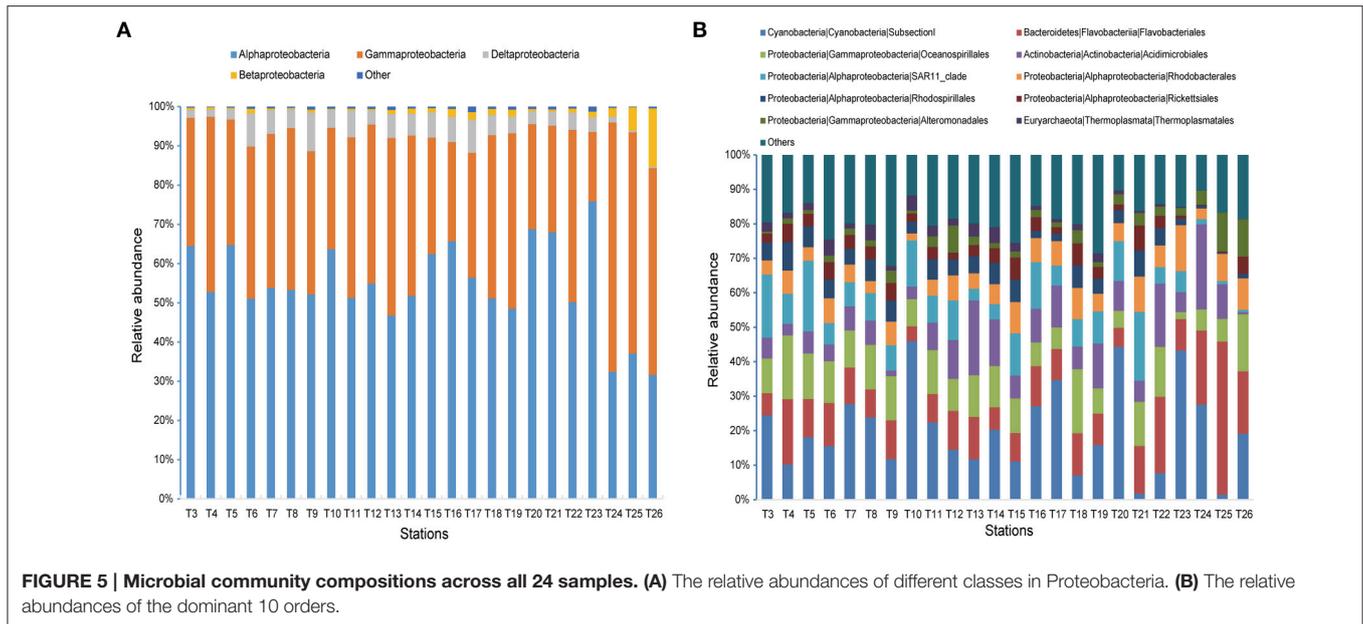
and Flavobacteriales (12.8%) were most abundant, followed by Oceanospirillales (10.7%), Acidimicrobiales (9.0%), and SAR11 clade (8.8%) (Figure 5B). At the genus level, most of the reads were assigned to uncultured genera (e.g., top 60 genera shown in the heat map in Figure S1), with those belonging to the genera *Prochlorococcus* and *Synechococcus* being the notable exceptions. This two representative cyanobacterial genera were observed to show a distinct distribution pattern (Figure 6). The relative abundance of *Prochlorococcus* was generally higher in the open sea samples than that in the coastal regions, while the reverse appeared to be true for the relative abundance of *Synechococcus*. The semi-closed marginal seas (areas from T18 to T22) were not only an intermediate region geographically, but also a transition zone in community composition between oceanic and coastal areas (Figure 6), which was also suggested by NMDS analysis (Figure 3).

Differences in Prokaryotic Community Composition among Regions

The Bray-Curtis dissimilarity analysis revealed different community compositions between the coastal and the open sea waters and between different water masses in Indian Ocean. These differences were not readily observed at the phylum level, but were found at the lower taxonomic levels (Table S2). Gammaproteobacteria were less abundant than Alphaproteobacteria in all samples except for those from the Chinese coastline (T24, T25, and T26) (Figure 5A). The abundance of Betaproteobacteria at the three coastal sites was also slightly lower than that at the other sites. In addition, Deltaproteobacteria showed the lowest abundance at the three



sites. By comparison, the coastal waters near Sunda Strait (T16 and T17) and in Southwest Taiwan Strait (T23) appeared to be similar to the open sea sites in Deltaproteobacterial and Betaproteobacterial abundance (Figure 5A). The SAR11 clade was predominant in oceanic water (up to 20.4% of quality reads at T5) (Figure 5B). These organisms were nearly undetectable at the sampling sites in Chinese coastal water, especially at the Changjiang estuary and the Shandong Peninsula Coastline, but accounted for as high as 13.5% of total quality reads at the coastal site (T16) in Sunda Strait. On the other hand, the abundance of Flavobacteriales was found to be higher at the Changjiang



estuary (T25) than other sampling sites. Moreover, members of the class Sphingobacteriia were low in abundance in all samples (<1%, Table S3), but were relatively more abundant at the Chinese coastal sites.

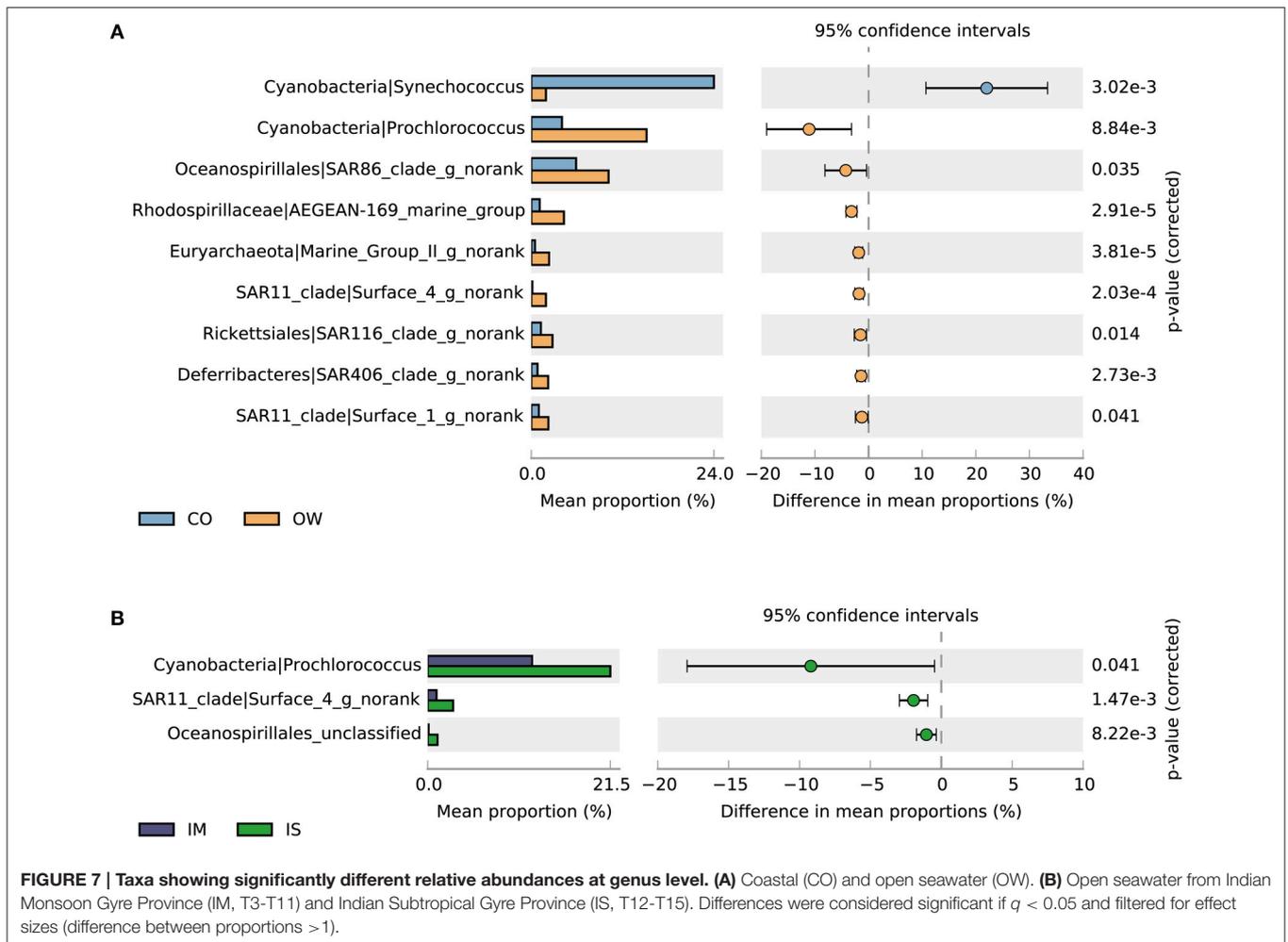
The STAMP program (Parks et al., 2014) was employed to determine taxa that contributed most significantly to the observed differences between the coastal and the open sea surface waters at the genus resolution (Figure 7A). We found that a total of nine genera showed significant variations between the coastal and the open sea surface waters. Eight

of them were more abundant in the open sea waters than in the coastal waters. These include an archaeal genus (related to Marine Group II), a Deferribacteres genus (related to SAR406), a Gammaproteobacteria genus (related to SAR86), four Alphaproteobacteria genera (related to SAR11, SAR116, and AEGEAN-169_marine_group) and the genus *Prochlorococcus*. *Synechococcus* was the remaining genus which was significantly more abundant in the coastal waters than in the open sea waters. This distribution pattern of *Prochlorococcus* and *Synechococcus* agrees with the finding that the two genera were dominant photoautotrophs in oligotrophic and eutrophication marine ecosystems, respectively (Jiao et al., 2005; Lin et al., 2012). For the prokaryotic communities in IM and IS, variations in the abundance of SAR11 clade, *Prochlorococcus* and an unclassified Oceanospirillales were primarily responsible for the separation of the two groups (Figure 7B).

DISCUSSION

Overview of Abundant Taxa

In the present study, we showed that prokaryotic communities in surface seawaters, collected at various sites stretching from Indian Ocean to Chinese marginal seas, were dominated by Proteobacteria, Cyanobacteria, Bacteroidetes, and Actinobacteria. The observation was consistent with global surveys reported recently (Biers et al., 2009; Zinger et al., 2011; Sunagawa et al., 2015). Alphaproteobacteria and Gammaproteobacteria were the two most abundant classes in both coastal and open sea surface waters at our sampling sites, in agreement with the previous findings in Sargasso Sea (Sjostedt et al., 2014), Eastern Mediterranean Sea (Feingersch et al., 2010; Techtmann et al., 2015), and Eastern Atlantic Ocean (Friedline et al., 2012). Betaproteobacteria, a typical freshwater lineage (Zwart et al., 2002), comprised only of 0.8% of the



total quality reads, and existed primarily in the sampling sites along the Chinese coastline, raising the possibility that the distribution pattern of Betaproteobacteria may be attributed to the contribution of offshore freshwater inflow to the surrounding sea areas (Feng et al., 2009; Tseng et al., 2015).

Cyanobacterial sequences represented the second largest fraction (21.7%) of total quality reads, but the abundance of cyanobacteria varied drastically from 2.6 (T21 in SCS) to 46.7% (T10 in India Ocean). This huge variation in cyanobacterial abundance was also observed in surface seawater in other regions. For example, the abundance of cyanobacteria was only ~2% at a station near Cape Verde islands in Atlantic Ocean (Friedline et al., 2012) and in the Gulf of Finland in Baltic Sea (Laas et al., 2014), over 25% in Eastern Mediterranean Sea (Feingersch et al., 2010) and South Pacific Gyre (Yin et al., 2013), and up to 50% in Red Sea (Qian et al., 2011). We found no specific patterns of the abundance of cyanobacteria in coastal and open seas. However, *Prochlorococcus* and *Synechococcus*, the two representative cyanobacterial genera widely distributed in the ocean (Flombaum et al., 2013), showed a distinct distribution pattern of high relative abundance for *Prochlorococcus* in open seas and *Synechococcus* in coastal regions (Figure 6). With small

cell size (~0.6 μm) and resulting high surface/volume ratio, *Prochlorococcus* is capable of more efficient nutrient uptake than *Synechococcus* in oligotrophic open seas (Partensky et al., 1999; Partensky and Garczarek, 2010), partly explaining their distinct distribution patterns. In contrast to *Prochlorococcus*, *Synechococcus* with a comparatively larger genome (Partensky et al., 1999; Chung et al., 2014), has the potential to utilize complex organic matter and the ability to prosper in high organic coastal regions. Interestingly, the semi-closed marginal seas, including SCS and near regions, were found to be a transition zone between oceanic and coastal areas based on the distribution patterns of these two algae (Figure 6) and NMDS analysis (Figure 3), emphasizing the possibility of terrigenous influence in the community variation.

Biogeography of Microbial Communities and Correlation with Shaping Factors Coastal Regions

As revealed by our results, the microbial composition of coastal regions differed from that of the open sea regions. The difference may have resulted from the difference in nutritional conditions between the two regions, presumably, caused by the terrestrial

anthropogenic inflow. The relatively easy community exchanges between water and sediments in coastal regions (Feng et al., 2009) may also be responsible for the microbial composition differences between the coastal and the open sea regions.

Gammaproteobacteria, shown to be predominant in both seawater and sediments (Schauer et al., 2010; Haverkamp et al., 2014; Mohit et al., 2015), play an important role in the degradation of marine organic matter, such as polysaccharides (Edwards et al., 2010), hydrocarbon (Horel et al., 2014), and petroleum (King et al., 2015). The abundance of Gammaproteobacteria was higher than Alphaproteobacteria only in T24, T25, and T26 near the China coastline, suggesting a high level of organic pollution in these areas. Two of the nine genera primarily responsible for the community dissimilarities between coastal and open seas (Figure 7A) belong to the SAR11 clade (Alphaproteobacteria). This clade, estimated to account for 1/3 of the cells present in marine surface waters on average (Morris et al., 2002), made up ~20.4% of the quality reads at T5 (Figure 5B). However, SAR11 reads were particularly low (Figure 5B) at our sampling sites in the Chinese coastline. Since SAR11 is able to derive energy through the oxidation of methylated and one-carbon compounds (Sun et al., 2011; Tripp, 2013), but not the complex organic matters (Alonso and Pernthaler, 2006; Sun et al., 2011; Tripp, 2013), members of this clade may not be able to live in habitats rich in organic matters. The remaining seven genera, except for *Synechococcus*, were also less abundant in the coastal waters than in the open seas, and were auxotrophic for complex organic matter. Moreover, Sphingobacteriia, abundant only in the Chinese coastline, is able to degrade benzene, toluene, ethylbenzene, and xylenes (BTEX) (Li et al., 2012), and, therefore, most likely participated in the process of pollutant degradation in the polluted coastal regions. Based on these observations, we suggest that current pollution through the input of organic carbon, nutrients, or other contaminants may serve as a major determinant for the microbial community structure in the coastal water of China. By comparison, the community compositions of Sunda Strait were more similar to those of the open seas, as compared to those in the Chinese coastal water, indicating that the coastal water of China was possibly more seriously polluted than that of Indonesia.

Open Seas

Water samples from various sites in Indian Ocean showed a similar community structure. The similar microbial assemblage composition and a negative correlation with geographical distance appear to be caused mainly by the Indian Ocean Gyre, a large system of rotating ocean currents (Schott et al., 2009), which permit the distant transportation and mixing of the surface water. However, two discrete clusters of water samples from Indian Ocean were still observed (Figure 2), and they corresponded to the two biogeographic provinces (i.e., IM: Indian Monsoon Gyre Province and IS: Indian Subtropical Gyre Province), highlighting the tight coupling of microbial diversity in Indian Ocean with the physicochemical composition, especially the thermohaline properties and nutrient status of

water masses (Jeffries et al., 2015). IS was characterized with a higher salinity than IM (Table S1), indicating salinity is an important factor in determining the variation of microbial diversity in Indian Ocean. A strong correlation between microbial assemblage dissimilarities in IS with salinity is indeed illustrated by NMDS analysis (Figure 3). Although this major determinant of microbial community composition was also observed among various habitats (Lozupone and Knight, 2007), salinity was not shown to be the only major correlation factor in the open seas. While the microbial communities of IS were associated with the more saline conditions, the microbial communities of IM were positively correlated with the higher temperature (Figure 3 and Table S1), indicating temperature is another important factor. The temperature for IM, located near the equator, was 29.7°C on average, compared with 24.8°C for IS (Table S1). As a primary genus responsible for the community dissimilarities between IM and IS, *Prochlorococcus* was found to grow well at temperatures below 29.8°C (Lin et al., 2012), with an optimal temperature of 25°C (Johnson et al., 2006). Therefore, temperature may be a major factor in explaining the slightly higher abundance of *Prochlorococcus* in IS than in IM. Moreover, one surface four taxon in SAR11 clade, another genus responsible for the community dissimilarities between IM and IS showed a significant difference in abundance ($p = 1.47e-3$) (Figure 7B). The decrease of SAR11 clade (surface 4) in abundance in IM may be related to high levels of organic matters, brought by Indonesian Throughflow (Gordon et al., 1997; Han and McCreary, 2001; Schott et al., 2009) from the Indonesian seas. In addition, samples from semi-closed marginal seas clustered together with those from IM in Indian Ocean, reflecting their similar community compositions and therefore, growth conditions. The semi-closed marginal seas, especially SCS, would inevitably be affected by nutrient input and freshwater from the continent, which shape the microbial community structure as major drivers (Zhang et al., 2014; Xia et al., 2015).

In conclusion, we have surveyed and compared, for the first time, microbial communities in surface sea water across a large distance from Indian Ocean to Chinese marginal seas. A clear spatial heterogeneity was demonstrated in community composition in a large scale. The semi-closed marginal seas, i.e., SCS and nearby regions, displayed a transition in community composition between the oceanic and the coastal regions. Environmental factors, such as salinity, temperature, and geographic distance, correlated well with the community variation. These results permit a better understanding of the biogeographic patterns of prokaryotic communities and their underlying environmental determinants in surface sea water.

AUTHOR CONTRIBUTIONS

XZ provided funding for research, performed research, analyzed data, and wrote the manuscript. XD analyzed data. LH provided funding for research, laboratory equipment and space for research to be conducted, interpreted data, and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2016.00017>

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