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Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations

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Abstract

Eddy pumping drives a set of biogeochemical processes by lifting deep waters into the euphotic zone. To address the potential effect of such physical processes upon the bacterial community, phylogenetic diversity was determined in two cold-core cyclonic eddies in the South China Sea. 16S rDNA terminal restriction fragment length polymorphism analysis of the microbial communities through the whole water column showed a wider depth range for the intermediate transition water mass at sites inside the eddies than for those outside. This water mass contained a relatively more complex community than the euphotic and deep-water zones. Stratification of prokaryotic populations between the surface and chlorophyll maximum layer of eddy-related sites versus homogeneity of communities in the euphotic zone of the reference site, revealed by statistical analysis of 16S rDNA libraries, is most likely a reflection of isopycnal displacement induced by differing water movement inside and outside eddies. Phylogenetic analysis revealed that eddy center sites were characterized by deep-water group Alteromonadales-affiliated clones, the psychrophilic genus *Octadecabacter* cluster and the nitrogen-fixing phototrophic Rhodospirillaceae cluster, while *Paracoccus*, an important functional group, abundantly existed at the reference site outside eddies. Our analysis revealed that bacterial community structure was significantly influenced by cyclonic eddy perturbations.

Keywords: Phylogenetic diversity; T-RFLP fingerprint; Bacterial community; Cyclonic eddy; Isopycnal displacement

1. Introduction

Mesoscale eddies are ubiquitous in the ocean and introduce spatial heterogeneity and temporal variability into a region. Eddy pumping induces isopycnal displacements that lift nutrient-replete waters into the euphotic zone, driving a set of biogeochemical processes. These processes include recycling of nutrients within the surface sunlit waters, physical transport of nutrients from nutrient-rich deep waters, productivity of autotrophic organisms, zooplankton grazing and the "biological pump". Most studies focus on these aspects (Benitez-Nelson et al., 2007; Benitez-Nelson and McGillicuddy, 2008; McGillicuddy et al., 2007), showing that biological and biogeochemical responses within eddies are quite complex due to a combination of variations in the magnitude, timing and duration of nutrient input caused by differences in eddy formation, intensity, age and movement (Benitez-Nelson and McGillicuddy, 2008).

Microbial plankton are centrally involved in fluxes of energy and matter and mediate all biogeochemical cycles in the oceans (DeLong et al., 2006). Perturbations of physical processes such as mesoscale eddy, upwelling and coastal jets usually modify chemical features in a region and introduce new microbial species, consequently probably modifying the function of the microbial community in the ecosystem. Regarding microbial responses to mesoscale eddies, recent studies report distinctly elevated heterotrophic prokaryotic biomass and production inside a cold-core eddy region compared to surrounding waters (Baltar et al., 2010; Thyssen et al., 2005; Zhang et al., 2009). Ewart et al. (2008) found an increase in prokaryotic heterotrophic production at the periphery of a cyclonic eddy relative to the eddy center in the Sargasso Sea. Baltar et al. (2010) reveal

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different prokaryotic community structure between mesoscale eddies and far-field stations in the epipelagic layer around the Canary Islands.

The South China Sea (SCS) with its deep basin is one of the largest marginal seas in the tropical Pacific Ocean. It is characterized by the relatively frequent passage of eddies (Hwang and Chen, 2000; Wang et al., 2003), which introduce spatial and temporal variability in the productivity of the western tropical Pacific Ocean. Here we report the phylogenetic diversity of the bacterial community in two cold-core cyclonic eddies in the SCS compared with reference sites at the eddy periphery and outside the eddies. This study is a contribution to the understanding of biological and biogeochemical consequences of a mesoscale cyclonic eddy in the SCS.

2. Materials and methods

2.1. Eddy tracking

Near-real-time satellite altimetry (http://argo.colorado.edu/~realtime/gsfc_global-real-time_ssh/) and shipboard acoustic Doppler current profile data from R.V. *Dongfanghong* #2 (14 August–14 September 2007) permitted accurate tracking of the eddies throughout the cruise, and allowed for high-resolution biogeochemical sampling across the eddies. For this study, four stations were chosen (Fig. 1). At each station, a vertical profile with nine or ten depths from surface to deep waters was sampled. Two stations were located inside cold-core cyclonic eddies (CE1 at 111.83° E, 14.25° N; CE2 at 111.03° E, 12.03° N), one station at the cold-core eddy periphery (CEP at 113.00° E, 15.00° N), and another at the Southeast Asia Time-Series Study station (SEATS at 115.96° E, 18.03° N) (Fig. 1). The water depths of the four sites were 2844, 2418, 2778 and 3839 m, respectively.

2.2. Sample collection

Sample water was collected via a SeaBird CTD-General Oceanic rosette sampler with Go-Flo bottles (SBE 9/17 plus, SeaBird Inc., U.S.A.). Two- to five-liter seawater samples, collected from 5 to 1500 m depths at CE1, CE2 and CEP and to 3500 m at SEATS, were filtered through 47-mm diameter 0.2-μm-pore-size polycarbonate filters (Whatman) at a pressure < 0.03 MPa. Filtered samples were immediately frozen and stored at −80 °C until DNA extraction.

2.3. Hydrographic parameters

Data for temperature, salinity, nutrients and chlorophyll-*a* were provided by the GOE (Group of Excellence, NSF of China) project. Samples for inorganic nutrients (nitrate + nitrite, phosphate) were filtered through 0.45-μm cellulose acetate filters and measured immediately onboard using a flow injection analyzer (Tri-223 autoanalyzer) and standard spectrophotometric methods. Samples for chlorophyll-*a* analysis were collected on 0.7-μm pore-size GF/F filters (Whatman) and chlorophyll-*a* was determined using a Turner-Designs Model 10 fluorometer.

2.4. DNA extraction

The polycarbonate filters were cut into pieces under sterile conditions. DNA extraction was performed using Mega Kit extraction (MoBIO Laboratories, Inc.) and the protocol of the manufacturer.

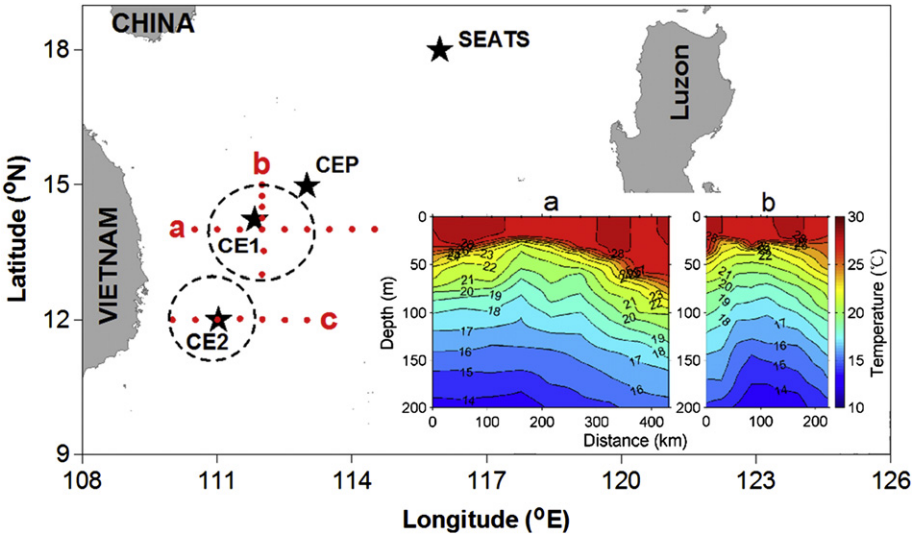

Fig. 1. Map of the SCS showing sampling stations (stars) for analysis of microbial community diversity and function. The red dots show the transects (a, b and c) of biogeochemical sampling across the eddies. Two dashed circles delineate the two cold-core cyclonic eddies observed during sampling. CE1: cold-core cyclonic eddy #1; CE2: cold-core cyclonic eddy #2; CEP: cold-core cyclonic eddy periphery; SEATS: a time series station in the SCS. Temperature sections along (a) and (b) transects are inlaid. Temperature section along (c) transect is shown elsewhere (J. Hu, submitted manuscript). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. PCR amplification and T-RFLP

Terminal restriction fragment length polymorphism (T-RFLP) fingerprinting determinations were performed on all samples. PCR conditions and chemicals were applied as described by Moeseneder et al. (1999). The universal bacterial primers 8F-FAM (5′-AGAGTTTGATCATGGCTCAG-3′) (Weisburg et al., 1991) and 927R (5′-ACCGCTTGTGCGGGCCC-3′) (Amann et al., 1995) were used. The PCR products were purified with the agarose gel DNA purification kit (TaKaRa). Fluorescently labeled PCR products were digested with the restriction enzyme RsaI (TaKaRa) at 37 °C overnight according to the manufacturer's protocol. Digested products were precipitated by adding two volumes of 100% cold ethanol, centrifuged at 15,000 × *g* for 20 min at 4 °C and rinsed with 100 μL of 70% cold isopropanol. After centrifugation, DNA pellets were dissolved in 20 μL of ultra-pure water and mixed with 0.5 μL of the internal size standard (ET ROX-900, Amersham). This mixture was denatured for 2 min at 95 °C and immediately chilled on ice. Fluorescently labeled fragments were separated and detected with a MegaBACE genetic analyzer (Amersham) operated in Genotyping mode (Zhang et al., 2008). Replicates were run for all samples to obtain a reproducible T-RFLP profile.

The electropherograms were analyzed using MegaBACE Genetic Profiler software (Amersham) to determine the peak height and area and for standardization using size markers. The output was transferred to T-REX software (http://trex.biohpc.org/) (Culman et al., 2009) for identification of true peaks over noise and to construct a two-way data matrix. The matrix obtained was further analyzed with the additive main effects and multiplicative interaction model (AMMI) (Culman et al., 2009) to determine similarities of the T-RFLP fingerprints between samples.

2.6. PCR amplification and clone library construction

A total of eight samples (from the 5 m depth and the chlorophyll maximum layer at the four sites) were used to construct clone libraries. PCR amplification and gel purification of PCR products were conducted as described above. The universal bacterial primers 27F and 1492R were used (Lane, 1991). The products were ligated into the pMD18-T vector (TaKaRa, Dalian, China) and then transformed into competent cells of *Escherichia coli* DH5α. The ampicillin-resistant clones were randomly picked and screened for inserts by performing colony PCR with M13 primers (Invitrogen, Shanghai, China) for the vector.

2.7. DNA sequencing and phylogenetic tree construction

A total of 767 clones with correct inserts (~1500 bp) were directly sequenced. Sequencing was carried out on an ABI model 3730 automated DNA sequence analyzer (Applied Biosystems, Perkin–Elmer) using M13 primers. All nucleotide sequences were manually checked for sequence quality and putative chimeras using CHIMERA CHECK (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU) (Cole et al., 2003). Six-hundred ninety-nine reliable sequences were grouped as 229 operational taxonomic units (OTUs) by 1% distance in the DOTUR program (Schloss and Handelsman, 2005). Representative sequences from each OTU were aligned using the ARB aligner, and added to the universal parsimony tree using "ARB parsimony" with a "positional variability by parsimony" filter for bacteria (Pruesse et al., 2007). Sequences were also compared to known 16S rDNA sequences in the database using the BLASTN search (http://www.ncbi.nlm.nih.gov/BLAST/). Around 180 reference sequences were retrieved, together with our representative sequences from each OTU, for phylogenetic reconstructions. Sequences were aligned and compiled using the BioEdit program and phylogenetic trees were constructed with the posterior probabilities algorithm using MrBayes (Version 3.1) (Huelsenbeck and Ronquist, 2001). The MrBayes trees were built according to the manual (http://mrbayes.csit.fsu.edu/manual.php). The General Time Reversible (GTR) model of nucleotide evolution was used. The program ran until the "average standard deviation of split frequencies" became less than or extremely near to 0.01. Then, the first quarter of the trees of the unstable generations was "burnt in" using "half-compat", and MrBayes consensus trees were constructed.

2.8. α-Diversity and β-diversity analysis of the clone libraries

α-Diversity (diversity within communities) was estimated as the number of species in a community (species richness). Sequences were assigned to major groups using BLAST similarities and the RDP Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp). Non-parametric coverage and phylotype richness estimators (ACE, Chao1, Shannon and Simpson) were calculated using DOTUR software (Schloss and Handelsman, 2005). β-Diversity (partitioning of diversity among communities) was estimated based on the number of shared species (Lozupone and Knight, 2008) using ∫-LIBSHUFF software (Schloss et al., 2004).

2.9. Nucleotide sequence accession numbers

Clone sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) under accession numbers GU061329 to GU062179.

3. Results

3.1. Cyclonic eddy perturbation

Two well-developed cold-core cyclonic eddies (CE1 and CE2) were identified and their development was tracked. The eddies were 100–150 km in diameter at the surface and their cores were located at approximately 112.5° E, 13.5° N and 111° E, 12° N. The acoustic Doppler current profile data documented counterclockwise currents of 0.7 m s^{-1} and 1.1 m s^{-1} at maximum speed in CE1 and CE2 respectively (J. Hu, unpublished data). Both the seasonal and permanent thermocline shoaled, causing negative sea-level anomaly (Zhang et al., 2009). Their altimetric history

suggested intensification of CE1 between 14 and 30 August and of CE2 between 26 August and 12 September. Observations were conducted during the intensification phases as samples were taken at CE1 on 26 August, at CEP on 28 August, at CE2 on 5 September and at SEATS on 13 September.

Eddies lifted isopycnals into the euphotic zone (Fig. 1 and Table 1), injecting nutrients into the nutrient-depleted surface waters that were rapidly utilized, resulting in the accumulation of phytoplankton biomass and organic matter. The subsurface waters at stations CE1 and CE2 exhibited lower temperature (Fig. 1), higher salinity, higher nutrient concentrations, higher chlorophyll-*a* concentrations and lower dissolved oxygen (Zhang et al., 2009) than at stations CEP and SEATS. Elevated heterotrophic prokaryotic biomass and production at CE1 and CE2 have been reported elsewhere (Zhang et al., 2009). The CEP station was located at the cold-core eddy periphery and hence, potentially influenced by the mesoscale features, whereas the SEATS station is located far outside the two eddy systems and is a representative outside-eddy station (Zhang et al., 2009).

3.2. Bacterial community composition analysis through the whole water column based on T-RFLP fingerprinting

T-RFLP patterns of the bacterial communities revealed, in total, 246 OTUs on the 16S rDNA level ranging from 57 to 939 bp fragments. Only six of the 246 OTUs (57, 77, 312, 313, 421 and 449 bp fragments) were present in more than 80% of all samples. The cluster plot generated by AMMI analysis of the T-RFLP fingerprints indicated three distinct clusters, a euphotic cluster, a deep-water cluster and an intermediate cluster (Fig. 2a). The intermediate layer as indicated by T-RFLP fingerprinting was wider at the CE1 and CE2 sites (100–800 or 1000 m depth) than the CEP (200–450 m depth) and SEATS sites (200 m depth) (Fig. 2b).

3.3. α-Diversity and β-diversity analysis of 16S rDNA clone libraries in the euphotic zone

A total of 699 reliable sequences from eight clone libraries (Table 2) were used for diversity analysis. These cloned sequences were designated as Site-Layer-*n*, in which *n* represents the number of the clone. Sequences were clustered into groups of defined sequence variation of $\leq$1% (or 3%) using DOTUR. These clusters served as OTUs for generating rarefaction curves and for making calculations with α-diversity indices, the Shannon and Simpson diversity indices (Magurran, 1988), the abundance-based coverage estimator ACE (Chao and Lee, 1992; Chao et al., 1993) and the Chao1 (Chao, 1984) estimator of species diversity (Table 2). Rarefaction curves described higher levels of microbial complexity for the samples at the chlorophyll maximum layers than at the surface layers except at site CE1 (Supplementary Fig. S1). The lowest diversity indices and the rarefaction curve in the surface water of CE2 indicated that its microbial community was composed of a few phylotypes, while in the surface water of CE1, the diversity indices showed a higher level of species richness. Table 2 also shows that, for both CE1 samples, the species diversity estimates obtained with ACE and Chao1 at the 1% distance level were almost twice as great as at the 3% distance level.

Table 1
Hydrographic characteristics of the different sites sampled in the SCS. Descriptions of stations are as in Fig. 1.

	CE1	CE2	CEP	SEATS
Depth of mixed layer (m)	25	15	30	40
Nitracline depth (m)	~20	~10	~45	~70
Depth of chlorophyll-*a* max (DCM) (m)	45	20	60	75
Chlorophyll-*a* at DCM (mg m^{-3})	0.46	0.49	0.27	0.26

The partitioning of bacterial diversity among communities, analyzed using ∫-LIBSHUFF software, revealed that there were no significant differences in phylogenetic composition between the surface waters of CE2 and CEP, nor between the surface water and the chlorophyll maximum layer of SEATS (Supplementary Table S1). The dendrogram which was constructed based on the ΔC_{xY} values (data not shown) derived from the ∫-LIBSHUFF analysis, revealed an interesting partitioning of bacterial diversity responding to environmental variations. The analysis using the unweighted pair group method with arithmetic mean joined the surface samples of CE1, CE2 and CEP and the chlorophyll maximum layer samples of CE1 and CE2 together in separate clusters, while both SEATS samples were joined together into another cluster (Fig. 3).

3.4. Bacterial community composition analysis of 16S rDNA clone libraries

The species composition of the clone libraries is shown in Fig. 4. All of our sequences fell into the nine major lineages of the bacterial domain: Alpha-, Gamma-, Delta- and Betaproteobacteria; Cyanobacteria; Bacteroidetes; Actinobacteria; Planctomycetes and Verrucomicrobia, as well as some clones originating from eukaryotic chloroplasts or plastids. The percentage of 16S rDNA sequences of each group in the total microbial rDNA pool indicated that the Cyanobacteria dominated the surface and the chlorophyll maximum layer of CE2 and the surface water of CEP (50–73%), while Alphaproteobacteria dominated the surface and the chlorophyll maximum layer of SEATS, the chlorophyll maximum layer of CEP and the surface water of CE1 (41–76%). The chlorophyll maximum layer of CE1 was codominated by Cyanobacteria (42%) and Alphaproteobacteria (36%) (Fig. 4). For the microbial communities dominated by Alphaproteobacteria, Rhodobacterales was the major group at SEATS, while SAR11 was the major group at other eddy-related sites. Gammaproteobacteria, Actinobacteria and Bacteroidetes also occurred in all of the samples but were not abundant. Deltaproteobacteria were not detected at the SEATS site nor in the surface water of CE2, and only very few Betaproteobacteria were detected at the CE2 site and in the chlorophyll maximum layer of the CEP site. Planctomycetes and Verrucomicrobia were also detected as minor groups in a few of the samples.

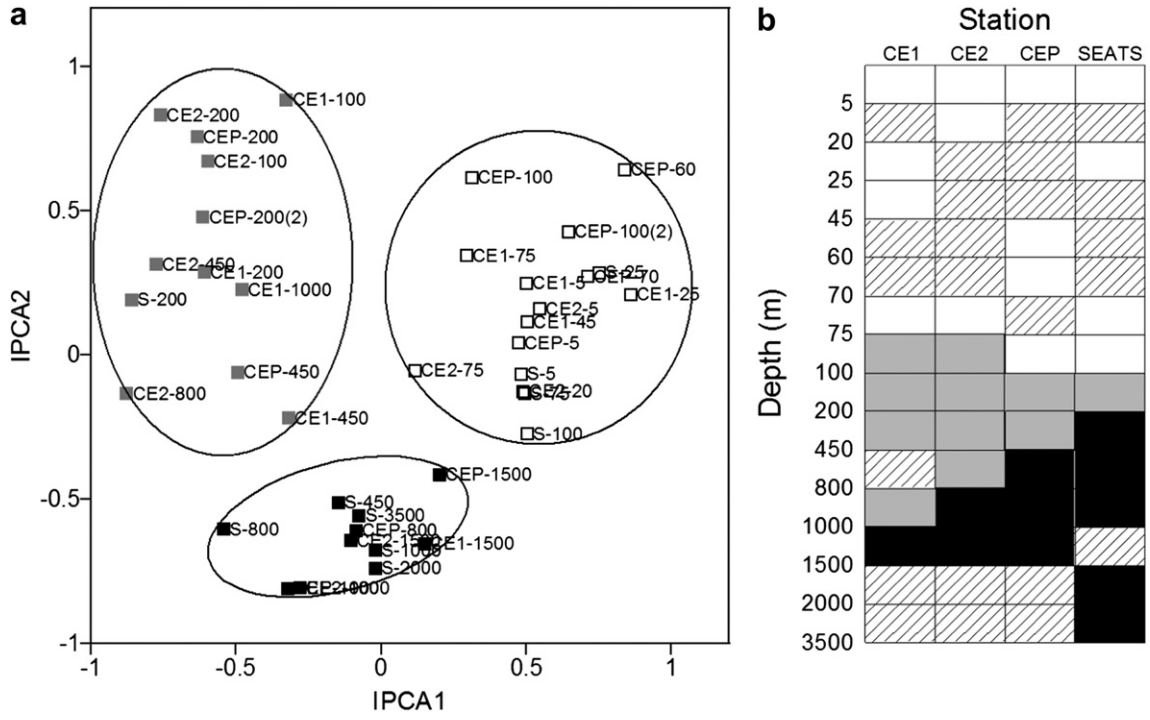

Fig. 2. Cluster plot (a) generated by AMMI analysis of the data matrix of presence/absence distribution of all individual bacterial OTUs. Descriptions of stations are as in Fig. 1. Numbers following stations indicate depth (meter). '(2)' indicates T-RFLP determination from the duplicate seawater sample. Stations and depths corresponding to white, gray and black squares in (a) are shown in (b). Diagonally hatched in (b) indicate no sample taken.

3.5. Phylogenetic analysis of gene sequences of 16S rDNA clone libraries

The highest diversity of phylotypes was found within the Alphaproteobacteria cluster, with a total of 280 sequences (40%), assigned to 105 OTUs, dominated by SAR11 sequences (50 OTUs, 125 clones). The SAR11 cluster was found in almost all samples we examined, except for the chlorophyll maximum layer sample of CE2 (Fig. 5a). The second most abundant cluster belonged to the Rhodobacterales. They were clustered with the genera *Paracoccus*, *Roseobacter*, *Sulfitobacter*, *Octadecabacter*, and *Hyphomonas*. The *Paracoccus* cluster was assigned to six OTUs and all the clones were from the SEATS site, especially in the surface water, where more than 50% of total sequences were classified as *Paracoccus* (Fig. 5a). They were clustered tightly with *Paracoccus denitrificans*, *Paracoccus zeaxanthinifaciens* and *P. sp*. 88/2-4 strains. PCR, cloning and sequencing analysis of the replicate sample revealed a similar species composition (Supplementary Fig. S2) and confirmed that *Paracoccus* was a major component in the surface water of SEATS. The *Octadecabacter* cluster was assigned to three OTUs and all 23 clone sequences were from both layers of CE1 and the chlorophyll maximum layer of SEATS. The third most abundant cluster belonged to the Sphingomonadaceae, dominated by the sequences belonging to the genus *Erythrobacter*. Additionally, five clone sequences, assigned to five OTUs, which were obtained from site CE1 were affiliated with the Rhodospirillaceae cluster in ARB (Fig. 5a). Eight clones were classified into the Rickettsiales in ARB. Only one clone was grouped into the *Hoeflea* genus. Seventeen other clones, assigned to nine OTUs, were related to unidentified alpha-affiliated sequences.

A total of 68 sequences (31 OTUs) were clustered into the Gammaproteobacteria. Most of these clones (52%) were

Table 2
Similarity-based OTUs and species richness estimates. Descriptions of stations are as in Fig. 1. DCM: depth of chlorophyll-*a* max. Simpson index is 1-D form.

Sample ID	No. of clones	DNA sequence dissimilarity							
		0.03				0.01			
		ACE	Chao1	Shannon	Simpson	ACE	Chao1	Shannon	Simpson
CE1-5m	92	114	88	3.14	0.94	207	158	3.58	0.97
CE1-DCM	86	65	73	2.84	0.93	106	136	3.33	0.96
CE2-5m	92	30	23	1.31	0.48	39	33	1.61	0.57
CE2-DCM	85	133	114	2.47	0.83	133	102	3.01	0.91
CEP-5m	88	49	59	1.99	0.77	85	52	2.71	0.86
CEP-DCM	80	69	65	2.94	0.93	79	78	3.67	0.98
SEATS-5m	89	51	56	2.25	0.76	83	89	2.39	0.77
SEATS-DCM	87	140	121	3.19	0.94	132	111	3.69	0.98

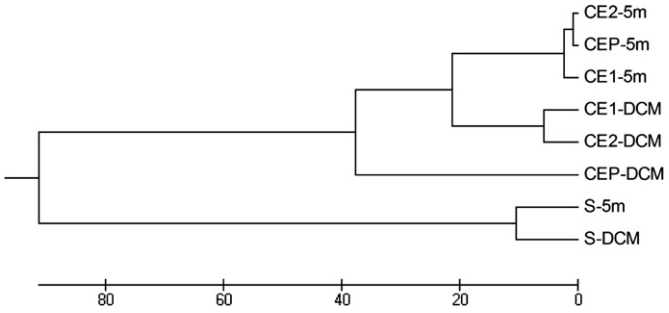

Fig. 3. The dendrogram constructed using the unweighted pair group method with arithmetic mean (MEGA software) based on the ΔC_{xY} values derived from ∫-LIBSHUFF analysis. Descriptions of stations are as in Fig. 1. DCM: depth of chlorophyll-*a* max. Scale bar indicates the proportion of samples changing along each branch.

related to unidentified gamma-affiliated sequences (Fig. 5b), suggesting that many not previously recognized bacterial groups exist in the SCS. The second dominant group (19 clones) was closely affiliated with the Alteromonadales or Alteromonadales-like cluster, and all clones were from both layers of CE1 and also the chlorophyll maximum layer of CE2 (Fig. 5b). The remaining clone sequences were grouped into the *Legionellales* (seven clones), *Halomonas* (three clones), *Acinetobacter* (two clones) and *Vibrio* (two clones).

A total of 203 clones (29%) classified into 25 OTUs were affiliated with the Cyanobacteria, in which 11 sequences were *Prochlorococcus*, and the others were *Synechococcus*. The clones affiliated with *Prochlorococcus* obtained from the surface waters of the four sites were most similar to the high-light-adapted ecotype *Prochlorococcus* strain, while others obtained from the chlorophyll maximum layers were most closely related to the low-light-adapted ecotype strains (Fig. 5c). A total of 53 clones classified into 12 OTUs were affiliated with the Actinobacteria. Most of them (57%) were related to unidentified Actinobacteria sequences. A total of 20 clones were affiliated with the Bacteroidetes, but a higher

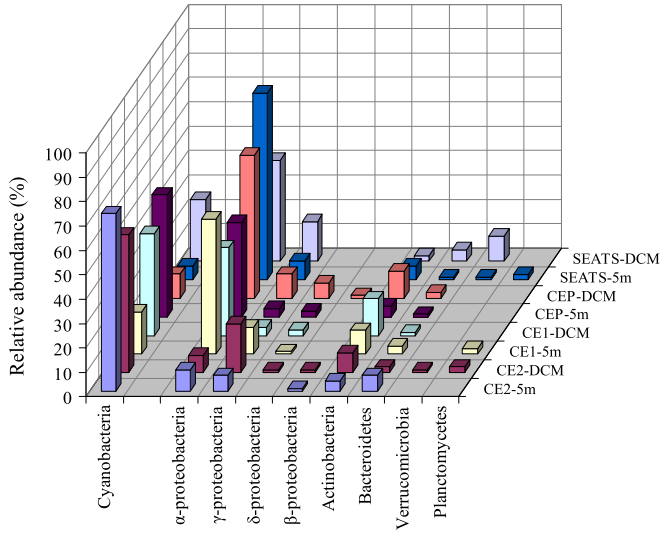

Fig. 4. Percent abundance of bacterial phylotypes as determined by 16S rDNA clone library statistics and phylogenetic analysis. Descriptions of stations are as in Fig. 1. DCM: depth of chlorophyll-*a* max.

diversity of phylotypes was found within this cluster with 16 OTUs. These clones were obtained from all the samples and some were closely related to functionally important bacteria, such as the predatory bacterium *Saprospira grandis* (Lewin, 1997) and the cohering bacterium *Lewinella cohaerens* (Fig. 5c). The remaining clones were grouped into the Deltaproteobacteria (12 clones/nine OTUs), the Betaproteobacteria (three clones/three OTUs), the Planctomycetales (six clones/four OTUs) and the Verrucomicrobia (11 clones/four OTUs). Clones originating from eukaryotic chloroplasts or plastids (43 sequences classified into 20 OTUs) are not shown in Fig. 5.

4. Discussion

Apparently, isopycnal displacement (Table 1) induced by different water mass movements inside and outside the cyclonic eddies leads to distinct temperature (Fig. 1), salinity, nutrient and chlorophyll characteristics (Zhang et al., 2009) and consequently, different hydrological and nutrient conditions modified the microbial community composition.

The composition patterns of the bacterial communities revealed by T-RFLP were classified into three distinct clusters, a euphotic cluster, a deep-water cluster and an intermediate cluster (Fig. 2a). Numerous reports show stratified microbial populations from euphotic to bathypelagic zones in the Atlantic and Pacific Oceans (DeLong et al., 2006; Fuhrman and Davis, 1997; Gordon and Giovannoni, 1996). Here, our comparative results between the sites revealed a wider intermediate zone at the sites inside rather than outside the cyclonic eddies (Fig. 2b), although a depth-related distribution pattern of bacterial communities through the whole water column was determined at all the sites. It is suggested that eddy-induced upwelling might widen the depth range of the intermediate transition water mass. Generally, the number of bacterial OTUs was almost twice as high in the intermediate layers (50 ± 10 OTUs) as in the euphotic (28 ± 10 OTUs) and deep-water layers (30 ± 7 OTUs), reflecting a higher level of microbial complexity for the intermediate transition water mass. Heterogeneity of available organic substrates potentially introduced by the export of both particulate and dissolved organic matter in combination with solubilization of sinking particles (Azam and Long, 2001; Azam and Malfatti, 2007) within the mesopelagic zone may have contributed to the greater diversity we observed in the bacterial community of the intermediate layers (Treusch et al., 2009).

Given that eddy perturbations usually introduce considerable spatial heterogeneity into the upper ocean due to the introduction of new nutrients into the euphotic zone from nutrient-rich deep waters (Becher and Rappè, 2006), our study focused on the bacterial community in the euphotic zone. Eight clone libraries from the 5 m and the chlorophyll maximum layers of the four sites were constructed in order to compare the phylogenetic diversity of the community in the upper ocean both inside and outside the eddies. Agreeing with previous studies (DeLong et al., 2006; Ma et al., 2009), the higher levels of microbial complexity at the chlorophyll


a
0.10
CE1-5m
CE1-DCM
CE2-5m
CE2-DCM
CEP-5m
CEP-DCM
SEATS-5m
SEATS-DCM
S-5m-1(GU061852) (42; 1)
S-5m-96(GU061914) (1)
Paracoccus sp. 88/2-4 (AJ313424)
Paracoccus denitrificans (AY157621)
S-DCM-62(GU061965) (1)
Paracoccus zeaxanthinifaciens (AF461158)
Paracoccus zeaxanthinifaciens (AM180732)
S-5m-48(GU061890) (1)
S-5m-26(GU061873) (1)
S-5m-12(GU061861) (2)
Paracoccus
CE1-5m-26(GU061341) (1)
Clone 4C229596(3336m)(EU802287)
Roseobacter sp. JL-131 (AY646162)
S-DCM-63(GU061966) (1)
CE1-5m-36(GU061348) (2)
Clone 1C226662(12m)(EU799101)
Sulfitobacter sp. SCSWC11 (FJ461445)
S-DCM-46(GU061958) (1)
CE2-5m-82(GU061513) (1; 1; 1)
Clone 1C227145(12m)(EU799534)
S-DCM-101(GU061976) (1)
Shimia sp. (FJ425589)
Shimia sp. (FJ425632)
Rhodobacterales bacterium HTCC2654 (AAMT01000002)
Maritimibacter alkaliphilus (DQ915443)
S-DCM-35(GU061952) (1)
Rhodobacteraceae
Clone 6C232002(2431m)(EU804110)
S-DCM-13(GU061945) (2)
CE1-5m-4(GU061330) (9; 4; 5)
CE1-5m-54(GU061355) (3)
Clone S23_909(EF572810)
O. arcticus strain 238(NR_026040)
Octadecabacter-like (ARB)
CE1-DCM-22(GU061424) (6; 1; 1)
CE1-DCM-45(GU061433) (1)
CE1-DCM-81(GU061447) (1; 1)
Clone ctg_CGOF033(DQ395891)
Caulobacter sp. MOLA 88 (AM990862)
Hyphomonas sp. OC5 (AB429068)
CE1-5m-63(GU061360) (1)
Hyphomonas
Hyphomonadaceae
Clone GWS-BW-H52M(AY515416)
S-DCM-74(GU061967) (1)
Hoeflea alexandrii (AJ786600)
Hoeflea
Phyllobacteriaceae
SAR11 (50 OTUs, 125 clones)
( )
SAR11
Unidentified group (7 OTUs, 15 clones)
( )
Unidentified group 1
CE1-5m-60(GU061358) (1; 1)
Clone C28(EU010183)
CE1-DCM-14(GU061423) (1)
Clone SPOTSMAY03_150m10(DQ009453)
Rickettsiales mitochondria (ARB) (3 OTUs, 3 clones)
( )
Unidentified group 2
CE1-5m-54(GU061361) (1)
Clone 6C231975(2431m)(EU804083)
CE1-DCM-56(GU061437) (1)
Clone 1C227594(12m)(EU799921)
CE1-5m-32(GU061345) (1)
Clone S25_668(EF574324)
CE1-DCM-80(GU061446) (1)
Clone C41(EU010192)
Rhodospirillaceae bacterium MC2UP-L3 (FJ463255)
CE1-5m-33(GU061346) (1)
Clone 4C229604(3336m)(EU802295)
Rickettsiales mitochondria (ARB) (4 OTUs, 5 clones)
( )
Rhodospirillaceae-like (ARB)
S-5m-101(GU061917) (1)
S-DCM-37(GU061953) (1)
Sphingomonas sp. Cep2 (EF462462)
Pseudomonas abikonensis (AB021416)
S-5m-6(GU061855) (2; 1)
CE2-DCM-87(GU061603) (1)
Novosphingobium sp. JEM-1 (DD224245)
Erythromicrobium ramosum (AF465837)
S-DCM-91(GU061974) (1)
Erythrobacter (8 OTUs, 22 clones)
( )
Sphingomonadaceae
b
0.10
Clone PLY-P1-108(AY354844)
CE2-DCM-102(GU061622) (1; 2)
CE1-5m-96(GU061386) (1)
Clone 1C227731(12m)(EU800042)
CEP-DCM-17(GU061821) (2)
Clone S25_1348(EF575004)
CE2-DCM-63(GU061617) (1)
Clone C55(EU010203)
CE1-5m-58(GU061385) (1; 3)
Clone 6C233323(2431m)(EU805320)
CE2-5m-27(GU061520) (1; 3; 1)
Clone S25_378(EF574034)
Clone S25_567(EF574223)
Unidentified group 1
CE1-5m-105(GU061387) (1)
Clone 6C232673(2431m)(EU804715)
CEP-5m-109(GU061719) (1)
Clone TLC-FL2-28(EF379476)
CE1-DCM-86(GU061453) (1)
Clone AD235-B9(EU686595)
Clone ZA3313c(AF382113)
CEP-DCM-70(GU061825) (1)
CE2-5m-4(GU061515) (5; 1; 2)
Clone P0X4b3C11(EU491445)
S-DCM-24(GU061979) (1)
S-DCM-71(GU061987) (1)
Clone C93(EU010224)
CEP-5m-65(GU061718) (1)
Clone B275(EU328006)
Unidentified group 2
CE1-5m-42(GU061382) (1)
S-DCM-65(GU061985) (1)
Clone 4C230497(3336m)(EU803079)
CE1-5m-5(GU061380) (1)
Clone 4C230126(3336m)(EU802753)
Clone S25_302(EF573958)
S-DCM-57(GU061984) (1)
Clone S23_59(EF571960)
Clone KTc0924(AF235121)
CE2-DCM-3(GU061605) (1; 1)
Metagenome ctg_1101668179621(AACY023372270)
CE2-DCM-38(GU061613) (1)
Legionellales
CE1-5m-110(GU061389) (1)
Clone B81(EU010152)
Uncultured Glaciecola sp. clone D1645(EU258752)
CE1-5m-52(GU061383) (3; 7)
Clone C22(EU010181)
CE1-DCM-73(GU061452) (1; 5)
CE2-DCM-1(GU061605) (1)
Clone EPR3967-O2-Bc43(EU491790)
Alteromonadales-like (ARB)
CEP-5m-110(GU061720) (1; 1)
Clone N67e_178(upwelling system)(EF646139)
Clone 1C227721(12m)(EU800034)
Unidentified group 3
Clone EF100-91A10(AY627370)
S-5m-85(GU061924) (1)
Unidentified group 4
Metagenome JCVI_SCAF_1096627306855(EQ003041)
S-DCM-34(GU061580) (1)
Halomonas sp. NIBH P1H14 (AB016051)
Halomonas
Oceanospirillales
CE1-5m-7(GU061381) (1; 2)
Acinetobacter sp. PmeaMuc16 (EU249988)
Acinetobacter
Pseudomonadales
S-5m-47(GU061921) (1; 1)
Vibrio sp. CAIM 1643 (DQ451212)
Vibrio
Vibrionales
S-DCM-44(GU061981) (2)
CE1-DCM-102(GU061454) (1)
Pseudoalteromonas sp. 8048 (AM111082)
Pseudoalteromonas sp. 8051 (AM111085)
Pseudoalteromonas
Alteromonadales
c
0.10
Flavobacteriaceae
(8 OTUs, 11 clones) ( )
Unidentified Bacteroidetes
(4 OTUs, 4 clones) ( )
Cytophagales bacterium clone 06(AF361205)
CE1-DCM-100(GU061493) (1)
CE2-5m-1(GU061329) (1)
Clone S23_600(EF572501)
CE2-5m-65(GU061364) (2)
Sphingobacteriales bacterium (EF092259)
Lewinella cohaerens (AB301614)
S-DCM-72(GU062025) (1)
Saprospira grandis (AY527408)
Saprospira sp. CNJ537 (AY527411)
Bacteroidetes
Mycobacterium poriferae (AF480589)
S-DCM-78(GU062027) (1)
Mycobacterium psychrotolerans (AJ534886)
Unidentified Actinobacteria (5 OTUs, 30 clones)
( )
Clone SHFG434(FJ203051)
CE2-5m-67(GU061598) (1; 1; 1)
Clone S23_1314(EF573215)
CE2-DCM-57(GU061677) (1)
Actinobacterium clone MD2.25(FJ403074)
Metagenome 1096626361887(AACY020288370)
CE1-DCM-6(GU061494) (7; 3; 1)
CE1-DCM-96(GU061506) (1)
Clone ZA3612c(AF382135)
CEP-DCM-48(GU061847) (3)
CE1-DCM-27(GU061498) (1; 1; 1)
Clone 6C233403(2431m)(EU805394)
Actinobacteria
CEP-DCM-5(GU061820) (1)
Clone BF23(DQ327689)
Aquabacterium sp. (AY569280)
CE2-5m-25(GU061519) (1)
Clone 1C227401(12m)(EU799756)
CE2-DCM-28(GU061611) (1)
Clone PEACE2006/40_P2(EU394539)
Betaproteobacteria
CE2-DCM-29(GU061672) (1)
Clone S25_1212(EF574868)
Clone Sylt 5(AM040101)
CEP-DCM-66(GU061838) (2)
Clone S25_1042(EF574698)
S-5m-64(GU061934) (1)
CE1-5m-104(GU061408) (1)
Clone S25_1336(EF574992)
Unidentified Deltaproteobacteria (5 OTUs, 7 clones)
( )
Deltaproteobacteria
CE1-5m-19(GU061406) (2; 1)
Clone S25_696(EF574352)
CE2-DCM-8(GU061671) (1)
Planctomycete 6FN (EF591887)
S-5m-40(GU061931) (1)
Clone c5LKS81(AM086149)
Clone LAgut-16S-P01(AY536217)
CE2-DCM-51(GU061674) (1)
Planctomycetes
Verrucomicrobiales (4 OTUs, 11 clones)
( )
Verrucomicrobia
CE1-5m-49(GU061395) (2; 1; 1; 1)
CEP-5m-33(GU061736) (1)
Metagenome 1096626829409(AACY020331499)
CEP-DCM-42(GU061830) (1)
Prochlorococcus marinus str. MIT 9313 (BX572095)
CE2-DCM-26(GU061633) (2; 2)
Prochlorococcus marinus str. MIT 9303 (CP000554)
Prochlorococcus
Synechococcus sp. WH 7805 (AAOK01000002)
Synechococcus sp. WH 8018 (AF311292)
Uncultured Cyanobacterium (EF123579)
Synechococcus (21 OTUs, 192 clones)
( )
Synechococcus

maximum layers than in the surface layers were revealed by rarefaction curves and diversity indices (Shannon, Simpson, ACE and Chao1) (Table 2 and Supplementary Fig. S1), except at site CE1, where the heterogeneity of available substrates and species introduced by cold eddy upwelling could have led to a higher diversity in the surface microbial community. However, at the other eddy center site CE2, the overwhelming cyanobacterial dominance (73%) (Fig. 4) led to quite low diversity in the surface bacterial community (Table 2). This was consistent with the higher surface chlorophyll concentration observed at site CE2 than at the other three sites (Zhang et al., 2009). The different responses of the phytoplankton and bacterial communities of the surface water between the two eddies could be explained by variations in the magnitude, timing and duration of nutrient input caused by differences in eddy formation, intensity, age and movement. In this study, CE2 was a more intense cyclonic eddy with greater current speed than CE1 and, therefore, more nutrient input supported more phytoplankton growth at CE2 (Table 1) (Zhang et al., 2009).

Interestingly, distinct partitioning of microbial diversity among all eight communities responding to environmental variations was revealed by ∫-LIBSHUFF analysis of the clone libraries (Fig. 3). The stratification of prokaryotic populations which existed between the surface and chlorophyll maximum layers of the eddy-related sites, compared to the homogeneity of communities in the euphotic zone of the reference site, was most likely a reflection of isopycnal displacement led by water upwelling inside the eddies. Cyclonic eddy isopycnal uplift resulted in shoaling of the mixed layer and the nitracline (Table 1) introducing a deep-water mass into the euphotic zone, and consequently separating the surface and chlorophyll maximum layers into different environments.

Phylogenetic analysis of gene sequences of 16S rDNA clone libraries revealed a difference in bacterial community composition inside and outside the eddies. The surface and the chlorophyll maximum layer of site SEATS were dominated by Alphaproteobacteria (76% and 41% respectively) (Fig. 4) with the major group being the Rhodobacterales (Fig. 5a). All 49 *Paracoccus*-affiliated clones were limited to the SEATS site, mainly at the surface, and closely related to functionally important bacteria, such as the nitrate-reducing lithotroph *P. denitrificans* (Ludwig et al., 1993) and the zeaxanthin-producing *P. zeaxanthinifaciens* (Berry et al., 2003) (Fig. 5a). Previous studies (and our unpublished studies) also show that *Paracoccus* strains are frequently isolated from the SCS (Du et al., 2006; Liu et al., 2008). These results indicated that *Paracoccus* might be an important functional group existing in the SCS.

Located within two cold-core cyclonic eddies, the CE1 site was dominated by Alphaproteobacteria, while the CE2 site was dominated by Cyanobacteria (Fig. 4). However, both of them were characterized by Alteromonadales-affiliated clones. As the second abundant group of gamma-affiliated sequences, Alteromonadales and Alteromonadales-like clusters were only limited to the two eddy center sites (Fig. 5b). The organisms within the Alteromonadales are prevalent in different oceanic regions (Biers et al., 2009; Ivars-Martinez et al., 2008), but seem to be more frequent in deep waters. Numerous studies report that many members of the Alteromonadales are often from deep waters (Bowman and McMeekin, 2005; DeLong et al., 2006; García-Martínez et al., 2002). Furthermore, this group is reported to be capable of adapting to the relatively phosphate-depleted marine environment (Long and Luo, 2009), which is consistent with the enhanced N:P ratio (>>16:1) observed within cyclonic eddies (M. Dai, personal communication). It is possible that heterogenous species were brought up from deep-water by cold eddy upwelling and adapted to the modified environment. In addition, most of the *Octadecabacter*-affiliated clones were also found to be limited to the CE1 site and a few were from the chlorophyll maximum layer of the SEATS site (Fig. 5a), members of which are obligate psychrophiles (Gosink et al., 1997), probably reflecting the response of microbial populations to relatively low temperature environments. Notably, Rhodospirillaceae-affiliated clones were limited only to the CE1 site (Fig. 5a). This family is the non-sulfur purple photosynthetic bacteria, and nitrogen-fixing ability is found in almost all species (Ludden and Roberts, 1995; Madigan et al., 1984). Studies suggest that the highest potential for nitrogen fixation occurs during the summer monsoon in the SCS (Loick et al., 2007), when the highest rates are measured (Voss et al., 2006). However, these bacterial nitrogen-fixing activities could be low during the cyclonic eddy, since dissolved inorganic nitrogen could be replenished in the subsurface layers through upwelling. Overall, considerable spatial heterogeneity in the bacterial community composition was apparent inside and outside the cold-core cyclonic eddies of the SCS.

As a result of cyclonic eddy perturbations in the SCS, the vertical water movement widened the mesopelagic zone characterized by a more complex community and, furthermore, brought deep-water into the upper ocean, causing a shift in the microbial community in the euphotic zone in response to environmental changes, such as nutrients and temperature. However, eddy perturbations are quite complex dynamic processes (Benitez-Nelson and McGillicuddy, 2008). To better understand the role of microbes in marine biogeochemical processes, we need more intensive microbial measurements

Fig. 5. Phylogenetic trees generated from alignments of 16S rDNA sequences from the SCS samples and representative references retrieved from the SILVA database of the ARB program and GenBank. The root was determined using 16S rRNA gene sequences of *Nitrosopumilus maritimus* SCM1 (DQ085097) and uncultured Crenarchaeote (EF597682) as an outgroup reference. Some groups in the trees were compressed using the ARB program with vertical height and horizontal width of the triangles representing OTU abundance and degree of divergence, respectively, within a given group. Clones from this study are indicated in boldface and designated as CE1-5m (or CE1-DCM, CE2-5m, CE2-DCM, CEP-5m, CEP-DCM, S-5m and S-DCM) -*n*, in which *n* represents the number of different clones. Accession numbers are shown in parentheses. Numbers following the symbols (CE1-5m ◬, CE1-DCM ▲, CE2-5m ▽, CE2-DCM ▼, CEP-5m ◈, CEP-DCM ◆, S-5m ◍, S-DCM ●) indicate occurrence frequency of OTUs in corresponding libraries. Values at each node indicate the posterior possibility. Scale bars represent the nucleotide substitution percentage. (a) Alphaproteobacteria. (b) Gammaproteobacteria. (c) Others.

using new technologies, e.g. (meta)genomic, (meta)transcriptomic, (meta)proteomic approaches and more effective coupling of the biological, physical and chemical processes.

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Appendix. Supplementary material

The supplementary data associated with this article can be found in the on-line version at doi:10.1016/j.resmic.2010.12.006.

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