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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.

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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,  $18.03^{\circ}$  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- $\mu$ m-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.

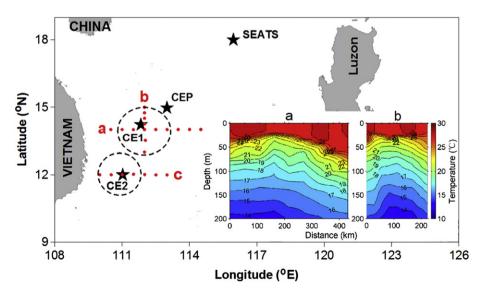


Fig. 1. Map of the SCS showing sampling stations (stars) for analysis of microbial community diversity and function. The red dots show the transects (a, b and c) of biogeochemical sampling across the eddies. Two dashed circles delineate the two cold-core cyclonic eddies observed during sampling. CE1: cold-core cyclonic eddy #1; CE2: cold-core cyclonic eddy 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.)

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