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Author(s)	Tada, Yuya; Shiozaki, Takuhei; Ogawa, Hiroshi; Suzuki, Koji
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Basin-scale distribution of prokaryotic phylotypes in the epipelagic layer of the Central South Pacific Ocean during austral summer

Yuya Tada^{*1}, Takuhei Shiozaki^{2, 3}, Hiroshi Ogawa², and Koji Suzuki¹

¹Faculty of Environmental Earth Science, Hokkaido University, North 10 West 5, Kita-ku, Sapporo, Hokkaido 060-0810, Japan

²Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba 277-8564, Japan

³Research and Development Center for Global Change, Japan Agency for Marine-Earth Science and Technology, 2-15, Natsushima-cho, Yokosuka, Kanagawa 233-0061, Japan

*Corresponding author: Yuya Tada

E-mail: yuyatada@ees.hokudai.ac.jp

Phone: +81-11-706-2246

Fax: +81-11-706-3026

Abstract

In the present study, we used catalyzed reporter deposition-fluorescence *in situ* hybridization to quantify the abundance of five bacterial (*Alphaproteobacteria*, SAR11, *Gammaproteobacteria*, SAR86, and *Bacteroidetes*) and two archaeal (*Crenarchaeota*

and *Euryarchaeota*) phylotypes in the epipelagic layer (0–200 m) of the Central South Pacific Ocean along 170°W from 0° to 40°S. We found that the distribution patterns of these phylotypes differed from each other. All phylotypes except *Gammaproteobacteria* were particularly abundant at the surface water of the equatorial region, whereas *Gammaproteobacteria* was relatively abundant in the area from the southern part of the South Pacific Ocean. SAR11, affiliated with *Alphaproteobacteria* was the dominant phylotype at all depths, throughout the study area. The abundance of SAR11 significantly increased with chlorophyll *a* concentration, suggesting that phytoplankton could affect their distribution pattern. There was a positive correlation between *Bacteroidetes* abundance and water temperature, suggesting that the temperature gradient could be a critical factor determining their distribution in the South Pacific Ocean. *Crenarchaeota* and *Euryarchaeota* were more abundant at the equatorial region than other study areas. *Euryarchaeota* abundance significantly decreased with depth, and increased with chlorophyll *a* concentration. It suggests that there was ecological interaction between *Euryarchaeota* and phytoplankton in the equatorial surface. Our data indicate that distinct hydrographic properties such as seawater temperature, salinity, and the concentrations of chlorophyll *a* and nutrients can principally control the basin-scale distribution of different prokaryotic phylotypes in the epipelagic layer of the Central South Pacific Ocean.

45 **Keywords:** Marine bacteria; Marine archaea; Fluorescence *in situ* hybridization;
46 Central South Pacific Ocean
47
48 **Heading:** Bacterial and archaeal distribution in the Central South Pacific Ocean

1. Introduction

Bacteria and Archaea are widely recognized as important players of oceanic biogeochemical processes that are involved in the organic matter and nutrient cycling in the ocean. Previous studies reported that prokaryotic biomass and production vary with certain basin-scale hydrographic gradients in the ocean (e.g., Teira et al. 2006a; Schattenuhofer et al. 2009; Yokokawa et al. 2013). In the Atlantic Ocean, vertical and horizontal distribution patterns of specific prokaryotic lineages have been characterized in several basin-scale studies (Teira et al. 2006a; Schattenuhofer et al. 2009; Morris et al. 2012). In these studies, SAR11 and *Prochlorococcus* were reported as dominant within prokaryotic assemblages at the surface, while SAR202 and *Crenarchaeota* were predominant in the meso- and bathypelagic layers, deeper than 200 m. With regard to horizontal distribution, the relative abundance of *Gammaproteobacteria* and *Bacteroidetes* increased in the Northern Atlantic Drift province (Schattenuhofer et al., 2009). This suggests that distinct hydrographic features of oceanic provinces (e.g. the South Atlantic Gyral, Western Tropical Atlantic, and North Atlantic Gyral provinces) could be important contributors to the distribution pattern of prokaryotic phylotypes in the Atlantic Ocean. However, the basin-scale distribution of bacterial and archaeal phylotypes has not yet been investigated in the epipelagic layer of the Central South Pacific Ocean.

The South Pacific Ocean is divided into five distinct provinces, called the Pacific Equatorial Divergence, the South Pacific Subtropical Gyre, the South Subtropical Convergence, the Subantarctic, and the Antarctic, based on prevailing

physical forces (Longhurst 2010). Primary productivity is generally high in the Pacific Equatorial Divergence due to the equatorial upwelling, while the South Pacific Subtropical Gyre contains a huge nutrient-limited ecosystem in the Pacific Ocean (Moore et al. 2001). As in the Atlantic Ocean, the Southern Pacific also has a steep latitudinal gradient of hydrographic features such as temperature, salinity, and concentrations of nutrients and chlorophyll *a* (Chl *a*), suggesting that these biogeographical features could affect the basin-scale distribution of bacterial and archaeal phylotypes.

During the last decade, genomic and isotopic studies have demonstrated that marine bacterial and archaeal phylotypes possess distinct metabolic systems to acquire energy from various sources. For instance, phylotypes of SAR11 (affiliated with *Alphaproteobacteria*), SAR86 (affiliated with *Gammaproteobacteria*), and *Flavobacterium* (affiliated with *Bacteroidetes*) are capable of harvesting energy from light using proteorhodopsin (Gómez-Consarnau et al. 2007; Campbell et al. 2008; González et al. 2008; Dupont et al. 2012; Yoshizawa et al. 2012). In addition, *Bacteroidetes* lineages possess several enzymes that degrade polymeric substrates (Bauer et al. 2006), and can proliferate in response to phytoplankton blooms in coastal waters (Pinhassi et al. 2004; Grossart et al. 2005; Tada et al. 2011). On the other hand, genomic studies on a marine non-extremophilic archaeon, *Nitrosopumilus maritimus*, suggest that the *Crenarchaeota* phylotype should be a chemoautotroph that can acquire energy through ammonia oxidation (Könneke et al. 2005; Walker et al. 2010). A previous metagenomics study also indicated that genes encoding rhodopsin are present

in marine *Euryarchaeota* (Iverson et al. 2012). In addition, isotopic studies showed that both archaeal lineages metabolized organic substrates such as amino acids in natural environments (Herndl et al. 2005; Teira et al. 2006b; Varela et al. 2008). These studies indicate that different prokaryotic phylotypes are possibly involved in distinct cycles of organic and inorganic matter in the ocean. Therefore, an investigation of the latitudinal and depth distribution of specific bacterial and archaeal phylotypes would facilitate a better understanding of the linkage between microorganisms and biogeochemical processes in the Central South Pacific Ocean.

The objective of this study was to quantify the abundance of five bacterial (*Alphaproteobacteria*, SAR11, *Gammaproteobacteria*, SAR86, and *Bacteroidetes*) and two archaeal (*Crenarchaeota* and *Euryarchaeota*) phylotypes along a transect, from the equator to the southern extremity of the Pacific Ocean. We collected samples from the study area in the austral summer of 2013 and performed catalyzed reporter deposition (CARD)-fluorescence *in situ* hybridization (FISH) (Pernthaler et al. 2002; Teira et al. 2004), which allows the identification and quantification of phylotype-specific prokaryotic cells using fluorescence-labeled oligonucleotide probes.

2. Materials and methods

2.1. Study sites and sample collection

Seawater samples were collected using 10-l Niskin bottles (General Oceanics, FL) at eight stations along a transect in the Central South Pacific Ocean along 170°W, from 0° to 40°S during the KH-13-7 (December 11, 2013–February 12, 2014) expedition of the

R/V *Hakuho-maru* (Fig. 1 and Table 1). Bacterial samples were collected from 0 or 5, 10, 20, 50, 100, and 200 m below the surface as well as the subsurface chlorophyll maximum (SCM) layers. Archaeal samples were collected from the same sampling layers for eubacteria, except for 0 or 5, and 20 m. Seawater samples were fixed with 2% (v/v) paraformaldehyde, and stored at 4°C for 2 h. Fixed seawater samples (10–30 ml) were then filtered through 0.2-µm pore size polycarbonate membrane filters (25 mm, type GTTP, Millipore, Cork, Ireland), and stored at –80°C until further analysis.

2.2. Environmental factors and total prokaryotic abundance

Environmental factors except for primary productivity were measured at 0, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, and 200 m depths and the SCM layer. Water temperature, salinity, and dissolved oxygen concentration were measured with a CTD system (SBE, Sea-Bird Electronics). Seawater samples were filtered at < 14 kPa through Whatman GF/F filters and Chl *a* was extracted from the filters with *N,N*-dimethylformamide at 4°C for 24 h in the dark (Suzuki and Ishimaru 1990). Chl *a* concentration was determined fluorometrically (Welschmeyer 1994). Samples for macronutrient analysis were collected in 10-ml acrylic tubes and stored at –20°C until further analysis on land, to estimate concentration of nitrate plus nitrite (NO₃ + NO₂), nitrite (NO₂), ammonium (NH₄), and phosphate (PO₄) using a segmented continuous-flow analyzer (QuAAtro, SEAL Analytical, Ltd.; Ogawa et al. 1999). The nitrate (NO₃) concentrations were obtained by subtracting NO₂ values from NO₃+NO₂ data. Primary productivity was estimated by ¹³C-labeling method (Hama et al. 1983) at

100%, 25%, 10%, 1%, and 0.1% photosynthetically active radiation (PAR) levels relative to the surface. Briefly, duplicate seawater samples were collected in acid-washed, 4.5-l polycarbonate bottles and ^{13}C -labeled sodium bicarbonate (99 atom % ^{13}C ; Cambridge Isotope Laboratories, Andover, MA) was added to each bottle at a final concentration of $10\text{ nmol}\cdot\text{l}^{-1}$. Bottles were then incubated for 24 h with adjusting light levels in an on-deck incubator cooled by flowing surface seawater. The analytical procedures were followed as previously described (Shiozaki et al. 2009). To quantify total prokaryotic abundance, filters (10 ml seawater filtration) were stained with 4',6-diamidino-2-phenylindole (DAPI) mix consisting of 5.5 parts (vol/vol) Citifluor (Citifluor Ltd., London, United Kingdom), 1 part Vectashield (Vector Labs, Burlingame, CA), and 0.5 parts phosphate-buffered saline (PBS) supplemented with $2\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ DAPI. Finally, images of the filters were captured using a BZ9000 epifluorescence microscope equipped with a CCD camera (Keyence, Tokyo, Japan).

2.3. CARD-FISH

The abundance of each phylotype was determined by CARD-FISH (Pernthaler et al. 2002; Teira et al. 2004). Briefly, filters (30 ml seawater filtration) were first embedded in 0.1% (w/v) low-melting point agarose (Nacalai Tesque, Kyoto, Japan), dehydrated for 1 min with 95% ethanol, and dried completely. To quench endogenous peroxidase, filters were treated with 0.01% HCl for 10 min at room temperature and then washed with 50 ml Milli-Q water for 15 min. Filters were then treated with $10\text{ mg}\cdot\text{ml}^{-1}$ lysozyme in Tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer (consisting of

10 mmol·l⁻¹ Tris-HCl and 1 mmol·l⁻¹ EDTA, pH 8.0) at 37°C for 60 min for bacterial samples, or with 10.9 mg·ml⁻¹ proteinase K in TE buffer at 37°C for 60 min for archaeal samples. The filters were then washed for 60 min with 50 ml Milli-Q water (three times for archaeal samples), dehydrated with 95% ethanol, cut into small pieces, and hybridized with oligonucleotide probes labeled with horseradish peroxidase. Probes were prepared at 0.28 ng·μl⁻¹ in 300 μl hybridization buffer, containing 900 mmol·l⁻¹ NaCl, 20 mmol·l⁻¹ Tris-HCl at pH 7.5, 10% (wt/vol) dextran sulfate, 0.02% (wt/vol) sodium dodecyl sulfate (SDS), 1% (vol/vol) blocking solution (blocking reagent in 100 mM maleic acid, 150 mM NaCl), and formamide (FA). The FA concentration appropriate for each probe was determined from probeBase (<http://www.microbial-ecology.net/probebase/>).

FISH probes were used that targeted bacteria affiliated with *Eubacteria* (Eub338; 35% FA) (Amann et al. 1990), *Alphaproteobacteria* (Alf968; 20% FA) (Neef 1997), SAR11 (SAR11-441 probe; 15% FA) (Rappé et al. 2002), *Gammaproteobacteria* (Gam42a; 35% FA) (Manz et al. 1992), SAR86 (SAR86-1249; 50% FA) (Eilers et al. 2000), *Bacteroidetes* (Cf319a; 35% FA) (Manz et al. 1996), *Crenarchaeota* (GI-554; 0% FA) (Massana et al. 1997) and *Euryarchaeota* (Eury806; 0% FA) (Teira et al. 2004) along with a negative control (Non338; FA, 35%) (Wallner et al. 1993). The Alf968 probe targeting *Alphaproteobacteria* detects only a few members of the SAR11 clade in the open ocean (SILVA TestPrime analysis; <http://www.arb-silva.de/search/testprime/>). Hybridization was performed at 46°C for 12–15 h.

After hybridization, filters were washed at 48°C for 15 min in 50 ml

pre-warmed buffer consisting of 20 mmol·l⁻¹ Tris-HCl pH 7.4, 5 mmol·l⁻¹ EDTA, 0.01% SDS, and NaCl at a concentration of 80 mmol·l⁻¹ (for Eub338, Non338, Gam42a, and Cf319a), 225 mmol·l⁻¹ (for Alf968), 318 mmol·l⁻¹ (for SAR11-441), 28 mmol·l⁻¹ (for SAR86-1249), or 900 mmol·l⁻¹ (for GI-554 and Eury806). EDTA was omitted for reactions with SAR11-441 and two archaeal probes. Subsequently, filters were washed with 50 ml of 0.05% (vol/vol) Triton X-100 in PBS (PBST) and then incubated at 46°C for 45 min in PBS containing 10% (wt/vol) dextran sulfate, 2 mol·l⁻¹ NaCl, 0.1% (vol/vol) blocking solution (blocking reagent in 100 mM maleic acid, 150 mM NaCl), 0.0015% (vol/vol) H₂O₂, and 0.7% (vol/vol) Alexa 488-labeled tyramide (amplification buffer). Filters were then washed for 15 min in 50 ml PBST, rinsed with Milli-Q water, air-dried, stained with DAPI mix, and photographed using epifluorescence microscope as described above.

2.4. Image analysis for cell counts

Epifluorescence microscopic images were stored as TIFF files and analyzed using the image analysis software Image J (version 1.48, National Institutes of Health, Bethesda, Maryland, USA) to obtain cell counts. Exposure times were optimized using samples with the negative control (Non338) probe. Image processing included three spatial filters: Laplacian (Kernel 5 × 5), Gaussian (Kernel 3 × 3), and median (radius 1) filters (Fazi et al. 2008). The newly created binary images for DAPI and FISH were edited in the overlay mode and overlapped cells were counted as FISH-positive cells. At least 10 images were analyzed for each sample.

203

204 **2.5. Phylotype abundance and water-column integrated cell number**

205 The each phylotype abundance was calculated using their proportion (i.e., percentage of
206 each phylotype to DAPI-stained cells determined with the CARD-FISH analysis) and
207 total prokaryotic abundance. Water-column integrated cell number of each phylotype
208 was calculated by using the phylotype abundance of each sampling depth (7 and 5
209 depths for Bacteria and Archaea, respectively).

210

211 **2.6. Statistical analysis**

212 Data were analyzed in R software, version 3.1.0 (R Development Core Team 2011),
213 using Spearman's rank correlation and multiple regression analyses to investigate the
214 relationship between phylotype abundance and environmental factors. For multiple
215 regression analysis, all data including the environmental parameters and the phylotype
216 abundance were log (x+1)-transformed to approximate normal distribution. We used the
217 Akaike information criterion (AIC: Akaike, 1974) for model selection, since the model
218 minimizes the value of AIC and equivalently maximizes that of the log-likelihood
219 minus the number of samples as a penalty. AIC model selection was performed using
220 the MASS package (Venables and Ripley, 2002) in R software. The environmental
221 factors used for statistical analysis were assumed to be independent of each other.

222

223 **3. Results**

224 **3.1. Environmental characteristics and total prokaryotic abundance**

Distribution of water temperature, salinity, dissolved oxygen, Chl *a*, primary productivity, concentrations of NO₃, NO₂, NH₄, PO₄, and prokaryote populations is shown in Fig. 2. Surface seawater temperature decreased from 30.3°C at Station 1 to 19.8°C at Station 9. Water temperature also generally decreased with depth, although the gradient was steeper at the equator than that in the south. An increase in salinity was observed with depth at Stations 3–4, reaching to > 36 at 100–200 m depth. Salinity decreased at Stations 8 and 9. Dissolved oxygen increased with latitude from north to south, and ranged from 3.9 ml·l⁻¹ at 5 m depth at Station 1 to 4.9 ml·l⁻¹ on the surface at Station 9. At Station 1, dissolved oxygen decreased sharply at depths of 100–200 m, presumably as a result of equatorial upwelling. The SCM layer declined from 41 m at Station 1 to 145 m at Station 5, but rose back to 82 m at Station 9. The highest Chl *a* concentration of 0.36 µg·l⁻¹ was observed in the SCM layer at Station 1, possibly caused by the equatorial upwelling. In addition, primary productivity was highest (10.8 µg C·l⁻¹·d⁻¹) at the surface of Station 1. Surface primary productivity reduced southward from Station 1 to 4, but increased again at Stations 5–7 (from 3.7 to 6.1 10 µg C·l⁻¹·d⁻¹). The highest concentrations of NO₃ and PO₄ (14.5 µM and 1.09 µM, respectively) were observed at 200 m depth at Station 1. NO₂ concentration was relatively high in the surface layer (0–50 m), ranging from 0.28 to 0.32 µM. On the other hand, NH₄ concentration was relatively high at depths of 100–200 m at Station 4, ranging from 0.29 to 0.35 µM.

Total prokaryotic abundance ranged from 1.0×10^5 to 7.9×10^5 cells·ml⁻¹.

Prokaryotes were the most abundant at 100 m depth at Station 7. As for latitudinal

distribution, abundance was higher at Stations 1 and 7 than at other stations.

3.2. Bacterial and Archaeal distributions

Eubacteria accounted for 27%–89% of DAPI-stained cells (Fig. 3A). Water-column integrated cell numbers of *Eubacteria* ranged from 4.42×10^{13} to 6.96×10^{13} cells·m⁻², the largest cell number was observed at Station 8 (Table 2). Eubacterial abundance significantly increased with water temperature, and decreased with depth and concentration of NO₃ and PO₄ (Table 3). As for latitudinal distribution, *Eubacteria* count decreased at Station 7 and accounted for 57%–65% of DAPI-stained cells (5–50 m depths).

Relative abundance of *Alphaproteobacteria* decreased with depth and was therefore, the most abundant at the surface, ranging from 0%–43% of DAPI-stained cells throughout the area investigated (Fig. 3B). Their water-column integrated abundance increased at Stations 1, 8, and 9 (1.70×10^{13} cells·m⁻², 1.93×10^{13} cells·m⁻², and 1.90×10^{13} cells·m⁻², respectively) (Table 2). The abundance significantly increased with dissolved oxygen concentration, and decreased with salinity and concentrations of NO₃ and PO₄ ($P < 0.05$, $n = 56$) (Table 3).

SAR11 clade was the most dominant bacterial lineage in the Central South Pacific Ocean and accounted for 10%–55% of DAPI-stained cells at all depths throughout the area investigated (Fig. 3C). This clade was particularly abundant at the surface, accounted for an average of $38\% \pm 11\%$ DAPI-stained cells. In addition, the abundance exceeded 50% between 0 and 100 m depths at Stations 1, 5, 6, and 9. The

water-column integrated cell number of SAR11 as well as that of *Alphaproteobacteria* increased at Station 8, and reached up to 3.90×10^{13} cells·m⁻² (Table 2). The SAR11 abundance significantly increased with Chl *a* concentration ($P < 0.05$, $n = 56$) (Table 3). In contrast, significantly negative correlations between their abundance and depth or nutrient levels (NO₃, NO₂, or PO₄ concentration) were noted.

Gammaproteobacteria was particularly abundant in surface waters up to 100 m depth and accounted for 0.6%–7.9% of DAPI-stained cells (Fig. 3D). The abundance at the surface layer increased southward from Station 4 and reached up to 7.9% at Station 8. In addition, their water-column integrated cell number increased at southern part of the South Pacific Ocean (0.36×10^{13} cells·m⁻² and 0.31×10^{13} cells·m⁻² at Stations 8 and 9, respectively) (Table 2). Gammaproteobacterial abundance was negatively correlated with depth, salinity, and concentration of Chl *a* and nutrients (except for NH₄) ($P < 0.01$, $n = 56$) (Table 3). In contrast, the abundance significantly increased with dissolved oxygen ($P < 0.01$).

SAR86 clade was also relatively abundant in surface waters at Stations 1, 8, and 9 (Fig. 3E). However, the abundance accounted for < 1% of DAPI-stained cells between 0 and 100 m depths at Stations 3–7. Remarkably, relative abundance of this clade increased at the SCM layer at Station 5 (2.0% of DAPI-stained cells). The water-column integrated cell number of SAR86 ranged from 0.02×10^{13} cells·m⁻² to 0.12×10^{13} cells·m⁻², and increased at Stations 1, 8, and 9 (Table 2). SAR86 abundance significantly increased with dissolved oxygen ($P < 0.01$, $n = 56$), and decreased with depth, salinity and NH₄ concentration ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively)

(Table 3).

Relative abundance of *Bacteroidetes* was clearly higher near the surface than in waters more than 100 m depth and ranged from 0.2%–17% of DAPI-stained cells (Fig. 3F). The proportion decreased southward from Station 1, increased at Station 8 and reached 17% of DAPI-stained cells at 20 m depth. The largest water-column integrated cell number was observed at Station 1 (0.70×10^{13} cells·m⁻²) (Table 2). Abundance of this phylotype was positively correlated with water temperature ($P < 0.05$, $n = 56$), and negatively correlated with depth, salinity, and concentrations of NO₃ and PO₄ ($P < 0.01$, $P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively) (Table 3).

Crenarchaeota was more abundant at Station 1 than any other station (ANOVA, $P < 0.01$; Tukey's post-hoc, $P < 0.01$), and accounted for 2.6%–5.7% of DAPI-stained cells (Fig. 4A). Their water-column integrated cell number increased at Stations 1 and 9 (each 0.19×10^{13} cells·m⁻²) (Table 2). However, the relative abundance of this phylotype was relatively stable at all stations except at Station 1.

Euryarchaeota accounted for 0.1%–3.9% of DAPI-stained cells (Fig. 4B) and was most abundant at 10 m depth, at Station 1. Also, their water-column integrated cell number increased at Station 1, and reached at 0.27×10^{13} cells·m⁻² (Table 2). Their abundance significantly decreased with depth ($P < 0.01$, $n = 40$), and increased with Chl *a* concentration ($P < 0.01$) (Table 3).

3.3. Interrelationship between distribution of phylotypes and environmental factors

Results of multiple regression analysis indicate that hydrographic features (water temperature, salinity, dissolved oxygen, Chl *a*, NO₂, HN₄, or PO₄) have a significant effect on the distribution patterns of bacterial and archaeal phylotypes (Table 4). Since there were strongly significant correlations between water temperature and depth ($R = -0.57$, $P < 0.001$, $n = 56$, in Pearson's correlation analysis), and NO₃ and PO₄ ($R = 0.96$, $P < 0.001$, $n = 56$), the factors selected for the regression model using the AIC included water temperature, salinity, dissolved oxygen, Chl *a*, NO₂, NH₄, and PO₄. These environmental factors explained 38%–63% of the distribution patterns of each phylotype in this study ($P < 0.001$).

4. Discussion

4.1. Distribution of bacterial lineages

Our investigation of prokaryotes in the epipelagic layer revealed that the abundance of bacterial and archaeal phylotypes in the South Pacific Ocean was significantly correlated with several environmental factors (i.e., water temperature, salinity, dissolved oxygen, Chl *a* and nutrients). This suggests that the hydrographic features are the critical factors shaping the bacterial and archaeal distribution patterns.

We characterized Bacteria and Archaea using CARD-FISH, and detected 29%–91% of DAPI-stained cells using the probes Eub338, GI-554, and Eury806. A possible reason for the relative inefficiency of detection is that many dead or decaying cells might still remain the genomic DNA after rRNA degradation (del Giorgio and Gasol 2008). Another possible reason is the limited range of Eub338 probe, which is

unable to detect *Planctomycetes* and *Verrucomicrobia*. These phylotypes can be detected using Eub338II and Eub338III, respectively (Amann and Fuchs 2008). Thus, the CARD-FISH method used in this study may have underestimated eubacterial abundance.

Our results showed that SAR11 clade was the dominant group of prokaryotes in the epipelagic layer (Fig. 3C). In addition, the abundance of this phylotype was significantly correlated with Chl *a* concentration (Tables 3 and 4). This implies that phytoplankton biomass could be a critical determinant of SAR11 abundance in our survey area. Additionally, these results are in agreement with data obtained from the Atlantic Ocean (Eiler et al. 2009; Schattenuhofer et al. 2009). Indeed, genomic and metabolic analyses suggest that SAR11 could degrade dissolved organic matter derived from oceanic phytoplankton. For example, the SAR11 genome reportedly contains a gene for the degradation of phytoplankton-derived dimethylsulfoniopropionate (Howard et al. 2006). Moreover, Nelson and Carlson (2012) used stable isotope probing method to report that some SAR11 subclades utilized organic substrates derived from *Synechococcus* in the open ocean. It suggested that SAR11 lineage were actively involved in the degradation of organic matter derived from phytoplankton in the South Pacific Ocean.

The cells detected by Alf968 probe accounted for $16\% \pm 10\%$ of DAPI-stained cells in the epipelagic layer, indicating that *Alphaproteobacteria*, except for the SAR11 clade also contribute significantly to the bacterial standing stock. In addition, alphaproteobacterial abundance was significantly correlated with salinity and

dissolved oxygen concentration, whereas environmental parameters barely affected SAR11 abundance (Table 3). These data suggest that environmental factors determining the distribution patterns of *Alphaproteobacteria* and SAR11 clade, differed. *Alphaproteobacteria* includes numerous lineages with functional characteristics distinct from SAR11, including *Roseobacter* and SAR116 clades, which are known as metabolic generalists with versatile mechanisms for energy acquisition from organic substrates (Newton et al. 2010; Oh et al. 2010). Thus, these functional differences may also affect distinct distribution patterns among alphaproteobacterial phylotypes in the Central South Pacific Ocean.

Some gammaproteobacterial lineages such as *Vibrio* and *Alteromonas*, are well known as copiotrophs, which can proliferate opportunistically in response to a supply of organic substrates from phytoplankton blooms (Riemann et al. 2000; Pinhassi et al. 2004; Tada et al. 2011; Teeling et al. 2012). However, *Gammaproteobacteria* includes some oligotrophic lineages such as the OM60/NOR5 clade, which represents aerobic anoxygenic photoheterotrophs (Yan et al. 2009) and cannot grow in nutrient-rich conditions (Cho and Giovannoni, 2004; Cho et al. 2007). In this study, the gammaproteobacterial abundance was negatively correlated with Chl *a* concentration and primary productivity (Tables 3, 4, and Supplementary Table 1), suggesting that the dominant gammaproteobacterial population in the southern part of South Pacific Ocean might be the oligotrophic lineages. In addition, our statistical analyses indicated that the dissolved oxygen concentration would be one of the primary factors affecting the gammaproteobacterial distribution (Tables 3 and 4). In previous report, the growth of

oligotrophic gammaproteobacterial lineages such as OM60/NOR5 strains was stimulated by oxygen availability (Spring and Riedel, 2013). These data suggested that dissolved oxygen concentration could be one of the primary factors determining the gammaproteobacterial distribution in the South Pacific Ocean.

As for the latitudinal distribution of *Gammaproteobacteria*, their proportion to the total bacteria and abundance drastically increased at Stations 8 and 9 (Fig. 3D, Supplementary Figure 1). The results of hydrographic features such as salinity and dissolved oxygen concentration (Fig. 2) indicated that the water mass belonging to Stations 8 and 9 (low salinity and high dissolved oxygen concentration) differed from those at other stations. Around this region, there is the eastward flow of the Eastern Australian Current from the coasts of Western Australia and New Zealand into the South Pacific Ocean (Godfrey et al. 1980; Oke et al. 2000; Tilburg et al. 2001). These data suggest that the increase in *Gammaproteobacteria* may be explained by an influence of the current system in this area. In addition, the western area of Station 8 was relatively productive based on weekly composite estimates of net primary production (Supplementary Figure 2), and that might affect the abundance of *Gammaproteobacteria*.

SAR86 is one of the ubiquitous clades of *Gammaproteobacteria* (Mary et al. 2006; Malmstrom et al. 2007), especially in the stratified euphotic layer (Morris et al., 2005; Treusch et al. 2009). The present study showed that the percentage of SAR86 relatively increased in the surface layers of Stations 8 and 9 where relatively high dissolved oxygen concentration and lower salinity and water temperature were observed

(Fig. 2). A previous metagenomic study showed that hydrographic features such as water temperature would be the important factor determining the distribution of SAR86 subgroups (Dupont et al. 2012). In this study, however, their abundance increased with dissolved oxygen concentration, and decreased with depth, salinity, and NH_4 concentration (Table 3), suggesting that the dissolved oxygen concentration, salinity, and nutrient concentration could affect the SAR86 distribution in the South Pacific Ocean. In addition, our multiple regression analysis showed that environmental factors accounted for 38% of the variation in SAR86 distribution (Table 4), indicating that external factors might also influence the spatial variability of SAR86.

Abundance of *Bacteroidetes* was positively correlated with water temperature and negatively correlated with depth, suggesting that temperature gradient with depth should be the critical factor determining the distribution pattern of *Bacteroidetes* in the Pacific Ocean. Some previous studies revealed that *Bacteroidetes* were more abundant in the surface ocean and their abundance decreased with depth (Schattenhofer et al. 2009; Gómez-Pereira et al. 2010). In addition, *Bacteroidetes* abundance significantly decreased with salinity in our survey area (Tables 3 and 4). In fact, the proportion and abundance of *Bacteroidetes* decreased from 100–200 m depths in the central part of the South Pacific Ocean (Stations 3 and 4), where there was a high salinity water body (Fig. 2A, B). A previous study in the Mediterranean Sea showed the contribution of *Bacteroidetes* lineage to the total community decreased with increasing salinity (Díez-Vives et al. 2014). These data suggested that the physical parameter such as the water temperature and salinity would be important factors determining the

distribution pattern of the *Bacteroidetes* in the South Pacific.

In the Atlantic Ocean, the contribution of *Bacteroidetes* to total prokaryotic population increased in the Northern Atlantic Drift where a phytoplankton bloom occurred (Schattenhofer et al. 2009). *Bacteroidetes* is known to be strongly associated with natural or artificial phytoplankton blooms in coastal waters (West et al. 2008; Tada et al. 2011; 2012). In our survey area, the *Bacteroidetes* abundance drastically increased in the nutrient-rich equatorial upwelling region (Table 2 and Supplementary Figure 1). However, our results showed that *Bacteroidetes* abundance was neither associated with Chl *a* concentration nor primary productivity (Tables 3, 4, and Supplementary Table 1). In fact, their relative abundance accounted for 5–10% of total cells even though in the oligotrophic gyre under nutrient-limiting conditions (Fig. 3F). Several genome analyses of marine *Bacteroidetes* lineages showed that they might possess the dual energy acquisition strategies for living in both nutrient rich and poor environments (Bauer et al. 2006; González et al. 2008; González et al. 2011; Fernández-Gómez et al. 2013). They can attach to the particles or phytoplankton cells to utilize the polymeric substrates under the nutrient-rich condition (Bauer et al. 2006). In contrast, in the nutrient poor water, they would obtain the energy from light using the proteorhodopsin gene, which produce ATP by the light-driven proton pump (González et al. 2008; 2011). Therefore, *Bacteroidetes* in the southern part of the South Pacific Ocean might be the distinct physiological state, which optimized to nutrient-limiting conditions.

4.2. Distribution of archaeal lineages

This study revealed that the distribution of marine *Crenarchaeota* varied with latitude, and this phylotype was the most abundant at 200 m depth at Station 1 (Fig. 4A). The results obtained are consistent with previous studies (Karner et al. 2001; Teira et al. 2004; Herndl et al. 2005; Kirchman et al. 2007; Schattenhofer et al. 2009). It should be noted that the probe GI-554 detects numerous chemoautotrophic lineages that can fix CO₂ using ammonia as the electron donor and energy source (Herndl et al. 2005; Francis et al. 2005), and thus could thrive in nutrient-rich layers (Könnecke et al. 2005; Varela et al. 2008). However, our correlation analysis did not show a positive relationship between crenarchaeotal abundance and the concentrations of NH₄ or NO₂ (Tables 2 and 3). In contrast, their abundance significantly increased with Chl *a* concentration (Table 3). Previous isotopic studies showed that marine *Crenarchaeota* possesses not only chemoautotrophic features, but heterotrophic as well (Teira et al. 2006b; Varela et al. 2008). In addition, the peaks in crenarchaeotal abundance and Chl *a* concentration were detected in the surface layer of the Gulf of California (Beman et al. 2008). These data suggests that *Crenarchaeota* could be actively involved in organic matter degradation around the euphotic zone in the Central South Pacific Ocean.

Euryarchaeota was relatively abundant within 100 m depth at Station 1, where Chl. *a* concentration and primary productivity were relatively high (Fig. 4B). In addition, the rank correlation and multiple regression analyses showed that the Chl. *a* concentration could positively affected the euryarchaeotal abundance (Tables 3 and 4). Our result is in good agreement with previous results obtained from the Mediterranean Sea and arctic environments (Galand et al. 2008; Galand et al. 2010). In addition, recent

study has revealed that the marine *Euryarchaeota* associated with particulate organic matter or phytoplankton cells (Orsi et al. 2015). These data suggested that there could be a strong ecological linkage between *Euryarchaeota* and phytoplankton, and marine *Euryarchaeota* could be involved in the degradation of organic matter derived from phytoplankton in the equatorial region in the South Pacific Ocean.

4.3. Contribution of environmental factors to phylotype distribution

The results of multiple regression analysis revealed that the interpretability with environmental factors for prokaryotic distribution patterns differed among distinct phylotypes (Table 4). Some previous studies about global distribution patterns of prokaryotic phylotypes showed that the relative abundances of specific phylotypes varied with different gradients of environmental factors in the ocean (Wietz et al., 2010; Lefort and Gasol, 2013). These data suggested that the hydrographical gradients differently affect the phylotype-specific distribution patterns.

In addition, our study showed that the environmental factors analyzed explained 38% to 61% of the phylotype distribution, but large proportion of variance (39% to 62%) remained to be inexplicable. One plausible reason for this result is the lack of information of dissolved and particulate organic matter concentrations, which should be important factors controlling the heterotrophic bacterial production and standing stock in the Pacific Ocean (Yokokawa et al., 2013; Hasumi and Nagata, 2014). Therefore, the input of these organic matter data to the statistical analyses could effectively explain the phylotype distribution patterns by environmental factors.

489

490 **5. Conclusions**

491 The results from CARD-FISH analysis in this study revealed the basin-scale
492 distribution of five bacterial and two archaeal phylotypes in the epipelagic layer of the
493 Central South Pacific Ocean. We found that the latitudinal and depth distribution
494 patterns of each phylotype differed in our survey area. Furthermore, Spearman's rank
495 correlation and multiple regression analyses showed that the abundance of specific
496 phylotypes was significantly correlated with several environmental factors. The SAR11
497 affiliated with *Alphaproteobacteria* was the dominant phylotype, and their abundance
498 increased with Chl *a* concentration, indicating an ecological linkage between SAR11
499 and phytoplankton assemblages in the study area. We also found that all bacterial
500 phylotypes, excluding *Gammaproteobacteria* but including SAR86, was more abundant
501 in the equatorial region than in all other studied area, presumably due to the equatorial
502 upwelling system. In contrast, *Gammaproteobacteria* and *Bacteroidetes* increased in the
503 southern part in the Southern Pacific Ocean. *Gammaproteobacteria* and *Bacteroidetes*
504 abundance significantly increased with dissolved oxygen concentration and water
505 temperature, respectively. *Crenarchaeota* and *Euryarchaeota* were more abundant in the
506 equatorial region, while they showed distinct vertical distribution patterns. In particular,
507 *Euryarchaeota* abundance significantly decreased with depth, and increased with Chl. *a*
508 concentration. These data suggest that hydrographic properties can principally
509 determine the distribution of bacterial and archaeal phylotypes in the Central South
510 Pacific Ocean.

511

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518

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750 **Figure legends**

751 **Fig. 1** Stations 1 through 9 along cruise KH-13-7, charted by the R/V *Hakuho-maru*
752 between December 2013 and February 2014

753

754 **Fig. 2** Water temperature (A), salinity (B), dissolved oxygen (C), chlorophyll *a*
755 concentration (D), primary productivity (E), concentrations of NO₃ (F), NO₂ (G), NH₄
756 (H), PO₄ (I), and total prokaryotic abundance (J) at various depths (y-axis) along a
757 transect from north to south (x-axis). Dots indicate sampling points

758

759 **Fig. 3** Latitudinal and depth distribution of *Eubacteria* (A), *Alphaproteobacteria* (B),
760 SAR11 (C), *Gammaproteobacteria* (D), SAR86 (E), and *Bacteroidetes* (F), as % of
761 DAPI-stained cells. Dots indicate sampling points

762

763 **Fig. 4** Latitudinal and depth distribution of *Crenarchaeota* (A) and *Euryarchaeota* (B),
764 as % of DAPI-stained cells. Dots indicate sampling points

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Fig. 1
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Fig. 2
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Fig. 3

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Fig. 4
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1 **Supplementary materials**
2 **Supplementary Figure 1.** Latitudinal and depth distribution of *Eubacteria* (A), *Alphaproteobacteria*
3 (B), SAR11 (C), *Gammaproteobacteria* (D), SAR86 (E), *Bacteroidetes* (F), *Crenarchaeota* (G) and
4 *Euryarchaeota* (H). Dots indicate sampling points
5

9 **Supplementary Figure 2.** Weekly composite estimates of net primary productivity (January 1–8,
10 2014), as derived from the Ocean Productivity database
11 (<http://www.science.oregonstate.edu/ocean.productivity/>), along the transect from 175°E to 160°W at
12 35°S.
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15
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Supplementary Table 1. Spearman’s rank correlation analysis of water-column integrated net primary productivity and abundance (depth-integrated values above 200 m) of total prokaryotes, *Bacteria*, and *Archaea* in the epipelagic zone of the central South Pacific.

Group and factor	<i>r</i> value ^b								
	Total prokaryotic abundance	<i>Eubacteria</i>	<i>Alphaproteo</i> <i>bacteria</i>	SAR11	<i>Gammaprot</i> <i>eobacteria</i>	SAR86	<i>Bacteroid</i> <i>etes</i>	<i>Crenarcha</i> <i>eota</i>	<i>Euryarcha</i> <i>eota</i>
Water-column integrated net primary production ^a	−0.21	0.17	0.31	0.24	0.12	0.19	0.38	−0.02	0.26

^aDepth-integrated from 0.1% to 100% light intensity depths

1 Table 1. Station numbers, sampling dates, latitudes, longitudes, and environmental factors in surface waters at 5 m depth monitored during the
 2 KH-13-7 cruise aboard the R/V *Hakuho-maru*.

Station number	Sampling Date	Latitude (°S)	Longitude (°W)	Water temperature (°C)	Salinity	Dissolved oxygen concentration (ml·l ⁻¹)	Chl. <i>a</i> concentration (µg·l ⁻¹)	Water-column integrated net primary production ^a (mg C·m ⁻² ·d ⁻¹)	Depth of SCM layer (m)
St. 1	23-Dec-13	0	170	27.3	35.5	3.9	0.25	464	41
St. 3	26-Dec-13	10	170	30.3	35.1	4.2	0.06	147	110
St. 4 ^b	2-Jan-14	15	170	29.0	35.2	4.3	0.07	186	135
St. 5	4-Jan-14	20	170	27.8	35.4	4.3	0.07	470	145
St. 6	7-Jan-14	25	170	26.3	35.5	4.4	0.07	339	113
St. 7	9-Jan-14	30	170	23.9	35.4	4.6	0.04	167	108
St. 8	12-Jan-14	35	170	22.0	35.2	4.8	0.03	216	130
St. 9 ^b	13-Jan-14	40	170	19.8	35.1	4.9	0.06	227	82

^aDepth-integrated from 0.1% to 100% light intensity depths

^bData of water temperature, salinity, and dissolved oxygen from 10 m depth, and that of Chl. *a* from 0 m depth

Chl *a*, chlorophyll *a*

SCM, subsurface chlorophyll maximum

4 Table 2. The water-column integrated cell number of each bacterial phylotype (depth-integrated values above 200 m) in the central South Pacific.

Station number	Water-column integrated cell numbers ($\times 10^{13}$ cells·m ⁻²)							
	Bacteria (5–200 m)						Archaea (10–200 m)	
	<i>Eubacteria</i>	<i>Alphaproteo bacteria</i>	SAR11	<i>Gammaproteo bacteria</i>	SAR86	<i>Bacteroidetes</i>	<i>Crenarchaeota</i>	<i>Euryarchaeota</i>
St. 1	6.07	1.70	3.42	0.18	0.07	0.70	0.19	0.27
St. 3	4.58	0.56	2.39	0.14	0.02	0.38	0.09	0.04
St. 4*	4.70	0.68	2.25	0.18	0.02	0.36	0.10	0.04
St. 5	4.58	0.73	2.39	0.20	0.03	0.45	0.06	0.05
St. 6	5.28	1.00	2.95	0.19	0.02	0.44	0.07	0.05
St. 7	4.42	0.88	2.49	0.26	0.04	0.55	0.07	0.04
St. 8	6.96	1.93	3.90	0.36	0.08	0.64	0.10	0.05
St. 9*	6.50	1.90	3.59	0.31	0.12	0.65	0.19	0.08

* Used the cell number at 0 m depth instead at 5 m depth

7 Table 3. Spearman's rank correlation analysis of environmental factors and abundance of total prokaryotes, *Eubacteria* and Archaea in the
8 epipelagic zone of the Central South Pacific Ocean.

Group and factor	Total prokaryotic abundance (n = 56)	<i>Rho</i> value ^a							
		Bacteria (n = 56)						Archaea (n = 40)	
		<i>Eubacteria</i>	<i>Alphaproteo bacteria</i>	SAR11	<i>Gammaproteobacteria</i>	SAR86	<i>Bacteroidetes</i>	<i>Crenarchaeota</i>	<i>Euryarchaeota</i>
Depth	−0.58**	−0.66**	−0.65**	−0.62**	−0.59**	−0.48**	−0.74**	−0.21	−0.64**
Water temperature	0.37**	0.32*	0.11	0.26	0.04	−0.1	0.30*	0.04	0.29
Salinity	−0.13	−0.23	−0.34*	−0.20	−0.40**	−0.39**	−0.28*	−0.34	−0.27
Dissolved oxygen conc.	0.16	0.23	0.29*	0.25	0.52**	0.37**	0.23	−0.02	0.24
Chl <i>a</i> conc.	0.21	0.23	0.22	0.27*	−0.47**	0.15	0.10	0.17	0.47**
NO ₃	−0.38**	−0.41**	−0.29*	−0.33*	−0.74**	−0.18	−0.41**	0.13	−0.30
NO ₂	−0.03	0.02	0.17	−0.34*	−0.37**	0.23	0.01	0.21	0.30
NH ₄	−0.07	−0.19	−0.23	0.13	−0.18	−0.31*	−0.21	−0.14	−0.06
PO ₄	−0.28*	−0.30*	−0.28*	−0.30*	−0.69**	−0.20	−0.37**	0.12	−0.13

^aLevel of significance: *, $P < 0.05$; **, $P < 0.01$

Chl *a*, chlorophyll *a*

11 Table 4. Multiple regression analysis of environmental factors and distribution of bacterial and archaeal phylotypes

Group and factor	Total prokaryotic abundance (n = 56)	Bacteria (n = 56)						Archaea (n = 40)	
		<i>Eubacteria</i>	<i>Alphaproteo bacteria</i>	SAR11	<i>Gammaprote obacteria</i>	SAR86	<i>Bacteroi detes</i>	<i>Crenarch aeota</i>	<i>Euryarch aeota</i>
Multiple <i>R</i> -squared	0.57	0.63	0.48	0.61	0.59	0.38	0.51	0.49	0.57
<i>F</i> -statistic	13.5	21.8	9.4	19.8	18.6	10.5	13.3	6.6	9.1
<i>P</i> -value for model	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Coefficient for each resource parameter ^a									
Water temperature	0.14***	0.13***	0.06***	0.12***	0.02***	NS	0.05***	0.02**	0.02***
Salinity	-0.04	-0.07***	-0.06**	-0.06**	NS	-0.01**	-0.03**	-0.01*	-0.01
Dissolved oxygen conc.	0.14**	0.10***	0.07***	0.09***	0.03***	0.01*	0.04***	0.01	0.03***
Chl <i>a</i> conc.	0.04*	0.07***	0.03	0.07***	-0.02**	NS	NS	0.01*	0.01**
NO ₂	NS	NS	0.89	NS	0.19	0.17***	0.57**	NS	NS
NH ₄	NS	NS	NS	NS	NS	NS	NS	NS	NS
PO ₄	0.05	NS	NS	NS	NS	NS	NS	0.02**	0.02***

^aLevel of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Chl *a*, chlorophyll *a*

NS, not selected by Akaike information criteria

To meet assumptions of regression analysis, all parameters were log(x+1)-transformed.