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Basin-scale distribution of prokaryotic phylotypes in the epipelagic layer of the Central South Pacific Ocean during austral summer

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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.
Keywords: Marine bacteria; Marine archaea; Fluorescence in situ hybridization;

Central South Pacific Ocean

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; Schattenhofer 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; Schattenhofer 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 (Schattenhofer 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 $\alpha$ (Chl $\alpha$), 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 archaean, *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 nmol·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 µg·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 mg·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.
2.5. Phylotype abundance and water-column integrated cell number

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

2.6. Statistical analysis

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

3. Results

3.1. Environmental characteristics and total prokaryotic abundance
Distribution of water temperature, salinity, dissolved oxygen, Chl a, primary productivity, concentrations of NO$_3$, NO$_2$, NH$_4$, PO$_4$, 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 NO$_3$ and PO$_4$ (14.5 µM and 1.09 µM, respectively) were observed at 200 m depth at Station 1. NO$_2$ concentration was relatively high in the surface layer (0–50 m), ranging from 0.28 to 0.32 µM. On the other hand, NH$_4$ 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 $1.0 \times 10^5$ to $7.9 \times 10^5$ 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 $4.42 \times 10^{13}$ to $6.96 \times 10^{13}$ 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 NO$_3$ and PO$_4$ (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 ($1.70 \times 10^{13}$ cells·m$^{-2}$, $1.93 \times 10^{13}$ cells·m$^{-2}$, and $1.90 \times 10^{13}$ cells·m$^{-2}$, respectively) (Table 2). The abundance significantly increased with dissolved oxygen concentration, and decreased with salinity and concentrations of NO$_3$ and PO$_4$ ($P < 0.05$, 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% ± 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 $3.90 \times 10^{13}$ 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 (NO$_3$, NO$_2$, or PO$_4$ 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 ($0.36 \times 10^{13}$ cells·m$^{-2}$ and $0.31 \times 10^{13}$ 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 NH$_4$) ($P < 0.01$, $n = 56$) (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 $0.02 \times 10^{13}$ cells·m$^{-2}$ to $0.12 \times 10^{13}$ 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 NH$_4$ concentration ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively).
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 ($0.70 \times 10^{13}$ 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 NO$_3$ and PO$_4$ ($P < 0.01$, $P < 0.05$, $P < 0.01$, 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 $0.19 \times 10^{13}$ 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 $0.27 \times 10^{13}$ 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, NH₄, 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 ($R = -0.57, P < 0.001, n = 56, \text{in Pearson’s correlation analysis}$), 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; Schattenhofer 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% ± 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.
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 (Diez-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 archaean 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 CO$_2$ 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 NH$_4$ or NO$_2$ (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.
5. Conclusions

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

We thank the captain and crew of the R/V *Hakuho-maru* for their support towards collecting samples for this study during cruise KH-13-7. This study was financially supported by Research Fellowships for Young Scientists (No. 13J04633 and No. 26740001) to Y. T. and Grants-in-Aid (No. 24121004) to K. S. from the Japan Society for the Promotion of Science.

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Figure legends

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

Fig. 2 Water temperature (A), salinity (B), dissolved oxygen (C), chlorophyll $a$ concentration (D), primary productivity (E), concentrations of NO$_3$ (F), NO$_2$ (G), NH$_4$ (H), PO$_4$ (I), and total prokaryotic abundance (J) at various depths (y-axis) along a transect from north to south (x-axis). Dots indicate sampling points.

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

Fig. 4 Latitudinal and depth distribution of Crenarchaeota (A) and Euryarchaeota (B), as % of DAPI-stained cells. Dots indicate sampling points.
Fig. 1

Tada et al. (2016)
Fig. 2

Tada et al. (2016)
Fig. 3
Tada et al. (2016)
Fig. 4

Tada et al. (2016)
Supplementary materials

Supplementary Figure 1. Latitudinal and depth distribution of *Eubacteria* (A), *Alphaproteobacteria* (B), SAR11 (C), *Gammaproteobacteria* (D), SAR86 (E), *Bacteroidetes* (F), *Crenarchaeota* (G) and *Euryarchaeota* (H). Dots indicate sampling points.
Supplementary Figure 2. Weekly composite estimates of net primary productivity (January 1–8, 2014), as derived from the Ocean Productivity database (http://www.science.oregonstate.edu/ocean.productivity/), along the transect from 175°E to 160°W at 35°S.
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.

<table>
<thead>
<tr>
<th>Group and factor</th>
<th>Total prokaryotic abundance</th>
<th>Eubacteria</th>
<th>Alphaproteobacteria</th>
<th>SAR11</th>
<th>Gammaproteobacteria</th>
<th>SAR86</th>
<th>Bacteroidetes</th>
<th>Crenarchaeota</th>
<th>Euryarchaeota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-column integrated net primary production(^a)</td>
<td>–0.21</td>
<td>0.17</td>
<td>0.31</td>
<td>0.24</td>
<td>0.12</td>
<td>0.19</td>
<td>0.38</td>
<td>–0.02</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\(^a\)Depth-integrated from 0.1% to 100% light intensity depths
Table 1. Station numbers, sampling dates, latitudes, longitudes, and environmental factors in surface waters at 5 m depth monitored during the KH-13-7 cruise aboard the R/V *Hakuho-maru*.

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

<sup>a</sup>Depth-integrated from 0.1% to 100% light intensity depths

<sup>b</sup>Data 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
Table 2. The water-column integrated cell number of each bacterial phylotype (depth-integrated values above 200 m) in the central South Pacific.

<table>
<thead>
<tr>
<th>Station number</th>
<th>Bacteria (5–200 m)</th>
<th>Archaea (10–200 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eubacteria</td>
<td>Alphaproteobacteria</td>
</tr>
<tr>
<td>St. 1</td>
<td>6.07</td>
<td>1.70</td>
</tr>
<tr>
<td>St. 3</td>
<td>4.58</td>
<td>0.56</td>
</tr>
<tr>
<td>St. 4*</td>
<td>4.70</td>
<td>0.68</td>
</tr>
<tr>
<td>St. 5</td>
<td>4.58</td>
<td>0.73</td>
</tr>
<tr>
<td>St. 6</td>
<td>5.28</td>
<td>1.00</td>
</tr>
<tr>
<td>St. 7</td>
<td>4.42</td>
<td>0.88</td>
</tr>
<tr>
<td>St. 8</td>
<td>6.96</td>
<td>1.93</td>
</tr>
<tr>
<td>St. 9*</td>
<td>6.50</td>
<td>1.90</td>
</tr>
</tbody>
</table>

* Used the cell number at 0 m depth instead at 5 m depth
Table 3. Spearman’s rank correlation analysis of environmental factors and abundance of total prokaryotes, *Eubacteria* and Archaea in the epipelagic zone of the Central South Pacific Ocean.

<table>
<thead>
<tr>
<th>Group and factor</th>
<th>Total prokaryotic abundance (n = 56)</th>
<th><em>Eubacteria</em></th>
<th><em>Alphaproteobacteria</em></th>
<th>SAR11</th>
<th><em>Gammaproteobacteria</em></th>
<th>SAR86</th>
<th>Bacteroidetes</th>
<th>Crenarchaeota</th>
<th>Euryarchaeota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>-0.58**</td>
<td>-0.66**</td>
<td>-0.65**</td>
<td>-0.62**</td>
<td>-0.59**</td>
<td>-0.48**</td>
<td>-0.74**</td>
<td>-0.21</td>
<td>-0.64**</td>
</tr>
<tr>
<td>Water temperature</td>
<td>0.37**</td>
<td>0.32*</td>
<td>0.11</td>
<td>0.26</td>
<td>0.04</td>
<td>-0.1</td>
<td>0.30*</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>Salinity</td>
<td>-0.13</td>
<td>-0.23</td>
<td>-0.34*</td>
<td>-0.20</td>
<td>-0.40**</td>
<td>-0.39**</td>
<td>-0.28*</td>
<td>-0.34</td>
<td>-0.27</td>
</tr>
<tr>
<td>Dissolved oxygen conc.</td>
<td>0.16</td>
<td>0.23</td>
<td>0.29*</td>
<td>0.25</td>
<td>0.52**</td>
<td>0.37**</td>
<td>0.23</td>
<td>-0.02</td>
<td>0.24</td>
</tr>
<tr>
<td>Chl a conc.</td>
<td>0.21</td>
<td>0.23</td>
<td>0.22</td>
<td>0.27*</td>
<td>-0.47**</td>
<td>0.15</td>
<td>0.10</td>
<td>0.17</td>
<td>0.47**</td>
</tr>
<tr>
<td>NO₃</td>
<td>-0.38**</td>
<td>-0.41**</td>
<td>-0.29*</td>
<td>-0.33*</td>
<td>-0.74**</td>
<td>-0.18</td>
<td>-0.41**</td>
<td>0.13</td>
<td>-0.30</td>
</tr>
<tr>
<td>NO₂</td>
<td>-0.03</td>
<td>0.02</td>
<td>0.17</td>
<td>-0.34*</td>
<td>-0.37**</td>
<td>0.23</td>
<td>0.01</td>
<td>0.21</td>
<td>0.30</td>
</tr>
<tr>
<td>NH₄</td>
<td>-0.07</td>
<td>-0.19</td>
<td>-0.23</td>
<td>0.13</td>
<td>-0.18</td>
<td>-0.31*</td>
<td>-0.21</td>
<td>-0.14</td>
<td>-0.06</td>
</tr>
<tr>
<td>PO₄</td>
<td>-0.28*</td>
<td>-0.30*</td>
<td>-0.28*</td>
<td>-0.30*</td>
<td>-0.69**</td>
<td>-0.20</td>
<td>-0.37**</td>
<td>0.12</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

*Level of significance: *, *P* < 0.05; **, *P* < 0.01

Chl a, chlorophyll a
Table 4. Multiple regression analysis of environmental factors and distribution of bacterial and archaeal phylotypes

<table>
<thead>
<tr>
<th>Group and factor</th>
<th>Total prokaryotic abundance (n = 56)</th>
<th>Bacteria (n = 56)</th>
<th>Archaea (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eubacteria</td>
<td>Alphaproteobacteria</td>
<td>SAR11</td>
</tr>
<tr>
<td>Multiple R-squared</td>
<td>0.57</td>
<td>0.63</td>
<td>0.48</td>
</tr>
<tr>
<td>F-statistic</td>
<td>13.5</td>
<td>21.8</td>
<td>9.4</td>
</tr>
<tr>
<td>P-value for model</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Coefficient for each resource parameter&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water temperature</td>
<td>0.14***</td>
<td>0.13***</td>
<td>0.06***</td>
</tr>
<tr>
<td>Salinity</td>
<td>−0.04</td>
<td>−0.07***</td>
<td>−0.06**</td>
</tr>
<tr>
<td>Dissolved oxygen conc.</td>
<td>0.14**</td>
<td>0.10***</td>
<td>0.07***</td>
</tr>
<tr>
<td>Chl a conc.</td>
<td>0.04*</td>
<td>0.07***</td>
<td>0.03</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>NS</td>
<td>NS</td>
<td>0.89</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Level 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.