The ISME Journal (2011) 5, 1077–1085

www.nature.com/ismej

ORIGINAL ARTICLE

Co-occurrence patterns for abundant marine archaeal and bacterial lineages in the deep chlorophyll maximum of coastal California

J Michael Beman, Joshua A Steele and Jed A Fuhrman
Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, CA, USA

Microorganisms remineralize and respire half of marine primary production, yet the niches occupied by specific microbial groups, and how these different groups may interact, are poorly understood. In this study, we identify co-occurrence patterns for marine *Archaea* and specific bacterial groups in the chlorophyll maximum of the Southern California Bight. Quantitative PCR time series of marine group 1 (MG1) *Crenarchaeota* 16S rRNA genes varied substantially over time but were well-correlated ($r^2 = 0.94$, $P < 0.001$) with ammonia monooxygenase subunit A (*amoA*) genes, and were more weakly related to 16S rRNA genes for all *Archaea* ($r^2 = 0.39$), indicating that other archaeal groups (for example, *Euryarchaeota*) were numerically important. These data sets were compared with variability in bacterial community composition based on automated ribosomal intergenic spacer analysis (ARISA). We found that archaeal *amoA* gene copies and a SAR11 (or *Pelagibacter*) group Ib operational taxonomic unit (OTU) displayed strong co-variation through time ($r^2 = 0.55$, $P < 0.05$), and archaeal *amoA* and MG1 16S rRNA genes also co-occurred with two SAR86 and two *Bacteroidetes* OTUs. The relative abundance of these groups increased and decreased in synchrony over the course of the time series, and peaked during periods of seasonal transition. By using a combination of quantitative and relative abundance estimates, our findings show that abundant microbial OTUs—including the marine *Crenarchaeota*, SAR11, SAR86 and the *Bacteroidetes*—co-occur non-randomly; they consequently have important implications for our understanding of microbial community ecology in the sea.
The ISME Journal (2011) **5,** 1077–1085; doi:10.1038/ismej.2010.204; published online 13 January 2011
Subject Category: microbial population and community ecology
Keywords: *Crenarchaeota*; SAR11; SAR86; *Bacteroidetes*; community assembly; time-series

Introduction

Microbial members of all three domains of life (*Archaea*, *Bacteria*, and *Eucarya*) are ecologically and biogeochemically important in the ocean. Microscopic primary producers such as *Prochlorococcus* carry out 50% of global primary production (Field *et al.*, 1998), while approximately half of marine primary production is transferred to the 'microbial loop'—within which energy may eventually be transferred to other trophic levels or organic material may be broken down and remineralized (Azam *et al.*, 1983). Certain processes are known to be important in the microbial loop—particularly heterotrophic respiration and nutrient-cycling processes such as nitrification—and a number of microbial groups are known to carry out these processes. However, early molecular studies uncovered substantial diversity within marine microbial assemblages (Giovanonni *et al.*, 1990; Fuhrman *et al.*, 1992), and recent metagenomic studies have revealed even greater diversity in the sea (Venter *et al.*, 2004; Sogin *et al.*, 2006).

Many of the groups identified in these studies have yet to be assigned to a particular ecological niche, although some potential functional roles have been identified. For example, the discovery of proteorhodopsin (PR; a putative light-driven proton pump) among the 'SAR86' group of alphaproteobacteria (Beja *et al.*, 2000, 2001) indicated that these organisms may use light as a source of energy. SAR86 constitute up to 10% of microbial assemblages in the ocean (Eilers *et al.*, 2000), and PR is now recognized to be present in a wide range of microbial groups—including the ubiquitous and abundant SAR11 or *Pelagibacter* clade (Giovannoni *et al.*, 2005a). *Pelagibacter* spp. respire organic carbon and seem to gain little benefit from light in laboratory experiments (Schwalbach *et al.*, 2005; Giovannoni *et al.*, 2005a), however, and a paradigm is emerging by which 'resourceful

Correspondence: JM Beman, Department of Biological Sciences and Wrigley Institute for Environmental Studies, School of Natural Sciences, University of California, Merced, 5200 North Lake Road, Merced, CA 95343, USA.
E-mail: jmbeman@gmail.com
Received 20 September 2010; revised 2 December 2010; accepted 2 December 2010; published online 13 January 2011

heterotrophs' such as the SAR11 group may use light to supplement conventional heterotrophy (Moran and Miller, 2007). Some copiotrophs may use PR to extend survival times during starvation in the light compared with dark (Gomez-Consarnau *et al.*, 2007), or PR may have an array of physiological roles, including sensory functions (Fuhrman *et al.*, 2008). PR was recently identified within marine group 2 *Euryarchaeota* (Frigaard *et al.*, 2006), yet PR phylogeny was inconsistent with 16S rRNA phylogeny, and *Euryarchaeota* at deeper depths did not seem to contain PR (Frigaard *et al.*, 2006).

In contrast to group 2 *Euryarchaeota*, marine group 1 (MG1) *Crenarchaeota* are distributed in large numbers below the euphotic zone, where they comprise ca. 30% of microbial communities (Karner *et al.*, 2001). Summed over the volume of the deep sea, MG1 *Crenarchaeota* constitute one the most abundant microbial groups in the ocean, and many of these organisms likely oxidize ammonia (reviewed by Francis *et al.*, 2007; Prosser and Nicol 2008; Erguder *et al.*, 2009). Several ammonia-oxidizing archaea have been isolated (Könneke *et al.*, 2005; de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008), and with a few exceptions (for example, Agogue *et al.*, 2008; but also see Beman *et al.* (2010), Church *et al.* (2010) and Santoro *et al.* (2010)), many studies have shown that most marine *Crenarchaeota* have the ability to oxidize ammonia (Francis *et al.*, 2005; Wuchter *et al.*, 2006; Mincer *et al.*, 2007), and that the MG1 *Crenarchaeota* actively do so in the ocean (Lam *et al.*, 2007, 2009; Beman *et al.*, 2008; Frias-Lopez *et al.*, 2008; Church *et al.*, 2010; Santoro *et al.*, 2010).

These and other studies have helped place abundant microbial groups such as SAR86, SAR11, *Euryarchaeota* and *Crenarchaeota* into prospective ecological niches, but how they may function as a community is poorly understood. Recent results from the San Pedro Ocean Time series (SPOT) site located off the coast of Southern California have combined automated ribosomal intergenic spacer analysis (ARISA) to 'fingerprint' bacterial communities, with terminal restriction fragment length polymorphism patterns for Eukaryotes, and quantitative PCR (QPCR) assays for archaeal groups to generate an ecological 'network' spanning all three domains of life (J Steele and JA Fuhrman, unpublished). In this study, we identify co-occurrence patterns for abundant archaeal and bacterial groups in the chlorophyll maximum at SPOT based on variation through time.

Materials and methods

Sample collection

Samples were collected at 33° 33′ N, 118° 24′ W at the San Pedro Ocean Time Series Microbial Observatory site located off the coast of Los Angeles, CA, USA. Oceanographic measurements have been regularly made since 1998, and a Microbial Observatory program was established for euphotic zone depths in 2000. Samples were collected at the deep chlorophyll maximum (DCM) on a nearly monthly basis from August 2000 to December 2004 using 20 L Niskin bottles deployed on a CTD rosette from the *R/V Sea Watch*. Water samples were sequentially filtered (Brown *et al.*, 2005) and DNA was extracted using previously described protocols (Fuhrman *et al.*, 1988). Further details of sampling are available in Brown *et al.*, 2005; Fuhrman *et al.*, 2006 and Beman *et al.*, 2010.

QPCR, automated ribosomal intergenic spacer analysis (ARISA), and local similarity analysis (LSA)

QPCR for the *Archaea* was performed using primers, probes, and conditions as reported by Takai and Horikoshi (2000). MG1 and archaeal *amoA* QPCR assays followed established protocols (Beman *et al.*, 2008, 2010), and all samples from 2000–2004 were analyzed in triplicate reactions. ARISA (Fisher and Triplett, 1999) was performed using primers and reaction conditions as previously described (Brown *et al.*, 2005; Fuhrman *et al.*, 2006, 2008); all samples from 2000 to March 2004 were analyzed. Identification of operational taxonomic units (OTUs) within ARISA profiles is based on extensive cloning a nd sequencing of the linked 16S-ITS-23S region detailed in Brown *et al.* (2005) and Fuhrman *et al.* (2006). Correlations among microbial groups and with environmental variables were identified over time using local similarity analysis (LSA) (Ruan *et al.*, 2006).

Oceanographic and biogeochemical data

Salinity, temperature, dissolved oxygen, chlorophyll fluorescence and concentrations of different nutrients (nitrite, nitrate, phosphate and silicate) were measured using standard oceanographic techniques as discussed in Fuhrman *et al.* (2006) and Beman *et al.* (2010). ^{3}H-Thymidine incorporation (Fuhrman and Azam, 1982) and ^{3}H-Leucine incorporation (Simon and Azam, 1989) were measured using previously published methods (Fuhrman *et al.*, 2006).

Results and discussion

Temporal dynamics of archaeal groups in the DCM

Chlorophyll fluorescence at SPOT varied significantly in space, time and magnitude (Figure 1a). Maximum fluorescence values ranged from 0–1.99 $\mu g\,l^{-1}$, and were typically higher in March–May when upwelling occurs along the CA coast. From 2000–2004, the depth of the DCM varied from 14 to 70 m, with a mean value of 32 m; the DCM was typically found at greater depths in the water column during summer months reflecting enhanced stratification. Time series were generated for archaeal groups at the chlorophyll maximum

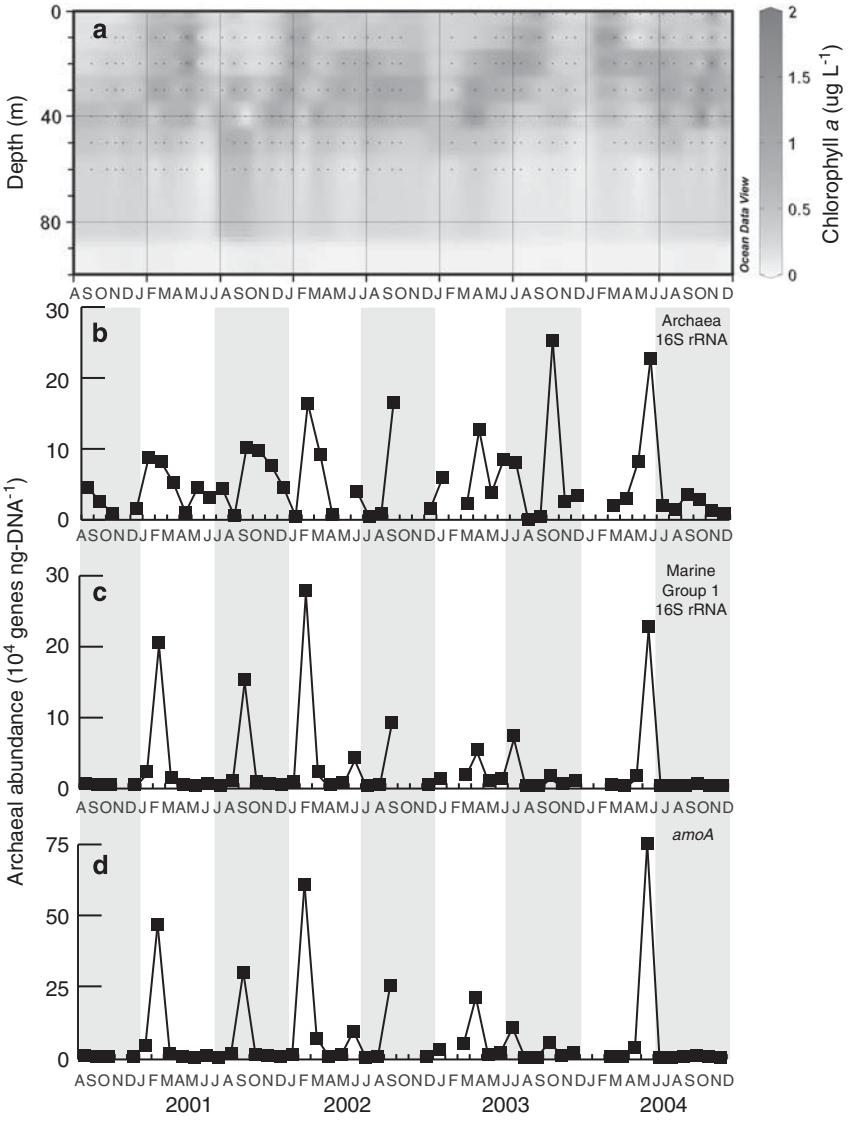

Figure 1 Times series from 2000–2004 of (**a**) chlorophyll from 0 to 100 m; (**b**) all archaeal 16S rRNA genes in the DCM; (**c**) MG1 16S rRNA genes in the DCM; and (**d**) archaeal *amoA* genes in the DCM. Shaded regions indicate the months from July to December.

using QPCR of 16S rRNA and ammonia monooxygenase subunit A (*amoA*) genes. Three data sets were independently generated: 16S rRNA genes of all *Archaea* using primers developed by Takai and Horikoshi (2000) (Figure 1b); 16S rRNA genes from MG1 *Crenarchaeota* (Mincer *et al.*, 2007; Beman *et al.*, 2008) (Figure 1c); and *amoA* genes from *Crenarchaeota* (Beman *et al.*, 2008) (Figure 1d). These three data sets may represent subsets of one another, in that the MG1 *Crenarchaeota* and *amoA*-containing crenarchaea both constitute a subset of the total archaeal community, whereas *amoA*-containing crenarchaea could form a subset of the marine MG1 community, may be essentially synonymous with MG1, or may include archaeal groups other than the MG1 crenarchaea (for example, the pSL12 clade identified by Mincer *et al.*, 2007).

These possibilities were evaluated based on the three data sets, and there were three instances where MG1 16S rRNA genes exceeded all archaeal 16S rRNA genes: these three discrepancies occurred during peaks in 16S rRNA gene copies in February 2001, September 2001 and February 2002 (Figure 1b and c). Although a recent review found the Takai and Horikoshi (2000) primer and probe set to be generally effective for quantifying MG1 16S rRNA genes (Teske and Sorensen, 2007), our findings indicate that they may not detect all MG1 *Crenarchaeota* present in the ocean water column. This was only the case when MG1 16S rRNA genes reached their highest levels ($>100\,000$ genes ng DNA^{-1}), and these datapoints fell just above a 1:1 line (Supplementary Figure S1). Other than these three exceptions, comparison of MG1 16S rRNA genes and those from all Archaea showed two broad patterns: (1) instances where data points fell on a 1:1 line, and the archaeal community was predominantly MG1 *Crenarchaeota*; and (2) those instances where

archaeal 16S rRNA genes exceeded those of crenarchaea (Supplementary Figure S1). This latter pattern represents 81% of samples, and is likely due to the presence of *Euryarchaeota* or crenarchaeal groups other than the MG1 *Crenarchaeota*.

MG1 16S rRNA genes were strongly correlated with archaeal *amoA* genes ($r^2 = 0.978$) over time in the DCM at SPOT (Supplementary Figure S2); this is consistent with a growing number of studies that have reported clear correspondence between *amoA* genes and crenarchaeal cell counts (Wuchter *et al.*, 2006), archaeal lipids (Leininger *et al.*, 2006), or MG1 16S rRNA genes (Wuchter *et al.*, 2006; Lam *et al.*, 2007; Mincer *et al.*, 2007; Beman *et al.*, 2008, 2010; Church *et al.*, 2010; Santoro *et al.*, 2010). Using a different archaeal *amoA* primer set, Agogue *et al.* (2008) reported discrepancies between MG1 16S rRNA genes and archaeal *amoA* genes, and inferred from this lack of correlation that low-latitude deep ocean crenarchaea lack *amoA*. In contrast, correlation between crenarchaeal 16S rRNA and *amoA* genes in the SPOT chlorophyll maximum indicates that all MG1 *Crenarchaeota* in the DCM are *amoA*-containing, and that *amoA*-containing crenarchaeal groups other than the MG1 are not present. This in turn suggests that differences between archaeal 16S rRNA genes and MG1 16S rRNA genes reflect the presence of 16S rRNA genes from marine *Euryarchaeota*.

Covariation among archaeal and bacterial groups

Archaeal QPCR time series were compared with ARISA bacterial community 'fingerprints' using LSA (Ruan *et al.*, 2006). Three archaeal time series were used for this comparison: archaeal *amoA*, MG1 16S rRNA genes, and the difference between archaeal 16S rRNA genes and MG1 16S rRNA genes (referred to as 'probable *Euryarchaeota*'). Comparisons with temperature, salinity, water density, chlorophyll concentrations, oxygen concentrations, nitrite concentrations, nitrate concentrations, phosphate concentrations, silicate concentrations, microbial cell counts and bacterial production were also assessed using LSA. All significant interactions (LSA $P < 0.05$) between archaeal time series and other parameter with a time delay of 1 month or less are reported in Table 1.

Both *amoA* and MG1 16S rRNA data were included and serve as an internal check given the strong correlation between these data sets. In fact, *amoA* and MG1 16S rRNA exhibited the highest LSA score (0.972), with a value similar to the Spearman's correlation reported in Supplementary Figure S2. In contrast, none of the archaeal time series exhibited significant relationships with measured physical or biogeochemical variables, including depth and density. LSA identified significant correspondence between *amoA* and MG1 16S rRNA genes and the same bacterial OTUs—for example, 525, 528, 633, 709 and 779 (Table 1). However, there were four instances when *amoA* was significantly related to a given bacterial OTU and MG1 16S rRNA was not. In these instances, LSA scores were slightly lower (0.373–0.410 compared with 0.407–0.438) and *P*-values were greater than 0.05 for the comparison between 16S rRNA and OTU 744 ($P = 0.056$), OTU 684 ($P = 0.061$), OTU 774 ($P = 0.098$) and OTU 654 ($P = 0.116$).

Table 1 Significant LSA relationships among archaeal data sets and bacterial OTUs identified in ARISA community fingerprints

Archaeal data set	*ARISA OTU*	*LSA score*	*LSA* P *value*	*Pearson's R*	*Pearson's* P *value*	*Spearman's rho*	*Spearman's* P *value*	*Clone-based ID in ARISA*
Archaeal *amoA* genes								
	528	0.47	0.001	0.68	6.40×10^{-6}	0.63	5.39×10^{-5}	SAR86 IIA
	709	0.40	0.022	0.49	0.0016	0.39	0.0209	Bacteroidetes
	654	0.36	0.047	0.47	0.0027	0.40	0.0038	SAR116
	525	0.35	0.003	0.61	8.7×10^{-5}	0.54	7.82×10^{-4}	SAR86 IIB
	744	0.35	0.042	0.47	0.0028	0.35	0.0404	Alpha-proteobacterium
	684	0.35	0.048	0.40	0.01	0.37	0.0294	SAR11–IB
	774	0.33	0.042	−0.45	0.0046	−0.36	0.0345	Unknown
	633	0.30	0.048	0.45	0.0042	0.36	0.0302	Unknown
	779	0.28	0.026	0.50	0.0016	0.48	0.0038	Bacteroidetes
MG1 16S rRNA genes								
	528	0.47	0.001	0.69	4.06×10^{-6}	0.63	4.27×10^{-5}	SAR86 IIA
	709	0.41	0.012	0.51	0.0011	0.42	0.0127	Bacteroidetes
	525	0.34	0.003	0.59	0.00014	0.51	0.0017	SAR86 IIB
	633	0.31	0.034	0.45	0.0045	0.36	0.0302	Unknown
	779	0.28	0.041	0.49	0.0017	0.48	0.0039	Bacteroidetes
Euryarchaeota 16S rRNA[a]								
	1051	0.39	0.009	−0.18	0.15	−0.25	0.1119	Synechococcus Grp. IV (+1 month)
	684	0.34	0.044	−0.29	0.05	−0.13	0.3546	SAR11–IB (+1 month)

Abbreviations: ARISA, automated ribosomal intergenic spacer analysis; OTUs, operational taxonomic units; LSA, local similarity analysis; MG1, marine group 1.
Values are sorted in order of LSA score.
[a]Euryarchaeota 16S rRNA = Archaeal 16S rRNA genes—Marine Group 1 16S rRNA genes.

LSA also identified three inverse relationships: archaeal *amoA* was inversely related to unknown bacterial OTU 774, and probable *Euryarchaeota* 16S rRNA genes were inversely related with OTU 684 (a SAR11 group) and OTU 1051 (a *Synechococcus* group; Table 1). Both of these relationships involved a month time lag, with increases in *Synechococcus* OTU 1051 and SAR11 OTU 684 leading decreases in *Euryarchaeota*. Archaeal *amoA* genes were positively related to SAR11 OTU 684 with no delay, the only case in which MG1 or *amoA* were related to the same OTU as were the probable *Euryarchaeota*. In total, the data presented here offer limited insight into the marine *Euryarchaeota*: few specific interactions were identified, none of which were positive and without time delay. This may be a function of the data set used for this analysis, which was generated by difference and captures all marine euryarchaeal groups (for example, groups 2, 3 and 4; Teske and Sorensen, 2007). Little is known about *Euryarchaeota* in the ocean other than the fact that group 2 is often abundant in surface waters (Massana *et al*., 1997; Frigaard *et al*., 2006) and some posses PR (Frigaard *et al*., 2006); results from LSA suggest that the abundance of *Euryarchaeota* may decrease following increases in SAR11 and *Synechoccocus* OTUs.

Cooccurrence patterns for Crenarchaeota *and abundant bacterial lineages*

MG1 and *amoA*-containing *Crenarchaeota* co-occurred with multiple bacterial OTUs identified in ARISA bacterial community fingerprints, including strong correspondence between *amoA* genes and OTU 684 from the SAR11 clade, SAR86 OTUs 525 and 528, and *Bacteroidetes* OTUs 709 and 779 (Figure 2). Genome streamlining in SAR11 is indicative of adaptation to oligotrophic conditions in the ocean (Giovannoni *et al*., 2005 b), and the dominance of this group of heterotrophs within microbial communities suggests that they are important in oceanic carbon cycling (Morris *et al*., 2002; Giovannoni *et al*., 2005a). The SAR86 clade remains uncultivated and very little is known about the physiology and biogeochemical activity of this group in the ocean—although they may constitute 10% of microbial assemblages (Eilers *et al*., 2000). *Bacteroidetes* commonly number 10–20% of bacteria and archaea in seawater (Glockner *et al*., 1999; Cottrell and Kirchman, 2000a), and can be particularly abundant following phytoplankton blooms (Riemann *et al*., 2000; Pinhassi *et al*., 2004) when they may specialize in the degradation of organic polymers (Cottrell and Kirchman, 2000b; Bauer *et al*., 2006; González *et al*., 2008; Kirchman, 2008). Some cultivated marine members of this group contain PR and are stimulated by light (Gomez-Consarnau *et al*., 2007; González *et al*., 2008), leading to the possibility of a 'dual life strategy' proposed by González *et al*. (2008) based on the genome of *Polaribacter* MED152—however, it is unclear whether *Bacteroidetes* OTUs 709 and 779 use a similar strategy, because they have not been cultivated and their genomes have not been sequenced.

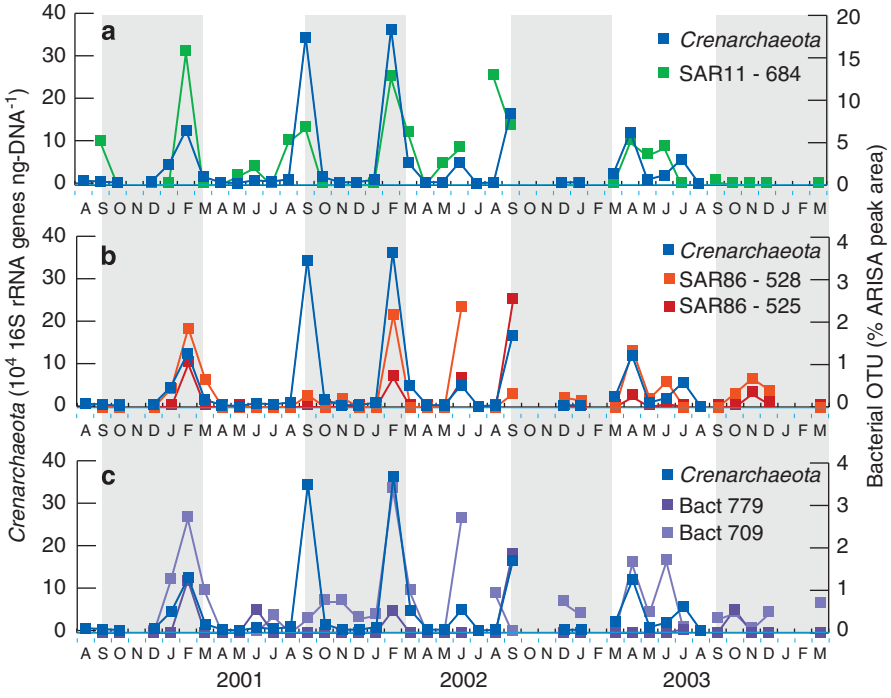

Figure 2 MG1 *Crenarchaeota* time series compared with (**a**) SAR11 OTU 684; (**b**) SAR86 OTUs 525 and 528; and (**c**) *Bacteroidetes* OTUs 709 and 779. MG1 *Crenarchaeota* data are expressed in 16 rRNA genes ng DNA^{-1} (left axis), and bacterial OTUs are expressed as percent of total ARISA peak area (right axis). Shaded regions correspond the months from September to March.

The relative abundance of *Bacteroidetes* OTUs 709 and 779 closely tracked SAR11 OTU 684, and were also correlated with MG1 *Crenarchaeota* over time (Figure 2a and c). Although SAR11 and the marine *Crenarchaeota* are typically characterized by different depth distributions—with SAR11 dominant in surface waters (Morris *et al.*, 2002) and *Crenarchaeota* at depth (Karner *et al.*, 2001)—SAR11 is comprised of multiple subclades that partition with depth in the marine water column (Field *et al.*, 1997; García-Martínez and Rodríguez-Valera, 2000; Morris *et al.*, 2002, 2005; Brown and Fuhrman 2005; Stingl *et al.*, 2007): SAR11 groups Ia and Ib are typically most abundant in surface waters and group II in deeper waters. *Crenarchaeota* also display depth-related partitioning based on 16S rRNA (Massana *et al.*, 1997; García-Martínez and Rodríguez-Valera, 2000), ITS (García-Martínez and Rodríguez-Valera, 2000), *amoB* (Mincer *et al.*, 2007) and *amoA* (Hallam *et al.*, 2006; Mincer *et al.*, 2007; Beman *et al.*, 2008; Santoro *et al.*, 2010) sequences. García-Martínez and Rodríguez-Valera (2000) in fact used 16S rRNA sequences and ITS sequences from SAR11 and MG1 *Crenarchaeota* to examine both groups in the same samples, and found that SAR11 and MG1 partitioned into different 'ecotypes.' Significantly, MG1 *Crenarchaeota* often exhibit peak absolute abundance (cells ml^{-1} or 16S rRNA genes ml^{-1}) and high temporal variability at the base of the euphotic zone (Karner *et al.*, 2001; Mincer *et al.*, 2007; Beman *et al.*, 2008, 2010), and SAR11 may share these properties (Eiler *et al.*, 2009). In the SPOT chlorophyll maximum, SAR11 OTU 684 and *Crenarchaeota* were correlated with one another through time ($r^2 = 0.55$ for *amoA*, $P < 0.01$; $r^2 = 0.51$ for 16S rRNA, $P < 0.05$). Peaks in MG1 *Crenarchaeota* occurred in February and September 2001, February and September of 2002, and May 2003, whereas peaks in SAR11 OTU 684 occurred in February 2001 and February, August and September of 2002 (peaks defined as greater than one standard deviation above the mean; Figure 2).

OTU 684 represents SAR11 group 1b, and in the Sargasso Sea, group Ib was 'transitional': it was most abundant when the water column was not stratified, and hence was not divided into surface and deep niches occupied by different ecotypes (Ia and II; Morris *et al.*, 2005; Carlson *et al.*, 2009). Our results from SPOT are consistent with this, as we found group 1b to be periodically abundant, whereas other 'surface' SAR11 OTUs were dominant for most of the time series—SAR11 group 1a OTUs 666 and 669 together comprised 14–37% of ARISA peak area, averaged 21%, and were correlated with a *Prochlorococcus* OTU (J Steele and JA Fuhrman, unpublished data). Our data furthermore indicate that ammonia-oxidizing archaea are transiently abundant in the DCM and closely track SAR11 OTU 684 (Figure 2a). It is possible that similar covariation among these groups occurs in the Sargasso Sea, as nitrite is the product of ammonia oxidation and can accumulate in high concentrations during periods of winter mixing (Lomas and Lipschultz, 2006) when SAR11 group Ib is also abundant (Morris *et al.*, 2005; Carlson *et al.*, 2009). However, to date, ours is the only study to have examined temporal variability in both of these groups concurrently.

Strong seasonal variability is typical for the Southern California Bight, as productivity is greatest in the months of March, April and May due to shoaling of the nutricline (Hayward and Venrick, 1998). SPOT also experiences seasonal changes in the strength of the Southern California Eddy or Inshore Current; the Southern California Eddy is located inshore of the equatorward flow of the California Current (an eastern boundary current) and results in maximum poleward flow along the coast of southern California in summer through early fall and also in winter (and weakens considerably in spring) (Hickey, 1979; Lynn and Simpson, 1987). These seasonal changes are known to affect phytoplankton community composition: distinct phytoplankton assemblages vary in time and space, and include an inshore group of abundant species that 'reaches maximum abundances … in the spring, almost certainly responding to the increased nutrient concentrations' (Venrick, 2002). The fluctuations of this group are superimposed on a less abundant background assemblage that is more important in offshore regions, and a few shared species form a 'transitional' phytoplankton community (Venrick, 2002).

Co-occurrence of *Crenarchaeota*, SAR11 OTU 684, SAR86 OTUs 525 and 528, and *Bacteroidetes* OTUs 709 and 779 occurred at a transition between periods of lower microbial abundance, production and nutrient concentrations to more replete conditions (Figure 3). The depth of the chlorophyll maximum did not drive these dynamics, as it was only weakly correlated with *amoA* based on Spearman's ranked correlation ($r^2 = 0.18$, $P > 0.05$), and neither depth nor density was identified by LSA as a significant correlate for any of these microbial groups. Instead, this pattern of increased abundance, production and nutrient concentrations in spring and summer was consistent from year to year (Figure 3). Carlson *et al.* (2009) hypothesized that different forms of dissolved organic carbon affect the abundance of different SAR11 ecotyopes in the Sargasso Sea, whereas Eiler *et al.* (2009) modeled the abundance of all SAR11 clades using environmental data: in the North Pacific Subtropical Gyre, dissolved organic carbon, combined nitrate and nitrite concentrations, depth, silicate and phosphate were the best predictors of SAR11 abundance (Eiler *et al.*, 2009). Previous laboratory studies have also shown that the growth and activity of nitrifying bacteria may be enhanced in mixed cultures with heterotrophic bacteria (Gundersen, 1955; Clark and Schmidt, 1966; Steinmüller and Bock, 1976; Jones and Hood, 1980), and Jones and Hood (1980) specifically demonstrated that the

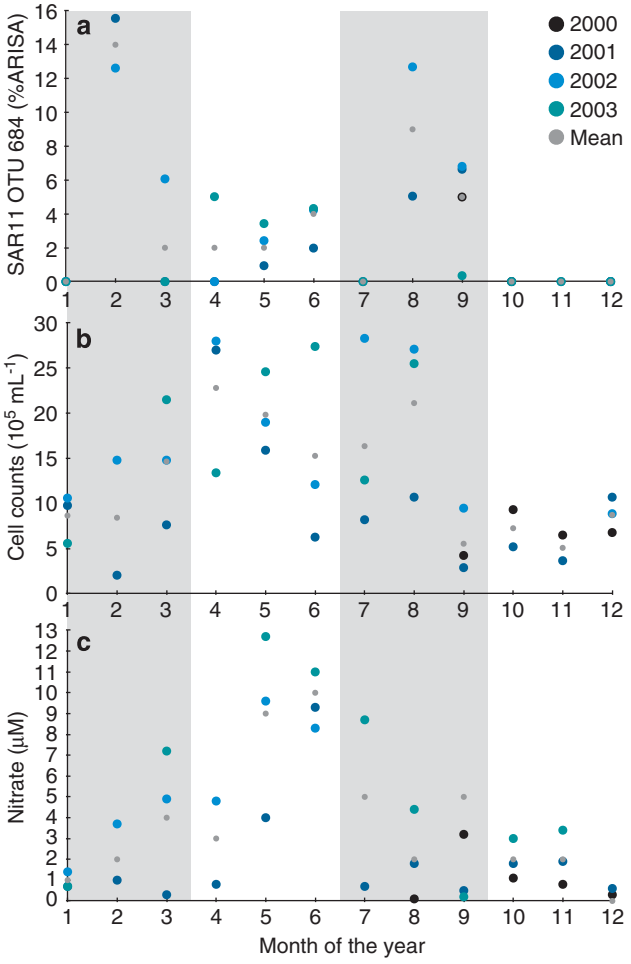

Figure 3 Time series for SAR11 OTU 684 (**a**) compared with (**b**) cell counts and (**c**) nitrate concentrations for each month of the year at SPOT. Smaller gray symbols show the mean values averaged for each month; values for individual months in each year are shown in different colors.

ability of *Nitrosomonas* spp. to oxidize ammonia increased by 50–150% in the presence of two heterotrophs (whose growth was in turn enhanced by as much as an order of magnitude).

In the DCM off the Southern California Bight, we propose that ammonia-oxidizing archaea, and specific SAR11, SAR86, and *Bacteroidetes* OTUs constitute a specialized microbial loop that is present during seasonal transitions. Together these groups may be involved in the breakdown of organic material and recycling of nutrients, and given the similarities in the behavior of the SAR11 group 1b off the coast of Southern California and in the Sargasso Sea, we hypothesize that this community may assemble (that is, become relatively more abundant and active) during similar transition periods in other areas of the ocean. Such co-occurrence patterns are observationally identifiable using the time series approaches that we have used here, and interactions among these groups could be examined experimentally using stable isotope probing or similar techniques. In addition to examining the physiological properties that have made these microorganisms successful ecological competitors in the sea (Giovannoni *et al.*, 2005b; González *et al.*, 2008; Martens-Habbena *et al.*, 2009), future research should determine whether similar 'community assembly rules' (Fuhrman, 2009) apply to these groups elsewhere in the ocean, and what ecological and biogeochemical processes shape these patterns.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Troy Gunderson, Rohan Sachdeva, Anand Patel, Cheryl Chow and Mahira Kakajiwala for help in the field and lab, and the officers and crew of the *R/V Sea Watch*. This work was supported by US National Science Foundation awards OCE-0623575 and 0703159, and the USC Wrigley Institute for Environmental Studies.

References

Agogue H, Brink M, Dinasquet J, Herndl GJ. (2008). Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. *Nature* **456**: 788–791.

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. (1983). The ecological role of water column microbes in the sea. *Mar Ecol Prog Ser* **10**: 257–263.

Bauer M, Kube M, Teeling H, Richter M, Lombardot T, Allers E *et al.* (2006). Whole genome analysis of the marine Bacteroidetes Gramella forsetii reveals adaptations to degradation of polymeric organic matter. *Environ Microbiol* **8**: 2201–2213.

Beja O, Spudich EN, Spudich JL, Leclerc M, DeLong EF. (2001). Proteorhodopsin phototrophy in the ocean. *Nature* **411**: 786–789.

Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP *et al.* (2000). Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea. *Science* **289**: 1902–1906.

Beman JM, Sachdeva R, Fuhrman JA. (2010). Population ecology of nitrifying Archaea and Bacteria in the Southern California Bight. *Environ Microbiol* **12**: 1282–1292.

Beman JM, Popp BN, Francis CA. (2008). Molecular and biogeochemical evidence for ammonia oxidation by marine *Crenarchaeota* in the Gulf of California. *The ISME J* **2**: 429–441.

Brown MV, Fuhrman JA. (2005). Marine bacterial microdiversity as revealed by internal transcribed spacer analysis. *Aquat Microb Ecol* **41**: 15–23.

Brown MV, Schwalbach M, Hewson I, Fuhrman JA. (2005). Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to

show marine microbial diversity: development and application to a time series. *Environ Microbiol* **7**: 1466–1479.

Carlson CA, Morris R, Parsons R, Treusch AH, Giovannoni SJ, Vergin K. (2009). Seasonal dynamics of SAR11 populations in the euphotic and mesopelagic zones of the northwestern Sargasso Sea. *ISME J* **3**: 283–295.

Church MJ, Wai B, Karl DM, DeLong EF. (2010). Abundances of crenarchaeal *amoA* genes and transcripts in the Pacific Ocean. *Environ Microbiol* **12**: 679–688.

Clark C, Schmidt EL. (1966). Effect of Mixed Culture on Nitrosomonas europaea Simulated by Uptake and Utilization of Pyruvate. *J Bacteriol* **91**: 367–373.

Cottrell MT, Kirchman DL. (2000a). Community Composition of Marine Bacterioplankton Determined by 16S rRNA Gene Clone Libraries and Fluorescence *In Situ* Hybridization. *Appl Environ Microbiol* **66**: 5116–5122.

Cottrell MT, Kirchman DL. (2000b). Natural Assemblages of Marine Proteobacteria and Members of the Cytophaga-Flavobacter Cluster Consuming Low- and High-Molecular-Weight Dissolved Organic Matter. *Appl Environ Microbiol* **66**: 1692–1697.

de la Torre JR, Walker CB, Ingalls AE, Konneke M, Stahl DA. (2008). Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environ Microbiol* **10**: 810–818.

Eiler A, Hayakawa DH, Church MJ, Karl DM, Rappé MS. (2009). Dynamics of the SAR11 bacterioplankton lineage in relation to environmental conditions in the oligotrophic North Pacific subtropical gyre. *Environ Microbiol* **11**: 2291–2300.

Eilers H, Pernthaler J, Glockner FO, Amann R. (2000). Culturability and *In Situ* Abundance of Pelagic Bacteria from the North Sea. *Appl Environ Microbiol* **66**: 3044–3051.

Erguder TH, Boon N, Wittebolle L, Marzorati M, Verstraete W. (2009). Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiol Rev* **33**: 855–869.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. (1998). Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science* **281**: 237–240.

Field K, Gordon D, Wright T, Rappe M, Urback E, Vergin K *et al.* (1997). Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. *Appl Environ Microbiol* **63**: 63–70.

Fisher MM, Triplett EW. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol* **65**: 4630–4636.

Francis CA, Beman JM, Kuypers MMM. (2007). New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME J* **1**: 19–27.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci USA* **102**: 14683–14688.

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW *et al.* (2008). Microbial community gene expression in ocean surface waters. *Proc Natl Acad Sci USA* **105**: 3805–3810.

Frigaard N, Martinez A, Mincer TJ, DeLong EF. (2006). Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. *Nature* **439**: 847–850.

Fuhrman JA, McCallum K, Davis AA. (1992). Novel major archaebacterial group from marine plankton. *Nature* **356**: 148–149.

Fuhrman JA, Azam F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar Biol* **66**: 109–120.

Fuhrman JA. (2009). Microbial community structure and its functional implications. *Nature* **459**: 193–199.

Fuhrman JA, Schwalbach MS, Stingl U. (2008). Proteorhodopsins: an array of physiological roles? *Nat Rev Micro* **6**: 488–494.

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S. (2006). Annually reoccurring bacterial communities are predictable from ocean conditions. *Proc Natl Acad Sci USA* **103**: 13104–13109.

Fuhrman JA, Comeau DE, Hagstrom A, Chan AM. (1988). Extraction from Natural Planktonic Microorganisms of DNA Suitable for Molecular Biological Studies. *Appl Environ Microbiol* **54**: 1426–1429.

García-Martínez J, Rodríguez-Valera F. (2000). Microdiversity of uncultured marine prokaryotes: the SAR11 cluster and the marine Archaea of Group I. *Mol Ecol* **9**: 935–948.

Giovannoni SJ, Bibbs L, Cho J, Stapels MD, Desiderio R, Vergin KL *et al.* (2005a). Proteorhodopsin in the ubiquitous marine bacterium SAR11. *Nature* **438**: 82–85.

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG. (1990). Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**: 60–63.

Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D *et al.* (2005b). Genome Streamlining in a Cosmopolitan Oceanic Bacterium. *Science* **309**: 1242–1245.

Glockner FO, Fuchs BM, Amann R. (1999). Bacterioplankton Compositions of Lakes and Oceans: a First Comparison Based on Fluorescence *In Situ* Hybridization. *Appl Environ Microbiol* **65**: 3721–3726.

Gomez-Consarnau L, Gonzalez JM, Coll-Llado M, Gourdon P, Pascher T, Neutze R *et al.* (2007). Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* **445**: 210–213.

González JM, Fernández-Gómez B, Fernàndez-Guerra A, Gómez-Consarnau L, Sánchez O, Coll-Lladó M *et al.* (2008). Genome analysis of the proteorhodopsin-containing marine bacterium Polaribacter sp. MED152 (Flavobacteria). *Proc Natl Acad Sci USA* **105**: 8724–8729.

Gundersen K. (1955). Observations on mixed cultures of nitrosomonas and heterotrophic soil bacteria. *Plant Soil* **7**: 26–34.

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM *et al.* (2006). Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PLoS Biology* **4**: e95.

Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H *et al.* (2008). A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proc Natl Acad Sci USA* **105**: 2134–2139.

Hayward TL, Venrick EL. (1998). Nearsurface patterns in the California Current: coupling between physical and biological structure. *Deep Sea Res Part II: Top Stud Oceanogr* **45**: 1617–1638.

Hickey BM. (1979). The California current system—hypotheses and facts. *Prog Oceanogr* **8**: 191–279.

Jones RD, Hood MA. (1980). Interaction between an ammonium-oxidizer, *Nitrosomonas* sp., and two heterotrophic bacteria, *Nocardia atlantica* and *Pseudomonas* sp. A note. *Microb Ecol* **6**: 271–275.

Karner MB, DeLong EF, Karl DM. (2001). Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**: 507–510.

Kirchman DL. (2008). New light on an important microbe in the ocean. *Proc Natl Acad Sci* **105**: 8487–8488.

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543–546.

Lam P, Lavik G, Jensen MM, van dV, Schmid M, Woebken D *et al.* (2009). Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc Natl Acad Sci USA* **106**: 4752–4757.

Lam P, Jensen MM, Lavik G, McGinnis DF, Muller B, Schubert CJ *et al.* (2007). Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proc Natl Acad Sci USA* **104**: 7104–7109.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW *et al.* (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**: 806.

Lomas MW, Lipschultz F. (2006). Forming the primary nitrite maximum: nitrifiers or phytoplankton? *Limnol Oceanog* **51**: 2453–2467.

Lynn RJ, Simpson JJ. (1987). The California Current System: The Seasonal Variability of its Physical Characteristics. *J Geophys Res* **92**: 12947–12966.

Martens-Habbena W, Berube PM, Urakawa H, de lT, Stahl DA. (2009). Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* **461**: 976–979.

Massana R, Murray A, Preston C, DeLong E. (1997). Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Appl Environ Microbiol* **63**: 50–56.

Mincer TJ, Church MJ, Taylor LT, Preston CM, Karl DM, DeLong EF. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environ Microbiol* **9**: 1162–1175.

Moran MA, Miller WL. (2007). Resourceful heterotrophs make the most of light in the coastal ocean. *Nat Rev Micro* **5**: 792–800.

Morris RM, Vergin KL, Jang-Cheon Cho, Rappé MS, Carlson CA, Giovannoni SJ. (2005). Temporal and Spatial Response of Bacterioplankton Lineages to Annual Convective Overturn at the Bermuda Atlantic Time-Series Study Site. *Limnol Oceanogr* **50**: 1687–1696.

Morris RM, Rappe MS, Connon SA, Vergin KL, Siebold WA, Carlson CA *et al.* (2002). SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806–810.

Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol O, Malits A *et al.* (2004). Changes in Bacterioplankton Composition under Different Phytoplankton Regimens. *Appl Environ Microbiol* **70**: 6753–6766.

Prosser JI, Nicol GW. (2008). Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environ Microbiol* **10**: 2931–2941.

Riemann L, Steward GF, Azam F. (2000). Dynamics of Bacterial Community Composition and Activity during a Mesocosm Diatom Bloom. *Appl Environ Microbiol* **66**: 578–587.

Ruan Q, Dutta D, Schwalbach MS, Steele JA, Fuhrman JA, Sun F. (2006). Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors. *Bioinformatics* **22**: 2532–2538.

Santoro AE, Casciotti KL, Francis CA. (2010). Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. *Environ Microbiol* **12**: 1989–2006.

Schwalbach M, Brown MV, Fuhrman JA. (2005). Impact of light on marine bacterioplankton community structure. *Aquat Microb Ecol* **39**: 235–245.

Simon M, Azam F. (1989). Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* **51**: 201–213.

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR *et al.* (2006). Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci USA* **103**: 12115–12120.

Steinmüller W, Bock E. (1976). Growth of *Nitrobacter* in the presence of organic matter. *Arch Microbiol* **108**: 299–304.

Stingl U, Tripp HJ, Giovannoni SJ. (2007). Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. *ISME J* **1**: 361–371.

Takai K, Horikoshi K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative pcr using fluorogenic probes. *Appl Environ Microbiol* **66**: 5066–5072.

Teske A, Sorensen KB. (2007). Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J* **2**: 3–18.

Venrick EL. (2002). Floral patterns in the California Current System off southern California: 1990–1996. *J Mar Res* **60**: 171–189.

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA *et al.* (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–74.

Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P *et al.* (2006). Archaeal nitrification in the ocean. *Proc Natl Acad Sci USA* **103**: 12317–12322.

Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)