RESEARCH ARTICLE

Variations in spatial and temporal distribution of Archaea in the North Sea in relation to environmental variables

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Abstract

The spatial and temporal distribution of pelagic Archaea was studied in the southern North Sea by rRNA hybridization, sequencing and quantification of 16S rRNA gene and membrane lipid analyses and related to physical, chemical and biological parameters to determine the factors influencing archaeal biogeography. A clear temporal variability was observed, with marine *Crenarchaeota* (Group I.1a) being relatively more abundant in winter and *Euryarchaeota* dominating the archaeal assemblage in spring and summer. Spatial differences in the lateral distribution of *Crenarchaeota* were also evident. In fact, their abundance was positively correlated with the copy number of the gene encoding the α subunit of crenarchaeotal ammonia monooxygenase (*amoA*) and with concentrations of ammonia, nitrate, nitrite and phosphorus. This suggests that most *Crenarchaeota* in the North Sea are nitrifiers and that their distribution is determined by nutrient concentrations. However, *Crenarchaeota* were not abundant when larger phytoplankton ($>3\,\mu m$) dominated the algal population. It is hypothesized that together with nutrient concentration, phytoplankton biomass and community structure can predict crenarchaeotal abundance in the southern North Sea. Euryarchaeotal abundance was positively correlated with chlorophyll *a* concentrations, but not with phytoplankton community structure. Whether this is related to the potential of *Euryarchaeota* to perform aerobic anoxygenic phototrophy remains to be shown, but the conspicuous seasonal distribution pattern of *Crenarchaeota* and *Euryarchaeota* suggests that they occupy a different ecological niche.

Introduction

For decades, Archaea were thought to only dwell in extreme environments hostile to Eukarya and Bacteria, but in the early 1990s, using rRNA hybridization and cloning and sequencing approaches, Fuhrman *et al.* (1992) and DeLong (1992) detected planktonic Archaea in the marine-oxygenated water column. Since then, *Euryarchaeota* and *Crenarchaeota*, the two main groups of marine Archaea, have been found in many different oceanic regions (DeLong *et al.*, 1994; Murray *et al.*, 1999; Crump & Baross, 2000; Pernthaler *et al.*, 2002). In fact, *Crenarchaeota* seem ubiquitous and abundant, constituting about 20–30% of total prokaryotic abundance in the aphotic zone of the global ocean, while *Euryarchaeota* are dominating the archaeal assemblage in surface waters (Massana *et al.*, 2000; Karner *et al.*, 2001; Herndl *et al.*, 2005). These differences in the relative contribution of *Crenarchaeota* and *Euryarchaeota* on the total archaeal biomass between surface and deeper waters suggest that *Euryarchaeota* and *Crenarchaeota* probably occupy different ecological niches.

Crenarchaeotal chemoautotrophy, whereby dissolved inorganic carbon (DIC) is used as a carbon source, was first suggested from compound-specific stable isotope and radiocarbon analyses of their specific membrane lipids (Hoefs *et al.*, 1997; Pearson *et al.*, 2001) and was confirmed by

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in situ labeling (Wuchter *et al.*, 2003) and microautoradiography experiments (Herndl *et al.*, 2005). However, interestingly, evidence for the uptake of dissolved organic substrates, including amino acid, also exists (Herndl *et al.*, 2005; Ingalls *et al.*, 2006; Teira *et al.*, 2006), suggesting a chemoheterotrophic or at least mixotrophic capability. In fact, based on the natural distribution of radiocarbon in their membrane lipids, Ingalls *et al.* (2006) were able to quantitatively partition autotrophy and heterotrophy in pelagic *Crenarchaeota* of the subtropical North pacific Gyre, with crenarchaeotal autotrophy representing 83% at 670 m. Recent findings have also suggested that *Crenarchaeota* are involved in nitrification. Crenarchaeotal genes for putative ammonia monooxygenase A (*amoA*) catalyzing the oxidation of ammonia to hydroxylamine, which is then further converted to nitrite, have been found to be present in seawater from widely different geographic regions (Venter *et al.*, 2004; Francis *et al.*, 2005). The actual ability of, at least, some *Crenarchaeota* to oxidize ammonia to nitrite was clearly established in pure (Könneke *et al.*, 2005) and enriched cultures of *Crenarchaeota* and in field samples (Hallam *et al.*, 2006; Wuchter *et al.*, 2006, Mincer *et al.*, 2007). At present, the metabolic requirements of *Euryarchaeota* remain enigmatic. It is clear that they are also able to assimilate amino acid (Herndl *et al.*, 2005; Teira *et al.*, 2006), thus suggesting heterotrophy. However, in North Atlantic deep waters up to 23% of euryarchaeotal cells took up DIC (Herndl *et al.*, 2005). Thus, in common with *Crenarchaeota*, there is evidence for chemoautotrophy and heterotrophy also for *Euryarchaeota*. Furthermore, very recently, genes encoding proteorhodopsin, a protein that catalyzes light-driven proton transfer across the cell membrane, have been detected in *Euryarchaeota* living in the photic zone but not in the deep waters of the North Atlantic Subtropical Gyre (Frigaard *et al.*, 2006), hence indicating a potential for phototrophy.

Seasonal dynamics in archaeal abundance have been described for several marine environments (Murray *et al.*, 1998; Church *et al.*, 2003; Wuchter *et al.*, 2006). An extensive time-series data set obtained from the coastal southern North Sea surface waters showed that *Crenarchaeota* dominated the archaeal community from autumn to early spring, while *Euryarchaeota* were more abundant in summer and early autumn (Wuchter, 2006; Wuchter *et al.*, 2006). In addition, peaks in crenarchaeotal cell numbers coincided with high concentrations of ammonium in seawater and also with high copy numbers of the crenarchaeotal genes encoding the alpha subunit of the putative ammonia monooxygenase (*amoA*). This distinct seasonal distribution pattern indicates that marine *Crenarchaeota* may be involved in nitrification in the coastal southern North Sea (Wuchter *et al.*, 2006). Because the study of Wuchter *et al.* (2006), which focused on *Crenarchaeota*, was only carried out at one site in the southern North Sea located in the Marsdiep tidal inlet between the southern North Sea and the western Wadden Sea, a more extensive sampling in the southern North Sea was performed to fully assess temporal and spatial differences in the archaeal community in this region. The distribution of *Euryarchaeota* and *Crenarchaeota* in surface and bottom waters was examined at eight different stations in the southern North Sea and this was related to physical, chemical and biological parameters. The sampling time was carefully chosen to best represent the seasonality in the region; February characterizing the winter time, with low water temperatures, and low chlorophyll and nutrient concentrations; April typifying the peak of the algal bloom, with still relatively low water temperatures, high phytoplankton abundances and lower nutrient concentrations; and August exemplifying the late summer period, with elevated water temperatures, phytoplankton abundances varying with sites and low nutrient concentrations.

Materials and methods

Sampling in the southern North Sea was carried out at eight stations (Fig. 1) during three cruises, with the R.V. *Pelagia* in February 2003 and April 2004 and with the R.V. *Alkor* in August/September 2004.

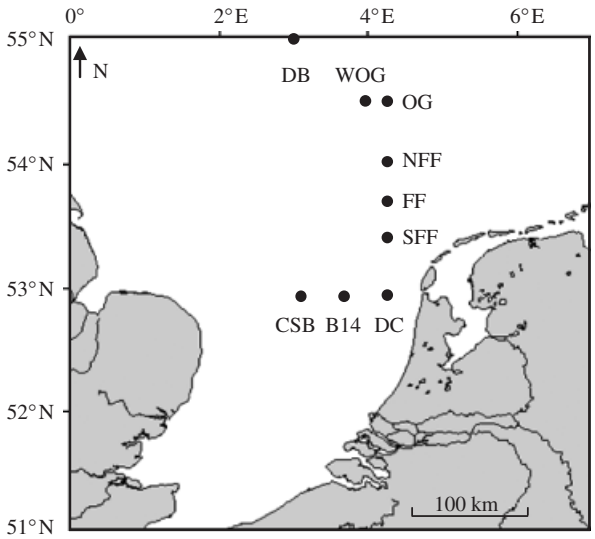

Fig. 1. Map showing the location of the study sites in the southern North Sea. DC, Dutch Coast; CSB, Central Southern Bight; B14, Breeveertien; SFF, South Frisian Front; FF, Frisian Front; NFF, North Frisian Front; OG, Oyster Grounds; WOG, West Oyster Grounds; DB, Dogger Bank. The geographical position of Breeveertien was slightly modified to 52 : 48N and 3 : 33E during the spring and summer cruises to ensure sampling in the correct water mass.

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Environmental parameters

All sampling was conducted following the time-tested and standardized instructions of experienced NIOZ personnel. All analyses were conducted at the NIOZ by the same experienced personnel who routinely perform these measurements at the institute. Seawater salinities and temperatures were obtained using a conductivity–temperature–depth sensor. To determine nutrient concentrations, 3 mL of freshly collected seawater was passed through a 0.2-μm Acrodisc filter and kept frozen until processed. These were later analyzed colorimetrically using a segmented continuous flow analyzer (TRAACS 800 autoanalyzer, Bran & Luebbe). To evaluate field precision, 10 nutrient replicates bottles were collected and measured during the initial winter cruise at the first station (Dutch Coast). This showed that the variability was $< 4\%$ for all nutrients. Given the typical low nutrient concentrations found in summer, all samples were collected and measured in duplicate in August. For quantification of dissolved organic nitrogen and phosphorus (DON and DOP), 25 mL of seawater was filtered through a 0.2-μm Acrodisc filter and kept frozen until analysis. Total dissolved phosphorus was estimated by acidified persulfate UV-destruction, while total dissolved nitrogen was analyzed by alkaline persulfate UV-destruction. DON and DOP were calculated as the difference between total and inorganic nitrogen and phosphorus, respectively. For dissolved organic carbon (DOC) determinations, 8 mL of seawater was directly added to a combusted glass ampoule and immediately acidified with a few drops of 45% H_3PO_4, sealed and kept at $-20\,^{\circ}C$ until analysis. DOC concentrations were determined by high-temperature catalytic oxidation on a modified Total Organic Carbon Analyzer (Shimadzu TOC-5000A) linked to an external infrared cell (LiCor Model LI-6252) for CO_2 detection. As quality control of the DOC determinations, the Consensus Reference Material (Batch 4) provided by Hansell and Chen (University of Miami) was used. Duplicate measurements of DOC were performed. For DIC measurements, seawater was collected into 2-mL glass bottles and stored at $4\,^{\circ}C$ until analysis. DIC concentrations were determined on the TRAACS 800 autoanalyzer (Bran & Luebbe). For chlorophyll *a* measurements, 100–500 mL of seawater was filtered through a GF/F filter (Whatman, 25 mm filter diameter) and kept frozen until analysis. Following extraction in 90% acetone in the dark at $-20\,^{\circ}C$ for 24 h, chlorophyll *a* concentrations were determined on a fluorometer F-2000 (Hitachi). Flow cytometry analyses of phytoplankton were performed in duplicate and performed on a Coulter XL-MCL as described in Veldhuis & Kraay (2004).

Crenarchaeotal membrane lipids

Suspended particulate organic matter for lipid analysis was collected by filtering 40 L of seawater onto preashed 0.7-μm pore-size glass fiber filters (47 mm, GF8 Schleicher & Schuell). These were kept frozen at $-20\,^{\circ}C$ until extraction. During the first cruise, seawater was sequentially filtered onto a 0.7-μm Glass Fiber filter and a 0.2-μm Cellulose Acetate filter. This showed that $< 5\%$ of the total amount of archaeal membrane lipids was recovered with the 0.2-μm filter. Hence, given that the cellulose acetate filters tend to cause problems with the HPLC/MS analysis, the 0.2-μm filtration stage was subsequently omitted. Freeze-dried filters were processed and measured as described in Herfort *et al.* (2006). Only peak areas of the isoprenoid glycerol dialkyl glycerol tetraethers found in the membrane of *Crenarchaeota* were integrated (*m/z* 1302.3, 1300.3, 1298.3, 1296.3, 1292.3) and used to give an estimate of crenarchaeotal membrane lipid concentrations. Peak areas were always at least one order of magnitude higher than background noise. Calibration was performed by running along with the samples known concentrations of purified crenarchaeol.

Catalyzed reported deposition FISH (CARD-FISH)

Samples for CARD-FISH and 4′,6′-diamidino-2-phenylindole (DAPI) counts were only taken during the April and August cruises. Seawater (15 mL) was fixed with formaldehyde (final concentration 4%) at room temperature for 1 h and filtered onto 0.2-μm pore-size polycarbonate filters (Millipore, 25 mm diameter) using 0.45-μm pore-size cellulose nitrate supporting filters. Archaea were stained using the improved CARD-FISH protocol described by Teira *et al.* (2004). Probes specific for Group I.1a *Crenarchaeota*, Cren537 (5′-TGACCACTTGAGGTGCTG-3′) and Group II *Euryarchaeota*, Eury806 (5′-CACAGCGTTTACACCTAG-3′), were used (Teira *et al.*, 2004). Both probes were tested for their specificity before this study. Picoplankton cells were counterstained with DAPI (Teira *et al.*, 2004). Archaea and Bacteria were counted using an epifluorescence microscope (Zeiss Axioplan 2) equipped with a 100 W Hg lamp as well as the appropriate filter sets for DAPI and Alexa488 fluorescence. To determine archaeal and total cell abundances, a minimum of 200 cells were counted per filter. The average counting error in cell abundance, expressed as a percentage of SE, was 26% for DAPI staining and *Euryarchaeota* and 40% for *Crenarchaeota*. The larger average counting error for *Crenarchaeota* is associated with extremely low cell numbers at the North Frisian Front in April.

Nucleic acids extraction

For all DNA analyses, 1 L of seawater was filtered onto 0.2-μm pore-size polycarbonate filters (47 mm, Schleicher & Schuell) and stored at $-80\,^{\circ}C$ until extraction. Total DNA was extracted according to Wuchter (2006). This

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extraction yielded a cell lysis efficiency of about 90% (see Wuchter *et al.*, 2006 for more details).

Archaeal 16S rRNA gene

Partial archaeal 16S rRNA genes (420 bp) were amplified using the general archaeal PCR primers Parch 519f (5′-CAG CCGCCGCGGTAA-3′) and Arch915r (5′-GTGCTCCCCCG CCAATTCCT-3′) with the protocol described previously by Coolen *et al.* (2004). Partial archaeal *amoA* genes (256 bp) were amplified by PCR using the specific primers developed by Wuchter *et al.* (2006), Arc-*amoA*-for (5′-CTGAYTGGGC YTGGACATC-3′) and Arch-*amoA*-rev (5′-TTCTTCTTTGT TGCCCAGTA-3′) and using the same thermo cycling conditions described in Wuchter *et al.* (2006). All amplicons were separated according to the GC content and secondary structure by denaturing gradient gel electrophoresis (DGGE) using a linear denaturing gradient of 30–60% for 6 h at 200 V for Archaea according to Coolen *et al.* (2004) and of 10–50% at 200 V for 3 h for archaeal *amoA* DGGE (Wuchter *et al.*, 2006). Gels were stained for 20 min with SYBR gold and documented with a Fluor-S Multi Imager (BioRad). DGGE bands were excised and each one was eluted in sterile 10 mM Tris-HCl (pH 8.0) at 4 °C for 24 h. Sequencing reactions were performed as described in Wuchter *et al.* (2006).

Sequencing

Following DNA quantification with PicoGreen, reamplified DGGE bands were processed as described Wuchter *et al.* (2003). Nucleotide sequences were determined by automated sequencing using a 310 Genetic Analyzer capillary sequencer (Applied Biosystem).

Phylogenetic analyses

Sequence data were compiled using ARB software (Ludwig *et al.*, 2004) and aligned with complete length sequences of closest relatives obtained from the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) using the ARB FastAligner utility. Using ARB, the phylogenetic trees of Figs 3 and 5 were first generated with the aligned, almost complete length sequences of closest relatives from the NCBI database using the neighbor-joining method (Saitou & Nei, 1987) and the Jukes & Cantor (1969) correction. Then, the short-aligned DGGE sequences were added to the trees using the maximum parsimony option implemented in ARB. The sequences obtained in this study have all been deposited in NCBI (accession numbers EU239431 to EU239463 and EU244456 to EU244461).

Real-time quantitative PCR

Copy numbers of the archaeal *amoA* gene were quantified using the above-mentioned primer sets. For the quantification of crenarchaeotal 16S rRNA gene, the specific primers used by Wuchter *et al.* (2006) (122 bp) were employed: MCG-1 391F (5′-AAGGTTARTCCGAGTGRTTTC-3′) and MCG-1 554R (5′-TGACCACTTGAGGTGCTG-3′). qPCR was carried out in an iCycler system (BioRad, Hercules, CA) as described by Wuchter *et al.* (2006). Calibration was performed by running a qPCR along with the samples of known copy numbers (between 10^{-2} and 10^{7}) of PCR-amplified crenarchaeotal 16S rRNA gene from the North Sea enrichment culture (Wuchter *et al.*, 2006) or archaeal *amoA* also originating from the North Sea enrichment culture (Wuchter *et al.*, 2006). Negative controls and blanks were also subjected to qPCR. Aliquots of these qPCR products were run on an agarose gel in order to identify unspecific PCR products such as primer dimers or bands with unexpected fragment lengths. Additional proof for the specificity of the *amoA* primers used during qPCR came from sequence analysis of *amoA*-DGGE bands (as described above) that were generated during PCR using the same primer set.

Statistical analyses

Data were divided into two main groups, surface and bottom waters, within which statistical analysis was carried out over the whole data set and for each month. Abundances of *Crenarchaeota* and *Euryarchaeota* were compared with each other and with other environmental variables by Spearman's rank correlation coefficient (r_s) analyses. Only significant correlations ($P < 0.05$) are reported.

Results and discussion

Archaeal seasonal and spatial distribution

The southern North Sea is a highly dynamic shallow shelf sea (maximum depth 50 m) characterized in most parts by a mixed water column throughout the year. This is reflected in the DGGE banding pattern, which was very similar at all depths for all sites, indicating a similar archaeal community composition in surface and bottom waters (Fig. 2). This was even true for the Oyster Grounds in August where the water column was thermally stratified (Tables 1 and 2). It is, however, important to note that the lack of saline stratification (Tables 2 and 3) suggests that the thermal stratification was a recent event probably due to the heavy storms that occurred in the southern North Sea in August 2004. However, DGGE fingerprinting of PCR-amplified archaeal 16S rRNA gene clearly indicated a seasonal variation in archaeal distribution in the southern North Sea. Most recovered and sequenced DGGE bands from February 2003 belonged to the Group I.1a *Crenarchaeota*, while in April and August 2004, the Group II *Euryarchaeota* dominated the archaeal assemblage in this region (Figs 2 and 3). This overall

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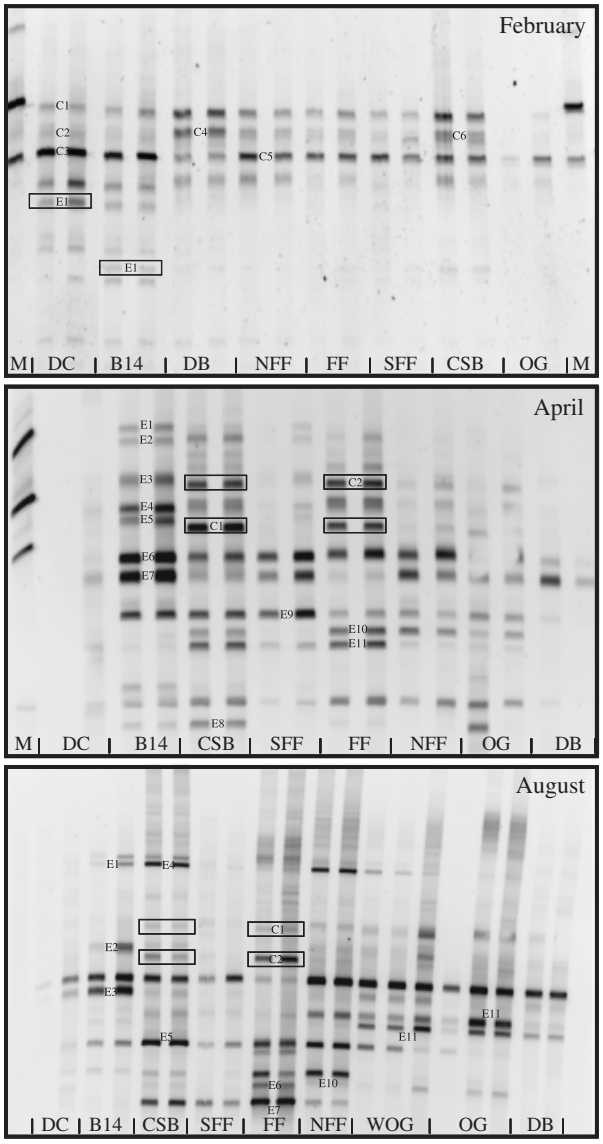

Fig. 2. DGGE images with archaeal 16S rRNA gene bands for the surface and bottom waters of eight sites in the southern North Sea at three different seasons. Each station is represented by two lanes with surface water on the left and bottom on the right, except for OG and WOG in August, which have an additional middle line that denotes the archaeal population at the subsurface chlorophyll maximum. Sequenced DGGE bands are numbered and a 'C' or an 'E' was added to denote *Crenarchaeota* and *Euryarchaeota*, respectively. Rectangles indicate bands related to *Euryarchaeota* in February and to *Crenarchaeota* in April and August. The DGGE marker (M) is a PCR product obtained by Wuchter *et al*. (2006) during a mesocosm experiment and serves as a position marker. Station abbreviations are given in Fig. 1.

seasonal pattern is consistent with the results of the time-series analysis of Wuchter (2006) and Wuchter *et al.* (2006), who found a similar succession in archaeal phylotypes in the coastal southern North Sea. This also agrees with the FISH results obtained in the German Bay of the North Sea by Pernthaler *et al*. (2002). DGGE bands affiliated to the Group II *Euryarchaeota* were also retrieved in February at the stations Dutch Coast and Breeveertien (Figs 2 and 3). Several water masses converge in this part of the southern North Sea, with Continental Coastal and Channel waters present at the Dutch Coast and Breeveertien, respectively (Lee, 1980). In February, salinity values of 29.7 and 33.7 for the Dutch Coast and Breeveertien (Table 1), respectively, indicate that at the Dutch Coast station Continental Coastal water mass was present, while at the Breeveertien station Channel and Continental Coastal water masses were mixed. *Euryarchaeota* Group II were only recovered at these two sites in February 2003, illustrating the low contribution of *Euryarchaeota* to the coastal archaeal community in winter. Interestingly, in contrast to the results of Wuchter (2006) and Wuchter *et al*. (2006), DGGE bands affiliated to the Group I.1a *Crenarchaeota* were obtained in April and August 2004 at the Central Southern Bight and the Frisian Front (Figs 2 and 3), indicating spatial heterogeneity in the distribution of *Crenarchaeota*.

This temporal and spatial distribution pattern was confirmed by quantitative analyses of crenarchaeotal membrane lipids and 16S rRNA gene, and by direct cell counts with CARD-FISH. Overall, higher concentrations of crenarchaeotal membrane lipids (Sinninghe Damsté *et al.*, 2002) were measured in February than in April and August (Table 1). Crenarchaeotal 16S rRNA gene (copies mL^{-1}) was also two to four times more abundant in February than in April and August at the Central Southern Bight (Table 1). This seasonal occurrence of *Crenarchaeota* is illustrated by the inverse correlation found between *Crenarchaeota* abundance and seawater temperature when all three seasons are considered together.

However, in good agreement with the fingerprinting data, all three quantitative approaches revealed a higher abundance of *Crenarchaeota* during spring and summer in the surface waters of the Central Southern Bight and the Frisian Front than at the other stations (Table 1 and Fig. 4). These temporal and spatial variations were also observed in bottom waters (Table 2). Because at all sites the water column is relatively shallow (< 42 m) and well mixed (apart from the Oyster Grounds in August), these data at depth constitute a confirmation of the surface findings of temporal and spatial variations in crenarchaeotal abundances. No rRNA-based analysis was carried out on the underlaying sediments, and hence it cannot be ruled out that *Crenarchaeota* living in the sediment may have resuspended in the water column at these two sites leading to the observed horizontal heterogeneity. The authors do not, however, believe that it is the case here because water column depths (34–47 m) and sediments characteristics (organic matter content and silk %) are similar at the Oyster Grounds, North Frisian Front, Frisian Front and Central Southern

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Table 1. Biological, chemical and physical parameters measured in the surface waters of eight sites in the southern North Sea at three different seasons

	February								April							August								
Parameters	DC	B14	CSB	SFF	FF	NFF	OG	DB	DC	B14	CSB	SFF	FF	NFF	OG	DB	DC	B14	CSB	SFF	FF	NFF	OG	DB
Biological																								
Archaeal membrane lipids (ng L^{-1})	12.4	NA	13.5	2.1	6.5	1.2	4.0	2.0	0.6	1.4	10.9	3.0	8.3	0.7	0.3	0.3	1.1	0.5	2.7	0.9	3.3	0.9	0.2	0.7
DAPI (10^5 cells mL^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	8	14	5	7	4	3	2	6	19	12	5	14	6	7	8	12
Euryarchaeota (10^3 cells mL^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	18	18	9	25	7	2	1	1	17	4	7	27	6	9	2	1
Crenarchaeota (10^3 cells mL^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND	1.8	ND	4.7	0.4	ND	ND	ND	ND	1.6	ND	1.4	ND	ND	ND
Crenarchaeotal 16S rRNA gene (10^3 copies mL^{-1})	2.1	1.5	12.1	2.0	6.6	12.8	7.4	0.3	0.02	0.3	5.1	0.01	11.7	1.1	0.1	0.3	0.01	0.1	1.6	0.03	8.0	0.4	0.01	0.03
Crenarchaeotal *amoA* (10^3 copies mL^{-1})	7.6	5.4	53.0	7.7	18.1	32.8	20.2	1.0	0.1	0.6	13.6	0.1	55.0	10.5	1.3	0.2	0.04	0.1	5.6	0.1	29.2	0.2	0.1	0.1
Chlorophyll *a* (μg L^{-1})	0.3	0.5	0.2	0.1	0.5	0.2	0.2	0.5	2.3	1.6	1.1	2.3	0.3	0.2	0.8	0.4	4.1	0.9	1.7	2.5	1.2	1.0	0.4	0.5
DOC (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	140	97	103	107	94	90	93	95
DON (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	48	7	4	7	7	5	10
DOP (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.18	2.43	0.16	ND	0.08	0.13	0.16	0.01
Chemical																								
Ammonium (μmol L^{-1})	3.0	0.7	0.1	2.0	1.4	0.3	0.2	0.3	0.9	0.4	0.4	0.3	0.7	0.3	0.3	0.2	0.3	0.2	1.3	0.3	2.4	0.4	0.2	0.1
Nitrate (μmol L^{-1})	45.8	18.2	10.6	40.1	31.2	11.3	8.1	5.0	12.4	2.6	10.0	4.8	8.2	4.0	3.1	0.03	0.1	0.1	3.8	0.1	0.8	0.1	0.02	0.02
Nitrite (μmol L^{-1})	0.42	0.22	0.07	0.40	0.30	0.12	0.05	0.08	0.22	0.06	0.17	0.12	0.23	0.13	0.17	0.01	0.01	0.00	0.39	0.00	0.23	0.01	0.00	0.00
Phosphate (μmol L^{-1})	0.85	0.57	0.66	0.84	0.74	0.60	0.55	0.45	0.03	0.02	0.46	0.21	0.54	0.39	0.37	0.15	0.14	0.05	0.16	0.06	0.31	0.18	0.07	0.06
DIC (mmol L^{-1})	2.16	2.10	2.08	2.16	2.13	2.09	2.08	2.07	2.12	2.14	2.18	2.13	2.19	2.19	2.19	2.17	2.14	2.13	2.13	2.11	2.12	2.14	2.11	2.12
Physical																								
Temperature (°C)	3.9	5.0	5.0	3.2	3.4	4.8	5.7	5.3	8.9	8.5	7.7	8.1	7.8	9.5	7.7	7.8	18.8	18.6	17.6	17.2	17.6	17.5	16.9	16.3
Salinity	29.7	33.7	34.3	30.7	31.9	34.2	34.6	34.6	32.1	35.0	34.7	34.8	34.6	34.8	34.8	35.0	32.8	34.8	34.3	34.2	34.0	34.7	34.6	34.9

Station abbreviations are given in Fig. 1.

NA, not available; ND, not detected; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; mDOP, dissolved organic phosphorous; DAPI, 4′,6′-diamidino-2-phenylindole.

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Table 2. Biological, chemical and physical parameters measured in the bottom waters of eight sites in the southern North Sea at three different seasons

	February								April								August							
Parameters	DC	B14	CSB	SFF	FF	NFF	OG	DB	DC	B14	CSB	SFF	FF	NFF	OG	DB	DC	B14	CSB	SFF	FF	NFF	OG	DB
Biological																								
Archacal membrane lipids (ng L^{-1})	NA	NA	17.1	5.9	12.3	1.4	4.7	2.3	4.5	3.5	13.5	4.3	16.2	1.9	5.8	0.9	2.9	0.7	5.5	1.9	22.0	2.2	3.2	0.4
Crenarchaeotal 16S rRNA gene (10^3 copies mL^{-1})	7.4	10.7	0.3	12.8	NA	0.8	20.8	3.4	0.1	0.2	8.3	0.2	3.7	1.7	2.1	0.02	0.1	0.1	3.2	0.2	5.6	0.4	2.4	0.05
Crenarchaeotal *amoA* (10^3 copies mL^{-1})	28.5	37.8	1.4	50.0	NA	2.2	73.0	11.3	1.0	0.8	32.2	0.9	19.9	10.7	9.1	0.1	0.3	0.1	9.0	0.3	15.0	0.4	2.4	0.1
Chlorophyll *a* (μg L^{-1})	0.6	0.7	0.2	0.3	0.2	0.2	0.2	0.6	6.6	1.6	1.8	6.3	0.4	0.2	1.1	0.4	4.7	1.0	2.4	3.2	1.1	0.6	0.4	0.3
DOC (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	130	96	95	104	97	93	88	91
DON (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	55	10	7	12	6	3	3	4
DOP (μmol L^{-1})	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.33	0.03	0.18	0.29	0.37	ND	0.01	0.03
Chemical																								
Ammonium (μmol L^{-1})	0.8	0.7	0.1	0.3	0.2	0.2	0.1	0.3	0.5	0.6	0.7	0.5	0.6	0.8	1.1	0.2	0.3	0.3	1.3	0.4	2.9	0.7	1.7	0.2
Nitrate (μmol L^{-1})	18.8	18.7	10.3	11.7	11.4	7.4	8.5	4.9	2.0	2.7	10.1	4.9	8.0	4.4	4.8	0.03	0.1	0.04	3.3	0.1	1.3	0.1	0.4	0.03
Nitrite (μmol L^{-1})	0.22	0.22	0.06	0.15	0.10	0.07	0.05	0.07	0.08	0.06	0.18	0.12	0.22	0.14	0.19	0.01	ND	ND	0.30	ND	0.36	0.01	0.06	ND
Phosphate (μmol L^{-1})	0.56	0.57	0.63	0.62	0.63	0.58	0.57	0.46	0.08	0.02	0.48	0.21	0.55	0.44	0.51	0.11	0.12	0.05	0.13	0.07	0.57	0.21	0.36	0.14
DIC (μmol L^{-1})	2.09	2.10	2.08	2.10	2.10	2.08	2.08	2.06	2.09	2.15	2.19	2.16	2.19	2.21	2.21	2.17	2.14	2.13	2.14	2.11	2.13	2.14	2.20	2.12
Physical																								
Temperature (°C)	5.7	5.0	5.1	5.4	5.5	5.6	5.7	5.3	8.1	8.5	7.7	8.0	7.7	7.1	6.8	7.8	18.8	18.6	17.6	17.2	17.4	17.5	13.4	16.3
Salinity	33.7	33.7	34.4	34.4	34.5	34.6	34.6	34.6	34.1	35.0	34.7	34.8	34.6	34.8	34.8	35.0	32.8	34.8	34.3	34.2	34.0	34.7	34.6	34.9

Station abbreviations are given in Fig. 1.

NA, not available; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorous; DIC, dissolved inorganic carbon.

Table 3. Summary of Spearman rank correlation coefficient (r_s) analyses carried out over the whole data set and on a monthly basis in the surface and bottom waters of eight stations in the southern North Sea showing statistical correlations ($^*P < 0.05$; $^{**}P < 0.01$) between abundances of *Crenarchaeota* 16S rRNA gene (Cren) (copies mL^{-1}) and *Euryarchaeota* 16S rRNA gene (Eury) (cells mL^{-1}) with biological, chemical and physical parameters

	Surface						Bottom			
	All	February	April		August		All	February	April	August
Parameters	Cren.	Cren.	Cren.	Eury.	Cren.	Eury.	Cren.	Cren.	Cren.	Cren.
Biological										
Archaeal membrane lipids (ng L^{-1})	0.71**						0.63**	ND	0.73*	0.85*
DAPI (cells mL^{-1})	NA	NA					NA	NA	NA	NA
Crenarchaeota 16S rRNA gene (copies mL^{-1})	ND	ND	ND		ND		ND	ND	ND	ND
Crenarchaeotal *amoA* (copies mL^{-1})	0.97**	0.97**	0.97**		0.90**		0.95**	1**	0.88**	0.97**
Chlorophyll a (μg L^{-1})	−0.61*			0.79*		0.88**				
DOC (μmol L^{-1})	NA	NA	NA	NA			NA	NA	NA	NA
DON (μmol L^{-1})	NA	NA	NA	NA			NA	NA	NA	NA
DOP (μmol L^{-1})	NA	NA	NA	NA			NA	NA	NA	NA
Chemical										
Ammonium (μmol L^{-1})					0.78*				0.79*	0.95**
Nitrate (μmol L^{-1})	0.65*						0.76**		0.85*	0.90**
Nitrite (μmol L^{-1})	0.56*				0.73*		0.65**		0.85*	0.83**
Phosphate (μmol L^{-1})	0.78*						0.71**		0.76*	
DIC (mmol L^{-1})			0.76*							
Physical										
Temperature (°C)	−0.62*						−0.51*			
Salinity										

Nonsignificant correlations are not reported and r_s values are given for significant correlations, with negative values indicating inverse relationships. Station abbreviations are given in Fig. 1.

NA, not available; ND, not detected; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorous; DIC, dissolved inorganic carbon.

Bight (Herfort *et al.*, 2006) while *Crenarchaeota* was only detected in the water column at the later two sites. Future studies analyzing together the pelagic and benthic marine archaeal population are nevertheless still necessary to test this. Euryarchaeotal cells were abundant in April and August, amounting up to 2×10^4 cells mL^{-1} at the Frisian Front and representing up to 4% of the total prokaryotic community estimated by DAPI counts (Table 1). As mentioned above, the seasonal distribution of *Crenarchaeota* and *Euryarchaeota* in the surface waters of the North Sea has been described before (Pernthaler *et al.*, 2002; Wuchter, 2006; Wuchter *et al.*, 2006), but to the authors' knowledge, this is the first time that small-scale horizontal heterogeneity in crenarchaeotal abundance is reported. Furthermore, this study has shown that *Crenarchaeota* can in fact be more abundant in spring and summer than in winter (e.g. Frisian Front, Fig. 4). It is at present uncertain whether high crenarchaeotal abundance is a characteristic feature of the water column at the Frisian Front and Central Southern Bight, because a detailed time-series analysis would be necessary to verify this. However, the observed horizontal heterogeneity in crenarchaeotal abundance gives a unique opportunity that previous studies did not provide to examine factors influencing their distribution.

It is also important to note that all the crenarchaeotal sequences recovered using general archaeal primers during PCR were marine *Crenarchaeota*, now classified as Group I.1a *Crenarchaeota* (DeLong, 1998), and shared 94% of sequence similarity, whereas the euryarchaeotal sequences clustered in the Group II *Euryarchaeota* and shared only 85% sequence similarity. This agrees very well with recent data obtained in the coastal southern North Sea, with a sequence similarity of 97% for *Crenarchaeota* and 85% for *Euryarchaeota* (Wuchter, 2006). These results are also consistent with other studies carried out in different oceanic regions indicating that Group II *Euryarchaeota* are phylogenetically more diverse than Group I.1a *Crenarchaeota* (Massana *et al.*, 2000; Bano *et al.*, 2004). Moreover, the present data support the conclusions drawn by Massana *et al.* (2000), who analyzed more than 2000 archaeal rRNA gene clones and showed that only a few phylotypes dominate the oceanic planktonic archaeal assemblage worldwide.

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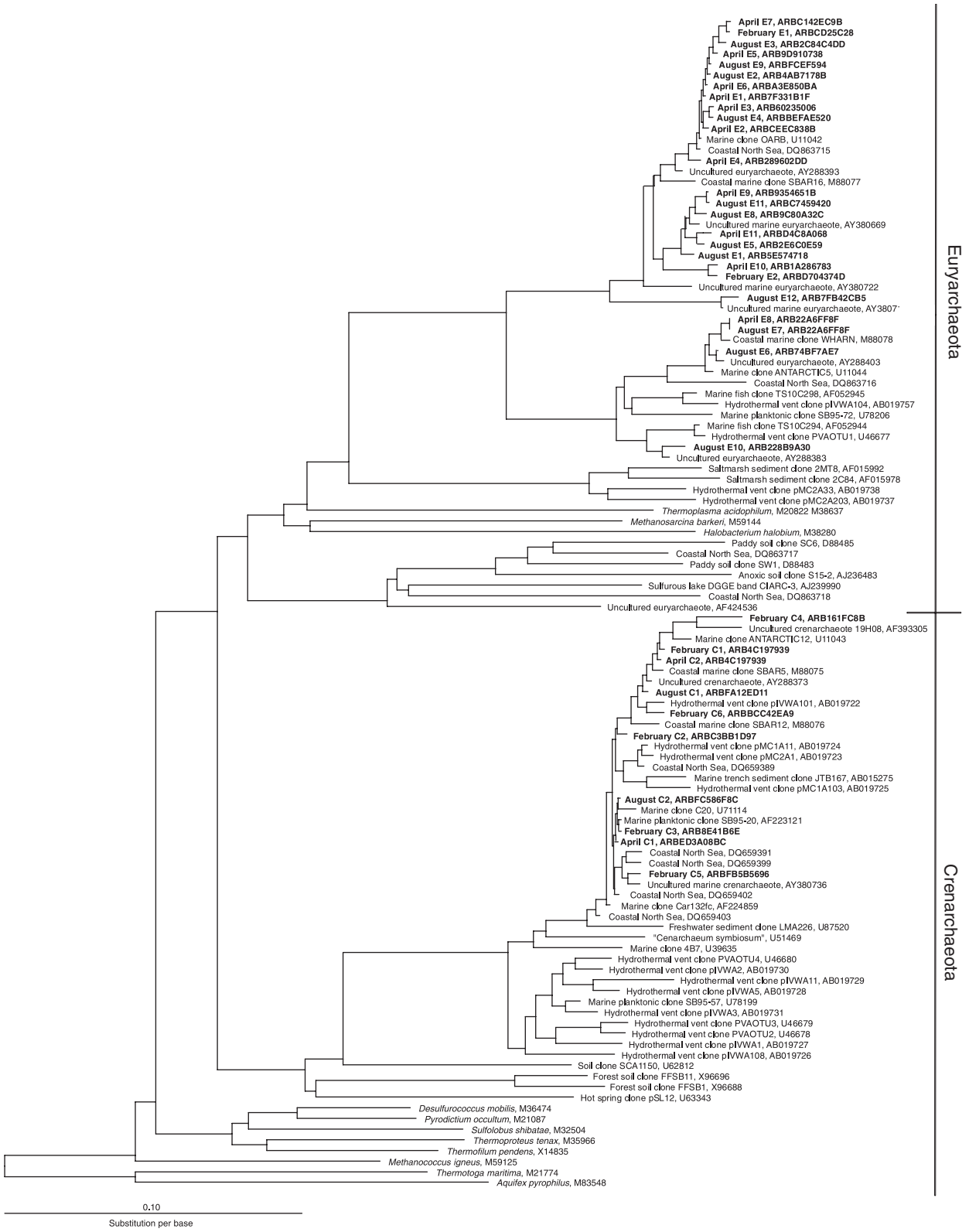

Fig. 3. Neighbor-joining phylogenetic tree showing the affiliation of the archaeal 16S rRNA gene bands recovered from the southern North Sea at three different seasons to archaeal sequences from the NCBI database. Sequences retrieved in this study are indicated in bold and are numbered according to the excised DGGE bands.

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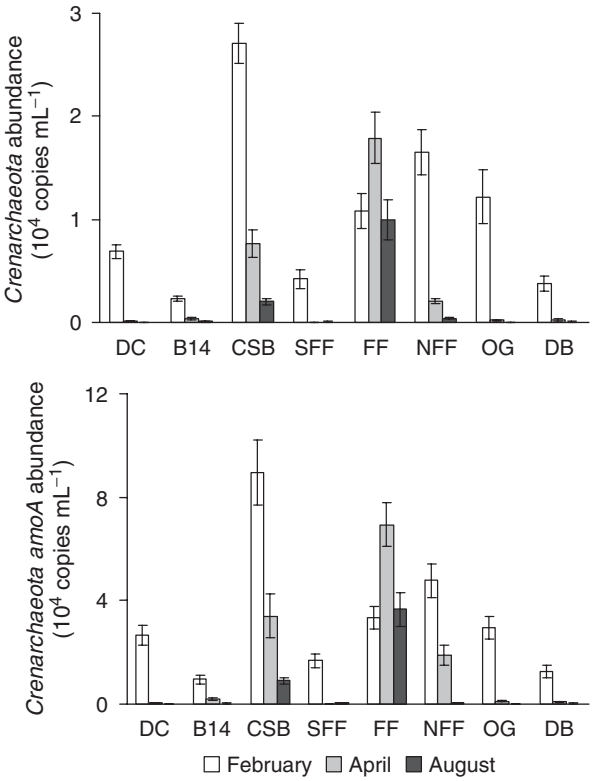

Fig. 4. Abundance of *Crenarchaeota* and crenarchaeotal *amoA* in the surface waters of eight sites in the southern North Sea at three different seasons. Station abbreviations are given in Fig. 1. Vertical bars indicate SE.

Nevertheless, supporting the idea of Dolan (2005) of emerging patterns in microbial biogeography, as explained above, the results demonstrate the existence of small-scale horizontal heterogeneity in archaeal community structure in terms of DGGE-banding patterns, CARD-FISH counts and crenarchaeotal lipids in the southern North Sea. This is consistent with recent studies that have investigated bacterial assemblages within a few kilometers (Suzuki *et al.*, 2001; Pinhassi *et al.*, 2003; Ghiglione *et al.*, 2005). Here, it is shown that these variations are also occurring among archaeal assemblages. Hence, the present data support the emerging view that prokaryotic distribution patterns vary over different spatial scales (Seymour *et al.*, 2005).

Crenarchaeotal nitrification

To investigate the potential for nitrification among the *Crenarchaeota* in the eight study sites in the southern North Sea, functional gene analysis of crenarchaeotal *amoA* was carried out by DGGE and sequencing for all samples that showed high crenarchaeotal abundance, i.e. at all sites in February and at the Central Southern Bight and Frisian Front in April and August. This yielded four highly similar *amoA* nucleotide sequences (99% sequence homology) that were assigned to the marine *Crenarchaeota*. The four *amoA* nucleotide sequences were closely related to the crenarchaeotal *amoA* sequence detected at one location in the coastal southern North Sea (Wuchter, 2006) (Fig. 5). At the amino acid level (data not shown), the resemblance is even more striking because the sequences and that from the coastal southern North Sea were in fact identical, indicating that nucleotide differences occur on the third base pair of each codon. The crenarchaeotal *amoA* nucleotide sequences were all closely related, with 94% and 92% sequence homology, to that of the only pelagic *Crenarchaeota* thus far in pure culture, *Candidatus* '*Nitrosopumilus maritimus*' (Könneke *et al.*, 2005), and with the enriched culture of a crenarchaeote from the coastal southern North Sea (Wuchter *et al.*, 2006) (Fig. 5). Considering that these cultured crenarchaeotes have been clearly characterized as nitrifiers (Könneke *et al.*, 2005; Wuchter *et al.*, 2006), it seems likely that at least some of the *Crenarchaeota* of the southern North Sea obtain their energy through nitrification. Interestingly, *amoA*-like sequences were retrieved at all stations where elevated crenarchaeotal abundances were found, i.e. at all sites in February and at the Central Southern Bight and the Frisian Front in April and August. In addition, quantification of crenarchaeotal *amoA* by qPCR showed exactly the same seasonal dynamics and spatial heterogeneity as that observed for crenarchaeotal 16S rRNA gene (Tables 1 and 2; Fig. 4). These two qPCR-based data sets were highly correlated ($P < 0.01$) (Table 3), with a slope of 3.4 ($r^2 = 0.96$, $n = 48$). This indicates that in this study each crenarchaeotal cell contained 3.4 copies of the *amoA* gene. This estimation should, however, be considered with caution as it is at present unknown whether different ecotypes of Group I.1a *Crenarchaeota* carry identical numbers of *amoA* copies per cell. It is nonetheless noteworthy that the estimate is only slightly higher than the two to three copies per cell reported previously by Wuchter *et al.* (2006) for the coastal North Sea. This slight difference may reflect inaccuracy in the qPCR approach or more probably a variability in *amoA* copies in *Crenarchaeota* similar to what has been observed for ammonia-oxidizing bacteria (AOB) (Norton *et al.*, 2002). A good correlation was also found between nutrient concentrations and crenarchaeotal abundances ($P < 0.05$ for nitrate, nitrite and phosphate when all data from the different seasons were pooled) (Table 3). This is in agreement with data obtained by Murray *et al.* (1999), who found a good correlation between *Crenarchaeota* and nitrite concentrations in the Santa Barbara Channel. *Crenarchaeota* and ammonium concentrations were also significantly correlated in August in surface waters and in February and April in bottom waters (Table 3), and the high levels of crenarchaeotal *amoA* gene copy number measured in August in surface waters were associated with elevated nutrient concentrations (Fig. 6). In August, in the stratified water

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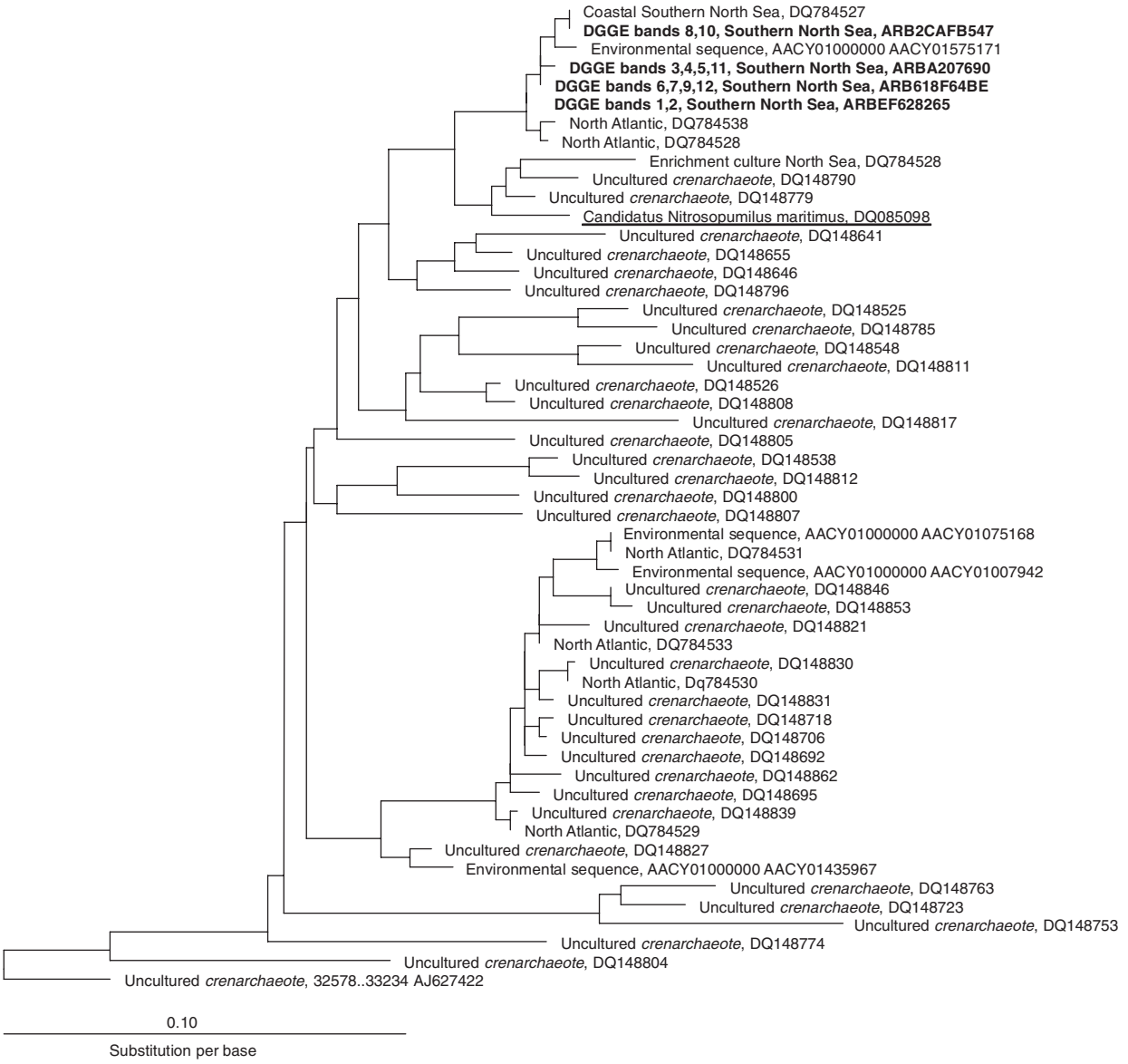

Fig. 5. Neighbor-joining phylogenetic tree of 256-bp long *amoA* nucleotide sequences according to Francis *et al*. (2005) showing the affiliation of the archaeal *amoA* recovered from the southern North Sea at three different seasons to archaeal *amoA* sequences from the NCBI database. Sequences retrieved in this study are indicated in bold and numbers correspond to the excised DGGE bands. The only pelagic marine *Crenarchaeota* thus far in pure culture, *Candidatus* '*Nitrosopumilus maritimus*' (Könneke *et al*., 2005), is underlined.

column of the Oyster Grounds the elevated crenarchaeotal abundances found in bottom waters were also associated with higher nutrient concentrations (Tables 1 and 2). Taken together, all the above-presented data suggest that most of the *Crenarchaeota* detected in the southern North Sea gain their energy by converting ammonium into nitrite and that this ability to perform nitrification explains their distribution in the southern North Sea. It is important to note that the presence of *amoA* genes is only an indication of a potential for nitrification and does not alone establish that this process is taking place. Only process-oriented studies, such as mRNAs analysis, can alone prove that crenarchaeotal nitrification is carried out in the marine environment. Unfortunately, the samples were not preserved in such a way that makes *amoA* transcript analysis possible. However, the authors' reasoning is not only based on the presence of *amoA* genes but also on the good correlation between *amoA* genes and crenarchaeotal cell counts and with nutrient concentrations. Archaeal nitrification is now a well-established idea and these results show that this ability to nitrify may explain their seasonal and spatial (horizontal) distribution. It may be that marine *Crenarchaeota* do have other

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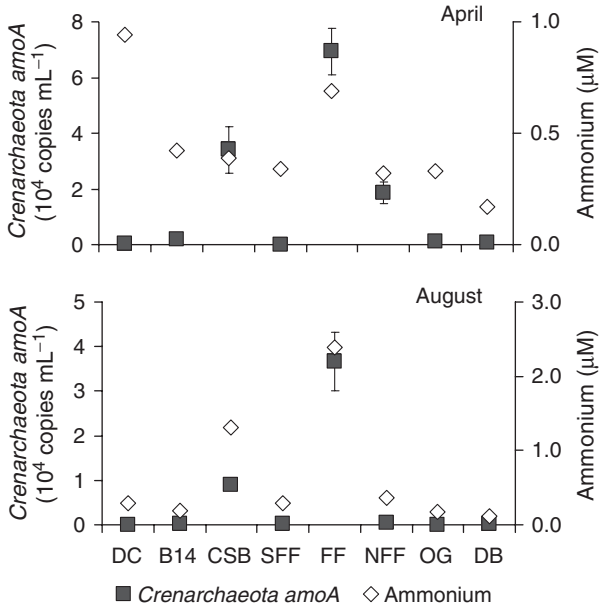

Fig. 6. Crenarchaeotal *amoA* abundance and ammonium concentrations in the surface waters of eight sites in the southern North Sea in April and August. Station abbreviations are given in Fig. 1. Vertical bars indicate SE.

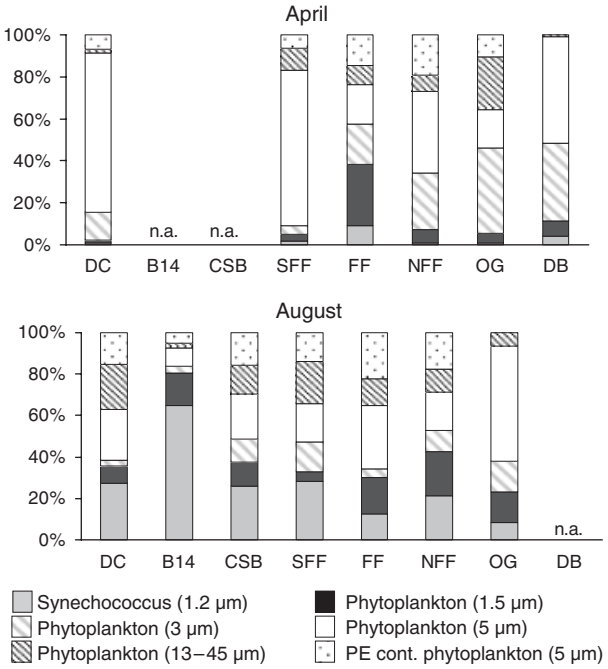

Fig. 7. Flow cytometry analysis showing the composition of the phytoplankton assemblage in the surface waters of eight sites in the southern North Sea in April and August. NA, not available. Station abbreviations are given in Fig. 1.

alternative sources of metabolic energy because for instance concentrations of nitrate, nitrite and even phosphate were more consistently correlated with crenarchaeotal abundance than ammonium (Table 3), but taken together the data suggest that the substrate for nitrification appears to be an important determinant of crenarchaeotal distribution. The inverse correlation observed between temperature and crenarchaeotal abundances when all three seasons are pooled (Table 3) is thus likely the result of the low nutrient concentrations usually found during spring and summer.

There are, however, some exceptions to the general observation made above. In April, elevated ammonium concentrations in surface waters were measured at the stations Dutch Coast, Central Southern Bight and Frisian Front, but *Crenarchaeota* were only abundant at the two latter sites (Table 1 and Fig. 6). This discrepancy may be explained by the high chlorophyll *a* concentrations present at the station Dutch Coast (Table 1). A significant inverse relation ($P < 0.05$) was found in surface waters between chlorophyll *a* concentrations and crenarchaeotal abundances when data from all seasons were pooled (Table 3). Possibly, the predominant phytoplankton bloom present at the Dutch Coast station in April prevented *Crenarchaeota* from flourishing despite elevated levels of ammonium. An inverse correlation between phytoplankton and *Crenarchaeota* is a well-established idea because contrasting seasonality between chlorophyll *a* and *Crenarchaeota* has been reported previously for different oceanic regions including the coastal southern North Sea (Murray *et al.*, 1998; Wuchter, 2006), but this is the first time that this inverse correlation has been observed in space. Hence, the horizontal heterogeneity in crenarchaeotal abundance observed in this study gave a unique opportunity to indicate that crenarchaeotal abundance is directly or indirectly related to the presence of phytoplankton and nutrient availability in the southern North Sea. This is in agreement with the recent findings of Ward (2005), who studied bacterial nitrification in the Pacific off Monterey Bay and showed that biological interactions, including phytoplankton, had the most impact in determining the composition of the nitrifying bacterial assemblage.

In addition to total phytoplankton biomass, its composition may also be affecting crenarchaeotal abundance. Flow cytometry analyses revealed that in the southern North Sea, a considerable variability in phytoplankton composition existed between sites and seasons (Fig. 7). For example, in April at the Frisian Front, where ammonium concentrations were high and *Crenarchaeota* was abundant, picophytoplanktons (*Synechoccocus*, phytoplankton $< 1.5\,\mu m$ and phytoplankton of 1.5–3 μm; Fig. 7) represented 40% of all photosynthetic organisms, while at the Dutch Coast, where nutrient levels were high but crenarchaeotal cell numbers were low, the larger size-classes (5 μm) dominated the phytoplankton assemblage. In August, picophytoplankton were abundant at all sites, constituting, on average, 46% of

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the photosynthetic community. Apparently, *Crenarchaeota* were thus abundant only at sites with high nutrient levels where larger phytoplankton were not dominating the assemblage. Whether this observation indicates that phytoplankton community structure directly or indirectly influences crenarchaeotal abundance will need to be examined by future experiment-based studies. This observation that *Crenarchaeota* and picoplankton abundance are correlated is nonetheless surprising because the high surface to volume ratio of picoplankton (prokaryotes' and eukaryotes' component of the phytoplankton community $< 3\,\mu m$) enables very efficient uptake of nutrients (Veldhuis *et al.*, 2005), suggesting a more pronounced competition for nutrients between prokaryotes and picoplankton than with larger phytoplankton. While the direct influence of the phytoplankton community composition on prokaryotic community composition is not yet well known, field observations and experimental evidence suggest that a shift in the phytoplankton community induces changes in the bacterial assemblage (Arrieta & Herndl, 2002; Pinhassi *et al.*, 2004). When Spearman's rank correlation coefficient analysis was performed on pooled April and August data, an inverse correlation ($\alpha = 0.05$) was found between DAPI counts and the copy numbers of crenarchaeotal 16S rRNA gene ($r_s = -0.626$, $n = 16$). Bacterial population may thus, at least in part, influence crenarchaeotal distribution in the southern North Sea. *Crenarchaeota* might constitute the steady component of the microbial assemblage while Bacteria form the dynamic one and as such control the relative contribution of *Crenarchaeota* to the total prokaryotic community. Recently, using high-resolution molecular fingerprinting, Fuhrman *et al.* (2006) have not only demonstrated that the temporal distribution and abundance of the marine microbial taxa of the southern California coast is strongly patterned but also that these patterns can be predicted from abiotic and biotic factors. In this context, based both on the time-series analysis of Wuchter (2006) conducted at one site in the coastal southern North Sea and on the present data set obtained at different locations in the southern North Sea, it is suggested that high nutrient concentrations and relatively low phytoplankton biomass (with perhaps also low percentage of large phytoplankton) may predict for high crenarchaeotal abundance.

Euryarchaeotal physiology and environmental gradients

In contrast to the *Crenarchaeota*, factors influencing euryarchaeotal distribution are less clear. Several indirect and direct evidences suggest that *Euryarchaeota* are capable of heterotrophy and/or autotrophy (Herndl *et al.*, 2005; Teira *et al.*, 2006; Wuchter, 2006). In this study, euryarchaeotal abundance was related to chlorophyll *a* concentrations

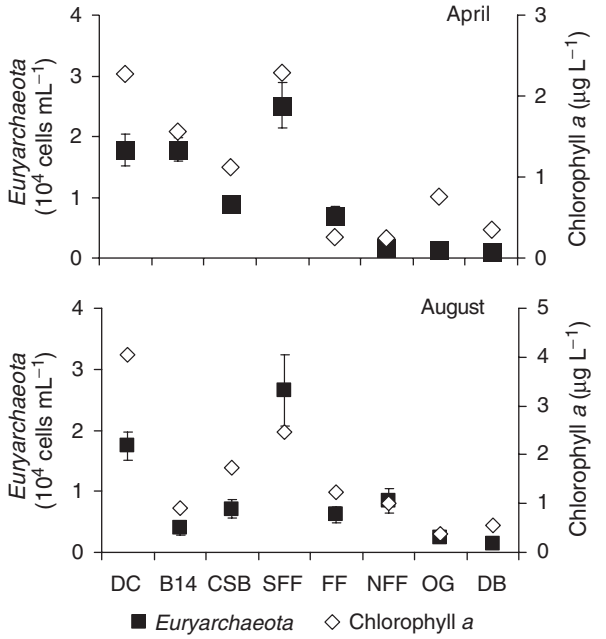

Fig. 8. *Euryarchaeota* abundance and chlorophyll *a* concentrations in the surface waters of eight sites in the southern North Sea in April and August. Station abbreviations are given in Fig. 1. Vertical bars indicate SE.

($P < 0.05$) (Table 3 and Fig. 8). Such a relationship with phytoplankton has been described previously for the coastal southern North Sea and has been interpreted as an indication of euryarchaeotal heterotrophy (Wuchter, 2006). Euryarchaeotal abundance does not seem to be associated with any given phytoplankton assemblage because, for example, phytoplankton larger than 5 µm were the dominant group at the Dutch Coast station in April but not at the South Frisian Front in August, while *Euryarchaeota* were abundant at both sites (Figs 7 and 8). This suggests that other factors probably determine the covariance between euryarchaeotal abundance in the surface seawater and phytoplankton biomass. Recently, proteorhodopsin has been detected in *Euryarchaeota* living in the photic zone of the North Atlantic Subtropical Gyre (Frigaard *et al.*, 2006). Their ability to use photons to generate energy may explain the covariance between *Euryarchaeota* and phytoplankton, i.e. both need sufficient light and nutrients. The exact function of this proteorhodopsin as well as its potential contribution to the overall energy requirements of proteorhodopsin-harboring cells are still uncertain. Some proteorhodopsins have also been described as having sensory capacity rather than being an energy source (Wang *et al.*, 2003) and experimental evidence showed that darkness had little impact on bacterial populations even when cyanobacteria were strongly affected (Schwalbach *et al.*, 2005). Nevertheless, the present study lends indirect support to the emerging notion that aerobic anoxygenic phototrophy may be important among

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prokaryotes and potentially also in *Euryarchaeota* (Beja *et al*., 2000; Kolber *et al*., 2000; Venter *et al*., 2004). However, more work is needed to better understand the physiological requirements of pelagic *Euryarchaeota* and, thus, to elucidate their ecological role in marine systems.

Summary and conclusions

This study has shown that pelagic Archaea constitute a dynamic component of the prokaryotic community in the southern North Sea. A clear temporal variability was observed, with *Crenarchaeota* being relatively more abundant in February and *Euryarchaeota* dominating the archaeal assemblage in April and August. Spatial differences in the lateral distribution of *Crenarchaeota* were, however, evident because over all seasons they remained abundant at two sites: the Frisian Front and the Central Southern Bight. This constitutes the first report of small-scale horizontal heterogeneity in Crenarchaeotal abundance in the marine environment. The abundance of *Crenarchaeota* was positively correlated with the copy number of the gene encoding the α subunit of crenarchaeotal ammonia monooxygenase (*amoA*) and with concentrations of ammonia, nitrate, nitrite and phosphorus, suggesting that most *Crenarchaeota* are nitrifiers and consequently that nutrient concentrations affect their distribution. The data also suggested that large phytoplankton abundance is negatively related to crenarchaeotal abundance, most probably via the influence of the bacterial community associated with large phytoplankton blooms. This implies that nutrient concentration as well as phytoplankton abundance and perhaps also community structure may predict crenarchaeotal abundance. In spring and summer, even though *Euryarchaeota* dominated the archaeal assemblage they constituted $< 4\%$ of the total prokaryotic community. Their abundance also showed spatial differences in lateral distribution, correlating with chlorophyll *a* concentrations but interestingly not with phytoplankton community structure.

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References

Arrieta JM & Herndl GJ (2002) Changes in bacterial β-glucosidase diversity during a coastal phytoplankton bloom. *Limnol Oceanogr* **47**: 594–599.

Bano N, Ruffin S, Ransom B & Hollibaugh JT (2004) Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with Antarctic assemblages. *Appl Environ Microbiol* **70**: 781–789.

Beja O, Aravind L, Koonin EV *et al*. (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* **289**: 1902–1906.

Church MJ, DeLong EF, Ducklow HW, Karner MB, Preston CM & Karl DM (2003) Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula. *Limnol Oceanogr* **48**: 1893–1902.

Coolen MJL, Hopmans EC, Rijpstra WIC, Muyzer G, Schouten S, Volkman JK & Sinninghe Damsté JS (2004) Evolution of the methane cycle in Ace Lake (Antarctica) during the Holocene: response of methanogens and methanotrophs to environmental change. *Org Geochem* **35**: 1151–1167.

Crump BC & Baross JA (2000) Archaeaplankton in the Columbia River, its estuary and the adjacent coastal ocean, USA. *FEMS Microbiol Ecol* **31**: 231–239.

DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* **89**: 5685–5689.

DeLong EF (1998) Everything in moderation: Archaea as 'non-extremophiles'. *Curr Opin Genet Dev* **8**: 649–654.

DeLong EF, Wu KY, Prézelin BB & Jovine RVM (1994) High abundance of Archaea in Antarctic marine picoplankton. *Nature* **371**: 695–697.

Dolan JR (2005) Biogeography of aquatic microbes. *Aquat Microb Ecol* **41**: 39–48.

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.

Frigaard NU, 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.

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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.

Ghiglione J-P, Larcher M & Lebaron P (2005) Spatial and temporal scales of variation in bacterioplankton community structure in the NW Mediterranean Sea. *Aquat Microb Ecol* **40**: 229–240.

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM & DeLong EF (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *PLOS Biol* **4**: 520–536.

Herfort L, Schouten S, Boon JP, Woltering M, Baas M, Weijers JWH & Sinninghe Damsté JS (2006) Characterization of transport and deposition of terrestrial organic matter in the southern North Sea using the BIT index. *Limnol Oceanogr* **51**: 2196–2205.

Herndl GJ, Reinthaler T, Teira E, van Aken H, Veth C, Pernthaler A & Pernthaler J (2005) Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Appl Environ Microbiol* **71**: 2303–2309.

Hoefs MJL, Schouten S, deLeeuw JW, King LL, Wakeham SG & Sinninghe Damsté JS (1997) Ether lipids of planktonic archaea in the marine water column. *Appl Environ Microbiol* **63**: 3090–3095.

Ingalls AE, Shah SR, Hansman RL, Aluwihare LI, Santos GM, Druffel ERM & Pearson A (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proc Natl Acad Sci USA* **103**: 6442–6447.

Jukes T & Cantor C (1969) Evolution of protein molecules. *Mammalian Protein Metabolism* (Munro HN, ed.), pp. 121–132. Academic Press, New York.

Karner MB, DeLong EF & Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **109**: 507–510.

Kolber ZS, van Dover CL, Nlederman RA & Falkowski PG (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407**: 177–179.

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.

Lee AJ (1980) North Sea: physical oceanography. *The North-West European Shelf Seas: the Sea Bed and the Sea in Motion. II. Physical and Chemical Oceanography, and Physical Resources* (Banner FT, Collins MB & Massie KS, eds), pp. 467–493. Elsevier, Amsterdam, The Netherlands.

Ludwig W, Strunk O, Westram R *et al.* (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* **32**: 1363–1371.

Massana R, DeLong EF & Pedrós-Alió C (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Appl Environ Microbiol* **66**: 1777–1787.

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

Murray AE, Preston CM, Massana R, Taylor LT, Blakis A, Wu K & DeLong EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. *Appl Environ Microbiol* **64**: 2585–2595.

Murray AE, Wu K, Moyer CL, Karl DM & DeLong EF (1999) Evidence for circumpolar distribution of planktonic Archaea in the Southern Ocean. *Aquat Microb Ecol* **18**: 263–273.

Norton JM, Alzerreca JJ, Suwa Y & Klotz MG (2002) Diversity of ammonia monooxigenase operon in autotrophic ammonia-oxidizing bacteria. *Arch Microbiol* **177**: 139–149.

Pearson A, McNichol AP, Benitez-Nelson BC, Hayes JM & Eglinton TI (2001) Origins of lipid biomarkers in Santa Monica Basin surface sediment: a case study using compound-specific Delta C-14 analysis. *Geochim Cosmochim Acta* **65**: 3123–3137.

Pernthaler A, Preston CM, Pernthaler J, DeLong EF & Amann R (2002) Comparison of fluorescence labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine Bacteria and Archaea. *Appl Environ Microbiol* **68**: 661–667.

Pinhassi J, Winding A, Binnerup SJ, Zweifel UL, Riemann B & Hagström Å (2003) Spatial variability in bacterioplankton community composition at the Skagerrak-Kattegat Front. *Mar Ecol Prog Ser* **255**: 1–13.

Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol Ò, Malits A & Marrasé CL (2004) Changes in bacterioplankton composition under different phytoplankton regimens. *Appl Environ Microbiol* **70**: 6753–6766.

Saitou N & Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**: 406–425.

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

Seymour JR, Seuront L & Mitchell JG (2005) Microscale and small-scale temporal dynamics of a coastal planktonic microbial community. *Mar Ecol Prog Ser* **300**: 21–37.

Sinninghe Damsté JS, Schouten S, Hopmans EC, Van Duin ACT & Geenevasen JAJ (2002) Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. *J Lipid Res* **43**: 1641–1651.

Suzuki MT, Preston CM, Chavez FP & DeLong EF (2001) Quantitative mapping of bacterioplankton populations in seawater: field tests across am upwelling plume in Monterey Bay. *Aquat Microb Ecol* **24**: 117–127.

Teira E, Reinthaler T, Pernthaler A, Pernthaler J & Herndl GJ (2004) Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. *Appl Environ Microbiol* **70**: 4411–4414.

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Teira E, van Aken H, Veth C & Herndl GJ (2006) Archaeal uptake of enantiometric amino acids in the meso- and bathypelagic waters of the North Atlantic. *Limnol Oceanogr* **51**: 60–69.

Veldhuis MJW & Kraay GW (2004) Phytoplankton in the subtropical Atlantic Ocean: towards a better assessment of biomass and composition. *Deep-Sea Res I* **51**: 507–530.

Veldhuis MJW, Timmermans KR, Croot P & van der Wagt B (2005) Picoplankton: a comparative study of their biochemical composition and photosynthetic properties. *J Sea Res* **53**: 7–24.

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

Wang WW, Sineshchekov OA, Spudich EN & Spudich JL (2003) Spectroscopic and photochemical characterization of a deep ocean proteorhodopsin. *J Biol Chem* **278**: 33985–33991.

Ward BB (2005) Temporal variability in nitrification rates and related biogeochemical factors in Monterey bay, California, USA. *Mar Ecol Prog Ser* **292**: 97–109.

Wuchter C (2006) Seasonal dynamics of marine Archaea in coastal North Sea waters determined by different molecular approaches. pp. 41–64. In Ecology and membrane lipid distribution of marine Crenarchaeota: Implication for the TEX_{86} paleothermometry. PhD thesis, University of Utrecht, the Nederlands.

Wuchter C, Schouten S, Boschker HTS & Sinninghe Damsté JS (2003) Bicarbonate uptake by marine Crenarchaeota. *FEMS Microbiol Let* **219**: 203–207.

Wuchter C, Abbas B, Coolen MJL *et al.* (2006) Archaeal nitrification in the ocean. *Proc Natl Acad Sci USA* **33**: 12317–12322.

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