

Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer

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ABSTRACT: The dynamics of the abundance and activity of selected heterotrophic prokaryotic groups were determined in the coastal North Sea during a coastal spring bloom dominated by the prymnesiophyte *Phaeocystis globosa* and in the subsequent spring and summer season using catalyzed reporter deposition-fluorescence *in situ* hybridization combined with microautoradiography (MICRO-CARD-FISH). Both Crenarchaea and Euryarchaea were detected throughout the study period, albeit never at levels that exceeded 2% of the total prokaryotic abundance, corresponding to a maximum abundance of 2.8×10^8 Archaea l⁻¹. Euryarchaea were generally more abundant than Crenarchaea. On average, 21% of the Crenarchaea and 35% of the Euryarchaea were taking up leucine throughout the study period. Members of the Bacteroidetes were abundant during the *P. globosa* bloom period. At the senescent stage of the bloom, Bacteroidetes comprised up to 63% of the prokaryotic community. The abundances of members of the *Roseobacter* clade as well as the SAR86 cluster were low during the phytoplankton spring bloom period (mean 2% each), but increased during August. On average, 51% of the *Roseobacter*, 38% of the Bacteroidetes and 39% of the SAR86 cluster were taking up leucine. The percentages of active Bacteria increased during the decline of the *P. globosa* spring bloom. The Bacteroidetes cluster showed the strongest increase, indicating that members of this cluster are likely to play a major role in the degradation of organic matter produced in the *P. globosa* spring bloom.

KEY WORDS: Marine Archaea · Bacteroidetes · *Roseobacter* · North Sea · CARD-FISH · Microautoradiography · Bacterial succession · *Phaeocystis*

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INTRODUCTION

Coastal areas cover only around 7% of the global ocean's surface, but contribute 30% to global marine primary production (Gattuso et al. 1998). In these waters, a significant part of the annual primary production is produced during phytoplankton spring blooms initiated when high nutrient concentrations coincide with increased solar radiation. In the temper-

ate southern North Sea, *Phaeocystis globosa* (Prymnesiophyceae) typically dominates the phytoplankton community in spring and forms dense blooms with cell abundances as high as 10^8 cells l⁻¹ (Cadée & Hegeman 1986, Cadée 1992, Brussaard et al. 1996). These blooms may account for as much as 65% of the local annual primary production (Joiris et al. 1982).

Phytoplankton produce labile dissolved organic carbon (DOC) that is directly utilizable by hetero-

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phic prokaryotes (Carlson et al. 1998). During growth, phytoplankton release a fraction of their photo-assimilated carbon as DOC (Teira et al. 2001). In addition, DOC is released by metazooplankton through 'sloppy feeding' on phytoplankton (Møller et al. 2003). With regard to *Phaeocystis* blooms, the collapse of the bloom can be sudden, with cell lysis potentially being an important source of mortality (Brussaard et al. 1995, 1996, 2005). Through cell lysis, phytoplankton biomass will be partly converted into dissolved and colloidal matter, and subsequently recycled by heterotrophic prokaryotes rather than transferred to higher trophic levels (Brussaard et al. 1996, Fuhrman 1999). Phytoplankton blooms in general, and *Phaeocystis* blooms in particular, lead to an increase in the abundance and production of heterotrophic prokaryotes (Laanbroek et al. 1985, Blight et al. 1995, Brussaard et al. 1996, Becquevort et al. 1998).

Phytoplankton blooms impact the composition of the heterotrophic bacterioplankton community (Arrieta & Herndl 2002, Pinhassi et al. 2004); however, few data exist on the response of individual prokaryotic groups to phytoplankton blooms. Studies of marine bacterioplankton diversity have shown that essentially 3 clusters of Bacteria dominate marine bacterioplankton communities. Bacteria belonging to the polyphyletic group Cytophaga-Flavobacteria-Bacteroides, now termed Bacteroidetes, the *Roseobacter* clade of the Alphaproteobacteria, and the SAR86 cluster of the Gammaproteobacteria are not only present in neritic seas such as the North Sea (Eilers et al. 2000, Alonso & Pernthaler 2005) and the Mediterranean (Acinas et al. 1999), but also dominate open oceans such as the Pacific and Atlantic (Mullins et al. 1995). *Roseobacter* is closely associated with phytoplankton blooms, and members of this group utilize DMS(P) (Malmstrom et al. 2004).

Besides heterotrophic Bacteria, non-thermophilic pelagic Archaea are abundant throughout the marine environment as well. The 2 major groups of Archaea, Crenarchaea and Euryarchaea, have been detected in the open ocean (Fuhrman et al. 1992, Karner et al. 2001), coastal waters (DeLong 1992, Preston et al. 1996, Massana et al. 2001), and salt marshes (Munson et al. 1997). In the open ocean, Crenarchaea and Euryarchaea may account for more than half of the prokaryotic abundance at the base of the euphotic zone and in the dark ocean, and play a significant role in the oceanic carbon cycle because they are able to utilize both dissolved organic and inorganic carbon (Herndl et al. 2005, Teira et al. 2006a,b). In addition, Crenarchaea may play an important role as ammonia oxidizers (Könneke et al. 2005, Wuchter et al. 2006). Despite this recent progress in elucidating the potential carbon and energy sources of non-thermophilic

planktonic Archaea, their contribution to the marine biogeochemical cycles remains largely unknown. Specifically, the dynamics of the abundance and activity of Archaea in response to phytoplankton blooms are unspecified. We used combined microautoradiography with catalyzed reporter deposition fluorescence *in situ* hybridization (MICRO-CARD-FISH) to determine the dynamics in abundance and activity of selected groups of Bacteria and Crenarchaea and Euryarchaea in the coastal North Sea during a *Phaeocystis*-dominated phytoplankton bloom and the subsequent spring and summer season.

MATERIALS AND METHODS

Sampling and analyses of phyto- and heterotrophic prokaryotic plankton. Surface water of the coastal North Sea was collected with an acid-rinsed bucket from the Royal Netherlands Institute for Sea Research (NIOZ) jetty located at the southern entrance of the North Sea into the Dutch Wadden Sea (53° 00' 18" N, 04° 47' 42" E) between March and August 2004. Samples from this tidal channel were collected at high tide in order to sample water originating from the Dutch coastal North Sea. Samples were taken from about 0.5 m depth, which was regarded as representative of the whole water column because the water of this tidal channel is generally well-mixed. Samples were taken twice a week during the spring bloom and at weekly intervals during the summer.

Phytoplankton abundance and species composition were determined on Lugol (non-acid)-preserved samples under a Zeiss inverted microscope using 3 or 5 ml counting chambers. Total and free-living prokaryotes were enumerated in unfiltered and in 3 µm-filtered samples (Millipore, polycarbonate filter), respectively. Water samples (1 ml) were fixed with 2% formaldehyde (final concentration), incubated at 4°C in the dark for 30 min, frozen in liquid nitrogen and stored at -80°C until analysis. After thawing, the samples were stained in the dark with SYBR Green I solution (Molecular Probes, 2.5 µmol l⁻¹ final concentration) for 15 min and subsequently enumerated on a FACScalibur (Becton Dickinson) flow cytometer (FCM) with a laser set at 488 nm wavelength (Brussaard 2004). Samples were run at medium speed (40 µl min⁻¹) and data were acquired in log mode until a minimum of 10 000 events had been recorded. The rate of particle passage in the capillary was always maintained below 1000 events s⁻¹. Cell concentrations were determined from the flow rate and by using a known concentration of 1 µm-diameter fluorescent latex beads (10⁵ beads ml⁻¹) as an internal standard (Polyscience). Prokaryotes were detected by their signature of side scatter (SSC)

versus green fluorescence (FL1) as described elsewhere (Gasol & del Giorgio 2000, Brussaard 2004).

For MICRO-CARD-FISH analyses of the free-living prokaryotes, water samples were filtered through polycarbonate filters (Millipore, 3 μm) in order to remove most of the non-prokaryotic biota and the particle-attached prokaryotes. Two to 5 ml of the filtrate was spiked with ^3H -leucine (specific activity 160 Ci mmol^{-1} , 20 nM final concentration; Amersham). Controls were fixed with paraformaldehyde (2% final concentration) 10 min prior to adding ^3H -leucine. Samples were incubated in the dark at *in situ* temperature for 3 to 9 h depending on the *in situ* water temperature, subsequently fixed with paraformaldehyde (2% final concentration), and stored at 4°C in the dark for 12 to 18 h. Thereafter, samples were filtered through polycarbonate filters (Millipore, 0.2 μm) supported by cellulose acetate filters (Millipore, 0.45 μm), washed twice with Milli-Q water, dried, and stored at -20°C until further processing as described below.

For chlorophyll *a* (chl *a*) determination, water samples were filtered onto Whatman GF/F filters, extracted in 90% (v/v) acetone and analyzed fluorometrically.

CARD-FISH analysis. CARD-FISH analysis was performed as described by Teira et al. (2004). Briefly, the cells retained on the polycarbonate filter were embedded by dipping the filter in low-gelling-point agarose (0.1% [w/v] in Milli-Q water), dried at 37°C, and subsequently dehydrated in 95% (v/v) ethanol. For cell wall permeabilization, filters hybridized with bacterial probes were incubated in a buffer (0.05 M EDTA, 0.1 M Tris-HCl [pH 6]) containing lysozyme (10 mg ml^{-1} ; Sigma), whereas filters for hybridization with archaeal probes were incubated in a buffer containing proteinase *K* (1844 U mg^{-1} , final concentration 2.18 $\mu\text{g ml}^{-1}$; Fluka) at 37°C for 1 h. Thereafter, filters were washed with Milli-Q water (once for filters incubated in lysozyme solution, 3 times for filters incubated in proteinase *K* solution) and incubated in 0.01 M HCl at room temperature for 20 min. Thereafter, filters were washed twice with Milli-Q water, dehydrated with 95% ethanol, and dried.

Filters were cut in sections for hybridization with HRP-linked oligonucleotide probes Eub338I-III (Bacteria), Non 338 (negative control), CF319 (many groups belonging to the Cytophaga-Flavobacteria cluster of the Bacteroidetes) (Amann et al. 1995), Ros537 (members of the *Roseobacter-Sulfitobacter-Silicibacter* clade) (Eilers et al. 2001), SAR86 (SAR86 cluster of Gammaproteobacteria) (Eilers et al. 2000), Cren537 (Marine Group I Crenarchaeota), and Eury806 (Marine Group II Euryarchaeota) (Teira et al. 2004). The HRP-probe was added at a final DNA concentration of 0.28 ng μl^{-1} to 300 μl of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.5],

10% [w/v] dextran sulfate, 0.02% [w/v] sodium dodecyl sulfate, 1% [w/v] blocking reagent [Boehringer Mannheim], and 55% [v/v] formamide [for Eub338, Non338, CF319, Ros537, Sar86] or 20% [v/v] formamide [for Cren537, Eury806 and Non338]). Hybridization was performed at 35°C for 12 to 15 h. Thereafter, filter sections with probes targeting Bacteria were washed in 50 ml pre-warmed washing buffer (5 mM EDTA [pH 8], 20 mM Tris-HCl [pH 7.4 to 7.6], 0.01% [w/v] sodium dodecyl sulfate) containing 13 mM NaCl at 37°C for 15 min. Filter sections with probes targeting Archaea were washed in 50 ml pre-warmed washing buffer containing 145 mM NaCl. Filter sections were then transferred to phosphate-buffered saline (PBS) (145 mM NaCl, 1.4 mM NaH_2PO_4 , 8 mM Na_2HPO_4 [pH 7.6]) that contained 0.05% Triton X-100 (PBS-T) in the dark at room temperature for 15 min. Subsequently, filter sections were transferred to 493 μl amplification buffer (10% [w/v] dextran sulfate, 2 M NaCl, 0.1% [w/v] blocking reagent, and 0.0015% [v/v] H_2O_2 in PBS) containing 5 μl of tyramide-Alexa488 (1 mg ml^{-1}), and incubated at 37°C for 30 min. After amplification, filter sections were washed in PBS-T at room temperature for 15 min, rinsed with Milli-Q water and dehydrated in 95% ethanol. Finally, filter sections were air dried and stored at -20°C until further processing.

Microautoradiography. Microautoradiography was performed in a dark room kept at 15°C (Teira et al. 2004). The photographic emulsion (type NTB-2; Kodak) was melted in a water bath at 43°C for 1 h. The emulsion was then mixed with ultra-pure water (Sigma) in a 1:3 (v/v) ratio, divided into aliquots (10.5 ml each) and stored at 4°C. The hybridized filter sections were transferred upside down onto slides coated with the diluted photographic emulsion melted at 43°C for 30 min. The slides were dried on ice-cold aluminum plates for 5 min, placed in a light-tight box containing silica gel as a drying agent and kept at 4°C for 24 h for exposure. Slides were then developed and fixed according to the specifications of the manufacturer (Kodak). Before slides were completely dry, filter sections were peeled off from the slide and the cells that were embedded in the photographic emulsion on the slides were counterstained with a DAPI mix (5.5 parts Citifluor [Citifluor], 1 part Vectashield [Vector Laboratories], 0.5 parts PBS with DAPI at a final concentration of 1 $\mu\text{g ml}^{-1}$).

Slides were examined under a Zeiss Axioplan 2 microscope equipped with a 100-W Hg-lamp and appropriate filter sets for DAPI and Alexa488. The presence of silver grains around the cells was checked by switching to the transmission mode of the microscope (Carman 1993). More than 200 leucine-positive cells or more than 800 DAPI-stained cells were counted per sample.

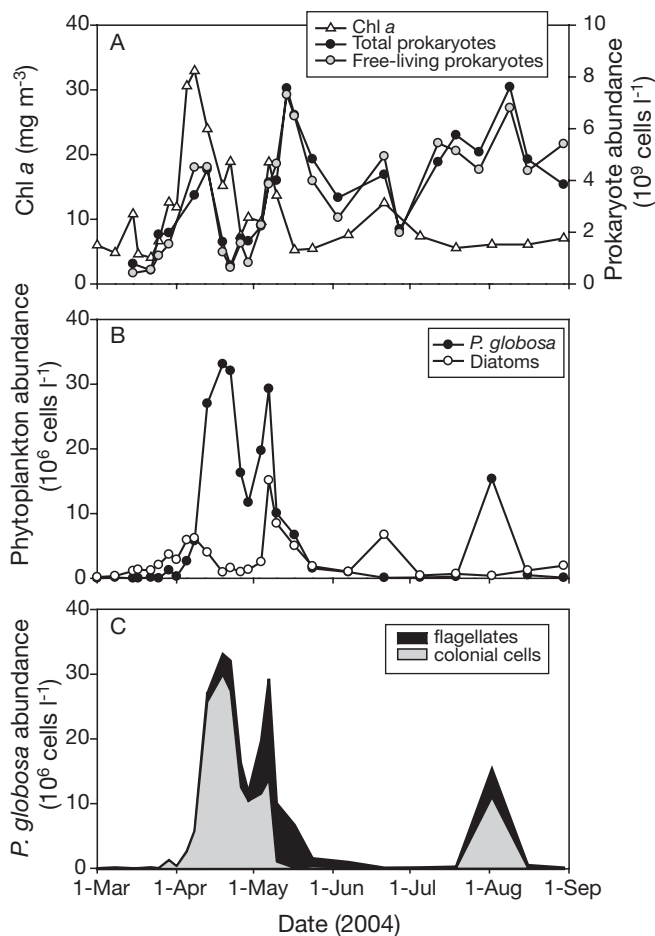


Fig. 1. Dynamics of (A) phytoplankton biomass, as indicated by chl *a* concentration and total and free-living prokaryotic abundance determined by flow cytometry, (B) abundance of diatoms and *Phaeocystis globosa*, and (C) abundance of *P. globosa* colonies and flagellated cells in spring and summer 2004

RESULTS

Temporal dynamics of phyto- and prokaryotic plankton

In the spring of 2004, chl *a* concentrations reached 32.9 mg m⁻³ (Fig. 1A). The spring phytoplankton bloom was dominated by colonial *Phaeocystis globosa* that attained a maximum abundance of 33 × 10⁶ cells l⁻¹ (Fig. 1B). At this time, *P. globosa* constituted 89% of the phytoplankton present, as revealed by enumeration of Lugol-fixed samples under the inverted microscope. From 22 April onward, the *P. globosa* bloom collapsed and single-cell *P. globosa* developed (Fig. 1C). The increase in flagellated, single *P. globosa* cells coincided with an increase in diatoms (Fig. 1B).

The abundance of prokaryotes increased about 4-fold during the development of the *Phaeocystis globosa* bloom, but declined again after the *P. globosa* bloom reached its peak (Fig. 1A). The total prokaryotic abundance increased again during the collapse of the *P. globosa* bloom, reaching a maximum abundance of 7.6 × 10⁹ cells l⁻¹ (Fig. 1A). After the spring bloom, chl *a* concentrations remained fairly constant at an average of 5 mg m⁻³, and the total prokaryotic abundance varied between 2.1 and 7.6 × 10⁹ cells l⁻¹, followed by another peak in early August (Fig. 1A). On average, the free-living prokaryotes (<3 μm fraction) accounted for 93% of the total prokaryotic community (Fig. 1A).

Archaeal versus bacterial abundance and activity

Using the oligonucleotide probes Eub338I-III, Cren537 and Eury806, we recovered >96% of DAPI-stained cells on the filters examined prior to transfer onto the coated slides for autoradiography (n = 5 randomly picked filters, results not shown). A high percentage of DAPI-stained cells hybridizing with HPR-labeled oligonucleotide probes indicates a low percentage of dead cells lacking RNA, confirming results of a previous report on coastal North Sea bacterioplankton by Pernthaler et al. (2002a). However, transfer of the cells from the filters onto the coated slides was incomplete. The efficiency of the recovery of DAPI-stained cells from the coated slides with the Eub338I-III, Cren537 and Eury806 probes was on average 60% (range 46 to 76%). Comparing the results of the hybridizations on the filters with those on the coated slides, no specific bias was found, i.e. none of the studied clusters were selectively lost. Therefore, the sum of cells hybridized with the Eub338I-III, Cren537 and Eury806 probe was used to calculate the percent contribution of the specific groups to the total prokaryotic abundance.

Over the entire spring and summer period, Bacteria (detected with the Eub338I-III probe) comprised on average 98% of the prokaryotic abundance (range 92 to 100%) (Fig. 2A). Although both Crenarchaea and Euryarchaea were present throughout the sampling period, their combined contribution to the total prokaryotic community (DAPI-stained cells) was on average 2%, and their abundance never exceeded 2.8 × 10⁵ cells ml⁻¹. Euryarchaea were generally more abundant than Crenarchaea (Fig. 2A).

The fraction of Bacteria taking up leucine did not statistically differ from the fraction of DAPI-stained cells taking up leucine from 24 March onwards, when more than 10% of both groups were leucine-positive (paired *t*-test, p = 0.68; results not shown). Bacteria made up on average 98% of the DAPI-stained cells, thus indicating

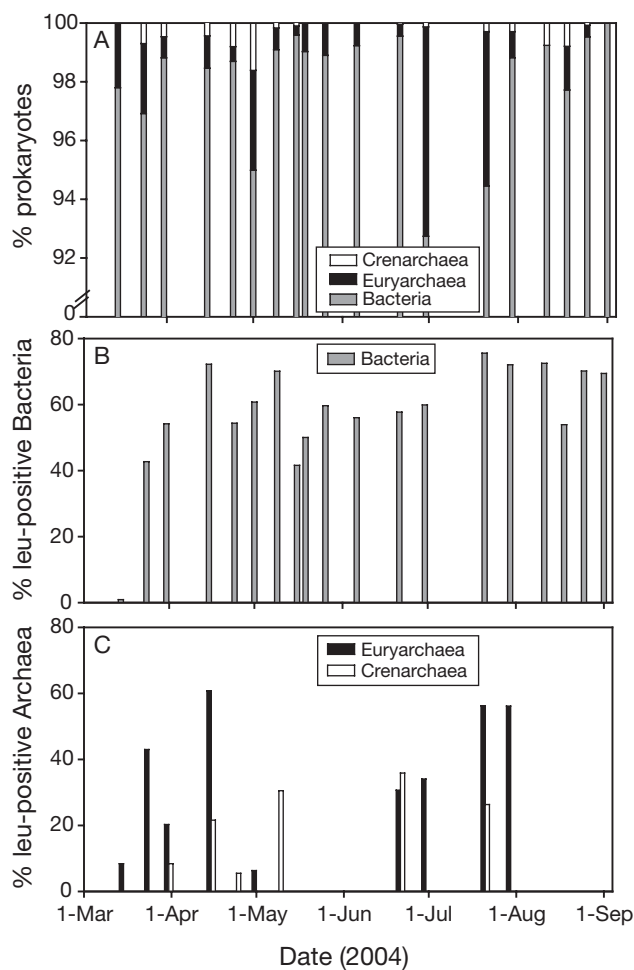


Fig. 2. Dynamics in the contributions (%) of (A) Bacteria, Crenarchaea and Euryarchaea to free-living prokaryote cells, (B) leucine-positive (leu-positive) Bacteria to total Bacteria, and (C) leu-positive Crenarchaea and Euryarchaea to total Crenarchaea and Euryarchaea. The % of leucine-positive Archaea was only included if >50 cells were detected

that our hybridization treatment did not affect the activity determination. In the first sample (15 March), only 2% of the Bacteria were leucine-positive (Fig. 2B). Over the course of spring the percentage of active Bacteria increased, reaching 73% by 13 April. This increase coincided with an increase in the fraction of high nucleic acid (HNA) prokaryotes from 43% in March to 56% on 13 April (data not shown), as determined by flow cytometry. During the remaining spring and summer season, the percentage of Bacteria taking up leucine remained high, on average 63% (range 42 to 73%). The fraction of HNA prokaryotes in this period was on average 63% (range 49 to 81%). Over the whole study period, an average of 35% of the Euryarchaea were active (range 6 to 61%; Fig. 2C), which was significantly lower than the percentage of active Bacteria (paired *t*-test, $p < 0.05$). Only on the first sam-

pling date (15 March) was the fraction of leucine-positive Euryarchaea higher than that of leucine-positive Bacteria. The percentage of active Crenarchaea was on average 21% (range 0 to 35%; Fig. 2C), which was significantly lower than the fraction of active Euryarchaea (paired *t*-test, $p < 0.05$).

Abundance and activity of Bacteroidetes, *Roseobacter* and the SAR86 cluster

Using oligonucleotide probes targeting Bacteroidetes, *Roseobacter* and the SAR86 cluster, we recovered on average 29% (range 8 to 64%) of all the Bacteria stained with the Eub338I-III probe (Fig. 3A). Over the whole study period, the Bacteroidetes cluster was the most abundant of the studied clusters. Bac-

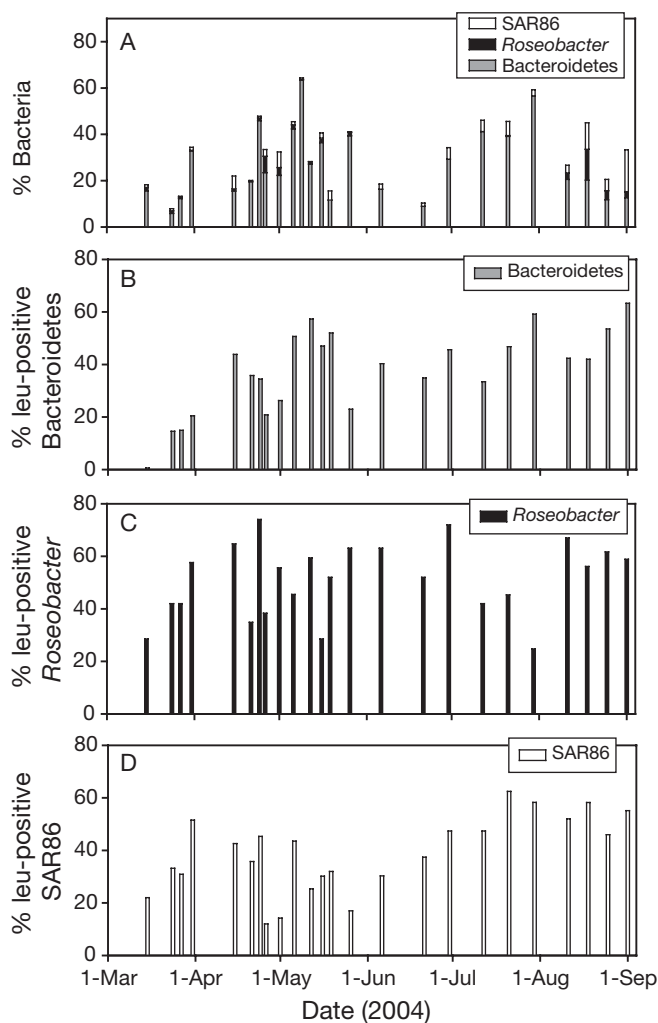


Fig. 3. Dynamics in the contributions (%) of (A) Bacteroidetes, the *Roseobacter* clade and SAR86 cluster to total bacterial abundance, (B) leucine-positive (leu-positive) Bacteroidetes to total Bacteroidetes, (C) leu-positive *Roseobacter* to total *Roseobacter*, and (D) leu-positive SAR86 to total SAR86

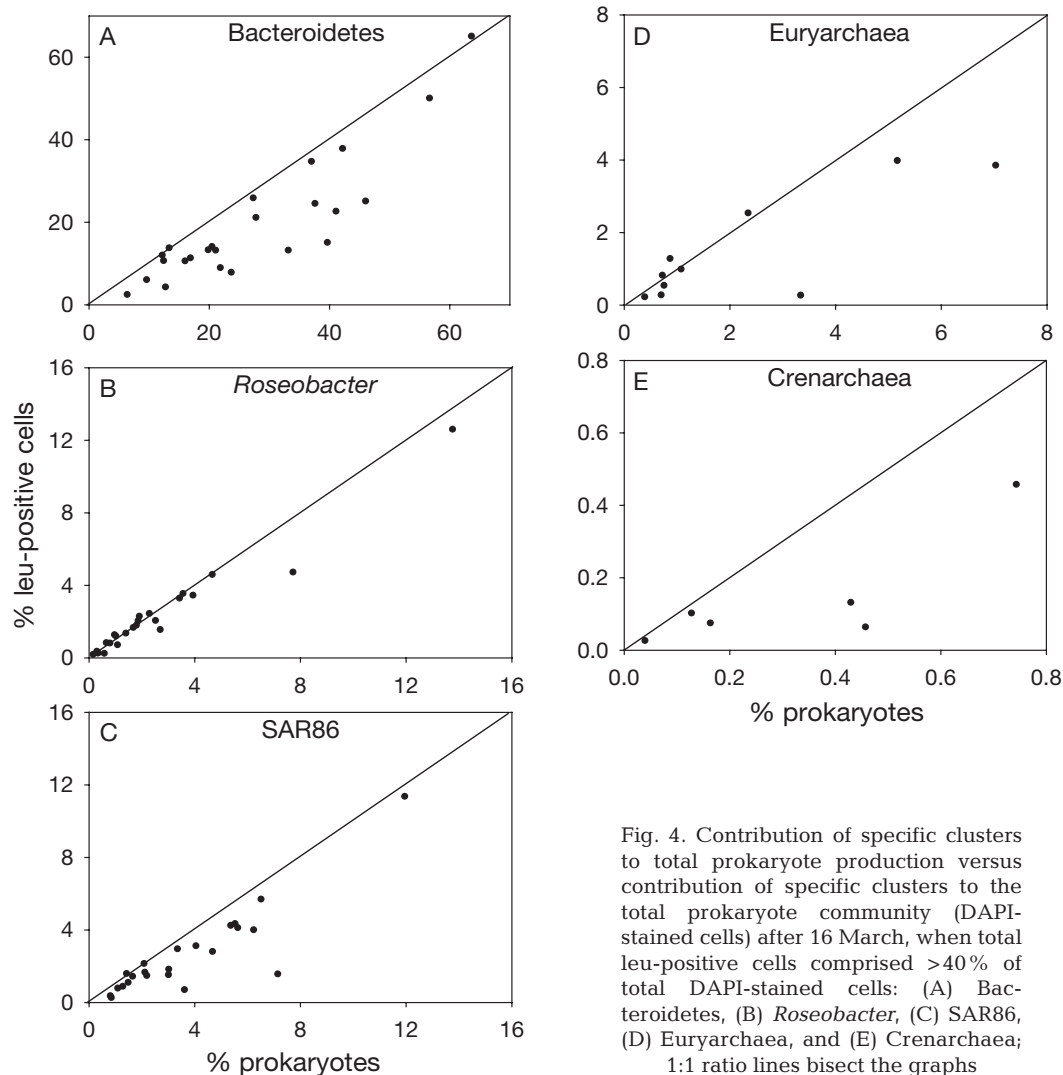


Fig. 4. Contribution of specific clusters to total prokaryote production versus contribution of specific clusters to the total prokaryote community (DAPI-stained cells) after 16 March, when total leu-positive cells comprised >40% of total DAPI-stained cells: (A) Bacteroidetes, (B) *Roseobacter*, (C) SAR86, (D) Euryarchaea, and (E) Crenarchaea; 1:1 ratio lines bisect the graphs

teroidetes comprised on average 27% of the bacterial community (range 5 to 63%). During early spring (until 1 April), which was the onset of the *Phaeocystis globosa* bloom, the Bacteroidetes cluster comprised on average 17% (range 6 to 33%) of the bacterial community (Fig. 3A). During the subsequent decay of the spring phytoplankton bloom (1 April to 15 May), the contribution of Bacteroidetes to the bacterial community increased until reaching a maximum contribution of 63% in mid May (Fig. 3A), when bacterial abundance was also highest (see Fig. 1A). After the spring phytoplankton bloom collapsed, the contribution of Bacteroidetes remained high (mean 26%, range 9 to 57%; Fig. 3A). A high contribution of Bacteroidetes (57%) on 1 August coincided with a peak in bacterial abundance, similar to the peak in bacterial abundance during the decline of the spring phytoplankton bloom. Thus, members of the Bacteroidetes were apparently dominating bacterioplankton blooms (Fig. 1A cf. Fig. 3A).

The contribution of the *Roseobacter* cluster to the bacterial community over the whole study period was on average 2% (range 0 to 13%). During early spring (until 1 April), *Roseobacter* contributed on average 0.5% (range 0 to 2%) to the bacterial community (Fig. 3A). During the subsequent decay of the spring phytoplankton bloom (1 April to 15 May), the contribution increased slightly during the second half of April (7% on 26 April), but nevertheless remained low (mean 3%, range 0 to 7%; Fig. 3A). After the decay of the spring bloom, the contribution of *Roseobacter* to the total prokaryotic community was <0.5% during June and July. During August, the contribution of *Roseobacter* increased to 13% on 16 August (August mean = 4%).

The contribution of the SAR86 cluster to bacterial abundance over the whole study period was on average 4% (range 0 to 18%). In early spring (until 1 April), the SAR86 cluster contributed on average 1% (range 0

to 2%) to the bacterial community (Fig. 3A). During the subsequent decay of the spring phytoplankton bloom (1 April to 15 May), the contribution of SAR86 remained low (mean 2%, range 0 to 7%; Fig. 3A). After the disappearance of the spring bloom, the contribution of SAR86 to the bacterial community was on average 5% (range 0 to 18%) over the remaining spring and summer season.

Although not abundant, *Roseobacter* showed the highest fraction of leucine-positive cells of the studied clusters over the entire study period (mean 52%, range 29 to 82%; Fig. 3C). The fraction of active Bacteroidetes was low at the beginning of March (1%) and ranged from 15 to 67% (mean 40%) over the rest of the studied period. High fractions of active Bacteroidetes coincided with high prokaryotic and Bacteroidetes abundance. The fraction of active SAR86 cells ranged from 12 to 71% (mean 40%) over the study period. For the period after 16 March, when leucine-positive cells comprised more than 10% of the DAPI-stained cells, the *Roseobacter* cluster contributed proportionally to the abundance of leucine-positive prokaryotic cells (paired *t*-test, $p = 0.05$; Fig. 4B). This suggests that the contribution of *Roseobacter* to the overall bacterial activity is generally equivalent to what would be predicted based on their abundance. The contribution of both Bacteroidetes and the SAR86 cluster to leucine-positive prokaryotic cells was significantly lower than their contribution to prokaryotic abundance (paired *t*-test, $p < 0.0001$; Fig. 4A,C). While Euryarchaea contributed roughly equally to prokaryotic abundance and leucine-positive prokaryotic cells (paired *t*-test, $p = 0.14$; Fig. 4D), the fraction of Crenarchaea taking up leucine was significantly lower than their contribution to total prokaryotic abundance (paired *t*-test, $p < 0.05$; Fig. 4E).

DISCUSSION

Both Crenarchaea and Euryarchaea comprised only a minor fraction of the prokaryotic community in the coastal North Sea during the spring and summer season. Cultivation-independent techniques have revealed the presence of Archaea in virtually every ecosystem investigated (Olsen 1994, Stein & Simon 1996), including coastal seas like the North Sea (Pernthaler et al. 2002b, Wuchter et al. 2003, 2006). In addition, recent evidence suggests that Crenarchaea may also play a role as ammonia oxidizers (Francis et al. 2005, Könneke et al. 2005) at the study site in the coastal North Sea during fall and winter (Wuchter et al. 2006). However, use of the quantitative MICROCARD-FISH shows that although both Crenarchaea and Euryarchaea are present and taking up leucine

throughout the spring and summer season, neither one of these 2 major groups of Archaea is likely to be a major player in the biogeochemical cycles at the study site during spring and summer.

Bacteria belonging to the Bacteroidetes were abundant throughout spring and summer, and dominated the peak in prokaryote abundance following the *Phaeocystis globosa* spring bloom (Fig. 3A). Generally, Bacteroidetes are abundant in coastal and temperate surface waters (Glöckner et al. 1999, Eilers et al. 2001, Kirchman 2002, Cottrell & Kirchman 2003). Previous studies have shown that members of the Bacteroidetes cluster respond to phytoplankton blooms with an increase in abundance (Riemann et al. 2000, Pinhassi et al. 2004). Therefore, members of this group are thought to be the main consumers of high-molecular weight dissolved organic matter (DOM) released during the wane of phytoplankton blooms (Kirchman 2002). *Phaeocystis globosa* is known to produce large amounts of carbohydrates during spring blooms. Their colony matrix consists of complex mucopolysaccharides released into the environment during the wane of the bloom (Janse et al. 1996). Degradation of such complex carbohydrates often requires specific extracellular enzymes. Members of the Bacteroidetes are chemo-organotrophic, known for their capacity to degrade complex carbohydrates such as pectin, cellulose and chitin (Reichenbach & Dworkin 1991, Cottrell & Kirchman 2000, Kirchman 2002). Members of this group were detected in microbial enrichments that were degrading *Phaeocystis* mucopolysaccharides (Janse et al. 2000).

The *Roseobacter* clade has been frequently found to be associated with high primary production and algal blooms (Zubkov et al. 2001, Pinhassi et al. 2004, Alonso & Pernthaler 2005, 2006a). A member of the *Roseobacter* clade was also observed in the bacterial community degrading *Phaeocystis* mucopolysaccharides (Janse et al. 2000). In addition, *Roseobacter* take up DMSP (Zubkov et al. 2001, Malmstrom et al. 2004), and DMSP is released during the collapse of *Phaeocystis globosa* blooms (Liss et al. 1994). Yet, in this study, members of the *Roseobacter* clade formed only a minor part of the bacterial community during the collapse of the *P. globosa* spring bloom (Fig. 3A). Brussaard et al. (2005) observed repression of a member of the *Roseobacter* clade during the collapse of a *P. globosa* bloom in a mesocosm. They argued that the disappearance of the *Roseobacter* member was related to the predominance of disintegrating colonies and accompanying transparent exopolymer particles (TEP), because *Roseobacter* remained abundant in a mesocosm in which only a small number of colonies disintegrated. In agreement, we found that members of the *Roseobacter* clade constituted up to 18% of the bacterial community in August (Fig. 3A) when *P. globosa* was present, but did

not dominate the phytoplankton community. We speculate that during this period, the number of disintegrating colonies was lower than that during the spring bloom. Although constituting only a relatively small fraction of the bacterial community, members of the *Roseobacter* clade were the most active group investigated based on the percentage of cells taking up leucine throughout the spring and summer season (Fig. 3C).

Members of the SAR86 cluster are cosmopolitan members of the Gammaproteobacteria, and are abundant in the free-living communities of marine bacterioplankton (Mullins et al. 1995, Acinas et al. 1999, Eilers et al. 2000). We observed a low, but persistent fraction of SAR86 throughout the season.

Using oligonucleotide probes to target Bacteroidetes, *Roseobacter* and the SAR86 cluster, we recovered a high percentage of Bacteria during periods of high prokaryotic abundance and activity after the phytoplankton bloom and during summer (mean 40%, range 28 to 64%). Recovery was lower in early spring and June, when prokaryotic abundance and activity were lower (mean 17%, range 8 to 34%). In addition to the groups targeted in this study, the SAR11 cluster of the Alphaproteobacteria comprises a major fraction of Bacteria in the coastal North Sea (Alonso & Pernthaler 2006a). Alonso & Pernthaler's (2006a) study was conducted in the coastal pelagic North Sea, off the coast of Helgoland (54° 11' N, 7° 54' E), thus further offshore than our study. Nevertheless, SAR11 could also be an abundant cluster at our site. Alonso & Pernthaler (2006a,b) studied the contribution of different bacterial groups to glucose uptake in March as well as in May during the development of a *Phaeocystis* sp. bloom. Similar to our results, *Roseobacter* formed a small, but active part of the microbial population that was taking up glucose and leucine at both times. The fraction of SAR11 cells taking up glucose was always low; however, because of their high abundance, their contribution to total glucose uptake by the prokaryotic community was significant (Alonso & Pernthaler 2006a). In contrast to our results, both the abundance and fraction of active Bacteroidetes cells in the coastal pelagic North Sea were always low (<25%). This may indicate spatial heterogeneity in the composition of the prokaryotic community in the North Sea. However, it should be noted that the peak of the *Phaeocystis* sp. bloom occurred approximately 3 wk later at the Helgoland location than at our study site. In our study, the strongest increase in Bacteroidetes abundance and activity occurred after the disappearance of the *P. globosa* bloom, and thus might have occurred at the Helgoland site as well.

The contribution of the *Roseobacter* clade and the Euryarchaea to the leucine-positive cells was propor-

tional to their contribution to prokaryotic abundance (Fig. 4B,D). The other groups investigated all contributed less to the fraction of leucine-positive cells than predicted from their contribution to prokaryotic abundance (Fig. 4A,C,E). However, the activity level on a single-cell basis also influences the contribution of a specific cluster to the activity of the prokaryote community. Some individual cells or groups might be more active than others, thereby affecting their contribution to total leucine incorporation by the prokaryotic community. In addition, the contribution of different groups may depend on the substrate concentration (Alonso & Pernthaler 2006a,b). In addition, the contribution of specific clusters to substrate-positive cells may vary for different substrates (Malmstrom et al. 2005). These differences were not taken into account in this study.

CONCLUSIONS

Both Crenarchaea and Euryarchaea were present in the coastal North Sea throughout the spring and summer season, but were never abundant. Although Archaea were taking up leucine, the percentage of cells taking up leucine was disproportionately lower than their corresponding contribution to total prokaryotic abundance. Thus, we conclude that Archaea do not play a major role in the biogeochemical cycles of the coastal North Sea during spring and summer.

Bacteria belonging to the Bacteroidetes were dominant during the spring and summer season, particularly during the collapse of *Phaeocystis globosa* spring bloom. Certain taxa of this cluster are likely to be involved in the degradation of mucopolysaccharides, as previously reported for mucopolysaccharide-degrading bacterial communities. Members of the *Roseobacter* clade formed a minor part of the bacterioplankton community during the spring and summer season, but generally exhibited the highest percentage of leucine-positive cells.

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