The improved MICRO-CARD-FISH method of Teira et al. (2004) was applied to study the dynamics in abundance and activity of prokaryotes in the coastal North Sea over the course of a spring and summer season. During spring, a phytoplankton bloom of the Prymnesiophyte *Phaeocystis globosa* dominated the primary production. Both cren- and euryarchaea were detected throughout the study period. Their combined contribution was on average 2% of the total prokaryotic community and their abundance was always less than $2.8 \times 10^5$ cells ml$^{-1}$. 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 wax and wane of the *P. globosa* bloom. At the senescent stage of the bloom, Bacteroidetes comprised up to 63% of the bacterial community. The abundance of both members of the *Roseobacter*-clade and the SAR-86 cluster was low during this period (on average 2 % each), but increased during August. Members of the *Roseobacter*-clade were generally more active than the other two groups; on average 51% of the *Roseobacter*, 38% of Bacteroidetes and 39% of the SAR-86 cluster were taking up leucine. The highest percentages of active cells were detected during and shortly after the decline of the *P. globosa* bloom, and during August. We conclude that due to their high abundance, members of the Bacteroidetes are likely to play a role in the degradation on the carbohydrate rich, high molecular weight, dissolved organic matter that is released during the *P. globosa* bloom.
INTRODUCTION

Coastal areas cover only approximately 7% of the global ocean surface, but contribute 30% to the global marine primary production (Gattuso et al. 1998). In these waters a significant part of the local annual primary production is produced during phytoplankton spring blooms, which typically occur when high nutrient concentrations coincide with increased solar irradiance. In the temperate southern North Sea, *Phaeocystis globosa* (Prymnesiophyceae) typically dominates the algal community in spring, and forms dense blooms with cell abundances as high as $10^8$ l$^{-1}$ (Cadée and 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 are the main producers of labile dissolved organic carbon (DOC) that can directly be utilized by heterotrophic prokaryotes for respiration and growth (Carlson et al. 1998). During growth, phytoplankton release part of their photoassimilated carbon as DOC (Teira et al. 2001). In addition, DOC is released by “sloppy feeding” by planktonic grazers on phytoplankton (Møller et al. 2003). In the case of *Phaeocystis* blooms, the collapse can be sudden, with cell lysis being an important source of mortality (Brussaard et al. 1995, 1996, 2005b). 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).

The importance of bacteria in marine biogeochemical cycles is now well established, and since the development of culture independent detection techniques based on the 16S rDNA gene detection, phylotypes of archaea have been detected throughout the marine environment. The two major groups of archaea, crenarchaea and euryarchaea, were 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 marches (Munson et al. 1997). In the open ocean crenarchaea and euryarchaea, may account for one-third of the prokaryote cells (Karner et al. 2001) and may play a significant role in the oceanic carbon cycle (Herndl et al. 2005). In addition, crenarchaea may play an important role as ammonia oxidizers (Könneke et al. 2005). The role of archaea in coastal biogeochemical cycles is however unknown.

Studies of marine bacterioplankton diversity have shown that essentially three clusters of bacteria dominate marine bacterioplankton communities. Bacteria belonging to different phylogenetic groups of the *Cytophaga-Flavobacteria-Bacteroidetes*, now termed Bacteroidetes, the *Roseobacter* clade of the α-proteobacteria, and the SAR86 cluster of the gamma γ-proteobacteria are present in coastal sea such as the North Sea (Eilers et al. 2000; Alonso and Pernthaler 2005), the Mediterranean (Acinas et al. 1999), but also dominate the open oceans such as the Pacific and Atlantic (Mullins et al. 1995). Phytoplankton blooms likely have an impact on the bacterioplankton community composition (Arrieta and Herndl 2002; Pinhassi et al. 2004), but few data exist on the response of members of the three dominant clusters. Since most marine bacteria that are abundant in the marine environment still resist cultivation in the laboratory (Eilers et al. 2000), little is known about their
physiology. Combining the use of oligonucleotide probes in fluorescence in-situ hybridization (FISH) with microautoradiography (Micro-FISH), allows determination of the abundance of specific groups of bacteria and archaea at a given time and site, and determination of their specific uptake of a given substrate. The recently developed protocol for catalyzed reporter deposition micro-FISH method (MICRO-CARD-FISH) (Teira et al. 2004), allows the use of oligonucleotide probes labeled with horseradish peroxidase, resulting in an enhanced sensitivity and a higher detection rate of archaea. Using this technique investigated the dynamics of archaea in the highly productive coastal surface waters of the coastal North Sea. In addition, we determined the group-specific dynamics in abundance and activity of prokaryotes over the spring and summer season, thus during the wax and wane of a Phaeocystis bloom.

MATERIALS AND METHODS

Sampling, phyto- and prokaryotic plankton analysis

Coastal North Sea surface water samples were collected with an acid-rinsed bucket from Marsdiep tidal inlet, south of the island Texel, The Netherlands, from March until August 2004. Samples were collected at high tide, twice a week during the spring bloom and weekly during the summer.

Phytoplankton abundance and species composition was determined on Lugol preserved samples under a Zeiss inverted microscope using 5 ml counting chambers as described by Philippart et al. (2006). Prokaryote abundance was counted in unfiltered samples and in samples filtered through polycarbonate filters (3 µm; Millipore). Samples (1 ml) each were fixed in glutaraldehyde (0.5% final concentration), incubated for 30 min at 4°C in the dark, flash frozen in liquid nitrogen and stored at –80°C until analysis as described by Brussaard (2004). After thawing, the prokaryote samples were stained for 15 min in the dark with SYBR Green I solution (Molecular Probes, at 2.5 µM final concentration) and subsequently enumerated on a FACScalibur (Becton Dickinson) flow cytometer (FCM) with a laser emitting at 488 nm wavelength. Samples were run at high speed for (57 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 500 events sec⁻¹. Cell concentrations were determined from the flow rate, calculated by weighing a sample before and after a 10 to 15 min run in the FCM. Fluorescent 1 µm latex beads (10⁵ beads ml⁻¹) were added to the prokaryote samples as an internal standard (Polyscience, Inc., Europe). Prokaryotes were detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1) as described elsewhere (Gasol and Del Giorgio 2000; Brussaard 2004).

For MICRO-CARD-FISH analyses, water samples were filtered through polycarbonate filters (3 µm; Millipore) and 2 to 5 ml of the filtrate was spiked with ³H-leucine (specific activity 160 Ci mmol⁻¹; 10 nm final concentration; Amersham) and incubated in the dark at in-situ temperatures for 3 to 9 h. Controls consisted of samples killed with formaldehyde (final concentration 2%) prior to addition of ³H-leucine. Incubations were terminated by adding formaldehyde (final concentration 2%), and the samples were fixed at 4°C in the dark for 12 to
18 h. Thereafter, samples were filtered through polycarbonate filters (0.2 µm, Millipore) supported by cellulose acetate filters (0.45 µm, Millipore), washed twice with Milli-Q water, dried, and stored at -20°C until further processing.

For chlorophyll a (Chl a) analysis, water samples were filtered onto Whatman GF/F filters, extracted in 90% (v/v) acetone and analysed fluorometrically according to Riegm an et al. (1993).

**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 to be 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 to be hybridized with archaeal probes were incubated in a buffer containing proteinase K (1,844 U mg⁻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. Next, 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 Eub338 (bacteria), Non 338 (negative control), CF319 (most of the phylotypes belonging to the Bacteroidetes phylum, formerly known as the CFB-cluster) (Amann et al. 1995), Ros537 (members of the *Roseobacter-Sulfitobacter-Silicibacter* clade) (Eilers et al. 2001a), SAR86 (SAR 86 cluster of γ-proteobacteria) (Eilers et al. 2000), Cren537 (marine crenarchaea), and Eury806 (marine euryarchaea) (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, Mannheim, Germany], 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 7.4-7.6], 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 NaH₂PO₄, 8 mM Na₂HPO₄ [pH 7.6], containing 0.05% Triton X-100 (PBS-T), in the dark, at room temperature for 15 min. Next, 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] H₂O₂ in PBS) containing 5 µl of tyramide-Alexa488 (1 mg/ml) and incubated at 37°C for 30 min. After amplification, filter sections were washed in PBS-T at room temperature, for 15 min, Milli-Q water and 95% dehydrated in ethanol. Finally, filter sections were air dried and stored at -20°C until further processing.
[Figure 1. Trends in phytoplankton and prokaryotic numbers during the 2004 spring and summer season. Chl a concentration, total and free-living prokaryotic cell numbers (A), Diatom and *P. globosa* cell numbers (B), and *P. globosa* colonial and flagellate cell numbers (C).]
Microautoradiography

Microautoradiography was performed in a dark room, kept at 15°C as described by 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 photographic emulsion that was melted at 43°C for 30 min. The slides were dried on ice-cold aluminum plates for 5 min. and 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 and the cells remaining in the photographic emulsion were counterstained with DAPI mix (5.5 parts Citifluor [Citifluor, Ltd.], 1 part Vectashield [Vector Laboratories, Inc.], 0.5 parts PBS with DAPI at a final concentration of 1 µg 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 using the transmission mode of the microscope (Carman 1993). More than 200 positive cells, or more than 800 DAPI cells were counted per sample.

RESULTS

Temporal dynamics of phyto- and prokaryotic plankton

In the spring of 2004 the phytoplankton community in the Marsdiep was dominated by a *P. globosa* colony bloom, reaching densities of densities of 33 x 10⁶ cells l⁻¹ (Figure 1B). At this time *P. globosa* cells constituted 89% of the phytoplankton cells. Chl *a* concentrations reached 32.9 mg m⁻³ (Figure 1A). From 22 April onward the *P. globosa* bloom collapsed and flagellate *P. globosa* cells developed (Figure 1C). The increase in flagellate cells coincided with the increase in diatoms. During the development of the *P. globosa* bloom the number of prokaryotes increased 4-fold, but declined as the bloom reached its peak. During the collapse of the *P. globosa* bloom the total prokaryotic abundance increased again with a maximum abundance of 7.6 x 10⁹ cells l⁻¹ (Figure 1A). After the spring bloom, Chl *a* concentrations remained rather constant at an average of 5 mg m⁻³ and the total prokaryotic abundance varied between 2.1 and 7.6 x 10⁹ cells l⁻¹, with a peak in early August (Figure 1A). On average, the free-living prokaryotes (<3 µm fraction) accounted for 93% of the total prokaryotic community.

Archaeal versus bacterial dynamics

Over the whole spring and summer period bacteria accounted for the bulk of the prokaryote cells (Figure 2A). Although both crenarchaea and euryarchaea were detected throughout the spring and summer season, their combined contribution was on average 2% of the total prokaryotic community and their abundance was never higher then 2.8 x 10⁵ cells ml⁻¹. Euryarchaea were generally more abundant then crenarchaea (Figure 2A).
Figure 2. Percentages of bacteria, crenarchaea and euryarchaea normalized to the free-living prokaryotic cells (A), percentage of bacteria taking up leucine normalized to the total number of bacteria (B), and the percentage of crenarchaea and euryarchaea taking up leucine normalized to the total number crenarchaea and euryarchaea (C) during the spring and summer season of 2004. The percentage of archaea taking up leucine was only included if more than 50 cells could be detected.
The percentage prokaryotic cells taking up leucine was low at the beginning of spring with 8\% of DAPI-stained cells. This percentage increased gradually during the course of the spring, reaching 66\% on 13 April. During the rest of the spring and summer season, the percentage of DAPI-stained cells taking up leucine ranged between 53 and 72\%, with an average of 61\%. The percentage of bacteria taking up leucine followed a similar trend (Figure 2B). On 15 March, only 2\% of the bacterial cells were taking up leucine. This percentage increased during early spring reaching a maximum of 73\% on 13 April. During the rest of the spring and summer season, the percentage of bacterial cells taking up leucine remained high, between 42 and 73\%, with an average of 63\%. The percentage of euryarchaea taking up leucine was variable over the whole spring and summer season varying between 6 and 61\%, with an average of 35\% (Figure 2C). The percentage of active euryarchaea was always lower than the percentage of active *Bacteria*, except for 15 March. No active crenarchaea were detected on 15 March, whereas during the rest of spring and summer the percentage of crenarchaea taking up leucine varied between 6 and 35\%, with an average of 21\% (Figure 2C).

**Dynamics of Bacteroidetes, Roseobacter and SAR-86 cluster.**
The percentage of bacteria hybridizing with any of the three cluster specific probes varied during the spring and summer season between 8 and 64\%. During the build-up of the *Phaeocystis* bloom, bacteria from the Bacteroidetes-cluster comprised a substantial part of the bacteria (between 6 and 33\%, average 17\%; Figure 3A). Bacteria from both the SAR86 and the *Roseobacter* cluster comprised a small part of the bacterial community during this period, SAR86 at most 6\% with an average of 2\%, and *Roseobacter* at most 2\% with an average of 1\%. Although not abundant, *Roseobacter* was the most active group during this period (Figure 3C). On average, 45\% of *Roseobacter* cells were taking up leucine (Figure 3C), which is similar to the average of 44\% of total bacterial cells taking up leucine. Both the Bacteroidetes- and SAR86 cluster were less active, with 22 and 36\%, respectively, taking up leucine (Figure 3B and D).

During the collapse of the *Phaeocystis* bloom bacteria from the Bacteroidetes-cluster comprised a substantial fraction of the bacteria, with a maximum contribution of 63\% (average of 37\%; Figure 3A). The contribution of the SAR86 and *Roseobacter*-cluster remained low, at most 7\% with an average of 2\% for both clusters (Figure 3A). During this period, *Roseobacter* was the most active group, with on average 55\% of the cells taking up leucine (Figure 3C), which was similar to the average of 61\% of bacterial cells taking up leucine. The percentage of cells belonging to the Bacteroidetes-cluster taking up leucine increased to 38\% (Figure 3B), whereas the percentage of SAR86 cells taking up leucine decreased slightly to an average of 28\% (Figure 3D).

From 14 May onward, after disappearance of the spring bloom, the contribution of bacteria from the Bacteroidetes-cluster to the total bacterial abundance varied between 9 and 57\% (Figure 3A). During the first part of June the contribution was low, increasing during July to a maximum of 57\% on 28 July. During August, the contribution decreased again. The contribution of the SAR86 cluster varied between 1 and 6\% during June and July, and increased during August, reaching a maximum of 18\% at 30 August. The contribution of the
Figure 3. Percentages of the Bacteroidetes, *Roseobacter*-clade, and SAR 86-cluster normalized to the bacteria (A) and the percentage of Bacteroidetes, *Roseobacter*-clade, and SAR 86-cluster taking up leucine normalized to the total number of Bacteroidetes, *Roseobacter*-clade, and SAR 86-cluster (B, C, D).
**Chapter 5**

*Roseobacter* cluster was low during June and July (less than 0.5%), but increased during August, reaching a maximum of 13% at 16 August (Figure 3A). After the spring bloom, members of the *Roseobacter*-clade remained the most active group, on average 49% of the cells were taking up leucine, increasing to 61% during August. This was slightly lower than the percentage of bacteria taking up leucine; on average 59% after the spring bloom, increasing to 66% during August. The percentage of cells belonging to the Bacteroidetes- and SAR 86-cluster taking up leucine were similar with 42% and 49%, respectively, following the spring bloom, and 50% and 53%, respectively, during August.

**DISCUSSION**

We applied the improved MICRO-CARD-FISH method of Teira et al. (2004) to samples from the coastal North Sea, in order to study the dynamics and activity of the prokaryote community during the wax and wane of a *Phaeocystis* bloom. A crucial step in this method is the transfer of the prokaryotic cells hybridized with the HRP-oligonucleotide probes from the polycarbonate filter to the glass slide coated with photographic emulsion. During this step up to 50% of the cells remained on the filter during this study and thus escaped analysis. When examining the polycarbonate filters prior to transfer to the glass slides we found that >96% of DAPI cells hybridized with either the bacterial, euryarchaeotal, or crenarchaeotal probe (n=5, results not shown), whereas upon examination of the glass slides this percentage varied between 39 and 76%. Comparing the results of the hybridizations on the filters with those on the glass slides, we did not find a bias, i.e. none of the studied clusters were preferentially transferred to the glass slide. Therefore we used the sum of the cells hybridized with the bacterial, euryarchaeotal, or crenarchaeotal probe as the total abundance of prokaryotic cells in Fig. 2. A high percentage of DAPI-stained cells hybridizing with oligonucleotide probes was also reported for North Sea samples by Pernthaler et al. (2002a). This indicates a low percentage of dead cells, which was confirmed by a similar percentage of active DAPI-stained cells and bacterial cells taking up leucine (results not shown).

In our study we analyzed the free-living prokaryotic community. Although they formed generally the bulk of the total prokaryotic community, it should be noted here that there may be major differences in community structure and activity of particle-attached versus free-living bacteria (Becquevort et al. 1998; Moeseneder et al. 2001). Therefore, particle attached bacteria will likely have an impact on the dynamics of the prokaryotic community which we did not take into account in this study.

Cren- as well as euryarchaeae formed a minor fraction of the prokaryotic community during the spring and summer season. Cultivation independent techniques have revealed the presence of archaea in virtually every ecosystem investigated (Olsen 1994; Stein and Simon 1996) including coastal seas like the North Sea (Pernthaler et al. 2002b; Wuchter et al. 2003). Few studies, however, used a quantitative approach to define the contribution of archaea to biogeochemical cycles, and even fewer have studied the *in-situ* uptake of substrates by archaea (Ouverney and Fuhrman 2001; Herndl et al. 2005). A quantitative study using rRNA targeting fluorescent probes showed that in the open ocean (Pacific, Hawaii) euryarchaeae were found in
low numbers throughout the water column, whereas crenarchaea were found to be as abundant as bacteria at depths below 1000 m (Karner et al. 2001). Herndl et al. (2005), using the same MICRO-CARD-FISH technique as applied in this study, found that archaea are actively taking up substrate (amino acids and bicarbonate) in deep waters of the Atlantic Ocean. Therefore, archaea may play a significant role in the oceanic carbon cycle. In addition, recent evidence suggests that crenarchaea may play a role as ammonia oxidizers at the study site in the coastal North Sea in the fall and winter (Wuchter et al. submitted), as indicated by the close correlation between crenarchaeotal abundance and the copy number of the amoA gene as determined by quantitative PCR corroborating the findings of Köneke et al. (2005). Our results, however, show that although cren- and euryarchaea are present and taking up leucine throughout the spring and summer season, neither one of these two 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 the spring and summer season and became the dominant group following the P. globosa spring bloom. Generally, Bacteroidetes are abundant in seawater (Glöckner et al. 1999; Eilers et al. 2001b; Kirchman 2002; Cottrell and Kirchman 2003), and previous studies have also shown a response to phytoplankton blooms of diatoms (Riemann et al. 2000; Pinhassi et al. 2004) and P. globosa (et al. 2005b). Therefore, members of this group are though to be the main consumers of high molecular weight DOM released during the wane of phytoplankton blooms (Kirchman 2002). P. globosa is known to produce large amounts of carbohydrates during spring blooms. Their colony matrix consists of complex mucopolysaccharides, that are released into the environment during the wane of a bloom (Janse et al. 1996). Degradation of such complex carbohydrates often requires specific extracellular enzymes, and therefore bacteria, or consortia of bacteria especially adapted to these complex biopolymers will develop. Members of the Bacteroidetes are chemo-organotrophic, and known for their capacity to degrade complex carbohydrates such as pectin, cellulose and chitin (Reichenbach and Dworkin 1991; Cottrell and Kirchman 2000; Kirchman 2002). Members of this group were detected in microbial enrichments degrading Phaeocystis mucopolysaccharides (Janse et al. 2000). In a P. globosa dominated mesocosm, a member of the Flexibacter, belonging to the Bacteroidetes, was disappearing as disintegrated colonies became abundant (Brussaard et al. 2005b). This illustrates the diversity within the group of Bacteroidetes that hybridizes with our probe.

A large fraction of bacteria belonging to the Roseobacter clade have been associated with high primary production and algal blooms (Zubkov et al. 2001; Pinhassi et al. 2004; Alonso et al. 2005). In addition, a member of the Roseobacter clade was observed in the bacterial community degrading mucopolysaccharides that was mentioned above (Janse et al. 2000). Yet, during and after the spring bloom, only a minor fraction of prokaryotes were affiliated to the Roseobacter clade. Roseobacter are regarded as key participants in dimethylsulfoxonium propionate (DMSP) assimilation (Malmstrom et al. 2004; Zubkov et al. 2001). DMSP is released during the collapse of P. globosa blooms (Liss et al. 1994), yet members of the Roseobacter clade formed only a minor part of the bacterial community during the collapse of the P. globosa spring bloom. Brussaard et al. (2005b) 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), since the member stayed present in a mesocosm where only a low amount of colonies disintegrated. In concordance with this, we found members of the *Roseobacter* clade constituting up to 18% of the bacterial community in August, coinciding with another small *P. globosa* bloom. We speculate that during this bloom the number of disintegrating colonies was lower than during the spring bloom. Although forming a low percentage of the bacterial community, members of the *Roseobacter* clade were the most active clade based on the percentage of cells taking up leucine throughout the spring and summer season.

Members of SAR86 cluster are cosmopolitan members of the gamma proteobacteria, and are abundant in the free-living communities of marine bacterioplankton (Mullins et al. 1995; Acinas et al. 1999; Eilers et al. 2000). We found low, but consistent percentages of cells throughout the season.

**Conclusions**

Both crenarchaea and euryarchaea were present in the coastal North Sea throughout the spring and summer season, but never abundant. Archaea were taking up leucine, although the percentage of cells taking up leucine was lower than the percentage of total bacterial cells. We conclude that archaea do not play a major role in biogeochemical cycles of the coastal North Sea during spring and summer.

Bacteria belonging to the *Bacteroidetes* were dominant during the spring and summer season, but especially during the collapse of *P. globosa* spring bloom. Probably certain taxa of this cluster are involved in degradation of mucopolysaccharides as previously reported in mucopolysaccharide degrading bacterial communities. Members of the *Roseobacter* clade were forming a minor part of the bacterioplankton community during the spring and summer season, but generally exhibited the highest percentages of cells taking up leucine.

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