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Research Article

RESEARCH ARTICLE

Annual dynamics of North Sea bacterioplankton: seasonal variability superimposes short-term variation

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One sentence summary: Bacterioplankton succession in response to phytoplankton blooms is indirectly affected by temperature, and thus temperature-dependent guilds are formed during spring and summer phytoplankton blooms.
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ABSTRACT

The dynamics of coastal marine microbial communities are driven by seasonally changing abiotic and biotic factors as well as by rapidly occurring short-term changes such as river fresh water influxes or phytoplankton blooms. We examined the variability of the free-living bacterioplankton at Helgoland Roads (German Bight, North Sea) over a period of one year with high temporal and taxonomic resolution to reveal variation patterns and main influencing factors. 16S rRNA gene tag sequencing of the bacterioplankton community hints at annual recurrence and resilience of few main taxa belonging to *Alphaproteobacteria, Betaproteobacteria, Flavobacteriia, Acidimicrobiia* and *Thermoplasmata*. Multiple regression analyses with various environmental factors revealed changes in water current patterns and resulting phytoplankton blooms as the main driving factors for short-term variation and temperature as the overlying factor for seasonal variation. Comparison of bacterioplankton successions during spring and summer phytoplankton blooms revealed the same dominating *Flavobacteriia* operational taxonomic units (OTUs) but shifts in *Roseobacter* related OTUs (*Alphaproteobacteria*) and SAR92 clade members (*Gammaproteobacteria*). Network analysis suggests that during spring and summer phytoplankton blooms temperature-dependent guilds are formed. In conclusion, our data imply that short-term bacterioplankton successions in response to phytoplankton blooms are indirectly affected by temperature, which is a major niche-defining factor in the German Bight.

Keywords: 16S rRNA tag sequencing; North Sea; bacterioplankton community composition; bacterioplankton succession; phytoplankton bloom

INTRODUCTION

Studies that examined the bacterioplankton response to changing environmental factors in diverse marine environments identified a range of oceanographic, physicochemical and biotic factors that influence variations in bacterioplankton community composition (BCC) (Fuhrman *et al.* 2006; Gilbert *et al.* 2009;

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Fortunato *et al.* 2012). In particular, dissolved and particulate organic matter released by phytoplankton strongly shapes the BCC; however, it has been suggested that in highly dynamic systems such as estuaries of continental shelf seas, the influence of primary producers on microbial dynamics is less important compared with that of abiotic factors (Kirchman *et al.* 2005; Teira *et al.* 2008). Concerning the variability of environmental conditions in different oceanic regions, one would expect individual combinations of environmental factors that are driving changes in the bacterial community composition at different sites.

The North Sea is a semi-enclosed continental shelf sea. Especially its southeastern region, the German Bight, is highly influenced by the runoff from the rivers Elbe and Weser and thereby constantly supplied with nutrients, making it a very productive area. The mixing of fresh and marine water typically leads to high spatial variability with respect to environmental parameters such as temperature, salinity, pH and organic loads (Atlas and Bartha 1987). Changes in these parameters, the biota and current patterns have been continuously monitored for more than five decades around Helgoland Island in the German Bight (54°11.3′ N, 7°54.0′ E), known as the Helgoland Roads time series (Wiltshire *et al.* 2008). This comprehensive long-term data set makes Helgoland Roads an optimal study site to investigate how environmental parameters shape bacterioplankton communities in coastal oceanic environments. The BCC in the German Bight has been well described, particularly during spring phytoplankton blooms using different molecular biological approaches like DGGE, RISA (Sapp *et al.* 2007), CARD-FISH (Alderkamp, Sintes and Herndl 2006) and 16S rRNA gene tag sequencing (Teeling *et al.* 2012; Wemheuer *et al.* 2014). Previous studies at Helgoland Roads also demonstrated seasonality of bacterioplankton communities driven by different environmental factors or phytoplankton abundances. However, these investigations were done with either limited temporal (Gilbert *et al.* 2009) or taxonomic resolution (Gerdts *et al.* 2004), and thus are lacking to uncover the complexity and diversity of the microbial community that has been described for other oceanic sites by high-throughput sequencing techniques (Fuhrman *et al.* 2006; Gilbert *et al.* 2012).

In this study, we examined the bacterioplankton community at Helgoland Roads at both high temporal and taxonomic resolution. To unravel whether the BCC is changing constantly throughout the year or if stable communities displace each other due to abrupt environmental changes, we assessed the BCC at Helgoland Roads on a weekly basis over a period of one year. To further elucidate these changes in community structure and define the succession of distinct dominating key taxa in different seasons and phytoplankton blooms, we used 16S rRNA gene tag sequencing of the free-living bacterioplankton fraction (0.2–3 μm). Multivariate statistics and network analyses were applied to determine which environmental parameters exert the strongest influences on the bacterial community and thus, shape the ecological niches that the defined key players occupy. The combination of high temporal and taxonomical resolution methods allowed a detailed understanding of possible controls of the BCC at Helgoland Roads and can serve as basis for future functional approaches.

MATERIALS AND METHODS

Sample collection and environmental parameter measurements

A total of 42 surface seawater samples (1 m depth) were collected weekly from 1 March 2012 to 28 February 2013 at Helgoland Roads (North Sea, Germany, 54°11.3′N, 7°54.0′E). The sampling site is located approximately 60 km off the German coastline. Total water depth varies between 7 and 10 m depending on the tides. Environmental data including dissolved organic carbon (DOC), dissolved inorganic nitrogen (DIN = NO_2^- + NO_3^- + NH_4^+), silicate (SiO_2), phosphate (PO_4^{3-}), salinity (S), water temperature (T), chlorophyll *a* (Chl *a*) and counts of phytoplankton groups (diatoms, dinoflagellates, flagellates, cilliates) were obtained in parallel as part of the Helgoland Roads time series (Wiltshire *et al.* 2008). Flagellate cell counts included also counts for heterotrophic nanoflagellates.

Hydrodynamic variability in the German Bight was assessed using current velocity fields from the model BSHcmod (Dick *et al.* 2001) operated by the Bundesamt für Seeschiffahrt und Hydrographie (BSH). First, current velocities of high temporal resolution (15 min) were averaged to obtain weekly mean vector (i.e. u, v) fields for the period March 2012–February 2013. Second, Empirical Orthogonal Function (EOF) analysis (von Storch and Zwiers 1999) was applied to identify dominant modes of spatially coherent variability in these current patterns. These EOFs reflect anomaly patterns with regard to the mean current conditions for the selected period with the first EOF covering the highest amount of explained variance in the simulated transport fields. The explained variance of the two leading EOFs is more than 85% (Fig. S2, Supporting Information). The corresponding principal components PC1 and PC2 (Fig. S3, Supporting Information) provide information about the sign and the amplitude of the EOFs as a function of time. The vector fields shown by the EOFs represent weighting factors (loadings) which are used for mapping each weekly mean current field to one data point of the corresponding principal component time series. See Callies and Scharfe (2015) for a comparable analysis based on the decadal scale.

Sample preparation and DNA extraction

Approximately 500 ml of each sample were subjected to fractionating filtration using 10, 3 and 0.2 μm pore size polycarbonate membrane filters (Millipore, Schwalbach, Germany), to separate particle-attached bacteria (3–10 μm) from free-living bacteria (0.2–3 μm). For cell counts, 4 ml of each filtrate obtained by filtration through 3 μm pore size filters were fixed with formaldehyde (1% [w/v] final concentration) for 1 h at room temperature and subsequently stored at –80 °C until further processing. Cell counts were determined as described in Krause *et al.* (2012) using an Accuri C6 flow cytometer (BD Accuri Cytometers, Ann Arbor, MI, USA). The threshold on FL1-H was set to 700. DNA of free-living bacteria was extracted from filters as described previously (Sapp *et al.* 2007). Briefly, cells were lysed using lysozyme/SDS, DNA was obtained by phenol–chloroform extraction and subsequent isopropanol precipitation. DNA concentration per sample was quantified using the Invitrogen (Carlsbad, CA, USA) Quant-iT PicoGreen ® dsDNA Reagent as per manufacturer's instructions.

16S rRNA V4 amplicon sequencing

16S rRNA gene tag sequencing was performed at the US Department of Energy Joint Genome Institute (JGI, Walnut Creek, CA, USA). Community DNA samples were sent to JGI in a 96-well plate for generation of 16S V4 rRNA amplicon libraries for Illumina sequencing. Sample preparation was performed on a PerkinElmer (Waltham, MA, USA) Sciclone NGS G3 Liquid Handling Workstation capable of processing 96 plate-based samples in parallel, utilizing 5 PRIME (Gaithersburg, MD

20878, USA) HotMasterMix amplification kit and custom amplification primers targeting the V4 region of the 16S rRNA gene using 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso *et al.* 2011). Primers also contained the Illumina adapter sequence and a unique barcode index. PCR reactions were set up in 75 μl total with 1x HotMasterMix (5 PRIME) with final concentrations of 0.4 μg μl^{-1} BSA and 0.2 μM of each primer. This total volume was split into triplicate 25 μl reactions for independent amplification and then pooled to reduce PCR bias. Prepared amplicon libraries were normalized and multiplexed into a single pool of amplicons per plate. 16S V4 rRNA amplicon library pools were quantified using the KAPA Biosystems (Wilmington, MA, USA) next-generation sequencing library qPCR kit and run on a Roche (San Francisco, CA, USA) LightCycler 480 real-time PCR instrument. The quantified pool was loaded on an Illumina (San Diego, CA, USA) MiSeq sequencer using 2 × 250 bp chemistry. The 16S rRNA gene tag sequences are available from the DOE-JGI website GOLD database (Project ID: Gp0056779) as part of the community sequencing project COGITO (Coastal Genomic & Taxonomic Observatory).

Raw paired-end reads were merged and filtered using scripts from illumina-utils (https://github.com/meren/illumina-utils) to retain only those sequences without mismatches in the overlapping region. These high-quality tags were processed through the SILVAngs pipeline (Quast *et al.* 2013). Sequences were dereplicated at 100% identity and then clustered within each individual sample at 98% similarity to reduce computational demands for classification. Representative sequences from operational taxonomic unit clusters (OTUs) were classified up to genus level against the SILVA v115 database using BLAST as described by Ionescu *et al.* (2012). Genus-level classifications were used in the final abundance matrix for downstream analyses. Each classification contained the sum of all sequences represented by OTUs with the same taxonomic path. For the purposes of this study, we were not interested in diversity calculated at the level of 98% clustered OTUs but rather used BLAST identities as our OTU. From this point on, we define these taxa as OTUs for simplicity. Therefore, in our study, OTU refers to a unique taxonomy and not a cluster of sequences defined by percent similarity. Eukaryotic, chloroplast and mitochondria-derived OTUs were removed from the resulting OTU matrix. To account for variation in total bacterial abundance over the year (Table S1, Supporting Information), OTU abundances were weighted by multiplying the relative OTU abundances with total bacterial cell counts according to Andersson, Riemann and Bertilsson (2009). We were interested in analysing which environmental parameters drive the ecologically most important microbial taxa. Since the most abundant microbial taxa are also thought to be the most active ones, contributing the most to biomass production and are most important in fluxes of dissolved organic matter (Cottrell and David 2003; Zhang *et al.* 2006), we decided to omit the 'rare biosphere' and focus on OTUs with an annual average relative abundance ≥0.1%. This 'trimmed data set' was used for further analyses.

Statistical analyses

To reveal patterns in bacterial community composition, principal coordinates analysis (PCoA) of all samples was carried out using Hellingers distance (D17; Legendre and Legendre 1998), which uses square-root-transformed relative abundances of sequence read numbers for distance matrix calculation. Analyses were carried out with the Primer v.6 software package (PRIMER-E, UK).

The relationship between environmental parameters and bacterial community structure was statistically analysed in SigmaPlot (Systat, Version 11). Multiple stepwise forward regressions were calculated using above-mentioned PCoA scores of the first two PCoA axes as dependent variables and all measured environmental parameters as independent variables. Since temperature and DIN were highly correlated ($R = -0.803$), this suggests a large shared contribution to the model. To disentangle unique and shared contributions, we individually regressed DIN against temperature and replaced original DIN values with the residuals of that regression. Multiple regression analysis (MRA) was then accomplished with all parameters and replaced DIN values. Only variables that significantly ($p < 0.05$) added to the prediction of the dependent variables were used to build the multiple regression model. Residual analyses of the regression models were carried out to investigate the difference between observed and predicted scores in detail.

Correlations between all environmental parameters were determined using Spearman rank order correlations applying a significance level of $p < 0.05$. Additionally, correlations between relative abundances of all OTUs and scores of the first two PCoA axes were calculated. To visualize the relationship between OTUs and PCoA axes, OTUs that were statistically significantly correlated ($p < 0.05$) with one or both PCoA axes were used to perform interaction network analysis using Cytoscape version 3.2.0 (Shannon *et al.* 2003).

RESULTS

Environmental conditions at sampling site

Concurrent with water sampling, physicochemical parameters were recorded and current components were calculated (Figs S1–S3 and Table S1, Supporting Information). Spearman rank order correlation analysis revealed statistically significant correlations ($p < 0.05$) between abiotic parameters (Table S2, Supporting Information) but only few had particularly high correlation coefficients ($R > 0.6$) such as DIN and temperature ($R = -0.803$), DOC and salinity ($R = 0.633$) and DIN and SiO_2 ($R = 0.634$) (Table S2, Supporting Information).

Two Chl *a* peaks were measured in April and August and were referred to as spring and summer phytoplankton blooms, respectively (Fig. 1). The spring bloom was dominated by a combination of dinoflagellates and flagellates, whereas the summer bloom seemed to be more diverse and was characterized by high diatom, ciliate, flagellate and dinoflagellate cell numbers (Fig. 1).

Bacterial community structure in relation to environmental parameters

The bacterioplankton community at Helgoland Roads was assessed by 16S rRNA gene tag sequencing at weekly time intervals over a period of one year. After exclusion of eukaryote and organelle sequences and OTUs with an average relative abundance ≤0.1% of the total community, 4739 551 high-quality sequences were retained that represented 116 different OTUs (Table S3, Supporting Information).

PCoA of the bacterial communities revealed a strong seasonal pattern (Fig. 2). A spring cluster was followed by rapidly changing bacterial community compositions with high week to week variance (rapid change phase), which passed into a relatively stable summer community. At the end of August, a second rapid change phase occurred, which led to a stable autumn community. During winter, the community structure changed slowly

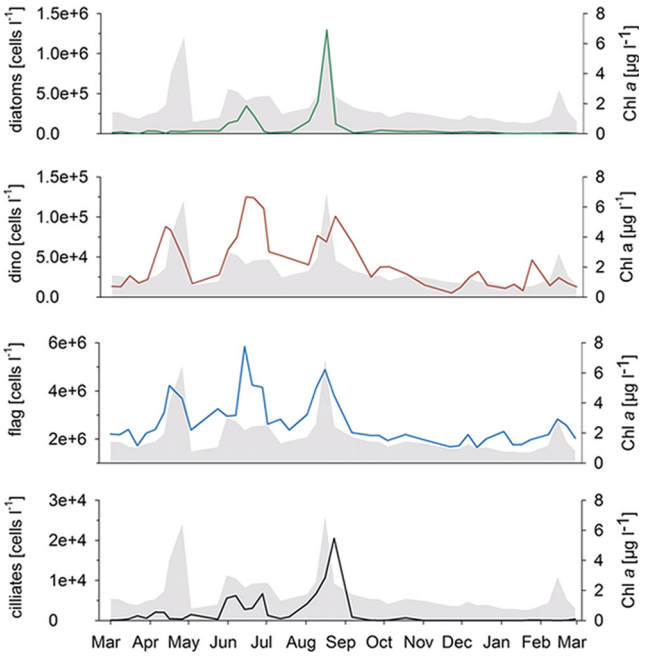

Figure 1. Phytoplankton abundances recorded from 1 March 2012 to 28 February 2013 at Helgoland Roads. Cell numbers per litre are depicted for diatoms, dinoflagellates, flagellates and cilliates. Chlorophyll *a* concentrations ranging from 0.74 to 6.8 μg l^{-1} are depicted as grey area.

and returned to the previous years spring community. Rapid changes of the community structure followed the first principal coordinate axis (PCoA1), whereas the more gradual changes within the summer cluster and from the autumn to the spring community occurred along the second PCoA axis (PCoA2). MRA with all environmental parameters as independent and scores of each PCoA axis as dependent variables revealed that PCoA1 was best explained by a combination of salinity, SiO_2, flagellates and PC2 (Fig. 3A and Table S4, Supporting Information), whereas the regression model for PCoA2 was significantly influenced by temperature, SiO_2, DIN, flagellates and PC1 (Fig. 3B and Table S4, Supporting Information). The fits of both models were statistically significant ($p < 0.001$). Residuals depicted for the models (Fig. 3A and B) displayed the largest differences between calculated and predicted PCoA scores from June until August.

Community composition and succession during phytoplankton blooms

The bacterial community at Helgoland Roads (Fig. S4, Supporting Information) was dominated by *Proteobacteria* (mainly *Alpha- and Gammaproteobacteria*) with an annual mean of 60.1% of the trimmed tag data. *Bacteroidetes* were represented almost exclusively by *Flavobacteriia* and accounted for 24.7% of the trimmed tag data. Other phyla that were present throughout the year and reached relatively high abundances were *Actinobacteria* (5.3%) and *Euryarchaeota* (5.2%).

A seasonal succession of six main OTUs that dominated the community (i.e. had highest relative abundance of all OTUs in a particular sample) at defined periods in time, i.e. at least two weeks in a row, was observed (Fig. 4). On average, these six OTUs represented 27.4% of the trimmed tag data. In addition to the seasonal succession pattern, periods of rapid shifts of these dominating groups were observed during the spring and summer blooms (Fig. 4). Spring bloom dominating OTUs belonged to the *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteriia*. In comparison, higher diversity was found during the

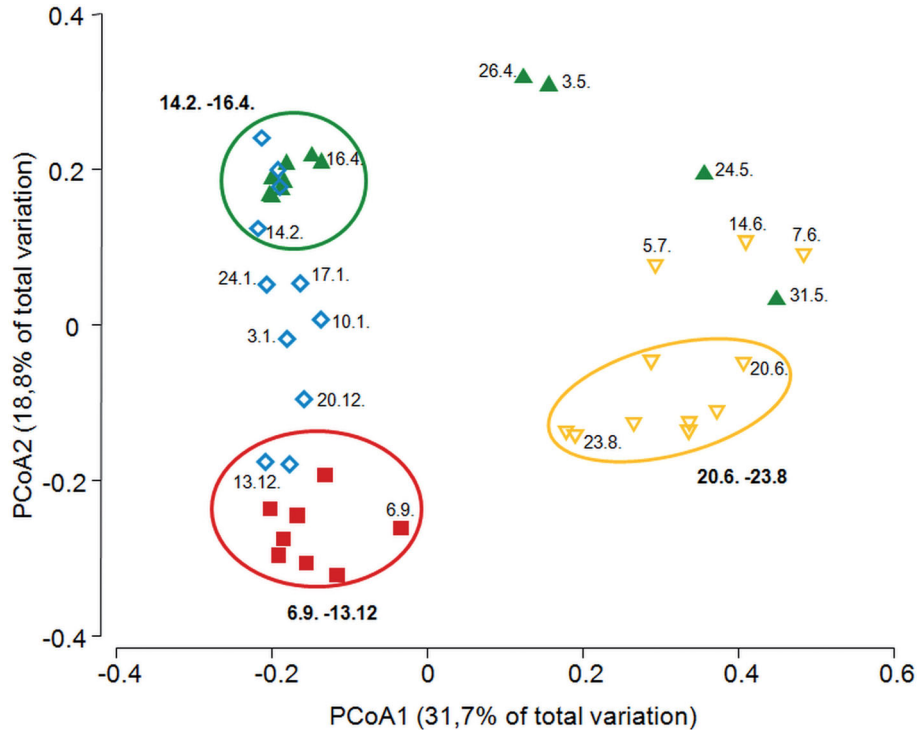

Figure 2. PCoA of bacterial communities using Hellingers distance. Symbols represent bacterial communities at sampling dates. Symbols are colour coded according to season. Seasons are defined according to meteorological definition. Green: spring (1 March–31 May); yellow: summer (1 June–31 August); red: autumn (1 September–30 November); blue: winter (1 December–29 February). Spring, summer and autumn clusters are indicated by ellipses on a distance level of 0.42. Numbers represent sampling dates and time frames that are covered by the clusters, respectively. Within each cluster, the first and last sampling dates are given.

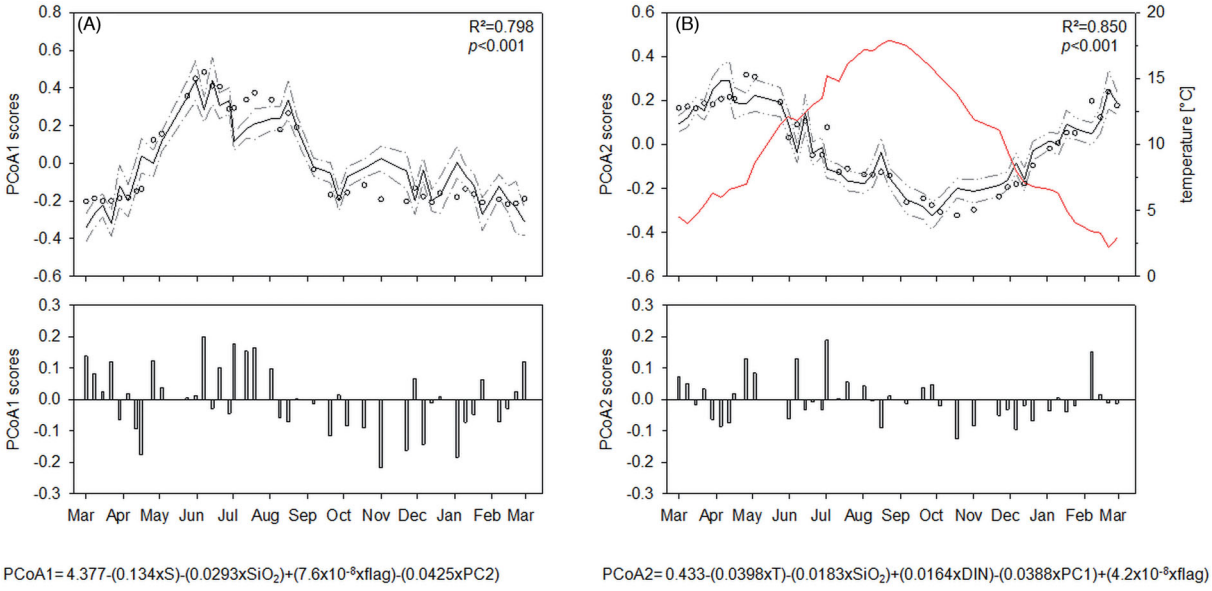

Figure 3. Stepwise forward multiple linear regression analyses. **(A)** prediction for PCoA1, **(B)** prediction for PCoA2. Open circles: calculated PCoA scores; black line: predicted PCoA scores; dashed lines: 95% confidence interval; red line in B: temperature. R^2, p values and formulas are displayed for the models. Only parameters with $p < 0.05$ were considered for model building. Corresponding residuals for models are displayed as bar charts below the models. S: salinity; T: temperature; SiO_2: silicate; DIN: dissolved inorganic nitrogen (DIN = $NO_2^- + NO_3^- + NH_4^+$); Chl *a*: chlorophyll a; PC1, PC2: Principal coordinates of EOFs; flag: flagellates.

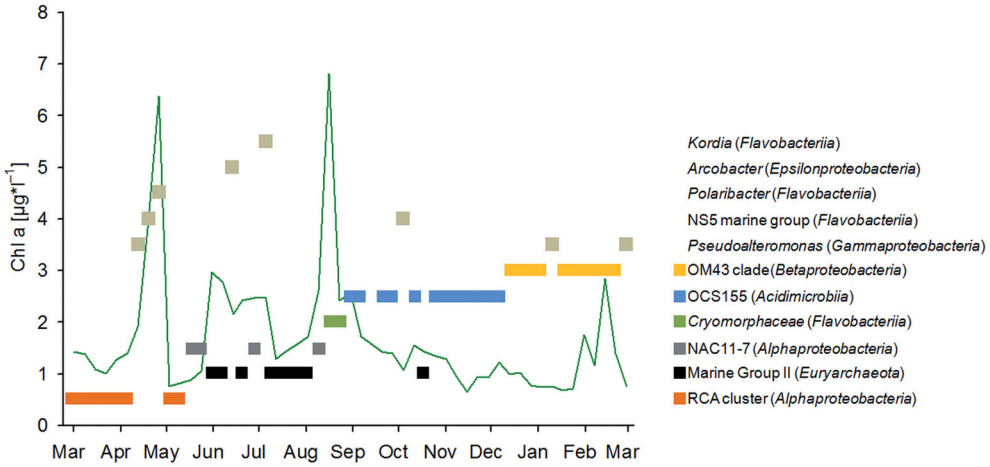

Figure 4. Annual succession of dominating OTUs. OTUs that exhibited highest relative abundance of all OTUs in a particular sample were considered to dominate the community at that time point. OTUs that dominated defined periods (at least 2 weeks in a row) are colour coded. OTUs that dominated the community in single samples only, were kept in beige. Green line: Chlorophyll *a* concentration.

summer bloom with dominating OTUs belonging to the *Alphaproteobacteria* and *Gammaproteobacteria*, *Flavobacteriia*, *Acidimicrobiia* (OCS155 marine group) and *Thermoplasmata* (Marine Group II; *Euryarchaeota*).

We observed swift successions of distinct OTUs during the spring and summer bloom phases and examined the dominant OTUs within the prominent classes of *Alphaproteobacteria, Gammaproteobacteria* and *Flavobacteriia* (Fig. 5). During the spring bloom, *Alphaproteobacteria* (*Roseobacter* related DC5-80-3 lineage, referred to as *Roseobacter* clade affiliated (RCA) cluster and NAC11–7 lineage) increased in relative abundance upon bloom decay (Fig. 5A). *Gammaproteobacteria* showed a succession with *Pseudoalteromonas* peaking twice in abundance, once early in the bloom phase and again late after the bloom phase when the Chl *a* concentration began to increase again. During the Chl *a* maximum the SAR86 clade dominated the *Gammaproteobacteria*, whereas the SAR92 clade responded more to bloom decay (Fig. 5B). *Flavobacteriia* were dominated by the NS5 marine group in the early bloom phase. *Polaribacter* increased simultaneously with increasing Chl *a* concentration and peaked during the Chl *a* maximum. In contrast, a *Cryomorphaceae* cluster exhibited higher relative abundances after the bloom decay (Fig. 5C).

During the summer bloom, we observed a different succession pattern within the prominent classes (Fig. 5D–F). Succession of *Alphaproteobacteria* was led by *Roseobacter* NAC11–7 members during the early bloom and the Chl *a* maximum. Upon algal decay, the relative abundance of SAR116 clade increased slightly, whereas the OCT lineage decreased in relative abundance (Fig. 5D). Within the *Gammaproteobacteria*, OM60/NOR5 clade members responded to the early bloom. As the bloom commenced, members of the ZD0405 clade increased in relative abundance and SAR86 clade members dominated during

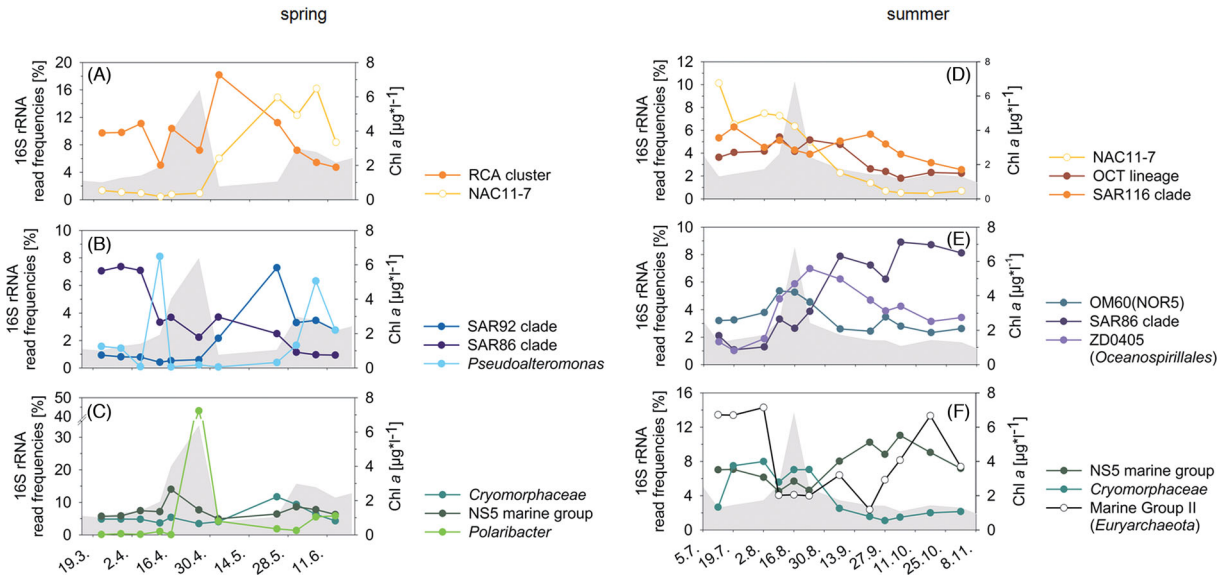

Figure 5. Short-term succession of dominant OTUs within the prominent classes of *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteriia* during the spring bloom and summer bloom. **(A)** *Alphaproteobacteria*, **(B)** *Gammaproteobacteria*, **(C)** *Flavobacteriia*, **(D)** *Alphaproteobacteria*, **(E)** *Gammaproteobacteria*, **(F)** *Flavobacteriia* and *Thermoplasmata* (Marine Group II). Chlorophyll *a* concentration is indicated as grey area.

the late bloom phase (Fig. 5E). Succession of *Flavobacteriia* was again governed by *Cryomorphaceae* until the bloom began to collapse and NS5 marine group members notably increased in relative abundance during bloom decay.

Clustering of OTUs according to response to environmental parameters

To elucidate how single OTUs were influenced by environmental conditions, we selected OTUs that were significantly ($p < 0.05$) correlated with one or both PCoA axes, assuming that these contributed significantly to the explained variation in community structure. These OTUs were subjected to network analysis and sorted according to their response to phytoplankton blooms (Fig. 6). We observed distinct correlation patterns for different groups. Most interestingly, OTUs that responded to the spring bloom were positively correlated with PCoA2 (i.e. temperature, see Fig. 3B), whereas OTUs responding to the summer bloom were negatively correlated with PCoA2. In both groups, strong positive correlations with PCoA1 (i.e. phytoplankton, see Fig. 3A) were observed. OTUs without a response to any of the bloom phases were in general negatively correlated with PCoA2.

DISCUSSION

Seasonal variation in North Sea bacterioplankton

We observed a pronounced seasonal pattern, which agrees with other studies on the seasonality of bacterioplankton communities (Fuhrman *et al.* 2006; Fortunato *et al.* 2012; Gilbert *et al.* 2012). The seasonal variation in this study is largely driven by temperature as revealed by multiple regression analysis and reflected in the shape of our model which is inversely proportional to the measured temperature curve. This temperature dependence is also supported by other authors who describe temperature as the main determining driver of community composition (Pommier *et al.* 2007; Gilbert *et al.* 2009; Chow *et al.* 2013).

The seasonal variation in community structure is governed by a few OTUs from different taxonomic classes, several of which have been identified as dominant community members in previous studies. Within the *Alphaproteobacteria*, especially the *Roseobacter* RCA cluster and NAC11–7 lineage were highly abundant with up to 18 and 15% of the trimmed tag data, respectively. These high abundances are in line with reports by Selje, Simon and Brinkhoff (2004), Giebel *et al.* (2011) and Teeling *et al.* (2012), who reported similar high abundances of these taxa in the North Sea during several years. Using 16S rRNA gene sequencing, Wemheuer *et al.* (2014) determined the *Betaproteobacteria* OM43 clade as a major bacterioplankton OTU in the North Sea. Our study also identified OM43 as a prominent clade, particularly during winter when up to ~13% of the trimmed tag data affiliated with this clade. The *Actinobacteria*-related OCS155 marine group is one of the five most abundant and persistent OTUs identified over 10 years of a long-term study in the Southern California Bight (Chow *et al.* 2013). In congruence with that study, we found OCS155 marine group sequences at an annual average abundance of ~4% with maximum abundances of 13.8% during autumn. During summer the Marine Group II (*Euryarchaeota*) became a dominant group, exhibiting relative abundances of up to 15.6%. Pernthaler *et al.* (2002) even determined that this group accounted for >30% of the total picoplankton in North Sea surface waters during spring and summer using poly-FISH. Taken together, these findings demonstrate resilience of a few bacterial core taxa as was also reported for the English Channel by Caporaso *et al.* (2012). Multiple regression analyses also suggest that the seasonal succession of dominating bacterial OTUs reflects successions of their corresponding niche optima, which in this study are mainly defined by temperature. This temperature-driven succession of core taxa is interrupted during short-term events such as phytoplankton blooms. We suggest that enhanced substrate supply during these blooms favours taxa capable of a feast-and-famine lifestyle resulting in short-term peaks of these taxa.

Short-term variation during spring and summer blooms

Short-term succession is not only driven by phytoplankton but also influenced by changes in hydrographic currents.

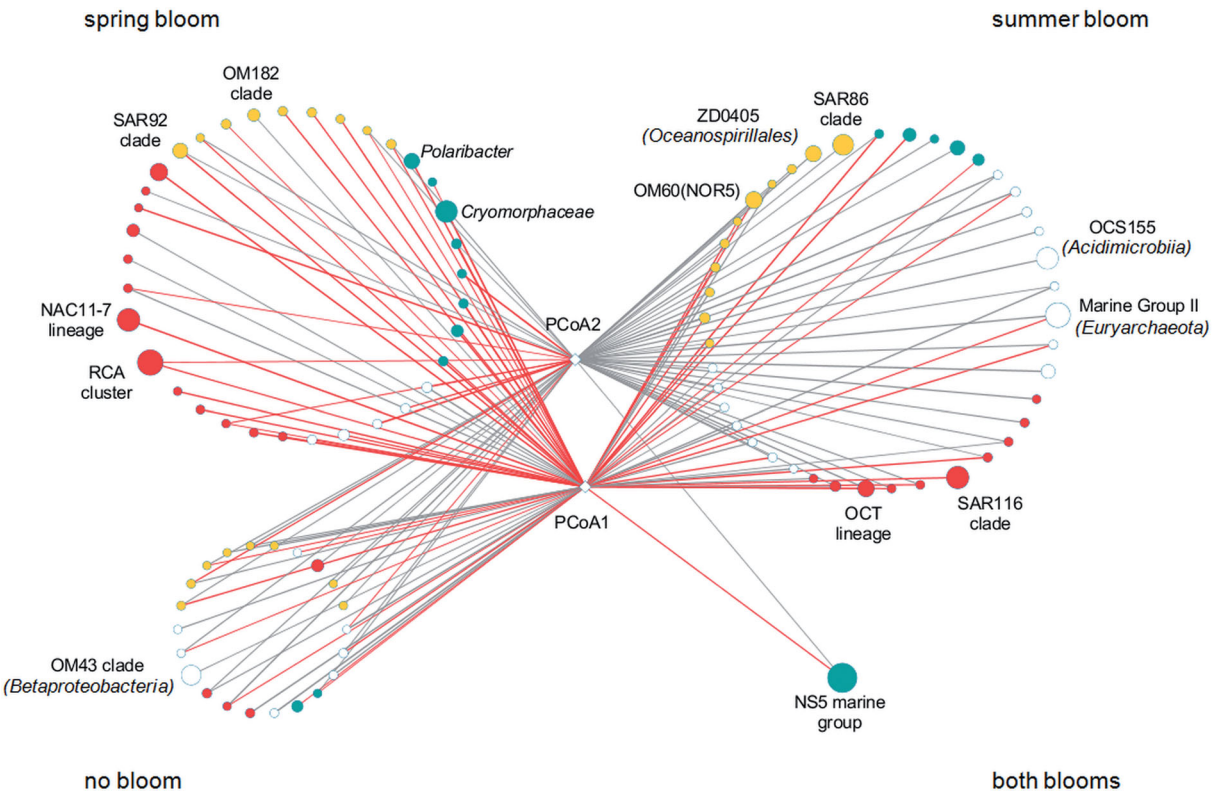

Figure 6. Interaction network analysis of OTUs that were significantly correlated ($p < 0.05$) with PCoA axes. Positive correlations are indicated in red, negative correlations in grey. Line width is set proportional to correlation strength. Mean annual OTU relative abundance is set proportional to node size. OTUs belonging to *Alphaproteobacteria*, *Gammaproteobacteria* or *Flavobacteriia* are color coded, remaining OTUs are kept in white. Red nodes: *Alphaproteobacteria*; yellow nodes: *Gammaproteobacteria*, blue nodes: *Flavobacteriia*. OTUs were split into groups: (i) spring bloom: OTUs with average abundance during spring bloom > annual average abundance, (ii) summer bloom: OTUs with average abundance during summer bloom > annual average abundance, (iii) both blooms: OTUs with average abundance during spring and summer bloom > annual average abundance and (iv) no bloom: OTUs with average abundance during blooms ≤ average annual abundance.

Hydrographic conditions at Helgoland Roads are governed by an inflow of marine waters from the north-west off the island (Fig. S2 B, Supporting Information). This current pattern is related to positive amplitudes of PC2 (Fig. S3, Supporting Information). Shortly before both phytoplankton blooms in April and August, PC2 exhibited negative amplitudes which indicate a reversed current pattern and thus, an inflow of nutrient-rich coastal waters that boosted the phytoplankton blooms at Helgoland Roads. A similar situation has been observed at Helgoland Roads during a spring phytoplankton bloom in 2009 (Teeling *et al.* 2012). However, the effect of coastal water inflow during summer observed in this study seemed not to be as strong as in spring. Phytoplankton is generally considered as the dominant source of bioavailable DOM in ocean surface waters (Hedges 1992), and heterotrophic bacteria strongly rely on this DOM (Baines and Pace 1991). In conjunction with the above-mentioned overall temperature dependence of the North Sea bacterioplankton, a comparison of bacterioplankton assemblages during spring and summer blooms is particularly interesting. This study is investigating the free-living fraction of the bacterial community only. It is noteworthy that in marine coastal environments and especially during phytoplankton blooms a large fraction of the bacterial community may be attached to particles (e.g. Simon *et al.* 2002). Lots of studies examined particle-attached and free-living communities in different aquatic environments and found that the free-living bacteria are often more abundant (Ghiglione *et al.* 2007), but particle-attached communities are more active (e.g. Crump and Baross 2000; Ghiglione *et al.* 2007). However, comparison of the community composition of free-living and particle-attached bacteria using high-throughput 16S rRNA gene sequencing methods revealed minor differences between both fractions. Campbell and Kirchman (2013) for instance reported that the free-living and particle-attached bacteria along a salinity gradient clustered together and shared similar abundances of most bacteria groups. This is also supported by Ortega-Retuerta *et al.* (2013) who found that the community composition of both fractions is similar especially at higher oceanic salinities.

In contrast to Teeling *et al.* (2012) who observed a diatom-dominated spring bloom in 2009, the spring bloom during our study was dominated by dinoflagellates and flagellates. Diatoms reached their maximum abundance during the summer bloom in August. Spring and summer blooms were dominated by *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteriia*. These classes have been consistently found to dominate bloom-associated bacterial communities as reviewed by Buchan *et al.* (2014). However, we did observe differences between the two blooms at higher taxonomic resolution.

During the spring bloom, dominating OTUs within the *Alphaproteobacteria* included the RCA cluster and NAC11–7 lineage. The RCA cluster tag sequences exhibited higher relative abundances than the NAC11–7 lineage during the beginning of the bloom and the Chl *a* maximum. In response to bloom decay, relative abundance of the RCA cluster tag sequences increased from 5 to 17% of the trimmed tag data. This is consistent with

Giebel *et al.* (2011) who reported relative abundances of the RCA cluster of 15% during a phytoplankton bloom in the southern North Sea via quantitative PCR. The NAC11–7 lineage exhibited an even stronger response to algal decay, increasing in read frequency from ∼1 to 15% and took over dominance following the RCA cluster after the bloom decay. Conversely, Teeling *et al.* (2012) observed a succession of *Roseobacter* clade members, with the NAC11–7 lineage dominating the early bloom phase and the RCA cluster dominating the late bloom phase. Dominating spring bloom *Flavobacteriia* were *Polaribacter*, NS5 marine group members and a *Cryomorphaceae* related cluster, all of which are known to react to phytoplankton blooms where they are likely involved in biopolymer degradation (Lau *et al.* 2005; Gómez-Pereira *et al.* 2012; Teeling *et al.* 2012; Xing *et al.* 2015). *Alteromonadales* (SAR92 clade, *Pseudoalteromonas*) and SAR86 clade members were the dominating *Gammaproteobacteria* during the spring bloom. Consistent with our study, SAR92 phylotypes have been demonstrated to react to a phytoplankton bloom decay during spring in 2009 at Helgoland Roads (Teeling *et al.* 2012). *Pseudoalteromonas* phylotypes are well known to produce exoproteases that enable them to degrade complex algae-derived organic matter (Holmström and Kjelleberg 1999; Lee *et al.* 2000; Ivanova *et al.* 2002; Vázquez, Hernández and Mac Cormack 2008). The ability to rapidly react to enhanced substrate supply during phytoplankton blooms is reflected in the short-term peaks of *Pseudoalteromonas* during the spring bloom in our study.

During the diatom-dominated summer bloom, the most abundant *Alphaproteobacteria* tag sequences affiliated with the *Roseobacter* clade NAC11–7 and OCT lineages as well as the SAR116 clade. *Roseobacter* clade members are found to associate with phytoplankton blooms and are particularly important for the degradation of dimethylsulfoniopropionate (DMSP), an abundant algal osmolyte (Buchan, González and Moran 2005). The SAR116 clade is an ubiquitous marine bacterioplankton lineage (Giovannoni and Rappé 2000). The first cultivated SAR116 strain was shown to possess the *dmdA* gene, responsible for DMSP demethylation (Oh *et al.* 2010) indicating possible association with phytoplankton blooms. *Flavobacteriia* during the summer bloom were dominated by the NS5 marine group and *Cryomorphaceae*. Both of these clades were also abundant during the spring bloom when nutrient concentrations were higher as compared to the summer bloom. This suggests that members of these clades can cope with a broad range of nutrient concentrations as well as DOM from different phytoplankton species. Summer bloom *Gammaproteobacteria* were dominated by the NOR5 lineage, ZD0405 (*Oceanospirillales*) and the SAR86 clade. The NOR5 lineage has been found to be able to cope with both, nutrient-poor and nutrient-rich conditions and to occur in pronounced association with phytoplankton blooms (Eilers *et al.* 2001; Yan *et al.* 2009). However, in this study the NOR5 lineage becomes dominating during the summer bloom only. A similar situation was observed for the SAR86 clade that dominated during both blooms but exhibited much higher relative abundances during the summer bloom. Concerning that both the NOR5 lineage and the SAR86 clade were positively correlated with temperature, this hints at the importance of temperature as an influencing factor for the response of bacterial OTUs to phytoplankton blooms and thus, points to its potential as a main niche builder.

Comparison of spring and summer blooms revealed similar successions on class level, with *Alphaproteobacteria* dominating the early bloom phase, *Flavobacteriia* increasing in relative abundances as the bloom commences and *Gammaproteobacteria* increasing as the bloom decays. The same succession of bacteria classes was reported for the 2009 spring bloom at Helgoland Roads (Teeling *et al.* 2012). However, the relative abundances of *Alphaproteobacteria* and *Flavobacteriia* were much higher during the spring bloom (32.9 and 30.4%) compared to the summer bloom (27.2 and 20.8%), when *Gammaproteobacteria* increased strongly in relative abundances to ∼48% and even dominated the whole trimmed community during the summer bloom decay. From the spring to the summer bloom, the temperature increased by about 8.8°C, while the proportion of *Flavobacteriia* was lower during the summer bloom as compared to the spring bloom. This is also supported by our network analysis, which revealed that all significant correlations of *Flavobacteriia* with PCoA2 (i.e. temperature) were negative. This agrees with Tada *et al.* (2013) who stated that growth of *Bacteroidetes* is positively influenced by the quantity and quality of organic matter concentrations, but their contribution to organic matter cycling is larger at colder conditions. We additionally found an increase of low-abundance OTUs; most noticeably, we found the *Thermoplasmata*-related Marine Group II (*Euryarchaeota*) as a dominating group. We hypothesize that the capability of the Marine Group II to positively respond to phytoplankton blooms is triggered by temperature. This is supported by measurements of the consumption of proteins and lipids during a spring bloom in the northwestern Pacific which indicated a potential interaction between diatoms and members of the Marine Group II (Iverson *et al.* 2012). Additionally, the Marine Group II is known to have a cosmopolitan distribution in marine surface waters and to be abundant during summer months (Pernthaler *et al.* 2002; Herfort *et al.* 2007).

Multiple regression models exhibited especially large residuals during summer (Fig. 3), when ciliates and flagellates exhibited pronounced peaks in abundance. There is evidence that the community structure of pelagic bacterial assemblages can be shaped by size-selective protistan predation, which might lead to profound shifts in community composition as reviewed in Pernthaler (2005). Although heterotrophic nanoflagellates smaller than 5 μm account for about 80% of total bacterivory (Unrein *et al.* 2007), the relative importance of grazing by ciliates seems to be especially high in coastal and estuarine systems (Sherr and Sherr 1987; Simek *et al.* 2000). It is noteworthy that in this study heterotrophic nanoflagellate cell numbers are included in 'flagellate' cell numbers. Thus, the potential impact of these grazers is already considered in the regression model. However, ciliate cell numbers did not contribute significantly to the regression model but might explain the large difference between observed and predicted values.

Temperature as major constraint for ecological niches

Growth and activity of heterotrophic bacteria are fuelled by enhanced DOM supply as found during phytoplankton blooms. Bacterial metabolic processes, such as the decomposition of organic matter, are also enhanced by increasing temperature (Pomeroy and Wiebe 2001; Kirchman, Moran and Ducklow 2009). Thus, increasing water temperatures lead to a tighter coupling of phyto- and bacterioplankton as shown in Hoppe *et al.* (2008) and Wohlers-Zöllner *et al.* (2012), but it may also result in shifts of bacterial community composition due to different temperature optima of distinct bacterial taxa. We assume that the enhanced supply of DOM by phytoplankton results in successional patterns of taxa that have different niches with respect to organic matter decomposition. This kind of nutrient partitioning was shown during a comprehensive metagenomic and metaproteomic study on the 2009 spring phytoplankton bloom at

Helgoland Roads (Teeling *et al.* 2012). However, in our study we observed succession of different dominant OTUs during the spring and summer blooms. Network analysis revealed group formation of these OTUs, exhibiting specific correlation patterns with temperature. This and the finding that all dominant OTUs found during bloom successions were present throughout the whole year suggest that there is a resident pool of bacterial taxa. This is supported by Caporaso *et al.* (2012) who demonstrated in a comprehensive taxonomic survey in the English Channel that the vast majority of taxa identified are always present in differing proportions that are predictable. We assume that the different OTUs we found during spring and summer blooms have redundant functional capacities, but are favoured either during spring or summer blooms, based on their ecological niche affiliations, which again seems to be largely defined by temperature. This notion is for instance supported by the dominance of Marine group II *Euryarchaeota* and NOR5/OM60 clade members only during the summer bloom. According to Yan *et al.* (2009) and Pernthaler *et al.* (2002), both taxa exhibit especially high abundances (>30% and up to 13% of total picoplankton communities, respectively) during summer and autumn. Von Scheibner *et al.* (2014) conducted a mesocosm experiment with incubation of natural plankton communities from the Baltic Sea during a phytoplankton bloom at *in situ* and increased temperatures. They reported an influence of both the phytoplankton bloom phase and temperature on the bacterial community composition. They found that bacterial communities incubated at warmer temperatures were enriched by additional taxa compared to the communities at lower temperatures. Other studies also suggest that the influence of physicochemical factors (e.g. day length, salinity, temperature and nutrients) on the microbial diversity in highly dynamic systems such as estuaries of continental shelf seas is more important than biotic factors (Kirchman *et al.* 2005; Teira *et al.* 2008; Gilbert *et al.* 2012; Sintes *et al.* 2013).

Although our results suggest a direct impact of temperature on the bacterial community structure at Helgoland Roads, we must consider additional linkage to other factors not examined during this study. For example, different taxa dominated the phytoplankton during spring and summer blooms. The release of distinct organic matter in dissolved and particulate form by different phytoplankton species and the differences in the capacity of heterotrophic bacterial populations to consume these differing substrates stimulate discussion about the influence of the phytoplankton composition on changes in bacterial community compositions (e.g. Pinhassi *et al.* 2004; Rooney-Varga *et al.* 2005; Sarmento and Gasol 2012; Becker *et al.* 2014). The spring bloom in this study was dominated by a combination of dinoflagellates and flagellates. In contrast to our study, Teeling *et al.* (2012) investigated a diatom-dominated spring bloom in 2009 at the same sampling site. Although the dominating phytoplankton groups differ between the two studies, similar dominating bacterial taxa (NAC11–7 lineage, RCA cluster, *Polaribacter*, SAR92 clade) have been found during the blooms. This might support the notion that heterotrophic bacteria react to the general substrate supply during phytoplankton blooms independently of the phytoplankton composition (Rooney-Varga *et al.* 2005; Rink *et al.* 2007). However, since we cannot provide detailed data on the phytoplankton species composition, assumptions on the coupling of specific OTUs and the phytoplankton composition would be speculative. Another important factor that shapes the BCC is the cell lysis by viruses. Viruses are well known to primarily affect the largest, most rapidly growing bacterial populations and by this suppress particular bacterial species (Thingstad 2000). The released DOM again favours the surviving bacterial species. All of the above-mentioned abiotic and biotic factors also exhibit interactions and thus affect each other. Therefore, changes in bacterial community composition are likely controlled by complex combinations of these factors rather than by single parameters.

Nonetheless, the temperature signal that was captured by our statistical analyses was significant, as was the influence of phytoplankton blooms. Hence, both of these factors exert a major influence on the bacterioplankton community at Helgoland Roads in the North Sea. We found a pronounced seasonal pattern and indications that this pattern might be annually recurring, which however needs to be evidenced with studies that span multiple years. The pronounced formation of temperature-dependent guilds during spring and summer phytoplankton blooms lets us conclude that short-term bacterial succession in response to phytoplankton blooms is indirectly affected by temperature as a major factor for the formation of ecological niches, resulting in distinct bacterial communities during colder spring bloom phases and warmer summer bloom phases. For future analyses, access to representative strains of relevant bacterial clades is needed for comprehensive examination of functional capacities under defined experimental conditions.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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