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ORIGINAL ARTICLE

Analysis of bacterial core communities in the central Baltic by comparative RNA–DNA-based fingerprinting provides links to structure–function relationships

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Understanding structure–function links of microbial communities is a central theme of microbial ecology since its beginning. To this end, we studied the spatial variability of the bacterioplankton community structure and composition across the central Baltic Sea at four stations, which were up to 450 km apart and at a depth profile representative for the central part (Gotland Deep, 235 m). Bacterial community structure was followed by 16S ribosomal RNA (rRNA)- and 16S rRNA gene-based fingerprints using single-strand conformation polymorphism (SSCP) electrophoresis. Species composition was determined by sequence analysis of SSCP bands. High similarities of the bacterioplankton communities across several hundred kilometers were observed in the surface water using RNA- and DNA-based fingerprints. In these surface communities, the RNA- and DNA-based fingerprints resulted in very different pattern, presumably indicating large difference between the active members of the community as represented by RNA-based fingerprints and the present members represented by the DNA-based fingerprints. This large discrepancy changed gradually over depth, resulting in highly similar RNA- and DNA-based fingerprints in the anoxic part of the water column below 130 m depth. A conceivable mechanism explaining this high similarity could be the reduced oxidative stress in the anoxic zone. The stable communities on the surface and in the anoxic zone indicate the strong influence of the hydrography on the bacterioplankton community structure. Comparative analysis of RNA- and DNA-based community structure provided criteria for the identification of the core community, its key members and their links to biogeochemical functions.

Introduction

Marine bacterioplankton is a natural microbial community of global relevance, and its taxonomic structure and biogeochemical functioning is of great importance to the whole biosphere (Azam and Malfatti, 2007; Fuhrman and Steele, 2008; Höfle et al., 2008; DeLong, 2009; Jiao et al., 2010). On a basin scale, the horizontal extent and vertical distribution of its community structure and activity has gained much attention in the main ocean basins, such as the Pacific and Atlantic (for a review see

Treusch et al., 2010). The vertical extent of the bacterioplankton community structure in these oceans has been studied extensively using 16S ribosomal RNA (rRNA) gene-based methods (Hewson et al., 2006b; Treusch et al., 2009). The horizontal extent of the bacterioplankton community structure on a large scale, that is, in the order of kilometers, was studied in detail in various marine ecosystems, such as the Pacific (Hewson et al., 2006a) and the Chesapeake Bay (Kan et al., 2006). Contradictory results have been reported ranging from substantial changes within a few kilometers to very slight changes over long distances (Hewson et al., 2006a; Kan et al., 2007).

Central to the biogeochemical functioning of bacterioplankton is a set of genes providing the essential biochemical pathways necessary for the major elemental fluxes, such as carbon, nitrogen and sulfur (Falkowski et al., 2008). These pathways

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are provided by the prokaryotic taxa representing the core community (Höfle *et al.*, 2008). This core community is composed of the abundant taxa and is in continuous exchange with the 'seed bank', that is, the rare taxa, depending on the environmental conditions (Pedrós-Alió, 2006). Currently, we understand the threshold between rare and abundant taxa in terms of their relative abundances in the bacterioplankton community in the range 2–0.5% (Höfle *et al.*, 2008). An operational criterion for this threshold is lacking and might vary from habitat to habitat. Therefore, one of the aims of this study is to provide such operational criteria for identifying the threshold and the relevant taxa of the core community.

The central Baltic Sea is characterized by a pronounced salinity gradient (60–80 m depth) that inhibits vertical mixing (Stigebrandt, 2001). Oxygen deficiency and sulfide accumulation occurs in the deep water below the halocline affecting primarily the large basins of the central Baltic Sea, such as the Gotland Deep, due to the combined effect of hydrography and anthropogenic pollution (Wulff *et al.*, 2001). On the other hand, this halocline provides very stable physicochemical conditions in the deep water below the halocline of the central Baltic. Horizontally the Baltic is characterized by a slow but steady increase of salinity in the surface water from the Northern Bothnian Bay to the Baltic proper from 2 to 8 per mille (PSU; Stigebrandt, 2001). Therefore, we will use the central Baltic as a model ecosystem in terms of horizontal changes and vertical stability below the halocline.

Molecular analyses of bacterioplankton communities started in the late eighties of the last century using environmental rRNA and substantially improved by the introduction of high-resolution electrophoresis in the nineties (DeLong *et al.*, 1989; Höfle, 1989; Muyzer *et al.*, 1993; Prosser *et al.*, 2010). The first combined analysis using DNA- and RNA-based community fingerprinting of bacterioplankton was carried out in 1996 (Teske *et al.*, 1996). Currently, DNA-based fingerprints are assumed to reflect the taxa present in a community, whereas RNA-based fingerprints reflect the active members of the community (Logue and Lindström, 2010; Prosser *et al.*, 2010). A seminal experimental study combining nucleic acid-based fingerprints with stable isotope probing by Mahmood *et al.* (2005) showed the validity of this concept by finding that six out of seven major RNA-based phylotypes in soil microcosms were actively degrading pentachlorophenol. This led us to the assumption that a comparison of RNA-based community fingerprints of bacterioplankton with DNA-based fingerprints from the same samples could provide criteria to identify the active members of the core community. We will call this approach in the following COmparative RNA–DNA-based Analysis of Fingerprints (CORDAF), including the identification of single taxa by sequencing of the major bands in the fingerprints.

In this study we will test the hypothesis if CORDAF of bacterioplankton can provide criteria for identifying the most abundant and active members of the core community. To this end, we studied the spatial variability of the bacterioplankton community structure and composition across the central Baltic Sea at four stations, which were up to 450 km apart and at a depth profile in the deepest central part, the Gotland Deep, a station representative for the central Baltic. Bacterial community structure was followed by 16S rRNA and 16S rRNA gene-based fingerprints using single-strand conformation polymorphism (SSCP) electrophoresis. The CORDAF analysis was assessed to provide an overview of the present and active bacterial core community in horizontal and vertical direction. We demonstrated that a large fraction of the bacterial core community, that is, 44% of all phylotypes, would have been missed without RNA-based analyses. Overall, CORDAF of bacterioplankton communities has the potential to identify the core community, reveal its active members and provide clues about their biogeochemical functions.

Materials and methods

Study site, sampling and environmental background parameters

All seawater samples were obtained from the following four stations: BY15, named G in this study (Gotland Deep, 57. 1920°N, 20.3020°E), Teili, named T1 (central Baltic, 59.2607°N, 21.3002°E), LL12 (Finnish Bay, 59.2900°N, 22.5398°E), SR5, named Bot1 (Bothnian Bay, 61.0499°N, 19.3499°E) in the Baltic Sea, on 15 to 19 September 1998 using Niskin PVC bottles (Hydro-Bios, Kiel, Germany) mounted on a CTD rosette (Table 1). Sampling, sample handling and physicochemical analysis are described in more detail elsewhere (Brettar and Rheinheimer, 1991). Inorganic nitrogen, oxygen and H_2S were determined aboard RV Aranda according to Grasshoff *et al.* (1983) directly after sampling. Total bacterial counts and bacterial production were determined as described by Weinbauer *et al.* (2003). Colony-forming units were determined using the spread plate technique on a one-fourth dilution of marine broth (Difco 2216, Lawrence, KS, USA) solidified with 2% agar and an incubation time of 2 weeks at room temperature. Bacterial biomass of the water samples was harvested by filtration on a sandwich of a glass-fiber filter (90 mm, Whatman GF/F, Dassel, Germany) on top of a polycarbonate filter (Nucleopore, Whatman International, Kent, UK, 0.2 μm pore size) and stored frozen (−70 °C) for later analysis. All surface samples were prefiltered through a polycarbonate (Nucleopore, Whatman International) filter with a pore size of 3 μm. All bacterial biomass samples were obtained as three to five replicates.

Table 1 Summary of the sampled stations in the central Baltic Sea: location, physicochemical parameters and diversity assessments of the surface samples (5m) studied

Station (int. name)	Location	Sampling date	Temperature, °C	Salinity, ‰	Richness S DNA	Shannon index H DNA	Evenness E DNA	Richness S RNA	Shannon index H RNA	Evenness E RNA
G1 (BY15) Gotland Deep	57.1920 N 20.3020 E	18 September 1998	14.8	6.7	49	3.2241	0.8284	54	3.7028	0.9282
T1 (Teili) Central Baltic	59.2607 N 21.3002 E	19 September 1998	15.3	6.6	41	3.1348	0.8441	57	3.6813	0.9119
LL12 Finnish Bay	59.2900 N 22.5398 E	15 September 1998	15.0	6.0	47	3.3795	0.8777	51	3.3217	0.8448
Bot1 (SR5) Bothnian Bay	61.0499 N 19.3499 E	19 September 1998	14.5	4.0	49	3.1816	0.8175	70	3.9145	0.9214

Nucleic acid extraction, community fingerprints by SSCP analysis of 16S rRNA PCR and RT-PCR amplicons and sequencing

Nucleic acid extraction and quantification from frozen filters was performed by parallel extraction of RNA and DNA as described by Weinbauer et al. (2002). Nucleic acid concentrations were determined by spectrophotometric fluorescence (Weinbauer and Höfle, 2001) using either RiboGreen (RNA quantification kit; Molecular Probes, Invitrogen, Carlsbad, CA, USA) or PicoGreen (dsDNA quantification kit; Molecular Probes) for RNA or DNA quantification, respectively. Prior to quantification, RNA extracts were purified from contaminating traces of DNA by incubation with DNAse I (Roche Diagnostics, Mannheim, Germany) for 60 min at 37 °C followed by phenol/chloroform (3:1) purification of the RNA and an ethanol precipitation. Purified RNA was resuspended in diethyl pyrocarbonate-treated distilled water and stored frozen until analyzed. For DNA, we included an additional WizardPrep DNA purification step (Promega, Madison, WI, USA) of a 50 µl aliquot. Although reducing the yield of total DNA, this additional purification step substantially increased the PCR efficiency for the DNA extracted.

Primers used for 16S rRNA gene amplification from environmental DNA/RNA are given by Schwieger and Tebbe (1998) (primer set Com1/Com2 amplifying positions 519 to 926 of *E. coli* numbering of 16S rRNA gene). PCR conditions were according to Eichler et al. (2006). Reverse transcription (RT)-PCR amplification was carried out using the *C. therm.* polymerase one-step RT-PCR System (Roche Diagnostics) and the protocol provided by the manufacturer. A quantity of 5 ng of environmental RNA was used as template for this RT-PCR. For the preparation of single-stranded DNA (ssDNA) and community fingerprints, a variant of the protocol described by Eichler et al. (2006) was applied. Briefly, magnetic streptavidin-coated beads (Promega) were applied to obtain ssDNA from the PCR amplicons. Quantification of the obtained ssDNA was performed on a 1.5% agarose gel by comparison with a low-molecular-weight marker (Invitrogen low-DNA-mass ladder, Carlsbad, CA, USA). For SSCP fingerprinting analysis, 25 ng of the obtained ssDNA was mixed with gel loading buffer (95% formamide, 10 mM NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol) in a final volume of 7 µl. After incubation for 3 min at 95 °C, the ssDNA samples were stored on ice, loaded onto a nondenaturing polyacrylamide-like gel (0.6 ×, MDE gel solution; Cambrex BioScience, Rockland, ME, USA) and electrophoretically separated at 20 °C at 400 V for 18 h on a Macrophor sequencing apparatus (Amersham Pharmacia Biotech, GE Healthcare, Little Chalfont, UK). The gel was silver stained according to the method described by Bassam et al. (1991). Reamplification of individual bands excised from the SSCP gels was performed as described by Eichler

et al. (2006), followed by cycle sequencing (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA, USA) including the primers applied before.

For all samples, at least three replicate SSCP analyses were achieved. The replicate fingerprint patterns and the sequences per sample were highly comparable and pooled for final analysis.

Statistical analysis of SSCP fingerprints

SSCP fingerprints were analyzed using the GelCompare II software package (Applied Maths, Kortrijk, Belgium) after digitalization of the SSCP gels by an Epson Expression 1600 Pro scanner (EPSON DEUTSCHLAND GmbH, Meerbusch, Germany). Only bands with an intensity $>0.1\%$ of the total lane were considered for further statistical analysis. Similarity coefficients were calculated using Pearson's correlation based on the densitometric curves of the lanes. Dendrograms were constructed based on the unweighted pair-group method using arithmetic averages. All richness and diversity indices were calculated using the 0.1% threshold for relative abundances of single phylotypes because without such a detection limit these indices do not make sense (Höfle *et al.*, 2008).

Comparative and phylogenetic analysis of 16S rRNA gene sequences

The obtained sequences for both forward and reverse reactions were checked for accuracy using the Sequencher software package (http://www.gene codes.com). Sequences were then included in a local database containing every public 16S rRNA bacterial gene sequence already aligned and analyzed by phylogeny. Each band sequence was aligned and compared with its most similar sequences. A band sequence was rejected if it contained numerous errors (differences at positions otherwise conserved in every other sequence), if it was shorter than 250 nt and finally if it was suspected to be of chimeric origin (5′ and 3′ parts, respectively, closest to sequences having different taxonomic designation). Every alignment was finally checked by eye using SeaView (Galtier *et al.*, 1996). Single phylotypes were defined as sequences of a maximum of 5 to 6 differences corresponding to a 16S rRNA gene sequences similarity of about 98%. For the similarity tables, each band sequence was blasted (with options no filter and $W = 7$) first against a database of sequences obtained from validly described species and second against the entire database of sequences. Sequence similarity values between two sequences were calculated as the numbers of identical nucleotides within obtained local alignments divided by the length of the shorter sequence, which therefore corresponds to the most conservative similarity estimate. For the closest described cultured species we used a 80% limit because often closest relatives are in different phyla.

Phylogenetic analyses were conducted after assigning the single sequences to large taxonomic units such as phyla and classes. For each band sequence, the two most similar public sequences were included as well a number of sequences representative either of well-established species or of some clone sequences known to be frequently isolated from water. Distances were calculated according to Kimura's two-parameters method (Phylogeny Inference Package, version 3.63, distributed by J Felsenstein, Department of Genetics, UW, Seattle, WA, USA) and using parts of the sequences common to all sequences analyzed. Phylogenetic trees were constructed according to three different methods (BIONJ using Kimura's two-parameters correction, ML: maximum likelihood using the Global option and MP: maximum parsimony). The BIONJ program from Gascuel (1997), DNADIST, ML and MP programs from PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J Felsenstein, Department of Genetics, UW, Seattle, WA, USA) were used.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences obtained from the SSCP fingerprints have been deposited under accession numbers FR714941 to FR715024.

Results

In-situ *conditions of the central Baltic Sea during sampling*

We studied the central Baltic during 5 days in late summer across a north-south transect of about 450 km, using four different stations during stable meteorological conditions to analyze the horizontal variability of the bacterioplankton community structure (Figure 1). Temperature at the surface (5 m) was around 15 °C and salinity decreased from south to north from 6.7‰ to 4.0‰ (Table 1). One station (G) at the deepest part of the central Baltic, the Gotland Deep, was used to understand the vertical variability of the bacterioplankton community structure. The physicochemical parameters at this station can be considered typical for the central Baltic in periods of stagnant deep water (Brettar and Rheinheimer, 1991). The central Baltic is characterized by a permanent halocline (60–80 m) that inhibits annual vertical mixing deeper than 80 m. Below 80 m, exchange processes rely on horizontal exchange processes that are rare events with deep water stagnation periods for up to a decade (Wulff *et al.*, 2001). Because of this hydrography, the water was well oxygenated above the halocline and showed pronounced physicochemical gradients below (Figure 2). Below 80 m, a strong decrease of oxygen occurred and an increase in nitrate with a maximum around 90 m (Figures 2a and b). At 130–140 m, an oxic–anoxic interface was observed. The anoxic zone below 130 m was characterized by a strong
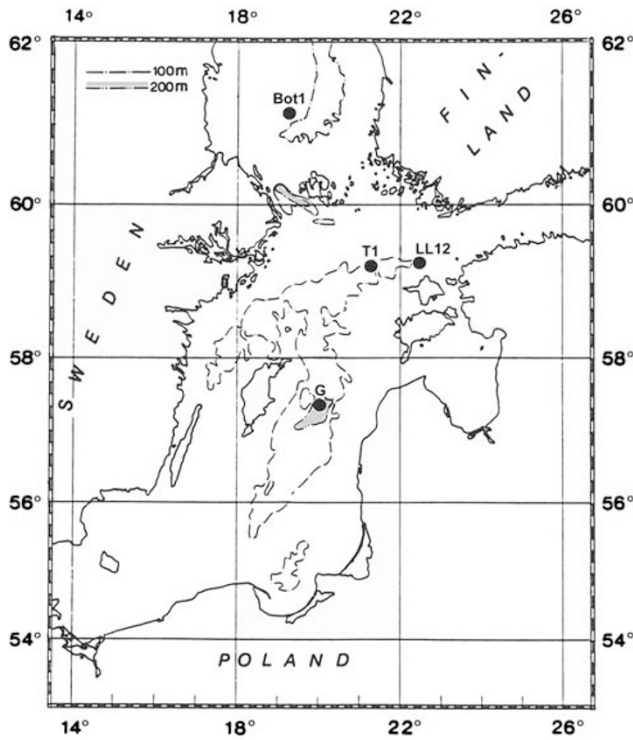
Figure 1 Location of the four stations in the central Baltic Sea used for the assessment of the horizontal and vertical extent of community structure of the bacterioplankton. Station G (Gotland Deep) was used for the assessment of the vertical extent with a depth profile to 225 m. Areas below 200 m are shaded gray.

increase in ammonium and hydrogen sulfide toward the sediment (Figures 2a and b). Bacterial numbers and production were highest in the surface water, and showed another increase at the oxic–anoxic interface (Figure 2c). Bacterial numbers were high in the anoxic water. Heterotrophic plate counts (colony-forming units) showed a maximum at 30–50 m, indicating the sedimentation of a plankton bloom caused by a stormy period 2 days before sampling. More details on the *in-situ* conditions and the biogeochemical processes associated are found elsewhere (Wulff *et al.*, 2001; Brettar *et al.*, 2006).

Overall community structure of bacterioplankton based on 16S rRNA and 16S rRNA gene fingerprints
We used SSCP fingerprints to assess the community structure of the bacterioplankton in the central Baltic and compared DNA- with RNA-based fingerprints to understand differences in the active versus the present bacterial taxa. A representative SSCP fingerprint comparing DNA- with RNA-based fingerprints is given in Supplementary Figure S1. The lane by lane comparison of the DNA- and RNA-based fingerprints revealed the following: first, in the surface samples of all stations, the DNA-based fingerprints were very different from the RNA-based fingerprints; second, the DNA-based fingerprints of the surface samples were very similar as were the RNA-based fingerprints; third, in the low-oxygen
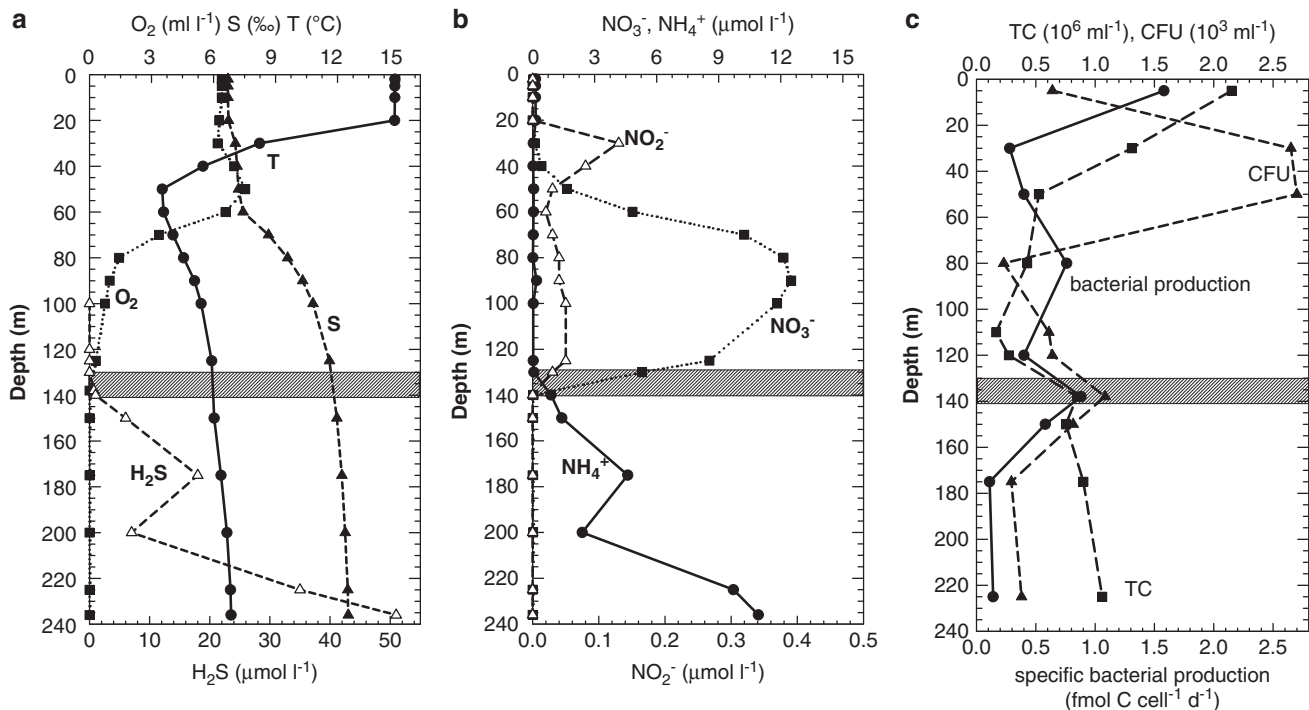

Figure 2 Depth profiles of physical, chemical and microbiological background parameters in the Gotland Deep (station G) on 17 September 1998. (**a**) Temperature, salinity, oxygen and hydrogen sulfite concentrations. (**b**) Concentrations of the major inorganic nitrogen compounds nitrite, nitrate and ammonium. (**c**) Major bacterial parameters: TC, total bacterial cell counts; CFU, colony-forming units on Baltic seawater agar, and bacterial production, details are given in Materials and Methods.

part of the water column (80–120 m, RNA data not shown in Supplementary Figure S1), the DNA and RNA-based fingerprints were becoming more and more similar; and fourth, in the anoxic part (138–225 m), the DNA and RNA-based fingerprints became almost identical. Cluster analyses of the fingerprints from the surface samples and the whole sample set were performed to substantiate these observations (Figure 3). The cluster analysis of the surface samples supported the first and the second finding and the fingerprints of the closer stations, that is, T1 and G showed a similarity of more than 80% for the DNA and the RNA-based fingerprints, whereas the least saline station Bot1 was substantially less similar (Supplementary Figure S2). The former was also the case for the fourth station LL12 in the Finnish bay (data not shown). The cluster analysis of all fingerprints substantiated the third and the fourth finding (Figure 3). The DNA-based fingerprints from the low oxygen samples (80–120 m) showed a distinct subcluster well separated from the anoxic samples. Most DNA- and RNA-based fingerprints of the same depth in the anoxic part were highly similar at around 80% similarity. Only the RNA-based fingerprint from 120 m was clustering among the anoxic fingerprints, indicating the transition of RNA-based fingerprints toward similarity with DNA-based fingerprints when approaching anoxic conditions. The most distant clusters of the DNA- and the RNA-based fingerprints of the surface samples also indicated that the deeper (30 and 50 m) samples still clustered together with the respective surface samples.
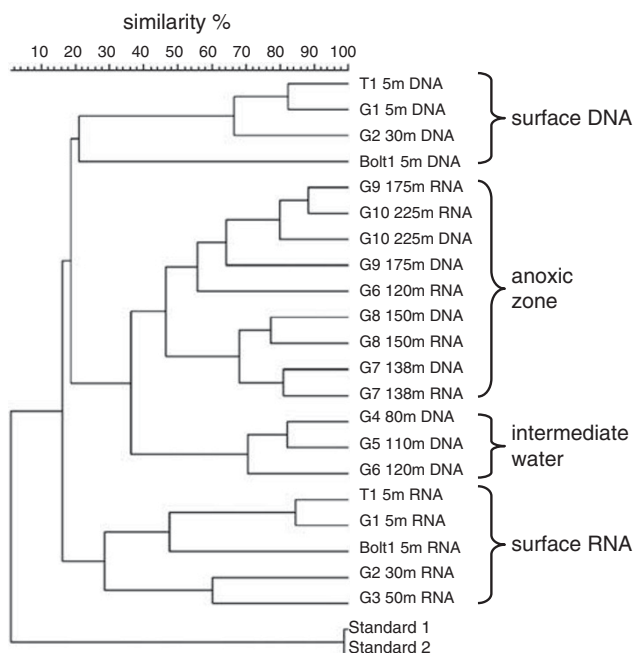
The relative intensity of the single bands from the fingerprints can be used to calculate the relative abundance of the single taxa present in the analyzed communities (Höfle et al., 1999). Using these relative abundances, rank-abundance curves allow the evenness to be evaluated, that is, the distribution of relative abundances of the single taxa within a community (Figure 4). Comparing the rank-abundance curves of surface communities (Figure 4a) with anoxic communities (Figure 4b) two observations were made: first, the number of phylotypes detected was almost twice as high in the surface as in the anoxic community, and second, DNA-based abundances in the surface were more pronounced than RNA-based abundances, that is, the first 5–8 most abundant DNA-based phylotypes were more dominant than the RNA-based phylotypes, as exemplified for station G in Figure 4a.

Richness, that is the number of taxa per sample, can be determined from SSCP fingerprints for bacterioplankton communities using a relative abundance of 0.1% as detection limit for a significant band in the SSCP fingerprints (Table 1). Below this detection limit, often a drop in the relative abundance is detectable in the rank-abundance curves as can be seen in Figure 4b. Comparing all surface samples, the DNA-based richness had on the average 47 taxa, and the RNA-based richness was on the average 58 taxa, that is, about 10 taxa higher than the DNA-based richness (Table 1, Figure 5). These differences between DNA- and RNA-based diversity estimates were also reflected in the Shannon index H and the evenness E (Table 1). The DNA-based Shannon index H for all surface samples was on the average 3.23, whereas the RNA-based Shannon index H was 3.65. DNA-based evenness E was on the average 0.84, whereas the RNA-based evenness E was 0.90. Comparing the four anoxic samples (135–225 m) with the surface indicated a lower diversity index H of 2.78 and an evenness E of 0.88 for the DNA-based estimates. The RNA-based diversity H for the anoxic samples was slightly higher than DNA-based with an average 3.27, whereas the RNA-based evenness was identical with the DNA-based evenness.

In surface samples, the RNA-based richness showed a less steep decrease in the rank-abundance curve than the DNA-based richness, as can be seen on Figure 5a. Along the depth profile in the Gotland Deep, a fairly constant richness was observed with the DNA-based fingerprints above the oxic–anoxic interface with an average of 53 taxa (Figure 5). In contrast, the RNA-based richness showed a higher variability in the upper water column ranging from 36 to 66 taxa. The anoxic part of the water column showed a strong decrease for both types of richness assessments with an average of 24 taxa for the DNA-based and 36 taxa for the RNA-based richness. Here the RNA-based richness was, similar to the surface samples, about 10 taxa higher than the DNA-based richness but with a rather similar rank-abundance curve (Figure 4b).
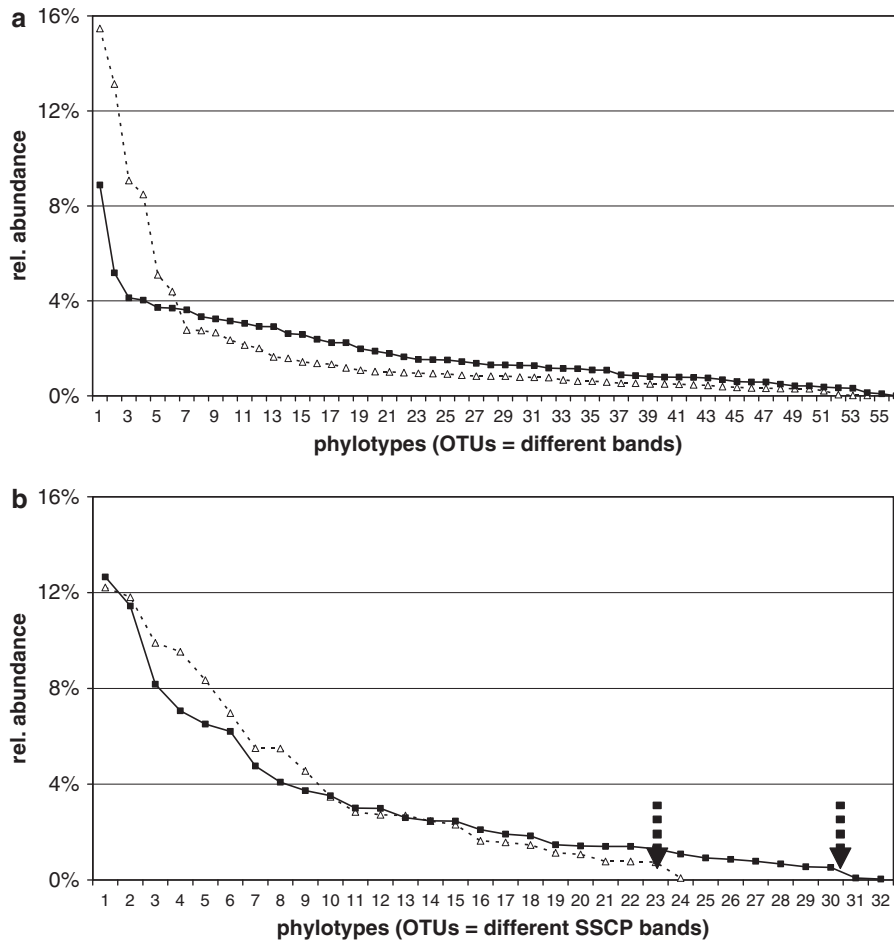

Figure 3 Cluster analysis of SSCP fingerprints from the different samples shown in Supplementary Figure S1. For all samples the station and sampling depth are indicated.

Figure 4 Comparison of rank-abundance curves of phylotypes from 16S rRNA (closed squares) and 16S rRNA gene (open triangles) based SSCP fingerprints from different bacterioplankton samples of the central Baltic Sea. (**a**) Gotland Deep (station G) 5 m, (**b**) 138 m (anoxic). Arrows indicate the respective detection limit of 0.1%.

For the assessment of the similarity of the RNA-based fingerprints with the DNA-based fingerprints, we used Pearson's coefficient for each fingerprint pair of the same depth (Figure 5). At the surface this similarity was about 20%, and it gradually increased to the oxic–anoxic interface where it reached a maximum of 81%. This similarity stayed high in the anoxic zone ranging from 65% to 77%. Comparable results were obtained with the Bray–Curtis coefficient (data not shown).

Phylotype composition of bacterioplankton based on 16S rRNA sequence analyses
More than 750 different bands from the 16S rRNA- and 16S rDNA-based SSCP fingerprints were sequenced in both directions with an average length of 380 nt to assess the community composition of the bacterioplankton in the central Baltic. From these sequences, 84 unique phylotypes were derived representing the detected taxa of the bacterioplankton community above the detection limit (Supplementary Table S1). These taxa belonged to 10 bacterial

phyla and the *Crenarchaeota*. As detailed in Figure 6, 22 phylotypes were detected on the DNA-based fingerprints, 37 phylotypes were detected on the RNA-based fingerprints and 25 phylotypes were detected on both types of fingerprints. Overall, this means that a combined DNA- and RNA-based analysis enabled the retrieval of 44% more phylotypes compared with a restricted analysis using only DNA-based fingerprints. *Actinobacteria* were primarily detected on DNA-based fingerprints, whereas *Deltaproteobacteria, Chloroflexi, Cyanobacteria* and *Deferribacteres* were only detected on RNA-based fingerprints or on both types of fingerprints. Several phyla were primarily found in the surface samples, such as *Actinobacteria, Cyanobacteria, Alpha-* and *Betaproteobacteria*. Other taxonomic groups, such as the *Delta-, Epsilonproteobacteria* and *Crenarchaeota*, primarily occurred in the suboxic to anoxic part of the water column, that is, below 110 m (Supplementary Table S1). Other major groups, such as the *Bacteroidetes* and *Gammaproteobacteria* did not show such a distinct vertical preference, despite the fact that most of

these phylotypes occurred in the oxic part of the water column. The two *Planctomycetes* phylotypes (PT 115, 121) occurred only at the suboxic zone, where ammonium and nitrite co-occurred, thereby
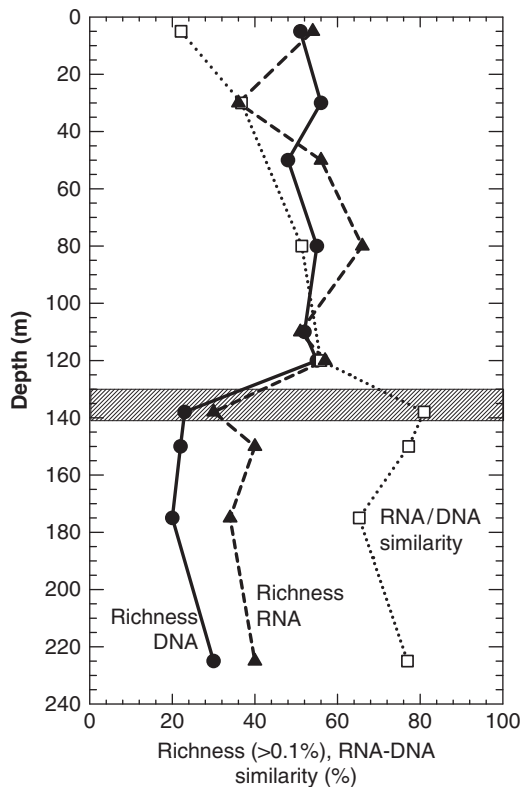

Figure 5 Comparison of RNA- (triangles) and DNA-based (dots) richness and the RNA to DNA similarity (open squares) calculated as described in the text from different bacterioplankton samples along depth at the Gotland Deep (station G).

pointing at their assumed biogeochemical function as catalysts of anaerobic ammonium oxidation. For a more detailed analysis of the bacterioplankton community composition, we will concentrate in the following on the surface and the anoxic community, because here the most significant differences occurred and functional details of these communities can be elucidated.

Horizontal surface community composition
A comparison of the three surface samples showed that the five most abundant DNA-based phylotypes (PT 5, 6, 10, 16, 49) comprised more than half of the community and belonged to only two taxonomic groups, that is, *Alphaproteobacteria* and *Actinobacteria* (Figure 7a, Table 2). In addition, none of these phylotypes was detected by the RNA-based fingerprints and they were all detected at more or less the same abundance at the three different stations studied in more detail, that is, G, T1 and LL12. The situation was very different for the community composition reflected by the RNA-based fingerprints. Here the five most abundant phylotypes (PT 14, 19, 43, 47, 87) were often only detected at a single station and were phylogenetically rather divers, that is, belonged to four different phyla. The two cyanobacterial phylotypes (PT 14, 16) demonstrated that the photosynthetic part of the bacterioplankton community at the surface was different between the two more central stations, G, T1, and the most eastern station LL12. The heterotrophic members of the surface community (PT 43, 47, 87) were more evenly distributed among the stations (Figure 7a), and were also represented by the DNA-based phylotypes with the exception of
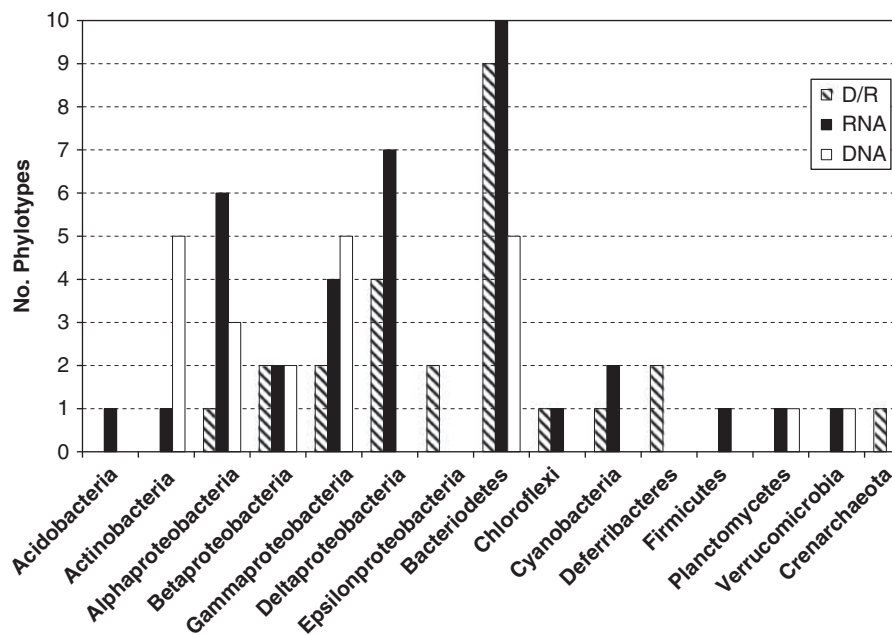

Figure 6 Taxonomic assignments of the phylotypes (Supplementary Table S1) detected in bands excised from DNA-(D) or RNA-(R) based fingerprints or that were recovered from both DNA- and RNA-based fingerprints (D/R).
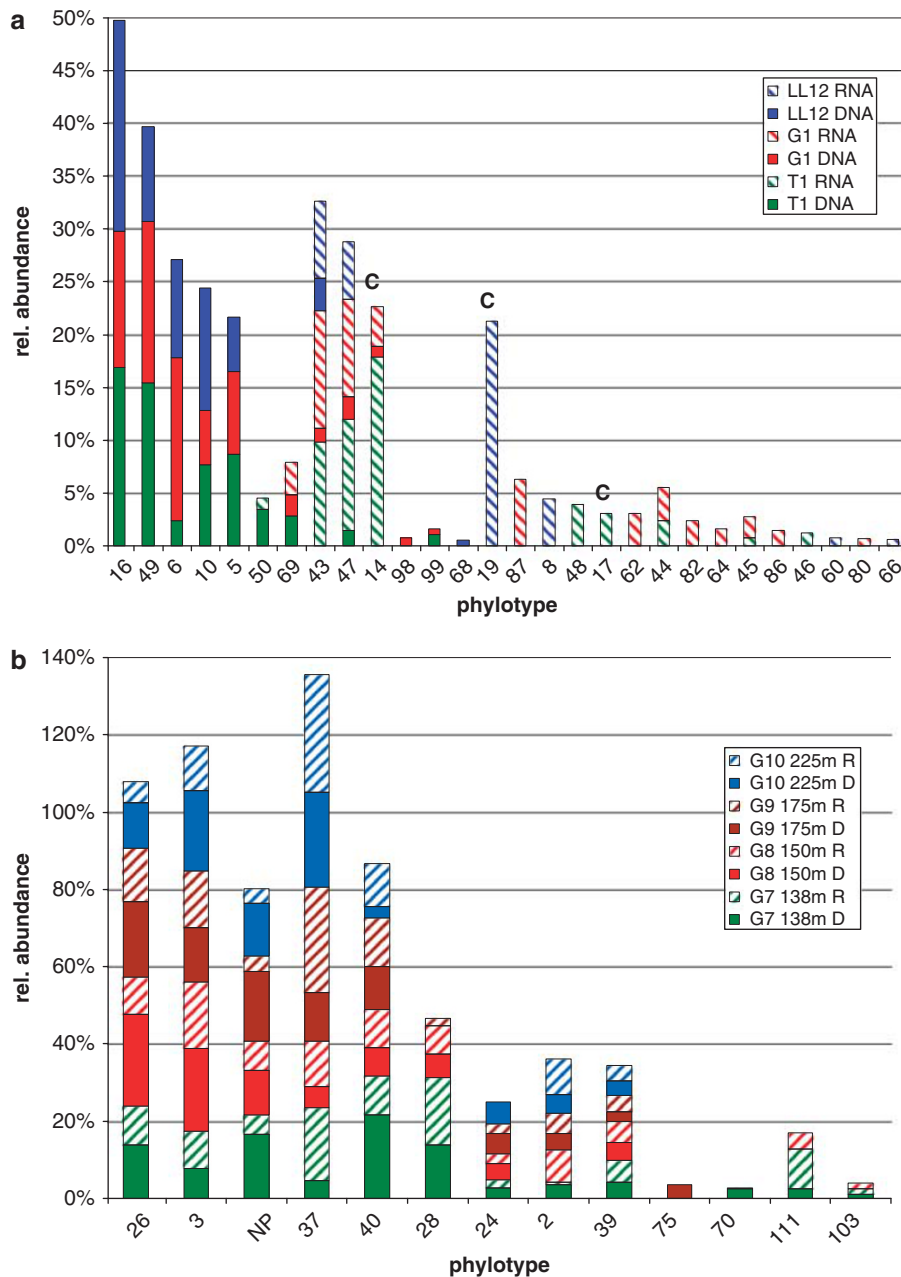
Figure 7 Relative abundances of the single major phylotypes in cumulative presentation sorted according to the criteria given in Tables 2 and 3. Averaged data are given in Tables 2 and 3, respectively. Full bars = DNA-based abundances, hatched bars = RNA-based abundances. (**a**) Surface stations G1, T1 and LL12 (Table 2); (**b**) anoxic samples of the Gotland Deep (station G) from G7 = 138, G8 = 150, G9 = 175 and G10 = 225 m (Table 3). Cyanobacterial phylotypes in Figure 7a are marked with a 'C'.

PT 87, which was only detected in the surface sample of the Gotland Deep (station G). Less abundant members of the community (below 2% relative abundance) representing the 'tail' of the rank-abundance curves (Figures 4a and b) were mostly detected on the RNA-based fingerprints.

Community composition in the anoxic deep water
The comparison of DNA- and RNA-based fingerprints by cluster analysis indicated a high similarity

of both types of fingerprints at and below the redox cline (Figure 3). This finding was corroborated by sequencing of the major bands, indicating that the anoxic community of the Gotland Deep consisted of a rather stable set of 8–10 key bacteria with no significant changes between 138 and 225 m (Figure 7b, Table 3). The detailed analysis of the relative abundances of these phylotypes revealed that most major phylotypes had a rather constant RNA to DNA ratio in all four anaerobic samples (Table 3). The RNA/DNA ratio varied for the single

Table 2 Major phylotypes of the bacterioplankton detected in the surface samples from the three stations, G=G1, T=T1 and L=LL12, of the central Baltic Sea above 0.5% relative abundance of the DNA-based fingerprints sorted according to their average relative abundances in DNA-based fingerprints followed by the RNA-based abundances

PT no.	Accession number	Closest described species	16S rRNA Sim. %	Phylum/class	Rel. average abundance DNA (%)	Rel. average abundance RNA (%)	RNA/DNA ratio	Sample/depth
16	FR714951	Pelagibacter ubique	92.4	Alphaproteobacteria	16.6	0.0	NA	T, L, G/5–120 m
49	FR714977	Pelagibacter ubique	85.8	Alphaproteobacteria	13.2	0.0	NA	T, L, G/5–120 m
6	FR714946	Planktophila limnetica	93.6	Actinobacteria	9.0	0.0	NA	T, L, G/5 m
10	FR714949	Pelagibacter ubique	98.8	Alphaproteobacteria	8.2	0.0	NA	T, L, G/5–120 m
5	FR714945	Planktophila limnetica	95.6	Actinobacteria	7.2	0.0	NA	T, L, G/5 m
50	FR714978	Chthoniobacter flavus	91.7	Verrucomicrobia	3.4	1.1	0.3	T
69	FR714988	Aureimarina marisflavi	96.0	Bacteroidetes	2.4	3.0	1.3	L, G/5 m
43	FR714971	Burkholderia sordidicola	93.9	Betaproteobacteria	2.2	9.4	4.3	T, L, G/5 m
47	FR714975	Coccinistipes vermicola	92.6	Bacteroidetes	1.8	8.4	4.7	T, L, G/5 m
14	FR714950	Synechococcus rubescens	99.0	Cyanobacteria	1.0	10.8	10.8	T, L, G/5–30 m
98	FR715004	Gracilimonas tropica	86.6	Bacteroidetes	0.8	0.0	NA	G/5 m
99	FR715005	Formosa crassostrea	93.9	Bacteroidetes	0.8	0.0	NA	G/5 m
68	FR714987	Gordonia sputi	84.4	Actinobacteria	0.5	0.0	NA	L
19	FR714954	Snowella litoralis	99.0	Cyanobacteria	0.0	21.3	NA	L, G/5–80 m
87	FR715003	Methylobacterium brachiatum	99.7	Alphaproteobacteria	0.0	6.3	NA	G/5 m
8	FR714948	Roseivirga ehrenbergii	99.3	Bacteroidetes	0.0	4.4	NA	L, G/5–80 m
48	FR714976	Thalassobaculum litoreum	94.4	Alphaproteobacteria	0.0	3.9	NA	T, L
17	FR714953	Synechococcus rubescens	97.6	Cyanobacteria	0.0	3.1	NA	L, G/5 m
62	FR714981	Gilvibacter sediminis	92.6	Bacteroidetes	0.0	3.1	NA	L, G/5 m
44	FR714972	Rhodothermus marinus	93.6	Bacteroidetes	0.0	2.8	NA	T, G/5–110 m
82	FR714998	Prolixibacter bellariivorans	86.8	Bacteroidetes	0.0	2.4	NA	G/5 m
64	FR714983	Aquamonas fontana	98.8	Betaproteobacteria	0.0	1.6	NA	L, G/5 m
45	FR714973	Jannaschia helgolandensis	96.8	Alphaproteobacteria	0.0	1.4	NA	T, G/5–110 m
86	FR715002	Owenweeksia hongkongensis	91.1	Bacteroidetes	0.0	1.4	NA	G/5 m
46	FR714974	Owenweeksia hongkongensis	88.9	Bacteroidetes	0.0	1.3	NA	T, L, G/5–120 m
60	FR714980	Nitrobacter novellus	99.3	Alphaproteobacteria	0.0	0.7	NA	L
80	FR714997	Owenweeksia hongkongensis	87.9	Bacteroidetes	0.0	0.7	NA	G/5 m
66	FR714985	Methylophilus methylotrophus	98.5	Betaproteobacteria	0.0	0.6	NA	L

Abbreviations: NA, not applicable; Rel., relative; Sim., similarity.

Table 3 Major phylotypes of the bacterioplankton detected in the anoxic part of the water column (138–225 m) of the central Baltic at station G above 1% relative abundance of the DNA-based fingerprints

PT	Accession numbers	Closest cultivated species[a]	16S rRNA Sim. %	Class/phylum	Average abundance DNA (%)	Average abundance RNA (%)	RNA/DNA ratio	Depth range detected (m)	Putative biogeochemical functions
26	FR714959	Sulfurimonas denitrificans	95.8	Epsilonproteobacteria	17.3	9.7	0.56	120–225	Sulfite oxidation, denitrification
3	FR714943	Clade* SAR 406	97.3[b]	Deferribacteres	16.0	13.3	0.83	120–225	?
NP	FR715023	Nitrosopumilus marinus	98.0	Crenarchaeota	15.0	5.0	0.33	110–225	Ammonium oxidation
37	FR714967	Vesicomyosocius okutanii	96.1	Gammaproteobacteria	11.8	22.1	1.87	80–225	Sulfur oxidation
40	FR714969	Polaribacter glomeratus	92.1	Flavobacteria	10.7	11.0	1.02	80–225	Polymer degradation
28	FR714961	Lutibacter litoralis	98.5	Flavobacteria	10.0	6.7	0.67	120–150	Polymer degradation
24	FR714957	Clade* SAR 406	95.0[b]	Deferribacteres	4.5	2.4	0.53	135–225	?
2	FR714942	Clade* SAR 202	96.0[b]	Chloroflexi	4.1	5.9	1.44	135–255	Mineralization of amino acids
39	FR714968	Desulfobacula toluolica	96.8	Deltaproteobacteria	3.8	4.8	1.26	120–225	Sulfate reduction
75	FR714993	Acinetobacter johnsonii	99.5	Gammaproteobacteria	3.7	0.0	NA	80–175	?
70	FR714989	Arcobacter butzleri	96.8	Epsilonproteobacteria	2.5	0.1	0.04	5–138	?
111	FR715015	Desulfocapsa sulfexigens	96.4	Deltaproteobacteria	2.5	7.3	2.92	138–150	Sulfate reduction
103	FR715007	Clade* OCS116	89.5[b]	Alphaproteobacteria	1.1	1.5	1.36	138–225	?

Abbreviations: NA, not applicable; Rel., relative; Sim., similarity.
Sorted according to their average relative abundances in the DNA-based fingerprints.
[a]Taxa that have no close cultivated species are named after their closest marine clade and are labeled with an asterisk.
[b]Similarity of the closest environmental sequence.

phylotypes from 0.3 to 3, but remained rather constant for the single major phylotypes, that is, PT 2, 3, 24, 26, 28, 37, 39, 40 and NP (Table 3). These nine key prokaryotic phylotypes of the bacterioplankton community had rather high relative abundances, ranging from 4% to 17% for DNA-based and 2% to 22% for RNA-based fingerprints (Table 3). The eight bacterial and the archeal phylotype formed the core community in the anoxic water column. The other four phylotypes, that is, PT 70, 75, 103 and 111, were not as abundant and less often detected. Phylogenetically, the anoxic core community was very diverse comprising two *Deferribacteres*, two *Bacteriodetes*, one *Chloroflexi*, one *Gamma-*, one *Delta-*, one *Epsilonproteobacteria* and one archeon.

Discussion

The current discussion about the community structure of marine bacterioplankton is focusing on two key questions: (i) Is there a biogeography of bacterioplankton communities across marine ecosystems (Dolan, 2005; Pommier et al., 2007) and (ii) What are the environmental factors regulating the community structure of bacterioplankton (Galand et al., 2010). On top of these questions remain the detailed understanding of the overall community structure of bacterioplankton, that is, what are abundant and rare species (phylotypes) and how do they influence the overall biogeochemical functioning of this community (Pedrós-Alió, 2006). For this overall structure, the understanding of the core community with the core taxa performing all major biogeochemical functions is essential (Höfle et al., 2008).

Horizontal community structure of surface bacterioplankton
The three surface communities (samples G1, T1, Bot1) had a rather high similarity, as indicated by cluster analysis, and the presence of the five dominating phylotypes (PT 16, 49, 6, 10, 5) with the highest relative abundance in all surface samples (Supplementary Figure S2, Table 2). The similarity of the DNA- and RNA-based fingerprints was more than 80% for station G and T1, which are about 220 km apart (Supplementary Figure S2). The community of the most Northern station, Bot1, which is another 2° north of station T1, still had a recognizable similarity, that is, about 20% for the DNA-based and 45% for the RNA-based fingerprints in comparison with the two Southern stations.

Currently, no studies are available comparing the community structure of bacterioplankton in the surface waters of the central Baltic on a large scale across the ecosystem. Its stability over several hundred kilometers based on the CORDAF might

reflect the stable hydrographic conditions of the surface waters, typical for the central Baltic in late summer (Stigebrandt, 2001). A comparable high degree of similarity for surface bacterioplankton was also observed recently in the Bothnian Sea during summer, across about 200 km using DGGE fingerprints (Holmfeldt et al., 2009). More intriguing in our study is the large discrepancy of the RNA- and DNA-based community structure and the major taxa identified with only five phylotypes out of 28 observed in both types of fingerprints (Figure 7a). We offer two hypotheses that are not mutually exclusive: (i) the two taxonomic groups, *Actinobacteria* and *Alphaproteobacteria*, comprising the five core phylotypes (PT 16, 49, 6, 10, 5) have in general very little RNA in comparison with their DNA and (ii) the five core phylotypes are rather inactive compared with the other 23 phylotypes additionally observed by RNA-based fingerprinting (Table 2).

Vertical community structure of bacterioplankton in the Gotland Deep
The vertical extent of the bacterioplankton communities in the Gotland Deep has been studied first in 1995 using 5S rRNA fingerprinting (Höfle and Brettar, 1995). More recently, several studies were done focusing on the surface water or the redox cline of the central Baltic, but no complete depth profile was analyzed until now (Grote et al., 2007; Labrenz et al., 2007; Andersson et al., 2009). Our fingerprint analyses showed three clearly separated major communities: (i) the aerobic surface community (5–50 m), (ii) the intermediate water community (80–120 m) and (iii) the deep water community (138–225 m; Figure 3). These major communities related well to the hydrographic structure of the water column (Stigebrandt, 2001). The richness did not show this trichotomy, it only dropped when anoxic conditions were reached by more than half for DNA-based and about one-third for RNA-based fingerprints (Figure 5). The values for richness, ranging from 22 to 66, correspond well with other fingerprint-based studies (Kan et al., 2007; Treusch et al., 2009). The drop in richness in the deeper part of the water column of the Baltic is in contrast to the large ocean basins, where below the euphotic zone an increase of richness has been observed (Hewson et al., 2006a; Treusch et al., 2009). This increase has been explained by an increase in less abundant members of the bacterioplankton due to more diverse organic compounds generating more niches for aerobic heterotrophic bacteria. The decrease in richness in the anoxic part of the Baltic might be due to the limited groups of bacteria that could thrive without oxygen and use the available mix of electron donors and acceptors.

Various vertical major communities along the water column have been observed in many aquatic ecosystems, ranging from open oceans to small lakes (Moeseneder et al., 2001; Dominik and Höfle, 2002;

Treusch et al., 2009). In comparison with large ocean basins, the vertical extent of the central Baltic is more than an order of magnitude less. In addition, the uniqueness of the Baltic ecosystem, primarily the strong freshwater influence and the shallow, complex morphology, might ask for a specific analyses of the composition of the bacterioplankton (see below). In contrast, the overall community structure with three major communities was also observed in comparable ecosystems, such as the Black Sea and the Cariaco Basin (Vetriani et al., 2003; Lin et al., 2006; Grote et al., 2007). These overall similarities support the general hypothesis that the basic physicochemical features structure the bacterioplankton communities accordingly (Galand et al., 2010).

Composition of the core communities of bacterioplankton in the central Baltic Sea
The composition of a complex community is in general defined by the sum of all abundances of all taxa occurring in a given habitat. In operational terms for a seawater sample, we sequenced all major bands of a fingerprint and calculated their relative abundances from the band intensities above a certain threshold level of abundance (Tables 2 and 3). Understanding the core community of the anoxic zone is rather straightforward due to its limited number of taxa (Table 3, Figure 7b). The observed key taxa in the anoxic community of the Gotland Deep consisted of 12 bacteria and an archaeon with a rather balanced RNA to DNA ratio of about 1.2, if phylotype PT 75 and 70 were excluded, which had no significant amount of RNA (Table 3). These prokaryotes had a rather even distribution of RNA and DNA in the whole deep water and could form the core community (Figure 7b). The top six members of this community comprised about 80% of the bacterioplankton based on DNA fingerprints and had abundances of more than 10% each (Table 3). All six core phylotypes have been identified before in the central Baltic and at least two of them, closely related to *S. denitrificans* (PT 26) and *N. marinus* (PT NP), have a substantial role in the sulfur and nitrogen cycle of the deep water (Brettar et al., 2006; Labrenz et al., 2007, 2010). Are these six taxa the core of the core community or are the other seven bacterial taxa (PT 24 to 103) playing a major role as well? At least two phylotypes (PT 39, 111) represent well-known sulfate-reducing taxa and could function as sulfate reducers in the deep water (Muyzer and Stams, 2008). As these less abundant taxa add a substantial biogeochemical function to the bacterioplankton community, they are part of the core community. Among the DNA-based phylotypes with abundances above 1%, we observed only two phylotypes (PT 75, 70) with no or very low RNA-based abundances. This might be due to an inactive state in the anoxic zone, as can be assumed for phylotype 70 that mainly occurred in

the oxic part (Table 3) or that the phylotype was not sequenced due to low abundance in the RNA pattern (PT 75). Overall, we think that the 1% threshold for relative abundance in DNA-based fingerprints might be a robust criterion for identifying in operational terms members of the core community in the anoxic deep water, if they also were observed in the RNA-based fingerprints and were detected along the depth profile primarily in the anoxic part of the water column (Table 3).

In the surface water, the first five most abundant phylotypes (PT 16, 49, 6, 10, 5) comprised more than half of the bacterioplankton community (Table 2, Figure 7a), but were phylogenetically not very diverse, that is, they only belonged to two clades, SAR 11 and ac I (Supplementary Figures S3 and S4). The two actinobacterial phylotypes (PT 6, 5) were rather similar and their high abundance in the Northern Baltic was recently demonstrated by CARD FISH (Holmfeldt et al., 2009). The three SAR 11 phylotypes (PT 16, 49, 10) were phylogenetically rather diverse (Supplementary Figure S3) and might represent three different genera with only phylotype PT10 being a representative of the species *Pelagibacter ubique*. All information available about the biogeochemical functioning of both phylogenetic groups point into the direction of aerobic mineralization of small organic molecules, such as amino acids, at low concentrations (Malmstrom et al., 2007; Jezbera et al., 2009). There was a significant drop in relative abundances after these first five phylotypes, that is, all other phylotypes had on the average abundances lower than 4% (Table 2, Figure 4a). Only the next eight phylotypes (PT 50, 69, 43, 47, 14, 98, 99, 68) showed DNA-based abundances above 0.5% relative abundance (Table 2). These eight phylotypes added four new phyla/classes and half of them were *Bacteriodetes*. The four *Bacteriodetes* (PT 69, 47, 98, 99) were highly diverse and belonged to four distant clades, which were different at least at the family level (Supplementary Figure S5). Some of these bacteriodetal phylotypes have close cultivated relatives that degrade polymers, especially complex carbohydrates, and are known to be abundant in pelagic ecosystems (Höfle, 1992; Kirchman, 2002). In conclusion, the 13 most abundant DNA-based phylotypes in the surface bacterioplankton might do most of the aerobic mineralization of the fixed carbon, except *Synechococcus rubescens* (PT 14), which is one of the dominating phototrophic *Cyanobacteria* during late summer in the central Baltic (Stal et al., 2003)

The three abundant *Cyanobacteria* (PT 14, 17, 19) observed in the surface water all showed exceptionally high RNA content or were only seen by the RNA-based fingerprints, respectively (Table 2, Figure 7a). Such a high RNA content for *Cyanobacteria* has also been observed in the central Baltic by Labrenz et al. (2007) and in the coastal Mediterranean Sea (Lami et al., 2009). The high RNA to DNA ratio is a general feature of *Cyanobacteria* and might be explained either by their high growth rate (Binder and Liu, 1998) and/or by the storage function of RNA for nitrogen and phosphorus in these oxygenic phototrophs (Cuhel and Waterbury, 1984; Asato, 2003). All three *Cyanobacteria* belong to the picocyanobacteria that contribute more than 50% to the primary production of the central Baltic in late summer (Stal et al., 2003). Overall, we think that the major phylotypes observed in the surface bacterioplankton function as key players of the carbon cycle, that is, they catalyze primary production and mineralize low- and high-molecular organic compounds.

For the understanding of the biogeography of the observed phylotypes, we will compare their occurrence at the regional scale, that is, in all of the Baltic, and on a global scale, that is, in all pelagic communities studied. In the surface community, the occurrence of the three Pelagibacter-like taxa (PT 16, 49, 10) reflect the well-known global presence of this group in aquatic ecosystems, but only phylotype 10 represents a marine ecotype, whereas the other two phylotypes are freshwater or estuarine ecotypes (Carlson et al., 2008). The two actinobacterial phylotypes represent freshwater taxa that occur in dependence of the freshwater influence in a North-South gradient of the Baltic, as demonstrated in detail by Holmfeldt et al. (2009). These observations reflect the niche-ecotype correlation at the specific habitat level as pointed out by Koeppel et al. (2008).

Concerning the biogeography of the major phylotypes of the core community of the anoxic zone (Table 3), at least two of the most abundant phylotypes (PT 26 and NP) are specific for this anoxic marine environment (Brettar et al., 2006; Grote et al., 2008; Labrenz et al., 2010). Especially phylotype NP, despite the global occurrence of *Nitrosopumilis marinus*, was shown to belong to a distinctly different clade in the central Baltic, based on its *amoA* gene sequence (Labrenz et al., 2010). The representatives of the phyla *Deferribacteres* (PT 2, 24) and *Chloroflexi* (PT 2) belonged to the globally abundant clades SAR 406 and SAR 202, respectively. Both of these clades are encountered below the photic zone in meso-pelagic waters of marine ecosystems, but all three Baltic phylotypes represent deep routing members of these clades, which could be specific taxa for the Baltic Sea (Schattenhofer et al., 2009). Phylotype 37 (50–138 m) belongs to the gammabacterial sulfur oxidizers found in many oxygen minimum zones of pelagic marine ecosystems related to chemoautotrophic gill symbionts of deep-sea mussels (Walsh et al., 2009). The two flavobacterial phylotypes (40, 28) were phylogenetically rather unique and might represent taxa specific for the Baltic Sea, despite their high 16S rRNA gene similarity to cultured species (Supplementary Figure S5). The two deltaproteobacterial phylotypes (PT 39, 111) clustered closely with many environmental

sequences and cultured species of sulfate reducers. Phylotype 103 represented a deep routing alphaproteobacterium and can be considered unique to the anoxic Baltic, but its function remains unknown.

Concerning the biogeography and the biogeochemical function of the specific phylotypes observed in the central Baltic, the following caveats have to be made: (i) all relative abundance assessments for the phylotypes might be skewed due to well-known PCR and primer bias but highly reproducible if certain rules are followed (Engelbrektson et al., 2010); (ii) the taxonomic resolution of the 16S rRNA gene is limited to around the species level and might only resolve ecotypes in special cases (Höfle et al., 2008); (iii) the biogeochemical function of a specific phylotype can only be assessed if closely related (16S rRNA gene similarity 97–99%) species have been cultured and/or functional in-situ studies provided evidence for the specific function (Green et al., 2008). Given these caveats, we think that most of the major taxa (PT 26, 3, NP, 40, 28, 24, 2, 70, 103) observed in the deeper part of the central Baltic are indicative or highly specific for the anoxic zone, and the putative biogeochemical functions given in Table 3 provide reasonable guesses for future in-situ studies using functional and/or metagenomic approaches.

Utility of CORDAF for the understanding of bacterial communities
The central observation of this study was the large discrepancy between the RNA- and DNA-based fingerprints of the bacterioplankton at the surface that gradually changed along depth to almost complete identity of both types of fingerprints in the anoxic part of the Gotland Deep (Figure 5). The discrepancy between RNA- and DNA-based studies of surface bacterioplankton has been observed in marine and freshwater ecosystems (Moeseneder et al., 2001; Troussellier et al., 2002; Lami et al., 2009; Treusch et al., 2009; Logue and Lindström, 2010). Most studies explained this phenomenon by hypothesis 2, that is, some of the present bacteria are not as active as others due to the high dynamics of surface water in terms of the diurnal light cycle. This reflects the idea to explain the RNA/DNA paradox by understanding that 'RNA-based techniques may reflect a history of activity rather than indicate which organisms are currently active' as recently summarized by Prosser et al. (2010). This explanation might not be sufficient to explain that the five most abundant phylotypes were not detected by RNA-based fingerprints. In this case, hypothesis 1 is supported by the well-known small cell sizes of the SAR 11 and *Actinobacteria* group I and that the RNA to DNA ratio tends to increase with cell size (Kemp et al., 1993). This line of reasoning is supported by in-situ studies in drinking water, freshwater and seawater, where freshwater *Actinobacteria* and the SAR 11 group could be

detected by DNA-based but not by RNA-based methods (Kolmonen et al., 2004; Eichler et al., 2006).

More exceptional for aquatic ecosystems than the large 'RNA–DNA community discrepancy' at the surface was the gradual reduction of this discrepancy down to the anoxic zone, where almost all major phylotypes were detected at comparable amounts in both types of fingerprints in all anoxic samples studied (Figure 7b). This high similarity could indicate that most present core taxa were active and might perform their specific biogeochemical functions summarized in Table 3, as shown for phylotype 26 by experimental studies and MICRO-CARD-FISH (Brettar et al., 2006; Grote et al., 2008). The overall similarity of the RNA- and DNA-based structure of the bacterial community is exceptional for pelagic ecosystems, but common in soils and sediments (Koizumi et al., 2003; Prosser et al., 2010).

In summary, CORDAF of bacterial communities has the following advantages: (1) increased resolution of the community structure toward less abundant but more active taxa due to an amplified taxonomic signal by the rRNA; (2) provision of information of the overall activity of single taxa if the RNA to DNA ratio can be assessed; (3) CORDAF similarity indexes can be used as a global measure for the microbial community to assess the dynamics of a habitat; (4) assumptions about the biogeochemical function of the major taxa can be made if the phylogenetic information provides hints and the RNA to DNA ratio is high.

Potential role of oxidative stress as mechanism to increase bacterial RNA content in the anoxic zone
A high RNA content of bacterial cells has been observed previously in addition to the high similarity of RNA- and DNA-based fingerprints in the anoxic zone of the central Baltic (Brettar et al., 2006). Furthermore, low cellular activity was observed in the water below the interface from 150 m downward (Figure 2c). This low activity contrasts with a bacterial cell volume that is in the anoxic layer by a factor of three higher than in the oxic water (Gast and Gocke, 1988) coinciding with a high cell number. This increased 'anoxic' biovolume has also been reported from a survey of lakes, where in anoxic hypolimnia the bacterial biomass exceeded those of oxic hypolimnia by a factor of four, irrespective of environmental factors such as the trophic state or temperature (Cole et al., 1993). A different degree of oxidative stress for oxic and anoxic layers could contribute to the observed phenomenon of high cellular RNA content and biomass versus low activity.

Although many studies on the role of oxidative stress have been performed in medical microbiology, little attention has been attributed to oxidative stress in microbial ecology (Hassett and Cohen, 1989; Imlay, 2008). Oxygen is an excellent electron

acceptor for the use of organic substrates, but life in an oxic environment is not free of costs due to the inherent oxidative damages caused by reactive oxygen species (ROS; Hassett and Cohen, 1989; Touati, 2000; Imlay, 2008). The production of intracellular ROS is highly dependent on the oxygen content in the environment because oxygen passes easily the cell membrane. In addition, external sources, such as ROS produced by photochemical reactions (ultraviolet, visible light), protozoan grazing and competing bacteria, cause oxidative stress for bacterial cells. These ROS cause a large variety of damages to nucleic acids, lipids and proteins. Although a set of antioxidants, detoxifying enzymes and repair enzymes/systems present in 'oxic' microorganisms enable a response to the threat by ROS, the needed energy for this ROS defense is critical under conditions of nutrient limitations. A set of regulators (OxyR, PerR, SoxR) is inducible by ROS and is known to drive a rapid and comprehensive response to protect the bacterial cell from ROS damage (Imlay, 2008).

In general, a strong decline of bacterial biomass was observed in many studies under conditions of nutrient limitation and oxic starvation of bacteria (Matin et al., 1989; del Giorgio and Cole, 1998). This contrasts with observations under anoxic conditions. A number of studies on survival/starvation of oxic or facultative anoxic aquatic bacteria (water and sediment) is available that show an improved survival under anoxic compared with oxic conditions (Jörgensen and Tiedje, 1993; Roslev and King, 1995; Roslev et al., 2004). In these studies, the bacteria showed a rapid return to high-metabolic rates upon the end of starvation and very low degradation of cellular biomass under anoxic starvation, including nucleic acids. On the basis of their studies on oxic starvation, McDougald et al. (2002) assumed that oxidative stress is a phenomenon that is most relevant to survival of bacteria, especially under conditions of nutrient limitation. We therefore conclude that oxidative stress is an important factor for the reduction of bacterial biomass under low-nutrient conditions in oxic, but not in anoxic environments. We assume that growth without oxidative stress is more 'balanced', means that the bacterial biomass is not much reduced when periods of starvation occur, and growth is more rapidly resumed on new pulses of nutrients. In terms of RNA abundance we assume that, similar to all other cellular biomass components, the RNA is far less degraded during anoxic starvation, indicating that the RNA as a 'signal of activity' is kept longer after the onset of starvation leading to a prolonged stability of a relatively high RNA–DNA ratio (on average 1.2 in the anoxic deep water, Table 3, Figure 7b).

As the intracellular production of ROS is highly dependent on the oxygen concentration in the environment, we assume different oxidative stress for the three layers in the central Baltic, with high oxidative stress in the oxic surface layer, reduced oxidative stress in the low oxygen layer, and very low oxidative stress for the anoxic deep water. On the basis of these considerations, we assume that in the central Baltic the increase of the RNA–DNA community similarity, the high RNA content in the anoxic water coinciding with high biomass per cell and low bacterial production could be attributed, at least to some extent, to the reduced oxidative stress.

Conclusions

Our study demonstrated the stability of the structure and composition of bacterioplankton in the surface waters of the central Baltic across several hundred kilometers, and its gradual changes along depth until the anoxic zone where it remained constant. CORDAF of bacterial communities was introduced as an approach to obtain additional functional information on the single core taxa and provided clues on their biogeochemical functioning if integrated with the physicochemical conditions of the respective habitat. CORDAF of bacterioplankton revealed large discrepancy between the different types (RNA-, DNA-based fingerprints) of fingerprints in surface water, whereas the anoxic deep water showed an unexpected high similarity of both types indicating a stable and active core community in this layer. Low oxidative stress could be a conceivable mechanism explaining the increase of similarity between the RNA- and DNA-based community fingerprints in the anoxic zone.

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