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Diversity and activity of marine bacterioplankton during a diatom bloom in the North Sea assessed by total RNA and pyrotag sequencing

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ABSTRACT

A recent investigation of bacterioplankton communities in the German Bight towards the end of a diatom-dominated spring phytoplankton bloom revealed pronounced successions of distinct bacterial clades. A combination of metagenomics and metaproteomics indicated that these clades had distinct substrate spectra and consumed different algal substrates. In this study we re-analyzed samples from the initial study by total community RNA (metatranscriptomics) and 16S rRNA gene amplicon sequencing. This complementary approach provided new insights into the community composition and expressed genes as well as the assessment of metabolic activity levels of distinct clades. *Flavobacteria* (genera *Ulvibacter*, *Formosa*, and *Polaribacter*), *Alphaproteobacteria* (SAR11 clade and *Rhodobacteraceae*) and *Gammaproteobacteria* (genus *Reinekea* and SAR92 clade) were the most abundant taxa. Mapping of the metatranscriptome data on assembled and taxonomically classified metagenome data of the same samples substantiated that *Formosa* and *Polaribacter* acted as major algal polymer degraders, whereas *Rhodobacteraceae* and *Reinekea* spp. exhibited less specialized substrate spectra. In addition, we found that members of the *Rhodobacteraceae* and SAR92 clade showed high metabolic activity levels, which suggests that these clades played a more important role during the bloom event as indicated by their *in situ* abundances.

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1. Introduction

Phytoplankton accounts for less than 1% of the photosynthetic biomass on Earth, yet is estimated to contribute half of the world's net primary production (Field et al., 1998). A minor fraction of the phytoplankton biomass sinks to the sea floor and, if not decomposed in the sediment, can end up as kerogen, the source of future oil and gas reservoirs (Kirchman et al., 2009). The vast majority however is rapidly consumed by higher organisms such as protists, copepods and fish as well as by the prokaryotic fraction of the plankton, the so-called bacterioplankton. Bacterioplankton hence plays a pivotal role in the recycling of phytoplankton biomass and thus controls a substantial fraction of the global carbon flux (Kirchman et al., 2009) in a process that is known as the 'microbial loop' (Davies et al., 2012).

Marine phytoplankton is comprised of photosynthetic bacteria such as cyanobacteria, but the bulk of its biomass consists of uni- to pluricellular algae like diatoms and haptophytes. Bacterial communities that decompose algal biomass in the pelagic zone are diverse and consist of different heterotrophic taxa with varying ecological strategies (Giovannoni and Stingl, 2005). Several studies based on culture-independent 16S ribosomal RNA gene sequence (16S rDNA) analysis have provided insights into these communities in terms of composition (Gilbert et al., 2012; Romano et al., 2005; Verslyppe et al., 2010, 2013), but little is known about the dynamics and functional interactions within such communities. Transcriptome-based approaches have been used in several studies to tackle these questions (Gilbert et al., 2008; Hewson et al., 2009; Poretsky et al., 2005, 2009, 2010; Vila-Costa et al., 2010), but it is still not fully understood, how a multitude of eukaryotic and prokaryotic planktonic species coexist in a seemingly homogenous habitat with limited resources (Glöckner and Kottmann, 2011). The relationships between these species range from mutualism to competition and even predation (Romano et al., 2005). Algicidal bacteria are known to affect algal bloom dynamics (Mayali and Azam, 2004), and vice-versa algae release compounds that inhibit bacterial growth (Ribalet et al.,

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2008). Hence there are plenty of reasons why species get extinct by competition and only a limited number of highly competitive species should prevail. However, planktonic species exhibit a great level of diversity, which is known as 'the paradox of the plankton' (Hutchinson, 1961). It has been argued that this might be due to the dynamics of environmental changes, favoring certain species at certain time points.

In a previous study, we used an integrated proteogenomic approach to investigate the bacterioplankton's response to a diatom-dominated spring phytoplankton bloom in German Bight of the North Sea in the year 2009 (Teeling et al., 2012). We observed a tight succession of distinct blooming bacterial clades that was most pronounced for *Flavobacteria* (genera *Ulvibacter*, *Formosa*, and *Polaribacter*) and *Gammaproteobacteria* (genus *Reinekea* and SAR92 clade species) (Fig. 1). The combined analysis of metagenomes and metaproteomes from different time points throughout the succession uncovered differences in the gene repertoires and expression profiles of distinct clades.

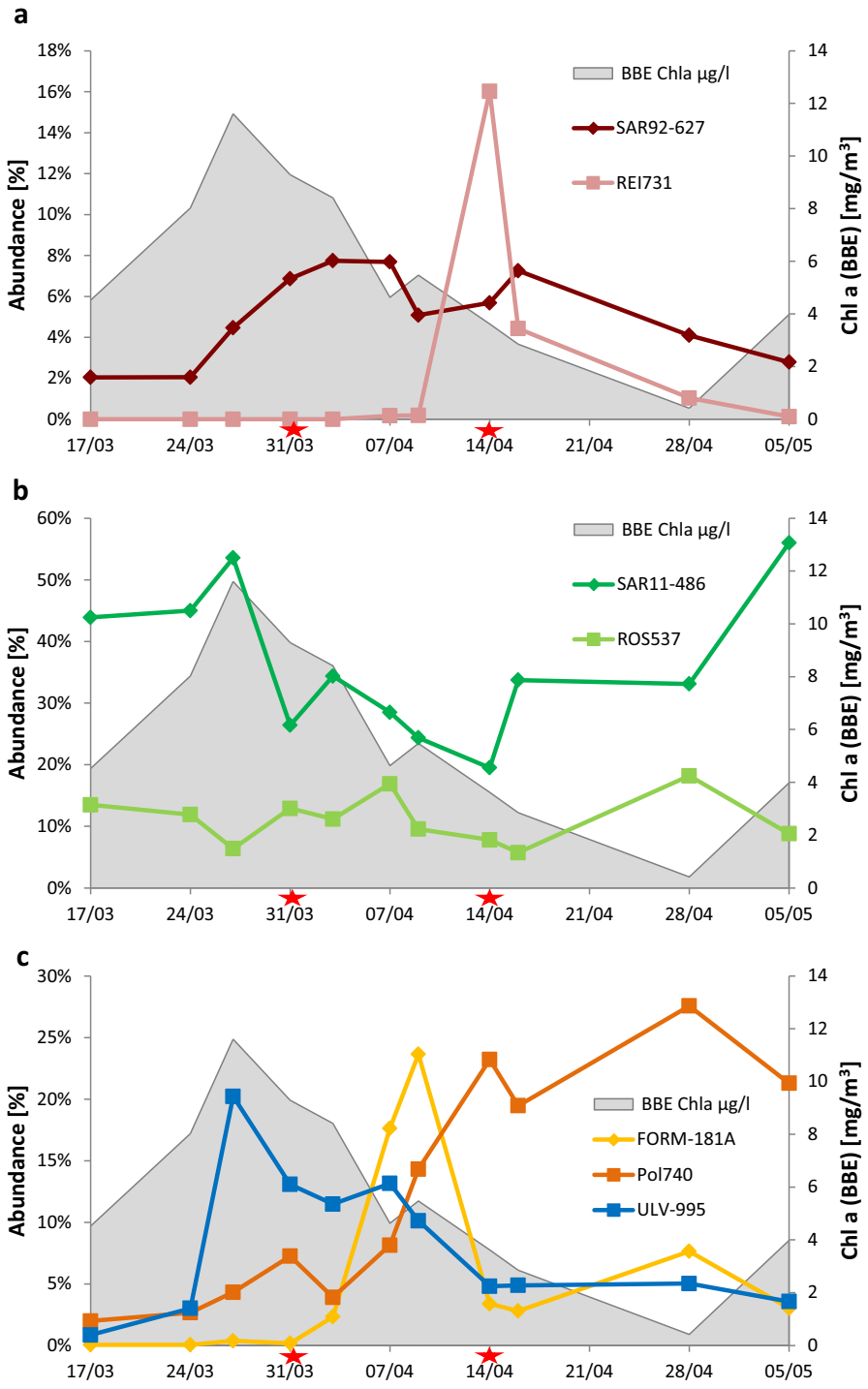

Fig. 1. Abundances of seven major bacterial populations during the bacterioplankton bloom as assessed by CARD-FISH and Chlorophyll a (Chl a) concentration (measured with a BBE Moldaenke algal group analyzer) (a) Relative abundances of selected *Gammaproteobacteria*: *Reinekea* spp. (probe REI731) and SAR92 clade (probe SAR92- 627). (b) Relative abundances of selected *Alphaproteobacteria*: SAR11 clade (probe SAR11-486) and Roseobacter clade (probeROS537). (c) Relative abundances of selected *Flavobacteria: Ulvibacter* spp. (probe ULV-995), *Formosa* spp. (probe FORM-181A), and *Polaribacter* spp. (probe POL740). Modified from Teeling et al. (2012). Red star indicates metatranscriptome sampling dates used in this study.

These differences suggest that the clades are specialized on different substrates, and that the succession was mainly a bottom-up controlled (i.e. substrate-driven) and not a top-down controlled (i.e. predator-driven) process.

In the current study we analyzed two metatranscriptomes from the same sampling campaign, one from before (31/03/2009) and one from amidst (14/04/2009) the phytoplankton bloom to provide complementary insights into the community composition, as well as the gene expression of dominating community members. We sequenced the metatranscriptomes (mRNA transcribed into cDNA) of both samples using Roche 454 pyrosequencing, and additionally the March sample was sequenced with Illumina technology. We furthermore compared metatranscriptome-based biodiversity estimates with biodiversity data derived from pyrotag sequencing and previous metagenome 16S rDNA analyses. In addition, we interrelated 16S rDNA expression levels with taxon abundance estimates of distinct taxa and used this as a proxy to identify the metabolically most active community members. Combining these analyses allowed us to reproduce the key aspects of the previous study and provided new insights into the ecological strategies of the most abundant community members.

2. Materials and methods

2.1. Sampling site and sample collection

Sample collection was carried out as part of the MIMAS (Microbial Interaction in Marine Systems) project as described previously (Teeling et al., 2012). In brief, surface water was collected from the site 'Kabeltonne' off the coast of the island Helgoland in the German Bight of the North Sea (54°11.18′N, 7°54.00′E) on 11/02/2009 and weekly from the 31/03/2009 until 24/11/2009. The samples (360 L) were collected at a depth of about 0.5 to 1 m, and processed immediately. The water samples were pre-filtered through 10 μm and 3 μm pore-size filters onto 0.2 μm pore-size filters, from which material was harvested for nucleic acids extraction. For DNA 25 L and for RNA 10 L of the original water sample were filtered on four filters each. All filters were stored at -80 °C until use. For this study we used sample material from the 31/03/2009 and 14/04/2009.

2.2. RNA extraction and mRNA enrichment

Filters were incubated in 10 mL of Chomczynski's Solution D (Chomczynski and Sacchi, 1987). The suspension was incubated for 5 min at room temperature (RT). Cells were lysed by beadbeating (lysing matrix B, material: 0.1 mm silica spheres; MPBiomedicals, Berlin, Germany) applying a FastPrep 24 automated homogenizer (MPBiomedicals). Three steps of 30 s (speed: 6 m/s) were performed, while cooling the tubes on ice in between beadbeating steps. After the third step, the beadbeater tubes were incubated on ice for an additional 10 min. Next, the tubes were centrifuged at 4 °C for 10 min (5415 C, Eppendorf, Hamburg, Germany; 13200 rpm, rotor FA-45-24-11). Supernatants (1 ml each) were transferred into RNase-free, sterile 1.5 mL Eppendorf vials. 200 μL of ice-cold chloroform were added per sample. Suspensions were thoroughly mixed by vortexing for 20 s, followed by a 2 min incubation step at RT. A further centrifugation step was carried out (4 °C, 15 min, 13,200 rpm). The aqueous upper phase was transferred into new RNase-free and sterile Eppendorf vials. 1 mL of 100% isopropanol was added, followed by 1 h incubation at -20 °C. Afterwards, a 30 min centrifugation step was performed (4 °C, 13,200 rpm). The supernatants were discarded and pellets were washed twice in 75% ethanol. Dried pellets were dissolved in 50–100 μl RNase-free water. Extracted RNA was cleaned using the RNeasy MinElute clean-up kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with the following modification: in the second step, 700 μl instead of 250 μl 96% ethanol were used. The eluted RNA was treated with TURBO™ DNase (Ambion, Austin, TX, USA) following the manufacturer's instructions to remove DNA contaminations. The concentration and quality of eluted RNA was determined using a NanoDrop® spectrophotometer (Thermo Fisher Scientific, Wilmington, MA, USA). The amount and quality of extracted and cleaned RNA was also documented by RNA agarose gel electrophoresis. Samples for 16S rDNA analysis from total RNA (16S cDNA) and for Illumina-based transcriptomics (31/03/2009) were used for cDNA synthesis immediately (Table 1), whereas samples for Roche 454-based transcriptomics (31/03/2009 and 14/04/2009; Table 1) had to undergo mRNA enrichment prior to cDNA synthesis using the mRNA-ONLY Prokaryotic mRNA Isolation Kit (Biozym Scientific, Oldendorf, Germany) and MICROB*Express*™ Bacterial mRNA Enrichment Kit (Ambion) according to the manufacturer's instructions. This procedure removes up to 90% of 16S and 23S rRNA from bacterial RNA, and thus results into a higher proportion of mRNA transcripts. The latter enables a more effective use of sequencing platforms with lower throughput.

2.3. cDNA synthesis

Synthesis of cDNA from both total RNA and mRNA-enriched RNA samples was carried out using the SuperScript® Direct cDNA Labeling System (Life Technologies, Darmstadt, Germany). The first strand cDNA synthesis reaction was followed by a second strand cDNA synthesis with polymerase (30 U) and 10x strand buffer and RNase H (1 U) (Fermentas, St-Leon-Rot, Germany). The reaction was carried out in a total volume of 100 μl at 15 °C for 2 h. Blunt ends were generated with T4 DNA polymerase (12.5 U) (Fermentas) at 15 °C for 5 min. The reaction was terminated with 0.5 M EDTA. The cDNA was subsequently purified with QIAEX II Gel Extraction Kit (Qiagen). The quantity and quality of the extracted cDNA were analyzed using ND-1000 spectrophotometer (Thermo Fisher Scientific) and by agarose gel electrophoresis. The cDNA was stored at -20 °C until use.

2.4. Roche 454 pyrosequencing

Pyrosequencing was carried out at LGC Genomics (LGC Genomics GmbH, Berlin, Germany). All sequencing reactions were based on FLX Titanium chemistry (Roche/454 Life Sciences, Branford, CT, USA) according to the manufacturers' protocols. Briefly, cDNA from total RNA and from mRNA-enriched samples were checked for quality on 2%

Table 1
Datasets in this study.

	Type of Sequence	Token	Reference	Study Accession Number[2]
taxonomic profiling	16S rDNA from directly sequenced cDNA[1]	16S cDNA	this study	ERP004166
	16S rDNA from PCR-amplified pyrotags	16S pyrotags	Klindworth et al. (2013)	ERP001031 and ERP004166
	16S rDNA fragments from metagenomes	16S metagenome	Teeling et al. (2012)	ERP001227
functional analysis	protein coding sequences from cDNA	454 metatranscriptome	this study	ERP004166
	protein coding sequences from cDNA*	Illumina metatranscriptome	this study	ERP004166
	protein coding sequences from genomic DNA	metagenome	Teeling et al. (2012)	ERP001227
	expressed protein sequences	metaproteome	Teeling et al. (2012)	ERP001227

[1] cDNA synthesized from an non-enriched total RNA sample.
[2] Sequences were submitted to INSDC (EMBL-EBI/ENA, Genbank, DDBJ).

agarose gels. 0.5 μg of each sample was used for the sequencing libraries. As a minor modification, size-selection of the fragments was omitted. The fragments were subjected to end repair and polishing. An extra adenine was added to the fragments' ends and the Roche Rapid Library adaptors were ligated to the fragments as described in the Roche Rapid Library Preparation Manual for GS FLX Titanium Series (version of October 2009, Rev. Jan. 2010). After subsequent emulsion PCR, the fragment libraries were processed and sequenced according to the Roche protocols. The resulting sequences were processed using the standard Roche software for base calling, and adaptor and quality trimming (Genome Sequencer FLX System Software Manual version 2.3). Each cDNA sample obtained from non-enriched total RNA was sequenced on 1/8th of a 454 picotiter plate (PTP), whereas a full PTP was used for cDNA from enriched mRNA samples. The sequencing statistics are summarized Supplementary Table S1. All sequences were submitted to the European Nucleotide Archive (ENA) with study accession numbers ERP004166. Metagenomic DNA as well as 16S rRNA gene pyrotags were sequenced as described previously (Teeling et al., 2012). For pyrotags, two distinct PCR reactions were sequenced per sample on 1/8th of a PTP (Klindworth et al., 2013). These sequences have been deposited at the ENA with the accession number ERP001031 and ERP004166. The sequence associated contextual (meta)data are MIxS compliant (Yilmaz et al., 2011).

2.5. Illumina sequencing

Illumina sequencing was carried out at LGC Genomics. Libraries were generated using the Illumina TruSeq DNA sample preparation kit (Illumina, Inc., San Diego, USA). In brief, cDNA samples were end-polished and the TruSeq adaptors were ligated. Sequences were size-separated on an agarose gel, and the band ranging from 250 bp to 350 bp was excised and purified using the MinElute Gel Extraction Kit (Qiagen). Library concentration was measured using the Qubit 2.0 fluorometer (Life Technologies) and the Agilent Bioanalyzer (Agilent, Waldbronn, Germany). Two libraries were constructed and each library was loaded to two channels each of a single-read flow cell. By using the cBot (Illumina) and the TruSeq SR Cluster Kit v2 – cBot–HS (Illumina) the libraries were hybridized to complementary adapter oligonucleotides of the flow cell and amplified isothermally and clonally to form clusters. Sequencing of 50 bp was performed using the TruSeq SBS Kit – HS chemistry (50 cycles) on an Illumina HiSeq 2000 resulting in 172 million single reads (Supplementary Table S1). These sequences are available from the ENA with the study accession numbers ERP004166. The sequence associated contextual (meta)data are MIxS compliant (Yilmaz et al., 2011).

2.6. Processing of 16S rDNA fragments

Extraction of 16S rDNA fragments from metatranscriptome and metagenome data as well as pyrotags, and their subsequent taxonomic assignments were done with the SILVA pipeline (Quast et al., 2013), which uses the SINA aligner (Pruesse et al., 2012). Details have been described elsewhere (Klindworth et al., 2013).

2.7. Processing of mRNA data

Messenger RNA reads were mapped with the short read mapper ssaha2 (Ning et al., 2001) onto metagenome sequences obtained from the same samples (Teeling et al., 2012). Pfam (Finn et al., 2010) and CAZy (Cantarel et al., 2009) hits with E-values below E-6 were used for functional analyses. In cases where a metatranscriptome read mapped to multiple genes, the least common denominator in terms of taxonomy and function was used. The Pfam analysis for the two 454 metatranscriptomes resulted in 39,518 hits (31/03/2009) and 33,215 hits (14/04/2009). The CAZy analysis revealed 1,210 hits (31/03/2009) and 1,010 hits (14/04/2009). The Illumina metatranscriptome showed 24,283,085 hits to the Pfam database and 602,359 to the CAZy database.

2.8. Data overview

In this study, we used novel data in conjunction with previously published data (Table 1). For taxonomic profiling, we used 16S rDNA reads from three different sources, (a) cDNA reads derived from total RNA (non mRNA-enriched), (b) pyrotag reads, and (c) shotgun metagenome reads. The cDNA and pyrotags datasets were on average 25 times larger than those from metagenomes. We have shown previously that results from larger datasets normally do not constitute artifacts of deep sequencing, and thus do not infringe on the comparability of the resulting taxonomic data (Klindworth et al., 2013). For functional profiling, we used metatranscriptome cDNA reads which were compared to the outcome from the metagenome and metaproteome analyses (Teeling et al., 2012). To facilitate traceability each of these datasets has been assigned a token that is used throughout the text (Table 1).

3. Results and discussion

3.1. Activity levels of abundant taxa

As described by Teeling et al. (2012) in 2009 a spring phytoplankton bloom started with increasing sunlight and temperatures in early March in the German Bight of the North Sea, which was most likely boosted by an influx of nutrient-rich estuaries waters. With a peak on 25/03/2009 the first algal bloom was dominated by the centric diatoms *Thalassiosira nordenskioeldii* as major key player and various other diatoms such as *Chaetoceros* ssp. and *Rhizosolenia* as minor contributors. Two weeks after the initial phytoplankton peak (07/04/2009), a second minor peak occurred dominated by a *Chattonella* related species. The algal activities lead to rapid exhaustion of nutrients that together with eukaryote grazing contributed to phytoplankton bloom termination. Subsequently, the increased algal mortality caused a massive amount of substrates to become available to the microbial community. In an integrated approach Teeling et al. showed that *Alphaproteobacteria* dominated during the pre-bloom phase comprising two thirds SAR11 clade and one third *Roseobacter* clade members (Fig. 1b). With the onset of the bloom, relative *Alphaproteobacteria* abundances diminished and *Flavobacteria* relative abundances increased and exhibited a notable succession of *Ulvibacter* spp., *Formosa* spp. and *Polaribacter* spp. (Fig. 1c). *Gammaproteobacteria* reacted later with increased relative abundances of SAR92 clade and *Reinekea* members (Fig. 1a). The latter reached high abundances within only one week, and peaked on the 14/04/2009.

The combination of CARD-FISH, pyrotag and metagenome analysis proved to be effective for characterizing the bacterioplankton composition, but none of these approaches allows to assess and compare the metabolic states of distinct bacterioplankton clades (Blazewicz et al., 2013). Frequency analysis of expressed rRNA sequences has been widely used as proxy to assess the most active fraction in environmental samples (Hunt et al., 2013; Männistö et al., 2012; Gentile et al., 2006), since metabolically active bacteria are considered to have higher rRNA expression levels than latent or starved cells (Kemp et al., 1993). However, Blazewicz et al. (2013) recently evaluated the limitations of rRNA levels as indicator of microbial activity and pointed out that cellular rRNA content reflects past, current and future activities and are also indicative of different life strategies. Nevertheless, expressed rRNA sequences can provide valuable hints on *in situ* microbial activity levels.

91% (31/03/2009) and 84% (14/04/2009) of the expressed 16S rDNA fragments from directly sequenced cDNA (16S cDNA) could be assigned to the dominant classes, *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria* (Fig. 2a), which mirrors the previous analysed community structure (Fig. 2b-c). *Rhodobacteraceae* appeared to express a higher

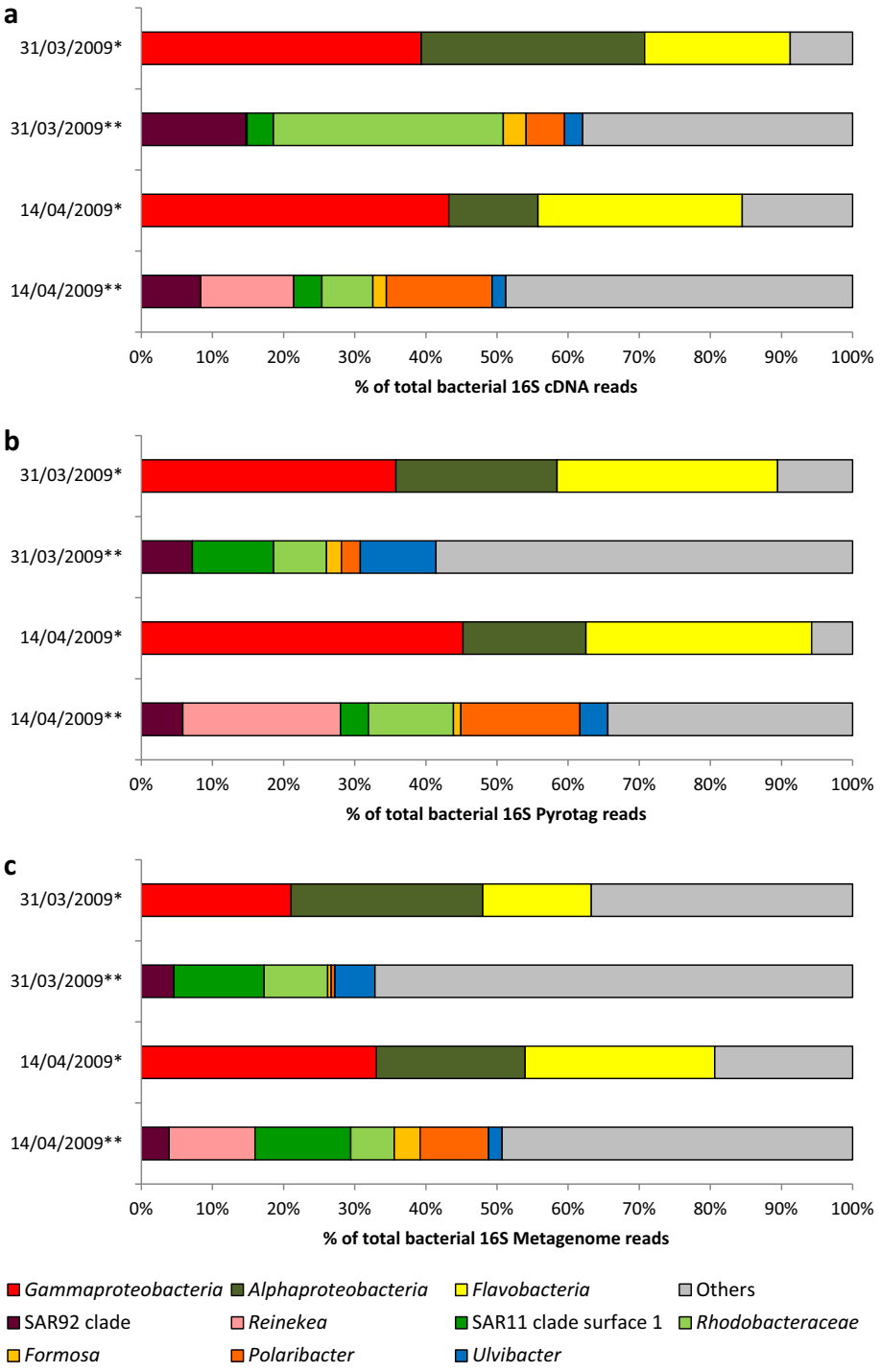

Fig. 2. Taxonomic profile of the bacterial community at Helgoland Roads on 31/03/2009 and 14/04/2009: (*) taxonomic profile on class level, (**) taxonomic profiles of selected dominant taxonomic groups on family and genus level. 16S rDNA reads were gained from (a) directly sequenced non-enriched cDNA (16S cDNA), (b) PCR amplified pyrotags (16S pyrotags) and from (c) metagenomes (16S metagenome).

amount of genes encoding for 16S rRNA in the earlier than in the late sample (Fig. 2a). Members of this family harbor up to five rRNA operons per cell (Moran et al., 2007), which most likely enables them to rapidly respond to changing nutrients conditions (Klappenbach et al., 2000). The distinct *Rhodobacteraceae* 16S cDNA peak in the early sample thus corroborates the hypothesis that members of the *Roseobacter* clade have the ability to rapidly shift metabolic functions in response to dynamic changes during phytoplankton blooms (Giebel et al., 2011), which enhances their competitiveness (Blazewicz et al., 2013). The gammaproteobacterial SAR92 clade were initially regarded to constitute a monophyletic clade of species with adaptations to oligotrophic conditions (Stingl et al., 2007). However, in comparison with the outcome of the 16S pyrotag and 16S metagenome analysis (Fig. 2b-c) we observed higher amount of expressed 16S rRNA sequences for the SAR92 clade on 31/03/2009 (Fig. 2a), suggesting an active role in the breakdown of algae-derived compounds as anticipated in the previous study (Teeling et al., 2012).

16S cDNA estimates for the SAR11 clade were notably depleted in the earlier sample (Fig. 2a) suggesting that SAR11 members cannot profit from abound substrates during algal blooms and thus were

outcompeted by other clades (Fig. 1). Pyrotag sequencing identified many SAR11 to consist of '*Candidatus* Pelagibacter' species. The well-studied representative '*Ca.* P. ubique' HTCC1062 has a rather small genome (1.3 Mbp) with a single rRNA operon (Giovannoni et al., 2005), and in terms of its genetic repertoire is perfectly adapted for the oligotrophic open ocean but not for coastal algae blooms.

3.2. Functional profile of the bacterial community

We compared two 454 metatranscriptome datasets from two different time points (Table 1). The 454 metatranscriptomes provided sufficient resolution down to class level when combined with the taxonomically classified metagenome. The most abundant transcripts with known functions were assigned to genes that are indicative of proliferating cells, such as elongation factors, DNA gyrases, sigma factors and chaperonins. For example, a total of 643 cDNA reads encoding for GTP-binding elongation factors (Pfam: *GTP_EFTU*) could be detected in the later sample (14/04/2009), which account for 2% of all Pfam annotations. With a 145-fold larger dataset, the Illumina metatranscriptome complemented the 454 data and allowed us to assign more reads on family and genus level; hence it allows us to make a clearer statement when combined with the metagenome data. In addition, the omission of mRNA enrichment provided a less biased picture.

3.2.1. Expression of CAZymes

The previously described pronounced peak in the abundance of carbohydrate-active enzymes [CAZymes (Cantarel et al., 2009)] during the bacterial succession (Teeling et al., 2012) was also detected in this study. The majority of CAZymes constituted glycoside hydrolases (GHs) and were expressed by *Flavobacteria* (mainly genera *Formosa*, and *Polaribacter*) which are known to harbor high proportions of GHs (Fernández-Gómez et al., 2013). However, transcripts for the degradation of complex polysaccharides were also detected to a lesser extent in *Gammaproteobacteria* — mostly in the SAR92-clade and some in *Reinekea*. The Illumina data provided additional results and revealed CAZyme expression of the α-glucan-degrading families GH13 and GH31 in *Reinekea* also on the 31/03/2009. While on 14/04/2009 454-data showed no expression of GH31, expression of GH13 was detected. The lack of GH31 expression may be due to the lower amount of the 454-reads, since *Reinekea*-assigned GH31 genes were present in the metagenome data of the same date (Teeling et al., 2012). This indicates that *Reinekea* specialized on the degradation of α-glucan polysaccharides in addition to the monomeric compounds that were inferred from previous data (Teeling et al., 2012).

3.2.2. Expression profile of uptake membrane transporter

Differences in substrate utilization spectra were also apparent in the expression profile of uptake membrane transporters. A large proportion of the abundant transcripts with Pfam annotations (31/03/2009: 3.2% of Pfam annotations; 14/04/2009: 2.7% of Pfam annotations) coded for different transporter types such as TonB-dependent receptors (TonBDR), starch utilization system proteins (SusD), and other low-molecular weight (LMW) transporters such as ATP binding cassette (ABC), tripartite ATP independent (TRAP) and tripartite tricarboxylate transporters (TTT). The transporter profiles in the 454 metatranscriptome were distinct for the dominant bacterial classes (Fig. 3a–b), which reflects differences in their nutritional ecological strategies as reported in Teeling et al. (2012). In our metatranscriptome datasets, the transporter profiles of *Flavobacteria* (*Ulvibacter*, *Formosa* and *Polaribacter*) and *Gammaproteobacteria* (Sar92 clade) were dominated by TonBDRs

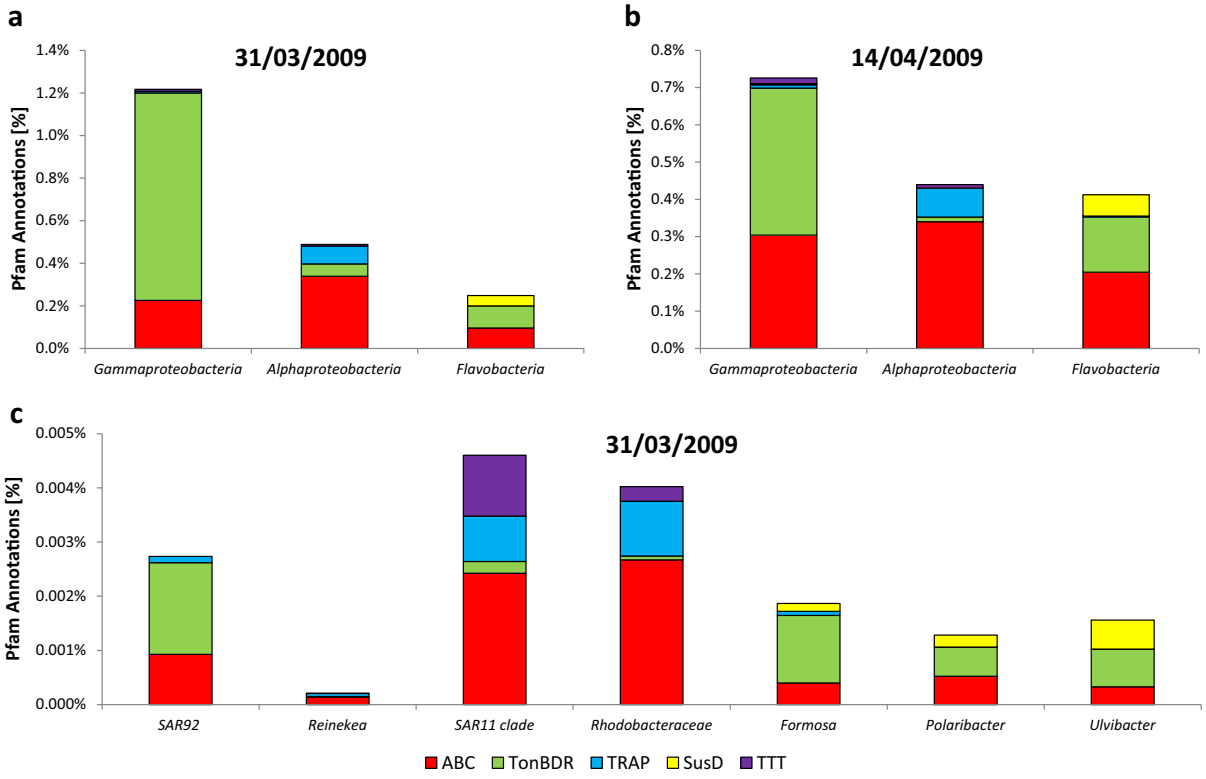

Fig. 3. Pfam annotations [%] of genes encoding for TonB-dependent receptors (TonBDR), starch utilization system proteins (SusD), ATP binding cassette (ABC), tripartite ATP independent (TRAP) and tripartite tricarboxylate transporters (TTT). (a) 454 metatranscriptome data from 31/03/2009. Pfam annotations [%] are shown for *Gammaproteobacteria*, *Alphaproteobacteria* and *Flavobacteria*. (b) 454 metatranscriptome data from 14/04/2009. Pfam annotations [%] are shown for *Gammaproteobacteria*, *Alphaproteobacteria* and *Flavobacteria*. (c) Illumina metatranscriptome from 31/03/2009. Pfam annotations [%] are show for selected abundant bacterial taxa: the gammaproteobacterial SAR92 clade and *Reinekea*, the alphaproteobacterial SAR11 clade and *Rhodeobacteraceae* and the flavobacterial *Formosa*, *Polaribacter* and *Ulvibacter*.

(Fig. 3) which play important roles in nutrient uptake including oligosaccharides (Fernández-Gómez et al., 2013; Tang et al., 2012). This corroborates a previous study (Tang et al., 2012), which revealed that the majority of the TonBDR sequences in the Global Ocean Survey (GOS) metagenomic data set (Rusch et al., 2007) originated from *Gammaproteobacteria* and the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group. In addition, other transcripts of TonB-dependent transport systems (TBDT) (*tonB*, *exbB*, *exdD*) were clearly dominated by *Flavobacteria* and *Gammaproteobacteria* (Supplementary Fig. S1a), and exhibited a peak in the early algae bloom phase simultaneously to the *tonBDR* expression maxima. Moreover, flavobacterial *tonBDR* transcripts were accompanied by *susD* expression with the highest levels in *Ulvibacter* (Fig. 3c). SusD-like proteins are outer membrane substrate-binding proteins that play a pivotal role in TBDT-mediated transport (Martens et al., 2009), including for starch and likely also other polysaccharides (Mackenzie et al., 2012).

In contrast, *Alphaproteobacteria* exhibited high expression levels for monomer transporters such as ABC and TRAP transporters (Fig. 3) and bacterial extracellular solute-binding protein (SBP) encoding genes (Supplementary Fig. S1b). SBP are known to be associated with ABC and TRAP transporters (Palmer et al., 2010; Janausch et al., 2002; Thrash et al., 2010) binding extracellular solutes for transport across the bacterial cytoplasmic membrane. This agrees with previous genomic studies on marine microbes (Moran et al., 2007; Tang et al., 2012; Pinhassi et al., 1997), which identified ABC transporters as the dominant transporter type in alphaprotobacterial species like *Roseobacter denitrificans* OCh 114 (*tonBDR*: 1 gene; *abc*: 110 genes) and '*Ca.* P. ubique' HTCC1062 (*tonBDR*: 0 genes; *abc*: 24 genes) (Pinhassi et al., 1997).

Overall, the metatranscriptome data support our previous metaproteomic analyses of membrane transporter expression profiles (Teeling et al., 2012). However, even though both methods agreed on class level, slight differences were detected on deeper taxonomic levels. Based on the metaproteome analysis (Teeling et al., 2012), members of the *Roseobacter* clade showed a higher expression of transporters than the more abundant members of the SAR11 clade, whereas our Illumina metatranscriptomic detected the opposite trend (Fig. 3c). Therefore we suggest that the lower amounts of detected transcripts for *Rhodeobacteraceae* might be a result of fast mRNA turnover coupled to high rRNA expression. This supports not only the cellular strategy to an environmental stimulus as described by Yu and Zhang (2012), but also provides another indicator that members of *Rhodobacteraceae* adapt readily to changing nutrient conditions induced by an algae bloom (Giebel et al., 2011).

3.2.3. Competitive strategy of Rhodobacteraceae

Rhodobacteraceae expressed a high amount of transcripts encoding SnoaL-like polyketide cyclases. SnoaL belongs to a family of small polyketide cyclases involved in nogalamycin biosynthesis (Sultana et al., 2004). Nogalamycin is a member of an anthracycline group (Arora, 1983) that intercalates into DNA and interacts with topoisomerase II (Sinha, 1995; Binaschi et al., 2001; Tran et al., 2011), thereby preventing transcription and subsequent protein synthesis. In research, nogalamycin has also been successfully used as antibiotic against algae (Guha-Mukherjeea and Keller, 1973). Considering that algae and bacteria most likely compete over the same limiting nutrients, SnoaL expression might confer a competitive advantage.

4. Conclusions

Frequency analysis of expressed rRNA sequences allowed us to interrogate the major findings of the Teeling et al. study down to genus level despite methodological differences. The results substantiated the view that the successive bacterioplankton bloom was largely governed by substrate availability. Expression of glycoside hydrolases most likely allowed *Formosa* and *Polaribacter* members to decompose complex algae polysaccharides resulting in an increasing availability of sugar oligomers and monomers. Algae-derived substrates provided a series of ecological niches for specific populations to bloom, and at the same time generated a selective advantage for bacteria with an opportunistic lifestyle like members of the *Roseobacter* clade. Furthermore, *Rhodobacteraceae* seemed to pursue a competitive strategy due to as yet unknown mechanisms, possibly by biosynthesis of algicidal polyketides. It is further interesting to note that SAR11 could not profit from the increased availability of nutrients in the decaying bloom while the activity of SAR92 suggests an active role in the breakdown of algae-derived compounds.

To conclude, the complementary interpretation of the diversity data with the reconstruction of the dominant metabolic processes allowed us (i) to monitor the dynamic response of planktonic bacterial taxa to a coastal phytoplankton bloom down to genus level and (ii) to elucidate the adaptive and distinct response to successive ecological niches that allowed prokaryotic planktonic species to coexist in great detail.

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