CONTENT

GENERAL INTRODUCTION

Study area: North Sea

The greater North Sea is located on the continental shelf of north-west Europe and can be divided into the shallow southern North Sea (depths on average <40 metres), the central North Sea, the Norwegian Trench and the Skagerrak (OSPAR 2000). The average temperature ranges between 6°C in winter and 17°C in summer, while salinity is varying between 15 – 25 in estuaries and 32 -35 in northern areas (OSPAR 2000). The seafloor topography of the North Sea is dominated by an ancient, north-south orientated continental drift depression. This depression is covered by terriginous sediment deposits, several kilometres thick, which consist mainly of sand and sandy silt (OSPAR 2000). Water movement in the North Sea is predominantly influenced by tidal, current and storm events as well as density gradients resulting from freshwater input (Howarth 2001). These motions are too weak to affect sediments characteristics in the central or northern North Sea. Contrary, in the shallow southern part of the North Sea, remarkable sediment transport processes occur. Principally, fine-grained material is transported over a considerable distance into deeper areas. Another source of sediment transports are estuaries along the coast. These transports are widely depending on estuary gradients and fluctuate according to tidal and seasonal changes (OSPAR 2000).

Estuary gradients

The river runoff in estuaries determines the deposition of sediments but also of nutrients (Atlas and Bartha 1987). Estuaries represent therefore highly productive areas. These regions are characterised by a multitude of environmental gradients. The mixing of freshwater and marine waters implies salinity and temperature gradients. Furthermore pH and organic loading fluctuate on tidal and seasonal ranges. Living in this highly variable environment

requires adaptations and eurytolerance to many environmental factors such as salinity, temperature or pH variations (Atlas and Bartha 1987).

The German Bight is situated in the southern part of the North Sea, predominantly influenced by the discharges of Elbe and Weser Rivers (Hickel *et al* 1993). This region represents probably the most eutrophied area in the North Sea. Due to the residual counter clockwise currents and the positioning of estuaries intense accumulations of eutrophying substances occur (Hickel *et al* 1993). Studies conducted in the past 50 years in the German Bight were addressing mainly eutrophication (Hickel *et al* 1993, Rachor 1990), pollution (Bester *et al* 1998, Gee *et al* 1992, Schwarzbauer *et al* 2000, Vauk and Schrey 1987) and storm surges (Woth *et al* 2006). Long term studies suggest changes of the hydrographic regime and biota around Helgoland coupled with increasing temperature (on average 1.67°C) and salinity (Wiltshire *et al* 2010). Hence, it becomes obvious, that the German Bight represents a highly anthropogenic influenced and variable ecosystem. On top of that, other anthropogenic activities as fishery, industry, shipping and the maintenance of coastal rivers and ports are affecting this region.

Anthropogenic perturbation in the North Sea

Human interventions in the marine ecosystem are manifold. Coastal areas are impacted by riverine input of contaminants and nutrients originating from industry, urbanisation and agriculture (Boetius *et al* 2000, Burak *et al* 2004, Witt and Trost 1999). Intensive fishing industry harms not only fish stocks but also the benthos by fishing practices such as trawling (Freese *et al* 1999). At the coastline, dredging procedures are possibly changing hydrodynamics in estuaries (Bale *et al* 2007). On the sea global shipping causes litter input, oil contamination and ballast water release (Balas *et al* 2006, Gundlach and Hayes 1978). Another issue of great concern represents waste disposal at sea. Different kinds of waste as for instance low-level radioactive waste (Phillips *et al* 2011), sewage sludge and dredged

sediment resulting from maintenance of navigation channels and port facilities (OSPAR 2009, Stronkhorst *et al* 2003) are deposited at sea. The impacts of these human induced perturbations are extensively studied but focus predominantly on higher organisms (Gee *et al* 1992, Mühlenhardt-Siegel 1981, Vauk 1984, Vethaak *et al* 1992).

Dumping activities in the German Bight

The history of dumping activities in the German Bight goes back to the 1960s. 20 000 m³ of sewage sludge from the city of Hamburg was dumped monthly into the eastern German Bight (Mühlenhardt-Siegel 1981). A reported decline in macrozoobenthic species richness led to a cessation of the dumping activities. Recently, in 2005, the city of Hamburg received permission to dump lowly polluted sediments in the same area (HPA 2005) and the dumping activities were resumed. Hence, in between the years 2005 and 2010 approximately 6 000 00 m³ sediment were removed from the Elbe River near the port area of Hamburg and dumped at the prescribed site. The dumping activity is accompanied by an elaborate monitoring program. Twice a year samples from 125 stations at the dumping and a reference site were taken in order to estimate the effect of the dumping activity. Bearing at the disposal site revealed that a main proportion of the sandy material forms a three meters high rising at the dumping centre. Fine-grained fractions were tracked with Acoustic Doppler Current Profiler (ADCP) recording that fine-grained material is transported up to eight kilometres until it reaches the seafloor (HPA 2005). In order to elucidate the impact of the dumping activity on the environment, contaminant content, macrozoobenthos and fish fauna data were recorded. Comparing measurements before and along the dumping activities revealed a significant increase in heavy metals, namely mercury, cadmium and zinc as well as organic pollutants, precisely poly aromatic hydrocarbons and organotin compounds. At the same time species richness and density of the macrozoobenthos decreased (HPA 2010). So far, bacterial community analyses have not been included in the monitoring program. To implement this

ecologically important group of organisms, we started an interdisciplinary project in 2009 with the Hamburg Port Authority, in charge for the monitoring, the environmental agencies of Schleswig-Holstein and Lower Saxony and the Federal Institute of Hydrology (BfG). This pilot study aimed to analyse the bacterial community with respect to the dumping activities and to examine the potential of bacterial community analyses to serve as a proxy for environmental perturbation. Participating in three monitoring campaigns, samples for community and functional structure of benthic bacteria were collected.

Bacteria in marine sediments

Marine sediments cover more than 70 % of the Earth's surface. Their physical and chemical conditions are unique in many ways. Grain size distributions are their most important physical characteristic (Sommer 2005). Typically, sediment is divided by its grain size into six fractions: < 4µm defined as clay, 4 – 63 µm defined as silt, three sand fractions and gravel (2 - 6 mm). The composition of these grain size fractions determines the distribution of physicochemical factors such as water or oxygen penetration. Both depend on the sediment porosity. The different sediment types offer further conditions: organic substances form aggregates in muddy sediments (containing a high proportion of silt and clay) and are more available to microorganisms than in sandy sediments (Sommer 2005). Contrary, sandy sediments are much looser than muddy sediments and allow a deeper water and oxygen penetration. In any case, the oxygen penetration stops at a certain depths. The abrupt lack of oxygen results in a very steep redox gradient. Predominantly, muddy sediments face chemical conditions changing across a few millimetres. While oxygen is used by organisms in the surface layers, nitrate and sulphate act as electron donors in deeper layers prior to the reduction zone. In the reduction zone compounds as sulphur, nitrogen, iron and manganese are present. Due to these manifold conditions sediments harbour the largest variety of metabolic types of microorganisms (Sommer 2005). Depending on the granular structure 10^8 -

10^{11} bacterial cells per millilitre can be observed in marine sediments. Thereby bacterial biomass is increasing with decreasing grain size. Generally, sandy sediments are less colonised since they offer less volume-specific surface area and less nutrients then muddy sediments (Yamamoto and Lopez 1985).

Because of their diverse metabolic capabilities and high enzymatic activities microbial communities play a crucial role in biogeochemical cycling (Pomeroy 1974). Principally, heterotrophic, phototrophic and lithotrophic bacteria can be found in sediments. Depending on the availability of electron donors and acceptors, various metabolic types exists. For instance: aerobic heterotrophs, aerobic and anaerobic chemolithotrophs, reducers and oxidisers of manganese, iron and sulphate, methanogens and methanothrophs, as well as fermentative bacteria (Nealson 1997). The metabolic processes of these bacteria result in a continuous release and resuspension of nutrients from the seafloor. Upwelling processes or storm events transport the nutrients in the photic zone where they stimulate phytoplankton and bacterial growth (Marcus and Boero 1998). These in turn stimulate the zooplankton and in this manner the whole food chain. Because not all organisms are consumed by planktonic grazers they eventually die and sink down on the seafloor. This process is known as benthic-pelagic coupling (Marcus and Boero 1998). The impact of organic material input on benthic bacterial communities was already subject in many studies (Franco *et al* 2007, Graf *et al* 1982). It was concluded that the input of organic matter leads to changes in the bacterial community structure, bacterial biomass and productivity (Franco *et al* 2007, Graf *et al* 1982, Meyerreil 1983).

Bacterial community composition

The bacterial community in marine sediments is dominated by gram-negative *Proteobacteria*. Moreover, members of the phyla *Bacteroidetes*, *Planctomycetes* and *Chloroflexi* contribute to the bacterial community of the marine benthos. The phylum of *Proteobacteria* includes

various metabolic types. Principally they are divided into five classes: *Alpha-*, *Beta-*, *Gamma -*, *Delta-* and *Epsilonproteobacteria.* In marine sediments *Delta-* and *Gammaproteobacteria* dominate the bacterial community. While *Deltaproteobacteria* constitute a physiological homogeneous group, comprising almost all sulphate reducing species, *Gammaproteobacteria* contain various physiological groups. Vital members of the chemoorganotrophic *Gammaproteobacteria* are the genera *Alteromonas* and *Pseudoalteromonas* as well as the genera *Oceanospirillum* or *Marinobacter* which form a separate clade. The phylum of *Bacteroidetes* contains the *Cytophaga-Flavobacteria* cluster as well as the *Bacteroides* subgroup. Firstly described by Winogradsky (1929), members of the *Cytophaga-Flavobacteria* cluster are characterised as unicellular, gliding, nonespore-forming rods. This group comprises members featuring various physiological capabilities, furthermore, they are adapted to a broad range of environmental conditions (Weller et al 2000). Generally, *Bacteroidetes* are strongly associated with the water column and marine aggregates. But some studies described their abundance also for aerobic and anaerobic sediments (Llobet-Brossa et al 1998, Ravenschlag et al 2001). *Flavobacteria* are believed to play a pivotal role in the degradation of organic matter since they own hydrolytic capabilities (Abell and Bowman 2005, Cottrell 2000). The phyla *Planctomycetes* and *Chloroflexi* occur in all natural environments and were detected also in North Sea sediments (Kittelmann and Friedrich 2008, Webster *et al* 2007). The phylum *Planctomycetes* represents in several ways an exceptional group. Members have cell walls that are not composed of peptidoglycan. Additionally, some species feature an intracellular compartment that contains DNA. Green non-sulfur bacteria or *Chloroflexi* comprise various phenotypes. Including species gliding filamentous and isolates that contain some sort of bacteriochlorophyll frequently arranged in chlorosomes (Rappe and Giovannoni 2003). The structure of benthic bacterial communities however is substantially determined by environmental conditions.

Bacterial communities in estuaries: Effects of physicochemical and biogeochemical variations and pollution

Studies conducted on benthic bacterial communities aim to investigate their community composition in variable or permanently cold marine habitats (Dale 1974, Llobet-Brossa *et al* 1998, Ravenschlag *et al* 1999), their ecological role in various nutrient cycles, examine the influence of organic material inputs (Duyl *et al* 1992, Meyer-Reil and Koster 2000) or contaminant input (Paisse *et al* 2008) on the bacterial community. Spatial investigations revealed that predominantly salinity, pH, and nutrients such as ammonium and phosphate shape the bacterial community assembly in estuaries (Sun *et al* 2011). Additionally, Bowen and co-workers (2009) suggested that habitat-specific forces determine the sediment bacterial communities in salt marsh environments. However, to date most studies focus on pelagic bacterial communities in estuaries (Bouvier and del Giorgio 2002, Crump *et al* 1999, Fortunato and Crump 2011, Fortunato *et al* 2012, Herlemann *et al* 2011, Selje and Simon 2003). Generally, pelagic bacterial communities appear to be rather influenced by spatial factors such as depths or salinity than by temporal factors. Herlemann and co-worker (2011) as well as Selje and co-workers (Selje and Simon 2003) observed distinct bacterial community cluster for marine, freshwater and brackish water environments. Spatiotemporal investigations of bacterial communities in estuaries, however, are rare. To our knowledge only Fortunato and co-workers (2012) considered the impact of spatial and temporal variations on pelagic bacterial communities in estuaries, while these studies are lacking for benthic bacterial communities.

Anthropogenic perturbation represents a major concern especially in coastal areas as already mentioned above. Hence, several studies have addressed the impact of perturbation on bacterial communities (Dean-Ross and Mills 1989, Gillan *et al* 2005, Roling *et al* 2001, Wang *et al* 2011). It was highlighted that bacterial communities react to physical disturbance, as sieving, with changes in community structure and reduced biomass (Findlay *et al* 1990).

Observations of the impact of heavy metal or oil contamination on bacterial communities revealed that the contamination affects the structure as well as the function of bacterial communities (dos Santos *et al* 2011, Gremion *et al* 2004, Suarez-Suarez *et al* 2011). Even the impact of ocean dumping on bacterial communities was studied, but predominantly in mesocosm experiments so far (Kan *et al* 2011, Nayar *et al* 2004, Toes *et al* 2008). Respective field studies are lacking until today.

To our knowledge, investigations of benthic bacterial communities of sublittoral sediments in the German Bight are scarce. More detailed information about benthic bacterial communities inhabiting sublittoral sediments will help us to better understand ecological processes and anthropogenic interferences in coastal environments.

Methodological approaches

Nowadays, the range of molecular approaches to describe microbial communities is extremely broad. Community structure and composition is mainly estimated by fingerprinting and sequencing approaches. Most applications, for both community analyses and phylogenetic studies, base on the highly conserved small-subunit (SSU) ribosomal genes. Apart from its highly conserved DNA sequence SSU ribosomal genes feature highly variables regions and finally the possibility to align the sequence information to a vast number of data bases in order to analyse phylogenetic relationships. Functional diversity of bacterial communities is addressed in RNA approaches and as newly invented microarray approaches targeting functional genes (He *et al* 2007).

Denaturing Gradient Gel Electrophoresis (DGGE) represents probably the most common fingerprinting method in microbial ecology. This method does not only provide insight into the community structure and dynamics, moreover the amplified and separated 16S rDNA fragments may be used in sequencing approaches to identify the represented phylotypes. Further applications are Amplified Ribosomal DNA Restriction Analysis (ARDA), Terminal

Restriction Fragment Length Analysis (T-RFLP), and Automated Ribosomal Intergenic Spacer Analysis (ARISA). In contrast to DGGE the ARISA fingerprinting bases on the length polymorphism of the intergenic spacer region located between 16S and 23S rDNA. Basically, the length of this region is species-specific and ARISA fingerprinting resolves therefore phylotypes more deeply compared to DGGE (Okubo and Sugiyama 2009). The method ARISA was applied in the presented thesis to investigate the structure of benthic bacterial communities.

In recent years sequencing methods faced a fast development of alternatives to the conservative sanger sequencing approach. 454 Life Sciences invented a new generation of sequencing, named often “high-through put sequencing” or “next generation sequencing”, capable of hundreds of thousands reads in parallel. The technique bases on an emulsion PCR. Simultaneously millions of PCR reactions, separated by oil droplets, are taking place. The advancement of sequencing methods offered new capabilities to explore microbial community composition (Schuster 2008).

Microarrays finally, enhanced the analysis of functional structures of microbial communities. The principle of microarray technology bases on small single stranded oligonucleotide probes (specific DNA sequences), which are immobilised on a solid phase (generally glass or silicon). For analysis of environmental samples fluorescently labelled single stranded DNA from a certain sample is applied on the microarray. Strong hydrogen bonds between complementary nucleotide base pairs hybridise the target DNA to the specific probe on the microarray. Non-specific bindings of probes are removed by washing steps and only strongly hybridised double strains remain. The signal generated by the labelled target DNA can be quantified. Generally, the signal intensity depends on the amount of target DNA bound to the probe. The functional gene array GeoChip, firstly introduced in 2007 (He at al 2007) encompasses probes from genes involved in key microbial mediated biogeochemical

processes (e.g. carbon, nitrogen and sulphur cycling as well as organic contaminant degradation and metal resistance and reduction. Initially invented for soil communities (He *et al* 2007), the GeoChip was recently implemented in marine (Wang *et al* 2009) and contaminated habitats (Liang *et al* 2009, Lu *et al* 2012). The latest version of this microarray, GeoChip 4.2, contains DNA probes targeting functional groups of carbon, nitrogen, sulphur and phosphorus cycling, metal and antibiotic resistance, energy process, organic contaminant degradation, stress and virulence. In the framework of this thesis we utilised the GeoChip 4.2 to analyses the functional structure of representative bacterial communities from a dumping site.

RESEARCH AIMS

The aim of this thesis was to gather more detailed information about benthic bacterial communities in the German Bight. Bacterial communities of sublittoral shelf sediments in the German Bight remain widely uncharacterised. Many questions concerning their distribution in particular, referring different environmental conditions, both spatial and temporal, remain unclear. We conducted monthly cruises along transects in the German Bight to observe bacterial community variations on temporal and spatial gradients. Another major part of this thesis focuses on the impact of dumping activities on benthic bacterial communities.

Spatiotemporal gradients influencing benthic bacterial communities in near and offshore regions in the German Bight

Spatial and temporal variations of benthic bacterial communities along three transects in the German Bight were characterised. Sediment samples were collected monthly over one year. Each transect offered unique geochemical and physicological conditions. Bacterial communities inhabiting the sediments along the transects were followed over a seasonal cycle. Simultaneously, physico-geochemical parameters such as grain size distribution, carbon content, temperature, salinity and *chlorophyll a* were recorded. Fingerprints of the bacterial community structure were obtained via Automated Ribosomal Intergenic Spacer Analyses (ARISA). The conjunction with environmental variables was realised in multivariate multiple regression analyses.

Characterisation of benthic bacterial communities at a dumping site: investigating bacterial community structure and function

Anthropogenic perturbation represents an issue of great concern also in regard to bacterial community response. We investigated the impact of an active dumping site on bacterial communities in the German Bight. For this approach we followed an existing sampling scheme for monitoring of geochemistry and macrozoobenthos. In three sampling campaigns sediment samples were obtained at 125 sampling positions. Bacterial community profiles were obtained via ARISA. Fingerprinting profiles were statistically analysed also related to environmental parameters. To yield information about the community composition representative samples were subjected to SSU ribosomal tag sequencing.

Our investigations of bacterial communities at a dumping site in the German Bight were completed by subjecting representative samples to functional gene arrays. We utilised the GeoChip 4.2, a gene array targeting functional genes of carbon, nitrogen, sulphur and phosphorus cycling, metal and antibiotic resistance, energy process, organic contaminant degradation, soil benefit, soil borne pathogens, stress and virulence. The functional structure of the microbial communities was furthermore related to environmental parameters (e. g. pollutans, grain size).

OUTLINE

This cumulative thesis consists of three chapters; each representing a stand-alone publishable manuscript

Chapter I (in preparation for submission to the *ISME Journal*)

Störmer, R., Wichels, A., Gerdts, G.

Spatiotemporal variations of benthic bacterial communities in the German Bight

The planning, analyses and manuscript writing were carried out by Rebecca Störmer under the guidance of Antje Wichels and Gunnar Gerdts. Sampling was conducted by Rebecca Störmer, Kristine Carstens, Sylvia Peters and Julia Haafke. CHN analyses were performed by Rebecca Störmer and Julia Haafke. Christian Hass assisted with the grain size analysis of the sediments. Salinity, temperature and *chlorophyll a* data were kindly provided by Karen H. Wiltshire.

Chapter II (submitted to the *Marine Pollution Bulletin*)

Impact of ocean dumping on bacterial communities I: Fine-scale investigations at a dumping site

Störmer, R., Wichels, A., Gerdts, G.

The planning, analyses and manuscript writing were carried out by Rebecca Störmer under the guidance of Antje Wichels and Gunnar Gerdts. Sampling was performed by Rebecca Störmer and the contextual data were kindly provided by the *Hamburg Port Authority*. Jörg Peplies assisted with the sequencing analyses.

Chapter III (submitted to the *Marine Pollution Bulletin*)

Impact of ocean dumping on bacterial communities II: GeoChip-based analysis of bacterial communities at a dumping site

Störmer, R., Wichels, A., Gerdts G.

The planning, analyses and manuscript writing were carried out by Rebecca Störmer under the guidance of Antje Wichels and Gunnar Gerdts. Sampling was performed by Rebecca Störmer and contextual data were kindly provided by the *Hamburg Port Authority*. Samples for functional gene analyses were conducted by Glomics, Inc.. Zhili He and Joy van Nostrand assisted with the interpretation of the data.

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CHAPTER I

Biogeography of benthic bacterial communities in the German Bight

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Abstract

Studies investigating the biogeography of benthic bacterial communities on spatiotemporal scales in different marine habitats are rare. This study presents spatiotemporal variations of benthic bacterial communities in near and offshore regions of the German Bight (North Sea). Bacterial community structure and diversity were estimated from Automated Ribosomal Intergenic Spacer Analysis (ARISA). Relationships between bacterial community structure and environmental factors were disentangled in multivariate multiple regression models. We observed temporal and spatial variations of bacterial communities in nearshore regions. Bacterial communities in offshore regions however, were highly dispersed and only the diversity showed seasonal variations. Temporal factors appeared to be most important in shaping the benthic bacterial communities in nearshore regions. Spatial variations of the bacterial communities were strongly linked to respective strong environmental gradients (sediment composition, salinity) occurring in the individual nearshore regions.

Introduction

Abundance and distribution of species and factors influencing them are crucial to understand ecosystem functioning and to predict environmental changes. It is widely agreed that bacterial biogeography is influenced by a multitude of biotic and abiotic factors (Fuhrman *et al* 2006, Graham 2004, Lozupone and Knight 2007, Yannarell *et al* 2003a). Fuhrman and co-workers (2006) stated that bacterial community composition is predictable from ocean conditions. They highlighted that bacterial community assembly in the ocean is depended on various abiotic and biotic factors but that temporal changes appear to be the most important ones. In contrast, considering various ecosystems (soil, marine, freshwater) Lozupone and Knight (2007) stated that most importantly salinity variations determine the bacterial community composition. The comparison of these two studies demonstrates the necessity of individual studies in individual ecosystems. Gaining more information about factors influencing bacterial community structure in individual ecosystems will help to model and predict bacterial community responses to certain environmental short time events (e.g. nutrient input) and to predict longterm consequences for instance in the context of climate change or environmental pollution.

Plenty of studies aim to determine spatial and temporal factors influencing bacterial community structure (Acinas *et al* 1997, Allan and Froneman 2008, Boer *et al* 2009, Ghiglione *et al* 2005). Most studies focus on specific environments such as estuaries (Fortunato and Crump 2011), coastal areas (Alonso-Saez *et al* 2007) or the open ocean (Fuhrman *et al* 2006). To our knowledge investigations of different environments, for instance offshore and nearshore regions on spatiotemporal scales remain scarce.

Seasonal variability has been explored extensively for marine bacterial communities (Alonso-Saez *et al* 2007, Boer *et al* 2009, Gerdts *et al* 2004). Environmental factors determined by the season such as temperature (Gonzalez-Acosta *et al* 2006), nutrient input (Jacquet *et al* 2002)

or primary production (Franco *et al* 2007, Meyerreil 1983) affect bacterial community structure, biomass and productivity.

Spatial variability was most likely investigated along estuaries. Estuarine environments are characterised by strong environmental gradients resulting from the mixing of fresh and marine water masses at river mouths. Many of these gradients, including salinity, nutrient concentrations and especially on the seafloor, sediment transports, may influence bacterial communities. The high variability of this ecosystem makes it a perfect model system to study spatial variations in community structure. Several studies described pelagic bacterial communities in estuaries (Crump *et al* 2004, Fortunato and Crump 2011, Selje and Simon 2003). These studies demonstrated that bacterial community variations depend on both abiotic and biotic gradients. However, most of them focus on the impact of salinity gradients on the bacterial communities. Herlemann (2011) and co-workers as well as Selje and Simon (2003) demonstrated the formation of distinct bacterial communities along salinity gradients. Both studies showed typical marine, freshwater and brackish water groups. Generally, regarding pelagic bacterial communities, the influence of spatial factors seems to overwhelm temporal impacts on the bacterial communities. Spatiotemporal variations of benthic bacterial communities in coastal areas remain widely uncharacterised. Especially in coastal areas benthic bacterial communities' contribute to a pivotal extent in remineralisation processes of organic matter (Atlas and Bartha 1987). The input of organic matter from the euphotic zone to the seafloor and the response of benthic communities is described by the term benthic-pelagic coupling. Benthic-pelagic coupling is important in coastal areas as well as open waters (Graf 1989, Marcus and Boero 1998). In any case the input of organic material, resulting from dying organisms in the water column, determines substantially the benthic community: To date detailed information of these processes regarding benthic bacterial communities in coastal areas are scarce.

The German Bight (North Sea) encloses the estuaries of Ems, Jade, Weser, Elbe, and Eider Rivers. Studies of the past 50 years carried out in the German Bight were addressing mainly eutrophication (Hickel *et al* 1993, Rachor 1990), pollution (Bester *et al* 1998, Gee *et al* 1992, Schwarzbauer *et al* 2000, Vauk and Schrey 1987) and storm surges (Woth *et al* 2006). Long term studies suggest a climate change in North Sea waters (Wiltshire *et al* 2010). Over the past 50 years distinct changes in the hydrography and biota around Helgoland, an island situated in the German Bight, going along with increasing temperature and salinity were recorded. Summarising these efforts it becomes obvious, that in particular the German Bight represents a highly variable ecosystem.

Pelagic bacterial communities in the German Bight were explicitly described in the last decades (Eilers et al 2000, Eilers *et al* 2001, Gerdts *et al* 2004, Oberbeckmann *et al* 2011, Sapp *et al* 2007, Teeling *et al* 2012). Benthic bacterial communities of the shelf sediments in the German Bight remain poorly characterised. Some effort has been made on characterising bacterial communities in the East Frisian Wadden Seas (Stevens *et al* 2005), subtidal sediments in the Sylt-Romo basin (Boer *et al* 2009) intertidal sand flats at Sylt (Musat *et al* 2006) and nearshore intertidal mud and sand flats of Dangast (Llobet-Brossa *et al* 1998). The community composition was concluded to be stable over time but differed for the individual investigated environments. Exceptional Boer and co-workers (2009) described a large depth and time related variation within bacterial communities in subtidal sediments in the Sylt-Romo basin. To our knowledge not a single study was conducted investigating benthic bacterial communities of the sublittoral shelf sediments in the German Bight. The lack of spatiotemporal investigations on benthic bacterial communities inhabiting different sublittoral shelf sediments in combination with existing knowledge about responses of pelagic bacterial communities in the corresponding pelagic habitats builds the basis for our investigation.

We hypothesise that the bacterial community structure is determined by individual environmental gradients in individual environments. Therefore spatiotemporal patterns of benthic bacterial communities along three, according to their biogeochemical and physicochemical parameters, unique transects were characterised. Geo- and physicochemical parameters, in particular grain size distributions, organic carbon content, temperature, salinity and *chlorophyll a* as a measure for phytoplankton abundances were recorded. Bacterial community structure was obtained via automated ribosomal intergenic spacer analysis (ARISA). The relationship between environmental factors and bacterial communities was investigated using multivariate multiple regression models.

Material and Methods

Location of transects and sampling

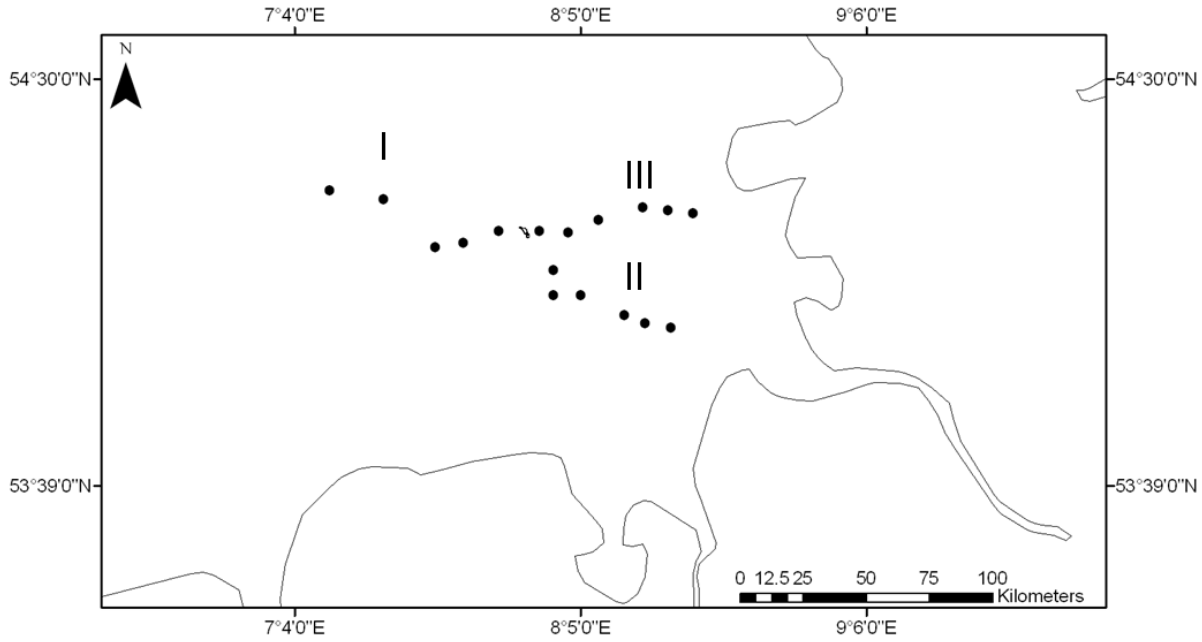

Fig. 1 Overview of the three investigated transects in the German Bight. I: P8 transect, II: Elbe transect, III: Eider transect.

Table 1 Sediment classification after Folk (1980).

Station	Sediment type	Station	Sediment type	Station	Sediment type
P8 II	Fine sand	Elbe II	Coarse silt	Eider I	Fine sand
P8 III	Fine sand	E3	(Very) Coarse silt	Eider II	Fine sand
P8 IV	Fine sand	Elbe III	(Very) Coarse silt	Eider III	Fine/Medium sand
P8 V	Very fine sand	Elbe IV	Very fine sand	Eider IV	Fine sand
P8 VI	Very fine sand	Elbe V	Fine sand	Eider V	Fine/Medium sand
		Elbe VI	Fine/Medium sand	Eider VI	Fine sand

Starting from the German island Helgoland, North Sea (54°10’ N, 7° 53’ E) three transects (Fig. 1, I: P8, II: Elbe and III: Eider) comprising in total seventeen stations were sampled. Water depths were ranging from 53 – 8 metres. Sediments were classified according to Folk (Folk 1980, Table 1). The sampling was performed monthly from September 2010 to July 2011. All sediment samples were taken with a van Veen grab (0.2m³). Onboard, the sediment was poured into a clean box and homogenised. To ensure coherent analyses, the samples for analyses of the microbial communities as well as the samples for CHN and grain size analyses were taken from this sediment homogenate. Temperature and salinity data were obtained from bottom water. For microbial community analysis, three subsamples were stored immediately after collection at -20°C in 50 ml falcon tubes.

DNA-Extraction and Quantification

For DNA-Extraction the PowerSoil Kit (MoBio Laboratories, Carlsbad, CA, USA) was used following the manufactures protocol. Per station three subsamples of 0.25g sediment each were subjected to the procedure. The extracted DNA was eluted in 50µl elution buffer. Genomic DNA concentrations were measured by photometry using the Infinite M200 (Tecan Austria GmbH, Gröding, Austria). The DNA was measured in duplicate. DNA was also controlled regarding the presence of proteins at 280nm (ratio >1.8).

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Automated ribosomal intergenic spacer analysis was performed as previously described (Störmer *et al* 2012).

OTU Definition for ARISA

ARISA fingerprint data were processed as previously shown (Störmer *et al* 2012). Fingerprinting profiles of each sample were converted to "consensus fingerprinting profile" (presence/absence) since environmental data were recorded only once per sample from each site. Calculating the "consensus fingerprinting profile", only fragments present in at least two of the subsamples were regarded as present.

Environmental data analysis

CHN analyses

For CHN analyses samples were dried in a freeze dryer. Afterwards the sediments were homogenised with a mortar. 30 mg sediment was filled in silver cups. In order to remove organic carbon compounds HCL was added. The filled silver cups were then dried again at 100°C over night. Before application the Vario MICRO cube (elementar, Hanau, Germany) the silver cups were encapsulated with tin cups in order to achieve optimal combustion conditions (Hedges and Stern 1984).

Grain size distribution

Sediments were treated with acetic acid (33%) and hydrogen peroxide (10%) in order to remove organic substances from the samples. The sediment was stored in water until analysis. Grain size analysis was performed via CILAS 1180 laser particle analyser as previously described (Dolch and Hass 2008).

Salinity, Chlorophyll a, Temperature

These data were obtained as part of the Helgoland Roads LTER series (Wiltshire *et al* 2008). The data set was kindly provided by Karen H. Wiltshire.

The Helgoland Roads time series is accessible via the open database Pangaea (http://www.pangaea.de).

Statistics

Univariate analysis

Differences regarding alpha diversity estimated from ARISA OTU numbers respecting spatial (site) and temporal (month) differences were tested using one-way analysis of variance (ANOVA, Statistica Version 7.1, StatSoft GmbH, Hamburg, Germany) for individual transects. A significance level of $p < 0.05$ was applied. Pairwise comparisons of the samples were tested in *post hoc* Tukey HSD tests ($p < 0.05$).

Pairwise correlations (Statistica Version 7.1, StatSoft GmbH, Hamburg, Germany) of all environmental variables were performed with Spearman´s rank correlation ($p < 0.05$).

Multivariate analyses

The PERMANOVA subroutine PRIMER v6 (Clarke and Gorley 2006) with fixed factors was employed to investigate "consensus fingerprinting profiles" of individual stations and months for significant differences regarding their community structure. A significance level of <0.01 and unrestricted permutation of raw data was applied.

Principal coordinates analysis (PCO) was performed to investigate inter-point dissimilarities between the "consensus fingerprinting profiles" of samples for each transect individually. The Jaccard index was applied to calculate the resemblance matrix for the "consensus fingerprinting profiles". The relationship between "consensus fingerprinting profiles" and environmental variables was investigated by distance-based multivariate multiple regression

(DISTLM). Environmental variables, precisely: TOC, *chlorophyll a*, temperature, salinity and grain size fractions were log transformed prior to the analysis. Jaccard Index was applied to calculate the resemblance matrix for "consensus fingerprinting profiles". The DISTLM model was built using stepwise selection, adjusted R^2 and applying 4999 permutations at a significance level of $p < 0.01$. Results were visualised by using distance-based redundancy analysis (dbRDA).

Results

Physicochemical and geochemical properties along the transects

Mean values and respective standard deviation for temperature, *chlorophyll a*, fine sand and salinity are depicted in the Figures 2-4 for the individual transects. All data were obtained from bottom water samples. Annual temperature and *chlorophyll a* followed a similar trend along all three transects. Highest temperature occurred in June and October (~ 15°C, Fig. 2A-4A). Highest *chlorophyll a* concentrations were observed in May along Eider and Elbe transect (Elbe :~ 20µg/l, Fig. 3B and Eider: ~ 10µg/l Fig. 4B). Spatial variations regarding fine sand and salinity are shown in the figures (2C and D – 4C and D). The Elbe transect displayed highest variations regarding fine sand distributions (Figure 3D). The sediment at the sampling sites I-III had almost no fine sand fractions while they increased up to 60 % at sampling site V (Fig. 3D).

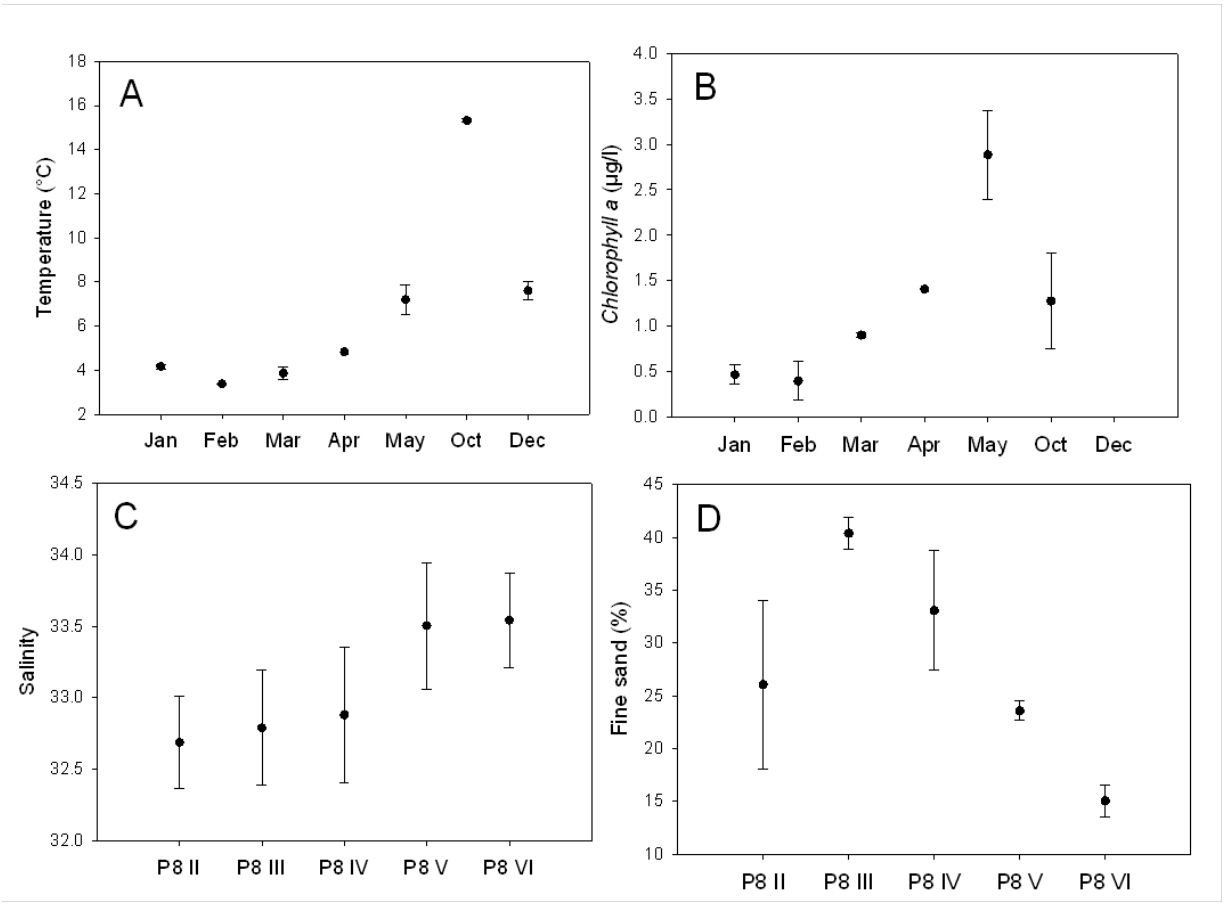

Fig.2 P8 transect: Means of annual temperature and c*hlorophyll a* variations (A,B) and spatial fine sand and salinity variations (C,D).

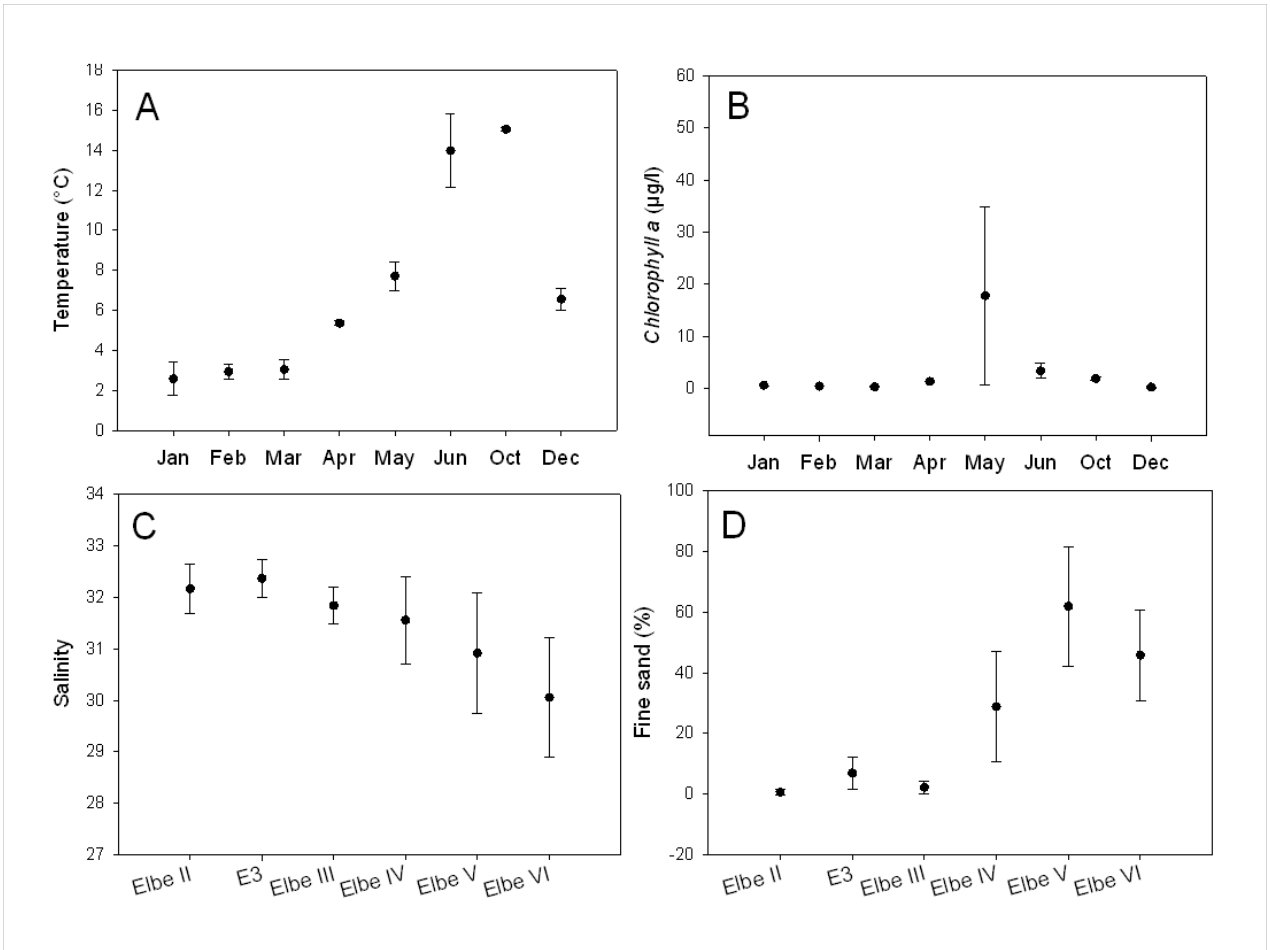

Fig.3 Elbe transect: Means of annual temperature and c*hlorophyll a* variations (A,B) and spatial fine sand and salinity variations (C,D).

Variations observed along the other transects were considerably lower ranging in general between 20 – 40 % fine sand for individual sampling sites (Fig. 2D-4D). The steepest salinity gradient was detected along the Eider transect (Fig. 4C). Salinity decreased from sampling site I to VI from ~ 33 to ~25. In contrast salinity was rather stable along both Elbe and P8 transect.

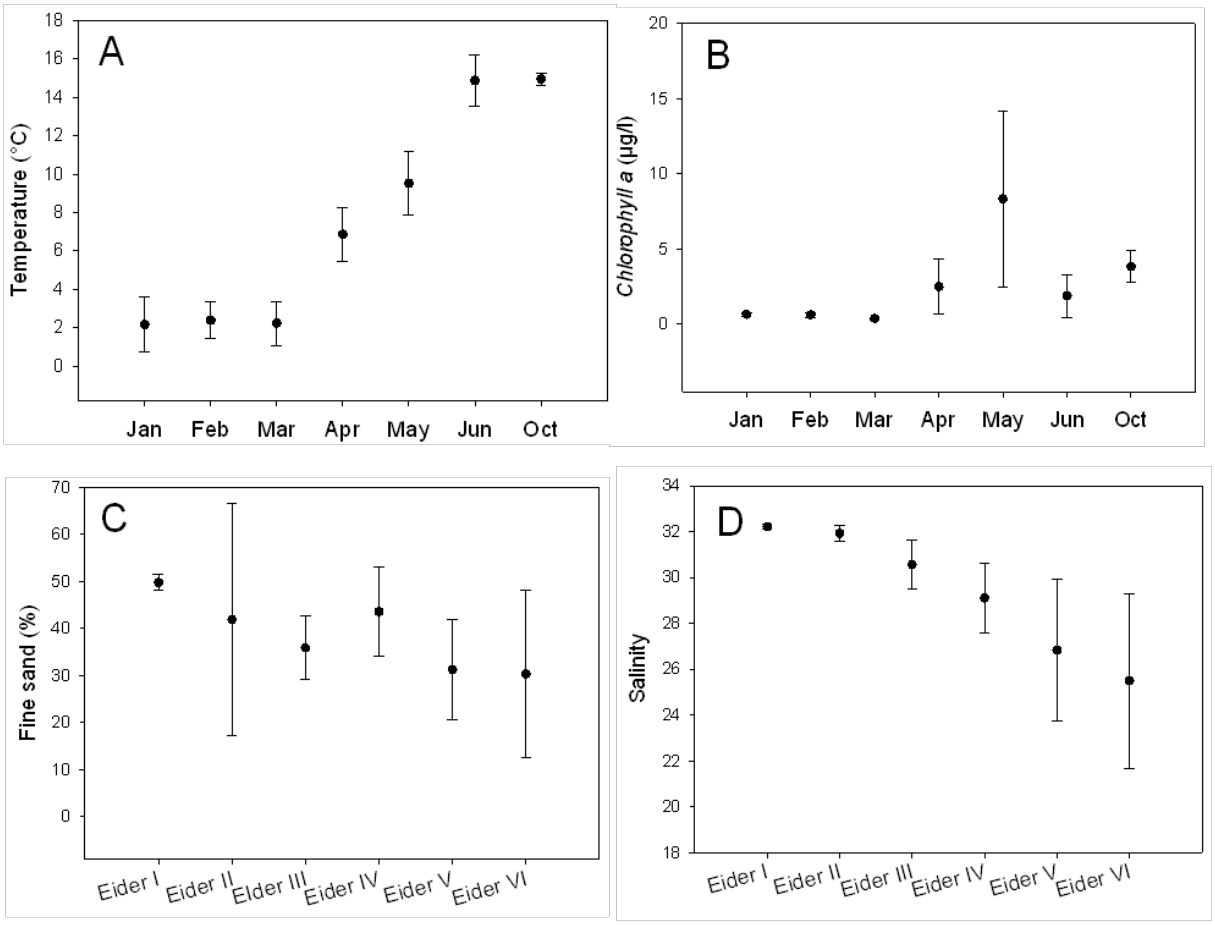

Fig.4 Eider transect : Means of annual temperature and c*hlorophyll a* variations (A,B) and spatial fine sand and salinity variations (C,D).

Bacterial community structure

The bacterial community structure based on "consensus fingerprinting profiles" was subjected to PERMANOVA and principal coordinates analysis (PCO) for each transect individually. PERMANOVA was performed to investigate bacterial community structure for significant differences respecting spatial (site) and temporal (month) factors. Furthermore alpha diversity estimated from ARISA OTU numbers was investigated for significant spatial and temporal differences. Certain months or sampling sites for individual transects are missing due to failed sampling cruises.

Figure 5B and 5C depict the PCO plots of bacterial communities along the P8 transect labelled according to respective sampling sites (Fig. 5B) and respective sampling months

(Fig 5C). The first two axes of the PCO for bacterial community structure along the P8 transect captured 30.8 % of the total variation. Neither distinct spatial nor temporal patterns within the bacterial communities were observed (Fig. 5B and 5C). Consistent with the results from the PCO, pairwise comparisons indicated no significantly different bacterial communities respecting the sampling site. However, respecting the temporal factor (month) significant differences comparing bacterial community structures from January and May were shown (PERMANOVA, $p < 0.01$).

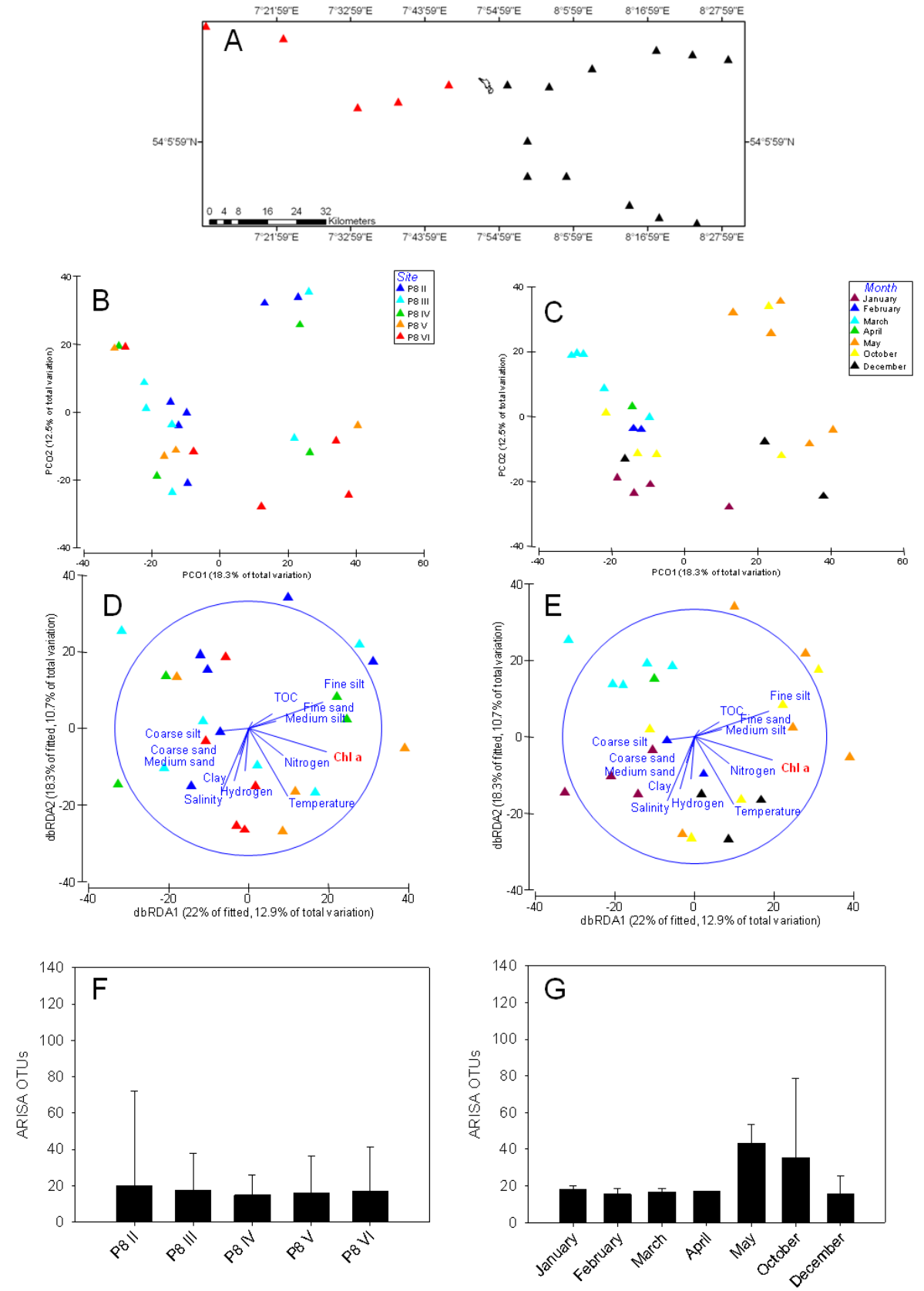

Fig. 5 Bacterial community analyses for the P8 transect. Location of the P8 transect (A), Plot of principal coordinates analyses (PCO) of bacterial community fingerprints based on the Jaccard index referring to sampling site (B) and month (C). Plots of distance-based redundancy analysis (dbRDA) of bacterial community fingerprints and environmental variables based on the Jaccard index referring to sampling site (D) and month (E). Significant environmental variables depicted in red ($p < 0.01$). Bar charts of means of ARISA OTU numbers and respective standard deviation referring to sampling site (F) and month (G).

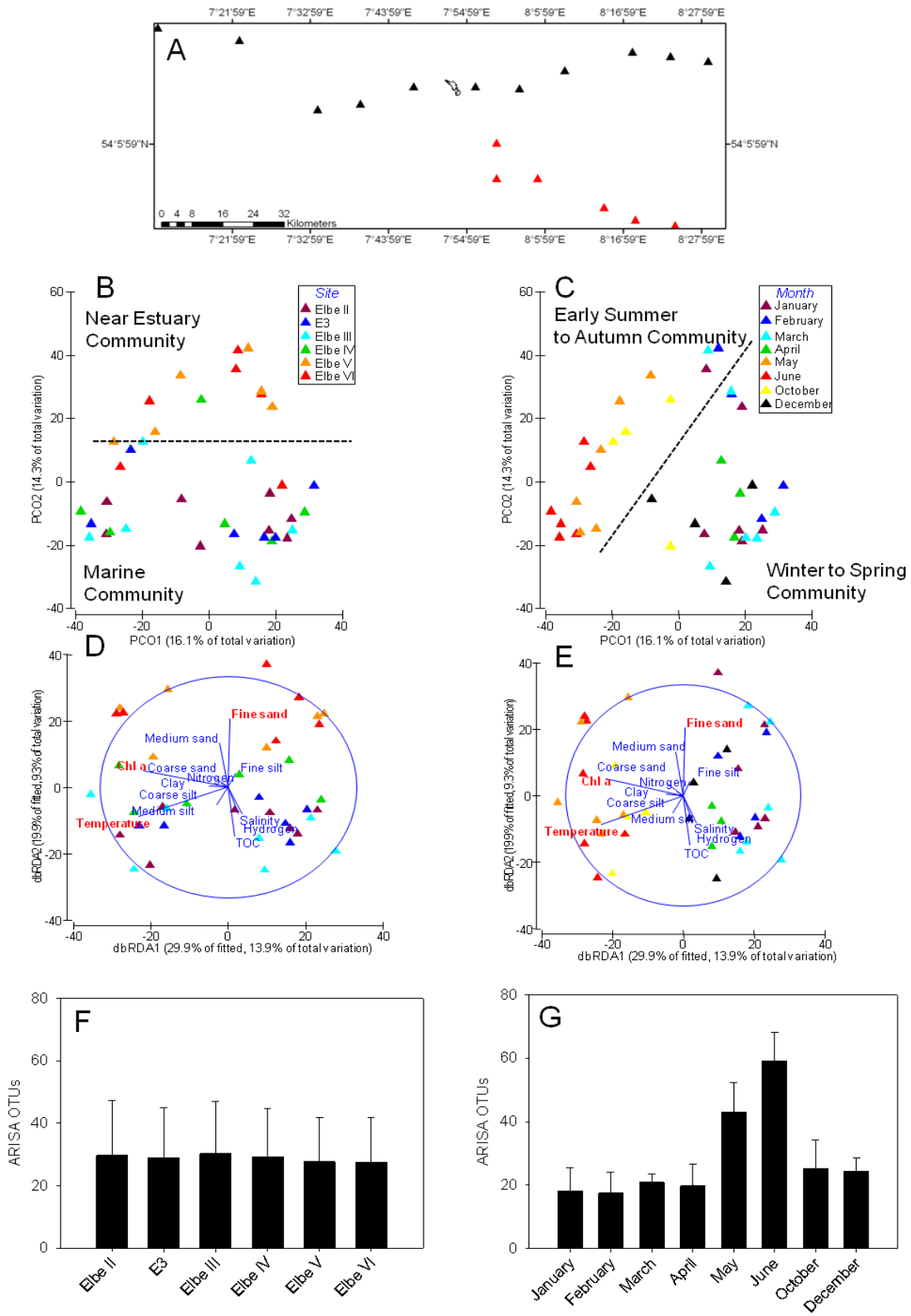

Fig. 6 Bacterial community analyses for the Elbe transect. Location of the Elbe transect (A), Plot of principal coordinates analyses (PCO) of bacterial community fingerprints based on the Jaccard index referring to sampling site (B) and month (C). Plots of distance-based redundancy analysis (dbRDA) of bacterial community fingerprints and environmental variables based on the Jaccard index referring to sampling site (D) and month (E). Significant environmental variables depicted in red ($p < 0.01$). Bar charts of means of ARISA OTU numbers and respective standard deviation referring to sampling site (F) and month (G).

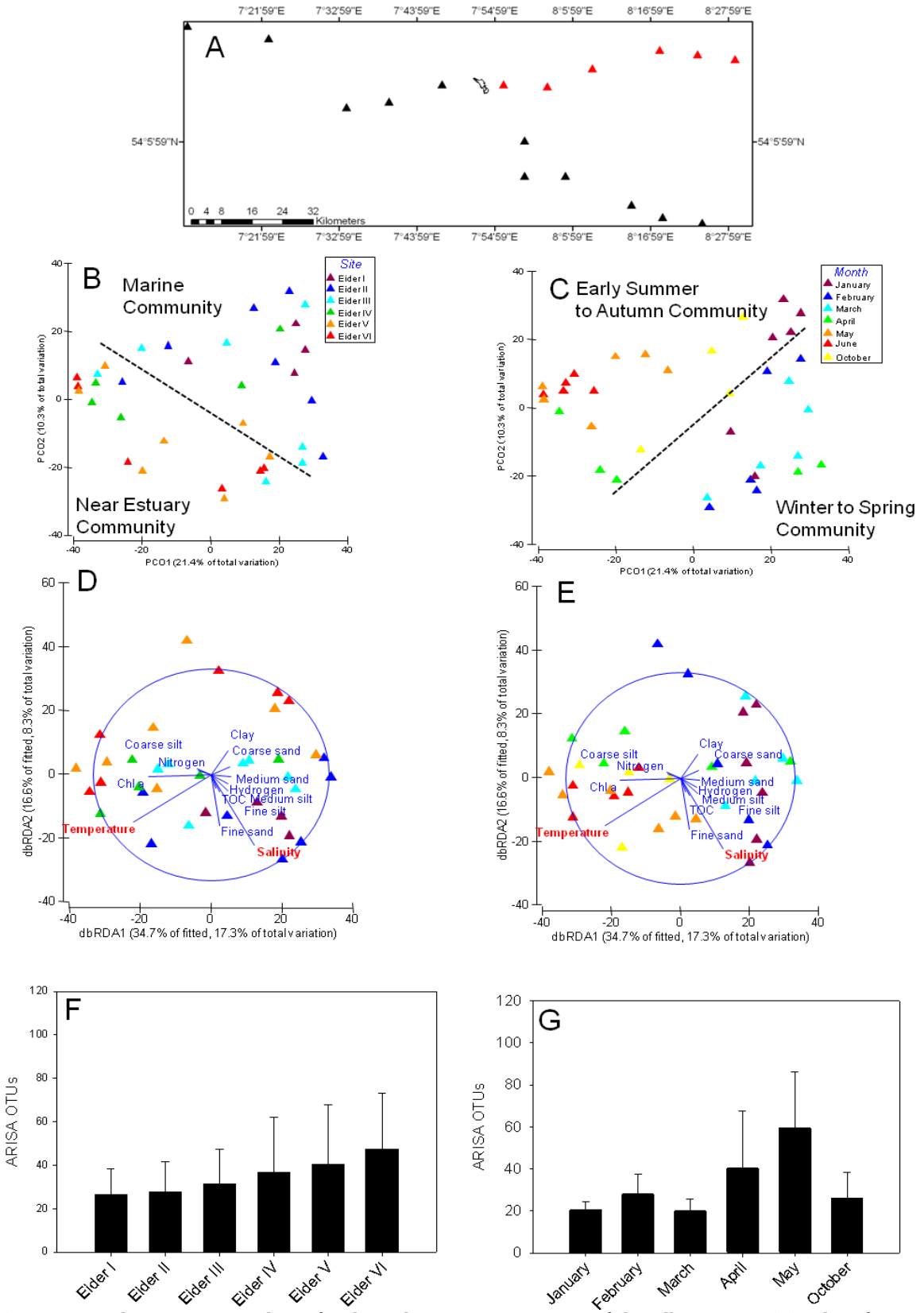

Fig. 7 Bacterial community analyses for the Eider transect. Location of the Elbe transect (A), Plot of principal coordinates analyses (PCO) of bacterial community fingerprints based on the Jaccard index referring to sampling site (B) and month (C). Plots of distance-based redundancy analysis (dbRDA) of bacterial community fingerprints and environmental variables based on the Jaccard index referring to sampling site (D) and month (E). Significant environmental variables depicted in red ($p < 0.01$). Bar charts of means of ARISA OTU numbers and respective standard deviation referring to sampling site (F) and month (G).

PCO plots of the bacterial communities along the Elbe transect are shown in Figure 6B and 6C. Regarding spatial variation bacterial communities from sampling sites near the estuary cluster together and are clearly separated from bacterial communities of the marine sampling sites (Fig. 6B). Respecting the sampling month bacterial communities obtained from early summer to autumn appear more similar when compared to the bacterial communities obtained from winter and spring (Fig. 6C). Pairwise comparisons of bacterial communities via PERMANOVA approach confirmed significant differences for spatial and temporal factors ($p < 0.01$).

Bacterial communities of the Eider transect are displayed in Fig. 7B and 7C. The first two axes of the PCO captured 31.7 % of the total variation. Generally, samples obtained near the estuary separated from those of marine sampling sites (Fig. 7B). Furthermore distinct community patterns were observed for the early summer to autumn and winter to spring period (Fig. 7C). Consistent with the PCO pairwise comparisons of bacterial communities revealed principally significant differences for marine and near estuary communities as well as for early summer to autumn communities and winter to spring communities (PERMANOVA, $p < 0.01$).

The alpha diversity is depicted as bar charts of mean values with respective standard deviation of ARISA OTU numbers (Fig. 5-7F and 5-7G). Significant spatial (site) and temporal (month) differences were tested with ANOVA and *post hoc* Tukey tests. We observed no significant spatial differences for the three transects (Fig. 5-7F). Generally, lowest alpha diversity was observed along the P8 transect. Means of ARISA OTU numbers ranged about 20 at the individual sites (Fig. 5F). Significantly higher ARISA OTU numbers (~ 50 ARISA OTUs, $p < 0.05$) were detected in May and October (Fig. 5G). As observed for the P8 transect significant higher ARISA OTU numbers were observed for May along the Elbe and Eider

transects. Moreover the Elbe transect showed significantly higher ARISA OTU numbers in June ($p < 0.05$).

Relation of bacterial community fingerprints to environmental data

We applied multiple regression analysis (DISTLM) to bacterial community data and environmental variables for the individual transects. Prior to the analysis spearman's rank correlation revealed that generally silt and clay fractions were significantly strong correlated with each other ($r_s > 0.87$, Supplement 1). The same was observed for nitrogen, hydrogen and TOC content ($r_s > 0.68$, Supplement 1). For the Elbe and Eider transect significant negative correlations for medium and fine sand fractions with silt and clay fractions were observed ($r_s > 0.69$, Supplement 1). Significant correlations were also observed for salinity, nitrogen, hydrogen and TOC with silt and clay fraction for both Elbe and Eider transects ($r_s > 0.34$, Supplement 1).

The results obtained by DISTLM are depicted in distance-based redundancy analyses (dbRDA, Fig. 5D and 5E). The first two axes of the dbRDA of the P8 transect explain 23.6 % of the total and 40.3 % of fitted variation. This indicates that the plot captures most of the salient patterns in the fitted model. Marginal and sequential tests indicated solely *chlorophyll a* concentrations to have a significant effect on the bacterial community structure (Table 2). However *chlorophyll a* contributes solely with 0.08 % to the model. The dbRDA shows, consistent with the results obtained from PCO and PERMANOVA no clear patterns of bacterial community structures. The results for the Elbe transect are displayed in Fig. 6D and 6E. Here, 23.2 % of the total and 49.8 % of the fitted variation are covered. Medium and fine sand as well as silt fractions and clay, temperature, salinity and *chlorophyll a* had a significant individual effect on bacterial community structure as revealed by marginal test in the DISTLM model. However regarding the sequential tests solely temperature, fine sand and *chlorophyll a* had significant effects (Table 2).

Table 2 DISTLM results for bacterial community data and environmental factors.

P8

Variable	Pseudo-F	P	Proportion of variance	Sequential test	Pseudo-F	P	Proportion of variance
Coarse gravel	0	1	0.000	**Chlorophyll a**	20,573	**0.0092**	0.082
Medium gravel	0	1	0.000	Nitrogen	15,993	0.0617	0.062
Fine gravel	0	1	0.000	Temperature	11,142	0.3351	0.043
Coarse sand	0.99048	0.4648	0.041	Medium sand	12,806	0.1882	0.049
Medium sand	0.90207	0.5728	0.038	Fine sand	10,496	0.3974	0.040
Fine sand	0.70197	0.8086	0.030	Salinity	0.90075	0.5591	0.034
Coarse silt	0.73036	0.7747	0.031	Coarse silt	0.8506	0.6175	0.033
Medium silt	0.68893	0.817	0.029	Fine sand	12,235	0.2462	0.047
Fine silt	0.72466	0.7769	0.031	Clay	1,049	0.3904	0.040
Clay	0.69247	0.8073	0.029	Hydrogen	0.86502	0.5824	0.033
Temperature	16,363	0.0493	0.066	TOC	13,366	0.2017	0.050
Salinity	0.8463	0.6443	0.035	Coarse sand	11,289	0.3468	0.042
Nitrogen	19,092	0.0133	0.077	Medium sand	0.75638	0.6767	0.029
TOC	0.58459	0.9132	0.025				
Hydrogen	13,384	0.1625	0.055				
Chlorophyll a	20,573	**0.0093**	0.082				

Elbe

Variable	Pseudo-F	P	Proportion of variance	Sequential test	Pseudo-F	P	Proportion of variance
Coarse gravel	0	1	0.000	**Temperature**	47,382	**0.0001**	0.114
Medium gravel	0	1	0.000	**Fine sand**	30,272	**0.0001**	0.069
Fine gravel	0	1	0.000	**Chlorophyll a**	20,515	**0.0016**	0.045
Coarse sand	15,685	0.0425	0.041	Salinity	16,793	0.0132	0.036
Medium sand	2,362	**0.0021**	0.060	Hydrogen	13,296	0.1106	0.029
Fine sand	26,317	**0.0007**	0.066	Coarse silt	11,434	0.2703	0.024
Coarse silt	26,109	**0.0012**	0.066	Medium sand	12,879	0.1348	0.027
Medium silt	25,044	**0.001**	0.063	Coarse sand	10,608	0.3847	0.022
Fine silt	23,395	**0.0024**	0.060	Fine silt	0.92303	0.5777	0.020
Clay	22,599	**0.0035**	0.058	Medium silt	12,812	0.1628	0.027
Temperature	47,382	**0.0001**	0.114	Clay	10,797	0.3552	0.023
Salinity	24,043	**0.0007**	0.061	Nitrogen	0.77095	0.7846	0.016
Nitrogen	16,197	0.0468	0.042	TOC	0.67199	0.8882	0.014
TOC	17,895	0.0219	0.046				
Hydrogen	19,452	0.0106	0.050				
Chlorophyll a	38,587	0.0001	0.094				

Eider

Variable	Pseudo-F	P	Proportion of variance	Variable	Pseudo-F	P	Proportion of variance
Coarse gravel	0	1	0.000	**Temperature**	48,452	**0.0001**	0.125
Medium gravel	0	1	0.000	**Salinity**	40,626	**0.0001**	0.096
Fine gravel	0	1	0.000	Coarse sand	15,623	0.0409	0.036
Coarse sand	12,317	0.1943	0.035	Medium sand	12,712	0.1559	0.029
Medium sand	14,631	0.0918	0.041	Fine sand	11,071	0.3112	0.025
Fine sand	17,225	0.0377	0.048	TOC	0.89848	0.6015	0.021
Coarse silt	12,322	0.1967	0.035	Nitrogen	10,825	0.3474	0.025
Medium silt	11,263	0.2823	0.032	Hydrogen	0.93452	0.538	0.022
Fine silt	10,064	0.4245	0.029	Clay	0.87536	0.6317	0.020
Clay	0.91645	0.5278	0.026	Coarse silt	15,997	0.0416	0.036
Temperature	48,452	**0.0001**	0.125	Medium silt	12,701	0.1845	0.028
Salinity	24,958	**0.0024**	0.068	Fine silt	0.83047	0.687	0.019
Nitrogen	0.65788	0.889	0.019	Chlorophyll a	0.65908	0.8647	0.015
TOC	12,657	0.181	0.036				
Hydrogen	0.82147	0.6721	0.024				
Chlorophyll a	4,022	**0.0001**	0.106				

The factors contribute with 22.8% to the model (Table 2). Observing the dbRDA plots temperature is rather associated with the first axis of the dbRDA while fine sand is correlating with the second axis (Fig. 6D and 6E). Regarding the spatial aspect (site, Fig. 6D); the environmental variable fine sand forms a strong gradient separating bacterial community structures from the stations Elbe V and Elbe VI from the other stations. Temperature on the other hand separates bacterial communities from May, June and October from the other months (Fig. 6E).

Bacterial community structure along the Eider transect however is significantly influenced by individual effects of temperature, salinity and *chlorophyll a* (Fig. 7D and 7E, Table 2). In the sequential tests significant effects for temperature and salinity were confirmed (Table 2). Both variables contribute with 22.1 % to the model. Again, temperature is rather correlated with the first axis forming a strong gradient which separates bacterial communities from April, May, June and October from the other months (Fig. 7E). The effect of salinity is rather spatial since bacterial communities from the sites Eider I – III correlate with increasing salinity (Fig. 7D).

Discussion

Temporal and spatial variations within bacterial communities are of great interest not only for microbial ecology but also for modelling ecosystem functioning on a global scale. To date most research focuses on either temporal (Fuhrman *et al* 2006, Kan et al 2006, Yannarell *et al* 2003a) or spatial variations within bacterial communities (Crump *et al* 2004, Herlemann *et al* 2011, Hewson *et al* 2007), nearly exclusively bacterioplankton communities were investigated. Temporal as well as spatial variations appear to influence the community structure tremendously (Fuhrman *et al* 2006, Herlemann *et al* 2011, Yannarell *et al* 2003b). As main driving factors predominantly temperature and salinity are mentioned. Several studies took both temporal and spatial scales into account (Fortunato *et al* 2012, Ghiglione *et al* 2005, Hewson *et al* 2006). To our knowledge not a single one concentrated on benthic bacterial communities. This study provides a unique perspective on how spatiotemporal gradients influence benthic bacterial communities in a coastal area in the German Bight. We investigated simultaneously benthic bacterial communities inhabiting near and offshore environments over an annual cycle. The biogeography was assessed via ARISA fingerprinting and main driving environmental factors were identified using multivariate multiple regression. We hypothesised that bacterial community structure is determined by individual environmental gradients in near and offshore regions.

Bacterial communities in near and offshore habitats

The three investigated transects in the German Bight differed greatly regarding influencing biogeochemical and physicochemical parameters such as sediment composition, temperature, salinity and *chlorophyll a* concentrations. We observed neither spatial nor temporal variation within bacterial communities along the P8 transect, located > 60 kilometres offshore the coastline. In contrast, bacterial communities along both Elbe and Eider transect varied significantly regarding their community structure on both, temporal and spatial scales. Both

transects ended around 25 kilometres near the coastline. The P8 transect exhibited rather stable conditions regarding recorded abiotic and biotic factors. Elbe and Eider transect in contrast, end in the near of their respective estuaries and our data showed that they are characterised by a high variability regarding physicochemical parameters such as salinity, temperature or organic loading (Fig. 2). Especially in nearshore regions local winds might lead to considerable shifts between coastal upwelling and downwelling conditions, while offshore regions are less affected by local winds and physicochemical conditions remain rather stable (Fig. 2). The pronounced spatial and temporal variations regarding physicochemical and biogeochemical parameters along the estuaries and the in contrast weak variations along the P8 transect suggest the presence of two regimes differently impacted by physical parameters. We assume that the distance to the coast and its implying different impact of physical forces epresents a major factor driving spatiotemporal variations within the communities.

To our knowledge studies investigating spatial variations of benthic bacterial communities comparing temperate nearshore and offshore habitats are scarce. Uthicke and co-workers (2007) examined bacterial communities in coral reef sediments and observed, in line with our investigation, significant differences between nearshore and offshore communities. The separation of nearshore and offshore communities was also described for pelagic communities (Fortunato and Crump 2011, Rink *et al* 2011).Only recently, Rink and co-workers (2011) published their investigations of regional patterns of pelagic bacterial communities in the German Bight. They assume that differences in the hydrographic and biogeochemical conditions affect the assembly of the bacterial communities. We assume that benthic bacterial communities display spatial variations but to a lesser extent compared to pelagic communities.

In contrast to bacterial community structure bacterial diversity appears not to be affected by spatial but explicitly by temporal factors. Principally, a significant higher alpha diversity was observed for the period from May to October. This finding is in line with observations made in Wadden Sea sediments in the Sylt- Romo basin (Boer *et al* 2009). Boer and co-workers reported a higher diversity in August when compared to the other sampling months. Especially in spring and autumn organic matter input can vary considerably due to changes in the primary production in the North Sea (Duyl and Kop 1994). The primary production is controlled by nutrients which resuspend from the seafloor into the photic zone. A high nutrient availability stimulates the phytoplankton production and phytoplankton blooms occur. These in turn stimulate the zooplankton and consequently the whole food chain. Organisms which are not consumed in the water column die and sink to the seafloor (approximately three metres per day in North Sea waters (Skogen *et al* 1995). Bacterial communities respond with increasing productivity to organic matter input for instance after phytoplankton blooms (Duyl and Kop 1994, Meyerreil 1983) and indications for increasing bacterial diversity coupled to organic matter input and nematode diversity were stated by Vanaverbeke and co-workers (2004). Bentho-pelagic coupling represents a crucial element for benthic life. The input of pelagic particles sinking to the seafloor determines substantially benthic communities. We assume that the increasing bacterial diversity is directly linked to these processes.

Main influencing gradients along the three transects

Chlorophyll a, fine sand, salinity and temperature were identified as main factors influencing the bacterial community structure along the individual transects.

Chlorophyll a concentrations had a significant effect on bacterial community structures along P8 and Elbe transects. *Chlorophyll a*, an indirect measure for phytoplankton, was considerably higher in May as compared to other sampling months (Fig. 2). As already

discussed in the previous section, phytoplankton blooms arise generally in spring and autumn in the shallower regions in the North Sea (Joint and Pomroy 1993). They are characterised by a distinct patchiness and are highly dynamic. Water movement, caused by currents and winds transport the phytoplankton bloom from shallower coastal regions into deeper offshore regions. Along with this movement productivity gradients from high productivity in nearshore regions to low productivity in offshore regions establish (Joint and Pomroy 1993). This productivity gradient of the phytoplankton was mirrored in our *chlorophyll a* data. We observed highest *chlorophyll a* concentrations at coastal sampling sites along Elbe and Eider transects compared to lower concentrations at offshore sampling sites along the P8 transect. In May, however, relatively high *chlorophyll a* concentrations in the bottom water were observed along all investigated transects representing probably a post-bloom signal. We observed significant different bacterial community structures in May when compared to bacterial communities in earlier months of the year. Again this might be an indication for benthic-pelagic coupling. We hypothesise, even though the *chlorophyll a* concentrations were measured in the bottom water, that the organic matter reached already the seafloor. Thus, the benthic bacterial community responded with a simultaneous increase in bacterial diversity and changes in the bacterial community structure to the input of organic material originating from decaying phytoplankton blooms.

Spatial differentiation was assigned to fine sand and salinity variations for the individual nearshore transects. While fine sand distributions affected bacterial communities along the Elbe transect, bacterial communities clustered according to salinity variations along the Eider transect. The Elbe transect passes a region which is characterised by (very) coarse silt at the sites Elbe II, E3 and Elbe III while the sediment at the sites Elbe IV, Elbe V and Elbe VI was composed of fine sand (Table 1, Fig. 2). The mud deposit in the south-east of Helgoland is well documented (Hebbeln *et al* 2003, Mühlenhardt-Siegel 1981, Puls *et al* 1997). The

continuous sedimentation in this area is caused by a small-scale eddy driven by the interaction of the longshore coastal current, the discharge of Elbe and Weser Rivers and tidal dynamics (Hebbeln *et al* 2003). Obviously, the bacterial communities in the sediments along the Elbe transect are affected by the resulting grain size gradient. This assumption bases on the significant individual effect of silt and clay fractions on the bacterial community variation as revealed by our marginal tests (Table 2) and the significant impact of fine sand distributions in the sequential tests coupled with the respective clustering of bacterial communities. Sediment composition represents a major driving factor for benthic bacterial community assembly (Dale 1974, DeFlaun and Mayer 1983). Only recently a study conducted at a dumping site which is included in the Elbe transect (site E3) revealed a strong gradient of grain size distributions which influenced significantly the bacterial community structure (Störmer *et al* 2012). Principally, sandy sediments harbour different bacterial groups than muddy ones (Llobet-Brossa *et al* 1998). We therefore conclude that bacterial community structure is highly influenced by the steep grain size gradient along the Elbe transect.

The steepest salinity gradient was recorded along the Eider transect. Salinity ranged between 33-22 and defined therefore marine and estuarine conditions. We observed a distinct clustering of bacterial communities according to this salinity gradient (Fig 5). Salinity gradients represent an important factor influencing bacterial communities in estuaries (Selje and Simon 2003). Salinity contributes to density gradients in coastal areas which separate water masses and their residential pelagic bacterial communities (Fortunato *et al* 2012). Among others Herlemann and co-workers (2011) reported defined bacterial communities for marine and fresh water as well as for the brackish water bodies. To our knowledge studies addressing benthic bacterial community changes in estuaries according to salinity gradients are little studied (Ikenaga *et al* 2010). However, Ikenaga and co-workers (2010) demonstrated recently that benthic bacterial communities cluster along a salinity gradient in the Everglades.

Temporal variation among the bacterial communities was mainly explained by temperature variations. For both nearshore transects (Elbe, Eider) seasonal changes in the bacterial community structure were resolved. Principally, bacterial communities clustered according to the early summer to autumn and winter to spring season. Regarding temperature these communities might also be considered as warm and cold period communities. The temperature effect implies in fact a multitude of other factors which change with temperature. In coastal areas for instance nutrient input, river discharge, upwelling and productivity are closely linked to temperature variations. Thus benthic bacterial communities might not be affected by temperature variations alone but by seasonal processes linked to temperature variations.

Temporal variations were shown to explain most of the variance in our models (Table 2). Therefore we assume that temporal variations are of great importance for benthic bacterial community assembly. The study of Boer and co-workers confirms this hypothesis (Boer *et al* 2009). Studies investigating pelagic bacterial communities demonstrated contrary, that spatial variations overwhelmed temporal factors (Fortunato *et al* 2012). Fortunato and co-workers found that salinity and depth influenced the bacterioplankton predominantly. Probably pelagic and benthic bacterial communities are affected by different factors in coastal regions. It would be worth approaching this hypothesis by studying pelagic and benthic bacterial communities simultaneously.

In summary our study allowed novel insights into bacterial community structure and diversity along spatial and temporal gradients in the German Bight. We showed that bacterial communities in offshore regions showed no clear temporal or spatial variations while their counterparts in nearshore regions exhibited distinct temporal as well as spatial patterns. Temporal variations were predominantly driven by temperature and of greater importance than spatial gradients.

Acknowledgments

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CHAPTER II

Impact of ocean dumping on bacterial communities

I: Fine-scale investigations at a dumping site

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Abstract

The impact of ocean dumping on benthic bacterial communities is not included in regular monitoring programs, yet. Hence, in 2009 and 2010, we initiated an extensive investigation of the spatial structure of the bacterial community at a dumping site in the German Bight using the fingerprinting method, Automated Ribosomal Intergenic Spacer Analysis. Using redundancy analyses, we aimed to identify the main environmental factors shaping the bacterial community. The phylogenetic composition was investigated via ribosomal tag sequencing for representative samples. Our results reveal significantly different bacterial communities when comparing dumping and a reference sites. Furthermore, ten months after dumping the dumping centre displayed a low alpha diversity. Typical freshwater bacterial phylotypes and *Desulfuromonadaceae* as well as *Flavobacteriaceae* were observed in considerably higher numbers at the dumping centre. We assume, that most likely the sediment granularity and to a lesser extent, pollutants, shape the bacterial community.

Keywords: ARISA/ dredged sediment / fingerprinting analysis / multivariate statistics/ pollution / 454 sequencing/

Introduction

Estuaries represent economically significant areas, which are exposed to many types of human interferences (Lotze 2010). Protective measures, such as dikes and the deepening of commercial shipping lanes, alter natural hydrodynamics of rivers and estuaries (Freitag *et al* 2008). Naturally high siltation rates in these areas exacerbate the increase in the amount of dredged material. Dumping sites for this dredged material and sewage sludge exist in many coastal zones worldwide (OSPAR 2009, Stronkhorst *et al* 2003, Tkalin *et al* 1993). International guidelines, advising the management of dredged material, recommend the assessment of physical, chemical and biological parameters such as fishes or macrozoobenthic communities (IMO 2000, OSPAR 2004). Dumping causes physical disturbance, burial of benthic organisms and a general change in substrate matter, which again may affect these benthic communities directly.

In the Elbe River altered hydrodynamics reinforced the accumulation of sediment in recent years (HPA 2005). The city of Hamburg received permission to dump lightly polluted sediment, characterised as muddy sand containing equal proportions of silt, very fine and fine sand into the German Bight (Folk 1980). The handling of dredged material and dumping activity is regulated by German guidelines in respect to London and OSPAR conventions (BfG 1999, BfG 2009). The actual dumping site measures 400 square metres. Bearing revealed a three metres high rising at the dumping site containing mainly sandy sediments as obtained by grain size analyses. Acoustic Doppler Current Profiler (ADCP) analyses recorded that upon dumping, fine-grained material drifts about eight kilometres until settling down (HPA 2005). The monitoring program at the dumping site targets among others the contaminant content of the sediments, the macrozoobenthic community and the fish fauna. During the dumping period from 2005 to 2010 a significant increase of organic pollutants, precisely poly aromatic hydrocarbons (PAH) and organotin compounds was reported for the

dumping site. Simultaneously, investigations of the macrozoobenthic communities revealed a decrease in species richness and density (HPA 2010). Thus far, analyses of bacterial communities are not implemented in monitoring programs.

Investigating the structure and composition of bacterial communities may be a promising tool to assess environmental changes within monitoring programs. Bacterial communities are the most abundant sediment organisms and regulate substantial functions such as nutrient cycling (Ramette *et al* 2009). Bacteria also cycle manganese, iron or even toxic metals (Ford and Ryan 1995). The integration of bacterial communities' in monitoring programs may allow for a faster and earlier assessment of environmental perturbation than well-established monitoring tools (such as investigating macrozoobenthos and fish populations).

Several studies have addressed the impact of perturbation on bacterial communities (Dean-Ross and Mills 1989, Gillan *et al* 2005, Roling *et al* 2001, Wang *et al* 2011). Bacterial communities react to physical disturbance, as sieving, with changes in community structure and reduced biomass (Findlay *et al* 1990). Observations of the impact of heavy metal or oil contamination on bacterial communities revealed that the contamination affects the structure as well as the function of bacterial communities (dos Santos *et al* 2011, Gremion *et al* 2004, Suarez-Suarez *et al* 2011). The deposition of polluted sediments has been predominantly investigated in mesocosm experiments (Kan *et al* 2011, Nayar *et al* 2004, Toes *et al* 2008). A comparison of polluted and non-polluted samples revealed different bacterial communities. However, the effect of heavy metal contamination on bacterial community structure is not always distinguishable from other environmental factors in the field (Dean-Ross and Mills 1989, Gillan 2004).

To our knowledge, fine-scale investigations, evaluating the spatial perturbation of dumping activity on the bacterial community remain lacking. In an interdisciplinary project we initiated fine-scale investigations of the benthic bacterial community at the dumping site in the

German Bight. The monitoring program itself was designed beforehand according to the German guidelines for dredged material handling (. 1999, BfG 1999) and GÜBAK-WSV (BfG 2009). The sampling scheme comprises 125 sampling stations grouped into *a priori* regions by distance to the dumping centre (e.g. 1 km, 1.5 km etc), including the dumping and reference sites. Bacterial community structure was estimated from a direct comparison to this reference site (12 km north off the dumping site and thus not affected by the dumping activity). We performed Automated Ribosomal Intergenic Spacer Analysis (ARISA), combined with ribosomal sequencing of representative samples to investigate bacterial community structures. Combining biotic information and geochemical data (including information on grain size fractions, several pollutants, elemental nitrogen, sulphur, phosphorus and organic carbon) was implemented using multivariate analysis, which is a feasible tool for predicting the causal factors of bacterial community structures (Cao *et at* 2006, Liu *et al* 2011).

The objectives of this study are as follow: a) to investigate bacterial communities in the *a priori* regions via ARISA fingerprinting, b) to compare bacterial community information with contextual environmental data, and c) to identify community members and structures in representative samples.

Materials and methods

Site description and sampling

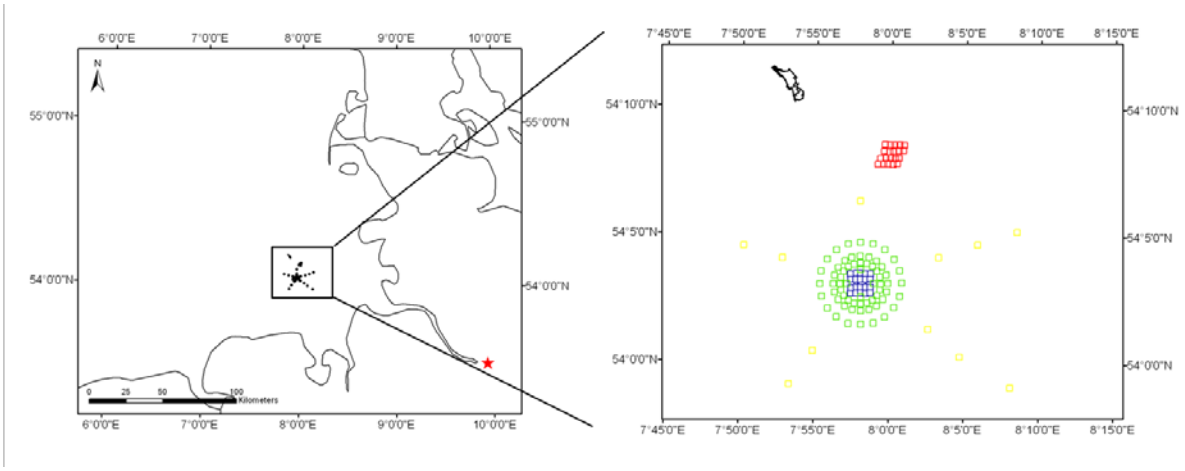

Fig. 1 Location of study site with sampling stations in the German Bight. Ten sampling stations are located at the immediate dumping centre, 24 others are situated in a range of 1 km. 20 sampling sites are located each at a distance of 1.5 km, 2 km and 3 km and another 11 are positioned on transects at 6, 9 and 12 km distance. The reference site consists of 20 sampling stations. The dredging zone is marked with a red star. The 34 sampling stations until 1 km were pooled as 'dumping site' (blue). The sampling stations arranged in circles up to 3km were grouped as 'surrounding' (green). The 'transects' are comprising sampling stations until 12 km distance (yellow) and finally the sampling stations of the 'reference site' (red) represent the fourth group.

Table 1 *A priori* regions, groups and sediment classification after Folk (1980).

Groups	*a priori* Regions	Sediment	April 2010	August 2010
dumping site	dumping centre (400m*400m)	clayey sand	sand	sand
	< 1 km	muddy sand	muddy sand	muddy sand
	1 km	sandy clay	sandy mud	clayey sand
surrounding	1.5 km	sandy mud	sandy mud	sandy mud
	2 km	sandy clay	sandy clay	sandy clay
	3 km	sandy clay	sandy mud	sandy clay
transects	6 km	clayey sand	muddy sand/sandy mud	muddy sand/sandy mud
	9 km	clayey sand	sandy mud	muddy sand
	12 km	muddy sand	muddy sand	muddy sand
reference	reference	sandy mud	sandy mud	sandy mud

The study site is located in the southern part of the German Bight (54°03´N 07°58´E). Water depths range between 20 and 35 m. Sediments were classified according to Folk (1980) (Table 1). Sediments at the study site are sandy at the immediate dumping centre, whereas the reference site consists of sandy mud. Sampling was performed in August 2009, April 2010

and August 2010. Dumping activities were conducted in October 2008 and from October 2009 to February 2010. Each sampling campaign consisted of 125 stations comprising dumping and a reference sites (Fig. 1). The sampling stations were grouped *a priori* into regions. Based on these *a priori* regions, we further categorised the sampling stations into four groups for visualisation (Fig. 1, Table 1): 'Reference site', 'transects', 'surrounding' and 'dumping site'. All sediment samples were collected with a van Veen grab (0.1 m^3). On board, the sediment was poured into a metal box and homogenised. To ensure coherent analyses, the samples for analyses of the bacterial communities as well as the samples for physicochemical analyses were taken from this sediment homogenate. For bacterial community analysis, three subsamples were stored immediately after collection at -20°C in 50 ml falcon tubes.

DNA extraction and quantification

DNA was extracted using the PowerSoil Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. Three subsamples of 0.25 g sediment were collected, and the extracted DNA was eluted in 50 µl elution buffer. Genomic DNA concentrations of the subsamples were measured by photometry using the Infinite M200 (Tecan Austria GmbH, Gröding, Austria). DNA was measured in duplicate.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

The intergenic spacer region (ITS) region of the bacterial genome was amplified with the primer set S-D-Bact-1522-b-S-20 [5´-TGC GGC TGG ATC CCC TCC TT-3´] and L—D-Bact-132-a-A-18 [5´-CCG GGT TTC CCC ATT CGG-3´] (Ranjard *et al* 2000). The forward primer was labelled with an infrared dye (IRD700). The PCR products of the subsamples were separated in a 5.5% polyacrylamide gel prepared following the manufacturer's protocol (LI-COR Biosciences, Lincoln, Nebraska, USA).

Ribosomal tag sequencing

Based on significant differences in community structure obtained by ARISA fingerprinting samples for ribosomal tag sequencing were selected. Genomic DNA from one subsample of the chosen The tag PCR approach as well as the sequencing approach were performed by LGC Genomics (Berlin, Germany). The V1-V6 region of the 16S RNA gene was amplified using the following primer set: forward GM3 5'-AGAGTTTGATCMTGGC-3' and reverse 907R 5'-CCGTCAATTCMTTTGAGTTT-3'. Sequencing was performed in a 454 Roche Genome Sequencer FLX + Titanium.

OTU definition for ARISA and ribosomal tag sequencing

ARISA fingerprints were all edited by BioNumerics Version 5.1 (Applied Maths NV, Sint-Martens-Latem, Belgium). Clustering of ARISA-OTUs (operational taxonomic units) (bands) into classes was performed as previously shown (Kovacs *et al* 2010). Peaks >1200 bp were negligible in the samples. ARISA-OTUs were analysed based on a constructed binary table (01).

Pyrosequencing data were processed for quality and barcode recovery with MOTHUR (Version 1.22.0) (Schloss *et al* 2009). Sequences were clustered at 97% similarity into 454-OTUs. Taxonomic information was obtained in parallel. For LIBSHUFF analysis, singletons were excluded from the data set using the subroutines split.abund (cutoff=1). Randomly, 6 950 sequences per sample were chosen.

Environmental data analysis

All environmental data (Table 2) were provided by the HPA. The total sediment was analysed following the HABAK guidelines (BfG 1999).

Table 2 Environmental data used in redundancy analysis (RDA) and variance partitioning. For RDA single values of grain size fractions, S, N, P, C and heavy metals were used; for PAH, PCB, HCH and DDX the sums of single values respectively. In variance partitioning variables were classified in grain size, S, N, P, C, organic pollutants (sums of PAH, PCB, HCH, DDX and hydrocarbons) and heavy metals.

Grain size fractions
< 20µm
20-63µm
63-100µm
100-200µm
200-630µm
630-1000µm
1000-2000µm
S, N, P, C
TOC (C)
nitrogen (N)
sulphur (S)
phosphor (P)
Hydrocarbons
Sum Polycyclic Aromatic Hydrocarbons (PAH)
naphthaline
fluorene
phenanthrene
anthracene
fluoranthene
pyrene
benz(a)anthracene
chrysene
benzo(b)fluoranthene
benzo(k)fluoranthene
benzo(a)pyrene
dibenz(ah)anthracene
benzo(ghi)perylene
indeno(1.2.3cd)pyrene
Sum Chlorinated Diphenyls (PCB)
PCB28
PCB52
PCB101
PCB118
PCB138
PCB153
PCB180

Sum Hexachlorocyclohexane (HCH)
alphaHCH
betaHCH
gammaHCH
deltaHCH
Sum Dichlorodiphenyldichloroethane (DDT) and metabolites
ppDDE
opDDD
ppDDD
opDDT
ppDDT
Sum Organotin Compounds
monobutyltin (MBT)
dibutyltin (DBT)
tributyltin (TBT)
tetrabutyltin
Heavy Metals
arsenic
lead
cadmium
chrome
copper
nickel
mercury
zinc

Statistics

Univariate Statistics

Pairwise correlations (Statistica Version 7.1, StatSoft GmbH, Hamburg, Germany) of all environmental variables were performed with a Spearman´s rank correlation coefficient. One-way factorial analysis of variance (ANOVA) was performed to test the effect of the *a priori* regions on ARISA-OTUs. Significant factors were then compared using a *post hoc* HSD test for unequal group size. All univariate statistical tests were tested at $\alpha = 0.05$.

Multivariate statistics

For non-metric multidimensional scaling (NMDS) (PRIMER Version 6, PRIMER-E Ltd, Lutton, UK) (Clarke and Gorley 2006), the Jaccard Index was applied in all cases. Analysis of similarities (ANOSIM) was employed in pairwise tests to assess the significant differences in groups of bacterial communities a) at each sampling station (testing similarity of replicates) and b) among different sites grouped according to their sampling regions (e.g. dumping centre, reference site, 1 km). The null hypothesises were a) "no differences with regard to sample position exist" and b) "no differences with regard to sample region exist". These analyses resulted in Global R values indicating the degree of separation. Values of $p < 0.1$ were considered significant.

The examination of relationships between bacterial community patterns and environmental data was conducted via CANOCO (Version 4.5; Biometris-Plant Research International, Wageningen, the Netherlands). First, detrended correspondence analysis (DCA) was performed to test whether linear or unimodal models were the best fit for the ARISA data set (Lepš and Šmilauer 2003). Redundancy analysis (RDA) was performed to test which environmental factors (Table 2) explain the significant variation in the bacterial communities. The data were not transformed prior to the RDA. Factors with a variance inflation factor > 15 were excluded (Legendre and Legendre 1998). The significance of the RDA models and the selected variables were determined by 499 Monte Carlo permutations at $p < 0.05$ for each group. The individual effects of factor groups (grain size; elemental composition, organic pollutants and heavy metals) on the variation in bacterial communities were further investigated by variance partitioning (Legendre and Legendre 1998).

Geostatistics

The software package ArcGIS Version 10 (ESRI Co, Redlands, CA, USA) was used to show spatial distributions of the data. Geostatistical analysis using the ordinary kriging subroutine,

mainly the spherical semivariogram model, was performed. Prediction errors, i.e., mean errors and mean standard errors, were adjusted to near zero. The root mean square error was adjusted close to 1. Moreover, root mean square and standard errors were highly similar. For grain size fractions and ARISA-OTUs, all 125 data points could be used; for heavy metals and organic pollutants, only 52 stations were used.

Phylogenetic Analyses

After sorting and quality control, the total number of OTUs was used for predictive rarefaction analysis and richness indices (invsimpson, ACE and Chao1). For the actual analysis rare species (n=1) were excluded. The MOTHUR subroutine LIBSHUFF was used to investigate significant differences within the whole community structure of the samples. A significance level of $p < 0.05$ was applied. The OTUs were subjected to cluster analysis (PRIMER Version 6, PRIMER-E Ltd, Lutton, UK) (Clarke and Gorley 2006) in order to investigate their community structure among the sampling sites. Therefore OUT data were log transformed and the Bray Curtis similarity index applied. Cluster analysis was performed using the group average.

Results

Geochemical characteristics of the sampling site

All parameters are summarised in Supplementary Table S1. The sampling site displayed strong grain size and elemental composition gradients that increased from the southwest to the northeast (Fig. 2).

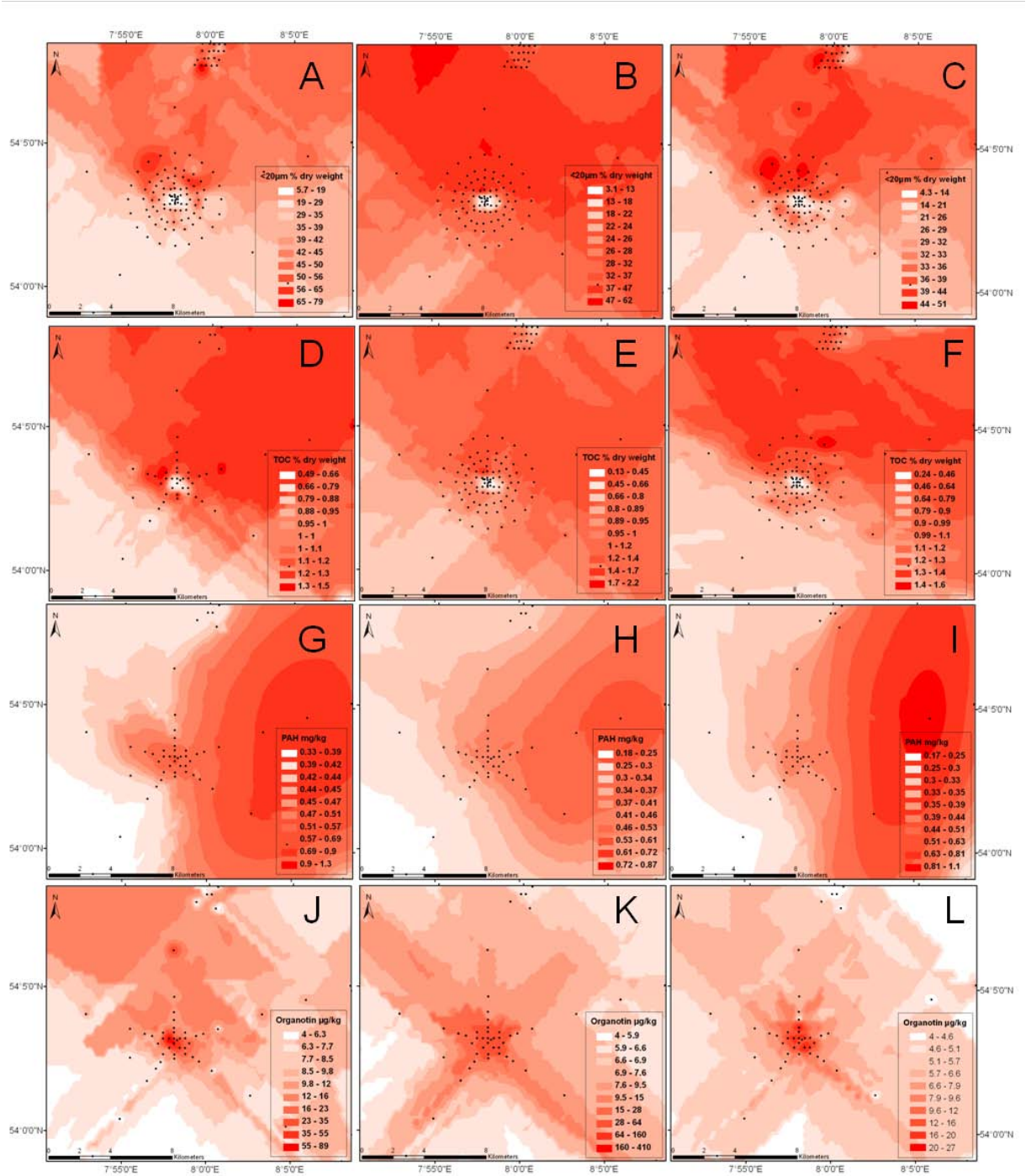

Fig. 2 Spatial distribution according to ordinary kriging of the fine grain fraction < 20 µm, total organic carbon (TOC), polycyclic aromatic hydrocarbons (PAH) and organotin compounds (Organotin) in August 2009 (vertical A; D; G; J), April 2010 (vertical B; E; H; K), August 2010 (vertical C; F; I; L). Dots represent the 125 and 52 sampling stations, respectively.

Fine grain fractions (< 20 µm) occurred predominantly in the northeastern region (Fig. 2 A-C; up to 50 % of the total grain size), whereas only 14 % of the sediment at the dumping centre harboured < 20 µm grain. The TOC content of the dumping centre was 0.5 % in August 2009 and approximately 0.3 % in April and August 2010 (Fig. 2 D-F). Approximately 1 % TOC was recorded at the reference site; additionally, high amounts of nitrogen (1000 mg/kg), sulphur (4000 mg/kg) and phosphorus (400 mg/kg) were detected. We observed lower values for these elements at the dumping centre. Predominantly nitrogen (166-491 mg/kg) and sulphur (420-860 mg/kg) content exhibited large differences compared to the stations at the reference site. Organic pollutants, such as poly aromatic hydrocarbons (PAH), could be detected at the whole study site. Organotin compounds were detected at the dumping centre at higher concentrations (79 µg/kg August 2009, 10 µg/kg in April and August 2010), whereas 5 µg/kg of organotin compounds were detected at the reference site (Fig. 2 G-L).

ARISA Fingerprints

Changes in the bacterial community structure in the *a priori* regions were investigated by ARISA fingerprinting. Prior to the analysis of the ARISA fingerprints, the similarity among replicates obtained from the same sampling station was tested indirectly via ANOSIM. Therefore sampling stations were tested for significant differences. In all cases, the Global R confirmed significant differences among all sampling stations (Supplementary Table S2). This result indicated high similarities among replicates. Thus, ARISA fingerprints results are based on one replicate per station. Figures 3-5 summarise all analyses performed for each sampling campaign.

Table 3 Results of analysis of similarities (ANOSIM) showing the global R of pairwise comparisons of *a priori* regions. Significant values bold ($p < 0.05$).

		dumping site			surrounding			transects			Reference
	Region	centre	< 1 km	1 km	1.5 km	2 km	3 km	6 km	9 km	12 km	reference
	August 2009										
dumping site	centre										
	< 1 km	0.015									
	1 km	0.357	-0.315								
surrounding	1.5 km	**0.783**	**0.194**	0.150							
	2 km	**0.505**	0.110	-0.054	0.100						
	3 km	0.245	0.098	-0.167	**0.217**	0.062					
transects	6 km	**0.790**	0.078	0.081	0.538	0.247	0.021				
	9 km	0.790	0.046	0.354	0.531	0.216	-0.074	0.125			
	12 km	0.562	-0.176	0.643	0.591	0.186	-0.127	0.036	-0.036		
	reference	**0.619**	0.344	0.214	**0.442**	**0.286**	0.127	**0.390**	0.271	0.333	
	April 2010										
dumping site	centre										
	< 1 km	**0.61**									
	1 km	0.61	0.03								
surrounding	1.5 km	**0.72**	**0.28**	0.01							
	2 km	**0.86**	**0.16**	0.34	**0.30**						
	3 km	**0.54**	**0.25**	-0.15	0.05	**0.26**					
transects	6 km	0.73	0.12	0.32	0.12	0.40	-0.11				
	9 km	**0.80**	0.14	0.17	0.37	0.51	0.16	0.20			
	12 km	0.80	0.25	0.33	0.32	0.52	0.01	-0.04	-0.24		
	reference	**0.80**	**0.50**	0.39	**0.36**	**0.55**	**0.34**	0.12	0.36	-0.26	
	August 2010										
dumping site	centre										
	< 1 km	**0.68**									
	1 km	0.75	0.50								
surrounding	1.5 km	**0.94**	**0.64**	-0.06							
	2 km	**0.84**	**0.39**	0.05	0.09						
	3 km	**0.45**	**0.40**	-0.18	0.10	0.04					
transects	6 km	**0.77**	0.11	0.20	0.35	-0.07	-0.18				
	9 km	**0.75**	0.42	-0.02	**0.47**	0.20	-0.07	-0.11			
	12 km	0.46	0.40	1.00	0.88	0.62	0.18	0.25	0.14		
	reference	**0.85**	**0.65**	0.12	**0.38**	**0.25**	**0.18**	0.17	0.32	0.68	

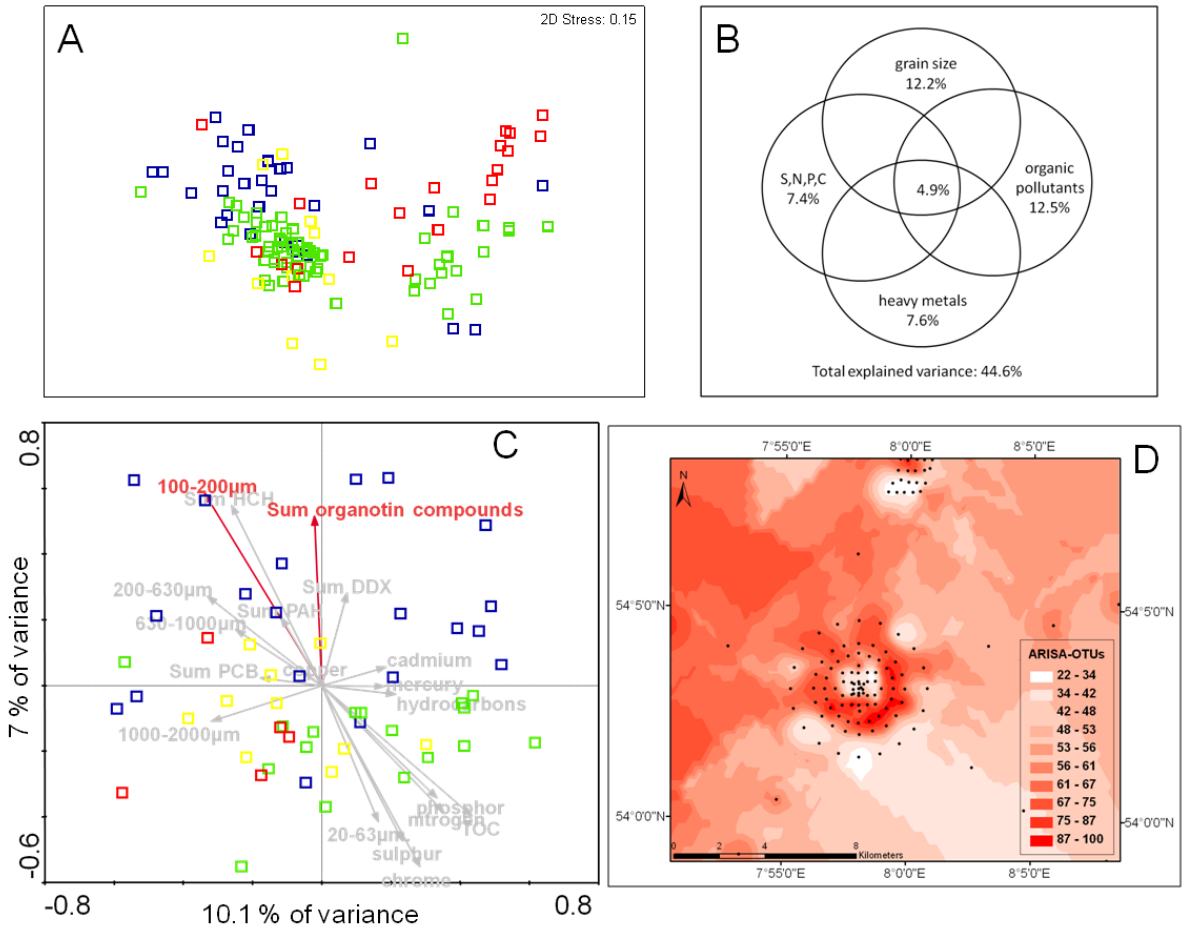

Fig. 3 Sampling campaign August 2009. **A** Non-metric multidimensional scaling (NMDS) plot of the Automated Ribosomal Intergenic Spacer Analysis (ARISA) profiles based on the Jaccard Index. Each square represents a profile of a sampling station belonging to the group 'dumping site' (blue), 'surrounding' (green), 'transects' (yellow), 'reference site' (red). **B** Partitioning of bacterial variation (%) into the relative effects of contextual factor groups as determined by 499 Monte Carlo permutations. **C** Redundancy analysis (RDA) biplot of bacterial communities and contextual parameters. Squares represent ARISA profiles colourised reffering to their associated group (see A). Significant environmental factors are displayed in red. **D** Spatial distribution of the sum of ARISA-OTUs of each sampling station as calculated by ordinary kriging. Dots represent the 125 sampling stations.

We used non-metric multidimensional scaling to display the ARISA fingerprints of all four groups ('dumping site', 'surrounding', 'transects', 'reference site') illustrated in Figure 1 (Fig. 3A-5A). ANOSIM was applied to identify differences in the bacterial communities (Table 3) between the *a priori* regions grouped by HPA (Table 1). In all cases, bacterial communities of the *a priori* dumping centre and reference site regions were significantly different (Fig. 3A-5A, Table 3). The non-metric multi-dimensional scaling

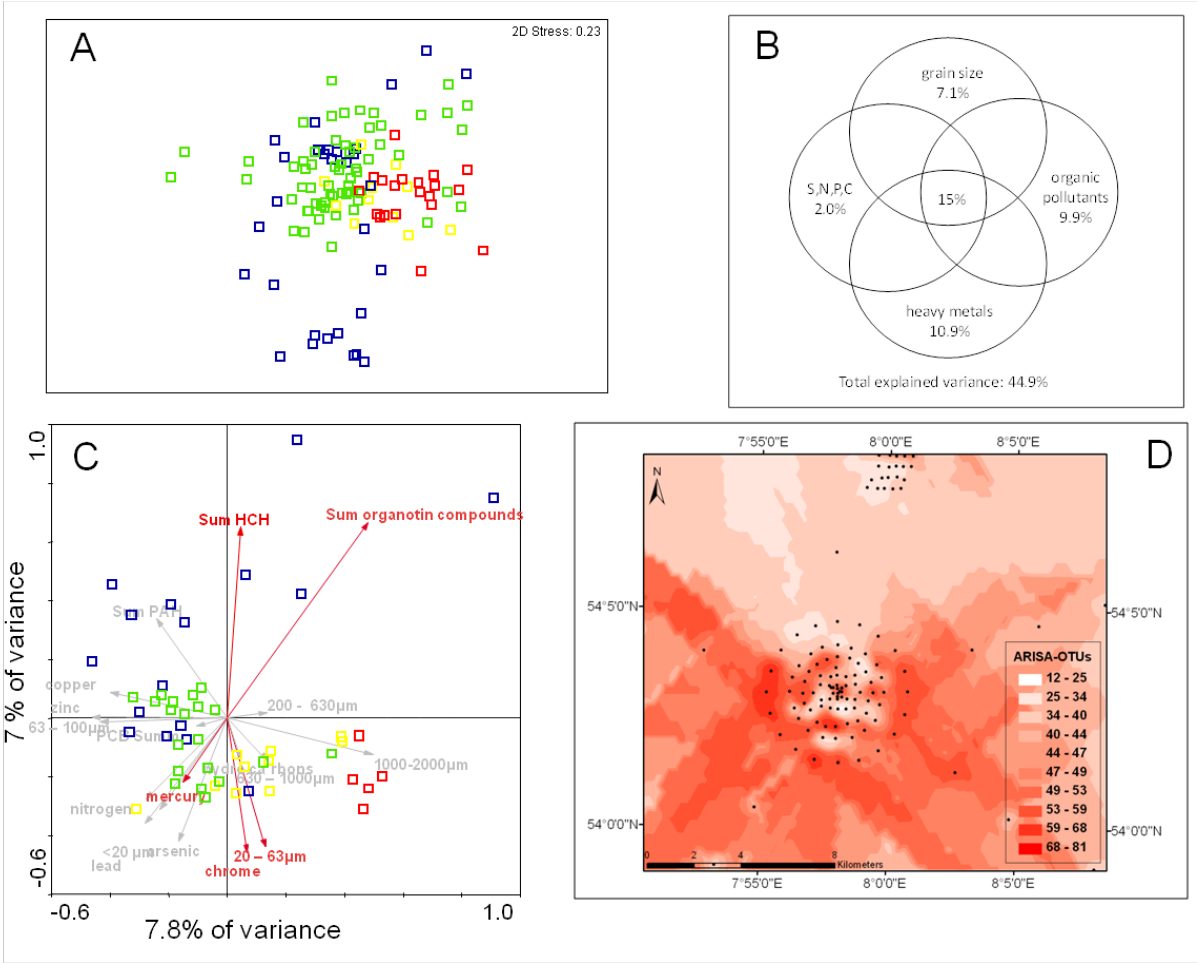

Fig.4 Sampling campaign April 2010**. A** Non-metric multidimensional scaling (NMDS) plot of the Automated Ribosomal Intergenic Spacer Analysis profiles based on the Jaccard Index. Each square represents a profile of a sampling station belonging to the group 'dumping site' (blue), 'surrounding' (green), 'transects' (yellow), 'reference site' (red). **B** Partitioning of bacterial variation into the relative effects of contextual factor groups as determined by 499 Monte Carlo permutations. **C** Redundancy analysis (RDA) biplot of bacterial communities and contextual parameters. Squares represent Automated Ribosomal Intergenic Spacer Analysis profiles colourised reffering to their associated group: 'dumping site' (blue), 'surrounding' (green), 'transects' (yellow), 'reference site' (red). Significant environmental factors are displayed in red. **D** Spatial distribution of the sum of ARISA-OTUs of each sampling station as calculated by ordinary kriging. Dots represent the 125 sampling stations.

of ARISA fingerprints in August 2009 displayed two clear subgroups among bacterial communities from the groups 'surrounding' and 'reference site' sampling stations (Fig. 3A).

The bacterial community structure of samples obtained at the 'dumping site', more precisely *a priori* centre and < 1 km, did not differ significantly in August 2009 (Table 3). In April and August 2010, we observed significant differences comparing the structure of these bacterial communities at the 'dumping site' (Fig. 4A and 5A, Table 3).

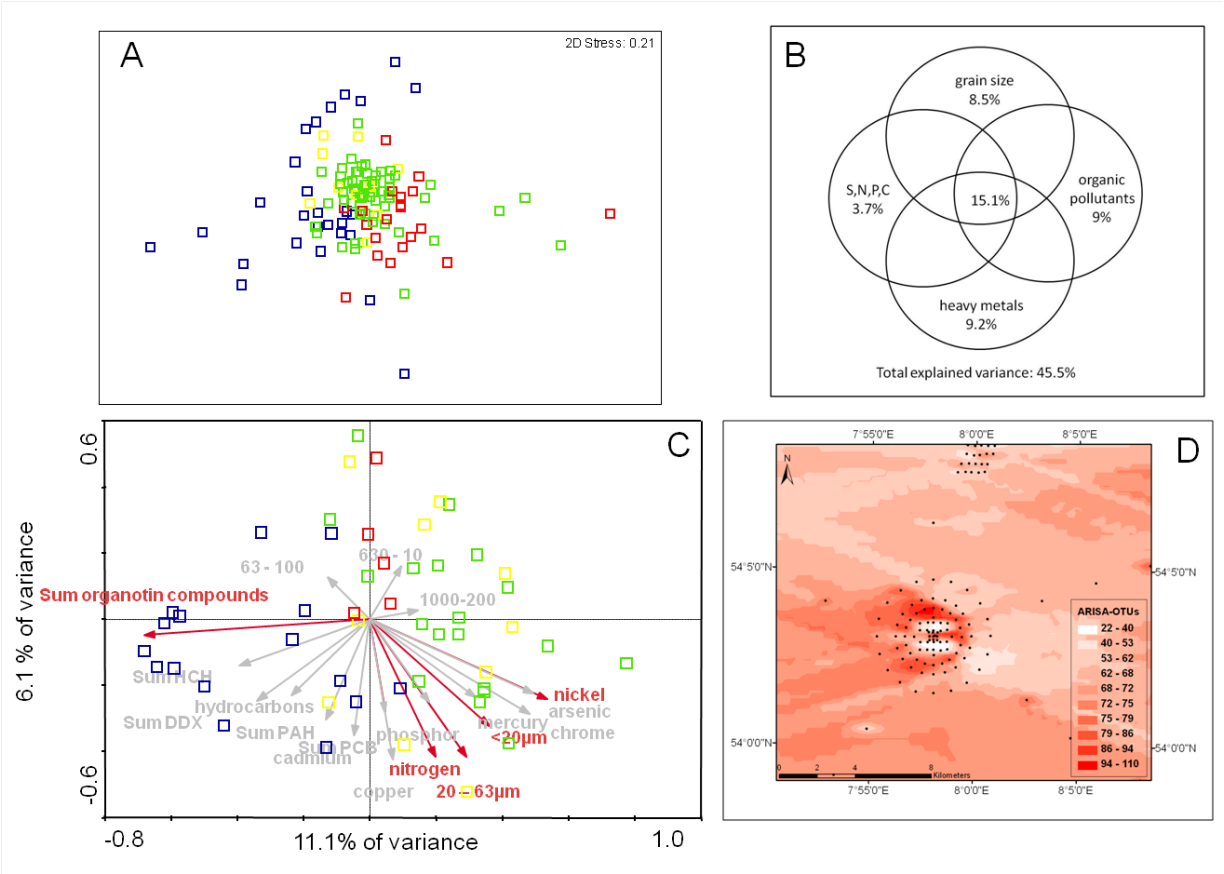

Fig. 5 Sampling campaign August 2010**. A** Non-metric multidimensional scaling (NMDS) plot of the Automated Ribosomal Intergenic Spacer Analysis profiles based on the Jaccard Index. Each square represents a profile of a sampling station belonging to the group 'dumping site' (blue), 'surrounding' (green), 'transects' (yellow), 'reference site' (red). **B** Partitioning of bacterial variation into the relative effects of contextual factor groups as determined by 499 Monte Carlo permutations. **C** Redundancy analysis (RDA) biplot of bacterial communities and contextual parameters. Squares represent Automated Ribosomal Intergenic Spacer Analysis profiles colourised reffering to their associated group: 'dumping site' (blue), 'surrounding' (green), 'transects' (yellow), 'reference site' (red). Significant environmental factors are displayed in red. **D** Spatial distribution of the sum of ARISA-OTUs of each sampling station as calculated by ordinary kriging. Dots represent the 125 sampling stations.

The alpha diversity, as estimated by ARISA-OTU numbers, was analysed by ordinary kriging and analysis of variance in respect to corresponding *a priori* regions (Table 1). Generally highest ARISA-OTU numbers were recorded in August 2009 and 2010 (22-107 OTUs; Fig. 3D and 5D), with significantly higher ARISA-OTU numbers in the *a priori* region 1.5 km when compared with *a priori* regions < 1 km and reference ($p < 0.001$, Fig. 3D and 5D). In April 2010, ARISA-OTUs ranged from 12 to 81 ARISA-OTUs at the different sampling positions (Fig. 4D).ARISA-OTU numbers at the *a priori* reference were significantly ($p < 0.001$) lower compared with the *a priori* regions dumping centre, 1.5 km and 3 km.

Relation to environmental data

Prior to the analysis environmental factors were investigated for correlation in order to consider these for the interpretation of results obtained via redundancy analysis. The Spearman's rank correlation revealed significant correlations among the fine grain size fractions, organic carbon, sulphur, phosphorus and nitrogen content and heavy metals, such as arsenic, lead, chrome, copper, nickel and zinc, for all sampling campaigns. Additionally, DDX sums correlated with the sums of HCH, PCB and organotin compounds.

We aimed to investigate the relationship between bacterial community structures as obtained via ARISA fingerprinting and simultaneously recorded environmental factors in redundancy analyses. Environmental parameters (Table 2) were recorded at 52 out of 125 sampling sites (Supplementary S3). A detrended correspondence analysis, as well as a redundancy analysis based on these data and the corresponding ARISA fingerprints, was performed.

A gradient length of < 2.5 for all first axes of the detrended correspondence analysis suggested a linear model, such as redundancy analysis, as the best method for analysing the data sets.

The first two axes of the redundancy explained between 14-17 % of the total variation (Supplementary S4). The first axis of the redundancy analysis of the data sets from August 2009 and April 2010 was associated with larger grain size fractions and heavy metals such as mercury, copper and zinc. The second axis was determined by a gradient formed by organic pollutants and fine grain fractions associated with sulphur, nitrogen, phosphorus, organic carbon and heavy metals such as chrome and arsenic. These axes were inverted for the data set from August 2010. Factors omitted from the analysis are shown in Table 4. Bacterial communities of the group 'reference site' correlated with larger grain sizes, whereas communities of the group 'dumping site' correlated with organic pollutants (Fig. 3C, 4C and 5C). Organotin compounds exhibited significant conditional effects in all analyses

Table 4. Conditional effects of forwardly selected environmental variables and variance inflation factor of excluded parameters as determined by RDA.

August 2009		
Environmental variable	Lambda-A	F ratio
100-200 µm	0.05	2.90**
Sum PAH	0.03	1.53NS
TOC	0.03	1.38NS
20-63 µm	0.03	1.5NS
1000-2000 µm	0.02	1.24NS
copper	0.02	1.24NS
Sum DDX	0.02	1.25NS
200-630 µm	0.03	1.16NS
Sum organotin compounds	0.02	1.65*
Sum PCB	0.03	1.26NS
phosphor	0.02	1.17NS
630-1000 µm	0.02	1.18NS
Sum HCH	0.02	1.13NS
hydrocarbons	0.02	1.1NS
chrome	0.02	1.18NS
cadmium	0.01	1.01NS
mercury	0.02	1.10NS
nitrogen	0.02	1.03NS
sulfur	0.02	0.84NS
20µm VIF >15		
63-100µm VIF >15		
arsenic VIF >15		
lead VIF >15		
nickel VIF >15		
zinc VIF >15		

April 2010		
Environmental variable	Lambda-A	F-ratio
Sum organotin compounds	0.06	2.99**
Sum HCH	0.03	1.56*
copper	0.02	1.44*
20 - 63 µm	0.04	2.06**
chrome	0.04	1.82**
mercury	0.03	1.78**
zinc	0.02	1.49NS
< 20 µm	0.02	1.18NS
1000 - 2000 µm	0.02	1.18NS
630 - 1000 µm	0.03	1.45NS
arsenic	0.02	1.25NS
nitrogen	0.02	1.18NS
TOC	0.02	0.98NS
Sum PAH	0.01	1NS
lead	0.02	0.93NS
63 - 100 µm	0.02	0.94NS
hydrocarabons	0.01	0.82NS
Sum PCB	0.01	0.77NS
TOC VIF >15		
100-200µm VIF >15		
sulphur VIF >15		
phosphor VIF >15		
Sum DDX VIF >15		
cadmium VIF >15		
nickel VIF >15		

August 2010		
Environmental variable	Lambda-A	F-ratio
Sum organotin compounds	0.06	2.98**
20 - 63 µm	0.04	1.81**
nickel	0.03	1.52*
nitrogen	0.03	1.49*
<20 µm	0.03	1.43*
copper	0.02	1.1NS
63 - 100 µm	0.02	1.04NS
arsenic	0.02	0.98NS
1000 - 2000 µm	0.02	1NS
630 - 1000 µm	0.02	1.02NS
Sum PAH	0.02	0.96NS
Sum DDX	0.02	1.09NS
Sum PAH	0.02	1.21NS
mercury	0.03	1.15NS
cadmium	0.02	0.97NS
chrome	0.01	0.84NS
phosphor	0.02	0.76NS
hydrocarbons	0.01	0.68NS
Sum HCH	0.01	0.71NS
TOC VIF >15		
100-200µm VIF >15		
200-630µm VIF >15		
sulphur VIF >15		
lead VIF >15		
zinc VIF >15		

Lambda-A represents the variance each variable explains in the model.Statistical significance is indicated by ** (P< 0.01), * (P<0.05), and NS (not significant) as determined by 499 Monte Carlo permutations.

(Table 4). The respective effects of each factor group were disentangled by variance partitioning analysis (Fig. 3D, 4D and 5D). The model was based on the complete data set (n=52). The environmental parameters were grouped according to grain size (< 20 µm,

20 - 63 µm, 63 - 100 µm, 100 - 200 µm, 200 - 630 µm, 630 - 1000 µm, 1000 - 2000 µm); organic pollutants (ΣPCB, ΣDDX, ΣHCH, Σorganotin compounds and Σhydrocarbons); S,N,P,C (sulphur, nitrogen, phosphorus, organic carbon) and heavy metals (arsenic, lead, cadmium, chrome, copper, nickel, mercury, zinc). Partitioning the variance of bacterial communities revealed that the highest proportion of variance was explained by organic pollutants and heavy metals in April and August 2010, while grain size explained a higher proportion as compared to heavy metals in August 2009 (Fig. 3-5B).

Phylogenetic- and 454-OTU-based analyses

The NMDS plot of ARISA profiles in August 2009 revealed subgroups of bacterial communities of the groups ‘surrounding’ and ‘reference site’. According to these subgroups, ANOSIM results suggested a high variability within the data set. For ribosomal tag sequencing, we chose one sample from the dumping centre, five from the ‘surrounding’, two from the ‘reference site’ (reference site 1 and reference site 2) and one sample from the dredging zone in the Elbe River (Supplementary S5).

In total, 669 647 sequences were retrieved from ribosomal tag sequencing, and 24 611 OTUs at a similarity level of 0.97 were detected. Rarefaction curves revealed similar profiles, which started to become asymptotic in all cases (Supplementary S6). We observed the highest richness for communities from the reference site and the lowest richness from the dumping centre (Supplementary S6).

We were interested in investigating solely abundant bacterial groups for differences regarding their community composition, consequently rare species were omitted. After removing singeltons (n=1) from the data set we observed 5627 OTUs which remained for the analysis. Lowest OTU numbers (~1000 OTUs) were detected in the Elbe and highest OTU numbers at the reference site (~2200 OTUs, Fig. 6A). In total, 16 phyla were observed (Fig. 6B). In all cases, sequences related to *Proteobacteria* dominated the samples (Fig. 6B). Highest

sequence numbers (~8500 sequences) were observed for the Elbe (Fig. 6B), contrary lowest sequence numbers were detected at the reference site (~3000 sequences). LIBSHUFF analyses revealed significant differences ($p < 0.0001$) in the bacterial community structure from Elbe and dumping centre as compared to all the others. Additionally, the reference site differed in bacterial community structure compared to surrounding 3 and reference 1 to surrounding 1. Subsequently we aimed to investigate differences among sampling sites regarding the most frequent phyla *Proteobacteria* and *Bacteroidetes*. In all cases dumping centre and Elbe shared highest similarities. *Alpha-* and *Betaproteobacteria* were detected only in considerable numbers in the Elbe and at the dumping centre (Fig. 6C and 6D). Whereby *Rhizobiales*, *Hypomicrobium* and *Methylocystaceae* from the *Alphaproteobacteria;* and *Burkholeriales* and *Hydrogenophilaceae* from the *Betaproteobacteria* were observed in both samples. The class of *Deltaproteobacteria* contained generally high sequence numbers for the marine sites (Fig. 6E). Fewest sequences affiliated to *Deltaproteobacteria* were reported for the Elbe (~ 700 sequences). The reference sites had only few sequences affiliated to *Deltaproteobacteria* (reference 1:~ 1600, reference 2: ~1900) as compared to dumping centre (~3200 sequences) and surrounding (~ 2300 sequences, Fig. 6E). The highest diversity however was observed in the Elbe, comprising 14 bacterial groups within the *Deltaproteobacteria*. The marine samples were dominated by *Desulfobulbaceae* and *Desulfuromonadaceae*. However, considerably higher sequence numbers were detected for *Desulfuromonadaceae* at the dumping centre when compared to all other sites (Fig. 6E). Unfortunately, *Gammaproteobacteria* comprised mainly unclassified sequences (Fig. 6F).

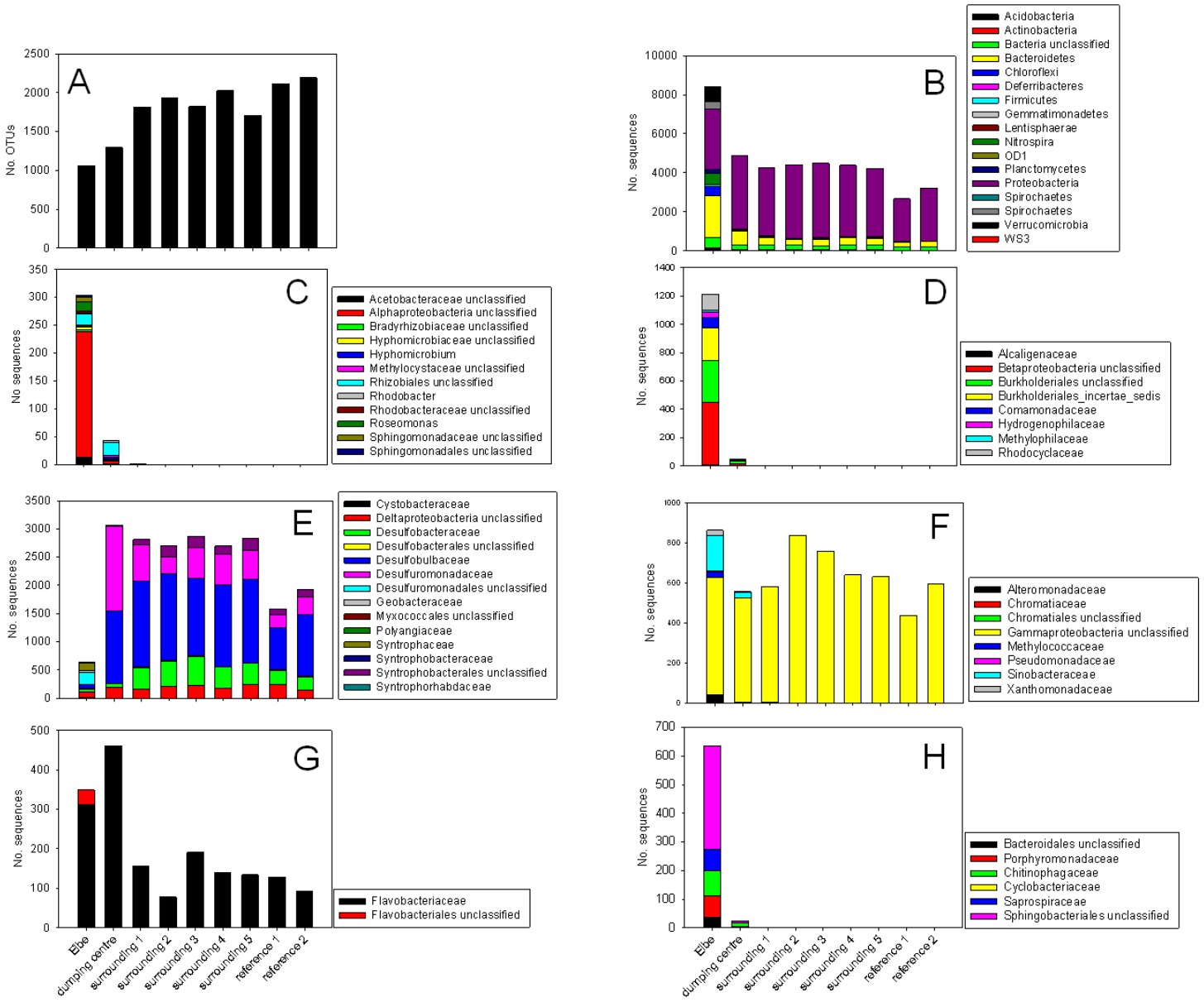

Fig. 6 Phylogenetic classification for the ribosomal tag sequencing results obtained by MOTHUR of nine representative samples based on OTUs (0.97) omitting singletons (n > 1). A total numbers of OTUs detected in the samples, **B** Stacked bars and cluster analysis on phylum level based on observed OTUs **C** *Alphaproteobacteria*, **D** *Betaproteobacteria*, **E** *Deltaproteobacteria*, **F** *Epsilonproteobacteria*, **G** *Flavobacteriales*, **H** *Bacteroidales* and *Sphingobacteriales*.

Investigating the phylum *Bacteroidetes* we recorded exclusively sequences affiliated to the *Flavobacteria* at the marine sites (Fig. 6G). The Elbe contained sequences affiliated to *Porphyromonadaceae*, *Flavobacteriaceae*, *Chitinophagaceae* and *Sphingobacterales.* Few sequences affiliated to *Porphyromonadaceae*, *Chitinophagaceae*, *Saprospiraceae* and *Sphingobacterales* were detected at the dumping centre (Fig. 6H).

Discussion

Investigations of dumping activities as stated by the OSPAR report draw the following consequences resulting from dumping activities: the increase of contaminants, a general change in substrate matter which may affect benthic communities directly, physical disturbance and burial of benthic organisms and the intrusion of foreign species (OSPAR 2004). Our results clearly confirm that changes in substrate matter as well as physical disturbance affected benthic bacterial community's structure. Additionally we showed that not only the dredged freshwater sediment but also typical freshwater bacteria were observed at the dumping centre even ten months after a dumping activity.

Changes in substrate matter

The monitoring design grouped sampling stations by distance into *a priori* regions (e.g. dumping centre, 1 km, 1.5km etc). We did not find any specific structures of bacterial communities (ARISA fingerprinting) referring to these *a priori* regions. Solely significant differences obtained between bacterial communities from the dumping centre and reference were observed in all sampling campaigns. The observation of a fundamentally different granular structure at the dumping centre (clayey sand –sand) as compared to the reference site (sandy mud) combined with lowest values for carbon, nitrogen, phosphorus and sulphur at the dumping centre leads to the assumption that these fundamentally different conditions might explain the different bacterial communities in these regions. Grain size distribution, in addition to other physicochemical factors, represents the main driving factor influencing bacterial communities (Dale 1974, DeFlaun and Mayer 1983). Sandy sediments harbour different bacterial groups than muddy sediments (Llobet-Brossa *et al* 1998). The sandy texture of the dumping centre contains, in comparison to the reference site, a low TOC and low sulphur, nitrogen and phosphorus content. Moreover, an increase of organotin and poly aromatic compounds in comparison to the reference site was reported (HPA 2005, HPA 2006,

HPA 2007, HPA 2008, HPA 2009, HPA 2010). Other studies have indicated a relationship between pollutant load in sediments and bacterial communities (Edlund *et al* 2006, Gremion *et al* 2004, Vishnivetskaya *et al* 2011, Zhang *et al* 2008). The impact of dredging on bacterial communities was investigated by Edlund and Jansson (2006). They reported a shift in community structure and composition before and after the intrusion in the dredging area and concluded that the pollutant load resulted in a change in the bacterial community (Edlund and Jansson 2006). We observed a strong gradient formed by sediment grain size fractions and organic pollutants in all redundancy analyses (Fig. 3-5). Organotin compounds had always a significant conditional effect. The gradient formed by the grain size fractions confirms our hypothesis that the geochemical conditions of the sediments are shaping the bacterial communities predominantly. However, the significant conditional effect of organotin compounds must be interpreted with caution. Our results show, that organotin compounds are correlated with ΣHCH, ΣPCB and ΣDDX. The distribution of organotin in the sediment, however, is rather heterogeneous because organotin compounds most likely reside as paint flakes in the sediment (Dowson *et al* 1993, Hoch 2001). A causal relationship between the metabolism of bacteria and the occurrence of organotin compounds cannot be determined. Moreover, a strong relationship among fine-grained sediments (<20 µm and 20-63 µm), elemental composition and several heavy metals, including arsenic, lead, chrome and nickel, was observed in all of the redundancy analyses. Other studies have revealed strong interactions between fine-grained sediments, nutrients and contaminants (Owens *et al* 2005), too. This strong correlation hinders the evaluation of single effects on bacterial community variation.

Variance partitioning (Fig. 3B-5B) was performed to assess the influence of grain size, elemental composition, organic pollutants and heavy metals. The results suggest, consistent with our previous hypothesis, the existence of a strong gradient formed by grain size

distribution and probably some organic pollutants. Variance partitioning revealed a complex framework between grain size, elemental composition, organic pollutants and heavy metals. It seems that the variability of bacterial communities cannot be assigned to a single factor or factor group. Thus, the combined effects explain more of the variance in the community structure than each group individually. Already other field studies failed in assigning changes in community structure to single factors in the field. Dean-Ross and co-workers (1989) as well as Gillian (2005) stated difficulties in distinguishing the impact of heavy metal pollution from other factors influencing bacterial community structure. One possible approach to overcome this problem in the future could be the linkage of laboratory experiments and field studies. In laboratory experiments single factors can be manipulated and observed in a controlled design. The results again can be linked to observations in the field in order to better estimate the true pollution impact.

Physical disturbance

An interesting finding of our study was the formation of significant different bacterial community structures at the dumping site in 2010 while these communities were rather similar in our sampling campaign in 2009 (Table 3). We hypothesise that the dumping activity between the sampling campaigns of August 2009 and April 2010 led to the recorded bacterial community shift at the ‘dumping site’. We observed a similar sediment texture at the ‘dumping site’ in 2009 (Table 1) but considerably higher sand proportions at the dumping centre in 2010. Due to transport and sedimentation processes, fine-grained material composed of biogenous and terrigenous particles accumulated in the undisturbed phase between the last dumping activity in October 2008 and our first sampling campaign in August 2009 at the centre. The dumping activity in 2010 led to a perturbation of the sediment. During the dumping process, sandy particles accumulate at the dumping centre, whereas fine-grained material spreads over a distance of up to 8 km (HPA 2005).

In our sampling campaigns 2010 we observed a higher alpha diversity at the dumping centre, as revealed by ARISA fingerprinting. Several studies dealing with the impact of perturbation on bacterial communities observed changes to the bacterial community structure. Generally, the communities respond to disturbance by decreasing community and functional diversity (Atlas *et al* 1991, Girvan *et al* 2005). In the present study, we observed a low alpha diversity at the dumping site as revealed by ARISA fingerprinting as well as ribosomal tag sequencing for bacterial communities in 2009, ten months after a dumping activity. Interestingly, two and six months after a dumping activity, as observed in 2010, the alpha diversity was higher at the dumping centre. Possibly, freshwater bacteria enter the marine system and survive for several months at the dumping site but vanish on a longer time scale. This may then in turn result in a lower alpha diversity at the dumping centre as observed for 2009. Contrary to the sequencing approach ARISA fingerprinting suggested a low alpha diversity for both the reference site and dumping centre, too. ARISA fingerprinting is known to possibly underestimate diversity since unrelated organisms may possess spacer regions of identical length (Fisher and Triplett 1999, Kovacs *et al* 2010). Certainly, the ribosomal tag sequencing approach is more accurate then the ARISA fingerprinting. The sequencing approach confirmed a low alpha diversity at the dumping centre. Contrary, diversity of the samples from the reference site was high (Fig. 6). Our sequencing results suggest that the diversity in samples of the reference site is most likely due to various rare species, here OTUs comprising only few sequences (Fig. 6A and 6B). Probably these rare species were not covered by the ARISA fingerprinting approach. To date several studies applying tag sequencing are distinguishing rare from abundant species and conclude possibly two sub communities (rare biosphere) (Galand *et al* 2009, Sogin *et al* 2006). Finally we suppose, concluding from our findings obtained by fingerprinting and sequencing that the dumping activity led to a less diverse bacterial community at the dumping site on the long term.

Intrusion of freshwater bacteria at the dumping centre

We identified fundamental differences in community structure and composition between fluvial and marine samples. The bacterial community of the fluvial sample represents a typical freshwater sediment community (Miskin *et al* 1999, Zwart *et al* 2002). Typical groups such as *Betaproteobacteria* or *Verrucomicrobia* were detected. The marine samples were dominated by *Proteobacteria* and *Bacteroidetes*. Thus far, bacterial communities of the sublittoral shelf sediments in the German Bight are poorly characterised. Studies conducted in coastal areas such as the Wadden Sea revealed the *Planctomycetes*, *Gammaproteobacteria* and *Cytophaga-Flavobacterium* cluster. Members of sulphate-reducing *Deltaproteobacteri*a were predominant, whereas *Alphaproteobacteria* were only observed in low numbers (Llobet-Brossa *et al* 1998, Musat *et al* 2006). Antarctic shelf sediment revealed high numbers of *Gamma-* and *Deltaproteobacteria* (Bowman and McCuaig 2003).

Principally, two findings can be drawn from the presented results: Firstly, even ten month after a dumping activity, typical freshwater bacteria such as *Rhizobiales*, *Hypomicrobium* and *Methylocystaceae* or *Burkholeriales* and *Hydrogenophilaceae* were detected in low numbers at the dumping centre. This finding might point to a successful establishment or long persistence of foreign bacterial groups at the dumping centre. Secondly, we observed highest abundances of *Desulfuromonadaceae* and *Flavobacteriaceae*. In the present study, *Deltaproteobacteria* dominated all marine samples. This observation was expected since this group inhabits anoxic environments (Jorgensen 1977, Kondo *et al* 2007, Mußmann *et al* 2005). The main proportion of shelf sediments is anoxic, where only the top millimetres are penetrated by oxygen (Schulz and Zabel 2006). We used a van Veen grab for sampling which penetrates the sediment up to 30 cm and contains therefore a main proportion of the anoxic sediment body. Less abundant but still considerable was the skewed distribution of *Bacteroidetes*, more precisely *Flavobacteriaceae*. This group comprises members featuring

various physiological capabilities, furthermore they are adapted to a broad range of environmental conditions (Weller *et al* 2000). Generally, *Bacteroidetes* are strongly associated with the water column and marine aggregates. However, some studies described their presence for aerobic and anaerobic sediments, too (Llobet-Brossa *et al* 1998, Ravenschlag *et al* 2001). *Flavobacteria* are believed to play a pivotal role in degrading organic matter since they own hydrolytic capabilities (Abell and Bowman 2005, Cottrell 2000). Nowadays *Flavobacteriaceae* are from great interest in the context of remediation of organic pollution. It was highlighted that the addition of complex organic substrates resulted in the growth of *Bacteroidetes* in anaerobic sediments (Rosselló-Mora *et al* 1999). In our study *Flavobacteriaceae* were observed in all samples, interestingly, at the dumping centre five times more sequences affiliated to *Flavobacteriaceae* were detected as compared to all other marine sites.

Both families, *Desulfuromonadaceae* and *Flavobacteriaceae* were already investigated in the context of environmental pollution. Recently, the response of sulphate-reducing bacteria to an artificial oil-spill was investigated in a mesocosm study by Suarez-Suarez (2011). The bacterial community from a pristine environment was exposed to increasing naphthalene and crude oil contents of up to 0.03 mg/kg. *Desulfuromonadaceae* and *Desulfobulbaceae* dominated oil and naphthalene treatments after incubation. Bissett and co-workers examined increasing numbers of *Flavobacteria* and community shifts within this group under regular inputs of highly labile organic carbon. They conclude that *Flavobacteria* may play an important role in the initial degradation of organic matter due to their positive response to organic pollution (Bissett *et al* 2008).

High PAH concentrations at the dumping centre may have led to an increase of *Desulfuromonadaceae* and *Flavobacteriaceae*. In our study, we detected naphthalene concentrations of approximately 0.2 mg/kg at the dumping centre. Additionally, poly aromatic

hydrocarbon (PAH) concentrations of 0.96 mg/kg were recorded at the dumping centre and in contrast ~0.4 mg/kg at the reference site. High PAH concentrations in the whole study area are likely caused by intensive dumping activity, shipping traffic and riverine input in the past century.

We showed that the community composition of essential bacterial groups differed at the dumping centre, suggesting that the dumping resulted in functional changes in the ecosystem. To further elucidate the consequences of dumping, more information about the interactions of these bacterial groups in the sediments of the German Bight is urgently needed.

Applicability in monitoring programs

This study aimed to assess the applicability of bacterial community analyses in beforehand designed monitoring schemes. We showed similar results for bacterial community response compared to the response of the macrozoobenthos. Both groups of organisms responded with a decrease of species richness at the immediate dumping centre. This finding itself demonstrates the capacity of bacterial community investigations for monitoring programs. A major obstacle of bacterial community analysis, however, is the great variability and diversity within bacterial communities. We recommend for future monitoring programs to integrate investigations of the diversity as well as composition of bacterial communities in comparison to a reference and optimally to communities at the dumping site before dumping activities take place. Diversity measures are still an index for the “fitness” of ecosystems. Additionally, investigations of the community composition might resolve changes in the communities and therefore possible responses to environmental pollution and intrusion of foreign communities.

The identification of environmental factors, influencing bacterial communities remains delicate. Strong correlations of pollutants with grain size fractions of the sediment or nutrients hinder an accurate analysis. As already mentioned above a combination of laboratory studies and field observations might be a wise elaboration. Additionally information about the redox

potential or other physicochemical parameters such as temperature, oxygen penetration or pH might be helpful to capture the complexity of the disturbed benthos.

In conclusion, our study provides for the first time fine-scaled spatial information about bacterial community structure under the influence of dumped polluted material. Bacterial community structure and diversity were affected even by lightly polluted material and ongoing dumping activity. This assumption is based on significant differences between bacterial community structure and alpha diversity at dumping centre and reference site in all sampling campaigns. Our correlation and redundancy analyses confirmed the high complexity of the underlying processes in the benthos. Single factors influencing the bacterial communities could not be identified adequately. Considering these analyses for monitoring programs further elaboration of these multivariate analyses is needed. However, we detected a unique bacterial community at the dumping centre composed of probably specialised marine groups and freshwater bacteria. These results significantly broaden the knowledge of how bacterial communities respond to dumping activities.

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CHAPTER III

Impact of ocean dumping on bacterial communities

II: GeoChip-based analysis of bacterial communities at a dumping site

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Abstract

In between the years 2005 to 2010 approximately 6 000 000 cubic metres sediment was dumped 15 kilometres south off the island Helgoland in the German Bight (North Sea). The accompanying monitoring program reported a decrease of macrozoobenthic species richness and density at the dumping centre. In a pilot study we complemented the monitoring program by analyses of the benthic bacterial community using functional gene arrays. We applied analysis of variance and hierarchical clustering to investigate differences between sampling sites. The relationship between functional genes and environmental factors was disentangled by distance-based multivariate multiple regression. The bacterial community at the dumping centre displayed significantly lower gene numbers compared to a reference site. Hierarchical clustering displayed distinct cluster for samples from Elbe River, dumping site and reference site. Conclusively, the dumping activity changed persistently the geochemical conditions of the area resulting in a less diverse bacterial community at the dumping centre.

Keywords: dredged sediment / pollution / multivariate statistics / functional gene arrays

Introduction

Estuaries represent economically valuable areas, constantly evolving and facing a wide range of natural and human-induced stresses. Worldwide ports and rivers located in estuaries suffer from high siltation rates caused by erosion and sedimentation. In addition the expansion of global trade requires increasingly large container ships and thus the constant deepening of waterways. Inevitably dredging procedures ensure therefore navigation in these areas (de Nijs *et al* 2009, McLoughlin 2000, Tanner *et al* 2000) and most likely the amount of dredged material will increase in the future (OSPAR 2000). Although most dredged material is uncontaminated; in some cases the application of major environmental constraints is required for contaminated material (IMO 2000, OSPAR 2004). International conventions, such as the London convention, regulate dumping activities in marine areas worldwide (Organization 2000). Additionally, regional conventions exist (OSPAR 2004). Guidelines for the management of dredged material recommend the assessment of physical, chemical and biological parameters of both dredged sediment and the dumping sites in order to estimate the impact of the disposal (OSPAR 2004, IMO 2000). Biological investigations focus in general on higher organisms such as fishes and macrozoobenthos and additionally, ecotoxicological assessments are frequently carried out. However, the execution of these directives depend on national politics of signatory countries (Bartels 2000). Recently, the OSPAR commission claims to invest more effort in investigating biological responses to the disposal of dredged material (Commision 2009).

The economic history of the Elbe River (Germany) goes back to the 12th century when the city of Hamburg received trade privileges. Today, the Elbe River and the port of Hamburg belong to the most important global trade routes. In the past years the amount of dredged material from the Hamburg port area increased. Already relocated sediment re-accumulates in the same water system and requires therefore multiple inconclusive dredging processes (HPA

2005). As a consequence the city of Hamburg applied for permission to dump lightly polluted river sediment at a dumping site in the German Bight. Hence, in between the years 2005 and 2010 approximately 6 000 000 m^3 sediment were removed from the Elbe River near the port area of Hamburg and were dumped at the prescribed site. German guidelines for the handling of dredged material regulate the dumping activity and base predominantly on London and OSPAR conventions (BfG 1999, BfG 2009). The dumping site measures 400 square metres. Recent bearing showed a three metres high rising at the dumping site consisting of sandy sediments as assessed by grain size analyses. Acoustic Doppler Current Profiler (ADCP) analyses revealed that fine-grained material drifts about eight kilometres until settling down on the seafloor (HPA 2005). The monitoring of the dumping site targets, among others, the respective contaminant content of the sediments, the macrozoobenthos and the fish fauna. During the dumping period from 2005 to 2010 a significant increase of organic pollutants, precisely poly aromatic hydrocarbons (PAH) and organotin compounds was reported by the Hamburg Port Authority (HPA). Simultaneously, investigations of the macrozoobenthos revealed a decrease in species richness and density (HPA 2010).

In fact dumping activity causes multiple implications. Beside the increase of contaminants, dumping causes physical disturbance, burial of benthic organisms and a general change in substrate matter, which again may affect benthic communities directly (OSPAR 2009).

Microbenthic communities (including bacteria) are currently disregarded by prescribed guidelines for dredged material handling. It is known that physical and chemical perturbation lead to changes in bacterial community structure and function (dos Santos *et al* 2011, Findlay *et al* 1990, Suarez-Suarez *et al* 2011). Against this background it appears obvious that microbenthic communities will be affected by dumping activities just like the macrozoobenthos. The response of microbenthic communities, as being far more complex than the macrozoobenthos, is expected to be as complex as the communities themselves.

Today, molecular approaches allow the assessment of microbenthic community's information. Therefore, it might be worth considering these approaches in future guidelines.

In an interdisciplinary project we initiated fine-scale investigations of benthic bacterial communities at the prescribed dumping site in the German Bight. The monitoring itself was designed beforehand according to the German guidelines for dredged material handling (BfG 1999) and GÜBAK-WSV (BfG 2009). It comprises 125 sampling stations including the dumping and a reference site. Our first sampling campaign was conducted ten months after a dumping activity in 2009 (Störmer *et al* 2012). Bacterial community structure, derived from 16S ribosomal gene analyses, displayed significant differences comparing dumping and reference sites (Störmer *et al* 2012). This study assumed that the dumping activity led to different bacterial communities and a lower alpha diversity at the dumping centre (Störmer *et al* 2012). However, information of the functional structure of these communities is still lacking.

In the past years functional gene arrays were applied in various environmental studies (He *et al* 2007, He *et al* 2010, Liu *et al* 2010, Wang *et al* 2009, Ward *et al* 2007, Wu *et al* 2008, Yergeau *et al* 2007, Zhou 2003) including heavily contaminated habitats (Lu *et al* 2012, Neufeld *et al* 2006, Van Nostrand *et al* 2009, Xie *et al* 2011). Nostrand and co-workers found an increase in diversity and overall gene numbers going along with the stimulation of uranium remediation by ethanol additions in an uranium contaminated aquifer (Van Nostrand *et al* 2009). Waldron and co-workers investigated a gradient of contaminant levels in groundwater. They observed that contamination affects functional gene diversity (e.g. reduced diversity) and heterogeneity (Waldron *et al* 2009). Only recently the GeoChip gene array was implemented in a study investigating microbial community response to the Deepwater Horizon oil spill. Lu and co-workers found a high enrichment of metabolic genes especially involved in hydrocarbon degradation in the plume (Lu *et al* 2012).

Here, we aimed to reveal the functional gene diversity of nine representative samples obtained in connection with the monitoring program in the dumping region in 2009 by using GeoChip 4.2 (Lu *et al* 2012). The functional gene array contains probes targeting among others the gene categories: carbon cycling, nitrogen cycling, and heavy metal resistance.The present study targets a) the diversity of functional genes and b) environmental factors influencing the functional gene structure in the bacterial community.

Material and methods

Site description and sampling

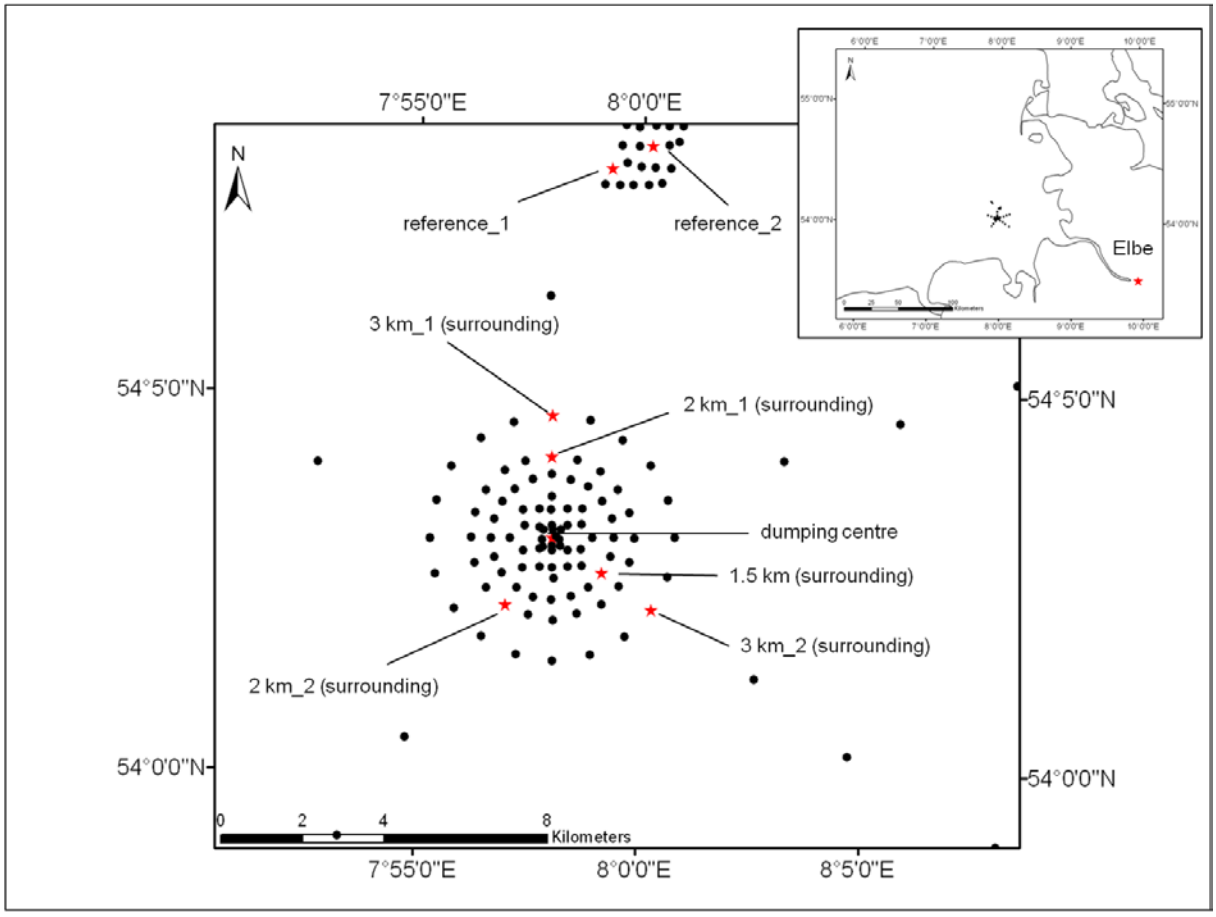

Fig. 1 Sampling scheme of the dumping site in the German Bight (54°03´N 07°58´E). Samples for GeoChip analyses are represented as red stars. One sample was taken at the dumping centre, five originate from the surrounding (1.5km, 2km_1, 2km_2, 3km_1, 3km_2) off the centre and two were chosen from the reference (reference_1, reference_2). Additionally one sample from the Elbe River (53°32' N 9°56' E) was chosen.

Table 1 Sediment characterisation of the samples after Folk (1980).

Samples	Sediment
Elbe	muddy sand
dumping centre	clayey sand
1.5km	sandy mud
2km_1	sandy clay
2km_2	sandy clay
3km_1	sandy clay
3km_2	sandy clay
reference_1	sandy mud
reference_2	sandy mud

The dumping site is located in the southern part of the German Bight (54°03´N 07°58´E) 15 kilometres south off the island of Helgoland. The current at the dumping site is cyclonic and influenced by east wind forcing (Staneva *et al* 2009) and the discharge of the adjacent rivers (Howarth 2001). Water depths range between 20 and 35 metres. Sediments of the dumping site are sandy whereas the reference consists of sandy mud (Table 1). The dredging zone in the Elbe River (53°32' N 9°56' E) features a depth of 13 metres and the sediment can be characterised as muddy sand (Table 1).

For this study nine representative samples, based on significant differences regarding their bacterial community structure were chosen (Störmer *et al* 2012) (Fig. 1). Eight samples were obtained from dumping site and reference, which is located 12 kilometres north off the dumping site. Moreover, one sample was taken in the dredging zone in the Elbe River (Fig. 1). Sampling took place in August 2009. A last dumping activity was executed in October 2008. All sediment samples were taken with a van Veen grab (0.1 m^3). Onboard the sediment was filled into a clean metal box and homogenised. For coherent analyses the samples for analyses of the bacterial communities as well as the samples for physicochemical analyses

were taken from the same sediment. For the analysis of bacterial communities three subsamples were stored immediately after sampling at -20°C in 50 ml falcon tubes.

Environmental data analysis

All environmental data were provided by the HPA (Störmer *et al* 2012). The total fraction of the sediment was analysed following the HABAK guidelines (BfG 1999).

DNA-Extraction, amplification and labelling

For DNA-Extraction the PowerSoil Kit (MoBio Laboratories, Carlsbad, CA, USA) was used following the manufactures protocol. Three subsamples of 0.25 g sediment were collected, and the extracted DNA was eluted in 50 µl elution buffer. Genomic DNA concentrations were measured in duplicate by photometry using the Infinite M200 (Tecan Austria GmbH, Grödig, Austria). Amplification and labelling were performed as described in Lu and co-workers (2012).

GeoChip 4.2 hybridisation and data pre-processing

Samples were analysed with GeoChip 4.2, which was updated from GeoChip 4.0 (Hazen *et al* 2010, Lu *et al* 2012) with more genes derived from fungi and soil borne pathogens. The gene array contains 103 666 probes targeting functional genes which are assigned to several gene categories (antibiotic resistance, bacteria phage interaction, energy process, fungi function, carbon, nitrogen, sulphur and phosphorus cycling, metal resistance, organic contaminant degradation, soil benefit, soil borne pathogen, stress and virulence (Table 2). Hybridisation and scanning were performed as previously described (Lu *et al* 2012). Singeltons, defined as positive probes detected solely in one of the subsamples, were removed prior to statistical

analyses in order to remove noise from the data set. All procedures were performed by Glomics Inc. (Norman, Oklahoma, USA).

Statistical analysis

Univariate statistics

Differences in the relative abundance of functional genes (percentage) among samples were tested using one-way analysis of variance (ANOVA, Statistica Version 7.1, StatSoft GmbH, Hamburg, Germany) for individual gene categories. For ANOVA tests the calculated percentage of functional genes were arcsin-square-root transformed and a significance level of $p < 0.05$ was applied. Pairwise comparisons of the samples were tested in *post hoc* Tukey HSD tests ($p < 0.05$).

Pairwise correlations (Statistica Version 7.1, StatSoft GmbH, Hamburg, Germany) of all environmental variables were performed with Spearman´s rank correlation ($p < 0.05$).

Hierarchical clustering

For individual gene categories cluster analyses (CLUSTER 3.0; http://www.eisenlab.org) were performed. The data were log transformed prior to the analysis. Euclidian distance was applied as similarity metric and as cluster method average linkage was chosen. The results were visualised using the TREEVIEW software (http://www.eisenlab.org) (Eisen *et al* 1999).

Multivariate statistics

Individual gene categories were compared by using 2STAGE analysis applying the PRIMER package (PRIMER Version 6, PRIMER-E Ltd, Lutton, UK) (Clarke and Gorley 2006). The resemblance matrices of individual gene categories were calculated applying Euclidean distance. Spearman's rank correlation was applied to correlate individual resemblance

matrices of the gene categories in pairwise comparisons. For cluster analysis the group average method was applied.

The relationship between functional genes and environmental variables was investigated by distance-based multivariate multiple regression (DISTLM). In order to perform DISTLM gene array subsamples were converted to binary values (presence/absence) since environmental data were recorded only once per sample from each site. Calculating the binary table, only genes present in at least two of the subsamples were regarded as present. Environmental variables were treated as follows: Grain size fractions, sulphur (S), nitrogen (N), phosphorus (P), carbon (C) and heavy metals were considered as single values, for PAH, PCB, HCH and DDX single compounds were summed-up in each category. Environmental data were log transformed prior to the analysis. Jaccard Index was applied to calculate the resemblance matrix for functional genes. The DISTLM model was built using stepwise selection, adjusted R^2 and applying 999 permutations at a significance level of $p < 0.05$. Results were visualised by using distance-based redundancy analysis (dbRDA).

Results

Geochemical description of the study sites

All parameters obtained from the total fraction of the sediments are summarised in the supplementary material (S1). The dumping centre had highest values of the grain size fractions 100-200 µm (33 %) and 200-630 µm (25 %). Organic pollutants, in particular polycyclic aromatic hydrocarbons (PAH, Elbe: 1.1 mg/kg, dumping centre: 1 mg/kg), polychlorinated biphenyls (PCB, Elbe: 9.6 mg/kg, dumping centre: 7 mg/kg) and organotin compounds (Elbe: 123.2 µg/kg, dumping centre: 78.7 µg/kg) were highest in the Elbe River and at the dumping centre. Contrary, concentrations of sulphur (860 mg/kg), nitrogen (491 mg/kg), carbon (0.6 mg/kg) and phosphorus (290 mg/kg) as well as heavy metals were lowest at the dumping centre. Highest TOC (1.8 mg/kg), nitrogen (2440 mg/kg) and phosphorous (840 mg/kg) concentrations were observed in the Elbe River. The reference site, characterised as sandy mud had highest values of the grain size fraction 20-63 µm (reference_1: 30.9 %, reference_2 30.5 %).

Overview of functional gene diversity

At first, we investigated the performance of the GeoChip 4.2 referring to gene overlap and number of detected genes. After removing 15 644 singeltons from the data set in total 18 787 genes remained for the further analyses. Subsamples (a,b,c) were compared for their similarity according to their gene overlap (S2). Generally, we observed high similarities (> 80 %) among subsamples. Furthermore we looked at individual gene categories in order to get an overview of the detected genes, also in relation to the total genes covered by the GeoChip 4.2 (Table 2). We observed the highest coverage for the gene categories: Organic remediation (25.03 %), energy process (23.93 %) metal resistance (23.23 %), soil benefit (21.11 %), phosphorous (20.61 %) and carbon cycling (20.35 %) (Table 2).

Table 2 Overview of total and detected probes and their percentage for individual gene categories as derived from the GeoChip 4.2.

Gene category	Antibiotic resistance	Bacteria phage	Carbon cycling	Energy process	Fungi function
No. total probes	3334	1071	11065	840	4557
No. Detected probes	698	109	2252	201	911
%	20.94	10.18	20.35	23.93	19.99
Gene category	Metal resistance	Nitrogen cycling	Organic Remediation	other category	Phosphorus
No. total probes	9272	12680	17061	3511	1349
No. Detected probes	2154	1234	4270	301	278
%	23.23	9.73	25.03	8.57	20.61
Gene category	soil benefit	soil borne pathogen	Stress	Sulphur	Virulence
No. total probes	3870	1454	21597	7101	3738
No. Detected probes	817	260	3825	775	678
%	21.11	17.88	17.71	10.91	18.14

Diversity of functional genes for individual gene categories

Diversity of functional genes was estimated by the percentage of detected genes for individual gene categories at the different sampling sites. The sampling sites were tested for significant differences by analysis of variance (ANOVA). Furthermore *post hoc* Tukey tests were applied for pairwise comparisons of the sampling sites (S3). Detailed tables for individual gene categories are provided within the supplemental material. Generally, the Elbe River had the significantly highest diversity of genes regarding all individual gene categories (S3, S4). The genetic diversity of the dumping centre was significantly lower when compared to reference site and Elbe River.

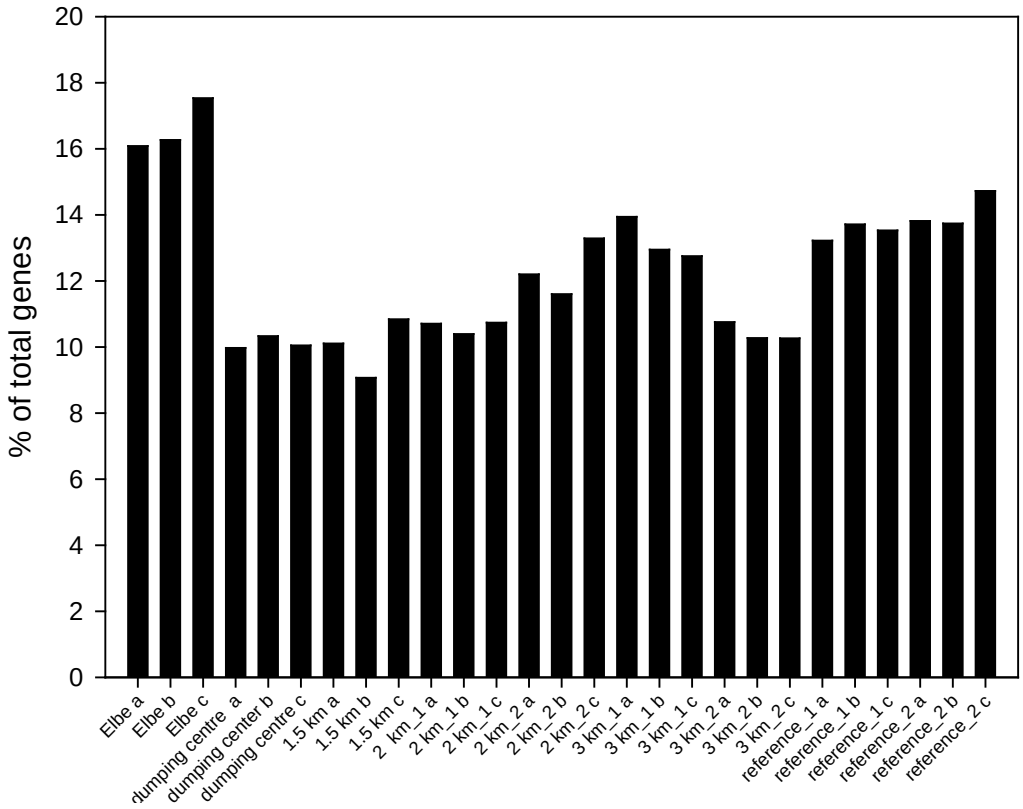

Fig. 2 Bar chart displaying the relative abundance of genes belonging to the gene category organic remediation in the different samples.

Exemplarily the distribution of functional genes involved in organic remediation is depicted in Figure 2. For each sample the percentage of detected functional genes in the three subsamples (a,b,c) is displayed. ANOVA and *post hoc* Tukey test indicated significant differences comparing the samples ($p < 0.05$, S3). Regarding organic remediation the Elbe River had significantly highest functional gene diversity comparing all samples. The dumping centre revealed significantly lower functional gene diversity compared to all samples except 1.5 km and 3 km_2 (Fig. 2, S3).

Hierarchical clustering of individual gene categories

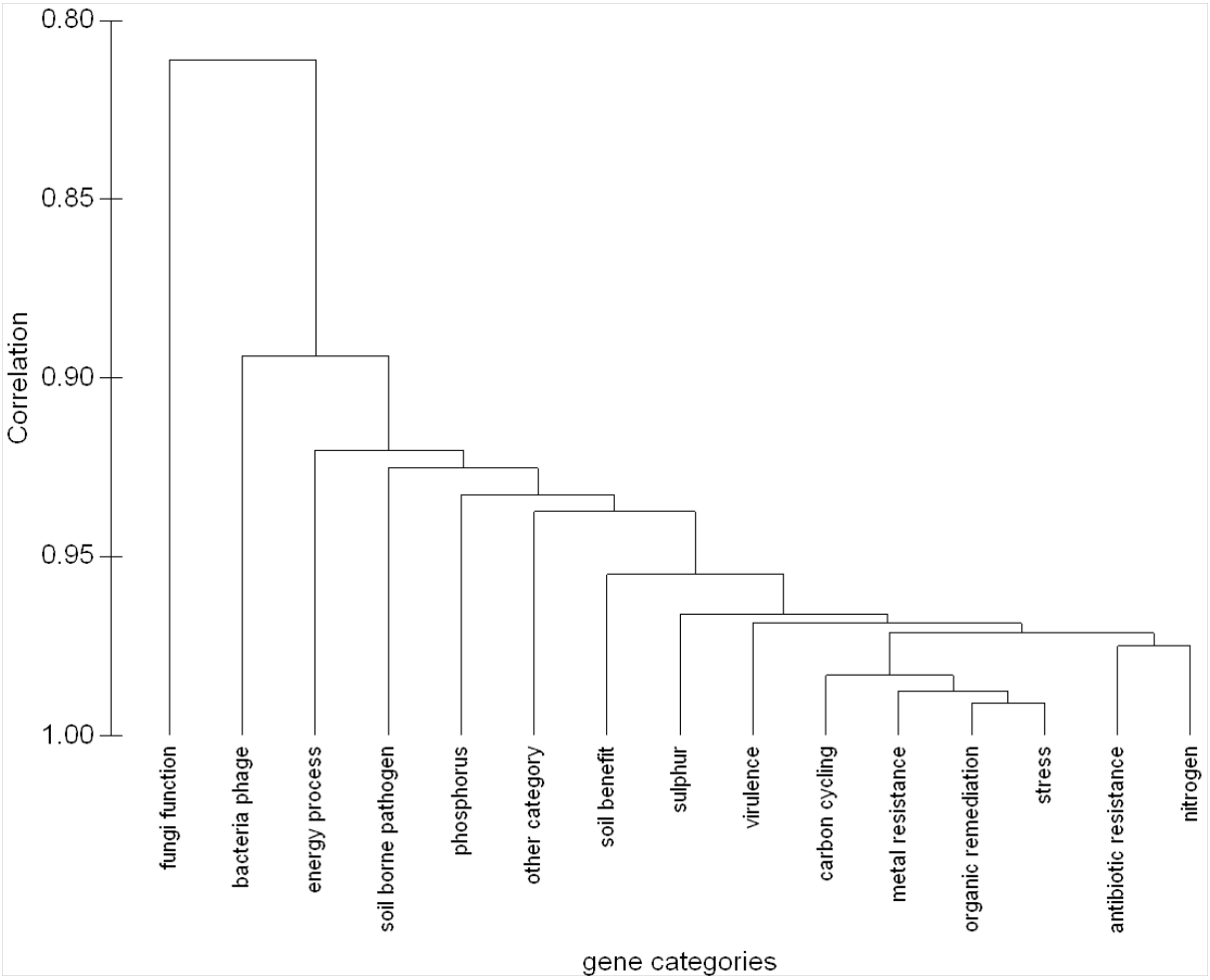

Fig. 3 2STAGE analysis of similarity matrices for all gene categories. Similarities were analysed in pairwise comparisons.

The sites were further compared using hierarchical clustering. We observed highly congruent pattern for all individual gene categories. Exemplarily, the cluster analysis for the gene category organic remediation is shown in Figure 4. 2STAGE analysis was utilised to compare individual gene categories in pairwise tests in order to elucidate congruency of patterns. Consistent with the results obtained from the individual hierarchical clustering, 2STAGE analysis indicated high similarities (r_s> 0.8) among individual gene categories (Fig. 3). Concerning the comparison of sites, each hierarchical clustering persistently displayed three cluster groups: I) samples from the Elbe River, II) samples from the dumping centre and part

of the surrounding (1.5km, 2km_1, 2km_2, 3km_2) III) samples from the reference site and 3km_1.

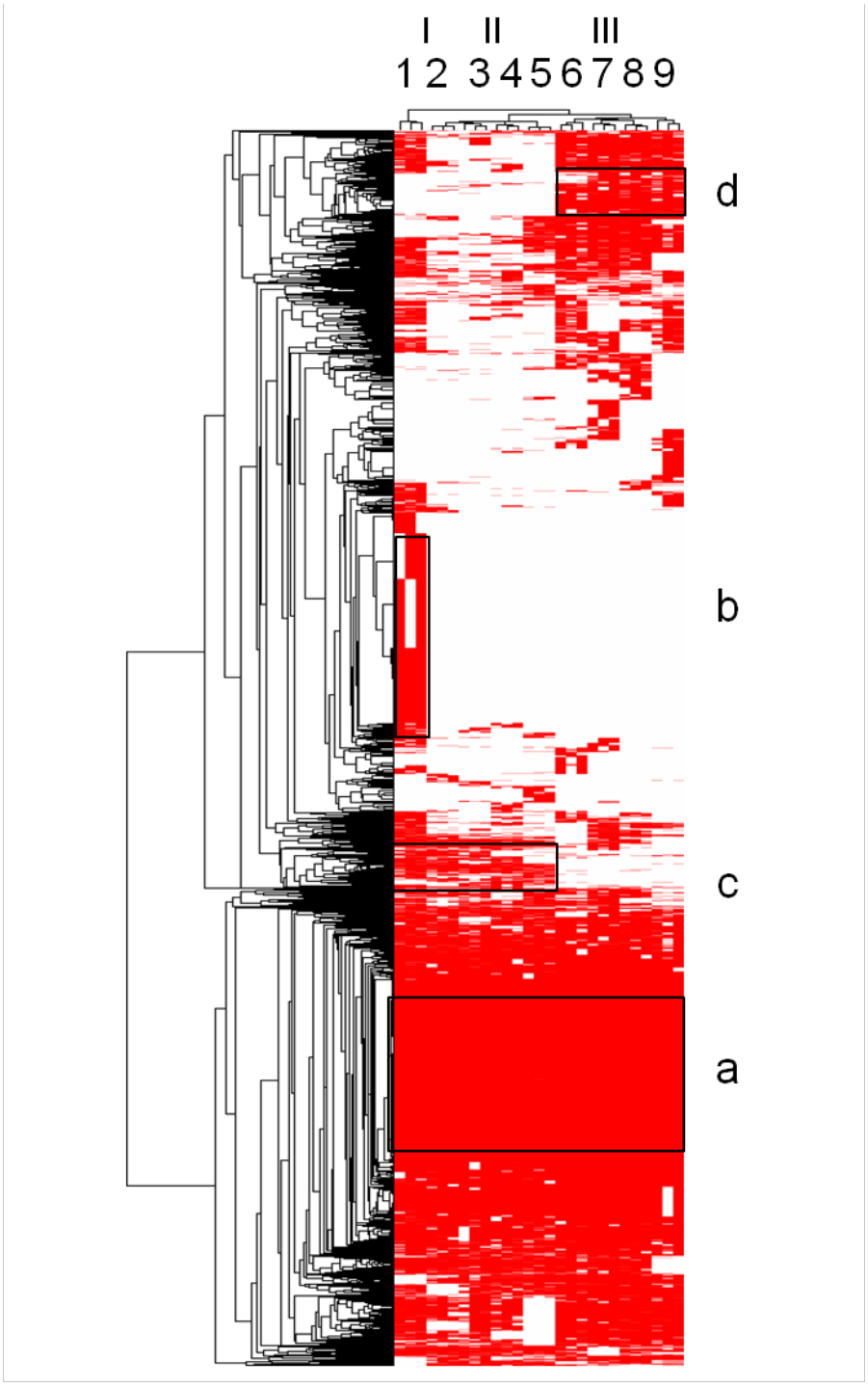

Fig. 4 Hierarchical clustering analysis of organic remediation genes based on hybridization signals using Euclidean distance. The figure was generated using CLUSTER and visualized with TREEVIEW. White represents no hybridization above background level and red represents positive hybridization. 1: Elbe River, 2: dumping centre, 3: 1.5 km, 4: 2 km_1, 5: 3 km_2, 6: 3 km_1, 7: reference_2, 8: reference_1, 9: 2km_2; I) samples from the Elbe River II) samples from dumping centre and surrounding (1.5km, 2km_1, 2km_2, 3km_2) III) samples from the reference site and 3km_1. a) genes which were detected in all samples b) genes which were detected only in the "Elbe" group, c) genes which were detected in the "dumping centre" group and d) genes which were detected in the "reference" group.

Concerning the clustering of genes, in general four patterns were observed for all gene categories (Fig. 4): a) genes which were detected in all samples b) genes which were detected only in the “Elbe” group, c) genes which were detected in the “dumping centre” group and d) genes which were detected in the “reference” group. Indeed, we did not observe differences regarding functional genes within the different pattern. However, we observed that genes in group I) were predominantly derived from *Betaproteobacteria*, while the other groups contained genes from *Delta*- and *Gammaproteobacteria* (data not shown).

Relation of environmental factors and functional genes

The relationship between functional genes and environmental factors was investigated by a distance-based multiple regression model (DISTLM). The influence of the dumping activity was estimated by comparing dumping and reference sites. The Spearman’s rank correlation of environmental variables revealed significant correlations among the fine grain size fractions, organic carbon, sulphur, phosphorus and nitrogen content and heavy metals, such as arsenic, lead, chrome, copper, nickel and zinc. Additionally, DDX sums correlated with the sums of HCH, PCB and organotin compounds.

The results obtained by DISTLM are depicted as distance-based redundancy analysis (dbRDA). The first two axes of the dbRDA explained 63.4 % of the total and 67 % of fitted variation (Fig. 5). This indicates that the plot captures most of the salient patterns in the fitted model. Marginal and sequential tests of the DISTLM revealed solely DDX sums to have a significant influence on the variation in functional genes (Table 3). Apart from that DDX sums formed a strong gradient separating samples from the dumping centre and close surrounding from samples of the reference site.

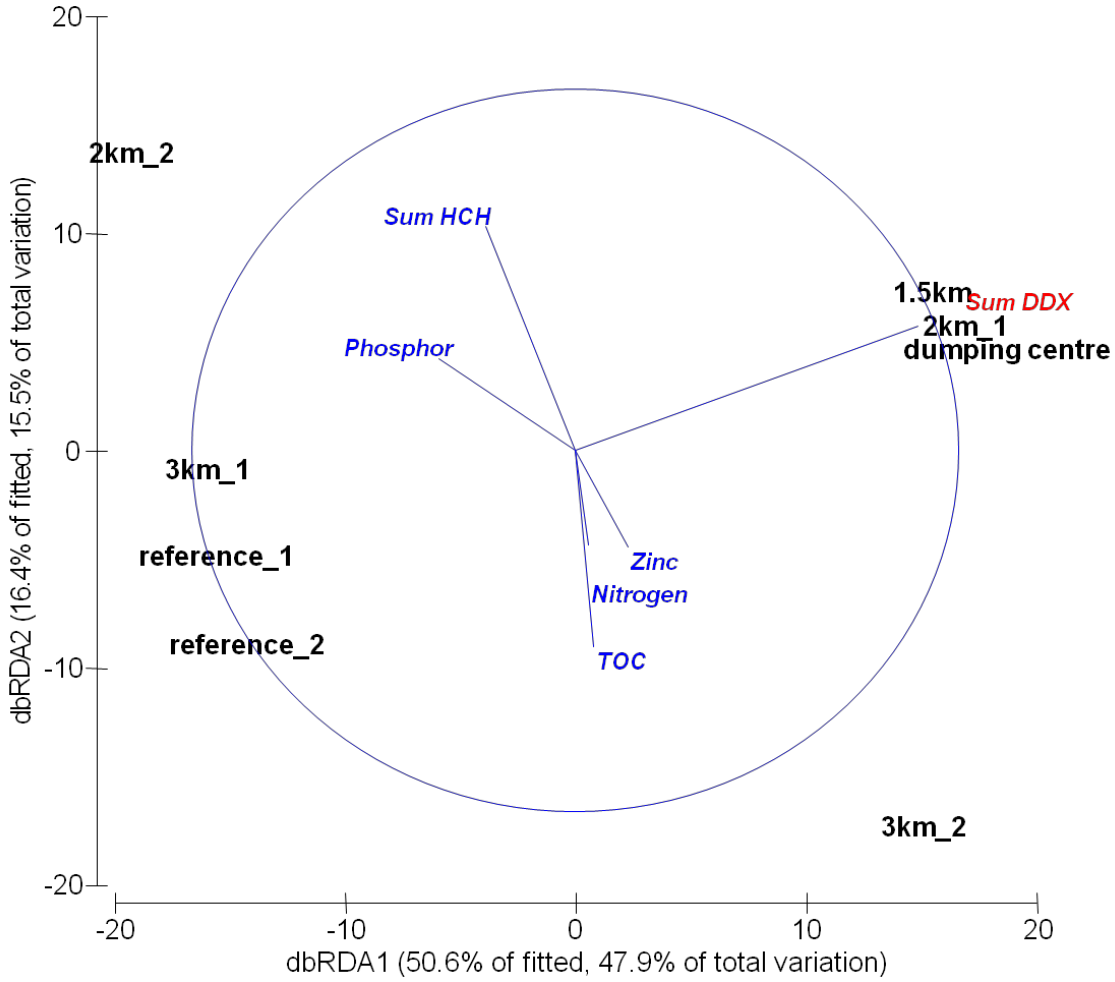

Fig. 5 Distance-based redundancy analysis (dbRDA) biplot displaying bacterial community and environmental variables. Significant ($p < 0.001$) environmental factors are displayed in red.

Table 3 DISTLM results for the sequential test after considering stepwise selection of environmental factors ($p < 0.05$). Significant factors bold.

Variable	Pseudo-F	P	Proportion of variance
Sum DDX	3.30	**0.004**	**0.355**
zinc	1.38	0.169	0.139
Sum HCH	1.22	0.321	0.118
TOC	1.35	0.292	0.120
phosphorus	1.84	0.213	0.128
nitrogen	1.67	0.348	0.087

Discussion

Ocean dumping represents a physical perturbation for ecosystems going along with a potentially increase in contaminants and changes in the substrate matter which may in turn influence benthic communities (OSPAR 2004). Investigations of macrozoobenthic communities are a fundamental element in monitoring programs to assess the impact of the dumping activity in the dumping area. Several investigations showed that macrozoobenthic communities respond to ocean dumping with decreasing diversity and density at dumping sites (HPA 2010, Mühlenhardt-Siegel 1981).

Our aim was to investigate the response of the marine microbenthic (in our case bacterial) communities to disturbance caused by the dumping of river sediment. We had two main expectations: a) a pollutant specific response (detection of functional genes involved in –for example- metal resistance or organic remediation) and b) differences in the functional structure of the whole community in the greater dumping area.

The GeoChip gene array was already successfully applied in several studies investigating environmental contamination in freshwater and marine habitats as well as in soils (Liang *et al* 2009, Lu *et al* 2012, Van Nostrand *et al* 2009, Waldron *et al* 2009). In a study on stimulated uranium bioremediation by ethanol, it could be shown by GeoChip analysis, that gene numbers and diversity increased due to the organic enrichment (Van Nostrand *et al* 2009). Investigations on the Deepwater Horizon oil spill revealed an enrichment of genes involved in aerobic and anaerobic hydrocarbon degradation in the plume (Lu *et al* 2012). In contrast to these studies, we did not detect an increase of specific functional genes related to heavy metal resistance or organic contaminant degradation at the dumping centre. This finding was contradictory to our expectations since the dredged and dumped sediment contained several organic pollutants (PAHs, organotin compounds) in relatively high concentrations.

However, we observed a general reduction in functional diversity regarding all gene categories represented by GeoChip 4.2 at the dumping centre, which reflects large differences in the community gene pool. This finding is explained by fundamentally different geochemical conditions at the dumping centre resulting from the dumping activity. Our sampling campaign took place in 2009 while several dumping campaigns were already conducted at the site since 2005. As a consequence the dredged sediment forms a three metres high rising on the seabed (recorded by bearing). Hence, the underlying seabed is buried permanently and was not accessed by our sampling activity. The dumping process goes along with a portioning of the introduced river sediment while passing the water column. Coarse sand fractions sediment immediately, while fine-grain fractions can be transported by currents up to eight kilometres until settling down on the seafloor (HPA 2005). This explains why the rising mainly consists of sandy sediments (grain size analyses) and is consequently relatively poor in sulphur, nitrogen, carbon and phosphorus as well as heavy metal concentrations. The portioning process has, as a matter of fact, consequences for sediment body or attached pollutants, organic material and organisms since different sediment fractions charge different loadings of pollutants, organic material and organisms (Llobet-Brossa *et al* 1998, Olsen *et al* 1982, Owens *et al* 2005). After dumping, the newly introduced sediment layers are exposed to the general hydrographical regime in this area. The current at the dumping site is cyclonic and influenced by east wind forcing (Staneva *et al* 2009) and the discharge of the adjacent rivers (Howarth 2001). It should be noted that the cyclonic nature of the current was the reason to choose this area for the dumping activities, since the dumped sediment was not expected to spread considerably on the seafloor. Nevertheless, also in this area, upper sediment layers are constantly relocated and mixed. The complexity of this system has to be taken into account, when interpreting the findings of the GeoChip analyses.

The majority of the former freshwater bacterial community is dispersed together with the smaller sediment fractions and those bacteria bound in biofilms to the sand particles have to cope with the fundamentally different marine conditions. Although the different bacterial community composition of marine and freshwater environments is well documented in a multitude of studies (Bowman and McCuaig 2003, Miskin *et al* 1999, Ravenschlag *et al* 2001, Zwart *et al* 2002), to the best of our knowledge no valid information exists which freshwater bacteria survive/adapt to the marine environment or coexist in both environments on the community level (not single species).

From our previous findings it can be emphasised that a least some freshwater bacteria (*Rhizobiales*, *Hypomicrobium* and *Methylocystaceae* or *Burkholeriales* and *Hydrogenophilaceae*) were still present even after 10 months exposure to the marine environment (Störmer *et al* 2012). Due to the low genetic diversity (richness) detected at the dumping centre, we emphasise, that, even after 10 months, the sediment is still in a process of physico-chemical equilibration also concerning the bacterial community, colonisation and differentiation. Furthermore, it can be assumed, that colonisation is further hampered by the sediment structure itself, since coarse sands are in general less colonised by bacteria since they offer less volume-specific surface area (Yamamoto and Lopez 1985).

In contrast to the dumping centre, the dumping surrounding was not covered by a thick layer of dredged sediment. Hence, samples from the surrounding, taken by the VanVeen grab consisted of dredged sediment as well as of the "original" marine sediment and thus were different regarding the sediment composition compared to that of the dumping centre. This condition supports our theory of the sediment partitioning process during dumping (see above). Interestingly some of the samples (3km_2) from the surrounding display the same low genetic diversity as the dumping centre clustering together regarding all gene categories

(Fig. 4). Other samples from the surrounding cluster together with the reference site (3km_1, Fig .4).

As already mentioned the hydrographical regime at the larger dumping area is cyclonic and influenced by east wind forcing and the discharge of the adjacent rivers. All sampling sites where defined at the beginning of the monitoring campaigns by the HPA (HPA 2005) and assigned to *a priori* areas (Fig. 1) with regard to distance to the dumping centre following a circular design. Since this grouping solely based on the distance to the dumping centre it is static and obviously not taking current driven relocation of sediments as well as different environmental conditions into account.

Hence, we used multi-linear regression models (DISTLM) to estimate which factors actually influence the functional structure of the bacterial community (Fig. 5, Table 3). In dbRDA two sites from the close surrounding (1.5km and 2km_1) grouped together with the dumping centre and were nicely separated from the reference sites. Interestingly, the sample 3km_2, clustering together with the dumping centre regarding hierarchical clustering does not cluster with the dumping centre in the DISTLM approach. This finding suggests that the low genetic diversity at these sites is driven by different environmental factors.

The only significant explanatory variable in DISTLM was ΣDDX. Several studies investigated the relationship between functional gene structure and environmental factors in multivariate analyses. These studies claim that functional genes are highly correlated to environmental factors (Waldron *et al* 2009, Xie *et al* 2011). Beforehand we tested our variables for correlation and observed significant correlations between fine-grained sediment fractions and various other factors such as nitrogen or carbon content and heavy metals as well as correlations between organic pollutants. These correlations hinder an accurate prediction of which factors influence the functional gene structure in particular. Therefore, the

significant effect of ΣDDX includes due to the high correlations ΣHCHs, ΣPCBs and Σorganotin compounds. A possible approach to disentangle these combined affects might be the combination of controlled laboratory experiments, manipulating single factors, with field studies. Furthermore one might consider obtaining additional, physicochemical parameters, such as temperature, pH, oxygen penetration or redox potentials in order to additionally estimate the bioavailability of pollutants for bacterial communities in future studies.

The results obtained from our DISTLM model suggest that ΣDDX together with the organic pollutants ΣHCH, ΣPCB and Σorganotin, form statistically a gradient, separating dumping and reference sites. However, our GeoChip analyses did not confirm this finding since we did not detect any positive functional response (presence of specific organic remediation genes) at the dumping centre observing the gene category "organic remediation". As observed for all other gene categories we detected less functional genes compared to the reference sites.

Beside the observed reduced functional diversity we made two other observations from hierarchical cluster analyses: The samples clustered generally for individual gene categories in the same manner, forming three distinct groups. However, phylogenetic differences could be observed. A considerable number of probes clustering in group I) were retrieved from *Betaproteobacteria*, while the other two groups contained probes from *Delta*- and *Gammaproteobacteria* (data not shown). *Betaproteobacteria* represent typical freshwater organisms (Miskin *et al* 1999, Zwart *et al* 2002) while *Delta*- and *Gammaproteobacteria* are predominantly found in marine sediments (Bowman and McCuaig 2003, Bowman et al 2005, Ravenschlag *et al* 1999). This observation is not surprising since it reflects the origin of the sediment. Group I), comprising samples from the Elbe representing a freshwater habitat. While the other groups included marine samples. Indeed this finding is in line with our results obtained from ribosomal tag sequencing in the framework of another study, where we subjected the same samples to a sequencing analysis. We found that community composition

of Elbe and marine stations differed significantly predominantly regarding *Beta-* and *Deltaproteobacteria* (Störmer *et al* 2012).

The GeoChip 4.2 contains an impressive number of gene probes covering several functional processes including heavy metal resistance and organic remediation. However, in our study no differences regarding pollutant specific genes were detected when comparing dumping and reference sites. Possibly, the pollutant load was not severe enough and a functional adaptation did not occur. However, to date many functional processes and relationships among bacterial communities are still not understood (Fuhrman 2009). Nowadays microbiologists face a wide range of molecular tools describing microbial community function and structure. Metagenomics, including here presented functional gene arrays, represent todays high-end technologies offering new insights into complex microbial networks (Fuhrman 2009). They allow for the assessment of complex systems as the bacterial response to phytoplankton blooms (Teeling *et al* 2012) or the description of the rare biosphere for instance in deep sea waters (Sogin *et al* 2006). Novel insights derived from studies like these will in turn improve out methodologies. In respect to the present study additional knowledge on the function of microbial community will lead to a further development of functional gene array as already happened in the past years (He *et al* 2007, He *et al* 2010, Lu *et al* 2012).

In summary our study presented novel insights into the functional response of bacterial communities to dumping activities. For the first time it was demonstrated that ocean dumping leads to a reduced functional diversity at the immediate dumping centre. This study contributed significantly to our understanding of ecosystem functioning and therefore further progression of metagenomic technologies.

Acknowledgements

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GENERAL DISCUSSION

Studying the abundance and distribution of species and factors influencing them are crucial to understand ecosystem functioning and to predict environmental changes. Bacterial communities are highly diverse and their biogeography depends most likely on specific habitat conditions (Fuhrman *et al* 2006, Hewson *et al* 2007). Based on existing literature it appears obvious that no general rules predicting the abundance or distribution of bacterial communities exist. Principally, the combination of various environmental factors and their characteristics determine bacterial communities in individual habitats (Fuhrman *et al* 2006). Current research aims to improve our understanding of ecosystem functioning (e.g. linking species data to environmental data) and -more importantly- to estimate or predict the influence of environmental changes (modelling approaches). Ecosystem modelling in turn requires the understanding of key processes in the respective environment. Coastal regions are highly productive (Atlas and Bartha 1987) and additionally heavily impacted by anthropogenic interferences (Lotze 2010). For these reasons modelling coastal environments is of great interest in order to estimate or even predict the impact of anthropogenic stress (Halpern *et al* 2008). Bacterial communities in coastal areas play a major role in essential mineralisation processes and are characterised worldwide (Allan and Froneman 2008, Crump *et al* 1999, Pernthaler *et al* 2002, Uthicke and McGuire 2007). Studies conducted on bacterial communities aim to investigate their ecological role in various nutrient cycles; examine the influence of environmental gradients such as salinity (Bouvier and del Giorgio 2002, Fortunato and Crump 2011, Herlemann *et al* 2011) or temperature as well as contaminant input (Paisse *et al* 2008). Currently, a main issue in microbial ecology represents the understanding of spatial and temporal dynamics of bacterial communities. To date most research concentrates on pelagic bacterial communities. Incomprehensively, knowledge on benthic bacteria in the sublittoral shelf sediments of the German Bight remains mainly scarce.

Some studies investigated bacterial community structure and composition in the Wadden Seas (Buhring *et al* 2006, Llobet-Brossa *et al* 1998, Musat *et al* 2006) or sub- and intertidal flats (Boer *et al* 2009, Musat *et al* 2006). To our knowledge the only study addressing spatial and temporal variations of benthic bacterial communities in the North Sea was investigating their productivity (Duyl and Kop 1994). Information about benthic bacterial community response to anthropogenic impacts in the German Bight does not exist. The purpose of this thesis was to give detailed insights into community structure and diversity of benthic bacterial communities in the German Bight. The determination of environmental factors, influencing their distribution and composition was another important goal. In the discussion section the following issues will be discussed in a general context: I) Spatiotemporal gradients influencing benthic bacterial communities in near and offshore regions in the German Bight II) Characterisation of benthic bacterial communities at a dumping site: investigating bacterial community structure and function

Spatiotemporal gradients influencing benthic bacterial communities in near and offshore regions in the German Bight

Spatiotemporal variations within bacterial communities represent a major issue in microbial ecology (Fuhrman *et al* 2006, Ghiglione *et al* 2005, Hewson and Fuhrman 2006, Murray *et al* 1998). Many studies aim to identify environmental factors influencing the distribution and assembly of bacterial communities. Seasonal changes were identified to influence bacterial communities in various ecosystems (Fuhrman *et al* 2006, Pietikainen *et al* 2005, Yannarell *et al* 2003a). Generally spoken these studies demonstrated that the occurrence of bacterial communities is predictable from environmental factors and vice versa. This finding implies that specific bacterial groups respond to specific environmental conditions. Fuhrman and co-workers (2006) showed that bacterial communities in the open ocean are annually reoccurring, while bacterial communities in lake systems follow a seasonal cycle but differ from year to year and were rather decoupled from environmental parameters (Yannarell *et al*

2003a). Lozupone and Knight (2007) on the other hand demonstrated for different ecosystems that salinity variations represent the main driving force shaping bacterial community assemblage. Summarising these efforts it becomes obvious that probably locally unique properties of these systems are responsible for these different observations.

To date only a handful of studies exist which take into account both, spatial and temporal dynamics of bacterial communities (Fortunato and Crump 2011, Fortunato *et al* 2012, Herlemann *et al* 2011, Selje and Simon 2003, Selje *et al* 2005). Studies investigating pelagic bacterial communities in coastal areas, more precisely estuaries, stated that spatial factors, such as salinity and depth overwhelmed temporal factors (Fortunato *et al* 2012, Selje and Simon 2003). Incomprehensively, spatiotemporal dynamics of benthic bacterial communities in coastal regions remain little studied. The importance of seasonal variations on the structure and productivity of marine benthic bacterial communities in the North Sea, however, was already highlighted (Boer *et al* 2009, Duyl and Kop 1994). The results yielded in the course of this thesis in conjunction with existing knowledge lead to the assumption that spatial and temporal factors are from different relevance for pelagic and benthic bacterial communities as well as for near and offshore habitats.

Our observations demonstrated that temperature effects shaped the benthic bacterial communities in the first place while spatial factors such as salinity or grain size gradients influenced them secondarily. Apparently, spatial variations of physicochemical factors, such as salinity, are predominantly influencing pelagic bacterial communities in coastal regions; contrary temporal factors are from major importance for their benthic counterparts. A possible explanation for this finding might be the different impact of physicochemical factors on pelagic and benthic habitats. Salinity gradients, for instance, imply density gradients which separate water masses and thus residential bacterial communities (Fortunato *et al* 2012). This impact is possibly less pronounced in sediments. Instead temporal fluctuations as

mineralisation processes after phytoplankton blooms might be, especially in highly productive areas as coastal ones, of greater importance for benthic bacterial communities. The results obtained in this chapter of the thesis indicated a significant impact of *chlorophyll a* concentrations on the diversity and structure of benthic bacterial communities. Bentho-pelagic coupling is a well known process in marine habitats (Buchanan 1993, Kirby *et al* 2007, Marcus and Boero 1998). But only some studies investigated the linkage between organic matter input and benthic bacterial communities. However, it was shown that organic material sinking to the seafloor determines bacterial community structure, biomass and productivity (Franco *et al* 2007, Graf *et al* 1982).

Further, we demonstrated that bacterial communities in offshore habitats (P8 transect) exhibited neither distinct spatial nor temporal variations regarding their community structure, while their counterparts in nearshore habitats (Elbe and Eider transect) showed both significant differences on spatial as well as temporal scales. We consider that nearshore and offshore habitats are facing different impacts of physical factors. While physical forces might have a great impact on nearshore habitats, offshore habitats are rather unaffected. This in turn might be reflected by the bacterial communities inhabiting these regions. We assumed that the distance to the coastline and the linked influences of physical processes such as wind forcing or tidal influences play a crucial role determining the variation in benthic bacterial communities. Comparing two nearshore habitats (Elbe and Eider) it became obvious that bacterial communities in the respective regions were influenced by different spatial gradients. Bacterial communities along the Elbe transect were spatially significant influenced by fine sand distributions. Bacterial communities of fine sand sediments differed significantly as compared to those from muddy sediments. Bacterial communities along the Eider transect however, were significantly influenced by salinity variations. Here, communities inhabiting marine sediments (salinity ~ 33) differed significantly to those of estuarine sediments (salinity

~22). Comparing physicochemical and biogeochemical conditions of both estuaries we observed that the estuaries different regarding their most prominent environmental gradients. Along the Elbe transect significant spatial differences in the sediment composition but not regarding salinity variations were observed. Contrary, along the Eider transect, salinity was varying significantly among the sampling sites and we observed a salinity gradient decreasing towards the estuary, while the sediment composition at the individual sampling sites was similar. This made us conclude that the benthic bacterial community is shaped by the respective strongest spatial environmental gradient(s) in their habitat. Both parameters, sediment composition and salinity are known to influence benthic bacterial communities considerably. Sandy sediments are principally less colonised by bacteria than muddy sediments since they offer less volume-specific surface area for bacteria which can be colonised (Yamamoto and Lopez 1985). Moreover it was highlighted that the bacterial community structure is dependent on the sediment composition (Dale 1974, DeFlaun and Mayer 1983). The impact of salinity variations on bacterial community assembly is well described for bacterioplankton communities in estuaries. Further, in line with our findings, Ikenaga and co-workers (2010) reported distinct cluster of sediment bacterial communities along a salinity gradient.

This first approach demonstrates the enormous complexity of benthic bacterial communities in the German Bight. Future studies should address the phylogenetic composition of the bacterial community in near and offshore regions as well as the functional capacity of these communities to get a more comprehensive picture of the factors, influencing benthic bacterial communities. Simultaneous investigations of pelagic and benthic bacterial communities will yield further insights in their respective, apparently contrary responses to environmental factors.

Impact of dumping activities on benthic bacterial communities

This thesis provides for the first time detailed insights into bacterial community response to dumping activities in the field. Ocean dumping, as well as other anthropogenic impacts on marine ecosystems, represents an issue of major concern. So far, investigations on higher organisms were conducted to estimate the impact of dumping activities on marine ecosystems (IMO 2000, Mühlenhardt-Siegel 1981). Most likely dumping activities resulted in a decrease of diversity of these communities at the immediate dumping area.

In an interdisciplinary project bacterial community analyses were realised at a dumping site in the German Bight. Bacterial communities of the dumping and a reference site were analysed and compared to estimate the impact of the dumping activity on the bacterial community. The dumping activity resulted in changes of the substrate matter at the immediate dumping site (HPA 2010). The dumping site consisted of sandy material while the reference region was characterised by sandy mud. This difference was mirrored in the bacterial community structure. Bacterial communities from dumping and reference sites differed significantly regarding their community structure. The steep grain size gradient appeared to be the strongest environmental gradient affecting significantly the bacterial community in this region. Obviously, the intrusion of this sediment represented a massive perturbation for the environment. This was shown when bacterial communities at the immediate dumping site were compared before and after a dumping activity. Before a dumping event bacterial community structures obtained at several sampling sites (centre and close surrounding) at the immediate dumping site were rather. After dumping, however, significant differences were observed when communities of the immediate dumping centre were compared to those of rather external sampling sites at the dumping site. The impact of perturbation events was already investigated in various contexts. Principally, changes in the bacterial community structure were reported (dos Santos *et al* 2011, Edlund and Jansson 2006, Edlund *et al* 2006,

Findlay *et al* 1990). In the present case multivariate analyses identified not only grain size distributions but also -to a lower extent- organic pollutants to be the main driving forces in shaping bacterial community structure in the investigated dumping area. The impact of organic pollutants was, due to high correlations among environmental variables, delicate to assess. Significant effects of organotin compounds as revealed in all redundancy analyses for instance, are predominantly a statistical artefact. Organotin compounds are highly correlated to other organic pollutants such as poly cyclic aromatic hydrocarbons, HCHs or PCBs. These correlations hinder a precise assessment of individual effects on the benthic bacterial community. Most of field studies face the problematic of correlations among pollutants with other environmental variables (Dean-Ross and Mills 1989, Gillan *et al* 2005). Moreover, since sufficient information about physicochemical parameters such as oxygen penetration and pH are missing, the bioavailability of these compounds is questionable. To further elucidate the individual impact of pollutants on benthic bacterial communities controlled laboratory studies need to be conducted. These studies should approach the disentangling of individual effects by manipulating single environmental factors (e.g. decoupling of grain size and pollutants).

Along with the freshwater sediment freshwater bacteria entered the dumping site and were still detectable ten months after the dumping activity. *Rhizobiales*, *Hypomicrobium* and *Methylocystaceae* or *Burkholeriales* and *Hydrogenophilaceae* seem to successfully establish at the dumping centre. Together with the observation of considerably higher numbers of *Desulfuromonadaceae* and *Flavobacteriaceae* these findings point to a formation of a specialised bacterial community at the dumping centre as a consequence of the dumping activity. To our knowledge, the establishment of bacterial freshwater communities in marine waters is little studied. The abrupt intrusion of freshwater communities into marine systems is naturally rather untypical. The marine-freshwater boundary represents under natural conditions a difficult barrier to cross and marine and freshwater phylotypes are believed to be

clearly evolutionary separated (Logares *et al* 2009). Our results demonstrate, however, that certain freshwater bacteria were still detectable ten months after the dumping activity, suggesting that these groups successfully established under marine conditions. Experimental studies could potentially provide further insight in the possible cross-colonisation of these two environments by testing physicochemical and ecological conditions (Logares *et al* 2009).

We demonstrated that the dumping activity affected bacterial community structure and diversity at the dumping site. To further elucidate the impact of the ocean dumping we aimed to investigate to which extent the functional diversity of the bacterial communities at the dumping site differs compared to those of a previously defined undisturbed reference site (HPA 2005) and those communities of the dredging zone in the Elbe River. To do so functional gene arrays were implemented to gain information about the functional structure of the bacterial communities. The applied gene array, GeoChip, was already utilised in various other environmental studies (He *et al* 2010, Lu *et al* 2012, Van Nostrand *et al* 2009, Xie *et al* 2011). The GeoChip 4.2 contains 103 666 probes of functional processes such as: antibiotic resistance, bacteria phage interaction, energy process, fungi function, carbon, nitrogen, sulphur and phosphorus cycling, metal resistance, organic contaminant degradation, soil benefit, soil borne pathogen, stress and virulence. For all these categories a significant lower functional diversity for the bacterial community at the dumping centre as compared to the reference site was observed. Hence, we suggest that the dumping activity led most likely to a reduction of the overall gene inventory at the dumping site. As already discussed in the previous section, due to the dumping activity fundamentally different geochemical conditions at the dumping site were created. The sandy, regarding sulphur, nitrogen and carbon content poor material harboured a less diverse bacterial community as compared to the reference. The low diversity of the bacterial community might in turn explain the lower functional diversity at the dumping site. The presence of few bacterial metabolic types implies also a lower

functional diversity. To date concrete investigations linking bacterial community diversity and functional diversity are lacking. One might argue that the observed lower functional diversity represents only an artefact of the GeoChip analysis. Possibly, functional genes are not covered by the array because they are not yet discovered. Undisputable many functional processes and relationships among bacterial communities are still not understood (Fuhrman, 2009). However, comparing the functional diversity of the bacterial communities at the dumping site to those of the reference it is valid to state that the diversity of investigated functional genes is significantly lower and most likely a result from the dumping activity.

Beside the reduced functional diversity of bacterial communities at the dumping site, hierarchical clustering of the samples showed similar distinct cluster for all individual gene categories. In all cases, samples originating from the Elbe River, dumping centre and reference site formed separate cluster. Interestingly, the clustering based not on different functional genes but on different phylotypes from which these genes were derived. Principally, the Elbe River cluster contained genes derived predominantly from freshwater bacteria, contrary mainly genes from marine phylotypes were detected in the reference group. The dumping site group however contained genes derived from both freshwater and marine bacteria. We assume that the bacterial community at the dumping site comprises a consortium of marine and fresh water phylotypes and that some marine functions may be replaced or in parallel executed by freshwater counter parts. The investigations of our sequencing approach are confirming this assumption since we detected freshwater and marine phylotypes at the dumping centre.

Since both approaches, sequencing and functional gene array base on DNA, no information about metabolically active bacterial communities is given. It might be considered to integrate further molecular approaches basing on RNA (Transcriptomics) or even proteins (Proteomics) to yield sufficient information about whether the detected freshwater groups are still

metabolically active. Metagenomics, including (Meta)transcriptomics and Meta(proteomics), represent today's high-end technologies offering new insights into complex microbial networks (Fuhrman, 2009). They allow for the assessment of complex systems as the bacterial response to phytoplankton blooms (Teeling *et al.*, 2012) or the description of the rare biosphere for instance in deep sea waters (Sogin *et al.*, 2006).

Conclusion

The outcome of this thesis significantly broadened our knowledge and understanding of benthic bacterial communities in the German Bight. Although bacterial community structures were highly variable; we were able to determine environmental factors influencing the bacterial community structure and diversity.

It was highlighted that the distance to the coast and implicated differences in the impact of physical factors and hydrographics form distinct regional regimes (offshore vs. nearshore). The amplitude of physicochemical and biogeochemical gradients in these regions was differently pronounced. In general, offshore regions were characterised by rather stable physicochemical and geochemical conditions and benthic bacterial communities rather disperse showing neither spatial nor temporal variations. In contrast the observed nearshore regions exhibited significant variations regarding physicochemical (namely salinity and temperature) and biogeochemical (grain size) parameters. Benthic bacterial communities varied significantly on both spatial and temporal scales. Temperature was considered to play the dominant role in shaping the bacterial communities in both nearshore regions. Spatially sediment composition and salinity were shown to influence the bacterial community structure whereas the respective strongest environmental gradient dominated. We therefore conclude that to understand the distribution and abundance of benthic bacterial communities the amplitude of physicochemical gradients and the impact of physical forces and hydrodynamics is most important and needs to be considered.

The dumping activity of lightly polluted sediment in the German Bight resulted in a fundamentally change of substrate matter at the immediate dumping site. The steep environmental gradient formed by grain size distribution significantly affected bacterial community structure and diversity as well as the functional diversity. Community and functional diversity of the bacterial community were significantly lower when compared to a reference site. Moreover we demonstrated that freshwater phylotypes were still detectable ten months after the dumping activity. The effect of pollutants however, was due to high correlations amongst each other delicate to assess. Remarkably higher numbers of *Flavobacteriaceae* and *Desulforomonadaceae* might be an indication for organic pollution at the dumping centre since both families were already named in this context. We therefore conclude that the dumping activity led to a specific environment inhabited by a adapted bacterial community. The impact of the dumping activity, however, was localised solely at the immediate dumping site.

This thesis comprises the most detailed investigations of benthic bacterial communities in the German Bight. Important knowledge on bacterial community structure and influencing environmental factors was presented. The insights gained by these investigations are crucial for our understanding of ecological process and anthropogenic impacts in coastal areas. We are convinced that the perceptions yielded by this will help estimating and predicting environmental changes in the German Bight.

SUMMARY

Bacterial communities are highly diverse and their composition and distribution depends most likely on individual habitat conditions. Hence, studying the abundance and distribution of species and factors influencing them are crucial to understand ecosystem functioning and to predict environmental changes. This thesis aimed to characterise benthic bacterial communities and environmental factors influencing them in the German Bight. The German Bight represents a highly variable and human-impacted system and to date only little is known about the respective consequences for benthic bacterial communities.

The biogeography of benthic bacterial communities in the southern German Bight was captured in monthly cruises along three transects obtaining simultaneously physicochemical and biogeochemical parameters. Ocean dumping activities performed in the German Bight represent a topic of actual concern and their impact on benthic bacterial communities was not included in regular monitoring programs so far. In a pilot study community analyses were implemented at an active disposal site for dredged sediments. Differences in the bacterial community structure and diversity were estimated in direct comparison to a reference site. Applying ARISA fingerprinting community structure and diversity of benthic bacteria were assessed. Sequencing of the SSU ribosomal DNA was implemented to determine the community composition. And finally, functional gene array analyses were utilised to investigate the functional structure of the bacterial communities. The bacterial community information was linked to the recorded contextual data via multivariate analyses in order to identify main factors shaping the community and functional structure.

Generally we observed highly diverse benthic bacterial communities. Bacterial communities inhabiting nearshore and offshore habitats in the German Bight were differently affected by respective environmental conditions. We demonstrated that bacterial communities in

nearshore habitats exhibited distinct temporal as well as spatial variations which were not observed for those inhabiting offshore regions. In any case temporal influences were determined to play the major role. The spatial variations were predominantly driven by respective strong environmental gradients, here grain size and salinity variations, occurring in two compared nearshore regions.

Our investigations at a disposal site demonstrated that the dumping activities affected benthic bacterial communities. Changes in the substrate matter at the dumping site determined a significantly different community structure and diversity of the benthic bacteria as compared to those at a reference site. We observed a considerably lower diversity regarding the community and functional level of the bacteria. Finally, our sequencing approach revealed that the bacterial community at the dumping site encompasses marine as well as fresh water phylotypes which were most likely introduced with the intrusion of dumped sediment. These findings leads us to conclude that the dumping activity created fundamentally different habitat conditions at the immediate dumping site and determined thereby an adapted bacterial community.

This thesis comprises the most detailed investigations of benthic bacterial communities in the German Bight. Important knowledge on bacterial community structure and influencing environmental factors was presented. The insights gained by these investigations are crucial for our understanding of ecological process and anthropogenic impacts in coastal areas. We are convinced that the perceptions yielded by this thesis will help in estimating and predicting environmental changes in the German Bight.

ZUSAMMENFASSUNG

Bakteriengemeinschaften sind sehr divers und ihre Zusammensetzung wird maßgeblich durch die Bedingungen in ihrem jeweiligen Habitat bestimmt. Die Untersuchung von Wechselbeziehungen zwischen Bakteriengemeinschaften und zugehörigen Umweltfaktoren ist deshalb essentiell um die Zusammenhänge zwischen dem Vorkommen bestimmter Arten und äußeren Einflüssen zu verstehen und gegebenenfalls das Ausmaß von Umweltveränderungen abschätzen zu können.

Die vorliegende Arbeit dokumentiert zum ersten Mal die Verbreitung benthischer Bakteriengemeinschaften in Abhängigkeit einflussnehmender Umweltfaktoren in der Deutschen Bucht (Nordsee). Die Deutsche Bucht selbst stellt eine sehr variable und durch anthropogene Einflüsse geprägte Region dar.

Die Zusammensetzung benthischer Bakteriengemeinschaften wurde in monatlichen Abständen entlang dreier Transekte in der Deutschen Bucht erfasst und mit gleichzeitig erhobenen physikochemischen und biogeochemischen Daten verschnitten. Umlagerungen von ausgebaggertem Elbsediment in die Deutsche Bucht im Zuge von Instandhaltungsarbeiten des Hamburger Hafens sind aktuell von großem, auch öffentlichem Interesse. Bisher wurden Gemeinschaftsanalysen von benthischen Bakterien nicht in das begleitende Monitoringprogramm integriert. In einer Pilotstudie wurden diese Analysen durchgeführt um den Einfluss der Verbringung auf die Bakteriengemeinschaften abzuschätzen.

Bakteriengemeinschaftsanalysen (Struktur und Diversität) wurden im Rahmen dieser Arbeit mittels der Fingerprintmethode Automated Ribosomal Intergenic Spacer Analysis (ARISA) durchgeführt. Darüber hinaus wurde die Zusammensetzung der Bakteriengemeinschaften mithilfe von Tiefensequenzierung einer ribosomalen DNA Sequenz ermittelt und die Funktion

mittels Mikroarrays (*functional gene arrays*) untersucht. In multivariaten Analysen wurden die Informationen der Gemeinschaftsanalysen mit kontextuellen Daten verschnitten.

Die Untersuchungen ergaben, dass die benthischen Bakteriengemeinschaften der Deutschen Bucht sehr divers sind. Der Einfluss von Umweltfaktoren auf die Bakteriengemeinschaften hängt dabei stark von den physikalischen Rahmenbedingungen (Wind, Tide) in den untersuchten Gebieten ab. Bakteriengemeinschaften der nahen Küstengebiete zeigten ausgeprägte saisonale sowie räumliche Abhängigkeiten auf, während Gemeinschaften in küstenfernen Gebieten weniger von Umweltfaktoren beeinflusst waren. In jedem Fall spielte die Temperatur bei der Ausprägung der Bakteriengemeinschaften eine entscheidende Rolle. Bezüglich der untersuchten Ästuare der Küstenregionen konnte gezeigt werden, dass der jeweils am stärksten ausgeprägte Umweltgradient, in diesem Fall Unterschiede des Salzgehaltes und des Feinsandanteils im Sediment, den größten Einfluss auf die Bakteriengemeinschaft nahm.

Die in der Deutschen Bucht stattfindenden Sedimentumlagerungen führten zu einer fundamentalen Veränderung der Sedimentstruktur an der Einbringstelle. Die Veränderung spiegelte sich auch in den vorkommenden Bakteriengemeinschaften wider. Die Gemeinschaftsstruktur unterschied sich signifikant von derer im Referenzgebiet. Die Diversität, im Hinblick auf Funktion und Artenniveau, war ebenfalls deutlich erniedrigt. Die Sequenzanalysen ergaben, dass neben marinen Arten auch Süßwasserbakterien an der Umlagerungsstelle nachgewiesen wurden. Insgesamt lässt sich feststellen, dass die Umlagerung zu einer deutlichen Veränderung der Habitatstruktur geführt hat die konsequenterweise zu einer angepassten Bakteriengemeinschaft führte.

Insgesamt bietet diese Arbeit einen detaillierten Einblick in die benthischen Bakteriengemeinschaften der Deutschen Bucht. Es konnte gezeigt werden welche und in welchem Ausmaß Umweltfaktoren diese Gemeinschaften beeinflussen und wie sich

Sedimentverbringungen im Besonderen auswirken. Die Erkenntnisse dieser Arbeit sind entscheidend um ökologische Prozesse und Veränderungen in Küstenregionen der Deutschen Bucht zu verstehen und abschätzen zu können.

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