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Title

The Bacterial Diversity in Marine Sediments of the German Bight:relation with carbon and nitrogen content

by

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Eidesstattliche Erklärung

Hiermit erkläre ich, Mathis von Ahnen, geboren am 13.10.1986 in Stade, dass diese Bachelorarbeit zur Vorlage beim Prüfungsamt der Hochschule Bremerhaven von mir in selbständiger Arbeit, unter Verwendung der angegebenen Quellen angefertigt wurde.

Helgoland, den

_____

Name

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Meiner Familie dafür, dass ihr immer hinter mir steht.

Abstract

Investigations have been carried out on the bacterial diversity in the southern North Sea at a position called *Tonne E3* where sediment originating from the Hamburg port area is dumped. An effect called *tidal pumping* causes the accumulation of sediment in the Port of Hamburg nowadays and gave occasion to the establishment of this dumping site in 2005. Bacteria react to changes in biotic and abiotic conditions very fast, e.g. due to high reproduction rates and the competence for gen transfer, which distinguishes them as qualified indicators for environmental impacts in general. Bacterial communities were investigated at the dumping site, a reference area and surrounding regions by genomic fingerprinting via Automated *Ribosomal Intergenic Spacer Analysis* (*ARISA*). This method focuses on the length polymorphism of the 16S-23S IGS in the bacterial genome thus providing estimates of bacterial diversity. Additionally sedimentary carbon and nitrogen contents were measured as they presumably affect bacterial abundances. It could be shown that in the immediate area of dumping a narrowing of the bacterial diversity had taken place compared to a reference area and surrounding regions. Carbon and nitrogen values were highly correlated with each other and positively correlated with the similarity among bacterial community patterns.

Zusammenfassung

Untersuchungen über die bakterielle Diversität in der südlichen Nord See bei *Tonne E3*, einer Position, die als Verbringungsstelle für Sediment aus dem Bereich des Hamburger Hafens dient, wurden durchgeführt. Ein Effekt namens *tidal pumping* führt heutzutage zur Anreicherung von Sediment im Haburger Hafen und gab den Anlass zur Etablierung dieser Verbringungsstelle im Jahre 2005. Bakterien reagieren aufgrund ihrer Eigenschaften, wie z.B. hohe Reproduktionsraten und der Fähigkeit zum Gentransfer, sehr schnell auf biotische und abiotische Veränderungen der Umwelt, was sie zu geeigneten Indikatoren für Umwelteinflüsse im Allgemeinen macht. Die Bakteriengemeinschaften der Verbingungsstelle, eines Referenzgebietes und umliegenden Regionen wurden untersucht, indem genomische Fingerabdrücke der Gemeinschaften mittels *Automated Ribosomal Intergenic Spacer Analysis* (*ARISA*) erstellt wurden. Diese Methode basiert auf dem Längenpolymorphismus des 16S-23S IGS im bakteriellen Genom und gibt so Aufschluss über die bakterielle Diversität. Zusätzlich wurden Kohlenstoff und Stickstoff Gehalte des Sediments gemessen, da sie vermutlich das Auftreten von Bakteriengemeinschaften beeinflussen. Es konnte gezeigt werden, dass im Vergleich zum Referenzgebiet und umliegenden Regionen eine Einengung der Bakteriendiversität an der Verbringungsstelle stattgefunden hat. Die Kohlenstoff- und Stickstoffgehalte waren hochgradig miteinander korreleiert sowie positiv mit der Ähnlichkeit unter den Bakteriengemeinschaften korreliert.

Abbreviations

Abbreviation	Meaning
αHCH	Alpha-Hexachlorocyclohexane
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
APS	Ammonium Persulfate
ARISA	Automated Ribosomal Intergenic Spacer Analysis
bp	Base pair
°C	Celsius
Cd	Cadmium
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
dH_2O	Distilled Water
dNTPs	Nucleoside Triphosphate
EDTA	Ethylenediamineteraacetic Acid
GDR	German Democratic Republic
HCB	Hexachlorobenzene
HCL	Hydrochloride Acid
Hg	Mercury
HPA	Hamburg Port Authority
IGS	Intergenic Spacer
IR	Infrared
LLUR	Landesamt für Landwirtschaft, Umwelt und ländliche Räume

	Schleswig-Holstein
nMDS	Non-Metric Multi-Dimensional Scaling
OTU	Operational Taxonomic Unit
PAH	Polycyclic Aromatic Haydrcarbons
PCB	Polychlorinated Biphenyl
PCR	Polymerase Chain Reaction
PeCB	Pentachlorobenzene
POM	Particulate Organic Matter
PON	Particulate Organic Nitrogen
PRIMER (software)	Plymouth Routines In Multivariate Ecological Research
SDS	Sodium Dodecyl Sulfate
TAE	Buffer Mixture of: Tris Base, Acetic Acid, EDTA
TBT	Tributyltin
TCD	Thermal Conductivity Detector
TEMED	Tetramethylethylenediamine
γHCH	Gamma- Hexachlorocyclohexane
V	Volt
Zn	Zinc

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1 Introduction

The impact of increasing economical growth on the North Sea, a shelf sea on the Northwest European continental shelf, is diverse nowadays. Among overexploitation of fisheries and eutrophication also the disposal of dredged material from the river Elbe effectuates the endangerment of this unique marine environment {Patsch 1997}.

Since about 50 years the fairway of the Lower and Outer Elbe has been constantly adapted to shipbuilding developments in order to ensure the competitiveness of the Port of Hamburg. Several river improvement measures such as deepening and straightening of the fairway or building of bank reinforcements affect the rivers hydrodynamic properties remarkably as well as the local fauna and flora. The deepening in 1999 increased the asymmetry of the tidal curve in the upstream half of the estuary which resulted in the so called "tidal pumping" effect. Strong currents during flood bring fine-grained sediment upstream to the harbour basins where it settles. This sediment stays unaffected by the tidal flow and needs to be dredged regularly. Due to tidal pumping in 2004 the annual amount of sediments dredged in the Hamburg harbour unexpectedly increased from about 4.3 mil. m^3 to over 8 mil. m^3 and remained that high in the following years {Kerner 2006}.

The capacity of on land storage of sediments will be exhausted in 6 years. Thus new sustainable long term solutions including treatment and new storage concepts for the sediment need to be developed {Leal 2006}. The Hamburg Port Authority (HPA) and the Waterway and Shipping Directorate (WSA Nord) have therefore developed a sediment management concept for the tidal Elbe which encompassed the storage of 4.5 mil. m^3 of dredged material in the North Sea at *Tonne E3* in the years 2005 to 2008.

The sea mark Tonne E3 is located approximately 15km in the southeast of the offshore island Heligoland at 54°03'N 07°58'E. As the restricted amount of disposable sediment was achieved in 2008 the LLUR permitted the disposal of another 6.5 mil. m^3 sediment until the end of 2011. Additionally the water resources act of the State of Schleswig Holstein permitted the disposal of 1.5 mil. m^3 material from the turning basins and mooring areas of the Port of Hamburg.

The contamination of these particular sediments with heavy metals (e.g. As, Cd, Hg, Zn) and organic contaminants (e.g. PCB, Dioxins, PAHs) results mainly from upstream industrial activities in the former GDR and Czech Republic (mining, chemical, pharmaceutical, pulp and paper, as well as leather-processing industries). This was shown to be mainly due to the contempt of environmental issues in former times {Heise 2005}. The HPA's annual report of the year 2007 exhibits that the contents of mercury, cadmium, zinc, PAH, αHCH, γHCH, DDT, TBT, HCB, PeCB and PCB increased at *Tonne E3* compared to values measured before the dredging campaign started in 2005. Likewise the grain size of the bottom material at *Tonne E3* increased as mainly sandy material is deposited. During the process of dumping, approximately 50-65% of the fine grain content (< 20 μm) drifts away {Hamburg Port Authority 2008}. The various investigations on the impact of dumping at *Tonne E3* (e.g. ecotoxicology, benthic invertebrates) that were established at first when the dredging campaign started didn't involve bacteria at all.

However, one of the most important findings in biological oceanography and aquatic ecology is that microbes, especially heterotrophic bacteria, are capital and essential components of food webs and nutrient cycles in the oceans and other aquatic systems {Ogunseitan 2005}. Microorganisms are presumed to be especially sensitive to the toxic effects of pollutants causing ecosystem functions that depend on microbial activities to suffer adverse impacts from repeated exposures {Domsch 1983}.

Bacteria play a key role in the mineralization of deposited organic material and its incorporation into the food chain including also higher trophic levels such as benthic fish through meio- and macrobenthic organisms {Billen 1990}. Due to high reproduction rates, the competence for gene transfer and a variable metabolism, most bacteria adapt to changing biotic and abiotic conditions very fast. Thus, investigating the bacterial community structure, the basis of a marine environment, enables following changes including various anthropogenic impacts in general.

This study is embedded in a PhD project dealing with the investigation of the benthic bacterial communities in the German Bight. The special focus lays on the impact of excavated material on these communities. First analyses of the bacterial communities via ARISA in August 2009 by the Biologische Anstalt Helgoland revealed the existence of three different community structures. One pattern occurred at the reference area and two at the dumping site

This study aims at analysing the bacterial communities occurring at the dumping site, its immediate environment and a reference area in an other resolution (see Appendix Fig. 6.1).

Many studies show a correlation between bacterial abundance and organic matter content of sediments {Hickel 1968, Dale 1974, Hargrave 1972, Reichgott 1978}. Therefore, relations with carbon and nitrogen values will be taken into account in order to possibly explain the occurrence of bacterial community patterns. The availability of nutrients could reveal possible geographical differences and sediment characteristics that may affect the bacterial communities of these marine sediments.

2 Fundamentals

2.1 Bacteria in Sediments

The most common species found in marine sediments belong to the class of bacteria (prokaryotes). They are perfectly featured to accomplish the mineralisation of deposited organic material, as they are the right size and dispose metabolic versatility to oxidize the organic carbon in a variety of different ways. In general diversity among bacteria is expressed in terms of metabolism rather than in structure and they have few modes of behaviour other than growth and division.

In sediments, the huge diversity in bacterial metabolisms is explained by redox gradients in the upper layers of sediments, and is of great importance for the functioning of marine ecosystems. According to {Lancelot 1988} oxygen is depleted below a depth of a few millimetres to a few centimetres dependant on the intensity of heterotrophic activity and oxygen supply. In further depths nitrate, manganese and iron oxides and sulfate are successively used as electron acceptors for respiration of organic substrates. The presence of high concentrations of reduced mineral substrates such as ammonium, reduced manganese and iron and sulfides in the redox gradients allows chemoautotrophic metabolisms to take place, like nitrification, manganese-, ferro- and sulfooxidation {Billen 1990}.

Energy in bacterial cells is conserved by the generation of a chemoosmotic gradient called the proton-motive force (pmf). The proton-motive force is generated by an electrochemical potential and a pH gradient. It is used to generate biologically useful energy in the form of adenosine triphosphate (ATP). A variety of electron donors,

both organic and inorganic, as well as many different alternative electron acceptors or "oxygen substitutes" for respiration are used by bacteria in the absence of molecular oxygen. This technique makes them extremely versatile with regard to energy (see Appendix Tab.6.1).

These microbial activities result in characteristic vertical nutrient profiles in sediments in which each nutrient is product or reactant of one or more metabolic groups {Nealson 1997}. Chemical reactions are strongly influenced by the surface to volume ratio (S/V) of the reactants. Bacteria have maximized this parameter and possess, at cell sizes of 0.5 to a few micrometers in diameter, S/V values 100-1000 times higher than eukaryotic cells ranging from 20μm to millimetres in diameter {Nealson 1997}. Thus even if prokaryotes may constitute only a few weight percent of the total biomass, they have a potential reactivity and environmental impact equal to that of the total. However, the small intracellular volume of bacterial cells also limit the availability of free protons and space and with that the chemical abilities. Moreover the rigid bacterial cell wall imposes certain restrictions on metabolism and the way in which bacteria interact with their environment. In general they possess two modes of nutrient modification and uptake: (a) the use of specific transport systems to move nutrients against concentration gradients into the cytoplasm and (b) the use of extracellular enzymes to convert large polymeric molecules into smaller oligomers and monomers. According to investigations of Burns (1980) in soil, extracellular enzymes released by bacteria into the surrounding medium may retain their activity through the formation of humic-enzymes complexes {Burns 1980}.

2.2 Carbon Cycling in the North Sea

Coastal and marginal seas like the North Sea reveal strong biological activity. These activities play an important role in the global carbon cycle by linking the terrestrial,

oceanic and atmospheric carbon reservoirs{Gattuso 1998}. The carbon content of the North Sea is dominated by the carbon exchange fluxes with the North Atlantic Ocean. Through its northern open boundary an anticlockwise "u-shaped" circulation of North Atlantic Ocean water enters the North Sea via the Shetland Channel and the Fair Island Channel. Its water is enriched with carbon, and leaves along the Norwegian Trench at the eastern boundary (Fig.2.1).

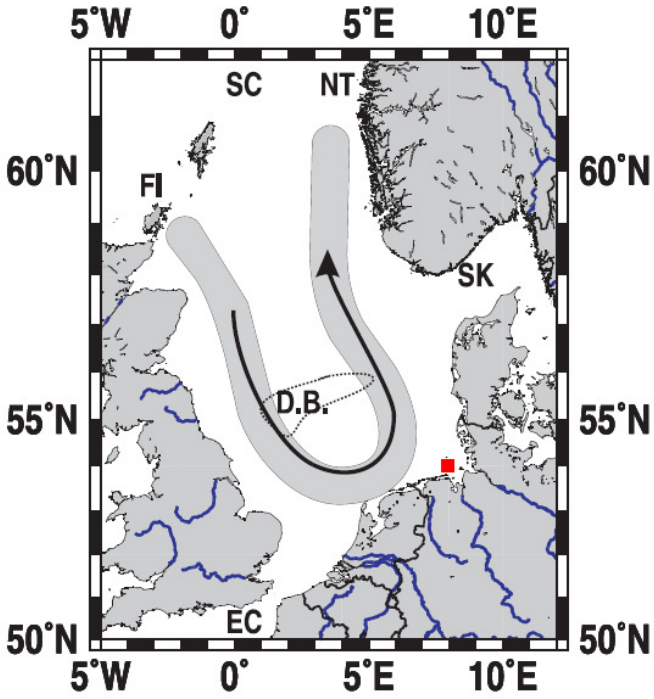


Fig. 2.1: The dominant current circulation in the North Sea. The red dot roughly marks the location of Tonne E3. Abbreviations show location of: English Channel (EC), Skagerrak (SK), Faire Island Channel (FI), Shetland Channel (SC), Norwegian Trench (NT) and Dogger Bank (D.B.) (according to H.Thomas et al., 2005)

By means of this current more than 99% of the total carbon is exported from the North Sea into the North Atlantic Ocean thus making the North Sea a highly efficient continental shelf pump for carbon. Less than 1% of primary production is buried into sediments which still might play a relevant role over geological time scales {Thomas 2005}.

The carbon content of the North Sea originates from many sources such as inputs from rivers, the Baltic Sea and the atmosphere. Within the upper water layers carbon dioxide is not only taken into solution but also incorporated into living organisms by photosynthetic activity of phytoplankton. High biological activity causes the drawdown of CO_2 from the atmosphere into the water column. Considering the partition of primary produced organic matter in the euphotic zone three 'concurrent' pathways exist: (i) direct grazing by zooplankters, (ii) uptake by planktonic bacteria and incorporation into the microbial loop involving both direct mineralization and bacterial biomass production; (iii) sedimentation and incorporation into the benthic food chains {Billen 1990}. Organic compounds are released or excreted by nearly all marine organisms. For instance, zooplankton excretes roughly one-third as particulate material (faecal pellets) and one-third as dissolved compounds {Steele 1974}. Faecal pellets produced during grazing of a phytoplankton bloom are still rich in utilizable organic matter {Brockmann 1990}. In the marine carbon cycle microorganisms fulfil an irreplaceable role for the sustainment of life by the mineralisation of organic compounds. The decay of the organic particles not only recycles carbon, but also the nutrients locked within.

The study site of this work belongs to the southern part of the North Sea which is strongly affected by terrestrial and anthropogenic nutrient inputs (e.g. agricultural run-off, sewage effluent) for a major part originated from the Weser and Elbe estuaries and the Wadden Sea {Brasse 1999}. In this part of the North Sea, most of the carbon fixed as POM by photosynthetic activity is recycled within the well mixed water column or within the sediment surface {Thomas 2005}.

2.3 The Marine Nitrogen Cycle

Biological production depends on nitrogen, as it is essential in building of cell components such as proteins and DNA. However, organisms are incapable of using

all nitrogen derivates and biological production is often limited by the non-availability of suitable forms or concentrations of nitrogen {Naqvi 2006}. The productivity in a marine ecosystem is therefore restricted by the available nitrogen present in the system. Riverine delivery of inorganic and organic nitrogen (N), including some N in particulate form, is the main source of combined nitrogen for coastal seas {Caraco 1999}. Nitrogen also arrives into the sea by wet and dry atmospheric deposition of inorganic and organic N. Wet atmospheric deposition occurs when compounds are absorbed by falling rain, while dry deposition is the direct adsorption of compounds to water. The conversion of different derivates of nitrogen is carried out by specialized microorganisms. In the ocean, nitrogen in the form of ammonium (NH_4^+) is mainly set free by the degradation of organic matter. It is terminally oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) by bacteria in a chemoautotrophic process called nitrification. Nitrifying bacteria consist of two groups, those who oxidize ammonia to nitrite and those who oxidize nitrite to nitrate. The first group—the ammonia oxidizers—include many species in the genera Nitrosobacter, Nitrosococcus, and Nitrosomonas. They are known for internal membranes and the presence of a primary amine oxidase (PMO) which catalyzes the oxidation of ammonia producing NADH + H^+. The second group (including many species in the genera Nitrobacter, Nitrococcus, and Nitrospira) are slow-growing specialists dependent on the supply of nitrite released by the first group. As the nitrate oxidizers, these bacteria provide internal membranes. Cell shape and internal membrane structure are often used for identification {Nealson 1997}.

In anaerobic environments denitrification takes place. This process involves the reduction of NO_3^- to N_2 with NO_2^-, nitric oxide (NO) and N_2O as intermediates. A large number of bacteria catalyze the reduction of nitrate to N2 gas in the denitrification process. Although nitrate is often present at low concentrations in the environment, these organisms are ubiquitous and play a role in the cycling of carbon and nitrogen in sedimentary systems. When oxygen is limited, nitrate is typically the

next major biological electron acceptor utilized. A large variation exists among organisms that accomplish nitrate reduction: some reduce the nitrate up to ammonia, known as dissimilatory nitrate reduction to ammonium (DNRA) and other bacteria reduce nitrate even in the presence of molecular oxygen {Kuenen 1988, Blackburn 1992}. Denitrification occurs in coastal areas and sediments and causing the gaseous evasion of nitrogen (N_2) from the nutrient cycle in the water column to the atmosphere {Brockmann 1990}. Nitrogen is also removed from the water column via sedimentation to the seafloor and biomass harvest. Another way that N_2 is produced in the ocean is the anaerobic ammonium oxidation (anammox; $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$) which is carried out by chemoautotrophic bacteria.

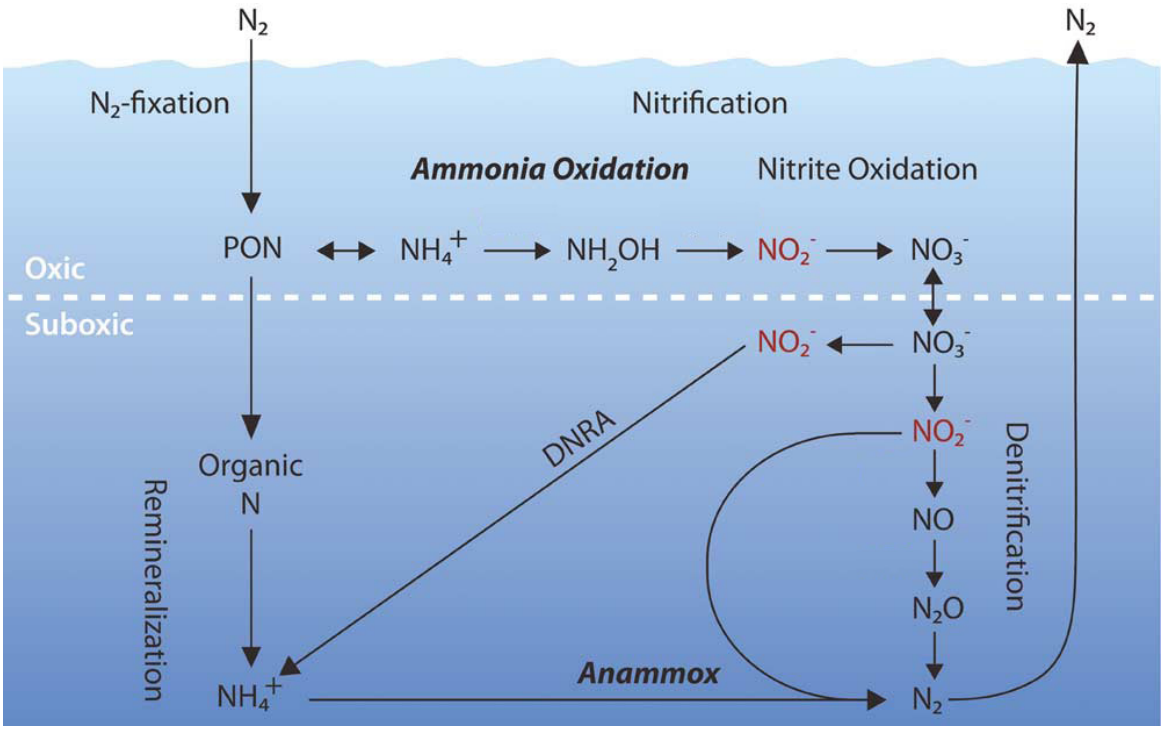


Fig. 2.2: The Marine Nitrogen Cycle. Microbial transformations above, below and across an oxic/suboxic interface in the marine environment (according to {Francis 2007}).

3 Material & Methods

3.1 Material

3.1.1 Scientific Instrumentation

Equipment	Term	Manufacturer
Agarose gel chamber		peQ Lab
Agarose gel supporter and combs		peQ Lab
Autoclav	5075 ELV	Tuttnauer
Autoclav	SANOCLAV	Adolf Wolf
Autoclav	VX-75, VX-150	Systec
Beaker	Different sizes	Duran, Brandt
Filtration units Bottletops	250 mL	Nalgene
Centrifuge	Centrifuge 5417 R	Eppendorf
Freezer (-20°C)	economic-super, economic-froster	Bosch
Freezer (-20°C)	öko Arctis	AEG
Freezer (-80°C)		
Fridge	electronic	Bosch
Fridge	öko santo super	AEG
Geldocumentation system	ChemiDoc™ XRS System	BioRad
Heating block	Thermomixer comfort	Eppendorf
Ice machine	Scotman AF 10	Bioblock scientific
Incubator	MIR - 252	
IR² 4200 DNA Sequencer Analyzer Gene Reader	Li-cor e-seq	LI-COR
Laboratory dishwasher	Professional G7883	Miele
Laboratory-type drying cabinet		Memmert
Measuring cylinder	Different sizes	Brandt
Microplate	NanoQuant Plate	Tecan
Microtiterplate		Sarstedt
Microwave	M6138	Samsung

Equipment	Term	Manufacturer
Pipettes	2 µL, 20 µL, 200µL, 1000 µL	Gilson
Power supply for gelelectrophoresis	Power pac 200	BioRad
Scale	LE 225D	Sartorius
Scale	BP 2100S, BP 6100	Sartorius
Scale	MC1 Research RC 210P	Sartorius
Sequencer (ARISA)	IR 4200	Licor
Software for Microplate reader	i-control	Tecan
Spectrophotometer	Infinite M200	Tecan
Clean bench	MDSA….01PU	Infralab
Thermocycler	Mastercycler	Eppendorf
Vortex	VF2	Jahnke & Kunkel
Water purification system	Milli-Q	Millipore

3.1.2 Consumables

Material	Term/Size	Manufacturer
Cellulose paper		Whatman
Falcon tubes	50 mL	Greiner
Gloves	Nitril	Roth
Gloves	Latex	Roth
Lint-free paper	Kimtech precision wipes	Kimberly-Clark
Paper tissue	Profix	Temca
PCR-reaction tubes	8-strip tubes	Eppendorf
Pipette tips	1000 µL, 200 µL, 20µL	Brandt/Sarstedt
Pipette tips	5 mL, 2 µL	Biozym
Reaction tubes	2 mL SafeLock	Eppendorf
Reaction tubes	1.5 mL, 0.5 mL,	Eppendorf
Tape for autoclaving	3m Comply™ Indicator	Steam

3.1.3 Reagents

Reagent	Abbreviation	Manufacturer
Agarose, DNA grade for Nucleic acids		Biomol
Desoxyribonucleotide	dNTP	Promega

Reagent	Abbreviation	Manufacturer
Ethanol		Merk
Ethidiumbromide		Calbiochem
Ethylenediaminetetraacetic acid	EDTA	Applichem
Hydrochloric acid	HCl	Merck
Isopropanol		Merk
Master-Enhancer		5Prime
Taq Buffer		5Prime
Taq Master PCR Enhancer, 5x		5Prime
TEMED		Sigma
Water Millipore		Autoclaved, strilfiltrated

3.1.4 Enzymes

Enzyme		Manufacturer
Taq Polymerase 5 U/µL		5Prime

3.1.5 Molecular Markers

Molekular Marker		Manufacturer
100 bp DNA ladder		Invitrogen
L1		Roche
Size Standard IRDYE700 50-1500bp		Li-Cor

3.1.6 Primer

Label	Sequence	Citation
S-D-Bact-1522-b-S-20	5‘-TGC GGC TGG ATC CCC TCC TT-3‘	Ranjard, 2000
L-D-Bact-132-a-A-18	5‘- CCG GGT TTC CCC ATT CGG-3‘	Ranjard, 2000

3.1.7 Solutions

Solution	Ingredients	Manufacturer
APS (Ammonium Persulfate)	10% (w/v)	
Ethidiumbromide solution [2ng/ml]	Aqua dest 1l Ethidiumbromide [20mg/ml] 100µl	
TAE Buffer (50x)	2 M Tris Acetate 50 mM EDTA	
TBE Buffer (10x)	0,089 M Tris base 0,089 M Borate 0,002 M Na2 EDTA	LI-COR
Stop-Mix 5 x	0.25 % Bromcresol purple 50 % Glycerine 0.05 M Trisbase pH 7,9 Steril filtrated (0.2 µm)	
Blue Stop Solution	EDTA <1% (w/v) Bromophenol Blue <1% (w/v) Water <14% (w/v) Formamide <87% (w/v)	
KB Plus 5,5% Gel Matrix	Acrylamide Urea TBE Buffer	LI-COR

3.1.8 Software

Application	Name	Manufacturer
DNA Quantification	i-control	TECAN
Gel Documentation	Quantity One	BioRad
DNA Sequencing	e-Seq	Li-cor
ARISA Gel Image Editing	BioNumerics 4.5	Applied Maths
Data Analysis	STATISTICA	StatSoft
Data Analysis	Primer 6	Primer-E

3.2 Methods

3.2.1 Study Site and Sample Collection

Samples were collected from 22nd – 24th of March in 2010 with the research vessel "Uthörn". The study site encompassed the reference area and the dumping site "Tonne E3" as well a chosen region in between (R1) and an area heading towards the Elbe estuary (R2) (see appendix Fig.6.1). The sediment was taken by a Van Veen grabber with a volume of 0.2m³. Water depth ranged from 20 – 35m and water temperature was about 4°C at that time. The sediment was homogenized and samples from 75 positions were filled into 50ml Falcon Tubes in triplicate and stored immediately at -20°C after collection. Back in the laboratory they were stored at -80°C until further processing.

The reference area and the dumping site are approximately 10km apart from each other. The sediment is dumped at Tonne E3 in an area of 400mx400m around the center (54°03'N 07°58'E) whereas the reference area was unaffected thus being representative for the former status of the dumping site.

3.2.2 DNA Extraction

DNA was extracted and purified by using the *PowerSoil™ DNA Isolation Kit* (*MO-BIO*) and following the *MO-BIO* protocol (for detailed protocol see appendix Fig.6.2-6.4). The method is based on a homogenization step which involves mechanical shaking and the use of SDS and other disruption agents to accomplish complete cell lyses. The reaction tubes contain beads which collide with microbial cells during the process of shaking causing cells to break open. Meanwhile the anionic detergent SDS breaks down fatty acids and lipids associated with cell membranes. In the following steps the addition of several solutions followed by centrifugation will remove non-DNA organic and inorganic material such as humic substances, cell debris and proteins. This method takes advantage of the fact that DNA absorbs onto a silica membrane in the presence of high salt concentrations. This interaction isn't

completely understood yet. After the lysate was centrifuged through the silica membrane DNA bound to silica. The impurities (residual proteins, salt) which remained on the membrane as well are washed away by an ethanol based solution. A subsequent centrifugation is essential for a clean elution as it removes the ethanol from the membrane. In the final step DNA is hydrated and thereby released from the membrane by the addition of a 10mM Tris buffer. The DNA extractions are subsequently stored at -20°C to prevent degradation.

3.2.3 Gel Electrophoresis

In order to check purity and concentrations of DNA extracts and PCR products, defined amounts were loaded on agarose gels. By electrophoresis DNA fragments are separated according to their fragment sizes. The DNA, consisting of negatively charged nucleic acids, migrates through the gel towards the anode when an electric field is applied. The speed of migration depends on the size and conformation of the DNA fragments. Small fragments move faster and thus farther than larger ones. The gel matrices consist of agarose in 1%TAE buffer. The percentage of agarose determines the pore size of the agarose matrix. For genomic DNA analysis a gel matrix containing 0.8% agarose was chosen for PCR products the gels contained 1.4% agarose. The DNA size markers were the L1 (*Roche*) for DNA extractions and 100bp (*Invitrogen*) marker for PCR products.

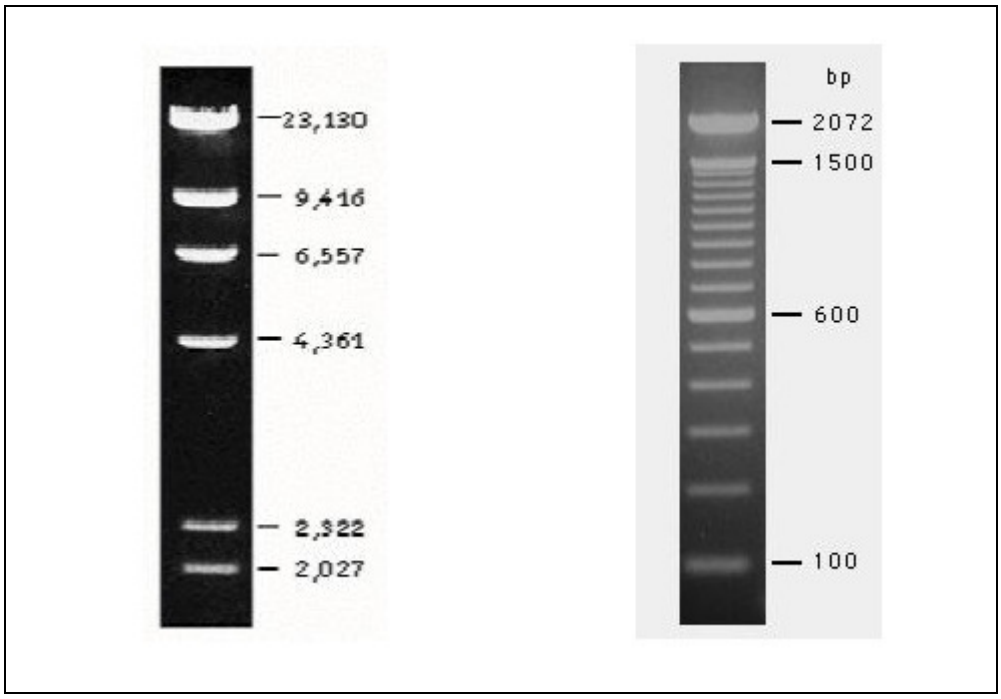


Fig. 3.1: Marker L1 (Roche) and 100bp marker (Invitrogen)

The samples were provided with stop mix which contains glycerine to make them sink into the wells. The loaded gels were placed into electrophoresis units filled with 1%TAE buffer. For DNA extracts as wells as PCR products the gel electrophoresis was performed at 100V for 60min.

After gel electrophoresis the gels were placed in an ethidium bromide bath [2ng/ml] for 5min. The staining is based on the intercalation of ethidium bromide with the DNA strands. After staining the gels were transferred in a water bath for 30 min to remove redundant ethidium bromide. Gels were imaged using UV light applying a standard amber UV filter with 580 nm emission. The gel documentation was performed with the *ChemiDoc™ XRS System (BioRad)* using a CCD camera. The images were recorded as digital images using the software *Quantity One*.

3.2.4 Spectrophotometer

The DNA amounts of each sample were determined using the spectrophotometer *TECAN Infinite M200 Nano Quant*. Samples were heated in a thermo cycler at 37°C for 1h so that DNA fragments are equally dispensed within the extracts. Absorbance measurements of 2µl dissolved DNA samples (double determination) were performed at 260 and 280nm in a *Nano Quant Plate*. In the further amplification process 10ng DNA were applied. The calculation based on the mean of the double determination for each sample. UV spectroscopic measurements of nucleic acids preparations give information about the amount of DNA as well as the purity. The ratio of the absorbance values at 260nm and 280nm is expected to be approximately 1.8 - 2.0 for pure DNA. The measurement at 280nm detects contaminations with proteins. Proteins Contain amino acid residues with a maximum absorbance at 280nm. In case proteins are present, the ratio will be significantly lower than 1.8 {Sambrook 1989}. After the measurement the software *i-control* displays the results in an *Excel* table.

3.2.5 Polymerase Chain Reaction (PCR)

Polymerase chain reaction enables the in vitro amplification of short DNA fragments up to 3000bp in size. The method is based on three main steps:

The reaction solution containing DNA molecules, Taq-polymerases, primers and nucleotides is heated at 95°C. The two complementary DNA strands separate because the hydrogen bonds between the bases break up. This is called denaturing.

The following step is called annealing. The temperature is lowered to 50°C allowing the primers to bind at the single DNA strands and build stable bonds to their complementary sequences. The polymerases binds to the 3' end of the primers and start DNA synthesis. Annealing temperature varies and is depending on the length and base composition of the primers.

The final elongation step is performed at 68°C, the ideal working temperature for the specific Taq-polymerases used in this study to synthesis the new DNA strands. Coincidental those bonds between Primers and DNA strands that are not fully complementary are broken.

Reaction mixtures were held at 95°C for 3min, followed by 30 cycles of amplification at 95°C, 50°C and 68°C for 1 min each and a final extension of 68°C for 5min. The amount of DNA strands doubles after each repetition of these three steps/one cycle leading to an exponential amplification of DNA segments.

In this study the **I**nter**g**enic **S**pacer (IGS) region was amplified (Fig. 3.2). The bacterial IGS is located between the small- and large-subunit rRNA genes were amplified by using the following primers: L-D-Bact-132f-a-A-18 (132r) (eubacterial rRNA large subunit, 5`-CCGGGTTTCCCCATTCGG-3´) and S-D-Bact-1522r-b-S-20 (1522f) (eubacterial rRNA small subunit, 5´-TGCGGCTGGATCCCCTCCTT-3´){Ranjard 2001}. These primers are complementary to the sequences at the DNA of 23S rDNA (1522) and at the beginning of 16S rDNA (132). As ARISA requires a fluorescent labeled primer for PCR, the forward primer was labeled with an infrared dye (IRD700). This dye fluoresces at a wavelength of 700nm and is sensitive to light therefore it has to be stored continuously in the dark. Reaction mixtures contained 5µl enhancer (*5Prime*), 2.5µl PCR buffer (*5Prime*), 0.75µl dNTPs (*Promega*), 0.7µl primer 1522, 0.7µl primer 132, 0.28µl Taq polymerase [5U/µl], approximately 10ng (estimated by results of spectrophotometer) of template DNA and xµl dH_2O in a final volume of 25µl. The reactions were applied into strip tubes and were briefly centrifuged to bring all components to the bottom of the tube. Every approach contained a negative control (dH_2O) and a postive control (DNA of an isolate) prepared of the same reaction mix used for the samples. The positive control is supposed to show a clear band in case the PCR was performed correctly, wheres if the negative control shows any bands this was an indication for the contamination of any reactant or equipment being used.

3.2.6 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA is a fingerprint method used for microbial community analyses providing in insight in microbial diversity and community. This method bases on PCR products which were obtained by olignucleotide primers targeting to conserved regions between the 16S and 23S genes (IGS) of the bacterial rDNA Operon.

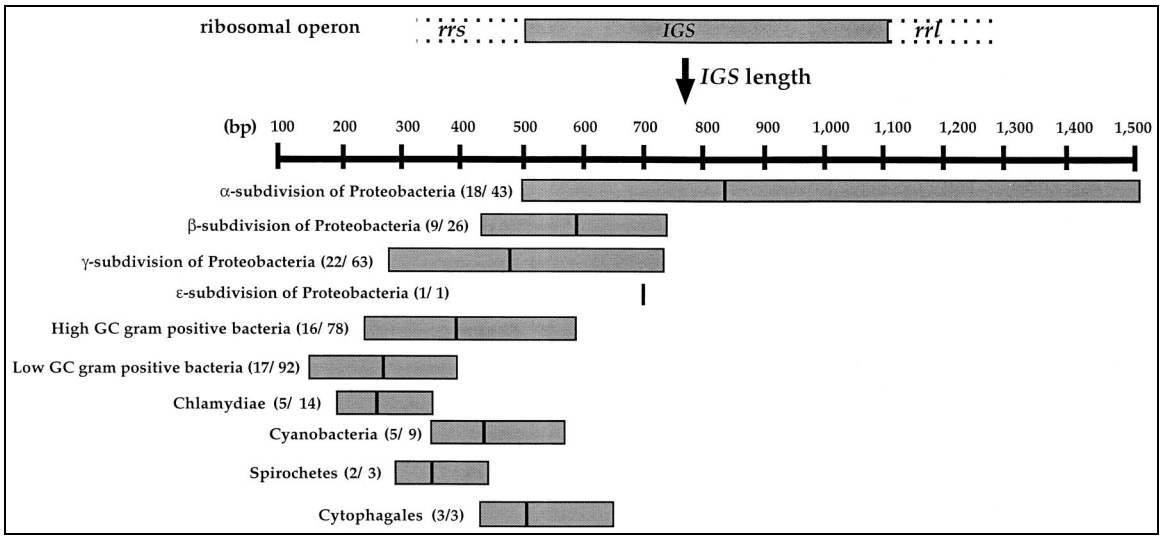


Fig. 3.2: Length distribution of bacterial IGSs between the rrs (16S rRNA) and rrl (23S rRNA) genes {Ranjard 2000a}.

It is known that the intergenic spacer encodes for tRNAs depending on the bacterial species. Moreover this region contains a significant heterogeneity in length among bacterial clades {Fisher 1999}. Taking advantage of the length polymorphism, the PCR product is seperated in a polyacrylamide gel to gain a complex band pattern of the bacterial community also known as a community fingerprint. In this community specific pattern, one band refers at least to one organism of the overall bacterial abundance of the sample. The resulting fingerprint is a densitometric record seen as a profile of peaks or bands, respectively.

The gel matrix contains 5.5% polyacrylamide. Polyacrylamide gels are generated in a vinyl addition polymerization of acrylamide and bis-acrylamide (N,N-methylene-

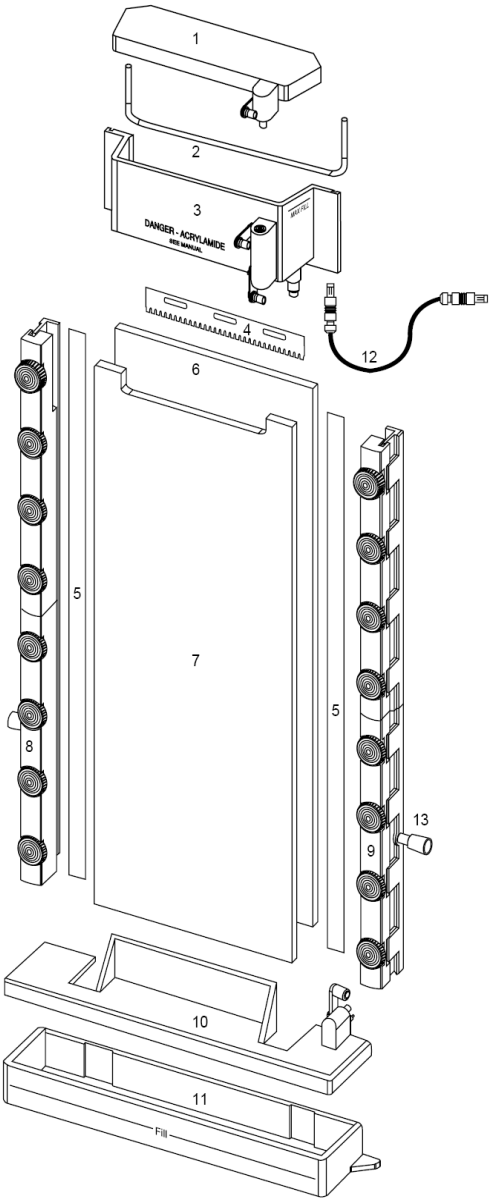
bisacrylamide) initiated by a free radical-generating system {Chrambach 1985}. APS provides a source of free radicals needed for polymerization of the gel {Sambrook 1989}. TEMED accelerates the rate of formation of free radicals from APS. The radicals convertacrylamide monomers to radicals which react with unactivated monomers to start the polymerization chain reaction {Shi 1998}. When acrylamide polymerizes, its monomers form a matrix in which the pore size depends on the concentration of acrylamide. The infrared labelled DNA fragments migrate through the gel matrix along an electric field and are detected by the scanning laser of the sequencer (*Li-cor IR² 4200 DNA Sequencer Analyzer Gene Reader*). The laser emits a wavelength of 700nm.

3.2.5.1 Gel Preparation

At first 35ml of the ready to use 5.5% gel matrix (*Li-Cor*) were stirred in a beaker at room temperature. Meanwhile the gel apparatus was assembled. The back and front glass plates as well as the two spacers (thickness: 0.25mm) were cleaned with dH_2O and isopropanol. After they were fixed by rail assemblies under a hood the apparatus was sloped by a reck to ensure that the gel matrix flows consistent from the top to the bottom in between the two glass plates. 23µl TEMED and 233µl of newly prepared APS were added to the gel matrix in the beaker. The matrix was ingested into a syringe which on which a filter (0.45µm) was screwed after. The syringe was placed on the top of the glass plates. The gel matrix was injected into the space between the glass plates avoiding the generation of any bubbles. They would affect the run of the samples. When the gel had reached the bottom, the plates were set horizontally for 2 hours to letting the gel polymerize completely.

After polymerization of the gel the apparatus was placed into the sequencer and the buffer tanks are filled with 1xTAE buffer(*Li-cor*). The sequencer is now ready for the

pre-run which lasts 30min. The pre-run is necessary to adjust the laser to the middle of the glas plates, thus ensuring ideal condition for the scanning process.



#	Description
1	Upper buffer tank lid
2	Silicone tubing gasket
3	Upper buffer tank
4	Comb
5	Spacers
6	Back plate
7	Front plate
8	Left rail assembly
9	Right rail assembly
10	Lower buffer tank lid
11	Lower buffer tank
12	High voltage cable
13	Rail support pin

Fig. 3.3: Exploded view and parts list of the gel apparatus (Li-Cor 4200 Series Sequencing Manual)

3.2.5.2 Sample Preparation and Sequencer Run

For an optimal performance within the gel 3µl of each sample were diluted with 6µl stopmix (*Li-cor*), denatured at 95°C for 2min and stored on ice for 10min before loading.

After the pre-run of the sequencer gel rests were removed from the wells by rinsing via a syringe. The comb was inserted 1 to 2mm into the edge of the gel to establish proper spaces for the loading of the samples. Subsequently 0.75µl of each sample-mix was loaded on the gel. The used size standard (*Li-cor*) covers fragments lengths from 50 to 1500bp and fluoresces at 700nm wavelength as well as the primers within the samples.

The run was performed at following conditions:

Voltage	1500V
Current	35,0mA
Power	31,5W
Temperature	50°C
Run-Time	720min

The gel image was saved using the Software *e-Seq*.

3.2.7 C/N Analysis

One subsample of each of the 75 sample positions was utilized to gain information about the carbon and nitrogen content of the sediments. The samples were freeze dried at 0.37mbar and -30°C for 2 days, pestled and stored in compartment drier until further processing. Approximately 30mg of sediment was loaded onto silver capsules. Inorganic carbon was removed from the samples by decrease of the pH value via addition of 30µl dH_2O and 10µl of a 6 molar solution of HCL. The samples were subsequently incubated in compartment drier for at least 4hours. The C/N

analysis was performed with the *vario MICRO cube* (*Elementar*). This machine automatically loads one sample after another into a combustion zone. The flue gas pass through a reburn zone and subsequently through a reduction zone. The analysis gases N_2 and CO_2 are separated via the carrier gas helium and column temperature programming (principle of gas chromatography) and quantitatively measured by a thermal conductivity detector (TCD). The TCD consists of two compartments forming a testing bridge. Through one of them flows the flue gas and through the other pure helium. The thermal conductivity of the carrying gas helium is sensitive to the addition of the analysis gases N_2 and CO_2 resulting in a disruption of the testing bridge and the generation of an electric signal. A connected PC determines the concentrations of N and C from the detected signal and dry weight of the samples.

3.2.8 Image Processing and Digitalization

BioNumerics offers a variety of tools for postprocessing electrophoretic fingerprints for instance for statistical analyses. Prior to the editing in *BioNumerics*, the digital gel image has been compressed to 20% in height and enlarged to 400% in width via *Corel Paint*. The image was inverted and cut at 50bp and 1500bp according to the size standard's bands.

After importing and editing the gel image in the software the lanes of each sample were defined. Those lanes of samples showing no band pattern were manually excluded and did not enter the database. The remaining lanes were normalized by aligning them with an ideal run of a reference size standard deposited in the database. Subsequently the sensitivity of the band-search algorithm in regard to the intensity of detectable profiles was adjusted to 3%. After the automated band-search, incorrect signals defining bands were deleted manually. Sections were defined which unified bands of similar size in a step known as binning. Based on this allocation a

binary table was generated. Bands which were not occurring in a sample were indicated with a "0" (not abundant in a sample). Bands occurring in a sample were binned into groups and indicated by a "1" (abundant in a sample). Most of the groups encompassed 3 to 5 bp numbers. With increasing bp size the numbers of groups bps increased. Hence, this table contains qualitative information about bacteria abundances. The groups are from now on defined as ***O****perational* ***T****axonomic* ***U****nits* (OUT).

3.2.9 Statistical Analysis

The dataset of this study encompasses the binary table, containing information about the abundances of OTUs at each of the 75 sample positions as well as the carbon and nitrogen contents at those positions. Marine bacteria are known for containing IGSs larger than 250bp {Ranjard 2000a}. For this reason only OUTs containing information about larger base pair sizes were taken into account here. The occurrence of bacterial community structures was investigated by uni- and multivariate approaches. The analysis of data addresses either only community patterns or environmental variables (univariate) or both with regard to their relationship (multivariate). For most approaches sample positions were clustered into the regions they belong to (either: reference, R1, dumping site or R2; see appendix Fig.6.1). The software used was STATISTICA (*StatSoft*) and Primer 5 (*Primer-E*).

3.2.9.1 Non-metric multi-dimensional scaling (nMDS)

The purpose of nMDS is to represent the samples as points in an ordination plot. Thus the relative distances apart of all points simulate the differences in between the various samples. Calculating an nMDS needs a resemblance matrix first, generated out of the binary table. This matrix contains the percentages of conformity between

all samples according to the extend to what their OTU patterns match. The higher the degree of matching is, meaning the more similar the community structures are, the closer they are located to each other in the 2-d ordination plot and vice versa. Plots can be arbitrarily rotated or reflected in any of the axes without changing the results, because in nMDS, the relative spacing of the samples on the two dimensional plane is meaningful, not their position in relation to the arbitrary axes. The axes are of the same value and nondimensional.

Distances between the samples according to the binary table were calculated with the Jaccard index, a qualitative method for calculating dissimilarities regarding presence or absence of OTUs. In this case the Jaccard similarity (S_J) is defined as follows:

$$S_J = 100 \cdot \frac{a}{a+b+c}$$

With:

a = number of OTUs present in both samples

b = number of OTUs present in sample x but absent in sample y

c = number of OTUs absent in sample x but present in sample y

Distances between samples according to carbon and nitrogen values were calculated with the Euclidean distance (D), an appropriate measure for environmental data:

$$D = \sqrt{\sum_i (y_{i1} - y_{i2})^2}$$

With: y_{i1} and y_{i2} resulting from normalisation

nMDS uses an algorithm which successively refines the positions of points until they satisfy, as closely as possible, the dissimilarity between samples {Clarke 2001}. The algorithm is an iterative one and it is not guaranteed that the first result is the best. For this reason several restarts are performed. Stress values represent the ability of the ordination to accurately capture the multidimensional similarity matrix in two

dimensions. Stress values range from 0 - 1 whereas stress values under 0.1 are considered to be excellent.

3.2.9.2 MVDISP

The MVDISP algorithm was used to quantify the variability in each group and to calculate the Index of Multivariate Dispersion (IMD). This comparative Index contrasts the average rank of the similarities among samples of a certain group with the average rank of other sample groups.

3.2.9.3 Analysis of similarities (ANOSIM)

The basic idea of ANOSIM is that similarities among samples of the same group should be greater than similarities between groups if the assigned groups are meaningful. This method produces an Global R, which indicates the magnitude of difference among groups of sample units and is calculated as follows:

$$R = \frac{\bar{r}_B - \bar{r}_W}{n(n-1)/4}$$

With:

$\bar{r}_B$ = mean ranked similarity between groups

$\bar{r}_W$ = mean ranked similarity within groups

n = total number of samples

The value of R can range from +1 (the most similar samples are within the same groups) to -1 (the most similar samples are all outside of the groups). A R of 0

indicates that high and low similarities are perfectly mixed and reveal no relationship to the group. 999 permutations were computed to assess the significance. At each permutation, a R is calculated for samples that are randomly assigned to groups. The ranked similarity within and between groups is then compared to the R which occurs at random to see if it is significantly different. If R is significantly different, one can conclude that there is evidence that samples within groups are more similar than would be expected by random chance.

3.2.9.4 BEST Analysis

In this case the function of BEST (**B**io-**E**nv + **St**epwise) is to find the "best" match between the multivariate among the various bacterial community patterns and that from the environmental variables (C and N values) associated with those samples. The extent to which these two patterns match reflects the degree to which the environmental variables "explain" the occurrence of bacterial community patterns. In order to assess how well the relationship between the variables can be described by means of a monotonous function, the Spearman's rank correlation coefficient (r_s) was calculated:

$$r_s = 1 - \frac{6\sum_{i=1}^{n} d_i^2}{n(n^2-1)}$$

With:

d_i = differences of ranks

n = total number of samples

All values are ranked before the coefficient is calculated. A Spearman correlation of "+1" or "-1" indicates that each of the variables is a perfect monotonous function of the other whereas a "0" indicates the nonexistence of correlation. Due to the numerous variables included in the overall data set, the BVSTEP method was chosen which carries out a forward-stepping and backward-elimination stepwise procedure to arrive at a possibly optimal set.

3.2.9.5 Analysis of variance (ANOVA)

ANOVA is an univariate analysis that corresponds to a single target variable. The target variables in this study are the environmental variables (C/N values). They are the dependent variables and the four regions of the sample site are the independent variables (categories). ANOVA compares the variances of the dependent variables within the groups/regions to the variances between groups/regions. If variances within groups are more different than variances among groups, then the groups are significantly different.

4 Results

4.1 Sample Collection

During the sample collection it has been observed that silt dominated the reference area, R1, the outward positions of the dumping site and parts of R2. Whereas sandy material was excavated at the dumping centre and near the estuary of the Elbe, especially at the northern positions of R2 which are located within the fairway. In general the silty sediment was distinguished by a rather thin aerobic layer, a sulphurous smell and a variety of vermicular species. The sandy material was characterized by a considerable occurrence of sea urchins and common razor clams (Ensis arcuatus). Within the dumping site a remarkably number of shells belonging to the freshwater species of zebra mussels (Dreissena polymorpha) were found. A detailed protocol of the sample collection can be found in the appendix (Tab. 6.2).

4.2 DNA Extraction

The results of the DNA extractions were documented on 0.8% agarose gels. Figure 4.1 is illustrating the genomic DNA for some samples exemplarily. For most of the samples a high-molecular band was present representing the genomic DNA. Smaller fragments of DNA shaped the darker area below this band. In general sandy samples contained less DNA as they showed weaker results on the gel.

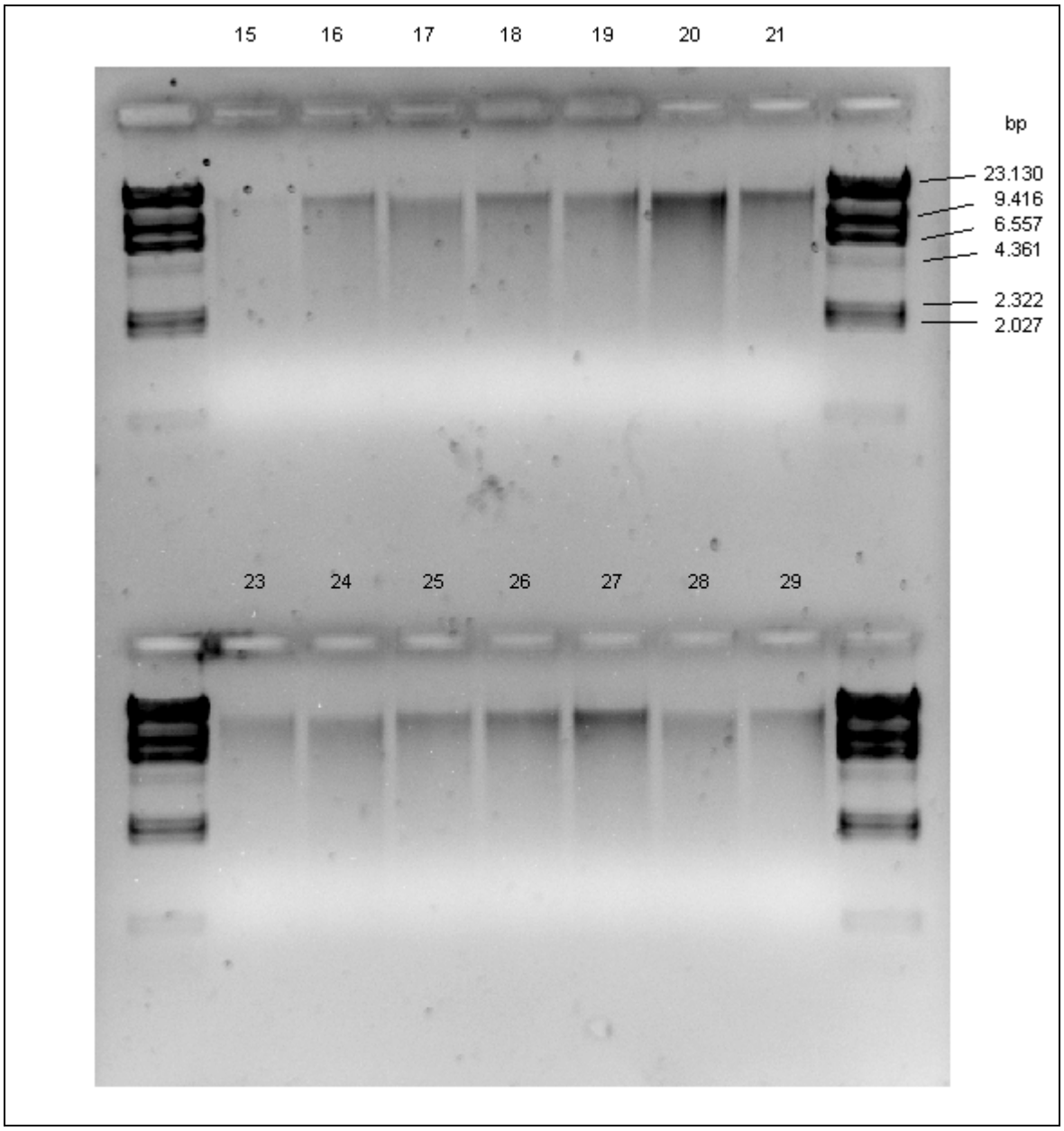


Fig. 4.1: Gel image of an electrophoresis performed at 100V for 60min showing the isolated genomic DNA of the samples 15 – 29. The gel contained 0.8% agarose. Base pair sizes of bands belonging to the marker (L1) are identified on the right side of the image.

Since sample number 15 yielded a very low DNA concentration, the DNA was extracted again from this sample as well as in other cases, too. Finally, DNA was extracted successfully from all 75 samples.

4.3 Intergenic spacer-PCR

The PCR method was performed as described in chapter 3.2.5. The PCR products visualized on a 1.4% agarose gel varied in terms of their intensity. The following image illustrates exemplarily the PCR products of the samples 1 – 6 originating from the reference area and show relatively intense results. Most of them contained DNA fragments between 500 and 1500 bp in length.

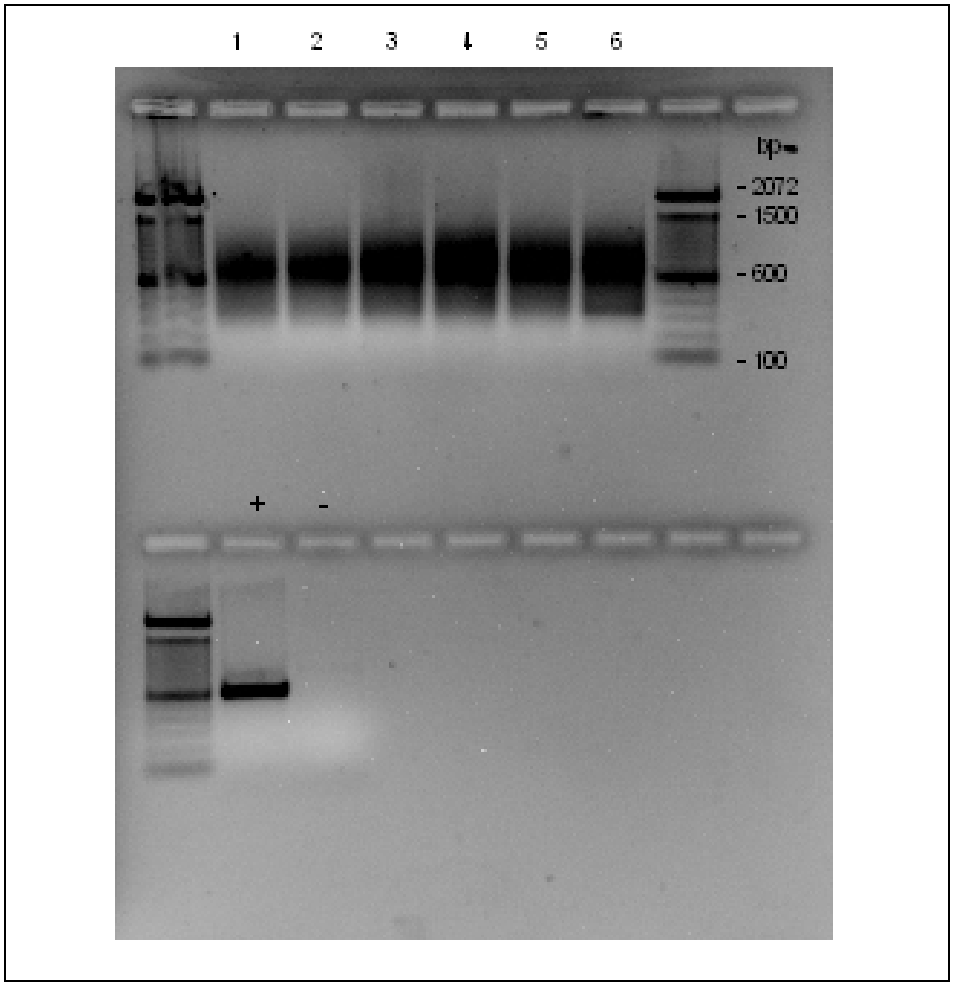


Fig. 4.2: Inverted image of PCR products of samples 1 – 6 on a 1,4% agarose gel with positive ("+") and negative("-") control. The base pair (bp) sizes of the marker (100bp, *Invitrogen*) are defined on the right side. Electrophoresis was performed at 100V lasting 60min.

The positive control ("+") shows a distinct band whereas the negative control ("-") apparently doesn't contain any DNA which indicates a proper run of PCR. With

intergenic spacer–PCR, DNA fragments of all 75 extracts were successively amplified.

4.4 ARISA Profiles

The following image exemplarily displays the fingerprints of samples 40 - 75. Samples 40 – 52 appear to be highly similar whereas the other samples show slightly shifted compositions.

The ARISA Profiles showed that most of the bacterial IGS fragments vary in length between 250 and 600 bp. Some bands seem to occur in all samples but exceptional bands and slightly different compositions occur as well among samples. However, in general only few differences can be pointed out. A couple of samples didn't show clear profiles on the first electrophoresis and therefore had to be repeated. Finally the complete set of fingerprints was available for further data analysis.

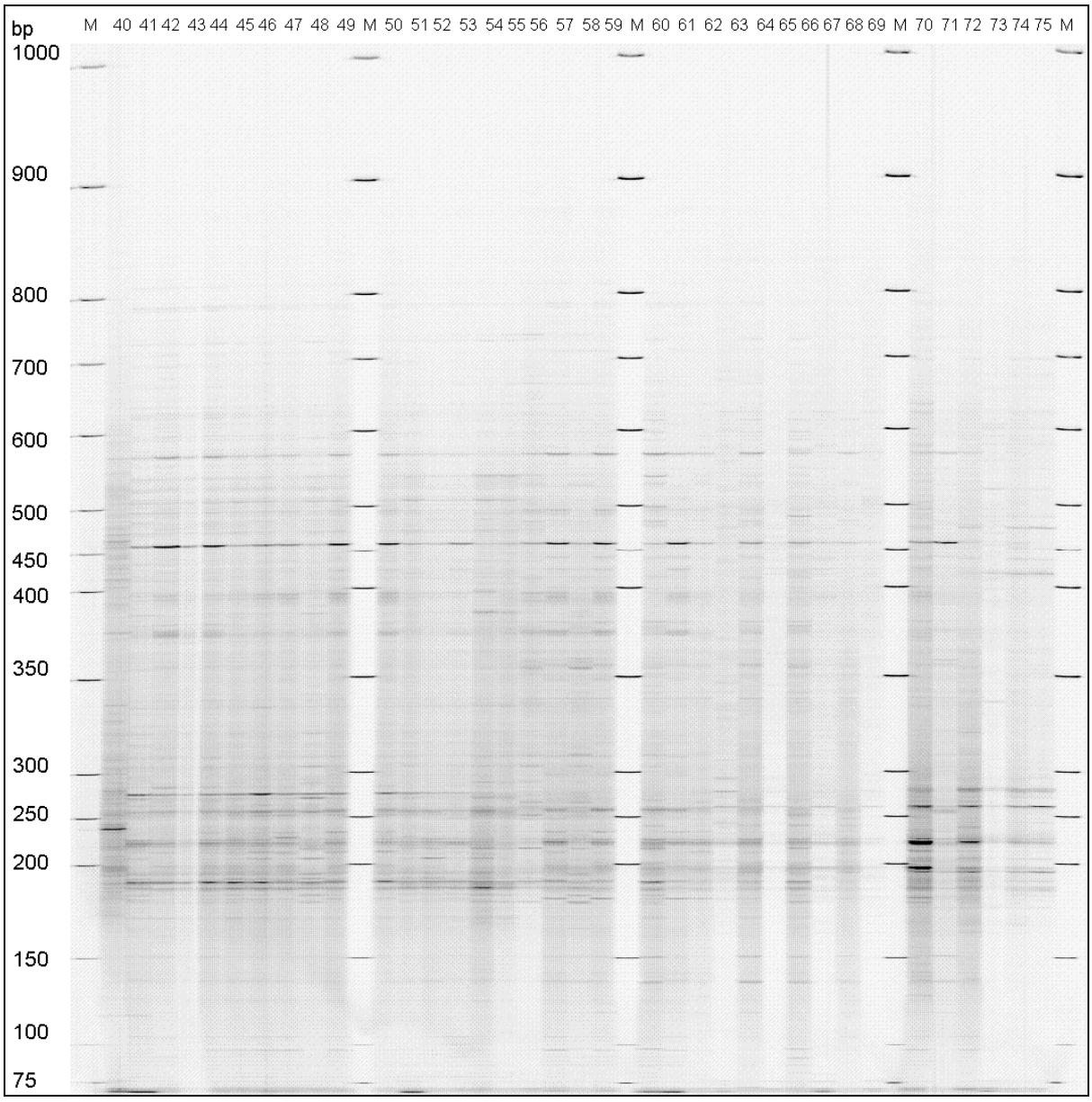


Fig. 4.3: Inverted image of an ARISA gel electrophoresis compressed to 20% in height and enlarged to 400% in width. Numbers on top represent the sample stations. The lanes of a marker are labelled by the letter "M". The numbers on the left are attributed to the bands of the marker, representing base pair sizes.

4.5 C/N Analysis

For silty sediment samples by approximation the percentages related to dry weight of nitrogen are 0.1% and for carbon 1.5% (Tab. 4.1). For sandy samples these values are considerably lower. Also the C/N ratios are similar among most silty samples but shows lower values in sandy samples except for sample 40 (centre of dumping site).

Tab. 4.1: Results of C/N analysis. Nitrogen and carbon percentages related to dry weight and C/N ratio of sediment samples 1 – 75 including sediment types.

Sample	Sediment type	N%	C%	C/N	Sample	Sediment type	N%	C%	C/N
1	silt	0,11810	1,37713	13,698	39	silt	0,10376	1,28806	14,588
2	silt	0,10566	1,39231	15,476	40	sand	0,02152	0,31938	17,794
3	silt	0,11357	1,59266	16,460	41	silt	0,10061	1,05668	11,970
4	silt	0,08913	1,78704	23,520	42	silt	0,09282	1,02063	12,535
5	silt	0,10741	1,64963	18,023	43	silt	0,11836	1,61247	15,486
6	silt	0,10554	1,51274	16,828	44	silt	0,07715	1,12513	16,618
7	silt	0,10169	1,39247	16,088	45	silt	0,07460	0,92747	14,185
8	silt	0,11177	1,64695	17,292	46	silt	0,05922	0,74238	14,330
9	silt	0,11919	1,51880	14,961	47	silt	0,05976	0,95787	18,286
10	silt	0,10070	1,30837	15,264	48	silt	0,07716	0,97580	14,425
11	silt	0,11495	1,52600	15,586	49	silt	0,06689	0,86895	14,833
12	silt	0,10526	1,26681	14,146	50	silt	0,05921	0,69504	13,430
13	silt	0,09810	1,22191	14,641	51	silt	0,07183	0,88937	14,131
14	silt	0,12511	1,49480	14,030	52	silt	0,07670	1,11541	16,568
15	silt	0,09048	1,23629	16,059	53	silt	0,05408	1,00633	21,219
16	silt	0,10951	1,73744	18,614	54	silt	0,09133	1,02571	12,799
17	silt	0,09916	1,31078	15,531	55	silt	0,08252	1,11930	15,452
18	silt	0,11884	1,61250	15,923	56	silt	0,12665	1,59335	14,300
19	silt	0,12398	1,47214	13,942	57	silt	0,03746	0,40949	12,603
20	silt	0,12028	1,62145	15,819	58	silt	0,13864	1,42041	11,652
21	silt	0,10564	1,26576	14,079	59	silt	0,04987	0,51032	11,754
22	silt	0,12257	1,56927	15,027	60	silt	0,09859	0,89265	10,330
23	silt	0,12423	1,72663	16,306	61	silt	0,04005	0,40861	11,762
24	silt	0,12528	1,70232	15,942	62	sand	0,02854	0,22292	9,154
25	silt	0,12302	1,64345	15,676	63	sand	0,00969	0,04278	5,758
26	silt	0,12069	1,69156	16,445	64	silt	0,09822	0,80521	9,362
27	silt	0,11187	1,36256	14,308	65	sand	0,00743	0,05296	7,779
28	silt	0,13789	1,56796	13,346	66	silt	0,09160	0,82935	10,336
29	silt	0,12597	1,75476	16,343	67	sand	0,00197	0,06337	13,024
30	silt	0,12830	1,48185	13,561	68	sand	0,06756	0,59431	10,086
31	silt	0,13431	1,83557	16,032	69	sand	0,00555	0,04796	7,995
32	silt	0,11739	1,49111	14,916	70	sand	0,01312	0,09677	9,065
33	silt	0,12521	1,69123	15,851	71	sand	0,05555	0,45115	9,349
34	silt	0,12847	1,67226	15,275	72	sand	0,01028	0,06489	8,036
35	silt	0,12618	1,81026	16,832	73	sand	0,01107	0,03677	4,568
36	silt	0,09988	1,11355	13,112	74	sand	0,01863	0,09614	6,328
37	silt	0,08639	1,14399	15,576	75	sand	0,01232	0,05271	5,611
38	silt	0,15205	2,01785	15,561					

As one might estimate from the marginally fluctuating C/N ratios, an existing correlation between both values can be shown when plotted against each other (Fig. 4.4). The coefficient of determination is close to 1 ($r^2 = 0.91$) which implies the probability of a linear relationship.

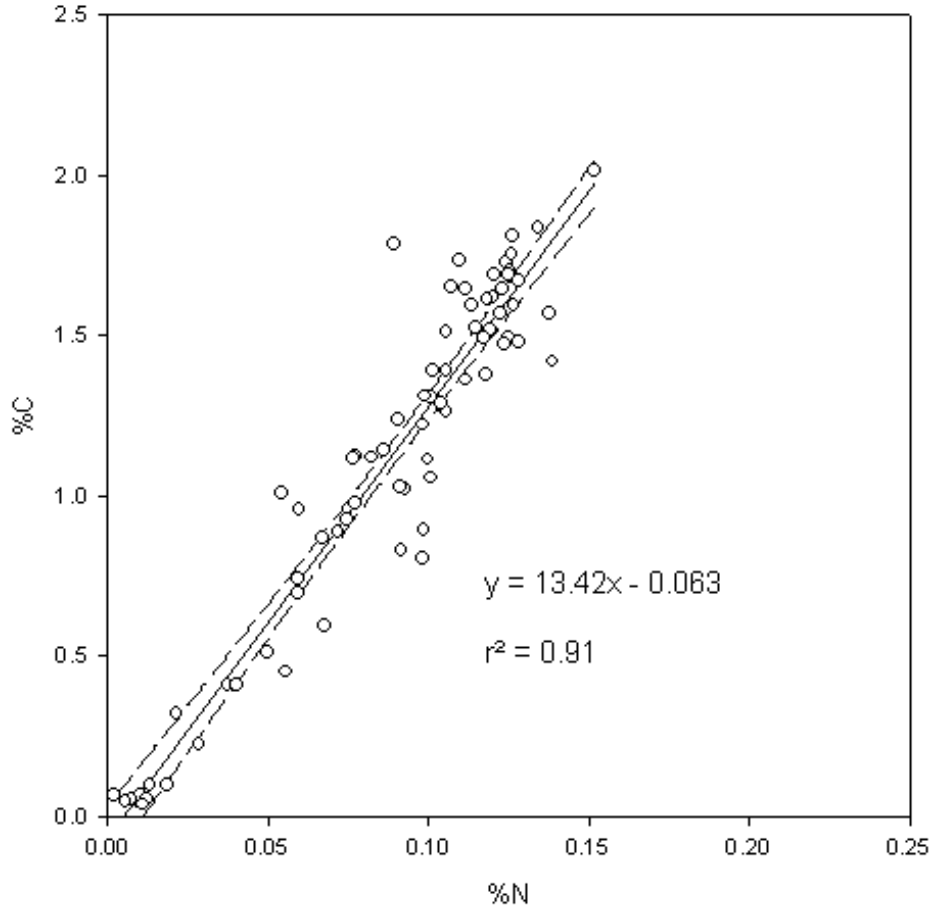


Fig. 4.4: Carbon contents plotted against Nitrogen contents. Contents are expressed as percentage related to dry weight of sediment. Additionally the equation of regression line and the coefficient of determination (r^2) are represented.

4.6 Statistical Analysis

For a statistic analysis the bacterial community fingerprint data (biota) from ARISA was converted into resemblance matrix. In the following chapters this data and the

data of C and N values (environmental) will be examined independently and in relation with each other.

4.6.1 Analysis of Biota Data

In the following nMDS plot similarities among samples are expressed by a distance matrix. Each dot corresponds to a sample position. The dots are coloured according to the region they belong to.

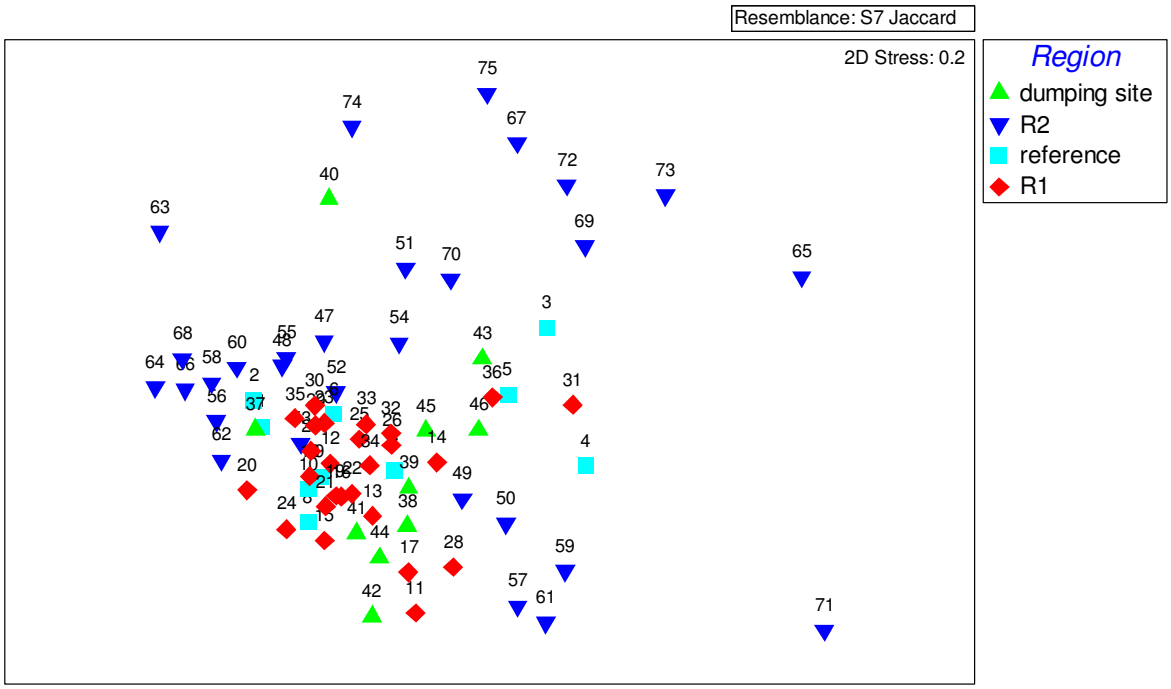


Fig. 4.5: nMDS of ARISA fingerprints for samples 1 – 75. Similarities were calculated according to Jaccard Index.

From Figure 4.5 it can be evaluated that it is a rather disordered arrangement and the stress value of 0.2 is relatively high. However, samples belonging to R2 differ from the other groups but show only few similarities among each other as well. Sample number 40 which is located in the centre of the dumping site is arranged aloof from samples belonging to the same group. Samples of R1 are considerably grouped with few exceptions.

The data was further investigated using the MVDISP algorithm (Tab.4.2). The highest variability was found in the samples of R2 while the lowest variability occurred in the samples of R1.

Tab. 4.2: MVDISP of community structures according to regions.

Factor	Dispersion
R1	0.576
Reference	0.786
Dumping site	1.011
R2	1.362

Regarding the Indices of Multivariate Dispersion (IMDs) of pairwise comparisons between groups a negative value for the comparison between the dumping site and R2 (Tab. 4.3) is noticeable. This implies that only in this case similarities among samples of the same group are greater than similarities of samples between different groups.

Tab. 4.3: Indices of Multivariate Dispersion. Pairwise comparisons between sample similarities of regions

Factors	IMD
Dumping site, R2	- 0.449
Dumping site, reference	0.212
Dumping site, R1	0.516
R2, reference	0.583
R2, R1	0.773
Reference, R1	0.215

Based on the OTUs the following box plot was created (Fig. 4.6). It shows the OTU-richness valus of samples for each region as box and whisker plots. A box plot is a summary plot that plots graph data as a box representing statistical values. The boundary of the box closest to zero indicates the 25th percentile, a line within the box

marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. The average richness and especially the range of richness values that occur among samples of the dumping site are remarkably low. The greatest variability in OUT-richness is found among samples of R2.

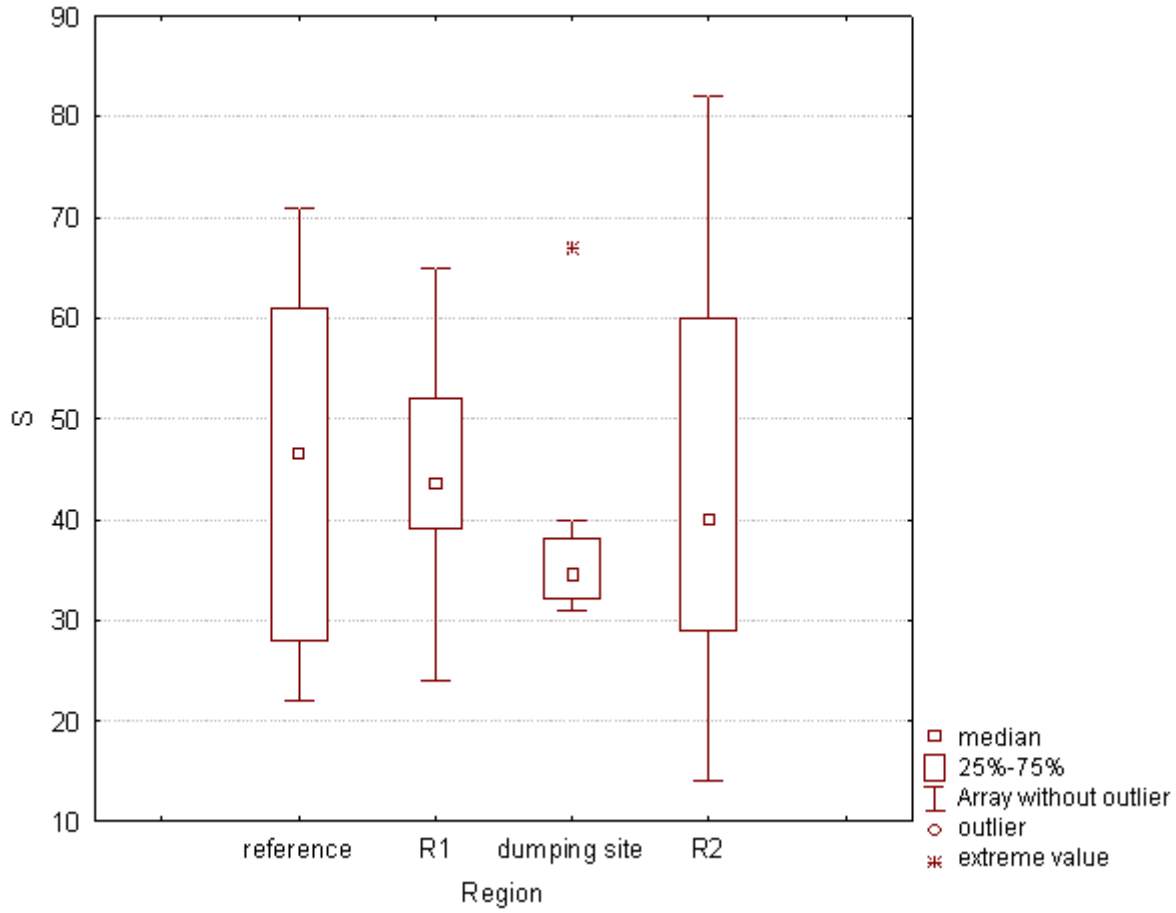


Fig. 4.6: Box plot of species richness (S). Samples are classified into regions.

4.6.2 Analysis of Environmental Data

In figures 4.7 and 4.8 the values of nitrogen and accordingly carbon contents measured within the four regions are illustrated via box plots. The reference area, R1 and dumping site show similar nitrogen and carbon contents whereas R2 shows lower contents in general and a higher variance. The discrimination of R2 to the other regions referring to carbon and nitrogen values is underlined by a post hoc tests, namely the Unequal N HSD test (Tab.4.4 and 4.5).

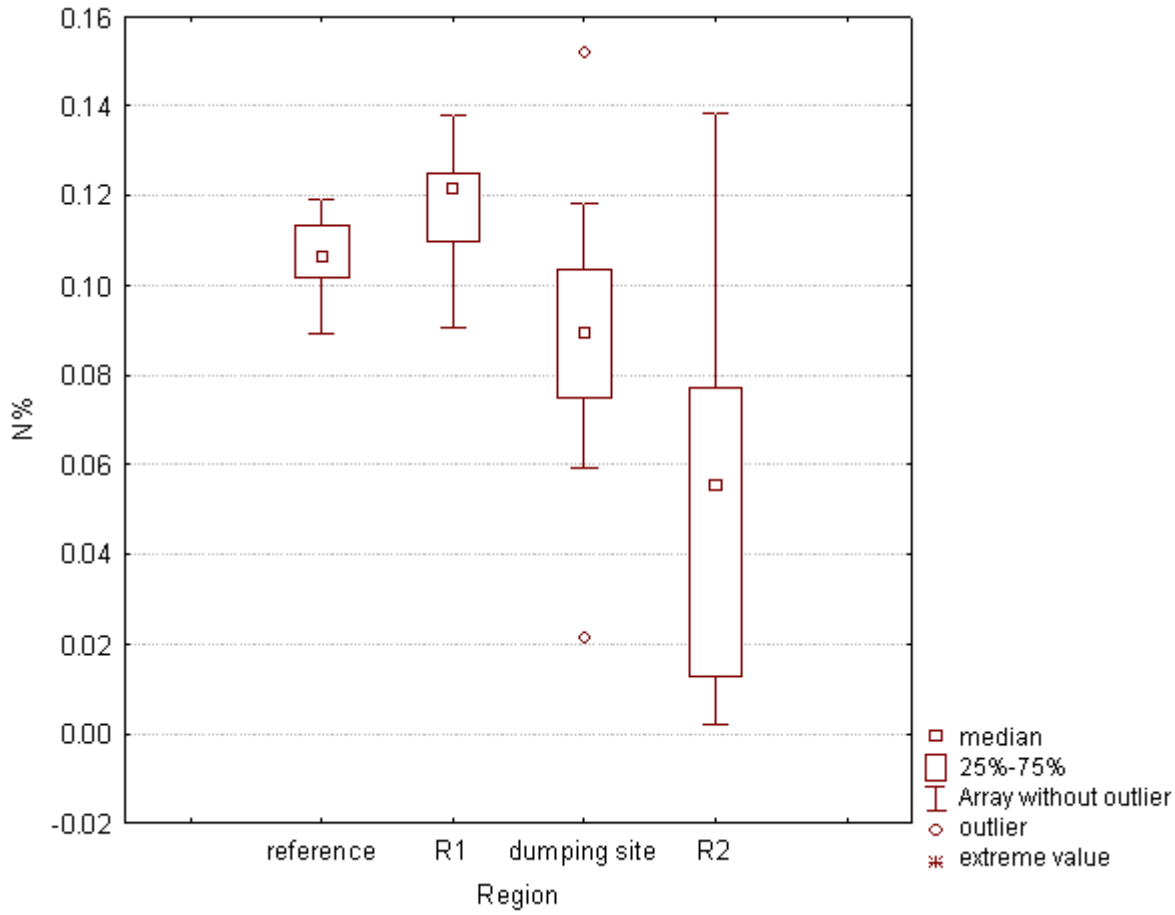


Fig. 4.7: Box plot of measured nitrogen contents classified into regions. The nitrogen content is expressed as percentage related to dry weight of sediment.

Tab. 4.4: Unequal N HSD Test. Determination of significant differences in group means of nitrogen contents. Significant differences are coloured red.

Region	reference	R1	dumping site	R2
reference		0.9465	0.5821	0.0009
R1	0.9465		0.2710	0.0001
dumping site	0.5821	0.2710		0.0381
R2	0.0009	0.0001	0.0381	

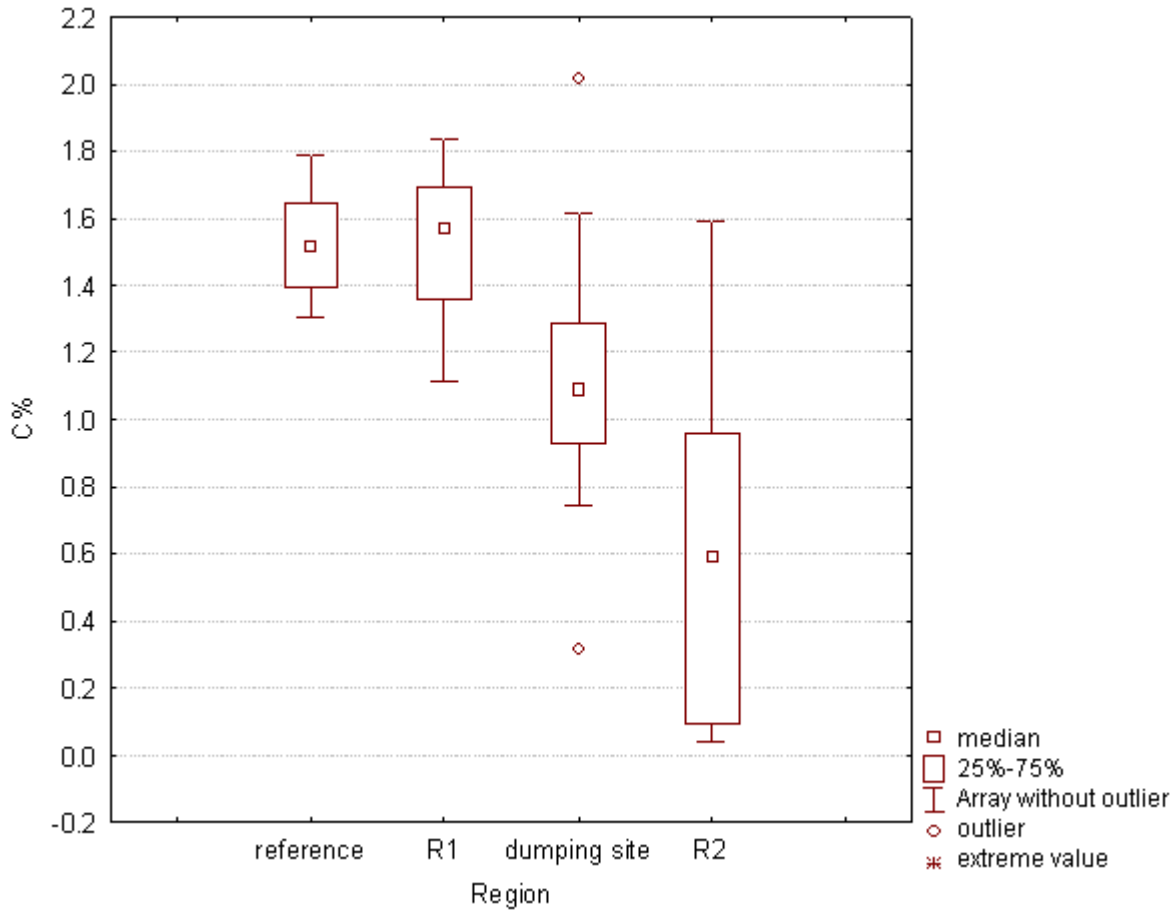


Fig. 4.8: Box plot of measured carbon contents classified into regions. The carbon content is expressed as percentage related to dry weight of sediment.

Tab. 4.5: Unequal N HSD Test. Determination of significant differences in group means of carbon contents. Significant differences are coloured red.

Region	reference	R1	dumping site	R2
reference		0.9999	0.2807	0.0001

R1	0.9999		0.2583	0.0001
dumping site	0.2807	0.2583		0.0102
R2	0.0001	0.0001	0.0102	

The following nMDS shows similarities among sample positions in terms of carbon and nitrogen values using the Euclidean distance. As the carbon and nitrogen values are not on comparable ranges they were normalized first.

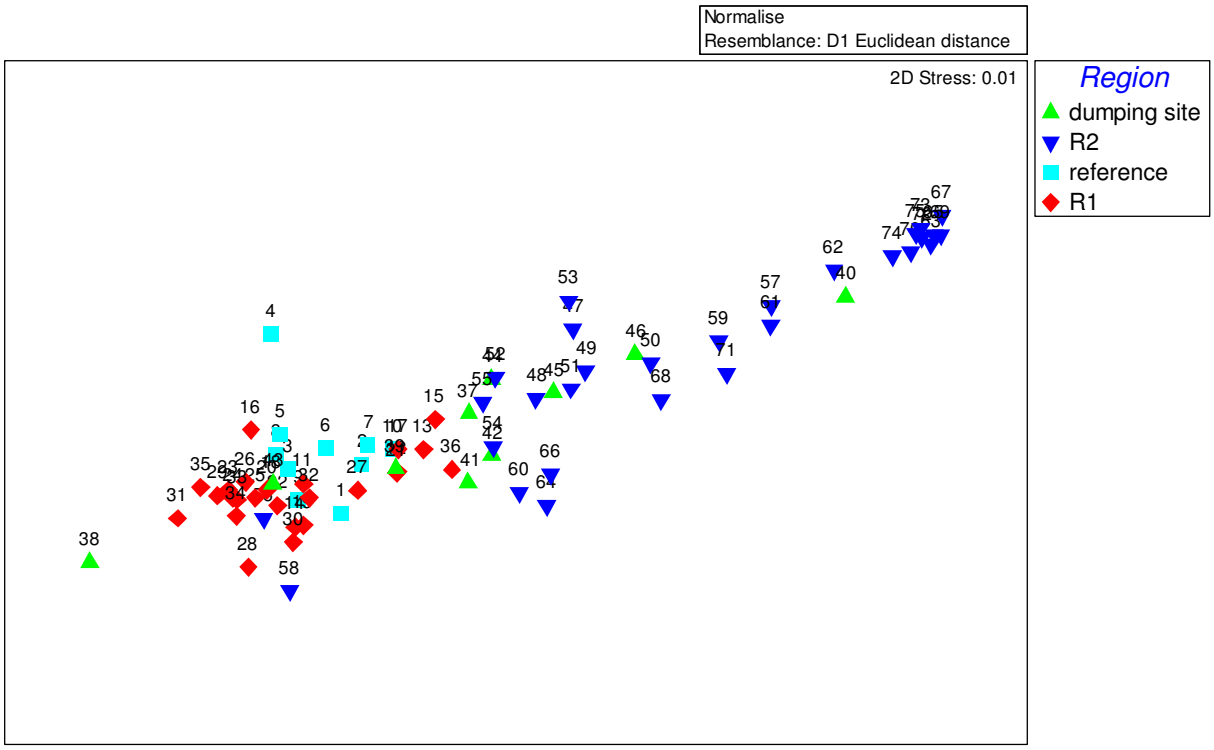


Fig. 4.9: NMDS of sample positions in regard to normalized carbon and nitrogen values using Euclidean distance.

Along this carbon and nitrogen gradient (decreasing from bottom left to top right) a nearly hierarchic occurrence of regions can be pointed out except for parts of R1 and the dumping side which spreads over the entire range. This reveals as well as the results shown in figures 11 and 12 that a distinguishable difference among the regions in terms of carbon and nitrogen contents does exist.

4.6.3 Interrelationship of Environmental and Biota Data

Finally the Global BEST match test was carried out in order to investigate to which extend the C and N values "explain" the occurrence of bacterial community patterns.

Tab. 4.6: Results of Global BEST Match Test for environmental (C/N values) and biota (community fingerprints) data.

Sample statistic (Rho)			0.554
Significance level of sample statistic			0.1%
Number of permutations			999 (Random sample)
Number of permuted statistics greater than or equal to Rho			0
Best results			
Multiple	No.Vars	Corr.	Selections
1	2	0.554	All

The outcome of the Global BEST Match Test is a positive correlation (Corr. = 0.554) between the C/N values and the similarities among bacterial community fingerprints.

5 Discussion

Results must always be considered in regard to the limitations which are inevitably involved in the methods being used. The following chapter will address these limitations first and will subsequently interpret the results.

5.1 Methods

5.1.1 DNA Extractions

In general the isolation of bacterial DNA is difficult to obtain for marine sediments. This is due to the large variety in physical and chemical parameters (e.g. silty or sandy consistencies, high amounts of humic acids) which exists among sample stations. To obtain optimal results different isolation kits for DNA isolation from sediment samples were tested in a former study. The kit producing the most reliable, clean and intact DNA fragments were used in this study.

The cell lyses step is critical in DNA extractions. Results have shown that the amounts of extracted genomic DNA were related to the grain size of the samples, thus sandy samples contained less DNA. Eventually this is the result of solid sand particles colliding with genomic DNA during the process of shaking. This might cause damages of DNA double strands, breaking into small fragments. This may ultimately affect the results of the PCR for the ARISA fingerprints as large fragments can't be amplified anymore and are thereby excluded from the analysis. For sandy

samples the duration of this extraction step was therefore shortened from 10 to 5min but a noticeable improvement of the results couldn't be observed anyway.

5.1.2 Intergenic spacer-PCR

The PCR products visualized on agarose gels varied in terms of their intensity. It was aspired to provide an equal template of 10ng DNA for each sample which may indicate that other factors such as a possible inhibition of the PCR process are responsible. Residual substances which may cause such an inhibition may for example be humic acids.

The number of PCR cycles have been set to 30 in this study. Other studies show that ARISA patterns were similar between PCRs performed with varying numbers of amplification cycles {Fisher 1999}.

5.1.3 ARISA

The results obtained with ARISA should be cautiously interpreted. As a molecular technique that relies upon total community DNA extraction and PCR amplification, ARISA involves the usual systematic biases of these two procedures. Additional specific biases are associated with the amplification of the 16S-23S IGS. On the one hand ARISA fingerprints may underestimate diversity because strains within species belonging to different phyla often have identical spacer length as shown by several authors {Aubel 1997, Garcia-Martinez 1996, Jensen 1993}. On the other hand depending on the species not only one rRNA operon may be present in the bacterial genomes, but several copies. These copies may also contain length heterogeneity leading to more than one signal for a single organism in the community profile {Fisher 1999}. As this method uses electrophoresis to separate nucleic acid fragments on the basis of size this is also a limitation because imprecise determination of

fragment sizes reduces confidence in the estimates of sequence diversity. In several cases it is difficult to distinguish sample fragments from background fluorescence. Although an algorithm is used to detect the peaks, there is still a level of uncertainty involved. In samples which showed PCR products of relatively low intensity, diversity may be underestimated because small peaks don't exceed the threshold of detectability.

The IGS shows a length polymorphism among bacterial clades (Fig. 3.2). which allows estimates of diversity of community structures. However there are several studies showing that also closely related clades possess a great variability in IGS length. Meanwhile not related clades may possess IGSs of identical length {Fisher 1999}. Therefore it is not possible to make any assessment about the taxonomic composition of a bacterial community via ARISA. However this may change in the future as there are already some IGS sequences (mainly of medically important organisms) available in the GenBank database.

5.1.4 Non-metric Multi-dimensional Scaling

The outcome of an nMDS is dependant on the similarity index which is used to calculate distances. In this study the Jaccard index was chosen because the binary biota data required a qualitative analysis. If for example community structures were analyzed quantitatively, the Bray-Curtis-Index would be more suitable. Distances among environmental data were calculated quantitatively with the Euclidean Distance. This approach compares distances directly. As C values were about 10 times higher than N values, they had to be normalized first. The choice of similarity indices is therefore strongly dependant on how data is present.

The stress value for biota data (0.2) was relatively high in the 2D – ordination plot which indicates that the information of the similarity matrix couldn't be captured

accurately. Lower stress values can be generated by increasing the dimensions of the plot but it this makes the result more difficult to visualize properly in 2-D.

5.2 Results

Compared to investigations of bacterial communities by the Biologische Anstalt Helgoland in August 2009 the community patterns of this study showed relatively few differences among each other. Also the intensities of peaks on ARISA gels were lower in this study compared to former investigations in August 2009. According to {Billen 1990} seasonal variations of microbial benthic activities exist. Peaks of activity occur during and at the end of spring blooms due to increasing deposition of fresh planktonic material {Graf 1982}. Sample collection for this study took place in March, before the spring phytoplankton bloom occurred. This time of the year may be characterized by low bacterial activity due to few amounts of deposited material and low water temperature. At this point further investigations are needed to reveal how the community patterns are affected by seasonality and temperature.

Investigations on the regional differences in OTU-richness of samples revealed the lowest values and variance for the dumping site. Being conscious about the contamination of the dumped sediment, this may support the hypothesis that toxic chemical exposures tend to narrow the spectrum of microbial diversity. Bacteria that can't resist the toxic effects either die or enter a static metabolic phase, leaving those that have evolved resistance metabolisms to proliferate and become dominant members of the impacted ecosystem {Ogunseitan 2005}.

Results have shown an obvious relationship between the carbon and nitrogen contents and the consistence of marine sediments (either silty or sandy). The existence of a significant correlation between organic matter and mud content

(particles smaller than 50μm) in sediments were also shown by investigations of {Creutzberg 1984}. Organic matter has a high affinity for fine-grained sediment because it absorbs to mineral surfaces {Hedges 1995}. The simultaneously observed lower abundances of bacteria in sandy sediments could therefore not only be a result of nutrient shortage but also of smaller particle surfaces available for microbial fixation as well {Billen 1990}. Thus, the results of this study show how strong the bacterial diversity in sediments is affected by hydrological conditions which either favour the deposition of fine particles (possibly: reference, R1, dumping site) or lead to sandy sediments (R2 near Elbe estuary) due to high currents. According to {Creutzberg 1979} the settling of mud is restricted to areas where maximum surface current velocities do not exceed a critical value of about 15cm/sec at 15cm above the bottom. However it shall be mentioned here that the simple settling process is not the only way that organic particles reach the benthos. Furthermore, among others, a mechanism occurs especially in sandy sediments, which involves transient surface deposition during periods of slack current, followed by burial to at least 5cm through the process of ripple formation during ebb current periods {Billen 1990}. Therefore a point of further investigation to this study site could be the measurement of prevailing currents near the bottom.

The hypothesis that carbon and nitrogen are highly correlated with each other is supported by investigations of {Anderson 1981}. The variances of carbon and nitrogen contents showed significant differences among the regions. This has to be interpreted with care because the regions are not of the same size. R2 shows the highest dispersion of values but encompasses also the biggest area. A measure which would implement a more reasonable illustration of the values would be the exposure of the values related to their distances from the Elbe estuary for example.

The C/N values were positively correlated with the similarity among bacterial community patterns. A positive correlation implies that an increase of the independent variable leads to an increase of the dependent variable as well. In this

case it means that higher similarities in terms of bacterial community structures are found among samples with high C/N values than among samples with low C/N values.

6 Appendix

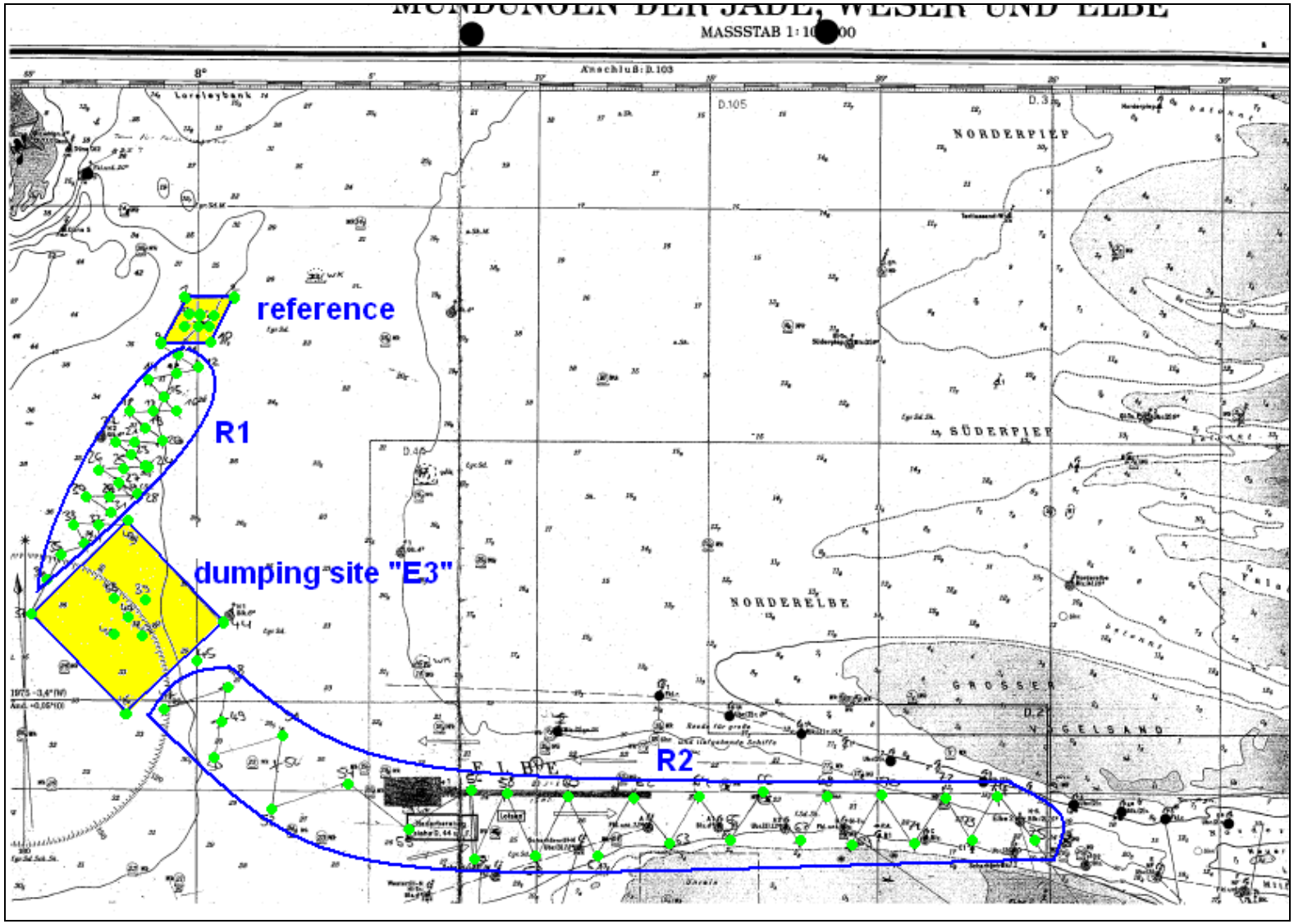


Fig. 6.1: Nautical chart illustrating the course of the research vessel Uthörn in March 2010 with each green dot representing one of the 75 sampling stations

Tab.6.1: Metabolic types of prokaryotes {Nealson 1997 #7}.

General type	Carbon source	Energy source	Electron donor	Electron acceptor
Heterotroph	Organic C	Organic C		
Aerobes			Organic C	O_2
Denitrifiers			Organic C	NO_3^-
Mn reducers			Organic C	Mn (IV)
Fe reducers			Organic C	Fe (III)
SRBs			Organic C	$SO_4^=$
Sulfur reducers			Organic C	S°
Methanogens			Organic C/H_2	CO_2
Syntrophs			Organic C	Organic C
Acetogens			Organic C/H_2	CO_2
Fermentors			Organic C	Organic C
Phototroph	CO_2	Light		
Cyanobacteria			H_2O	
Photosynthetic			S compounds,	
bacteria			H_2, Organic C	
Lithotroph	CO_2/organic C	Inorganics		
H_2oxidizers			H_2	O_2, NO_3^-, Mn (IV), Fe(III), $SO_4^=$, CO_2
Fe oxidizers			Fe (II)	O_2, NO_3^-
S oxidizers			H_2S, S°, $S_2O_3^=$	O_2, NO_3^-
N oxidizers			NH_3, NO_2^-	O_2
CH_4 oxidizers			CH_4	O_2

1. To the **PowerBead Tubes** provided, add 0.25 grams of soil sample.

   What's happening: After your sample has been loaded into the PowerBead Tube, the next step is a homogenization and lysis procedure. The PowerBead Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids and (c) protect nucleic acids from degradation.

2. Gently vortex to mix.

   What's happening: Gentle vortexing mixes the components in the PowerBead Tube and begins to disperse the sample in the PowerBead Solution.

3. **Check Solution C1**. If **Solution C1** is precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   What's happening: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

4. Add 60 µl of **Solution C1** and invert several times or vortex briefly.

5. Secure **PowerBead Tubes horizontally** using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. **Note:** If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.

   Note: *The vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-4 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.*

   What's happening: The MO BIO Vortex Adapter is designed to be a simple platform to facilitate keeping the tubes tightly attached to the vortex. It should be noted that although you can attach tubes with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower yields. Therefore, the use of the MO BIO Vortex Adapter is a highly recommended and cost effective way to obtain maximum DNA yields.

6. Make sure the **PowerBead Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.

7. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).

   Note: *Expect between 400 to 500 µl of supernatant at this step. The exact recovered volume depends on the absorbancy of your starting material and is not critical for the procedure to be effective. The supernatant may be dark in appearance and still contain some soil particles. The presence of carry over soil or a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry over soil and coloration of the mixture.*

Fig. 6.2: MO-BIO User Protocol for DNA Extractions Part 1

8. Add 250 µl of **Solution C2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

   What's happening: Solution C2 is patented Inhibitor Removal Technology® (IRT). It contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

10. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean **2 ml Collection Tube** (provided).

    What's happening: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

11. Add 200 µl of **Solution C3** and vortex briefly. Incubate at 4°C for 5 minutes.

    What's happening: Solution C3 is patented Inhibitor Removal Technology® (IRT) and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

13. Transfer up to 750 µl of supernatant to a clean **2 ml Collection Tube** (provided).

    What's happening: The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

14. Shake to mix Solution C4 before use. Add 1.2 ml of **Solution C4** to the supernatant (be careful solution doesn't exceed rim of tube) and vortex for 5 seconds.

    What's happening: Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

15. Load approximately 675 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature.
    Note: A total of three loads for each sample processed are required.

    What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

16. Add 500 µl of **Solution C5** and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

    What's happening: Solution C5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

Fig. 6.3: MO-BIO User Protocol for DNA Extractions Part 2

17. Discard the flow through from the **2 ml Collection Tube**.

    What's happening: This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

18. Centrifuge at room temperature for 1 minute at 10,000 x *g*.

    What's happening: This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

19. Carefully place Spin Filter in a clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution C5** onto the **Spin Filter**.
    Note: *It is important to avoid any traces of the ethanol based wash solution.*

20. Add 100 µl of **Solution C6** to the center of the white filter membrane.

    Note: *Placing the Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris) which lacks salt.*

    Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10). Solution C6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

22. Discard the **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20° to -80°C). **Solution C6** does not contain any EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

Fig. 6.4: MO-BIO User Protocol for DNA Extractions Part 3

Tab. 6.2: Protocol of the sample collection in March 2010. Position, time and properties of the sediment are displayed for each sample position.

Sample	Latitude	Longitude	Date	time (hh:mm)	Sediment	Comments
1	54° 8.4000' N	7° 59.5000' E	23.03.2010	08:42	silt	
2	54°8.4000' N	8°0.8000' E	23.03.2010	08:55	silt	distinst aerobic layer (stench of sulfur)
3	54°8.2000' N	7°59.8000' E	23.03.2010	09:00	silt	
4	54°8.2000' N	8°0.0600' E	23.03.2010	09:05	silt	
5	54°8.2000' N	8°0.5000' E	23.03.2010	09:12	silt	
6	54°7.9000' N	7°59.5000' E	23.03.2010	09:25	silt	
7	54°7.9000' N	7°59.9000' E	23.03.2010	09:35	silt	half aerobic / half anaerobic
8	54°7.9000' N	8°0.2000' E	23.03.2010	09:40	silt	
9	54°7.7000' N	7°59.0000' E	23.03.2010	09:50	silt	half aerobic / half anaerobic
10	54°7.7000' N	8°0.4000' E	23.03.2010	10:00	silt	
11	54°7.4000' N	7°59.5000' E	23.03.2010	10:10	silt	
12	54°7.1000' N	8°0.0000' E	23.03.2010	10:20	silt	

13	54°7.0000' N	7°59.4000' E	23.03.2010	10:30	silt	
14	54°7.0000' N	7°58.6000' E	23.03.2010	10:35	silt	
15	54°6.6000' N	7°59.0000' E	23.03.2010	10:40	silt	comapct consistency, aerobic layer hardly noticable
16	54°6.4000' N	7°59.3000' E	23.03.2010	10:45	silt	comapct consistency, aerobic layer hardly noticable
17	54°6.4000' N	7°58.7000' E	23.03.2010	10:55	silt	comapct consistency, aerobic layer hardly noticable
18	54°6.4000' N	7°58.0000' E	23.03.2010	11:03	silt	comapct consistency, aerobic layer hardly noticable
19	54°6.1000' N	7°58.4000' E	23.03.2010	11:10	silt	comapct consistency, aerobic layer hardly noticable
20	54°5.9000' N	7°59.0000' E	23.03.2010	11:20	silt	comapct consistency, aerobic layer hardly noticable
21	54°5.9000' N	7°58.1000' E	23.03.2010	11:25	silt	comapct consistency, aerobic layer hardly noticable
22	54°6.0000' N	7°57.5000' E	23.03.2010	11:30	silt	comapct consistency, aerobic layer hardly noticable
23	54°5.8000' N	7°58.0000' E	23.03.2010	12:15	silt	comapct consistency, aerobic layer hardly noticable
24	54°5.6000' N	7°58.6000' E	23.03.2010	12:20	silt	comapct consistency, aerobic layer hardly noticable
25	54°5.5000' N	7°57.9000' E	23.03.2010	12:30	silt	comapct consistency, aerobic layer hardly noticable
26	54°5.4000' N	7°57.0000' E	23.03.2010	12:40	silt	very comapct consistency, aerobic layer hardly noticable

27	54°5.3000' N	7°57.5000' E	23.03.2010	12:45	silt	comapct consistency, aerobic layer hardly noticable
28	54°5.1000' N	7°58.3000' E	23.03.2010	12:55	silt	comapct consistency, aerobic layer hardly noticable
29	54°5.0000' N	7°57.5000' E	23.03.2010	13:00	silt	comapct consistency, aerobic layer hardly noticable
30	54°5.0000' N	7°56.8000' E	23.03.2010	13:05	silt	comapct consistency, aerobic layer hardly noticable
31	54°4.8000' N	7°57.4000' E	23.03.2010	13:15	silt	comapct consistency, aerobic layer hardly noticable
32	54°4.6000' N	7°57.0000' E	23.03.2010	13:25	silt	comapct consistency, aerobic layer hardly noticable
33	54°4.5000' N	7°56.3000' E	23.03.2010	13:40	silt	compact consistency, coarse black particles in sediment, little aerobic layer
34	54°4.3000' N	7°56.8000' E	23.03.2010	13:50	silt	compact consistency, coarse black particles in sediment, little aerobic layer
35	54°4.0000' N	7°56.0000' E	23.03.2010	13:55	silt	compact consistency, coarse black particles in sediment, little aerobic layer
36	54°3.5000' N	7°55.8000' E	23.03.2010	14:05	silt	compact consistency, coarse black particles in sediment, little aerobic layer
37	54°3.0000' N	7°55.4000' E	23.03.2010	14:15	silt	compact consistency, coarse black particles in sediment, little aerobic layer
38	54°3.4000' N	7°57.4000' E	23.03.2010	14:25	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer
39	54°3.4000' N	7°58.4000' E	23.03.2010	14:35	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer
40	54°3.0000' N	7°58.0000' E	23.03.2010	14:45	sand	bright sediment, lots of sea urchins

41	54°2.6000' N	7°57.5000' E	23.03.2010	14:50	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer, stench of sulfur
42	54°2.8000' N	7°57.5000' E	23.03.2010	15:05	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer, stench of sulfur
43	54°4.6000' N	7°58.0000' E	23.03.2010	15:20	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer, stench of sulfur
44	54°3.0000' N	8°0.8000' E	23.03.2010	15:40	silt	compact consistency, layer of fine-grained sediment on top, coarse black particles in sediment, little aerobic layer, stench of sulfur
45	54°2.1000' N	8°0.2000' E	24.03.2010	09:30	silt	thin aerobic layer, coarse black particles
46	54°1.3000' N	7°58.0000' E	24.03.2011	09:50	silt	thin aerobic layer, coarse black particles
47	54°1.2000' N	7°59.0000' E	24.03.2012	10:30	silt	aerobic layer approximately 5cm
48	54°1.8000' N	8°0.5000' E	24.03.2013	10:35	silt	compact consistency, 5cm of aerobic layer
49	54°1.1000' N	8°0.6000' E	24.03.2014	10:50	silt	compact consistency, 5-6cm of aerobic layer
50	54°0.6000' N	8°0.4000' E	24.03.2015	11:00	silt	compact consistency, 10cm of aerobic layer, seashells
51	54°1.1000' N	8°2.2000' E	24.03.2016	11:05	silt	compact consistency, 10cm of aerobic layer, seashells
52	54°0.4000' N	8°2.2000' E	24.03.2017	11:15	silt	compact consistency, 10cm of aerobic layer, seashells
53	53°59.7000' N	8°1.7000' E	24.03.2018	11:30	silt	compact consistency, 10cm of aerobic layer, seashells
54	54°0.2000' N	8°4.3000' E	24.03.2019	11:45	silt	compact consistency, 10cm of aerobic layer

55	53°59.4000' N	8°6.2000' E	24.03.2020	12:05	silt	compact consistency, 10cm of aerobic layer
56	54°0.6000' N	8°8.1000' E	25.03.2010	10:55	silt	compact consistency, 10cm of aerobic layer
57	53°58.9000' N	8°8.2000' E	24.03.2020	12:15	silt	thin aerobic layer, stench of sulfur
58	53°59.9000' N	8°8.6000' E	25.03.2010	10:45	silt	thin aerobic layer, stench of sulfur
59	53°58.9000' N	8°10.0000' E	24.03.2020	12:35	silt	thin aerobic layer, stench of sulfur
60	53°59.9000' N	8°0.6000' E	25.03.2010	10:35	silt	half aerobic / half anaerobic
61	53°58.9000' N	8°11.6000' E	24.03.2020	12:45	silt	soft sediment, lots of worms
62	53°59.9000' N	8°12.6000' E	25.03.2010	10:20	sand	lots of common razor shells
63	53°59.0000' N	8°13.7000' E	24.03.2020	12:55	sand	bright sand, aerobic
64	53°59.9000' N	8°14.6000' E	25.03.2010	10:05	silt	bright sand, aerobic
65	53°59.0000' N	8°15.5000' E	24.03.2020	13:05	sand	bright sand, aerobic
66	53°59.9000' N	8°16.4000' E	25.03.2010	09:55	silt	lots of common razor shells
67	53°59.3000' N	8°17.6000' E	24.03.2020	13:15	sand	thin anaerobic layer
68	53°59.9000' N	8°18.3000' E	25.03.2010	09:45	sand	half aerobic / half anaerobic

69	53°59.3000' N	8°19.2000' E	24.03.2020	13:25	sand	half aerobic / half anaerobic
70	53°59.9000' N	8°20.1000' E	25.03.2010	09:35	sand	half aerobic / half anaerobic
71	53°59.2000' N	8°21.0000' E	24.03.2020	13:35	sand	5cm of aerobic layer
72	53°59.9000' N	8°21.9000' E	25.03.2010	09:25	sand	
73	53°59.3000' N	8°22.7000' E	24.03.2020	13:55	sand	bright sand, aerobic
74	53°59.9000' N	8°23.5000' E	25.03.2010	09:15	sand	
75	53°59.4000' N	8°24.5000' E	25.03.2010	09:05	sand	bright sand, aerobic

Tab. 6.3: Results for DNA quantification with the spectrophotometer TECAN Infinite M200 Nano Quant. The necessary input in PCR to assure a template of 10ng DNA was calculated on the basis of the mean DNA concentration for each sample.

Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
1	0,0076	0,0045	7,60	1,69	8,30	1,20
	0,0090	0,0051	9,00	1,76		
2	0,0052	0,0029	5,20	1,79	6,10	1,64
	0,0070	0,0039	7,00	1,79		
3	0,0114	0,0062	11,40	1,84	11,00	0,91
	0,0106	0,0056	10,60	1,89		
4	0,0132	0,0071	13,20	1,86	11,25	0,89
	0,0093	-0,0020	9,30	1,83		
5	0,0093	0,0053	9,30	1,75	9,35	1,07
	0,0094	0,0053	9,40	1,77		
6	0,0070	0,0026	7,00	2,69	6,55	1,53
	0,0061	0,0025	6,10	2,44		
7	0,0085	0,0049	8,50	1,73	7,90	1,27
	0,0073	0,0042	7,30	1,74		
8	0,0075	0,0045	7,50	1,67	6,70	1,49
	0,0059	0,0037	5,90	1,59		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
9	0,0098	0,0055	9,80	1,78	9,90	1,01
	0,0100	0,0053	10,00	1,89		
10	0,0094	0,0048	9,40	1,96	8,70	1,15
	0,0080	0,0039	8,00	2,05		
11	0,0116	0,0058	11,60	2,00	10,95	0,91
	0,0103	0,0056	10,30	1,84		
12	0,0104	0,0060	10,40	1,73	8,05	1,24
	0,0057	-0,0043	5,70	1,75		
13	0,0115	0,0062	11,50	1,85	10,25	0,98
	0,0090	0,0046	9,00	1,96		
14	0,0077	0,0037	7,70	2,08	7,80	1,28
	0,0079	0,0041	7,90	1,93		
15	0,0159	0,0014	15,90	11,36	16,60	0,60
	0,0173	0,0033	17,30	5,24		
16	0,0095	0,0051	9,50	1,86	10,20	0,98
	0,0109	0,0061	10,90	1,79		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
17	0,0155	0,0083	15,50	1,87	16,15	0,62
	0,0168	0,0094	16,80	1,79		
18	0,0178	0,0090	17,80	1,98	16,55	0,60
	0,0153	0,0082	15,30	1,87		
19	0,0162	0,0088	16,20	1,84	16,30	0,61
	0,0164	0,0091	16,40	1,80		
20	0,0245	0,0135	24,50	1,81	21,45	0,47
	0,0184	0,0027	18,40	1,83		
21	0,0202	0,0115	20,20	1,76	18,65	0,54
	0,0171	0,0087	17,10	1,97		
22	0,0080	0,0039	8,00	2,05	7,35	1,36
	0,0067	0,0038	6,70	1,76		
23	0,0119	0,0064	11,90	1,86	12,65	0,79
	0,0134	0,0078	13,40	1,72		
24	0,0178	0,0098	17,80	1,82	14,80	0,68

	0,0118	0,0068	11,80	1,74		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
25	0,0167	0,0092	16,70	1,82	15,15	0,66
	0,0136	0,0077	13,60	1,77		
26	0,0186	0,0101	18,60	1,84	16,95	0,59
	0,0153	0,0085	15,30	1,80		
27	0,0103	0,0058	10,30	1,78	10,55	0,95
	0,0108	0,0061	10,80	1,77		
28	0,0124	0,0072	12,40	1,72	9,60	1,04
	0,0068	-0,0033	6,80	1,74		
29	0,0158	0,0090	15,80	1,76	15,10	0,66
	0,0144	0,0071	14,40	2,03		
30	0,0114	0,0060	11,40	1,90	11,35	0,88
	0,0113	0,0063	11,30	1,79		
31	0,0134	0,0075	13,40	1,79	11,45	0,87
	0,0095	0,0053	9,50	1,79		
32	0,0113	0,0066	11,30	1,71	11,70	0,85
	0,0121	0,0071	12,10	1,70		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
33	0,0104	0,0056	10,40	1,86	10,50	0,95
	0,0106	0,0055	10,60	1,93		
34	0,0127	0,0062	12,70	2,05	11,60	0,86
	0,0105	0,0054	10,50	1,94		
35	0,0265	0,0139	26,50	1,91	23,65	0,42
	0,0208	0,0111	20,80	1,87		
36	0,0123	0,0068	12,30	1,81	8,75	1,14
	0,0052	-0,0043	5,20	1,85		
37	0,0108	0,0063	10,80	1,71	10,60	0,94
	0,0104	0,0054	10,40	1,93		
38	0,0068	0,0035	6,80	1,94	6,35	1,57
	0,0059	0,0028	5,90	2,11		
39	0,0030	0,0010	3,00	3,00	3,30	3,03
	0,0036	0,0023	3,60	1,57		
40	0,0046	0,0030	4,60	1,53	4,45	2,25
	0,0043	0,0027	4,30	1,59		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
41	0,0111	0,0061	11,10	1,82	10,45	0,96
	0,0098	0,0052	9,80	1,88		
42	0,0239	0,0051	23,90	4,69	22,95	0,44
	0,0220	0,0060	22,00	3,67		
43	0,0127	0,0070	12,70	1,81	9,70	1,03
	0,0067	-0,0041	6,70	1,84		
44	0,0126	0,0070	12,60	1,80	13,30	0,75
	0,0140	0,0074	14,00	1,89		
45	0,0112	0,0058	11,20	1,93	11,00	0,91
	0,0108	0,0063	10,80	1,71		
46	0,0116	0,0062	11,60	1,87	12,60	0,79
	0,0136	0,0071	13,60	1,92		
47	0,0072	0,0039	7,20	1,85	7,45	1,34
	0,0077	0,0048	7,70	1,60		
48	0,0145	0,0082	14,50	1,77	13,10	0,76
	0,0117	0,0062	11,70	1,89		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
49	0,0095	0,0054	9,50	1,76	9,75	1,03

	0,0100	0,0055	10,00	1,82		
50	0,0255	0,0058	25,50	4,40	24,70	0,40
	0,0239	0,0050	23,90	4,78		
51	0,0191	0,0036	19,10	5,31	17,15	0,58
	0,0152	-0,0066	15,20	5,11		
52	0,0168	0,0098	16,80	1,71	15,20	0,66
	0,0136	0,0068	13,60	2,00		
53	0,0121	0,0064	12,10	1,89	11,95	0,84
	0,0118	0,0062	11,80	1,90		
54	0,0094	0,0049	9,40	1,92	9,25	1,08
	0,0091	0,0053	9,10	1,72		
55	0,0218	0,0040	21,80	5,45	21,90	0,46
	0,0220	0,0050	22,00	4,40		
56	0,0228	0,0027	22,80	8,44	22,85	0,44
	0,0229	0,0023	22,90	9,96		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
57	0,0145	0,0078	14,50	1,86	14,80	0,68
	0,0151	0,0082	15,10	1,84		
58	0,0141	0,0075	14,10	1,88	12,85	0,78
	0,0116	0,0062	11,60	1,87		
59	0,0150	0,0083	15,00	1,81	13,15	0,76
	0,0113	-0,0013	11,30	1,82		
60	0,0270	0,0142	27,00	1,90	27,40	0,36
	0,0278	0,0143	27,80	1,94		
61	0,0178	0,0094	17,80	1,89	16,00	0,63
	0,0142	0,0074	14,20	1,92		
62	0,0269	0,0141	26,90	1,91	27,75	0,36
	0,0286	0,0153	28,60	1,87		
63	0,0169	0,0008	16,90	21,13	17,30	0,58
	0,0177	0,0016	17,70	11,06		
64	0,0195	0,0106	19,50	1,84	18,65	0,54
	0,0178	0,0093	17,80	1,91		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
65	0,0154	0,0013	15,40	11,85	16,15	0,62
	0,0169	0,0019	16,90	8,89		
66	0,0186	0,0103	18,60	1,81	17,95	0,56
	0,0173	0,0095	17,30	1,82		
67	0,0187	0,0017	18,70	11,00	16,55	0,60
	0,0144	-0,0084	14,40	10,80		
68	0,0066	0,0039	6,60	1,69	6,85	1,46
	0,0071	0,0034	7,10	2,09		
69	0,0166	0,0006	16,60	27,67	16,90	0,59
	0,0172	0,0012	17,20	14,33		
70	0,0032	0,0016	3,20	2,00	4,25	2,35
	0,0053	0,0033	5,30	1,61		
71	0,0113	0,0061	11,30	1,85	10,50	0,95
	0,0097	0,0059	9,70	1,64		
72	0,0068	0,0036	6,80	1,89	6,70	1,49
	0,0066	0,0034	6,60	1,94		
Sample	260	280	Conc [ng/µl]	Ratio	mean conc [ng/µl]	input PCR [µl]
73	0,0143	0,0007	14,30	20,43	14,55	0,69
	0,0148	0,0017	14,80	8,71		
74	0,0156	0,0013	15,60	12,00	16,35	0,61
	0,0171	0,0022	17,10	7,77		

75	0,0191	0,0020	19,10	9,55	17,35	0,58
	0,0156	-0,0082	15,60	8,10		

References

Anderson J., Boonruang P., Meadows P. (1981) Interrelationships between Chlorophylls, Carbon, Nitrogen and Heterotrophic Bacteria in an Intertidal Sediment Transect. Marine Ecology - Progress Series:277–283

Arrigo K. (2005) Marine micro-organisms and global nutrient cycles. Nature:349–355

Aubel D., Renaud F., Freney J. (1997) Genomic diversity of several Corynebacterium species identified by amplification of the 16S-23S rRNA gene spacer regions. Int. J. Syst. Bacteriol.:767–772

Billen G., Joiris C., Meyer-Reil L., Lindeboom H. (1990) Role of Bacteria in the North Sea Ecosystem. Netherlands Journal of Sea Research:265–293

Blackburn T., Blackburn N. (1992) Model of Nitrification and Denitrification in marine sediments. FEMS Microbiol. Ecol.:517–522

Brasse S., Reimer A., Seifert R., Michaelis W. (1999) The influence of intertidal mudflats on the dissolved inorganic carbon and total alkalinity distribution in the German Bight, southeastern North Sea. Journal of Sea Research:93–103

Brockmann U., Laane R., Postma H. (1990) Cycling of Nutrient Elements in the North Sea. Netherlands Journal of Sea Research:239–264

Burns R.G. (1980) Microbial adhesion to soil surfaces: consequences for growth and enzyme activities

Caraco N., Cole J. (1999) Human impact on nitrate export: an analysis using major world rivers. Ambio:167–170

Chrambach A. (1985) The Practice of Quantitative Gel Electrophoresis, Deerfield Beach

Clarke K., Gorley R. (2001) Primer Version 5, Plymouth, UK

Creutzberg F., Postma H. (1979) An experimental approach to the distribution of mud in the southern North Sea. Netherlands Journal of Sea Research:99–116

Creutzberg F., Wapenaar P., Duineveld G., Lopez Lopez N. (1984) Distributin and density of the benthic fauna in the southern North Sea in relation to bottom characteristics and hydrographic conditions.:226–233

Dale N. (1974) Bacteria in intertidal sediments: Factors related to their distribution. Limnol. Oceanogr.:509–518

Domsch K., Jagnow G., Anderson T. (1983) An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms

Fickert M., Strotmann T. (2007) Hydrodynamische Entwicklung der Tideelbe. Coastline Reports:59–68

Fisher M.M., Triplett E.W. (Received 26 April 1999/Accepted 14 July 1999) Automated Approach for Ribosomal Intergenic Spacer Analysis of Microbial Diversity and Its Application to Freshwater Bacterial Communities. Applied and Environmental Microbiology

Francis C.A., Beman J.M., Kuypers M.M. (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. The ISME Journal:19–27

Garcia-Martinez J., Martinez-Murcia A., Anton A., Rodriguez-Valera F. (1996) Comparison of the small 16S-23S intergenic spacer region (ISR) of the rRNA operons of some Escherichia coli strains of the ECOR collection and E.coli K-12. J. Bacteriol.:6374–6377

Gattuso J., Frankignoulle M., Wollast R. (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. Annual Reviews of Ecological Systems:405–434

Graf G., Bengston W., Diesner U., Schulz R., Theede H. (1982) Benthic response to sedimentation of a spring phytoplankton bloom: process and budget. Mar. Biol.:201–208

Hamburg Port Authority (2008) Umgang mit Baggergut aus dem Hamburger Hafen. Teilbericht Umlagerung von Baggergut zur Tonne E3 über den Zeitraum 1.1.2007 - 04.01.2008

Hargrave B. (1972) Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Oceanogr.:583–596

Hedges J., Keil R. (1995) Sedimentary organic matter preservation: An assessment and speculative hypothesis. Marine Chemistry:81–115

Heise S. (2005) Studie zur Schadstoffbelastung der Sedimente im Elbeinzugsgebiet. Ursachen und Trends. Hamburg Port Authority:195

Hickel W., Gunkel W. (1968) Untersuchungen über die Häufigkeit der Bakterien in der obersten Sedimentschicht der Deutschen Bucht in Beziehung zu den Substrateigenschaften. Helgol. Wiss. Meeresunters.:213–231

Hunter E.M., Mills H.J., Kostka J.E. (2006) Microbial Community Diversity Associated with Carbon and Nitrogen Cycling in Permeable Shelf Sediments. Applied and Environmental Microbiology:5689–5701

Jensen M., Webster J., Straus N. (1993) Rapid identification of bacteria on the basis of polamerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Applied and Environmental Microbiology:945–952

Kerner M., Jacobi A. (2006) Ausbau- und Unterhaltungskosten für die deutschen Seehäfen. Report for the World Wide Fund for Nature (WWF-Deutschland), Internationales Zentrum für, Meeresschutz, Hamburg:23 pp.

Kuenen J., Robertson L. (1988) Ecology of nitrification and denitrification.

Lancelot C., Billen G., Mathot S. (1988) Ecophysiology of phyto- and bacterioplankton growth in the Pryds Bay area during Austral summer 1987. Part 1: Modelling phytoplankton growth. Proceedings of the Belgian National Colloqiums on Antarctic Research:116–132

Leal W., Holda A., Juurikas J., Lucius I., Krahn D., Quereshi A. (2006) The River Elbe in Hamburg. A description of the issus surrounding the dredging of the River Elbe and possible means of conflict resolution. Case study report within the Coastman project

Leyer I., Weschke K. (2008) Multivariate Statistik in der Ökologie. Springer

Meyer-Reil L.A. Enzymatic decomposition of proteins and carbohydrates in marine sediments: methodology and field observations during spring. Kieler Meeresforsch., Sonderh.:311–317

Naqvi W., Gattuso J. (2006) Marine Nitrogen Cycle. Encyclopedia of Earth

Nealson K.H. (1997) Sediment Bacteria: Who's There, What Are They Doing, and What's New? Annu. Rev. Earth Planet. Sci.

Ogunseitan O. (2005) Microbial Diversity. Form and Function in Prokaryotes. Blackwell Publishing

Patsch J., Radach G. (1997) Long-term simulation of the eutrophication of the North Sea: temporal developments of nutrients, chlorophyll and primary production in comparison to observations. Journal of Sea Research:275–310

Ranjard L., Brothier E., Nazaret S. (2000a) Sequencing Bands of Ribosomal Intergenic Spacer Analysis Fingerprints for Characterization and Microscale Distribution of Soil Bacterium Populations Responding to Mercury Spiking. Applied and Environmental Microbiology:5334–5339

Ranjard L., Poly F., Lata J., Mougel C., Thioulouse J., Nazaret S. (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. Applied and Environmental Microbiology

Reichgott A., Stevenson L. (1978) Microbiological and physical properties of salt marsh and microecosystem sediments. Applied and Environmental Microbiology:662–667

Sambrook J., Fritsch E., Maniatis T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, NY

Schink B. (1991) Syntrophism among prokaryotes

Schlegel H.G. (1992) Allgemeine Mikrobiologie, 7th edn. Thieme, Stuttgart

Shi Q., Jackowski G. (1998) One-dimensional polyacrylamide gel electrophoresis, Oxford University Press

Thomas H., Bozec Y., de Baar H.J.W., Elkalay K., Frankignoulle M., Schiettecatte L., Kattner G., Borges A.V. (2005) The carbon budget of the North Sea. Biogeosciences