Benthic bacteria in the German Bight:

Characterising community structure and influencing environmental factors

or

‚Living along gradients‘

Outline

Introduction

Methods

Results

- Spatiotemporal variations of benthic bacterial communities in the German Bight

- Impact of ocean dumping on benthic bacterial communities

Conclusions

Future perspectives

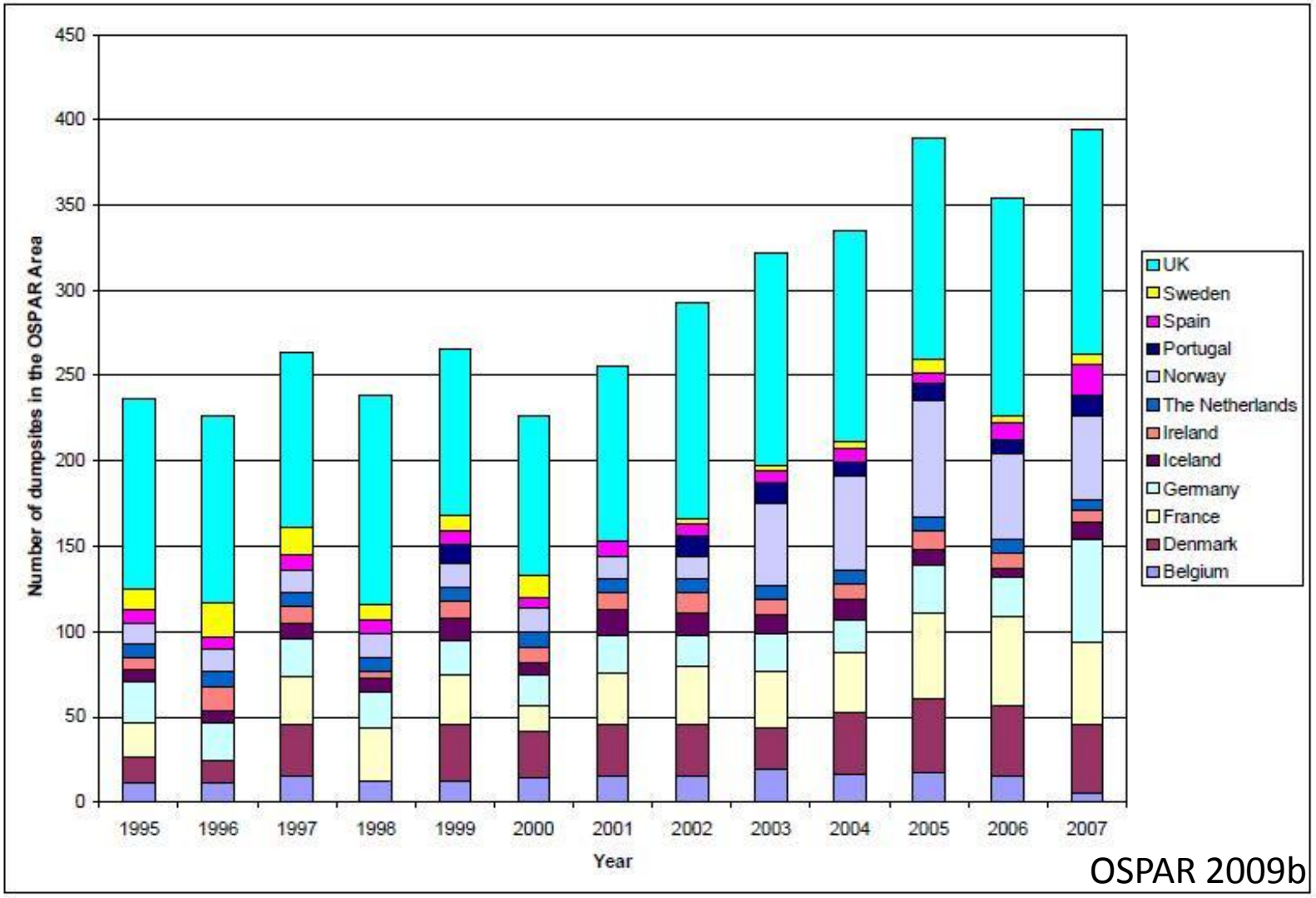
Motivation: Ocean dumping

- deposition of waste at sea
- waste includes:
  - liquid wastes (dilute acid, sludge)
  - construction waste
  - dredged material (harbours, rivers)

➡ Threat for ecosystems

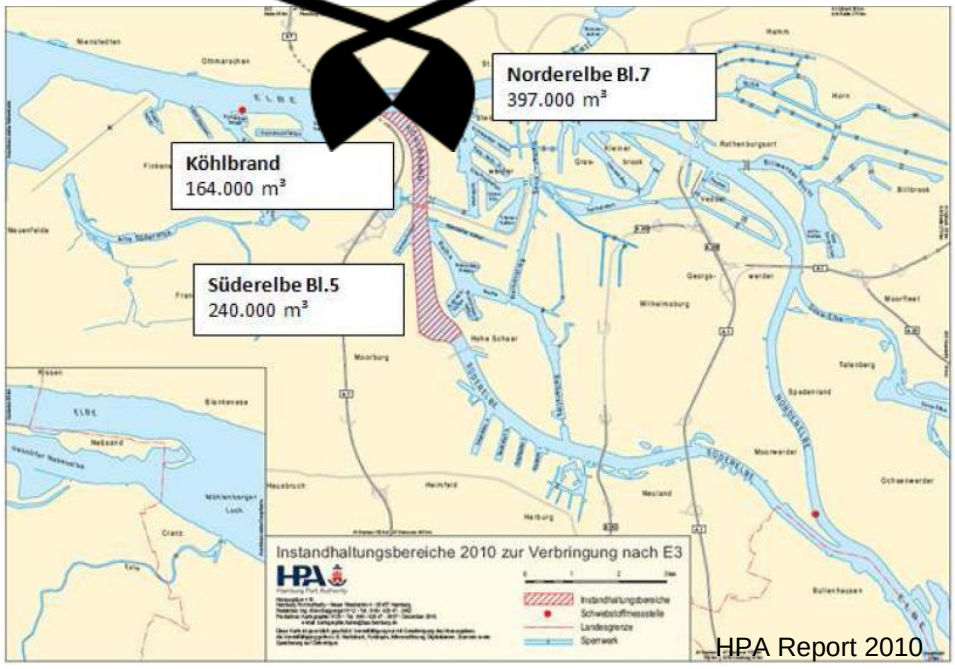
Motivation: Ocean dumping

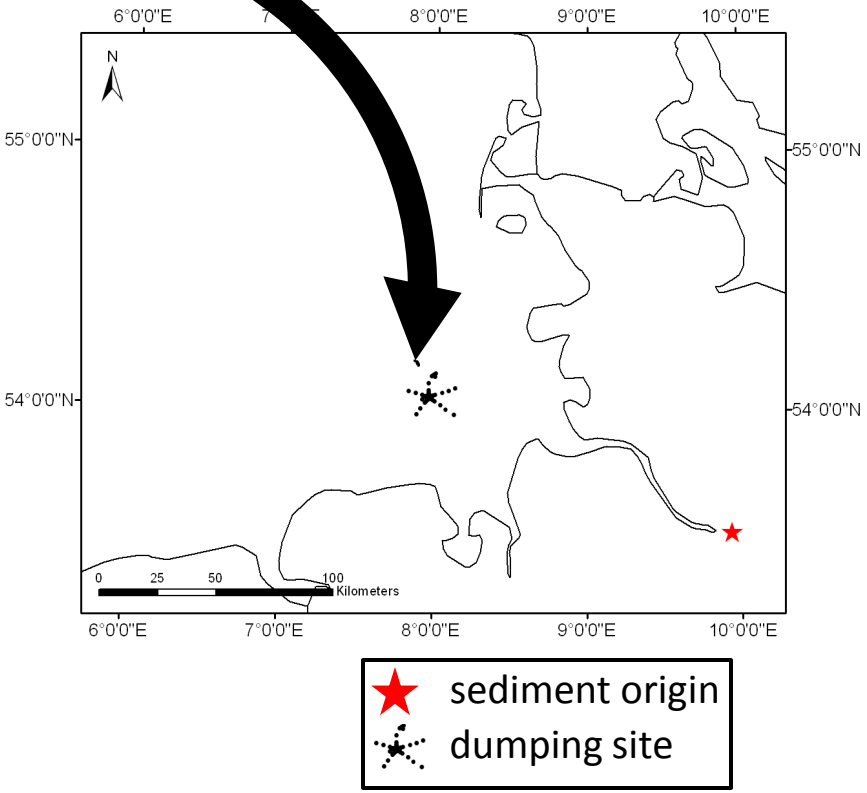
Overview of the number and distribution of dumpsites within the OSPAR area



OSPAR 2009b

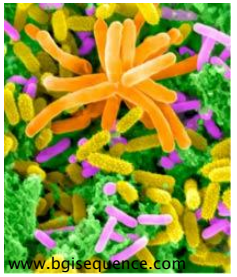
Introduction





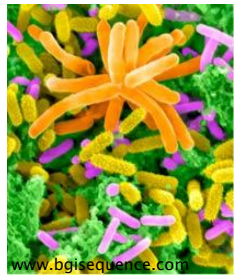
since 2005: ~ 6 mio m³
3 m high rising containing mainly sand

Motivation



- monitoring focuses on macrozoobenthic communities and flatfishes
- *“Progress on investigating biological responses to the disposal of dredged material has been slow in OSPAR and more effort is needed for a wider and more systematic application of bioassays in the testing of dredged sediments.“ (OSPAR 2009b)*
- bacterial community structure affected by
  - physical disturbance (Findlay *et al* 1990)
  - heavy metal and oil contamination (Gremion *et al* 2004, dos Santos *et al* 2011)

Motivation



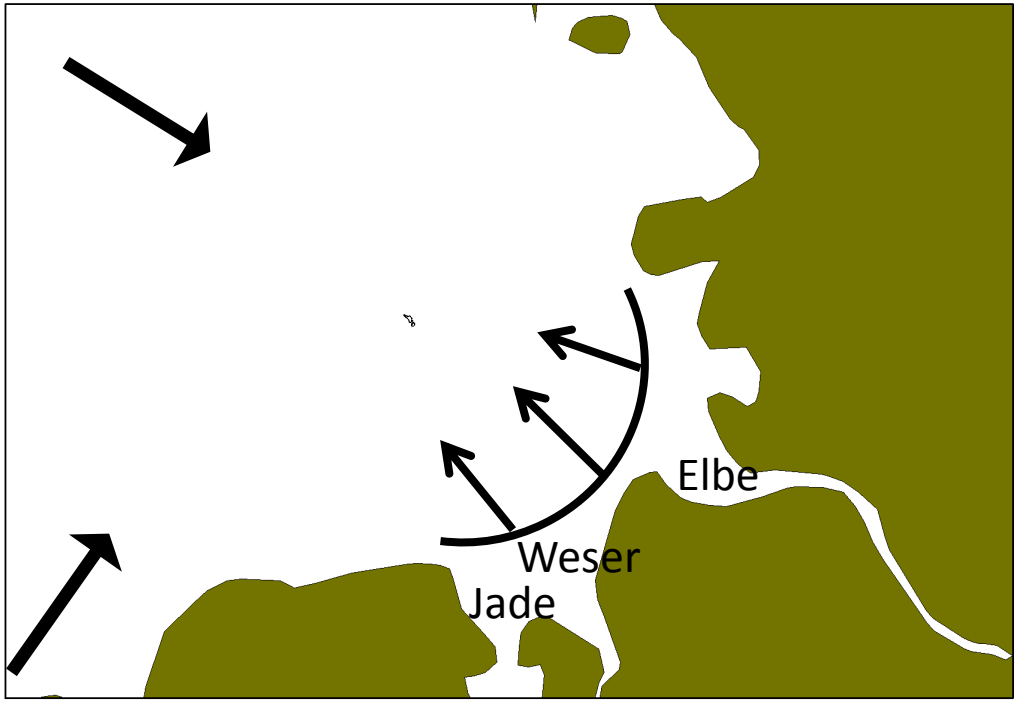
- benthic bacterial communities:
  - largest variety of metabolic types
  - heterotrophic, phototrophic and lithotrophic bacteria
  - highly abundant: 10^8 - 10^{11} cells per ml

Are bacterial community analyses a useful supplement for monitoring programs at dumping sites?

Problem: sublittoral benthic bacterial communities uncharacterised in the German Bight

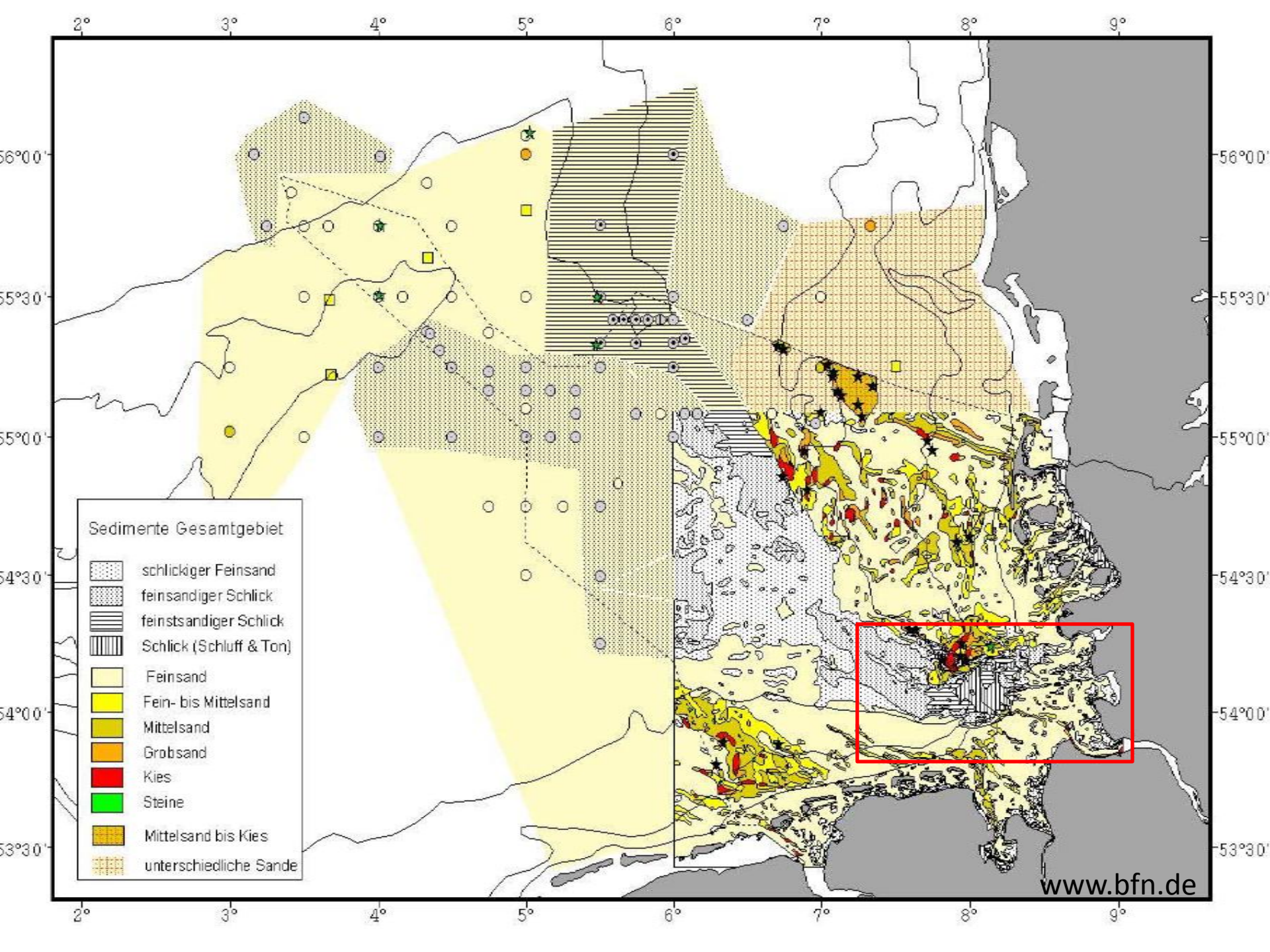
German Bight

- hydrographic regime pre-dominantely influenced by discharges of Elbe and Weser River
- most euthrophied region in the North Sea
- shallow part of the North Sea with max. dephts ~ 50 m
- Helgoland



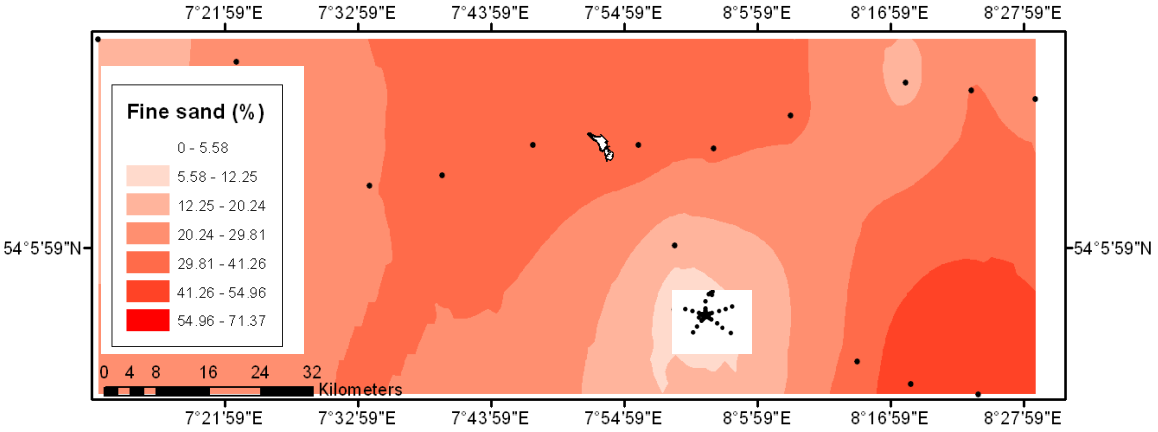
Sediments in the German Bight

- Grain size fractions
  - Clay particles < 4 µm
  - Silt particles 4 - 63 µm
  - Fine sand particles 63 – 200 µm
  - Medium sand particles 200 – 630 µm
  - Coarse sand particles 630 – 2000 µm
  - Gravel particles 2000 – 6000 µm
- Proportion of each grain size fraction determines the classification into sandy or muddy sediments

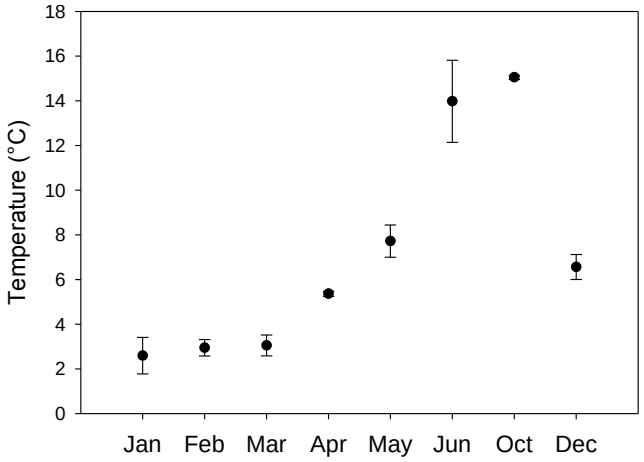

2°
3°
4°
5°
6°
7°
8°
9°
56°00'
55°30'
55°00'
54°30'
54°00'
53°30'
Sedimente Gesamtgebiet
schlickiger Feinsand
feinsandiger Schlick
feinstsandiger Schlick
Schlick (Schluff & Ton)
Feinsand
Fein- bis Mittelsand
Mittelsand
Grobsand
Kies
Steine
Mittelsand bis Kies
unterschiedliche Sande
www.bfn.de

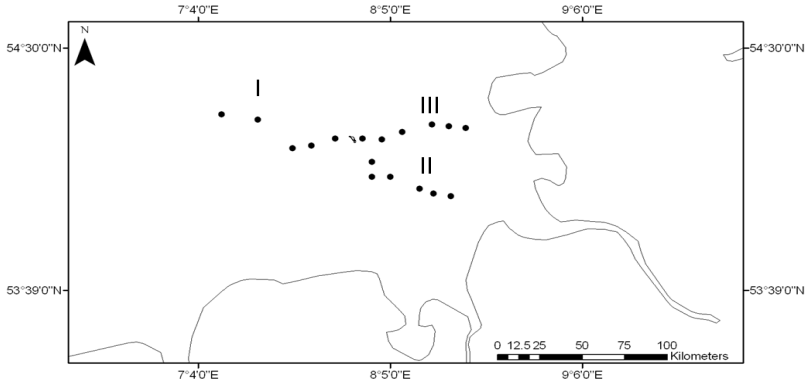
Environmental gradients

Spatial gradients



Temporal gradients





Characterising sublittoral benthic bacterial communities

I: Spatiotemporal variations influencing benthic bacterial communities in the German Bight

II: Impact of ocean dumping on benthic bacterial communities

a) Community structure

b) Community function

Data analysis

- Bacterial community analysis
  - ARISA fingerprinting
  - 16S pyrosequencing
  - functional gene arrays

- Environmental data (additional data provided by Prof. Karen Wiltshire and HPA)
  - CHN analysis
  - particle size analysis

- Statistical analysis
  - univariate and multivariate statistics
  - Geostatistics

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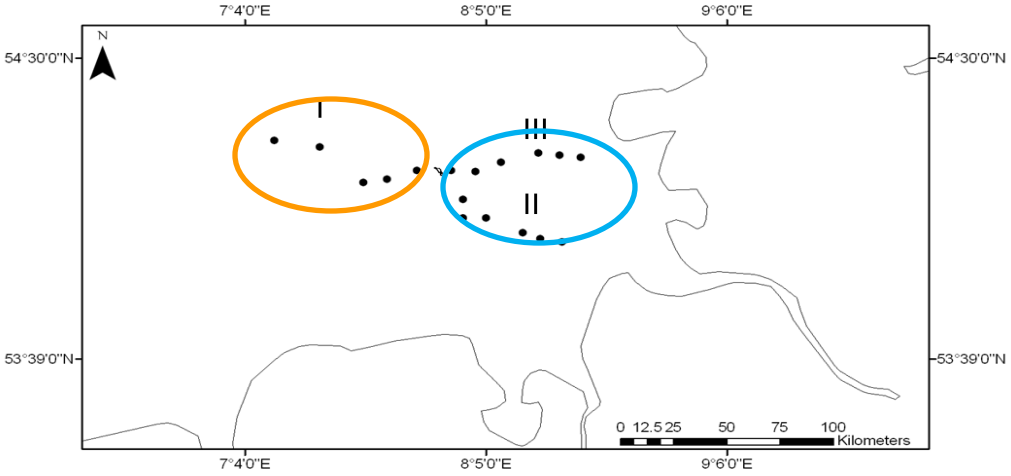
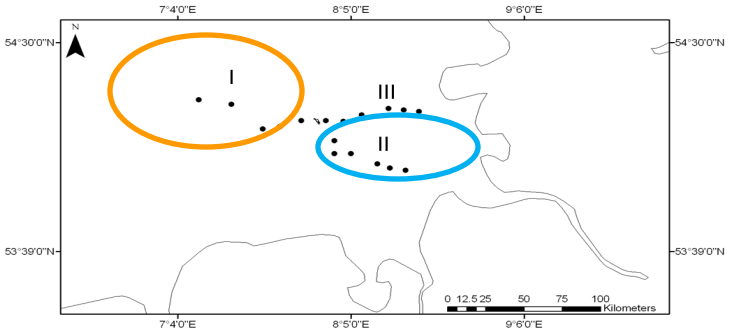


offshore transect

nearshore transects

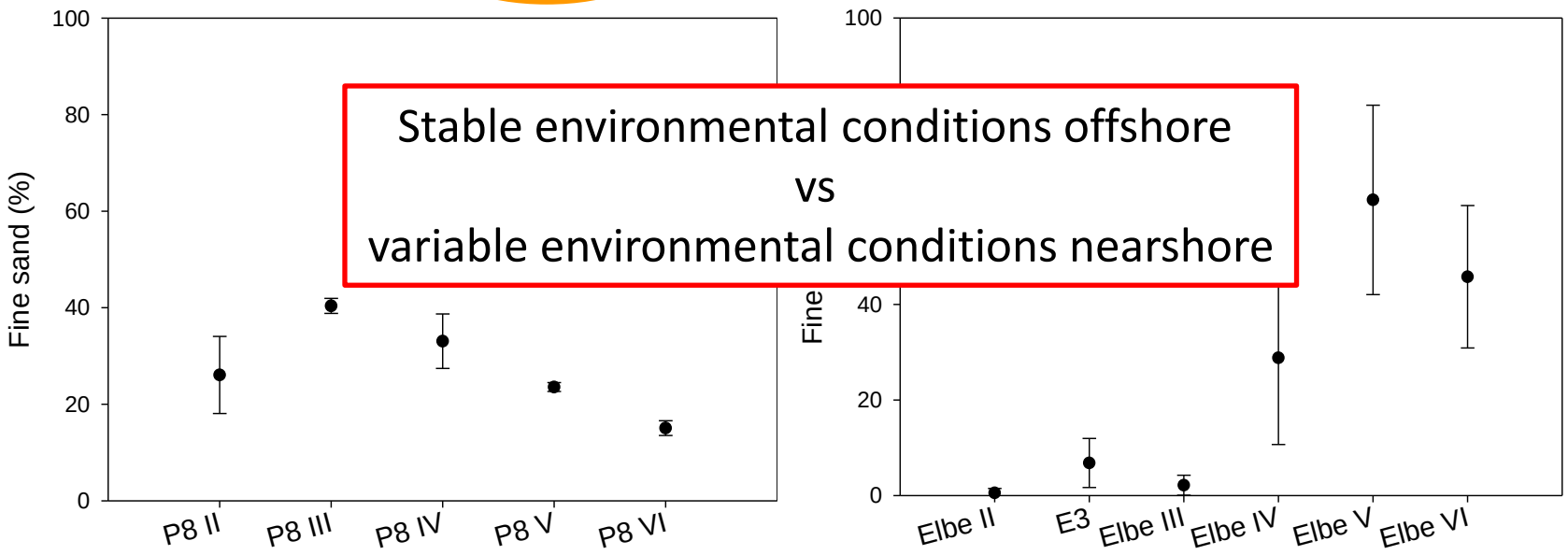
- monthly sampling from September 2010 to August 2011
- three replicates for bacterial community analysis (ARISA)
- environmental data includes:
  - temperature
  - salinity
  - chl a
  - CHN
  - particle size of the sediments

I: Spatiotemporal variations influencing benthic bacterial communities



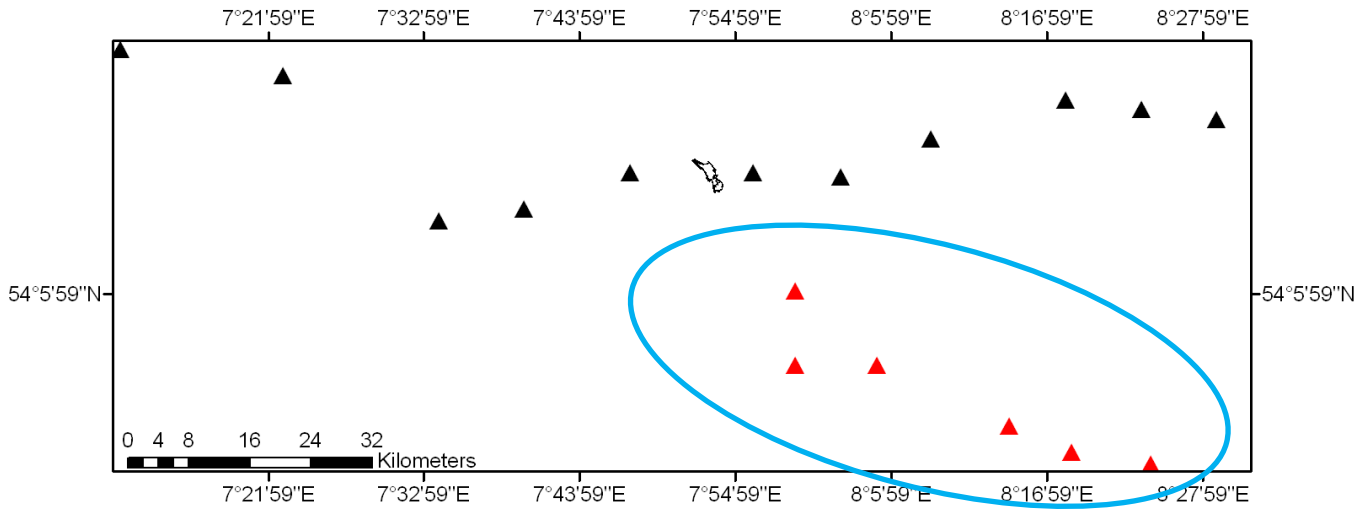
Transect I Offshore

Transect II Nearshore



Stable environmental conditions offshore
vs
variable environmental conditions nearshore

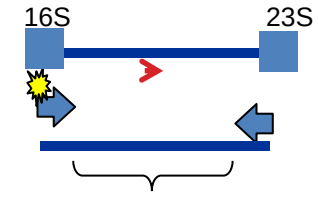
I: Spatiotemporal variations influencing benthic bacterial communities

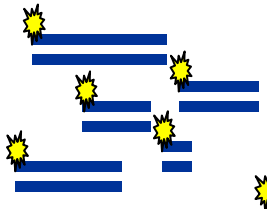


I: Spatiotemporal variations influencing benthic bacterial communities

Fingerprinting via Automated Ribosomal Intergenic Spacer Analysis (ARISA)

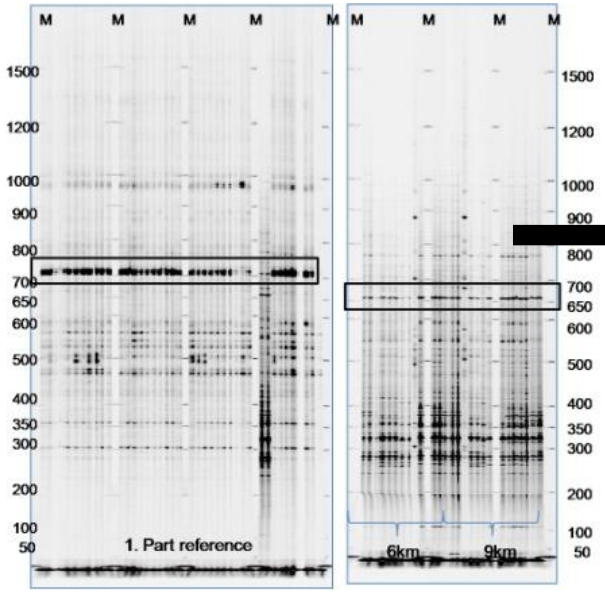
Ribosomal Operon of Bacteria





Infrared labeled tag

Community profiles



Conversion of community profiles into numeric data

Sample	262	265	268	271	274	277	280
1	1	1	1	1	1	1	1
3	1	0	1	1	1	1	0
5	0	1	1	1	1	1	1
7	0	0	0	0	0	0	1
9	0	1	0	0	1	0	1
10	0	1	0	0	1	0	1
11	0	0	1	0	0	0	1
12	0	1	0	0	1	0	1
13	0	1	0	0	1	0	1
14	0	1	1	1	1	1	1
15	0	0	0	0	0	0	1
16	1	1	1	1	1	1	1
18	1	1	1	1	1	1	0
20	0	1	1	1	1	0	1
21	1	1	1	0	0	1	1
25	1	1	1	0	1	0	1
28	0	1	1	0	1	0	1
33	0	0	0	0	0	0	1
37	1	1	1	1	0	1	1
41	1	1	1	0	1	0	1
45	1	1	1	1	1	1	1
48	0	0	0	0	0	0	1
53	0	0	0	0	0	0	1
55	0	0	0	0	0	1	0

I: Spatiotemporal variations influencing benthic bacterial communities

Distance based multivariate multiple regression model (DISTLM)

Marginal test

Variable	Pseudo-F	P	Proportion of variance
Coarse gravel	0	1	0.000
Medium gravel	0	1	0.000
Fine gravel	0	1	0.000
Coarse sand	15,685	0.0425	0.041
Medium sand	2,362	**0.0021**	0.060
Fine sand	26,317	**0.0007**	0.066
Coarse silt	26,109	**0.0012**	0.066
Medium silt	25,044	**0.001**	0.063
Fine silt	23,395	**0.0024**	0.060
Clay	22,599	**0.0035**	0.058
Temperature	47,382	**0.0001**	0.114
Salinity	24,043	**0.0007**	0.061
Nitrogen	16,197	0.0468	0.042
TOC	17,895	0.0219	0.046
Hydrogen	19,452	0.0106	0.050
Chlorophyll a	38,587	0.0001	0.094

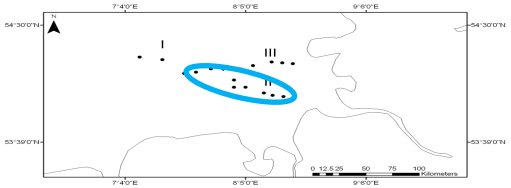
Sequential test

Variable	Pseudo-F	P	Proportion of variance
Temperature	47,382	**0.0001**	0.114
Fine sand	30,272	**0.0001**	0.069
Chlorophyll a	20,515	**0.0016**	0.045
Salinity	16,793	0.0132	0.036
Hydrogen	13,296	0.1106	0.029
Coarse silt	11,434	0.2703	0.024
Medium sand	12,879	0.1348	0.027
Coarse sand	10,608	0.3847	0.022
Fine silt	0.92303	0.5777	0.020
Medium silt	12,812	0.1628	0.027
Clay	10,797	0.3552	0.023
Nitrogen	0.77095	0.7846	0.016
TOC	0.67199	0.8882	0.014

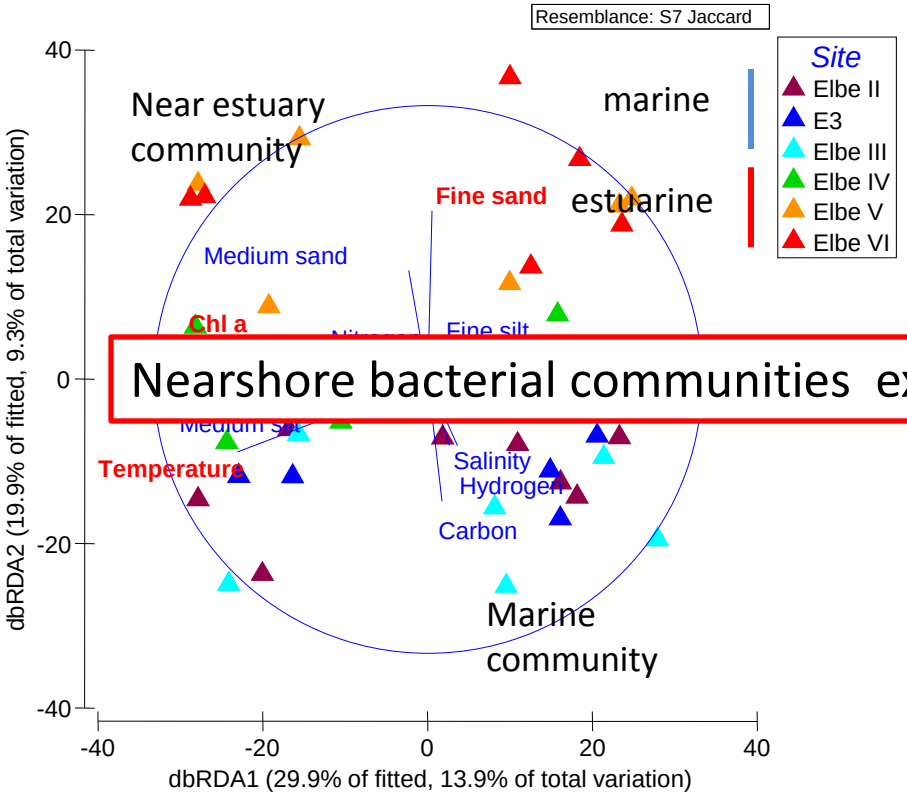
$p < 0.01$

I: Spatiotemporal variations influencing benthic bacterial communities

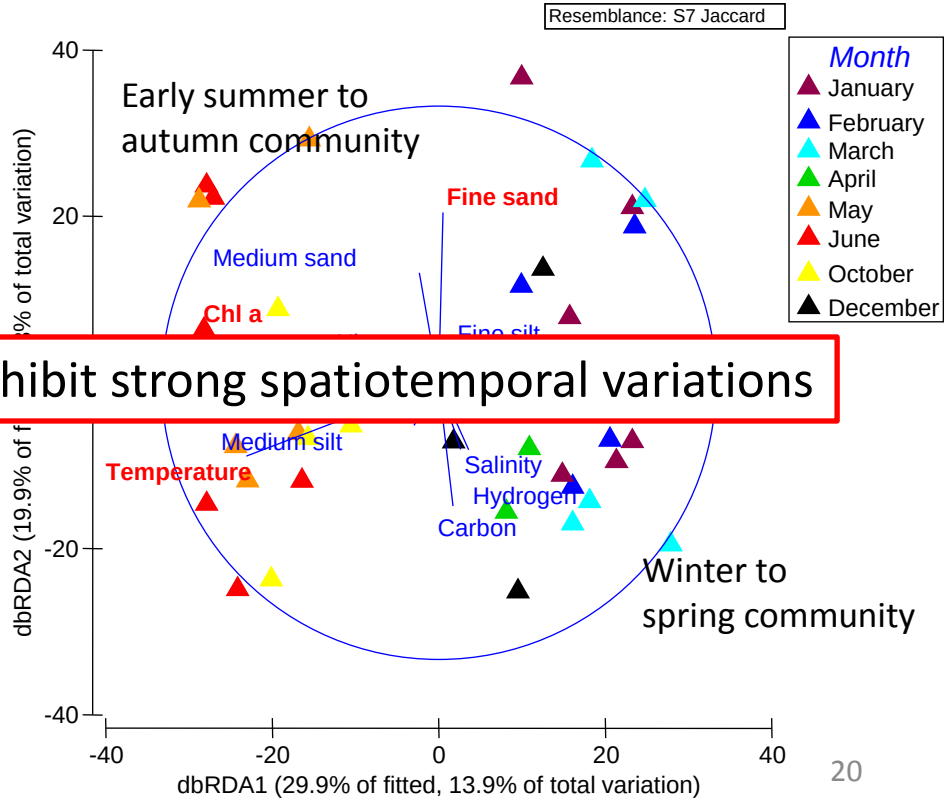
distance-based Redundancy analysis



Spatial

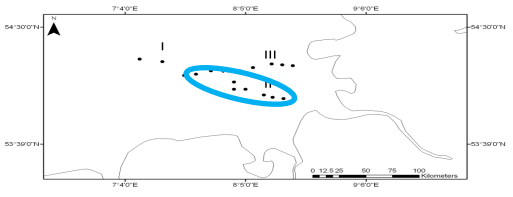


Temporal



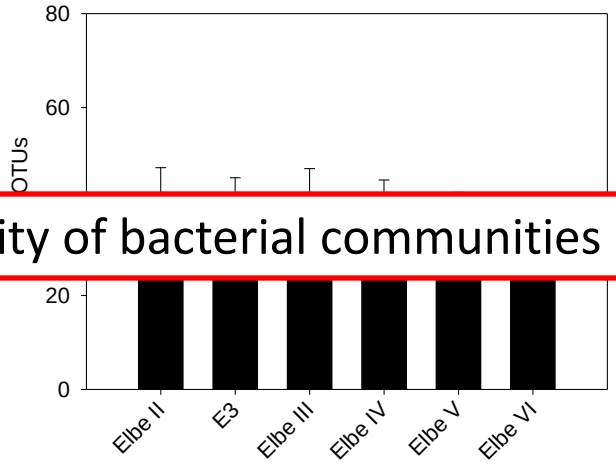
Nearshore bacterial communities exhibit strong spatiotemporal variations

I: Spatiotemporal variations influencing benthic bacterial communities

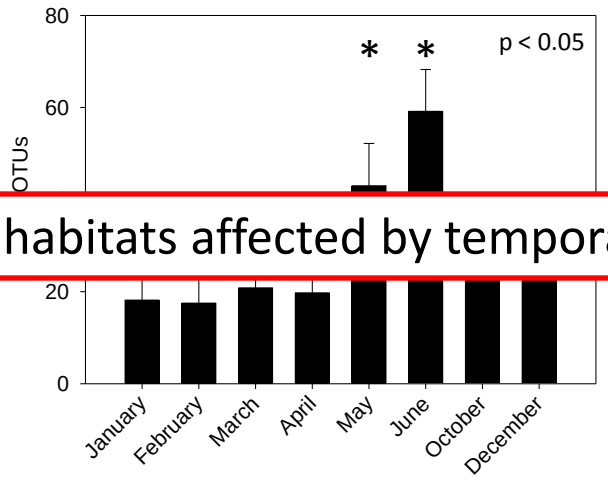


Distribution of operational taxonomic units (OTUs)

Spatial



Temporal



Diversity of bacterial communities in nearshore habitats affected by temporal variations

OTUs defined according to ITS fragment lenghts

I: Spatiotemporal variations influencing benthic bacterial communities

Main findings

- amplitude of environmental gradients determines the bacterial community structure
- temporal variations affect bacterial community structure and diversity

Characterising sublittoral benthic bacterial communities

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II: Impact of ocean dumping on benthic bacterial communities

a) Community structure

b) Community function

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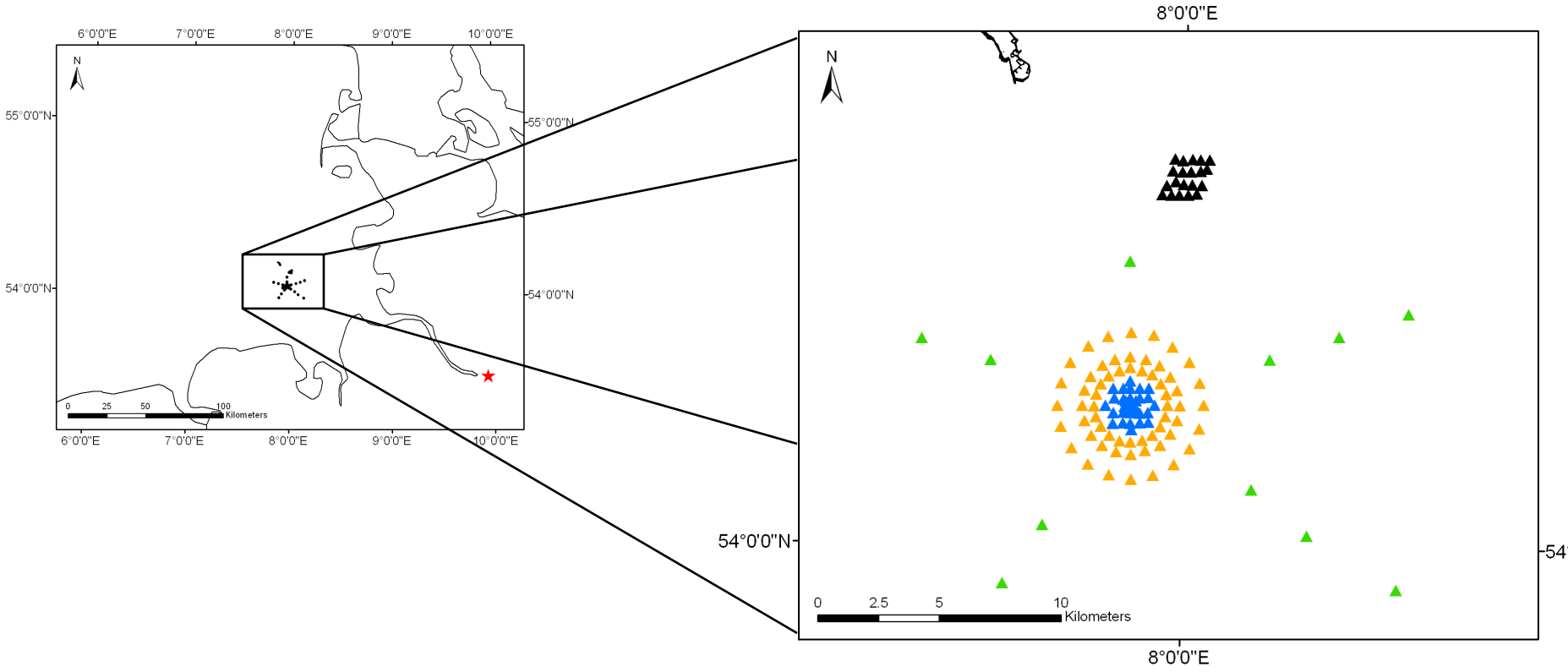
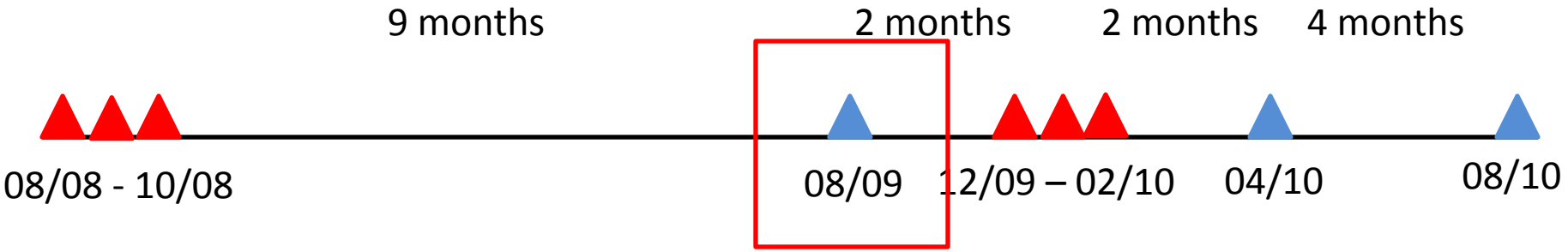


II: Impact of ocean dumping on benthic bacterial communities

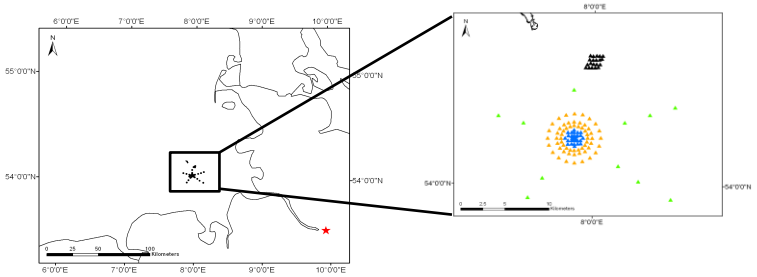
Timeline sampling campaigns



Dumping campaigns

Sampling campaigns

II: Impact of ocean dumping on benthic bacterial communities



- sampling campaigns in August 2009 and April and August 2010
- each campaign comprises 125 sampling sites
- three replicates for bacterial community analysis (ARISA)

Contextual data

Grain size fractions
- < 20µm
- 20-63µm
- 63-100µm
- 100-200µm
- 200-630µm
- 630-1000µm
- 1000-2000µm

S, N, P, C
- TOC (C)
- nitrogen (N)
- sulphur (S)
- phosphor (P)

Hydrocarbons

Sum Polycyclic Aromatic Hydrocarbons (PAH)
- naphthaline
- fluorene
- phenanthrene
- anthracene
- fluoranthene
- pyrene
- benz(a)anthracene
- chrysene
- benzo(b)fluoranthene
- benzo(k)fluoranthene
- benzo(a)pyrene
- dibenz(ah)anthracene
- benzo(ghi)perylene
- indeno(1.2.3cd)pyrene

Sum Chlorinated Diphenyls (PCB)
- PCB28
- PCB52
- PCB101
- PCB118
- PCB138
- PCB153
- PCB180

Sum Hexachlorocyclohexane (HCH)
- alphaHCH
- betaHCH
- gammaHCH
- deltaHCH

Sum Dichlorodiphenyldichloroethane (DDT) and metabolites
- ppDDE
- opDDD
- ppDDD
- opDDT
- ppDDT

Sum Organotin Compounds
- monobutyltin (MBT)
- dibutyltin (DBT)
- tributyltin (TBT)
- tetrabutyltin

Heavy Metals
- arsenic
- lead
- cadmium
- chrome
- copper
- nickel
- mercury
- zinc

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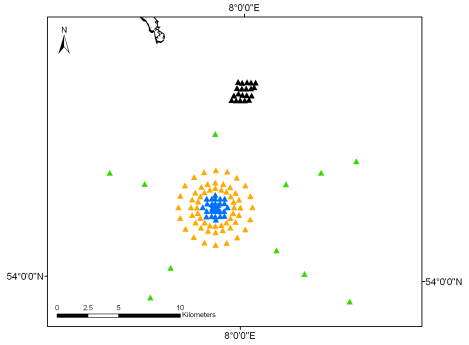
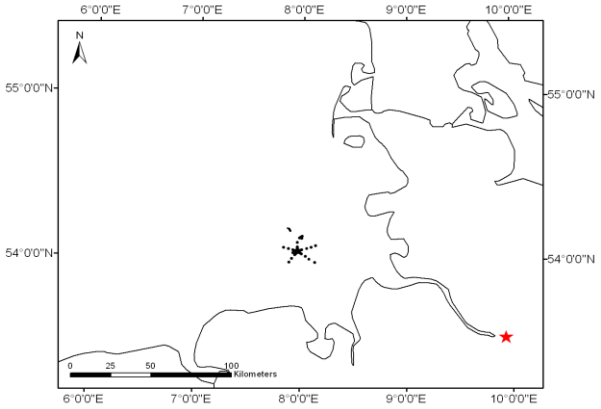
II: Impact of ocean dumping on benthic bacterial communities

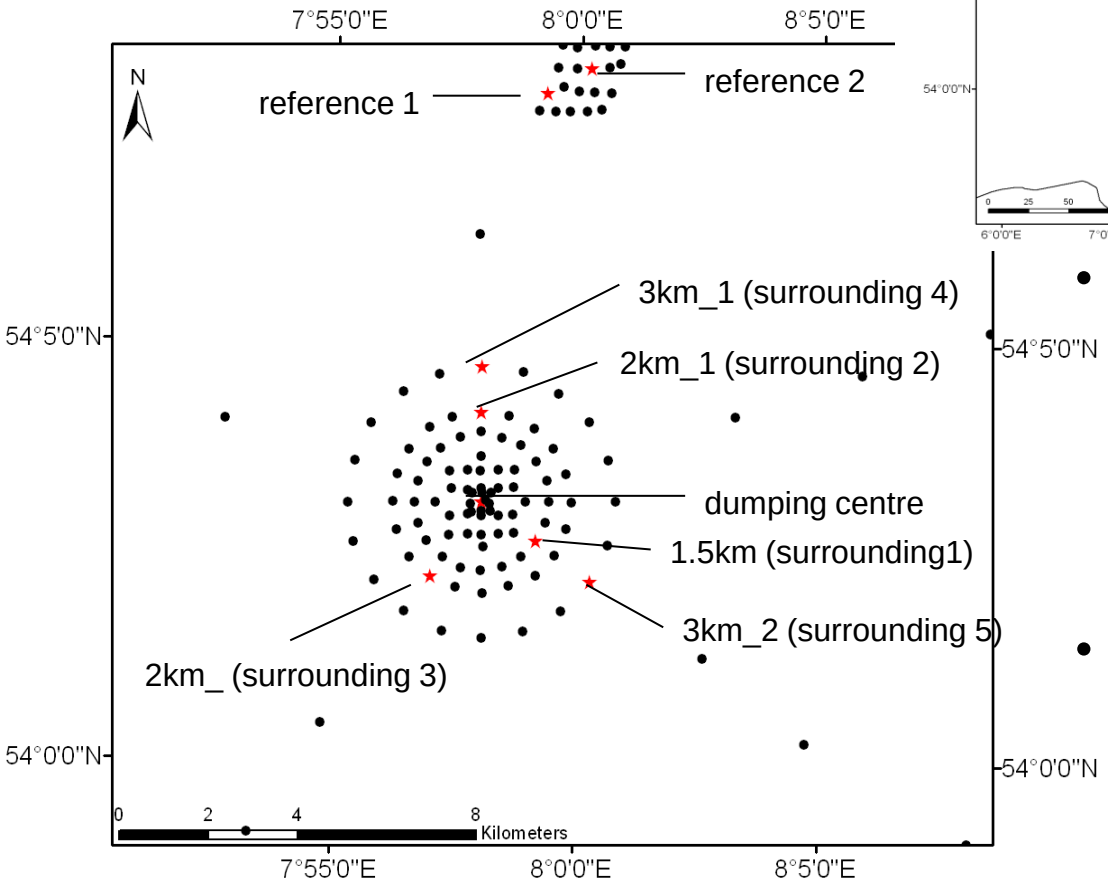
Biplot Redundancy analysis August 2009



Bacterial communities at the dumping site significantly affected by fine sand (100 – 200 µm) and organic pollutants

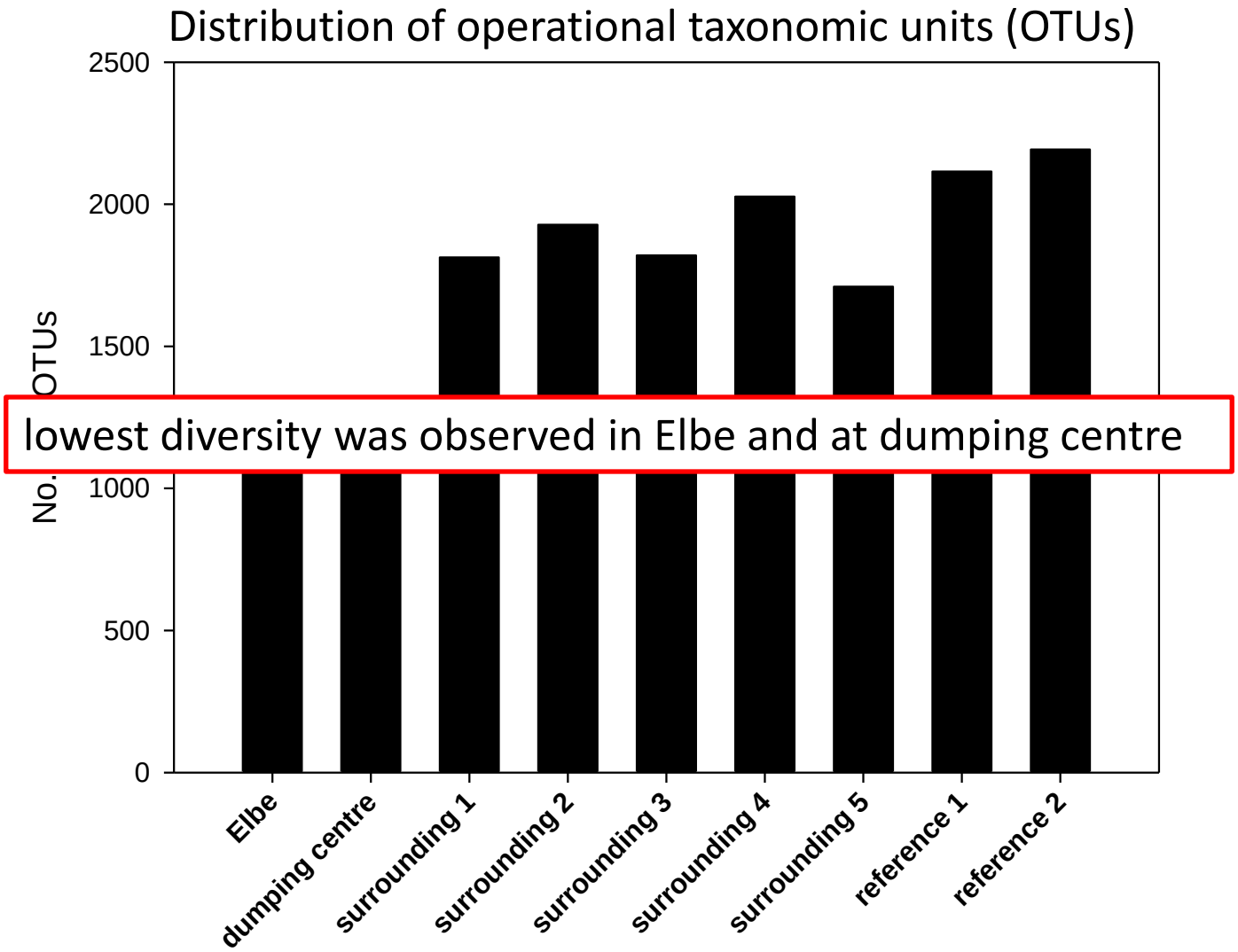
II: Impact of ocean dumping on benthic bacterial communities





- based on significant differences (analysis of similarities) in the communitity structure nine representative samples were selected
- via pyrosequencing the V1-V5 region of the 16S rDNA was sequenced

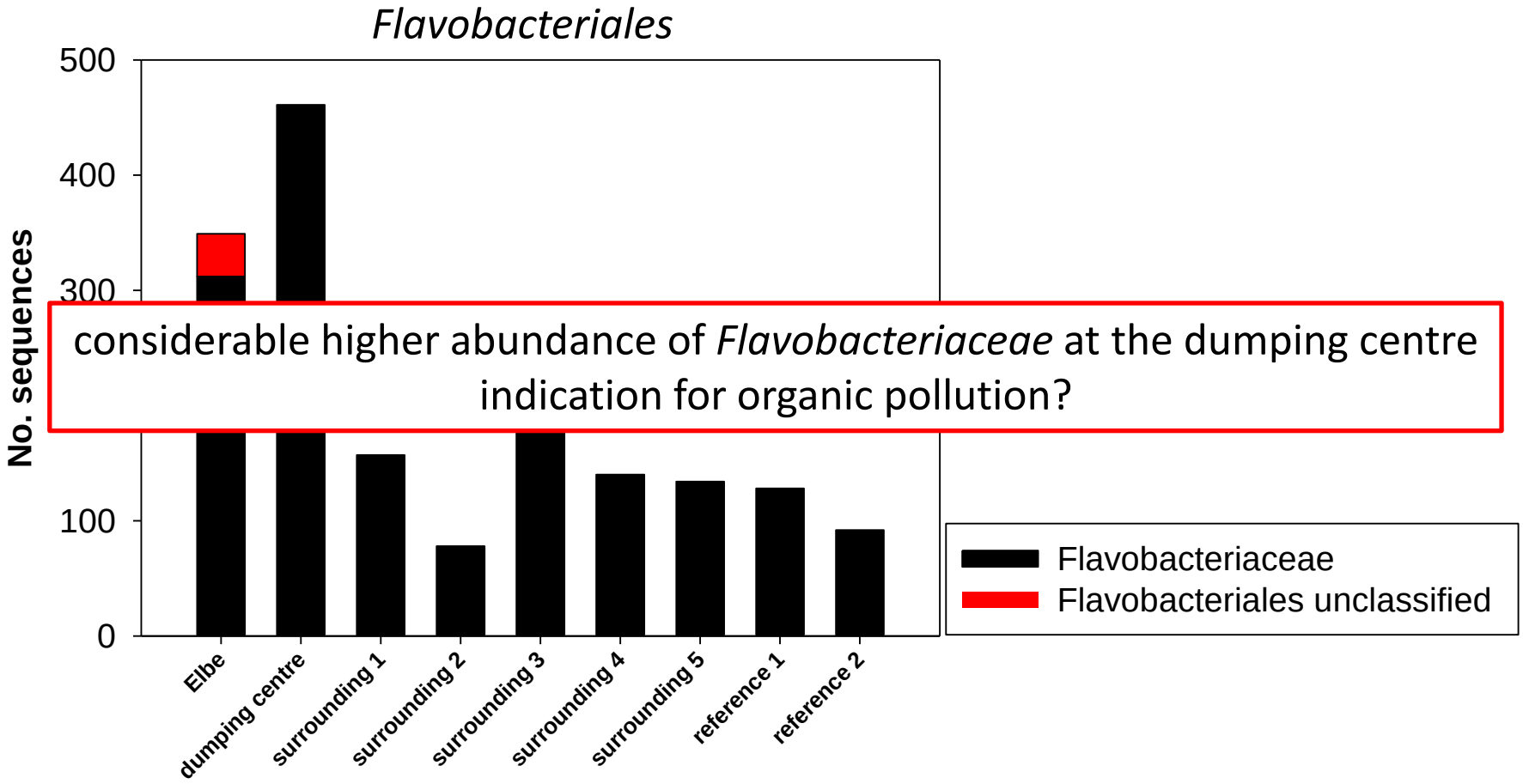
II: Impact of ocean dumping on benthic bacterial communities



lowest diversity was observed in Elbe and at dumping centre

OTUs = sequence similarity > 97 %
Singletons (n=1) were removed prior to the analysis

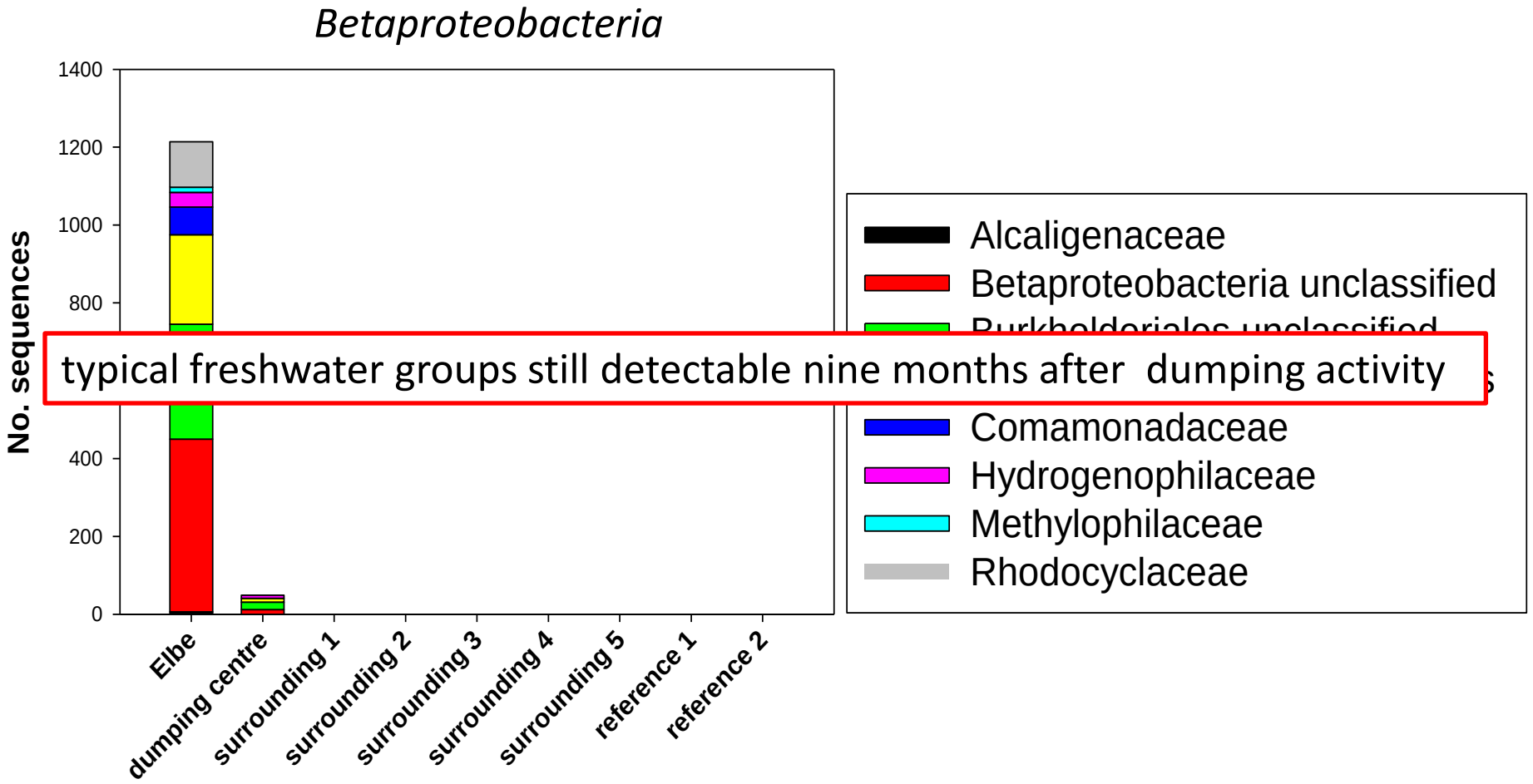
II: Impact of ocean dumping on benthic bacterial communities



considerable higher abundance of *Flavobacteriaceae* at the dumping centre
indication for organic pollution?

singletons (n=1) were removed prior to the analysis

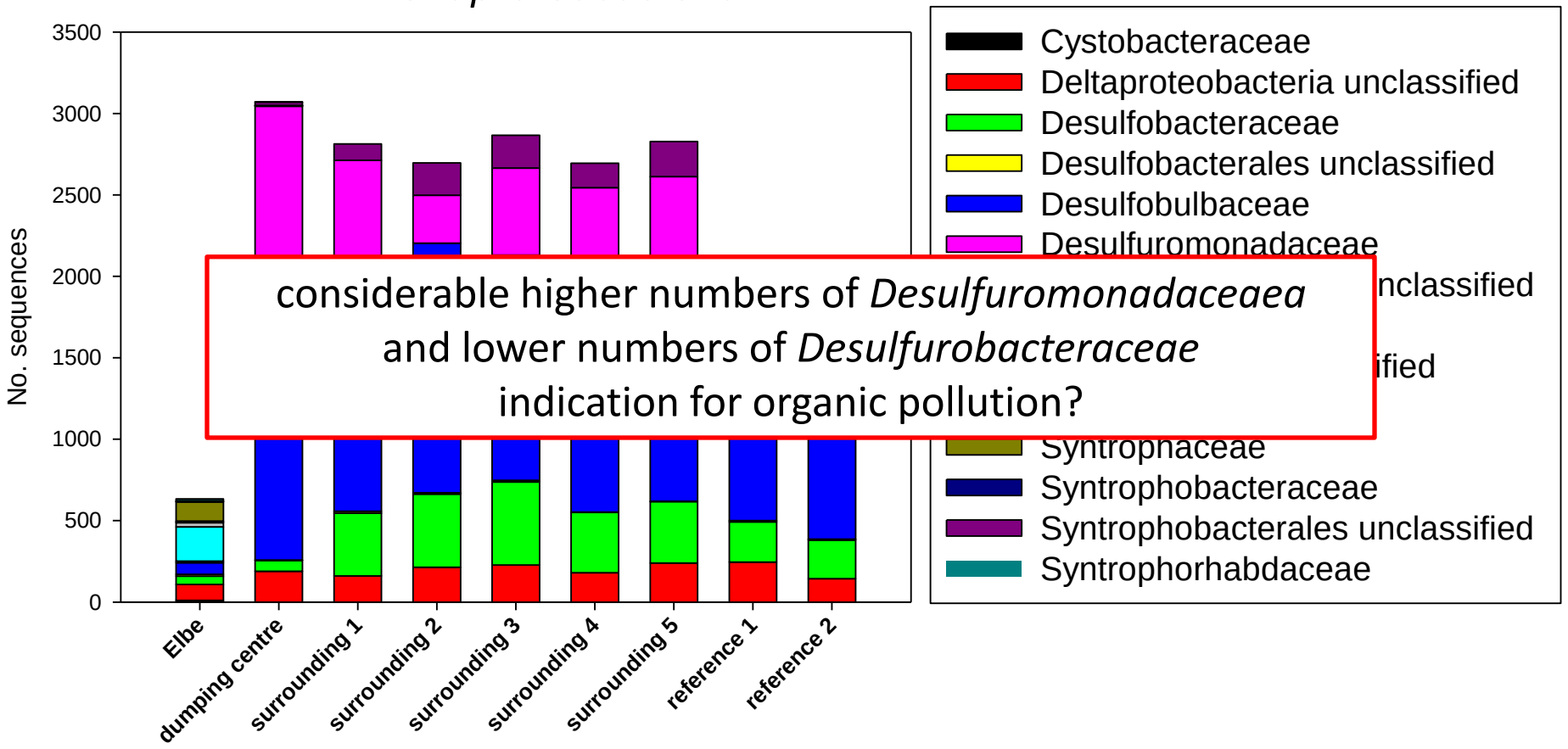
II: Impact of ocean dumping on benthic bacterial communities



typical freshwater groups still detectable nine months after dumping activity

singletons (n=1) were removed prior to the analysis

II: Impact of ocean dumping on benthic bacterial communities



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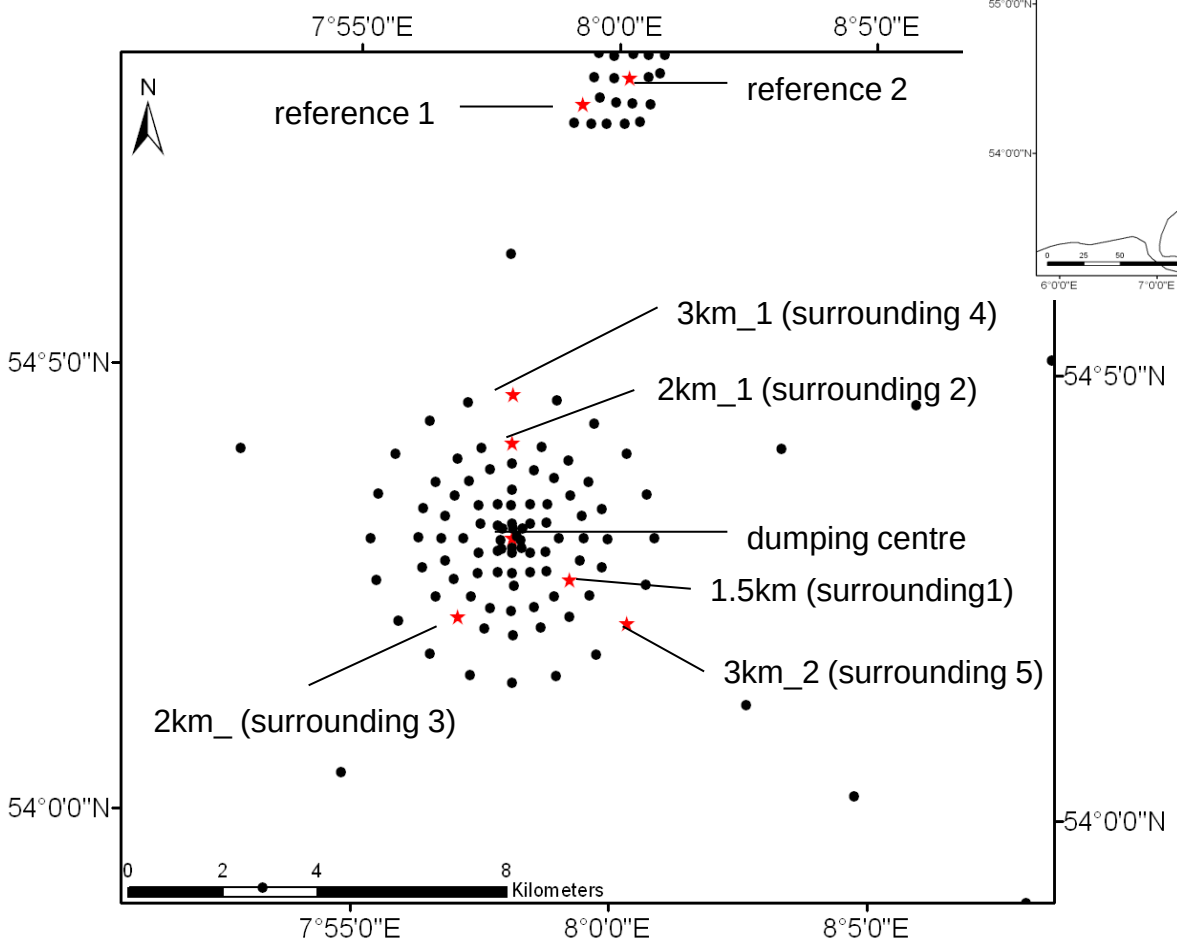
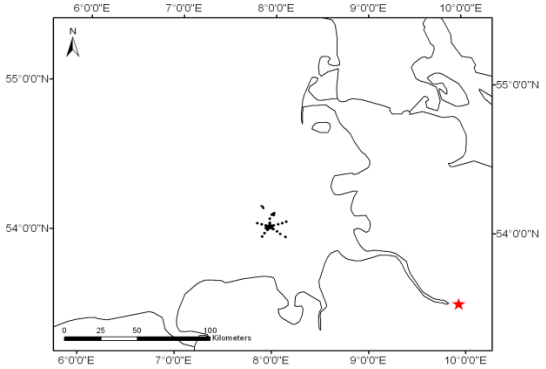
II: Impact of ocean dumping on benthic bacterial communities





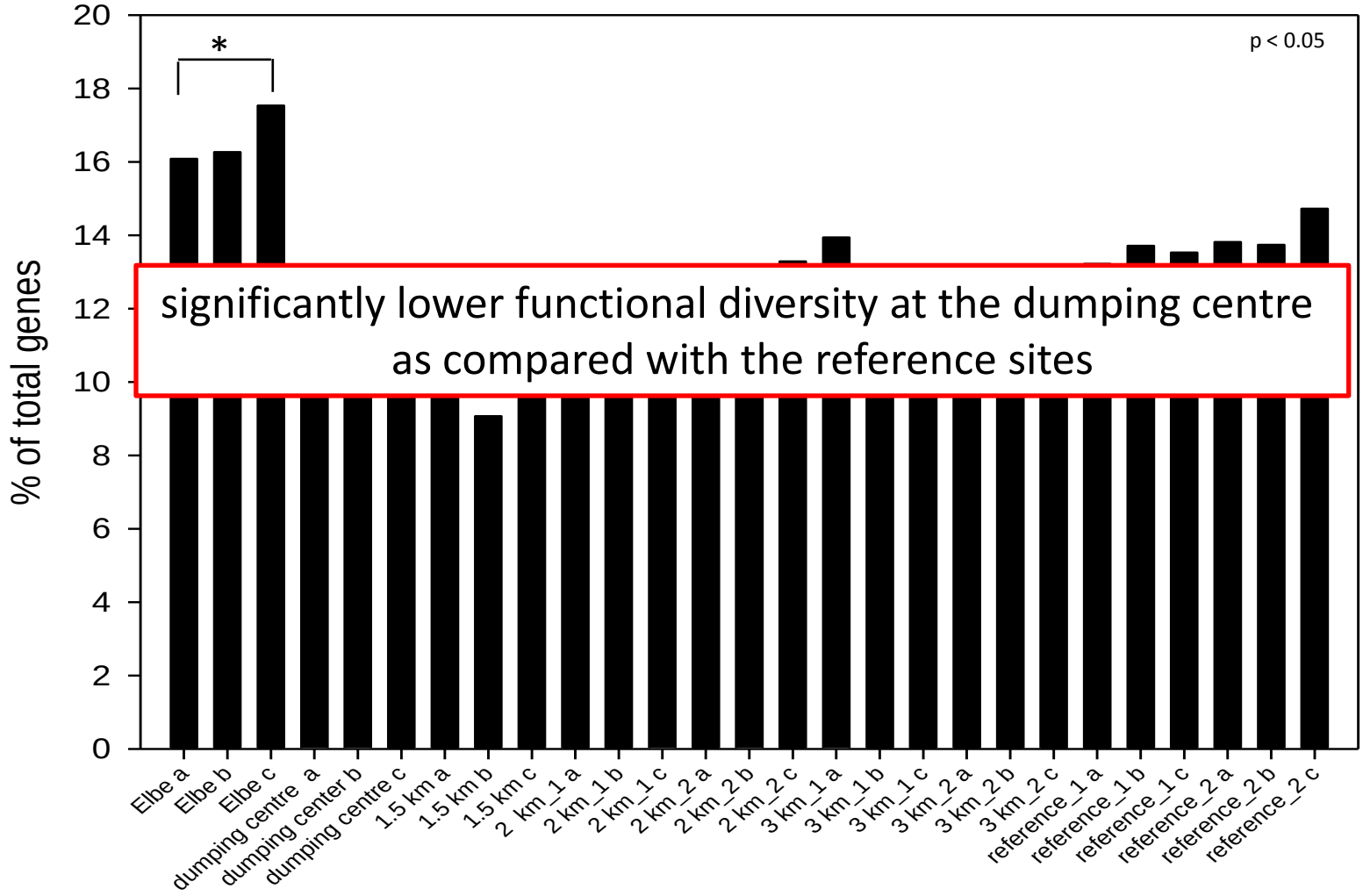
II: Impact of ocean dumping on benthic bacterial communities

GeoChip analysis

- GeoChip 4.2 (functional gene array) (He *et al* 2007, Lu *et al* 2012)
- contains 103 666 probes encoding for functional genes involved in biogeochemical key processes
- genes are categorised according to these processes e.g. sulphur cycling, heavy metal resistance, organic remediation

II: Impact of ocean dumping on benthic bacterial communities

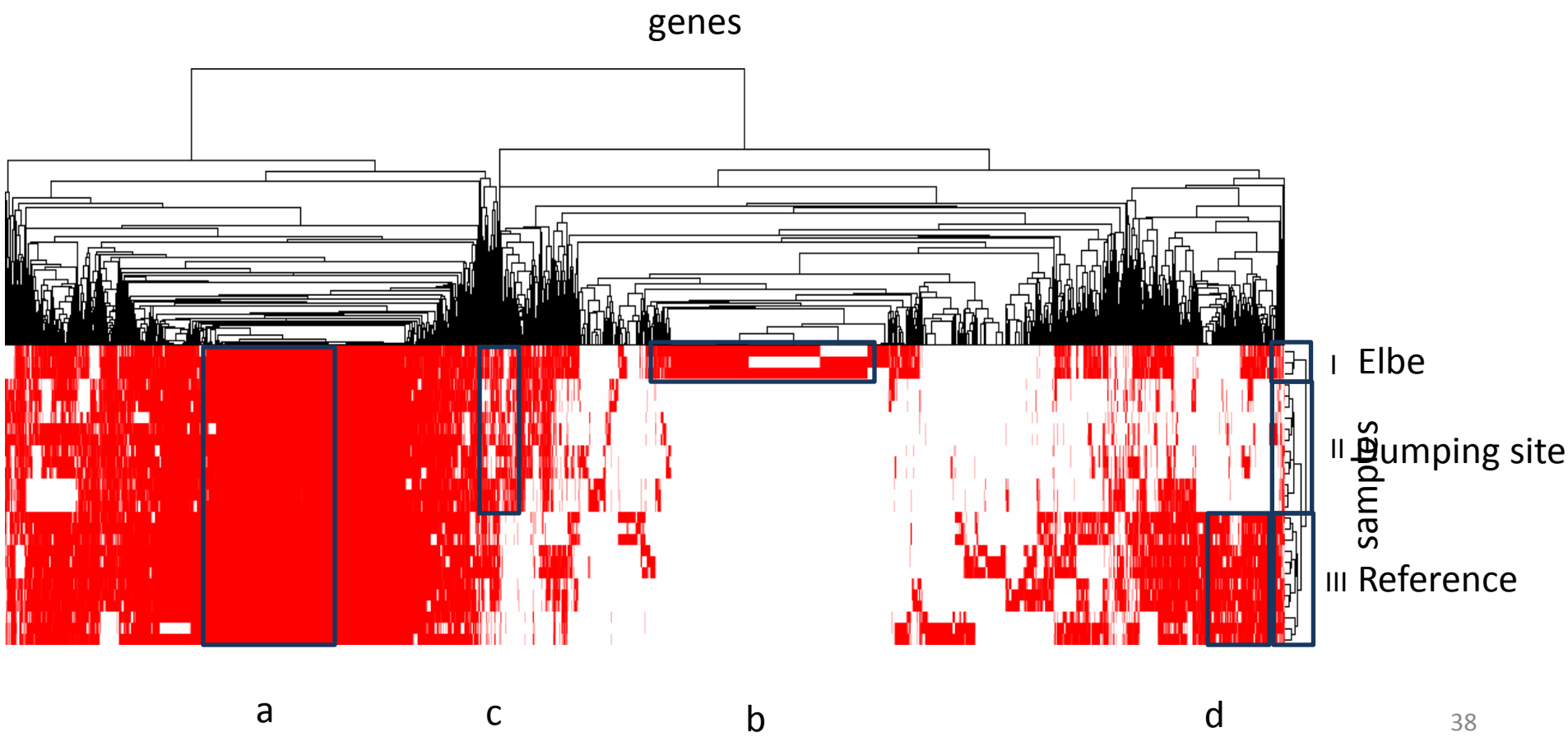
Distribution of functional genes of the gene category „organic remediation“



significantly lower functional diversity at the dumping centre as compared with the reference sites

II: Impact of ocean dumping on benthic bacterial communities

Hierachical clustering based on Euclidean distance
for the gene category „organic remediation“



Main findings

- similar results for all gene categories
- no accumulation of genes involved in pollution related processes at the dumping centre detectable
- significant lower functional diversity at the dumping centre
- differences among detected gene groups based on phylogenetic background

Main conclusions

- most pronounced environmental gradients affect the bacterial community structure significantly
- bacterial community structure at the dumping site was significantly influenced by the dumping activity
  - lower alpha and functional diversity
  - mix-community containing fresh water and adapted marine bacteria
- bacterial community analysis represent a useful supplement for monitoring programs
- **But: further elaboration is needed!!**

- deepening the knowledge about benthic bacterial communities in the German Bight
  - identifiying community composition and function „Metagenomic approaches“
  - simultaneous investigation of pelagic and benthic bacteria „Benthopelagic coupling“
- adaptation of monitoring conditions and experimental set up for the inclusion of bacterial community analysis
  - controlled experiments focusing on the impact of relevant pollutants on the bacterial communities
  - identifying indicator organisms
  - inclusion of physicochemical parameters such as pH, oxygen penetration, bioavailability of pollutants

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