Development of Ecologically Relevant Quality Indicators for Sediment Microbial Communities

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Abstract

With the implementation of European Water Framework Directive, good chemical status for all water bodies and good ecological status for all natural waters are to be reached by 2015. Considering sediments as an inseparable part of river basins, current sediment quality guidelines derived only from chemical concentrations can not guarantee the ecological services of sediments in supporting aquatic ecosystems. There is therefore a need to develop an integrated quality indicator (IQI) addressing the ecological relevance to assist the chemically dominant sediment assessment for a better river basin management.

A weight of evidence approach integrating sediment ecotoxicities and functional diversity of sediment microbial community in nutrient cycling was employed. The sediment ecotoxicity describes the potential risk of contaminants on biota through bioassays, which integrate effects of pollutants and environmental conditions. Two standardised bioassays, algal growth inhibition test and bacterial contact assay, were applied for different exposure routes. The functional diversity of microbial community in nutrient cycling provides the supporting service in aquatic ecosystem, which is necessary for the production of other organisms and maintain the conservation of nature. The diversity in nutrient cycling was addressed by the heterotrophic carbon substrates utilisation patterns and autotrophic nitrification was tested as the key ecological function. In addition, substrates induced DMSO reduction method was proposed to characterise the microbial functional diversity including both heterotrophic and autotrophic activities.

A temporal investigation of sediment quality using these lines of evidence was carried out in the river Elbe to define a possible response range. The results were further integrated using a fuzzy rule based model taking biological uncertainties into account to develop quality classes and indices characterising sediment quality in ecotoxicities, functional stability (heterotrophic), and nitrification (autotrophic). All three quality classes and indices were combined and presented using quantitative triangles and linguistic tables as the final IQI.

The effect of aging and resuspension on sediment quality was evaluated with the developed IQI as an example to illustrate its unexpected impact on nutrient cycling despite the reduced risk from contaminants on biota. The IQI provides a transparent measure communicating the complex ecological relevant sediment quality with simplicity, which offers a clear overview coupling causes as well as actions for decision makers and can be explained to and understood by non scientists.

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Part I

Sediment as Source/Resource in River Basins

Chapter 1

Introduction

Sediment has been defined as "particulate matter such as sand, silt, clay or organic matter that has been deposited on the bottom of a water body and is susceptible to being transported by water" by WFD AMPS (2004). As an integral part of aquatic ecosystems, sediments provide several services. Four types of ecosystem services are defined by Millennium Ecosystem Assessment (2005) including provisioning, regulating, cultural, and supporting services. Provisionally sediments provide habitat for micro- to macrofauna. They regulate the flood plains with organic rich fertilisers, and most important of all, they support the ecosystem with the retention and cycling of essential nutrients.

However, the environmental quality has deteriorated since— as a consequence of anthropogenic activities— contaminants have been released in the rivers through direct emission from industries, mines, and urban wastewater as well as from diffuse sources such as surface runoffs from urban and agriculture areas. The strong capacity of sediments to bind contaminants makes them sinks as well as sources of contamination if they become resuspended (Förstner and Müller, 1974). As sediment layers from different years reflect the contamination at that time, they have been called the "long term memory of water quality" and "chemical time bomb" in the 1990s (Stigliani et al., 1991). The latter due to the potential risk from contaminants that lie preserved but potentially re-emerging in sediments.

On the whole, mobile sediment in river basins is an important and dynamic vector for contaminants and organic matter. Despite their functions as both source of contamination and resource for the ecosystem, sediments gained little attention in the European Water Framework Directive (WFD).

1.1 European Water Framework Directive

The European Water Framework Directive (WFD) has been regarded as "the most significant piece of European water legislation in more than 20 years" (Joziasse et al., 2007). With the purpose to prevent further deterioration and to pro-

tect and enhance the status of aquatic ecosystem, the European Water Framework Directive was implemented in 2000. The objective to achieve good water status is pursued for each river basin, so that measures in respect of surface water and groundwater belonging to the same ecological, hydrological, and hydrogeological systems are coordinated.

With the WFD, waters are managed for the first time in a transboundary basin scale including groundwater, instead of as separate compartment in local scale under different regulations (Heise and Apitz, 2007). The final aim of the WFD is to achieve a good chemical status in all water bodies and a good ecological status in all natural surface waters. The status would be assessed against a reference derived from the *natural unmodified conditions* for the water body type instead of status quo.

The ecological status of a river basin is defined by biological, hydromorphological, and physico-chemical quality elements in *Annex V*. For the river waters, the biological quality elements focus on the alteration of communities, including aquatic flora, benthic invertebrate fauna, and fish fauna. The hydromorphological quality elements prevent the possible lost of habitat and migration of organisms, while the physico-chemical quality elements measure several concentrations, such as oxygen, salinity, and nutrients, which support the biological elements.

Though ecological oriented, the ecological status is defined by the well-being of few aquatic communities disregarding the sediment microbial and microzoobenthos communities, who support the ecosystem with nutrient cycling. Sediments are only mentioned as habitat for macrozoobenthos community, and are not included as a management target as surface or ground waters, whose ecological and chemical status are defined and to be monitored (Joziasse et al., 2007).

For compliance monitoring for the chemical status as intended by the WFD, environmental quality standards (EQS) would have to be defined for sediments as they have been defined for dissolved substances. While the WFD states in general, that EQS can be set for water, sediment or biota, no sediment standards were actually included in the WFD Daughter Directive. As recounted by Crane and Babut (2007), who give an excellent report on the discussion of deriving EQS for sediments, the main criticisms for not applying EQS for sediments were concerns about the uncertainty connected with factors affecting, or at least influencing, sediment toxicity as well as compliance checking. The former group of concerns stems from sediment heterogeneity, confounding factors such as ammonium, uncertainties related to assessment or testing approaches, and ultimately to the lack of unambiguous relationships between toxic effect endpoints and (individual) chemical concentrations in sediment (Crane and Babut, 2007).

Nevertheless, for management purposes, quality standards have been developed according to different approaches in Europe. An overview has been given by Den Besten et al. (2003) and Den Besten (2007).

1.2 National Sediment Quality Guidelines, Germany as an Example

On national level, several different approaches assessing sediment quality are implemented among European countries. In Germany, three different sediment quality criteria based mainly on chemical data exist and were summarised by Den Besten (2007): Joint Water Commission of the Federal States, (LAWA, Länderarbeitsgemeinschaft Wasser), the Elbe Water Quality Board (ARGE ELBE, Arbeitsgemeinschaft für die Reinhaltung der Elbe), and the index of geoaccumulation (Müller, 1979). The LAWA and ARGE ELBE systems classify sediment qualities according to ecotoxicological effect levels of contaminants, while the index of geoaccumulation considers only the geochemical data.

The most widely used LAWA system includes 7 heavy metals, 28 organic substances, nutrients, salts, and 11 sum parameters. The quality targets, which are correspondent to the good environmental quality category of WFD, were however adopted from soil limit values under Sewage Sludge Ordinance. Though these quality targets were derived from the lowest of ecotoxicological "No Observed Effect Concentrations" of 4 bioassays with a safety factor of 0.1, they were not designate to protect sediment dwelling organisms.

Similar to the LAWA system, the ARGE ELBE system includes 27 Elbe associated priority substances and uses target values decided upon the International Commission for the Protection of the Elbe.

Though sediments are taken as target of management on national level, the management is only aimed at prevention of contamination. Furthermore, the solely chemical quality targets derived from ecotoxicology do not include possible synergistic effect of contaminants or environmental factors; as it has been pointed out by O'Connor and Paul (2000) that except possibly in cases of extreme contamination, chemical data should not be used to predict hazard.

A famous approach to assess sediment quality and the risk from it while addressing the aforementioned uncertainties is the sediment quality triad (SQT) approach which was proposed by Chapman (1990). It assesses the possible ecological impairments using chemical, ecotoxicological, and ecological criteria.

1.3 Sediment Quality Triad

To have an integrated assessment of sediment quality, towards the assessment of ecological risk, a sediment quality triad (SQT) approach was proposed (Chapman, 1990; Long and Chapman, 1985) consisting three components: sediment chemistry to determine chemical contamination; sediment bioassays to determine toxicity; and benthic community structure to determine the status of resident fauna. This approach provides a weight-of-evidence (WOE) framework (Chapman et al., 2002) for an integrated assessment of ecological impairments by contaminations,

and has been widely applied in scientific studies in marine (Long and Chapman, 1985), freshwater (Hollert et al., 2002), and soil studies (Rutgers et al., 2002).

Nowadays, the SQT is seldom applied with the same components, or so-called lines of evidence (LOE), especially the component of benthic community structure due to the intensive labour and complex dataset. The assessment of ecological impairments employs rather the WOE framework with altered or additional LOE according to the stressors and receptors of potential concern (Chapman and Hollert, 2006). Recently, recommendations for German Sediment Quality Criteria using an integrated stepwise approach employing sediment quality triad were proposed, where bioassays are used as trigger step for screening (Ahlf et al., 2002, 2008). However, the ecological analysis of benthic community remained a weak point for the guidance to be included as regulatory tool. Except being habitat for benthic community, are there other important ecological services that sediment provides?

1.4 Ecological Services and Functions

As defined by the Millennium Ecosystem Assessment, the ecosystems provide provisioning, regulating, cultural and supporting services. Among all the services, the supporting services, such as nutrient cycling in aquatic ecosystem, are necessary for the production of all other services. The cycling of important nutrients, such as C and N, is a complex process carried out by a cooperative consortium of organisms with different functions, where microbial communities play a crucial role.

In the cycling of carbon, after the organic polymers were broken down by the shredding invertebrates and fungi, the benthic "microbial loop" takes up the non-refractory dissolved organic carbon to build up microbial biomass and complete the carbon food web (Pomeroz, 1974; Azam et al., 1994). The nitrogenous compounds on the other hand are cycled exclusively by several groups of autotrophic and heterotrophic microorganisms in both oxic sediment water interface and in anoxic sediments. While the microbial loop in carbon cycle is composed of a variety of heterotrophic microorganisms, the nitrification step in nitrogen cycle is carried out exclusively by two groups of bacteria. The importance of nitrogen in all living systems and environmental issues as well as the exclusiveness of its cycling make nitrogen cycle the most sensitive nutrient cycle (Galloway et al., 2004).

The nutrient cycling is a crucial supporting service in the aquatic ecosystem and requires a variety of microorganisms with different functions, which are exclusive in some cases. It is an example of "functional diversity" in action and any lost of functional diversity indicates an impacted ecological quality. The current assessment of nutrient cycling with nutrient concentrations neglected the importance of each important ecological function and its interaction.

1.5 Aim and Outline of the Thesis

Considering sediments as an inseparable part of river basin which plays a major role in the functions that the ecosystem provides, an assessment of sediment quality and its integration into monitoring schemes is of high importance. In many regulations, also in Germany, sediment quality guidelines are based on chemical sediment quality criteria. But do these chemical criteria provide enough measure to achieve well-functioning ecosystems? What are the functions and services provided by sediments in the river basins and which of them could be used in the assessment of sediment quality? Is it possible to develop an integrated but simple indicator to describe the ecological quality of sediments?

The European Water Framework Directive posed a strong driving force to reach a "good ecological status" for all natural surface waters without specifying sediment as one of the management target. Mainly chemical criteria, as in some national regulations, disregard the ecological functions and the sediment dwelling communities. Though the sediment quality triad provides an integrated weight of evidence framework, there is still a gap between the aim of a good ecological status of surface waters and the easily applicable ecological tool such as the chemical criteria for decision makers.

There is therefore a need for ecologically relevant indicators to supplement currently dominant chemical criteria in assessing sediment quality. As a starting point, a well studied and continuously monitored river basin, the river Elbe, was chosen as the main investigation basin. The contamination regime known from previous studies as well as the resulting sampling strategies are summarised in Chapter 2.

In the second part, indicators for sediment quality, two supplementary lines of evidence, ecotoxicity and functional diversity, for the chemical criteria are demonstrated. In Chapter 3, the direct application of sediments on approved bioassay test batteries to assess ecotoxicological quality is presented, showing the spatial distribution and temporal variation of bioavailable sediment contaminations in the river Elbe (partly published in Hsu et al. (2007)). The crucial and complex microbial functions and their diversity in the context of nutrient cycling consortium is further illustrated in Chapter 4. The sensitive autotrophic nitrogen cycling is considered the key ecological function and measured with microsensors showing spatial interactions of nitrogen cycling. The influence of environmental factors as well as lost key function on heterotrophic carbon cycling is then measured with carbon substrates utilisation patterns showing their relative structure and therefore functional stability. A measurement employing DMSO reduction is proposed at the end aiming at simultaneous indication of both autotrophic key function and heterotrophic functional capability.

The final aim is to **develop ecologically relevant quality indicators for sediments with regard to the ecotoxicities and functional diversity in nutrient cycling in order to assist the river basin management aiming for good status of rivers**. This is accomplished and demonstrated in Part III.

The integration from ecotoxicity and functional diversity data to quality indicators is summarized applying fuzzy logic theory in Chapter 5. The conceptual integrated quality indicators (IQIs) are presented in classes representing level of contamination impact and community resistance, which represents the ecological importance when combined. The developed IQIs are at the end applied in Chapter 6 on aged sediments, evaluating the impact of sediment resuspension on the ecological quality as a confirmation and pragmatic example of the developed IQIs.

Chapter 2

Elbe as Investigated Basin

There are several transboundary rivers in Germany, including the Elbe, the Rhine, and the Danube. Among these major rivers, the river Elbe originates in the Czech Republic, after entering Germany it receives waters from tributaries Saale and Mulde in the former East Germany, and finally flows in the North Sea. The river Elbe has a total length of 1094 km and catchment area of 148,268 km^2 and is the most important river in the northern Germany (FGG Elbe, 2004).

In the last half century, large quantities of heavy metals and organic contaminants have been discharged from a number of historic sources including mines in the Ore Mountain area, a number of chemical industries in the Czech Republic and mining and industrial activities in the Mulde and Saale subcatchments (Ahlf and Gratzer, 1999; Förstner et al., 2004). Since the German reunification in 1990, the water quality has improved significantly, including the recovery of aquatic communities (Schöll, 1999). In 2002, the first international Elbe swimming day was organised to celebrate the safe-for-swimming water quality. However, despite the improvement in water quality, large amounts of pollutants are still present in contaminated sediments or are still being released into the river basin from legacies of the past. The extensive range of "substances of concern" transported by sediments along the River Elbe include PCBs, dioxins, HCH, HCB, arsenic, cadmium and mercury (Heise et al., 2005).

The sediment quality of the river Elbe is monitored monthly at 12 sites with the 27 Elbe associated contaminants by the Federal States, coordinated by ARGE-ELBE and IKSE. With the continuous monitoring data from the ARGE-ELBE and several thorough studies (Heise et al., 2005; Netzband et al., 2002) in the Elbe basin, the contamination regime in the Elbe basin is well described. Heise et al. (2007) summarised recent contamination pattern along the river Elbe. Figure 2.1 depicts the contaminant concentrations in sediments exceeding target values for protection of aquatic communities from the 12 monitoring sites along the river Elbe between 2000 and 2006. The contamination patterns from Schmilka at German Czech Border to Magdeburg were similarly dominated by high concentrations of DDT and its derivatives (collectively DDX). The exceedance level

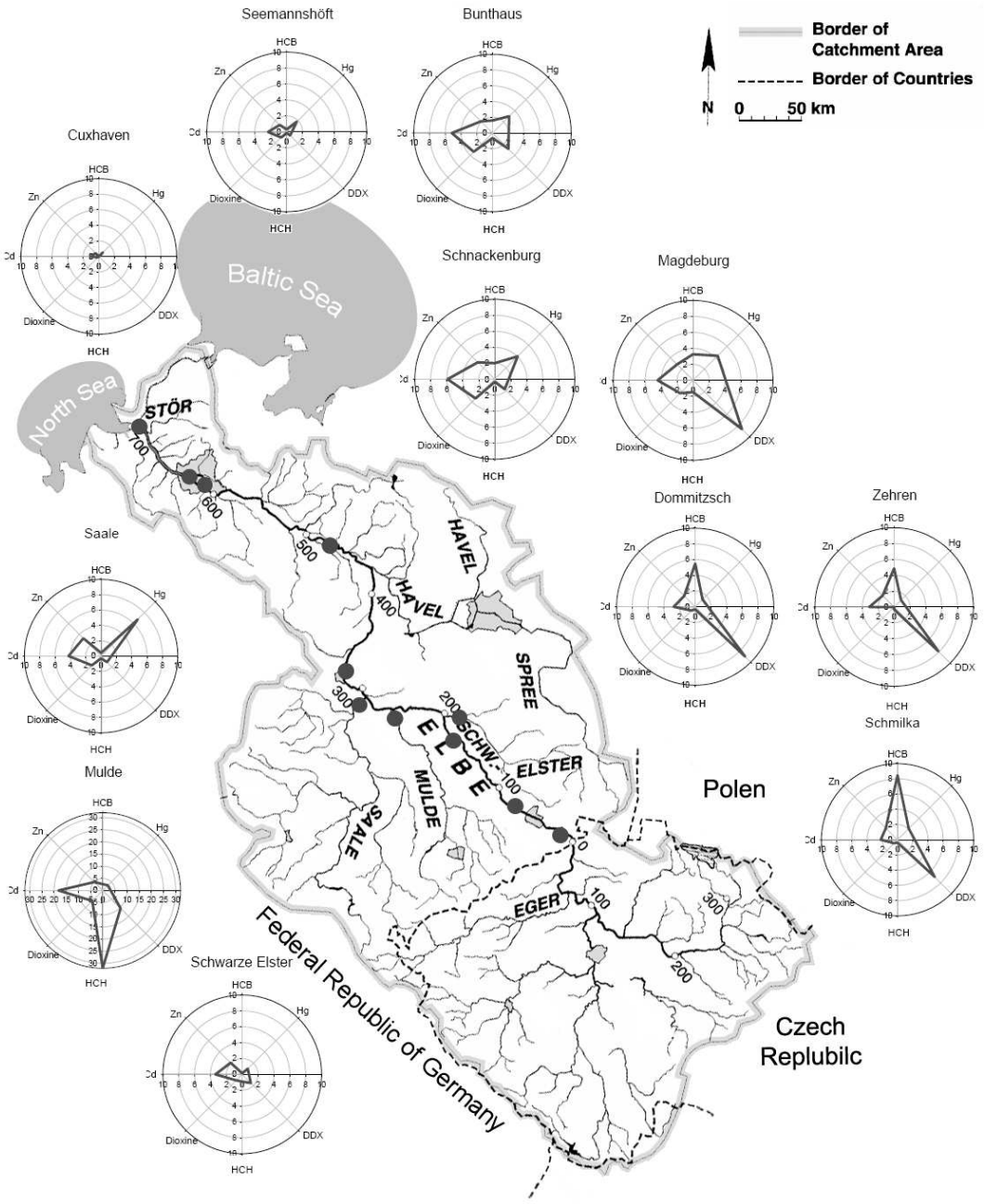


Figure 2.1: Concentrations of contaminants in sediments exceeding target values for protection of aquatic communities along the Elbe between 2000 and 2006. Values were average concentrations divided by target values. Figures modified from Heise et al. (2007)

of the DDX concentrations from Schnackenburg to Cuxhaven decreased with the flow and showed strong influence of tidal dilution downstream from Bunthaus. The contamination patterns from the tributaries exhibited high cadmium exceedance in the Mulde and Saale. Sediments in Saale had high exceedance levels of mercury, while sediments in Mulde had extreme high concentrations of HCH. Though the high load of HCH in Mulde was only slightly transported and observed in the Elbe, the importance of the contaminants transported by sediments are not negligible.

To further investigate the ecological quality of sediments in the river Elbe, two sampling strategies were used in this thesis. First, a spatial sediment sampling along the river Elbe was conducted to screen the input of contamination from tributaries with toxicity bioassays. Second, a temporal sediment sampling was performed at a downstream site near Hamburg to verify the natural variations of toxicities and microbial communities in sediments related to hydrological and biological events. The spatial and temporal sediment sampling provided a range of possible responses for further development of quality indicators. Finally the impact of the lost key function, nitrification, on sediments was investigated with sediments near Hamburg.

2.1 Sampling Sites

Considering the possible sources of contamination originated from the tributaries Saale and Mulde, sites before and after the inflow of the tributaries were selected. Sediments and water samples were collected at Roßlau, before the Mulde inflow; at Muldestein in the Mulde; at Saale inflow; at Barby, downstream from Saale inflow; and Schönberg, downstream at the Elbe meander (see Figure 2.2). These samples were applied on bioassays to verify and compare the potential hazards measured chemically.

For the development and confirmation of the quality indicators, temporal variations of ecotoxicities as well as the carbon substrates utilisation profiles of the microbial community were monitored to observe the potential variation and range of responses. To achieve an easy access for monthly sampling, the downstream site Over (10.03598 E, 53.44628 N) was chosen near Hamburg, where the monitoring station Bunthaus is located only 2 km downstream. The possible influence of another surface water type among European rivers was compared with one site at Liskesbrug (5.38147 E, 51.27700 N) in the stream Dommel, tributary of Meuse in the Netherlands (Figure 2.3). Over is located at the Elbe downstream at 607 stream km from the German border with a river width of 400 m. Liskesbrug on the other hand is located in front of a weir, where fine grained organic rich sediments can be found, in the upper reaches of the headwater stream Beekloop of the river Dommel. Sediment and water samples were collected monthly between January and September 2005. At Over, fine sediments can be found in groyne fields, which

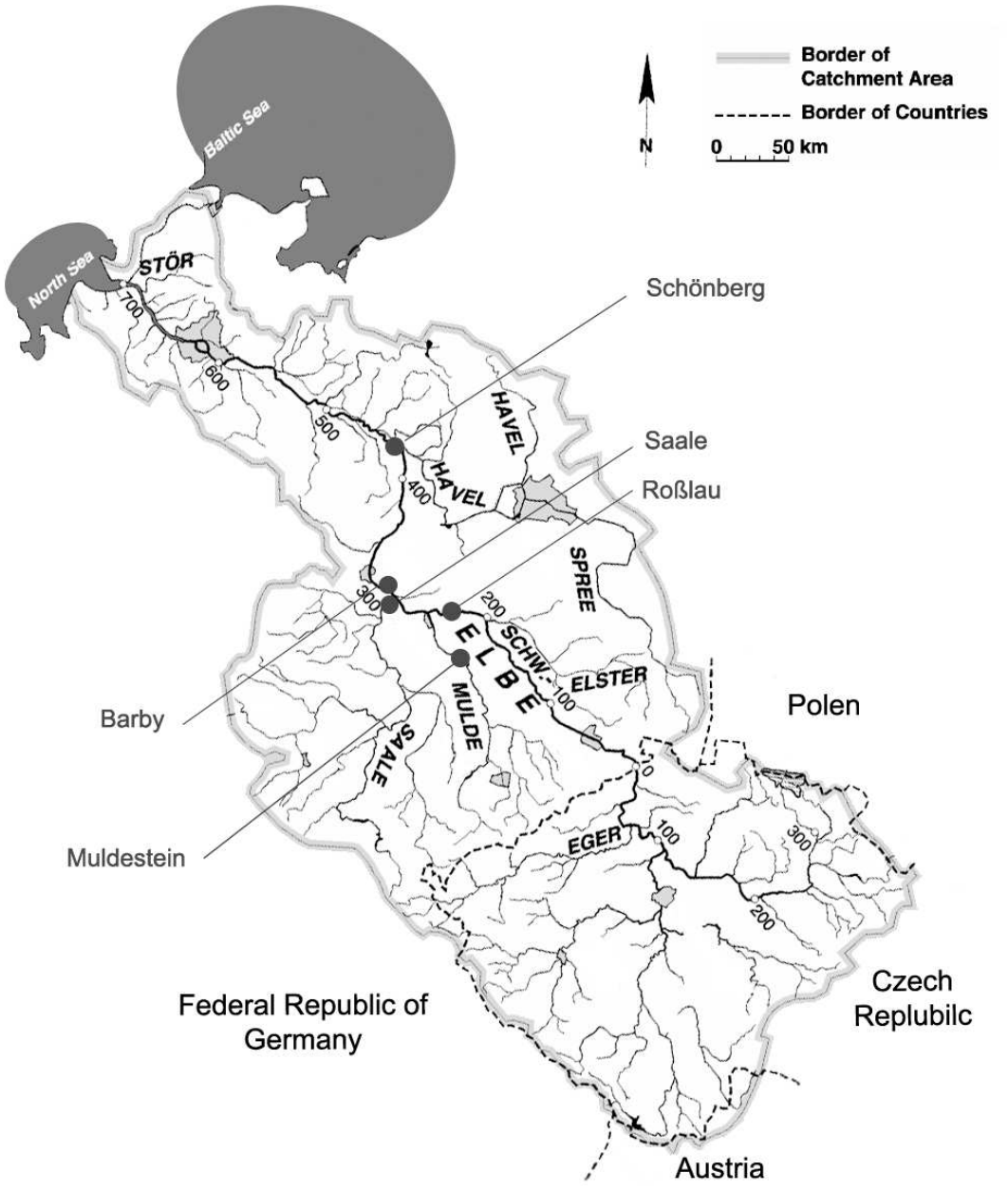


Figure 2.2: Sampling sites to determine spatial toxicity distribution in the River Elbe. (modified from IKSE)

have been constructed along the Elbe to facilitate navigation. Sediment and water samples were collected from a boat. At Liskesbrug sediment and water samples were transported to the laboratory directly after sampling like in Hamburg.

Further sediment samples to investigate the influence of nitrification as the key ecological function on ecological quality of the sediments were collected at Over in the river Elbe, where the range of responses is well known.

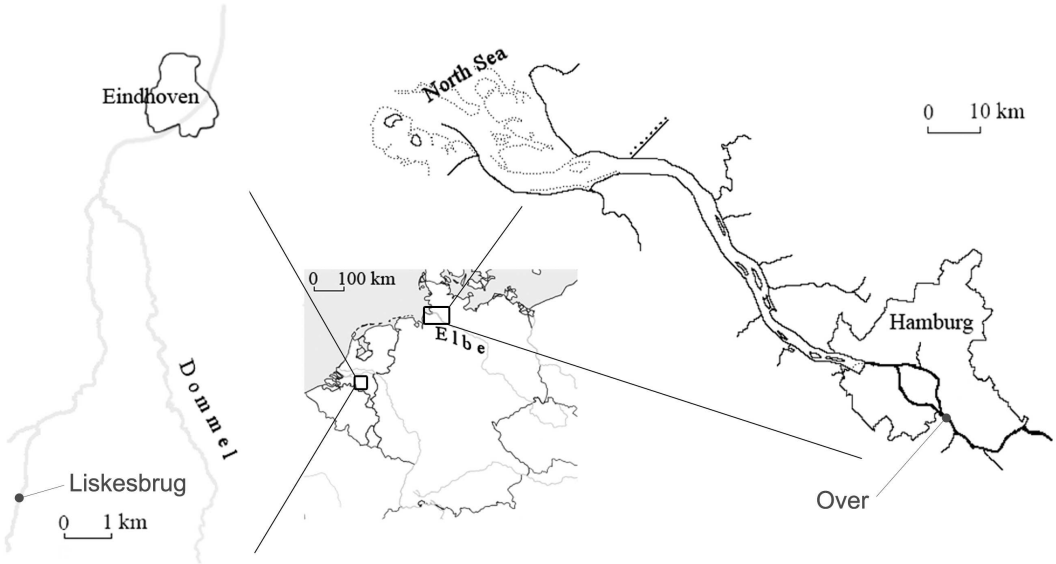


Figure 2.3: Sampling sites: Over at the River Elbe near Hamburg, and Liskesbrug at the River Dommel near Eindhoven.

2.2 Sampling Methods

Layered sediments were collected at all sites so that the historic contamination could be compared with current ones. Depending on the sediment depth, sediments of 0-5 cm (at all sites) and 10-15 cm (at Liskesbrug and along the Elbe) or 15-20 cm (at Over) depth from sediment water interface were collected using a sediment corer with diameter of 10 cm. Three cores were collected and combined to obtain a representative sediment sample. Water samples were collected at the same sites as sediment. All samples were stored at 4℃ to minimise microbial activities during transport before tested within 4 weeks. Air and water temperatures were measured on site, while pH value, redox potential, dry weight and organic content were measured in the laboratory before further handling.

Part II

Indicators for Sediment Quality

Chapter 3

Sediment Ecotoxicology

The term "ecotoxicology" is first proposed by Truhaut (1977) and defined as "the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context". The discipline begins with the study of the effect of toxicants on single species under environmental conditions and is moving nowadays towards an effect prediction for ecosystems. It is the study of "ecology at the presence of toxicants" (Chapman, 2002) with the objective to understand and predict effects of chemicals on natural communities under realistic exposure conditions by extrapolating effects of stressors from single species to the ecosystem.

The toxic effect of a chemical on organisms in sediments depends strongly on the bioavailability. Due to different hydrophobicity of chemicals, the proportion of chemicals adsorbed on sediment particles to those dissolved in pore water differs. It has been assumed in sediment quality criteria for non-ionic organic (US EPA, 1993) that only the soluble fraction of a chemical would be accessible to biota (O'Connor and Paul, 2000). However, due to various exposure routes, for example uptake via food, also the adsorbed fraction of chemical may become bioavailable (Harkey et al., 1994; Liss and Ahlf, 1997). Furthermore, the bioavailability of a chemical has been shown to change with varying environmental conditions, such as pH and redox potential (Griscom et al., 2000).

In contaminated sediments, chemical cocktails instead of single chemical are present. It is difficult to extrapolate from measured environmental chemical concentrations and laboratory chemical toxicity data to potential impairment of biota (risk) in the environment. Therefore bioassays testing directly on sediments was recommended by Burton et al. (2002), and has been used e.g. in Canada to derive national criteria for remediation of contaminated sites (Van Gestel, 1997).

3.1 Bioassays

Bioassays are biological testing systems measuring chemical toxicities on living organisms, whose growth and activity can become impacted by chemical effects and environmental conditions. They are widely applied to assess chemical products as regulated by the new European chemical legislation REACH (**R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical substances) and environmental risks. In environmental studies, bioassays provide a more direct measure of environmentally relevant toxicity than chemical analysis do since they integrate environmental variables and contaminants (Keddy et al., 1995). Bioassays applied on organisms range from bacteria to fish. Endpoints from the level of molecular biomarkers up to population indexes are available for sediments. Due to the diverse organisms present in sediments and the differences in their sensitivities, a battery of bioassays is usually applied. By using bioassay combinations exposing organisms with different sensitivities via various exposure routes, test batteries can cover a broad range of possible adverse effects of pollutants (Dutka et al., 1989).

A minimum set of four bioassays (algal growth inhibition test, bioluminescence inhibition test, bacterial contact assay, and nematode test) is shown necessary to characterise most of the sediment toxicity in the Elbe River Basin, and is suitable for the assessment spatial distribution of sediment ecotoxicities along the river Elbe. This may be reduced to two bioassays (algal growth inhibition test and bacterial contact assay) if only a small area such as the Hamburg Harbour is monitored (Ahlf et al., 2002) due to the stable contamination pattern.

For the assessment of temporal variations of sediment ecotoxicities at Over, the set of two bioassays is therefore enough. In order to characterise spatial sediment toxicities and be efficient in measuring temporal variations of sediment toxicities of single site, the time-consuming and not-yet standardised nematode test was removed from the set of four bioassays. As a result, the following set of three bioassays is applied to assess the ecotoxicities of the sediments, elutriates and water samples in this study.

3.1.1 Elutriates

In the algal growth inhibition test and bioluminescence test, test organisms are exposed via water phase (sediment elutriate, water samples). Water elutriates were prepared from sediment samples by adding 10 ml sediment and 40 ml deionised water in a 500 ml PE bottle. The mixture was shaken overhead for 24 hours at 70 rpm and then centrifuged at 10,000 rpm for 15 min. The supernatant was removed with a pipette and further used for bioassays within 48 hours.

3.1.2 Algal Growth Inhibition Test (AGI)

The inclusion of algae in a test battery has been recommended by Giesy and Hoke (1990), because they represent the primary producers in the aquatic environment and are sensitive to specific chemicals, such as herbicides. Inhibition in algal growth inhibition test can be interpreted as an adverse (direct or indirect) effect on photosynthetic activity of the algal cells. For this study, the algal growth inhibition test of the fresh water green algae *Pseudokirchneriella subcapitata* with elutriates was chosen, because it has been standardized internationally (DIN ISO 38412-33). The *P. subcapitata* culture was obtained from German culture collection SAG (strain number 61.81).

A miniaturised method using 24 well cell culture plates was employed. Serial dilution of the sediment elutriates were prepared with bi-distilled water. For the test, 0.2 ml of a pre-incubated 3 day old algal culture with a density of 10^4 cells/ml was added to 1.6 ml of the elutriate or its dilutions. 0.2 ml of 10-times concentrated ASTM medium was added for nutrient supply. This set-up resulted in exposure of the organisms to elutriates of 12.5 to 80%. For the test, the algae were incubated under continuous light (3000 Lux) at 20℃. The autofluorescence emitted by chlorophyll α was measured every 24 hours, and the growth rate was calculated over 72 hour incubation time. Bi-distilled water was taken as control and 10 mg/L 3,5-DCP (dichlorophenol) was taken as positive control to verify the sensitivity of lab culture.

3.1.3 Bioluminescence Inhibition Test (LB)

The standardised DIN EN ISO 11348-1-3 bioluminescence inhibition test of *Vibrio fisheri*, or so-called Microtox® test, with elutriates is widely used to screen chemical toxicities (Kwan and Dutka, 1990). This marine bacterium emits luminescence during metabolic activity, which can be adversely influenced by chemicals.

The freeze dried marine bacterium was obtained from Hach Lange GmbH. Sediment elutriates were first adjusted to pH of 7.0±0.2 and salinity of 2.0±0% for the marine bacterium. Elutriates were further diluted with artificial seawater using an automated dilutor to obtain a final test concentration of 80 to 16.67% elutriate. 50 µl activated bacteria was exposed to 150 µl elutriates for 30 minutes at 4℃. Artificial sea water and 5 mg/L 3,5-DCP were taken as negative and positive controls respectively. The luminescence was measured before and after the exposure to the elutriates, and the growth factor over 30 minutes was calculated as endpoint.

3.1.4 Bacterial Contact Assay (BCA)

The bulk sediment test of *Arthrobacter globiformis* according to DIN 38412-48 was chosen for its direct exposure of organisms to sediments. *A. globiformis* is a common soil bacterium with an affinity to surfaces, which facilitates the assessment of particle-bound contaminants. The test is based on the measurement of dehydrogenase activity. Dehydrogenase is an important enzyme of bacterial metabolism, involved in respiratory processes. Its activity is measured using the redox-indicating fluorescent dye resazurin (Rönnpagel et al., 1995). Fluorometric calibration was performed to account for possible quenching effects from sediment (Heise and Ahlf, 2005). The culture of *A. globiformis* was obtained from German collection of microorganisms and cell cultures DSMZ (strain number 20124).

A miniaturised method using 24 well cell culture plates was employed. 0.6 g sediments were first homogenised and aerated with 0.6 ml deionised water with horizontal shaker at 200 rpm for 48 hours. After pasteurisation of sediment slurries, 0.4 ml of *A. globiformis* was added at exponential growth phase and incubated for 2 hours in the dark at 30℃. 0.8 ml of redox indicating dye resazurin in 0.1 M Mops buffer (pH= 8.2) was added and the reduced fluorescent dye product resorufin was measured with a plate fluorometer every 10 minutes up to 40 minutes. The calibrated slope of dehydrogenase kinetic was calculated as endpoint. Quartz sand W4 with 50% water content was used as reference sediment (Heise and Ahlf, 2005). Reference sediment containing 6 mg/kg BAC (benzalkonium chloride) was taken as positive control.

3.1.5 Validation of Results

The results of bioassays were calculated as percent inhibition compared to the negative controls. Three replicate samples and controls were taken for algal growth inhibition test and bacterial contact assay, whereas two replicates were used for the highly reproducible bioluminescence test (De Zwart and Slooff, 1983). The variability of the bioassays can be calculated from the responses of each positive and negative control. Variability of bioassays on environmental samples is often considered to be high due to the heterogeneity of environmental material and biological variability of test organisms. From results of bioassays previously performed with samples from the river Elbe, a standard deviation of 1 to 5% is observed between replicates in the positive and negative controls (Ahlf and Heise, 2005). The standard deviation between positive controls in different tests lies between 10 to 15% for the conducted bioassays (Ahlf and Gratzer, 1999). Therefore, depending on the bioassays employed, a variability of 10% to 20% can be characterised, indicating the range of uncertainty for further interpretation (Ahlf and Heise, 2005).

Extreme values above 100% or below 0% are possible and not related to low data precision. Inhibition of more than 100% can be explained by a die off

of organisms below the original inoculation number. For example, in the algal growth inhibition test, the fluorescence at day three can become smaller than that at day zero under those circumstances. Consequently, the growth rate of the tested sample would be negative and an inhibition of more than 100% would be measured. Negative inhibition, or the "stimulation of algae", can be a result of tested material with a higher nutrient content (Dubé and Culp, 1996) or stress induced fluorescence per cell. In the case of the bacterial contact assay, a strong stimulation was found in the sediments rich in organic matters. It is shown by Stuijfzand et al. (2000) that rapidly biodegradable organic substrates stimulate the growth of invertebrates in sediments. The same phenomenon can also be expected in bacterial contact assays when such substrates are constituents of the sediment and add valuable substances to the test medium, which is already rich in organic matter. *A. globiformis* is able to degrade sorbed organic substances, which are present in the sediments. This results in a measured stimulation, when a nutrient-lacking control such as quartz sand is used.

3.2 Sediment Ecotoxicities in River Basins

The sediment ecotoxicities in river basins are greatly influenced by the distribution of contaminants and their interaction within the river-sediment-soil-groundwater system. Following the sampling strategies, sediment toxicities are described according to first the spatial distribution indicating source of contamination in the Elbe, second the temporal variations in rivers Dommel and Elbe indicating baseline responses, and at the end the influence of inhibited ecological function.

3.2.1 Spatial Distributions

The spatial distribution of the sediment toxicities are shown in Figure 3.1. Generally the distribution of toxicities was similar in upper and deeper sediments with higher toxicities in the upper ones. Sediments from Saale and Barby induced the highest toxicities.

Upper sediments at all sites except for Muldestein exhibited algal toxicities, with highest toxicity in Barby, shortly after Saale inflow. Bioluminescence toxicity of upper sediments, on the other hand, was only observed in Muldestein at 50%, while the other sites showed around 20% inhibition. The toxicities of bacterial contact assay increased downstream from Roßlau to Barby, and decreased again at Schönberg.

The deeper sediments showed algal toxicities only after Saale inflow. The bioluminescence toxicities in deeper sediments were similar to those in upper ones with lower magnitude. The toxicities of bacterial contact assay were found only in Saale deeper sediments.

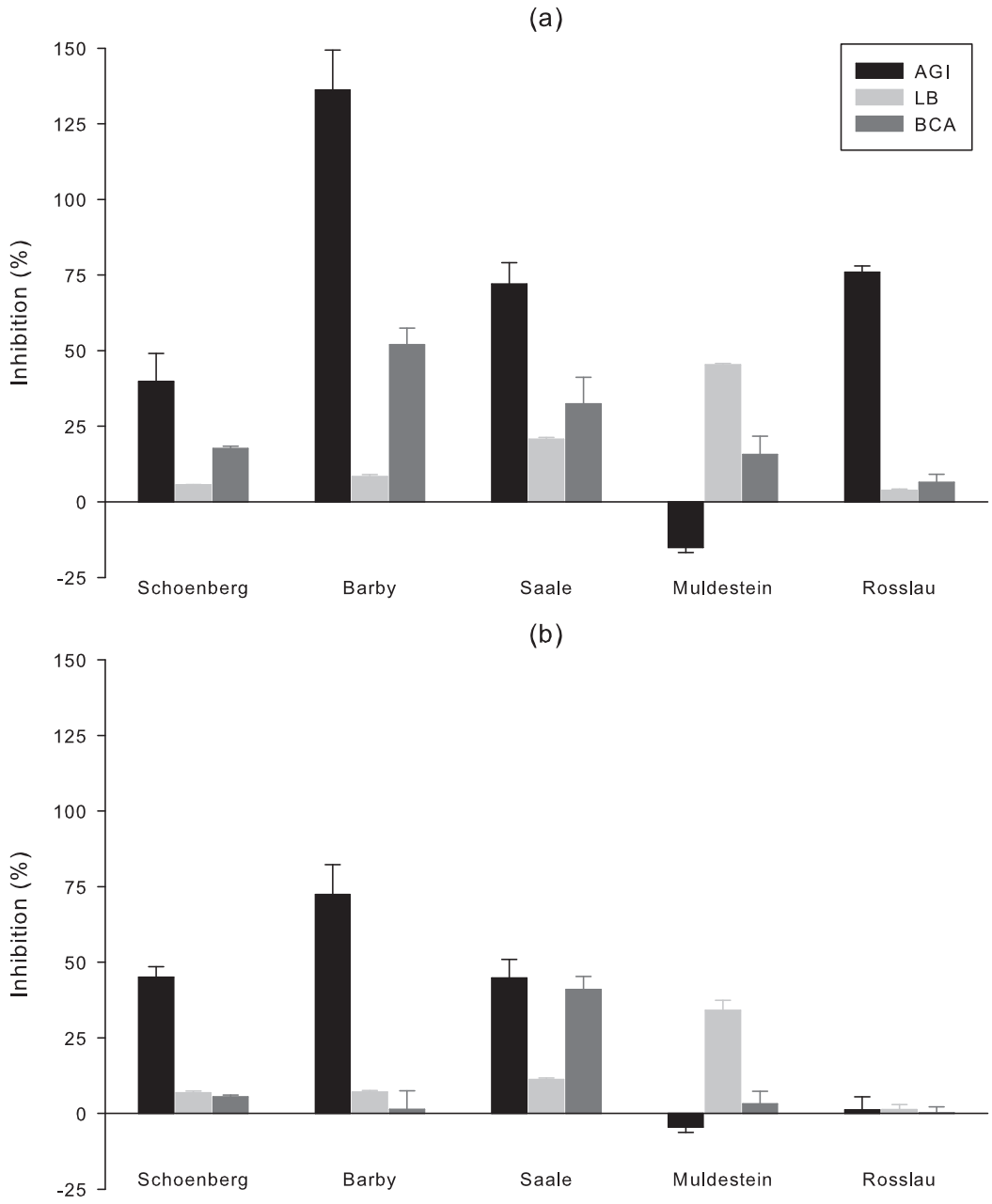


Figure 3.1: Spatial distributions of ecotoxicities in Elbe upper (a) and deeper (b) sediments. AGI represents algal growth inhibition test, LB bioluminescence inhibition test, and BCA the bacterial contact assay.

In principle, suspended matter reflects the current contamination situation. In sediment cores, dating of different sediment layers which increase in age with depth give ambient contamination profiles over time given the local deposition rate. Prominent examples of those profiles are the concentrations of lead or DDT in sediment cores, which show peaks in sediment layers resulting between 1960 and 1975 when both were extensively released to the environment (Götz et al., 2007; Klös and Schoch, 1993). The top sediment layer usually consists of freshly settled suspended material, which has been eroded from sediments upstream, emitted from upstream regions (e.g. soil erosion) or produced by phytoplankton (Graf et al., 1982). Due to its adsorption properties, suspended material will reflect the current contamination pattern in the basin.

In this study, different sediment layers (upper and deeper) were sampled in order to assess availability and effect of those contaminants which are currently still transported through the basin, and those, which are stored in deeper sediment. The latter may become less available according to aging processes (Alexander, 2000) though highly contaminated. They will also not become easily resuspended and therefore may not be of transregional importance, but their contaminant depot may impact the local biological community. Therefore, a measured hazardous effect in the upper sediments indicated the presence of contaminants in recently transported sediments. A measured inhibitory effect in the deeper sediments, on the other hand, reflected previous situation of contamination in the sediments under aging processes. The previous contamination could be a result from extensive diffusive source and transport, such as DDT and lead, or from a distinctive local point source, such as effluent of chemical plants.

In the Saale and Mulde tributary, due to historic chemical industries, a variety of contaminants are released to river water and sediments (Netzband et al., 2002). These historic contaminations were reflected by a distinct bioluminescence toxicity of deeper sediments in Muldestein as well as distinct bulk sediment toxicity of deeper sediments in Saale. The toxic effects in deeper sediments were observed only in tributaries, indicating an historic local contamination. The algal toxicities in deeper sediments were observed downstream from the Saale confluence, showing possible historic downstream transport of certain contaminants. The results indicated two clear historic sources of contamination from Mulde and Saale tributaries. The generally lower toxicities in the deeper sediments than in the upper ones may be the result of aging processes, which reduces the availability of contaminants to biota.

Assuming that the toxic effects in upper sediments is related to more recent contaminations, another source for algal toxicities was observed upstream from Roßlau, causing around 70% of algal toxicity in upper sediments. It would be expected that the dilution would lead to lower algal toxicities downstream, if there was only release from upstream of Roßlau. However, similar toxicity was observed in the Saale mouth, causing elevated algal toxicity shortly downstream from the Saale inflow. A dilution effect on algal toxicity was only observed 150

km downstream in Schönberg. Similar toxicities of bioluminescence and bulk sediment were still present in Saale and Mulde. Saale sediments remained a source of inhibitory effect for algae from bulk sediments decades after the end of industrial activities in the Saale tributary. The results indicated that the sources causing the historic contaminations were still releasing contaminants to sediments. Furthermore, contaminants causing bulk sediment toxicities were no longer restricted to the Saale tributary and were transported down to Schönberg. It is shown by Baborowski et al. (2007) and Heise et al. (2003) that at high flood events, these contaminations can be found in both Elbe floodplain near Schönberg and Hamburg Harbour.

The results coincided with the study from Grote et al. (2005), who performed a transect survey of sediment contaminants and ecotoxic effects of surface sediments (0-10 cm) at 13 sites in the river Elbe before the flood in 2002. He measured more than 70% algal toxicities at Königstein (near the Czech Boarder), Wittenberg (upstream from Mulde inflow), Spittelwasser in Mulde, Barby and Magdeburg, as well as more than 70% of bioluminescence toxicity at Spittelwasser in the Mulde. He further developed a partial causal relationship of PAHs on the algal inhibition. High DDT concentrations ($>$ 1.5 mg/kg) were surprisingly measured at Czech Boarder (Grunewald et al., 2004), which was attributed to a leaking deposit of chemical production waste in the Czech Republic and could contribute to the algal toxicity upstream from the Mulde inflow.

The spatial distribution of sediment toxicities in the river Elbe was shown to be an adequate indicator to screen for impacts caused by contaminants from different sources at different times. The Saale sediments seemed to provoke sediment-bound bacterial toxicity, while the Mulde sediments induced strong bioluminescence toxicity. This corresponds well to the contamination regime depicted in Figure 2.1 that sediments from the Mulde and the Saale tributaries demonstrated distinct patterns of contaminants, which are different from the Elbe main stream, and a dilution effects between Barby and Schönberg can be seen. A different source causing algal toxicities was implied to be upstream from Roßlau. The baseline pattern of toxicities can be expected in sedimentation areas, including flood plains and groyne fields downstream from Saale tributary, where river Elbe receives the majority of the contaminations. At flood events, the contaminated sediments could be transported and relocated downstream causing elevated toxicities in surface sediments as well as in river waters.

3.2.2 Seasonal Variations

Previous studies of temporal quality changes in sediments mostly involved either long term chemical monitoring data or intensive short term ecotoxicological assessments in areas of concern. Though chemical analysis shows a long term trend of decreasing concentrations of contaminants in water and sediments of the river Elbe (Netzband et al., 2002), the environmental risk of contaminated sediments

transported downstream during high flood events is still present (Heise et al., 2003). The risk may be elevated under more frequent drought and flood events caused by global climate change. In most cases, ecotoxicological parameters are not included in monitoring programmes. Here the toxicities of sediments and water were analysed for 10 months in rivers Elbe and Dommel. The aim was to observe temporal variations of river water and sediment quality using ecotoxicological endpoints, which represent the possible impact on the biota and its relation to sediment transport along the river. Furthermore, the results provide a better understanding of the behaviour of sediment toxicity and important factors influencing it, thus aiding further river basin risk assessment and management.

River Dommel

The river Dommel is a small lowland stream fed mainly by rainwater and spring rains. It is characterised by a sandy bottom with discharge ranging between 0.4 and 5 m^3/s. The sampling site Liskesbrug is located upstream in the headwater stream Beekloop in front of a weir, which regulates for constant flow velocity. As illustrated in Figure 3.2, ecotoxicities did not vary much in the sampling period. However, there was a clear pattern of different sensitivities of bioassays applied to water and sediment samples. In the water phase (Figure 3.2a), only inhibition of algal growth was observed. It was highest in April and lowest in January. A slight stimulation on bacterial dehydrogenase activity was also observed in March. The responses of the three bioassays on the upper and deeper layers of sediment were comparable. One can not differentiate the responses between freshly deposited upper sediments and older deeper sediments, due to the fast flowing river water and thin sediment layers in the river Dommel. In Figures 3.2b and c, a strong stimulation effect on the bacterial contact assay is demonstrated. This coincided with relatively low inhibition of algal growth and bioluminescence. The weakest stimulation effects on bacteria in the contact assay were observed in June in both layers of sediments. This coincided with significantly higher metal concentrations measured in sediments (Poot et al., 2007).

In the river Dommel at the Liskesbrug sampling site, no clear seasonal variability was observed. The hydrology and organic input were relatively constant, due to the stream size and sampling location in front of a weir.

River Elbe

In the Elbe River, sampling site Over is located downstream in a deposition groyne field, where fresh sediments has been deposited for decades. The variation of water discharge in Elbe downstream ranged between 265 and 2280 m^3/s during the sampling period as a result of both seasonality and upstream activities. The sampling of river water and sediments taken from two depths enabled the estimation of seasonal interactions between upper sediment layer and water

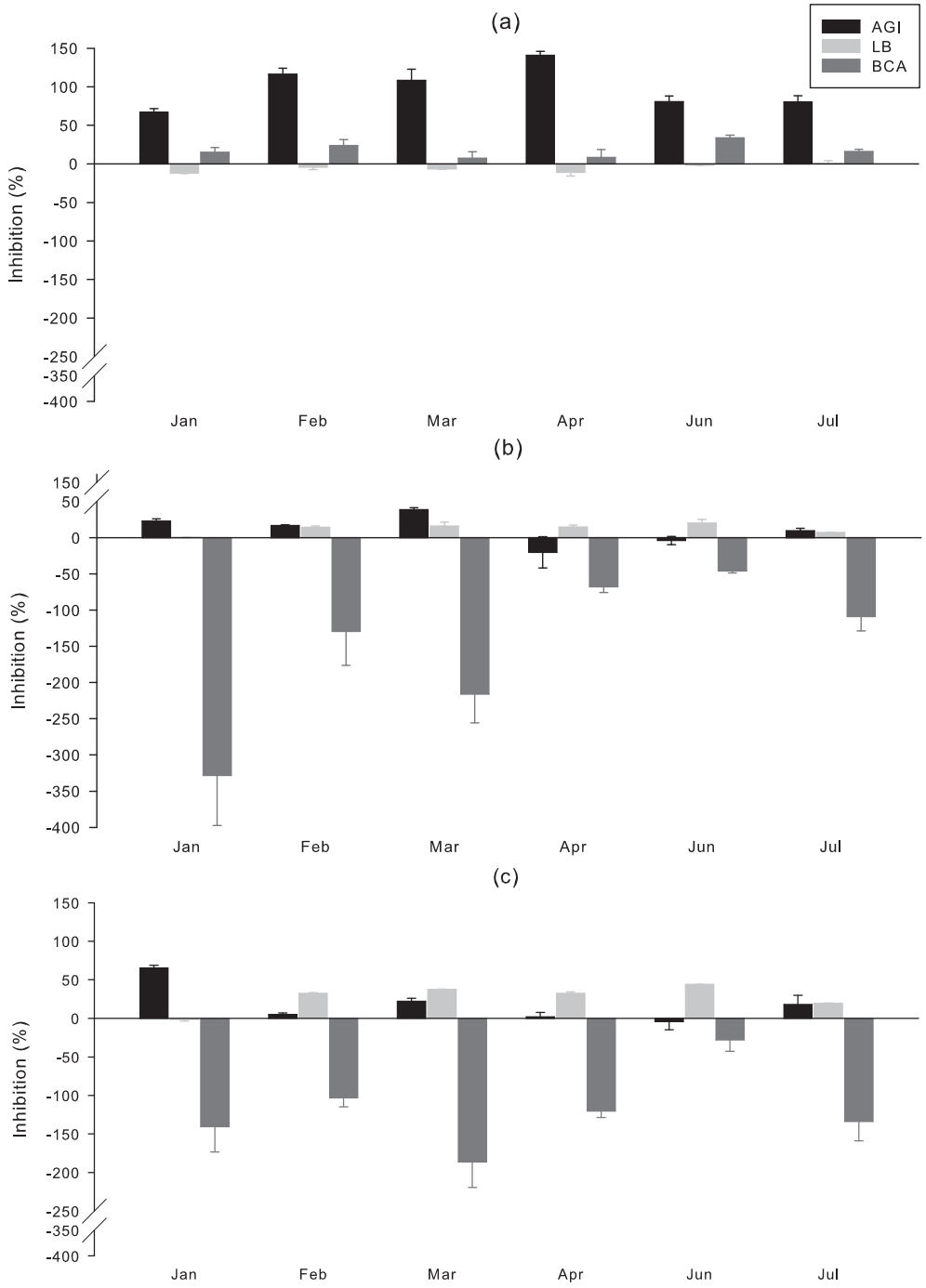


Figure 3.2: Seasonal variations of toxicities in Dommel water (a), upper (b), and deeper (c) sediment samples. AGI represents algal growth inhibition test, LB bioluminescence inhibition test, and BCA the bacterial contact assay.

phase. In opposite to the river Dommel, a clear seasonal variation of toxicity decreasing from river water via the upper layer to the deeper layer of the sediment is depicted in Figure 3.3.

In the river water (Figure 3.3a) algal growth were completely inhibited during the sampling year except for a strong decrease in April and May. Moreover, algal growth stimulation was observed in May, when algal bloom is usually observed in nature. The inhibition of microbial bioluminescence and bacterial dehydrogenase activities on the other hand only varied in a narrow range. Elutriates of the upper layer of sediment (0-5 cm) showed a trend of algal growth inhibitions, which is similar to that of water phase in a smaller magnitude, and which decreased from 70% to 5% in May (Figure 3.3b). However, the inhibitions of bacterial sediment toxicity varied with high peaks in February, March, May, and September from 50% to 70%, with slight stimulations in December, April, and July of 25%. The measured toxicities were in similar range compared to the study from Ahlf and Gratzer (1999), who reported a 97% algal inhibition, 26% bioluminescence inhibition, and 60% inhibition of bacterial dehydrogenase activity (of *Bacillus cereus*) of surface sediment at Geesthacht, 10 km upstream from Over, in 1995.

Changes of microbial bioluminescence inhibition between different months were not obvious in the upper sediment. A seasonal variation on a higher toxic level was found only in the deeper sediment (Figure 3.3c). The inhibition of bacterial dehydrogenase activity in the deeper layer of sediment showed a similar pattern compared to the upper one but to a lesser degree. Inhibitions of bioluminescence were similar to that of the dehydrogenase activity, whereby the lowest inhibition was found in July.

In the river Elbe at the Over site, a strong temporal variation of toxicities, particularly in river water and the upper sediment, was observed in April and May. Quality of river water as well as of freshly sedimented material is in the Elbe strongly influenced by water discharges. During single high discharge events in the Mulde and Saale, e.g. 70 to 75% of the annual load of suspended particulate matter (SPM) is transported (Heise et al., 2007). Though this material is partly contaminated due to erosion from polluted zones of the river bed or from flooded areas, non-contaminated particles tend to decrease the contaminant concentration per mass suspended matter. Additional complexity is added due to autochthonous phytoplankton bloom in the Elbe: Böhme et al. (2006) assume, that 66 to 82% of the SPM load in Schnackenburg in the vegetation period is produced in the Elbe itself, providing plenty new surfaces, to which contaminants can bind.

Little is known though, how bioavailabilities of bound contaminants vary during different discharge periods and whether partitioning between dissolved and solid phases are influenced. During the sampling period, two peaks of high discharge (1971 m^3/s at the end of February and 2417 m^3/s at the end of March) were measured at Zollenspieker (few kilometres upstream from Over), while the average discharge during the sampling period was 788 m^3/s (Figure 3.4). The

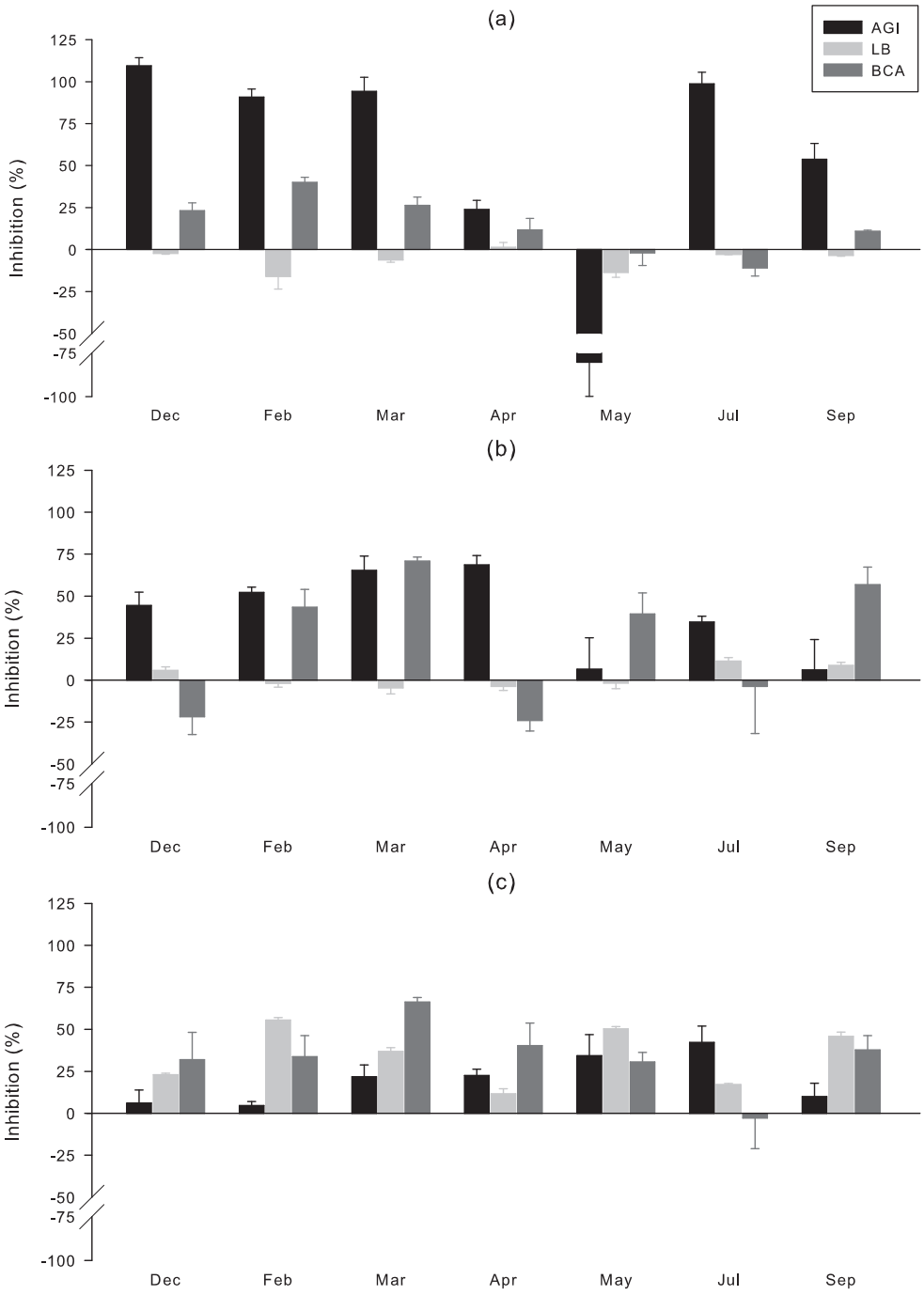


Figure 3.3: Seasonal variations of ecotoxicities in Elbe water (a), upper (b), and deeper (c) sediment samples. AGI represents algal growth inhibition test, LB bioluminescence inhibition test, and BCA the bacterial contact assay.

March samples taken after the first high discharge indicated a slight increase of inhibition for all bioassays in all matrices. This could be a result of the flood event, connected with increased dissolved contaminant loads and the deposition of higher amounts of contaminated suspended matter from the areas upstream of Over, where the freshly deposited sediment toxicity is significantly higher e.g. in the Magdeburg area (Matthäi et al., 2007).

When the April samples were taken a month after the highest peak of water discharge they showed a decrease of inhibitions for all bioassays in all matrices, except for algal inhibition from surface sediment. Presumably, a very high discharge increased the mixing processes of river water and sediment phase and/or increased transport of upper sediments with the receding flood wave. A possible mechanism is that the additional particle surfaces bind dissolved contaminants due to an increase of sorption sites. This process could explain the decrease of inhibition in the water phase and the increase of labile bound pollutants in freshly deposited sediment.

Another more profound reason for the decrease of water toxicity may be the annual phytoplankton bloom in the river Elbe during spring and summer, since the water discharge reduced to average discharge level (760 m^3/s) before the April sampling. The phytoplankton bloom is coupled with an increase of the pH value in the river water, due to an increase of CO_2 uptake for photosynthesis. The high

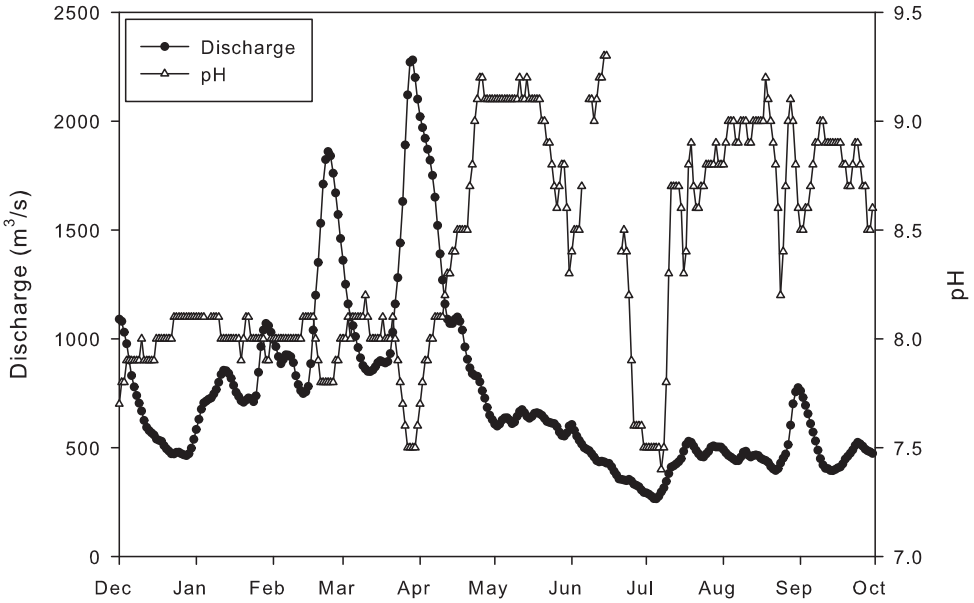


Figure 3.4: Discharge and pH value of the river Elbe near Over from December 2004 to October 2005.

pH value of 9.44 measured in April indicated the occurrence of a phytoplankton bloom. By means of the same process described above, water soluble contaminants were likely taken up in or adsorbed on the cells during the bloom, thus causing a decrease of water toxicity in April and May. During the active growth of the phytoplankton, a variety of photosynthetic products including easily assimilated low molecular weight substrates are excreted into the water (Chróst and Faust, 1983). These substrates support the growth and metabolisms of bacteria and may explain the stimulation effect of *A. globiformis* in the surface sediment in April. These substrates could also be the cause for the stimulating effect of *P. subcapitata* in the river Elbe water in May. When the phytoplankton growth peaked in May, some senescent algae with incorporated or adsorbed contaminants formed additional suspended particular matter and settled as sediments causing inhibition of *A. globiformis* in surface sediment.

Matrices Effect

A clear difference of ecotoxicological responses among layered sediment and water samples is illustrated in Figures 3.2 and 3.3. The water samples caused the strongest inhibition of the alga *P. subcapitata*. The bacteria *A. globiformis* with direct contact to solid phase responded stronger to sediments than to water samples. Furthermore, the deeper sediments from the river Elbe caused the highest inhibition of bioluminescence with *V. fisheri*. This matrices effect was closely related to the different exposure routes and the sensitivities of test organisms to different chemicals.

Algae, as a monitoring target of river water quality, are considered to be especially sensitive to metals and herbicides (Blanck, 1984), the latter being transported e.g. from agricultural areas by rain water into the river. The increased growth inhibition of *P. subcapitata* in the water phase compared to that in sediment elutriates (Figure 3.3) illustrated that river water was the main medium for those contaminants causing algal toxicity in nature, e.g. herbicides. On the contrary, microbial bioluminescence inhibition was highest in elutriates of the deeper sediment. Burton et al. (2001) demonstrated an elevated bioluminescence toxicity in sediments at a depth of 5 cm or greater in lake Orta and Suominen et al. (1999) further displayed the inhibited bioluminescence corresponding to high organic halogen contaminants at a sediment depth of 4-6 cm in lake Saimaa. The bioluminescence inhibition test is more sensitive to a wide range of organic substances and less sensitive to metals (Munkittrick, 1991). Heininger et al. (2004) summarised the cause of bioluminescence toxicity in Elbe sediment to be PAHs from chemical and ecotoxicological survey of surface sediment between 1992 and 2001. The results therefore indicated a reduction of sediment-bound contaminants causing bioluminescence toxicity in the river Elbe in the last decades.

The low inhibition of bioluminescence in river water and surface sediments did not indicate a risk of these contaminants even when surface sediments were resus-

pended. In the bulk sediment test with *A. globiformis*, on the other hand, both surface and layered sediments showed responses, either inhibition or stimulation, while water samples did not. The bulk sediment bioassay best represents the exposure of particle bound contaminants in the field, which can hardly be elutriated and resolved into the water phase (Liss and Ahlf, 1997). The effects of sediments from the rivers Elbe and Dommel were completely different that sediments from the former caused inhibition and from the latter stimulation. One possible explanation for this difference, considering the contaminants present in the river Dommel, was that the high content of organic material here masked any toxic effect. The matrices effects measured with the bioassays indicated possible distribution of contaminants in different matrices in the river water-sediment system. Our approach demonstrated that using combination of these three bioassays, a broad spectrum of behaviour and distribution of pollutants was covered.

3.2.3 Influence of Key Function

Nitrification is regarded as the "key ecological function" in this study due to its ecological importance and exclusiveness of organisms carrying it out. It is therefore important to investigate whether an inhibited nitrification in the samples could have directly or indirectly (e.g. via ammonium concentration) influenced the biotest results and therewith the ecotoxicological assessment of the sediment qualities.

In laboratory tests, the artificial inhibition of nitrification in sediments was done by addition of nitrapyrin. Nitrapyrin is known as a specific inhibitor of ammonium oxidation and has no toxic effect at amended concentration of 50 µM (Tate, 1977). However, the inhibition of nitrification may result in the increase of ammonium concentration, which could cause algal toxicity. Abeliovich and Azov (1976) reported the inhibited photosynthesis and growth of green algae *Scenedesmus obliguus* at concentrations over 2 mM and pH values over 8 in an oxidation pond. A 10 times lower tolerance of marine algae to ammonium was concluded by Kallqvist and Svenson (2003). Therefore the effect of nitrapyrin and the solvent DMSO was examined with algal growth inhibition test and bacterial contact assay.

The differences in toxicities are shown in Figure 3.5. The algal growth of *P. subcapitata* seemed to be slightly inhibited by the addition of DMSO and slightly induced by the addition of nitrapyrin. The differences of algal toxicities between control and treated sediments were tested with student t-test and shown to be not significant ($p=0.303$ with DMSO and $p=0.144$ with nitrapyrin), while the differences between addition of DMSO and nitrapyrin were significant ($p=0.048$). No significant differences were found in bacterial toxicities.

Though a slightly lower algal toxicity was caused by the addition of nitrapyrin compared to the addition of DMSO, the range of differences lay within the variances of bioassays. Variances of 10 to 15% for algal growth inhibition test and 15

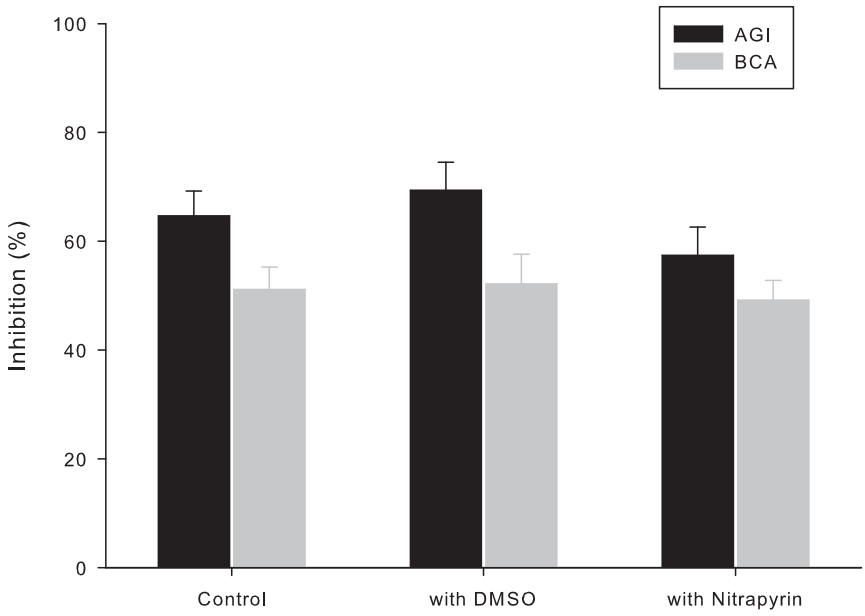


Figure 3.5: Effect of inhibited nitrification on ecotoxicities in sediments.

to 20% for bacterial contact assays are reported due to the biological and sample heterogeneity (Ahlf and Heise, 2005). Therefore, at the added concentration of the specific inhibitor nitrapyrin in solvent DMSO, no toxic effect was concluded with the bioassays.

3.3 Conclusions

Bioassay test batteries are widely used to screen potential environmental risk of sediments. The applied test battery seemed suitable for the purposes of screening contaminated sites for effects as well as discriminating effects (by interpreting a response pattern) which may be caused by differently distributed pollutants.

The measured spatial distribution of ecotoxicities in the river Elbe demonstrated potential source of contamination in the river Elbe, which can be easily related to sediment contamination patterns (Heise et al., 2007) along the Elbe. The results also indicated downstream transport of the contaminants with sediments and historic local contaminations in the tributaries.

The measured temporal variations of the ecotoxicities in the rivers Elbe and Dommel demonstrated the influence of hydrological and biological events. First, contaminated sediments were transported and settled at downstream area at high hydrological discharges. Local biological activity such as algal blooms could dominate the distribution of pollutants between water phase and solid matter.

The connection of hydrological and ecological events appeared to be the main cause of the ecotoxicological quality changes in the river Elbe, while no temporal variation was observed in the river Dommel. The toxicity and potential risk on biota in sediments were results of complex interactions between nutrients, hydrology, and pollutants. The use of solely chemical criteria could only give a reduced picture of the environment if those factors, which influence greatly the bioavailability and environmental risks, are not integrated in the assessment.

Available effects from the contamination regime and environmental risks were covered with bioassays, showing impact of chemical stressors under environmental conditions. However, impact of ecological functions was not covered by bioassays, showing the possible lack of ecological relevance. Therefore, the bioassays should be included as one "line of evidence" describing the impact of contaminants on sediment biota, but they should not be used as the only indicator for sediment quality. The well-being of the sediment dwelling organisms is still to be assessed to obtain the clear picture of the sediment quality.

Chapter 4

Ecological Functional Diversity

Recycling of nutrients by microorganisms is an essential ecological service generating important inorganic nutrients from organic matter and hence keeping the ecosystem in function. Nutrient cycling involves a variety of metabolic reactions carried out by consortia of microorganisms in various matrices. The term "ecological functional diversity", as used in this chapter, refers to this variation of metabolic reactions of microbial community which are essential for the functioning (nutrient cycling) of the ecosystem.

The metabolic diversity of the microbial community comprises a wide range of energy producing processes, such as phototrophic, chemolithotrophic, and chemoorganotrophic pathways, where light or oxidation of inorganic or organic substances are the energy-generating processes. The chemoorganotrophic organisms acquire carbon for the production of organic matter from organic sources and are called *heterotrophs*, while phototrophs and chemolithotrophs obtain carbon from CO_2 and are called *autotrophs*. The recycling of organic matter is carried out mainly by the chemoorganotrophs. Recycling of other nutrients, such as nitrogen and sulphur, involves the chemolithotrophs, which obtain electrons from inorganic forms of the oxidised nutrients.

The diversity of metabolic reactions, the utilisation of different electron acceptors, and the relation to nutrient cycling in sediments are summarised (Madigan et al., 2003) and depicted in Figure 4.1. The decomposition of organic carbon from dead cells in sediments is taken as the starting point to understand the complex metabolic diversity. The organic carbons such as starch and cellulose provide both high energy bounds as electron donor and source of carbon for microbial growth. Depending on the redox potential in sediments and electron acceptors available, different levels of energy yield are obtained. With the same electron donor, the higher the reduction potential of the electron acceptor is, the more energy is yielded. And most microorganisms use electron acceptors according to this hierarchy of energy yield. Several important electron acceptors present in the river water sediment interface are illustrated showing the decrease of reduction potential with decrease of redox condition. Oxygen is the electron acceptor yield-

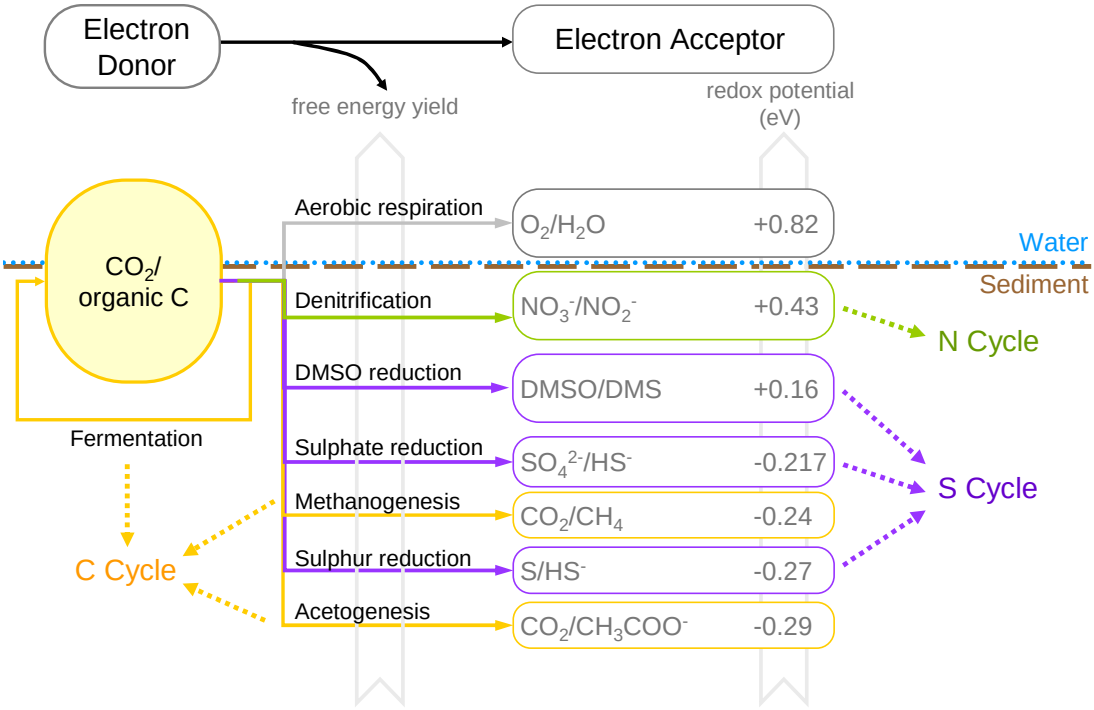


Figure 4.1: The hierarchy of electron acceptors and energy yields in water sediment interface associated with the redox gradients and the relation to important nutrient cycling.

ing the highest energy and is consumed at the water sediment interface. After the depletion of oxygen in sediments, other electron acceptors such as nitrate, DMSO, sulphate, are used. The degradation of organic carbons to CO_2 using electron acceptors other than oxygen is known as anaerobic respiration.

These electron acceptors used for anaerobic respiration are mostly products from autotrophic processes in nutrient cycling. For example, nitrate, DMSO and sulphate all play important roles in nitrogen and sulphur cycles. The cycling of carbon in sediments is closely related to these important nutrient cycles (Figure 4.1). This linkage suggests that disruption of one cycle will have an impact on the other processes. Especially those reactions that are carried out by only a few species are of special importance as they deliver essential nutrients for other organisms (Section 4.1). A change in the diversity of heterotrophic processes, which can be assessed relatively easy, can then be either a consequence of other impaired processes and therewith an indirect indicator for an occurred impact or it can reflect direct effects on the degradation potential of the sediment microbial community.

The measure of this “potential” as opposed to “acute” or “executed” functional diversity is in this study considered to be more relevant for assessing long-term impairments of environmental quality, because it takes into account the

abilities of a microbial community to adapt to changing circumstances such as chemical concentrations.

The discussion of functional diversity as an indicator of sediment quality in this chapter comprises three parts: First, the importance of the autotrophic nitrification in the nitrogen cycle and the reason for considering it a "key ecological function" are presented. The discussions move on to the diversity of the heterotrophic carbon cycling. What are the factors influencing the diversity in carbon cycling in natural sediments? Are there negative influences of inhibited nitrification on the heterotrophic carbon cycling? At the end, a hypothesis is proposed using substrates induced DMSO reduction as an indicator showing capability of both heterotrophic carbon cycling and important autotrophic processes.

4.1 Key Function in Nitrogen Cycling

The element Nitrogen exists in a number of oxidation states, which are involved in the nitrogen cycle, including N_2, NH_4^+, NO_3^-, and NO_2^-. Their roles in the complex nitrogen cycle associated with the changes of redox conditions has been well described by Revsbech et al. (2006) and depicted in Figure 4.2.

The most abundant nitrogen species in the N-cycle is the nitrogen gas N_2, which makes up 78% of air. But only few microbes can perform **"nitrogen fixation"**, which reduces nitrogen gas to ammonium. Hence, most of the abundance nitrogen is unavailable to most organisms. A lot of ecosystems of the world are nitrogen-limited (Vitousek et al., 2002) with organisms that are adapted to use the available nitrogen efficiently. Nitrogen fixation is wide spread in the aquatic environment as well as in rhizospheres. Ammonium, produced from decomposition of organic cells and tissues as well as from nitrogen fixation, can be either assimilated in cell structure, or further oxidised. Under aerobic condition, ammonium is oxidised in a two step reaction. It is first oxidised to nitrite by ammonium oxidising bacteria then further oxidised to nitrate by nitrite oxidising bacteria. The complete reaction is called **"nitrification"**. Nitrate can be either assimilated into the cell structure under oxic condition, or reduced under anoxic condition through different pathways. First, nitrate can be reduced through nitrite to nitrogen gas in anaerobic condition, called **"denitrification"**. The coupling of nitrification and denitrification can remove nitrogen from the system, which is important in wastewater treatment. Second, the reaction of dissimilatory nitrate reduction to ammonium **"DNRA"** may occur. It has been shown by Tiedje (1987) that the rate of DNRA is higher than denitrification under organic rich conditions with high oxygen consumption. Another recycling pathway of combined nitrogen to atmospheric nitrogen gas pool is the **"anammox"**, anaerobic oxidation of ammonium to nitrogen gas with nitrite. This pathway has been directly proved first in a wastewater treatment plant in Delft, NL (Mulder et al.,

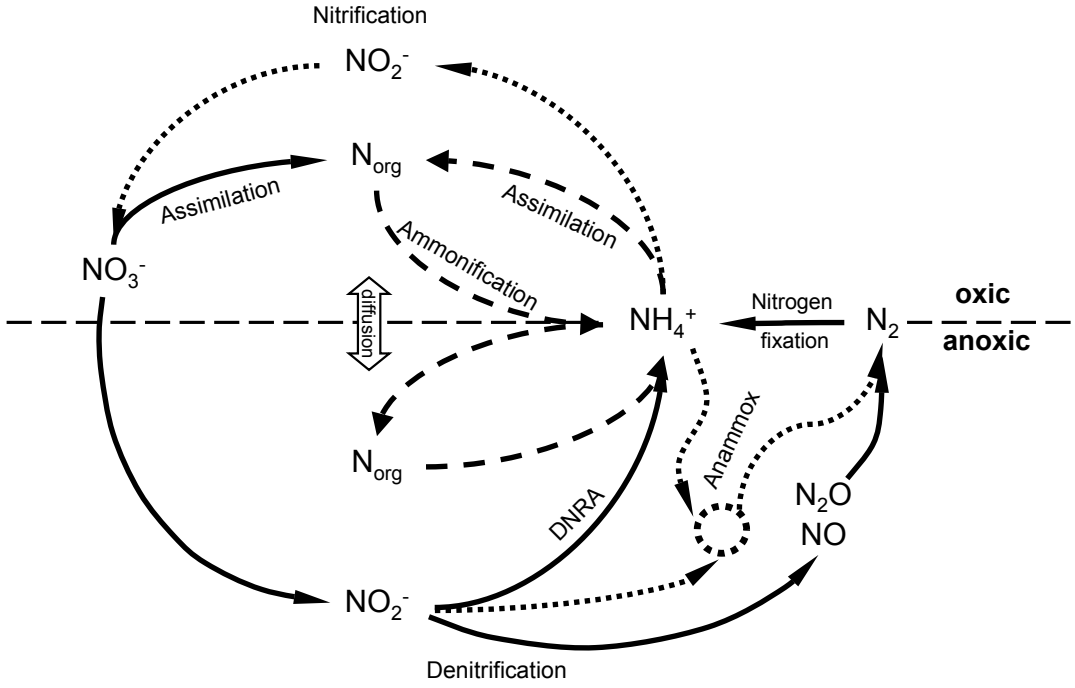


Figure 4.2: Nitrogen cycle associated with oxic, anoxic interface. The dotted lines show oxidation reaction, the solid line reduction reaction.

1995). The significance of anammox on N_2 production differs from site to site, and depends mostly on the supply of NO_2^- in the suboxic zone.

In the stratified freshwater sediments with redox gradients, the transformation of nitrogen species across the oxic/anoxic interface is of great ecological importance. The organic nitrogen from dead algae and periphyton cells add to the ammonium pool in the water column and surface sediment. The ammonium and organic nitrogen pool provide the driving force of the nitrogen cycle through nitrification to produce nitrate, which is an alternative electron acceptor in anoxic sediment and can be reduced through different pathways. There are several groups of heterotrophic microbes, who are able to use nitrate as electron acceptor in the anoxic sediments, while the oxidation of ammonium to nitrite, the fist step of nitrification, is carried out almost exclusively by one group of autotrophic bacteria, *Nitrosomonas* and *Nitrobacter* (nitrifiers). In addition to the autotrophic nitrification, where energy is gained, some heterotrophic bacteria can also carry out nitrification without gaining energy from the reaction. The physiological role of heterotrophic nitrification and phylogenetic diversity of the microorganisms are not yet clear (Hatatsu et al., 2008), but it is mostly ecologically relevant in acidic soils (Jordan et al., 2005) and has not yet been observed in freshwater sediments.

The nitrification reaction in the nitrogen cycle was taken as the "key ecological function" for its exclusiveness and sensitivity. It is exclusively carried out by the

nitrifiers, which have been shown by testing the toxicity of 50 to 100 chemicals to be more sensitive than aerobic heterotrophs and methanogens (Blum and Speece, 1991). The impairment of nitrification therefore can serve as an early indication of impacted ecological function. The nitrification activity was inhibited with the specific inhibitor "nitrapyrin" to mimic the loss of sensitive ecological function.

The nitrification in sediment is a well researched topic and there are several methods to measure the activity, including measuring concentrations, fluxes, and rates (Ward and O'Mullan, 2005). To obtain a high resolution of the process and an overall activity in the sediment, two methods were used in this study: microsensors and potential nitrification rate. The microsensors provide a high spatial resolution of in-situ oxygen, ammonium, and nitrate concentration; while the potential nitrification rate provides a standardised method in measuring overall potential activity (Jensen et al., 1993). These methods were applied to investigate the impact of inhibited nitrification on the conversion of nitrogen compounds at the water sediment interface.

4.1.1 Depth Profile of Microsensors

Microsensors are electrodes with fine tip size of 1 to 100 µm; therefore it is possible to measure sediment in-situ concentrations with minimal disturbance.

Figure 4.3 illustrates two types of microsensors measuring different electrochemical properties: potentiometric electrodes and amperometric electrodes. The potentiometric electrodes measure the electrochemical potential differences across a liquid membrane, also called **liquid ion exchange (LIX)** sensor. The LIX sensors are usually used to measure ions, such as H^+, NH_4^+, NO_3^-, and NO_2^-. The presence of other ions may interfere with the results, but these sensors have been demonstrated as a useful tool to measure processes in microscale in freshwater biofilms and sediment (De Beer and Sweerts, 1989). The amperometric sensors on the other hand measure the current generated by reduction of substrates at the cathode of the sensor. This type of sensor is usually used to measure gases, such as O_2, N_2O, and H_2S, and has the advantage of a short reaction time and of being little effected by other compounds.

The microsensors have been proved to be sensitive to measure nitrogen cycle in freshwater sediments (Jensen et al., 1993; Stief et al., 2003), where redox gradients corresponding to different reactions in nitrogen cycle are present. In natural sediments oxygen is present at the water sediment interface, where nitrification would take place producing nitrate. In the anoxic zone below, nitrate is consumed for denitrification. With the microsensor as a tool, the increase of nitrate concentration and decrease of ammonium concentration can be taken as indicator of occurrence of nitrification, which can be verified by increased oxygen consumption. The microsensors therefore provide high resolution depth profiles of the distribution of different reactive nitrogen species in natural sediments.

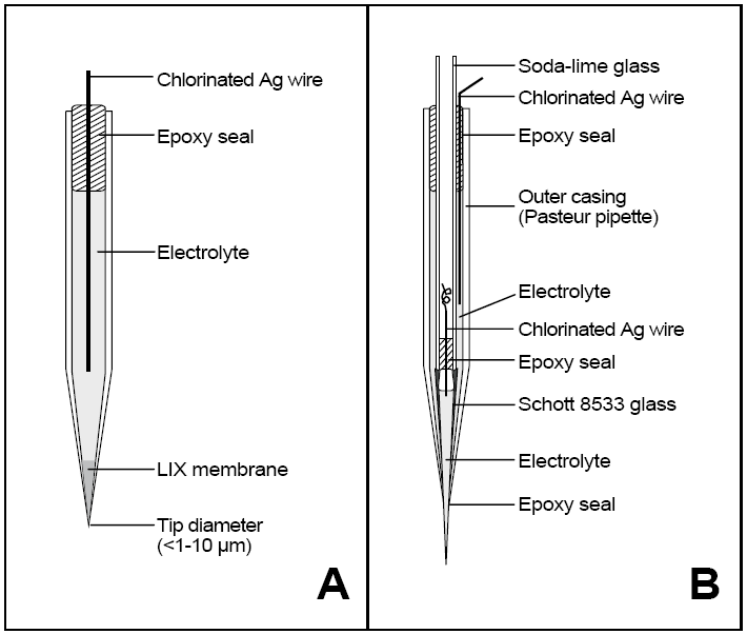


Figure 4.3: Detailed view of microsensors. A shows the LIX sensor and B the oxygen sensor. (Kühl and Revsbech, 2001)

4.1.1.1 Materials and Methods

Microsensors Three different sensors were used in the study. Two LIX sensors measuring both substrate NH_4^+ and product NO_3^- of nitrification together with one oxygen sensor were used at the same time. The microsensors were self-constructed according to De Beer et al. (1997) and Revsbech (1989) by Dr. Peter Stief from Max-Planck Institute for Marine Microbiology in Bremen. Both LIX sensors were calibrated before and after measuring a sediment profile. The sensors were calibrated from 0 to 500 µM of NH_4Cl and $NaNO_3$ at 15℃ and three measurements were taken per calibrating concentration. Due to the possible presence of ammonium and nitrate in the tap water for calibration, further determination of ammonium and nitrate in tap water was done in the central laboratory with photometric and ion chromatographic methods. The non linear calibration curve of the LIX sensor was fitted with a polynomial curve of two degrees.

Sediments Three sediment cores of 0-10 cm for each treatment were taken from Over in March 2007. Each core was sieved through 1 mm mesh and refilled in a 1 L glass beaker. The beakers were then filled with river water to 1 L mark. The sieving homogenised the sediments and removed miscellaneous matters, which might destroy the sensor. The sieved sediments were incubated at 15℃ with slight aeration for two weeks to re-develop the oxic layer and nitrifying community in the sediments. One intact core was also taken and incubated under the same

condition without sieving. The sediment beakers were treated either with 1 ml of 50 µM nitrapyrin dissolved in DMSO to inhibit nitrification or 1 ml of DMSO as control 3 days before microsensor measurement.

Measuring Profiles All three microsensors (oxygen, ammonium, and nitrate) were mounted on a computer controlled micromanipulator with the smallest moving step of 50 µm. The sensors were positioned with horizontal distances not exceeding 10 mm with the help of a binocular. The profiles were measured from 2 mm above to 5-6 mm below the water sediment interface with resolution of 100 µm. Ten measurements were taken at each depth and sensors were moved to the next depth after 30 seconds resting time. Three profiles at different position were measured in one sediment beaker, and three sediment beakers for one treatment, resulting in 9 profiles for one treatment. Sensors were calibrated before and after measuring profiles of each beaker. One control beaker and one treatment beaker were measured per day to avoid usage of different sensors. Three profiles were also measured in the undisturbed intact core at the end of the experiment with 200 µm resolution down to 10 mm depth. During the two week incubation time, ammonium in the overlying water was depleted in control beakers. The average ammonium concentration in the river Elbe near Over in 2006 was around 15 µM, therefore ammonium was added to the control beakers to a final concentration of 20 µM.

Nine replicates for each treatment were obtained and the average concentration and the standard deviation were depicted as averaged concentration depth profile. The profiles can be further converted to flux of local mass transfer and local volumetric conversion rates of relevant solute. The concentration profiles were considered steady when conversion rates equals transport rate. In the sediment cores, it is assumed that the only transport mechanism is diffusion. Therefore, based on Fick's first law, a one-dimensional diffusion-reaction model has been developed (Revsbech and Jørgensen, 1986; De Beer and Stoodley, 1999) to determine the conversion rate of the solute and flux for each point in the profile. The flux J was calculated as

$$J = -D_s \frac{\delta c}{\delta x} \ , \tag{4.1}$$

where D_s is the diffusivity of the sediment, and the $\frac{\delta c}{\delta x}$ is the concentration gradient between depths x_i and x_{i+1}.

From the depth profile of the flux, the local volumetric conversion rate was calculated (De Beer and Stoodley, 1999) as

$$R = \frac{J_{ab} - J_{bc}}{0.5(x_a + x_b) - 0.5(x_b + x_c)} \ , \tag{4.2}$$

where R is the local volumetric conversion rate . This conversion rate can then be compared with actual nitrification rates provided that the sediment density is known.

4.1.1.2 Results

Concentration Depth Profiles Average (shown as symbols) and standard deviation (shown as horizontal line) of the 9 profiles from each treatment were calculated. The averaged depth profiles of the sediments treated with nitrapyrin and DMSO are illustrated in Figure 4.4.

In the control profiles, where DMSO was added as solvent, no distinct peak but a smooth curve showing steady nitrate concentration in the 0-2 mm sediment depth was measured, while a clear minimum of ammonium concentration was observed at the same depth. The oxygen concentration penetrated to around 2 mm below sediment water interface. The treatment beaker with the nitrification inhibitor showed on the other hand a continuous decrease of nitrate concentration and increase of ammonium concentration starting directly at the sediment water interface. Oxygen was measured down to 3 mm below the sediment water interface. The concentration of the ammonium was higher in the treatment beakers than in the control beakers, while the nitrate concentration was lower. The reproducibility of the profiles was high as shown by the low standard deviations.

Each concentration profile in the intact core (Figure 4.5) was depicted separately and seemed more irregular. Though it is clear to see a significant peak of

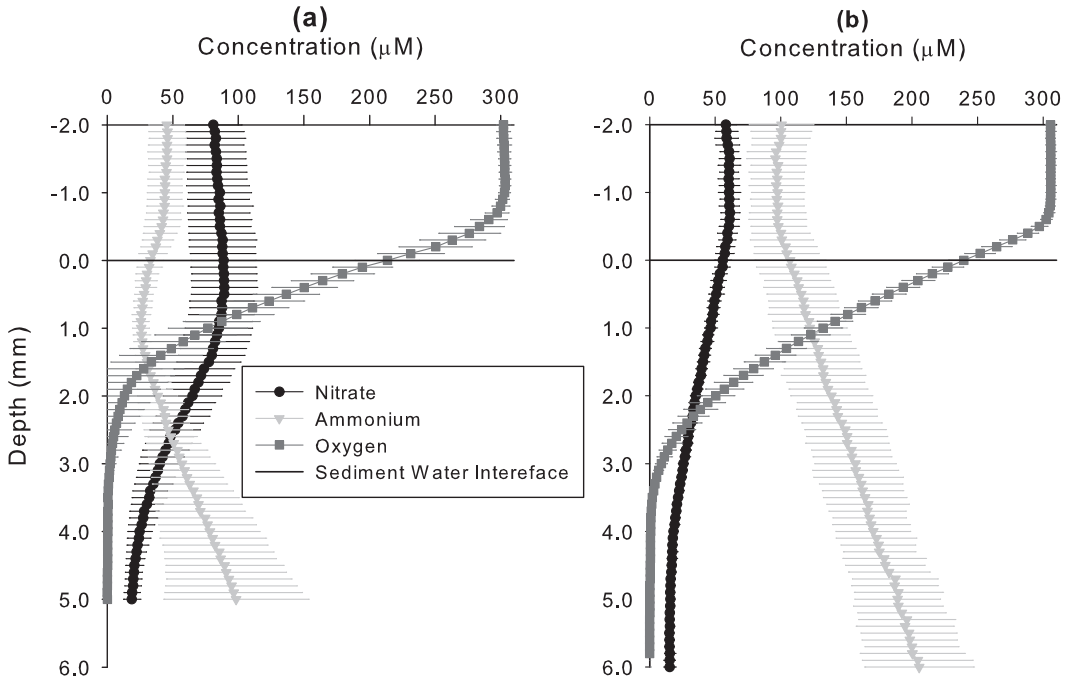


Figure 4.4: Averaged concentration depth profiles of oxygen, nitrate and ammonium in sediments. Figure (a) shows the profiles of control and Figure (b) the treatment with nitrapyrin.

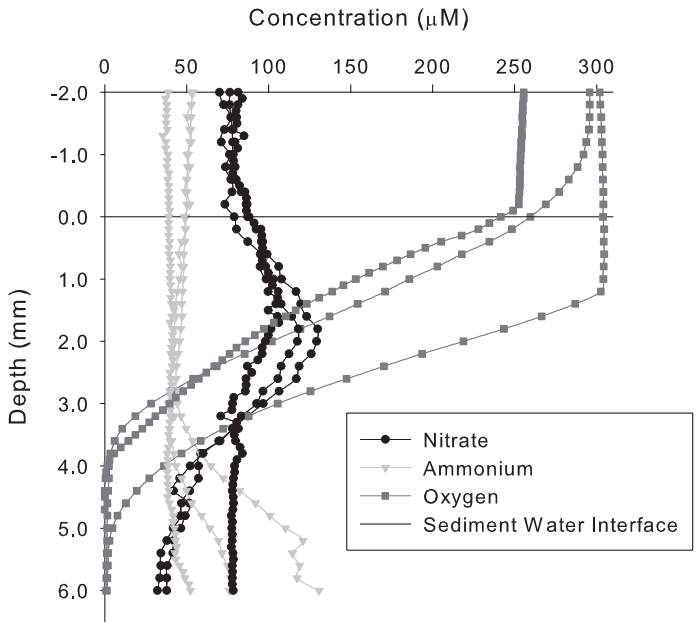


Figure 4.5: Concentration depth profiles of oxygen, nitrate and ammonium in sediment intact core.

increased nitrate concentration at around 2 mm depth, no concave of ammonium concentration was observed together with the peak of nitrate concentration. The oxygen concentration became depleted at around 3 mm depth, and the increase of ammonium concentration began at 3-5 mm below interface.

Local Conversion Rate In calculating local conversion rates (Equation 4.1; 4.2), statistical noise may be found due to the differentiation process in calculation, and is improved by averaging a zone (0.5 mm) of local conversion rates of the relevant solute.

The conversion rates of each measured solute from both treatments are illustrated in Figure 4.6. A clear nitrate production in the upper 2.5 mm of sediments followed by nitrate consumption in the layers deeper than 2.5 mm in control beakers was observed. Ammonium consumption corresponded in the 0-2 mm depth sediments. The oxygen was consumed to a great extent to 2 mm depth, while complete oxygen depleting was observed at 3 mm. In the treatment beakers, no significant nitrate production or consumption was found down to 5 mm depth. The ammonium conversion rates were somehow irregular and the oxygen consumption was depleted at 4 mm depth.

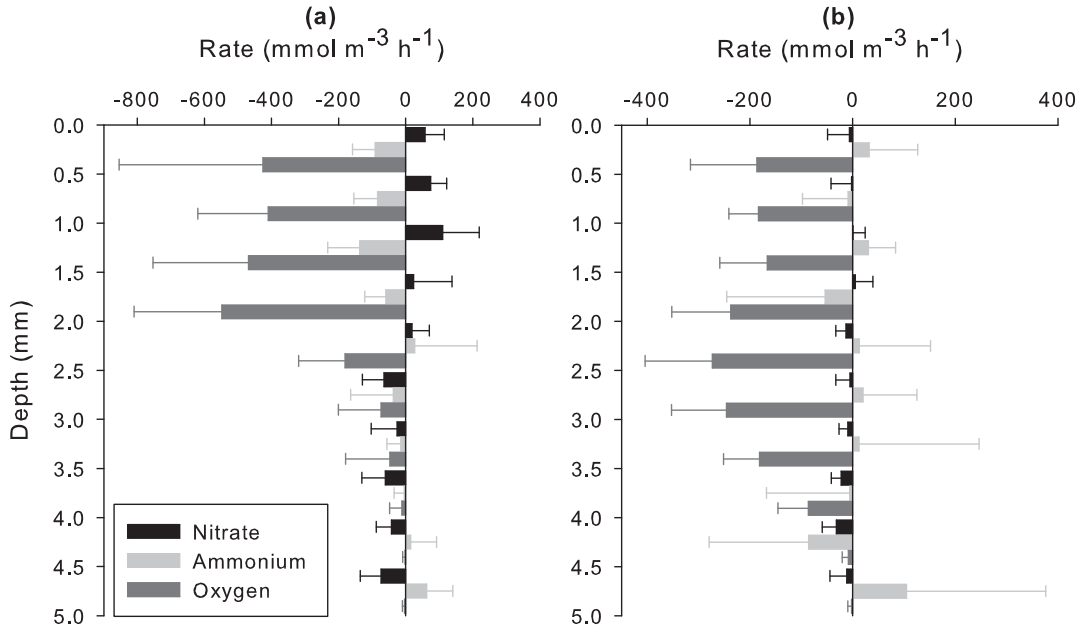


Figure 4.6: Averaged local conversion rates of oxygen, nitrate and ammonium in control (a) and treatment (b) sediments.

4.1.1.3 Discussion

Effect of Nitrapyrin The nitrification inhibitor nitrapyrin inhibits the ammonium oxidation to nitrite. Therefore it is assumed that the sediments treated with nitrapyrin would have higher ammonium and lower nitrate concentrations in the upper sediments and this can be seen in the depth profiles in Figure 4.4 and in the conversion rates in Figure 4.6. In opposite to the control sediments without nitrapyrin, no increase of nitrate or decrease of ammonium concentration was observed. The oxygen penetrated deeper in the sediment due to the lack of oxygen consumption from nitrification. The results corresponded well with the study from Jensen et al. (1993), who reported a decline of nitrate peak from 100 to 0 µM and deeper oxygen penetration depth from 1.5 to 5 mm two hours after treating sediments with another nitrification inhibitor C_2H_2. A more pronounced nitrate peak was illustrated in this study due to the induction of added ammonium concentration of 300 µM, which induced the nitrification activity.

In control and nitrapyrin treated sediments, small nitrate gradients (slow decrease of concentration with depth) were probably caused diffusion of nitrate from the water into the sediment, followed by denitrification in the deeper anoxic zone. Hence, in the sediments amended with nitrapyrin, the highest concentration was at the sediment water interface, while in the control sediments a pronounced decline began at 2 mm sediment depth, corresponding with the depletion of oxygen. This once more confirmed the production of nitrate in the 0-2 mm

sediment depth, which shifted down the diffusion of nitrate for denitrification in the anoxic zone.

Differences between sieved and unsieved cores The microsensors measure the concentration of the relevant solute in the sediments as a result of net conversion. The main difference in the profiles between the intact core and sieved sediment treated with DMSO was the distinct nitrate peak in sediment at 3-5 mm below sediment water interface. Two possible explanations are the sieving and the effect of solvent.

The sieving process removed the organic matters and the stratification of natural sediments. The organic matter contains organic-N and ammonium, which are the substrates for nitrification. The profiles measured in the control sediment are similar to those measured by Stief et al. (2003), who illustrated no notable nitrate peak in the organic-poor sediment before ammonium enriched (50 µM) incubation and in the organic-rich sediment after two week ammonium enriched incubation. The former was a result of removal of stratification, and the later a result of greater anoxic zone and nitrate consumption. No ammonium was added to the control sediments in this study during the incubation time. The continual consumption of ammonium to develop a nitrifying community may result in ammonium depletion and a smaller redeveloped nitrifying community. Jensen et al. (1993) also reported a distinct nitrate peak of 50 µM at 1 mm depth in the intact core without ammonium addition, while the homogenised sediment with no ammonium addition showed only a smooth nitrate curve of 20 µM at 2 mm depth. The solvent DMSO added in the control sediments may also result in a decreased nitrifying activity, though the DMSO at added concentration is reported not to inhibit nitrification in sediments (Roy and Knowles, 1995).

In the profiles of intact cores, the oxygen diffusion boundary layers did not correspond well with the set sediment water interface. This was probably caused by the irregular surface of the intact core. Therefore the depth shown in Figure 4.5 should not be taken as absolute value.

4.1.2 Potential Nitrification Rate

The measurement of nitrification rate for determining nitrification activity in soil and sediment is a standardised and widely accepted method. Different protocols describe the measurement of either the decrease of substrate ammonium concentration, or of the increased product nitrite or nitrate. For the measuring of nitrification potential, nitrification activity is actively induced by addition of ammonium and incubation at optimum conditions, e.g. under aeration and at ideal temperature (25-30℃). In this study, the ISO 15685 method for measuring potential nitrification rate in soil was used as modified by Frühling (2004). Hereby, the oxidation of nitrite to nitrate is inhibited by sodium chlorate and the

accumulated nitrite concentration is measured colourimetrically. Under high ammonium concentration, the differences of nitrite concentrations before and after incubation then equal the nitrification activity over time. This method provides a measure of the maximum capacity of nitrification activity in the sediment. For measuring the actual nitrification rate no ammonium substrates are added.

4.1.2.1 Materials and Methods

Potential nitrification rates were measured 3 days after spiking of DMSO and nitrapyrin in fresh sediments and sieved sediments after the measurement with microsensors. A comparison of the measurement before and after the microsensor application indicated possible changes of nitrification rate with incubation time and disturbance caused by microsensor measurements.

5 g of sediment was added to a 50 ml sterile centrifuge tube and 20 ml of 1 mM $(NH_4)_2SO_4$ substrate and 0.1 ml of 0.5 M $NaClO_3$ inhibitor were added. The tubes were closed and incubated at 30℃ with 200 rpm horizontal shaking for 24 hours. The same set up was prepared for the blank value and stored at -20℃. After the incubation, 5 ml of 2 M KCl was added to both test and blind tubes to terminate the bacterial reaction. The tubes were then centrifuged at 3000 rpm for 5 min and supernatant was removed for photometric nitrite determination.

The actual nitrification rates of sediments after microsensor measurements were also measured to have a closer comparison with the local conversion rates obtained from microsensor measurements.

The nitrite concentration in supernatant was determined after Nicholas and Nason (1957) that 200 µl of supernatant was removed from each centrifuged tube and pipetted in a 96 well microplate. 20 µl of 2% Sulphanilamide solution in acid was added to each well, after 2 minutes reaction time 20 µl of 0.1% N-(1-Naphthyl)ethylenediamine dihydrochloride was added. After further 10 minutes reaction time, nitrite was determined as absorbance at 540 nm with a plate photometer.

The absorbance was calibrated with standard nitrite solution from 20 µM to 1 mM. The nitrification rate was subsequently calculated as ng nitrite per g sediment per hour.

4.1.2.2 Results

The actual and potential nitrification rates of the sediments were calculated as ng NO_2^-/g sediment/hr in Figure 4.7 and Table 4.1. Nitrapyrin dissolved in DMSO inhibited around 40% of the potential nitrification rate, while around 20% inhibition was also measured in sediments amended with DMSO as solvent.

The potential nitrification rates of the sieved sediments after two weeks incubation time showed activities of around only 50% of fresh sediments, and a higher inhibition of nitrapyrin was observed. In the fresh sediments, the inhibition of

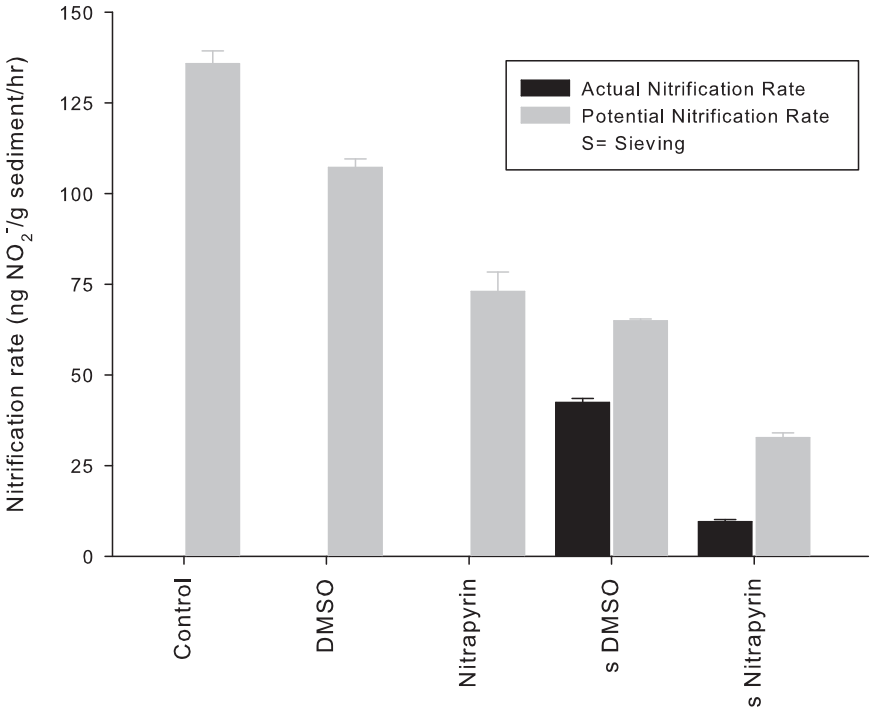


Figure 4.7: Actual and potential nitrification rates of sediments amended with solvent DMSO and nitrapyrin.

Table 4.1: Nitrification rates and inhibitions by solvent DMSO and nitrapyrin.

samples	potential nitrification			actual nitrification	
	rate ($ng\ g^{-1}h^{-1}$)	inhibition to control	inhibition to DMSO	rate ($ng\ g^{-1}h^{-1}$)	inhibition to DMSO
control	135.8±3.6				
DMSO	109.3±1.7	20%			
Nitrapyrin	75.8±6.3	40%	31%		
DMSO sieved	64.9±0.5			42.4±1.4	
Nitrapyrin sieved	32.7±1.3		50%	9.5±0.6	77%

nitrapyrin compared to solvent was around 30%, while after sieving the inhibition raised to 50%. The actual nitrification rates of the sieved sediments, showed an even higher but not complete inhibition caused by nitrapyrin compared to sediments amended with solvent.

4.1.2.3 Discussion

The potential nitrification rate of an unaffected soil is considered to lie between 200 to 800 ng NO_2^-/g soil/hr by German standard. The nitrification rates measured in the sediments were much lower than the standards for soil. This can be explained by a much lower oxygen concentration in the sediment compared to soils or an impacted nitrification activity. A previous study by Fischer et al. (2005) reported the range of potential nitrification rates between 10 and 221 ng NO_2^- cm^{-3} h^{-1} in surface sediments of the middle Elbe.

In the freshly collected sediments without sieving, a relatively high potential nitrification rate could still be observed when nitrapyrin was added (70% compared to solvent), even though the applied concentration of 50 µM was considered to inhibit more than 90% of the autotrophic nitrification (Roy and Knowles, 1995). It can not be excluded that a certain amount of heterotrophic microbial activity may account for the nitrite produced. Tate (1977) showed that the heterotrophic *Arthrobacter* spp. were able to produce nitrite and nitrate from reduced nitrogen species with a four fold increase when ammonium and sodium acetate were added. Excess ammonium was also added in the experiments of this study for measuring potential nitrification in order to induce the maximum rate of autotrophic nitrification, and this may also have induced heterotrophic nitrification. It is, however, unlikely that the heterotrophic nitrification alone accounted for 50% of the whole nitrification activities in the sediments, since higher heterotrophic nitrification than autotrophic one was only reported in acidic soils and pure cultures (Revsbech et al., 2006). An incomplete inhibition of nitrification or other pathways adding to the nitrite pool such as denitrification and dissimilatory nitrate production were more likely to account for the high measured nitrite concentration which was then interpreted as potential nitrification rate in the sediments.

A 50% reduction of potential nitrification rate in sediments amended with DMSO after sieving and two weeks incubation was measured. Both sieving and incubation under substrate limited conditions may have influences on nitrification activities in sediments. First, sieving removes organic matter, which would otherwise be metabolized by heterotrophic bacteria, including the ones able to perform heterotrophic nitrification as described by Strauss and Lamberti (2000). Sieving further destroyed the natural stratification and the oxic zone, which is crucial for the nitrifying community as shown in Figures 4.4 and 4.5 and discussed in paragraph 4.1.1.5. Second, the incubation of the sediments was done without addition of ammonium, which would have induced the nitrification activ-

ities. During the two weeks incubation time, the autotrophic nitrifiers' activities did not increase greatly due to the limited substrate ammonium. These two factors together resulted in a reduced potential nitrification rates in both sediments amended with DMSO and nitrapyrin.

In the actual nitrification rates measurement, a much higher inhibition (77%) of the nitrification rate by nitrapyrin was observed compared to the effect on potential nitrification (50%). When no ammonium was added, the actual nitrification rate was closer to in-situ real activity of nitrification as measured by microsensors compared to potential nitrification rate. Similar to the microsensor experiments it was expected that all nitrification would be inhibited by nitrapyrin, especially as with actual nitrification no stimulation had been provoked as no ammonium was added. Accordingly, a very low nitrification rate of 9 ng NO_2^- g^{-1} h^{-1} was measured whereby the detection limit of nitrite is about 5 ng NO_2^- g^{-1} h^{-1}. This low but measurable nitrification rate can probably be assigned to the incubation at high temperature and continuous shaking resulting in higher oxygen availability in sediments.

4.1.3 Summary

Nitrification is a sensitive part of nitrogen cycling. It is therefore considered a key ecological function in the study. This section displayed the actual and potential nitrification activities and profiles in sediments and described the effects on the nitrogen cycle, when autotrophic nitrification was shut down by adding the inhibitor nitrapyrin.

Microsensor measurements of nitrate, ammonium and oxygen profiles in sediments which were sieved and incubated for 2 weeks, indicated nitrification activity in the upper 2 mm of sediment as ammonium and oxygen were consumed while nitrate was produced (Fig.4.4). Below this layer, oxygen concentration was not detectable any more. Nitrate consumption was calculated from the microsensor measurement, pointing to denitrification processes under anoxic conditions. In an undisturbed core, the nitrate concentration showed a more pronounced peak compared to the sieved core. This indicated a more active nitrification activity which can be explained by the established, undisturbed stratification of the core. In order to investigate the effects of an interruption of the nitrogen cycle in the sediment by shutting down autotrophic nitrification activity, nitrapyrin was added.

In the microsensor measurements, a complete inhibition of nitrification activity was observed in the sieved and incubated sediments, overlaid with water containing relatively high ammonium concentrations (100 µM), when amended with nitrapyrin. The actual nitrification rate of the same material also showed a very low actual nitrification activity of around 9 ng NO_2^- g^{-1} h^{-1}. When ammonium was added to the sediment amended with nitrapyrin in order to measure the potential nitrification rate, a three to four fold increase of nitrification rate

was measured. One explanation for this failure of nitrapyrin to induce a situation with very low nitrite concentrations could be the presence of heterotrophic nitrifiers. Nitrapyrin only inhibits autotrophic nitrification process. Heterotrophs, however, which oxidise reduced organic nitrogen and ammonium to nitrite from metabolism of organic carbohydrates, would not be affected. Heterotrophic nitrification, however, has been assumed to have significant activity only in acidic forest soils (Joo et al., 2005) or in pure culture (Dalsgaard et al., 1995) and to be negligible in freshwater sediments compared to autotrophic nitrifiers due to its low affinity to ammonium. The addition of high quantities of ammonium to the sediment when intending to measure potential nitrification, however, could provide favourable conditions to these heterotrophic organisms compared to autotrophs, especially because of their higher growth rate. On the other side, also an incomplete inhibition of autotrophic nitrification activity could result in the measurable nitrite concentrations. Even though the microsensor set ups seem to indicate a complete inhibition by nitrapyrin, net concentrations were measured in the profiles. The observed complete inhibition of nitrification may be a result of coupled nitrification-denitrification processes, where part of the produced nitrate was used directly by denitrifiers. Therefore, only decrease of nitrate concentration and no net production rate of nitrate can be measured.

It can be summarised that nitrogen cycling is a critical key function in sediment ecology. In the microsensor measurements, the fine spatial distribution of different nitrogen species within one centimetre of sediment depth was described. The nitrification occurred only in the oxic zone of the sediments when no inhibitor was added. The solvent DMSO exhibited some adverse effect on the nitrification activity. In the potential nitrification rate the maximum activity under high substrate concentration was measured. The addition of nitrapyrin did not inhibit the nitrification activity completely. The remaining nitrite production could have been a combined result of incomplete inhibition of nitrification and activity of heterotrophic bacteria, which oxidise ammonium during metabolism of carbohydrates. Robertson and Groffsman (2007) summarised that the autotrophs are poor competitors for ammonium in soils, therefore if there are heterotrophic communities and a surplus of ammonium, the heterotrophs may be much productive in nitrite compared to autotrophs.

Results in this section confirmed the assumption of nitrification being one key ecological function in river sediments. Its activity in stratified freshwater sediment was shown with microsensors to be very sensitive to alterations of environmental conditions such as oxygen, ammonium concentrations as well as removal of organic matters. Its potential under ideal conditions of high substrate concentrations and optimal temperatures was however less impacted as demonstrated with potential nitrification rates. Considering the importance of nitrate in carbon cycling as an alternative electron acceptor, what is the influence of an inhibited nitrification on heterotrophic carbon cycling?

4.2 Carbon Substrates Utilisation

Carbon is a major nutrient and its organic form is the basis of all known life. Organic carbon is synthesised on Earth via photosynthesis and chemo-synthesis. Plants and algae in aquatic environment take up energy from light and CO_2 in the atmosphere or dissolved in water to form organic carbon. The organic carbon is then decomposed by microbes to CO_2 or CH_4 completing the carbon cycle, see Figure 4.8. The decomposition or respiration of organic carbon by microbes in both oxic and anoxic conditions is important. The diverse range of organic carbon from macromolecules to carboxylic acids which microbial community can utilise for energy production is the base for the functional diversity in carbon cycling and is taken as an indicator in this study.

Garland and Mills (1991) first introduced the assessment of the heterotrophic functional diversity of environmental microbial community with the commercially available BIOLOG MicroPlate system. The BIOLOG Microplates are 96 well microtiter plates containing up to 95 different carbon substrates and the redox indicating dye tetrazolium. When microbes are inoculated into the wells, they start to mineralise the carbon sources available to them and thereby reduce the tetrazolium violet redox dye, which turns purple. As not all microbes are capable of mineralising all carbon substrates, the developed pattern of purple wells among a plate reflects a "substrates utilisation fingerprint", which can be easily

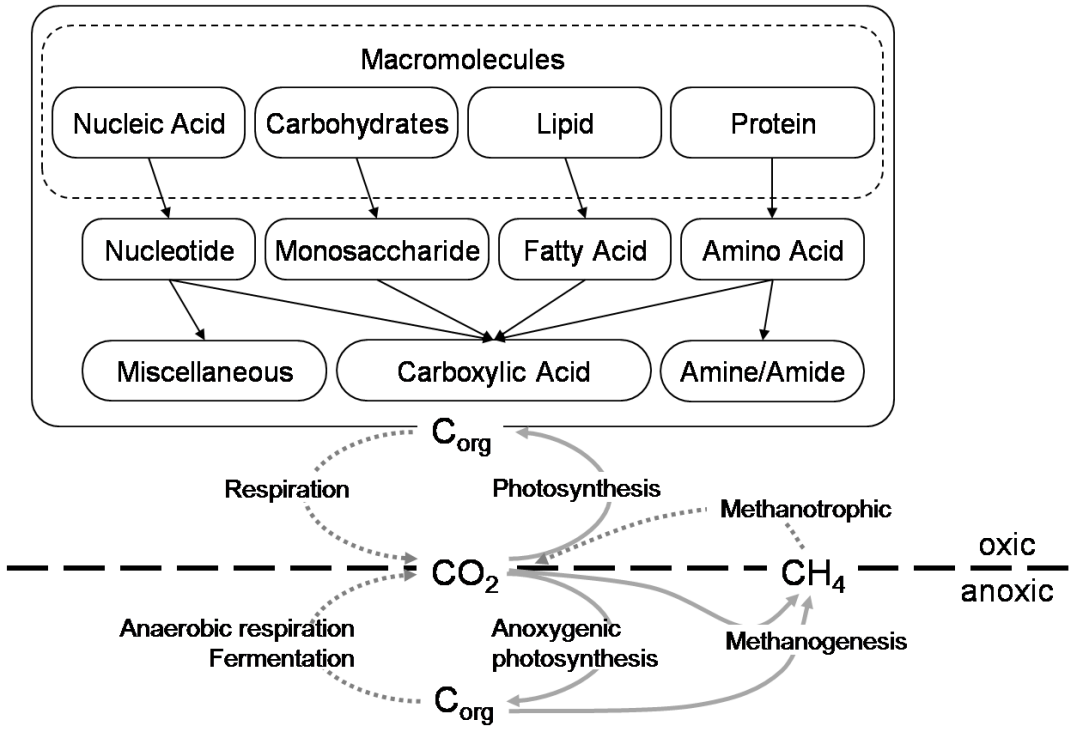


Figure 4.8: Cycling of carbon at different substrates level.

measured photometrically. The BIOLOG plates were first developed for pure cultures to identify different bacteria strains from the unique substrates utilisation fingerprint with 95 different substrates for Gram positive or negative bacteria in the laboratory. Since Garland and Mills (1991) reported the MicroPlates as a successful tool in distinguishing microbial communities from different rhizospheres, over 120 scientific papers have been published using the method within 10 years (Preston-Mafham et al., 2002).

For better application on and distinguishing between environmental samples containing a variety of different species, EcoPlates containing triplicates of 31 ecologically relevant structurally diverse compounds (listed in Appendix A.1) instead of 95 substrates have been introduced. It has been used, e.g. in the investigation of the pollution induced community tolerance, as a tool for differntiation of physiological profiles on the community level in soil microcosms (Schmitt et al., 2006).

Though BIOLOG plates have the advantage to generate a great amount of data relatively easy within a short time compared to molecular methods, there are also several limitations concerning the method. In their review, Preston-Mafham et al. (2002) pointed out that inherent biases in data interpretation may rise from the extraction, inoculation and incubation of microbes in the laboratory. Appropriate exploratory data analysis from ordination, clustering to indexes has been applied to overcome these problems but still poses several uncertainties. Therefore, it has been suggested to be used only to compare different communities, but not to characterise the communities. A substrates induced respiration method with similar principle has been developed measuring CO_2 production in 6 hours without extraction of microbes (Degens and Harris, 1997), which reduced the mentioned inherent biases greatly. However, with the high water solubility of CO_2, application of the method is limited to soil and seems less suitable for sediment microbial communities.

Since the aim of the study is to develop site-specific or basin-specific indicators showing alteration of sediment quality caused by natural or anthropogenic activities (e.g. for comparison), the "substrates utilisation fingerprint" from BIOLOG EcoPlate was applied to display the potential functional diversity of the sediment microbial community. The "functional diversity" in this section refers to the variety of carbon substrates, which can be utilised by the microbial communities. First, in order to obtain an overview of a possible natural response range of the "substrates utilisation" in carbon cycling, the EcoPlates were applied to sediment and water samples from Over and Liskesbrug (Fig.2.3). The data were analysed by a kinetic model, which supplies information about population growth influenced by seasonality as well as its resulting contaminant distribution (Section 3.2.2). Second, to investigate the possible impact of the loss of key ecological function, nitrification, on the functional diversity of carbon cycling, nitrification in Over surface sediment was shut down by the specific inhibitor nitrapyrin. The data were analysed by dilution to extinction of community functions (Rutgers

et al., 2006), which estimates the relative structural diversity and therefore functional stability altered by the inhibited nitrification.

4.2.1 Seasonal Variation in Functional Diversity

In Chapter 3, a dramatic seasonal variation of sediment and river water toxicity in the river Elbe was observed and related to annual hydrological and biological events. The algal bloom adsorbed contaminants in the water phase, reducing the toxicity in the water column when they sink to the bottom. Toxicities were then measured in the surface sediments as the algal cells were degraded. An adapted seasonal variation of microbial functional diversity in the surface sediments is very probable. The seasonal variation of the microbial functional diversity from the same seasonal survey can offer an insight into the possible range of responses in carbon cycling at the locations.

4.2.1.1 Material and Methods

The microbes in sediments were extracted according to Frühling (2004) on the sampling day for Elbe samples, and on the sample arrival day for Dommel samples. 10 g of sediments was added to 100 ml 0.85% of Tetrasodium-pyrophosphate solution and shaken horizontally at 150 rpm for 30 minutes. It was then treated with ultrasound at 10% intensity for 30 seconds to separate the microbes from the sediment particles. The sediment medium mixture was then left standing for 10 minutes before the surface supernatant was removed and diluted 10 times with 0.2% sodium chloride solution. A single dilution concentration instead of same inoculum density was used, since the same sampling sites were compared. The diluted sediment extract was then pipetted in the BIOLOG EcoPlate with triplicates. The river water on the other hand was pipetted with triplicates to the EcoPlates directly. The plates were incubated at 25℃ and measured with a plate photometer at 590 nm every 24 hours up to 7 days.

4.2.1.2 Data Transformation and Analysis

A large amount of data can be generated quickly using BIOLOG: 31 (substrates) times 8 (measurements) matrix showing O.D. readings with time is produced for each sample. The extraction of important information from the huge data set and the interpretation of the data need extra caution. To summarise multivariate data, the statistical approach of principal component analysis (PCA) is usually used to extract multivariate variances with few principal components. Different approaches are used to choose data feed for PCA. Two commonly suggested data transformations, well colour development (WCD) and kinetic parameters were used for the seasonal data to compare results.

Well Colour Development The raw O.D. values from each replicate were first corrected by subtracting the O.D. value from blank well, where no carbon substrates but water is present. The corrected O.D. values of each substrate from the triplicates were averaged, and this averaged subtracted value was defined as "well colour development" (WCD) value of each substrate for further analysis. In the early incubation period, negative WCD as a result of variances in bacterial extract turbidity may occur and was assigned zero. For each sample, an "average well colour development" (AWCD) was calculated as the average of WCDs from the 31 substrates. The AWCD can be considered to reflect the total respiratory activities of the extracted microbial community. To avoid biases caused by not standardised inoculum density, WCDs at the same AWCD (0.3 or 0.8) are usually taken for further statistical analysis. However, the AWCD from the deeper sediments were too low to reach possible AWCD for all samples. The WCDs were normalised through division by AWCD as utilisation efficiency. The substrates utilisation efficiencies at day three were then analysed statistically for the variances and grouping.

Kinetic Model The colour development of each substrate over time follows an asymptotic sigmoidal curve, which describes the population growth of the microbial community utilising the specific substrate. Therefore, the WCD curves with time were analysed by a kinetic approach according to Lindstrom et al. (1998). The corrected WCD values over time were fitted with a density dependent logistic growth equation

$$Y = WCD = \frac{K}{1 + e^{-r(t-S)}} \ , \tag{4.3}$$

where K is the carrying capacity; r determines the exponential growth rate; t is the time course; and S is the time at the midpoint of exponential growth phase. The fitting of the colour development curves were performed using the software GraphPad Prism. With the kinetic model, the substrates utilisation time course was minimised to 3 kinetic parameters, which represent growth factors for principal component analysis (PCA). For substrates not utilised at all during the incubation period, no kinetic fitting could be found and the substrates were discarded for PCA. In some deeper sediment, where microbes grow anaerobically, the WCDs were extremely low. The inclusion of the samples would result in discarding more than half of the substrates. Therefore, the Elbe deeper sediments from April to September and Dommel deeper sediment from August were not included for PCA.

Principal Component Analysis (PCA) Principal component analysis is a powerful tool in reducing number of variables from multivariate data set. It extracts the variances from variables and delivers few principal components as

combination of the original variables. The principal components serve as new variables, which explain most of the variances from the original variables. The correlations between original variables and the derived principal components are described by the **factor loadings** with values from -1 to 1. Original variables with factor loadings greater than 0.7 or smaller than -0.7 were considered to contribute significantly to the principal components. The factor loadings of each substrate on the principal components can be interpreted as the composition of principal components from the substrates. The measure of each sample with the principal components was described by the **sample scores**, which were used to distinguish between samples. The normalised WCD at day 3 and the kinetic parameters were fed to principal components analysis using the software STATISTICA with varimax ordination. Two principal components were extracted for each data set to show the substrates utilisation fingerprints.

4.2.1.3 Results and Discussion

The seasonal variation of the microbial communities of water and sediment samples is presented in two parts. First, the total respiration activity of the microbial community of the samples is shown as AWCD time curves and kinetic parameters. The results will give an overview of differences in respiration activities of microbial community extracted from different seasons and matrices. Second, the functional diversity was shown with principal component analysis using the substrates utilisation efficiencies and kinetic growth parameters. The ordination analysis will display the similarities and differences of microbial community in utilising these environmental substrates.

Total Respiration Activity To give a general overview over the total respiration activity (AWCD) between two sampling sites as well as the matrices, the

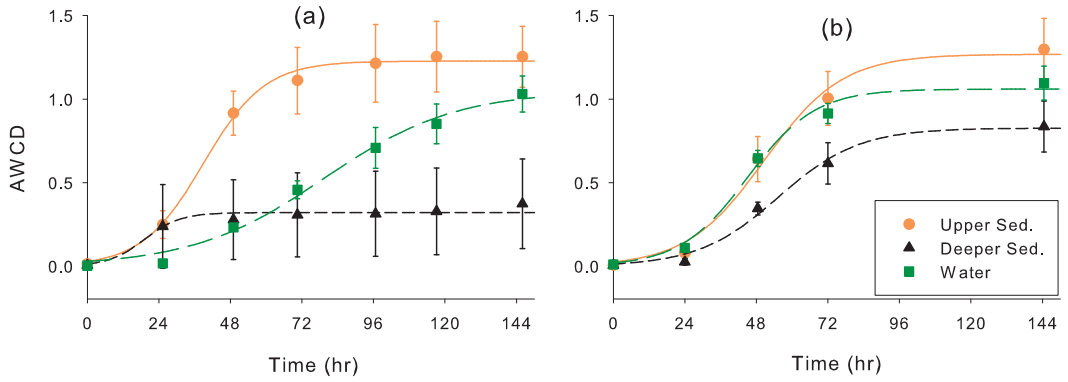


Figure 4.9: Average well colour development and kinetic fitting of March samples from (a) Elbe and (b) Dommel.

AWCD curves of March samples with their kinetic fitting are plotted in Figure 4.9. Generally the activities of upper sediments were higher than deeper sediments for both sites. This could be due to either a higher cell density or a more active respiration under oxic conditions. The same colour development in the first 24 hours of Elbe deeper sediments as in the upper sediments indicated a short active respiration period of the microbial community from the deeper sediment. This is once more confirmed by the bigger differences between upper and deeper sediments from Elbe than from Dommel. The shallow sediment depth (deeper sediment of 5-10 cm instead of 15-20 cm) in the Dommel probably led to less anoxic conditions in the deeper sediments. The microbial activity of water is more similar to the one of upper sediment, with the difference that the upper sediments reached the maximum growth faster than the waters.

The seasonal variation of the AWCD curves of upper sediments from both sites is shown in Figure 4.10. With regard to the Elbe upper sediment, the AWCD curves of July and September samples showed lower total respiration rates, which

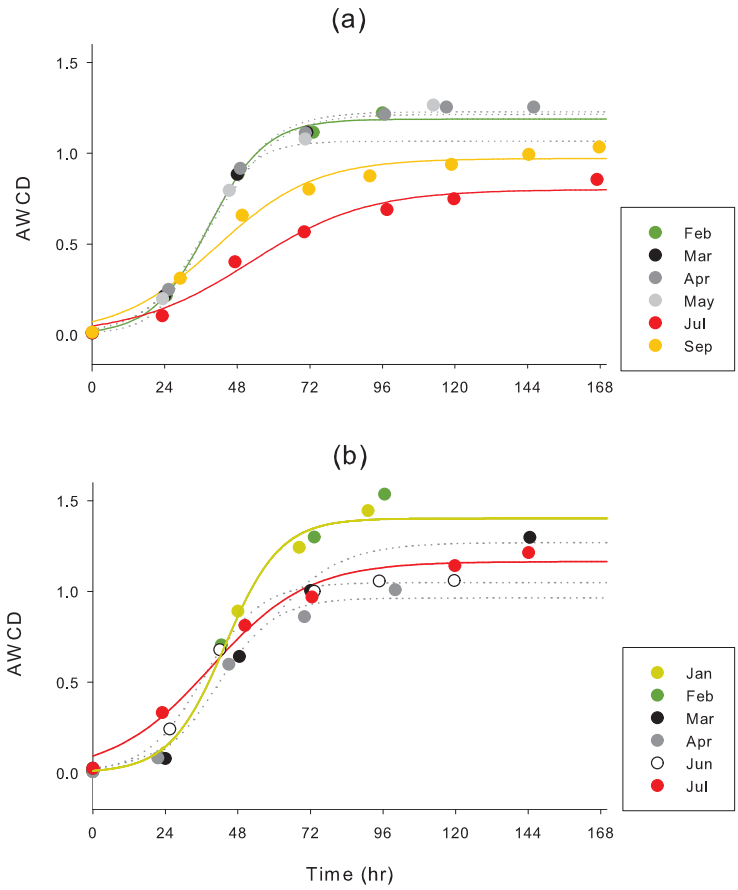


Figure 4.10: Average well colour development and kinetic fitting of upper layer sediments from (a) Elbe and (b) Dommel.

are significantly different from those of other months. In the Dommel upper sediments, the AWCD curves showing total respiration activities of January and February samples were significantly higher than in other months. Interestingly, the sediment microbial respiration activities under laboratory incubation in the winter/spring were higher than in the summer time. This was different from the expectation of higher microbial activity at higher temperatures in nature. The decrease of respiration activities in the Elbe may be caused by the increase of organic material in the upper sediments from dead algae and periphyton cells resulting in more anoxic conditions after algal bloom in May in the upper layer. The anoxic conditions in the upper sediments may result in less active microbes under aerobic laboratory incubation. In the Dommel, on the other hand, no algal bloom or distinct organic input was observed, due to the location of sampling site. In addition, the incubation temperature of 25℃ instead of temperature closer to field temperature may result in an induced growth of microbial communities in winter months and reduced growth in summer months (Christian and Lind, 2006; Dobranic and Zak, 1999).

The total respiration rates of the extracted microbial community demonstrated a clear difference between the sample matrices. The seasonality of the respiration activity was on the other hand not observed as a result of incubation at ideal instead of close to in-situ temperature.

The three kinetic parameters from AWCD time curve are plotted in a 3D scatter plot (Figure 4.11). Lindstrom et al. (1998) reported the meaning and interpretation of these parameters as follows. The S parameter, which indicates the lag time, provides information about the initial inoculum density. This is negatively correlated: the lower the cell density, the longer the time it takes to reach half of the exponential growth, the higher is S. The r parameter, which indicates the growth rate supplies information about how rapidly the substrates can be utilised by the community. The K parameter, which indicates the asymptote, is limited by the exhaustion of carbon substrates, and is probably less useful.

The Elbe deeper sediments (lower left circle) showed lower K, S and higher r value, and indicated the communities with high inoculum density, growth rate, but low level of substrates utilisation. It is usually assumed that the deeper layer of sediment has a lower microbial density compared to the upper layer of sediment. The S of Elbe deeper sediments, however, were low (Figure 4.11) which implied a considerable inoculum applied in the BIOLOG system, resulting in relatively short lag time and high growth rate. However, during the incubation period in oxic conditions, the microbes seemed unable to continue metabolism of the given substrates in the wells, which resulted in low carrying capacity (K) of the growth curve. The long lag times and low growth rates observed in the Elbe waters (upper right circle) indicated that the cell densities in Elbe river waters were lower than in sediments. No clear seasonal variations of the kinetic parameters (listed in Appendix A.2) could be related to ecotoxicities or temperature changes.

The total respiration activities of the extracted microbial communities demon-

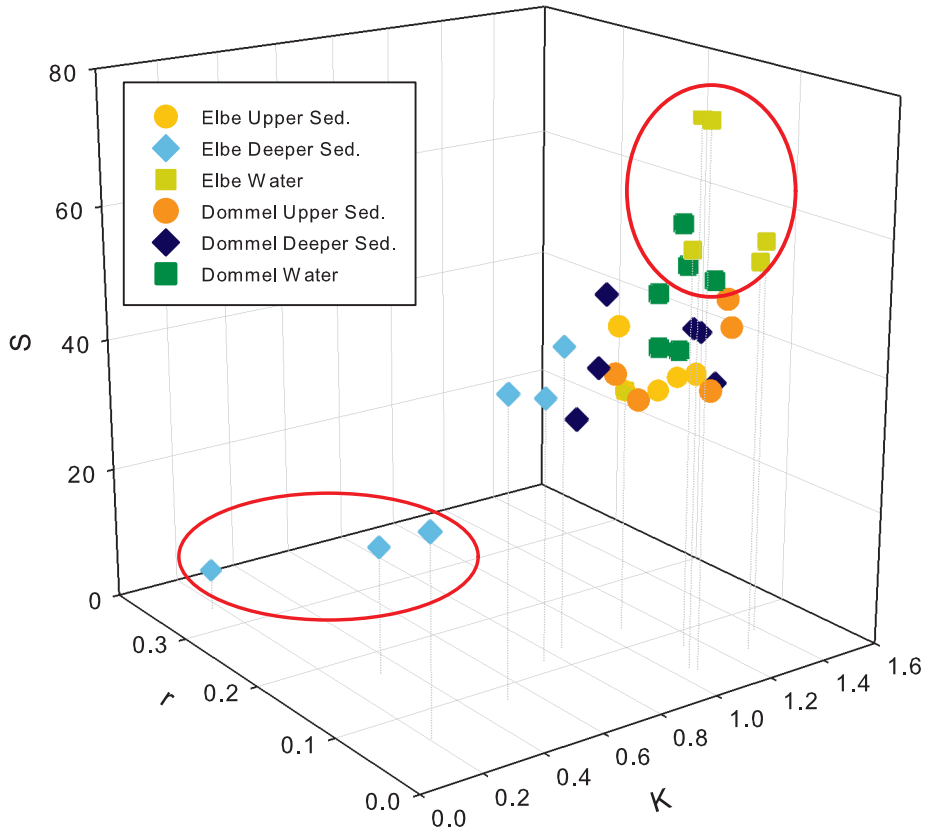


Figure 4.11: Kinetic parameters of AWCD curves from all samples. K, represents the carrying capacity, r the exponential growth rate, and S the middle time point of exponential growth phase.

strated the effect of matrices clearly but no seasonal variations, partly caused by the incubation under laboratory conditions. The application of a kinetic model, however, could assist the identification of these inherent biases. The ability of the extracted microbes to metabolise substrates under oxic conditions determined the total respiration activities measured with BIOLOG (Christian and Lind, 2006).

Functional Diversity The differences of the microbial communities in their ability to utilise carbon substrates, or so called functional diversities in this study were depicted as sample scores on the two principal components. In this section, only the groupings of microbial communities in their substrates utilisation fingerprints were discussed. The substrates contributing to the groupings and their factor loadings were listed in Appendix A.

Figure 4.12 shows the two principal components and sample scores using sub-

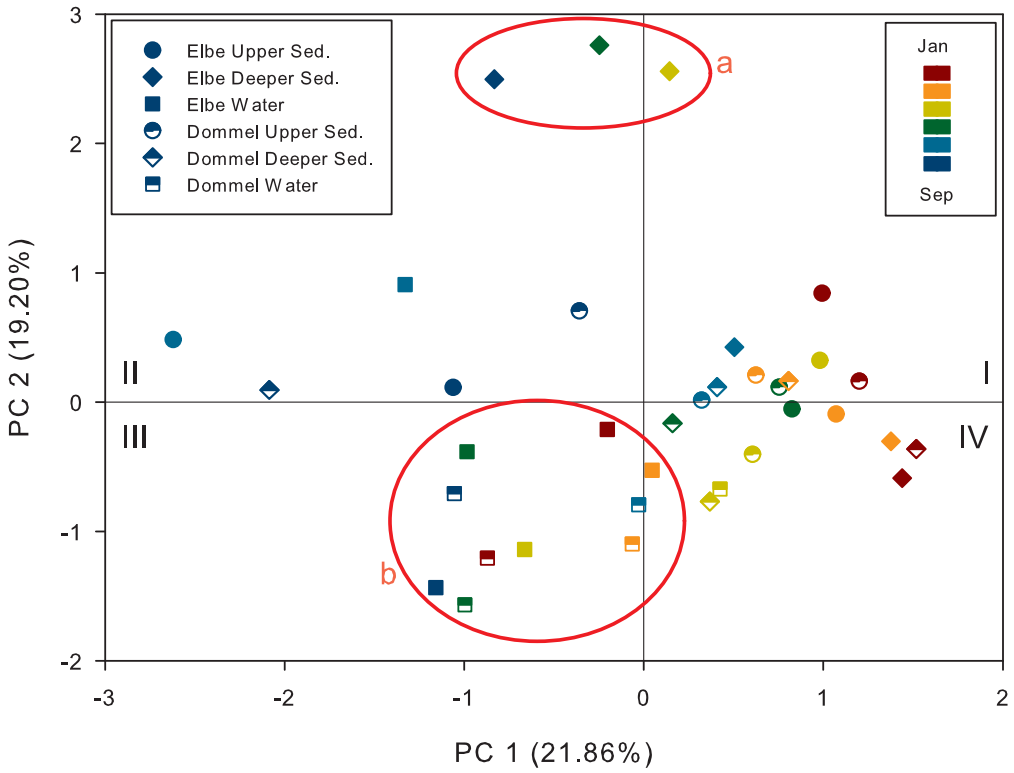


Figure 4.12: Sample scores of upper, deeper sediments and water from the Rivers Elbe and Dommel on principal components calculated for WCDs at day 3 as data feed. I, II, III, IV denote the quadrants.

strates utilisation efficiency at day 3 as feed data. The first principal component separated basically the sediments and water samples with few outliers. The second principal component on the other hand distinguished practically the Elbe deeper sediments with lowest activities (circle a) from the other samples. In quadrant III, only water samples (circle b) were found. The winter and spring samples were roughly at the lower right and summer samples at upper left. The different sample matrices as well as seasons were roughly separated by the principal components, though no obvious differences between Elbe and Dommel samples were demonstrated. The two components explained around 40% of the total variances from the utilisation fingerprint of 31 substrates at day 3.

The results showed that after 3 days incubation of the extracted microbial communities under laboratory conditions, the main differences in substrates utilisation fingerprints were observed between sediment ands water samples. In addition, the Elbe deeper sediments from summer months also displayed a different pattern of substrates utilisation compared to all other samples.

Before the kinetic parameters of the WCD time curves from all samples and substrates were analysed by PCA, some of the substrates were removed because they were not utilised and no curve fit could be found. Therefore, data showing WCD less than 0.2 and with extreme kinetic parameters were discarded before the PCA. As a result, Elbe deeper sediments from April to September and Dommel deeper sediment from August were not included. Furthermore, 22 instead of 31 substrates were included. (Included substrates were listed together with factor loadings in Appendix A.3.) The sample scores on two principal components for each kinetic parameter are shown in Figure 4.13.

The K and S parameters did distinguish between the water and sediment samples better than the parameter r. Though more variances (70%) were extracted from the r parameter, it only showed that the main variances were contributed by the outliers. The principal components resulting from carrying capacity (K) separated the water from sediment samples diagonally (marked with dashed line) with a total of 40% explained variances. The water samples have higher scores on component 1 and lower scores on component 2; while the sediment samples have lower scores on component 1 and higher scores on component 2. K indicated the carrying capacity, and the ordination of K demonstrated the differences in maximal substrates utilisation level of the water samples from the sediment ones.

The PCs that resulting from the exponential growth rate (r) showed a large group of samples with upper Dommel sediment in May and deeper Dommel sediment in July as outliers. Within the big group of samples, the Dommel samples were grouped more to the lower right, while the Elbe samples upper left. The results demonstrated that the substrates, which could be utilised rapidly by Elbe and Dommel microbial communties, were ifferent. In other words, those microbial communities extracted from Dommel and Elbe were different in how reapidly they could metabolise certain substrates. Furthermore, the two outliers could probably indicate input of certain substrates to Dommel sediments in May, causing microbial communities adapted to metabolise them quickly.

The PCs that resulting from the lag time (S) separated the water (marked with circle) and sediment samples clearly with the first component and outliers of Elbe upper sediments from July and September with the second PC and explained in total 64% of the variances. The lag time S provides information about inoculum density of microbes able to utilise certain substrates. The results indicated that the extracted microbial communities from the water and sediment samples had different composition. The microbial communities in the water phase had different proportion (density) of microbes able to utilise certain substrates.

Altogether, the PCA displayed the water and sediment microbial communities to be different in their substrates utilisation fingerprints. The microbial communities extracted from water and sediments were different in their proportion of microbes able to metabolise certain substrates as well as in the substrates able to deliver their maximal growth. The substrates readily utilised by the microbial communities on the other hand were more site dependent. No clear seasonal

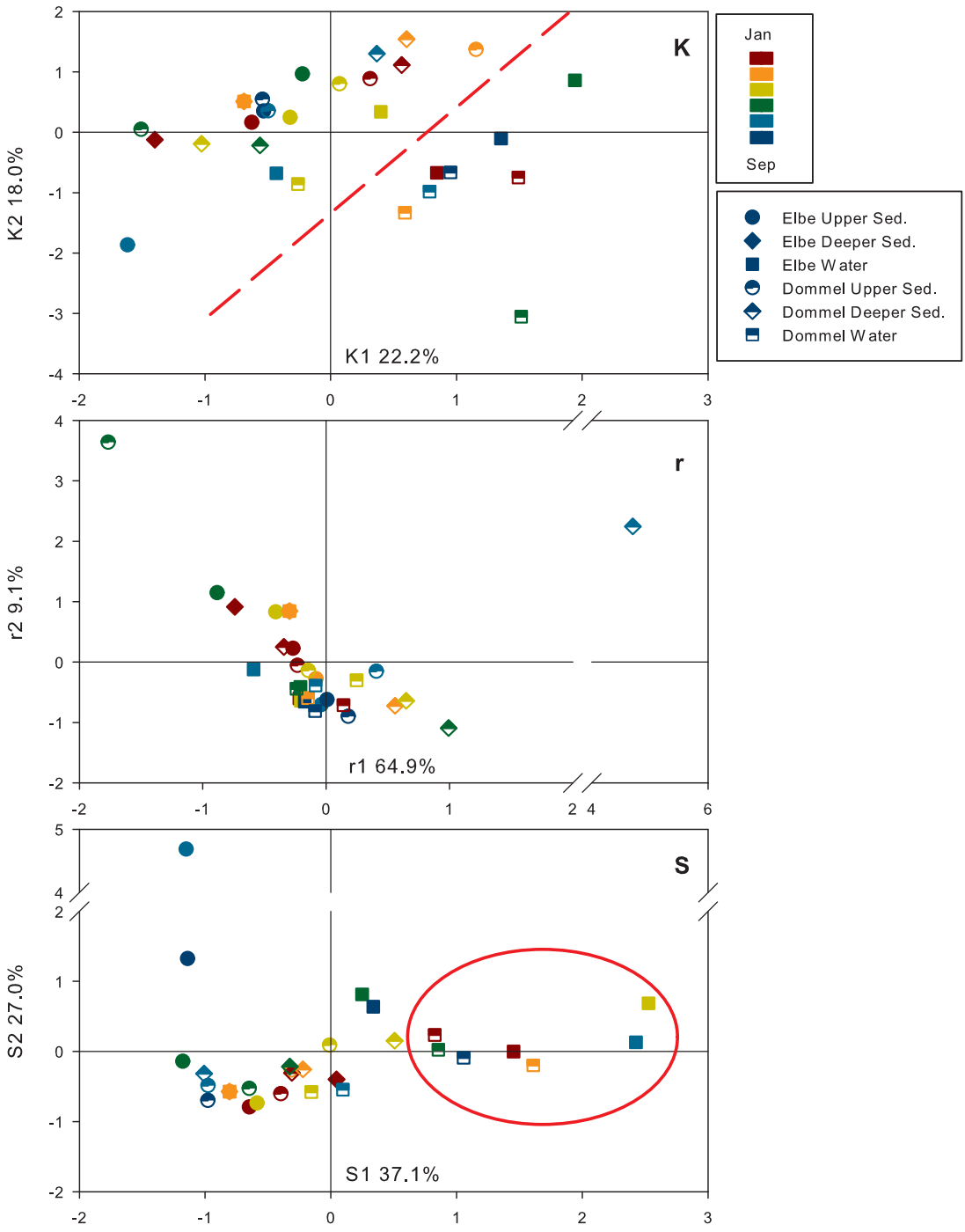


Figure 4.13: Sample scores of upper, deeper sediment and water from the Rivers Elbe and Dommel on principal components calculated for kinetic parameters.

variations could be observed.

Seasonal variation of ecotoxicities was shown (Section 3.2.2) in the river Elbe contributed by the hydrological and biological events. The activities and functional diversities of the microbial communities, however, did not vary accordingly. The total respiration activities of the microbial communities depended greatly on the sampling matrices, especially the ability to respire aerobically. Higher activities were observed in winter than in summer, but as a result of incubation under laboratory conditions (Christian and Lind, 2006). The functional diversity of the microbial communities also differed between the sampling matrices, but from sediment phase to water phase. There was also a tendency of different readily metabolisable substrates from the two sampling sites. The functional diversity of microbial communities with regard to carbon cycling is probably so prevalent and adapted in the environment that the seasonal events as well as redistribution of contaminants can hardly have influence on its potential, especially when it is determined by utilisation of 31 substrates under laboratory conditions. The kinetic modelling provides the differentiation of microbial functional diversity according to the population growth factor, but may be insufficient to be taken as indicator for quality of heterotrophic carbon cycling, since the result was shown very site specific and a reference response may be impossible to define.

4.2.2 Effect of Nitrapyrin with Dilution to Extinction

The previous section demonstrated that the BIOLOG microplates can differentiate the potential functional diversity of microbes in sediments. It distinguished between different sample matrices and possible influences from sites. The next question is how this heterotrophic carbon cycling relates to the key ecological function nitrification. The microsensor profiles in section 4.1 illustrated the nitrification activity within few mm of sediment depth. The nitrification activities in sediments depend strongly on the oxygen (and in the case of heterotrophic nitrification on the organic content) of the sediments, which are both important for the heterotrophic carbon cycling. Could inhibited nitrification pose an adverse impact on heterotrophic carbon cycling? The influence of a lacking ecological function on the functional diversity was investigated with the nitrification inhibitor, nitrapyrin.

To extend the differentiating power and to overcome the uncertainties of single inoculation density, the relative structure diversity was assessed by dilution to extinction of community functions according to Garland and Lehman (1999) and Rutgers et al. (2006). Except for the substrates utilisation fingerprints, this approach also provides information about the richness, evenness, redundancy and stability of the microbial community, which will be discussed with the results. The examination of community functional diversity across a series of dilution has shown a strong discriminating power between microbial communities in different soil types and soil management (Breure and Rutgers, 2000; Schouten et al., 2003).

This approach required three EcoPlates for one sample and was not applied in the investigation of seasonal variation for the efficiency in analysing monthly samples.

4.2.2.1 Material and Method

Sediment cores of 0-10 cm were collected at Over in March 2007. The sediment cores were mixed and 100 ml of sediment each were moved to another box and further spiked with 100 µl of 50 µM nitrapyrin to a final concentration of 50 nM or 100 µl DMSO three days before extraction of sediment microbes. Furthermore, the sieved sediments after microsensor measurements (see Section 4.1.1) were also extracted and analysed three weeks after sampling. Differences in potential nitrification rates were observed from the sediments before and after the sieving and microsensor measurement (Section 4.1.2). To confirm the possible influence of sieving and time interval incubated in laboratory on microbial communities, the heterotrophic functional diversities before and after the treatment were also investigated.

The extraction and dilution of the sediment microbial communities followed the instruction by Schmitt et al. (2006) with different extraction and dilution medium compared to those in Section 4.2.1 for the dilution plating. The microbes in the sediments were extracted by adding 3 g of sediment and 27 ml phosphate buffer (100 mM, pH = 7.0) in a 50 ml centrifuge tube. The sediment buffer slurry was incubated at 25℃ 150 rpm for 30 minutes followed by 10 second ultrasound to separate the microbes from the sediment particles. The slurry was then centrifuged at 500 rpm for 3 minutes to remove turbidity caused by humic substances. The supernatant was then removed for inoculation in BIOLOG plates. The microbial extracts were further diluted with 10 mM phosphate buffer 3 folds up to 3^{-8} times of original cell density. 100 µl of the 9 dilutions of microbial extracts were then pipetted with no replicate into 3 EcoPlates. The BIOLOG plates were then incubated at 25℃ and optical density of colour development was measured every 8 to 24 hours up to 7 days.

4.2.2.2 Data Transformation and Analysis

For the sediments amended with inhibitor, the dilution plating method was used, which is independent from inoculum density. The measured O.D. was corrected as in Section 4.2.1.2. The area under colour development curve of each substrate at each dilution concentration was taken as the integrated response instead of normalised WCD or kinetic parameters and calculated with Prism. The areas under curves (AUCs) were then plotted against the dilution factor, which is correlated to inoculum cell density. This response curve was then fitted with a log normal distribution according to Garland and Lehman (1999) to determine the dilution factor, at which 50% of the responses were lost. The log normal

distribution of the AUC was described as

$$AUC = \frac{t}{1 + 10^{h(logCFU_{50} - logCFU)}} \ , \tag{4.4}$$

where t is the asymptotical maximum at infinite cell concentration, h is the hill slop and the $log(CFU_{50})$ is the logarithm of the dilution factor, at which 50% of the responses, in this case area under curve, were lost. For substrates easily utilised by the extracted microbial community, the $log(CFU_{50})$ is lower, meaning the community can utilise the substrates at lower cell density. By comparing the $log(CFU_{50})$ of each substrates and the averaged $log(CFU_{50})$, which is the $log(CFU_{50})$ of the AWCD, the relative abundance (RA) of microbes utilising a specific substrate can be calculated as

$$RA = log \frac{CFU_{50_s}}{CFU_{50_{average}}} \ . \tag{4.5}$$

For substrates not utilised at highest cell density, -2 was assigned; and for substrates resulting in high colour development at lowest cell density, +2 was assigned to avoid extreme values in ordination analysis.

In addition to the above mentioned analysis of the relative structure diversity, the WCD time curves of each substrate at each dilution concentration can also be analysed as described in Section 4.2.1.2 for comparison, which was not included under the scope of the study.

4.2.2.3 Results and Discussion

The influence of inhibited nitrification by specific inhibitor nitrapyrin on the relative structure diversity was investigated. Figure 4.14 shows the relation between the averaged AUC and dilution factor of all samples with the log normal fitting. Generally, the spiking with DMSO and nitrapyrin did not change the structure diversity of the extracted microbial communities. However, after the sieving and two weeks incubation, lower functional activities were measured from log dilution factor of minus one.

Fonseca and Ganade (2001) illustrated the relationships between functional groups of microbial community and random extinction of species using a probabilistic framework to predict its stability. The concept was taken and adapted to the relationship between functional activities and extinction from dilution in this study. Figure 4.15 illustrates the dilution-function extinction curves of communities with varying functional richness, functional evenness, and species richness. The **richness** is defined here as the number of functions or species and the **evenness** as the distribution of species across functions. Though functional richness would equal to number of metabolised substrates in terms of BIOLOG responses, it was highly correlated to the AUC with the time interval (168 hours), when

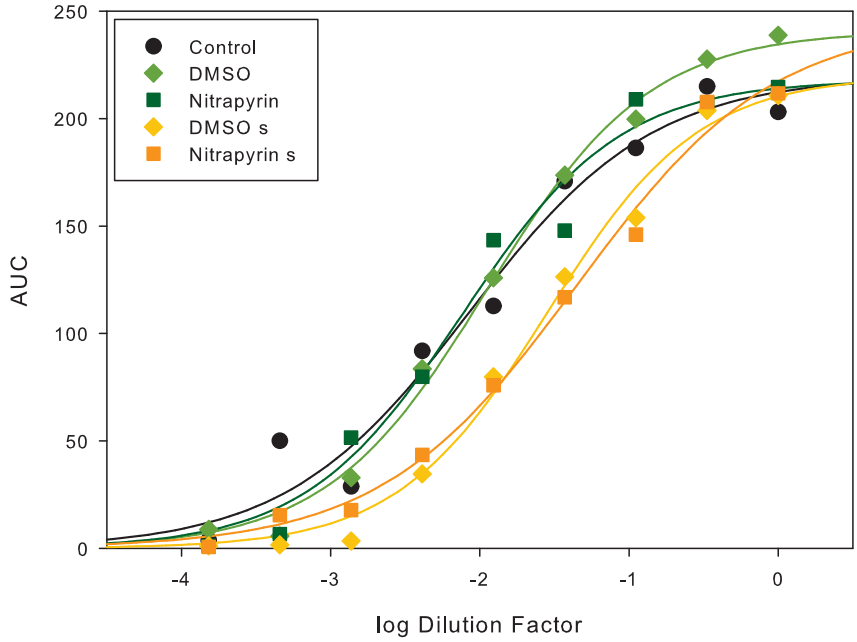


Figure 4.14: Area under curve of AWCDs of freshly collected and incubated Over sediments with dilution of inoculum cell density and fitting of log normal curve. The s at the end means sieved and incubated sediments.

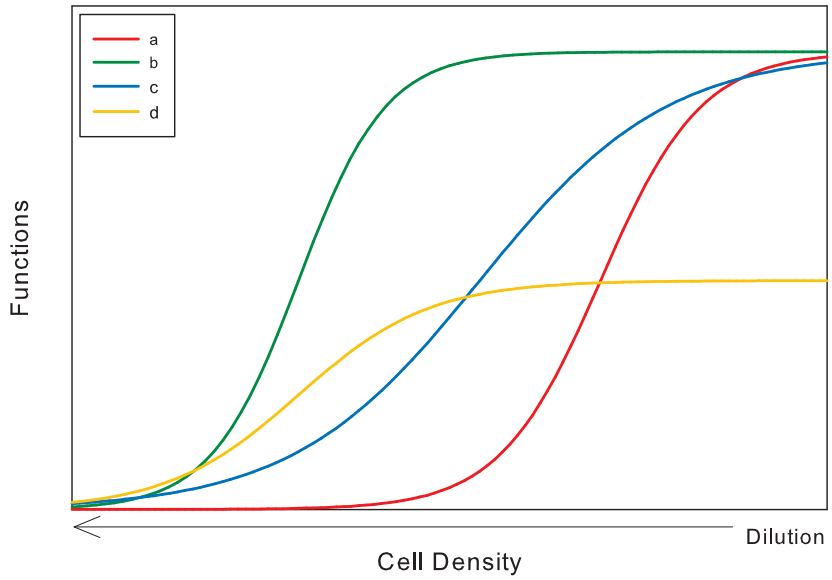


Figure 4.15: Relation of ecological function and cell density of microbial communities. Community a has high functional richness, high functional evenness, but low species richness. Community b has high functional richness, high functional evenness, and high species richness. Community c has high functional richness, low functional evenness, and high functional species. Community d has low functional richness, high functional evenness, and high species richness.

stationary growth of the diluted microbial community can be reached. Communities with high species richness or high functional evenness tend to have high functional redundancy, given that the other parameters are the same. As a result, a community with low functional evenness, i.e. most of the species perform few functions and the other functions were carried out by few species, would start losing functions, which were carried out by one or few species, with the slightest dilution, given that the other parameters were the same (community c compared to b in Figure 4.15). A community with low species richness, on the other hand, would lose half of the functions with less dilution (community a compared to b in Figure 4.15). Therefore, the parameter t of the log normal fitting indicated the functional richness, h indicated the functional evenness, and the $log(CFU_{50})$ reflected the redundancy contributed by both functional evenness and species richness of the microbial community.

Table 4.2 lists the log fitting parameters from sediments illustrated in Figure 4.14, and shows that the functional richness (t) was similar for all samples. The undiluted community from all samples had similar AUCs. The evenness (h) was also similar with all samples, except for those of the control and the sieved sediment samples spiked with nitrapyrin were slightly lower. The $log(CFU_{50})$ showed the dilution factor, at which half of the response faded out. The higher $log(CFU_{50})$, i.e. higher cell density at which half of the functions were lost, observed for sediments sieved and incubated indicated that the sieving and incubation processes decreased the functional redundancy of the microbial community. Given that the functional evenness and functional richness of the sieved sediments were similar to those of un-sieved ones, it was most probably that the species richness of the communities was reduced. Altogether, the sieving and incubation removed certain microbial species, which were able to carry out several redundant functions, and the addition of nitrapyrin in sieved sediments further reduced microbial species, which were able to carry out less redundant functions.

The dilution-function (AUC) extinction relations indicated that the solvent

Table 4.2: Log normal fitting parameters of the Over sediment samples.

samples	Regression parameters		
	t functional richness	h functional evenness	$log(CFU_{50})$ redundancy
control	219.6	0.7082	-2.027
DMSO	240.9	0.8036	-1.950
Nitrapyrin	217.9	0.8248	-2.117
DMSO sieved	220.2	0.8609	-1.544
Nitrapyrin sieved	245.3	0.6610	-1.348

and nitrification inhibitor in fresh sediments did not change the relative structure of the community, as none of the community measures were significantly different from each other. However, the sieving and incubation process decreased the redundancy of the community most probably through reducing several functional redundant species. The nitrapyrin in the sieved sediments further diminished the functional evenness through removing less functional redundant species. The sieved sediments seemed to be less stable and more susceptible to the loss of key function, nitrification.

The functional diversity with regard to the substrate utilisation fingerprints of these sediments can be shown as the relative abundance (RA) calculated from Equation 4.5, which indicates the potency to convert a specific substrate compared to the average of the extracted microbial community. The RAs of the above analysed samples are illustrated in Appendix A.2. The functional diversity was analysed with PCA using log relative abundance, normalised O.D. at day 3 and the kinetic parameters (K, r, S) from the inoculum density closest to the $log(CFU_{50})$ from each substrates. The variances were extracted from a total of 13 Elbe surface sediment samples, which were applied with dilution plating method. The results from different feed data showed similar results and only results from relative abundance are illustrated. Figure 4.16 displays the func-

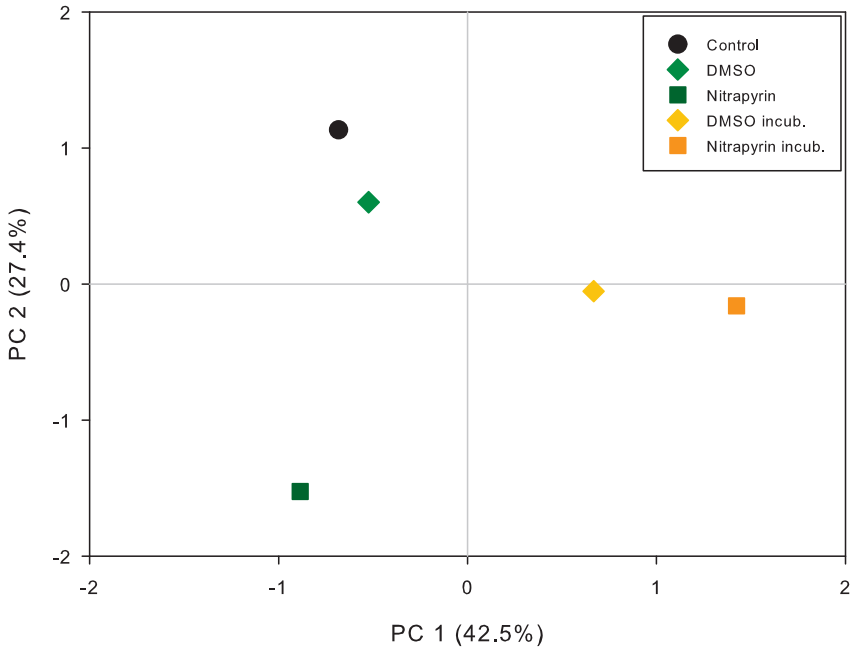


Figure 4.16: Sample scores of Over fresh and incubated sediments amended with DMSO and Nitrapyrin on principal components calculated with log relative abundance (n=13). incub. represents sieving and incubation.

tional diversity using relative abundance of 31 substrates. The first component distinguished clearly the microbial communities before and after sieving. The second principal component distinguished mainly the sediment amended with nitrapyrin from the control and DMSO amended fresh sediments with a total of 70% variances explained.

No differences in total activity but slight differences in relative structure of the microbial community as well as substrates utilisation fingerprints due to sieving and addition of nitrapyrin were observed. The inhibited autotrophic nitrifying activity did not seem to influence the heterotrophic utilisation capability of carbon substrates directly. However, the sieving and incubation had greater influence on the functional redundancy of the microbial community by making it more susceptible to loss of key function.

4.2.3 Summary

Carbon is a major nutrient, and the cycling of its organic form by heterotrophic microbes is of central ecological importance. The aerobic heterotrophic microbes are able to utilise a diverse range of carbon substrates, exhibiting a potential heterotrophic functional diversity which can be estimated by applying a BIOLOG system. However, the BIOLOG system is better used to compare than to characterise microbial communities extracted from the environment due to the inherent bias from extraction and incubation of microbes under laboratory conditions (Preston-Mafham et al., 2002). The possible application of the method as one indicator of the functional diversity of sediment microbial community was investigated comparing samples taken at different seasons and with regard to the importance of nitrification for heterotrophic activity.

Concerning the seasonal variation of the potential diversity of the sediment microbial communities, a pronounced variation of total respiration activity was observed. The low total respiration activities of sediments from summer were related primarily to the low redox condition and cultivable cell density. The seasonal variation of functional diversity was only roughly identified, while the functional diversity between different sample matrices was shown clearly. No clear link between the functional diversities and ecotoxicities shown in Chapter 3 was observed. The difficulty in distinguishing seasonal samples in this study was caused by the incubation at ideal instead of close to field temperature, resulting in a temperature induction of growth. The anoxic condition in the deeper sediments also contributed to a smaller cultivable community. Further application of BIOLOG on environmental samples should keep laboratory conditions as close as possible to in-situ conditions concerning oxygen level and temperature. Though identifying functional diversities from different sample matrices, the application of BIOLOG with single inoculum density did not provide a sensitive indicator for the heterotrophic functional diversity in carbon cycling.

A dilution to functional extinction method, which can be used to predict the

stability of a microbial community, is therefore more suitable to serve as a quality indicator. The method was applied to investigate the relative structure of the heterotrophic cycling of carbon concerning the influence of inhibited nitrification on the heterotrophic functional diversity. A more distinct difference was caused by sieving and two weeks incubation than by the specific inhibitor nitrapyrin. The inhibition of nitrification activity did not show any effect on total respiratory activity of the heterotrophic community. The lack of nitrification processes in the sediments did not lead to a higher heterotrophic activity due to more available oxygen or a lower functional diversity. The sieving and incubation processes on the other hand reduced the functional redundancy of the microbial community by decreased species richness while remaining the functional evenness. The lower functional redundancy of the sieved sediments lead to lower stability and stress resistance of the microbial communities, which was more susceptible to the loss of nitrification as shown with lower functional evenness.

The BIOLOG method offers a quick indication of potential functional diversity of microbial communities in heterotrophic carbon cycling. Despite different approaches in data analysis and interpretation, the long incubation time under laboratory conditions pose methodological bias. As a result, the environmental conditions such as redox potential, temperature, and organic content showed a greater influence on the potential functional diversity in sediments than seasonality or key ecological function. Despite the above mentioned disadvantages, the dilution to functional extinction method provided an indication about the relative structure and therefore stability of the cultivable heterotrophic community. This indication of stability may not be related to the functional diversity of the heterotrophic carbon cycling directly, but can serve as a good indicator of the susceptibility of the carbon cycling.

4.3 Substrates Induced DMSO Reduction

The functional diversity of a microbial community includes both autotrophic and heterotrophic activities, which relate closely to each other in nutrient cycling. The autotrophic key function of nitrification was shown in section 4.1 to be sensitive to oxygen content within a few millimetres, and the potential could recover at ideal environmental conditions. The heterotrophic functional diversity of carbon cycling was redox and temperature dependent and did not reflect an inhibition of nitrification as the key ecological function. To further investigate the relationship between the autotrophic and heterotrophic nutrient cycling as part of the functional diversity of sediment microbial community, the method of DMSO reduction was applied, which can indicate respiratory activity under aerobic as well as anaerobic conditions, of autotrophs and heterotrophs.

Dimethylsulphoxide (DMSO) is a natural organic sulphur compound, which can be taken as electron acceptor in substrates degradation. It is reduced to

dimethylsulphide (DMS), which is the most abundant organic sulphur compound and plays an important role in sulphur cycle globally. The reduction reaction shown in equation 4.6 has redox potential of +0.16 eV, makes it possible to be carried out in both aerobic and anaerobic conditions.

$$H_3C - \underset{\underset{\|}{\mathrm{O}}}{\mathrm{S}} - CH_3 + 2e^- + 2H^+ \leftrightharpoons H_3C - S - CH_3 + H_2O \qquad (4.6)$$

In the marine environment, up to 45 million ton DMS was produced annually from degradation of dimethylsulphoniopropionate (DMSP), a major osmoregulatory solution in marine algae. In fresh water sediment, DMS is produced by reduction of DMSO in the anoxic environment, where DMSO was used as electron acceptor. And the produced DMS can be further used:
(1) in methanogenesis, yielding CH_4 and H_2S;
(2) as electron donor for phototrophic purple bacteria, yielding DMSO; and
(3) as well for chemolithotrophs and chemoorganotrophs, yielding DMSO. The use of DMSO reduction as an indicator for microbial activity in soil has first been suggested by Alef (1990). He measured the microbial turned-over DMS in bulk soils with GC within few hours and reported that more than 95% of the microbes isolated from the soil and water were able to use DMSO as electron acceptor. Griebler (1997) further examined its application on freshwater sediments and pointed out that the DMSO reduction rate was significantly correlated to the electron transport system activity. Therefore DMSO reduction rate can be taken as an alternative to measure microbial activity to BIOLOG, respiration rates, and radioactive methods. It has several advantages in studying microbial activity in sediment such as no extraction, high sensitivities, and short experimental time (Griebler, 1997). The methodological comparison of DMSO reduction, BIOLOG, and substrates induced respiration (SIR) rate, which is an alternative method measuring microbial respiration activity (production of CO_2) to BIOLOG proposed by Degens and Harris (1997), is listed in Table 4.3.

Though Griebler and Slezak (2001) investigated several aspects concerning the competition of DMSO with electron acceptors and the correlation of DMSO with cell production, no study was published so far concerning the theoretical turn-over rate of DMSO induced by substrates at the presence of preferred electron acceptors. The addition of different carbon substrates is likely to induce the DMSO reduction rate, rate of DMSO taken as electron acceptor, in a way different from the principle of BIOLOG, growth metabolism, and substrates induced respiration rates. In this chapter, the substrates induced DMSO reduction rate as a measure of microbial functional diversity is proposed. The potential information gained for both heterotrophic respiration diversity and nitrification activity from DMSO reduction rate is discussed in "Theory and Hypothesis" and further investigated by experiments.

Table 4.3: Methods measuring microbial functional diversities.

	BIOLOG	SIR*	DMSO
Extraction	yes	no	no
Incubation			
method	aerobic	in-situ	in-situ
time	5 - 7 days	6 hours	3 hours
Determination			
method	photometric	photometric	GC-FID
limits	$>10^4$ cells	CO_2 solubility	

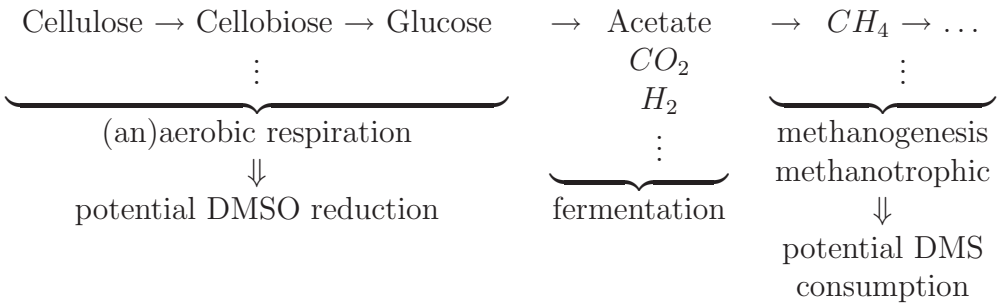
$*$ substrates induced respiration.

4.3.1 Theory and Hypothesis

To start the discussion, we have to come back to the principle of energy production by microbes. Figure 4.1 in the beginning of the chapter illustrated the variety of electron acceptors utilised by microbes under different environmental conditions. Figure 4.8 further depicted the huge range of organic substrates taken as electron donors. The energy released from substrate metabolism depends on the electron donor/electron acceptor couple. The higher the energy differences between electron donor and acceptor are, the more energy the metabolism will release and the more it is preferred by microbes. Electron donors are compounds from macromolecules to carboxylic acids that can be easily oxidised. Electron acceptors with highest redox potentials, i.e. highest energy yields, are oxygen, followed by nitrate and DMSO. Here several questions regarding the substrates induced DMSO reduction rate are discussed theoretically: At which environmental conditions is DMSO utilised as electron acceptor? How are different substrates coupled to DMSO reduction? How do autotrophic activities such as nitrification affect the DMSO reduction rate?

4.3.1.1 Scheme of Substrates Metabolism

For demonstration of the varieties of possible electron donors and acceptors, the degradation of natural substrates and polymers in the environment is a good example. It is seldom performed solely by a single bacterium group but rather by a large number of species working closely together in a community therewith showing a high metabolic diversity. The polymers are likely to be oxidised or hydrolysed to monomers using different electron acceptors present in the sediments. The monomers are then likely to be glycolysed to pyruvate or fermented to alcohols, acids, H_2, etc. The acetate and H_2 provide substrates for the anoxic sulphate reducing bacteria and syntrophic community with methanogenic and acetogenic bacteria.



Using DMSO as electron acceptor for energy production would occur in the first step of substrates degradation. The fermentation and methanogenesis would occur at anoxic condition when other electron acceptors were absent. When excess DMSO was added to the sediment system, the degradation with respiration will most probably compete off the fermentative microbes, due to the higher energy yield. The activity of substrates induced DMSO reduction depends on the couple of electron donor and acceptor, which will be discussed in detail. Methanogenic bacteria are able to use DMS as electron donor in anoxic condition; however they are unlikely to contribute significantly to reducing the DMS concentration within 3 hours due to their relatively slow growth rate. The rate of DMSO reduction can thus be considered equal to microbial respiratory activity.

4.3.1.2 Kinetic Calculation

In order to elucidate the effect of DMSO addition on the competing reductions of "natural" electron acceptors, the theoretical DMSO reduction rate was calculated based on enzymatic reactions. Several models have successfully simulated the depth profiles of several electron acceptors in fresh water sediments (Van Cappellen and Wang, 1996; Dale et al., 2006). However, due to the low natural concentrations of DMSO and DMS in freshwater systems, they were not included in existing models. The models calculated the reduction or production rate of electron acceptors based on pure enzymatic biochemical reactions. To calculate the theoretical DMSO reduction rates at given substrate concentrations in relation to other electron acceptors and eventually to the activity of nitrification, the enzymatic reaction principles were considered.

The primary substrates degradation reactions referring to respiration in sediments and secondary redox reactions referring to autotrophic activities are listed according to energetic hierarchy in Table 4.4. The substrate was formulated to include not only carbohydrates but also proteins, which could serve as electron donor and deliver capability of metabolic functions. According to the differences in redox potential of the electron acceptor pairs, aerobic degradation (Equation 4.7, +0.82 eV) will yield maximum energy, followed by denitrification (Equation 4.8, +0.43 eV) and then DMSO reduction (Equation 4.9, +0.16 eV). Since DMSO would be added in excess concentrations, the utilisation of other electron acceptors having lower energy yield such as sulphate is not considered. Therefore, the

Table 4.4: Important reactions involved in substrates degradation with DMSO.

primary degradation reaction:

$$(CH_2O)_x(NH_3)_y \ + \ (x+2y)O_2 \ + \ yHCO_3^- \xrightarrow{R_1/x} (x+y)CO_2 \ + \ yNO_3^- \ + \ (x+2y)H_2O \quad (4.7)$$

$$(CH_2O)_x(NH_3)_y \ + \ (\frac{4x+3y}{5})NO_3^- \xrightarrow{R_2/x} (\frac{x-3y}{5})CO_2 + (\frac{2x+4y}{5})N_2 \ + \ (\frac{3x+6y}{5})H_2O \ + \ (\frac{4x+3y}{5})HCO_3^- \quad (4.8)$$

$$(CH_2O)_x(NH_3)_y \ + \ (2x+4y)C_2H_6SO \ + \ yHCO_3^- \xrightarrow{R_3/x} (x+y)CO_2 \ + \ yNO_3^- \ + \ (2x+4y)C_2H_6S \ + \ (x+2y)H_2O \quad (4.9)$$

secondary redox reaction:

$$NH_4^+ \ + \ 2O_2 \ + \ 2HCO_3^- \xrightarrow{R_4/x} 2CO_2 \ + \ NO_3^- \ + \ 3H_2O \quad (4.10)$$

electron acceptors included in the calculation are O_2, NO_3^-, and DMSO. There are further redox reactions involving the chemolithotrophic activity of the microbes, where the electron donors in these reactions are no more carbohydrates but certain inorganic ions. The nitrification reaction 4.10 is included since its product nitrate is involved as an electron acceptor.

When expressed in first order enzymatic reactions, the reaction rate (R_i) depends only on the degradable (m) substrate concentration, if the electron acceptor is not limited and enough activation energy is given

$$R_c = -\frac{d[CH_2O]}{dt} = k_c[CH_2O]_m. \quad (4.11)$$

The total rate of substrate degradation (R_c) is therefore sum of reactions 4.7 to 4.9:

$$R_c = \sum_{i=1}^{3} Ri, \ R_i = f_i R_c \quad i = O_2, NO_3^-, DMSO. \quad (4.12)$$

Two limiting factors influence the proportion of each degradation pathway: the presence of a more energetically favoured pathway and the concentration of the electron acceptor (EA). For each electron acceptor exists a limiting concentration, which describes the concentration of electron acceptor with maximum

reaction rate. The rate of primary degradation pathways can be written as

$$R_i = R_{imax}\frac{[EA]}{[EA]_{lim}} \ . \tag{4.13}$$

According to the enzyme kinetics, the rate of primary degradation reaction and nitrification is calculated according to Table 4.5. Since excess DMSO is added to the system, the reaction rate of using DMSO as electron acceptor is not dependent on the DMSO concentration and should remain at maximum reaction rate, given that the other thermodynamically preferred electron acceptors do not exist.

As a summary, the utilisation rates of the important electron acceptors are listed in Table 4.6. It shows that the oxygen reduction rate depends strongly on the oxygen concentration in the system and that one folds of the nitrification rate contribute to two fold of the oxygen reduction rate. Nitrate is produced through degradation of nitrogen containing substrates as well as nitrification and reduced as electron acceptors at the same time. The reduction rate of DMSO (production rate of DMS) is negatively dependent on the proportion of substrate degradation coupled with oxygen and nitrate as electron acceptor, and the N containing substrates lead to proportionally higher DMSO reduction rate. Therefore, DMSO reduction rate would decrease at high oxygen or nitrate concentration. The degradation of N containing substrates leads to overall higher reduction of electron acceptors compared to carbohydrates without N.

Reaction 4.10 shows that it took two oxygen to produce one nitrate. When nitrification occurred, two folds of oxygen reduction will lead to one fold of nitrate production. The rate of utilising oxygen and nitrate as electron acceptor in degrading substrates depends directly on the oxygen and nitrate concentration, since these two electron acceptors are usually limited in the top milimetres of sediments (Chapter 4.1.1). At the end, the occurrence of nitrification will lead to a lower sum of substrates degradation by oxygen and nitrate and a higher proportion of substrates degraded using DMSO as electron acceptor.

Table 4.5: The reaction rates of substrates degradation and nitrification.

$$R_1 = R_{O_2max}\frac{[O_2]}{[O_2]_{lim}} \tag{4.14}$$

$$R_2 = R_{NO_3^-max}\frac{[NO_3^-]}{[NO_3^-]_{lim}} \tag{4.15}$$

$$R_3 = R_{DMSOmax} = R_c - R_1 - R_2 \tag{4.16}$$

$$[DMSO] \geq [DMSO]_{lim}$$

$$R_4 = k_4[NH_4^+][O_2] \tag{4.17}$$

Table 4.6: Net rate of the electron acceptors.

$$
\begin{aligned}
R_{O_2} &= -\frac{x+2y}{x}R_1 - 2R_4 \\
&= -\frac{x+2y}{x}R_{1max}\frac{[O_2]}{[O_2]_{lim}} - 2k_4[NH_4^+][O_2] \qquad (4.18)\\
R_{NO_3^-} &= \frac{y}{x}R_1 - \frac{4x+3y}{5x}R_2 + \frac{y}{x}R_3 + R_4 \\
&= \frac{y}{x}R_1 - \frac{4x+3y}{5x}R_2 + \frac{y}{x}(R_c - R_1 - R_2) + k_4[NH_4^+][O_2] \\
&= \frac{y}{x}R_c - \frac{4x+8y}{5x}R_{2max}\frac{[NO_3^-]}{[NO_3^-]_{lim}} + k_4[NH_4^+][O_2] \qquad (4.19)\\
R_{C_2H_6SO} &= -\frac{2x+4y}{x}R_3 \\
&= -\frac{2x+4y}{x}R_{DMSOmax} \\
&= -\frac{2x+4y}{x}(R_c - R_1 - R_2) \qquad (4.20)
\end{aligned}
$$

When N is present in substrates, higher reduction rates of all electron acceptors would be needed to degrade the same concentration of substrates. At the same time, more nitrate will be produced resulting in higher nitrate concentration and therefore higher denitrification rate, which out competes DMSO as an electron acceptor. As a result, the lower the C/N ratio at the same numbers of carbon in substrate, the lower the DMSO reduction rate.

4.3.1.3 Hypothesis

Based on the conclusion made from the kinetic calculations, the DMSO reduction rate can not only reflect the metabolic capabilities in both oxic and anoxic zones of sediment, it can also indicate a possible loss of the key ecological function nitrification. The hypothesis is therefore to simultaneously indicate both heterotrophic metabolic capabilities and autotrophic activities with the substrates induced DMSO reduction rate method. The hypothesis will be tested in the next section first by addition of nitrate, a competing electron acceptor, which is considered to decrease the DMSO reduction rate, and then by shutting down the nitrification activity with the specific inhibitor, nitrapyrin, which is assumed to increase the DMSO reduction rate.

4.3.2 Laboratory Confirmation

The hypothesis of using substrates induced DMSO reduction rate as indicator of microbial functional capabilities was tested with natural sediments. The effect of a preferred electron acceptor on substrates induced DMSO reduction rate was first investigated to confirm the enzyme kinetic calculations according to the hierarchy of energy yield. The inhibition of nitrification with a specific inhibitor was then tested as a further confirmation of its impact on functional capability.

4.3.2.1 Material and Methods

Selection and Preparation of Substrates Several important and common carbon substrates from categories of carbohydrates, amino acids, carboxylic acids, and environmental chemicals were chosen. Carbohydrates obtain high energy and are utilised almost by all microbes. Amino acids are N-containing substrates, and several carboxylic acids are important metabolic intermediates. The inclusion of environmental chemicals can give indication of a pollution adapted community. The concentration of added substrates was suggested by Degens and Harris (1997) to be 30 mg C/g sediment. For substrates with low solubility, concentration of 7.5 mg C/g sediment was used. To avoid possible toxic effects from the environmental chemicals, concentrations of 5 mM were prepared (Kaufmann et al., 2006). All substrate solutions were prepared with bi-distilled water and filter sterilised.

Addition of Nitrate To confirm the influence of electron acceptors in the hypothesis, substrates induced DMSO reduction rates from sediments amended with or without nitrate were investigated. Sediments were collected in Over in August 2007. Six different substrates including two sugar, two amino acid, and two carboxylic acids were added. Substrates included were D-glucose ($C_6H_{12}O_6$), D-xylose ($C_5H_{10}O_5$), acetic acid ($C_2H_4O_2$), malic acid ($C_4H_6O_5$), L-arginine ($C_6H_{14}N_4O_2$), and L-asparagine ($C_4H_8N_2O_3$). Nitrate was added as KNO_3 with an end concentration of 500 µM.

Addition of Nitrapyrin The effect of inhibited nitrification in natural sediments was then investigated with eight substrates. Sediments were collected in Over in March 2007. The inhibition of nitrification was done by adding a specific inhibitor, nitrapyrin, three days before the experiment at an end concentration of 50 µM dissolved in DMSO. The substrates included were three carbohydrates, D-glucose, D-cellobiose ($C_{12}H_{22}O_{11}$) and N-acetyl-D-glucosamine ($C_8H_{15}NO_6$); two carboxylic acid, acetic acid and malic acid; one amino acid, L-arginine; and two environmental chemicals, 1,1,2,2-tetra-chloroethane ($C_2H_2Cl_4$) and hexadecane ($C_{16}H_{34}$).

DMSO Reduction Rate The measurement of DMSO reduction rate followed the method used by Frühling (2004). 2 g sediment was weighed in glass vials, 2 ml of substrate solution and 0.5 ml of 6% DMSO solution were added to the vials and sealed with a gas tight rubber stopper. The glass vial was incubated at 30℃ in the dark for 3 hours. The glass vials were rigorously shaken after sealing and before the headspace measurement with GC-FID. The area under the GC peak was calculated and calibrated with standard DMS solutions. DMSO reduction rate without addition of substrates were the only samples measured in replicates. Sterile bi-distilled water was added to control vials.

4.3.2.2 Results and Discussions

Addition of Nitrate To confirm the hypothesis and to illustrate the influence of preferred electron acceptors in the system, the effect of nitrate addition on utilisation of different substrates coupled to DMSO reduction is summarised. The experimental conditions were kept close to the in-situ conditions; therefore both oxic and anoxic environments were present. Considering the short penetration depth of oxygen in the sediment samples, the degradation of organic substrates coupled with oxygen would be limited and the level would be the same with or without addition of nitrate. When the thermodynamically preferred electron acceptor nitrate was added, it was expected from the hypothesis that the DMSO reduction rate would decrease. Figure 4.17 illustrates the effect of nitrate and that this is the case in control sediments (water instead of substrates). Reduction rates after 3 hours were significantly higher without nitrate addition than with.

Similar differences in DMSO reduction rates were also found when substrates glucose, xylose, and asparagine were added. The DMSO reduction rates induced by arginine with and without addition of nitrate were almost equal, showing that nitrate had no measurable influence on the DMSO coupled degradation rates. This may be an effect of nitrate production when arginine was degraded. It was however unexpected that the addition of nitrate induced further DMSO reduction with acetic acid and malic acid.

The unexpected result was likely contributed by degradation pathways not included in the kinetic calculation. There are several possible degradation pathways for acetic acid and malic acid. In natural sediments, malic acid and acetic acid are present in the anoxic layers of sediment as product of aerobic/anaerobic respiration or fermentation. Malic acid is either fermented to acetate or taken directly by some of the sulphate reducers as electron donor and degraded to acetate. Acetic acid is mostly degraded by sulphate reducing bacteria to CO_2 or methenogenating bacteria to CH_4. Malic acid and acetic acid (after the activation to acetyl-CoA) can also enter citric acid cycle, which can be carried out under anaerobic conditions using nitrate, sulphate, sulphur and thiosulphate as terminal electron acceptors citeDANSON. Due to the slow growth rate of the

methanogenating bacteria, processes important for the acetate and malate degradation under experimental conditions were fermentation (malate only), citric acid cycle, and sulphate reduction. The reduction of DMSO as electron acceptor could occur in part of the citric acid cycle or by some of the sulphate reducing bacteria but not in fermentation process.

There are several enzymes included in the citric acid cycle which have the possibility to use nitrate and DMSO as terminal electron acceptor. Each genus of sulphate reducing bacteria utilises specific electron donors and acceptors, and altogether the sulphate reducing bacteria can utilise a diverse range of electron donors and acceptors. It is not possible to extrapolate from pure culture result to obtain a holistic picture of potential DMSO reduction in these processes. One possible explanation for the induced DMSO reduction rate with nitrate addition was suggested.

In the case of reduction through citric acid cycle, DMSO can not be taken the as sole electron acceptor for the complete cycle under anaerobic condition but nitrate can. Therefore, the addition of nitrate could lead to malate and acetate degradation via citric acid cycle, whereas DMSO could be utilised partly. In the case of degradation by sulphate reducing bacteria, DMSO was reported to couple the degradation of acetate only at the presence of H_2 (Zinder and Brock, 1978). Nitrate on the other hand can be utilised by several sulphate reducers as alternative electron acceptors, including the degradation of acetate to CO_2 and H_2. Therefore, the addition of nitrate may lead to acetate degradation by sulphate reducers with the end product of H_2, which could serve as electron donor for acetate degradation using DMSO as electron acceptor. In both degradation pathways, the addition of nitrate could increase the DMSO reduction rate.

The metabolic capability of substrates utilisation coupled with DMSO reduction is shown as differences between bars and horizontal line marking DMSO reduction rates without additional substrates in Figure 4.17. When DMSO was added as the sole electron acceptor, the DMSO reduction rates were increased by glucose, xylose, and asparagine; while decreased by acetic acid, malic acid, and arginine. It was expected in the hypothesis that a lower level of substrates induced DMSO reduction would be observed when amino acids containing N was added. The production of nitrate through degradation with oxygen and DMSO acted as competing electron acceptor during degradation. The reduced DMSO reduction rates by acetate and malate were probably the result of H_2 limited sulphate reduction and reduced activity of DMSO reductase enzyme at slightly acidic pH values.

Addition of all substrates resulted in increased DMSO reduction rates when nitrate was added. The addition of malic acid resulted in higher DMSO reduction than addition of acetic acid. The results correspond well to Gocke (1976)'s study. He investigated the dissimilatory to assimilatory rates of 9 dissolved organic compounds in 5 different fresh and marine waters. The highest respiration rates were observed with malic acid and aspartic acid measured as $\%\ ^{14}C$ in CO_2.

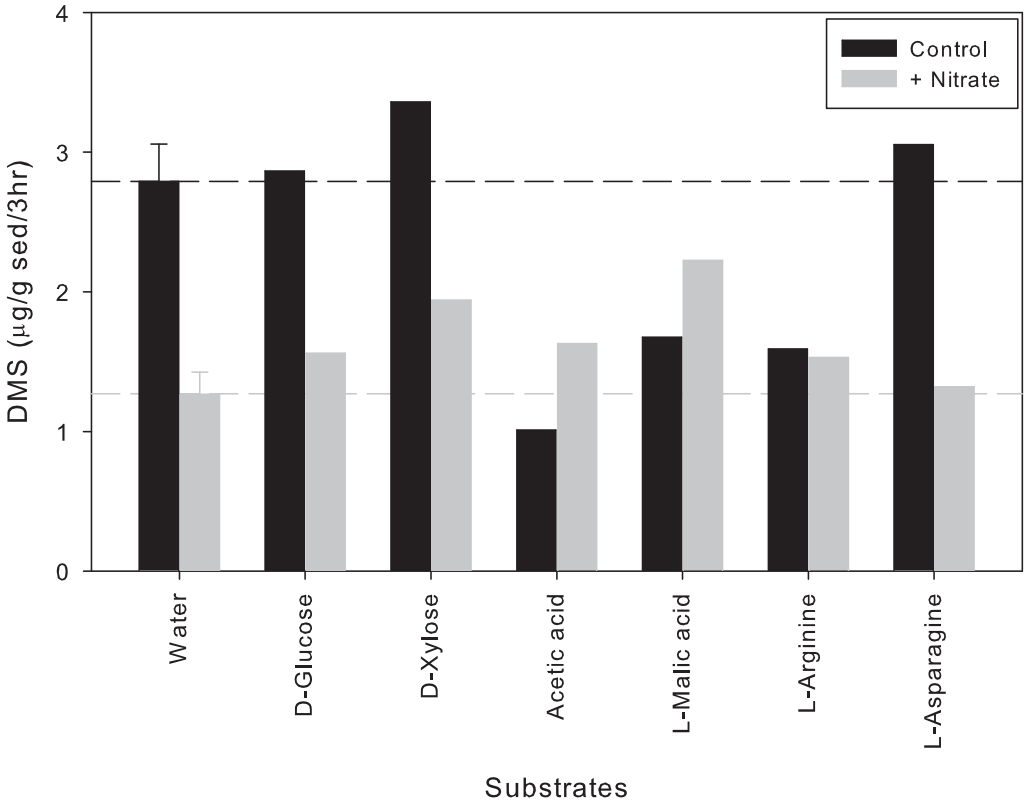


Figure 4.17: DMSO reduction rates of Over sediments coupled with carbon substrates with/without addition of nitrate.

He explained the higher dissimilatory to assimilatory metabolism of the C_4 substrates such as malic acid and asparagine by their presence in citric acid cycle. It was expected that the C_4 asparagine would result in higher DMSO reduction rate than arginine as malic acid did. However, due to the low water solubility and concentration of added asparagine, the DMSO reduction rate of asparagine may not be much higher. Furthermore, at the added nitrate concentration of 500 µM, the nitrate production through degradation using oxygen and DMSO as electron acceptor was considerably low, therefore the rate using DMSO as electron acceptor was not influenced.

Different correlations between DMSO reduction rate and respiration or cell production activity are reported. Griebler and Slezak (2001) showed a higher correlation of DMSO reduction rate and the glucose incorporation rate than glucose respiration rate in water samples. However the results were obtained with low substrates concentration (10 nM) from bacterial community at exponential growth phase, when more assimilation for cell material and reproduction dominates the utilisation of substrates. Previous experiments by Griebler (1997) in sediments with no addition of substrates showed that the DMSO reduction rates

were more correlated to ETS (electron transport system) and extracellular enzyme α-glucosidase activities than incorporation rate of [methyl-^{3}H] thymidine into DNA. Lopez and Duarte (2004) also exhibited that the DMSO reduction rates in marine sediments are highly correlated to the organic content and α-glucosidase activity. It is considered that though assimilation of substrates also contributed to DMSO reduction, at optimal substrates concentration for respiration (Degens and Harris, 1997) the DMSO reduction rate represents mostly the respiration activity of the microbial community.

Overall, at the presence of nitrate, the energetically favoured electron acceptor, lower DMSO reduction rates were observed due to the competition of electrons between nitrate and DMSO. The substrates utilisation of the carboxylic acid was different from the hypothesis due to less energetic degradation pathways involved. The addition of nitrate could trigger the citric acid cycle under anaerobic condition as well as the production of electron donor needed for DMSO reduction through sulphate reduction pathway and therefore higher DMSO reduction rate.

Nitrapyrin The decreased DMSO reduction rates were shown with the addition of a significant amount of nitrate as example of competition between electron acceptors. The next question is if similar results can be observed when natural nitrification activity is inhibited. The substrates induced DMSO reduction rates in the control and nitrapyrin amended sediments are illustrated in Figure 4.18. The percentage standard deviation was calculated from the replicates where no substrates were added and plotted for all substrates.

It was expected that the addition of nitrapyrin would result in lower nitrate concentration and therefore higher DMSO reduction rates. However, no significant differences can be observed in sediments with or without addition of nitrapyrin due to the high standard deviation when nitrapyrin dissolved in DMSO was amended. Due to the initially added DMSO as solvent for nitrapyrin in sediments, DMSO reduction occurred before the measurements. The produced DMS was trapped in the sediment water film and was released to the headspace during measurements at high temperature. A preliminary test without incubation time and DMSO only present as solvent showed already measurable DMS concentrations (data not shown). Therefore the DMSO reduction rates coupled with substrates can not be compared directly. The substrates induced DMSO reduction rates in both treatments showed an increase coupled with glucose and malic acid, and a decrease when coupled with acetic acid and arginine.

The results of control sediments indicated that addition of glucose induced the DMSO reduction. However, the addition of other carbohydrates, such as D-cellobiose and N-acetyl-D-glucosamine, did not lead to significant induction of DMSO reduction. The decreased DMSO reduction rates due to addition of arginine can be considered as the effect of higher N content in arginine ($C_6H_{14}N_4O_2$). As discussed in Section 4.3.1.1 that, the N content in the substrate contributes

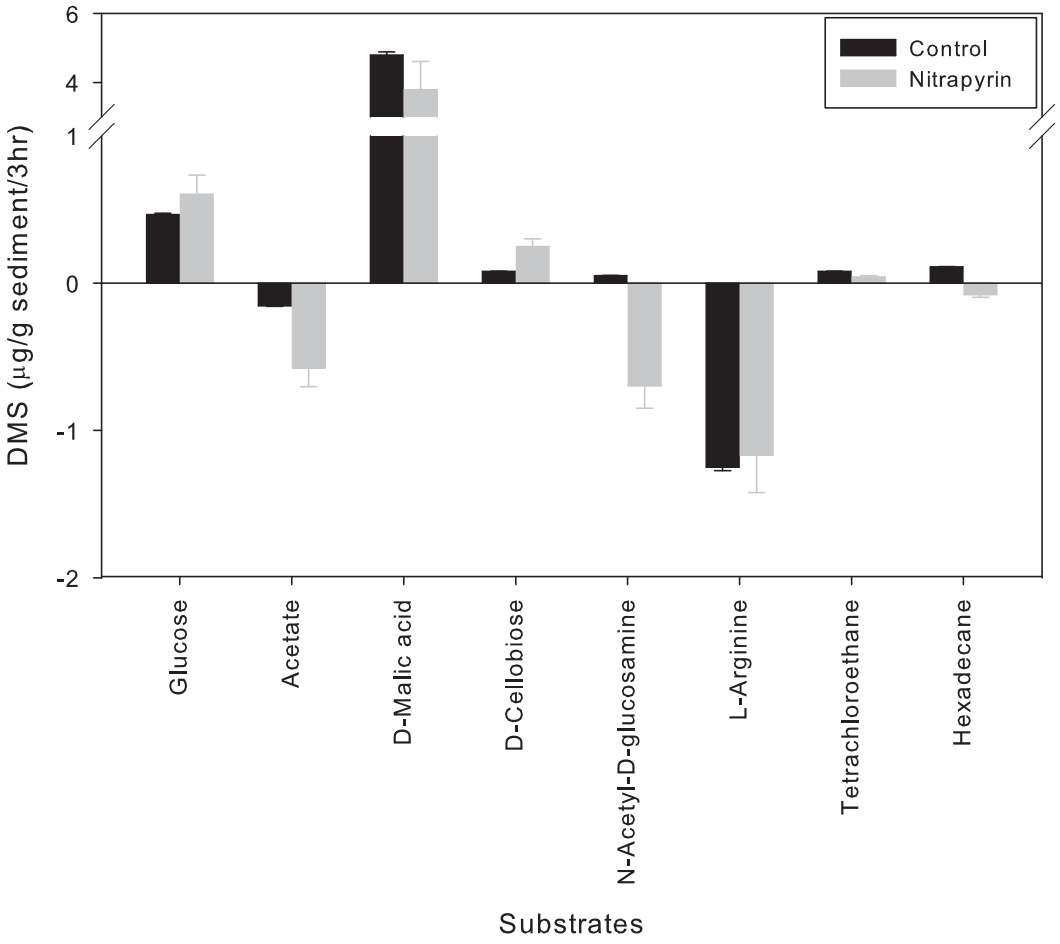


Figure 4.18: Substrates induced DMSO reduction rates of Over sediments coupled with carbon substrates with/without addition of nitrapyrin.

to the higher nitrate concentration and therefore reduces DMSO reduction rate. While in the other N containing N-acetyl-D-glucosamine ($C_8H_{15}NO_6$) the DMS reduction rate was slightly induced in control sediments.

The addition of two carboxylic acids lead to a very different result. The acetic acid decreased the DMSO reduction while the malic acid induced the DMSO reduction up to 5 fold higher than glucose. It was expected as in previous section that the C_4 malic acid was used more for dissimilative respiration than assimilative cell production than acetic acid and even glucose were. The utilisation of the two environmental chemicals, tetrachloroethane and hexadecane could demonstrate a pollution adapted community. However, no significant differences could be observed.

Comparing the substrates induced DMSO reduction rates in Figures 4.17

and 4.18, both control sediments showed a decrease when coupled with acetate and arginine and an increase when coupled with glucose. Significantly different was the DMSO reduction rate coupled with malic acid. The decreased DMSO reduction rate by malic acid in Figure 4.17 was observed in sediments collected in August, while the highly increased DMSO reduction rate in Figure 4.18 was obtained from March sediments. It is considered that in summer, due to the high organic input from descent periphyton, much higher organic content was found in surface sediments. The degradation of these organic content resulted in faster depletion of oxygen and nitrate in sediment and a more anoxic condition (-154 eV in July and -39 eV in March). The microbial community is more active in fermentation and sulphate reduction than respiration, resulting in reduced DMSO reduction rates with malic acid in summer and induced DMSO reduction rates in spring.

4.3.3 Summary

Using DMSO reduction rate as an indicator for microbial activities in sediments was suggested by Griebler (1997) who described the advantage of a short incubation time, no extraction of bacteria, and the preservation of redox conditions. However no further research coupling the DMSO reduction rate with substrates or other electron acceptors was carried out. Through the enzyme kinetic calculations, the hypothesis of using substrates induced DMSO reduction rate as an indicator for microbial functional diversity of both heterotrophic and nitrification activities was proposed.

The proposed hypothesis stated that in sediments with redox and oxygen gradient the substrates induced DMSO reduction rate represents the respiration capability of microbial community. Furthermore, an inhibition of key ecological function nitrification will lead to an overall increased DMSO reduction rate.

The assumption of competition of electron acceptors due to hierarchy of energy yield in hypothesis was partly confirmed by the reduced DMSO reduction rate with addition of nitrate. Furthermore, the sensitive alteration of metabolic pathways by presence of thermodynamically preferred electron acceptor, nitrate, was shown. It seemed that the substrates induced DMSO reduction rates not only reflected the possible effect of nitrification, but also other processes, such as sulphate reduction and methanogenesis, under more anoxic conditions. The inhibited nitrification unfortunately did not lead to further confirmation of the hypothesis due to the experimental bias of DMSO added as solvent.

The hypothesis and laboratory confirmations were the first steps in understanding the complex microbial metabolism and functional capability of both heterotrophic and autotrophic activities. In natural freshwater sediments, the environmental conditions and biochemical reactions are much more complex than those included in the calculation. Alterations of electron acceptor concentrations may lead to very different degradation pathways. Further investigations

of all possible substrates utilisation pathways with different available electron acceptors, their energy yields and occurring redox conditions related to DMSO reduction are needed to define the DMSO reduction rates in sediments better.

The application of substrates induced DMSO reduction rate as the only indicator of microbial functional diversity is not yet possible. In any case, it demonstrated the close interaction of heterotrophic and autotrophic activities in freshwater sediments.

4.4 Conclusions

The nutrient cycling by microorganisms is one essential ecological service maintaining the web of life on earth. The cycling of nutrients is carried out by a consortium of microorganisms, who exhibit a variety of metabolic reactions, which is defined as functional diversity in this study. Describing functional diversity related to nutrient cycling of sediment microbial community as one quality indicator is one of the most challenging tasks due to the complexity of metabolic pathways under gradients of redox potential, available electron acceptors, etc. Several important processes indicating impairment of ecosystem were selected, including autotrophic nitrification and heterotrophic carbon cycling. Considering the importance and sensitiveness of nitrogen cycling, the functional diversity here was studied with the inhibition of key ecological function, nitrification, using specific inhibitor nitrapyrin.

The autotrophic nitrification in the nitrogen cycling is carried out exclusively by *Nitrosomonas* and *Nitrobacter*, which have been shown to be highly sensitive to chemicals (Blum and Speece, 1991). The microsensor profiles exhibited the nitrification activity in natural stratified sediments in the top 5 mm of sediment depths where oxygen is available. Nitrapyrin inhibited the nitrification activity in stratified sediments completely with no production of nitrate observed. The activity was only 50% inhibited when ideal conditions such as high substrate concentration and optimal temperature were present.

The heterotrophic carbon substrates utilisation fingerprint under aerobic condition showed an overview over the physiological capabilities in carbon cycling but no influence of inhibited nitrification in the relative structure diversity of microbial community. Due to the long incubation time under aerobic condition with the BIOLOG method, the results indicated more impact from geochemical parameters such as redox potential than seasonality. When incubated at close to in-situ conditions with the dilution to extinction approach, the BIOLOG can provide the relative structure diversity of the microbial community in carbon cycling.

As the first attempt to describe both heterotrophic functional capability and nitrification activity using one parameter, the substrates induced DMSO reduction rate was proposed. Its advantage of measuring microbial respiration activity

at in-situ condition in a short time makes it ideal for sediment studies. The results indicated its sensitiveness to the available electron donors and acceptors. Though a lot more questions concerning its application as indicator for both autotrophic and heterotrophic functional capabilities are still open, with more experiments being performed, it may be possible to identify the response patterns of substrate induced DMSO reduction to a set of selected substrates, and to conclude an information on the functional diversity and the functional stability from this pattern.

There is no simple and easy method so far to describe the microbial functional diversity as a whole including the microbial service in nutrient cycling. Its importance in maintaining ecosystem and global climate change is not negligible, since several intermediates of nutrient cycling such as CO_2, NO_x, and CH_4 are important green house gases and its disruption will lead to destruction of life on earth. It is therefore important to include the microbial functional diversity as one quality indicator in river basin management.

Part III

Integrated Quality Indicators

Chapter 5

Integration of Indicators

An indicator is an index, an instrument, and (or) (statistical) value that provides a measure of condition or direction. Its main function is communication with simplicity, which enables and promotes information exchange regarding the issue it addresses (Smeets and Weterings, 1999). The environmental indicators have become indispensable for policy makers and are used for three major purposes (Smeets and Weterings, 1999):

1. to supply information on environmental problems, in order to enable policy-makers to value their seriousness;
2. to support policy development and priority setting, by identifying key factors that cause pressure on the environment; and
3. to monitor the effects of policy responses.

In part I, it was pointed out that to reach the "good ecological status" of a water body aimed by the European WFD; ecologically relevant indicators for sediment are needed. In part II, several important parameters indicating potential hazard (ecotoxicity) and ecological function (functional diversity) of the sediment communities were presented showing complexity in data interpretation. How can we use indicators as a tool to communicate these complex parameters for sediment quality with simplicity?

The challenge to combine information from all parameters for a management plan is huge in ecological assessments for two reasons (Silvert, 1997). First, the parameters are often multidimensional and may sometimes even contradict to each other. Second, high degree of uncertainties is often present in ecological parameters. The complexity of the ecosystem makes it difficult for policy and decision makers, who need an integrated indicator to decide upon their actions. The "ecological status" of the European WFD is an example of an integrated indicator. It is a result of 10 quality elements (in rivers) describing hydromorphological, biological, and physico-chemical quality of a water body. Each quality element is further determined by several parameters, for example temperature, oxygen balance, pH, etc. are all used to describe the physico-chemical quality element of general conditions.

So how can the parameters be integrated and simplified without losing important information as the famous quote from Albert Einstein "*Everything should be made as simple as possible, but not simpler*"?

Crane et al. (1997) summarised the application of multivariate analysis in environmental studies for reduction of ordination to simplify interpretation (principal component analysis), for assessment of differences between groups (cluster analysis) and for examination of relationships between different groups (redundancy analysis). The PCA is of great importance because it can extract information and reduce number of parameters. However the variances extracted are usually limited and the different importance between the parameters are not considered. Several numerical models such as artificial neuron network (ANN) can learn from given data set for pattern recognition and further classification of new data points. However, the underlying classification principles are unknown and unclear.

Silvert (1997) first proposed the classification of ecological impact using fuzzy set theory (Zadeh, 1965). Fuzzy sets, in contrast to crisp sets, enable processing of imprecise information by means of membership function (between 0 and 1). Chemical criteria are a clear example of crisp sets. Figure 5.1 illustrates the difference between a crisp set and a fuzzy set using Cd concentration in sediment as an example. A "Class II (corresponding to good chemical status)" measured Cd concentration in sediments is defined as concentrations lower than 1.2 mg/kg

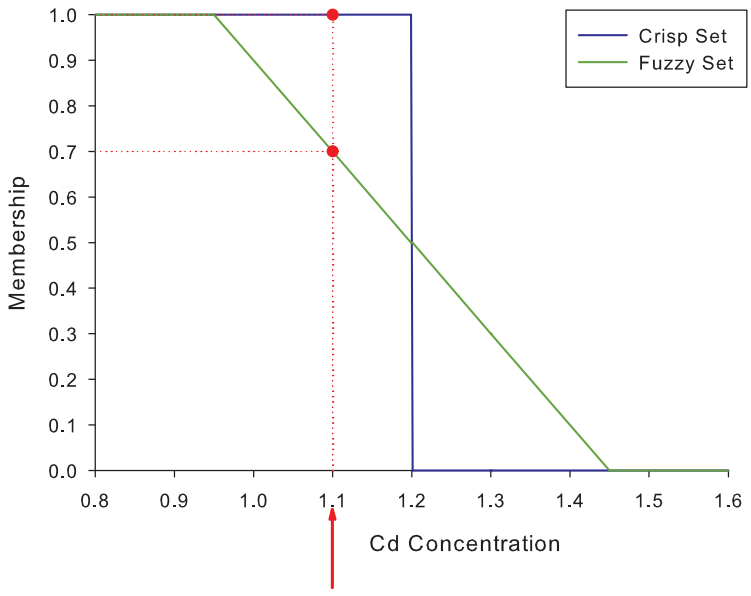


Figure 5.1: Comparison of the acceptable Cd concentration in sediments of a crisp set, as in chemical criteria, and a fuzzy set. The arrow pointed at the Cd concentration of 1.1 mg/L).

according to the ARGE-Elbe classification. Though the potential risk of a measured Cd concentration of 1.1 mg/kg may pose slight concern, it is completely acceptable according to the regulation (a crisp set). In fuzzy sets, a range of uncertainty can be appointed as the overlap of transient acceptance. For example, the measured Cd concentration of 1.1 mg/kg could be appointed to have 0.7 degree to be acceptable. The range of overlap could be assigned from biological variability and therefore taking the ecological uncertainties into account instead of treating it as low data accuracy.

Adriaenssens et al. (2004) further displayed the rule-based fuzzy models as a logical, reliable and transparent information flow from data collection to usage in decision-making. The if-then rules in the fuzzy classification enable the expert knowledge to interpret data in linguistic format with transparency for decision makers. The biggest advantage of a fuzzy system is the linguistic interpretability (Seines et al., 1998). In WFD, the ecological status of a water body is defined to be classified as "high status", "good status", or "moderate status". The if-then rules in the fuzzy classification enable the expressions of concepts important for ecological assessment such as the concept of **"good ecological status"** in WFD. All together the fuzzy set theory offers a methodology to deal with multidimensional dataset incorporating ambiguity and convert environmental acceptability from a strictly yes/no dichotomous classification to continuous one (Silvert, 2000).

An absolute fuzzy logic classification of toxicities for sediment and dredged material has been developed and applied successfully by Ahlf and Heise (2005) to Elbe estuary sediments before and after the flood event in 2002 (see box 5.1). In this approach, results of five bioassays are integrated as five classes with increasing ecotoxicological hazards. Fuzzy logic in this case provide a transparent method for ranking and classification in a way that the vagueness of datasets was taken into account and the classification was performed according to user defined rules.

Considering the advantages of a rule-based fuzzy integration dealing with uncertainties, the fuzzy set theory seemed ideal for the development of integrated sediment quality. This chapter describes the integration of parameters to integrated quality indicators (IQIs) concerning following questions: what are the parameters characterising sediment quality concerning the ecotoxicities and functional diversities? How are the parameters defined as fuzzy sets? What are the rules for classification? Finally, how are the results presented and what is the ecological relevance?

Box 5.1

Fuzzy Logic Classification of Sediments and Dredged Materials by Ahlf and Heise (2005)

A hazard assessment of sediments based on combination of bioassays was presented. About 250 sediment samples were collected from two rivers and evaluated with four bioassays: algal growth inhibition, bioluminescence inhibition, bacterial contact assay, and nematode test.

Categorisation of Responses The test specific response spans for each bioassay was assessed for further interpretation. It is assumed that the data sets along the whole risk scale, and that 25% of sediments are slightly toxic. 50% have moderate risk, and 25% pose a substantial environmental risk according to the response distribution. The estimation of accuracy and uncertainty of different test systems were set as the fuzzy vagueness.

Classification The pattern within the large database was identified with cluster analysis and k-means analysis followed by ranking. The outcome of the procedure was 5 effect classes with an increasing potential of hazard. The classes were described as follows:

Class 1: No test shows any response;
Class 2: at least one test shows low responses, no test shows moderate response or more;
Class 3: at least one test shows moderate response, no test shows high response;
Class 4: one test shows high or 3 tests show at least moderate response;
Class 5: at least 2 tests show high or 4 or more tests show at least moderate toxicity.

Application and Conclusions The toxic effect classes were applied on Elbe estuary sediment before and after the Elbe flood in September 2002. The effect classes before flood were Class 2 or 3 among the 10 sampling sites and turned to Class 4 and 5 only after the flood.

The effect classes represent a logical procedure of deduction. It is transparent giving an increased degree of certainty. Finally, the 5 classes could be used for sediment monitoring and facilitate fast interpretation.

5.1 Input Variables

The two main lines of evidence in this study assisting the chemical dominant sediment assessment are the sediment ecotoxicology and sediment functional diversity. The sediment ecotoxicology were described by three standardised bioassays, which are approved for the river Elbe (Ahlf et al., 2002). The seasonal variations of ecotoxicities of Elbe sediments further showed that the algal growth inhibition test and bacterial contact assay characterised most of the measured toxicities in Chapter 3. Therefore, inhibitions of sediments on algal growth with *P. subcapitata* and bacterial activities in direct contact with *A. globiformis* were taken as the two parameters for the sediment quality in ecotoxicities.

The functional diversity on the other hand was described in Chapter 4 by the key nitrification activities, respiration rates, substrates utilisation finger prints and relative structure from BIOLOG, and the substrates induced DMSO reduction rate. The attempt to describe both key ecological function and carbon substrates utilisation with DMSO reduction rate was unfortunately not yet understood fully for the application. Considering the importance of both carbon and nitrogen cycling for the functional diversity, the nitrification activity and the relative structure of carbon cycling are each taken as one line of evidence.

The nitrification activity is described by the potential nitrification rate, showing capability of nitrification at ideal environmental condition. Since it is taken as the only parameter for the function in nitrogen cycling and the limited sample numbers, no further integration using fuzzy sets was necessary. The potential nitrification rates were standardised through division by maximal potential nitrification rate measured and reported in the literature from the river Elbe (175 ng/g sed./hr) (Fischer et al., 2005).

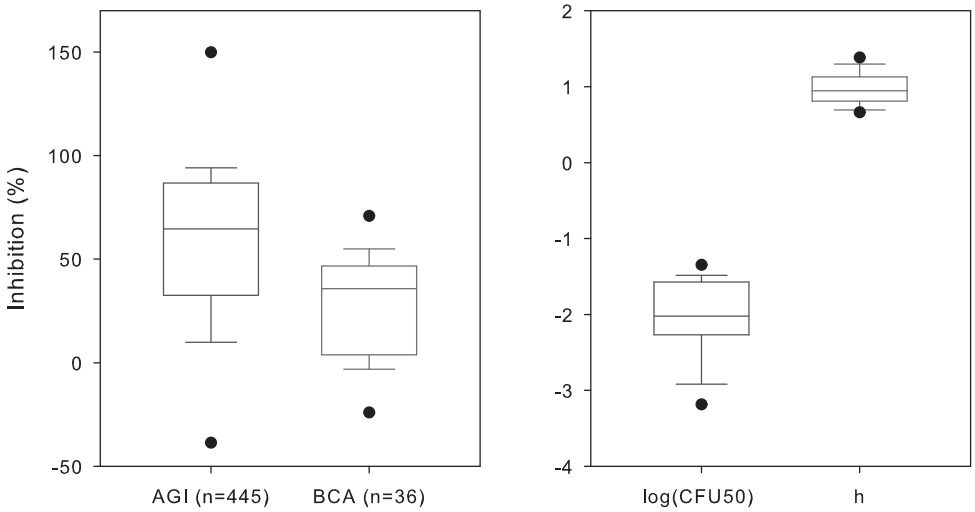
For the carbon substrates utilisation measured with BIOLOG, several input variables are possible, including the utilisation efficiencies from 31 substrates, total respiration rates, scores on principal components, and relative abundance. Considering possible bias from the method of single inoculant's density, the relative structure of the microbial community from the method of dilution to functional extinction has higher data accuracy for further integration. The functional capability in carbon cycling is therefore described as the relative structure by the parameter of functional evenness (h) and redundancy ($log(CFU_{50})$), both showing how fast does a community loose its metabolic functions. The higher the functional redundancy is i.e. lower the $log(CFU_{50})$, the higher is the stability of a community (losing half of function only at higher loss of cell density). The lower the evenness is, the easier the community loses function with dilution (losing functions at lower dilution).

The level of responses from each variable can be then categorised as high or low by the membership function according to expert knowledge.

5.2 Membership Functions and Fuzzy Sets

The membership functions describe the belongingness of data to fuzzy sets, which are the base for further integration. To define the membership functions and fuzzy sets in a transparent way, the distribution of the responses of each parameter was taken into account. Figure 5.2 depicts the distribution of responses in a box-whisker plot from all available data. One can conclude from Figure 5.2a that a 60% inhibition of algal growth represents a response between 25^{th} and 50^{th} percentile, while the same 60% inhibition in bacterial contact assay means a response higher than 90^{th} percentile of total responses. The inhibition values should not be taken as absolute values for comparisons.

The number and membership functions of the fuzzy sets can then be defined using expert knowledge. Cornelissen et al. (2001) consider the membership function as the strongest point for its definition of soft threshold and weakest point for its subjective construction in fuzzy set theory. Three fuzzy sets for each bioassay can be constructed and defined following the example in Box 5.1 with the assumption that the lowest 25% of the measured responses represent low or no toxicities, the middle 50% of the responses are moderate, and highest 25% of the responses show high toxicities. The membership function defining the uncertainties between two fuzzy sets takes the standard deviation and variances of bioassays into account. Ahlf and Heise (2005) summarised 15% variances for the



(a) Ecotoxicology (b) Functional Stability (n=16)

Figure 5.2: Distribution of responses with each measured parameters. The bar depicts the 10^{th} and 90^{th} percentile, and the box 25^{th} to 75^{th} percentile with median. The dots outside the bars are the maximum and minimum values.

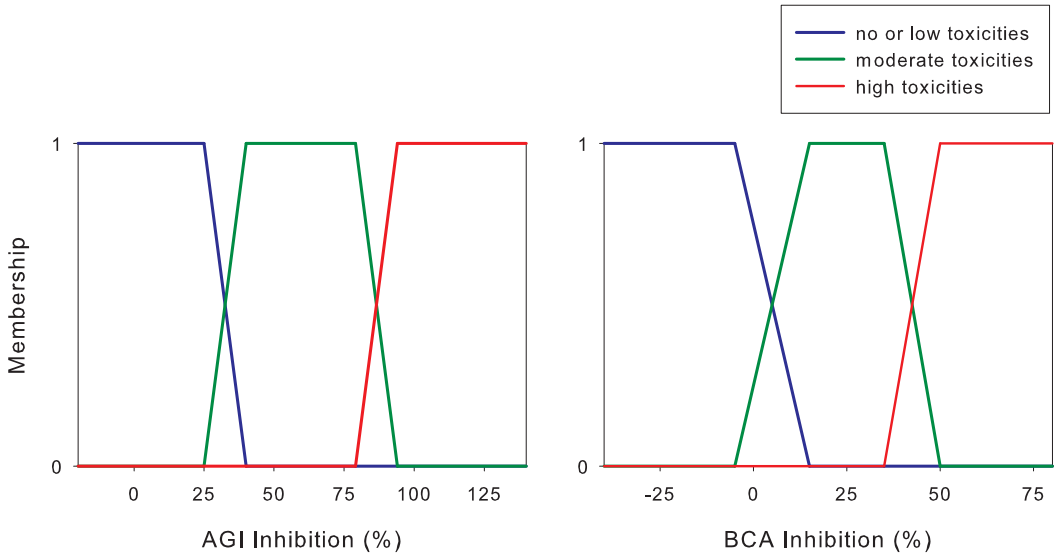


Figure 5.3: Membership functions of the fuzzy sets for algal growth inhibition test (AGI) and bacterial contact assay (BCA).

algal growth inhibition test and 20% for bacterial contact assay. The membership functions of the bioassays describing the overlaps are therefore defined and depicted in Figure 5.3. Consequently a measured 30% AGI inhibition would have memberships of both low or no toxicity and moderate toxicity.

There is however no successful example to assist the definition of membership functions of fuzzy sets for parameters showing relative structure of carbon cycling. Besides, the small sample numbers, lack of information about possible variances, and narrow data distribution all contribute to the difficulties in defining the membership functions. It is first assumed that 50% of the sampled community has high functional stability and the other 50% has low functional stability. Not knowing the variability of the responses, it is further assumed that the 5^{th} to 95^{th} percentile of the responses belonged to the uncertainties between high and low community stability. Only the highest 5% of the responses show total membership of low stress resistance and the lowest 5% of responses total membership of high stress resistance. The higher the $log(CFU_{50})$, cell density at which half of the function are lost, and the lower the h, evenness, the faster is the loss of measured heterotrophic function with dilution and lower stability. As a result, the two fuzzy sets describing high or low stress resistance for each parameter were defined and depicted in Figure 5.4. The wide overlap of the membership functions represented that most of the sampled community were not subjected to have full membership of high or low functional stability, but different degrees to belong to both.

The responses from each parameter could be now transformed as membership to fuzzy sets, describing low to high toxicities and functional stability.

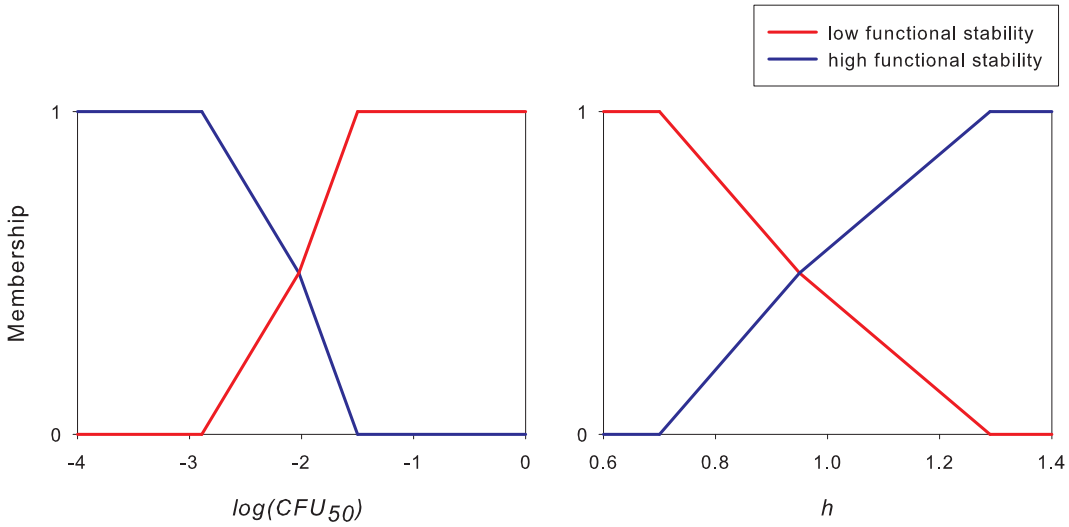


Figure 5.4: Membership functions of the fuzzy sets for cell density losing half function ($log(CFU_{50})$) and community evenness (h).

5.3 Fuzzy Rules for Classes

The integration from fuzzy sets to effect classes followed again the expert knowledge. In the example provided in Box 5.1, the rule for classification was based on the clustering of the five measured sample responses (Ahlf and Heise, 2005). In this study, however, only two parameters were to be integrated for ecotoxicity and functional stability. The integration can follow a simple matrix showing possible combinations and interpretation for it.

Different from the chemical quality classes and the ecotoxicological classes described in Box 5.1, where increasing class number reflects increasing environmental risk, the quality classes were showing increasing quality with numbers. This reversed numbering system is to avoid confusion with presentation in summary indices (more details in Section 5.4.1).

The derived rules and resulted five classes showing the quality of ecotoxicity with increasing goodness are:

Class 1: high toxicities, when both bioassays have high toxicities,
Class 2: mixture toxicities, when both bioassays have at least moderate toxicities,
Class 3: mobile toxicities, when algal growth inhibition shows moderate to high toxicity and bacterial contact assay shows low or no toxicities,
Class 4: particle-bound toxicities, when bacterial contact assays show moderate to high toxicity and algal growth inhibition shows no or low toxicity, and
Class 5: healthy, when both bioassays have low or no toxicities.

If considering only the categories of responses but not character of the two bioassays, the classes of mobile toxicities and particle-bound toxicity should be

taken as parallel classes. The ranking of qualities between the algal inhibition and bacterial contact assay is based on the bioavailability and therefore risk and impact of certain toxicities. The algal growth inhibition was measured using sediment elutriate as exposure route, while bacterial contact assay measured the bulk sediment toxicities. The elutriate represents the fraction of toxicities in the pore water, which is more bioavailable and are easier transported in the river basins. Therefore the algal toxicities were considered to cause higher risk and lower ecotoxicological quality.

The relationship between cell density and ecological functions as well as the meaning of the functional evenness (h) and redundancy ($log(CFU_{50})$) were illustrated in Figure 4.15 and described in details in Section 4.2.2.3. The h indicates the evenness i.e. distribution of species across functions. A community with high functional evenness is more stable, since the distribution of species across functions is even i.e. more redundant. A community with low functional evenness then again would have functions carried out only by one or two species, whose removal from the system would result in loss of functions. The $log(CFU_{50})$ is negatively correlated to the functional redundancy of a microbial community, which is contributed by both functional evenness and species richness. A community with high functional redundancy is more stable, since removal of species, which are functionally redundant, would have less or no impact on the total functions. Therefore, the lower the $log(CFU_{50})$ and the higher the h, the higher is the functional stability of a community.

The quality of carbon cycling concerning its stress resistance was therefore classified into four classes using following rules showing increasing functional stability of a community:

Class 1: very low functional stability, when redundancy and evenness are both low i.e. functions were not evenly distributed and not redundant,
Class 2: low functional stability, when redundancy is low but evenness is high i.e. species richness is low, each function is carried out by lower number of functionally redundant species,
Class 3: moderate functional stability, when redundancy is high but evenness is low i.e. species richness is high, some functions are carried out by more and some functions are carried out with less functionally redundant species,
Class 4: high functional stability, when both functional redundancy and evenness are high i.e. there are several species performing redundant functions.

Similar to the classes of ecotocixities, the functional redundancy was giver higher importance in maintaining functional stability. Therefore, community with high functional redundancy but low functional evenness was appointed to Class 3.

5.4 Presentation and Interpretation of Conceptual IQI

The results were transformed to quality classes with increasing ecological status, but how can the classes be further presented as integrity for decision makers? Chapman (1996) presented three approaches interpreting results in sediment quality triad, and Burton et al. (2002) further recommended different approaches in a weight-of-evidence approach. Generally the results are presented either as qualitative terms and combined using a tabular decision matrix, or as quantitative numbers and combined in numerical indices. The numerical presentation gives a visualised overview of quality in each line of evidence, and the tabular decision matrix link the results to interpretation and action directly. Therefore, the conceptual integrated quality indicator (IQI) is presented both in summary indices and in a tabular listing its ecological relevance and possible action for decision makers.

5.4.1 Quantitative Summary Indices

Chapman (1996) suggested the quantification of each line of evidence by normalising the values to a reference value (ratio-to-reference (RTR) determination). However, the results are not independent and may not apply in a big scale. A defuzzification procedure can transform the quality classes to comparable quantitative indices without conflicting the process of fuzzy classification (Silvert, 1997). For the quantitative summary indices, a numerical value representing increasing quality is the aim. The centre of gravity method could deliver values between classes, which is ranked according to quality. The quality classes of ecotoxicity and functional stability were defuzzified to numerical index with values between 0 and 1. A different scale can be taken if one line of evidence is to be emphasised.

The three quality indices (ecotoxicology, functional stability, and nitrification rate) are here plotted and visualised by a triangle as illustrated in Figure 5.5. The area within the triangle is taken for the final ranking of the sediment quality as working basis for decision makers. There are a vast number of possible results. Four of them are depicted on the conceptual quality indices in Figure 5.5. When all three quality indices are high, quality of the sediment community is considered healthy, while when all three quality indices are low, the quality of the community is impacted. When the ecotoxicological quality is high showing no toxicities measured, and the functional stability and/or the nitrification quality is low, the sediment community is probably sensitive to other environmental stressors. On the contrary, if the ecotoxicological quality is low, but the functional stability from microbial community and nitrification activities are high, the sediment community is adapted to the chemical stresses from contamination.

In the triangle visualisation of the summary indices, the ecological relevance

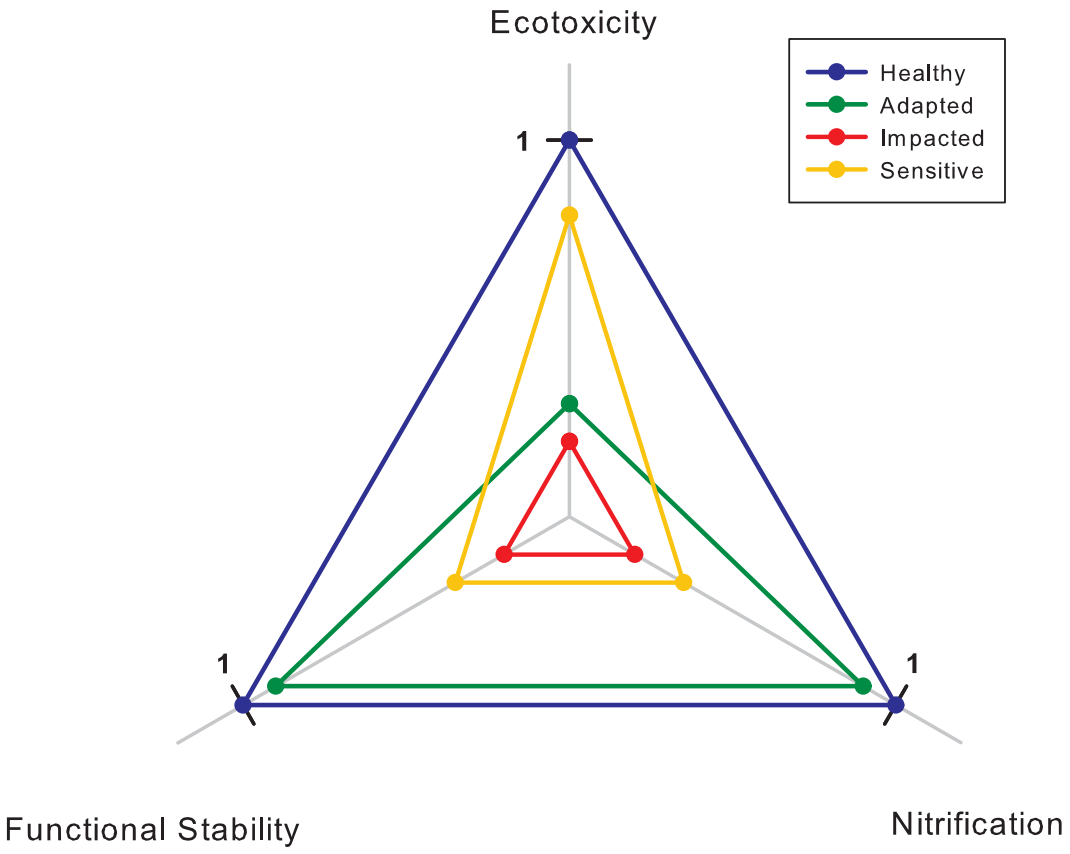


Figure 5.5: Triangular presentation of conceptual IQI using ecotoxicity, functional stability and nitrification rate as three LOE.

and interpretation depends on the shape of the triangle. It provides simple and highly visual data presentation, which can be explained to and understood by non-scientists. A general overview of each quality index with quantitative measures is given and the area within offers a final ranking system for decision makers.

5.4.2 Linguistic Tabular Decision Matrix

In some cases, numerical indices may seem artificial for decision makers and linguistic terms such as “good” or “acceptable” quality are preferred and introduced into Fuzzy Logic. In this case, a tabular decision matrix can offer clear interpretation of possible cause. For the tabular matrix, the classes representing specific characteristics of ecotoxicities and functional stability are used directly. When a sediment showing ecotoxicological classes between mobile toxicity (class 3) and healthy (class 5), the defuzzification using centre of gravity method may appoint this sediment to have class 4 of particle-bound toxicity, which does not describe the responses of bioassays. The centre of gravity method addresses the

level of increasing quality well, but could ignore the specific meaning given to the classes. Therefore, the maximum of output method was used in presenting results as classes to avoid this situation.

Traditionally in a tabular decision matrix, only yes/no attributes instead of levels of acceptance are used. This can be improved by using fuzzy rules to combine membership from each quality class to a final IQI with interpreted ecological relevance and actions. Table 5.1 lists the decision matrix using traditional yes/no attributes as a starting point to illustrate eight out of more than 20 possible combinations of the IQI and possible causes.

The four illustrated scenarios in quantitative summary indices were presented in corresponding colours. The sensitive community is discussed further in detail to demonstrate the two ways of presentations. When quality in ecotoxicity is high, but the quality in functional stability and nitrification is low, the community is sensitive to stressors other than chemicals. There is possibly an alteration in sediment habitat, which causes the decreased functional stability and nitrification activity. Furthermore, the decrease of quality in carbon and nitrogen cycling may

Table 5.1: Tabular decision matrix for linguistic IQI.

Quality			IQI	Causes
Ecotox.	Stab.	Nitri.	Ecological Relevance	
+	+	+	**healthy**	as reference site
+	+	−	**sensitive nitrification** to e.g. temperature	alteration in geochemical properties or presence
+	−	+	**sensitive carbon cycling** to e.g. organic matter	of specific pollutants at low concentrations
−	+	+	**contaminants present** but microbial communities ecologically adapted	long term contamination, possible impact on higher tier communities
+	−	−	**sensitive nutrient cycling** to e.g. habitat change	alteration of habitat, presence of pollutants
−	+	−	**impacted key function** but high functional stability	presence of pollutants, possible pollution-
−	−	+	**impacted carbon cycling** but high key function	induced tolerance in microbial communities
−	−	−	**ecological impacted** by contaminants	highly contaminated, consider remediation

have impact on higher tier organisms such as periphyton and macrozoobenthos communities, which should be monitored and analysed in case of further ecological impact. A second possible explanation would be that the bioassays are not sensitive to certain contaminations causing alterations in microbial communities.

The conceptual IQI and its interpretations presented in this chapter is not an absolute way of integration, but is an example demonstrating a method incorporating lots of expert knowledge in each step of integration and interpretation. It is an attempt to integrate multiple parameters in a very simple summary index or a linguistic IQI, which is easily understood by non-specialist and can be used directly by the decision makers. Here the IQI is developed as an example to describe ecologically relevant sediment quality apart from the chemical criteria. Various other IQIs can be developed integrating expert knowledge in the classification and interpretation following the same framework according to the user's need.

Chapter 6

IQI Evaluation of Aging in Sediments

Aging (or weathering) of chemicals is a well known phenomenon in soils and sediments, showing increasing resistance of contaminants to biodegradation and extraction with prolonged contact time in soils and sediments (Hatzinger and Alexander, 1995). The partitioning of organic substances in the humic matter and their diffusion followed by entrapment into pores of soil aggregates have been suggested as possible mechanisms of the aging phenomenon (Alexander, 2000). It has been shown that organic pollutants become biologically unavailable due to the aging phenomenon, decreasing the ecological risks, even though chemical analysis using rigorous extraction with solvent may show high concentrations of organic compounds (Alexander, 2000).

More recently, a dual-mode sorption concept was reviewed by Cornelissen et al. (2005) relating the distribution of organic compounds to two domains of organic matter in soils and sediments. The first domain of linear and noncompetitive **absorption** consists of amorphous organic matter (OM) such as humic/fulvic substances, lipoproteins, and lignin (Huang et al., 1997); while the second domain of nonlinear and extensive **adsorption** consists of more condensed moieties, such as black carbon, coal, and kerogen, collectively termed "carbonaceous geosorbents (CG)" (Pignatello and Xing, 1996). The adsorption of organic compounds to CG is generally 10 to 100 times higher than absorption to amorphous OM. Cornelissen et al. (2005) further suggested that the adsorption to CG is so strong that only the freely dissolved concentrations of organic contaminants reflect the risk on organisms in soils and sediments. Although the mechanism of absorption to AOM was regarded as diffusive dissolution in the organic matrix, absorption could also mean uptake via skin or membrane by organisms in toxicology. The term **adsorption** was therefore often used to refer sorption of contaminants to both domains in soil and sediment as in this study.

In river Elbe, contaminants were released decades ago and have been sorbed to and aged in sediments ever since. Following the long term adsorption in sedi-

ments, the risk of these contaminants is supposedly limited to the freely dissolved concentrations. However, under alterations of hydrological conditions such as at flood events, where the conditions for the sorption changes as well, contaminated sediments are remobilised and transported downstream. As a result, Heise et al. (2003) reported elevated sediment toxicities at Elbe estuaries after flooding in summer 2002. So what is the effect of aging of contaminants in sediments? Can we expect decreased environmental risk from it? Could it be reversed? And what are the impacts on microbial community? Though the term aging is used strictly referring to the advanced sorption of freshly added chemicals in soils and sediments under no perturbations, and the contaminants in rivers are usually not traceable in their contact time as well as endured perturbations, the term aging is used in this study as the prolonged undisturbed contact time of contaminants with sediments, which may still reduce the contaminant bioavailability and therefore environmental risks. The process of aging and resuspension of contaminated sediments was therefore taken as an example of evaluation using IQI to illustrate alteration of ecological relevance caused by the process.

6.1 Materials and Experimental Design

The natural aging and remobilisation of contaminated sediments in rivers is illustrated in Figure 6.1. In order to display the possibly reduced bioavailability of present contaminants in sediments due to aging, sediments were stored without contact to water phase, which simulated the process of freshly deposited sediments becoming layered aged ones (A to B). The fate of the aged sediments and reduced bioavailability under changing hydrological conditions was further tested under static contact to water phase (B to C), as simulation when overlying sediments were remobilised, and under resuspension (B to D), as simulation when aged sediments become remobilised too.

The well studied and monitored site Over at river Elbe was taken as the investigation site. The possible temporal responses of bioassays and carbon substrates utilisation profiles were characterised in this study and the 27 Elbe priority substances were monitored in sediments monthly by ARGE-ELBE. 0-15 cm of sediments and river water were collected in Over (see Figure 2.3) in September 2005. Concentrations of the contaminants exceeding Sediment Quality Classes II, which is correspondent to the aim of good chemical status in WFD, are listed in Table 6.1. The investigated Over sediments contained a mixture of both heavy metals and organic pollutants.

The sediments were filled with no headspace in 1 L PP jars and stored at 4℃, at which microbial activities such as biodegradation are minimal, for 1 year. After one year storage (aging), 40 ml of sediments and 160 ml of river water were added in a 250 ml PP flask and further incubated at 4℃, 20℃, and 30℃ for 2 weeks. The sediment and water samples before and after the incubation

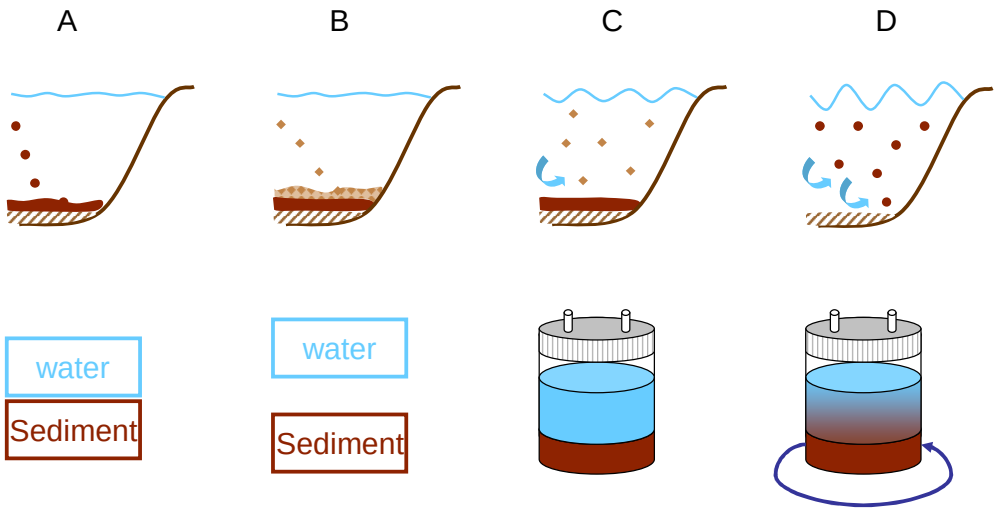


Figure 6.1: Schematic diagram of sediment aging in river basins and laboratory simulations. A deposition of fresh sediments. B aging process of deposited sediments in a deeper layer. C remobilisation of surface sediment into the water phase. D remobilisation of aged sediments.

at different temperatures were applied to the algal growth inhibition test and bacterial contact assay.

Six months later, the aged sediments were further reconstituted with river water and incubated under slight resuspension (circular shaking at 50 rpm) for two weeks at 4℃, 20℃, and 30℃. The sediments before and after resuspension were applied to both bioassays measuring toxicities. The BIOLOG method measuring the relative structure of the carbon substrates utilisation profile, and the potential nitrification rates measuring activities of key ecological function were applied only on the resuspended sediments.

The results of each line of evidence were presented in values and summary index or classes. At the end, the overall impacts of aging and resuspension on the sediment quality and its ecological relevance were evaluated with the IQI developed in Chapter 5.

Table 6.1: Concerned chemical concentrations in Over sediments August 2005.

	Metals [mg/kg]					Organics [μg/kg]	
	Hg	Cd	Zn	Cu	As	HCB	AOX
SQG II Concentration	0.8	1.2	200	60	20	40	50000
Measured Concentration	2.4	7.4	950	91	30	65	97000

6.2 Lines of Evidence

6.2.1 Ecotoxicities

The sediments and river water before and after one year storage at 4℃ were applied to both algal growth inhibition test and bacterial contact assay. The results are depicted in Figure 6.2a showing alterations of ecotoxicities due to one year storage. The toxicities in the river water did not change after one year storage, while the bacterial toxicities decreased from 50% to around 0% in the sediment. The algal toxicities also decreased in sediments, but the differences were not significant.

The results indicated a great reduction of particle-bound toxicities within one year storage. Part of the contaminants with higher water solubility remained available during the one year storage. The freely dissolved concentrations of contaminants causing algal toxicities did not decrease due to prolonged contact time. Sorption of these contaminants reached equilibrium likely before the aging process started. The initially sorbed organic compounds however became less available for bacteria with high particle affinity. Two possible explanations of the decreased bulk sediment toxicity are microbial or physical degradation of toxic contaminants or stronger sorption onto sediment particles. Considering the low microbial activities at 4℃ under anoxic conditions, significant biodegradation is not expected to reduce 50% of bacterial toxicity. Progressive absorption or adsorption of contaminants was more likely to decrease the bioavailability.

After the aged sediments were in static contact with river water for two weeks at different temperatures, the reduced bacterial toxicities were unexpectedly reversed in sediments (Figure 6.2b). The algal growth inhibition in sediments also increased by around 30% at 4 and 20℃, and up to 90% at 30℃. In the water phase, the toxicities did not vary much after incubation with sediments, except for the incubation at 30℃. The algal toxicities after two week incubation at 30℃ increased from 60% to 120%.

The increase of algal toxicity from sediment was probably caused by the diffusion of contaminants from river water into sediment pore water. As a result, similar algal toxicities were observed in water phase and in sediments, where much lower algal toxicities were present before the static contact experiment. The increased bacterial toxicities in sediments indicated that the previously observed aging effect was reversible at temperature of 4℃ and was significantly higher at 30℃.

A desorption caused by extraction such as elutriation process could be the reason of increased toxicity. Weissenfels et al. (1992) reported that the aged PAHs can become bioavailable for microbial degradation when the extracted PAHs were added back to soil. However, before the sediments were applied to bioassays, elutriation (mild extraction) or homogenisation were performed and desorption of contaminants would also be observed before the incubation.

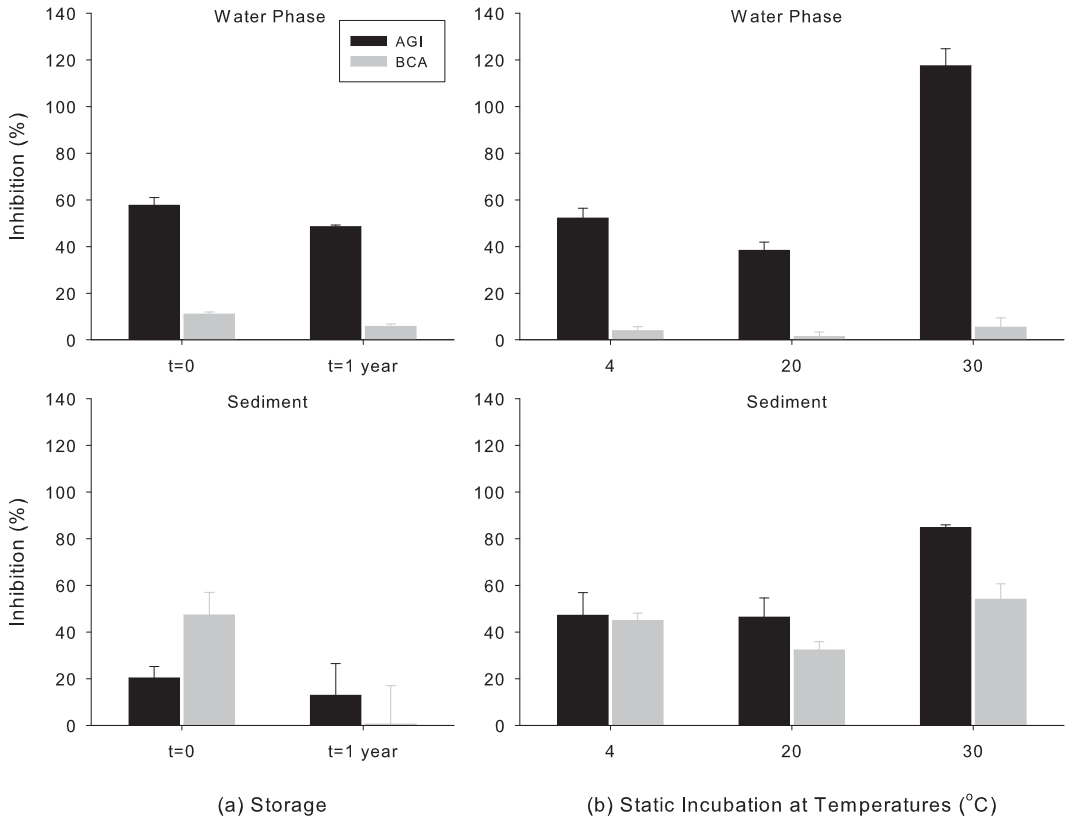


Figure 6.2: Inhibitions of bioassays in water and sediments (a) after one year storage at 4℃ and (b) under static contact with river water for two weeks at different temperatures.

The reversed bacterial toxicity was most likely caused by desorption of the contaminants. Organic contaminants adsorbed to the CG part of sediments have very low biota to sediment accumulation factor and limited potential for microbial degradation. Therefore the adsorbed organic contaminants are considered not bioavailable. A desorption of organic pollutants from amorphous OM and metals due to varying redox potential were more likely to cause the increased toxicities within two week time.

Cornelissen et al. (2005) described the overall sorption with the following equation:

$$C_S = f_{AOC} K_{AOC} C_W + \sum_X f_X K_{F,X} C_W^{n_{F,X}}, \tag{6.1}$$

where C_S is the sorbed concentration on a whole sediment basis, f_{AOC} is the fraction of non-CG amorphous organic carbon, K_{AOC} is the linear AOC-water partitioning coefficient, C_W is the aqueous concentration. The second part of

the equation denotes the sorption onto the different CGs. Generally, the sorbed concentration depends on the fraction of sorbents, the partitioning coefficient, and the concentration in the water phase. When concentration in the water phase is higher than the equilibrium concentration, for example when adding contaminants to nonpolluted sediments; the contaminants will be sorbed. When concentration in the water phase is lower than the equilibrium concentration, for example when contaminated sediments are extracted with solvent, the contaminants will be desorbed.

Over is a sedimentation groyne field, where sediments are transported through the Elbe and finally settled. The contaminated sediments have reached equilibrium of CG sorption since the contaminants were released decades ago. During the transport of contaminated sediments downstream, the absorbed part of contaminants have greater surface for exchange and the equilibrium moved towards desorption. When sediments were stored with no contact of river water, the equilibrium moved back to absorption on organic matter, resulting in decreased bacterial toxicity. The static contact of the aged sediments and water again push the equilibrium toward desorption due to the lower concentration of organic contaminants in river water than in pore water. The high algal toxicities observed at 30℃ could be resulted from increased solubility of contaminants or their degradation products by autochthonous microbial activity.

Slightly higher toxicities in sediment and water phase were observed six months later (Figure 6.3) before the experiment of resuspension was performed. A strongly reduced bacterial toxicity was still significant in the aged sediment. The resuspension of the surface sediment did not increase bacterial toxicity of *A. globiformis* significantly in the water phase at different temperatures. The algal growth in the water phase, on the other hand, was inhibited when incubated at 20℃ and 30℃ to more than 90% inhibition, while slightly induced to 30% inhibition at 4℃. The resuspension of sediment at 4℃ caused a decrease of algal toxicity, while in the static contact no significant differences were observed. The bacterial toxicities in sediments were reversed at all temperatures as measured in the static experiment of around 40% inhibition. The algal toxicities on the other hand reached 75% at all temperatures, while in the static experiment, such high algal toxicities occured only at 30℃.

The resuspension not only enabled the exchange of sediment pore water and river water, but also increased the area of sediment water interface and therefore sites for sorption/desorption. As a result of river water/pore water exchange, elevated algal toxicities were observed in sediments incubated at all temperatures. Interestingly, at 4℃ the algal toxicity in water phase was lower than in sediments, which may be caused by sorption of certain contaminants to sediment particles. The overall higher algal toxicities may be caused by the degradation products, which were produced by microbial degradation at elevated temperatures.

The strong sorption of contaminants on sediment particles are generally believed to reduce environmental risks (Alexander, 2000); however the results showed

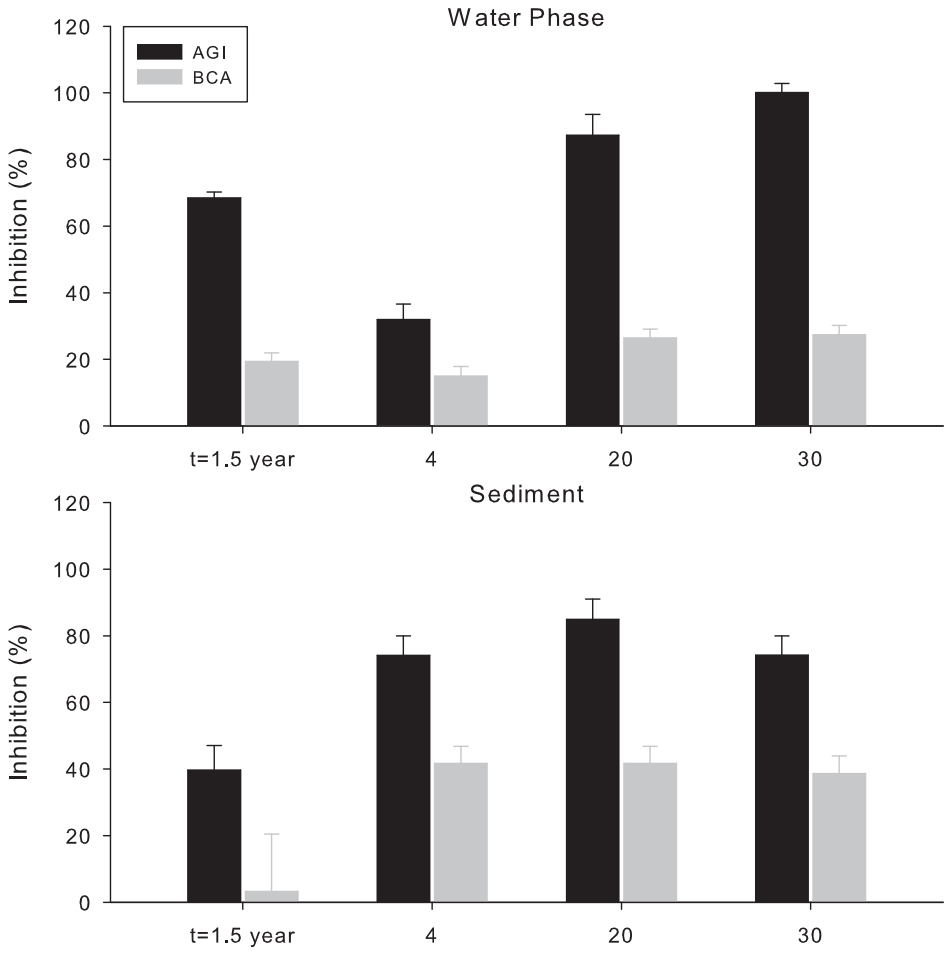


Figure 6.3: Inhibition of bioassays on aged sediment resuspended with river water at different temperatures.

that the sorbed contaminants may be more mobile than expected. Simple static contact for two weeks at low temperature showed already the reversible particle bound toxicity and at elevated temperatures water soluble toxicities could exceed the expected toxicity range. The two week incubation time was shown to approach desorption equilibrium. A more frequent sampling within two weeks may reveal the desorption kinetics of the contaminants and corresponding toxicities. At slight resuspension, such unexpected toxicities could be observed at low temperature as well. In the natural river sediments, due to the continuous changing of hydrological conditions, the risks of the layered (aged) contaminated sediments being resuspended under high discharge may be much higher than expected.

The results of ecotoxicological bioassays were summarised using fuzzy logic classification as described in Chapter 5 taking both response range and biological

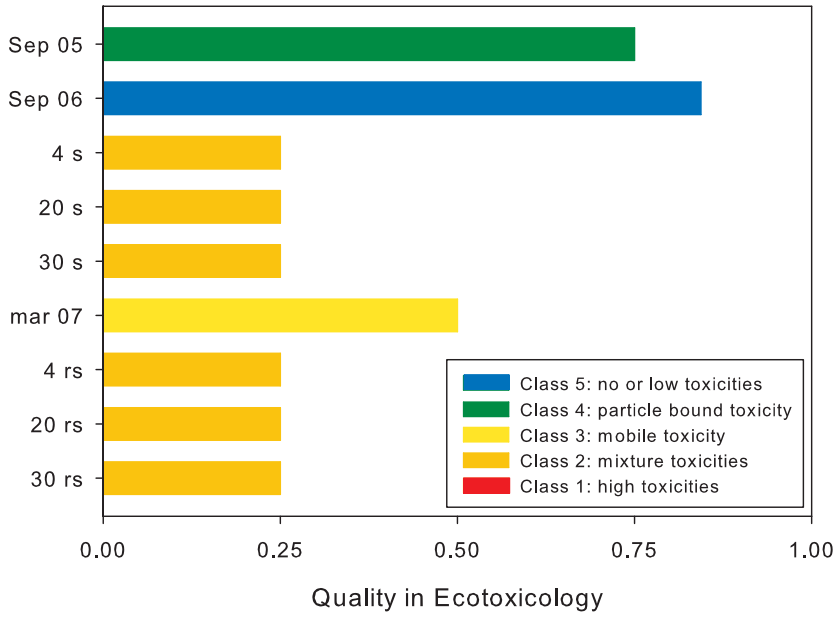


Figure 6.4: Ecotoxicological quality of aged sediments using fuzzy logic. The s represents static experiment and the rs the resuspension experiment.

variability into consideration. The resulting quality classes in ecotoxicity and the summary index of sediments undergoing aging and resuspension were depicted in Figure 6.4. The one year aging (storage) increased the ecotoxicological quality of the sediments slightly from class 4 to class 5, while the further incubation led to lower quality (class 2) indicating elevated toxicities in mobile fraction (elutriable) in sediments and therefore higher impacts. The incubation at different temperatures and with or without resuspension did not lead to different quality classes in toxicities.

6.2.2 Functional Stability

No significant differences of algal and bacterial toxicities were observed in sediments after resuspension at different temperatures. The sediments after resuspension were applied to BIOLOG EcoPlates with dilution to functional extinction method to observe possible effects on the relative structure and stability of the microbial community in carbon cycling. The colour development of resuspended community was compared with freshly collected sediments (September 2005), which was applied with single dilution with the factor of 10^{-1}. The aged and incubated sediments with dilution factor of 3^{-2} was closest to the dilution factor used for freshly collected sediments and were therefore taken to fit on the kinetic model for population growth.

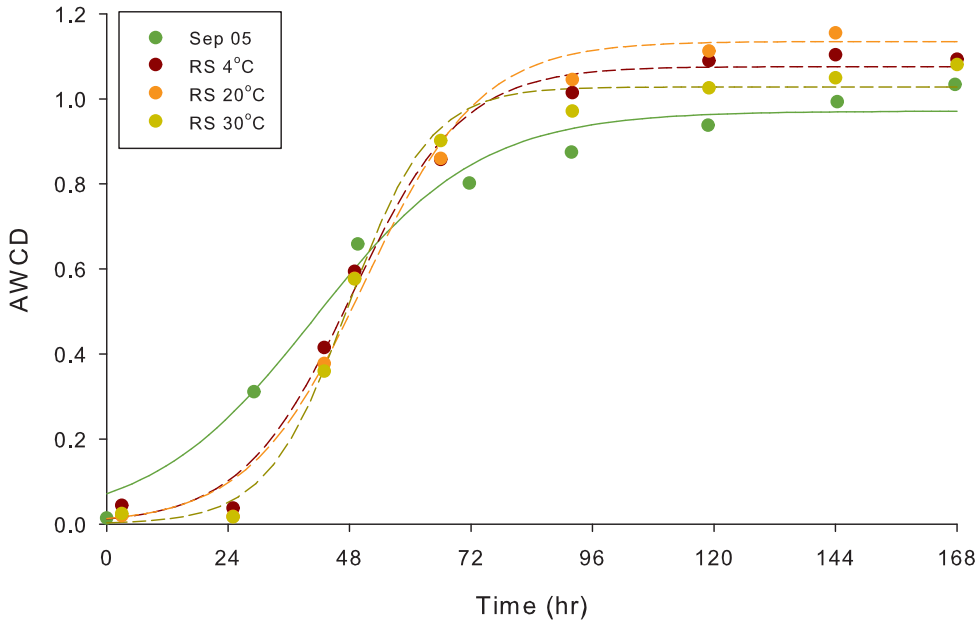


Figure 6.5: Averaged well colour development of sediments before and after aging and resuspension at 4℃, 20℃, and 30℃.

Table 6.2: Kinetic fitting parameters of the sediment samples before and after aging and resuspension at 4℃, 20℃, and 30℃

Samples	Regression parameters		
	K (carrying capacity)	r (growth rate)	S (lag time)
Sep. 05	0.971	0.06159	41.15
4℃	1.076	0.09268	48.44
20℃	1.135	0.08792	50.98
30℃	1.028	0.12490	47.80

Figure 6.5 illustrates the averaged well colour development of the extracted sediment microbial communities with time and the corresponding kinetic fitting parameters are listed in Table 6.2. Generally the freshly collected sediments had lower growth rate (slope) and shorter lag time compared to aged and resuspended sediments. The results indicated that the total respiration activities (AWCDs) were similar between all sediments after aging and resuspension. It was pointed out in Section 4.2.1 that the lag time is negatively correlated to the cell density. Therefore the microbes extracted from fresh sediments had probably higher cell density, which was reflected with the shorter time to reach half of the exponen-

tial growth. The aging and incubation under resuspension probably had some selection effect on the microbial communities. The substrates utilisation fingerprints analysed with principal component analysis also demonstrated differences between the aged and resuspended sediments from the freshly collected sediments (data not shown).

The relative structure of aged and resuspended sediments incubated at different temperatures are depicted as the relationships between the area under curve and dilution factor in Figure 6.6 and the logistic fitting parameters are listed in Table 6.3. Generally sediments resuspended at 4℃ had highest functional redundancy, sediments resuspended at 20℃ had the highest functional evenness, and sediments resuspended at 30℃ had lowest functional richness. The high functional redundancy (low $log(CFU_{50})$) indicated that at 4℃ the microbial community is most functionally stable, when species were diluted out. The high evenness of community resuspended at 20℃ displayed even distribution of species among functions. The low functional richness showed that at 30℃ some of the functions (ability to utilise carbon substrates) were lost.

The results displayed interestingly how the microbial community changed at different temperatures. The reduced functional redundancy and induced func-

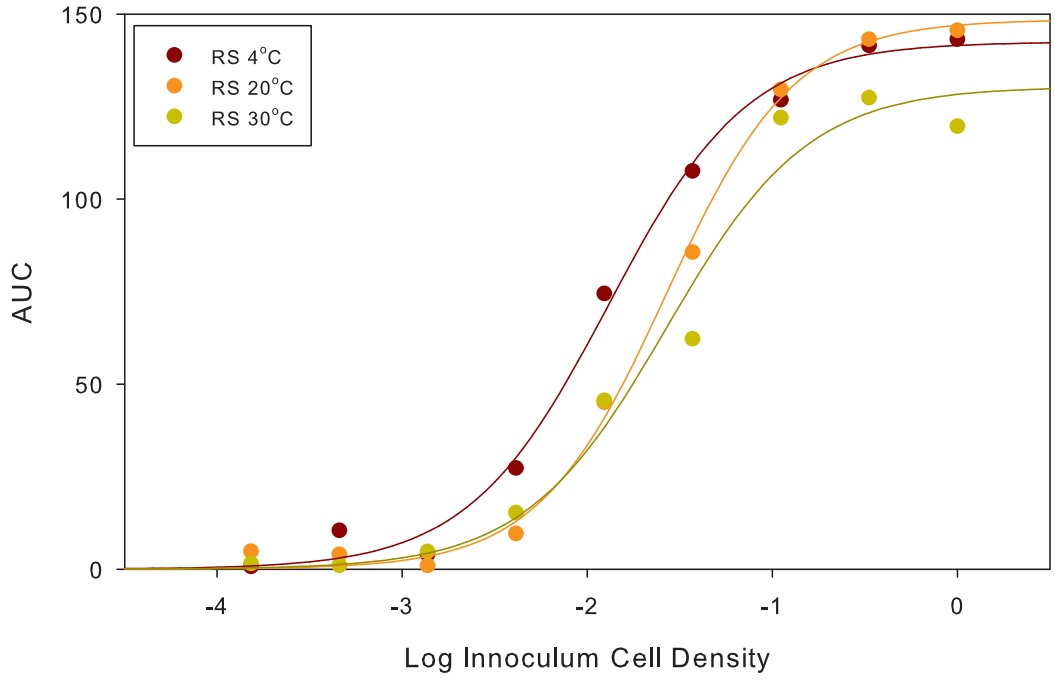


Figure 6.6: Area under curve of AWCDs of freshly collected and incubated Over sediments after slight resuspension at 4, 20 and 30℃with dilution of inoculum cell density and fitting of log normal curve.

Table 6.3: Logistic fitting parameters of the sediment samples after slight resuspension at 4, 20 and 30℃.

Samples	Regression parameters		
	t functional richness	h functional evenness	$log(CFU_{50})$ functional redundancy
4℃	142.5	1.147	-1.887
20℃	148.5	1.259	-1.574
30℃	130.4	1.134	-1.572

tional evenness of sediments resuspended at 20 and 30℃ were probably results of reduced species richness (see Section 4.2.3). After storage at 4℃, the microbial community had probably adapted to the low temperature, resulting in loss of species when the sediments were further incubated and resuspended at 20 or 30℃. When resuspended at 20℃, the regrown community had probably loss only functionally redundant species. As a result, the functional richness remained, while functional redundancy was reduced. When resuspended at 30℃, the regrown community had probably greater loss of species including both functionally redundant ones, and functionally specific ones. The community had at the end lower functional richness, functional evenness, and functional redundancy. The reduced functional stability of microbial communities resuspended at higher temperatures could be caused by a low temperature selected community and/or impacted by the higher ecotoxicities when resuspended at higher temperatures (see Section 6.2.1.).

To conclude the results, the aging (storage) of sediments obviously had selection effect of the regrown microbes. Communities with lower density were reflected in the longer lag time for colour development in aged sediments compared to freshly collected ones. The resuspension at different temperatures pose further influence on the relative structure and functional stability of the regrown communities. After aging at 4℃, several functionally redundant species were not capable to regrow at 20℃ and 30℃ which was reflected by the high $log(CFU_{50})$. Whether these differences were caused simply by incubation at different temperatures or were related to availability of contaminants were not clear. However differences in the functional stability of resuspended community were observed when no differences in ecotoxicities were measured.

The parameters describing the functional stability of the microbial communities were further summarised in quantitative index and quality classes as described in Chapter 5, showing classes and indices with increasing functional stability contributed by the functional redundancy and evenness. The resulting quality classes and index in functional stability of microbial community in car-

bon cycling after aging and resuspension at different temperatures are illustrated in Figure 6.7. The microbial communities extracted from aged and resuspended sediments all showed low functional stability with quality classes 2 for community with low species richness. The resuspension at 20℃ and 30℃ resulted in microbial community with lower functional stability shown in index. The aging and resuspension had adverse influence not only on ecotoxicities, but also on microbial functional stability in carbon cycling. The risk of a microbial community with low functional stability was elevated when aged sediments were resuspended at altered temperatures.

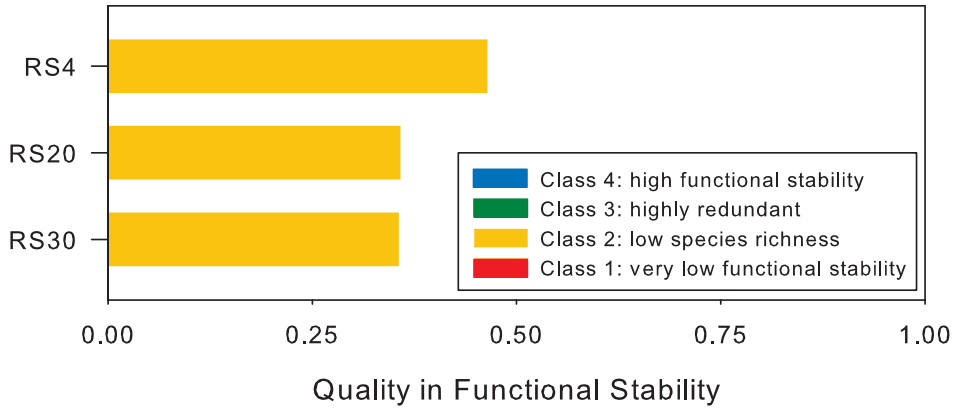


Figure 6.7: Quality of functional diversity using fuzzy logic showing sediment incubated at different temperatures.

6.2.3 Key Nitrification Activities

The key function of nitrification in the aged sediments was measured with potential nitrification rates, showing the capability of nitrification activities at ideal conditions.

Figure 6.8 illustrates the potential nitrification rates of sediments after one year aging and after resuspension at different temperatures. All sediments except for sediments resuspended at 4℃ displayed high potential nitrification rates comparable to potential nitrification rates measured in fresh sediments (see Section 4.1.2). Sediments resuspended at 4℃ showed a 40% inhibition of potential nitrification rate compared to one year aged sediments at 4℃. It was reported by Antoniou et al. (1990) that activities of autotrophic nitrifying bacteria are inhibited at temperatures lower than 15℃. The potential nitrification rates of aged sediments exhibited slightly higher potential nitrification rates than freshly collected sediments in spring months (135 ng/g sed./hr at temperature < 10℃).

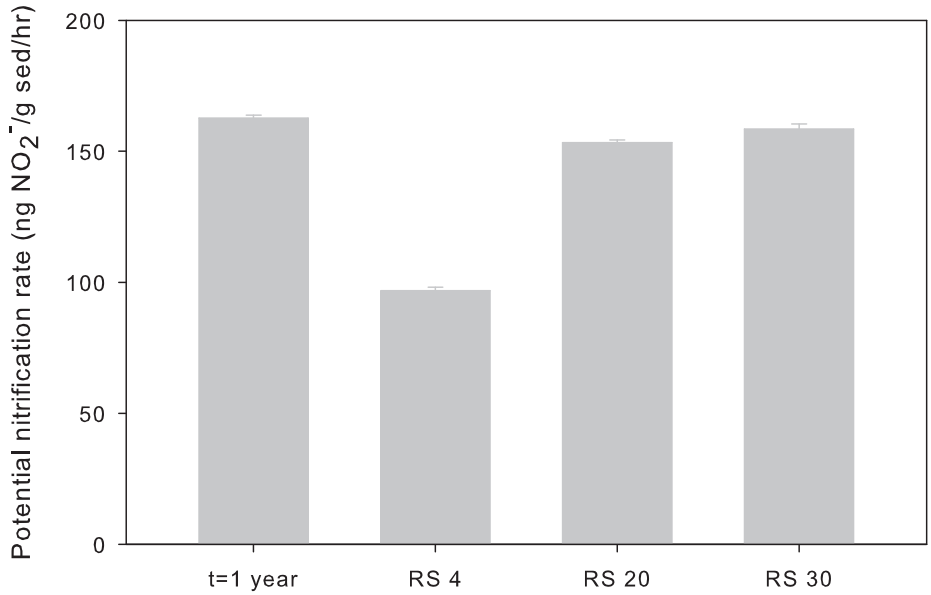


Figure 6.8: Potential nitrification rates of aged sediment (at 4℃) before and after resuspended at different temperatures.

The aging did not seem to influence the potential nitrification activity, though incubation at 4℃ did.

The potential nitrification activity seemed more sensitive to temperature than aging and resuspension. The potential of nitrification was readily redeveloped given appropriate temperatures within 2 weeks.

6.3 IQI Evaluation

The detailed effects of aging and resuspension on each line of evidence of the quality indicators were described and summarised as quality index and classes. The evaluation of sediment quality with IQI was taken as an exercise of the IQI application. It can be concluded that aging did contribute to the strong sorption of chemicals and therefore decreased the toxicities of the contaminated sediments. The resuspension however could reverse the reduced risk and even induce higher toxicities after aging. The microbial communities also demonstrated lower functional stability and cell densities after aging and resuspension. The key ecological function nitrification was influenced more by temperature than aging and resuspension processes. To have a picture of the impact from aging and resuspension on the ecological relevance of these sediments as a whole, the three lines of evidence were combined and presented with the developed IQI both quantitatively and linguistically. The results were compared with sediments impacted

Table 6.4: Results and scores of aged sediments and sediments amended with Nitrapyrin on different quality parameters and indicators.

LOE	Inhibited Nitrification			Aging & Resuspension		
	Control	DMSO	Nitrapyrin	4℃	20℃	30℃
Ecotoxicities						
AGI	64.6	69.4	57.4	74.1	84.9	74.2
BCA	51.1	52.2	49.2	41.7	41.7	38.6
Q. Class	**2**	**2**	**2**	**2**	**2**	**2**
Q. Index	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Functional Stability						
$log(CFU_{50})$	-2.07	-1.95	-2.12	-1.89	-1.57	-1.57
h	0.71	0.80	0.82	1.15	1.26	1.13
Q. Class	**3**	**1**	**3**	**2**	**2**	**2**
Q. Index	**0.50**	**0.46**	**0.51**	**0.46**	**0.36**	**0.36**
Key Function						
P.N.Rate	136	101	73	97	153	159
Q. Index	**0.78**	**0.61**	**0.41**	**0.55**	**0.88**	**0.91**

with a specific nitrification inhibitor to illustrate the different IQIs of sediments influenced by the contaminant availability and lost key function.

Results of each input parameters as well as the integrated quality index and classes from each line of evidence are listed in Table 6.4. The algal growth inhibition (57-85%) and bacterial contact inhibition (39-52%) were all categorised as moderate toxicities (see Figure 5.3). The quality classes and index in ecotoxicities were therefore the same for all sediments, showing similar level of hazardous effect from contaminants. The quality in functional stability and key function nitrification on the other hand varied. In general, fresh sediments had higher functional redundancy and lower functional evenness, and the aged and resuspended ones the other way round. The fresh sediments had therefore higher quality in functional stability even with addition of nitrification inhibitor nitrapyrin. The quality in key function was inhibited by the specific inhibitor and low incubation temperature. The overall ecological relevance were evaluated and presented as quantitative and linguistic IQI.

6.3.1 Quantitative Summary Index

To have an overview of the sediment quality and its ecological relevance, each quality index were plotted in a triangle showing all quality indices together. Figure 6.9 illustrates the quality indices of the six investigated sediments.

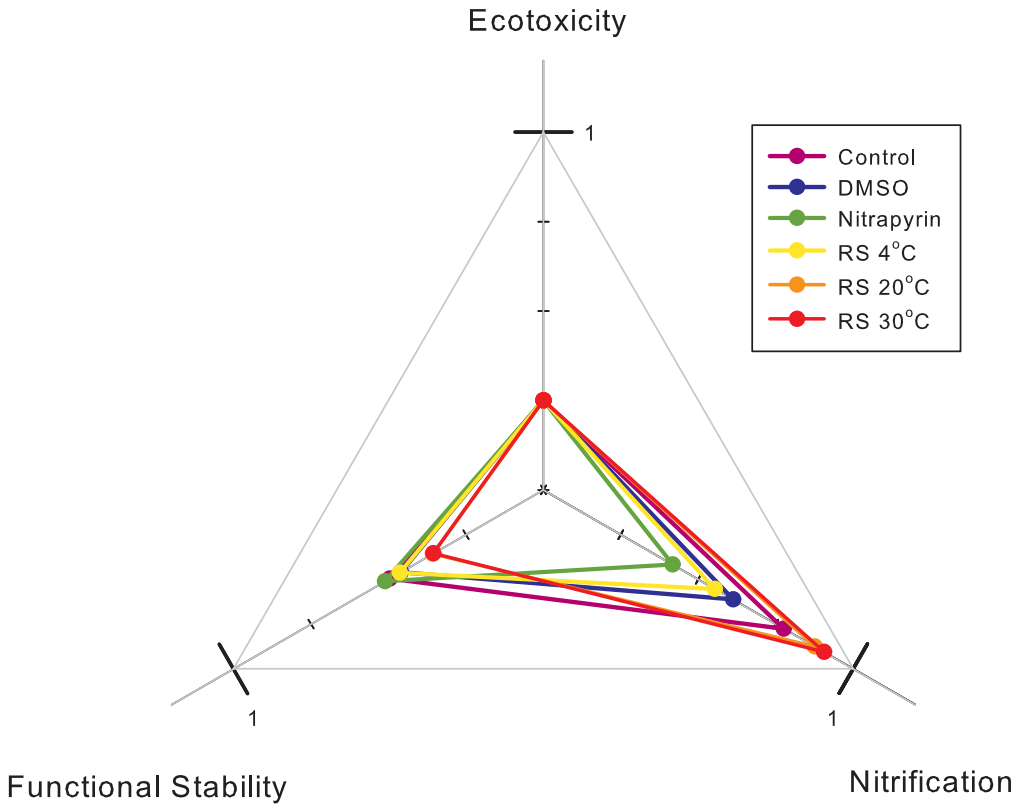


Figure 6.9: Integrated quality indicator presented as summary indices of sediments treated with nitrification inhibitor and aging with resuspension.

First of all, all sediments had low quality in ecotoxicities with different levels of quality in functional stability and key ecological function. This indicated that the sediment communities were most probably adapted to the level of contamination in the sediments. Second, all sediments had shape of a scalene triangle, indicating different qualities in functional stability and nitrification activity. The aged and resuspended sediments at 20 and 30℃ showed highest quality in nitrification, while fresh sediments had higher quality in functional stability in carbon cycling. The developed IQI distinguished clearly the fresh and aged sediments in their functional stabilities.

The area of triangle can be further calculated to rank the overall quality. The overall quality from high to low were ranked as following: Control > RS 30℃ > RS 20℃ > DMSO > RS 4℃> Nitrapyrin.

The graphical quantitative presentation of the IQI gave a simple overview of the overall quality, which can be easily understood by and explained to non scientists. It also presented the quality as a sum with the area within the triangle and facilitates intuitive ranking. However, the quantitative index did not characterise the underlying meaning of the toxicities and functional stabilities, which can be

described more in detail using the following tabular decision matrix.

6.3.2 Linguistic Tabular Decision Matrix

The underlying meaning of the quality classes of each line of evidence can be listed in a tabular decision matrix, which offers a direct link of the results and ecological relevance as well as possible cause of it.

Table 6.5 listed the quality classes of the six investigated sediments and conclusion on its ecological relevance. The three freshly collected sediments all had elevated risk from contaminants present in both pore waters and sediment particles. The control sediment displayed a community with high functional redundancy with moderate to high nitrification activities. The relatively good quality in functional stability and key function compared to the aged sediments demonstrated the Over sediments as a moderately contaminated site with adapted microbial community who exhibit high level of nutrient cycling. The addition of solvent DMSO and specific inhibitor nitrapyrin impacted the microbial community in its nutrient cycling. At the addition of solvent and nitrapyrin, the adapted sediment microbial community started losing functions in nutrient cycling, especially in nitrogen cycling.

The aged and resuspended sediments displayed the same level of risk from contaminants in both pore water and sediment particles. Theses sediments exhibited however low level of functional stability as a result of reduced species richness and temperature dependent nitrification activities. After resuspension, the decreased risk from contaminants in ecotoxicities was reversed to the same level of ecotoxicities. The functional stability in carbon cycling of these sediments was impacted, while the key ecological function nitrification were recovered at optimal temperature range. The sediment microbial community became sensitive from the aging and resuspension processes at recovery of different nutrient cycling. When aged and resuspended at 4℃, functional stability were sensitive and key ecological function was inhibited by low temperature. When aged at 4℃ and resuspended at 20℃ or 30℃, the functional stability was similarly sensitive though key ecological function was recovered. The aging generally reduced the functional stability of microbial community and the resuspension at different temperatures offer conditions for recovery of different functions in nutrient cycling.

Overall, the quantitative summary indices presented as a triangle offer an overview of the quality from each line of evidence and are easy for ranking. To understand the underlying meaning of the quality indices and combined conclusion as well as the ecological relevance, the linguistic tabular matrix offer simple and clear explanation and are easier coupled with possible cause and corresponding actions for decision makers.

Table 6.5: Tabular decision matrix for linguistic IQI.

	Quality			IQI Conclusions
	Ecotox.[a]	FS[b]	Nitri.	
Control	2	3	136	Elevated risk from contaminants, community adapted with high redundancy, moderate to high nitrification activities. **Contaminants adapted community.**
DMSO	2	1	101	Elevated risk from contaminants, community impacted with reduced redundancy, moderate nitrification activities. **Impacted but moderate key function.**
Nitrapyrin	2	3	73	Elevated risk from contaminants, community adapted with high redundancy, partly impacted nitrification activities. **Adapted with reduced key function.**
Adapted Community Losing Functions.				
RS 4℃	2	2	97	Elevated risk from contaminants, community with low species richness, partly impacted nitrification activities. **Ecologically impacted by contaminants and aging/resuspension.**
RS 20℃	2	2	153	Elevated risk from contaminants, community with low species richness, high nitrification activities. **Carbon cycling impacted but recovery of nitrification.**
RS 30℃	2	2	158	Elevated risk from contaminants, community with low species richness, high nitrification activities. **Carbon cycling impacted but recovery of nitrification.**
Impacted Community at Recovery.				

[a]Quality Class in Ecotoxicity:
- 5: no or low toxicity
- 4: particle-bound toxicity
- 3: mobile toxicity
- 2: mixture toxicity
- 1: high toxicity

[b]Quality Class in Functional Stability:
- 4: high functional stability
- 3: functionally redundant
- 2: low species richness
- 1: low functional stability

6.4 Conclusion

The sorption desorption characteristics of organic chemicals on soil and sediments have been well studied and several models describing the kinetics were published and reviewed by Cornelissen et al. (2005). These models assisted the prediction of risk and bioavailability more precisely with chemical analysis for contaminated sites. However, the presence of contamination cocktails and the continuous variation of environmental conditions make the sole chemical analysis insufficient in assessing risk. The aging and resuspension is therefore an ideal example to demonstrate the importance of an ecological relevant quality indicator.

The quality classes in ecotoxicities of the Over sediments increased from Class 4, showing only particle bound toxicity, to Class 5, showing no or low toxicities after one year of aging. These results corresponded well with the well known phenomenon of aging, which describes the increasing resistance of contaminants to biodegradation and extraction with the prolonged contact time. Though in this study, the experimental setup was represented probably aging of already aged contaminants in sediments, the reduced ecotoxicities still showed the process of reduced bioavailability due to prolonged contact time.

The increased quality of aging in sediment, however could not guarantee the reduced ecological risk mentioned sometimes as natural attenuation. The quality in ecotoxicities decreased dramatically to Class 2, showing mixture toxicities in both pore water and particles, after slight resuspension of the sediments. Moreover, the quality of functional stability in carbon cycling was decreased to Class 2, showing low species richness, by the aging and resuspension processes. The considered most sensitive ecological function of nitrification on the other hand could recover easily after incubation at ideal range of temperatures. The easily recovered nitrification activities did not contradict to its sensitiveness. The nitrifying community is very sensitive to contaminants and environmental conditions, but the community did not extinct and can recover easily at ideal conditions. This is probably why the nitrification activity is still wide spread in environment despite the exclusiveness and sensitiveness of nitrifying communities.

To summarise the effects of aging on each line of evidence (ecotocixicity, stress resistance, and nitrification), the aged and resuspended sediments were evaluated using the integrated quality indicators (IQI) developed in Chapter 5 and compared with freshly collected sediments. An overview of the IQI was presented using summary indices illustrated in a triangle. It was demonstrated that the aging and resuspension at elevated temperatures reduced the quality in functional stability clearly. To further describe the underlying meaning and concluded ecological relevance, the tabular decision matrix listed the aged and resuspended sediments as sensitive communities at different level of recovery, while the freshly collected sediments as adapted community at different level of losing functions.

The evaluation of aging in sediments using IQI provided supplementary ecological relevance to assist presently chemical dominant criteria in assessing sedi-

ment qualities. The sensitive and crucial microbial nutrient cycling gave insight in the possible impairment of ecological services and demonstrated the unexpected hazard on microbial nutrient cycling despite the decreased risk from contaminants on biota. The development of the IQI was done according to specific rules, and took ecological uncertainties into account in categorising effects, which improves transparency. The presentation of IQI in quantitative triangle and linguistic tables further facilitates communication with decision makers.

As conclusion, the important ecological service of nutrient cycling provided by microbial community was successfully integrated together with ecotoxicities as an ecologically relevant integrated quality indicator, which provides a framework in assessing sediment quality and eventually improving the river quality.

Chapter 7

Concluding Summary

To reach the "good ecological status" of all surface waters as requested by the European Water Framework Directive by 2015, sediment should become a management object. Up to date sediment quality is mostly assessed using chemical criteria in Europe disregarding the ecological services it provides. Therefore an integrated quality indicator addressing the ecological relevance of sediment in river basins was developed using the weight of evidence approach including sediment ecotoxicity, heterotrophic functional stability in carbon cycling, and key ecological function nitrification as three lines of evidence.

The quality of ecotoxicities describes the potential risk of contaminants on biota and was demonstrated sensitive to changes of hydrological and biological conditions such as flood and algal bloom. It indicates the bioavailable contaminants in the sediments. The quality of functional stability addresses the relative structure of microbial community in heterotrophic carbon cycling in response to dilution and was shown dependent on geochemical conditions such as redox potential. It serves as reference of the susceptibility of microbial community in carbon cycling. The quality of nitrification represents the most sensitive and exclusive step in nitrogen cycling and was displayed only in the small oxic zone at the sediment water interface. It exhibits the functioning nitrogen cycle. Characterisation of both heterotrophic carbon cycling and autotrophic nitrification activity with substrates induced DMSO rate was hypothesised, discussed theoretically and examined with experiment. However, the complex and sensitive metabolic pathways involved needs further investigation before possible application.

The three lines of evidence were integrated using rule based fuzzy model, which addresses the biological uncertainties as overlaps of fuzzy sets and classifies results according to expert knowledge based if-then rules. The results were presented using quantitative indices to give an overview and linguistic tables coupling results to conclusion on ecological relevance and causes. The example of IQI evaluation of aging and resuspension demonstrated the unexpected reversible impact of reduced risk of contamination on the sensitive and crucial nutrient cycling.

An IQI was developed addressing ecologically relevant sediment quality which will in the future need to be considered when aiming for a good status of rivers. The IQI demonstrated the advantage over the decision-making framework using pass/fail attributes of each LOE (Grapentine et al., 2002) with the fuzzy rule-based model, which integrated the biological uncertainties and allowed a finer classification of quality. The three included LOE also showed focus on the sediment ecology. First, the exclusion of chemical criteria enabled status and effect assessment without being contaminants oriented as in many ecological risk assessments (Hollert et al., 2002). Second, the assessment of microbial communities with nutrient cycling instead of molecular biomarkers (Kostanjšek et al., 2005; Lachmund et al., 2003) facilitated the interpretation of results. This study is an attempt to include complex microbial nutrient cycling in an indicator, which needs further validation and optimisation with a larger data set. Recently, a fuzzy index of ecosystem integrity (Java Programme available online) was developed to detect the biological element of the ecological quality status of lagoon environments into the five classes according to WFD (Mistri et al., 2008). It is foreseeable that with an immense dataset, such index could be developed for the IQI. In any case, it suggests a conceptual framework of a transparent and user defined integration of uncertain biological data for communication with simplicity for decision makers and river basin managers.

Bibliography

Abeliovich, A. and Y. Azov (1976). Toxicity of ammonia to algae in sewage oxidation ponds. *Applied and Environmental Microbiology 31*, 801–806.

Adriaenssens, V., B. De Baets, P. L. M. Goethals, and N. De Pauw (2004). Fuzzy rule-based models for decision support in ecosystem management. *Science of the Total Environment 319*, 1–12.

Ahlf, W., T. Braunbeck, S. Heise, and H. Hollert (2002). *Environmental Monitoring Handbook*, Chapter 17 Sediment and Soil Quality Criteria, pp. 17.1–17.18. New York: McGraw-Hill.

Ahlf, W., U. Feiler, P. Heiniger, and S. Heise (2008). *The Water Framework Directive - Ecological and Chemical Status Monitoring Water Quality Measurements*, Chapter 6.2 Monitoring sediment quality using toxicity tests as primary tools for any risk assessment, pp. 255–270. Whiley.

Ahlf, W. and H. Gratzer (1999). *Entwicklung methodischer Ansätze*, Chapter Erarbeitung von Kriterien zur Ableitung von Qualitätszielen für Sedimenten und Swebstoffe TV2, pp. 1–168. Berlin: Umweltbundesamt.

Ahlf, W. and S. Heise (2005). Sediment toxicity assessment - rationale for effect classes - dedicated to Prof. Dr. Ulrich Förstner on his 65th birthday. *Journal of Soils and Sediments 5*, 16–20.

Ahlf, W., H. Hollert, H. Neumann-Hensel, and M. Rickling (2002). A guidance for the assessment and evaluation of sediment quality: A german approach based on ecotoxicological and chemical measurements. *Journal of Soils and Sediments 2*, 37–42.

Alef, K. (1990). Bestimmung mikrobieller Aktivität und Biomasse in Boden und Kompost mit der Dimethylsulfoxid-Reduktion. *Umweltwissenschaften und Schadstoff-Forschung 2*, 76–78.

Alexander, M. (2000). Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science & Technology 34*, 4259–4265.

Antoniou, P., J. Hamilton, B. Koopman, R. Jain, B. Holloway, G. Lyberatos, and S. A. Svoronos (1990). Effect of temperature and ph on the effective maximum specific growth rate of nitrifying bacteria. *Water Research 24*, 97–101.

Azam, F., D. C. Smith, G. F. Steward, and A. Hagström (1994). Bacteria-organic matter coupling and its significance for oceanic carbon cycling. *Microbial Ecology 28*, 167–179.

Baborowski, M., O. Büttner, P. Morgenstern, F. Krüger, I. Lobe, H. Rupp, and W. V. Tümpling (2007). Spatial and temporal variability of sediment deposition on artificial-lawn traps in a floodplain of the River Elbe. *Environmental Pollution 148*, 770–778.

Blanck, H. (1984). Species dependent variation among aquatic organisms in their sensitivity to chemicals. *Ecological Bulletin 36*, 107–119.

Blum, D. J. W. and R. E. Speece (1991). A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. *Research Journal of the Water Pollution Control Federation 63*, 198–207.

Böhme, M., H. Guhr, and K. Ockenfeld (2006). *Stoffdynamik und Habitatstruktur in der Elbe*, Chapter Phytoplanktondynamic, pp. 33–43. Berlin: Weißensee Verlag.

Breure, A. M. and M. Rutgers (2000). The application of Biolog plates to characterise microbial communities. In *Proceedings of the COST Action 831, Joint Working Group Meeting Biotechnology of Soil, Monitoring, Conservation and Bioremediation*, Rome, Italy, pp. 179–185.

Burton, Jr., G. A., R. Baudo, M. Beltrami, and C. Rowland (2001). Assessing sediment contamination using six toxicity assays. *Journal of Limnology 60*, 263–267.

Burton, Jr., G. A., P. M. Chapman, and E. P. Smith (2002). Weight-of-evidence approaches for assessing ecosystem impairment. *Human and Ecological Risk Assessment 8*, 1657–1673.

Chapman, P. M. (1990). The sediment quality triad approach to determine pollution-induced degradation. *Science of the Total Environment 97*, 815.

Chapman, P. M. (1996). Presentation and interpretation of sediment quality triad data. *Ecotoxicology 5*, 327–339.

Chapman, P. M. (2002). Integrating toxicology and ecology: putting the "eco" into ecotoxicology. *Marine Pollution Bulletin 44*, 7–15.

Chapman, P. M. and H. Hollert (2006). Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? *Journal of Soils and Sediments 6*, 4–8.

Chapman, P. M., B. G. McDonald, and G. S. Lawrence (2002). Weight-of-evidence issues and frameworks for sediment quality (and other) assessments. *Human and Ecological Risk Assessment 8*, 1489–1515.

Christian, B. W. and O. T. Lind (2006). Key issues concerning Biolog use for aerobic and anaerobic freshwater bacterial community-level physiological profiling. *International Review of Hydrobiology 91*, 257–268.

Chróst, R. H. and M. A. Faust (1983). Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. *Journal of Plankton Research 5*, 477–493.

Cornelissen, A. M. G., J. Van Den Berg, W. J. Koops, M. Grossman, and H. M. J. Udo (2001). Assessment of the contribution of sustainability indicators to sustainable development: A novel approach using fuzzy set theory. *Agriculture, Ecosystems and Environment 86*, 173–185.

Cornelissen, G., O. Gustafsson, T. D. Bucheli, M. T. O. Jonker, A. A. Koelmans, and P. C. M. van Noort (2005). Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environmental Science & Technology 39*, 6881–6895.

Crane, M. and M. Babut (2007). Environmental quality standards for water framework directive priority substances: challenges and opportunities. *Integrated Environmental Assessment and Management 3*, 290–296.

Crane, M., P. M. Chapman, and T. H. Sparks (1997). Multivariate methods in environmental toxicity and chemistry. *Toxicology and Ecotoxicology News/Reviews 4*, 164–164.

Dale, A. W., P. Regnier, and P. Van Cappellen (2006). Bioenergetic controls on anaerobic oxidation of methane (AOM) in coastal marine sediments: A theoretical analysis. *American Journal of Science 306*, 246–294.

Dalsgaard, T., J. Dezwart, L. A. Robertson, J. G. Kuenen, and N. P. Revsbech (1995). Nitrification, denitrification and growth in artificial thiosphaera-pantotropha biofilms as measured with a combined microsensor for oxygen and nitrous-oxide. *FEMS Microbiology Ecology 17*, 137–147.

De Beer, D., A. Schramm, C. M. Santegoeds, and M. Kuhl (1997). A nitrite microsensor for profiling environmental biofilms. *Applied and Environmental Microbiology 63*, 973–977.

De Beer, D. and P. Stoodley (1999). *The Prokaryotes: An Elvolving Electronic Resource for the Microbiological Community*, Chapter 267 Microbial Biofilms, pp. 904–937. New York: Springer.

De Beer, D. and J. P. R. A. Sweerts (1989). Measurement of nitrate gradients with an ion-selective microelectrode. *Analytica Chemica Acta 219*, 351–356.

De Zwart, D. and W. Slooff (1983). The microtox as an alternative assay in the acute toxicity assessment of water pollutants. *Aquatic Toxicology 4*, 129–138.

Degens, B. P. and J. A. Harris (1997). Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology & Biochemistry 29*, 1309–1320.

Den Besten, P. J. (2007). *Sediment Risk Management and Communication*, Chapter 5 Risk Assessment Approaches in Europe, pp. 153–206. Amsterdam: Elsevier.

Den Besten, P. J., E. De Dekere, M. P. Babut, B. Power, A. Del Valls, C. Zago, A. M. P. Oen, and S. Heise (2003). Biological effects-based sediment quality in ecological risk assessment for European waters. *Journal of Soils and Sediments 3*, 144–162.

Dobranic, J. K. and J. C. Zak (1999). A microtiter plate procedure for evaluating fungal functional diversity. *Mycologia 91*, 756–765.

Dubé, M. G. and J. M. Culp (1996). Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. *Environmental Toxicology and Chemistry 15*, 2019–2027.

Dutka, B. J., T. Tuominen, L. Churchland, and K. K. Kwan (1989). Fraser river sediments and waters evaluated by the battery of screening tests technique. *Hydrobiologia 188-189*, 301–315.

FGG Elbe (2004). *Zusammenfassender Bericht der Flussgebietsgemeinschaft Elbe über die Analysen nach Artikel 5 der Richtlinie 2000/60/EG. (A-Bericht).* Magdeburg: Flussgebietsgemeinschaft Elbe.

Fischer, H., F. Kloep, S. Wilzcek, and M. Pusch (2005). A river's liver microbial processes within the hyporheic zone of a large lowland river. *Biogeochemistry 76*, 349–371.

Fonseca, C. R. and G. Ganade (2001). Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology 89*, 118–125.

Förstner, U., S. Heise, R. Schwartz, B. Westrich, and W. Ahlf (2004). Historical contaminated sediments and soils at the river basin scale - examples from elbe river catchment area. *Journal of Soils and Sediments 4*, 247–260.

Förstner, U. and G. Müller (1974). *Schwermetalle in Flüssen und Seen als Ausdruck der Umweltverschmutzung.* Berlin-Heidelberg-New York: Springer.

Frühling, W. (2004). *Funktionale und Strukturelle Untersuchungsparameter für mikrobielle Bodenbiozönosen und ihr ökotoxikologischer Nutzen.* Ph. D. thesis, Technische Universität Hamburg-Harburg.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl, A. F. Michaels, J. H. Porter, A. R. Townsend, and C. J. Vörösmarty (2004). Nitrogen cycles: Past, present, and future. *Biogeochemistry 70*, 153–226.

Garland, J. L. and R. M. Lehman (1999). Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. *FEMS Microbiology Ecology 30*, 333–343.

Garland, J. L. and A. L. Mills (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology 57*, 2351–2359.

Giesy, J. P. and R. Hoke (1990). *Sediments: Chemistry and Toxicity of In-Place Pollutants*, Chapter Freshwater Sediment Criteria Toxicity Assessment, pp. 265–348. Boca Barton: Lewis.

Gocke, K. (1976). Respiration von gelösten organischen Verbindungen durch natürliche Mikroorganismen-Populationen. Ein Vergleich zwischen verschiedenen Biotopen. *Marine Biology 35*, 375–383.

Götz, R., O. H. Bauer, P. Friesel, T. Herrmann, E. Jantzen, M. Kutzke, R. Lauer, O. Paepke, K. Roch, U. Rohweder, R. Schwartz, S. Sievers, and B. Stachel (2007). Vertical profile of PCDD/Fs, dioxin-like PCBs, other PCBs, PAHs, chlorobenzenes, DDX, HCHs, organotin compounds and chlorinated ethers in dated sediment/soil cores from flood-plains of the river Elbe, Germany. *Chemosphere 67*, 592–603.

Graf, G., W. Bengtsson, U. Diesner, R. Schulz, and H. Theede (1982). Benthic response to sedimentation of a spring phytoplankton bloom: Process and budget. *Marine Biology 67*, 201–208.

Grapentine, L., J. Anderson, D. Boyd, G. A. Burton, Jr., C. DeBarros, G. Johnson, C. Marvin, D. Milani, S. Painter, T. Pascoe, T. Reynoldson, L. Richman, K. Solomon, and P. M. Chapman (2002). A decision making framework for sediment assessment developed for the Great Lakes. *Human and Ecological Risk Assessment 8*, 1641–1655.

Griebler, C. (1997). Dimethylsulfoxide (DMSO) reduction: a new approach to determine microbial activity in freshwater sediments. *Journal of Microbiological Methods 29*, 31–40.

Griebler, C. and D. Slezak (2001). Microbial activity in aquatic environments measured by dimethyl sulfoxide reduction and intercomparison with commonly used methods. *Applied and Environmental Microbiology 67*, 100–109.

Griscom, S. B., N. S. Fischer, and S. N. Luoma (2000). Geochemical influences on assimilation of sediment-bound metals in clams and mussels. *Environmental Science & Technology 34*, 91–99.

Grote, M., R. Altenburger, W. Brack, S. Moschutz, S. Mothes, C. Michael, C. Narten, A. Paschke, K. Schirmer, H. Walter, R. Wennrich, K. Wenzel, and G. Schuurmann (2005). Ecotoxicological profiling of transect river Elbe sediments. *Acta Hydrochimica et Hydrobiologica 33*(5), 555–569.

Grunewald, K., C. Unger, H. J. Brauch, and W. Schmidt (2004). Elbe flood 2002 in retrospect: Pollution of sediments due to the extreme flood situation around Dresden (Saxony, Germany). *Umweltwissenschaften und Schadstoff-Forschung 16*, 7–14.

Harkey, G. A., P. F. Landrum, and S. J. Klaine (1994). Comparison of whole-sediment, elutriate and porewater exposures for use in assessing sediment associated organic contaminants in bioassays. *Environmental Toxicology and Chemistry 13*, 1315–1330.

Hatatsu, M., M. Saito, and K. Tago (2008). Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science & Plant Nutrition 54*, 33–45.

Hatzinger, P. B. and M. Alexander (1995). Effect of aging of chemicals in soil on their biodegradability and extractability. *Environmental Science & Technology 29*, 537–545.

Heininger, P., J. Pelzer, E. Claus, and S. Pfitzner (2004). Results of long-term sediment quality studies on the river Elbe. *Acta Hydrochimica et Hydrobiologica 31*, 356–367.

Heise, S. and W. Ahlf (2005). A new microbial contact assay for marine sediments. *Journal of Soils and Sediments 5*, 9–15.

Heise, S. and A. E. Apitz (2007). *Sediment Risk Management and Communication*, Chapter 1 The Role of Risk Management and Communication in Sustainable Sediment Management, pp. 1–8. Amsterdam: Elsevier.

Heise, S., E. Claus, P. Heiniger, T. Krämer, F. Krüger, R. Schwartz, and U. Förstner (2005). *Studie zur Schadstoffbelastung der Sedimente im Elbeeinzugsgebiet: Ursachen und Trends*. Commissioned by the Hamburg Port Authority. 181.

Heise, S., S. Höss, and W. Ahlf (2003). Ökotoxikologische Befunde aus dem Wattenmeer. In *Proceedings BMBF-Verbundprojekt "Schadstoffbelastung im Mulde- und Elbe-Einzugsgebiet nach dem Augusthochwasser 2002"*, Freiberg, pp. 64–64.

Heise, S., F. Kruger, U. Förstner, M. Baborowski, R. Götz, and B. Stachel (2007). *Bewertung von Risiken durch feststoffgebundene Schadstoffe in Elbeeinzugsgebiet*. Hamburg: Comissioned by the Flussgebietsgemeinschaft Elbe and the Hamburg Port Authority, compiled by the Beratungszentrum fur integriertes Sedimentmanagement (BIS/TuTech) at the Hamburg University of Technology. 349 pages.

Hollert, H., M. Dürr, H. Olsman, K. Halldin, B. Van Bavel, W. Brack, M. Tysklind, M. Engwall, and T. Braunbeck (2002). Biological and chemical determination of dioxin-like compounds in sediments by means of a sediment triad approach in the catchment area of the River Neckar. *Ecotoxicology 11*, 323–336.

Hollert, H., S. Heise, S. Pudenz, R. Brüggemann, W. Ahlf, and T. Braunbeck (2002). Application of a sediment quality triad and different statistical approaches (hasse diagrams and fuzzy logic) for the comparative evaluation of small streams. *Ecotoxicology 11*, 311–321.

Hsu, P., A. Matthäi, S. Heise, and W. Ahlf (2007). Seasonal variation of sediment toxicity in the Rivers Dommel and Elbe. *Environmental Pollution 148*, 817–823.

Huang, W., T. M. Young, M. A. Schlautman, H. Yu, and W. J. Weber (1997). A distributed reactivity model for sorption by soils and sediments. 9. general isotherm nonlinearity and applicability of the dual reactive domain model. *Environmental Science & Technology 31*, 1703–1710.

Jensen, K., N. P. Revsbech, and L. P. Nielsen (1993). Microscale distribution of nitrification activity in sediment determined with a shielded microsensor for nitrate. *Applied and Environmental Microbiology 59*, 3287–3296.

Joo, H. S., M. Hirai, and M. Shoda (2005). Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* no. 4. *Journal of Bioscience and Bioengineering 100*, 184–191.

Jordan, F. L., J. J. L. Cantera, M. E. Fenn, and L. Y. Stein (2005). Autotrophic ammonia-oxidizing bacteria contribute minimally to nitrification in a nitrogen-impacted forested ecosystem. *Applied and Environmental Microbiology 71*, 197–206.

Joziasse, J., S. Heise, A. Oen, G. J. Ellen, and L. Gerrits (2007). *Sediment Risk Management and Communication*, Chapter 2 Sediment Management Objectives and Risk Indicators, pp. 9–76. Amsterdam: Elsevier.

Kallqvist, T. and A. Svenson (2003). Assessment of ammonia toxicity in tests with the microalga, *Nephroselmis pyriformis*, *Chlorophyta*. *Water Research 37*, 477–484.

Kaufmann, K., S. J. Chapman, C. D. Campbell, H. Harms, and P. Hohener (2006). Miniaturized test system for soil respiration induced by volatile pollutants. *Environmental Pollution 140*, 269–278.

Keddy, C. J., J. C. Greene, and M. A. Bonnell (1995). Review of whole-organism bioassays - soil, fresh-water sediment, and fresh-water assessment in Canada. *Ecotoxicology and Environmental Safety 30*, 221–251.

Klös, H. and C. Schoch (1993). Historical trends of sediment loads: Memory of an industrial region. *Acta Hydrochemica et Hydrobiologica 21*, 32–37.

Kostanjšek, R., A. Lapanje, D. Drobne, S. Nikcević, A. Perović, P. Zidar, J. Štrus, H. Hollert, and G. Karaman (2005). Bacterial community structure analyses to assess pollution of water and sediments in the Lake Shkodra/Skadar, Balkan Peninsula. *Environmental Science and Pollution Research 12*, 361–368.

Kühl, M. and N. P. Revsbech (2001). *The Benthic Boundary Layer*, Chapter 8 Biogeochemical microsensors for boundary layer studies, pp. 180–210. New York: Oxford University Press.

Kwan, K. K. and B. J. Dutka (1990). Simple two-step sediment extraction procedure for use in genotoxicity and toxicity bioassays. *Toxicity Assessment 5*, 395–404.

Lachmund, C., B. Köcher, W. Manz, and P. Heininger (2003). Chemical and microbiological in situ characterization of benthic communities in sediments with different contamination levels. *Journal of Soils and Sediments 3*, 188–196.

Lindstrom, J. E., R. P. Barry, and J. F. Braddock (1998). Microbial community analysis: A kinetic approach to constructing potential C source utilization patterns. *Soil Biology & Biochemistry 30*, 231–239.

Liss, W. and W. Ahlf (1997). Evidence of whole-sediment, porewater, and elutriate testing in toxicity assessment of contaminated sediments. *Ecotoxicology and Environmental Safety 36*, 140–147.

Long, E. R. and P. M. Chapman (1985). A sediment quality triad: Measures of sediment contamination, toxicity and infaunal community composition in puget sound. *Marine Pollution Bulletin 16*, 405–415.

Lopez, N. I. and C. M. Duarte (2004). Dimethyl sulfoxide (DMSO) reduction potential in mediterranean seagrass (*Posidonia oceanica*) sediments. *Journal of Sea Research 51*, 11–20.

Madigan, M., J. Martinko, and J. Parker (2003). *Brock Biology of Microorganisms*, Chapter 17 Metabolic Diversity, pp. 547–613. Upper Saddle River: Pearson Education, Inc.

Matthäi, A., P. Hsu, and W. Ahlf (2007). *Sediment Dynamics and Pollutant Mobility in Rivers*, Chapter 10.2 Evaluation of Sediment Toxicity in the Elbe River Basin, pp. 391–400. Berlin Heidelberg: Springer.

Millennium Ecosystem Assessment (2005). *Ecosystems and human well-being: wetlands and water synhesis.* Washington, DC.: World Resources Institute.

Mistri, M., C. Munari, and A. Marchini (2008). The fuzzy index of ecosystem integrity (FINE): A new index of environmental integrity for transitional ecosystems. *Hydrobiologia 611*, 81–90. http://web.unife.it/progetti/FINE/.

Mulder, A., A. A. Vandegraaf, L. A. Robertson, and J. G. Kuenen (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *FEMS Microbiology Ecology 16*, 177–183.

Müller, G. (1979). Schwermetalle in den Sedimenten des Rheins - Veränderungen seit 1971. *Umschau 79*, 779–783.

Munkittrick, E. A. P. (1991). The relative sensitivity of microtox, daphnid, rainbow trout, and fathead minnow acute lethality tests. *Environmental Toxicology & Water Quality 6*, 35–62.

Netzband, A., H. Reincke, and M. Bergmann (2002). The River Elbe - a case study for the ecological and economical chain of sediments. *Journal of Soils and Sediments 3*, 112–116.

Nicholas, D. J. and A. Nason (1957). *Methods in Enzymology*, Chapter Determination of nitrate and nitrite, pp. 981–984. New York: Academis Press Inc.

O'Connor, T. P. and J. F. Paul (2000). Misfit between sediment toxicity and chemistry. *Marine Pollution Bulletin 40*, 59–64.

Pignatello, J. J. and B. Xing (1996). Mechanisms of slow sorption of organic chemicals to natural particles. *Environmental Science & Technology 30*, 1–11.

Pomeroz, L. R. (1974). The ocean's food web, a changing paradigm. *BioScience 24*, 499–504.

Poot, A., F. Gillissen, and A. A. Koelmans (2007). Effects of flow regime and flooding on heavy metal availability in sediment and soil of a dynamic river system. *Environmental Pollutants 148*, 779–787.

Preston-Mafham, J., L. Boddy, and P. F. Randerson (2002). Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles - a critique. *FEMS Microbiology Ecology 42*, 1–14.

Revsbech, N. P. (1989). An oxygen microsensor with a guard cathode. *Limnology and Oceanography 34*, 474–478.

Revsbech, N. P. and B. B. Jørgensen (1986). Microelectrodes: their use in microbial ecology. *Advances in Microbial Ecology 9*, 293–352.

Revsbech, N. P., N. Risgaard-Petersen, A. Schramm, and L. P. Nielsen (2006). Nitrogen transformations in stratified aquatic microbial ecosystems. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 90*, 361–375.

Robertson, G. P. and P. M. Groffsman (2007). *Soil Microbiology, Biochemistry, and Ecology*, Chapter Nitrogen transformations, pp. 341–364. New York: Springer.

Rönnpagel, K., W. Liß, and W. Ahlf (1995). Microbial bioassays to assess the toxicity of solid-associated contaminants. *Ecotoxicology and Environmental Safety 31*, 99–103.

Roy, R. and R. Knowles (1995). Differential inhibition by allylsulfide of nitrification and methane oxidation in fresh-water sediment. *Applied and Environmental Microbiology 61*, 4278–4283.

Rutgers, M., A. M. Breure, and H. Insam (2006). *Microbiological Methods for Assessing Soil Quality*, Chapter Substrate utilisation in Biolog plates for analysis of CLPP, pp. 212–227. Wallingford: CAB International.

Rutgers, M., J. H. Faber, J. F. Postma, and H. Eijsackers (2002). *Site-specific ecological risks: A basic approach to the function-specific assessment of soil pollution.* Wageningen: Programme Bureau Soil Research. Reports of the Programme on Integrated Soil Research. 28.

Schmitt, H., B. Martinali, P. Van Beelen, and W. Seinen (2006). On the limits of toxicant-induced tolerance testing: Cotolerance and response variation of antibiotic effects. *Environmental Toxicology and Chemistry 25*, 1961–1968.

Schöll, F. (1999). Neues Leben im Fluss? *Wasserwirtschaft Wassertechnik mit Abwassertechnik 7*, 15–18.

Schouten, T., J. Bloem, W. A. M. Didden, M. Rutgers, H. Siepel, L. Posthuma, and A. M. Breure (2003). Development of a biological indicator for soil quality. *Setac Globe*, 30–33.

Seines, M., R. Babuska, and H. B. Verbruggen (1998). Rule-based modeling: Precision and transparency. *IEEE Transactions on Systems, Man and Cybernetics Part C: Applications and Reviews 28*, 165–169.

Silvert, W. (1997). Ecological impact classification with fuzzy sets. *Ecological Modelling. 96*, 1–10.

Silvert, W. (2000). Fuzzy indices of environmental conditions. *Ecological Modelling. 130*, 111–119.

Smeets, E. and R. Weterings (1999). *Environmental indicators: Typology and overview*. Denmark: European Environmental Agency.

Stief, P., A. Schramm, D. Altmann, and D. De Beer (2003). Temporal variation of nitrification rates in experimental freshwater sediments enriched with ammonia or nitrite. *FEMS Microbiology Ecology 46*, 63–71.

Stigliani, W. M., P. Doelman, W. Salomons, R. Schulin, G. R. B. Smidt, and S. E. A. T. M. Van der Zee (1991). Chemical time bombs. predicting the unpredictable. *Environment 33*, 5, 26–9, 30.

Strauss, E. A. and G. A. Lamberti (2000). Regulation of nitrification in aquatic sediments by organic carbon. *Limnology and Oceanography 45*, 1854–1859.

Stuijfzand, S. C., M. Helms, M. H. Kraak, and W. Admiraal (2000). Interacting effects of toxicants and organic matter on the midge *Chironomus riparius* in polluted river water. *Ecotoxicology and Environmental Safety 46*, 351–356.

Suominen, K. P., C. Wittmann, M. Liukkonen, M. A. Kahkonen, and M. S. Salkinoja-Salonen (1999). Ecotoxicological assessment of a recipient lake sediment of bleached-kraft pulping discharges. *Environmental Toxicology and Chemistry 18*, 2262–2267.

Tate, III., R. L. (1977). Nitrification in histosols: a potential role for the heterotrophic nitrifier. *Applied and Environmental Microbiology 33*, 911–914.

Tiedje, J. (1987). *Biology of Anaerobic Microorganisms*, Chapter Ecology of denitrification and dissimilatory nitrate reduction to ammonium, pp. 179–244. New York: Wiley.

Truhaut, R. (1977). Ecotoxicology: objectives, principles and perspectives. *Ecotoxicology and Environmental Safety 1*, 151–173.

US EPA (1993). *Technical basis for establishing sediment quality criteria for nonionic organic contaminants for the protection ofbenthic organisms by using equilibrium partitioning.* Washington, DC.: US Environmental Protection Agency, Office of Science and Technology, Health and Ecological Criteria Devision.

Van Cappellen, P. and Y. Wang (1996). Cycling of iron and manganese in surface sediments; a general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron, and manganese. *American Journal of Science 296*, 197–243.

Van Gestel, C. A. M. (1997). *Ecological Risk Assessment of Contaminants in Soil*, Chapter 2 Scientific basis for extrapolating results from soil ecotoxicicity tests to field conditions and the use of bioassays, pp. 25–50. London: Chapman & Hall.

Vitousek, P. M., S. Hättenschwiler, L. Olander, and S. Allison (2002). Nitrogen and nature. *AMBIO: A Journal of the Human Environment 31*, 97–101.

Ward, B. B. and G. D. O'Mullan (2005). Community level analysis: Genetic and biogeochemical approaches to investigate community composition and function in aerobic ammonia oxidation. In *Environmental Microbiology*, Volume 397 of *Methods in Enzymology*, pp. 395 – 413. Academic Press.

Weissenfels, W. D., H. J. Klewer, and J. Langhoff (1992). Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. *Applied Microbiology and Biotechnology 36*, 689–696.

WFD AMPS (2004). *Discussion Document on Sediment Monitoring Guidance for the EU Water Framework Directive. Version 2.* Working group on Analysis and Monitoring of Priority Substances, Subgroup on Sediment.

Zadeh, L. A. (1965). Fuzzy sets. *Information and Control 8*, 338–353.

Zinder, S. H. and T. D. Brock (1978). Dimethyl sulfoxide as an electron acceptor for anaerobic growth. *Archives of Microbiology 116*, 35–40.

Appendix A

Supplementary Data of Carbon Substrates Utilisation

The carbon substrates utilisation fingerprint was measured using commercial BIOLOG EcoPlate containing triplicate of 31 environmentally relevant substrates in one microtiter plate. The substrates included in the EcoPlate are listed in Table A.1. The data was presented according to data extraction approaches.

Table A.1: Substrates included in EcoPlate.

Functional groups	Substrates	
Amino Acids	L-arginine	L-asparagine
	glycyl-L-glutamic acid	L-phenylalanine
	L-serine	L-threonine
Carbohydrates	D-cellobiose	i-erythritol
	D-galactonic acid-γ-lactone	N-acetyl-D-glucosamine
	glucose-1-phosphate	β-methyl-D-glucoside
	D,L-α-glycerol phosphate	α-D-lactose
	D-mannitol	D-xylose
Carboxylic Acid	γ-hydroxy-butyric acid	α-ketobutyric acid
	D-galacturonic acid	D-glucosaminic acid
	itaconic acid	D-malic acid
	pyruvic acid methyl ester	
Amines	phenylethylamine	putrescine
Phenols	2-hydroxybenzoic acid	4-hydroxybenzoic acid
Polymers	α-cyclodextrin	glycogen
	Tween 40	Tween 80

A.1 Seasonal Variations

The AWCD time curve, which illustrates the population growth of microbes utilising substrates, indicated the total respiration activity of the extracted microbes. The curve was fitted with the density dependent logistic growth equation

$$Y = AWCD = \frac{K}{1 + e^{-r(t-S)}} \tag{A.1}$$

as described in Section 4.2.1. The kinetic parameters K, r, and S indicate the carrying capacity, exponential growth rate, and lag time respectively. The lag time is in particularly negatively correlated with the initial inoculum density.

The seasonal variation of the kinetic parameters of water and sediments collected in rivers Elbe and Dommel are illustrated in Figure 4.11 and listed in Table A.2.

Table A.2: Kinetic parameters showing population growth of extracted microbial communities.

	Elbe			Dommel		
Time	Water	Upper Sed.	Deeper Sed.	Water	Upper Sed.	Deeper Sed.
	K Carrying Capacity					
Jan				1.165	1.402	1.331
Feb	1.136	1.188	0.842	1.071	1.402	1.245
Mar	1.066	1.066	0.747	1.060	1.269	0.827
Apr	1.051	1.228	0.321	1.010	0.963	0.906
May	1.375	1.214	0.170			
Jun				1.101	1.048	1.270
Jul	1.062	0.800	0.511	1.162	1.164	0.651
Sep	1.274	0.971	0.202			
	r Growth Rate					
Jan				0.090	0.110	0.123
Feb	0.077	0.106	0.125	0.068	0.110	0.106
Mar	0.127	0.127	0.115	0.088	0.077	0.074
Apr	0.045	0.097	0.165	0.075	0.106	0.107
May	0.068	0.093	0.334			
Jun				0.079	0.106	0.088
Jul	0.039	0.052	0.079	0.060	0.062	0.049
Sep	0.045	0.062	0.059			

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	Elbe			Dommel		
Time	Water	Upper Sed.	Deeper Sed.	Water	Upper Sed.	Deeper Sed.
	S Lag time					
Jan				55.83	43.72	42.99
Feb	58.88	38.53	45.30	63.50	43.72	45.20
Mar	36.62	36.62	39.30	45.02	50.71	55.40
Apr	79.41	38.90	19.19	53.90	41.18	42.56
May	58.54	39.59	6.35			
Jun				44.71	36.54	37.83
Jul	78.92	52.32	44.29	55.09	39.10	40.97
Sep	57.48	41.15	29.66			

The substrates utilisation fingerprints of the 31 substrates were further summarised with principal component analysis and the sample scores showing the substrates utilisation of the samples using the principal components as summarised parameters are illustrated in Figures 4.12 and 4.13. The substrates contributing to the principal components are further listed as factor loadings in Table A.3. Factor loadings are the correlation between substrates and the principal components. Therefore substrates showing factor loadings greater than 0.7 or less than -0.7 were considered to contribute significantly to the principal components.

Table A.3: Factor loadings of substrates on the principal components.

Substrates	WCD		K		r		S	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
pyruvic acid methyl ester	-0.45	-0.28	0.64	-0.41	**0.81**	0.09	0.33	0.49
Tween 40	**-0.71**	0.30	0.66	-0.10	**0.88**	0.41	**0.78**	0.02
Tween 80	-0.40	-0.19	0.46	0.14	**0.87**	0.42	**0.85**	-0.05
α-cyclodextrin	0.06	0.17	-0.04	0.18	0.37	-0.02	-0.07	-0.01
glycogen	-0.32	-0.55	0.62	0.21	**0.86**	0.44	**0.88**	0.11
D-cellobiose	-0.48	-0.54	**0.88**	-0.20	**0.82**	0.33	**0.85**	0.14
α-D-lactose	-0.35	-0.20						
D-fructose	-0.66	-0.50	**0.73**	-0.15	0.63	0.23	**0.86**	0.11
D-xylose	0.60	-0.11						
i-erythritol	0.22	0.73	-0.17	0.02	**0.87**	0.42	-0.24	**0.75**

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Substrates	WCD		K		r		S	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
D-mannitol	-0.33	-0.44	**0.76**	-0.16	**0.89**	0.40	**0.94**	0.11
N-acetal-D-glucosamine	-0.24	**-0.71**	**0.78**	0.05	**0.85**	0.34	**0.79**	0.24
D-glucosaminic acid	0.65	0.19	0.25	0.06	**0.80**	0.37	-0.04	**0.89**
glucose-1-phosphate	-0.41	0.33						
D,L-α-glycerol phosphate	-0.24	0.53						
D-galactonic acid-γ-lactone	0.05	-0.63	0.50	0.35	0.48	0.51	0.00	**0.84**
D-galacturonic acid	0.47	0.20	-0.05	**0.87**	0.29	**0.82**	0.33	**0.86**
2-hydroxybenzoic acid	0.15	**0.73**						
4-hydroxybenzoic acid	0.47	**-0.05**	0.42	0.64	**0.84**	0.48	0.20	**0.91**
γ-hydroxybyturic acid	**0.71**	0.28	-0.30	-0.35	0.49	0.48	-0.22	**0.90**
itaconic acid	**0.73**	-0.04	0.17	0.47	0.25	**0.85**	0.44	0.54
α-ketobutyric acid	-0.59	0.33						
D-malic acid	0.52	-0.15						
L-arginine	0.27	0.13	0.29	-0.55	0.62	0.69	0.42	0.05
L-asparagine	-0.05	0.35	-0.04	**0.75**	0.40	**0.79**	0.62	0.38
L-phenylalamine	0.22	0.62	0.28	-0.57	**0.72**	0.11	0.17	0.02
L-serine	0.06	0.27	-0.03	0.63	0.52	**0.70**	**0.89**	0.14
L-threonine	-0.23	**0.73**	-0.18	0.13	**0.85**	0.44	-0.01	-0.04
glycyl-L-glutamic acid	-0.43	0.63						
phenylethylamine	0.65	0.17						
prutescine	-0.19	-0.10	0.07	0.66	-0.12	**0.79**	0.31	0.63

A.2 Relative Structures

To show the relative structure of the microbial communities and their functional stability, BIOLOG plates were inoculated with series dilution of extracted microbes. The area under WCD time curve (Area Under Curve) was taken as an integrated parameter of the substrate utilisation. The relationship between the AUC and the log dilution factor was fitted with a log normal distribution curve

$$AUC = \frac{t}{1 + 10^{h(logCFU_{50} - logCFU)}} , \tag{A.2}$$

where $log(CFU_{50})$ indicates the dilution factor at which only half of the substrates utilisation level can be reached. By comparing $log(CFU_{50})$ of a specific substrate and that of AWCD, the relative abundance (RA) to utilise the specific substrates is calculated as

$$RA = log\frac{CFU_{50_s}}{CFU_{50_{average}}} \tag{A.3}$$

and illustrated in Figures A.1, A.2, A.3.

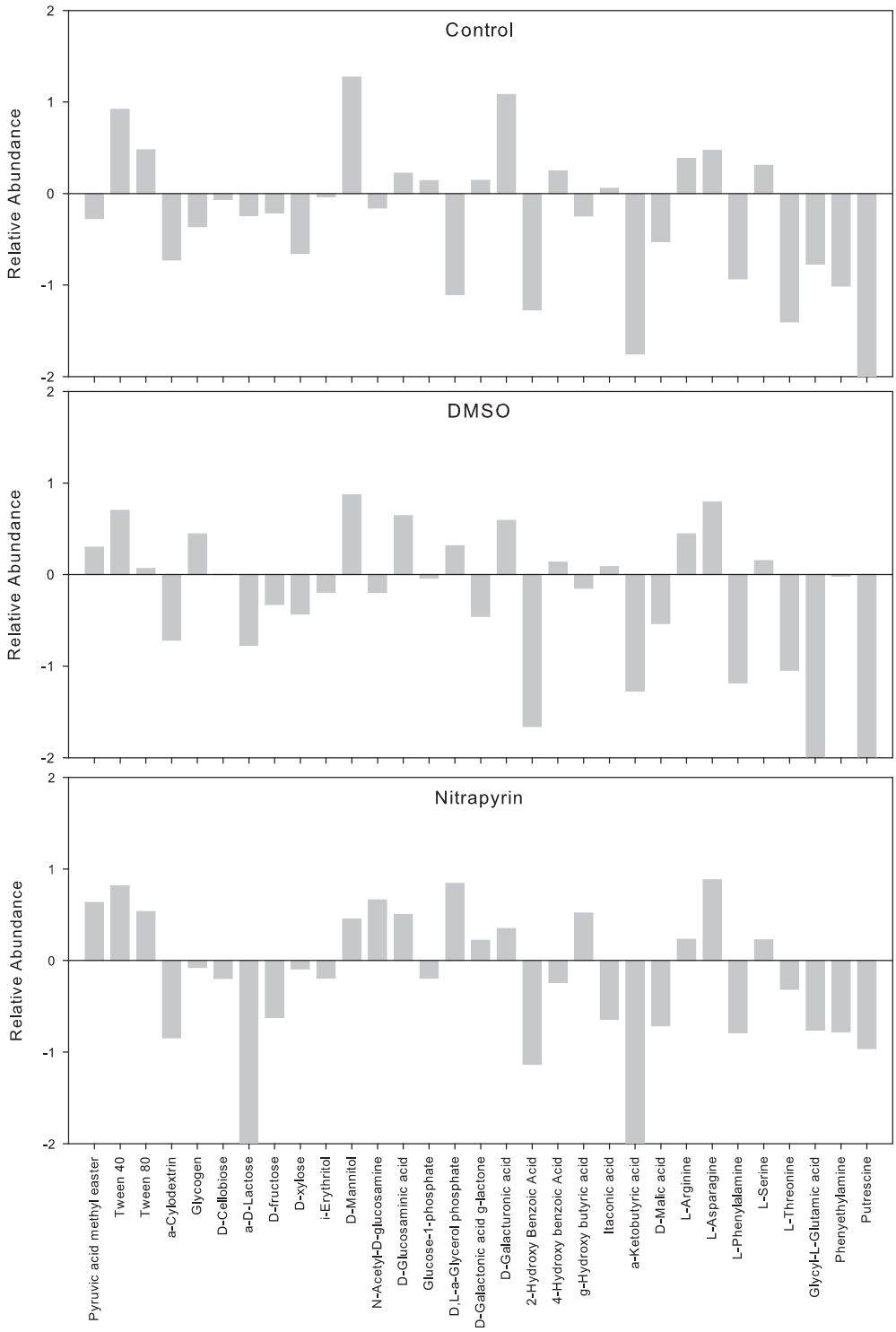

Figure A.1: Relative Abundance of microbial communities extracted from fresh sediments, fresh sediments amended with DMSO, and nitrapyrin.

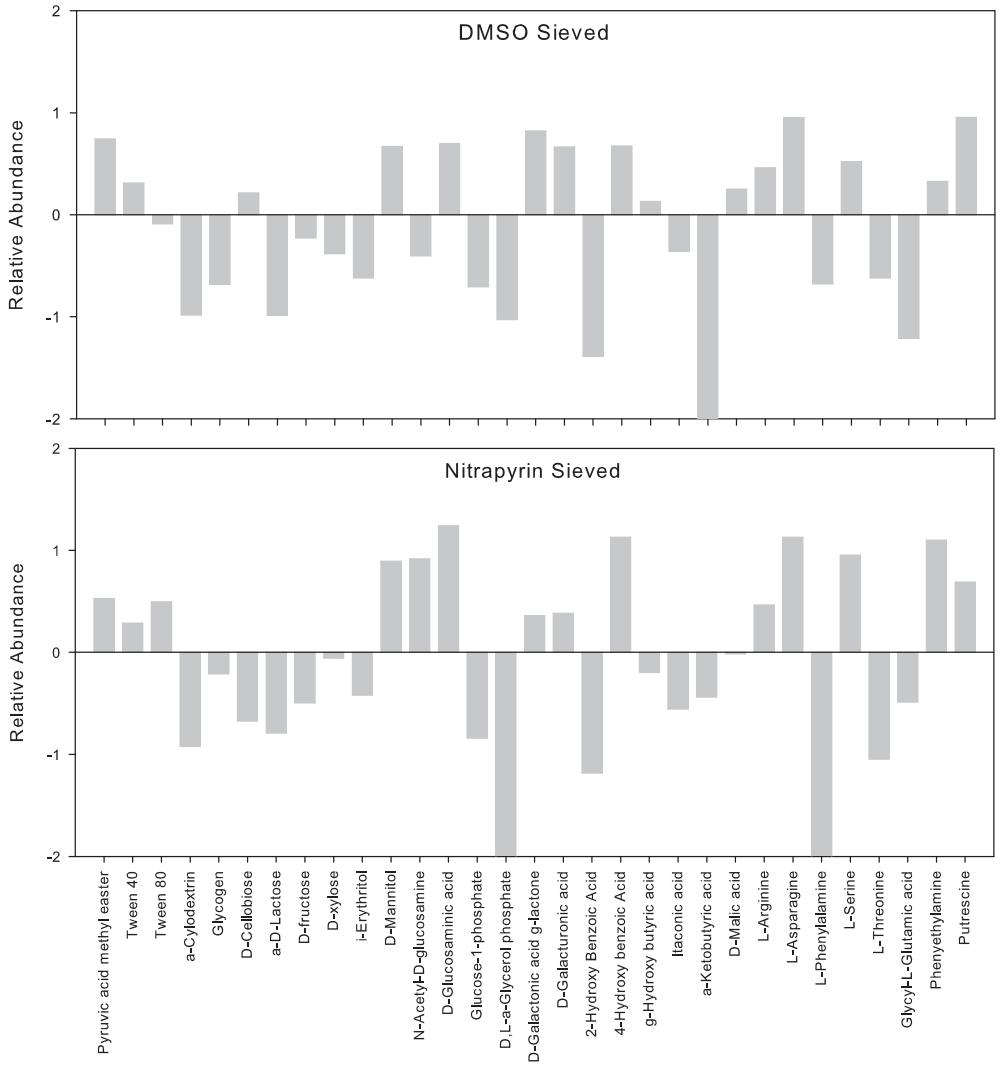

Figure A.2: Relative Abundance of microbial communities extracted from sediments amended with DMSO and nitrapyrin after sieving and incubation at 15℃ for two weeks.

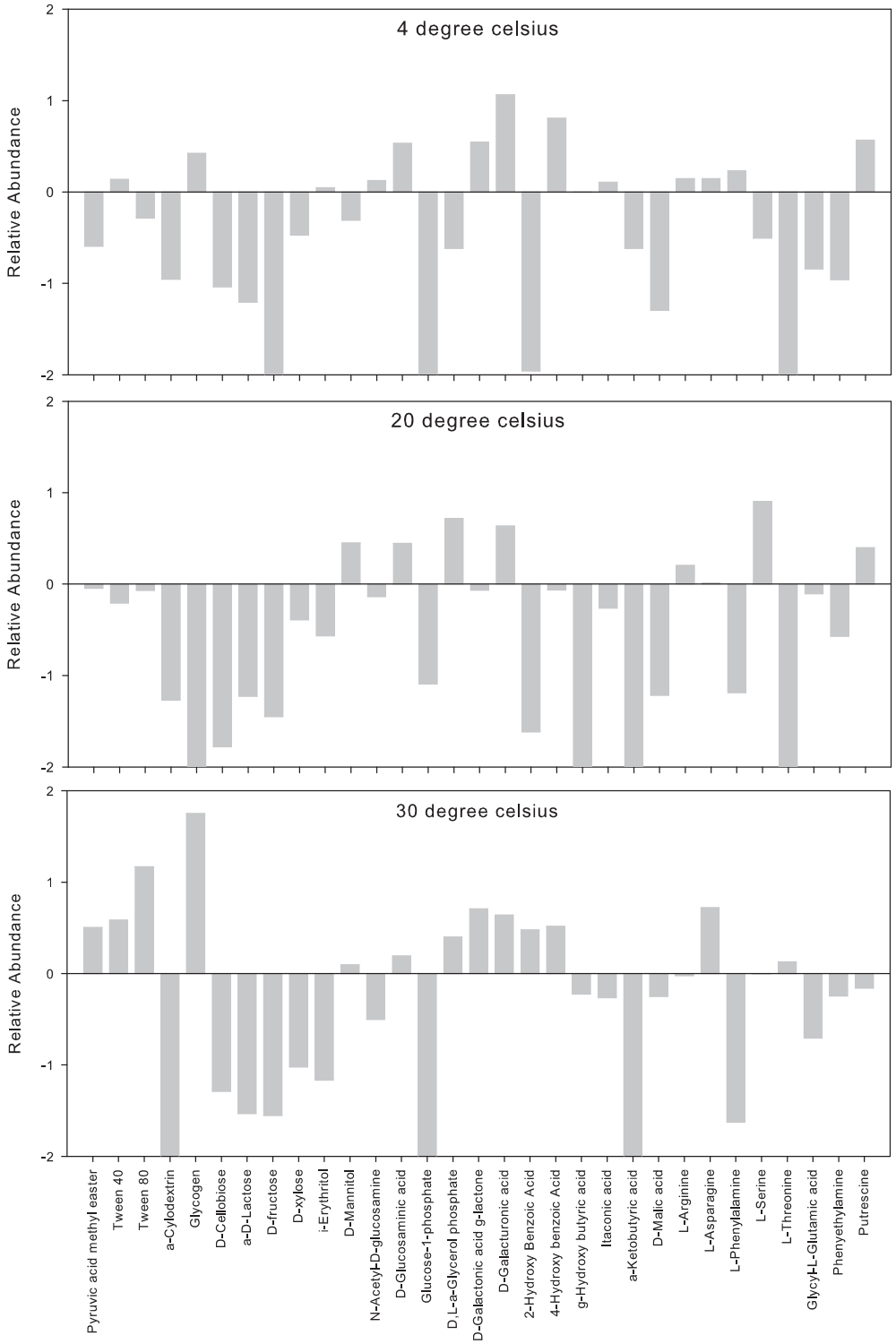

Figure A.3: Relative Abundance of microbial communities extracted from aged and resuspended sediments at 4℃, 20℃, and 30℃.

Curriculum Vitae

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1985 - 1991 Tien-Mu Elementary School, Taipei.

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1997 - 2001 National Taiwan University, Taipei.
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