Environmental filtering of bacteria in low productivity habitats

INGA RICHERT
Microbes fulfill important ecosystem functions by contributing as drivers of global nutrient cycles. Their distribution patterns are mainly controlled by environmental heterogeneities. So far, little is known about the mode of action of particular environmental drivers on the microbiota, particularly in low productivity habitats.

The aim of this thesis was to investigate the relationships between local environmental drivers and the microbial responses at the level of communities, individuals and realized function, using three structurally different model habitats sharing the feature of overall low productivity. Using a hypothesis-based approach and extensive 16S rRNA amplicon mapping of bacterioplankton colonizing the polar Southern Ocean, I identified how the seasonal formation of open-water polynyas and coupled phytoplankton production affected the diversity of surface bacterial communities and resulted in a cascading effect influencing the underlying dark polar water masses. Additional laboratory experiments, with cultures exposed to light, resulted in reduction in alpha diversity and promoted opportunistic populations with most bacterial populations thriving in the cultures typically reflected the dominants in situ.

Furthermore it was experimentally tested how induced cyclic water table fluctuations shaping environmental heterogeneity in a constructed wetland on temporal scale, by directly affecting redox conditions. Twelve months of water table fluctuations resulted in enhanced microbial biomass, however a shift in community composition did not lead to a significant increase in pollutant removal efficiency when compared to a static control wetland. I detected phyla that have previously been proposed as key players in anaerobic benzene break-down using a protocol that was developed for single cell activity screening using isotope-substrate uptake and microautoradiography combined with taxonomic identification based on fluorescent in situ hybridization targeting the 16S rRNA. Eventually, I provide an example of how anthropogenic pollution with polyaromatic hydrocarbons induced a strong environmental filtering on intrinsic microbial communities in lake sediments.

In conclusion, my studies reveal that microorganisms residing in low productivity habitats are greatly influenced by environmental heterogeneity across both spatial and temporal scales. However, such variation in community composition or overall abundance does not always translate to altered community function.

Keywords: bacteria, environmental filtering, diversity, ecosystem service, hydrocarbon utilization, Southern Ocean, sediment

Inga Richert, , Department of Ecology and Genetics, Limnology, Norbyvägen 18, Uppsala University, SE-75236 Uppsala, Sweden.

© Inga Richert 2014

ISSN 1651-6214
ISBN 978-91-554-8986-1
urn:nbn:se:uu:diva-229144 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-229144)
To my family
Supervisors
Dr. Annelie Wendeberg
Department Environmental Microbiology
Centre for Environmental Research
Leipzig, Germany

Prof. Stefan Bertilsson
Limnology Department
Evolutionary Biology Centre
Executive Manager, SciLifeLab
Uppsala, Sweden

Opponent
Prof. Lise Øvreas
Marine Microbiology
Department of Biology
University of Bergen
Bergen, Norway
List of Manuscripts

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


List of additional Manuscripts

Contents

Introduction .................................................................................................................. 11
   The microbial landscape...................................................................................... 11
   Microorganisms in focus.................................................................................... 11
   Environmental heterogeneity ........................................................................... 12
   Identifying the functional role of specific microorganisms......................... 13
   Biogeochemical relevance of bacteria........................................................... 15
   Nutrient turnover and transformation ............................................................. 15
   Microbes involved in carbon re-cycling ......................................................... 15
   Microbial model systems.................................................................................. 16
   The Southern Ocean Polynyas: the window of opportunity ......................... 16
   Contaminated wetland sediment: coping with hydrodynamics..................... 17
   Polluted freshwater sediments: bridging the redox gradient....................... 18

Objectives of the thesis .......................................................................................... 19

Methods ...................................................................................................................... 20
   Sampling and incubation.................................................................................... 20
   Environmental characterization ........................................................................ 20
   Molecular analysis of bacterial communities and populations................. 20
   Bacterial abundance and biomass .................................................................... 22
   Tracer experiments.............................................................................................. 22
   Statistical approaches......................................................................................... 22

Results & Discussion ............................................................................................... 23

Summary in Swedish................................................................................................. 30

Zusammenfassung in Deutsch.................................................................................... 33

Acknowledgments .................................................................................................... 35

References .................................................................................................................. 39
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>Amundsen Sea Polynya</td>
</tr>
<tr>
<td>BCC</td>
<td>Bacterial Community Composition</td>
</tr>
<tr>
<td>BTEX</td>
<td>Benzene, Toluene, Ethylbenzene, Xylene</td>
</tr>
<tr>
<td>CARD FISH</td>
<td>Catalyzed Reporter Deposition Fluorescence <em>in Situ</em> Hybridization</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved Organic Matter</td>
</tr>
<tr>
<td>MAR</td>
<td>Microautoradiography</td>
</tr>
<tr>
<td>MTBE</td>
<td>Methyl <em>tert</em>-butyl ether</td>
</tr>
<tr>
<td>OTU</td>
<td>Operational Taxonomic Unit</td>
</tr>
<tr>
<td>PAH</td>
<td>Polyaromatic Hydrocarbons</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>Bacterial Small Subunit Ribosomal Ribonucleic Acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1. Scheme on the interplay between environmental heterogeneity and microbial communities.........................................................24

Figure 2. Relationship between BCC in the Amundsen Sea Polynya (ASP) of different depth classes and apparent environmenta gradients.................26
Introduction

The microbial landscape

Microorganisms in focus

Microorganisms, which are for the most part not visible for the human eye, shape the world surrounding us. They make up an immense amount of biomass, ubiquitously colonizing all biomes of Earth and account for half of the overall biotic respiration (Pomeroy, 1974). With their ecological role as decomposers, microorganisms are driving the global nutrient cycles by channeling the nutrient fluxes due to their metabolic activity contributing essential to the overall ecosystem functioning (Arrigo, 2005; Falkowski et al., 2008).

Their cumulative gene pool for protein and enzyme encoding genes of the active and dormant members of natural microbial communities represent a comprehensive library of possible metabolic traits (Philippot et al., 2010; Martiny et al., 2012). For instance, microbes harbor a tremendous potential for the transformation and degradation of recalcitrant organic matter and xenobiotics. Thus, microbes provide essential ecosystem service (Costanza et al., 1997). These small and single-celled organisms reproduce asexually by binary fission and can exchange genes - even across phyla - by lateral gene transfer or homologous recombination, for example to acquire antibiotic resistance genes (Ochman et al., 2000) for genes for specific metabolic processes (Lorenz and Wackernagel, 1994; Fraser et al., 2007). As part of the food web, microbes contribute to complex interactions between nutrient sources, viruses, grazers, phytoplankton and organism of higher trophic levels (Azam et al., 1983).

With their relatively short generation time (as short as 10 minutes), microbes are ideal model organisms to experimentally study general ecological mechanisms and processes. The development of cultivation-independent molecular methods during the last decades has opened the door to a yet unknown world, allowing us to shed light into the “functional black box” - as microorganisms have been referred to in the past (Ogilvie and Hirsch, 2012).
Box 1: Species concept in Microbial Ecology

The classical species concept of macroecology is hardly applicable to microbial ecology (Ward, 1998; Cohan, 2002; Kämpfer and Glaeser, 2012; Rosselló-Móra, 2012). Species are defined as discrete populations that share ecological and phenotypic properties, are genetically similar by having a common ancestor and that can interbreed (Mayr, 1942; De Queiroz, 2007). In the microbial world, features such as asexual reproduction and lateral gene transfer interfere with this concept. Often, closely related populations – as based on bacterial small subunit ribosomal ribonucleic acid (16S rRNA) sequences - do not necessarily share phenotypic and ecological properties. Microbial relatedness is often classified based on sequence similarities of the 16S rRNA gene, and sequence similarities of greater than 97% commonly defines an operational taxonomic unit (OTU) (Handelsman, 2004).

Environmental heterogeneity

Most of the World’s ecosystems are highly complex and composed of spatial and temporal variations in environmental conditions caused by abiotic factors (e.g. nutrient availability, physical and chemical environmental conditions and climate) and biotic factors (e.g. predation, intra- and interspecies interactions) (Billen et al., 1988; Pernthaler, 2005). In natural systems, these factors always co-occur in immense variation and consequently influence the amount of habitat types and the available niche space by forming environmental gradients in time and space (Stein et al., 2014), consequently causing filtering processes (or species sorting according to (Van der Gucht et al., 2007) that act on all organisms living within this ecosystem. The heterogeneity allows more species to coexist and results in spatial distribution patterns, from a micrometer to a kilometer scale (microbial biogeography, Martiny et al., 2006; Nemergut et al., 2011; Ghiglione et al., 2012). Research in aquatic microbiology has focused on how environmental factors drive variation in diversity in composition (Lindström and Langenheder, 2012; Székely et al., 2013) and phenotypes (Comte and Giorgio, 2013), as well as evolution (Kassen, 2002).
Box 2: Important community assembly mechanisms

The metacommunity concept for instance, integrates processes that act on the community assembly (Leibold et al., 2004). Here, additional to environmental filtering (also referred to as species sorting) created by environmental heterogeneity in biotic and abiotic factors, the metacommunity concept also includes neutral processes, which involves stochastic processes resulting in a random distribution of organisms (Woodcock et al., 2007) as well as dispersal-driven mass effects and the maintenance of diversity in refuges driven by patch dynamics (Bahlo et al., 2012). Historical factors such as dispersal limitation, past environmental conditions and evolution (Martiny et al., 2006), also have a major impact on the contemporary community composition. The communities’ abilities to respond to disturbances of all kinds, involves mechanisms regarding resilience, resistance and redundancy in the assembly process (Allison and Martiny, 2008).

Identifying the functional role of specific microorganisms

Ecosystem functioning and microbial diversity are coupled, though the degree of this connectedness depends very much on the microbial community at hand, the phylogenetic level used to characterize the communities, and the functional and metabolic traits considered. Diversity most often has a positive effect on the stability of ecosystem functioning (Cardinale et al., 2006). High diversity may maintain functionality in case of biodiversity loss, but can also enhance overall functions because of complementarity of functions stored in the genomic pool (Loreau and Hector, 2001; Allison and Martiny, 2008). A major goal in contemporary microbiology is the ability to link a genotype appearing in a natural community to a specific metabolic potential as well as the contemporary functional role it might occupy in the ecosystem. Such information will eventually enable accurate predictions of the performance with regards to processes such as nutrient cycling in a broad range of habitats based on knowledge of the combined biotic and abiotic factors and microbial community structure.

One approach to address this is by assigning taxa (species concept in microbiology see box 1) to functional guilds, defined as groups of phylogenetic independent taxa, which exploit the same resources under similar abiotic conditions, and consequently share broader ecological niches (Simberloff and Dayan, 1991). In microbiology, functional guilds are often related to the appearance of local redox condition that favor a certain type of energy metabolism, such as oxic, denitrifying, sulfate reducing, methanogenic conditions (Megonigal et al., 2003; Schink, 2006) but can also be related to specific carbon pools (Sinsabaugh and Foreman, 2003).
Individual microbial populations express different degrees of functional plasticity, i.e. the ability to adapt to changing environmental conditions (Comte and Giorgio, 2013). A possible way to systematically describe this in the context of a largely heterogeneous environment is to categorize the plasticity of individual populations into metabolic types related to the degree of heterogeneity in the ecosystem. Roughly, microbes can be characterized as either generalists or specialists (Kassen, 2002). This concept implies that the ecological niche of a genotype evolves depending on the degree of environmental heterogeneity, with highly heterogeneous habitats selecting for generalists that exhibit a wide niche, and more homogeneous habitats selecting for specialists with a narrower niche.

Another differentiation that can be made is related to affinity for substrates. Here, organisms can be classified as either copiotrophs or oligotrophs. While copiotrophs are specialized towards high nutrient availability in combination with high maximum substrate and population turnover rates, oligotrophs are adapted to nutrient limited conditions and exhibit low maximum turnover rates (Klappenbach et al., 2000; Lauro et al., 2009).

$K$- and $r$-strategists differ by their maximum specific growth rate and their substrate utilization efficiency. While $r$-strategists grow rapidly on easily available substrates, the $K$-strategists exhibit low maximum growth rates and use the resources slowly but more efficiently, even in the absence of limitation (Pianka, 1970; Andrews and Harris, 1986). Some bacterioplankton phyla are hypothesized to feature a reduced and streamlined genome size due to natural selection processes, which eventually reduce metabolic cost of DNA synthesis. The alphaproteobacteria Pelagibacter is for instance described as a streamlined genotype (Giovannoni et al., 2014).
Biogeochemical relevance of bacteria

Nutrient turnover and transformation

Because of their interactions with the environment in combination with their high biomass, microbes contribute as an important controlling factor for global nutrient fluxes. Microbial cells consist of macromolecules such as nucleotides, proteins or enzymes, which in turn consist of the major elements carbon, nitrogen and phosphorus - all acquired from the external environment. The cycling of matter resulting from this coupling between microbes and their abiotic environment has been widely studied in marine (Azam et al., 1994; Azam, 1998; Azam and Malfatti, 2007), freshwater (Vadstein et all 2000), and terrestrial (Swift et al., 1998) environments.

Microbes involved in carbon re-cycling

Microbes mediate the major fluxes between different carbon reservoirs consisting of different species of organic and inorganic carbon. From a biogeochemical perspective, microbes remineralize organic carbon to more oxidized intermediates or all the way to CO$_2$. Microbial dissolved organic matter (DOM) consumption accounts for half of the total global respiration (Pomeroy, 1974), which emphasizes the importance of their role as decomposers. Microbial respiration is also coupled to net primary productivity. A regression analysis across biomes showed a positive correlation between respiration rate and net primary productivity (Kirchman, 2011). The carbon and energy of microbial biomass is also transferred into higher trophic levels within the microbial food web (Azam et al., 1983, also referred to as the microbial loop), largely via the grazing activity of small protozoans.

Degradation and re-mineralization rates vary between habitats and depend on available carbon sources and energetic constraints in a given local environment. The carbon pool usually consists of a mix of labile carbon, such as polysaccharides, amino acids and small organic acids, as well as recalcitrant carbon sources, such as lignin, humic acids, and sometimes anthropogenic introduced hydrocarbons from oil production or chemical industry (see box 3). (Thurman, 1985; Sinsabaugh and Foreman, 2003)

A natural microbial community consists of various co-existing metabolic types that prefer different types of carbon sources (Peter et al., 2011). Due to their higher bioavailability, labile carbon sources are mostly favored over recalcitrant carbon in microbial utilization (Sinsabaugh and Foreman, 2003) and have been suggested to support the mineralization of recalcitrant carbon in a process called "priming" (Guenet et al., 2010).

Microbial carbon utilization is energetically constrained by local redox conditions, in particular by the availability of oxygen. Microbial respiration
with oxygen as the terminal electron acceptor facilitates the energy-demanding break-down of large organic molecules. Oxygen is a rate-limiting factor of hydrocarbon break-down, as aerobically acting enzymes are involved, as has been show for the utilization of alkanes and aromatic compounds (Megonigal et al., 2003).

### Box 3: Ecosystem Service

Marine and freshwater ecosystems are critical for the sustainability of life on earth (Arrigo, 2005; Williamson et al., 2008). To find strong arguments in protecting and maintaining natural ecosystems, efforts have been made in categorizing the world’s ecosystem services and it’s economical benefits for humankind (Costanza et al., 1997). The intrinsic capacity of microbes to transform and break anthropogenic pollutants is an important ecosystem service, which receives attention in chapter III and IV. Other ecosystem services include climate regulation, disturbance regulation, refugia, food production, water supply, and pollination.

### Microbial model systems

Microbes face very complex combinations of factors controlling their community composition and functioning. Three very different ecosystems are presented as model systems to illustrate how environmental heterogeneity shape the microbial community in composition and activity and how different environmental forces can act. All three sites share the characteristic of relatively low microbial turnover and productivity.

### The Southern Ocean Polynyas: the window of opportunity

Prokaryotic life in the worlds oceans consist of either planktonic forms inhabiting the pelagic water column and scavenging dissolved nutrients in the sea (Pernthaler and Amann, 2005) or dense biofilms on suspended detritus particles (Azam et al., 1994). With their large pelagic water bodies, the base of the food web of oceanic ecosystems is largely internally produced organic matter from primary production. Any other nutrient sources from continental sediment run-off or from atmospheric deposition may locally promote nutrient supply (Liu et al., 2010). Even though independent from their source, the spatial distribution of organic and inorganic nutrients creates gradients on a horizontal and vertical scale. Currents create a patchy habitats structure mostly based on gradients in temperatures and salinity (Galand et al., 2010).
Polar marine systems, such as the Southern Ocean, provide an extraordinary habitat for microorganisms (Ducklow and Yager, 2007; Convey et al., 2014). Due to the constant cold, the Southern Ocean harbors psychrophilic microbial communities with low turnover rates that are adapted to the low temperatures and which have their optimum growth temperature at around zero degrees Celsius (Rivkin et al., 1996). Organisms that colonize the Southern Ocean are confronted with partial permanent ice cover that disconnects and isolates the sea from the atmosphere. Due to long periods of darkness, sunlight-driven primary productivity and overall ecosystem productivity are low.

However, during austral summers - characterized by long day light hours - parts of the Southern Ocean are freed of the ice cover and so-called polynyas – ice-free lagoons - are formed (Smith et al., 1990). Then, the once-dark sea is exposed to sunlight and nutrients from melting pack ice, triggering an increasing productivity across all trophic levels. This re-occurring seasonal event may have a huge impact on the prokaryotic community.

Contaminated wetland sediment: coping with hydrodynamics

Wetlands bridge terrestrial with aquatic ecosystems, connecting groundwater transported through aquifers with the open water of lakes, ponds or rivers. In contrast to marine habitats, wetlands lack the extensive open water phase and provide abundant solid surfaces for growth of dense microbial biofilms. Abiotic factors that influence the wetland ecosystem are related to sediment structure, redox condition in combination oxygen supply and hydrodynamics. Wetlands experience changes in flow dynamics and water saturation that can lead to regular temporal changes in redox conditions, oxygen supply and distribution of nutrients (Odum et al., 1995; Williams and Oostrom, 2000). Generally, dissolved nutrients are transported with, and hence constantly provided by, the water flow. Sediment particles adsorb and release a large fraction of organic and inorganic nutrients, leading to a constant and slow supply of compounds to the water phase.

Anaerobic processes are often dominant in wetland systems and control turnover of organic matter, even though the appearance of rooted vascular plants can oxygenize deeper sediment horizons (Williams, 2002). In contrast to pelagic environmental gradients relevant for effecting microbial diversity and activity at the scale of cm to km in pelagic water, these gradients occur at mm to µm scale in wetlands. Due to these heterogeneous microhabitats, different functional guilds of microbes (such as microaerophiles, methanogens, sulfur reducers, iron reducers or fermenters) co-exist at a very small spatial scale.

From a global perspective, this combination of abiotic and biotic characteristics contribute largely to the turnover of organic matter (Odum et al.,
Microbes colonizing wetland sediments have an enormous capacity to transform and degrade pollutants, such as petroleum hydrocarbons, chlorinated hydrocarbons or pharmaceuticals, hence providing an important ecosystem service (see box 3). Accordingly, artificial wetlands are constructed to actively remove pollutants (Kuschk et al., 2012).

Polluted freshwater sediments: bridging the redox gradient

Sediments of freshwater lakes and rivers often exhibit great biological activity and biomass because of nutrients and organic substrates delivered by sedimenting particles from the overlying water. In contrast to wetlands with dynamic flow regimes that create heterogeneity on a temporal scale, lake sediments seem to be more stable over time, but feature predictable and strong gradients in oxygen and redox across depth profiles. If not disturbed by biotic perturbation, these gradients can typically be observed at the scale of a few millimeters where narrow horizons of fully oxidized and more reduced sediment layers are connected by a diffusive boundary layer (Brune et al., 2000). This depth stratification is controlled by the amount of oxygen and other oxidized compounds diffusing into deeper sediment layers and the rate at which these electron acceptors are being consumed for respiration. The diffusive boundary layer facilitates a direct exchange of oxidized and reduced compounds supporting a close association of different functional guilds that can profit from an exchange of metabolites. One particular example of such links are microaerophilic bacteria that oxidize ammonia (NH$_4^+$) to nitrate (NO$_3^-$), and obligate anaerobes that reduce nitrate to gaseous nitrogen by denitrification (Brune et al., 2000). Other examples include redox pairs of, for instance, oxidized and reduced sulfur, iron, manganese or organic compounds that are metabolized by microbes.

The steepness of the gradient is controlled by the availability of organic matter and nutrients. In contrast to marine systems with a major fraction of internally produced organic matter from primary production, lakes receive a lot of allochthonous organic material from the surrounding catchment area. High concentrations of organic matter in the sediment result in high respiration rates due to biotic and abiotic decomposition processes and hence lead to high rates of oxygen consumption. Our model lake habitat in particular is affected by high loads of recalcitrant polycyclic aromatic hydrocarbons from industrial tar production. Here, high loads of organic matter persist in the sediment, forming steep interfaces as described above.
Objectives of the thesis

The different chapters of this thesis address the relationship between local environmental drivers and the response of microbes on the levels of community, population, and specific metabolic traits. Using three structurally different model biomes to investigate such relationships in particular low productivity ecosystems, this work sheds light on the complex process of bacterial community formation and population dynamics, and resolves putative filtering mechanisms acting within contrasting habitats where microorganisms face a broad range of limitations and disturbances.

Chapters I and II address bacterioplankton in a polar marine habitat located in the Southern Ocean. The studies use both, an observational field survey (chapter I) and controlled incubation experiments (chapter II). Chapter III and IV addresses factors that induce patchiness and spatial as well as temporal community and population dynamics of microbial communities colonizing habitats with high petroleum hydrocarbon loads, but also the ecosystem services that these microbes provide by removing these petroleum hydrocarbons. The following main questions are addressed:

- **Chapter I** What are the most important environmental drivers causing the habitat heterogeneity in the Amundsen Sea Polynya (ASP) and how do they affect changes in diversity on the community level?

- **Chapter II** What is the effect of light, grazing pressure, and primary production on the bacterioplankton community as a whole, on selected populations, and cell proliferation rates?

- **Chapter III** How does the microbial community in a constructed wetland respond to water table fluctuations and how do such fluctuations affect individual and community benzene removal efficiency?

- **Chapter IV** What impact do high loads of polyaromatic hydrocarbons have on bacterial communities?
Methods

Sampling and incubation
Three geographically different habitats were sampled for the purpose of conducting either field surveys or incubation experiments or both: (i) the marine ASP, located in the Southern Ocean, (ii) a constructed wetland in Leuna, Germany, which was receiving the water from a benzene, toluene, ethylbenzene and xylene (BTEX) and Methyl tert-butyl ether (MTBE) contaminated groundwater aquifer and (iii) tar contaminated lake sediment in Grötingen of Jämtland in Central Sweden.

Environmental characterization
For each habitat, abiotic factors known to be influential and identified as potentially critical for the microbiota were traced. The concentrations of organic and inorganic compounds dissolved in the water were measured analytically.

Molecular analysis of bacterial communities and populations
To identify the diversity and composition of bacteria on the community and population level, cultivation-independent approaches were applied that target the 16S rRNA gene. Ribosomes have a highly conserved molecule structure and – as a result of being essential for protein synthesis - occur in high numbers in every cell, with approximately $10^2$ - $10^5$ molecules per microbial cell (Olsen et al., 1986). During the recent years, especially for the small subunit rRNA as a phylogenetic marker, comprehensive published databases have been established and made available, providing a vast comprehension on bacterial phylotypes inhabiting the various worldwide habitats (Maidak et al., 1997; DeSantis et al., 2006; Quast et al., 2013) and which provide reference data bases to previously undescribed habitats.

Barcoded amplicon sequencing
To study the overall bacterial community structure in environmental samples, 454 pyrosequencing (Margulies et al., 2005; Sogin et al., 2006; Andersson et al., 2008) and paired end pyrosequencing using the Illumina MiSeq technology (Bartram et al., 2011; Caporaso et al., 2012; Degnan and Och-
man, 2012) were used to sequence variable regions of the 16S rRNA gene (Herlemann et al., 2011). Such high throughput sequencing combined with the application of uniquely barcoded primers allowed parallel sequencing of multiple samples simultaneously with high sequencing accuracy and high sequencing depth for each of the analyzed samples.

**Whole genome sequencing**

In chapter IV taxonomic and functional identification was additionally done based on whole genome sequencing with Illumina HiSeq from DNA extracts, which was aligned and annotated using the automated metagenome analysis server MG Rast (Meyer et al., 2008).

**Single cell detection by CARD-FISH**

CARD FISH (catalyzed reporter deposition fluorescence in situ hybridization) was applied for identifying bacteria at the single-cell level, by targeting specific sections on the ribosomal ribonucleic acid (RNA) using labeled oligonucleotide probes. The main advantage of direct cell identification - compared to PCR based methods - is that the cell morphology and often, physical cell-cell associations remain preserved throughout the analytical procedure. As no PCR amplification step is required, biases introduced by PCR are avoided. The method is established and commonly applied for all kinds of habitats, including marine (Alonso-Sáez et al., 2008), freshwater (Sekar et al., 2003) and soil ecosystems (Pernthaler et al., 2002) and still undergoes constant development (Amann and Fuchs, 2008).

Even the FISH technique with the highest sensitivity to date, (CARD FISH) require sufficient rRNA copies in the target cells. The rRNA content of microbial cells is somewhat related to growth-activity simply because ribosomes are responsible for protein biosynthesis. Active cells with high turnover rates are thus more readily stained using FISH and CARD FISH than cells with low metabolic activity. However, ribosomes also act as C-, N-, and P-storage molecules (DeLong et al., 1989) and in that case an inactive cell will appear “active.” Yet, hybridization-positive cells in habitats with low community turnover, such as the open ocean, remain difficult to study in this way because of the overall low cellular rRNA copy numbers. Another hindrance is the still incomplete set of probes to target specific taxa of interest. In chapter III, a protocol and a probe set was applied that was only recently established and adapted for bacterial communities colonizing BTEX contaminated aquifer sediment (Tischer et al., 2012). When combining CARD FISH with the incubation and detection of tracer molecules (see below), functional consortia and guilds could be identified, which is of great interest for reconstructing the connection between microbial diversity and function.
Bacterial abundance and biomass

The bacterial abundance was measured by either flow cytometry (chapter I and II) or by automated image analysis (chapter III).

Tracer experiments

Rates of bulk hydrocarbon-uptake of the microbial community were measured using short term incubations of sediment samples with radio-labeled substrates (chapter III: $^{14}$C-benzene) followed by scintillation counting. Single-cell $^{14}$C-benzene uptake was visualized by microautoradiography (MAR) coupled to CARD FISH (Amann and Fuchs, 2008). This technique allows the identification of populations that incorporate select radio-labeled substrates into their biomass. To quantify cell proliferation rates of populations of interest, we pulsed the microbial community with the thymidine analogue bromodeoxyuridine (BrdU), which is readily incorporated into newly synthesized DNA. The tracer can then be detected immunochemically on the single-cell level (Pernthaler and Pernthaler, 2005) (chapter II).

Statistical approaches

Compositional and functional changes detected from 16S gene and metagenome based phylogenetic identification were specifically addressed by estimating the local diversity (alpha diversity) and the turnover or variation between communities from separate temporal or spatial locations (beta diversity) (Whittaker, 1972; Hill et al., 2003; Legendre et al., 2005; Anderson et al., 2011) and assessing the response of these diversity features to environmental changes using linear regression (chapter I). Ordination of a multivariate dataset was furthermore applied to explore the shift in bacterial community composition (BCC) in the environment (chapter I, III and IV) and for identifying group-specific beta dispersal, which represent a metric of beta diversity on homogeneity of bacterial communities (chapter I).
Results & Discussion

The three system studied here, differ in their scales, location, and fundamental characteristics shaping BCC in composition, metabolic response, and performance (illustrated in Figure 1).

Low productivity habitats
Environmental filtering along gradients of spatial and temporal abiotic factors shape the composition, interaction and metabolic activity of bacteria worldwide. Often, microbial productivity appears to be constraint due to particular local conditions, resulting in low turnover rates of days or even months. Microbes, and in particular bacteria, respond sensitively to environmental changes even in habitats with low turnover rates.

Amundsen Sea Polynya
The Southern Ocean is such a low turnover rate environment. Microcosm experiments with the natural bacterioplankton community at in situ conditions barely resulted in one complete doubling of microbial cells after one week of incubation (chapter II). These characteristics are result of the environmental features bacterioplankton has to face in the polar sea (Kirchman et al., 2009), characterized by a combination of very low water temperature and extended periods of limited light, nutrients, carbon sources, and trace elements.

Constructed wetland sediment
Clear signs of low-productivity anoxic sediment were also found in the constructed wetlands (chapter III), such as low biomass, low CARD FISH detection rates, and hardly any detectable biotic utilization of benzene. It remains methodological difficult to assess at the single-cell level a physiological trait such as benzene utilization. In consequence, scientists believed for a long time that microbial break-down of recalcitrant carbon under anoxic conditions does not exist (Atlas, 1981; Lovley, 1997).
Community level

Environmental forces evidently control the abundances of microbial populations, resulting in spatial diversity pattern due to either suppression, enhancement, removal or replacement of particular taxonomic groups (Fierer and Jackson, 2006; Martiny et al., 2006; Nemergut et al., 2011; Logares et al., 2013).

Amundsen Sea Polynya

Numerous environmental gradients were identified within the Amundsen Sea Polynya that act on bacterial alpha diversity and cause spatial variation within the bacterial community (chapter I). Here, the bacterioplankton community structure shifted relative to phytoplankton biomass, biotic and abiotic nutrients concentration, and gradients of temperature and salinity (Figure 2). Some factors are tightly correlated and co-vary, such as light, phytoplankton biomass and ice cover. By assessing the strength of seasonal processes in the surface water of the polynya, which were related to ice retreat, increased irradiance and a phytoplankton bloom which also increased the beta diversity across the sampling sites at the level of communities and populations across the polynya (chapter I and II). I found that processes acting within the photic surface were related to the level of phytoplankton biomass which caused a strong filtering effect, lowering bacterioplankton community richness while bacterioplankton abundance and phytoplankton biomass increased. This is in agreement with findings from enrichment cultures, which were exposed to light. Already solar radiation alone caused a

Figure 1. Scheme on the interplay between environmental heterogeneity and microbial communities.
reduction in alpha diversity and the promotion of opportunistic populations. This effect was seen in all experimental microcosms regardless of inoculum (chapter II).

The low species richness in the emerging communities reduces the gene pool in these regions, implying an important role of dispersal from underlying water masses, the melting sea ice or other dispersal routes (Székely et al., 2013). From these experiments I conclude that the community in the photic zone represents a seasonally reformed subset of communities residing in the underlying dark water masses.

The high phytoplankton biomass in the surface water represents an important pool of organic matter and inorganic nutrients, fueling the underlying dark water with nutrients in a cascading effect. In contrast to the community response in shallower water, the bacterioplankton community at the bottom of the phytoplankton biomass increased in diversity as phytoplankton biomass in overlying waters increased, while deeper waters remained largely unaffected.

These combined results point to the close connectivity between the different water masses starting from processes affecting the surface water by the seasonally reoccurring ice retreat combined with long day light, nutrient supply from the melting ice and phytoplankton bloom, which connects the surface water with the deeper dark water by sedimentation of detritus, fueling the depth with nutrients.

The high phytoplankton biomass in the photic surface represents an important pool of organic matter and inorganic nutrients, fueling the underlying dark water with nutrients in a cascading effect; In contrast to the community response in shallower water, the bacterioplankton community at the bottom of the phytoplankton biomass increased in diversity as phytoplankton biomass in overlying waters increased, while deeper waters remained largely unaffected.

These results in combination point to the close connectivity between the different water masses starting from processes affecting the surface water by the seasonally reoccurring ice retreat combined with long day light, nutrient supply from the melting ice and phytoplankton bloom, which connects the surface water with the deeper dark water by sedimentation of detritus, fueling the depth with nutrients.
Figure 2. Relationship between BCC in the Amundsen Sea Polynya (ASP) and apparent environmental gradients.

**Constructed wetland habitats**

In contrast, abiotic conditions in the mostly anoxic wetland sediment are often characterized by steep gradients in redox conditions or changes on a temporal scale (chapter III). Redox conditions have a great impact on the local resource availability, as they control the redox state of compounds. In the studied site, water table fluctuations caused alternating redox conditions that resulted in an increase of microbial biomass and a compositional shift.

**Lake sediment**

In chapter IV I illustrate how high input of polyaromatic hydrocarbons (PAH) exhibit a filtering effect on the pristine lake community resulting in a specialized community of typical tar-colonizing taxa. This filtering effect could be related to a compositional shift, rather than a reduction in taxa richness. Interestingly the compositional and functional beta diversity were tightly correlating, which may indicate the selection of specialized microbial consortia at the factory outlet, that carry functional features adapted to high tar loads in the sediment (Kassen, 2002).

When comparing the three studied sites, two contrasting mechanisms of how bacteria respond to environmental changes were observed: (A) by shifting community structure with steady diversity levels, or (B) by increasing the abundance of a few functionally dominant species (Langenheder et al., 2012). In the Antarctic community I identified an examples were environmental filtering under the influence of light and phytoplankton induced the reduction of diversity towards a community with low species richness. I observed in contrast compositional shifts, rather then the reduction of species
richness, due to environmental filtering acting on the lake sediment and on constructed wetland community.

Functional guilds

Amundsen Sea Polynya

Chapter II provides evidence for a shift between different metabolic types, in particular relatively fast growing copiotrophs in the photic surface, while oligotrophs dominated in the dark waters. Adapted from (Grzymski and group (2012), who separated the polar bacterioplankton community by phototrophs in the seasonal photic surface and chemoheterotrophs in the dark winter waters. I identified taxa, which are described as phototrophs such Polaribacter or the Roseobacter clade as well as typical chemoheterotrophs, such as the streamlined alphaproteobacteria SAR11 (Giovannoni et al., 2005) or a group of oligotrophic marine gammaproteobacteria (OMG) (Cho and Giovannoni, 2004).

Constructed wetland sediment

The wetland sediments (chapter III) provide a habitat were different functional guilds can co-occur and evidently utilize benzene under anaerobic conditions were proven for various redox conditions selecting for particular functional guilds involved in benzene break-down such as methanogenic, sulfate-reducing, ferric iron-reducing, denitrifying, and oxic (Lovley, 2000; Vogt et al., 2011). By introducing changes in the water level, we may have induced a shift in functional guilds that dominantly catalyze benzene break-down in the wetland sediment. Specialized consortia are proposed for anaerobic benzene break down, with each member of the consortia fulfilling a particular step in the benzene break down (Herrmann et al., 2009). Testing the occurrence and contribution in benzene contaminated wetland. Identified taxa with MAR CARD FISH that were proposed to be involved in the initial activation, and partly suggested as keyplayers. Concerning monaromatic hydrocarbon mineralization the importance of keyplayers, which catalyze the initial energy demanding activation reaction depends on the presence of phylotypes that carry that particular trait (Kleinsteuber et al., 2012). Alternatively, depending on the degree of functional redundancy within the community the loss of particular function may be compensate by another member of the community (Gamfeldt et al., 2008). Nevertheless, several taxa were identified in active benzene utilization, which give evidence for a certain degree of redundancy of benzene utilizing bacteria in the wetlands.
Trait performance

Heterogeneity can enforce shifts in trait performance. Such trait performance may be related to physiological aspect related to the fitness of an individual cell or to microbes efficiency in transformation or remineralization of nutrient and organic matter. Identifying how environmental heterogeneity influences the nutrient cycling processes is of particular interest for modeling global nutrient fluxes of, for instance, fluxes between the different carbon pools mediated by microbes.

Amundsen Sea Polynya

In the studied polar water masses different rates of cell proliferation were observed. Here, bacterioplankton adapted to environmental gradients by shifting cell proliferation rates. The highest cell proliferation rates were found in surface waters, when compared to hardly detectable rates in the deeper dark water masses.

Constructed wetland

I studied anaerobic benzene break down, with the prior assumption that biotic microbial benzene break-down may be enhanced due to a shift in composition and metabolic activity, and for understanding the ecology of functional consortia that might be involved in anaerobic benzene mineralization. Nevertheless, even twelve months of operation did not lead to a significant increase of pollutant removal efficiency. Despite a variation in community composition or overall abundance did not translate to altered community function.
Conclusion & Perspective

My studies provide a variety of examples of how spatial and temporal patterns of microbiota are shaped by the local and regional environmental heterogeneities. I also show that such variations in community composition or overall abundance does not always translate to altered community function. From a global perspective, microbes are all controlled by ecological mechanisms that drive community assembly and functioning, even though each of the Earth’s ecosystems is influenced by individual sets of abiotic and biotic factors, whose complex interactions hinder a clear mechanistic understanding.

By integrating structurally very different ecosystems across aquatic and terrestrial habitats, all characterized by low productivity, my work contributes to an emerging conceptual framework for modeling community assembly processes and ecosystem functioning on a global scale. Even so, it remains challenging to identify the actual mechanisms that cause compositional and functional shifts, because various factors interact or co-vary at different levels in natural environments.

Despite all methodological developments, there is no ideal way to quantify the true microbial diversity of complex natural communities. There is a need to develop a concept of diversity characterization that integrates taxonomy and the metabolic potential at the level of individuals as well as communities. In my opinion, characterizing microbial communities with single-cell techniques and thereby pinpointing identity, functional role and/or metabolic potential without destroying physical associations valuably complements destructive molecular methodologies.

Future research should aim at identifying the interplay between key-players and functional guilds by assessing the degree of functional redundancy at microbial scales. By identifying the direction in which abiotic factors might act on the microbial communities, mathematical models can be generated that allow to predict the diversity and functioning in a particular habitat with a given combination of abiotic factors. One example is the prediction of roles played by different microorganisms in carbon cycling and how they, in turn, affect global estimates of nutrient fluxes. Such information can of course also be used for understanding and developing ecosystem-specific bioremediation strategies following pollutant contamination. This requires a systematic integration of taxonomy and the metabolic potential of individual genotypes and more integration of conceptual models by generating hypothesis that can be answered with large data sets, generated now in the era of omics.
Summary in Swedish

Hur formar miljön bakteriesamhällen i lågproduktiva habitat?

Mikroorganismer spelar en central roll i våra ekosystem. Genom sin unika funktion som nedbrytare driver de bland annat på omsättningen av näringsämnen i biosfären och bidrar till långsiktigt vidmakthåll en produktivitet. Dessa extremt talrika men mikroskopiska organismer upphäver en enorm mångfald och allt tyder på att olika populationers utbredning i tid och rum främst kontrolleras av miljöns variabilitet. Det har sedan länge funnits en ambition inom forskarsamhället att identifiera och förklara hur miljön påverkar mikrobiella samhällens artssammansättning, men hittills har man inte lyckats särkilt väl med att beskriva och förutsäga vilken specifik påverkan olika miljöparametrar, som till exempel temperatur, näringstillgång, solstrålning och syresättning har på dessa komplexa, artrika och höggradigt interagerande samhällen och deras biogeokemiska funktion. Detta gäller i hög grad de ekosystem och habitat som kännetecknas av låg produktivitet, där det dessutom tillkommer analytiska utmaningar när man som forskare ska beskriva deras artssammansättning, metabola funktion och miljöpåverkan. Denna avhandling har därför som övergripande mål att undersöka hur olika miljöfaktorer påverkar mikroorganismer i lågproduktiva miljöer. Genom att utnyttja ny avancerad metodik för att studera bakteriesamhällens artssammansättning och funktion samt långtgående karakterisering av den omgivande miljön, kunde tre olika modellsystem som kännetecknas av låg produktivitet studeras i detta avseende.

I Södra Ishavet, nära den Antarktiska kontinenten, bildas under sommaren stora öppna vattenområden som omges av sammanhängande is. Precis som resten av detta sydliga hav kännetecknas dessa isomgärtrade vatten av låga temperaturer och brist på olika spårämnen och näringsämnen som begränsar den biologiska produktionen, men den korta isfria perioden innebär också en dramatiskt ökad solinstrålning som vid lokalt god tillgång på näringsämnen och spårämnen kan leda till dramatiskt ökad produktion. Med utgångspunkt från tidigare studier av huvudsakligt bakterier i dessa vatten ställde jag upp ett antal hypoteser om hur den ökade solinställningen och lokalt förhöjda växtplankontillväxten som observerades under den korta sommarperioden, påverkade artssammansättningen i lokala bakteriesamhällen. Genom att applicera ny kraftfull sekvenseringssteknik för karakterisering av den välstu-
derade taxonomiska markörgen som kodar för 16S rRNA, kunde bakteriesamhället beskrivas på ett mycket detaljerat sätt och det var möjligt att identifiera hur förändrad tillgång på ljus och växtplanktonproducerade kolföreningar vid ytan gynnade vissa typer av bakterier i dessa ytvatten, men också i de underliggande vattenmassorna. Jag kunde sedan i experiment visa hur bakteriesamhällen, som under den långa vintersäsongen anpassats till ett liv i mörker, reagerade på plötslig ljusexponering liknande den som sker under sommarperioden. Den ökade ljustillgången gynnade vissa opportunistiska bakteriepopulationer på bekostnad av andra, med minskad artrikedom som följd. Dessa opportunister visade sig också vara framgångsrika och talrika i det isfria innanhavet vilket understryker solljusets betydelse för bakterier i dessa lågproduktiva havsmiljöer.

Lågproduktiva habitat som uppvisar en hög grad av spatiell struktur och miljövariabilitet i mikroskopisk skala innebär helt andra utmaningar och begränsningar för de mikroorganismer som befinner sig där. En faktor som primärt begränsar deras aktivitet och tillväxt i denna typ av system är tillgång på syrgas och andra lämpliga elektronmottagare för effektiv heterotrof tillväxt med organiska substrat som energikälla. Detta kan även begränsa mikroorganismernas möjlighet att bryta ner olika miljöföroringar såsom olika aromatiska kolföreningar av antropogent ursprung. Sådana mikrobiella ekosystemtjänster är av stor betydelse i miljöförvaltning och kan även utnyttjas i tekniska system för att komma tillrätta med olika föroreningssamtal.


Olyckor och industriell verksamhet leder ibland till att sediment i kustnära vatten och sjöar förörenas av organiska miljöföroringar. Dessa miljöföroringar kan dröja sig kvar i sedimenten under lång tid, men
kan också till viss del brytas ner av naturligt förekommande mikroorganismer. Även i dessa system spelar tillgången på näringsämnen och syrgas en avgörande roll för bakteriesamhällets sammansättning och funktion, inklusive deras förmåga att bryta ner organiska miljögifter. Genom att jämföra mikrobiella samhällen i sediment uppströms och nedströms en tidigare tjärfabrik studerades hur höga halter polyaromatiska kolväten (PAHs) påverkade samhällets sammansättning. Genom replikerad direkt sekvensering av DNA från det mikrobiella samhället (metagenomik) kunde påverkan av föroreningarna på olika organismgrupper jämföras kvantitativt och i för- längningen även kopplas till funktionell potential. Både artsammansättningen och förekomsten av olika funktionella grupper av gener var tydligt avvikande i de förorenade sedimenten jämfört med påverkade sediment uppströms och nedströms den tidigare fabriken. Samtliga sediment (inklusive de förorenade) dominerades av Cyanobakterier, olika typer av Proteobakterier, Verrucomicrobia och Actinobakterier. Vidare var det en tydlig koppling mellan artsammansättning och funktionell potential.

Sammanfattningsvis visar mina studier att mikroorganismer i lågproduktiva habitat i hög grad påverkas av miljöfaktors variabilitet i rum och tid, men att skillnader i art sammansättning inte alltid leder till förändrad funktion på samhällsnivå.
Zusammenfassung in Deutsch


In einer Feldstudie wurden die Bakteriengemeinschaften identifiziert, welche die Amundsen See im Südpolarmeer besiedeln. Während der Probenahme zur Sommerzeit war eine relativ große Region der Amundsen See frei von Packeis und formte die Amundsen See Polynja. Diese jährlich wiederkehrende Bildung von Polynjas führt zur auch zur Erhöhung der Heterogenität an abiotischen Umweltfaktoren im Meer und beeinflusst die räumliche Ver-


Zusammengefasst enthält diese Arbeit eine Reihe von Beispielen, wie Bakterien durch die Heterogenität ihrer Umwelt beeinflusst werden und sich in ihrer Artenzusammensetzung und ihrer funktionellen Aktivität den jeweiligen Bedingungen anpassen. Es wird insbesondere deutlich, auf welch unterschiedlichen räumlichen und zeitlichen Ebenen abiotische Faktoren wirken können.
First of all I’m grateful to my supervisors Annelie and Stefan, you initiated this opportunity to me to run my PhD program at these two great places. Annelie, you established a very unconventional way of working environment, what I highly appreciate, especially in this bureaucratic German culture of science. You were always very positive and encouraging. I learnt from you how to write, how to stain cells and you helped immensely with all the corrections and editing of this thesis.

Stefan it’s unbelievable what connections and collaborations you have, that provided me all these opportunities, sending me to the Southern Ocean or developing collaborations. Despite being occupied in so many directions, you were always very supportive and positive and provided me with valuable and constructive feedback on planning my experiments, sampling campaigns and writing. What accounts for both of you: I’m also very grateful for all your editing all the manuscripts in the final, very crazy and hectic final weeks during any daytime.

The limnology department: When I arrived in the limnology department as an intern, still at the BMC, I first shared an office with Janne and later on we taught together the Limno I chemistry lab. Thank you Janne, for helping with all these little things in the lab, in the garage, for sampling preparation, handling instruments and the microscope and while teaching. I definitely have to acknowledge the “old” generation of PhDs with Sara, Hannes, Pia, Philip, Cristian, Göran, Jürg, Merce, Frida und Lorena. I learnt so much about teamwork, mutual support and friendship, I won’t forget. Your laid back attitude was very infectious, taking a little bit of the pressure of me of this monster challenge of doing a PhD. Sara, I worked for you as an intern. Your way of conceptual way of thinking and your confidence in your ideas influenced me as well, while I was making my first own experience with scientific working. Merce, you accompanied me during the whole period, I always looked up to you, your very balanced way of living, your accurate way of organizing your work and your statistical knowledge always impressed me. Hannes, you made me feel confident, that I actually could accomplish a PhD thesis and I always enjoyed the very warm and familiar dinners at your home, also with Kathi. Thank you for that. Lorena and Frida, I enjoyed our little chats and the discussions during our small reading club so much.
Many thanks to the PhD crew in Leipzig. Thanks to Julia and Karin, my first office mates. Karolin, Kathleen, Ute and Martha from the MES group. Karolin, you became a good friend and thanks for all the explanation and discussion about anaerobic BTEX degraders and introducing me to the your CARD FISH protocol. Kathleen, thank you for valuable and conceptual discussion on how to approach the tricky MAR with anaerobic samples. Ute, thanks for all your help in the lab, doing all these hybridizations for me… all these little filter pieces can drive you crazy.

Later also Tom, Annelie, Jule, Sally, Babette, my UFZ lunch crew. Tom, thanks for all your help on Leuna and microscopy and Micha, thanks for taking the time introducing me to cellprofiler and geographic mapping with R. You both in combination, Tom and Micha, so much fun! Franeseca, thanks for all your support in Leuna while sampling or in the car on the way to Leuna under all good and bad experimental conditions. Eva, Mareike, and Florian, thanks for good times at the UFZ. Eva, you supported me especially during the last days of writing, as a good friend and by giving needed advices. Thanks to Martin, for our two amazing trips to Northern Sweden and to Susi and Lars for being such good friends.

This PhD wouldn’t be accomplished without the white disc; playing Ultimate after a frustrating day of non-working experiments is the best thing to do. Thanks to the Leipziger park team: “Leipzig Ultimate Frisbee Dudes” and of course our little semi-professional team “Leipziger Allerlei” and later the Leipzig Ultimate club “Saxy Divers”. You are all wonderful people. Thanks also to Uppsala Frisbee with Severin, Sara, Simon and all the others having a good time with playing with during practice and the little tournaments all these years! Keep up the spirit!

Julie and Ramiro, we went together on the cruise to the Southern Ocean and made some extraordinary experiences with all the beauty of Antarctica, living two months onboard of a research vessel and the stressful times during sampling and running our experiments. Lasse, I’m grateful for all your feedback on the manuscript.

Adila, I won’t forget living with you and your lovely family.

Thanks to you Torsten and Valerie, my Leipzig-connections in Uppsala. Valerie, you “Paradiesvogel”. It is so much fun having you around and sharing all our work and life experiences.

Jerome, Charles, Nuria, remember our little road trip to Copenhagen. It was so much fun. Charles, Crazy you! Jerome, I miss the times in Eriksberg. You are a good friend and a researcher I look up to. The same to you Nuria (Muuuuuah 😊) and Ina. It was good times having you around.

Then there was this generation shift, which brought new air to the department with all PhD students, excuse me if get things wrong with the order. Yhinghua, great times sharing the apartment with you. My lovely and smart office mates Monica and Blaize. I enjoyed the time sharing the office with you. Thanks for everyone else in the Limno department, creating this good
atmosphere and sharing good times outside of science: Roger, Martin, Anne, Hannah, Jovana, Rhiannon, Alina, Jingying, Leyden, Maria, Anastatsia, all the PostDocs Omneya, Claudia, Birgit, Francois, Xavier, Simone and Anna. Andrea, you are such a positive person! Dolly, I won’t forget our field trip to Jämtland with Torsten and Monica. Lucas and Moritz for all your support and patience with handling the sequence data and with scripting. Lars, I’m grateful for your support and encouragement especially in times when it didn’t move forward with writing. Silke and Eva, you were setting common ground for my microbial ecology knowledge and the good teaching times during the Baltic Sea course, Alex, thanks for challenging me with the sequencing data analysis. Thanks to all the other seniors I forgot to mention, you all created that nice working environment in the department and were always encouraging. Eventually I want to acknowledge Douglas, who became some kind of mentor during the last months in developing research proposals, during conceptual discussions and who made me finally finding a way in accessing R and starting scripting.


Inga Richert, August 2014
References


**Sekar, R., A. Pernthaler, J. Pernthaler, F. Warnecke, T. Posch, and R. Amann.** 2003. An improved protocol for quantification of freshwater Actino-


Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1157

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology*. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)