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Links Between Structure and Function of Heterotrophic Aquatic Bacterial Communities

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Abstract
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Heterotrophic bacteria utilize dissolved organic matter, and the carbon flow through an ecosystem depends on the fractions of the utilized carbon that is either respired or transferred to higher trophic levels. The major aim this thesis is to investigate 1) the relationship between composition and functioning in heterotrophic bacterioplankton communities and 2) the influence of environmental conditions on both parameters. I set up several batch culture experiments, where lake water filtrates containing bacteria but no grazers were inoculated into sterile freshwater medium to investigate the importance of the origin of the source community (the inoculum) versus the environmental conditions (the medium) for the composition and functional performance of bacterial communities. In some experiments the medium was manipulated to simulate changes in salinity, pH and dissolved organic matter quantity and quality. Functional parameters (biomass yield, respiration, growth efficiency and enzyme activities) and the genetic composition of the emerging bacterial communities were determined.

When bacterial inocula obtained from different habitats were re-grown under identical conditions, differently composed communities emerged. This indicates that the history and distribution of taxa within the inoculum was an important regulating factor of community composition. The coupling between community composition and functioning was not very tight, and there was functional equivalency with respect to aggregated functions important at the ecosystem scale (e.g., biomass production and respiration). The functional performance of bacterial communities could to a large extent be predicted from the medium alone, except when it deviated strongly from the ambient settings. When bacterial communities were exposed to dilution, a strong change in pH or an increase in salinity, growth of structurally and functionally distinct communities occurred. I therefore suggest that it depends on the disturbance regime how bacterial community structure and function are related to each other.

Keywords: heterotrophic bacteria, dissolved organic matter, diversity, community composition, ecosystem functioning, salinity

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To the memory of my mother

List of papers

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

I Langenheder, S., Lindström, E.S. & Tranvik, L.J (2005) Weak coupling between community composition and functioning of aquatic bacteria. *Limnology & Oceanography* (in press)

II Langenheder, S., Lindström, E.S. & Tranvik, L.J. Do environmental conditions determine bacterial community composition and functioning? A test of the ubiquity of aquatic bacteria. *Submitted manuscript.*

III Eiler, A., Langenheder, S., Bertilsson, S., & Tranvik, L.J. (2003). Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology* 69: 3701-3709.

IV Langenheder, S., Kisand, V., Wikner, J. & Tranvik, L.J. (2003). Salinity as a structuring factor for the composition and performance of bacterioplankton degrading riverine DOC. *FEMS Microbiology Ecology* 45: 189-202.

V Langenheder, S., Kisand, V., Lindström, E.S., Wikner, J. & Tranvik, L.J (2004). Growth dynamics within bacterial communities in riverine and estuarine batch cultures. *Aquatic Microbial Ecology* 37: 137-148.

Contents

Abbreviations

Abund	Bacterial abundance
ANOVA	Analysis of variance
BCC	Bacterial Community Composition
BGE	Bacterial Growth Efficiency
BGR	Bacterial Growth Rate
BR	Bacterial respiration
DGGE	Denaturing Gradient Gel Electrophoresis
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
NMDS	Nonmetric Multidimensional Scaling
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
QDH	Quantitative DNA-DNA hybridization
t-RFLP	Terminal Restriction Fragment Length Polymorphism
RDOM	Riverine dissolved organic matter
UPGMA	Unweighted Pair-Group Average
μ	Bacterial Growth Rate
Y_B	Biomass Yield

Introduction

General Background

During the last decade the relation between biodiversity and ecosystem functioning and stability has attracted considerable attention (Loreau 2000) and evolved into a new ecological paradigm combining the discipline of community and ecosystem ecology (Naeem 2002, Fig.1). This was stimulated by the insight that increasing habitat destructions and fragmentation can lead to species extinctions and hence to a decrease in biodiversity. Therefore the question of potential consequences for the magnitude and stability of important ecosystem processes (e.g. primary productivity, nutrient retention, decomposition, climate) arouse.

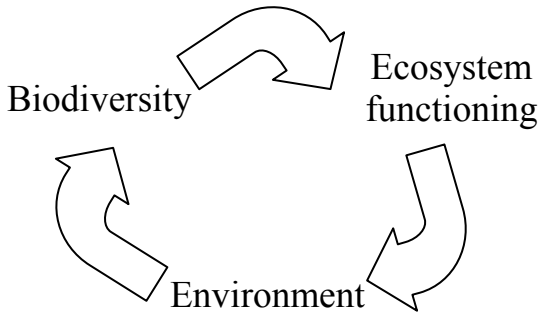

Figure 1. The biodiversity-ecosystem paradigm. Biodiversity (i.e. species richness and composition) is not simply a dependant of the environment (i.e. abiotic conditions and biotic complexity), but forms a link to ecosystem function that feeds back to the environmental conditions (Naeem 2002).

Biodiversity refers to all biotic variation from the level of genes to ecosystems. It has many facets, including species richness as well as evenness (i.e. the relative abundance of species) and different spatial and temporal scales (Purvis and Hector 2000). Ecosystem functioning is the biogeochemi-

cal activity of an ecosystem or the flow of materials (nutrients, water, atmospheric gases) and processing of energy. Bacteria are of special interest, because they conduct most of the biogeochemical transformation. Moreover, they are extremely abundant and equivalent in biomass to plants (Whitman et al. 1998) and with an evolutionary age of 300 billion years (compared to 100 for eukaryotes) they inhabit any imaginable habitat on Earth (Horner-Devine et al. 2004a). For aquatic ecologists, heterotrophic bacteria are of major interest since they utilize dissolved organic matter (DOM). This means that the carbon flow through an ecosystem such as a lake depends strongly on the fractions of the utilized carbon that are either respired directly, or transferred to higher trophic levels within the food chain.

Most studies done on the interaction between plant diversity and ecosystem functioning and stability are almost exclusively from terrestrial systems (Naeem et al. 1994; Tilman et al. 1996). Evidence from these studies is non-conclusive (i.e. different relationships between biodiversity and ecosystem functioning have been found, Schwartz et al. 2000), even though it is accepted that there is a positive relationship between plant diversity and plant biomass (Loreau et al. 2001). Even though the majority of studies focused on species richness as a predictor of ecosystem functioning and stability, there are studies that draw the attention to other factors, such as species composition (Hooper and Vitousek 1997; Mikola and Setälä 1998; Symstad et al. 1998), environmental conditions (Cardinale et al. 2000) or ecological history (Sankaran and Mc Naughton 1999). In general, it is assumed that a high biodiversity works as an insurance or buffer against environmental perturbations (Yachi and Loreau 1999). Accordingly, it is beneficial for an ecosystem to maintain a high level of diversity. Even though species might be redundant (i.e. functionally equivalent) *at* a given time, they might not be so *through* time because different species respond differently to environmental changes.

It is important to extend studies on the biodiversity – ecosystem functioning relationship to aquatic systems (Giller et al. 2004). In addition, to study how microbial and especially bacterial diversity is related to the functioning of ecosystems is one of the major challenges of current ecological research (Loreau et al. 2001). The major obstacles in this endeavor have been and still are methodological difficulties to study bacterial diversity. Traditionally, microbial ecologists relied on cultivation of bacteria from environmental samples. This method allowed identification of approx 1 % of the existing bacterial diversity, since most bacteria in the environment are not culturable (Amann et al. 1995). The dogma of the low culturability of natural bacteria is still valid even though considerable progress in the cultivation of ecologically relevant groups has been made in recent years (Rappe et al. 2002; Hahn et al. 2003). Most progress in gaining information on bacterial diversity and community composition has, however, been made during the last two dec-

ades, due to the use of evolutionary marker genes, like the 16S ribosomal RNA gene (Woese 1987). Since then, knowledge about bacterial diversity and the dominating types of bacteria in natural systems has increased rapidly (Pace 1997). However, there are still no reliable estimates of the total number of bacterial species. Estimates vary from a couple of thousands in lakes to a few millions in oceans and are generally higher for soils compared to planktonic systems (Curtis et al. 2002; Torsvik et al. 2002). Of interest to a limnologist is that Curtis et al. (2002) estimated that a lake of a volume of 1000 m^3 with 10^{15} individuals harbors approx. 8000 distinct taxa.

The "16S rRNA approach" has led to the development of several different methodological approaches to study bacterial diversity at different scales of resolution (Dahllöf 2002).

At present it is difficult for microbial ecologists to investigate relationships between prokaryote diversity (i.e. species richness and evenness) and ecosystem functioning since determination of diversity is a time consuming process that it unrealistic to apply to a large set of samples (but see Curtis et al. 2002; Dunbar et al. 2002 for promising approaches to mathematically tackle these problems). Difficulties also arise from the lack of a reliable species concept for prokaryotes (Rossello-Mora and Amann 2001). However, the use of molecular fingerprinting methods enables investigation of how community composition is related to ecosystem functioning and stability. These tools allow to follow rapid changes in bacterial community composition (Forney et al. 2004). Therefore, they provide the opportunity to investigate how bacterial community composition and functioning are related and if and how bacterial communities change in composition along an environmental gradient or in response to a stress factor.

Mechanisms determining bacterial community composition at the local scale

The general patterns behind microbial diversity and mechanisms regulating bacterial community composition at the local scale are currently under debate. Moreover it is discussed whether bacteria exhibit similar biogeographical patterns as plant and animals, i.e. whether species are endemic to certain geographic regions or whether they are cosmopolitans. Broadly spoken there are two opposing views: (1) the ubiquity concept and (2) the metacommunity concept.

(1) The ubiquity concept

The traditional view among microbiologist can be dated back to the beginning of the last century and more specifically to Baas-Beckings' famous statement that "everything is everywhere, the environment selects" (Baas-Becking 1934).

This view was reinforced by a number of field and laboratory studies performed on protozoa (Fenchel et al. 1997; Finlay and Clarke 1999). The rationale behind the ubiquity concept is that microorganisms are extremely abundant, proliferate rapidly, disperse easily and are unlikely to go extinct (Fenchel 1993; Pedrós-Alió 1993; Whitman et al. 1998). Hence, the distribution of bacteria and other microbes may be largely independent of geographic barriers (Finlay 2002) and there is a high local but low global diversity. Moreover, the ubiquity concept implies that, although not actively growing, the majority of all globally occurring species are present as a "seed bank" at any local site. The prevailing environmental conditions act as a filter selecting taxa from this so-called "cryptic" species pool.

As already mentioned, these ideas got support from studies on eukaryotic microorganisms. For example, Finlay and Clarke (1999) found that a small sediment sample from an English pond contained remnants of as much as 80% of all globally identified species within the diverse flagellate genus *Paraphysomonas*. Fenchel et al. (1997) found that a small sediment sample contained an appreciable fraction of the globally occurring species of free-living ciliates. Most of these species were not detectable in the original sample, but could be triggered to growth by a variety of enrichment techniques, such as heating and substrates additions.

On the other hand, field studies that settled out to investigate the global distribution patterns of prokaryotic taxa give a deviating picture. Globally occurring 16S rRNA sequence clusters have been found repeatedly (Glöckner et al. 2000; Zwart et al. 2002; Hahn 2003; Hahn et al. 2003) indicating that cosmopolitan distribution of taxa might occur. Recently, however, it was shown that species-area relationships exist even for bacteria and ascomycete fungi (Green et al. 2004; Horner-Devine et al. 2004b) even though the slope of the species-area relationship was less steep compared to values found for plant communities (Horner-Devine et al. 2004b). These results support findings of geographic isolation in microorganisms (Oda et al. 2003; Papke et al. 2003; Whitaker et al. 2003). Papke et al. (2003) studied hot-spring cyanobacterial communities and found that phylogenic patterns corresponded to geographic distribution but did not correspond to the chemical conditions. Hence, this study provides a clear example that local community structure is regulated by geographic isolations (probably a result of restricted dispersal) and not by the local environmental conditions. Oda et al. (2003) studied the biogeography of the soil bacterium *Rhodomonas palustris*

along a sampling transect and found that there was a decreasing genotype similarity with increasing distance between sites. This suggests that multiple microhabitats exist and that each of them selected for distinct genotypes. Baas Becking might have put it like this: "everything is *not* everywhere, *because* the environment selects". Similar patterns of geographic separation have even been found in the homogenous water column of the open ocean (de Vargas et al. 1999; Selje et al. 2004) but seem to occur at much larger spatial scales. What is becoming increasingly obvious is that the answer to the question about ubiquitous distribution of microorganism depends on the phylogenetic resolution (Cho and Tiedje 2000) and the question might not be *if* "everything is everywhere" but down to which phylogenetic resolution ubiquitous distribution of microbes occurs.

In contrast to the metacommunity concept, which will be described in the next section, the ubiquity concept focuses on the importance of the local environmental conditions as the major regulating factor of BCC. It is closely coupled with the assumption that there is one niche for every taxa and assumes that there is hardly any niche overlap, niche facilitation and functional redundancy. In the most extreme case it is assumed that any ecological niche becoming available is going to be occupied exclusively by one superior organism with a global distribution.

(2) The metacommunity concept

The opposing view that has recently also been introduced to the field of microbial community ecology is that bacterial community composition at the local scale is influenced by scale-related processes (Curtis and Sloan 2004). Hence, local communities (such as communities in a lake) cannot be seen as closed and isolated entities, but are influenced and shaped by regional (metacommunity) processes (see Leibold et al. 2004 for detailed review). A metacommunity can be defined as a set of local communities that are linked by dispersal of their constituent members (Holt 1991). Dispersal acts as a homogenizing factor increasing similarity in species composition among local sites within the region (or metacommunity).

Curtis and Sloan (2004) suggest – based on the neutral theory of biodiversity (Hubbell 2001) – that local bacterial community composition is a product of random events in connection to the recruitment of specific bacteria from the surrounding regional species pool. It is assumed that bacteria within functional groups are redundant, i.e. different species carry out the same or at least similar functions. Hence, a species carrying out a certain function in a local community is not necessarily the only one able to "do the job" but the one that happened to fill the niche first when it became available. Curtis and Sloan (2004) put it like this: "If random invasion from outside has a role the characteristics of any given community will be influenced by i) the size of

the reservoir or metacommunity or diversity from which it is drawn, ii) the distribution of taxa within the metacommunity, iii) the rate of carriage of bacteria from the reservoir to the source community, iv) the size of the source community and v) the spatial structure of the community."

The major difference in contrast to the ubiquity concept is that the metacommunity concept is much less deterministic and assumes that BCC is - at least to a certain extent - regulated by chance.

Factors controlling BCC

It has been shown that DOM quality (Cottrell and Kirchman 2000; Covert and Moran 2001) and quantity (paper III), inorganic nutrients (e.g. Fisher et al. 2000), primary productivity (Horner-Devine et al. 2003; Yannarell and Triplett 2004) and grazing (e.g. Jürgens and Matz 2002) are major regulating factors of BCC in aquatic environments. The importance of viruses is controversial. They are generally believed to be drivers of bacterial diversification, but the underlying mechanisms and the importance of lytic versus lysogenic phages remains unclear (Weinbauer and Rassoulzadegan 2004). Additionally, osmotic conditions, e.g. salinity (see section below) and pH (Lindström and Leskinen 2002) seem to be important as well.

Focus on Salinity: Estuaries as transition zones between terrestrial and marine systems

Estuaries are of major interest as transition zones between limnic and marine environments and are characterised by steep environmental gradients, high bacterial activities and rapid nutrient turnover (Day et al. 1989; Goosen et al. 1997; Cunha et al. 2000; Selje and Simon 2003). Bacterial production in estuaries is fuelled by DOM derived from primary production on one hand and by allochthonous inputs from rivers on the other (Raymond and Bauer 2001). Estuaries are generally considered to be net-heterotrophic (Findlay et al. 1991; Goosen et al. 1997). Especially in boreal systems, the allochthonous DOM-pool is often dominated by terrestrially derived organic substances and of humic nature (Pettersson et al. 1997). It has been shown that this DOM can be used as an energy source for bacterial production (Coffin et al. 1989; Moran and Hodson 1994; Zweifel et al. 1995) and can even be transferred to higher trophic level (Rolff and Elmgren 2000). Hence, estuaries are important links between terrestrial and marine environments and to study the fate of riverine DOM in estuaries is of fundamental importance for understanding the destiny of allochthonous DOM in marine system. Moreover, microbial utilization together with flocculation and sedimentation

might account for the low concentration of allochthonous DOM in the open ocean (Hedges et al. 1997). The relatively high respiration rates in estuaries (e.g. Wikner et al. 1999) might contribute to the high carbon dioxide emissions from estuaries (Frankignoulle et al. 1998).

The River Öreälven with its adjacent estuary discharging into the Northern Baltic Sea is a typical example for such a system. This system is, however, different from most other estuaries viewed in a global perspective, because the change in salinity that freshwater bacteria are exposed to is rather benign. The annual mean salinity in the Northern Baltic Sea is approximately 4 (compared to 35 in the open ocean).

Salinity has shown to be a strong regulating factor of bacterial community composition and there are several studies showing a gradual change in community composition along salinity gradients in estuaries (Troussellier et al. 2002; Hewson and Fuhrman 2004). Salinity even shapes bacterial communities on a broad phylogenetic level: Beta-proteobacteria are generally much more abundant and active in freshwater environments, whereas the opposite seems to occur for alpha-proteobacteria (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2003). The composition of estuarine communities appears to be a mix of freshwater and marine communities (Crump et al. 1999; Rappé et al. 2000) and there are indications that indigenous estuarine communities develop depending on water residence times in the estuary (Crump et al. 2004). Even though there is a gradual change in many environmental parameters along the estuarine gradient from the freshwater to marine end members, the studies cited above strongly suggest that salinity *per se* is of major importance. However, little is know about the fate of freshwater bacteria during the transport along the estuarine salinity and dilution gradient. The transition from freshwater to estuarine conditions is mediated by loss of activity, injury and even cell death of bacteria (del Giorgio and Bouvier 2002) supporting earlier studies that riverine bacteria die when exposed to increasing salinities (Valdés and Albright 1981). However, it has also been found that riverine bacteria can survive at least in oligohaline environments up to a salinity of 10 (Painchaud et al. 1995). In agreement with this, estuarine bacteria are able to utilize riverine DOM (RDOM) and it has also been suggested that these RDOM degrading bacteria in estuaries are successful riverine immigrants (Kisand et al. 2002; Kisand and Wikner 2003; Kisand et al. in press). Cunha et al. (2000) also proposed that carbohydrate degradation in a south European estuary is mostly due to a limnic source of bacteria. However, there is still a lack of knowledge on how riverine bacteria are affected by increases in salinity and whether they are the main consumers of RDOM even in estuaries.

The role of microbial diversity and community composition for ecosystem functioning

(a) General concepts

Different theoretical concepts have been developed to describe the potential importance of biodiversity for ecosystem functioning. (1) *Redundant species hypothesis:* Species within functional groups have broad fundamental niches allowing them to modify their realized niches when biotic interactions change. Hence, if one species disappears the open niche will be filled immediately by a co-existing species from the same functional group, biomass or density compensation will occur and no effect on function will be observable (Walker 1992; Lawton 1994). (2) *Predictable change or "rivet" hypothesis*: there is niche differentiation among species, i.e. loss of species will always lead to a loss in function (Lawton 1994). (3) *Idiosyncratic hypothesis*: The functioning of a system changes whenever species disappear, but the direction or magnitude of the response is unpredictable since it depends on the identity of the lost species.

There are only few studies relating changes in microbial diversity or community composition to the functioning of the system. In the two first studies that were done, McGrady-Steed et al. (1997) and Naeem and Li (1997) could show that an increase in microbial diversity reduced the variability of ecosystem functions, such as biomass and CO_2 production, thereby increasing stability and predictability of the system. Hence, even though diversity did not enhance the magnitude of the ecosystem function, the presence of 'redundant' species led to more consistent ecosystem function and thereby, in accordance with the insurance hypothesis (Yachi and Loreau 1999), increased the stability of the system. However, Petchey et al. (2002) observed no stabilizing effect of species richness on total community biomass.

It is commonly assumed that the strongest effects of microbial diversity on ecosystem functioning should be observable when either keystone species or complete functional groups are lost (Chapin et al. 1997). In agreement with this, Hodgson et al. (2002) found that the relationship between diversity and productivity for the soil bacterium *Pseudomonas fluorescens* was driven by functional group rather than genotypic diversity. Griffiths et al. (2000) could show that well-defined microbial functions such as nitrification and methane oxidation, carried out by a limited microbial sub-set, were more sensitive to a decrease in soil microbial diversity than general functions, such as respiration and decomposition, carried out by a wide range of organisms. Another study that investigated diversity effects on functioning within

a well-defined functional group of cellulose degraders (Wohl et al. 2004) found an asymptotic relationship between diversity and cellulose degradation rate, suggesting that coexistence of functionally redundant species promotes ecosystem function. Similar results were obtained for saprophytic fungi degrading recalcitrant substrates (Setälä and McLean 2004). However, in both studies, positive relationships between diversity and functioning only applied at low diversities. As soon as a certain minimal diversity was reached further addition of species did not yield any further increase in ecosystem function. Bärlocher and Corkum (2003) demonstrated that nutrient supply outweighed fungal species effects during the degradation of leaf litter, hence demonstrating that in this case the functional performance of the system was limited by the environmental conditions more than by the composition or diversity of the fungal degrader community.

(b) The role of environmental changes and disturbance

Studies investigating the relationship between bacterial diversity and ecosystem functioning are rare due to difficulties to determine bacterial species richness in nature. Even though promising first attempts have been made (Carney et al. 2004), most studies rely on experiments with rather limited significance. A constructive approach to create a diversity gradient from a pool of cultured species where diversity levels were low has been applied (McGrady-Steed et al. 1997; Naeem and Li 1997; Mikola and Setälä 1998; Bärlocher and Corkum 2003; Setälä and McLean 2004; Wohl et al. 2004). Alternatively a destructive approach was used, where manipulations of diversity were done by diluting or fumigating complex natural communities whereby less abundant species gradually go extinct (Griffiths et al. 2000; Franklin et al. 2001). The major disadvantage of the latter approach is that diversity is not defined, but only categorized on a gradient from ‘high’ to ‘low’.

The majority of studies focus on the subsequent effect of environmental conditions or perturbations/stress on microbial community composition and functioning to gain insight into the importance of microbial diversity for ecosystem functioning. Key questions that can be answered with this approach are: (1) Do similar environments harbour similarly composed communities and similar functions? And (2) To what extent can microbial communities buffer environmental perturbations without any effect on ecosystem functioning and stability? The conceptual outline presented above makes sense in the context of these studies as well. According to the *redundant species concept*, we would predict a weak coupling between structure and function including the possibility that similar environments can have different BCC. Moreover, we would expect a system to be highly resistant to envi-

ronmental stress since replacements among redundant species should not have any effect on ecosystem functioning. According to the *predictable change hypothesis* similar environments should have similar BCC and function. Moreover, bacterial communities should be quite sensitive to environmental changes with potential effects on functioning as well.

Are similar environments similar in terms of bacterial community composition and functioning?

Several studies investigated whether lakes similar in water chemistry have similarly composed bacterial communities (e.g. Lindström and Leskinen 2002; Yannarell and Triplett 2004), but to my knowledge field studies looking at concomitant changes in community functioning (such as e.g. short term respiration or enzyme activities) in aquatic systems are missing. Investigating whether lakes similar in water chemistry harbour similar bacterial communities conducting similar functions is, however, a difficult task, because top-down effects exerted by grazing and viral infection have strong impact as well and might masks bottom-up effects presuming they exist. Sites with different soil types have been shown to harbour structurally and physiologically distinct bacterial communities (Girvan et al. 2003; Rich et al. 2003), but even for terrestrial systems it is not clear how this relates to important ecosystem functions such as nutrient retention, decomposition and denitrification. At present, experimental studies, like presented in my thesis, are better and easier tools to study the impact of similar environmental conditions on bacterial community composition and functioning.

How do environmental changes and perturbation influence bacterial community composition and functioning?

Studies investigating how bacterial community composition and functioning vary along environmental gradients or as response to environmental perturbations are numerous. They are either field studies investigating changes in BCC and functioning along gradients of various abiotic factors, or experimental studies where environmental conditions are manipulated and the resulting changes in community composition and functioning followed.

From the viewpoint of a limnologist, studies that followed parallel changes in BCC and community functioning resulting from substrate amendments or changes in DOM sources are of outstanding interest. Such studies have show that changes in DOM trigger changes in both BCC and function (Findlay et al. 2003; Kirchman et al. 2004). In contrast to this, metabolic changes following DON additions were not paralleled by changes in BCC (Findlay and Sinsabaugh 2004) and there are also studies that report a remarkable stability in BCC despite alterations in environmental conditions and productivity (Riemann and Middelboe 2002; Stepanauskas et al. 2003). These latter findings correspond well with the observation that highly dy-

namics communities can maintain a stable performance in methanogenic reactors (Fernandéz et al. 1999).

Other studies found that salinity (del Giorgio and Bouvier 2002), physical removal in case of attached communities (Ferris et al. 1997), temperature stress (Wu et al. 2002), heavy metal contamination (Müller et al. 2002; Massieux et al. 2004), heat (Griffiths et al. 2004), pH (Princic et al. 1998) and substrate shocks (Fernandéz et al. 2000; Hashsham et al. 2000) influence the structure and function of prokaryotic communities in various environments. Most of these studies focus on the questions whether microbial community composition and/or functioning is resistant to a disturbance, and only few studies consider resilience, i.e. the potential of the community to recover and to return to the original state after a perturbation. However, Fernandéz et al. (2000) demonstrated that changes in BCC induced by a substrate shock were reversible. Princic et al. (1998) could show similar patterns of reversibility for nitrifying bacteria that were perturbed by substrate shocks and oxygen depletion but not for pH perturbations. Thus, it is generally unclear how the structure and function of bacterial communities is affected by a disturbance and to what degree communities are resistant or resilient to environmental perturbations.

Aims of the thesis

This thesis addresses 2 fundamental questions:

1. How are bacterial community composition and functioning related to each other? (papers I and II)

2. How do bacterial community composition and functioning respond to environmental changes? (papers III-V)

The conceptual outline of the thesis is summarized in Figure 2.
Paper I investigates if there is a tight coupling between bacterial community composition and functioning. Moreover, we ask whether the environmental conditions select similar bacterial communities from different source communities, because the assumption "everything is everywhere – the environment selects" is valid. **Paper II** compares more explicitly the "ubiquity concept" and the "metacommunity concept" and investigates, if differently composed communities can maintain similar functions or not. In **papers I and II,** I also address the role of pH in regulating BCC. **Paper III** looks at changes in bacterial community composition and functioning along a DOM dilution gradient. **Papers IV and V** deal with the impact of salinity on community composition and functioning and temporal dynamics of riverine and estuarine bacteria during DOM degradation, respectively.

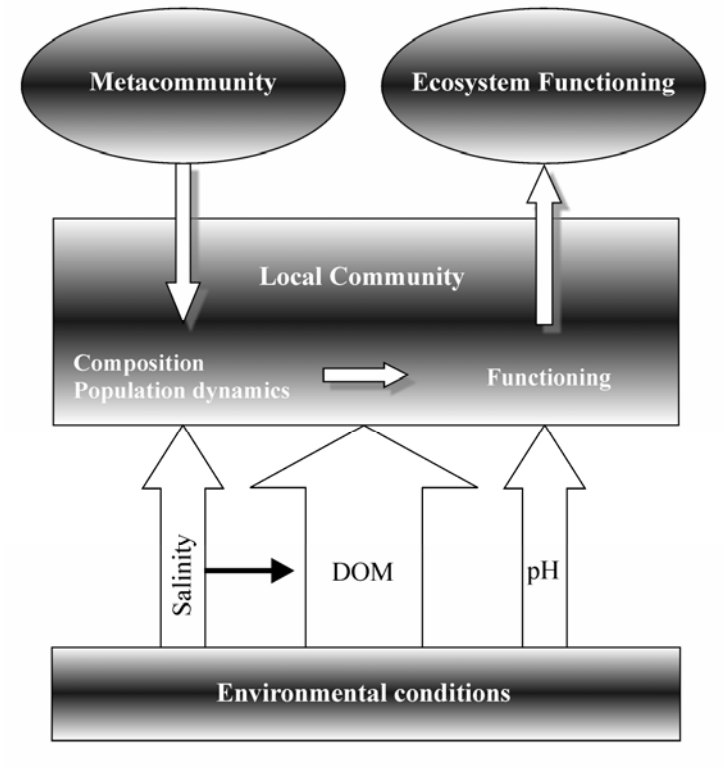

Figure 2. Conceptual outline of the thesis. Community composition and population dynamics are influenced by local processes (i.e. the environmental conditions) and by regional processes (i.e. the so-called metacommunity), and determine the functioning of the local community as well as the ecosystem. Environmental conditions investigated in the thesis are salinity (which additionally to direct effects also can have an indirect effect due to modifications of the DOM pool), DOM quality and quantity, and pH.

Methods

All results presented in this thesis are based on growth or enrichment experiments using batch cultures. To set-up such experiments a grazer free filtrate containing bacteria but no grazers (the "inoculum") is inoculated into sterile water (the "medium" or "DOM fraction"). Such cultures, which often are referred to as dilution cultures were originally used to assess growth rates of bacteria (Kirchman et al. 1982) and are common tools to assess the bioavailability of DOM in aquatic systems (del Giorgio and Davis 2003, and references therein). In the context of my studies, they are tools to investigate the importance of the origin of the source community (i.e. the inoculum) versus the environmental conditions (i.e. the medium) for the composition and functional performance of bacterial communities.

In **paper I**, both the source of the medium and the inoculum was varied, and I used an experimental set-up where sterile medium and source communities obtained from 4 different lakes were mixed in all possible combinations. In **paper II** different source communities were grown on the same medium. In **paper III** we applied the same inoculum to cultures with the same carbon source provided in different concentrations, but otherwise identical. In **paper IV and V**, two media based on the same DOM but adjusted to different salinities were inoculated in all possible combination with two different inocula (riverine or estuarine bacteria). In all cases inorganic nutrients were added in access to be sure that bacterial growth was most likely C-limited.

Growth of bacterial communities in batch cultures follows a typical pattern (Wanner and Egli 1990; Mason and Egli 1993): after an initial lag phase, bacterial cell numbers increase exponentially, since each single cell is initially encountering a surplus of substrates and nutrients. The exponential growth phase descends into the stationary phase as soon as resource depletion occurs. At this stage the experiments were stopped and the following parameters determined to obtain an estimate about the functional performance of the bacterial communities growing in the cultures: bacterial abundance, bacterial biomass yield (Y_B), respiration (BR), bacterial growth efficiency (BGE) and DOC-utilization. In **paper II** we also measured the activities of two extra-cellular enzymes (beta-glucosidase and leucine-aminopeptidase) and the ability to utilize benzoic acid. Benzoic acid utilization measurements included additions of two types of differently radioac-

tively labeled benzoic acid molecules, one where the ^{14}C atom was located in the carboxy-side chain and another one where it was within the ring structure. We measured both respiration and biomass production during benzoic acid utilization.

Bacterial community composition (BCC) was also determined at the final stage of the experiments. As an exception to this, **paper V** includes a more elaborated sampling schedule since this study specifically addressed temporal changes within bacterial communities during batch culture growth.

For addressing BCC we used two different molecular fingerprinting methods, either terminal restriction fragment length polymorphism analysis (t-RFLP, Liu et al. 1997, **papers I and II**) or denaturing gradient gel electrophoresis (DGGE, Muyzer et al. 1993, **papers III, IV and V**). In t-RFLP, 16S rDNA is amplified by PCR with a 5' fluorescent primer, the amplicons digested (i.e. cut into fragments of different length) with restriction enzymes, which are then separated by gel electrophoresis. DGGE relies on melting point variations of 16S rDNA sequences differing in their GC-content, which can be separated on a gel with a denaturing gradient. In combination with multivariate statistical approaches both methods provide powerful tools for comparative studies of BCC with a high number of samples (Forney et al. 2004). In **paper IV and V** we also used whole genome DNA-DNA hybridization (Voordouw et al. 1993; Pinhassi et al. 1997) to assess differences in BCC, following individual bacterial groups, in dependence on the source of the inoculum and salinity. In **paper IV** we blotted an aliquot of DNA extracted from a total number of 68 previously isolated strains (Kisand et al. 2002) and used radioactively labeled community DNA, which we obtained from the final stage of a batch culture experiment, as a probe to detect the relative abundance of the isolates in our samples (reverse DNA-DNA hybridization). In **paper IV and V** we used quantitative DNA-DNA hybridization (QDH) to follow the growth patterns of 7 selected strains during the experiment. In the case of QDH, the procedure is reverse to that described above. Hence, in this case community DNA is blotted onto a membrane and labeled DNA from isolated strains is used to determine their abundance in the sample.

Results

Coupling between structure and functioning: how important are environmental conditions as a selective factor? (paper I)

In order to test how important the environmental conditions are compared to the source community in determining bacterial community composition and functioning, I performed a batch culture experiment with a factorial design where sterile water and bacterial assemblages from 4 lakes were set-up in all possible combinations.

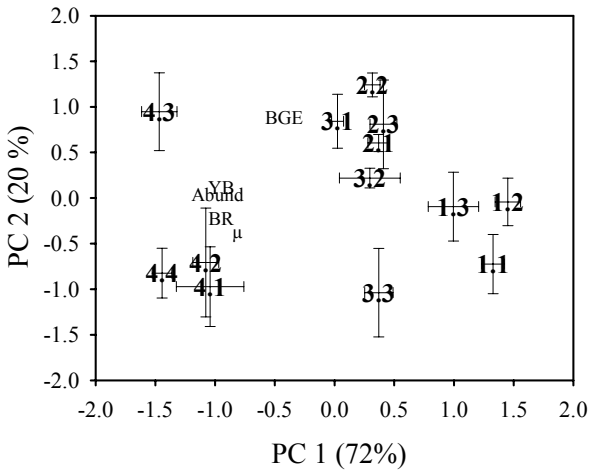

Figure 3. Principal components analysis (PCA) with functional parameters. The distribution (Scores) of treatments along the first 2 principal components is shown. Loadings of functional variables included in the analysis (Abund, YB, BGE, μ, and BR, see table of abbreviations for further explanation) are shown as well. All symbols represent mean values ± SD calculated from replicate cultures. For treatment assignment, the first digit refers to the medium, while the second one refers to the inoculum (see table 1 in paper I for further information).

The functional performance (biomass yield, respiration, growth rate, and growth efficiency) of bacterial communities growing in the cultures depended primarily on the type of the medium and to a much lesser extent on the origin of the source community (Fig. 3). Functional changes were, however, only partly paralleled by changes in bacterial community composition. NMDS analysis of t-RFLP patterns suggested that the patterns underlying BCC in the cultures are rather complex (Fig. 4). Hence, there were significant effects of the source of the medium as well as the source of the inoculum on BCC. The strongest effect from the medium was found in cultures that were based on DOM obtained from a polyhumic lakes, which differed considerable in DOM quantity and quality compared to the other media.

One interesting exception from this overall pattern was, that the bacterial source community originating from a slightly acidic polyhumic lake was not able to rapidly adjust to changes in environmental conditions going along with increasing pH and an altered DOM pool.

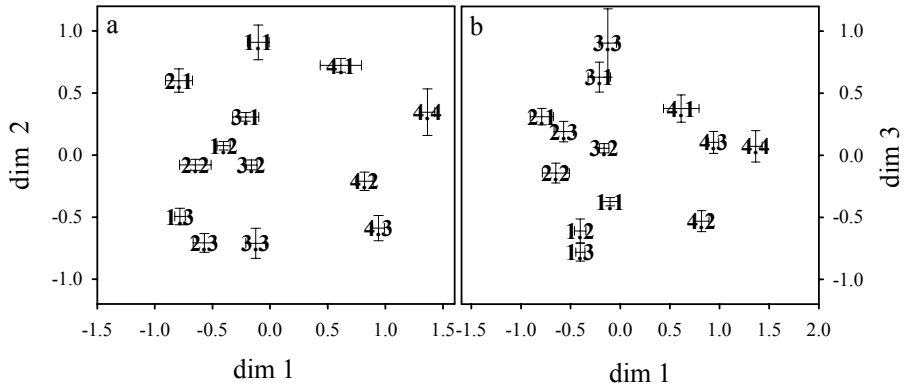

Figure 4. Results from a 3-dimensional nonmetric multi-dimensional scaling analysis using t-RFLP data. (a) Dimension 1 versus dimension 2, (b) dimension 1 versus dimension 3. Stress = 0.125. All symbols represent mean values ± SE calculated from replicate cultures. For treatment assignment, the first digit refers to the medium, while the second one refers to the inoculum (see table 1 in paper I for further information)

Composition and functioning of bacterial communities developing from different source communities: Ubiquity versus metacommunity concept (paper II)

The aim of this study was to test the two major hypotheses concerning the regulation of BCC at the local scale, the "ubiquity concept" versus "the metacommunity concept" (see Introduction for background information). Bacterial inocula that were collected from 8 different aquatic habitats were regrown in batch cultures under identical conditions and community composition and functioning of the selected communities were assessed. In the light of the conceptual framework presented in the Introduction, the sterile media represent vacant (or unexploited) habitats to be invaded and the inocula represent the source communities from which taxa can be "drawn". Hence, we wanted to test whether different source communities would converge towards higher similarity due to similar selection pressure upon growth under identical conditions. If this would be the case, we would favor the "ubiquity concept", if not, evidence would point towards the direction of the "metacommunity concept".

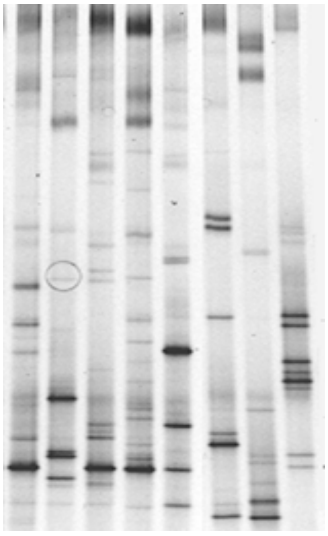

Figure 5. DGGE gel showing banding patterns from cultures receiving different source communities as inocula. Lanes from left to right represent INOC 1 – INOC 8 in numerical order. Only one of 4 replicate cultures was analyzed by DGGE. The more complete statistical analysis of BCC was done by t-RFLP as described in **paper II**.

Differently composed communities emerged from the different source communities (Fig. 5) and there was no indication that the selected communities converged towards higher similarities compared to the ambient situation. Source communities from acidic sites (pH < 6.5) had extended lag phases before growth commenced indicating that they did not harbor (or contained only few) cell that could grow under the new conditions. In general bacterial communities growing in these treatments (6-8) were functionally distinct compared to the remaining treatments (Fig. 6).

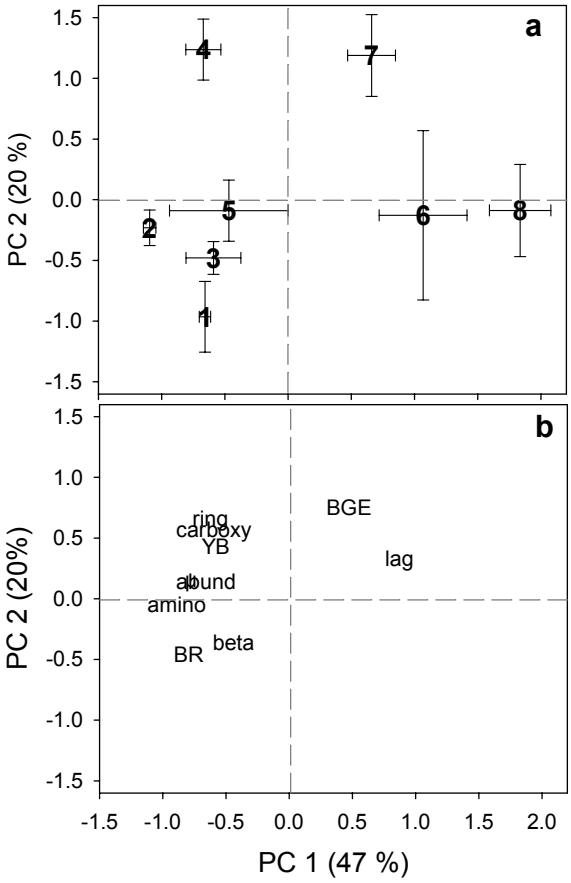

Figure 6. PCA with functional parameters. Parameters included in the analysis were: Bacterial abundance (abund), Biomass yield (Y_B), bacterial respiration (BR), bacterial growth efficiency (BGE), length of lag phase (lag), maximum intrinsic growth rate (μ), beta-glucosidase activity (beta), amino-leucine peptidase activity (amino), ^{14}C-carboxy-benzoic acid uptake (carboxy), ^{14}C-ring benzoic acid uptake (ring). All values were log (x+1) transformed, except for μ (untransformed) and BGE, which was arcsin square root transformed.
a. distribution of treatments (mean ± SE, n=4) along the first 2 principal components
b. loadings of parameters included in the analysis. "μ" is hidden behind "abund".

This demonstrates that the "environmental conditions" (and in particular pH) were important for the regulation of the overall growth patterns and growth ability of the bulk bacterial community. Apart form the deviating behavior of source communities from acidic sites, specific functions (such as enzyme activities and benzoic acid utilization) were much more strongly affected by the source of the inoculum community than "broad" functions such as biomass production and respiration (Table 2 in **paper II**).

Bacterial community structure and functioning at different natural organic matter concentrations: is dilution a stressor for bacterial community composition and functioning? (paper III)

The major aim of this study was to investigate changes in bacterial community composition and functioning along a DOM concentration gradient. The experimental set-up included 7 concentrations of the same aged humic DOM extract ranging from 0.5 to 30 mg L^{-1}.

Y_B and BR increased linearly with increasing substrate concentrations. In contrast, μ (or BGR as used in paper III) and BGE showed an asymptotic relationship to DOM concentration. Hence, at low DOM concentration, μ and BGR increased linearly with increasing concentration and then "leveled off" at higher concentrations. Substrate concentration seemed to influence BGE and μ only at DOC concentrations lower than 6 mg L^{-1}. There was a gradual change in bacterial community composition along the dilution gradient as investigated by using DGGE in parallel with changes in functional parameters (Fig. 7).

The highest degree of change in DGGE banding patterns with concentration was observed at the diluted end the concentration gradient That is, a doubling of DOC concentration from 1 to 2 mg L^{-1} resulted in clear changes in BCC whereas doubling of DOC concentration from 15 to 30 mg L^{-1} did not induce a change in BCC.

There was also some indication that changes in BCC along the dilution gradient were accompanied by large shifts in the phylogenetic structure of the communities. DGGE-bands that were excised, cloned and sequenced from treatments with high DOC concentrations were affiliated with either the Bacteriodetes or beta-proteobacteria, whereas the one distinct band that was sequenced from a treatment with low DOC concentration was affiliated with the alpha-proteobacteria.

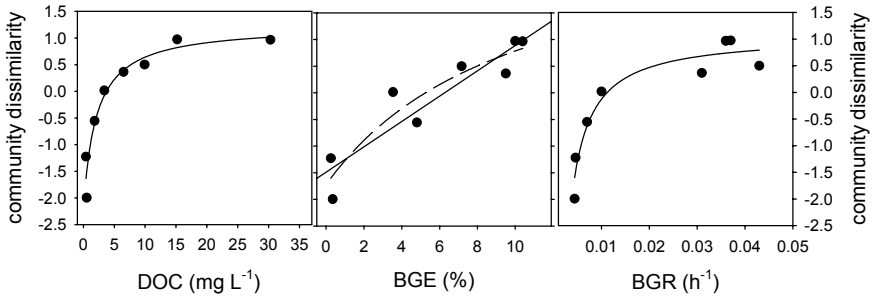

Figure 7. (a) Relationship between community dissimilarity and DOC concentration in the cultures. (b) Relationship between community dissimilarity and BGE. (c) Relationship between community dissimilarity and BGR. Community dissimilarity was calculated from a binary matrix of DGGE banding pattern where the multidimensional dissimilarity matrix was reduced to 1 dimension using NMDS. Community dissimilarity values are plotted against mean values calculated from 3 replicate cultures for all functional parameters. Different regression model fitted to the curves are shown as well.

Salinity as a selective force for the structure and functioning of bacterial communities degrading riverine DOM (paper IV)

To test how salinity influences community composition and functioning, we performed a re-growth experiment based on riverine DOM from Öreälven. The cultures were adjusted to riverine (0 ‰ salinity) or estuarine salinity (4 ‰) and inoculated with bacteria from these two environments. Hence, we could compare functional parameters related to RDOM utilization by riverine and estuarine bacteria under natural conditions. More important, we could also study the effect of salinity changes on riverine bacteria and estuarine bacteria. The combinations of inoculum and salinity that constituted a disturbance of original conditions, i.e. when the salinity of the cultures did not match the salinity at the site where the inoculum originated, typically deviated from the undisturbed situation with regard to biomass yield, BGE and growth rates. This was especially the case for riverine bacteria, which were confronted with an estuarine salinity level, whereas, interestingly, estuarine bacteria were less affected by a change in salinity. The composition of the bacterial communities developing in the batch cultures differed, as shown by 16S rDNA DGGE, depending on the origin of the inoculum and salinity (Fig. 8). Reverse and direct DNA-DNA hybridization performed on a collection of strains previously isolated from estuarine RDOM enrichment

cultures (Kisand et al 2002) revealed salinity optima in the growth of specific bacterial strains as well as broader phylogenetic groups. Strains belonging to the α- and β-*Proteobacteria*, *Actinobacteria* and γ-*Proteobacteria* other than the genus *Pseudomonas* showed higher relative abundance under freshwater conditions, whereas strains of the genus *Pseudomonas* and the *Bacteriodetes* group were favored by estuarine conditions (Fig. 9).

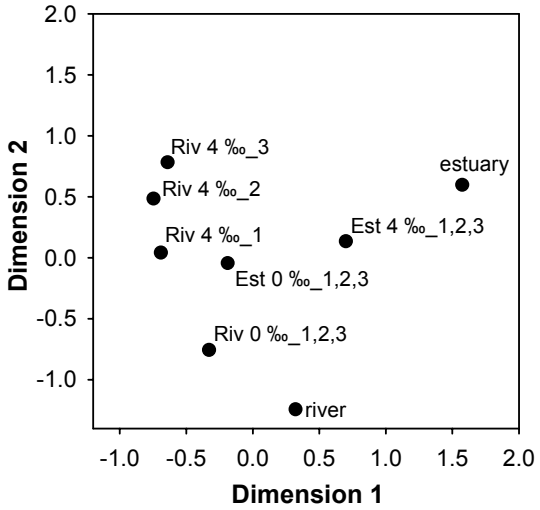

Figure 8. Results from a NMDS analysis on DGGE banding patterns. Treatment assignments are as follows: RIV 0 ‰: riverine bacteria at 0 ‰, RIV 4 ‰: riverine bacteria at 4 ‰, EST 0 ‰: estuarine bacteria at 0 ‰, EST 4 ‰: estuarine bacteria at 4 ‰. For RIV 4 ‰ the three replicate cultures were analyzed separately because they differed from each other. "estuary" and "river" refer to the communities from the original sampling sites

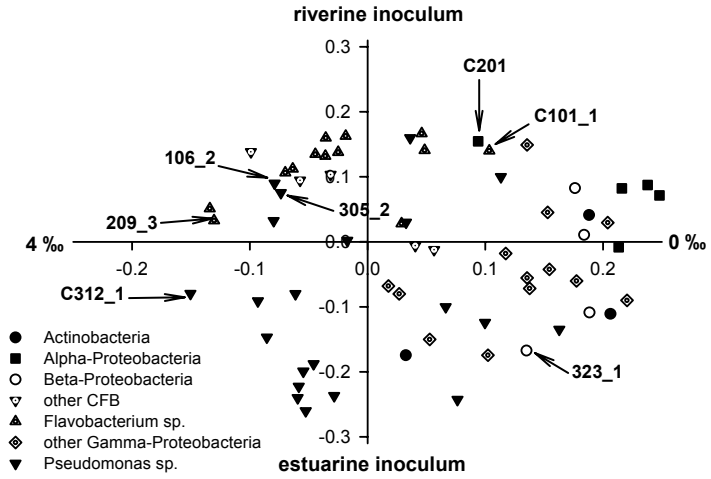

Figure 9. Results of a multivariate discriminant analysis using the relative abundance of strains obtained by reverse DNA-DNA hybridization. The position of the strains is plotted in relation to salinity (horizontal axis) and inoculum source (vertical axis), and are grouped into larger phylogenetic units (see legend). Positions of strains that were followed by quantitative DNA-DNA hybridization are marked by arrows. **209_3**: *Flavobacterium* sp. strain GOBB3-209-3; **C101_1**: *Flavobacterium* sp. strain GOBB3-C101-1; **305-2**: *Pseudomonas* sp. strain GOBB3-305-2; **C312_1**: *Pseudomonas* sp. strain GOBB3-C312-1; **106_2**: *Pseudomonas* sp. strain GOBB3-106-2; **323_1**: *Rhodoferax* sp. strain GOBB3-323; **C201**: *Zymomonas* sp. strain GOBB3-C201). For details see **paper IV**.

Growth dynamics within estuarine and riverine bacterial communities: temporal patterns during DOM utilization (paper V)

This paper describes results from the same experimental set-up as **paper IV.** The major aim of this paper was to investigate if different growth rates and growth dynamics could be observed for taxa enriched in the cultures and whether these could be related to potential changes in the labile DOM pool similarly to the study of Tranvik and Höfle (1987). Moreover we wanted to investigate whether the growth patterns are influenced by environmental stress (i.e. a change in salinity) and/or the source of the inoculum.

Temporal patterns in BCC were compared by DGGE and by following the abundances of seven preselected strains over the time interval from late exponential growth phase to stationary phase. DNA-DNA hybridization results showed that the source of the inoculum (riverine vs. estuarine) was a

stronger regulating factor for temporal dynamics within the studied bacterial communities than salinity. In contrary, only salinity, but not the origin of the inoculum, had an effect on the overall cell yields reached by some of the strains (compare with **paper IV**). This indicates that changes in environmental conditions, such as salinity might operate on a general level and regulate the biomass accumulation of different bacteria, whereas growth dynamics may be mainly regulated by interactions between bacterial community members (e.g. competition). DGGE, in contrast, revealed a gradual increase in band number during the observed time period, which was more pronounced in cultures that received a riverine inoculum compared to those that received an estuarine inoculum. In accordance with the DNA-DNA hybridization results such patterns are most likely due to differences in the initial abundance of different taxa in the inoculum communities that became detectable by DGGE at different stages. However, it cannot be excluded that there was a differentiation into fast- and slow growing types of taxa.

Cloning and sequence analysis of dominant DGGE bands revealed that most taxa were closely related to representatives of the Bacteriodetes and beta-proteobacteria from environments influenced by allochthonous DOM. None of the sequenced DGGE bands matched any of the seven highly abundant strains that were followed by DNA-DNA hybridization.

Note: in paper IV and V the same seven selected strains were investigated by quantitative DNA-DNA hybridisation. There are, however, the following differences strain assignments: Zymomonas sp. GOBB3-C201 ↔ Sphingomonas sp. GOBB3-C201; Pseudomonas sp. GOBB3-106-2 ↔ Pseudomonas sp. GOBB3-101

Discussion

Ubiquity versus metacommunity concept

In the experimental study described in **paper II** we compared two theoretical concepts about the regulation of bacterial community composition at the local scale, the "ubiquity" and the "metacommunity" concept. Differently composed communities emerged from different inocula indicating that - in line with the metacommunity concept (Curtis and Sloan 2004) - the history and distribution of taxa within the source communities was an important regulating factor of BCC. If, as suggested by the "ubiquity concept" (Fenchel and Finlay 2004), certain taxa selected from a single ubiquitous species pool were superior to cope with the conditions in the cultures, we would expect the local communities in the cultures to show larger similarities to each other, as compared to the similarities at the original sampling sites. However, this was not the case. Apparently, the source communities were highly adapted to the sites they originated from and the ability to adjust and grow under the new conditions in the cultures depended on differences in pH. In **paper II** we argue that the extended lag phase before detectable growth commenced is due to a small number of bacteria in the source communities obtained from acidic site being able to grow under the new conditions. Turning the tables, it can also be argued that it takes a long time for rare or cryptic species to reach a detectable level, even if they are growing fast and steadily (**paper II**). Previous experiments that provided convincing evidence for the ubiquity concept (Fenchel et al. 1997) included incubations of a range of enrichment strategies and extended over several month in order to provoke the growth of very rare cryptic species. Hence, the success of rare species might be restricted to environments with stable environmental conditions. These considerations imply that the "metacommunity concept" and the "ubiquity concept" are not mutually exclusive, but rather apply to different time scales and ecological conditions. It appears that the metacommunity concept applies to the time scales that are realistic for pelagic communities and other microbial communities that experience frequent disturbance.

How are bacterial community composition and functioning related to each other?

My thesis suggests that the coupling between bacterial community composition and functioning does not need to be very tight. **Paper I** shows that functional properties were mostly influenced by the source of the medium whereas BCC was influenced by the medium as well as by the origin of the inoculum. This was corroborated by the findings in **paper II**, where different communities that were enriched under identical conditions were similar in their functional performance with regard to carbon utilization. However, as soon as the environmental conditions deviated considerably from the ambient conditions in terms of pH, growth patterns deviated resulting in prolonged lag phases. Hence, in this case there was a more pronounced relationship between community composition and functioning. Additionally, in **paper I** growth on DOM from a polyhumic lake led to the selection of structurally and functionally distinct communities. Apart from this exception, the results tend to support studies showing that similar function can be maintained by differently composed communities (Fernandéz et al. 1999; Findlay and Sinsabaugh 2004).

It is striking that, as long as no extreme deviations occur, the environmental conditions are apparently much more important in determining community functioning compared to BCC. Also, my results suggest that BCC is not very important for processes that are relevant on the ecosystem scale, such as biomass production and respiration. This might be due to the high degree of functional redundancy within the heterotrophic bacteria (Groffman and Bohlen 1999). However, it seems contradictory that bacterial communities that are distinct in composition and differ in their degradative capability, as indicated by ectoenzymatic activities and the ability to utilize benzoic acid, are similar in terms of broader aggregated functions. Similar patterns were however found in zooplankton communities, where community functions, such as productivity, respiration and nutrient retention were similar despite differently composed communities and the authors concluded that the coupling between structure and function is dependent on the scale at which the different parameters are measured (Jenkins and Buikema 1998). Another explanation might be that the low bioavailability of DOM limits the window of opportunity for functional differences. As an example, using the values for biomass production and respiration from **paper II**, we can estimate that roughly 10 %, i.e. 1 mg of the DOC was labile in all cases. This low amount of utilizable DOC may therefore mask any clear effect of BCC on bacterial biomass production and respiration. That is, the same amount of DOC can be consumed in different ways by different bacteria, but due to common

physiological constraint (bacteria grow and respire in a similar manner) we observe no measurable effect on broad-scale functions such as biomass production and respiration. Therefore it seems possible that the size of the labile DOM pool determines bacterial biomass production and respiration and that these functions are to a large extent independent of community composition. Put in another way, the high recalcitrance of natural DOM might account for the stability of ecosystems (Tranvik 1998). All this might delight biogeochemists working with models of global carbon flux, because it indicates that it might be sufficient for their purposes to treat bacteria as a black box to answer questions related to e.g. the effect of climate change on carbon dioxide emissions from lakes.

Many studies using similar re-growth experiments as I did, found weak inoculum effects on community functioning and much stronger effects of the medium (i.e. the "environmental conditions") (Tranvik and Höfle 1987; Fuchs et al. 2000; Gasol et al. 2002). The lack of an inoculum effect was often implicitly interpreted as a sign for bacterial communities in systems with similar function being similar in composition (e.g., Findlay et al. 2003). My results show that this is not necessarily the case.

How is this possible, if other studies show clear changes in BCC after DOM amendments (Pinhassi et al. 1999; van Hannen et al. 1999; Riemann et al. 2000; Burkert et al. 2003; Crump et al. 2003) and clear preferences for utilization of different carbon sources even on a higher phylogenetic level (Cottrell and Kirchman 2000; Covert and Moran 2001)?

I suggest that the effect of an environmental change on BCC depends on the character and magnitude of the perturbation, i.e. the disturbance regime. A low disturbance regime (Fig. 10 A) is characterized by relatively benign changes in environmental conditions. Transferred to our experimental systems it means that the conditions in the cultures are not deviating too much from the ambient conditions. In such systems BCC is to a large extent determined by the inoculum, i.e. the source community, and it depends on the history, distribution of taxa and interactions among taxa within the source communities, which ones are selected (Curtis and Sloan 2004). This may explain, why, like in **paper II**, differently composed bacterial communities are selected from different source communities even under identical conditions. In the "low disturbance scenario" (Fig. 10 A) "ecosystem functioning" (such as DOM utilization, bacterial biomass production and respiration) is determined by the environmental conditions and mostly by the quantity and quality of the DOM. The coupling between bacterial community composition and ecosystem functioning is relatively weak since differently composed bacterial communities are functionally equivalent to each other (Fig. 10 A).

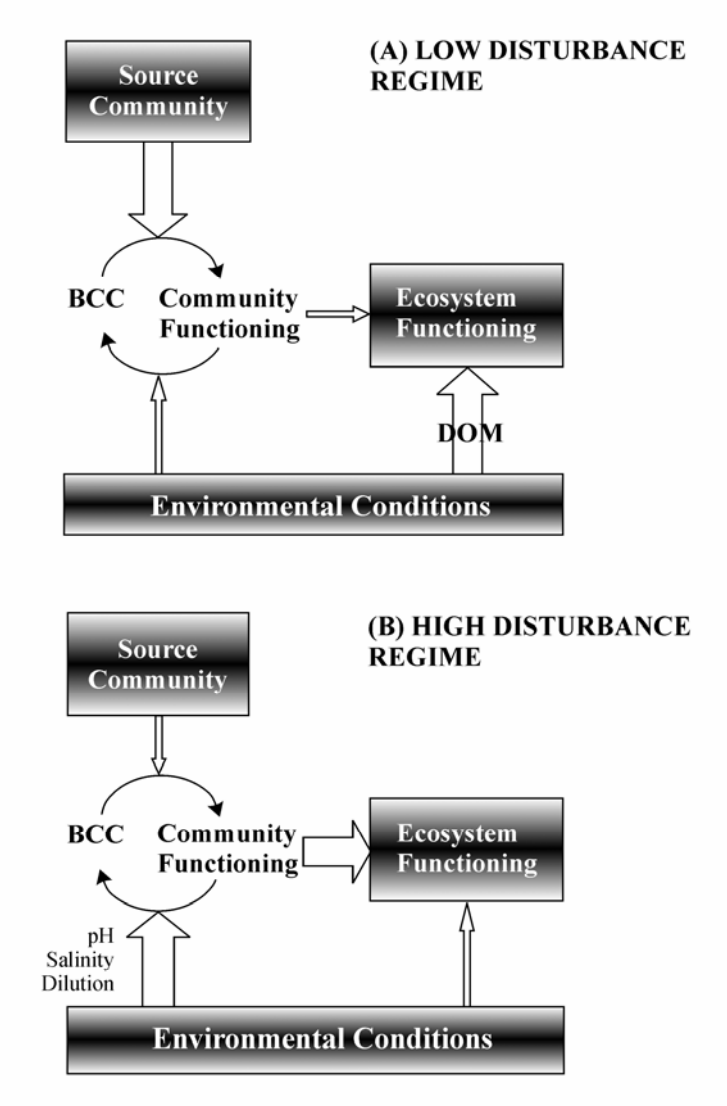

Figure 10. Conceptual model highlighting the coupling between community composition and functioning under different disturbance regimes. Under a **low disturbance regime (A)** BCC is mostly influenced by metacommunity processes and not by the environmental conditions. Ecosystem functioning can be predicted from the environmental conditions and the coupling between BCC and ecosystem functioning is weak because differently composed communities are functionally equivalent to each other. Under a **high disturbance regime (B)** the environmental conditions constitute a strong selective factor for BCC and there is a direct link between BCC and ecosystem functioning. See text for further explanations.

A different scenario arises under a high disturbance regime when the environmental conditions deviate considerably from the original setting (Fig. 10 B), for instance when bacterial communities are exposed to growth at different pH (**paper I and II**), salinity (**paper IV and V**), substrate concentrations (**paper III**) or a clearly differently composed DOM pool (treatments 4.1 – 4.4 in **paper I**). In this case bacterial community composition and functioning are to a much larger extent determined by the environmental conditions. Moreover, changes in BCC as a result of a perturbation are likely to result in changes in ecosystem functioning, e.g. due to higher bacterial respiration rates as a stress response (del Giorgio and Cole 1998).

Figure 10 shows a simple model explaining the results from my culture experiments. This model might also be applicable to a more natural situation such as the regulation of BCC in lakes and other aquatic environments assuming that a disturbance is defined as a clear change in environmental conditions such as eutrophication, pollution or acidification.

Many studies have shown that spatial and even temporal variations in BCC are quite low even when environmental conditions differ or fluctuate (Riemann and Middelboe 2002; Stepanauskas et al. 2003). Hence, the level of disturbance in these natural systems may be within the low-disturbance regime of my conceptual model where the source community is largely shaping BCC (Figure 10 A). Therefore, even if frequent environmental fluctuations occur, they are uncoupled from fluctuations in BCC. A real turnover in BCC within a system might only occur as a result of drastic external influences (e.g. pollution, eutrophication) or between seasons, i.e. for example when temperature decreases drastically during winter (a scenario corresponding to the conceptual model in Fig. 10 B). For instance it is often observed that BCC in the same system in consecutive years in highly variable (Lindström 1998; Crump et al. 2003; Yannarell et al. 2003).

Hence we might speculate that a bacterial community resembles a pack of cards that is regularly reshuffled (after a string perturbation). It then depends on processes occurring on the metacommunity level which cards will be uncovered in the next round. This might indicate that bacteria exhibit similar successional patterns as phytoplankton, where there is a recurrence on the functional group- but not on the species level (Reynolds et al. 2002; Scheffer et al. 2003).

In nature, this model is complicated by the fact that lakes and estuaries are open systems where the relationship between the composition of local and regional bacterial communities depends on the water residence time. Studies have shown that only systems with long water retention times develop their "own" communities in response to local environmental conditions (Crump et al. 2004; Lindström and Bergström 2004). In general, these studies collaborate a strong external control of local community composition.

In **papers I and II** we used t-RFLP to assess the bacterial community composition without obtaining information about the phylogenetic relationships among community members. Even though communities seem to be different from each other, they might still contain highly related, though different, representatives belonging to the same bacterial cluster. It is tempting to speculate that differences in BCC under a low disturbance regime occur on the microdiversity level, i.e. among closely related taxa that are presumably functionally equivalent, whereas larger phylogenetic shifts occur often after more drastic disturbance events. In agreement with these ideas, large bacterial groups exhibit preferences for certain environments, e.g. most beta-proteobacteria prefer non-saline environments (Glöckner et al. 1999) and the Bacteriodetes seem to be effective utilizers of high molecular weight DOM (Kirchman 2002). However, in opposition to the hypothesis presented above it has been shown that closely related or even taxa with identical 16S rRNA sequences can be functionally distinct and do not necessarily belong to the same "ecotype" (Jaspers and Overmann 2004).

Response of community composition and functioning to changes in environmental conditions

In my thesis I focused on two abiotic parameters that are generally thought to be important factors regulating BCC: osmotic pressure (i.e. salinity and pH, **paper I, II, IV and V**) and substrate concentration (**paper III**). The results show that changes in all parameters are important for bacterial community composition and functioning.

Paper III shows that DOM concentrations below 6 mg DOC L^{-1} led to clear changes in BCC and affected bacterial growth efficiency and growth rate. The experiment was performed with a very recalcitrant carbon source, suggesting that in natural systems with a more available DOM the threshold concentration for effects on BCC may be considerably lower. This indicates that exposure to low substrate concentrations comprises a significant perturbation for bacterial communities. Additionally, in such diluted environments, bacterial communities responded quite drastically, both in terms of changes in BCC and functional parameters to even minor changes in DOM concentration. This indicates that such system might be particularly susceptible to changes in environmental conditions.

Paper I and II show that pH seems to be an important factor for BCC and functioning since in particular communities from acidic sites were affected by changes in pH and grew unsteadily and deferred when exposed to neutral

or alkaline conditions. Field studies suggested pH to be an important factor regulating BCC (e.g. Lindström and Leskinen 2002) and my cultures studies confirm this and suggest that pH is a major regulator of BCC in freshwaters.

Paper IV shows that even minor changes in salinity may be a stress-factor for riverine bacterial communities, resulting in changes in BCC, decreased functional stability and lower BGE.

Estuaries are suitable environments to study effects of environmental changes on bacterial community composition and functioning. Riverine bacteria that are transported downstream to the estuary are exposed to steep environmental gradients, including the parameters studied in this thesis (DOM concentration, pH, salinity) but also temperature and nutrient concentrations (Day et al. 1989). Generally little is known about which environmental factors regulate the commonly observed shift in BCC along the estuarine gradient, even though salinity is believed to be of major importance (Bollmann and Laanbroek 2002; Bouvier and del Giorgio 2002; Selje and Simon 2003). **Paper IV** provides one of the first explicit tests of the effect of changes in salinity of free-living estuarine and riverine bacterial communities. The results show that salinity is an important factor determining bacterial community composition and functioning, even though it is not clear to what extent this is a direct effect of salinity acting on the activation/inactivation of certain bacteria groups or an indirect effect mediated by DOM-transformations or modifications in enzyme activities (Stepanauskas et al. 1999a).

DOM concentration usually decreases along the estuarine gradient due to e.g. flocculation of DOM with increasing salinity (Sholkovitz et al. 1978) and subsequent sedimentation as well as dilution due to mixing with marine waters. Generally estuarine DOC concentrations are often (or at least in different seasons) below the threshold level for which DOC concentration is expected to affect bacterial growth rate, growth efficiency and BCC (del Giorgio and Davis 2003, **paper III**). This agrees with seasonal studies showing that bacterial production and growth efficiency can be limited by organic substrates during certain times of the year and some parts along the estuarine gradient (Murrell 2003; Smith and Kemp 2003). DOM quality and composition might also change from the river to the estuary, due to mixing with autochthonous DOM and salinity mediated transformations of allochthonous DOM (Stepanauskas et al. 1999a). An enhancement of biovailability of land-derived DOM under estuarine or marine conditions has been observed (Stepanauskas et al. 1999a; Stepanauskas et al. 1999b; Wikner et al. 1999). In **paper IV** there was no enhancement of DOM bioavailability, and the functional performance of riverine and estuarine bacteria degrading riverine DOM under ambient condition was comparable indicating that estua-

rine bacteria are as efficient degraders of riverine DOM as riverine bacteria. This is contrary to findings by Wikner et al. (1999) for the Öre estuary since they found lower BGE when estuarine bacteria are degrading RDOM compared to limnic bacteria. Potential reasons for these deviating results might have been differences in the DOM-pool or inoculum communities and are discussed in **paper IV**. Generally, growth efficiencies are often found to be higher in estuaries compared to rivers (del Giorgio and Cole 1998). However, it might not be possible to generalize growth efficiencies for estuaries since they are highly dependent on where along the estuarine gradient they are measured. In **paper IV** I found a decreasing BGE when riverine bacteria were exposed to elevated salinities. Moreover, riverine bacteria grew at higher rates and reached lower biomass yields as compared to ambient conditions. Lower growth efficiencies may at least partly reflect the higher energy requirements for the regulations of cellular pH and osmotic pressure as well as membrane energization of the cells (del Giorgio and Cole 1998). A similar pattern was observed by del Giorgio and Bouvier (2002) within the fresh- to saltwater transition zone in a North American estuary. They found changes in single cell activities and cell mortality along with decreased BGE and increased growth rates on the community level. Moreover, Troussellier et al. (2002) observed a decrease in bacterial abundance in the low salinity range of the gradient that coincided with low activities.

Generally, it appears that there is a phylogenetic replacement of bacterial groups along the estuarine salinity gradient that does, however, not result in predictable changes in community functioning, growth and ecophysiology of the community members since these are also regulated by other environmental factors such as temperature, substrate-and nutrient availability and grazing (Cunha et al. 2001; Selje and Simon 2003; Smith and Kemp 2003; Yokokawa et al. 2004).

Figure 11 shows a conceptual model of what **paper IV** implies for the fate of riverine bacteria when confronted with elevated salinities during transport to the estuary. The riverine bacteria community is composed of 1) halo-sensitive bacteria and 2) halo-tolerant bacteria. When exposed to higher salinities a typical estuarine community develops, consisting of halotolerant riverine species and indigenous brackish bacteria. This is consistent with results from e.g. clone library studies showing that estuarine bacterial communities contain a mix of freshwater, marine and indigenous sequences (Crump et al. 1999; Rappé et al. 2000). Moreover, for the Northern Baltic Sea, Kisand et al. (in press) could demonstrate that several species that were abundant and active in the estuary originated from the river. When exposed

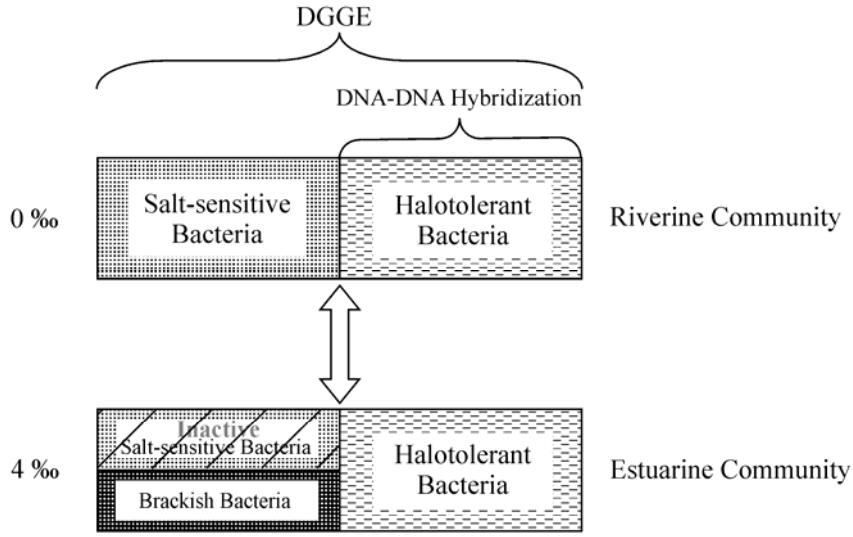

Figure 11. Conceptual model highlighting the fate of riverine bacteria during transport from the river (salinity 0) to the estuary (salinity 4). The riverine bacterial community is composed of salt-sensitive bacteria and halotolerant bacteria. Halotolerant bacteria are not affected by the higher salinities in the estuary but the salt-sensitive fraction is inactivated upon transport to the estuary and replaced by an indigenous brackish bacterial community. DNA-DNA hybridization detected the halotolerant bacteria able to grow under both freshwater and estuarine conditions, whereas DGGE detected all types of community members.

to elevated salinity, salt-sensitive riverine bacteria get inactivated, but can be revitalized when exposed back to freshwater conditions. The later conclusion is drawn based on results found for the treatment in which an estuarine inoculum was grown at 0 ‰, since here DGGE banding patterns showed that the enriched communities had similarities with all other treatments. Almeida et al. (2001) demonstrated that there was a reversible positive response in abundance, activity and production when marine bacteria were exposed to nutrient-rich brackish conditions. In contrary, brackish bacteria that were exposed to marine conditions showed an increase in activity that was irreversible. Even though the underlying factors regulating those patterns cannot be determined, it is tempting to speculate that the increase in activity was at least partly triggered by the increase in salinity. Thus, the study of Almeida et al. (2001) fits into my conceptual model even though it addressed the brackish-marine end of the gradient.

In summary, the results from **paper IV and V** confirm that estuarine bacteria are a mix of immigrating freshwater and indigenous populations (Crump

et al. 1999; Cunha et al. 2000), but also that phylogenetic shifts that occur from low to high salinities along the estuarine gradient include salinity driven inactivation or death of some cells (del Giorgio and Bouvier 2002).

Reverse DNA-DNA hybridization showed that there were preferences for either freshwater or estuarine salinities among 68 strains isolated from estuarine RDOM enrichment cultures. A more detailed analysis following the growth patterns of 7 out of the 68 strains revealed that there were stronger salinity effects on the carrying capacities than on the growth dynamics (**paper V**). This disagrees with studies that showed clearly different growth patterns in co-cultures of marine isolates (Pernthaler et al. 2001) and even among broader phylogenetic groups (Pinhassi and Berman 2003). Among the 7 strains were 3 representatives of the genus *Pseudomonas* belonging to the gamma-proteobacteria, which are generally believed to be opportunistic species favoured by high substrate concentrations (Eilers et al. 2000). These strains did not grow faster compared to the other strains, which suggests that it is not possible to generally classify the gamma-proteobacteria as "fast growing" (Cottrell and Kirchman 2003; Yokokawa et al. 2004). Another example suggesting that variability in growth patterns within bacterial groups might exceed that between groups can be drawn from a comparison of **paper III and V**. It has been argued that alpha-proteobacteria belonging to the genus *Sphingomonas* are oligotrophic slow-growing bacteria (Pinhassi and Berman 2003) which corresponds well with the fact that *Sphingomonas*-related sequences were obtained from the high dilution treatment in **paper III**. On the other hand, **paper V** showed that *Sphingomonas sp*. GOBB3-C201 had a similar growth pattern compared to the other strains. Hence our results do not support that there are intrinsic differences in the growth between the general taxonomic groups. An obvious and likely explanation for the lack of differences in growth dynamics between the 7 strains was that all of them represented the same opportunistic growth type favoured in batch cultures (Eilers et al. 2000) irrespectively of their phylogeny.

There was a rather striking mismatch between the patterns shown by DGGE and by DNA-DNA hybridisation, which are discussed in **paper V** and even in more detail by Kisand et al. (2003). One additional explanation for this deviation that applies especially to the culture study described in **paper IV and V** might be that the two methods detect different fractions of the total bacterial communities (Fig. 11): whereas the DGGE analysis detects changes in total BCC, including salt-sensitive riverine bacteria, the hybridisation approach detects only changes within the halotolerant bacteria since the 68 strains that were investigated by DNA-DNA hybridisation were all isolated from estuarine enrichment cultures. Hence, it becomes understandable why clear difference in BCC were observed with DGGE but not with reverse or quantitative DNA-DNA hybridisation. However, in contrast to DGGE, which is fairly unspecific, since it only potentially detects dominant

community members (Muyzer et al. 1993), the hybridisation approach allowed us to resolve salinity preferences within the “halotolerant groups” and to follow growth dynamics for some group members. Hence, both methods have their justification, but a direct comparison is difficult.

Conclusions and perspectives

Briefly, the following can be concluded form the papers presented in this thesis:

1. Differently composed bacterial communities emerged from different inoculum communities indicating that the history and distribution of taxa within the source community was important in regulating BCC. Even though the "metacommunity" and "ubiquity concept" are not mutually exclusive, my results suggest that the "metacommunity" concept is more relevant in systems that are subject to frequent but benign perturbations. Hence, there is a significant stochastic element in the determination of BCC at the local scale and it cannot be predicted from the environmental conditions alone how bacterial communities are composed.

2. The coupling between bacterial community composition and functioning is not necessarily very tight, i.e. differently composed bacterial communities accounted for similar levels of aggregated functions important at the ecosystem scale (such as biomass production and respiration). However, strong environmental changes, such as exposure of inoculum communities to dilution, changes in pH or salinity and a clearly differently composed DOM pool triggered changes in both community composition and functioning. This indicates that major shift in environmental conditions leads to concomitant changes in structure and function.

3. Exposure to increasing salinity and pH can result in structurally and functionally different, less stable and less predictable communities.

To study how bacterioplankton communities assemble is in my opinion essential to uncover the relationship between community composition and ecosystem functioning. It is important to study bacterial diversity at different scales (α, β- and γ diversity), i.e. to include aspects of regional and local diversity. This will enhance the understanding how important environmental conditions are compared to regional "metacommunity" processes for the determination of bacterial diversity at the local scale. Moreover, the approaches recently applied to soil and salt marsh communities (Green et al. 2004; Horner-Devine et al. 2004b) should employed for the study of plank-

tonic communities as well, to study whether species area relationships exist for aquatic prokaryotes. Such research would aim at confirming/rejecting the hypothesis that spatially structured communities only occur in terrestrial habitats (including sediments), but that free-living bacteria have a global distribution. To include measures of diversity at different levels of taxonomic resolution as a standard into future research might also help to unveil at which taxonomic level cosmopolitan versus endemic distribution patterns occur.

Chase (2003) suggests that environmental conditions are less important for the diversity of local plankton community composition (excluding microbes) in systems (ponds) that show low levels of disturbance. This is in congruence with Conclusion 1 as outlined above and suggests that similar processes are involved in the assembly of micro- as well as "macroorganism" communities. This conceptual framework, however, applies to relatively closed water bodies (or culture systems) and needs to be expanded to integrate both lakes and other water bodies and their catchment areas.

It has been suggested that phytoplankton species composition is unpredictable, but that predictability increases with the aggregation level (Scheffer et al. 2003, and references therein). Hence, it cannot be predicted from the environmental conditions, which species would occur at a certain point in time and space. However, it is possible to make successful predictions at higher taxonomic levels as well as in terms of e.g. biomass production. Therefore it would be essential for the understanding of the connection between microbial diversity and ecosystem function to identify adequate predictable functional groups also for e.g. heterotrophic bacteria. This is a demanding task, but fortunately, there are several recent approaches, including new methods such as metagenomics, proteomics and stable isotope probing, that will certainly enhance our understanding about "who's doing what and under which conditions". Application of these and other approaches will help to identify microbial key players in ecosystem processes, as well as the taxonomic level at which microbial diversity matters in terms of ecosystem functioning.

Studies should also continue aiming at defining to which extent changes in environmental conditions constitute a "disturbance" for microbes and how resistant and resilient microbial communities are. This would considerably increase our knowledge about functional redundancy and the intrinsic value of microbial diversity for ecosystem functioning.

Swedish Summary (Sammanfattning)

Heterotrofa bakterier är avgörande för ekosystems funktion, inte minst genom att de bryter ned organiska ämnen. Beroende på hur effektivt organiskt kol inkorporeras i bakteriernas celler kommer proportionen mellan den mängd kol som respireras, och släpps ut som koldioxid till atmosfären, och den mängd som transporteras vidare genom näringsväven att variera. Vi vet mycket lite om vilka bakterier som finns i sjöar och vad de gör. Detta beror mest på att det tidigare saknades metoder för att studera bakteriers mångfald och bakteriesamhällens sammansättning i naturen. Under senare år har dock stora framsteg gjorts genom användningen av molekylärbiologiska metoder som baserar sig på evolutionära markörgener som t.ex. 16S rRNA-genen. Genom att använda dessa metoder är det nu möjligt att identifiera bakterier direkt i prover från naturen och att studera kopplingen mellan deras diversitet och ekosystems funktion.

Målet med min avhandling är att studera hur ett akvatiskt bakteriesamhälles genetiska sammansättning hänger ihop med de funktioner de har, t ex deras förmåga att bryta ned organiska ämnen. Dessutom studerade jag hur ändringar i miljöbetingelser påverkar bakteriernas sammansättning och funktion.

Jag genomförde olika **experimentella studier** där jag tillsatte bakterier från olika akvatiska miljöer till ett sterilt naturligt medium. Syftet var att studera hur viktigt bakteriernas ursprung är jämfört med miljöfaktorer för bakteriesamhällens struktur och funktion. Mediet manipulerades i vissa försök med hänsyn till pH, salinitet och mängden och sammansättningen av organiskt material. Jag mätte olika funktionella parametrar (till exempel biomassa, respiration, kolnedbrytning, enzymaktiviter) och bestämde bakteriesamhällets sammansättning med hjälp av molekylärbiologiska metoder.

Om det inte sker stora förändringar i miljön kan kopplingen mellan den genetiska strukturen hos bakteriesamhället och funktionen vara ganska begränsad. Det betyder att bakteriesamhällen som domineras av olika arter kan ha liknande funktion på ekosystemnivå, till exempel vad det gäller den uppnådda biomassan och respirationen. Det betyder till exempel att bakteriesamhällets sammansättning är relativt oviktigt för mängden CO_2 som släpps ur en sjö. Detta verkar istället mest bero på det organiska materialets kvali-

tet och den mängd som finns tillgänglig för bakterierna. I min avhandling föreslår jag att detta scenario av funktionell redundans (dvs. att det finns flera olika bakterier som kan utföra samma processer) mellan olika bakteriesamhällen är begränsat till miljöer som är utsatta för frekventa men förhållandevis små störningar. Till exempel så kan det vara relevant för frilevande bakterier i sjöar under stora delar av året. I sådana system behöver inte bakteriesamhällets sammansättning bara bero på de lokala miljöfaktorerna (vattenkemi, predation osv.) utan också på så kallade ”metacommunity”-processer, dvs. slumpmässigt rekrytering från sjöns omgivning.

Om det sker stora ändringar i miljön finns det däremot en tydligare koppling mellan sammansättningen och funktionen. Jag kan till exempel visa att ändringar i pH, salinitet och kolkällans mängd och sammansättning resulterar i samhällen som är strukturellt och funktionellt distinkta. Dessa samhällen är oftast mindre stabila.

Jag genomförde bland annat ett försök för att studera hur älvbakterier från Öreälven i Västerbotten påverkas av höjningen i salinitet när de transporteras ut i Östersjön. Det visade sig att en del av älvbakterierna påverkades negativt av salinitetsökningen, men också att vissa saltresistenta sötvattensbakterier är framgångsrika invandrare i Östersjön.

Sammanfattningsvis så tyder mina resultat på att det kan bero på störningsnivån hur viktigt bakteriesamhällets genetiska sammansättning är för bakteriernas funktion i ekosystemet.

German summary (Zusammenfassung)

Heterotrophe Bakterien sind wichtig für die Funktion eines Ökosystems. In aquatischen Systemen bauen sie im Wasser gelöste organische Kohlenstoffverbindungen ab, und die Effizienz, mit der sie dieses tun, entscheidet über das ”Schicksal” des Kohlenstoffs auf seinem weiteren Weg durch das Ökosystem. Der Kohlenstoff kann in bakterieller Biomasse gebunden und dann eventuell durch Bakterienkonsumenten höheren trophischen Ebenen wie dem tierischen Plankton verfügbar gemacht werden. Die Alternative ist, dass der Kohlenstoff zum größten Teil veratmet wird und als Kohlendioxid in das Wasser und schließlich auch in die Atmosphäre abgeben wird. Wissenschaftler, die sich mit der Ökologie von Mikroorganismen befassen, wissen sehr wenig darüber, welche Bakterien in unseren Gewässern vorkommen und was deren jeweilige Funktion ist. Hauptursache dafür ist, dass es bis vor kurzem kaum Methoden gab, um die Diversität oder die Zusammensetzung einer Bakteriengemeinschaft in der Natur zu bestimmen. Im Laufe der letzten 20 Jahre hat man jedoch Methoden entwickelt, die sich sogenannte evolutionäre Markergene, wie zum Beispiel die 16S rRNA, zu Nutzen machen. Damit ist es jetzt möglich, die Vielfalt von Bakterien („Bakteriendiversität“) und ihren Einfluss auf wichtige Ökosystemfunktionen zu erforschen.

Das Ziel meiner Doktorarbeit war es zu ermitteln, wie sich die Zusammensetzung aquatischer, heterotropher Bakteriengemeinschaften auf die Funktionalität eines Ökosystems auswirkt. Die Kernfragen meiner Arbeit waren: 1) Führt eine Veränderung in der Zusammensetzung zwangsläufig zu einer funktionellen Veränderung und 2) wie wirken sich Veränderungen in den Umweltbedingungen auf die Zusammensetzung und Funktionalität von Bakteriengemeinschaften aus?

Um diese Fragen zu beantworten, habe ich eine Anzahl **experimenteller Studien** durchgeführt. Dazu wurden Verdünnungskulturen verwendet, in denen Bakterien aus unterschiedlichen Lebensräumen auf sterile Seewassermedien angeimpft wurden. Somit war es möglich, den Einfluß des Inokulums (d.h. der ”Bakterienquelle”) im Vergleich zum Medium (d.h. den Umweltbedingungen) zu untersuchen. Die Bedingungen im Medium wurden in einem Teil der Versuche im Vergleich zu den Ausgangsbedingungen verändert. Dabei handelte es sich um Änderungen des pH-Wertes, der Salinität und der Menge und Zusammensetzung der organischen Kohlenstoffquelle. Verschiedene funktionelle Parameter (Biomasseertrag, Wachstumrate, Re-

spiration, Enzymaktivitäten) wurden gemessen und die genetische Zusammensetzung der Bakteriengemeinschaft mit Hilfe verschiedener molekularbiologischer Methoden ermittelt.

Der Zusammenhang zwischen der Zusammensetzung und Funktionalität von Bakteriengemeinschaften ist schwach, wenn die **Umweltbedingungen relativ stabil sind** und nur geringe Schwankungen aufweisen. In diesem Fall ist es möglich, dass Bakteriengemeinschaften von unterschiedlichen Arten dominiert werden, die alle sehr ähnliche Ökosystemfunktionen ausführen, d. h. zum Beispiel ähnliche Biomasseerträge und Respirationsraten aufweisen. Die Zusammensetzung der Bakteriengemeinschaft könnte somit z. B. relativ unwichtig für die Menge an CO_2 sein, die aus einem See freigesetzt wird, da diese demnach von der Menge und Qualität des gelösten organischen Materials, das den Bakterien zur Verfügung steht, bestimmt wird und nicht von der Struktur der Bakteriengemeinschaft. In meiner Doktorarbeit schlage ich vor, dass dieses Szenario der funktionellen Gleichartigkeit unterschiedlich zusammengesetzter Bakteriengemeinschaften auf Lebensräume beschränkt ist, die relativ stabile Umweltbedingungen aufweisen. Dies könnte z. B. für aerobe, freilebende Bakterien in Gewässern über den größten Teil des Jahres zutreffen. In solchen und ähnlichen Lebensräumen wird die Zusammensetzung der Bakteriengemeinschaft nicht nur von den jeweiligen lokalen Umweltbedingungen bestimmt (Wasserchemie, Nährstoffe, Freßfeinde usw.), sondern auch von sogenannten ”Metacommunity”-Prozessen. Dieses bedeutet, dass auch die umgebenen Lebensräume wichtig sind und dass die lokale Bakteriengemeinschaft zu einem gewissen Teil zufallsgesteuert aus der Umgebung rekrutiert wird.

Wenn allerdings die Bakterien **größeren Veränderungen in den Umweltbedingungen** ausgesetzt sind, gibt es eine deutlichere Verknüpfung zwischen Gemeinschaftsstruktur und Funktionalität. In meiner Arbeit konnte ich zeigen, dass größere Veränderungen im Hinblick auf pH, Salinität und der Menge und Zusammensetzung des organischen Kohlenstoffes zu deutlichen Änderungen in der Zusammensetzung der Bakteriengemeinschaften führten, die sich in diesem Fall auch auf die Funktionalität und Stabilität des Systems auswirkten. Somit scheint es von der Stärke einer ”Störung” abzuhängen, ob und wie sich eine Veränderung in der Zusammensetzung einer Bakteriengemeinschaft in einer funktionellen Änderung widerspiegelt.

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