

Processes and factors governing benthic community dynamics—environmental change in the Baltic Sea

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Abstract

As drivers of biogeochemical cycles and nutrient recycling, such as carbon turnover, the microbial community is essential in sustaining functioning ecosystems. Together with the metazoan community, the microbial community constitute the majority of all life in the benthos. Environmental change in biotic and abiotic factors may influence the dynamics of these communities, for example through a sorting or driving effect on the community structure through assembly processes. Environmental change, *e.g.* change in dissolved oxygen concentration, salinity and temperature, can directly or indirectly affect community composition. How, in what way, and to what extent, benthic bacterial and meiofaunal community composition in the eutrophied, brackish benthic environments, in the Baltic Sea sub-basin the Baltic Proper, respond to environmental change is understudied, both at local and seascape scale. This thesis aimed to study and understand the effects of environmental variation on the diversity and biogeographic patterns of Baltic Sea sediment bacterial and meiofaunal communities. A further aim was to understand the links between the different community levels by studying the interaction between meiofaunal- and macrofaunal communities in relation to environmental variation. Community diversity was analysed along a latitudinal transect of national environmental monitoring stations in the Baltic Proper using a framework of metapopulation and metacommunity theory. The analyses were based on environmental genomics, with high-throughput sequencing, bioinformatics and statistics. The total community genome was analysed using phylogenetic marker gene fragments as a proxy for taxonomic diversity, to investigate diversity, community structure and dynamics. Salinity and oxygen were found to be the main abiotic environmental drivers of benthic community composition and alpha- and beta-diversity patterns. Furthermore, macrofauna-meiofauna interactions were significantly more complex in higher salinity environments. Results also showed that both enhanced environmental gradients and dispersal following a major inflow of saline and oxygenated water from the Atlantic Ocean, influenced the composition of sediment bacterial communities at the seascape scale of the Baltic Sea, as shown by a reduced beta-diversity and increased alpha-diversity, and the development of a significant distance-decay of community similarity. This study also identified strong metapopulation dynamics of the benthic sediment bacterial communities with many satellite and a few core taxa. The outcomes from this study contribute to the understanding of how environmental variation and environmental change relate to changes in Baltic Sea benthic community diversity and composition, and important factors and processes governing community dynamics.

Keywords

Benthic communities, Baltic Sea, biodiversity, community composition, environmental change, environmental genomics, microbial ecology, Major Baltic Inflow, meta-population

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List of papers

This licentiate thesis is based on the following papers:

1. **Sommer C.**, Hu Y., Nascimento F., Gunnarsson J., Dinnézt P., Sjöling S. Reduced large-scale beta-diversity and changes in metapopulation patterns of sediment bacterial communities following a major inflow into the Baltic Sea. *Manuscript submitted to Environmental Microbiology*.
2. Broman E., Raymond C., **Sommer C.**, Gunnarsson J., Creer S., Nascimento F. Salinity drives meiofaunal community structure dynamics across the Baltic ecosystem. *Molecular Ecology*; *epub ahead of print*
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Author contribution to papers

Paper 1.

Sommer C. planned the study, performed parts of the sampling and all of the laboratory work. Furthermore, **Sommer C.**, performed the bioinformatics and statistical analysis and wrote the manuscript.

Paper 2.

Sommer C. co-planned the study, performed parts of the sampling and assisted in the laboratory work. Additional contributions were performed in bioinformatics and statistical analysis, and with proofreading and editing the manuscript.

Abbreviations

ASV = Amplicon sequence variant
DADA2 = Divisive amplicon denoising algorithm 2
DNA = Deoxyribonucleic acid
HTS = High-throughput sequencing
MBI = Major Baltic inflow
NBP = Northern Baltic Proper
NGS = Next-generation sequencing
NMDS = Non-metric multidimensional scaling
OFD = Occupancy-frequency distribution
OM = Organic matter
OMZ = Oxygen minimum zones
OTU = Operational taxonomic unit
PCR = Polymerase chain reaction
ppt = parts per thousand
RNA = Ribonucleic acid
rRNA = Ribosomal RNA
SBP = Southern Baltic Proper
SBS = Sequencing by synthesis

Introduction

Factors and processes affecting benthic community compositions

Biodiversity is essential for sustaining many essential ecosystem functions, such as nutrient cycling, and services, like food availability and the provision of clean water (Pan et al., 2013). Microbial communities have a key role in these functions in all ecosystems, including soils, aquatic environments and the human gut, particularly in terms of nutrient turnover, organic matter transformation, oxygen production, pollution degradation and water purification (Falkowski et al., 2008; Konopka, 2009). Despite the existing knowledge of the importance of the benthic microbial community compositions for aquatic ecosystem functions, fundamental questions still remain unresolved (Salazar and Sunagawa, 2013). Also, the effect on microbial communities from major environmental changes, such as climate change and eutrophication (*e.g.* de Vries and Shade, 2013), is significantly understudied. Meiofauna (animals < 1 mm), a very abundant and diverse metazoan group, have been shown to play important roles in a number of ecosystem processes (Bonaglia et al., 2014; Nascimento et al., 2012; Näslund et al., 2010), such as stimulating degradation of organic matter (OM) and bacterial denitrification (Bonaglia et al., 2014). As with benthic microbial communities, there exist important knowledge gaps regarding factors and processes structuring meiofaunal communities, and how the diversity and structure of these communities are affected by environmental change (Bik et al., 2012). This results in uncertainty that increases the risk of underestimating the effects of environmental change on biodiversity at all trophic levels.

Dynamics in microbial composition and assembly processes are often studied at the level of communities, due to microorganism's enormous diversity and the spatial and temporal scales over which they function (Nemergut et al., 2014). Within microbial ecology, microbial communities can be defined as local assemblages of populations of microorganisms that interact with, or influence, other organisms and the environment (Konopka, 2009). The obstacles in analysing microbial communities of high diversity and complexity is reflected in the treatment of microorganisms in ecosystem models, often being lumped together and hidden as kinetic constants in thermodynamic equations in a "black box" manner (Allison and Martiny, 2008). To many researchers, the fields of microbial ecology and environmental genomics aim at filling some of these knowledge gaps while entering the black box.

In this thesis, the main focus is on bacteria and to understand factors and processes that structure bacterial community composition patterns. This is important in order to better predict ecosystem response to environmental change (Martiny et al., 2011). The factors and processes affecting the assembly of bacterial communities can be considered to be acting at a mix of local- and regional spatial scales (Hanson et al., 2012; Lindström and Langenheder, 2012; Nemergut et al., 2013). In 1934, the Baas-Becking hypothesis was formulated as "*everything is everywhere: but the environment selects*" (Baas-Becking, 1934). This species sorting, or selection driven by local contemporary environmental conditions, later became an important part in *e.g.* the metacommunity framework (Leibold et al., 2004; Holyoak et al., 2005; Vellend, 2010), and has repeatedly been

shown to play a major role determining the assembly of microbial communities (Logue and Lindström, 2008). More examples of species sorting include recent studies of marine and brackish environments that have provided a better understanding of how bacterial community composition in the open water co-varies with environmental variables, such as water temperature, oxygen, salinity, habitat type and geographical distance (Herlemann et al., 2011; Amend et al., 2013; Herlemann et al., 2016; Hu et al., 2016; Lindh et al., 2017; Thompson et al., 2017). However, research regarding the understanding of factors behind benthic and brackish community taxonomic diversity variations and dynamics is scarcer (Thompson et al., 2017). Metapopulation dynamics of a collection of subpopulations is considered to mainly be determined by the rate of extinction of subpopulations and colonisation of uninhabited patches (Hanski, 1999). At a large spatial scale, local populations of microbial communities can also be affected by regional processes such as dispersal from surrounding communities (Leibold et al., 2004). The dynamics of the factors and processes described above are still poorly understood and closing this knowledge gap is an important task for microbial ecologists.

Interactions between microorganisms and other levels of life

Another important aspect of any ecosystem is interactions between organisms in direct and in-direct contact (Prosser, 2012). Benthic microbial communities are not only affected by the heterogeneity of the environment and different anthropogenic disturbances related to abiotic factors, but simultaneously also by biotic factors (Louati et al., 2013). Interactions among different benthic organisms have the possibility to influence the composition, diversity and ecosystem functions of microbial communities. Meiofauna have been shown to affect *e.g.* community organic matter mineralisation (Nascimento et al., 2012), bacterial denitrification (Bonaglia et al., 2014) and pollution degradation (Näslund et al., 2010). As an example, infaunal invertebrate's (*i.e.* macrofauna) bioturbation and bioirrigation activities contribute to temporal heterogeneity in oxic and anoxic zones (Bertics and Ziebis, 2009; Pischedda et al., 2008), organic matter (OM) availability (Levin, 1997) and the distribution of metabolic electron acceptors (Fanjul et al., 2007).

Microbial and meiofaunal communities in sediments

Aquatic environments, ranging from small freshwater reservoirs to the immense open ocean, are often endpoints for pollutants from both aquatic and terrestrial anthropogenic activities (*e.g.* Shahidul Islam and Tanaka, 2004), and help buffer the effects of climate change (*e.g.* CO₂-levels and global temperature) (Reid et al., 2009). Microorganisms have been shown to inhabit all domains of aquatic environments, from the photic surface layer all the way down to the aphotic sediment bottoms of the deep ocean (Zinger et al., 2011), and are therefore likely susceptible to being affected in some way. Furthermore, microbial biogeochemical cycling and transformation processes, such as oxygen production or consumption, energy transformation, degradation of pollutants and carbon and nitrogen turnover, sustain life and climate in any aquatic ecosystem (Smil, 2003), including the Baltic Sea.

Knowledge gap

The Baltic Sea is one of the world's largest brackish water bodies. It is surrounded by a large and heavily populated drainage area and has well-documented problems of eutrophication (Andersen and Laamanen, 2009; Carstensen et al., 2014; Fleming-Lehtinen et al., 2015), pollution (*e.g.* Elmgren et al., 2015) and ecosystem modifications (*e.g.* overfishing (Aps and Lassen, 2010)). There are a number of studies of the diversity patterns of microorganisms in the Baltic Sea and the link to environmental factors (*e.g.* Lundberg et al., 2005; Edlund, 2007; Andersson et al., 2009; Tamminen et al., 2011; Herlemann et al., 2011; Thureborn et al., 2013; Lindh et al., 2014, 2015; Bergen et al., 2018; Klier et al., 2018). However, the microbial community diversity and composition under the influence of large-scale environmental change remain understudied (Naeem et al., 1998; Shade et al., 2012; Abram, 2015). This is especially true for benthic communities, where the sediment community has been shown to differ in community structure and functional capacity from water column communities (Thureborn et al., 2013). Moreover, in the Baltic Sea, links and interactions between microbial communities and other trophic levels (macro- and meiofauna) exposed to environmental change must still be considered underexplored. These knowledge gaps are also bottlenecks for succeeding in strong ecosystem simulation models (Bardgett et al., 2008) and practical monitoring applications (Strong et al., 2015).

Study system – the Baltic Sea

Today, the Baltic Sea is the largest brackish water body on the planet with a surface area of 415 000 km². Brackish can loosely be defined as sea water with a dissolved salt concentration between 0,5–30 ppt. This relatively shallow and semi-enclosed sea has a watershed populated by more than 85 million people in 14 countries, nine of these are coastal states, the others have at least rivers that drain into the Baltic Sea. The drainage area is roughly four times larger than the surface area of the sea and has outlets of numerous major rivers creating a surplus of freshwater input annually (Leppäranta and Myrberg, 2009). The Baltic Sea can be divided into the six different major sub-basins, Kattegat, the Danish straits, the Baltic Proper, the Gulf of Riga, the Gulf of Finland and the Gulf of Bothnia Sea (Andersen and Laamanen, 2009). The basins are divided by sills that limit water mixing.

The inflow of water through the Danish Belts and the Swedish Sound from Kattegat to the Baltic Sea are forced by sea level differences, caused by air pressure and wind forcing (Frank et al., 1987). These inflows constitute the source of saline marine water with higher levels of dissolved oxygen, that have the possibility to change the situation dramatically, if the inflows are large enough, from anoxic to oxic in the deeper parts of the Baltic Sea (Mohrholz et al., 2015). Periods between larger inflows are indicative of a more stagnant state (Mohrholz et al., 2015; Gräwe et al., 2015). Inflow events that carry a large enough volume of saline water to reach the bottoms of the central basins of the Baltic sea are called Major Baltic inflows (MBI). Major Baltic inflow events are rare (Mohrholz et al., 2015), but can have a dramatic impact on environmental conditions, especially in deep oxygen-depleted waters (Carstensen et al., 2014). Besides the effect on oxygen content, an increase in salinity (Gräwe et al., 2015) and nutrients (Feistel et al., 2008) have also been described. The first major inflow event for more than two

decades, and the third-largest in 100 years occurred on December 2014. Research describing this event from an oceanographic viewpoint have been published (Mohrholz et al., 2015; Gräwe et al., 2015; Rak, 2016; Neumann et al., 2017; Mohrholz, 2018), and recently also focusing on the dispersal of bacterioplankton communities (Bergen et al., 2018). However, to my knowledge, the effects of this MBI on the benthic ecosystem, and specifically the diversity patterns and composition of the microbial community is not yet described.

The inflow regimes of saline and fresh water may create a latitudinal salinity gradient of surface water, with decreasing salinity from south to north (Matthäus, 2006; Zillén et al., 2008). Density differences between the denser saline water and freshwater results in a permanently stratified water column, and a halocline (often found at depths ranging from 40–80 m), which hinders vertical mixing and thereby oxygenation of the deep hypoxic or anoxic bottom waters (Matthäus and Schinke, 1999).

Other major factors to consider when describing the chosen study system are, of course, climate change and the result of human activities. A study from 2011 based on physical-biogeochemical models showed that under a warming climate, the hypoxic and anoxic area in the Baltic Sea would very likely increase, due to *e.g.* increased nutrient loads and temperatures (Meier et al., 2011). Anthropogenic eutrophication and pollution are especially apparent in the semi-enclosed Baltic Sea with its long retention time (Reissmann et al., 2009; Lehtonen et al., 2010). Furthermore, model studies have projected a decrease in salinity due to increased freshwater run-off, with potentially large effects on taxa at many different levels (Vuorinen et al., 2015). The effect of climate change on the frequency of MBIs is still a debated topic, with both support for a decrease in frequency (Fischer and Matthäus, 1996) and support for no significant long-term effect (Mohrholz, 2018). The high anthropogenic load of nutrients and pollutants (*e.g.* heavy metals, persistent aromatic hydrocarbons and pharmaceuticals) reaching the Baltic Sea via run-off and treated/non-treated sewage water has in a relatively short time period, created what many consider one of the world's most eutrophied and contaminated seas (Elmgren, 1989; Lehtonen et al., 2010).

Eutrophication literally means “well-nourished” and is caused by a high anthropogenic load of nutrients, or internal loading, which leads to high microbial primary productivity. Microbial heterotrophic respiration of dissolved organic matter, primarily from primary production, consumes oxygen, which leads to oxygen depletion in deep waters and sediments (Cederwall and Elmgren, 1990). Hypoxic and anoxic regions (called oxygen minimum zones or OMZ) now make up a major part of the Baltic Sea deep waters and sediments, a situation that is mainly concentrated to the sub-basin the Baltic Proper (Carstensen et al., 2014). OMZ are often considered devoid of life, but are in fact, teeming with microbial organisms displaying a remarkable diversity performing vital ecosystem functions (*e.g.* Wright et al., 2012). However, as mentioned earlier, more knowledge is needed on how environmental change impacts these communities to fully understand if there is in fact, a negative or positive trend in the future.

The choice of the Baltic Sea, especially sampling sites (**Figure 1.**) in the Baltic Proper sub-basin, as the study system in this thesis rests on the following reasons; the characteristics of the Baltic Sea described previously highlight the uniqueness of this aquatic system which makes it an important study subject, and the Baltic Proper sub-basin, with widespread and increasing OMZ, might be the region of the Baltic Sea facing most of the problems at ecosystem level. Even though bacterial community studies in recent years have investigated transitions of bacterioplankton community composition along a salinity gradient (Herlemann et al., 2011; Hu et al., 2016), there have been no studies based on investigations with a larger number of sediment sampling sites along a longitude transect.

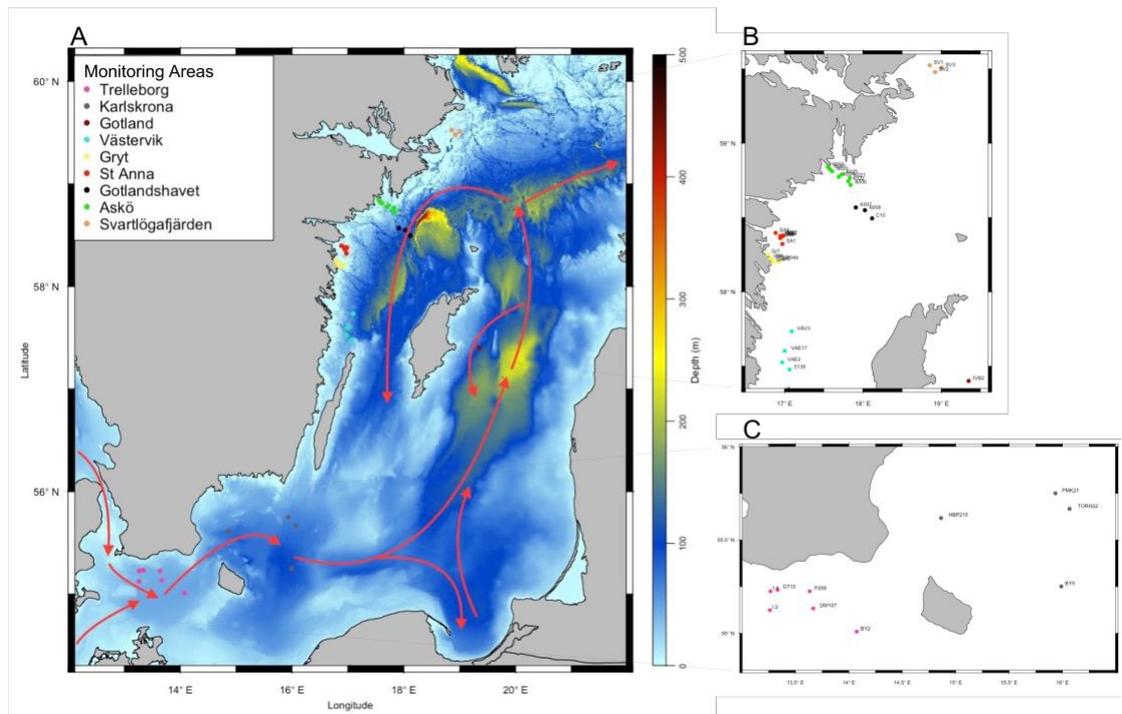


Figure 1. A. Sampling stations of the monitoring areas in the Baltic Proper. Red arrows show the approximate sub-halocline circulation patterns following an MBI, redrawn from Meier, 2007. B-C. Sampling stations in the northern and southern monitoring areas, respectively.

Problem formulation, aims of the thesis and research questions

Problem formulation

Understanding how environmental variation and environmental change affect benthic sediment bacterial diversity patterns, assembly processes and meiofaunal diversity patterns in the Baltic Sea, is still limited. Any adverse effects that impact these communities' ecosystem functions will most likely, in the end, degrade ecosystem goods and service, and ultimately human well-being. It is therefore of vital importance to fill the knowledge gap and improve understanding of Baltic Sea benthic bacterial and meiofaunal community diversity, composition and co-variation with other benthic biota under environmental change.

Aims

The main aim of this thesis is to understand the processes behind brackish benthic community diversity patterns and composition using a framework from metapopulation and metacommunity theory (Nekola and White, 1999; Hanski et al., 1991; Vellend, 2010; Nemergut et al., 2013), with the following objectives:

- to study and understand the effects of environmental variation and environmental change on the processes that drive diversity-, composition- and biogeographic patterns of Baltic Sea benthic bacterial communities.
- to study and understand the effects of environmental variation on the processes that drive composition and diversity patterns of Baltic Sea benthic meiofaunal communities.
- to understand the links between the different benthic community levels by studying the co-variation between bacterial-, meio- and macrofaunal communities under environmental change.

Research questions

To meet the objectives, the following research questions were addressed:

RQ1. How does environmental variation at different geographic scales affect the benthic community composition and diversity patterns in the Baltic Proper?

RQ2. How does large-scale environmental change, exemplified by an MBI, affect benthic bacterial community composition, diversity, and biogeographic patterns of the Baltic Proper?

RQ3. What interactions can be found between meio- and macrofaunal communities in the Baltic proper sediments and are these interactions affected by environmental variation?

Methodological approach and methods

Theoretical framework

The processes that create diversity patterns and affect the assembly mechanisms of microbial communities are potentially diverse and complex and can be studied at many different temporal- and spatial scales. Local processes, such as selection caused by environmental conditions, diversification, interactions with other biota and ecological drift, can affect the assembly of the communities (*e.g.* Leibold et al., 2004; Vellend, 2010; Nemergut et al., 2013). On a larger, regional scale, metacommunity processes, such as dispersal, might be important (Leibold et al., 2004). The influence of historical versus contemporary processes on the communities, *i.e.* along the temporal scale, must also be considered. Several studies have verified this complexity and showed that the microbial communities are assembled and governed by a blend of many of these processes

(Hanson et al., 2012; Lindström and Langenheder, 2012; Nemergut et al., 2013). The dynamics of these processes—the when, why and level of importance—are still poorly understood.

This thesis addresses the knowledge gaps in understanding the processes behind brackish benthic community diversity patterns and composition by using a framework based on metapopulation- (Hanski et al., 1991; Nekola and White, 1999;) and metacommunity theory (Vellend 2010; Nemergut et al., 2013). This framework will help to interpret the impact of local and regional processes, during more stagnant periods of fairly stable environmental conditions and periods of large-scale environmental change. See the introduction of paper 1 for a description of the framework.

Sampling design

This study analysed sediment sampled from 44 monitoring sampling stations in the Baltic Proper region of the Baltic Sea, spanning from outside Trelleborg in the south to Stockholm in the north (**Figure 3**), sampled at two different time points. In some cases, *e.g.* when comparing bacterial diversity patterns and community composition between 2010 and 2015, samples from 42 monitoring stations were used. The sampling design includes a mix of coastal and open ocean bottom sites, and thereby spans a salinity gradient in the Baltic Sea from roughly 5–20 ppt and covers environments above and below the halocline. All sediment and water samples included in this study were gathered and analysed within the Swedish National and Regional Benthic Monitoring Program at Stockholm University.

Diversity analyses

Conventional bacterial diversity analyses, *i.e.* the analysis of the taxonomic diversity of a total community, the sequencing of a phylogenetic marker gene, or a fragment of the gene (metabarcoding for Eukaryota) is a convenient approach. It can include targeted high-throughput sequencing of amplicons of a region of the small ribosomal subunit gene, the 16S rRNA gene, a suitable phylogenetic marker gene (*e.g.* Klindworth et al., 2013). This approach requires less data capacity and offers simpler, cheaper and faster ways of studying the diversity of microbial communities and at a high resolution.

First described back in 1977 (Woese and Fox, 1977), the classification of ribosomal rRNA genes has revolutionised molecular taxonomic research. With the development of the polymerase chain reaction, amplicon sequencing of the small ribosomal subunit rRNA gene (16S rRNA gene in Bacteria and Archaea and 18S rRNA gene in Eukarya) has been successfully used with different methods to study composition and spatiotemporal patterns of microbial communities, *i.e.* for more than three decades (Sinclair et al., 2015). With the new technique of high-throughput sequencing (HTS), sometimes referred to as next-generation sequencing (NGS) or third-generation sequencing, higher throughput and resolution have made it possible to study microbial communities from thousands of samples simultaneously over much finer spatiotemporal scales than previously (Sinclair et al., 2015).

For this study, 16S rRNA gene fragment and 18S rRNA gene fragment analysis using the Illumina Miseq sequencing platform was used. Illumina sequencing technology is based on solid-surface PCR amplification to create clusters of fragments followed by Sequencing by Synthesis (SBS).

Workflow 16S and 18S rRNA gene amplicon sequencing

The process of performing amplicon sequencing of the 16S rRNA gene from start to finish can be divided into several steps. After collection of the sediment samples from either the monitoring stations or controlled experiments, DNA is extracted directly from the sample followed by DNA purification. The extraction method, for nucleic acids from predominantly sediment, used in this thesis work is bead-beating with a special lysing matrix using MP Biomedicals FastDNA and FastRNA spin kits for soil with the FastPrep© instrument ("FastDNA™ SPIN Kit for Soil DNA Extraction (116560200) - MP Biomedicals"; Mincer et al., 2005). The kits have been shown to extract nucleic acids from hard to lyse organisms and show better than, or comparable results with other manufactureres kits (Cruaud et al., 2014). Then two sets of primers are designed, the first set targeting a hypervariable region of the 16S rRNA gene. The hypervariable region V3-V4 of the 16S rRNA gene is a suitable choice (no region with "complete" coverage is known yet) for studying bacterial communities (Cruaud et al., 2014), with the primer pair Bakt_341F (with the degenerate sequence CCTACGGGNGGCWGCAG) and Bakt_805R (with the degenerate sequence GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011; Hugerth et al., 2014). The first primer set is also complemented with necessary Illumina adapters. The second set of primers consists of Illumina sequencing adapters and the very important barcodes (also called indices). Barcoding is a way of tagging amplicons, and thus the reads, from a specific sample with an identifiable marker. This enables identification of reads coming from the different sample stations during data analysis, even if all samples are pooled together prior to sequencing. The DNA and primers are then used in library preparation where two consecutive PCRs amplify and barcode the targeted region. After post-PCR normalisation of DNA concentrations to meet the Illumina sequencing criteria and pooling of the samples to a single tube for easier transport, Miseq sequencing commences at a sequencing facility (SciLifeLab/NGI in Solna, Sweden). For a full description of the procedure, see paper 1.

Amplicon sequencing of the 18S rRNA gene fragment for the analysis of meiofaunal communities, in general, follows the same steps as with 16S rRNA amplicon sequencing, but with some modifications. The modifications are mainly in the DNA extraction and in the use of different PCR primers. Meiofaunal organisms are first separated from the sediment particles using the procedure described by Nascimento, Karlson, and Elmgren (2008), where the sediment first is sieved through a sterilised 40 µm sieve and the retained meiofauna isolated by density extraction performed with three different settling times. For a full description of the procedure, see paper 2 (Broman et al., 2019). DNA from the meiofauna isolates are then extracted with the PowerMax Soil DNA Isolation Kit (Cat#12988; MOBIO) and purified with PowerClean Pro DNA Clean Up Kit (Cat#12997-50, MOBIO). The metabarcoding primers TAREuk454FWD1 (5'-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGAT(C/T)(A/G)A-3') (Stoeck et al. 2010) are then used with Q5 HS

High-Fidelity Master Mix (2x) (New England Biolabs) to amplify by PCR the 18S rRNA gene region, targeting fragments between 365 and 410 bp excluding adaptors and barcodes.

Sequence analysis using bioinformatics and statistics

For the analysis of sequence data both bioinformatics and statistical tools are important. The results from the above-described sequencing are millions of raw paired-end reads (aiming for at least 100 000 reads per sample). From here on, bioinformatic tools and pipelines are used to process the raw sequence data with the aim of both determining the frequency of the unique sequences in the samples (as a proxy for abundance) and assigning a taxonomic affiliation of all reads. I use a recently developed cluster-free method for analysing amplicon sequencing data, called DADA2 that has shown good performance compared to methods based on clustering of operational taxonomic units, OTUs (Callahan et al., 2016).

DADA2, a cluster-free method for Illumina sequenced reads, has been shown to outperform the most commonly used previously mentioned methods in mock community identification comparisons (Callahan et al., 2016). This method deals with amplicon errors differently from other often used methods, which tries to remove them by filtering out low-quality reads and lumping similar sequences together into OTUs. DADA2 infers the exact sample sequences, often called amplicon sequence variants, ASVs, by using the Illumina error profiles, and resolves differences between sequences of as little as one nucleotide. The result is larger numbers of identified real sequence variants (equivalent to species or OTU), and far less spurious sequences than other methods (Callahan et al., 2016). DADA2 is an open-source R-package with a comparably low computational power demand, which cuts the need to run the analysis at supercomputer clusters. The DADA2 workflow can be summarised in the following steps: filtering, dereplication, sample inference, chimera identification, merging of paired-end reads, construction of sequence table and taxonomic assignment. The output of the DADA2-pipeline is an ASV table (similar to an OTU table), where all the abundances of each found ASV for each sample is shown together with the taxonomic assignment. For more details on the complete workflow of DADA2, see (Callahan et al., 2016).

The output from DADA2 together with environmental variables data from each sampling station is further analysed with multivariate statistical analysis tools such as the phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2016) R-packages. The focus of the analysis is to reveal patterns in benthic community composition under environmental change and variation using *e.g.* alpha- and beta-diversity indices, ordinations and correlations with environmental variables. Describing all statistical analyses used in this thesis in detail lies outside the scope of this text, but the statistical analyses used were mainly multivariate statistical analyses, such as NMDS-ordinations, permutational multivariate analysis of variance (PERMANOVA) and analysis of multivariate homogeneity of group dispersions (function *betadisper* in R package *vegan*). Additionally, linear-mixed models and multiple linear regressions were used.

Critical evaluation of methods

Validity and reliability of the methodology and methods that were applied in this thesis, and hence the results, depend on several aspects. Basically, all steps, from the study design and retrieving the actual sample are possibly adding bias that influences the end result of the community composition study, e.g. number of replicates, sampling technique, storage of samples, nucleic acid extraction protocol, PCR, sequencing, bioinformatics and chosen statistical analysis.

Since most microorganisms in an environmental sample are un-culturable or at least hard to culture, environmental genomics is dependent on extracting DNA (or RNA) from ideally all microorganisms in the sample to proceed with the downstream analyses. DNA/RNA extraction biases, meaning that all DNA/RNA might not be extracted or that certain taxa's DNA/RNA are over- or underrepresented, therefore, constitutes the first and perhaps most important limitation to consider in an environmental genomics project (see *e.g.* Feinstein et al., 2009; Willner et al., 2012; Rubin et al., 2014; McCarthy et al., 2015). One of many examples introducing bias during extraction is that certain species are harder to lyse than others, due to the formation of resting cells, cysts or spores (Valentine, 2007). The problem with extraction-biases is also more limiting when focussing on rare taxa (Schloss et al., 2011). The extreme bias associated with culture-dependent approaches (*i.e.* another alternative) must, however, be kept in mind when describing the limitations with extraction-based methods. They often underestimated microorganism numbers and diversity by several orders of magnitude (DeLong, 2009).

Because of their larger size, meiofaunal organisms are traditionally identified by morphological taxonomic techniques. Lately, however, more and more studies of meiofaunal diversity use environmental genomics and HTS of taxonomic marker genes, such as the 18S rRNA gene (Bik et al., 2012; Carugati et al., 2015; Peham et al., 2017). This increases the processing time while requiring no specific knowledge of meiofaunal morphological taxonomy (Carugati et al., 2015). Conversely, both microbial and meiofaunal studies using HTS suffer from the same drawbacks, such as using read frequency as a proxy for abundance instead of absolute abundance, and sometimes poor taxonomic annotation due to limitations of the available reference databases (Carugati et al., 2015; Park and Won, 2018).

If the sample is destined to be analysed by 16S rRNA or 18 S rRNA gene amplicon sequencing, PCR constitutes the next source of possible bias. Some cycles of PCR are almost always included somewhere in the analysis of samples in any environmental genomics project. First of all, the sample source can have a large impact on the result of the PCR. Sediment samples contain known PCR inhibitors such as humic acids (Juniper et al., 2001; Schrader et al., 2012), either affecting enzymatic processes or demanding elaborate purification steps than can introduce their own bias, *e.g.* they often diminish the nucleic acid content with the possibility of stochastically removing material from certain organisms.

The 16S rRNA gene is composed of conserved and up to nine hypervariable regions (V1-V9) (Peer et al., 1996; Ibarbalz et al., 2014). The conserved regions facilitate using so-called universal primers, hopefully, binding to also unknown microorganisms, while the hypervariable region makes species (or another taxonomic level) separation possible. Forward and reverse primers create fragments consisting of both the conserved and the hypervariable region, and these fragments are then later sequenced (Peer et al., 1996). However, the hypervariable regions show varying degrees of variability between taxonomic groups and levels, and there is likely no region that can be used to discriminate between all known microbial lineages (Cruaud et al., 2014). As mentioned earlier, the hypervariable region V3-V4 of the 16S rRNA gene is used during amplicon sequencing in this thesis work. This region has been used successfully by many other studies (Caporaso et al., 2011; Herlemann et al., 2011; Sinclair et al., 2015).

The continued analysis with sequencing of the amplified fragments, bioinformatics and choices made during the statistical analysis are also possible sources of bias. Describing the almost infinite number of choices that can be made during these steps and the effects they might have lies, however, outside the scope of this text and is here just summarised briefly. The Illumina MiSeq platform offers paired-end sequencing runs of up to $\sim 2 \times 300$ bp, with a rapid turnaround time, comparably affordable ultra-deep amplicon sequencing (Glenn, 2011), and with a very low rate of sequencing errors ($< \sim 1$ % per nucleotide) (Knight et al., 2018). There are, however, other platforms that *e.g.* results in longer reads, which could be beneficial if several variable regions of the targeted marker gene are of interest. The bioinformatic pipeline and all the steps therein can have dramatic effects on the targeted end result, *i.e.* a matrix with the abundances of all the sequence variants (taxonomically annotated or not) in all the samples of the study. The problem with sequencing errors was, until recently, handled by clustering similar sequences (usually with a 97 % similarity threshold) into single features, so-called Operational Taxonomic Units, OTUs. This procedure might, however, lead to researchers missing fine-scale biological sequence variation, such as single nucleotide polymorphisms, SNPs, which could possibly affect ecological patterns of diversity (Callahan et al., 2017). This thesis used a clustering-free method that resolves differences between sequences of as little as one nucleotide (DADA2, which is described above). This method, and other similar methods such as Deblur (Amir et al., 2017), is now being recommended by influential microbial ecologists to replace OTU clustering methods because of the higher resolution they offer, and the lower frequency of spurious sequences they generate (Callahan et al., 2017; Thompson et al., 2017; Knight et al., 2018). The table of the abundances of all the sequence variants in all samples is then used, together with sample metadata such as measured environmental variables, to study patterns of alpha- and beta diversity over different scales. Here, the chosen statistical analyses might require different transformations of the dataset to minimise the effect of *e.g.* differences in sampling depth (library size) between the samples (Ramette, 2007; McMurdie and Holmes, 2014; Weiss et al., 2017; Knight et al., 2018).

Central results and Discussion

RQ1. How does environmental variation at different geographic scales affect the benthic community composition and diversity patterns in the Baltic Proper?

Paper 1: Reduced large-scale beta-diversity and changes in metapopulation patterns of sediment bacterial communities following a major inflow into the Baltic Sea.

In paper 1, we analysed the bacterial community composition in sediment sampled at 42 monitoring stations from 9 monitoring areas in both 2010 and 2015 (**Figure 1, Paper 1**). This study showed that local environmental conditions are important in structuring benthic bacterial communities, as community compositions were strongly correlated with salinity and dissolved oxygen content. NMDS-ordination of all monitoring stations based on Bray-Curtis dissimilarities showed clear geographical clustering of the monitoring areas both in 2010 and 2015 along mainly salinity and oxygen driven axes (**Figure 4, Paper 1**). The community compositions of the southern monitoring areas of Trelleborg and Karlskrona were clearly separated along the salinity driven axes from the more northern monitoring areas. Within sampling years, community composition of the monitoring areas was significantly different (PERMANOVA, 2010: $F=5.4$, $p<0.01$; 2015: $F=6.1$, $p<0.01$). This corroborates results from previous studies where salinity was identified as the main driver in structuring both Baltic Sea bacterioplankton communities (Herlemann et al., 2011; Dupont et al., 2014; Hu et al., 2016) and sediment bacterial communities (Thureborn et al., 2013; Klier et al., 2018). Furthermore, results showed bacterial communities with many rare amplicon sequence variants indicating strong meta-population and meta-community dynamics with many satellite and a few core taxa as shown by the strongly single-modal OFDs with an α -mode in the left-most class (**Figure 2, paper 1**). Occupancy-abundance plots showed that ASVs with the highest occupancy also had the highest abundance (**Figure S3, supplementary information paper 1**). The Baltic Sea sediment bacterial community thereby consisted of mainly rare ASV with low abundance, and only a few common ASV were present at all sampling stations. OFD with satellite modes are often found when sampling large areas with large environmental variation (McGEOCH and Gaston, 2002). This pattern is commonly found in aquatic bacterial communities (Rappé et al., 2000; Pedrós-Alió, 2006; Nemergut et al., 2013; Walsh et al., 2016), and it is also characteristic for many other types of organisms (Hanski, 1982; Gaston et al., 2000). A recent spatiotemporal study found that Baltic Sea bacterioplankton communities showed significantly bimodal OFD (Lindh et al., 2017), in line with the core-satellite hypothesis proposed in (Hanski, 1982). A possible explanation of the difference in OFD between benthic bacterial and bacterioplankton communities is the increased dispersal and colonisation rates expected in pelagic ecosystems due to water mixing. This would affect both the dispersal units and the physical environment, and would enhance the development of the right mode in OFD, *i.e.* bimodal OFD.

Paper 2: Salinity drives meiofaunal community structure dynamics across the Baltic ecosystem.

In paper 2, we analysed benthic meiofauna community structure along a salinity gradient in the Baltic Sea proper using metabarcoding of the 18S rRNA gene. Results showed that salinity is the main driver influencing benthic meiofauna diversity and community composition. NMDS-ordination, using Bray-Curtis dissimilarities, and PERMANOVA analysis showed that the majority of the sampling stations were separated in two significantly different clusters, one for stations in the lower salinity northern Baltic Proper (NBP) and a second cluster for the higher salinity southern Baltic Proper (SBP) (**Figure 2a, Paper 2**, $R_2 = 0.35197$, $F = 22.812$, $p < 0.01$). Alpha-diversity was found to be significantly higher in sampling stations in the SBP compared to the NBP (**Figure 3, Paper 2**, One-way ANOVA, $p < 0.01$ for all tested indexes (ACE, Chao1, Shannon diversity)). Looking at all data from all stations, the abiotic variables that best explained the beta-diversity were longitude, latitude and salinity ("bioenv" test in R package vegan, $\rho = 0.73$), further highlighting the importance of salinity in structuring the Baltic Sea benthic meiofaunal communities. When looking at the SBP and NBP separately, the oxygen content was found to be an important abiotic variable, together with latitude and sediment water content in the SBP ("bioenv" test, $\rho = 0.57$) and together with longitude and water depth in the NBP ("bioenv" test, with low correlation $\rho = 0.32$). Corroborating our results, salinity has previously also been shown to influence meiofaunal community structure in other marine environments (Coull, 1988; Lallias et al., 2015). Oxygen is vital for survival for the majority of meiofaunal organisms (Braeckman et al., 2013), and oxygen availability has been shown to cause shifts in community composition in certain meiofaunal taxa, e.g. nematodes (Nguyen et al., 2018). As mentioned above, we found significantly different meiofaunal communities at large-scale when comparing the regions SBP and NBP, others have also found this type of large-scale heterogeneity in other marine environments (Fonseca et al., 2014). The described differences could be caused by e.g. specific salinity preferences of different meiofaunal taxa, and that salinity could act as a dispersal barrier for Baltic Sea benthic meiofaunal species, limiting dispersal of marine species to the north and of fresh water species to the south. In the Baltic Sea, regions with low salinity (<6 ppt) have increased since the 1970s and are predicted to further increase with climate change due to increased freshwater run-off from the large catchment area (Vuorinen et al., 2015). The results from our study indicate that that scenario will lead to a decrease in meiofaunal community diversity.

RQ2. How does large-scale environmental change, exemplified by an MBI, affect benthic bacterial community diversity, composition and biogeographic patterns of the Baltic Proper?

Paper 1: Reduced large-scale beta-diversity and changes in metapopulation patterns of sediment bacterial communities following a major inflow into the Baltic Sea.

In paper 1, we sampled sediment from monitoring stations in 2010 during the stagnant period between 2003-2014 (Gräwe et al., 2015; Mohrholz et al., 2015), when no MBI occurred. Later, in 2015, we sampled the same stations again, but after (or during

depending on the station) the largest MBI in 60 years which started in December 2014 (Gräwe et al., 2015). We analysed the change in bacterial community composition from 2010-2015 using a theoretical framework based on meta-population and meta-community theory predicting a transition from a more heterogeneous community pattern driven by local colonisation-extinction dynamics, towards a more pronounced environmental gradient but with lowered beta diversities. More specifically, we predicted that during periods of fairly stable environmental conditions (*i.e.* the stagnant period) we would expect a decrease in alpha-diversity and an increase in beta-diversity due to ecological drift, but also through local adaptation to habitat-specific environmental conditions (Nemergut et al., 2013; Zhou and Ning, 2017). If environmental conditions are similar at a large scale but locally variable, we would also expect a very weak distance-decay relationship, where local conditions are more important than large-scale environmental gradients.

A large-scale environmental shift in the form of an MBI could change this, both by creating a more pronounced horizontal environmental gradient and by distributing new bacterial taxa to local habitats. According to these theories, we would expect an increase in alpha- and gamma-diversity and a decrease in beta-diversity in response to an MBI-caused distribution of fairly common taxa. Due to the environmental impact of the MBI, we would also expect a more pronounced distance-decay pattern after an inflow. If the MBI is important for dispersal, we would predict a homogenisation of the community composition, *i.e.* that all sites become more similar in community composition. If the MBI also enhances the Baltic Sea oxygen- and salinity gradients, there would be a stronger spatial autocorrelation of environmental conditions.

The results showed that the MBI affected the bacterial community compositions and diversity patterns along the lines of our predictions. Firstly, sampling stations with bacterial communities that underwent the largest changes from 2010 to 2015 were those with the lowest oxygen concentration and salinity in 2010 (**Figure 7 and 8, Paper 1**, Salinity: 2nd order polynomial regression, adjusted $r^2=0.53$, $p<0.01$; Oxygen: linear regression, $r^2=0.24$, $p<0.01$). This indicates a strong effect of the 2014 MBI, both from selection caused by the environmental change instigated by the inflow of saline and oxygenated water, and by increased dispersal and colonisation by taxa from more saline and oxygen-rich environments. The diversity of the bacterial communities was quantified with alpha-diversity using Shannon diversity, beta-diversity using the Bray-Curtis dissimilarity index and using the total number of unique ASV among all sampling stations within a year as gamma-diversity. Results showed that gamma-diversity of the sampled areas of the Baltic Sea increased, from 2010 to 2015. There was also a significant increase in alpha-diversity (**Figure 3, paper 1**, linear mixed-model, $z= 21.98$, $df=7$, $p<0.01$). The increase in gamma-diversity indicates an inflow of new taxa with the MBI in 2014. The simultaneous increase in alpha-diversity supports our prediction that a general increase in dispersal rates following the MBI will proliferate the distribution of common taxa, which would reduce beta-diversity among sampling sites in the Baltic Sea. Since gamma-diversity is often described as the result of alpha- and beta-diversity either added or multiplied (Whittaker, 1972), we predicted that beta-diversity would decrease in 2015 if both alpha-diversity and gamma-diversity increased. This was the result with a significantly reduced beta-diversity in 2015 compared to 2010 (**Figure 5B**,

Paper 1, $\chi^2= 4.41$, $df=1$, $p=0.036$). Some experimental field studies have shown that large-scale environmental change or disturbances can decrease beta-diversity by filtering out taxa unfavoured by the new environmental conditions (Chase, 2007; Berga et al., 2015). If the environmental change could lead to increased dispersal possibilities, as is the case with an MBI, it could increase colonisation by taxa adapted for the new environmental conditions, thus also leading to decreased beta-diversity (Berga et al., 2017).

The biogeographic pattern of distance-decay, *i.e.* the decreasing similarity in community composition with increasing spatial or environmental distance, or both, was not found during the stagnant period in 2010, but after the MBI in 2015 these patterns had developed. The significant distance-decay relationships were verified with both Mantel tests (**Figure 6B, paper 1**, spatial distance: Mantel statistic $r=0.70$, $p<0.01$, permutations=10⁴; **Figure 6D, paper 1**, environmental distance: Mantel statistic $r=0.53$, $p<0.01$, permutations=10⁴) and when controlling for spatial autocorrelation (Multiple linear regression, adjusted $r^2=0.62$, $\beta_{\text{geographical distance}}=0.56$, $\beta_{\text{environmental distance}}=0.36$). During periods with low dispersal rates, local environmental conditions will be more important than large-scale environmental gradients. With an MBI, there will be more pronounced large-scale environmental gradients and increased dispersal rates. Therefore, we expected a pronounced distance-decay relationship after an inflow. As mentioned above, we found no distance-decay relationship in the metacommunity in the late part of the stagnant period in 2010, but we found a significant distance-decay relationship after the MBI in 2015. The distance effect in 2015 was persistent even after controlling for the influence of environmental conditions. Geographical distance explained more of the variation in community similarity than did environmental distance. This agrees with our prediction of an absent distance-decay relationship when the environmental conditions are fairly similar over a large seascape but locally variable.

RQ3. What interactions can be found between meio- and macrofaunal communities in the Baltic proper sediments and are these interactions affected by environmental variation?

Paper 2 Salinity drives meiofaunal community structure dynamics across the Baltic ecosystem.

In paper 2, we conducted correlation network analysis (Röttjers and Faust, 2018) to study the potential biotic interactions between macro- and meiofauna communities, and possible community-based niches based on the measured abiotic environmental variables. We normalised sequence counts as proportions per sample and used spearman correlations with ρ thresholds ≤ -0.7 or ≥ 0.7 , and Fisher's z p -value threshold <0.05 with Bonferroni adjustment for multiple-test correction. We identified salinity as the main driver of beta-diversity of the meiofaunal communities, and the sampling stations were significantly separated into the clusters we called NBP and SBP. To strengthen the correlation network analysis, therefore, we divided the dataset in these two clusters to remove influences of heterogenous local environmental factors in a large ecosystem-wide dataset (Röttjers and Faust, 2018). The correlations networks were strikingly different between NBP and SBP, with NBP showing very few significant

correlations (**Figure 7a, paper 2**) while the SBP showed a complex web of significant correlations among abiotic variables, macrofauna and meiofauna (especially Nematoda genera) (**Figure 7b, paper 2**). In the NBP, the only correlations between macro- and meiofauna were a cluster of two Mollusca species, the bivalve *Mya arenaria* and low abundant meiofaunal nematodes and arthropods (**Figure 7a, paper 2**). Conversely, in the SBP many positive correlations between macro- and meiofauna were identified, such as between the Nematoda genus *Microlaimus* and the macrofauna crustacean *Diastylis rathkei*, between the Platyhelminthes genera *Odontorhynchus* and the macrofauna *Nephtys caeca* and between Annelida macrofauna species such as *Pygospio elegans*, *Polydora quadrilobata*, and *Heteromastus filiformis* with low abundant meiofauna genera (**Figure 7b, paper 2**). Furthermore, bottom oxygen content was one of the central nodes in the SBP correlation networks, showing the well-known importance of oxygen for macro- and meiofaunal diversity (**Figure 7b, paper 2**). Our results also showed that macrofauna species richness and meiofaunal diversity were both higher in the SBP (**Figures 6 and 3, respectively, paper 2**). Macrofaunal activities, such as bioturbation, have been shown to increase meiofaunal diversity possibly by creating more habitable niches and providing a higher variety of food types (Meysman et al., 2006). Many of the identified correlations in the SBP were with Annelida and crustacean macrofaunal species, that have been identified as bioturbators (Krantzberg, 1985). The burrows made by annelids or other macrofauna could also possibly increase bacterial growth, thereby making niches favourable for meiofauna (reviewed in Olafsson, 2003). We have not yet looked into the possible interactions between the three trophic levels bacteria, meiofauna and macrofauna, which is a possibility because of the same sediment samples being used in paper 1 and paper 2 (see Future outlook below), but this could help shed light on the processes and factors affecting community assembly on all these levels of life. Taken together, our results indicate that there are fewer meiofauna-macrofauna interactions in areas of the Baltic Sea with lower salinity, and that meiofauna-macrofauna interactions were found to play a main role in structuring and increasing benthic diversity in regions with higher salinity. Our results show that, in the Baltic Sea and similar coastal systems, a decrease in salinity resulting from accelerated climate change will likely lead to a decrease in benthic biodiversity, and cause profound changes in benthic communities, with potential consequences for ecosystem stability, functions and services.

Conclusions

This thesis is based on a large-scale environmental field study in the Baltic Sea investigating the effects of environmental variation and environmental change on benthic bacterial and meiofaunal community diversity patterns and composition. Using state-of-the-art environmental genomic techniques and advanced multivariate statistical analyses, I show the immense diversity of the studied communities at the highest resolution. Salinity and oxygen were found to be the main abiotic environmental drivers of community composition and alpha- and beta-diversity patterns. Macrofauna-meiofauna interactions were significantly more complex in higher salinity environments. The Baltic Sea, with already well-known problems of bottom areas with increasing hypoxia and anoxia, and with predicted future lower salinities due to increased freshwater run-off instigated by climate change, is thereby at risk of reduced

diversity in the benthic communities. Major inflows of saline marine water from the Atlantic Ocean are considered to be a force that can mitigate the decrease in diversity. Therefore, I also studied how the benthic bacterial community diversity patterns and composition were affected by the largest MBI during the last 60 years. I found that both enhanced environmental gradients, and dispersal following an MBI, influenced the composition of sediment bacterial communities at the seascape scale of the Baltic Sea, as shown by a reduced beta-diversity and increased alpha-diversity, and a significant distance-decay of community similarity. This study also identified strong metapopulation dynamics of the benthic sediment bacterial communities with many satellite and a few core taxa. The fact that the identified community diversity patterns changed after the stagnant period in 2010 shows the importance of movements of large-scale water bodies affecting benthic bacterial communities through changes in large-scale environmental gradients and dispersal patterns. This thesis work contributes novel knowledge important for research on the effects of environmental variation on benthic meiofaunal and bacterial diversity patterns and composition, and of large-scale environmental change on benthic bacterial community compositions and diversity patterns. With the improved understanding of the diversity patterns and metapopulation dynamics of Baltic Sea benthic communities, and the integration of microbial community sampling efforts with Baltic Sea monitoring programs pioneered in this thesis work, more reliable future predictions and ecologically relevant scenarios can be made on how these communities may change as a consequence of environmental variation. This will be important in light of the predicted effects that climate and oxygen changes may have on the aquatic ecosystem, in particular, the Baltic Sea.

Future outlook

The data generated in paper 1 is very information-rich and there are still many potential questions that could be answered with this data. Questions such as; were different taxonomic groups introduced to the Baltic Sea by the MBI, or looking even more at the beta-diversity patterns using the Raup-Crick beta-diversity metric to try to even more disentangle the importance of different assembly processes such as ecological drift, dispersal limitation, selection and mass effects based on the procedure described by Stegen et al. (2013).

Furthermore, in collaboration with co-authors at Stockholm University, there is a possibility to investigate the potential co-variation of bacterial community composition with meio- and macrofaunal communities, using the same Baltic Proper sediment samples of May 2015. An aim would be to test the hypothesis that bacterial diversity provides meio- and macrofaunal diversity. Analyses of co-variation in community structure between trophic levels and response to environmental change may advance our understanding of the tangled web of interactions among trophic levels of the benthos.

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