Simone I. Böer

Investigation of the distribution and activity of benthic microorganisms in coastal habitats

Bremen, September 2008

Investigation of the distribution and activity of benthic microorganisms in coastal habitats

Dissertation
zur Erlangung des Doktorgrades der Naturwissenschaften
- Dr. rer. nat. -

Dem Fachbereich Biologie/Chemie
der Universität Bremen
vorgelegt von

Simone I. Böer

Bremen
September 2008

Die vorliegende Arbeit wurde in der Zeit von März 2004 bis November 2008 am Max-Planck-Institut für marine Mikrobiologie in Bremen angefertigt.

Gutachter:

Prof. Dr. Kai Bischof (Erstgutachter)
Prof. Dr. Antje Boetius (Zweitgutachterin)

Prüfer:

Prof. Dr. Wilhelm Hagen
Dr. Alban Ramette

Weitere Mitglieder des Prüfungsausschusses:

Christina Bienhold
Petra Pop Ristova

Datum des Promotionskolloquiums: 24. November 2008

Acknowledgements

This thesis would not have been possible without the support of many helping hands I would like to acknowledge here:

I would like to thank my advisor Prof. Dr. Antje Boetius for accepting me as a PhD student, for the opportunity to carry out this work at the Max Planck Institute and for supporting me throughout my work.

The reviewers of my thesis and members of my defense committee are gratefully acknowledged.

I am very indebted to Alban Ramette for introducing me into the (no longer) secret world of multivariate statistics and ARISA and his invaluable support in improving this thesis, including critical manuscript reading. He always found time to answer my numerous questions.

Very special thanks to Carol Arnosti for giving me the possibility to do part of the research in her laboratory at the University of North Carolina in Chapel Hill and for her very motivating support with the Hausstrand activities manuscript in times I thought I could never finish the piles of work.

Michael Böttcher and Abdul Al-Raei are thanked for splendid sampling campaigns at the Königshafen field site and many interesting scientific discussions.

I would further like to acknowledge Justus van Beusekom for hosting me in the Hafenlabor during Hausstrand field campaigns and for valuable insights into Wadden Sea ecology.

Very, very special thanks to Stefanie Hedtkamp for letting me know everything about the work with sands, for shivering with me in the field and cold room, for a fun time during my stays on Sylt, both within and outside the lab, for uncountable cups of fresh hot tea or coffee, for constant provision of cookies, chocolate and meals and everything else I may have forgotten. This thesis would not have been possible this way without her invaluable support.

Many, many thanks also to Susanne Menger for her wonderful technical support in the field and laboratory on Sylt, for a fun time during long car rides and during work and for her delicious spaghetti with tons of garlic.

Hannelore Halliger, Tatyana Romanova and the other technical staff on Sylt are gratefully acknowledged for the logistical and technical support during field campaigns, Tanya particularly for the nutrient analyses.

Nils Volkenborn is kindly acknowledged for introducing me into the lugworm exclusion experiment.

Thanks also to all students on Sylt for their hospitality during long Sylt sampling campaigns, particularly to Tina and Poldi for coffee breaks and dog walks on the beach. Many thanks also to Steff, Nils, Imme, David, Norma and Amanda for enjoyable dinner breaks in between sampling points and their hospitability in the “Gemeindehaus-WG” when guest houses were again overcrowded. They made my Sylt stays an unforgettable time.

Gabi Schüßler, Imke Müller, Tomas Wilkop, Viola Beier, Martina Alisch, Sabine Kühn, Silke Wetzel, Axel Nordhaus and all the other technicians are gratefully recognized for their invaluable help in the laboratory, particularly Gabi, Sabine and Silke who performed part of the bacterial growth measurements and fragment analyses, respectively. Furthermore, Bernd Stickford is acknowledged for his help in the library, and for ordering many books and articles not available. Also, all the people in the administration and computer department are thanked for their organizing abilities.

Very, very special thanks to my wonderful office mates (and friends) Gunter and Jan who accompanied me throughout my entire Ph.D. and were a never ending source of information about nearly everything, including Japanese knife forging, philosophy, all sorts of technical equipment I had a hard time to even remember the names of, sports and everything else one could imagine. Thanks for the scientific and non-scientific discussions, the deep insights into men and especially for the fun time!

I would further like to thank all my other friends, colleagues and former office mates at the institute: Enoma, Phyllis, Alban, Janine, Anna, Moritz, Hang, Friederike, Matthias, Angélique, Kyriakos, Lars, Sergio, Elsabé, Fanni, Felix, Perran, Hans, Frank, Steffi and all the others I may have forgotten. Thanks for the pleasant time, nice working atmosphere, fruitful discussions and all the support.

Special thanks also to all my friends outside the MPI, Maike, Julia, Daniel, Konstanze, Yvonne, redredred and the O-town A.M. for reminding me that there is a life besides science and for always keeping the faith in me.

I am deeply grateful to Matthias who accompanied me with his love, patience and never ending support.

Zuletzt möchte ich meiner Familie danken, meinen Eltern und meinen Geschwistern für ihre stetige Unterstützung während meiner gesamten Ausbildung und dafür, dass sie immer an mich geglaubt haben.

Table of Contents

Abstract……I

Zusammenfassung……..……..III

Chapter 1: Introduction……..……1

1.1 The Continental Shelf.....1

1.2 Cycling of organic matter in the shelf seas…….4

1.3 Particle transport and solute exchange processes over the sediment-water interface…….9

1.4 The special role of sandy sediments in coastal benthic ecology……...15

1.5 Diversity of plants and animals and their distribution……...16

1.6 Temporal community patterns of plants and animals……19

1.7 Microbial diversity and distribution……..22

1.8 Temporal patterns in aquatic microbial communities……...24

1.9 Temporal microbial community patterns in marine sediments…….26

1.10 Objectives of this thesis…….28

1.11 Materials and methods……...32

Chapter 2: Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment…..……49

Chapter 3: Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments……..67

Chapter 4: Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sandy sediments…….101

Chapter 5: Spatial and temporal variations in bacterial activities and community structure associated with faunal exclusion experiments in intertidal sands of the North Sea (Sylt)……..133

Chapter 6: Summary and outlook……..……169

Appendix……...…...187

Abstract

Coastal sandy sediments play an important role for global carbon and nutrient cycles. Key organisms in coastal sands are the microphytobenthos and diverse heterotrophic bacteria. Unfortunately, the understanding of temporal and spatial patterns in microbial carbon turnover and the link to dynamics in community structure in coastal sands is very limited. In this dissertation thesis, temporal and spatial variations in bacterial activities and community structure were investigated in subtidal permeable sediments of the temperate German Wadden Sea. The first part (Chapter 2 and 3) focuses on the coupling between bacterial activities and primary production of the microphytobenthos (MPB), in the second part temporal and spatial variations in bacterial community structure were investigated via molecular fingerprinting (ARISA), and the environmental drivers of these variations were identified by multivariate statistics.

Microphytobenthic and bacterial production were tightly coupled both in subtidal and intertidal sandy sediments. The addition of a $H^{13}CO_3^-$ pulse and subsequent uptake of ^{13}C by bacteria and MPB was tracked in a mesocosm experiment. Large amounts of the carbon fixed by the MPB were excreted in the form of carbohydrates and rapidly utilized by the heterotrophic bacterial community. Under nitrogen-limited conditions maximum amounts of carbohydrates were excreted through an "overflow metabolism" and the coupling to heterotrophic activity was very tight. However, even under nutrient-replete conditions, carbohydrates were excreted in high amounts.

This tight coupling between the MPB and the benthic bacteria was confirmed *in situ* by sampling of subtidal sandy sediments at the so-called *Hausstrand* site and in intertidal sandy sediments in the *Königshafen* bay (both on Sylt). The sediments showed strong vertical gradients in bacterial abundances, bacterial carbon production and extracellular enzymatic activities. These vertical gradients were tightly correlated to MPB biomass. Despite the low organic carbon content typical for surge-exposed sandy sediments, high extracellular enzymatic activities and bacterial carbon production rates indicated a very active heterotrophic bacterial community. At *Hausstrand*, a gross secondary productivity of 30-180 mmol C m^{-2} d^{-1} was estimated with a bacterial biomass turnover time of 2-18 days. The data suggests that this high activity was supported by the rapid flux of carbohydrates from MPB primary productivity to the heterotrophic bacteria. The average total bacterial carbon demand of 120 mmol C m^{-2} d^{-1} (peaking in summer with up to 360 mmol C m^{-2} d^{-1}) could be covered by a daily turnover of on average 6% of the total carbohydrate pool, or 11% of the MPB biomass. The potential extracellular enzymatic hydrolysis of carbohydrates could supply

approximately 110% of the total bacterial carbon demand at *Hausstrand*. Benthic oxygen consumption rates and bacterial activities strongly varied with temperature whereby the enhanced activities in summer are presumably the result of a more productive MPB community at higher temperatures, a pattern observed also at the *Königshafen* site. In contrast, a lack of temporal variation in MPB and bacterial biomass was observed at both sites, suggesting that grazers control bacterial and MPB biomass in coastal sands.

The vertical and temporal changes in bacterial activities at both sites were accompanied by significant shifts in bacterial community structure. The vertical shifts could mainly be related to the vertical gradients in MPB-associated labile organic carbon availability and redox potentials. At *Hausstrand*, almost half of the operational taxonomic units (OTU) occupied the entire depth range (15 cm), while smaller numbers of OTUs were apparently restricted to the specific biochemical and physical conditions prevailing in specific sediment horizons. The bacterial diversity increased with depth, presumably as a result of the strong physical disturbances at the sediment surface.

The temporal changes in bacterial community structure at *Hausstrand* were investigated over two years. It did not reflect the cyclical seasonal patterns in temperature, substrate availability and bacterial activities, but was rather unpredictable; no specific OTUs were detected exclusively associated with summer or winter sampling months. The temporal dynamics in the bacterial community structure were very high with an OTU replacement of 47% over two years. Community structure was most dynamic within the first 6 months of the sampling period and stabilized towards the end.

At the *Königshafen* site, the bacterial community structure was investigated in different plots of a large-scale exclusion experiment of the lugworm *Arenicola marina*. The bacterial community structure varied not only temporally but also spatially. The observed variations were mainly linked to shifts in MPB abundances and productivity and thus by the availability of MPB-associated labile organic carbon in the sediment. The spatial variations were thereby related to the spatial variations in sediment composition. Large-scale exclusion of the lugworm *Arenicola marina* enhanced the biomasses and activities of the bacterial and MPB community; however, it did not affect bacterial community structure.

Zusammenfassung

Sandige Küstensedimente spielen eine wichtige Rolle für die globalen Kreisläufe von Kohlenstoff und anderen Nährstoffen. Schlüsselorganismen für zahlreiche biochemische Umwandlungsprozesse in marinen Sanden sind Mikroorganismen, vor allem das Mikrophytobenthos und eine Vielfalt von Bakterien. Leider gibt es bisher nur wenig Kenntnis über den Zusammenhang zwischen der zeitlichen und räumlichen Dynamik der Struktur mikrobieller Gemeinschaften und ihrer Funktion und Aktivität im Ökosystem. In dieser Doktorarbeit wurden zeitliche und räumliche Veränderungen in der Aktivität und Zusammensetzung von Bakteriengemeinschaften in subtidalen permeablen Sedimenten des klimatisch gemäßigten Deutschen Wattenmeeres sowohl experimentell als auch durch Feldbeobachtung und –beprobung untersucht. Der erste Teil der Arbeit (Kapitel 2 und 3) konzentriert sich auf die Kopplung zwischen bakterieller Aktivität und mikrophytobenthischer Primärproduktion, im zweiten Teil wurde mittels molekularem Fingerprinting (ARISA) untersucht, wie die Zusammensetzung der bakteriellen Gemeinschaft zeitlich und räumlich variiert und mittels multivariater statistischer Analysen jene Umweltparameter identifiziert, die für diese Veränderungen verantwortlich sind.

Die mikrophytobenthische und die bakterielle Produktion waren sowohl in subtidalen als auch in intertidalen sandigen Sedimenten eng aneinander gekoppelt. Die Aufnahme von ^{13}C durch Bakterien und Mikrophytobenthos nach Zugabe von $H^{13}CO_3^-$ wurde in einem Mesokosmos-Experiment verfolgt. Große Menge des von den mikrophytobenthischen Algen fixierten Kohlenstoffs wurden in der Form von Kohlenhydraten ausgeschieden und schnell von der heterotrophen bakteriellen Gemeinschaft verwertet. Unter Stickstofflimitierung wurden in Folge eines „Überfluß-Stoffwechsels“ maximale Mengen an Kohlenhydraten ausgeschieden und die Kopplung zur bakteriellen Aktivität war sehr eng. Jedoch auch unter Nährstoffsättigung wurden große Mengen an Kohlenhydraten abgesondert.

Diese enge Kopplung zwischen Mikrophytobenthos (MPB) und benthischen Bakterien konnte im Rahmen von zwei *in situ* Studien in subtidalen Sandsedimenten am sogenannten *Hausstrand* als auch in intertidalen Sandsedimenten in der *Königshafen* Bucht (beide auf Sylt) bestätigt werden. Die Sedimente zeigten starke vertikale Gradienten in Bakterienanzahl, bakteriellem Wachstum und extrazellulären enzymatischen Aktivitäten, welche eng an die mikrophytobenthische Biomasse gekoppelt waren. Trotz des für sandige Sedimente typischen geringen organischen Kohlenstoffgehaltes, wiesen hohe extrazelluläre Enzymaktivitäten und bakterielle Wachstumsraten auf eine sehr aktive heterotrophe Bakteriengemeinschaft hin. Für

den Untersuchungsstandort *Hausstrand* wurde eine Gesamtsekundärproduktivität von 30-180 mmol C m^{-2} d^{-1} mit einer bakteriellen Generationszeit von 2-18 Tagen geschätzt. Die Daten legen nahe, dass diese hohe Aktivität durch den schnellen Fluß von Kohlenhydraten aus der mikrophytobenthischen Primärproduktion zu den heterotrophen Bakterien gefördert wurde. Der durchschnittliche Gesamtkohlenstoffbedarf der Bakterien in Höhe von 120 mmol C m^{-2} d^{-1} (mit einem Höchstwert von bis zu 360 mmol C m^{-2} d^{-1} im Sommer) konnte potenziell durch einen täglichen Umsatz von 6% der Gesamtkohlenhydrate oder 11% der MPB Biomasse gedeckt werden. Die potenzielle extrazelluläre enzymatische Hydrolyserate von Kohlenhydraten konnte durchschnittlich circa 110% des bakteriellen Gesamtkohlenstoffbedarfs am *Hausstrand* decken. Benthische Sauerstoffzehrungsraten und bakterielle Aktivitäten veränderten sich stark mit der Temperatur, wobei die erhöhten Aktivitäten im Sommer wahrscheinlich das Ergebnis einer produktiveren mikrophytobenthischen Gemeinschaft sind; ein Schema, das auch an der *Königshafen* Station beobachtet werden konnte. Im Gegensatz zu den saisonalen Schwankungen der Aktivitäten zeigten die mikrophytobenthischen und bakteriellen Biomassen an beiden Stationen keine zeitliche Variabilität, was vermuten lässt, dass diese durch Grazer reguliert werden.

Einhergehend mit den vertikalen und zeitlichen Veränderungen der bakteriellen Aktivität veränderte sich die Zusammensetzung der Bakteriengemeinschaft an beiden Stationen signifikant. Die vertikalen Veränderungen konnten überwiegend mit der vertikal abnehmenden Verfügbarkeit des mit dem MPB assoziierten labilen organischen Kohlenstoffs und der Veränderung der Redoxpotenziale in Verbindung gebracht werden. Fast die Hälfte der am Hausstrand vorkommenden operationalen taxonomischen Einheiten (OTU) kamen in der gesamten untersuchten Sedimenttiefe vor (15 cm), während eine kleinere Anzahl OTUs offensichtlich an die spezifischen biochemischen und physikalischen Bedingungen in bestimmten Sedimenthorizonten gebunden war. Die bakterielle Diversität nahm mit der Sedimenttiefe zu, vermutlich als Ergebnis der verringerten physikalischen Störungen im Vergleich zur Sedimentoberfläche.

Die zeitliche Veränderung der über zwei Jahre beobachteten, sehr dynamischen Bakteriengemeinschaft am Hausstrand spiegelte nicht die saisonalen Schwankungen in Temperatur, Substratverfügbarkeit und bakteriellen Aktivitäten wider, sondern war nicht vorhersagbar; es konnten keine spezifischen OTUs ausschließlich mit Sommer- oder Wintermonaten in Verbindung gebracht werden. Die zeitliche Dynamik war insgesamt sehr stark, 47% der OTU wurden über einen Zeitraum von 2 Jahren ausgetauscht. Die Dynamik

der Bakteriengemeinschaft war innerhalb der ersten 6 Monate am stärksten und stabilisierte sich zum Ende hin.

An der *Königshafen* Station wurde die Dynamik der bakteriellen Gemeinschaft an einer Reihe von im Feld angelegten Ausschlussexperimenten des Wattwurms *Arenicola marina* untersucht. Hier veränderte sich die Zusammensetzung der Bakteriengemeinschaft nicht nur zeitlich, sondern auch räumlich, wobei diese Veränderungen im Wesentlichen mit Unterschieden in der MPB-Biomasse und –Produktivität und somit mit der Verfügbarkeit des mit dem MPB assoziierten labilen organischen Kohlenstoffs in Verbindung gebracht werden konnten. Die räumliche Variabilität stand dabei mit räumlichen Unterschieden in der Zusammensetzung des Sediments im Zusammenhang. Großflächiger Ausschluß des Wattwurm *Arenicola marina* erhöhte Biomasse und Aktivität der Bakterien und des MPB, hatte jedoch keinen Einfluß auf die Zusammensetzung der Bakteriengemeinschaft.

Chapter 1

Introduction

1 Introduction

Sands are the dominant sediment type on the continental shelf. Microbes in the shelf sediments undertake unique biochemical transformation processes and thus play a major role for global carbon and nitrogen cycles. The distribution of microbes in space and time, however, is not well understood. This dissertation thesis deals with microbial activity and bacterial diversity in coastal sandy sediments and focuses on their temporal (seasonal) and spatial variations.

In the following introduction, the habitat will briefly be characterized, followed by a description of the most important biochemical and physical processes which shape the environmental conditions for the microbes in this habitat. General patterns in animal and plant diversity will be depicted and an overview will be given on what is hitherto known about microbial diversity. After the objectives of this thesis have been clarified, the sampling schemes will shortly be described and a brief introduction will be given on the automated ribosomal intergenic spacer analysis (ARISA) fingerprinting approach which was used in this thesis to investigate spatial and temporal bacterial community patterns.

1.1 The Continental Shelf

The continental shelf separates the continents from the open ocean. In this transition zone, the continental crust gently slopes seawards from the shoreline to the shelf break before it steeply falls off towards the deep sea along the continental slope and rise (Fig 1). The shelf break is typically found between 100 and 150 m water depth, but can be as deep as 400 m in Polar Regions, where the widest stretches of continental shelves are situated. The width of the continental shelf is 85 km on average, but may reach up to 1500 km in the Arctic Ocean, while stretching across only a few kilometers at tectonically active ocean margins as they are commonly found in the Pacific Ocean (Wollast 2002).

About 70% of the continental shelf is covered by coarse sediments, out of which approximately 50% comprise so-called relict sands while the remaining 20% are the more recently deposited sediments that can particularly be found in the Arctic shelf areas (Johnson & Baldwin 1986). The coarse relict sediments were deposited on today's continental shelf during the long glacial periods within the late Quaternary when the sea level was much lower than it is today (~125 m lower during the last glacial maximum) and vast parts of the shelf areas were exposed to terrestrial conditions (Fleming et al. 1998). These glacial periods were

interrupted by rather short sequences of deglaciation with high water levels; the present exceptionally high sea level was reached only ~7000 years ago (Lambeck & Chapell 2001). During these sea-level maxima, when marine conditions prevailed on the shelf, the fine-grained fraction of the terrigenous deposits was largely flushed out and exported to the upper continental slope so that coarse, well sorted sediment deposits remained on the shelf (Milliman et al. 1972, Milliman & Summerhayes 1975). These periods, however, were not sufficiently long to allow a sedimentary equilibrium to be reached between burial and erosion of the coarser deposits.

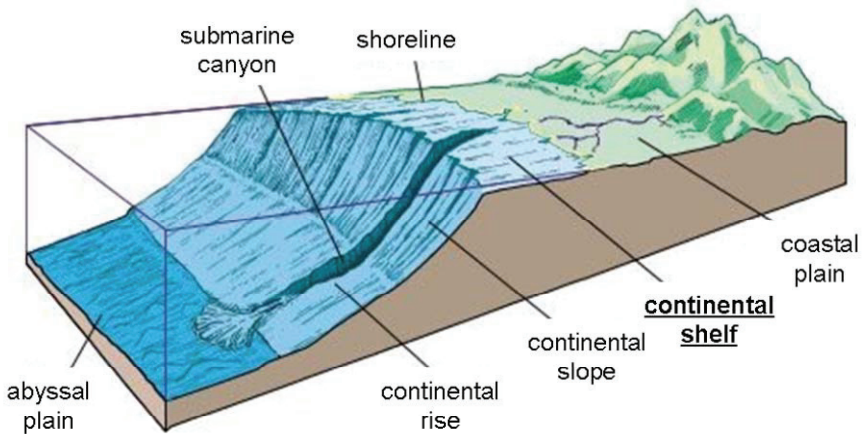

Fig. 1: Schematic overview over the ocean margin system (modified from Encyclopaedia Britannica Inc., 2006)

Up to 90% of the sediment that is generated by erosion on the continental land masses today, is deposited in the shelf areas, predominantly along the river deltas (Gattuso et al. 1998). Recent coarse sand deposition mainly takes place in shallow areas where the strong hydrodynamic forces of wind-induced waves and tidal currents winnow small and light particles and promote their transport (Gätje & Reise 1998, McCave 2002). Meanwhile, fine-grained deposits usually accumulate in distinct mud belts close to river mouths, in deeper waters with reduced hydrodynamics and on mudflats in areas of large tidal range (McCave 1972, de Haas et al. 2002). A large fraction of the fine-grained sediments is further transported to the open ocean (Harris & Wiberg 2002).

Sediment transport and composition as well as seabed topography of the continental shelf is mainly controlled by unidirectional as well as wave-induced currents. Wind stress at the sea-air interface imparts kinetic energy to the water in form of ocean currents and surface gravity waves. Where these physical forces act on the bottom-boundary layer, they cause

shear stresses that are able to erode and transport sediment particles (Precht 2003). Surface gravity waves start to generate oscillatory currents at the sea floor when water depths fall below half the wave length; however the bottom current velocities increase as the waves move into shallower coastal areas (Denny 1988, Kawamata 1998). Also tidal forcing usually increases towards the shore (Bearman 1989, Chamley 1990). Thus hydrodynamic forcing by waves and tides is particularly strong on the inner shelf, where it may cause substantial sediment transport (Nittrouer & Wright 1994).

1.2 Cycling of organic matter in the shelf seas

The shelf seas are one of the most active sites for biological and geochemical processes in the world (Gattuso et al. 1998). Although they comprise less than 8% of the oceanic surface area, the shelf seas account for 18-33% of the global marine primary production. Between 6 and 8 billion tons of carbon are generated in these areas each year, corresponding to more than 10% of the primary production of the biosphere as a whole (Wollast 1991, Falkowski et al. 1998). This high productivity of the shelf seas results from the considerable inputs of nutrients from upwelling deep-sea waters along the shelf break, as well as large amounts of terrestrial organic matter and nutrients through run-off and groundwater discharge from the near-by continental land masses. In these regions of high nutrient input, primary productivity is often locally enhanced (de Haas et al. 2002).

About 75% of the planktonic carbon fixation in the shelf seas is performed by diatoms (Tréguer et al. 1995). In temperate areas and high latitudes, the shelf seas are subject to strong seasonal changes in light availability and temperature which largely influence pelagic productivity. In the North Sea, the typical production cycle starts with a “spring outburst” of diatoms in March or April (Zijlstra 1988), when the water column becomes stratified upon heating of the surface waters (Colebrook 1979) and so light can penetrate deeper into this more stabilized water column (Gieskes & Kraay 1975). The diatom spring bloom is succeeded in most areas by a bloom of flagellates, typically *Phaeocystis* spp. in the southern North Sea. In some years a second, usually lower, diatom bloom may occur in autumn (Zijlstra 1988).

The diatom spring blooms often contribute not only to the bulk of the annual primary production of the entire shelf (Goering et al. 1973), but also to the main part of organic carbon in the benthos (Conley & Johnstone 1995). Only minor portions of the newly produced carbon are grazed upon by the zooplankton (Wollast 1991). Instead, between 20 and 60% of the carbon fixed in the water column is deposited on the shelf sediments due to the shallowness of

the shelf seas (Jørgensen et al. 1990, Wollast 1991, Gattuso et al. 1998). The diatoms sink to the sea floor upon nutrient depletion, aggregation or death, and may be actively flushed into the coarser-grained sediments by advection (Smetacek 1985, Passow 1991, Ehrenhauss et al. 2004).

For decades, it has been believed that most of the organic matter newly produced in the shelf seas is exported from the shelf and deposited in the ocean basins. This view was supported by the discovery of depocenters on the upper continental slope (Walsh et al. 1985). Nevertheless, more recent studies suggest that only a small fraction of this primarily produced carbon (12%) leaves the shelf areas (Liu et al. 2002). At the same time, despite the hydrodynamic and topographic conditions may sometimes encourage an accumulation of organic carbon on the shelf (e.g. at river deltas or in upwelling regions), the actual burial of organic matter in shelf sediments seem rather unimportant (Walsh et al. 1981, de Haas et al. 2002). Instead, more than 95% of the organic carbon on the continental shelf, either autochthonous or allochtonous, is remineralized within the shelf sediments or, to a minor degree, within the water column of the shelf seas (de Haas et al. 2002).

1.2.1 Benthic primary production

The relative proportions of planktonic and benthic primary production to the overall organic carbon synthesis in the shelf seas are difficult to assess. However, Gattuso et al. (2006) were able to show that more than 33% of the continental shelf might receive sufficient light to promote benthic primary production, implying that the contribution of benthic carbon fixation to the overall primary productivity of the shelf may have been largely underestimated in the past. The occurrence of benthic macrophytes, such as macroalgae and seagrasses, is usually locally restricted to specific areas along the coast, such as rocky shores, salt marshes and mangrove forests. Thus they only form a moderate contribution to the overall benthic primary productivity. The benthic microalgae (MPB), however, constitute a significant fraction of the photoautotrophic benthic community in terms of biomass and area cover (Duarte & Cebrian 1996, Middelburg et al. 2005, Middelburg & Soetaert 2005), and have been shown to contribute substantially to the primary production of intertidal and subtidal shelf areas (MacIntyre et al. 1996, Underwood & Kromkamp 1999, Jahnke et al. 2000, Glud et al. 2002). The MPB mainly consists of diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*) and cyanobacteria, which grow in the upper millimeters of photic sediments (MacIntyre et al.

1996, Brotas & Plante-Cuny 2003). Cahoon (1999) proposed that an estimated 0.5 billion tons of carbon could be annually generated by these organisms worldwide.

Organic matter deriving from photosynthesis, either as settling phytodetritus or benthic photosynthetic products, is the predominant food source for sediment bacteria (Goto et al. 2001, Rusch et al. 2003). In the intertidal and subtidal zones, radiolabeled tracer studies revealed that sediment carbon flow from the MPB to the bacteria was significant and rapid (Middelburg et al. 2000, Cook et al. 2007). This carbon flow was found to be primarily mediated by the secretion of extracellular polymeric substances (EPS) by the MPB. EPS mainly consists of high-molecular-weight polysaccharides and may be secreted in substantial amounts by the MPB to promote migration, adhesion to sediment particles (Hoagland et al. 1993, Underwood & Paterson 2003), or under nutrient-depleted conditions to maintain cell-nutrient balances (Ortega-Calvo & Stal 1994, Smith & Underwood 1998). Consequently, up to 70% of the carbon fixed by the MPB may not lead to cell growth but rather to production of EPS (Middelburg et al. 2000). Such EPS represent an easily accessible growth substrate for the benthic microorganisms and meiofauna (Smith & Underwood 1998, de Brouwer & Stal 2001, Köster et al. 2005, van Oevelen 2006).

Hence, the benthic microalgae stand at the beginning of a long food chain and provide an important source of energy for a rich biological community that includes various kinds of fish, shrimp, birds and certain mammals on the entire coastal shelf (e.g. Reise 1977, Bertness & Leonard 1997).

1.2.2 Benthic organic carbon mineralization

Complex interactions between various types of benthic organisms, involving a vast array of aerobic and anaerobic redox processes, result in high organic carbon mineralization rates in shelf sediments. The build-up of microbial or metazoan biomass is thus finally released as CO_2 and inorganic nutrients. Remineralization rates largely depend on the quality of the organic matter. The general formula for organic matter is $(CH_2O)_x(NH_3)_y(H_3PO_4)_z$, whereby x, y and z may vary depending on its age and origin. Fresh marine phytoplankton typically has a stoichiometry ratio of 106:16:1 according to Redfield (1958), while organic matter deriving from river discharge may have a ratio of 119:8.9:1 (Meybeck 1982).

Aerobic respiration is the only process that allows complete mineralization of biopolymers to inorganic nutrients, CO_2 and H_2O, using oxygen as the terminal electron acceptor. Oxygen availability, however, is generally limited to a mm-to-cm thick surface layer

in shelf sediments. While metazoans are restricted to aerobic respiration, microorganisms are metabolically highly versatile and may use a large variety of electron acceptors other than oxygen for organic carbon oxidation. Anaerobic mineralization is accomplished stepwise by mutualistic consortia of different functional types of microbes, as no single type of anaerobic organism is capable of complete oxidation of particulate organic polymers (Canfield et al. 2005).

As microbes can only transport substrates with a molecular weight of less than 600 Da across their cell membranes (Weiss et al. 1991), the crucial step for microbial organic carbon remineralization and nutrient cycling in the sediments is the initial hydrolysis of large molecular weight organic carbon such as polysaccharides, proteins, nucleic acids and lipids into low-molecular-weight organic matter. This degradation process is initiated by the release of extracellular enzymes which hydrolyze the macromolecules into oligomers and monomers that are suitable for uptake into the cell. In order to limit the expenditure of energy, carbon and nitrogen by the cell, only low levels of enzymes are excreted constitutively, and their production is up- and down-regulated by substrate induction, catabolite competition and catabolite repression. In addition, the enzymes are substrate specific. Hydrolysis rates have been shown to respond to changes in the amount of organic carbon, the polymeric composition and thus bioavailability of the organic matter present in the sediment (Meyer-Reil 1987, Boetius & Lochte 1996, Boschker & Cappenberg 1998). Different bacterial species may be specialized for different types of polymeric substrates (Martinez et al. 1996, Alderkamp et al. 2007), while it is mainly aerobic microbes that possess the ability to degrade complex organic matter. The aerobic *Cytophaga-Flavobacterium* group, for example, is specialized on the degradation of high-molecular-weight molecules and was found to comprise between 15 and 20% of prokaryotes in Wadden Sea sediments (Llobet-Brossa et al. 1998).

The products of enzymatic hydrolysis are either aerobically respired or taken up by fermenting bacteria and further degraded into a narrower spectrum of fermentation products, including primarily volatile fatty acids such as formate, acetate, propionate and butyrate, as well as H_2, lactate, some alcohols and CO_2 (Jørgensen 2000). Thereby, the used organic compounds serve both as electron donor and electron acceptor (Werner 2005).

The organic compounds and hydrogen released during hydrolysis and fermentation are completely oxidized to H_2O and CO_2 by a number of aerobically and anaerobically respiring microbes, using a variety of inorganic compounds as electron acceptors (Canfield et al. 2005). The general depth sequence of oxidants used in carbon mineralization is $O_2 \rightarrow NO_3^- \rightarrow$

Mn(IV) → Fe(III) → SO_4^{2-} → CO_2^-, involving anaerobic respiration processes such as denitrification, manganese-reduction, iron-reduction, sulfate-reduction and methanogenesis (Table 1).

Table 1: Pathways of organic matter oxidation, hydrogen transformation and fermentation in marine sediments and their standard free energy yields, ΔG^0, per mol of organic carbon (Jørgensen 2000)

Pathway and stoichiometry of reaction	ΔG^0 (kJ mol^{-1})
Oxic respiration:	
$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-479
Denitrification:	
$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4\ HCO_3^- + CO_2 + 3H_2O$	-453
Mn(IV) reduction:	
$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-349
Fe(III) reduction:	
$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- + 3H_2O$	-114
Sulfate reduction:	
$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-77
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	-152
$CH_3COO^- + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + HS^- + 2H_2O$	-41
Methane production:	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-136
$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-28
Acetogenesis:	
$4H_2 + 2CO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	-105
Fermentation:	
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$	-10
$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+$	-77

The sequence of oxidants corresponds to a gradual decrease in redox potentials and thus to a decrease in the free energy yield of the respective metabolic pathways (Froelich et al. 1979). The most favourable electron acceptor will be used until depleted before the next favourable will be used. However, the zones may overlap and fluctuate depending on the input of organic matter, light availability or sediment mixing and flushing (Froelich et al. 1979). On the other hand, the spectrum of substrates that can be used by the microorganisms

becomes narrower with increasing depth. Denitrifiers still have a relatively broad metabolic versatility and may use a wide range of different substrates. In contrast, sulfate reducers are generally unable to respire hydrolysis products such as sugars or amino acids, but are greatly dependent on products of fermentation (Jørgensen 2000). In accordance with the decreasing energy yield and the more refractory nature of the organic matter, remineralization rates strongly decrease with increasing sediment depth (Kristensen & Holmer 2001).

Aerobic respiration and sulfate reduction are the quantitatively most important processes in shelf sediments. Due to the high sulfate concentrations in the marine environment, sulfate reduction may be responsible for 25-50% of the organic carbon remineralization in this habitat. Denitrification as well as manganese- and iron-reduction are usually of minor importance; however these processes may be stimulated upon deeper penetration of the respective oxidants into deeper sediment layers via faunal activities or advection (Aller 1990, Canfield et al. 1993).

1.3 Particle transport and solute exchange processes over the sediment-water interface

Organic matter supply to the sediment and organic carbon remineralization by the benthic microbial community is strongly influenced by the transport processes and the exchange of solutes and particles between the sediment-water interface, especially in coarse-grained sediments (Boudreau et al. 2001, Huettel & Webster 2001). In cohesive, fine-grained sediments, solute transport is mainly mediated by molecular diffusion, bioturbation and bioirrigation (Berner 1980, Aller 1982), whereas advective pore water flow is the most important transport process in the upper centimeters of coarse-grained sandy deposits (Huettel et al. 2003). While molecular diffusion describes a solute transport along concentration gradients without a net transport of water and sediment, bioturbation, bioirrigation and advection are mass flow processes that include a net water and particle transport and are independent of concentration gradients. The relative importance of these transport processes depends not only on the sediment composition, but also on sediment permeability, sediment depth and the abundance and activity of macrobenthic organisms, as has been depicted for coastal sediments by Huettel et al. (2003) (Fig. 2).

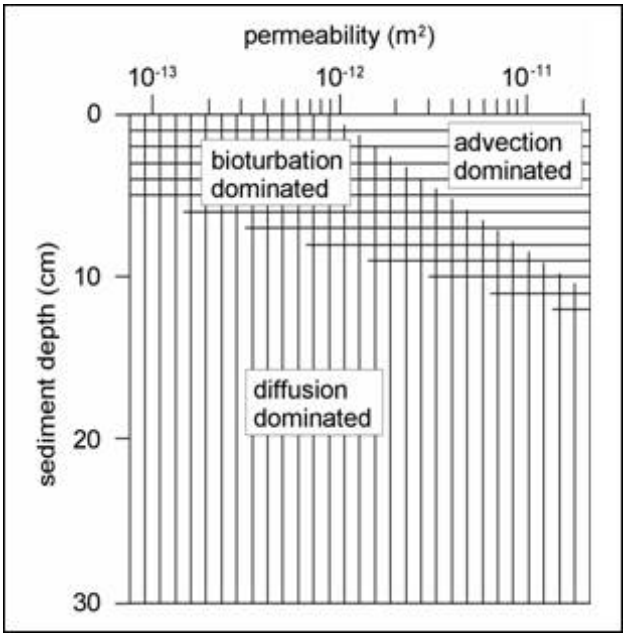

Fig. 2: The main transport processes in coastal marine sediments and their dominance according to sediment depth and permeability (figure derived from Huettel et al. 2003).

1.3.1 Molecular diffusion

Molecular diffusion takes place in cohesive, fine grained-sediments where benthic macrofauna is lacking. Due to the low permeability of these sediments, the pore water is more or less immovable. The physical solute transport is instead driven by thermally-induced random movement of solutes that leads to a net solute transport of molecules from sites of high concentrations to sites of low concentrations of the respective solute. Diffusion rates are dependent on the size of the molecules, salinity and temperature, and are proportional to the concentration gradient of the solute $\delta C / \delta x$, as described by Fick´s law:

$$J = -D_0 (\delta C / \delta x)$$

where J stands for the total diffusive flux (mol m^{-2} s^{-1}), D_0 for the substrate-dependent diffusion coefficient at a specific water temperature and salinity (m^{-2} s^{-1}) and $\delta C / \delta x$ for the concentration gradient. Sediment particles, however, enlarge the actual travel distance of solutes (Ullman & Aller 1982, Iversen & Jørgensen 1993), such that effective diffusion fluxes depend on the porosity of the sediment. Fick´s law was therefore modified for sediments:

$J = -\varphi Ds (\delta C / \delta x)$

where φ denotes the sediment porosity and *Ds* the effective diffusion coefficient in the pore water.

Molecular diffusion is only effective on small scales (µm to lower mm range) since the time a molecule needs to overcome a certain distance increases with the square of the distance. Thus, solute distributions in diffusion-dominated systems are relatively stable over time.

1.3.2 Biologically driven transport

The solute exchange and particle transport over the sediment-water interface may be largely increased by the activities of benthic macrofaunal communities. Depending on the reactivity of the solute, sediment composition and the infaunal community structure (Kristensen 2000, Kristensen 2001, Aller 2001), the biological enhancement of solute transport may exceed the transport of molecular diffusion by as much as an order of magnitude (Kristensen & Kostka 2004).

In particular, burrowing macrofauna may profoundly affect sediment biogeochemistry, solute exchange and particle transport across the sediment-water interface as the network of burrows can increase the sediment-water contact zone and thus the oxic surface area by 50 to 400% (Kristensen & Kostka 2004). Through their bioturbation and bioirrigation activities, these animals may rework considerable amounts of sediment and largely influence the organic matter input into the sediment and the vertical distribution of deposited organic matter within the sediment. Populations of the lugworm *Arenicola marina*, for example, may annually turn over a sediment layer of up to 40 cm (Kristensen 2001).

To cover their respiratory needs, burrowing animals actively ventilate their burrows with overlying sea water. This process of bioirrigation strongly alters the biogeochemical zonation which is typical for diffusion-dominated sediments (Fig 3). The availability of high-energetic electron acceptors, such as oxygen and nitrate, and the associated biogeochemical processes are largely extended into deeper, otherwise anoxic sediment strata that normally would be characterized by sulfate reduction. Meanwhile, inhibitory metabolic waste products are also more efficiently released into the water column. Iron reduction,which is usually of minor importance in shelf sediments, may sometimes become responsible for up to 100% of carbon oxidation in the presence of large infaunal communities (Kostka et al. 2002, Gribsholt et al. 2003).

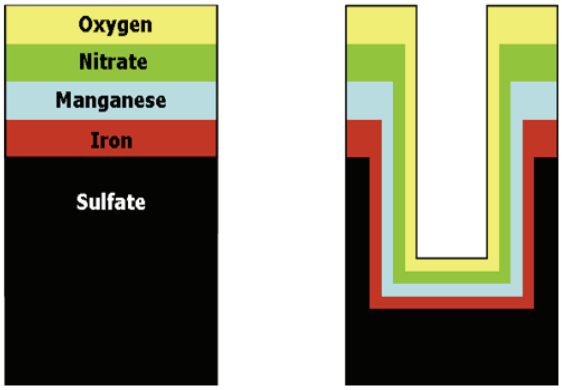

Fig. 3: Schematic presentation of the vertical distribution of electron acceptors for organic carbon mineralization in marine sediments. Left panel: idealized diffusion-dominated sediment without inhomogeneities; right panel: sediment with the presence of a biogenic structure like a polychaete burrow (modified figure from Canfield et al. 2005).

The enhanced biodeposition of labile organic matter via bioturbation and the improved supply of high-energetic oxidants by bioirrigation to the benthic microbes in deeper sediment layers lead to an overall stimulation of organic matter mineralization and nutrient recycling in the sediment (Kristensen & Kostka 2004) whereby the enhanced carbon oxidation rates are believed to be partly the result of co-mineralization of "older" more recalcitrant organic matter upon input of "fresh" more labile organic substrates (Welsh 2003). Accordingly, microbes were shown to be more active and abundant within worm burrow structures compared to bulk sediment (Aller & Yingst 1978, Aller & Aller 1986, Reichardt 1988). Also bacterial community structure and composition within worm burrows was found to be different from those in non-bioturbated bulk sediments (Steward et al. 1996, Matsui et al. 2004, Papaspyrou et al. 2007).

At sufficiently high densities, the effects of bioturbation and bioirrigation may not be limited to the vicinity of the burrows. In areas densely populated by *Arenicola marina*, Volkenborn et al. (2007) revealed that the effects of burrowing activities extend to the entire benthic boundary layer, preventing an accumulation of fine material in the sediment, enhancing permeabilities and thus additionally enlarging the effective range of advection.

1.3.1 Advective transport processes

The majority of the continental shelf sediments consist of coarse-grained sandy deposits. Unlike muddy sediments where solute transport is restricted to bioirrigation/bioturbation and molecular diffusion, advective pore water flow acts as the major transport mechanism in the upper sediment layers of sandy sediments (Webb & Theodor 1968, Huettel & Gust 1992).

Permeability is a measure of the ease with which fluids can flow through porous media and depends on sediment properties such as grain size and shape, sediment sorting and sediment porosity. Advection describes a mass pore water flow through the interstices of porous media, which is always driven by pressure gradients and leads to an effective exchange of solutes and particles over the sediment-water interface. Sandy sediments are such a porous medium that advective pore water flow through their interstices are permitted, resulting in a rapid exchange of pore water with the overlying water body, thereby enhancing the influx of oxygen, labile organic matter and electron acceptors into the sediment, as well as the efflux of metabolic waste products out of the sediment. Natural pressure gradients of less than 1 Pa are sufficient to cause advective pore water flow at sediment permeabilities larger than 10^{-12} m^2 (Huettel & Gust 1992). The pore water flow rates are reduced by frictions in the pore spaces which are dependent on the viscosity of the fluid μ and the permeability of the sediment. The pore water flow velocity u is thus proportional to the sediment permeability k and the induced pressure gradient Δp, according to Darcy´s law (Darcy 1856):

$$u = (k/\varphi\mu)\, \Delta p$$

whereby φ denotes the porosity of the sediment.

Pressure gradients may be generated in different ways. For instance, the deflection of unidirectional bottom water currents by uneven sediment topography (Thibodeaux & Boyle 1987), such as wave ripples (Fig. 4), fecal mounds, animals or mussel shells may generate horizontal pressure gradients. In the zone where the current is deflected upwards by the respective obstacle, a high-pressure region is created, while a low-pressure region is created directly downstream of the obstacle where the flow gains speed and detaches from the sediment surface. A second high-pressure region is generated downstream behind the obstacle where the flow reattaches to the sediment surface. In the zones of high pressure, bottom water intrudes into the sediment, while pore water is released from the sediment in the low-pressure

region, resulting in advective solute fluxes that may be up to three orders of magnitude higher than molecular diffusion (Huettel & Gust 1992).

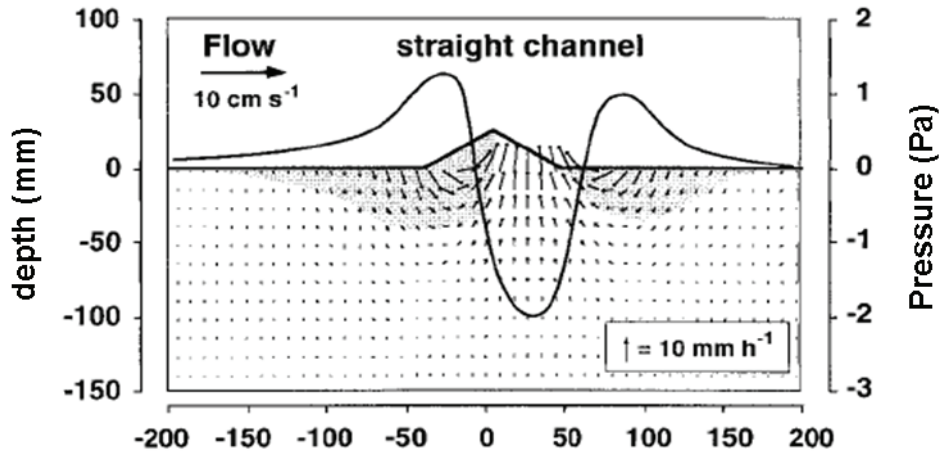

Fig. 4: Advective pore water flow fields under protruding sediment topography exposed to a unidirectional bottom current. The solid curved line represents the pressure distribution at the sediment-water interface. Arrows depict the direction and magnitude of the advective pore water flows in the sediment (reproduced from Huettel & Rusch 2000).

Pressure gradients may also be generated by unstable density differences in temperature (thermal convection) and/or salinity (haline convection) (Huettel & Webster 2001). Thermal convection may develop when warm pore water comes into contact with cooler bottom water, for example by flooding of an intertidal flat that was heated up during low tide exposure. Haline convection may by induced in areas where salinity of bottom waters is higher than the salinity of the pore water. This may be caused by submarine groundwater discharge or upon rainfall on an intertidal flat that is thereafter flooded with denser sea water. Solute fluxes caused by both haline and thermal convection may increase those of molecular diffusion by several orders of magnitude (Webster et al. 1996, Rocha 2000).

In addition, moving pressure gradients on the seafloor may be created by the passage of surface gravity waves over subtidal shelf sediments, leading to an interfacial pore water exchange, a process which is referred to as wave pumping or the “subtidal pump” (Webb & Theodor 1968, Riedl et al. 1972). The mechanism behind this process is based on hydrostatic pressure differences between the wave crests and troughs which move together with the wave propagation. In permeable sediments, these locally changing pressure gradients can cause

considerable undulating mass water flow over the sediment-water interface (Precht & Huettel 2003, Precht & Huettel 2004).

1.4 The special role of sandy sediments in coastal benthic ecology

Permeable sandy sediments are the dominant sediment type on continental shelves (Emery 1968). Especially coastal areas are often exposed to the strong hydrodynamic forces of wind-induced waves and tides which interact with the seafloor, winnow small and light particles and leave behind coarse-grained sandy deposits. These areas are highly valuable both from the economical and ecological point of view. Fishing, haulage, the extraction of oil, gas, minerals and fresh water as well as touristic recreation creates an enourmous global turnover of money every year. Also, these areas harbor an invaluable biological diversity; they not only provide space for a rich fish fauna and certain mammals, but serve as a resting, feeding and breeding area for a huge variety of native and migrating birds.

Due to their low organic carbon content, permeable sediments have long been considered as biochemically inert and hence been neglected in carbon turnover estimates (Boudreau et al. 2001). Within the last two decades, however, the importance of permeable shelf sediments for oceanic and global carbon and nutrient cycles has been increasingly recognized. More recent studies showed that sandy sediments in photic zones are sites of high benthic primary productivity (Billerbeck et al. 2007) and there is growing evidence that the low organic matter content at sandy sediment sites reflects high microbial turnover rather than sediment inactivity (Huettel et al. 1998, Boudreau et al. 2001, Dauwe et al. 2001, de Beer et al. 2005). This high microbial turnover of carbon seems to be stimulated by advection which allows a rapid exchange of solutes between the sediment-water interface and an effective transport of particles into the sediment (Huettel & Gust 1992, Forster et al. 1996, Precht & Huettel 2004, Reimers et al. 2004). The constant advective flushing of coastal sands accelerates the influx of labile organic matter, oxygen and other electron acceptors into the sediment (Lohse et al. 1996, Ziebis et al. 1996) and the efflux of metabolic waste products out of the sediment (Gehlen et al. 1995, Huettel et al. 1998). Detritus, bacteria and algae from the overlying water column are efficiently trapped within the sediment (Huettel & Rusch 2000, Rusch & Huettel 2000, Rusch et al. 2001, Ehrenhauss & Huettel 2004, Ehrenhauss et al. 2004). Thus deeper sediment layers are provided with fresh organic matter and high-energetic electron acceptors, leading to organic carbon mineralization rates that are comparable to or even higher than those in fine-grained, organic rich sediments (Forster et al. 1996, D'Andrea

et al. 2002, de Beer et al. 2005, Rusch et al. 2006). Billerbeck et al. (2005) showed that aerobic mineralization was the dominant process in an intertidal sand flat which was advectively flushed and thus efficiently supplied with oxygen.

Although total microbial abundance in sands was frequently found to be about an order of magnitude lower than that in muddy organic-rich sediments (Goñi-Urriza et al. 1998, Rusch et al. 2001, Rusch et al. 2003), recent studies revealed that they may be comparable at some localities (Ishii et al. 2004, Musat et al. 2006). Microbial abundances in permeable sands usually show a subsurface maximum at 1-3 cm depth, presumably as a result of the constant physical stress at the sediment surface. The constant physical mixing and flushing of the upper sediment centimeters with oxygen-rich water often leads to a lack of vertical stratification which is reflected in the rather stable bacterial abundances below the subsurface maximum (Llobet-Brossa et al. 1998, Rusch et al. 2001, Musat et al. 2006).

1.5 Diversity of plants and animals and their spatial distribution

Since the eighteenth century, biologists have investigated the geographic distribution of plant and animal diversity. They found that animal and plant species are not evenly distributed over the globe, but show general and species-specific distribution patterns that are the result of both deterministic (environmental) and stochastic (dispersal) processes (Green et al. 2008). While the interactions of a large number of physical, chemical and biological factors mainly determine distribution patterns on local scales, large-scale distribution patterns of plants and animals may often be traced back to geological history. Not only the composition but also the size of regional species pools is probably dependent on factors acting on larger scales, such as climate, while the size of local species pools is most likely influenced by local processes, such as biotic interactions between competitors or between predator and prey (Currie 1991).
Factors that have been suggested to influence species richness are (Currie 1991):

1) Climate: A mild climate is hypothesized to support higher species richness.
2) Climatic variability: A stable climate permits specialization.
3) Habitat heterogeneity: Physically or geologically complex habitats provide a higher number of ecological niches.
4) History: More time permits a more complete colonization of a habitat and the evolution of new species.

5) Energy: Species richness is limited by the partitioning of energy among species. Species richness will potentially be higher, the more energy is available.
6) Competition: a) Competition favors reduced niche breadth. b) Competitive exclusion eliminates species.
7) Predation: Predation slows down competitive exclusion.
8) Disturbance: Moderate disturbance slows down competitive exclusion.

One of the large-scale patterns often observed for species richnesses of plants (trees) and animals such as mammals, amphibians, reptiles and birds has been the strong relationship with latitude (Wallace 1878, Simpson 1964, Cook 1969). In a large-scale study of North America, including a large variety of different animal groups and trees, Currie (1991) found pronounced peaks for bird and mammal richnesses at 44° N and 39° N, respectively. Amphibian richness peaked more gently at 34° N while trees and reptiles decreased monotonically with latitude. Mid-latitude peaks of species richness seem to be a general phenomenon and were described earlier for birds and insects (Rabenold 1979, Janzen 1981). The species richness of trees is strongly related to environmental factors such as temperature, solar radiation, potential evapotranspiration (PET) and somewhat weaker to precipitation (Currie 1991). It was hypothesized that the latitudinal variations in animal species richness are either explained by the latitudinal variations in plant richness with which the animals are associated (MacArthur and MacArthur 1961) or by primary productivity (Wright 1983, Abramsky and Rosenzweig 1984, Owen 1988). Indeed, Currie (1991) was able to show that animal species richness was strongly correlated with the annual PET which he interpreted as a measure of integrated, crude, ambient energy and which depends mainly on the amount of energy available to evaporate water and on the relative humidity, thereby being closely related to latitude and to the variability in solar radiation.

Also species richness of organisms in the shallow-water benthos was shown to follow latitudinal gradients with high species richness on the tropical continental slopes and decreasing diversity towards the poles. It was suggested that these variations relate to the effect of solar radiation on temperature, productivity, and rates of evolution (Snelgrove 1999, Jablonski 1993, Poore and Wilson 1993, Rex et al. 1993, Roy et al. 1998). For some groups of marine organisms (e.g. nematodes, Boucher and Lambshead 1995) no latitudinal trend was observed, however, proposing that these groups may be affected by fundamentally different processes.

Over broad scales, geologic history plays a major role in patterns of species distribution. An increase in the number of taxa with increasing sample area has been observed repeatedly for numerous plant and animal taxa regardless of ecosystem type, referred to as the taxa-area relationship and indicating that on a very large scale the legacy of historical separation can overwhelm any effects of environmental factors (Hughes Martiny et al. 2006). One of the reasons for this relationship is reproductive isolation (for example between island- and mainland populations or by physical isolation due to mountain ranges) which leads to a local evolution of species driven by the adaption of organisms to their environment.

The area of distribution may vary considerably between organisms. While large organisms, such as elephants, will only disperse by active motion, others such as tree ferns, trees and giant clams may be passively dispersed over thousands of kilometers by air or water as spores, seeds and larvae and thus spread over larger scales (Horner-Devine et al. 2004).

At small spatial scales for which distance effects are negligible, environmental effects have been repeatedly shown to significantly influence the composition of faunal communities. This can mainly be explained by predator-prey relationships and competition among species for food and shelter and their specific tolerances to physical factors such as temperature. Diversity in marine benthic macrofauna, for example, is generally lowest in highly variable and physically extreme environments as most organisms have physiological constraints in terms of temperature, salinity and pressure which have to do with the maintenance of osmotic balances and enzyme functions (Snelgrove 1999). While on regional scales distribution patterns of individual species of shallow-water sedimentary fauna are determined by temperature, salinity, depth, surface productivity and sediment dynamics, distribution patterns on local scales are rather affected by biotic interactions, sediment geochemistry, and near-bed flow processes (Snelgrove 1999).

Diverging large- and small-scale distribution patterns have also been found for plants: Swensen & Enquist (2007) and Weiher & Keddy (1995) observed that on a global scale wood density was negatively correlated with latitude, whereas on local scales competition became increasingly important towards the equator.

One of the often picked up concepts in ecology that is based on competition among species is the “intermediate disturbance hypothesis” raised by Connell in 1978 for tropical trees and corals. He stated that “diversity is higher when disturbances are intermediate on the scales of frequency and intensity […] If disturbances continue to happen frequently, the community will consist of only those few species capable to quickly reaching maturity. As the interval between disturbances increases, diversity will also increase, because more time is

available for the invasion of more species". Likewise "diversity is highest when disturbances are intermediate in intensity or size, and lower when disturbances are at either extreme. For example, if a disturbance kills all organisms over a very large area, recolonization in the center comes only from propagules that can travel relatively great distances and that can then become established in open, exposed conditions […] If the disturbance was less intense so that some residents were damaged and not killed, in a large area recolonization would come both from propagules and from regeneration of survivors, so that diversity would be greater than was the case when all residents were killed and colonization came only from new propagules". This hypothesis is based on the assumption that local ecological communities seldom attain equilibrium, but rather change as a result of frequent disturbances (e.g. storms, forest fires, floods) or more gradual climatic changes. These changes interrupt competitive processes among species and maintain diversity by preventing the elimination of inferior competitors (Connell 1978). Although niche differentiation usually allows the coexistence of species in local areas and maintains some degree of diversity even under equilibrium conditions, the "intermediate disturbance hypothesis" has been proved to be valid for a large number of organisms and refuges, such as river macroinvertebrates (Townsend & Scarsbrook 1997), tropical forests of the Solomon Islands (Fox & Connell 1979), phytoplankton (Flöder & Sommer 1999), freshwater macrophytes (Bornette & Amoros 1996) and sessile rocky shore biota (Sousa 1979) besides others.

1.6 Temporal community patterns of plants and animals

Any naturally occurring community of organisms is not only distributed in space but also in time (Whitlatch 1977). The community composition and community structure of plants and animals may change on short (seasonal) and long-term (decades and more) scales.

In temperate areas, temperature fluctuations show marked temporal variations which are repeatable from year to year. Temperature is one of the controlling mechanisms for initiating reproductive activity in most species (Whitlatch 1977) and commonly considered to be the most important single factor influencing the distribution and reproduction of marine invertebrates in particular (Thorson 1946) as it controls the digestive system, the ontogeny and the gonadal maturation in marine organisms (Lange & Greve 1997). Different species, however, have different physiological requirements and specific preferences for temperature. Repeatable temperature variations thus often lead to a seasonal timing of populations (Greve et al. 2001, Lange & Greve 1997) and to seasonal patterns of species composition and/or

structure of marine communities in temperate areas. For marine subtidal areas, seasonal changes in community structure have been shown repeatedly (e.g. Tenore 1972, Boesch 1973) and restricted seasonal occurrence of particular groups of organisms have frequently been noted (Levings 1975, Muus 1967, Bodiou & Chardy 1973, Grassle & Smith 1976) which may partly be explained by latitudinal migration of (fully developed or developing) species. The temperature cycles, however, show some year-to-year fluctuations, influencing the exact timing of appearance of seasonal species groups (e.g. larvae in the marine benthos; Whitlatch 1977). Small changes in temperature extremes, annual means and/or patterns of monthly means often cause large changes in distribution, abundance and seasonal dynamics of marine species.

Annual community dynamics are not only influenced by temperature, but also by trophic linkages between populations. Greve et al. (2004) described an annual cycle of trophic succession in the plankton community of the Helgoland Roads in the North Sea, characterized by the synchrounous functionality of bottom-up and top-down control which is typical for prey-predator cycles (Greve and Reiners 1988). This trophic succession typically starts with the growth of the phytoplankton (plankton bloom) in weeks 16-21, followed by a drastic reduction in weeks 21-25, corresponding to a drastic population increase of small calanoid copepods in week 21-23. In weeks 24-25 the population collapses while the abundance of the ctenophore *Pleurobrachis pileus* simultaneously increases, thus shifting the system dominance from the phytoplankton to the secondary carnivore within 7 weeks. The reduction in herbivorous copepods leads to a second phytoplankton bloom period while the abundance of *Pleurobrachis pileus* is reduced by its specific predator *Beroe gracilis*. The release of the predation pressure on copepods leads to a second population maximum in week 32, followed by a collapse of the phytoplankton (Greve et al. 2004). This trophic succession is a typical example of the complex seasonal community changes in temperate marine ecosystems.
Although this annual succession is somewhat repeatable, in varies considerably from year to year with respect to timing and the abundance of single populations, sometimes representing a trend over a few or many years (Greve et al. 2004).

Contrastingly, non-systematic temporal changes in the distribution, abundance, biomass and assemblage structure of benthic macrofauna in a temperate estuarine, intertidal soft-sediment in the Schelde estuary (the Netherlands) were observed by Ysebaert & Herman (2002). Temporal variations appeared to be spatially and species-dependent. The magnitude of recruitment was extremely variable between years, and rarely showed covariation between

different species within a year, suggesting that the potential causes and consequences of this variability probably vary on a species-to-species basis.

Long-term changes are often assigned to gradual changes in climate which cause new species to enter the species pool. New species can immigrate into a locality either by introduction (e.g. by animal vectors) or by range extension. Dispersal of species into neighboring areas may lead to a permanent range extension of these species when the ecological conditions in these areas are changing on a long-term basis in such a way, that they meet the requirements of those respective species (Franke & Gutow 2004).

Long-term temporal changes in ecological communities have been gaining increasing attention in the past few years under the aspect of global change. Such long-term temporal scale changes have been observed for zooplankton in the North Sea (Greve et al. 2004; Fig. 5) and for macrozoobenthic communities of intertidal and subtidal hardbottom habitats around the island of Helgoland (German Bight), both regarding the species spectrum and the abundance of species. A shift in North Sea climate towards more oceanic conditions was suggested to be one of the most important factors driving the recent changes in the community structure (Franke & Gutow 2004).

In the same area, a long-term temporal increase in phytoplankton biomass has been tracked by Wiltshire & Dürselen (2004). Also, the spring diatom bloom in lakes has been shown to be shifted forward by up to one month by the overall global warming trend (Weyhenmeyer et al. 1999, Weyhenmeyer 2001). A meta-analysis of species and global warming data from a total of 143 studies revealed a consistent temperature-related shift in species ranging from mollusks to mammals and from grasses to trees, strongly suggesting that a significant impact of global warming is in fact discernible in animal and plant populations (Root et al. 2003).

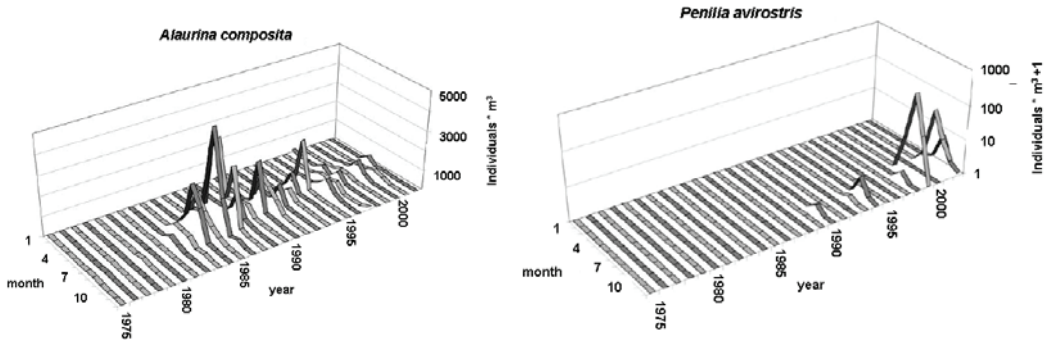

Fig. 5: Abundance distribution (monthly means 1975-2002) of exemplary zooplankton species in waters of Helgoland Roads (North Sea, Germany). Presented are dominance changes of *Alaurina composita* (left panel) and neozoan immigration of *Penilia avirostris* (right panel) (figure modified after Greve et al. 2004).

1.7 Microbial diversity and distribution

Despite their small size, microbes constitute an essential part of the earth's biota. The total amount of microbial carbon is 60-100% of the estimated total carbon in plants. In addition, microbes contain 85-130 Pg of nitrogen and 9-14 Pg of phosphate which is an estimated 10-fold more of these nutrients than contained in plants, thus representing the largest pool of these nutrients in living organisms (Whitman et al. 1998). Although microbes undertake unique and indispensable biochemical transformation processes and as such have a strong global impact on aquatic and terrestrial carbon and nutrient cycles and on the gaseous composition of the atmosphere (Whitman et al. 1998), the biodiversity of these organisms and their distributions in space and time are poorly understood. This lack of information is mainly due to the limitation of conventional cultivation techniques that could only identify perhaps about 1% of microbes in the environment. The recent advent of molecular ecological tools, such as ribosomal RNA sequence analyses, revolutionized microbial ecology and significantly expanded our knowledge of microbial phylogenetic diversity (Donachie et al. 2007, Woese & Fox 1977). Modern improvements in the resolving power of molecular ecological tools and the development of high-throughput methods (e.g. 454 tag-sequencing) allow us to identify large numbers of microbes within a relatively short time and have created a renewed interest in the field of microbial biogeography, which aims to examine patterns of microbes at local, regional and continental spatial scales, in time, and along environmental gradients (Ramette & Tiedje 2007a, Green et al. 2008).

One of the often cited concepts in microbial biogeography is the assumption that for microbes "everything is everywhere, the environment selects" (Baas-Becking 1934). The hypothesis that "everything is everywhere" is based on the belief that the small size and high abundance of microbes increases the rate and geographic distance of their dispersal to levels where dispersal limitation is nonexistent, thus leading to a "cosmopolitan" distribution, while the hypothesis of "the environment selects" describes that differentiation in microbial assemblages is driven by environmental heterogeneity. Similar microbial communities in similar habitats and differentiated microbial communities along environmental gradients would thus be expected (Finlay 2002, Fenchel & Finlay 2004, Green & Bohannan 2006). In contradiction to the hypothesis that microbial diversity is unlimited, recent studies found taxa-area relationships (an increase in species richness with sample size) for microbial communities, as they have often been described for plants and animals (Rosenzweig 1995, Green et al. 2004, Horner-Devine et al. 2004, Bell 2005). This implies that microbial

distribution is not exclusively governed by environmental heterogeneity, but that microbes are also subject to some degree of historical separation. Various barriers to microbial dispersal have been suggested, such as physical barriers (e.g. topography), physiological constraints (narrow temperature, salinity or pH tolerance range), ecological constraints (e.g. higher competition) or limited resource availability between favorable patches (Papke & Ward 2004, Ramette & Tiedje 2007a).

It was shown that microbes, like plants and animals, may follow latitudinal gradients (Pommier et al. 2006) and exhibit forms of endemism. One famous example of microbial endemism is that of *Synechococcus* and *Sulfolobus* assemblages of which certain morphotypes have been identified in North American hot springs, however not in other hot springs around the globe which was proposed to be a result of physical isolation and physiological constraints that prevent cell viability during dispersal (Papke et al. 2003, Whitaker et al. 2003, Ramette & Tiedje 2007a).

In addition, variations in bacterial community composition between mountain lakes from the Sierra Nevada (Spain) have been shown to be explained by geographic distance, rather than by environmental factors (Reche et al. 2005). Similarly, geographic distance was found to better predict turnover of desert ascomycete fungal communities than soil and vegetation type (Green et al. 2004).

Conversely, Horner-Devine and coworkers (2004) studied the spatial distribution of bacteria in New England salt marshes and although they found that bacterial communities located close together were more similar in composition than communities located farther apart, they were able to show that this taxa-area relationship was driven primarily by environmental heterogeneity and that sites that were similar in environmental characteristics were also similar in bacterial community composition. Likewise, Fierer and Jackson (2006) found bacterial diversity in soils across North and South America to be largely independent of geographic distance and to be neither related to site temperature, latitude or other variables which typically predict plant and animal diversity. Instead, they showed that bacterial diversity and richness were strongly linked to soil pH.

In aquatic ecosystems, a large range of environmental factors such as temperature (Yannarell & Triplett 2004, Sapp et al. 2007), inorganic nutrients (Muylaert et al. 2002, Pinhassi et al. 2004), pH (Yannarell & Triplett 2005), primary productivity (Horner-Devine et al. 2003), dissolved organic matter quality and quantity (Crump et al. 2003) and protozoan grazing (Jürgens et al. 1999, Hahn & Höfle 2001) have been suggested to be linked to microbial community composition and structure. Microbial communities in marine sediments

were found to be additionally influenced by benthic chlorophyll a (Polymenakou et al. 2005), sediment type (Franco et al. 2007) and sediment water content (Hewson et al., 2007).

In other words, the current belief in microbial ecology is such that microbial diversity and distribution are explained by both contemporary environmental heterogeneity and spatial distance as a proxy for past historical events and disturbances. Their relative contribution to microbial diversification and the extent of interactions between environmental factors and spatial distance, however, are still not well constrained. This is partly due to the difficulty of defining the spatial and taxonomic resolutions at which environmental heterogeneity and spatial isolation act on diversification (Ramette & Tiedje 2007b).

1.8 Temporal patterns in aquatic microbial communities

Similar to plants and animals, clear endemic, patchy, or cosmopolitan patterns have been described in several microbial taxa; yet, scant data exists on the stability of those patterns through time and seasons (Ramette & Tiedje 2007b).

Seasonal patterns in microbial activities and abundances have often been observed for temperate aquatic ecosystems. General microbial community functions such as bacterial production, bacterial community respiration and extracellular enzymatic activities have repeatedly been shown to be strongly related to temperature (e.g. Mayer 1989, Hubas et al. 2007) and seasonal inputs of organic carbon such as the spring phytoplankton bloom (e.g. Meyer-Reil 1987). However, these patterns have seldom been accompanied by investigations of the microbial community composition.

A rather small number of studies indeed showed that seasonal fluctuations in the microbial community composition and/or structure occur in natural ecosystems. A long-time series of microbiological parameters over more than 40 years since 1962 in waters of Helgoland Roads (German Bight) revealed reoccurring annual patterns in microbial abundances and activities, but also in the composition of the bacterioplankton (Gerdts et al. 2004). Microbial abundance in winter (November until February) generally reached only 25% of the counts in summer (June until September). ATP concentrations and aminopeptidase activities followed clear seasonal trends with highest values in summer and almost unmeasurable values in winter. ATP is a useful measure of microbial activities as it is continuously synthesized and degraded as long as the microbes are alive. After death, ATP is no longer produced and decomposed rapidly. ATP concentrations usually reached their peaks

in June, one month before the peak in microbial abundance (Fig. 1.7), whereas aminopeptidase activities were usually highest in mid summer (July, August).

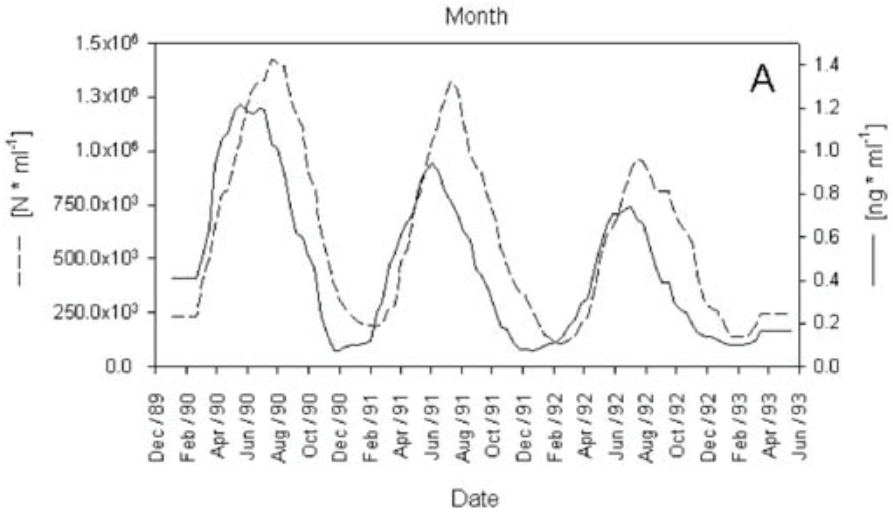

Fig. 6: Running average of ATP concentration (solid black line) and bacterial direct counts (AODC; dashed line) in waters of Helgoland Roads (North Sea, Germany) from January 1989 to June 1993 (figure derived from Gerdts et al. 2004).

Interestingly, the bacterial community composition was studied simultaneously by denaturing gradient gel electrophoresis (DGGE) weekly from 1996 until 1999, revealing a seasonal succession of bacterial operational taxonomic units. Discerned DGGE band patterns could be observed in three periods during the year: 1) October to March, 2) April to July and 3) August to September. While from October until March usually stable DGGE band patterns were observed, in March some of the formerly faint bands tended to increase in intensity, while others, formerly clearly visible bands, almost vanished. Changes in DGGE band patterns became dramatic between April and July (long before the temperature peak) and decreased again in August and September before they reached the stable winter situation again in October. In the same area, bacterioplankton dynamics were studied over the winter-spring transition by a combination of automated ribosomal intergenic spacer analysis (ARISA) and DGGE of 16S rRNA genes followed by DNA sequence analysis (Sapp et al. 2007). The bacterial community data was correlated to phytoplankton community data and a range of abiotic factors, whereby both temperature and phytoplankton composition were found to be responsible for temporal shifts in the bacterial community composition.

Comparable results were obtained by Rink et al. (2007) who also used the DGGE approach to study short-term temporal changes in the Wadden Sea and found that the bacterial

community composition of both free-living and aggregate-associated bacterial communities changed during the course of the phytoplankton succession in spring and early summer.

Fuhrman et al. (2006) sampled waters 20 km off the southern Californian coast monthly over 4.5 years. Bacterial community composition was studied via ARISA and a suite of oceanographic variables such as temperature, salinity, dissolved oxygen, chlorophyll a, bacterial and viral abundance, bacterial heterotrophic production and nutrients were measured in lockstep. Some bacterial taxa were detected rather inconsistently and occurred only a few times or sporadically in their data sets. However, they found several subsets of bacterial taxa that annually reoccurred, whereby this occurrence was predictable from seasonal ocean conditions.

Temporal variability in bacterial community composition has also been observed in a range of limnic ecosystems. For example, Yannarell et al. (2003) studied the bacterial community composition of three lakes in Wisconsin, USA, every 2 weeks for 2 years during the ice-free period. While the bacterial community was rather stable in fall and spring, a strong short-term temporal variability in community composition could be observed in summer. Kent et al. (2004) continued the sampling of one of the lakes for one more year and showed that, however, the bacterial community composition in the lake was rather dissimilar between years, suggesting that there were no repeatable seasonal patterns.

1.9 Temporal microbial community patterns in marine sediments

Only few studies tried to resolve temporal microbial community patterns in marine sediments. Phylogenetic studies based on 16S rRNA gene sequencing revealed that marine sediments indeed harbor a rich microbial diversity with sequences affiliated to α-, β-, γ-, δ-*Proteobacteria*, to *Planctomycetes*, to the *Cytophaga-Flavobacterium-Bacteriodetes* group, to *Verruco-microbiales*, and to the *Actinobacteria* (e.g. Rochelle et al. 1994, Gray & Herwig 1996, Cifuentes et al. 2000, Bowman et al. 2003).

Only two recent studies have been published that aimed to resolve temporal changes in microbial community composition and microbial activities in marine intertidal sediments, both using the FISH (fluorescent in situ hybridization) approach.

In a temperate intertidal mudflat of Dangast in the Jadebusen Bay, North Sea, these dynamics were assessed for a period of two years based on 6 sampling campaigns (Llobet-Brossa 2000). Microbial abundance was found to vary seasonally reaching its maximum in summer and the minimum in winter. It ranged between 4.3×10^9 and 5.8×10^9

cells cm^{-3} at the sediment surface and between 0.9 x 10^9 and 1.1 x 10^9 cells cm^{-3} at 9 cm depth below the sediment surface, thus also representing a strong vertical decrease which could be led back to the vertical decrease in organic carbon availability within the sediment. The temporal changes were linked to seasonal changes of temperature and biogeochemical activities such as sulfate reduction rates. The *Cytophaga-Flavobacterium* cluster of the CFB phylum was found to be the most abundant group of bacteria, constituting 15-20% of total detectable cells. The δ-proteobacterial sulphate reducing bacteria (SRB) were the second most abundant group, while *Planctomycetes*, α-, β-, γ-, ε-proteobacterial members as well as Gram-positives with high GC content were present throughout the entire vertical profile, however at relatively low abundances (1-6%). The *Cytophaga-Flavobacterium* population as well as the SRB population increased in abundance during summer, while lowest abundances were detected in winter. Also *Arcobacter* spp., a group detected in the upper 3 cm of sediment only, duplicated in summer with respect to winter abundances. It was suggested that the increase in the SRB population in summer largely depended on the supply of fermentative products by the *Cytophaga-Flavobacterium* population, a group specialized on the degradation of complex organic matter.

The second study focused on temporal changes in an intertidal sandy sand flat near the island of Sylt (German Wadden Sea). Thereby, observations were based on 3 field campaigns (Musat et al. 2006). The microbial community was dominated by members of the *Planctomycetes*, the *Cytophaga-Flavobacterium* group, γ-*Proteobacteria* and bacteria of the *Desulfosarcina-Desulfococcus* group, thus largely reflecting the community structure of intertidal mudflats observed by Llobet-Brossa (2000). Bacterial abundances with values up to 3 x 10^9 cells cm^{-3} were comparable to those of muddy sediments, however did not show any strong vertical variation, presumably due to the distinct hydrodynamics at the field site. Cell abundances were higher in July and October versus March. While the total abundance of *Planctomycetales* determined by FISH was significantly higher in July than in October or March, the *Cytophaga-Flavobacterium* group showed variable numbers only in the top sediment layer with enhanced numbers in July and October compared to March. In comparison, cell numbers of the *Desulfosarcina-Desulfococcus* group did not vary considerably, however RNA slot blot hybridization with the same probes retrieved high rRNA values in July, medium values in October and lowest values in March which was consistent with seasonal changes in sulfate reduction rates that were observed at the same sampling site by de Beer et al. (2005).

1.10 Objectives of this thesis

Continental shelf sediments play an important role for global carbon and nutrient cycles. Microbes possess metabolic capabilities that are often unique for living organisms and thus are the key players in various biochemical transformation processes on the shelf. Permeable sands are the dominant sediment type on continental shelves, but for a long time have been in a shadowy existence in microbial research. This is because they have a low organic carbon content and were thus believed to be biogeochemical deserts. More recent studies in temperate coastal ecosystems showed, however, that permeable sediments indeed are microbially highly active and may act as biocatalytical filters for the overlying water column. The high microbial activities in these permeable sediments were suggested to be the consequence of strong advective pore water flows which constantly supply the microbial community with labile organic matter and high-energetic electron acceptors on the one hand and of a strong coupling between the heterotrophic bacterial community and the benthic microalgae on the other hand. Meanwhile, almost nothing is known about the bacterial community structure and the spatial and temporal dynamics of microbial activities and community structure in these areas and how they are coupled to ecosystem functioning and stability.

The overall aim of this study was therefore to gain insight into the magnitude of variability in microbial activities and community structure in coastal sandy sediments in space and time and to determine how this variability is linked to external environmental factors. The first part of this thesis (Chapter 2 and 3) focuses on the coupling between microbial activities and the primary productivity of the MPB in a shallow subtidal sediment. The role of the MPB and MPB-derived carbon sources for the overall heterotrophic microbial metabolism was investigated and the influence of the MPB on temporal and vertical changes in microbial distributions and activities in the sediment was estimated. The second part of this thesis (Chapter 4 and 5) focuses on the bacterial community structure in coastal sandy sediments and how it varies in time and space. The community data was evaluated with regard to a large variety of contextual parameters in order to determine the main environmental drivers for variability in bacterial community structure. More specifically, the main objectives of this thesis were:

Part I:

- To explore the role of the microphytobenthos as a carbon and energy source for the bacterial community in coastal sandy sediments.

- To study vertical and seasonal changes in microbial activities in coastal sands.
- To determine the main environmental drivers for temporal and spatial variations in microbial activities in this ecosystem.

Part II:

- To gain an understanding of variations in bacterial diversity in coastal sandy sediments.
- To investigate vertical patterns in bacterial community structure in coastal sands and their causes.
- To study temporal dynamics in bacterial community structure and to explore whether they are linked to seasonal changes in microbial activities.
- To study spatial variability in bacterial community structure and activity in relation to macrofauna distribution
- To determine the main environmental drivers for temporal and spatial variations in bacterial community structure in coastal sandy sediments.

Chapter 2 describes a mesocosm study on carbon and nitrogen uptake by bacteria and microalgae in near shore sandy sediments under nutrient-limited and nutrient-replete conditions. Sediments for the experimental set-up were retrieved from the *Hausstrand*, a shallow subtidal sandflat in the North Frisian Wadden Sea, Germany. Sediment mesocosms received a pulse of $H^{13}CO_3^-$ and $^{15}NH_4^+$ after 2 weeks of incubation and the subsequent uptake of carbon and nitrogen by bacteria and the MPB was studied by analysis of ^{13}C and ^{15}N incorporation into hydrolysable amino acids, including the bacterial biomarker D-alanine. It was hypothesized that the MPB would excrete high amounts of newly-fixed carbon in the form of carbohydrates under nutrient-limited conditions compared to nutrient-replete conditions. The transfer of ^{13}C from the MPB to bacterial biomass was measured in order to assess if these carbohydrates are an important carbon source for the heterotrophic bacteria in these sediments and to quantify how much of the excreted carbohydrates would be utilized by the bacterial community.

Chapter 3 deals with temporal dynamics and vertical patterns of microbial abundances and activities on the *Hausstrand*, a shallow subtidal sand flat in the Sylt- Rømø Basin in the North Frisian Wadden Sea, Germany. Microbial abundances, microbial growth and potential extracellular enzymatic activities were measured during 6 field campaigns, covering a temperature range of 18°C. The ratio of bacterial turnover of MPB-derived carbon sources

was estimated for the different seasons, and the effects of temperature, sediment depth and other factors controlling microbial activity were explored by multivariate statistics. This study was intended to provide insight into the influence of the microphytobenthos on carbon cycling by heterotrophic benthic bacteria in a highly dynamic environment. The working hypothesis was that the vertical and temporal distribution of the MPB and MPB-derived carbon sources controls bacterial distributions and activities in the sediment. The major goal of this study was to quantify such an influence should it existed.

At the same sampling site, depth-related patterns and temporal changes in benthic microbial community structure were assessed by automated ribosomal intergenic spacer analysis (ARISA) over a 2-year period, involving data from 8 sampling campaigns and covering all seasons (Chapter 4). The community analysis was combined with a systematic measurement of 23 environmental and bacterial parameters and multivariate statistical analyses were applied in order to test whether 1) stable depth-related patterns in microbial activities and community structure exist despite the strong physical forces of waves and tides at the field site, 2) the strong seasonal dynamics in temperature and pelagic primary productivity are reflected in microbial activities and community structure and 3) depth-related and temporal changes in benthic microbial activities are reflected by changes in microbial community structure and 4) to identify the environmental parameters that best explain depth-related patterns and temporal changes in the microbial community structure of coastal sandy sediments.

Chapter 5 presents the results of a study that aimed to investigate spatial and temporal variations in microbial activities and community structure on an intertidal sand flat. The study was conducted in the framework of a large-scale *Arenicola marina* lugworm exclusion experiment in the Königshafen area, a semi-enclosed bay at the northern tip of the island of Sylt, Germany. The experimental set-up consisted of 6 experimental blocks, each comprising a lugworm exclusion plot and an ambient control plot 400 m^2 in size. The experimental blocks were arranged in a 2-factorial nested design so that 3 blocks were situated in the low intertidal, characterized by fine-grained sands, and 3 blocks in the mid intertidal, characterized by medium-sized sand. Samples were taken in March and September. The bacterial community structure was assessed via ARISA and a large variety of contextual parameters was measured in lockstep.

Interactions between biological, spatial, temporal and biochemical variables were investigated by Pearson's correlation analysis and variation partitioning was performed in order to

disentangle the respective effects of lugworm presence/absence, depth, space and time on variations in microbial community structure, microbial activities and sediment biochemistry.

The aim of this study was to explore which are the environmental drivers for variations in microbial community structure and activities in intertidal sands. It was hypothesized that differences in sediment characteristics due to the spatial setting of the blocks or due to the influence of bioturbation by *Arenicola marina* would have major effects on the microbial community.

1.11 Material and Methods

1.11.1 Study sites and sampling scheme

The Wadden Sea is one of the largest coherent tidal flat systems of the world and located at the southern and eastern coasts of the North Sea. It stretches from Den Helder in the Netherlands to Blåvands Huk in Denkmark and covers an area of roughly 13,000 km^2 (van Beusekom & de Jonge 2002). The Wadden Sea is characterized by a semi-diurnal tidal cycle with a tidal range of up to 3.5 m and strong seasonal changes in temperature, light availability and phytoplankton biomass (van Beusekom 2005). Tidal flats constitute about 70% of the North Frisian Wadden Sea (Reise & Riethmüller 1998) where the two *in situ* studies of this thesis took place (Fig. 7). The first site, the so-called "*Hausstrand*" site is a subtidal sandflat in close vicinity to the List ferry harbor at the eastern side of the German island of Sylt close to the Danish border. The site is characterized by the strong hydrodynamic forces of tides and wind-induced waves, with a tidal amplitude of ~2 m. Water depth ranges between 0.5-2.5 m, depending on weather and the tidal phase. Sediments consist of well to moderately well sorted silicate sand with a medium particle size of 350 µm and a permeability of 1.01×10^{-11} m^2 to 3.36×10^{-11} m^2 (upper 15 cm).

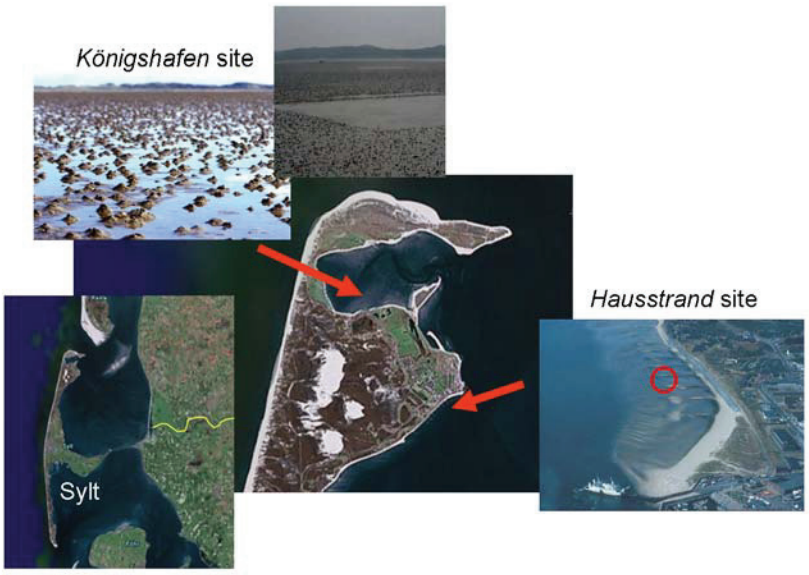

Fig. 7: Overview over the study area on the island of Sylt and the *Hausstrand* and *Königshafen* sampling sites (including one of the lugworm exclusion plots at the *Königshafen* site; satellite pictures derived from Google Earth).

The second study site, hereafter called "*Königshafen*", is an intertidal sand flat in the Königshafen bay at the northern tip of Sylt, characterized by medium to fine sand of low organic carbon content (< 1%) and densely populated by the lugworm *Arenicola marina*. The mean tidal range is 1.8 m. The study was undertaken in the framework of a large-scale lugworm exclusion experiment (see Volkenborn & Reise 2006). Briefly, the experimental set-up included 6 experimental blocks, each consisting of one *Arenicola marina* exclusion plot and one bioturbated control plot.

The total study period comprised 20 months, starting in August 2004 and ending in April 2006, thereby covering a temperature range of more than 20°C. At the surge-exposed *Hausstrand site*, sediment cores were collected in August and October 2004, February, April, July and November 2005 and at the beginning and end of March 2006 (Fig. 8). Data from February 2005 until end of March 2006 were incorporated in the first *in situ* study of this thesis (Chapter 3), dealing with temporal variations in microbial activities and carbon turnover, while all 8 sampling dates were included in the second *in situ* study (Chapter 4) which was concerned with time- and sediment depth-related variations in bacterial diversity.

At the more protected *Königshafen* site, sediment samples were collected on all plots by the use of cut-off syringes in September 2005 and March 2006 (Fig. 8) in order to investigate temporal, spatial and bioturbation effects on microbial activities and bacterial community structure (Chapter 5).

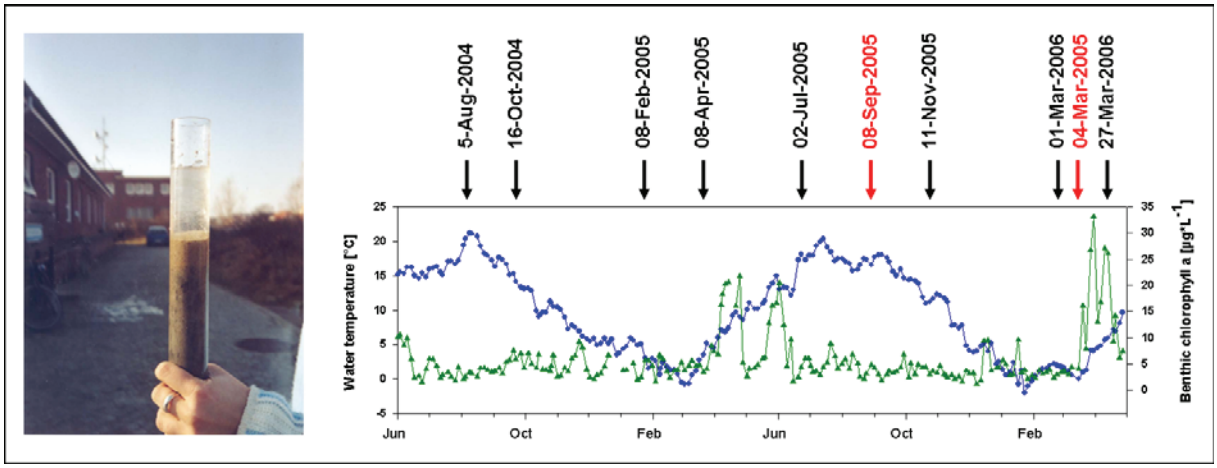

Fig. 8: Left panel: collected sediment core from *Hausstrand*; right panel: overview over the different sampling dates. Dates in black represent sampling campaigns at *Hausstrand*, dates in red represent sampling campaigns at the *Königshafen* site. In blue, water temperature is depicted, in green benthic chlorophyll *a* was plotted.

A detailed description of the study sites, sampling procedures and measurements is given in the respective chapters.

1.11.2 Automated ribosomal intergenic spacer analysis (ARISA)

The bacterial community structure analyses in this study were performed by automated ribosomal intergenic spacer analysis (ARISA). ARISA is a rapid and effective method for assessing microbial community diversity and structure and is especially useful at the fine spatial and temporal scales necessary in ecological studies. It is a qualitative culture-independent fingerprinting technique which allows estimating the microbial diversity and community structure in natural samples without the labor imposed by the construction of rRNA gene clone libraries. The method targets the intergenic transcribed spacer region (ITS) between the small (16S) and large (23S) subunit rRNA genes in the rRNA operon. This ITS region displays significant heterogeneity in both length and nucleotide sequence, however only the length heterogeneity is utilized in the ARISA method (Fisher & Triplett 1999).

Briefly, the DNA is extracted from a mixed bacterial community in an environmental sample. A polymerase chain reaction (PCR) is conducted with a universal and a eubacterial oligonucleotide primer targeted to conserved regions in the 16S and 23S rRNA genes, whereof the latter is fluorescently tagged. The amplification products are cleaned and analyzed via capillary electrophoresis comprising laser detection of fluorescent DNA fragments. Discrimination of fragment sizes is received by comparison with a base standard. Each peak in the electropherogram represents at least one operational taxonomic unit (OTU) (Fig. 9).

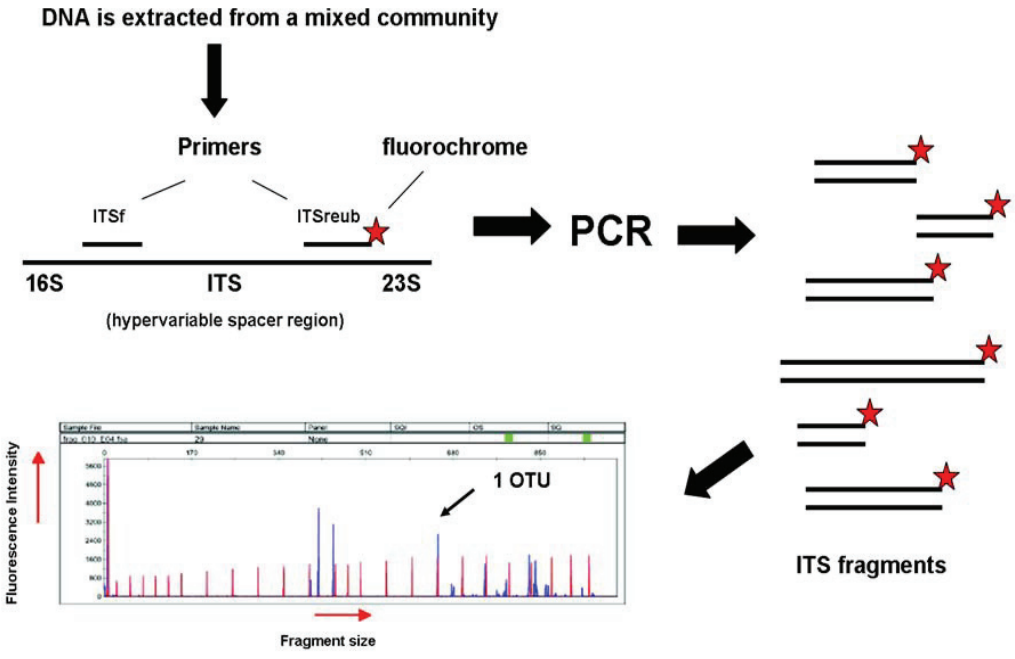

Fig. 9: Work flow of the ARISA approach.

The ARISA data may be easily analyzed by pair-wise comparisons of different samples, either based on the mere presence/absence of operational taxonomic units or including the relative fluorescence information from the electropherograms. Although ARISA profiles may be susceptible to methodological artifacts occurring during the amplification process, as they are typical for PCR-based approaches, ARISA patterns have been observed to be insensitive towards different numbers of PCR cycles (ranging from 15 to 30). Additionally, a number of studies have demonstrated that ARISA is very robust and produces equivalent profiles in both peak number and intensity from multiple amplifications in the same sample or in replicate samples (Fisher & Triplett 1999, Yannarell & Triplett 2005).

1.12 References

Abramsky Z, Rosenzweig ML (1984) Tilman`s predicted productivity-diversity relationship shown by desert rodents. Nature (London) 309:150-151

Alderkamp AC, van Rijssel M, Bolhuis H (2007) Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. FEMS Microbiol Ecol 59(1):108-117

Aller RC, Yingst JY (1978) Biogeochemistry of tube-dwellings: a study of the sedentary polychaete *Amphitrite ornate* (Leidy). J Mar Res 36:201-254

Aller RC (1982) The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: McCall PL, Tevesz MJS (eds.) Animal-Sediment Relations. Plenum Press. pp 53-102

Aller JY, Aller RC (1986) Evidence for localized enhancement of biological activity associated with tube and burrow structures in deep sea sediments at the Hebble Site, western North Atlantic. Deep-Sea Res 33(6):755-790

Aller RC (1990) Bioturbation and manganese cycling in hemipelagic sediments. Philos. Trans. R. Soc. London. Ser. A 331:51-68

Aller RC (2001) Transport and reactions in the bioirrigated zone. In: Boudreau BP, Jørgensen BB (eds.) The Benthic Boundary Layer. Transport Processes and Biogeochemistry. Oxford University Press. Oxford, UK. pp 269-301

Baas-Becking LGM (1934) Geobiologie of Inleiding to de Milieukunde. Van Stockum WP & Zoon NV

Bearman G (1989) Waves, tides and shallow-water processes. 1 ed. Pergamon Press

Bell T, Ager D, Song J-I, Newman JA, Thompson IP, Lilley AK, van der Gast CJ (2005) Larger islands house more bacterial taxa. Science 308(5730):1884

Berner RA (1980) Early Diagnesis – A Theoretical Approach. Princeton University Press.

Bertness MD, Leonard GH (1997) The role of positive interactions in communities: Lessons from intertidal habitats. Ecology 78:1976-1989

Billerbeck M, Røy H, Bosselmann K, Huettel M (2007) Benthic photosynthesis in submerged Wadden Sea intertidal flats. Est Coast Shelf Sci 71 (3-4):704-716

Bodiou JY, Chardy P (1973) Analyse en composantes principales du cycle annuel dùn peuplement de copepods harpactoides de sables fin infralittoraux de Banyuls-sur-Mer. Mar Biol 20:27-34

Boesch DG (1973) Classification and community structure of macrobenthos in the Hampton Roads area, Virgina. Mar Biol 20:27-34

Boetius A, Lochte K (1996) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. Mar Ecol Prog Ser 140:239-250.

Bornette G, Amoros C (1996) Disturbance regimes and vegetation dynamics: role of floods in riverine wetlands. J. Veg. Science 7:615-622

Boschker HTS, Cappenberg TE (1998) Patterns of extracellular enzyme activities in littoral sediments of Lake Gooimeer, the Netherlands. FEMS Microbiol Ecol 25:79-86

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, Nielsen P, Sansone F, Taghon G, van Raaphorst WR, Webster, I, Weslawski JM, Wiberg P, Sundby B (2001) Permeable marine sediments: overturning an old paradigm. EOS Trans. Am. Geophys. Union 82:133-136

Boucher G, Lambshead PJD (1995) Ecological biodiversity of marine nematodes in samples from temperate, tropical and deep-sea regions. Conservation Biology 9:1594-1604

Bowman JP, MacCammon SA, Gibson JAE, Robertson L, and Nicholas PD (2003) Prokaryotic metabolic activity and community structure in Antarctic Continental shelf sediments. Appl Environ Microbiol 69:2448−2462.

Brotas V, Plante-Cuny MR (2003) The use of HPLC pigment analysis to study microphytobenthos communities. Acta Oecol-Int J Ecol 24:109-115

Cahoon LB (1999) The role of benthic microalgae in neritic ecosystems. Oceanogr. Mar. Biol., Annu. Rev. 37:47-86

Canfield DE, Jørgensen BB, Fossing H, Glud RN, Gundersen JK, Ramsing NB, Thamdrup B, Hansen JW, Nielsen LP, Hall POJ (1993) Pathways of organic carbon oxidation in three continental margin sediments. Mar Geol 113:27-40

Canfield DE, Kristensen E, Thamdrup B (eds.) (2005) Heterotrophic carbon metabolism. In: Aquatic geomicrobiology. Elsevier Academic Press.

Chamley H (1990) Sedimentology. Springer

Cifuentes A, Antón J, Benlloch S, Donnelly A, Herbert RA, and Rodriguez-Valera F (2000) Prokaryotic diversity in *Zostera noltii*-colonized marine sediments. Appl Environ Microbiol 66:1715-1719.

Colebrook JM (1979) Continuous plankton records: zooplankton and environment, north-east Atlantic and North Sea, 1948-1973. Oceanol Acta 1:9-23

Conley DJ, Johnstone RW (1995) Biogeochemistry of N, P and Si in Baltic Sea sediments: response to a simulated deposition of a spring diatom bloom. Mar Ecol Prog Ser 122:265-276

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302-1310

Cook PLM, Veuger B, Böer S, Middelburg JJ (2007) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat Microb Ecol 40:165-180

Cook RE (1969) Variation in species density of North American birds. Syst Zool18:63-84.

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an Antarctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69(4):2253-2268

Currie DJ (1991) Energy and large-scale patterns of animal- and plant-species richness. Am Nat 137(1):27-24

D'Andrea AF, Aller RC, Lopez (2002) Organic matter flux and reactivity on a South Carolina sandflat: The impact of porewater advection and macrobiological structures. Limnol Oceanogr 47:1056-1070

Darcy H (1856) Les Fontaines Publiques de la Ville de Dijon. Dalmont

Dauwe B, Middelburg JJ, Herman PMJ (2001) Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Mar Ecol Prog Ser 215:13-22

de Beer D, Wenzhöfer F, Ferdelman TG, Boehme SE, Huettel M, van Beusekom JEE, Böttcher ME, Musat N, Dubilier N (2005) Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnol Oceanogr. 50(1):113-127

de Brouwer JFC, Stal LJ (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar Ecol Prog Ser 218:33-44

de Haas H, van Weering TCE, de Stieger H (2002) Organic carbon in shelf seas: sinks or sources, processes and products. Cont. Shelf Res 22:691-717

Denny MW (1988) Biology and the mechanics of the wave-swept environment. Princeton University Press

Donachie SP, Foster JS, Brown MV (2007) Culture clash: challenging the dogma of microbial diversity. The ISME Journal 1:97-99

Duarte CM, Cebrian J (1996) The fate of marine autotrophic production. Limnol Oceanogr 41:1758-1766

Ehrenhaus S, Huettel M (2004) Advective transport and decomposition of chain-forming planktonic diatoms in permeable sediments. J Sea Res 52:179-197

Ehrenhauss S, Witte U, Bühring SI, Huettel M (2004) Effect of advective pore water transport on distribution and degradation of diatoms in permeable North Sea sediments. Mar Ecol Prog Ser 271:99-111

Emery KO (1968) Relict sediments on continental shelves of world. AAPG Bulletin 52:445-464

Falkowski PG, Barber RT, Smetacek V (1998) Biogeochemical Controls and Feedbacks on Ocean Primary Production. Science 281:200-206

Fenchel T, Finlay BJ (2004) The ubiquity of small species: patterns of local and global diversity. BioScience 54:777-784

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. PNAS 103(3):626-631

Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. Science 296:1061-1063

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65(10): 4630-4636

Fleming K, Johnston P, Zwartz D, Yokoyama Y, Lambeck K, Chapell J (1998) Refining the eustatic sea-level curve since the last glacial maximum using far and intermediate field sites. Earth Planet Sci Lett 163:327-342

Flöder S, Sommer U (1999) Diversity in planktonic communities: An experimental test of the intermediate disturbance hypothesis. Limnol Oceanogr 44:1114-1119

Forster S, Huettel M, Ziebis W (1996) Impact of boundary layer flow velocity on oxygen utilisation in coastal sediments. Mar Ecol Prog Ser 143:173-185

Fox JF, Connell JH (1979 Intermediate-Disturbance Hypothesis. Science 204:1344-1345

Franco MA, de Mesel I, Demba Diallo M, van der Gucht K, van Gansbeke D, van Rijswijk P, Costa MJ, Vincx M, Vanaverbeke J (2007) Effect of phytoplankton bloom deposits on benthic bacterial communities in two contrasting sediments in the southern North Sea. Aquat Microb Ecol 48:241-254

Franke HD, Gutow L (2004) Long-term changes in the macrozoobenthos around the rocky island of Helgoland (German Bight, North Sea). Helgol Mar Res 58:303-310

Froehlich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P, Hammond D, Hartmann B, Maynard V (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxid diagenesis. Geochim Cosmochim Acta 43:1075-1090

Fuhrman JA, Hewson I, Schwalback MS, Steele JA, Brown MV, Naeem S (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. PNAS 103(35):13104-13109

Gätje C, Reise K (eds.) (1998) Ökosystem Wattenmeer: Ausstausch-, Transport- und Stoffumwandlungsprozesse. Springer Verlag. Berlin, Germany

Gattuso JP, Frankignoulle M, Wollast R (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. Annu Rev Ecol Syst 29:405-434

Gattuso JP, Gentili B, Duarte CM, Kleypas JA, Middelburg JJ, Antoine D (2006) Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. Biogeosciences 3:489-513

Gehlen M, Malschaert H, van Raaphorst WR (1995) Spatial and temporal variability of benthic silica fluxes in the southeastern North Sea. Cont Shelf Res 13:1675-1696.

Gerdts G, Wichels A, Döpke H, Klings KW, Gunkel WM, Schütt C (2004) 40-year long-term study of microbial parameters near Helgoland (German Bight, North Sea): historical view and future perspectives. Helgol Mar Res 58:230-242

Gieskes WWC, Kraay GW (1975) The phytoplankton spring bloom in Dutch coastal waters of the North Sea. Neth. J. Sea Res. 9:166-196

Glud RN, Kuhl M, Wenzhofer F, Rysgaard S (2002) Benthic diatoms of a high Arctic fjord (Young Sound, NE Greenland): importance for ecosystem primary production. Mar Ecol Prog Ser 238:15-29

Goering JC, Patton CJ, Shields WW (1973) Nutrient cycles. In: Hood EW, Shields WW, Kelley EJ (eds.) Environmental studies of Port Valdez. Inst Mar Sci Occ Publ. University of Alaska. Fairbanks, USA. pp 253-271

Goñi-Urriza M, de Montaudouin X, Guyoneaud R, Bachelet G, de Wit R (1999) Effect of macrofaunal bioturbation on bacterial distribution in marine sandy sediments, with special reference to sulphur-oxidising bacteria. J Sea Res 41:269−279.

Goto N, Mitamura O, Terai H (2001) Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J Exp Mar Biol Ecol 257:73-86

Grassle JF, Smith W (1976) A similarity measure sensitive to the contribution of rare species and its use in investigation of variation in marine benthic communities. Oecologia 25:13-22

Gray JP, Herwig RP (1996) Phylogenetic analysis of the bacterial communities in marine sediments. Appl Environ Microbiol 62:4049−4059.

Green JL, Holmes AJ, Westoby M, Oliver I, Briscoe D, Dangerfield M, Gillings M, Beattie AJ (2004) Spatial scaling of microbial eukaryote diversity. Nature:432:747-750

Green J, Bohannan BJM, Whitaker RJ (2008) Microbial biogeography: From taxonomy to traits. Science 320(5879):1039-1043

Greve W, Reiners F (1988) Plankton time-space dynamics in the German Bight – a system approach. Oecologia 77:487-496

Greve W, Lange U, Reiners F, Nast J (2001) Predicting the seasonality of North Sea zooplankton. In: Krönke I, Türkay M, Sündermann J (ed) Burning issues of North Sea ecology, Proceedings of the 14th International Senckenberg Conference North Sea 2000. Senckenb Marit 31:263-268

Greve W, Reiners F, Nast J, Hoffmann S (2004) Helgoland Roads meso- and macrozooplankton time-series 1974 to 2004: lessons from 30 years of single spot, high frequency sampling at the only off-shore island of the North Sea. Helgol Mar Res 58:274-288

Gribsholt B, Kostrka JE, Huettel M (2003) Impact of fiddler crabs and plant roots on sediment biogeochemistry in a Georgia saltmarsh. Mar Ecol Prog Ser 259:237-251

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113-121

Harris CK, Wiberg P (2002) Across-shelf sediment transport: Interactions between suspended sediment and bed sediment. J Geophys Res-C 107

Hewson I, Jacobson/Meyers ME, Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ Microbiol 9(4):923-933

Hoagland KD, Rosowski JR, Gretz MR, Roerner SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J Phycol 29(5):537-566

Horner-Devine MC, Leibold MA, Smith VH, Bohannan JM (2003) Bacterial diversity patterns along a gradient of primary productivity. Ecol. Lett. 6(7):613-622

Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM (2004) A taxa-area relationship for bacteria. Nature 432:750-753

Hubas C, Lamy D, Artigas LF, Davoult D (2007) Seasonal variability of intertidal bacterial metabolism and growth efficiency in an exposed sandy beach during low tide. Mar Biol 151(1):41-52

Huettel M, Gust G (1992) Impact of bioroughness on interfacial solute exchange in permeable sediments. Mar Ecol Prog Ser 89:253-267

Huettel M, Ziebis W, Forster S, Luther GW (1998) Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochim Cosmochim Acta 62:613-631

Huettel M, Rusch A (2000) Transport and degradation of phytoplankton in permeable sediment. Limnol Oceanogr 45:534-549

Huettel M, Webster IT (2001) Porewater flow in permeable sediments. In: Boudreau BP, Jørgensen BB (eds.) The benthic boundary layer: transport processes and biogeochemistry. Oxford University Press. pp 144-179

Huettel M, Røy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231-236

Hughes Martiny JB, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nature 4: 102-112

Ishii K, Mußmann M, MacGregor B, Amann R (2004) An improved fluorescence in situ hybridization protocol for the identification of bacteria and archaea in marine sediments. FEMS Microbiol Ecol 50:203−212

Iversen N, Jørgensen BB (1993) Diffusion-coefficients of sulfate and methane in marine sediments – Influence of porosity. Geochim Cosmochim Acta 57:571-578

Jablonski D (1993) The tropics as a course of evolutionary novelty through geological time. Nature 364:142-144

Jahnke RA, Nelson JR, Marinelli RL, Eckman JE (2000) Benthic flux of biogenic elements on the Southeastern US continental shelf: influence of pore water advective transport and benthic microalgae. Cont Shelf Res 20:109-127

Janzen DH (1981) The peak in North American ichneumonid species richness lies between 38° and 42° N. Ecology 62:532-537

Jørgensen BB, Bang M, Blackburn TH (1990) Anaerobic mineralization in marine sediments from the Baltic Sea - North Sea transition. Mar Ecol Prog Ser 59:39-54.

Jørgensen BB (2000) Bacteria and Marine Biogeochemistry. In: Schulz HD, Zabel M (eds.) Marine Geochemistry. Springer. Berlin-Heidelberg, Germany. pp 173-206

Johnson HD, Baldwin CT (1986) Shallow siliclastic seas. In: Reading HG (ed.) Sedimentary environments and facies. Blackwell Scientific Publications. pp 229-282

Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65(3):1241-1250

Kawamata (1998) Effect of wave-induced oscillatory flow on grazing by a subtidal sea urchin Strongylocentrotus nudus (A. Agassiz). J Exp Mar Biol Ecol 224:31-48

Kent AD, Jones SE, Yanarell AC, Graham JM, Lauster GH, Kratz TK, Triplett EW (2004) Annual patterns in bacterioplankton community variability in a humic lake. Microb Ecol 48:550-560

Köster M, Dahlke S, Meyer-Reil LA (2005) Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). Aquat Microb Ecol 39:69-83

Kostka JE, Gribsholt B, Petrie E, Dalton D, Skelton H, Kristensen E (2002) The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments. Limnol Oceanogr 47:230-240

Kristensen E, Holmer M (2001) Decomposition of plant materials in marine sediment exposed to different electron acceptors (O_2, NO_3^-, and SO_4^{2-}), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. Geochim Cosmochim Acta 65:419-433

Kristensen E (2000) Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1-24

Kristensen E (2001) Impact of polychaetes (*Nereis* spp. and *Arenicola marina*) on carbon biogeochemistry in coastal marine sediment. A review. Geochem Trans 2:92-104

Kristensen E, Kostka JE (2004) Macrofaunal Burrows and Irrigation in Marine Sediment: Microbiological and Biogeochemical Interactions. In: The Ecogeomorphology of Tidal Marshes. Coastal and Estuarine Studies 59. Am. Geophys. Union. pp 1-35

Lambeck K, Chapell J (2001) Sea level change through the last glacial cycle. Science 292:679-686

Lange U, Greve W (1997) Does temperature influence the spawning time, recruitment and distribution of flatfish via its influence on the rate of gonadal maturation? Dtsch Hydrogr Z 49:251-263

Levings CD (1975) Analysis of temporal variation in the structure of a shallow-water benthic community in Nova Scotia. Int Rev Gesamten Hydrobiol 55:449-469

Liu KK, Iseki K, Chao SY (2002) Continental margin carbon fluxes. In: Hanson RB, Ducklow HW, Field JG (eds.) The changing carbon cycle: A midterm synthesis of the Joint Global Ocean Flux Study. University Press. pp 187-239

Llobet-Brossa E, Rossellò-Mora R, Amann R (1998) Microbial community composition of Wadden Sea sediments as revealed by Fluorescence-*in-situ*-Hybridization. Appl Environ Microbiol 64:2691−2696

Llobet Brossa E (2000) Microbial community structure and dynamics of Wadden Sea sediments (Dangast). Dissertation Thesis. Universität Bremen. Bremen, Germany

Lohse L, Epping HG, Helder W, van Raaphorst W (1996) Oxygen pore water profiles in continental shelf sediments of the North Sea: turbulent versus molecular diffusion. Mar Ecol Prog Ser 145:63-75

MacArthur RH, MacArthur JW (1961) On bird species diversity. Ecology 42:594-598

MacIntyre HL, Geider RJ, Muller DC (1996) Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries 19:186-201

Martinez J, Smith DC, Steward GF, Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquat Microb Ecol 10:223-230

Matsui GY, Ringelberg DB, Lovell CR (2004) Sulfate reducing bacteria in tubes constructed by the marine infaunal polychaete Diopatra cuprea. Appl Environ Microbiol 70:7053-7065

Mayer LM (1989) Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnol Oceanogr 34(6):973-981

McCave IN (1972) Transport and escape of fine-grained sediment from shelf areas. In: Swift D, Duane D, Pilke O (eds.) Shelf sediments and transport: process and pattern. Hutchinson & Ross.

McCave IN (2002) Sedimentary settings on continental margins - an overview. In: Wefer G., Billet D., Hebbeln D., Jørgensen B. B., Schlüter M., van Weering T. C. E. (eds.) Ocean margin systems. Springer. pp 1-14

Meybeck M (1982) Carbon, nitrogen, and phosphorus transport by world rivers. Am J Sci 282:401-450

Meyer-Reil LA (1987) Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. Appl Environ Microbiol 53(8):1748-1755

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ 13C-labeling study. Limnol Oceanogr 45:1224-1234

Middelburg JJ, Duarte CM, Gattuso J-P (2005) Respiration in coastal benthic communities. In: del Giorgio PA, Williams PJB (eds.) Respiration in Aquatic Ecosystems. Oxford University Press. Oxford. pp 206-224

Middelburg JJ, Soetaert K (2005) The role of sediments in shelf ecosystem dynamics. In: Robinson AR, Brink KH (eds.) The Global Coastal Ocean: Multiscale Interdisciplinary Processes, Vol 13. Harvard University Press. Cambridge, USA. p 1033

Milliman JD, Ross DA, Pilkey OH (1972) Sediments of the continental margin off the eastern United States. Geological Society of America Bulletin 83:1315-1334

Milliman JD, Summerhayes CP (1975) Upper continental margin sedimentation off Brazil. Schweizerbart.

Musat N, Werner U, Knittel K, Kolb S, Dodenhof T, van Beusekom JEE, Dubilier N, Amann R (2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. Syst Appl Microb 29:333-348

Muus BJ (1967) The fauna of Danish estuaries and lagoons. Distribtion and ecology of dominating species in the shallow beaches of the mesohaline zone. Medd Dan Fisk Havunders 5:1-316

Muylaert K, van der Gucht K, Vloemans N, de Meester L, Gillis M, Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68(10):4740-4750

Nittrouer CA, Wright LD (1994) Transport of Particles across Continental Shelves. Rev Geophys 32:85-113

Ortega-Calvo JJ, Stal LJ (1994) Sulphate-limited growth in the unicellular cyanobacterium *Gloethece* (Nageli) sp. PCC 6909. New Phytol 128(2):273-281

Owen JG (1988) On productivity as a predictor of rodent and carnivore diversity. Ecology 69:1161-1165

Papaspyrou S, Kristensen E, Christensen B (2007) *Arenicola marina* (Polychaeta) and organic matter mineralization in sandy marine sediments: In situ and microcosm comparison. Est Coast Shelf Sci 72:213-222

Papke RT, Ramsing NB, Bateson MM, Ward DM (2003) Geographical isolation in hot spring cyanobacteria. Environ Microbiol 5:650-659

Papke RT, Ward DM (2004) The importance of physical isolation to microbial diversification. FEMS Microbiol Ecol 48:293–303

Passow U (1991) Species-specific sedimentation and sinking velocities of diatoms. Mar Biol 108:449-455

Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol Ò, Malits A, Marrasé C (2004) Changes in bacterioplankton composition under different phytoplankton regimens. Appl Environ Microbiol 70(11):6753-6766

Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Links between geographic location, environmental factors, and microbial community composition in sediments of the eastern Mediterranean Sea. Microb Ecol 49:367-378

Pommier T, Canväck L, Riemann L, Boström KH, Simu K, Lundberg P, Tunlid A, Hagström Å (2006) Global patterns of diversity and community structure in marine bacterioplankton. Mol Ecol 16(4):867-880

Poore GCB, Wilson GDF (1993) Marine species richness. Nature 361:597-598

Precht E (2003) Advective interfacial exchange in permeable sediments driven by surface gravity waves and its ecological consequences. Dissertation Thesis. Universität Bremen. Bremen, Germany.

Precht E, Huettel M (2003) Advective pore-water exchange driven by surface gravity waves and its ecological implications. Limnol Oceanogr 48:1674-1684

Precht E, Huettel M (2004) Rapid wave-driven advective pore water exchange in a permeable coastal sediment. J Sea Res 51:93-107

Rabenold KN (1979) A reversed latitudinal diversity gradient in avian communities of eastern deciduous forests. Am Nat 114:275-288

Ramette A, Tiedje JM (2007a) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. PNAS 104(8):2761-2766

Ramette A, Tiedje JM (2007b) Biogeography: An emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. Microb Ecol 53(2):197-207

Reche I, Pulido-Villena E, Morales-Baquero R, Casamayor EO (2005) Does ecosystem size determine aquatic bacterial richness? Ecology 86:1715-1722

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:206-222

Reichardt W (1988) Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. Mar Ecol Prog Ser 44:149-158

Reimers CE, Stecher III H A, Taghon, GL, Fuller CM, Huettel M, Rusch A, Ryckelynck N, Wild C (2004) In situ measurements of advective solute transport in permeable shelf sands. Cont Shelf Res 24:183-201.

Reise K (1977) Predator Exclusion Experiments in an Intertidal Mud Flat. Helgoländer Wiss Meeresunters 30:263-271

Reise K, Riethmüller R (1998) The Sylt-Rømø Bight in the Wadden Sea: an overview. In: Gätje C, Reise K (eds.) The Wadden Sea Ecosystem – Exchange, transport and transformation processes. Springer Verlag. pp 21-23

Rex MA, Stuart CT, Hessler RR, Allen JA, Sanders HL, Wilson GDF (1993) Global-scale latitudinal patterns of species diversity in the deep-sea benthos. Nature 365:636-639

Riedl RJ, Huang N, Machan R (1972) The subtidal pump: Mechanism of interstitial water exchange by wave action. Mar Biol 13:210-221

Rink B (2007) Structure and dynamics of heterotrophic bacterial communities in the German Wadden Sea and the German Bight. Dissertation Thesis. Universität Oldenburg. Oldenburg, Germany

Rocha C (2000) Density-driven convection during flooding of warm, permeable intertidal sediments: the ecological importance of the convective turnover pump. J Sea Res 43:1-14

Rochelle PA, Cragg BA, Fry JC, Parkes RJ, Weightman AJ (1994) Effect of sample handling on estimation of bacterial diversity in marine sediments by 16S rRNA gene sequence analysis. FEMS Microbiol Ecol 15:215-225.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. Nature 421:57-60

Rosenzweig ML (1995) Species diversity in space and time. Cambridge University Press

Roy K, Jablonski D, Valentine JW, Rosenberg G (1998) Marine latitudinal diversity gradients: Tests of causal hypotheses. PNAS 95:3699-3702

Rusch A, Huettel M (2000) Advective particle transport into permeable sediments – evidence from experiments in an intertidal sandflat. Limnol Oceanogr 45:525-533

Rusch A, Forster S, Huettel M (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry 55:1-27

Rusch A, Huettel M, Reimers CE, Taghon GL, Fuller CM (2003) Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sands. FEMSMicrobiol Ecol 44:89-100

Rusch A, Huettel M, Wild C, Reimers CE (2006) Benthic oxygen consumption ond organic matter turnover in organic-poor, permeable shelf sands. Aquat Geochem 12:1-19

Sapp M, Wichels A, Wiltshire KH, Gerdts G (2007) Bacterial community dynamics during the winter-spring transition in the North Sea. FEMS Microbiol Ecol 59:622-637

Simpson GG (1964) Species densities of North American mammals. Syst Zool 13:361-389.

Smetacek VS (1985) Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. Mar Biol 84:239-251

Smith DJ, Underwood GJC (1998) Exopolymer production by intertidal epipelic diatoms. Limnol Oceanogr 43:1578-1591

Snelgrove PVR (1999) Getting to the bottom of marine biodiversity: Sedimentary habitats. BioScience 49(2):129-138

Sousa ME (1979) Disturb in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. Ecology 60:1225-1239

Steward CC, Nold SC, Ringelberg DB, White DC, Lovell CR (1996). Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments. Mar Ecol Prog Ser 133:149-165

Swenson NG, Enquist BJ (2007) Ecological and evolutionary determinants of a key plant functional trait: wood density and its community-wide variation across latitude and elevation. Am J Bot 94:451-459

Tenore KR (1972) Macrobenthos of the Pamlico River estuary, North Carolina. Ecol. Monogr. 42:51-69

Thibodeaux LJ, Boyle JD (1987) Bedform-generated convective-transport in bottom sediment. Nature 325:341-343

Thorson G (1946) Reproduction and larval development of Danish marine bottom invertebrates. Medd Komm Fisk Havunders Copenhagen Ser Plankton 4:1-523

Townsend CR, Scarsbrook MR (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. Limnol Oceanogr 42(5):938-949

Tréguer P, Nelson DM, van Bennekom AJ, de Master DJ, Leynaert A, Quéguiner B (1995) The silica balance in the world ocean: a reestimate. Science 268:375-379

Ullman WJ, Aller RC (1982) Diffusion-coefficients in nearshore marine sediments. Limnol Oceanogr 27:552-556

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Advances in Ecological Research, Vol 29. Academic Press Inc. San Diego. pp 93-153

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. In: Callow JA (eds.) Advances in Botanical Research, Vol. 40. Advances in Botanical Research Incorporating Advances in Plant Pathology

van Beusekom JEE, de Jonge VN (2002) Long-term changes in Wadden Sea nutrient cycles: importance of organic matter import from the North Sea. Hydrobiologia 475:185-194

van Oevelen D, Soetaert K, Middelburg JJ, Herman PMJ, Moodley L, Hamels I, Moens T, Heip CHR (2006) Carbon flows through a benthic food web: integrating biomass, isotope and tracer date. J Mar Res 64:453-482

Volkenborn N, Reise K (2006) Lugworm exclusion experiment: Responses by deposit feeding worms to biogenic habitat transformations. J Exp Mar Biol Ecol 330:169-179

Volkenborn N, Polerecky L, Hedtkamp SIC, van Beusekom JEE, de Beer D (2007) Bioturbation and bioirrigation extend the open exchange regions in permeable sediments. Limnol Oceanogr 52(5):1898-1909

Wallace AR (1878) Tropical nature and other essays. Macmillan. New York.

Walsh JJ, Rowe GT, Iverson RL, McRoy CP (1981) Biological export of shelf carbon is a sink of the global CO_2 cycle. Nature 291:196-201

Walsh JJ, Premuzic ET, Gaffney JS, Rowe GT, Harbottle G, Stoenner RW, Balsam WL, Betzer PR, Macko SA (1985) Organic storage of CO2 on the continental slope off the Mid-Atlantic Bight, the southeastern Bering Sea, and the Peru Coast. Deep Sea Research Part a – Oceanic Research Papers 32:853-883

Webb JE, Theodor J (1968) Irrigation of submerged marine sands through wave action. Nature 220:682-683

Webster IT, Norquay SJ, Ross FC, Wooding RA (1996) Solute exchange by convection within estuarine sediments. Est Coast Shelf Sci 42:171-183

Weiher E, Keddy PA (1995) Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74:159-164

Weiss MS, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE (1991) Molecular architecture and electrostatic properties of a bacterial porin. Science 254:1627-1630

Welsh DT (2003) It´s a dirty job but someone has to do it: the role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. Chem Ecol 19(5):321-342

Werner U (2005) Microbial activity and transport processes in near-shore, permeable sediments. Dissertation thesis. Universität Bremen. Bremen, Germany

Weyhenmeyer GA, Bleckne T, Pettersson K (1999) Changes of the plankton spring outburst related to the North Atlantic Oscillation. Limnol Oceanogr 44:1788-1792

Weyhenmeyer GA (2001) Warmer winters: are planktonic algal populations in Sweden`s largest lakes affected? Ambio 30:565-571

Wiltshire KH, Dürselen CD (2004) Revision and quality analyses of the Helgoland Reede long-term phytoplankton data archive. Helgol Mar Res 58:252-268

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic Archaea. Science 301:976-978

Whitlatch RB (1977) Seasonal changes in the community structure of the macrobenthos inhabiting the intertidal sand and mud flats of Barnstable harbor, Massachusetts. Biol Bull (Woods Hole) 152:275-294

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. PNAS 95:6578-6583

Woese C, Fox G (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. PNAS 74:5088–5090

Wollast R (1991) The coastal organic carbon cycle: fluxes, sources, and sinks. In: Mantoura RFC, Martin JM, Wollast R (eds.) Ocean Margin Processes in Global Change. John Wiley & Sons. pp 365-381

Wollast R (2002) Continental Margins – Review of Geochemical Settings. In: Wefer G, Billett D, Hebbeln D, Jørgensen BB, Schlüter M, van Weering TCE (eds.) Ocean Margin Systems. Springer. pp 15-31

Wright DH (1983) Species-energy theory: an extension of species-area theory. Oikos 41:496-506

Yannarell AC, Kent AD, Lauster GH, Kratz TK, Triplett EW (2003) Temporal patterns in bacterial communities in three temperate lakes of different trophic status. Microb Ecol 46:391-405

Yannarell AC, Triplett EW (2004) Within- and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. Appl Environ Microbiol 70(1):214-223

Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. Appl Environ Microbiol 71(1):227-239

Ysebaert T, Herman PMJ (2002) Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. Mar Ecol Prog Ser 244:105-124

Ziebis W, Huettel M, Forster S (1996) Impact of biogenic sediment topography on oxygen fluxes in permeable seabeds. Mar Ecol Prog Ser 140: 227-237

Zijlstra JJ (1988) The North Sea Ecosystem. In: Postma H, Zijlstra JJ (eds.) Continental shelves, Ecosystems of the world, Volume 27. Elsevier. Amsterdam, The Netherlands. pp 273

1.13 Publications outline

This thesis comprises four manuscripts, presented as chapters. One article comprised a co-authorship and has been published in the international science journal *Aquatic Microbial Ecology*. One manuscript has been published shortly after submission of this thesis in the international science journal *Biogeosciences Discussions*. One manuscript has been submitted to *The ISME Journal* and is currently under revision. One manuscript is in preparation for submission.

Chapter 2:

Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment

by Perran L.M. Cook, Bart Veuger, Simone Böer and Jack J. Middelburg

The concept of the study was developed by Perran L.M. Cook, Bart Veuger and Jack J. Middelburg. Perran L.M. Cook carried out the mesocosm experiment and measured all data except for bacterial growth measurements which were performed by Simone Böer and isotope tracer incorporation measurements which were conducted by Bart Veuger. Perran L.M. Cook evaluated the data and wrote the manuscript with editorial help from all co-authors.

This manuscript has been published in *Aquatic Microbial Ecology*.

Chapter 3:

Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments

by Simone Böer, Carol Arnosti, Justus E.E. van Beusekom and Antje Boetius

This study was initiated by Simone Böer and Antje Boetius. Simone Böer organized and coordinated all field campaigns, obtained the samples, performed all measurements and evaluated the data except for the following: water-column data and part of the benthic oxygen consumption data was provided by Justus E.E. van Beusekom, the chromatographic measurement of FLA-labeled laminarin hydrolysis and subsequent hydrolysis rate estimations were conducted by Carol Arnosti. The manuscript was written by Simone Böer with editorial help and input from Carol Arnosti, Antje Boetius and Justus E.E. van Beusekom.

This manuscript has recently been published in *Biogeosciences Discussions*.

Chapter 4:

Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sands

by Simone Böer, Stefanie I.C. Hedtkamp, Justus E.E. van Beusekom, Jed A. Fuhrman, Antje Boetius and Alban Ramette

The concept of this study was developed by Simone Böer, Antje Boetius and Alban Ramette. Simone Böer organized and coordinated all field campaigns, obtained the samples (with help of Stefanie I.C. Hedtkamp at the beginning of the study), performed all measurements and evaluated the data except for the following: water column data and sedimentary nutrients data was provided by Justus E.E. van Beusekom, Stefanie Hedtkamp measured benthic oxygen consumption rates, carbohydrate concentrations and photopigment concentrations at the first three sampling dates of this study. Jed A. Fuhrman provided a training for the use of ARISA to Alban Ramette who established the method at the MPI. The concept for the statistical analysis of the data was developed by Alban Ramette with input by Simone Böer who also performed the analyses. Statistical scripts were written by Alban Ramette. Simone Böer wrote the manuscript with input from Alban Ramette and Antje Boetius and with editorial help from all authors.

This manuscript has been submitted to *The ISME Journal*.

Chapter 5:

Spatial and temporal variations in bacterial activities and community structure associated with faunal exclusion experiments in intertidal sands of the North Sea (Sylt)

by Simone Böer, Abdul Al-Raei, Michael Böttcher, Nils Volkenborn and Alban Ramette

The study was initiated by Simone Böer who also organized and coordinated the field campaigns in cooperation with Abdul Al-Raei and Michael Böttcher. The experimental plots were created by Nils Volkenborn. Samples were obtained by Simone Böer, Abdul Al-Raei and Michael Böttcher. Simone Böer conducted the measurements on bacterial diversity, bacterial abundances, extracellular enzymatic activities, photopigments and carbohydrates, Abdul Al-Raei and Michael Böttcher measured sulfate reduction rates, sediment biogeochemistry and porosity. The data was evaluated by the respective experimenters and compiled by Simone Böer according to the requisites of this study. All statistical analyses were performed by Simone Böer with input by Alban Ramette who also provided the statistical scripts. Simone Böer wrote the manuscript with editorial help from all authors.

This manuscript is in preparation for submission to *Environmental Microbiology*.

Chapter 2

Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment

P.L.M. Cook, B. Veuger, S.I. Böer, J.J. Middelburg

published in *Aquatic Microbial Ecology*

Vol. 49: 165–180, 2007
doi: 10.3354/ame01142

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published November 15

OPEN ACCESS

Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment

Perran L. M. Cook[1,3,*], Bart Veuger[2], Simone Böer[1], Jack J. Middelburg[2]

[1]Max Planck Institute for Marine Microbiology, Celsiusstr 1, Bremen 28359, Germany
[2]Netherlands Institute of Ecology (NIOO-KNAW), Centre for Estuarine and Marine Ecology, PO Box 140, 4400 AC Yerseke, The Netherlands
[3]*Present address:* Water Studies Centre, Monash University, Clayton, 3800 Victoria, Australia

ABSTRACT: Carbon and nitrogen uptake in a microbial community comprising bacteria and microalgae in a sandy marine sediment under nutrient-limited and -replete conditions was studied using a mesocosm approach. After 2 wk of incubation, a pulse of $H^{13}CO_3^-$ and $^{15}NH_4^+$ was added to the mesocosms, and subsequent uptake of ^{13}C and ^{15}N by bacteria and microphytobenthos (MPB) was traced by analysis of ^{13}C and ^{15}N incorporation into hydrolysable amino acids, including the bacterial biomarker D-alanine. The results confirm that MPB communities are capable of sustained high rates of photosynthesis despite nutrient limitation. Under these conditions cellular growth stops (as defined by the synthesis of chlorophyll *a* and amino acids) and the carbon fixed under such conditions consists predominantly of carbohydrates produced through 'overflow metabolism'. In the treatment with nutrient addition, algal growth was stimulated and label incorporation was more balanced, with carbohydrates accounting for a much smaller fraction of newly fixed organic carbon. There was close agreement between net C fixation based on O_2 fluxes and the increase of particulate organic carbon in the sediment under both nutrient-limited and -replete conditions. This finding suggests that very little fixed C was lost to the water column as dissolved organic carbon (DOC), consistent with direct measurements of DOC release using ^{14}C. There was a significant and rapid transfer of ^{13}C from the MPB to bacterial biomass in both treatments within 24 h of label addition, revealing that fixed carbon excreted by MPB was rapidly utilised by bacteria. In both treatments, bacteria incorporated a significant fraction of ^{15}N from $^{15}NH_4^+$, with the greatest incorporation being observed under nutrient-limited conditions.

KEY WORDS: Amino acid · Isotope label · Nitrogen · Carbon · Algae · Bacteria · Sediment · Excretion

INTRODUCTION

Primary production by phytoplankton is generally considered to underpin the productivity of oceanic and near-shore food webs, with nitrogen-rich biomass providing a rich resource for higher trophic levels. In near-shore euphotic sediments, microphytobenthos (MPB) may have similar, if not higher, rates of total primary production compared to phytoplankton (Underwood & Kromkamp 1999), and may also be a significant source of carbon for higher trophic levels (Kang et al. 2003). In contrast to pelagic algae, however, MPB appear to direct a large fraction of primary production into synthesis of carbohydrates, accounting for as much as 70% of this production (Goto et al. 1999, de Brouwer & Stal 2001). A number of functions of this carbohydrate production have been suggested. It is thought that extracellular polymeric substances (EPS) are produced for a number of reasons, including locomotion, adhesion to sediment particles and sediment cohesion (Underwood

*Email: perran.cook@sci.monash.edu.au

& Paterson 2003). Carbohydrate production may also play a role in the maintenance of cell nutrient balances by excreting and/or storing carbon assimilated in excess of the ratio to nutrients required for cell growth. This is consistent with the observation that both EPS and intracellular carbohydrate (ICH) production increase under nutrient-limited conditions (in particular N) in culture studies of benthic and pelagic algal species (Staats et al. 1999, 2000b, Engel et al. 2002).

The fact that a large fraction of carbon fixed by MPB is directed into EPS synthesis (a labile, carbon-rich, nitrogen-poor substrate; Biddanda & Benner 1997, Wetz & Wheeler 2003) has a number of profound ecological consequences relevant to our understanding of the coupling of the C and N cycles in near-shore benthic ecosystems. Firstly, one would expect the assimilation of dissolved inorganic C and N to be well above the expected 'Redfield' C:N ratios of cellular algal material (~7). Indeed, a recent field study of a shallow water mesotrophic ecosystem dominated by MPB suggested a significant deviation from Redfield C:N ratios in benthic primary production (Cook et al. 2004). It should be noted, however, that such estimates of N assimilation by MPB in a field setting are highly uncertain, because MPB derive a large fraction of N from within the sediment—a flux which is difficult to accurately constrain. Constantly changing light regimes in natural settings further complicate efforts to scale up the relative rates of CO_2 and N assimilation, because they are often temporally decoupled. As such, controlled experimental approaches are necessary to further elucidate the relative assimilation rates of C and N within benthic microbial communities.

Substrate C:N ratio will also affect the amount of N released upon mineralisation, and above a critical threshold of C:N ≈ 20 there will no longer be a net release of N (Blackburn 1986). For example, it has been shown that marine bacteria growing on labile, high C:N ratio substrates will assimilate inorganic nitrogen to synthesise their low C:N ratio biomass (e.g. Goldman & Dennett 2000), and, thus, it seems likely that EPS with high C:N ratios derived from MPB will have the same effect on sediment bacterial communities. Once again, this is consistent with field data from mesotrophic estuaries, which show an extremely low release of N from the sediment relative to C, compared to that expected from 'Redfield' stoichiometry (Cook et al. 2004, Ferguson et al. 2004). Thus, the input of EPS into the sediment is likely to give rise to a situation of intense microbial competition for available nitrogen. It has been suggested that low rates of nitrification in sediments colonised by MPB may be due to competition for NH_4^+ between nitrifiers and heterotrophic bacteria, possibly stimulated by the extracellular carbohydrate production (Risgaard-Petersen 2003). One might expect an ecologically paradoxical situation under nutrient-limited conditions, in which MPB create an intense competition for nitrogen between themselves and bacteria, because of increased rates of EPS production. In the pelagic environment, it has been found that bacteria assimilate inorganic nutrients and even outcompete algae under low nutrient conditions (Bratbak & Thingstad 1985, Goldman & Dennett 2001), confirming that nutrient status may play an important role in determining the coupling between C and nutrient cycling and in the pathways of these elements through the microbial food web. To date, however, no studies have investigated whether this may be the case within benthic algal and bacterial communities.

The lack of studies investigating the relative flows of C and nutrients through benthic microbial communities is partly due to difficulties in distinguishing between bacterial and algal pools of carbon and nitrogen and the rates of transfer between them. Fatty acid biomarkers have proved to be a useful tool for tracing C through the algal and bacterial compartments of food webs (Boschker & Middelburg 2002); however, they give no information about N flows. The recent development of a new method for stable isotope analysis of hydrolysable amino acids, including the bacterial biomarker D-alanine (D-Ala) (Veuger et al. 2005), now makes it possible to trace both C and N through the algal and bacterial compartments of benthic food webs. A further advantage of this method is that EPS extraction procedures are not needed, which may extract intracellular carbon as well as excreted carbon (Chiovitti et al. 2004).

The objective of the present study was to assess the effect of nutrient limitation and excess nutrients on the composition of organic matter generated by the benthic primary production, and the fate of C and N within the algal and bacterial pools in sandy sediment. Based on previously published findings addressing pelagic microbial communities, we tested the hypothesis that carbon fixed by microphytobenthos is rapidly transferred to the benthic bacterial community, with N limitation leading to production of extracellular polymeric substances depleted in N and a shift in the relative rate of N uptake from algae towards bacteria. To this end, we conducted a dual stable-isotope pulse-chase experiment in a mesocosm and followed the pathways of ^{13}C and ^{15}N within the bacterial and algal pools in the incubated near-shore sandy sediment.

MATERIALS AND METHODS

Experimental setup. At the study site on the Island of Sylt, Germany, surface sand (upper 5 cm) was collected during July 2004 from mobile sublittoral sands,

characterised by high rates of benthic primary production (~5 to 7 mmol m^{-2} h^{-1} O_2). For a detailed description of the study site see Cook & Røy (2006) and Cook et al. (2007). For convenience, the sand was stored for 2 mo at 4°C in the dark prior to use. This was justified on the basis that (1) *in situ*, MPB survive for many months buried in the sediment over winter, and (2) we found that areal rates of photosynthesis in (unsieved) sediment preserved in this way rapidly (1 d) returned to rates measured in the field (Cook & Røy 2006). The sediment was sieved (500 µm mesh, to remove macrofauna and large particles), rinsed (to remove detritus and non-attached cells) and transferred to 2 aquaria (390 × 230 mm) to form a ~5 mm layer of sand overlaid by 11 cm (10 l) of seawater collected from the North Sea. Microscopic analysis of the sand showed that the MPB community was dominated by episammic diatoms *in situ*; a list of the species present is given in Cook & Røy (2006). The aquaria were placed in a climate-controlled room (15°C) and illuminated at a uniform irradiance of ~300 µE m^{-2} s^{-1} (saturating in these sediments; F. Wenzhöfer unpubl. data) on a 12:12 h light:dark cycle. These temperature and light conditions were chosen as they were considered to be most representative of the regime occurring *in situ* during times of low nutrient concentrations in the water column and, hence, of nutrient limitation (late spring to early autumn). Water was recirculated through the aquaria at a rate of ~1 l min^{-1}, thus ensuring that the water column was well mixed. To 1 aquarium NH_4^+, $Si(OH)_4$ and HPO_4^- were added daily in amounts equivalent to 0.8 mmol of N and Si, and 0.05 mmol of P (enough to support a daily production of ~60 mmol C m^{-2} d^{-1} assuming Redfield C:N:Si:P stoichiometry of 106:16:16:1); this treatment is referred to as the (+) treatment. The other aquarium did not receive additional nutrients; this was referred to as the (–) treatment. No algal growth was observed in the water of the (+) treatment; 14 d into the incubation and at the start of the dark phase, pulses of $^{15}NH_4^+$ (100 µM final conc.) and $H^{13}CO_3^-$ (1000 µM final conc.) were added to the aquaria. (This timing was based on the observation that photosynthesis in the 2 treatments had diverged and stabilised at this point.) After a complete light:dark cycle (24 h) following the additions, the water in each aquarium was replaced with unlabeled fresh seawater containing 0.08 mmol Si, 0.14 mmol NO_3^- and 0.02 mmol P.

Primary production and respiration were measured by placing a lid over the water surface of the respective aquarium and measuring the O_2 concentration change over time. The O_2 fluxes were corrected for the O_2 leak rate for each aquarium, which was determined in aquaria filled only with water after O_2 had been depleted in the water by purging with N_2 gas. After primary production and respiration had been measured, a sediment sample equivalent to ~5% of the sediment surface area of the aquarium was taken in 1 spot from the top ~3 mm of sediment (using a spatula). The sample was homogenised and subsampled for chlorophyll *a* (chl *a*), bacterial production, extracellular carbon production rates, sediment carbohydrates, sediment hydrolysable amino acid (HAA) concentrations and label incorporation, total label incorporation and content analyses of the percent C and N in the sediment. Bacterial production and extracellular carbon production rates were measured immediately. Sediment samples for all other parameters were frozen at –20°C and then freeze dried before analysis within 6 mo.

Only 1 tank for each treatment was used, and, hence, there was no true replication in this experimental design. We justify this on the basis that nutrient addition to a phototrophic community will clearly elicit a growth response that is well known and was indeed observed. Accepting that the 2 tanks are different, our aim was to make measurements of a range of parameters which are less well understood in relation to algal growth and nutritional status, including carbohydrates, carbon excretion, bacterial production and the relative assimilation of C and N by algae and bacteria. These parameters were replicated over time in the aquaria, so we were able to test for significant differences between the tanks using a Wilcoxon matched pairs test. In doing this we assume that the difference in algal growth and nutrition between the 2 aquaria was the only variable giving rise to these differences. Other potentially important variables such as the light field, water circulation and temperature were carefully controlled to ensure that they were the same in both aquaria.

DOC production. In order to measure the proportion of fixed carbon lost from the sediment as dissolved organic carbon (DOC), duplicate ~0.5 g subsamples of sediment were placed in 15 ml clear polypropylene centrifuge vials with 3 ml of filtered seawater and $H^{14}CO_3^-$ tracer (~2 kBq). The vials were incubated under the same light field as the aquaria for 2 to 3 h, after which the samples were stored overnight in the dark at 4°C. The following day, the vials were centrifuged at 1000 × *g* for 1 min and a 2 ml sample of supernatant was acidified with 100 µl of 6 M HCl and purged with N_2 to remove all CO_2 before scintillation counting. For the determination of ^{14}C fixed into the sediment, the sediment was placed in 20 ml scintillation vials. All $^{14}CO_2$ remaining in the sample was removed as described above. Blanks (water samples to which only ^{14}C were added) were run to ensure the efficiency of this procedure. Scintillation cocktail (Ultima Gold) was added to the super-

natant and sediment samples, and the radioactivity was measured in a Packard Tri-Carb 2900TR or 2500TR liquid scintillation counter. Counts for the sediment samples were corrected for self quenching, which was determined by adding known amounts of radiation (^{14}C acetate) to sand from the same site. Photosynthesis rates were calculated using the ratio of radioactivity to TCO_2 (CO_2 + H_2CO_3 + HCO_3^- + CO_3^{2-}) concentrations measured in the tracer solutions. The fraction of DOC released from the sediment was then calculated in relation to total ^{14}C fixed into the sediment.

Bacterial production. Bacterial production was estimated by measuring ^{14}C leucine incorporation using L-[U-^{14}C]leucine (specific activity 303 mCi mmol^{-1}, Amersham). Triplicate samples of approximately 1 ml sediment were taken from each aquarium 3 to 4 h into the light phase of the daily cycle and mixed with 1 ml of sterile, filtered seawater. Then, 100 µl of aqueous leucine solution (containing 0.5 nmol ^{14}C-leucine and 399.5 nmol leucine) was added to each of the slurries, and they were incubated for 1 to 2 h at 15°C in the dark. The incubations were terminated upon the addition of 100 µl of 37% formalin, and the samples were stored at 4°C until analysis. An additional blank sample from each aquarium was supplied with 100 µl of 37% formalin prior to the addition of leucine.

Following the methodology of Findley et al. (1984) and Michel & Bloem (1993), samples were washed 3 times with 2 ml of sterile, filtered, 5% formalin seawater by mixing, centrifuging and decanting the supernatant. The sediment was resuspended in 2.5 ml of 0.5 M NaOH, and the samples were heated for 2 h in a water bath at 60°C. After centrifugation, the supernatant was decanted into ice-cooled tubes and the sediment was washed with 2.5 ml of 0.5 M NaOH. The supernatants were pooled, supplied with 480 µl of 3 M HCl, 150 µl BSA (50 mg ml^{-1}) and 800 µl of 50% TCA (trichloroacetic acid) and heated for 30 min in a water bath at 80°C. After cooling on ice, the samples were filtered through cellulose-nitrate filters (0.4 µm mesh size) and the filters were washed 5 times with 5% ice-cold TCA. The filters were transferred to scintillation vials, and 12 ml of Ultima Gold scintillation cocktail was added. After 12 h, the radioactivity was counted on a 2500TR liquid scintillation counter. Benthic bacterial production rates were estimated according to Kirchman (1993). This approach assumes that all leucine incorporation is by bacteria, which may not necessarily be the case (Veuger & Middelburg 2007). Uptake of leucine by algae would mean that the bacterial production rates, and hence bacterial production: primary production ratios, measured here were higher than the true values.

Hydrolysable amino acids. Samples were processed and analysed according to the protocol presented in Veuger et al. (2005). Briefly, samples (~1 g) of freeze-dried sediment were washed with HCl (2 M) and Milli-Q water (removing dissolvable HAAs), followed by hydrolysis of the sediment pellet in HCl (6 M) at 110°C for 20 h. After purification by cation exchange chromatography, amino acids were derivatized with isopropanol and pentafluoropropionic anhydride and samples were further purified by solvent extraction. Stable isotope ratios for carbon (R = ^{13}C:^{12}C) and nitrogen (R = ^{15}N:^{14}N) in the derivatized D- and L-amino acids were measured by gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS) and were used to calculate δ-values: $\delta X(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X = ^{13}C or ^{15}N. Standards were Vienna Pee Dee Belemnite and atmospheric nitrogen for ^{13}C and ^{15}N analyses, respectively. δX was used to calculate the atomic percent of X: at $\%X = [100 \times R_{standard} \times (\delta X_{sample}/1000) + 1]/[1 + R_{standard} \times (\delta X_{sample}/1000) + 1)]$, which was used to calculate excess X (absolute amount of incorporated ^{13}C or ^{15}N): excess X = $[(\text{at } \delta X_{sample} - \text{at.}\%X_{control})/100] \times \text{AA concentration}_{sample}$ (with amino acid [AA] concentrations expressed in mol C or N). Excess ^{13}C was calculated directly from δ^{13}C values for derivatized amino acids (using AA-C concentrations for derivatized amino acids), which bypasses correction of δ^{13}C values for added C during derivatization.

Bulk sediment and isotopic analysis. The carbon and nitrogen isotopic composition of freeze-dried sediment samples were analysed (single sample) using a Fisons CN elemental analyser coupled on-line via a Conflo 2 interface with a Finnigan Delta S mass spectrometer. Samples for C analysis were first acidified to remove carbonates. Bulk particulate organic carbon and nitrogen were analysed after acidification using a Fisons NA1500 elemental analyser.

Carbohydrates and chlorophyll *a*. Total sediment carbohydrates and EDTA-extractable (hereafter referred to as colloidal) carbohydrates were measured in triplicate on freeze-dried samples as described by Underwood et al. (1995). For total carbohydrate analysis, 2 ml of Milli-Q water was added to ~50 mg of sediment, followed by 1 ml of 5% phenol and then 5 ml concentrated sulphuric acid. The absorbance of the supernatant was measured at 485 nm and quantified against a glucose standard. Colloidal carbohydrates were extracted from 100 mg sediment with 3 ml of 100 mM EDTA for 15 min. After extraction the samples were centrifuged at 3000 × *g* for 15 min, then 2 ml of the supernatant was analysed for carbohydrates as described above. Chl *a* and pheo-pigments were measured (single sample) by colorimetry as described by Lorenzen (1967) using 0.5 ml of wet sediment.

Respiration, primary production and nutrient uptake. O_2 concentrations during the flux measurements were measured using Winkler titration (as described by Grasshoff 1983); the leak rate of O_2 into the aquaria was measured using a PreSens oxygen optode connected to a PreSens microx TX3 oxygen meter. Nutrient samples were filtered through 0.2 µm filters and frozen for later analysis using a Skalar Continuous-Flow-Analyser and the chemistry described by Grasshoff (1983). C assimilation rates were calculated assuming a 1:1 O_2:CO_2 stoichiometry, consistent with 1:1 stoichiometry measured at the site from which the sediment was derived (P. L. M. Cook unpubl. data). Net daily C fixation was calculated by subtracting dark respiration (12 h) from the net C fixation rate in the light (12 h). Daily gross C fixation rates were calculated by adding the dark respiration rate to the measured net light C fixation rate. Cumulative C fixation was calculated by adding net daily rates of C fixation. For days on which C fixation and respiration were not measured, rates were estimated as the average of the rates of the preceeding day and following day. Rates of N assimilation were calculated from daily measurements of NH_4^+ concentrations before and after NH_4^+ additions. We estimate the loss of N through nitrification and subsequent denitrification to be negligible, based on the fact that benthic algae inhibit nitrification (Risgaard-Petersen 2003); this is supported by our observation that no NO_3^- was detected in any of the samples analysed for nutrients. Furthermore, *in situ* rates of nitrification and denitrification at this study site are low (F. Wenzhoefer unpubl. data).

RESULTS

Chlorophyll *a*

The chl *a* content of the sediment in the (–) treatment remained within a relatively narrow range of 47 to 60 mg m^{-2} over the course of the experiment (Fig. 1a). For the (+) treatment, the chl *a* content of the sediment increased steadily from the start of the experiment to Day 16, when chl *a* content reached 270 mg m^{-2}. Pheophytin, a chlorophyll degradation product, was usually undetectable or it was only a small fraction of chl *a* (data not shown).

Total organic carbon

The organic carbon content of the sediment increased from an initial concentration of 600 mmol m^{-2} to 1000 and 1240 mmol m^{-2} at the conclusion of the experiment in the (–) and (+) treatments, respectively (Fig. 1b). In the (+) treatment, very high sediment carbon concentrations in excess of 1600 mmol m^{-2} were measured on Days 15 and 16, coinciding with the high chl *a* values observed at this time in a particularly dense patch of diatoms sampled on these dates. The average increases in sediment carbon content over the 21 d incubation calculated using linear regression were 470 ± 70 and 900 ± 200 mmol m^{-2} for the (–) and (+) treatments, respectively. These increases balanced closely with the calculated cumulative C fixation based on the O_2 flux measurements, assuming a production quotient of 1:1, which were 410 ± 30 and 1000 ± 70 mmol m^{-2} for the (–) and (+) treatments, respectively. The C:chl *a* ratio of the sediment also showed clear differences between the 2 treatments, with the ratio increasing up to ~300, just before the pulse in the (–) treatment, compared to a decrease to ~60 in the (+) treatment (Fig. 1c). The N:chl *a* ratio was always highest in the (–) treatment, generally varying between 20 and 30, while in the (+) treatment the N:chl *a* ratio generally remained between 10 and 20 (Fig. 1d). The C:N ratio of the (–) treatment increased markedly from a minimum value of 4 to 5 at the start of the experiment to up to 10 on Day 14, just before the pulse of $^{15}NH_4^+$ was added, after which the sediment C:N ratio dropped down to ~6. The sediment C:N ratio for the (+) treatment remained within the range of 4.5 to 6.5 (Fig. 1e).

Carbohydrates

Total carbohydrates in the sand increased steadily over the first 2 wk, reaching ~400 and 380 mmol C m^{-2} in the (–) and (+) treatments, respectively, just before the pulse of $^{15}NH_4^+$, after which the concentrations decreased, before increasing again on Day 21 (Fig. 2a); there was no significant difference between the 2 treatments ($p > 0.05$ Wilcoxon matched pairs test). Colloidal carbohydrates increased rapidly in the (–) treatment, reaching 120 to 140 mmol C m^{-2} between Days 7 and 14, after which the concentration dropped abruptly to ~70 mmol C m^{-2}, coinciding with the addition of the NH_4^+ pulse (Fig. 2b). In the (+) treatment, the colloidal carbohydrate concentrations increased more gradually and erratically to a maximum concentration of 120 mmol C m^{-2} on Day 11, before dropping rapidly to 90 mmol C m^{-2}, coincident with the addition of the ^{15}N pulse. There was no significant difference in the colloidal carbohydrate concentrations between the 2 treatments ($p > 0.05$, Wilcoxon matched pairs test). When the carbohydrate fractions are normalised to chl *a*, the differences between the 2 treatments are clear, with the carbohydrate to chl *a* ratio rapidly climbing in the (–) treatment, before dropping back

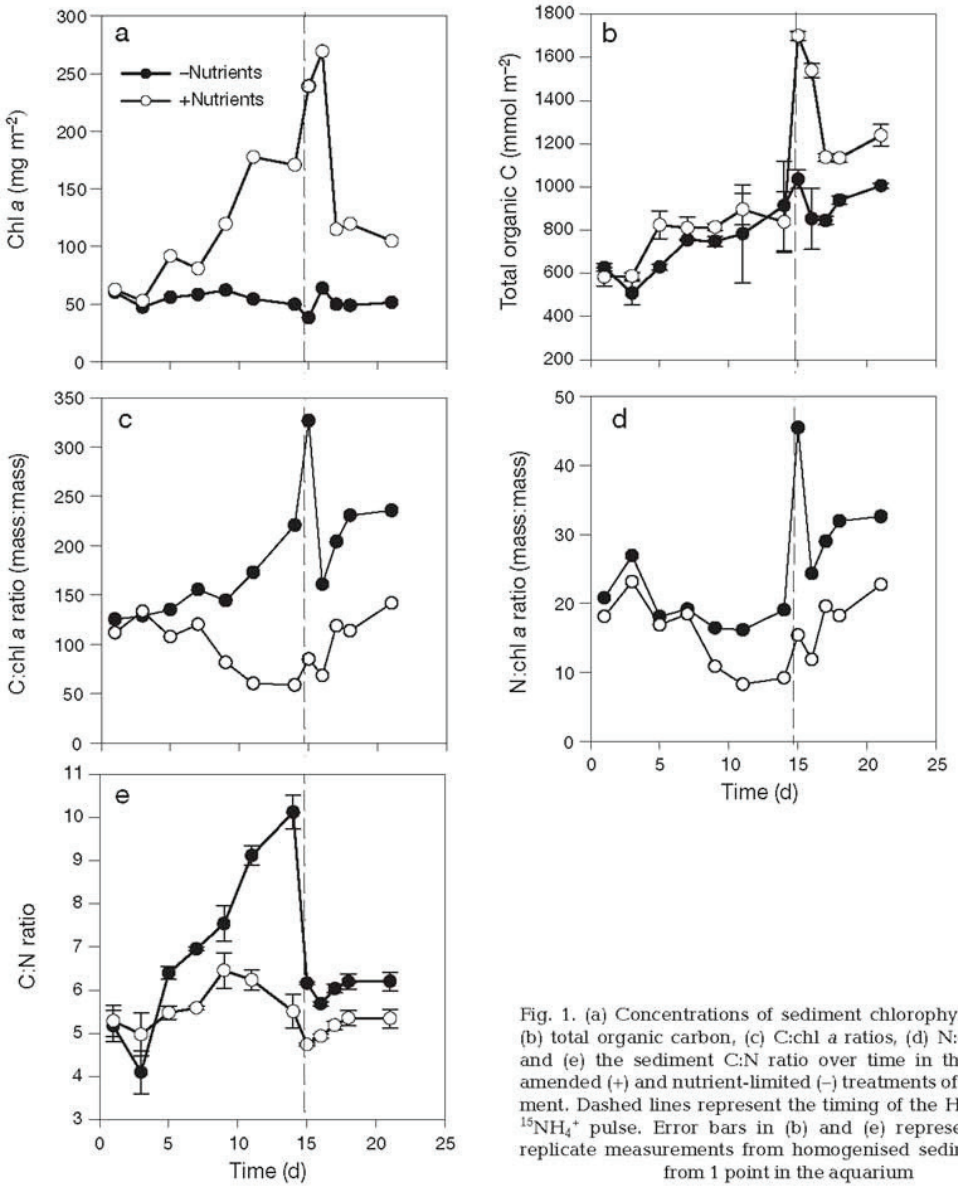

Fig. 1. (a) Concentrations of sediment chlorophyll *a* (chl *a*), (b) total organic carbon, (c) C:chl *a* ratios, (d) N:chl *a* ratios and (e) the sediment C:N ratio over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars in (b) and (e) represent ±SD of replicate measurements from homogenised sediment taken from 1 point in the aquarium

again after the $^{15}NH_4^+$ and nutrient pulse (Fig. 2c,d). The proportion of newly fixed carbon present as carbohydrates (the concentration of total C or carbohydrates at each time point minus the initial total C or carbohydrate concentration) was often 100% between the start of the experiment and Day 10 in the (–) treatment, before dropping to between 30 and 70% towards the end of the experiment (Fig. 2e). In the (+) treatment, carbohydrates generally made up between 20 and 50% of the newly fixed carbon.

Metabolism and nutrient assimilation

In the (–) treatment, net daily C fixation decreased from an initial rate of 28 to 9 mmol m^{-2} d^{-1}, just before the pulse of $^{15}NH_4^+$, after which C fixation increased to 31 mmol m^{-2} d^{-1} within 2 d (Fig. 3a). In the (+) treatment, net C fixation increased steadily over the first 9 d of the experiment, from an initial rate of 25 up to 40 mmol m^{-2} d^{-1}, after which the rates stabilised until the day after the pulse addition (Day 15), when the

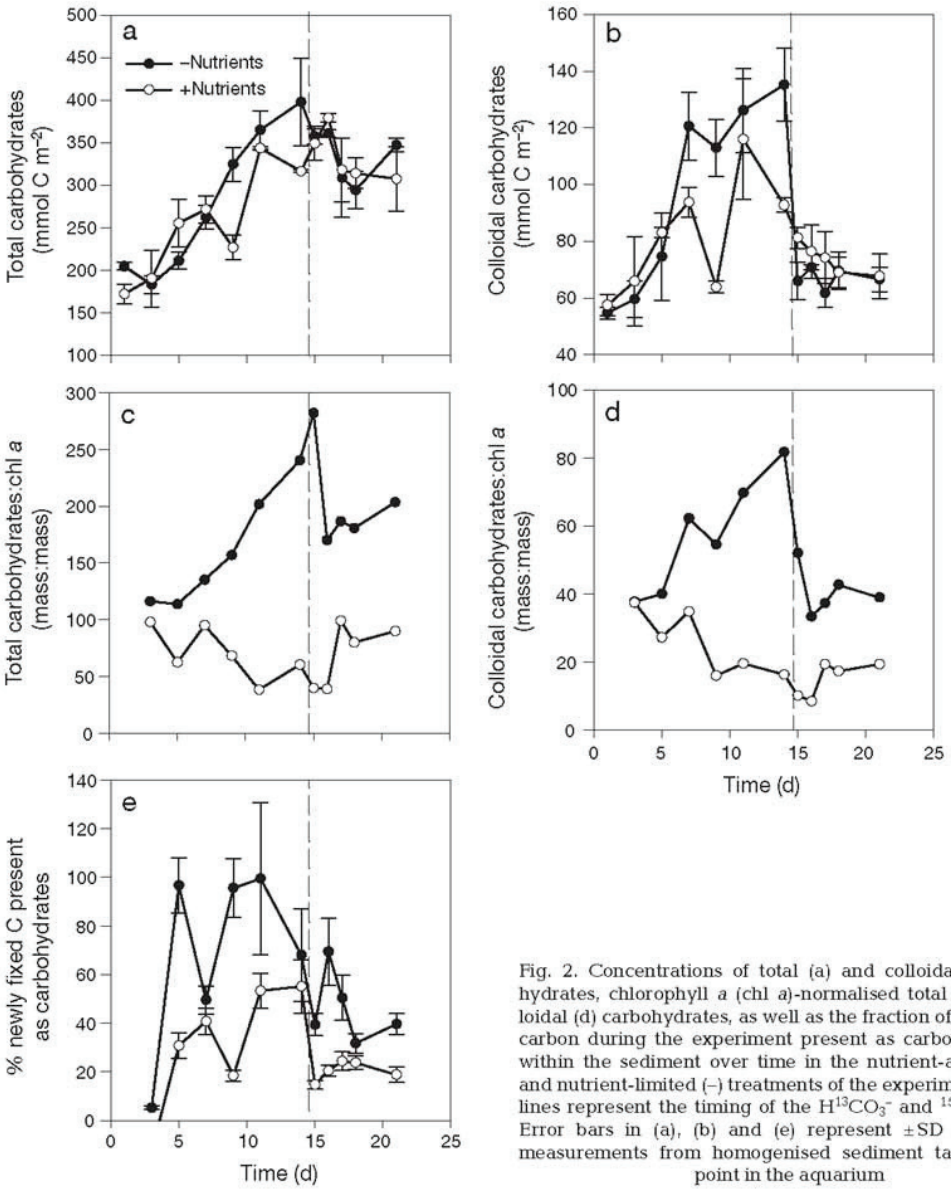

Fig. 2. Concentrations of total (a) and colloidal (b) carbohydrates, chlorophyll *a* (chl *a*)-normalised total (c) and colloidal (d) carbohydrates, as well as the fraction of newly fixed carbon during the experiment present as carbohydrates (e) within the sediment over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars in (a), (b) and (e) represent ±SD of replicate measurements from homogenised sediment taken from 1 point in the aquarium

rates of O_2 production jumped up to ~80 mmol m^{-2} d^{-1} and remained high until the conclusion of the experiment. Respiration in the (–) treatment followed no clear trend and generally remained between 10 and 23 mmol m^{-2} d^{-1} over the course of the experiment (Fig. 3b). For the (+) treatment, respiration increased dramatically over the first 5 d of the experiment, from an initial rate of 23 to up to 60 mmol m^{-2} d^{-1}, after which it stabilised, before increasing to 80 mmol m^{-2} d^{-1} on Day 21.

Nutrient measurements showed that the daily addition of N and P was assimilated in the (+) treatment, with concentrations of NH_4^+ dropping from 80 to between 0.5 and 6 µM and of PO_4^- from ~5 to ~0.05 to 1 µM in 24 h. Concentrations of $Si(OH)_4^-$ decreased to a much lesser extent, and never fell below 10 µM. However, there was no long-term build up, suggesting that the added Si was ultimately assimilated. In the (–) treatment, NH_4^+ and PO_4^- were generally <1 and 0.05 µM, respectively, and concentrations of Si remained <5 µM. Concentrations of NO_3^- remained <0.5 µM in both treatments.

Daily gross C to N assimilation stoichiometry in the (+) treatment increased to 10 within 5 d of the com-

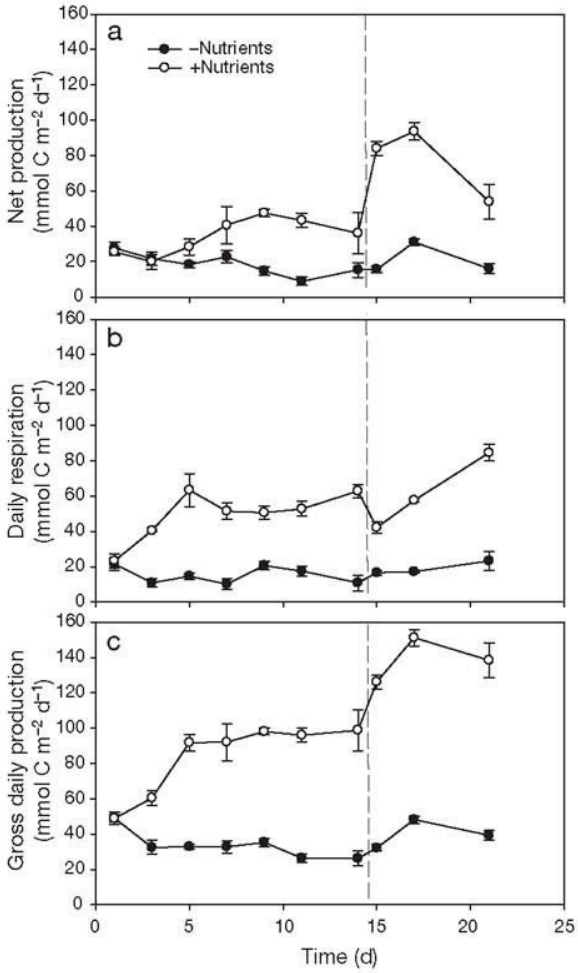

Fig. 3. Daily (a) net primary production, (b) respiration and (c) gross primary production in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars represent ±SE of the linear regression analysis of O_2 concentration changes versus time

mencement of the experiment, and subsequently increased to ~20 by Day 10, reaching its maximum value of ~40 on Day 18 (Fig. 4). Net C to N assimilation stoichiometry increased similarly, although the magnitude of the increase was much smaller, reaching a value of 13 on Day 14, when the isotope pulse was added, and climbing to as high as 27 by Day 18. The net cumulative C:N ratio of organic matter assimilated steadily increased from a value of ~3 at the start of the experiment to a value of ~5.5 at the point of the isotope pulse addition and to a maximum value of ~8 by the conclusion of the experiment.

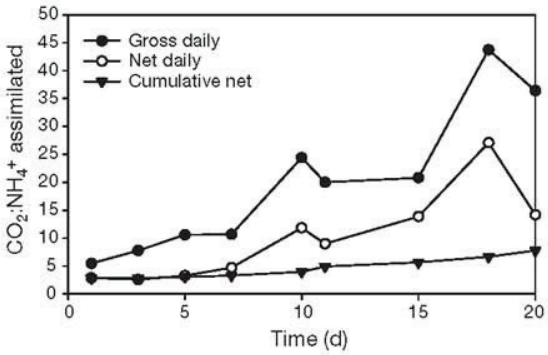

Fig. 4. Ratio of gross (Gross daily) and net (Net daily) CO_2 assimilation to NH_4^+ assimilation on a daily basis, as well as the cumulative ratio of net CO_2 to NH_4^+ assimilated over the course of the (+) nutrient experiment (Cumulative net)

Dissolved organic carbon release from the sediment

The loss of DOC from the sediment was assayed on 6 occasions for each treatment between Days 1 and 11. The loss of DOC from the sediment was very low, being 3 ± 2 and 1.4 ± 0.7 % of total C fixation for the (–) and (+) treatments, respectively.

Bacterial production

Bacterial production measured using the leucine incorporation method showed a very similar pattern for both treatments, with the rates of production being generally slightly higher in the (+) treatment, although this difference was not significant ($p > 0.05$, Wilcoxon matched pairs test). Rates of production showed an initial drop after Day 1, followed by a period of stable production (5 to 9 mmol m^{-2} d^{-1}) between Days 3 and 15, before bacterial production again rose in both treatments towards the end of the experiment (Fig. 5a). Bacterial production was generally 10 to 30 % of primary production, and this proportion was always higher in the (–) treatment, by a factor of about 2 for the majority of the experiment (Fig. 5b).

Label incorporation into hydrolysable amino acids

The total hydrolysable amino acid (THAA) content of the sediment ranged between 270 and 400 mmol C m^{-2} in the (+) treatment compared to between 140 and 204 mmol C m^{-2} in the (–) treatment (Fig. 6a). THAAs made up a smaller fraction of the carbon pool in the (–) treatment (16 to 22 %) compared to the (+) treatment (22 to 33 %; Fig. 6b). THAAs always comprised a

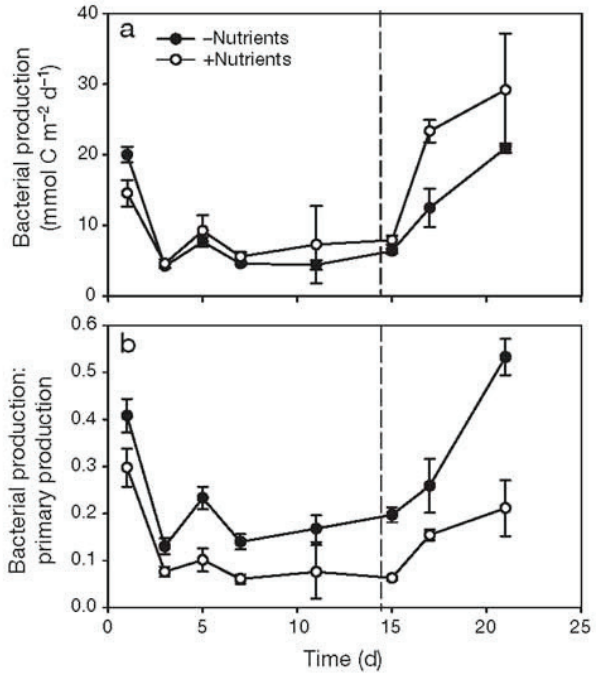

Fig. 5. (a) Bacterial productivity and (b) ratios of bacterial to primary production over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse. Error bars represent ± SD of 3 replicate analyses of sediment taken from 1 point in each aquarium

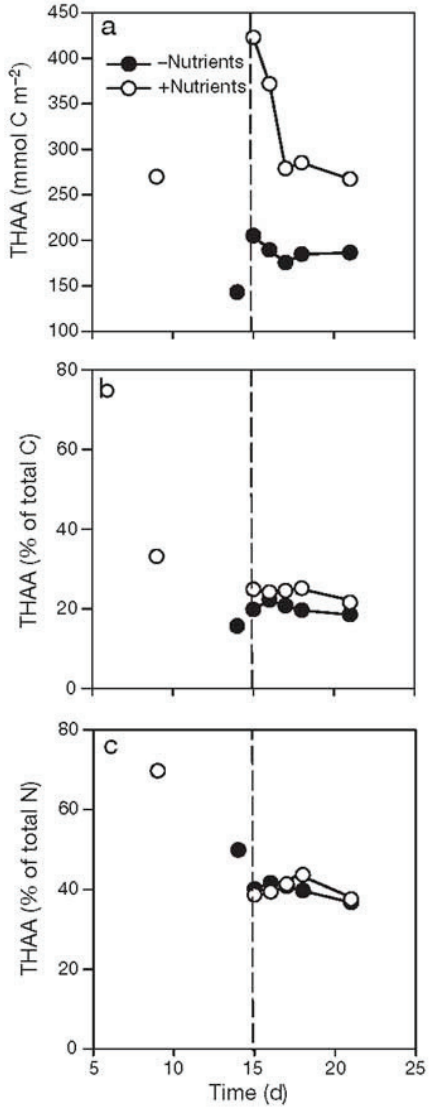

Fig. 6. (a) Concentrations of total hydrolysable amino acids (THAA), their contribution to (b) total C and (c) total N within the sediment over time in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. Dashed lines represent the timing of the $H^{13}CO_3^-$ and $^{15}NH_4^+$ pulse

greater fraction of total sediment N than C, with the highest fraction observed being 70% of total N in the (+) treatment on Day 9. After the isotope pulse, THAAs comprised just over 40% of total N for both treatments.

After ^{13}C label addition and illumination, the incorporation of ^{13}C into THAAs was higher in the (+) treatment (~6 to 12 mmol m^{-2}) compared to the (–) treatment (~2 mmol m^{-2}; Fig. 7a), consistent with the differences in net C fixation measured between the treatments during this phase of the experiment (Fig. 3a). By contrast, the initial incorporation of ^{15}N into THAAs in the dark was highest in the (–) treatment, where ~2 mmol m^{-2} of N was incorporated into THAAs compared to ~1 mmol m^{-2} for the (+) treatment (Fig. 7b). In general, however, no clear distinction could be made between the 2 treatments for $^{15}NH_4^+$ incorporation. The proportion of total ^{13}C in the sediment incorporated into THAAs after the light phase of the experiment was between 19 and 25% for the (–) treatment and between 25 and 43% for the (+) treatment (Fig. 7c). In general, a much greater fraction of ^{15}N was incorporated into THAAs, with 50 to 60% of the total label in the sediment being incorporated into THAAs within 2 d of the label addition (Fig. 7d). Excess ^{13}C and ^{15}N in D-Ala mirrored that for THAA (Fig. 7e,f). After the first illumination cycle, excess ^{13}C D/L-Ala ratios were 0.029 and 0.032 in the (+) and (–) treatments, respectively, followed by a gradual increase in both treatments, with the highest values being consistently observed in the (–) treatment (Fig. 7g). After 12 h in the dark, the excess ^{15}N D/L-Ala

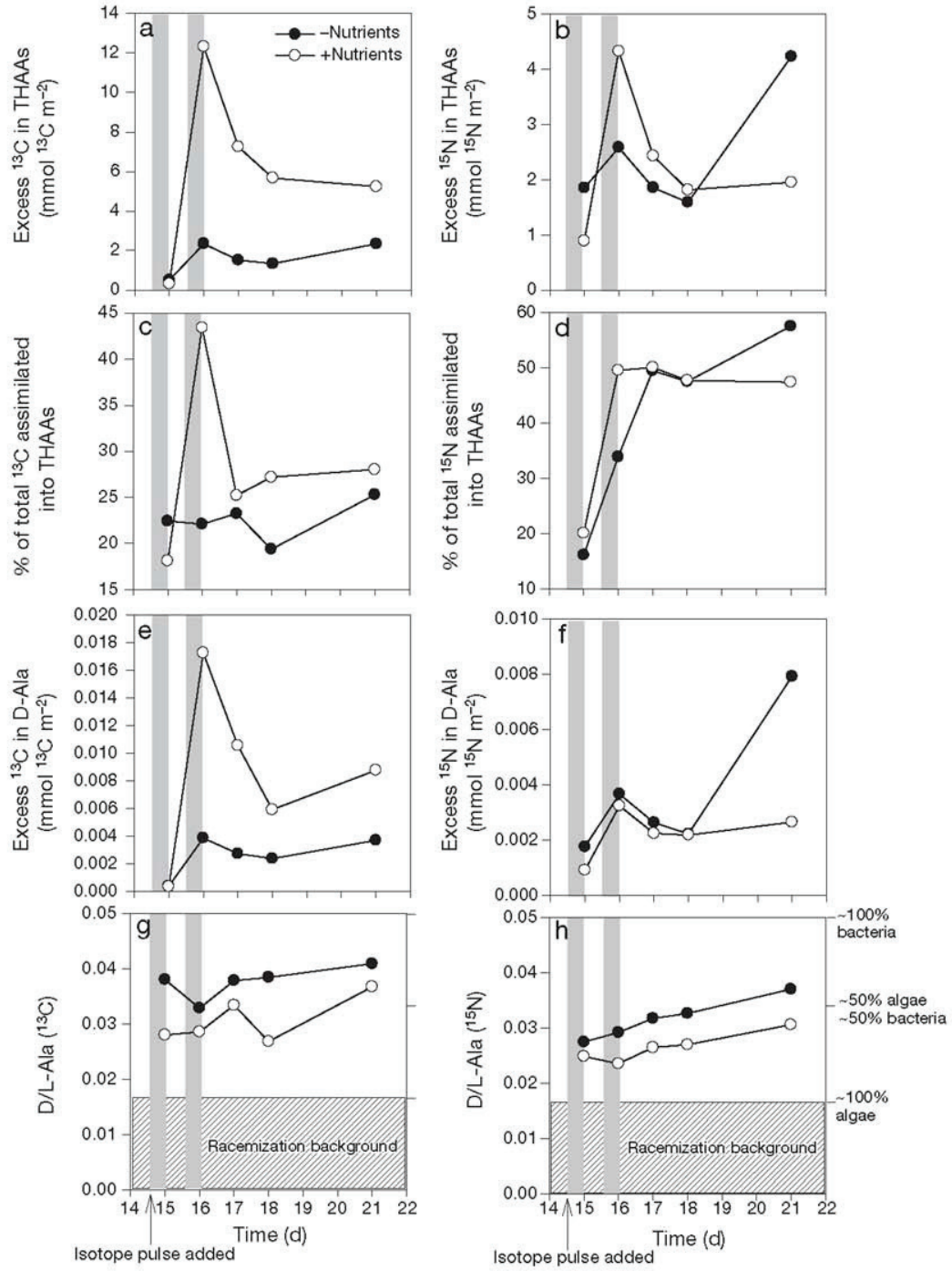

Fig. 7. Excess (a) ^{13}C and (b) ^{15}N in total hydrolysable amino acids (THAAs); the fraction of (c) total ^{13}C and (d) total ^{15}N in the sediment (or bulk ^{13}C and ^{15}N) incorporated into THAAs; excess (e) ^{13}C and (f) ^{15}N in D-alanine (D-Ala); and the excess label D/L-Ala ratio of (g) ^{13}C and (h) ^{15}N after the addition of a pulse of $H^{13}CO_3^-$ and $^{15}NH_4^+$ in the nutrient-amended (+) and nutrient-limited (–) treatments of the experiment. The approximate proportions of ^{13}C and ^{15}N incorporated into bacterial and algal biomass are shown on the *y*-axis in (g) and (h); details of this estimate are described in 'Discussion; ^{15}N and ^{13}C in D-alanine'. For clarity, the first 2 dark phases of the illumination cycle are denoted with grey bars. Hatched areas indicate racemization background

ratios were ~0.025 and 0.027 in the (+) and (–) treatments, respectively, followed by a gradual increase, with the highest ratios being consistently observed in the (–) treatment (Fig. 7h).

DISCUSSION

Conditions in mesocosms as used here may deviate significantly from *in situ* conditions in terms of grazing, advective porewater movement and re-suspension. Grazing was not quantified in these experiments; however, no macrofauna were present and the lack of pheophytin suggests that grazing rates were minimal. We cannot, however, rule out the presence of microscopic grazers such as ciliates, flagellates and meiofauna. Grazing may lead to cell lysis, releasing DOC, and represents an additional mechanism through which carbon fixed by photoautotrophs can be transferred to heterotrophic bacteria; we therefore refer to DOM (dissolved organic matter) release as opposed to excretion throughout this section. Grazing and resuspension may act in concert to remove MPB biomass, maintaining the algal cells in a constant phase of growth rather than forming a dense biofilm where growth becomes limited (Blanchard et al. 2001). Advective porewater transport may have a significant influence on the microbial community, for example, through enhanced supply of TCO_2 to primary producers, which may be limited by CO_2 in these sediments (Cook & Røy 2006). Advection and sediment resuspension may play an important role in the release of DOC from the sediment through mechanical action not captured in these experiments (see below). Despite these differences we note that the essential observations and conclusions of this study are the same as those from a similar study conducted at the same site run under close to *in situ* conditions (V. Evrard et al. unpubl. data). We are therefore confident that our results are ecologically meaningful.

The use of mesocosms, however, offers the distinct advantage of having well-controlled conditions, allowing budget calculations without interference from stochastic uncertainties, which occur *in situ*. Furthermore, mesocosms are well suited to the regular and simultaneous sampling of many parameters, which together give a deeper insight into the functioning of microbial communities. We first discuss the functioning of the algal compartment in terms of its loss of DOC to the water column and its response to nutrient limitation. We then address the use of D-Ala as a bacterial biomarker and its application to following the relative incorporation of ^{13}C and ^{15}N into bacteria and algae under N-limited and -replete conditions.

Loss of DOC to the water column

Microphytobenthos are known to excrete a large fraction of fixed carbon as low and high molecular weight compounds (Underwood & Paterson 2003). In short-term incubations of cultured benthic diatoms it has previously been shown that up to 60% of carbon assimilated may be excreted into the water column within 1 h (Smith & Underwood 2000). Our assays were methodologically very similar in terms of the incubation time frame and the extraction procedure. The major difference between these experiments was that the diatoms in our study were predominantly epipsammic as opposed to the epipelic species studied by Smith & Underwood (2000). The fact that very little (~1 to 5%) of the fixed ^{14}C in undisturbed sediment samples could be recovered in the dissolved phase, and the good agreement between the net particulate organic carbon fixation with that calculated from O_2 production in our experiment, indicates that virtually all of the excreted carbon is bound to the sediment and not released to the water column. Thus, it seems that the proportion of excreted carbon that becomes dissolved in the water column depends on the type of carbon excreted (i.e. algal type/species). We note also that the algal growth phase has a marked effect on the amount of carbon excreted, with the fraction of carbon excreted increasing markedly in the transition from the logarithmic to the stationary growth phase (Underwood & Paterson 2003). The DOC excretion incubations were terminated at 11 d at around the time the stationary phase of the (+) treatment was reached (see below); it is therefore possible that DOC excretion in the (+) treatment increased after 11 d. We consider this unlikely, however, because little excretion was observed in the (–) treatment, which was in the stationary phase when the DOC excretion incubations were undertaken. We also note that the conditions used here were very different to *in situ* in terms of hydrodynamic energy. *In situ* resuspension and advective processes may play an important role in disaggregating and dissolving EPS, which possibly lead to DOC release from the sediment.

Effect of nutrient limitation on algal growth dynamics

The effect of nutrient status on pelagic algal growth dynamics has received much attention, and sophisticated models of unbalanced algal growth have been developed to describe experimental observations (Van den Meersche et al. 2004). Under nutrient-replete conditions, virtually all of the carbon incorporated will be directed into cellular growth, and very little DOC will

be lost from the cell (the bloom phase). Upon exhaustion of the external nitrogen pool, carbon assimilation will continue, resulting in a gradual increase in the C:N ratio of the algal cellular material produced, concomitant with an increase in the fraction of C assimilation exuded as DOC (the intermediate phase). Eventually, the cellular C:N ratio will increase to a point (~20) where C acquisition becomes limited by the physiological condition of the cell, and most of the C fixed is exuded as DOC (the stationary phase), at this point the algal biomass decreases as grazing and mortality exceed growth (Van den Meersche et al. 2004).

For microphytobenthos grown in mesocosms, however, such growth dynamics are tempered by the changing physical environment of the growing biofilm, where CO_2 concentrations, light and space may eventually become limiting factors. As such, biofilm growth typically follows a sigmoidal curve, where a stationary phase is reached after 10 to 12 d and the community is not necessarily limited by nutrients (Blanchard et al. 2001, Morris 2005). During the first 16 d of the (+) treatment, chl *a* increased steadily, but after this, there was a marked drop, suggesting either senescence of the algae or a high degree of spatial variability. We ascribe this trend primarily to spatial variability, based on visual observations and the fact that no pheophytin was detected in any of the samples. Furthermore, rates of photosynthesis were highest on Days 15 to 21, supporting the contention that the algal community had not senesced. An alternative line of evidence to suggest that the community was in a stationary phase of growth after ~10 d comes from the C:chl *a*, N:chl *a* and total carbohydrate:chl *a* ratios, which all showed a minimum at 10 d followed by a gradual increase. These observations are all consistent with a decrease in the allocation of resources to cellular growth relative to C excretion after 10 d, suggesting that the cells had entered a more stationary phase of growth. It is unclear in our experiments whether the apparent slowing in biofilm growth was due to nutrient, light, or CO_2 limitation. However, it seems unlikely that the MPB community in our experiment were N limited, because the net cumulative ratio of C and N incorporated was 5.5, which is relatively low (Fig. 4); furthermore, the continued addition of nutrients after Day 15 should have allowed further cellular growth. Thus, at the point of the label addition, the MPB community was most likely in a stationary phase of growth, but not necessarily limited by nutrient availability. In the (–) treatment, no growth took place, as indicated by the relatively constant chl *a* concentrations, and, as such, this MPB community remained in a stationary phase due to severe nutrient limitation.

The different growth phases of the algae over the first 15 d of the experiment are also clearly seen in the ratios of C and carbohydrates to chl *a*, which climbed steadily in the (–) treatment, but dropped in the (+) treatment (Figs. 1 & 2). Virtually all of the C fixed in the (–) treatment was directed into carbohydrate synthesis compared to <50% in the (+) treatment (Fig. 2e). Thus, at the point of isotope addition, the 2 treatments produced 2 biofilms: one in a stationary phase severely limited by nutrients [(–) treatment], where there was a large relative build-up of high C:N ratio material such as carbohydrates, and the other also in a stationary phase of growth, but in a relatively nutrient-replete state [(+) treatment], with low cellular C:N ratios and carbohydrate concentrations (relative to biomass). Hence, the findings here should be considered relevant to relatively mature and undisturbed biofilms.

Interestingly, there was a rapid drop in the colloidal carbohydrate concentration upon the addition of the ^{15}N label (Fig. 2b,d), suggesting a rapid consumption of colloidal carbohydrates by either bacteria or algae. No jump in bacterial productivity was observed at the time of the drop in colloidal carbohydrates, suggesting that the consumption of this fraction was not by bacteria. It has been suggested that benthic diatoms may hydrolyse, then consume excreted carbohydrates; however, the ecological controls and relevance remain poorly understood (Staats et al. 2000a). In this instance, it is possible that the addition of NH_4^+ to the aquarium stimulated the algae to hydrolyse and/or consume the excreted carbon.

^{15}N and ^{13}C in D-alanine

As D-Ala is an amino acid unique to bacteria, label incorporation into D-Ala represents label incorporation by bacteria. The most useful information can be derived from the ratio between excess label in D-Ala versus that in L-Ala (a common HAA in all organisms), which provides a direct indication of the bacterial contribution to total microbial label incorporation. Quantitative interpretation of these data requires knowledge about the D/L-Ala ratio of the active bacterial community in the sediment. The D/L-Ala ratio of bacterial communities roughly ranges between 0.05 and 0.1, where relatively high values are associated with high abundance of Gram-positive bacteria and/or cyanobacteria (Veuger et al. 2007). Since the contribution of Gram-positive bacteria to the total bacterial community mainly seems to be relevant in deeper (anaerobic) sediment, while the present study concerned a thin layer of sandy (aerobic) sediment, the contribution of Gram-positive bacteria to the D/L-Ala ratio of the total bacterial community was probably negligible. Moreover, microscopic examination of the composition of the MPB community showed that cyanobacteria com-

prised only a minor fraction (<10%) of the benthic microbial community. Therefore, the D/L-Ala ratio of the bacterial community in the sediment (dominated by Gram-negative bacteria) was most likely around 0.05. This is supported by measured excess ^{13}C D/L-Ala ratios from additional sediment incubations with ^{13}C-labeled extract from the original labelled sediment, which yielded excess ^{13}C D/L-Ala ratios of ~0.05. Furthermore, the rates of bacterial production using ^{14}C leucine incorporation were consistent with the estimated uptake rates of ^{13}C by bacteria. Assuming a D/L-Ala racemization background of 0.017 and that a D/L-Ala ratio of 0.05 represents 100% bacterial C, then, based on the D/L-Ala incorporation ratios of 3.3 and 2.9 in the (–) and (+) treatments, respectively, after 24 h, we estimate that 48 and 35% of the ^{13}C uptake into amino acids was by bacteria in the (–) and (+) treatments, respectively (for a more detailed description of these calculations please see Veuger et al. (2007). Given a total ^{13}C assimilation into amino acids of 2.3 and 12.3 mmol ^{13}C m^{-2} d^{-1} for the (–) and (+) treatments, respectively, after 24 h, a ^{12}C:^{13}C ratio for dissolved inorganic carbon in the water column of 3.2 and assuming that amino acids comprise 50% of the bacterial biomass, this equates to a total bacterial C assimilation of ~7 and ~28 mmol C m^{-2} d^{-1} in the (–) and (+) treatments, respectively, 24 h after the start of the first illumination cycle following isotope addition. This compares to concurrently measured bacterial production rates of 6 to 12 and 8 to 23 mmol C m^{-2} d^{-1} on Days 15 and 17 for the (–) and (+) treatments, respectively, using the more conventionally employed leucine incorporation method. Thus, while these calculations are based on quite speculative assumptions, they give a reasonable agreement with more conventionally used methods.

Algal–bacterial interactions

Having established clear differences between the algal growth dynamics between the 2 treatments leading up to the isotope pulse, the question then arises as to how these differences affect the interaction between the bacteria and algae. According to the concepts put forward by Caron (1994), Legendre & Rassoulzadegan (1995), and Anderson & Ducklow (2001), one would expect that with increasing nutrient limitation there would be an increased coupling between algae and bacteria through the increased exudation of DOC by algae leading to a scenario resembling the 'microbial loop' (Legendre & Rassoulzadegan 1995). Consistent with this, the ratio of bacterial production to primary production (BP:PP) was generally twice as high in the (–) treatment (Fig. 5b), a difference which was significant at $p < 0.05$ (Wilcoxon matched pairs test). Evidence for the tighter coupling between algal and bacterial productivity in the (–) treatment also comes from the ^{13}C D/L-Ala ratios, which suggest that a significantly higher fraction (Wilcoxon matched pairs test $p < 0.05$) of the fixed C was initially transferred to the bacterial pool as indicated by the higher ^{13}C D/L-Ala ratios in this treatment (Fig. 7g). Furthermore, the excess ^{13}C D/L-Ala ratios also suggested that ~50 and 40% of the C incorporated by MPB was transferred to the bacteria in the (–) and (+) treatments, respectively, after the first illumination cycle (Day 16), and that there was a subsequent, albeit slower, transfer of C to the bacteria (Fig. 7g). This strongly suggests that the carbon release in this instance was ~50% of C assimilation under both nutrient-replete and -limited conditions. Such a high rate of EPS production in the (+) treatment is surprising given that the cumulative ratio of CO_2 and NH_4 incorporation was 5.5 at this point (Fig. 4). According to the model of Van den Meersche et al. (2004), excretion of fixed carbon would only become significant when the C:N ratio of the algal cells approaches 20. This discrepancy is, however, consistent with observations that MPB excrete a greater fraction of C-assimilated than of pelagic algae (Goto et al. 1999). Moreover, these results are also consistent with field and mesocosm studies of EPS dynamics, which show dramatic diurnal variations in EPS concentrations, suggesting a rapid production of EPS by MPB and consumption by bacteria (van Duyl et al. 1999). As already mentioned, it is also possible that the transfer of C from the algae to the bacteria is enhanced by cellular lysis through grazing, although we suggest that this is a minor contributor in this instance.

As for C, the N dynamics showed broad internal consistency with current concepts in microbial food web dynamics (sensu Caron 1994, Legendre & Rassoulzadegan 1995). Under N-replete conditions, the C:N ratio of excreted DOM is low and the bacteria utilising this DOM release NH_4, which then can be utilised again by the algae, resulting in a commensal relationship. Under N-limited conditions, the C:N ratio of exuded DOM increases, and bacteria using this high C:N ratio DOM will increasingly rely on inorganic nitrogen to meet their N requirements, putting them directly in competition with algae for available N (Bratbak & Thingstad 1985, Van den Meersche et al. 2004). The excess ^{15}N D/L-Ala ratios in our experiments suggested that bacteria initially incorporated ~20 and 30% of the ^{15}N added to the (+) and (–) treatments, respectively, after 12 h in the dark (Fig. 7h). This initial rapid enrichment was then followed by a slower transfer of ^{15}N from the algal to the bacterial pool in the days following the isotope pulse. About 50% of all ^{15}N in the sediment was in THAAs (Fig. 7d), indicating that all

the added $^{15}NH_4$ had been incorporated in biomass. It is unlikely that MPB could have first incorporated the added $^{15}NH_4^+$ and then released ^{15}N-DON (dissolved inorganic nitrogen) within 12 h in the dark. Thus, it seems that there was an initial direct assimilation of $^{15}NH_4^+$ by bacteria, followed by a slower continued assimilation of ^{15}N, most likely in the form of DON derived from algae.

In the case of the (+) treatment, we would expect the DOM released to have a low C:N ratio, resulting in bacteria being able to rely solely on this for their N source, according to the model and observations of Van den Meersche et al. (2004). The C:N ratio of DOM exuded by algae has rarely been directly measured. The limited data available suggest that under nutrient-replete conditions, the C:N ratio of DOM is quite variable (~5 to 14) (Biddanda & Benner 1997, Wetz & Wheeler 2003, Van den Meersche et al. 2004), but that it rapidly increases beyond 16 under nutrient-limited conditions (Wetz & Wheeler 2003, Van den Meersche et al. 2004). Other measurements suggest that protein and amino acids comprise only 5 to 10% of the DOM exuded by algae (Staats et al. 1999, Granum et al. 2002). Thus, the limited data available support our observation that the DOM derived from MPB can be relatively N poor, and that bacteria utilising this carbon source would most likely derive a large proportion of their N requirements through the direct assimilation of inorganic forms of N.

Additional support for the concept of MPB maintaining a low cellular C:N ratio by excreting high C:N ratio material, which is subsequently incorporated by bacteria, comes from an examination of gross C fixation to N assimilation ratios in the (+) treatment (Fig. 4). It can be seen that a large excess of C is fixed relative to N, but that, because a significant fraction of the gross C fixation is respired, the net ratio of C:N fixed is significantly lower. Assuming that diatoms respire on average 21% of the daily gross production (Langdon 1993), then, based on the gross primary production estimates, we can only account for 30 to 50% of the measured respiration rate, suggesting that bacteria account for 50 to 70% of the C respired. These estimates of bacterial respiration are also consistent with the measurements of bacterial production, suggesting an average bacterial growth efficiency of ~0.5 over the course of the experiment in the (+) treatment, which is quite high, but consistent with a value of ~0.6 expected for growth on excreted organic carbon (del Giorgio & Cole 1998). The implications of this are that, whilst MPB do produce low C:N ratio cellular material, the substrate used by bacteria may actually be of a much higher C:N ratio as a consequence of the exudation of high C:N ratio EPS. Therefore, the C:N ratio of the pools of organic matter present in the sediment gives limited information on the C:N ratio of the substrate being used by bacteria, because it is rapidly turned over. We suggest that, paradoxically, the C:N ratio of the substrate being used by bacteria may actually be higher than the bulk C:N ratio of the sediment, which is the opposite of what is conventionally assumed.

Comparing our results to other field studies is problematic because of the highly artificial system used here. Perhaps, most significantly, rates of grazing were low, owing to an absence of macrofauna as also indicated by the absence of pheophytin. Within the microbial loop, grazing has an important role in N recycling, particularly in the release of DON (Glibert et al. 1991). Indeed, an absence of grazing may explain the extremely low loss of DOC to the water column in our experiment, which is at odds with some field observations of high DON fluxes in systems with high MPB biomass (Cook et al. 2004, Ferguson et al. 2004). Furthermore, given that grazing and resuspension may maintain algae in a higher state of production (Blanchard et al. 2001), and, hence, possibly lower excretion rates, the extremely high transfer of C from the algae to the bacteria observed here may not be applicable to the field. Our results are, however, in broad agreement with previous studies, which indicate that bacteria in the surface sediment of intertidal flats rely heavily on MPB as a C substrate (Middelburg et al. 2000), and that the recycling of N relative to C is very conservative in highly productive sediments colonised by MPB (Cook et al. 2004, Ferguson et al. 2004). Furthermore, the findings here are consistent with the suggestion that the presence of MPB reduces nitrification and denitrification rates as a consequence of competition between nitrifiers and heterotrophic bacteria for available NH_4^+ (Risgaard-Petersen 2003).

Our experiments suggest that MPB release a large fraction of C assimilated under both nutrient-limited and -replete conditions, but that an insignificant fraction of the carbon released is lost to the water column when fluxes are dominated by molecular diffusion. Consistent with current concepts of microbial food web dynamics, N limitation led to a tighter coupling between algal and bacterial production and an increased competition for inorganic N. Our data suggest, however, that even under relatively nutrient-replete conditions, MPB exude a large fraction of the C assimilated.

Acknowledgements. We thank Markus Huettel for valuable comments on the manuscript, and Antje Boetius for useful discussions and support of this work. Susanne Menger and Helmy Cook provided assistance with sample analysis. This research was supported by a grant from the European Union (COSA EVK 3-CT2002-00076), a PIONIER grant from the Netherlands Organisation for Scientific Research (833.02.2002) and by the Max Planck Society. This is Publication Number 4151 of the Netherlands Institute of Ecology (NIOO-KNAW).

LITERATURE CITED

Anderson TR, Ducklow HW (2001) Microbial loop carbon cycling in ocean environments studied using a simple steady-state model. Aquat Microb Ecol 26:37–49

Biddanda BA, Benner R (1997) Carbon, nitrogen and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol Oceanogr 42:506–518

Blackburn TH (1986) Nitrogen cycle in marine sediments. Ophelia 26:65–76

Blanchard GF, Guarini JM, Orvain F, Sauriau PG (2001) Dynamic behaviour of benthic microalgal biomass in intertidal mudflats. J Exp Mar Biol Ecol 264:85–100

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol Ecol 40: 85–95

Bratbak G, Thingstad TF (1985) Phytoplankton–bacteria interactions: An apparent paradox? Analysis of a model system with both competition and commensalism. Mar Ecol Prog Ser 25:23–30

Caron DA (1994) Inorganic nutrients, bacteria and the microbial loop. Microb Ecol 28:295–298

Chiovitti A, Molino P, Crawford SA, Teng RW, Spurck T, Wetherbee R (2004) The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides. Eur J Phycol 39:117–128

Cook PLM, Røy H (2006) Advective relief of CO_2 limitation in highly productive sandy sediments. Limnol Oceanogr 51: 1594–1601

Cook PLM, Revill AT, Butler ECV, Eyre BD (2004) Benthic carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. Mar Ecol Prog Ser 280:39–54

Cook PLM, Wenzhöfer F, Glud RN, Janssen F, Huettel M (2007) Benthic exchange and mineralisation in two subtidal sandy sediments: effect of flow. Limnol Oceanogr 52: 1943–1963

de Brouwer JFC, Stal LJ (2001) Short-term dynamic in microphytobenthos distribution and associated extracellular carbohydrate in surface sediments of an intertidal mudflat. Mar Ecol Prog Ser 218:33–44

del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. Annu Rev Ecol Syst 29:503–541

Engel A, Goldthwait S, Passow U, Alldredge A (2002) Temporal decoupling of carbon and nitrogen dynamics in a mesocosm diatom bloom. Limnol Oceanogr 47:753–761

Ferguson AJP, Eyre BD, Gay JM (2004) Benthic nutrient fluxes in euphotic sediments along shallow sub-tropical estuaries, northern New South Wales, Australia. Aquat Microb Ecol 37:219–235

Findley SEG, Meyer JL, Edwards RT (1984) Measuring bacterial production via rate of incorporation of [^{3}H]thymidine into DNA. J Microbiol Methods 2:57–72

Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. Limnol Oceanogr 36:895–909

Goldman JC, Dennett MR (2000) Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. Limnol Oceanogr 45:789–800

Goldman JC, Dennett MR (2001) Rapid nitrogen uptake by marine bacteria. Limnol Oceanogr 46:1195–1198

Goto N, Kawamura T, Mitamura O, Terai H (1999) Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. Mar Ecol Prog Ser 190:289–295

Granum E, Kirkvold S, Mykelstad SM (2002) Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: diel variations and effects of N depletion. Mar Ecol Prog Ser 242:83–94

Grasshoff K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim

Kang CK, Kim JB, Lee KS, Lee PY, Hong JS (2003) Trophic importance of benthic microalgae to macrozoobenthos in coastal bay systems in Korea: dual stable C and N isotope analyses. Mar Ecol Prog Ser 259:79–92

Kirchmann DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 509–512

Langdon C (1993) The significance of respiration in production measurments based on oxygen. ICES Mar Sci Symp 197:69–78

Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41:153–172

Lorenzen CJ (1967) Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol Oceanogr 12:343–346

Michel PH, Bloem J (1993) Conversion factors for estimation of cell production rates for soil bacteria from [^{3}H]thymidine and [^{3}H]leucine incorportion. Soil Biol Biochem 25: 943–950

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: an *in situ* ^{13}C-labeling study. Limnol Oceanogr 45:1224–1234

Morris EP (2005) Quantifying primary production of microphytobenthos: application of optical methods. Rijks University, Groningen

Risgaard-Petersen N (2003) Coupled nitrification–denitrification in autotrophic and heterotrophic sediments: on the influence of benthic microalgae. Limnol Oceanogr 48: 93–105

Smith DJ, Underwood GJC (2000) The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. J Phycol 36:321–333

Staats N, de Winder B, Stal L, Mur LR (1999) Isolation and characterization of extracellular polysaccharides from epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur J Phycol 34:161–169

Staats N, Stal LJ, de Winder B, Mur LR (2000a) Oxygenic photosynthesis as driving process in exopolysaccharide production of benthic diatoms. Mar Ecol Prog Ser 193: 261–269

Staats N, Stal LJ, Mur LR (2000b) Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. J Exp Mar Biol Ecol 249: 13–27

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Nedwell DB, Raffaelli DG (eds) Advances in ecological research — estuaries, Vol 29. Academic Press, San Diego, CA, p 93–153

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. In: Callow JA (ed) Advances in botanical research, Vol 40. Academic Press, London, p 183–240

Underwood GJC, Paterson DM, Parkes RJ (1995) The measurement of microbial carbohydrate exopolymers from

intertidal sediments. Limnol Oceanogr 40:1243–1253
Van den Meersche K, Middelburg JJ, Soetaert K, Rijswijk P, Boschker HTS, Heip CHR (2004) Carbon–nitrogen coupling and algal–bacterial interactions during an experimental bloom: modelling a ^{13}C tracer experiment. Limnol Oceanogr 49:862–878
van Duyl FC, de Winder B, Kop AJ, Wollenzien U (1999) Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar Ecol Prog Ser 191:19–32
Veuger B, Middelburg JJ (2007) Incorporation of nitrogen from amino acids and urea by benthic microbes: role of bacteria versus algae and coupled incorporation of carbon. Aquat Microb Ecol 48:35–46
Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2005) Analysis of ^{15}N incorporation in D-alanine: a new method for tracing nitrogen uptake by bacteria. Limnol Oceanogr Methods 3:230–240
Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2007) Update of 'Analysis of ^{15}N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria' (Veuger et al. 2005, Limnol Oceanogr Methods 3: 230–240). Limnol Oceanogr Methods 5:192–194
Wetz MS, Wheeler PA (2003) Production and partitioning of organic matter during simulated phytoplankton blooms. Limnol Oceanogr 48:1808–1817

Editorial responsibility: Rutger de Wit, Montpellier, France

Submitted: April 12, 2007; Accepted: September 4, 2007
Proofs received from author(s): October 30, 2007

Chapter 3

Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments

S.I. Böer, C. Arnosti, J.E.E. van Beusekom and A. Boetius

published in *BiogeosciencesDiscussions*

Abstract

Temporal dynamics and vertical patterns in bacterial abundances and activities were studied in a shallow subtidal sand flat in the Sylt-Rømø Basin (North Frisian Wadden Sea, Germany.) Extracellular enzymatic activities, bacterial carbon production and community respiration showed strong (factor of 4-5) temporal variations that were mostly related to seasonal temperature change, but also to changes in substrate availability. These temporal patterns in activity were barely reflected in bacterial (200-400 mmol C m^{-2}) and microphytobenthic biomass (800-1500 mmol C m^{-2}) or the sedimentary carbohydrate inventory (1300-2900 mmol C m^{-2}), suggesting that grazing controls the standing stocks of the microphytobenthic and bacterial assemblages. Despite their exposure to strong hydrodynamic forces such as tidal currents and wind-induced wave surge, the subtidal sandy sediments showed persistent vertical gradients in bacterial abundances, bacterial carbon production and extracellular enzymatic activities at all times. The vertical distribution of these parameters was tightly coupled to that of the microphytobenthos, dominated by diatoms. Despite the low organic carbon content typical for surge-exposed sandy sediments, high extracellular enzymatic activities and bacterial carbon production rates indicate a very active heterotrophic bacterial community, with a gross secondary productivity of 30-180 mmol C m^{-2}, and a biomass turnover time of 2-18 days. Our data suggest that this high activity is supported by the rapid flux of carbohydrates from microphytobenthic primary productivity. Accordingly, the potential activities of enzymes hydrolyzing carbohydrates cover most of the total bacterial carbon demand during all seasons.

3.1 Introduction

Shelf sediments represent less than 10% of the ocean floor, but they contain the largest pool of particulate organic matter in the marine environment and play a major role in global carbon and nitrogen cycles (Jahnke 2004). Shallow coastal ecosystems contribute substantially to oceanic carbon fixation via pelagic and benthic primary productivity (Wollast 2002, Gattuso et al. 2006). The turnover of carbon in subtidal shelf sediments has been extensively investigated, however most studies focused on organic-rich, silty and muddy seafloors. Sandy sediments have long been considered as biochemically inert due to their low organic carbon content, and hence have been neglected in the marine carbon cycle (Boudreau et al. 2001). However, permeable sands are the dominant sediment type on continental shelves (Emery 1968, Hall 2002) and are important from biological, geological, and economic perspectives. Recent studies measured high primary productivity in sandy sediments (Billerbeck et al. 2007), and found that organic matter mineralization rates in sands are comparable to or even higher than rates in fine-grained, organic-rich sediments (D'Andrea et al. 2002, de Beer et al. 2005, Rusch et al. 2006).

The high mineralization rates are driven by photosynthetic production, either in the form of settling phytodetritus or via photosynthetic products excreted by the microphytobenthos (Goto et al. 2001, Rusch et al. 2003). Although burial of phytoplankton detritus has been shown to stimulate benthic microbial activities (Meyer-Reil 1987, van Duyl et al. 1992, Rooney-Varga et al. 2005), primary productivity by microphytobenthic algae (MPB) is recognized as the main source of organic carbon for benthic life in light-exposed coastal sediments (MacIntyre et al. 1996, Underwood & Kromkamp 1999).

Carbon flux from microphytobenthic algae to other benthic microorganisms can be significant and rapid, as has been demonstrated by studies using isotope tracer incubations (Middelburg et al. 2000, Cook et al. 2007a). The carbon flux includes large quantities of extracellular polymeric substances (EPS) secreted by the MPB to promote migration, adhesion to sediment particles and sediment cohesion (Hoagland et al. 1993, Underwood and Paterson 2003). In addition, unbalanced growth under nutrient-depleted conditions may considerably increase the release of carbohydrates by photoautotrophs in order to maintain cell-nutrient balances (Ortega-Calvo & Stal 1994, Smith & Underwood 1998). In consequence, up to 70% of the carbon fixed may be excreted as EPS, rather than allocated to algal growth (Middelburg et al. 2000, de Brouwer & Stal 2001). The MPB is often dominated by benthic diatoms, which form biofilms in association with bacteria (Huettel et al. 2003).

Degradation of polysaccharides by bacterial communities plays a key role in the dynamic environment of coastal sands. Previous works on bacteria and their activities in permeable sediments (e.g. Staats et al. 2001a, Staats et al. 2001b, Rusch et al. 2003, de Beer et al. 2005, Misic & Fabiano 2005, Misic & Harriague 2007) focused mostly on intertidal areas, and/or had limited time or depth resolution. Therefore, the dynamics of interactions between autotrophic and heterotrophic components of the microbial communities in sandy sediments and the distribution and availability of substrates, including potential variations in carbon inventories with time and depth, and responses to seasonal temperature changes are not well constrained.

We studied a shallow subtidal sand flat ("*Hausstrand*") in the Sylt - Rømø Basin of the North Frisian Wadden Sea, focusing on temporal and temperature-related variations in benthic microbial activity and the MPB-derived carbohydrate inventory. We also examined the extent to which depth gradients in microbial and MPB-related parameters persisted over time in this hydrodynamically active site. During 6 sampling periods over the course of more than a year, covering a water temperature range of 1.5–18°C, we measured bacterial abundances, bacterial carbon production, and potential activities of extracellular exoenzymes using several substrate proxies, and activities of endo-acting enzymes using fluorescently-labeled polysaccharides. We also measured photopigments (as a proxy for primary producer biomass), bacterial biomass, and total sedimentary carbohydrates as well as EDTA-extractable carbohydrates, which are believed to be the quantitatively most important substrates for the benthic microbial community of photosynthetically active sands. The ratio of bacterial turnover of MPB-derived carbon sources was estimated for different seasons, and the effects of temperature, sediment depth and other factors controlling microbial activity were explored by multivariate analysis. This suite of measurements was intended to provide insight into the influence of MPB on carbon cycling by heterotrophic bacteria in a highly dynamic environment. We hypothesized that the spatial and temporal distribution of the MPB and their carbohydrates are major controls on bacterial distribution and activity, and quantification of this influence was a goal of our study.

3.2 Materials and Methods

3.2.1 Study site

3.2.1.1 Site description

The study site *Hausstrand* (Fig. 1) is located on the island of Sylt (55° 00′47.7′′N, 008° 25′59.3′′E; Fig. 1), where the temperature difference between summer and winter can be more than 20°C (Hedtkamp 2005).

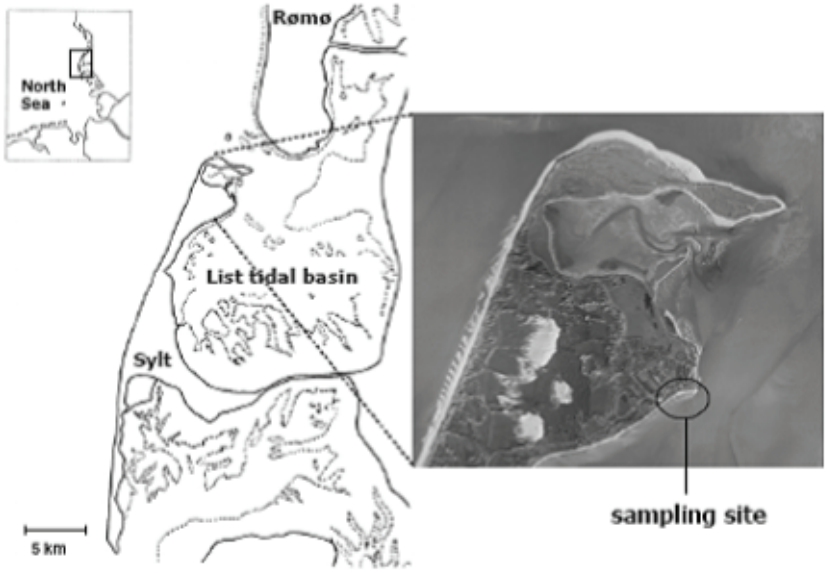

Fig.1: Map showing the location of the *Hausstrand* sampling site in the Sylt-Rømø basin (also known as the List tidal basin) close to the island of Sylt in the North Frisian Wadden Sea, Germany (picture obtained from the Wadden Sea National Park Office Schleswig-Holstein).

The site is characterized by the strong hydrodynamic forces of tides and wind-induced waves, with a tidal amplitude of ~2 m. Water depth ranges between 0.5-2.5 m, depending on weather and the tidal phase. Sediments consist of well to moderately well sorted silicate sand with a medium particle size of 350 µm. The permeability of the upper 15 cm of the sediments ranges between 1.01×10^{-11} m^2 and 3.36×10^{-11} m^2, with higher values in winter and spring compared to summer and autumn (Hedtkamp 2005). Since the islands of Sylt and Rømø are connected to the mainland by a causeway, the main water exchange takes place through the inlet to the North Sea. However, in comparison to other areas of the North Sea, the entry of fine material

is low (Köster & Müller 1998). The *Hausstrand* sands are organic matter impoverished, with an average organic carbon concentration of 0.13% (Hedtkamp 2005). Further information on the Sylt Rømø Basin (also known as the List Tidal Basin) are given by Gätje and Reise (1998) and van Beusekom and Reise (2008) and references therein.

3.2.1.2 Sampling and sample processing

Push cores (3.6 cm inner diameter, 15-20 cm length; cores for laminarin incubations with silicon-covered injection holes) were collected in February, April, July and November 2005, and March 1st and 27th (labeled March I and March II) 2006 during low tide. In April and November, sampling was performed by scuba-diving, due to stormy weather and high water level. In November, the retrieved sediment cores only included the top 10 cm layer. Replicate cores (8.4 cm inner diameter, 30 cm length) were also collected for measurement of benthic oxygen demand.

Ten replicate sediment cores from an area of < 20 m^2 were sectioned in 1-cm intervals down to 15 cm depth. Sediment slices from the same depth horizon were pooled and homogenized. The samples were processed in the laboratory adjacent to the *Hausstrand* field site (AWI station, List on Sylt) immediately after collection. Subsamples for the measurement of photopigments and carbohydrates were frozen immediately and stored at -20°C. The rest of the homogenized sediment was combined in 5-cm intervals (0-5, 5-10, and 10-15 cm). For bacterial cell counts sediment was fixed in 2% formaldehyde/seawater solution; subsamples for the measurement of extracellular enzymatic activities (EEA) and bacterial carbon production (BCP) were processed immediately. Cores used for laminarin incubation were injected with 100 µl of laminarin (1.9 µmol monomer equivalents l^{-1}) at depths of 1, 5, and 13 cm, and incubated at *in situ* temperature in the dark.

3.2.2 Rate Measurements

3.2.2.1 Bacterial carbon production (BCP)

BCP was estimated by measuring the rate of [Methyl-^{3}H] thymidine (TdR) incorporation into DNA (Fuhrman & Azam 1982, Moriarty & Pollard 1990). Sediment slurries (1:1 sediment: sterile-filtered seawater; n = 4-5 per depth interval) amended with TdR were incubated for 1.5-4 h at near *in situ* temperature, and TdR incorporation was stopped by adding 100 µl of

37% formaldehyde. Killed controls received formaldehyde prior to incubation. A time course and concentration experiment was conducted at the beginning of the study to ensure that all measurements were carried out in the initial linear period of incorporation of label into DNA and that isotope dilution by *de novo* synthesis of thymidine was insignificant (Moriarty 1990). Extractions followed a modification of Findley et al. (1984) and Michel & Bloem (1993). BCP was calculated using a conversion factor of $2x10^6$ cells produced per pmol thymidine incorporated (Findley 1993), and a carbon conversion factor of 20 fg C $cell^{-1}$ (Fuhrman & Azam 1982).

3.2.2.2 Extracellular enzyme activities (EEA)

Potential exoenzymatic activity

Hydrolysis rates were measured in slurries using 4-methylumbelliferone- (MUF-) and 4-methylcoumarinyl-7-amide- (MCA-) labeled model substrates, which capture the activities of exoenzymes that cleave the terminal ends of polymer chains. They are widely used (e.g. Hoppe 1983, Hoppe et al. 1988, Boetius 1995, Boetius & Lochte 1996a, 1996b), facilitating comparison between studies, but the extent to which they represent the activities of enzymes on true polymers is uncertain (Warren 1996). Potential hydrolytic activities of α-glucosidase, β-glucosidase, chitobiase, lipase, phosphatase and aminopeptiase were measured using the MUF-labeled substrate analogs MUF-α-D-glucoside, MUF-β-D-glucopyranoside, MUF-N-acetyl-β-glucosamine, MUF-stearate, MUF-phosphate and MCA-labeled leucine (all obtained from Sigma-Aldrich, Munich, Germany), respectively. For each depth interval (0-5, 5-10 and 10-15 cm) and substrate, five replicate 0.5-ml sub-samples of homogenized sediment from 10 pooled cores were diluted 1:10 with sterile filtered seawater. The substrates were dissolved in ethylene glycol monomethyl ether and added to the slurry at a final concentration of 0.5 mM. The samples were then agitated at *in situ* temperature in the dark. Shortly after the start of the time course experiment, 1 ml of the sample was removed and added to 2 ml of sterile-filtered seawater amended with 250 µl of borate buffer (pH 10.0). After centrifugation, the supernatant was transferred into disposable UV-cuvettes and the fluorescence of the enzymatically released MUF and MCA was measured (445 nm emission, 365 nm exitation). A second measurement was performed after 2-4 hours of incubation time. Pre-experiments were conducted prior to the start of the study to confirm that the final concentrations of MUF and MCA substrates generally represented substrate saturation levels and yielded maximum velocities. Hydrolytic activity was calculated from the increase of fluorescence over time

calibrated via standards of both MUF and MCA. Since the fractions of the bacterial community that produce specific enzymes are unknown, cell-specific EEA was calculated as extracellular enzymatic activity divided by the total number of cells.

Potential endoenzymatic activity

Fluorescently labeled (FLA-) macromolecules (e.g. polysaccharides) can be used to measure the activities of endoenzymes (mid-chain cleaving enzymes) (Arnosti 1995), and may better mimic the degradation of complex macromolecules in natural settings. The analytical procedure is time-consuming, however, restricting the number of analyses that can be performed. Here, hydrolysis of laminarin, an energy storage product of diatoms (Lee 1980, Painter 1983), was measured using the method of Arnosti (1995, 2003). Laminarin and fluorescenamine (isomer II) were purchased from Sigma, and fluorescently labeled laminarin was synthesized after Glabe et al. (1983) as described in Arnosti (1995, 2003). Potential hydrolysis rates of laminarin were measured in intact sediment cores by injection of 1.9 μmol monomer equivalents of laminarin at -1 cm, -5 cm and -13 cm depth. These measures were used to extrapolate to the average activity in the 0-5, 5-10 and 10-15 cm layer. One core was left without laminarin and served as a blank sample. All cores were incubated at *in situ* temperature in the dark in an upright position. After ~24, ~48 and ~72 hours incubation at *in situ* temperature, 2-3 replicate cores were sacrificed and sectioned in 2-cm intervals. Hydrolysis of substrate was found to be nearly complete by 24 h, so all of the rates reported here are derived from triplicate 24 h incubations. Pore water was purged from the sediment with nitrogen, as described by Billerbeck et al. (2006), filtered through 0.2 μm pore size filters, and stored at -20°C until analysis. One core was sectioned immediately after injection as the time zero sample. The molecular weight distribution of hydrolyzed polysaccharides was determined via low-pressure gel permeation chromatography (GPC) with fluorescence detection, as described in Arnosti (2003). Potential hydrolysis rates were calculated based on the molecular weight distribution of laminarin hydrolysis products in each sample as described by Arnosti (1995, 2003).

All laminarinase rates in this study may represent minimum potential rates, since the fluorescently labeled substrate compete with naturally occurring laminarin for enzyme active sites. Usually, FLA-laminarin is added equal to 100-500% of the total dissolved carbohydrates in order to measure hydrolysis at substrate saturation level. The concentration of dissolved interstitial carbohydrates in the sands was not measured in this study. FLA-laminarin was added in concentrations of 2.3-5.4 mmol glucose L-1, which represents 200-

800% of the pool of EDTA-extractable carbohydrates. However, since total carbohydrate concentrations were rather high (see results below), competition with naturally available laminarin cannot be excluded, especially in the diatom-dominated surface sediments.
Alderkamp et al. (2006) estimated that ca. 13% of the bacteria from North Sea coastal waters are capable of producing laminarinase. However, it is not known if this result also applies to sediments and how this ratio changes with time or location. In order to allow comparison with cell-specific MUF-/MCA-enzymatic activities, cell-specific laminarinase activities were calculated by dividing laminarinase activities by the total number of bacterial cells.

3.2.2.3 Benthic oxygen consumption

Gross benthic community respiration rates were estimated *ex situ* via incubation of intact sediment cores. The gas- and air-tight cores ("chambers") were fitted with sampling ports and a magnetic stirrer and contained approximately 1 L sediment and 0.75 L of overlying water. The cores were maintained at *in situ* temperature in a dark cold room. The overlying water in the cores was stirred carefully at a speed of ~20 rpm. Oxygen concentrations in the samples were determined by duplicate Winkler titrations (Winkler 1888, Grasshoff et al. 1983) according to the procedure of Strickland & Parsons (1972), of samples withdrawn from the chambers over a 5-point timecourse. The benthic oxygen consumption rates were calculated from the averages of oxygen decrease over time according to Thorbergsdóttir et al. (2004). As the stirring speed was at the low end of advection of oxygenated water through *Hausstrand* sands, the rates presented here likely represent an underestimation of *in situ* respiration rates.

3.2.3 Bacterial counts and concentration measurements

3.2.3.1 Cell numbers

Bacterial abundances were estimated by epifluorescence microscopy after staining with Acridine Orange (AO) using a modification of the method of Hobbie et al. (1977). Prior to staining, the fixed samples (0.5 ml sediment per replicate) were centrifuged for 15 min at 4000 rpm and the fixative was removed and set aside. One milliliter of the removed fixative was placed back onto the sample and the slurry was sonicated for 4 min with an ultrasonic disintegrator while keeping the vial on ice (van Duyl and Kop 1994). After the sand grains

settled, the supernatant containing the cells was transferred to another vial. The remaining sediment was washed six times, each time with 1 ml of the fixative, which was thereafter combined with the cell suspension. Two 1-ml sub-samples (1:1000 final dilution) were filtered onto 0.2 µm black polycarbonate filters and stained with AO solution (final concentration 0.01%) for 3 min. Subsequently, the filters were rinsed with 1 ml of citrate buffer and bacteria enumerated by epifluorescence microscopy (x1000). Final counts represent averages of 6 filters (2 filters from 3 replicate field samples), with 10-20 grids counted on each filter with at least 1000 cells per filter.

3.2.3.2 Determination of photopigments

Triplicates of approximately 1 g of freeze-dried and homogenized sediment from each sediment layer were mixed with 7.5 ml of 90% acetone and extracted in the dark at 4°C for 24 hours. Prior to measurement, the samples were centrifuged at 4000 rpm for 10 min. The supernatant was transferred to quarz cuvettes and the absorbance of the extract was measured at 665 and 750 nm before and after acidification with 10% HCl. Chlorophyll *a* and phaeophytine concentrations were calculated according to Lorenzen (1967). Based on known sediment water contents, concentrations were converted to µg cm^{-3} wet sediment. A carbon to chlorophyll *a* conversion factor of 34 (Dijkman, as cited in Evrard 2007) was used to estimate MPB biomass.

3.2.3.3 Carbohydrate analysis

Total and EDTA-extractable carbohydrates were quantified using the phenol-sulfuric acid assay (Dubois et al. 1956, Herbert et al. 1971), according to Underwood et al. (1995). All measurements were done in triplicates. For measurement of EDTA-extractable carbohydrates, 100 mg of lyophilized and homogenized sediment was extracted with 5 ml of 0.1 M Na_2-EDTA for 15 min at 20°C. Following centrifugation at 4000 rpm for 15 min, 2 ml of the supernatant was transferred to combusted glass vials and 1 ml of 5% aqueous phenol (w/v) was added, immediately followed by 5 ml of concentrated H_2SO_4. After 10 min, the samples were carefully homogenized and incubated another 30 min. Absorbance was measured against a reagent blank at 485 nm. Measurements were calibrated against a curve made using glucose as a standard, so concentrations are expressed as microgram glucose equivalents. For the measurement of total carbohydrates, 50 mg of lyophilized and

homogenized sediment were diluted in 2 ml of distilled H_2O, and then analyzed as above. Based on known sediment water contents, concentrations were converted to µg ml^{-1} wet sediment.

3.2.4 Statistical analysis

Error estimates represent standard deviations from the averages, based on replicate measurements as described above for each method. Correlations between all environmental variables were calculated using the Pearson's correlation coefficient for all samples that included carbohydrate measurements. All data, except for depth and temperature, were log_{10}-transformed to normalize their distribution. Data was normalized to standard mean = 0 and a standard deviation of 1 prior to computation of the Pearson's correlation coefficients. P-values were corrected for multiple testing using the Bonferroni correction (Ramette 2007).

The relationship between environmental variables was studied in more detail by multivariate statistics using the software package CANOCO for Windows 4.5 (terBraak and Smilauer 1998). A principal component analysis (PCA) was used to reduce the dimensionality of the complex data set. PCA is an orthogonal linear transformation method that converts a number of potentially correlated variables into a smaller number of independent variables, the so-called principal components, while maintaining most of the original variability in the data. The first principle component accounts for the greatest variability in the data and each succeeding component explains as much of the remaining variability as possible.

3.3 Results

3.3.1 Bacterial cell numbers and growth rates

Total cell numbers and bacterial carbon production (BCP) were highest in the upper 0-5 cm sediment layer and decreased with depth (Table 1). Cell numbers ranged between 1.7 and 2.9 10^9 cells cm^{-3} wet sediment in the 0-5 cm layer, representing a bacterial biomass of 2.8-4.8 mmol C L^{-1}, based on a conversion factor of 20 fg C $cell^{-1}$ (Fuhrman & Azam 1982). BCP ranged between 0.43 and 2.29 mmol C L^{-1}. Turnover time of the bacterial community biomass was calculated to be 2.1-8.6 d in the 0-5 cm layer, with fastest turnover in July and

slowest turnover in November. Bacterial biomass and BCP generally followed temperature, with maximum values in July (Fig. 2). However, despite the fact that BCP rates and bacterial turnover time in the upper sand layer were ca. 4 times higher in July than in preceeding or following sampling months, temporal changes in bacterial biomass were less pronounced and varied by less than a factor of 2.

Table 1: Depth-related and temporal changes in carbon fractions and enzymatic carbon hydrolysis in *Haussträand* sediments.

Date	Sed. layer (cm)	Bact. cell numbers ($\times 10^9$ cm^{-3})	Bacterial biomass (mmol C L^{-1}) [a]	Bact. C prod. (mmol C L^{-1} d^{-1})	Bact. turnover time (d)	MPB biomass (mmol C L^{-1}) [b]	Total carbs (mmol C L^{-1})	EDTA extr. carbs (mmol C L^{-1})	C-hydrolysis by Chitobiase (mmol C L^{-1} d^{-1}) [c]	C-hydrolysis by β-Glucosidase (mmol C L^{-1} d^{-1}) [d]	C-hydrolysis by α-Glucosidase (mmol C L^{-1} d^{-1}) [d]	C-hydrolysis by Laminarinase (mmol C L^{-1} d^{-1}) [e]*
8-Feb-05	0-5	2.4 ± 0.26	4.0	0.62 ± 0.07	6.5	16.4	33 ± 1.1	7.1 ± 0.40	0.38 ± 0.03	0.34 ± 0.01	0.22 ± 0.00	0.13 ± 0.002
(1.5°C)	5-10	1.3 ± 0.13	2.2	0.38 ± 0.19	5.8	4.6	25 ± 0.9	4.6 ± 0.31	0.55 ± 0.21	0.14 ± 0.03	0.08 ± 0.02	0.12 ± 0.007
	10-15	0.9 ± 0.07	1.5	0.23 ± 0.07	6.5	2.0	N.A.	N.A.	0.05 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0
8-Apr-05	0-5	2.0 ± 0.28	3.3	0.44 ± 0.06	7.5	19.2	25 ± 0.9	6.6 ± 0.59	0.36 ± 0.04	0.47 ± 0.04	0.25 ± 0.06	0.13 ± 0.006
(7°C)	5-10	1.4 ± 0.13	2.3	0.35 ± 0.05	6.6	7.0	17 ± 0.7	4.6 ± 1.18	0.23 ± 0.02	0.19 ± 0.01	0.09 ± 0.03	0.09 ± 0.003
	10-15	0.6 ± 0.08	1.0	0.23 ± 0.05	4.3	1.3	N.A.	N.A.	0.05 ± 0.01	0.05 ± 0.00	0.03 ± 0.01	0.01 ± 0.001
2-Jul-05	0-5	2.9 ± 0.17	4.8	2.29 ± 0.34	2.1	18.1	25 ± 2.0	4 ± 0.50	1.89 ± 0.51	1.13 ± 0.08	0.65 ± 0.06	0.26 ± 0.105
(18°C)	5-10	2.0 ± 0.28	3.3	1.32 ± 0.13	2.5	6.1	19 ± 1.4	1.9 ± 0.72	0.47 ± 0.33	0.67 ± 0.10	0.22 ± 0.03	0.25 ± 0.049
	10-15	1.6 ± 0.12	2.7	0.05 ± 0.08	5.4	1.4	14 ± 1.5	1.8 ± 1.01	0.12 ± 0.09	0.18 ± 0.04	0.05 ± 0.03	0.11 ± 0.067
9-Nov-05	0-5	2.3 ± 0.14	3.8	0.44 ± 0.02	8.6	17.5	24 ± 1.0	4 ± 0.64	0.68 ± 0.03	0.44 ± 0.05	0.28 ± 0.04	0.2 ± 0.041
(11°C)	5-10	1.7 ± 0.26	2.8	0.16 ± 0.05	17.5	7.5	18 ± 1.1	2.2 ± 0.27	0.38 ± 0.08	0.31 ± 0.03	0.13 ± 0.01	0.19 ± 0.064
	10-15	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
1-Mar-06	0-5	1.7 ± 0.61	2.8	0.43 ± 0.05	6.5	12.9	17 ± 1.8	5.9 ± 2.23	0.42 ± 0.03	0.15 ± 0.01	0.11 ± 0.03	0.14 ± 0.050
(2°C)	5-10	0.8 ± 0.07	1.3	0.30 ± 0.10	4.3	3.1	9 ± 1.0	3.6 ± 0.58	0.14 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	0.07 ± 0.004
	10-15	0.6 ± 0.06	0.9	0.17 ± 0.06	5.3	1.3	8 ± 0.9	2.9 ± 1.31	0.19 ± 0.03	0.04 ± 0.01	0.01 ± 0.00	0.08 ± 0.047
27-Mar-06	0-5	2.1 ± 0.16	3.5	0.46 ± 0.12	7.6	21.3	19 ± 0.9	2.9 ± 0.48	0.19 ± 0.01	0.24 ± 0.01	0.15 ± 0.01	0.07 ± 0.021
(4°C)	5-10	1.4 ± 0.18	2.3	0.30 ± 0.10	7.7	8.7	11 ± 0.9	1.7 ± 0.13	0.24 ± 0.05	0.08 ± 0.00	0.05 ± 0.00	0.16 ± 0.053
	10-15	0.5 ± 0.04	0.9	0.19 ± 0.06	4.7	1.1	4 ± 0.8	1.2 ± 0.33	0.04 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.06 ± 0.057

[a] Conversion factor: 20 fg cell^{-1} (Fuhrman & Azam 1982)
[b] Conversion factor: 34 (Dijkman, as cited in Evrard 2007)
[c] Carbon conversion factor: 8
[d] Carbon conversion factor: 6 (Hoppe 1993)
[e] Carbon conversion factor: 6 (Hoppe 1993)
* Rates measured at 0-2, 2-4 and 12-14 cm

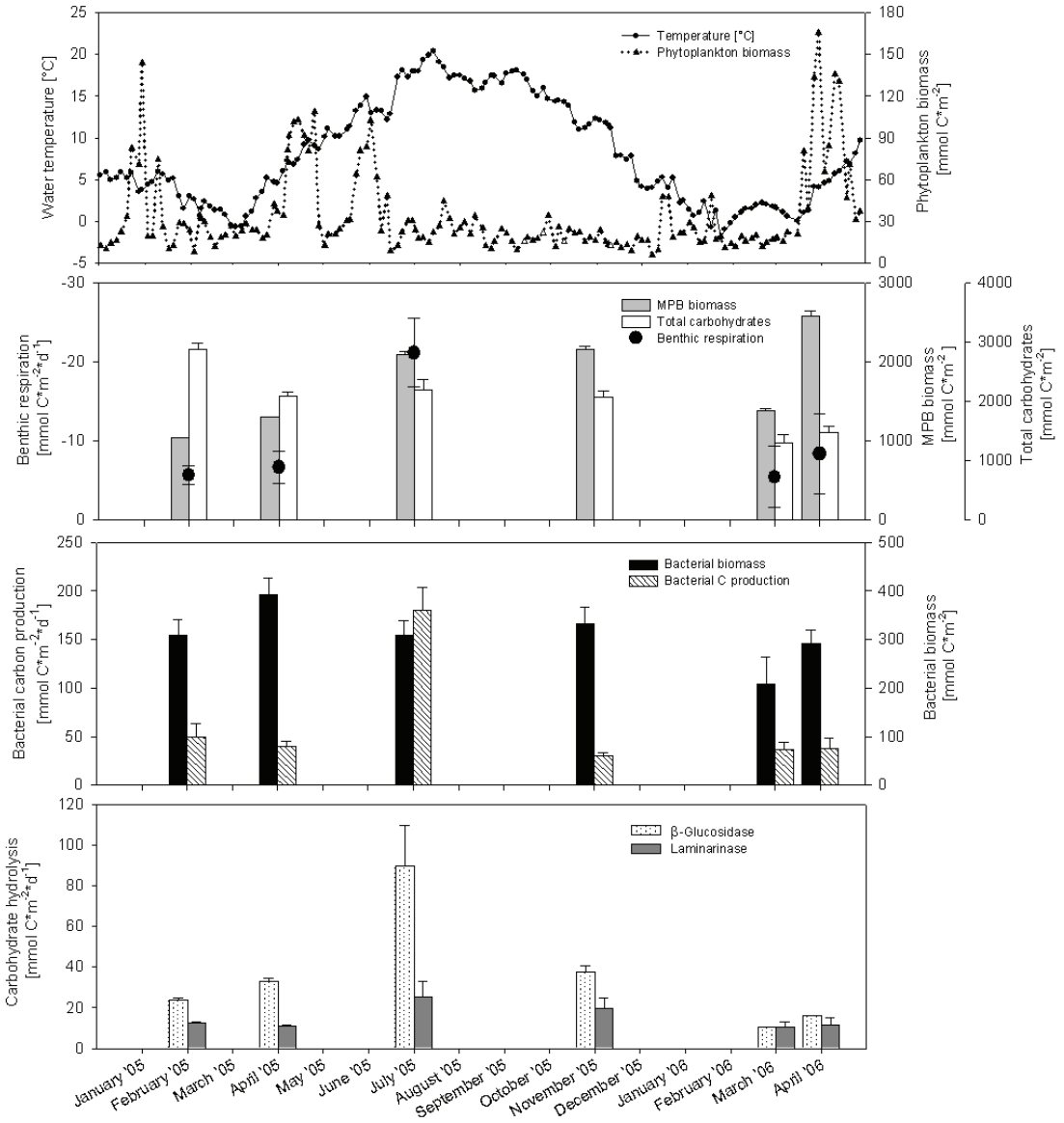

Fig. 2: Seasonal patterns of standing stocks and fluxes in the *Hausstrand* sands. All values were converted to carbon units and integrated over the upper 10 cm of sediment except for the water column data (integrated over the average depth of the water column of 2 m). Error bars represent standard deviations. **A**) Phytoplankton biomass (triangles) and water temperature (circles). **B**) MPB biomass (grey bars, n = 3), total carbohydrates (white bars, n = 3) and benthic oxygen consumption rates (circles, n = 4). **C)** Bacterial biomass (black bars, n = 6), bacterial carbon production rates measured via thymidine incorporation (striped bars, n = 4-5). **D)** C-hydrolysis by β-glucosidase activity (dotted bars, n = 5) and by laminarinase activity (dark grey bars, n = 2-3).

3.3.2 Potential extracellular enzyme activities

Enzyme activities measured using MUF- and MCA- substrate amended sediment slurries decreased in the order aminopeptidase > phosphatase > chitobiase, β-glucosidase > α-glucosidase > lipase. Hydrolysis rates in the upper sediment layer ranged between 0.7-4.5

μmol L^{-1} wet sediment h^{-1} (α-glucosidase), 1.1-7.8 μmol L^{-1} h^{-1} (β-glucosidase), 1-9.9 μmol L^{-1} h^{-1} (chitobiase) (Fig. 3A-C); 9-40 μmol L^{-1} h^{-1} (phosphatase), 0.03-0.8 μmol L^{-1} h^{-1} (lipase) and 13-101 μmol L^{-1} h^{-1} (aminopeptidase). Similar to bacterial abundances and bacterial growth rates, all EEA showed a clear depth trend. With few exceptions, hydrolytic activities decreased by factors of 1.5 to 6 with increasing sediment depth. The decrease was more pronounced between the middle and deepest layer than between the surface and middle layer. Only for chitobiase and lipase activity was this depth trend inverted in a few cases, such that the EEA was higher at 5-10 cm depth than at 0-5 cm depth. All EEA showed strong temporal patterns and generally followed the temperature curve, with highest activities measured in July and lowest activities at winter sampling dates (Table 1; Fig. 3).

However, in the top layers chitobiase (0-10 cm) and phosphatase (0-5 cm) activities were lower in April than in February despite a temperature increase of 5.4°C. Cell-specific MUF- and MCA-EEA ranged between 0.2–2.2 amol h^{-1} $cell^{-1}$ for chitobiase, between 0.12–0.98 amol h^{-1} $cell^{-1}$ for α-glucosidase, between 0.18–1.7 amol h^{-1} $cell^{-1}$ for β-glucosidase, between 0.02–0.24 amol h^{-1} $cell^{-1}$ for lipase, between 1.9–22 amol h^{-1} $cell^{-1}$ for aminopeptidase and between 1.24 and 8.8 amol h^{-1} $cell^{-1}$ for phosphatase, and generally showed comparable temporal and vertical patterns than non-specific rates; however the vertical decrease was less steep. Enzymatic hydrolysis of laminarin showed depth and seasonal trends comparable to hydrolytic rates of exoenzymes acting on carbohydrates (Fig. 3). Laminarin hydrolysis rates generally decreased with increasing sediment depth except for March II samples, where rates were higher at a depth of 5 cm than at 1 cm. Laminarin hydrolysis also followed the temperature curve and reached maximum values in July, although seasonal differences were not as pronounced as for MUF- and MCA-labeled substrates. Hydrolysis rates ranged between 0.5–1.8 μmol glucose L^{-1} wet sediment h^{-1} at 1 cm depth, 0.5–1.7 μmol glucose L^{-1} h^{-1} at 5 cm depth and 0-0.7 μmol monomer L^{-1} h^{-1} at 13 cm depth. Rates in intact cores were a factor of ~4 lower than exoenzymatic activities measured in sediment slurries (e.g. β-glucosidase), in accordance with the factor of 2 to 6 difference in hydrolysis rates in homogenized sediments compared to intact cores (Arnosti and Holmer, 2003). However, a high fraction of the injected FLA-polysaccharide was hydrolyzed after 24 h incubation: 20-70% in surface sediments, 19-68% in mid-depth sediments, and 0-29% in deep sediments, with highest fraction turned over in July and lowest in March. Hence, at least the rates in surface and mid-layer sediments may be significantly underestimated. Cell-specific laminarinase rates ranged between 0.25–0.61 amol h^{-1} $cell^{-1}$ at 1 cm depth, 0.44–0.86 amol h^{-1} $cell^{-1}$ at 5 cm depth and 0-0.97 amol h^{-1} $cell^{-1}$ at 13 cm sediment

depth, which is a similar range as for the exo-acting enzymes. No clear trend with depth was observed. Cell-specific laminarinase rates were higher at -5 cm than at -1 cm and in March also higher at -13 than at -5 cm sediment depth, underlining the potentially high competition with naturally occurring substrate at the sediment surface.

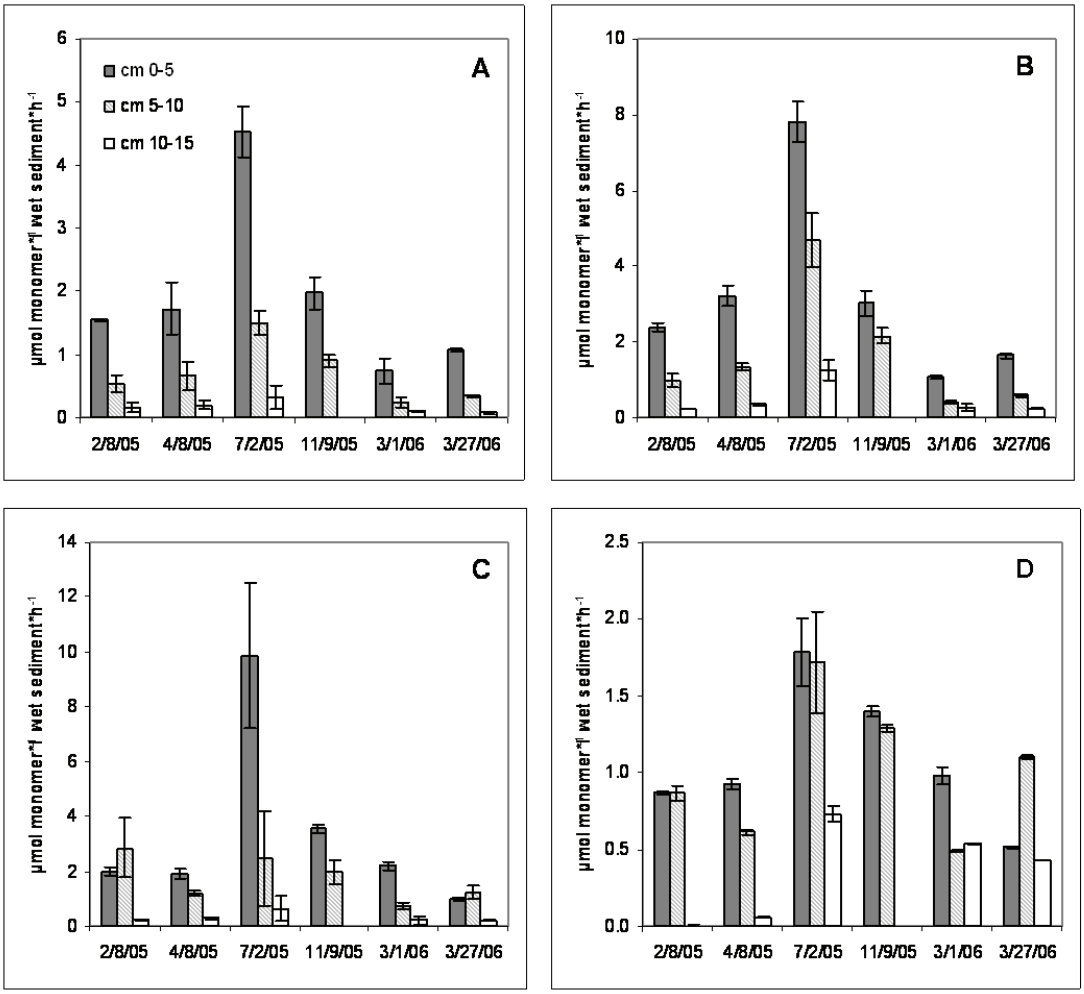

Fig. 3: Depth-related and temporal patterns in potential extracellular enzymatic activities of A) α-glucosidase (MUF-α-D-glucoside), B) β-glucosidase (MUF-β-D-glucoside), C) chitobiase (MUF-N-acetyl-β-glucosamine), and D) laminarinase (fluorescently labeled laminarin).

3.3.3 Chlorophyll pigments and microphytobenthic biomass

As derived from the chlorophyll *a* concentrations, a high MPB standing stock was present throughout the whole study period, with values ranging between 6.5-9.1 µg cm^{-3} wet sediment in the top centimeter. Based on average chlorophyll *a* concentrations between 4.5 and 7.5 µg cm^{-3} in the top 5 centimeters (Table1; Fig. 4A), a MPB biomass of 13-21 mmol C

L^{-1} was estimated. Highest concentrations were measured in April 2005 and in March (II) 2006. Both these sampling dates occurred at the beginning of a pelagic diatom spring bloom. However, the integrated standing stock in the water column is only 0.5-7% of the integrated standing stock of the MPB (Fig. 2). The benthic chlorophyll *a* peaks in spring thus most likely resulted from enhanced growth of the MPB rather than from phytodetritus sedimentation and burial. Over the investigated time period, temporal variation remained below a factor of 2 (Fig. 2). The vertical distribution was characterized by constantly decreasing but high chlorophyll *a* concentrations (> 1.5 µg cm^{-3} wet sediment) down to 5-8 cm. In deeper sediment layers chlorophyll *a* decreased to concentrations below 1.5 µg cm^{-3} wet sediment but was still detectable (Fig. 4A).

Phaeophytine also tended to decrease with depth, although there was no clear vertical profile. Concentrations stayed below 0.9 µg cm^{-3} wet sediment, and varied especially in the upper 10 cm of sediment. The ratio between chlorophyll *a* and phaeophytine ranged between 8 and 75 : 1 in the upper 5 cm of sediment and decreased to ratios between 1.7 and 11 : 1 in the 10–15 cm layer, indicating the dominance of living algae over phytodetritus. There was no obvious seasonal trend, but concentrations appeared to slighty decrease from the 1st to the last sampling date.

3.3.4 Benthic oxygen consumption

Total benthic oxygen consumption in dark incubations (gross respiration) was closely coupled to temperature, ranging between 5 and 21 mmol $C*m^{-2}*d^{-1}$ and varying seasonally with highest values in July 2005 (Fig. 2). The total benthic oxygen consumption measured here likely significantly underestimated *in situ* benthic oxygen consumption due to the low stirring speeds applied. Hence, seasonal trends can be compared, but carbon flux calculated from total oxygen consumption is underestimated (see below).

3.3.5 Carbohydrate inventory

Two carbohydrate fractions were measured, total carbohydrates, which include intracellular and extracellular carbohydrates (soluble as well as particle-bound), and the EDTA-extractable carbohydrate fraction that includes all extracellular soluble carbohydrates. Both fractions showed a clear depth trend (Table 1; Fig 4B; Fig. 4C). Concentrations of total carbohydrates were highest in the uppermost centimeter (4-6 µmol glucose equivalents cm^{-3}

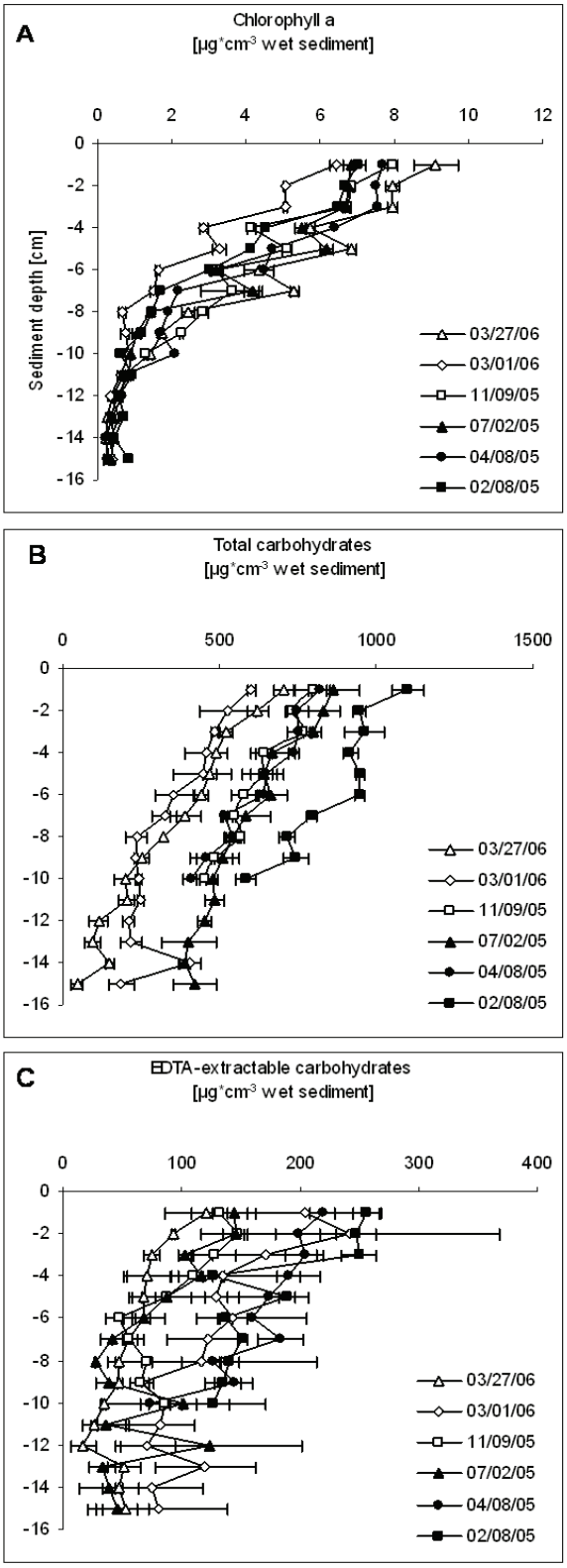

Fig. 4: Depth-related and temporal changes in A) benthic Chlorophyll a concentrations; B) total carbohydrate con-concentrations and C) EDTA-extractable carbohydrate concentrations

wet sediment) and decreased almost lineary to 10 cm depth (1-3 µmol glucose equivalents cm^{-3}) and 15 cm (0.3-2 µmol glucose equivalents cm^{-3}). There was no seasonal trend (Fig. 2B, 4B). Rather, total carbohydrate concentrations in the upper 10 cm constantly decreased from February 2005 until end of March 2006. EDTA-extractable carbohydrates followed the same vertical patterns, with values between 0.7 - 1.4 µmol glucose equivalents cm^{-3} wet sediment in the uppermost centimeter and between 0.2 - 0.7 µmol glucose equivalents cm^{-3} at 10 cm depth (0.2-0.4 µmol glucose equivalents cm^{-3} at 15 cm depth; Fig. 4C). The EDTA-extractable carbohydrates constituted between 13 and 37 % of the total carbohydrate pool in the upper 10 cm; this share did not change considerably with increasing sediment depth. The contribution of EDTA-extractable carbohydrates to the total carbohydrate pool was highest in April 2005 and in March (I) 2006, averaging 27% and 37%, respectively. These sampling dates, together with February 2005, were also the ones where the highest concentrations of EDTA-extractable carbohydrates were measured.

3.3.6 Statistical analysis of spatial and temporal variations

The permeable subtidal sands of the island of Sylt in the northern Wadden Sea constitute a highly dynamic environment, exposed to strong tidal currents and frequent storm events. Nevertheless, strong and persistent depth-patterns in bacterial abundances, BCP, EEA, and carbohydrate inventories were found over an annual cycle. Despite the low organic carbon content of the sediments, high extracellular enzyme activities and bacterial carbon production showed that the microbial community was very active. These high microbial activities were found to be tightly coupled to the productivity of the microphytobenthos. The dominating factors explaining the spatial (vertical) and temporal variations of bacterial activities were evaluated by Pearson Correlation (Table 2) and Principal Component Analysis (PCA; Fig. 5).

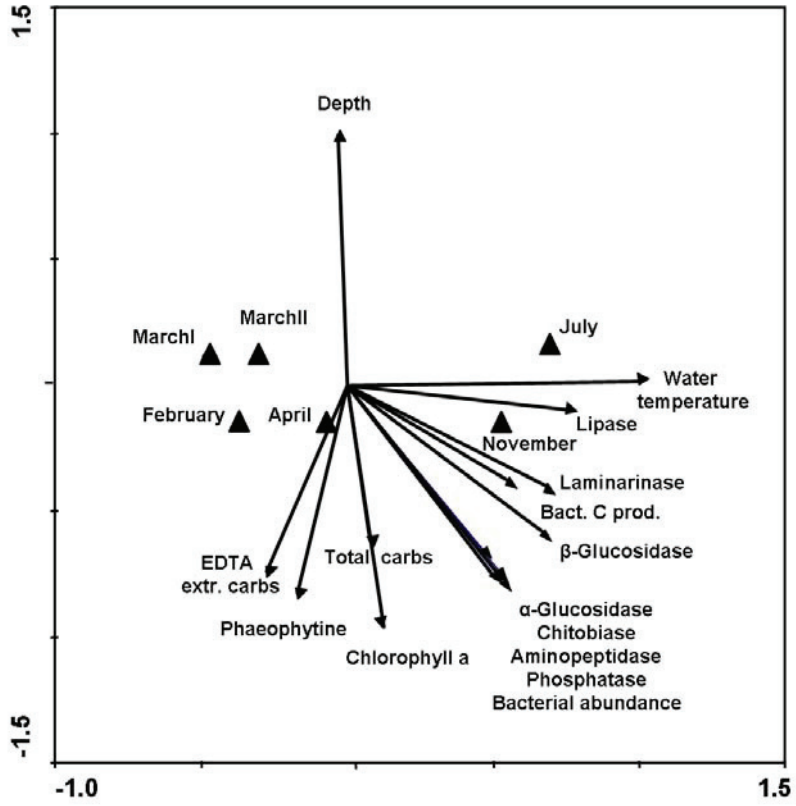

Fig. 5: PCA- (Principal component analysis) plot of major environmental variables at the *Hausstrand* based on an orthogonal linear transformation method that transforms a number of potentially correlated variables into a smaller number of independent variables, the so-called principal components. The magnitude of variation between the variables is depicted in a two-dimensional space. The plot represents 98% of the original variation in the data; axis 1 and 2 explain 76% and 22% of this variation, respectively.

Table 2: Pearson correlation coefficients at the Bonferroni-corrected level. Data comprises all samples that included carbohydrate measurements.

	Depth	Temp	Chl a	Phaeo	Lam	α-glu	β-glu	Chito	Phos	Lip	Pep	Abd	C prd	EDTA	Total
Depth		0.887	0.000	0.000	0.108	0.001	0.014	0.006	0.000	0.664	0.001	0.001	0.073	0.002	0.014
Temp.	**0.040**		0.865	0.333	0.010	0.052	0.004	0.098	0.065	0.000	0.056	0.070	0.014	0.260	0.963
Chl a	***-0.968**	**0.048**		0.000	0.069	0.000	0.007	0.007	0.000	0.592	0.000	0.000	0.081	0.012	0.014
Phaeo	***-0.819**	**-0.268**	***0.798**		0.270	0.093	0.219	0.056	0.014	0.677	0.032	0.006	0.493	0.004	0.000
Lam.	**-0.431**	**0.644**	**0.481**	**0.305**		0.002	0.000	0.000	0.001	0.001	0.002	0.005	0.009	0.780	0.269
α-gluc.	**-0.763**	**0.511**	**0.787**	**0.449**	**0.741**		0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.073	0.138
β-gluc.	**-0.616**	**0.691**	**0.663**	**0.337**	***0.802**	***0.955**		0.000	0.000	0.001	0.000	0.000	0.000	0.240	0.120
Chito.	**-0.677**	**0.443**	**0.664**	**0.503**	***0.832**	***0.887**	***0.833**		0.000	0.007	0.000	0.001	0.001	0.094	0.181
Phos.	***-0.810**	**0.488**	***0.829**	**0.617**	**0.758**	***0.944**	***0.924**	***0.872**		0.017	0.000	0.000	0.001	0.074	0.019
Lip.	**-0.122**	***0.878**	**0.151**	**-0.117**	**0.749**	**0.638**	**0.755**	**0.660**	**0.603**		0.031	0.046	0.000	0.597	0.898
Pep.	**-0.783**	**0.503**	***0.852**	**0.553**	**0.732**	***0.934**	***0.933**	***0.799**	***0.952**	**0.558**		0.000	0.002	0.143	0.033
Abund.	**-0.776**	**0.480**	***0.834**	**0.677**	**0.689**	***0.842**	***0.848**	**0.760**	***0.936**	**0.523**	***0.924**		0.005	0.095	0.004
Growth	**-0.475**	**0.620**	**0.464**	**0.192**	**0.645**	***0.795**	***0.820**	**0.744**	**0.743**	***0.833**	**0.725**	**0.686**		0.389	0.488
EDTA	**-0.734**	**-0.310**	**0.631**	**0.696**	**0.079**	**0.475**	**0.323**	**0.448**	**0.474**	**-0.149**	**0.397**	**0.447**	**0.240**		0.011
Total	**-0.617**	**-0.013**	**0.620**	***0.795**	**0.305**	**0.401**	**0.419**	**0.365**	**0.595**	**-0.036**	**0.551**	**0.693**	**0.194**	**0.637**	

Pearson correlation coefficients calculated on standardized data are shown in bold font in the bottom part of the table. P-values of correlations are shown in normal font in the upper part of the table; *denotes significant coefficients at the Bonferroni-corrected level ($p < 0.05/105 = 0.000476$).
Abbreviations: Temp = temperature, Chl *a* = benthic chlorophyll *a*, Phaeo = benthic phaeophytine, α-glu. = α-glucosidase, β-glu = β-glucosidase, Chito = chitobiase, Phos = phosphatase, Lip = Lipase, Pep = Aminopeptidase, Abd = bacterial abundance, C prd = bacterial carbon production, EDTA = EDTA-extractable carbohydrates, Total = Total carbohydrates.

Temporal variation was clearly related to temperature changes (1st axis); and spatial variations (vertical gradients) were best explained by the spatial distribution of the

microphytobenthos (2nd axis). Bacterial abundances, benthic chlorophyll *a*, and phaeophytine as well as total and EDTA extractable carbohydrates showed a strong negative relationship with sediment depth (statistically significant only for chlorophyll a and phaeophytine), likely a result of photosynthetic production being confined to the upper few millimeters of the sediment and mixing with depth. Bacterial abundances were significantly correlated with benthic chlorophyll *a* and hydrolytic activities of most extracellular enzymes (Table 2; Fig. 5). Phosphatase and aminopeptidase activities were also directly correlated with chlorophyll *a* and phosphatase also with depth, and most enzymes were correlated with each other. Variation in bacterial carbon production was significantly correlated to α-glucosidase, β-glucosidase and lipase activities. Lipase varied mainly temporally and this variation was strongly linked to the water temperature. The nature of the correlations identified via statistical analysis is examined in greater detail below.

3.4 Discussion

3.4.1 Benthic primary production and respiration

Permeable coastal sediments are characterized by a high biomass of benthic diatoms, which represent the main source of organic matter (Nelson et al. 1999, D'Andrea et al. 2004). At the *Hausstrand* site, benthic net primary productivity exceeds pelagic net primary productivity with rates around 53 mmol C m^{-2} d^{-1} (~19 mol C m^{-2} yr^{-1}; Wenzhöfer, pers. comm.) compared to 4-18 mmol C m^{-2} yr^{-1}(integrated over a mean water depth of 2 m) in the water-column of the Sylt-Rømø-basin (Asmus et al. 1998, Loebl et al. 2007). Even assuming a flushing rate between 160–500 l m^{-2} d^{-1} (de Beer et al. 2005) of the upper 5 cm of sands by the overlying water, the input of the pelagic spring bloom would be only less than a third compared to the microphytobenthic (MPB) standing stock. Furthermore, the MPB standing stock at the *Hausstrand* is relatively constant throughout the year (Hedtkamp 2005). It produces large amounts of extracellular polymeric substances (EPS), mainly composed of carbohydrates, which has been suggested to be the main source of energy and labile organic carbon to the bacterial community (MacIntyre et al. 1996, Underwood & Kromkamp 1999). In addition, the benthic meiofauna may benefit from these exudates (van Oevelen et al. 2006), and may also feed directly on the bacteria and diatoms (Epstein 1997, Cahoon 1999). The MPB, bacteria and other microorganisms form a biofilm community in the surface sands, presumably causing a tight coupling between variations in primary productivity, fluxes of

exudates and remineralization. This close linkage allows for a particularly rapid carbon flow from the autotrophic to the heterotrophic compartment at sunlight-exposed, sandy sediment sites (Middelburg et al. 2000).

Total benthic carbon metabolism can generally be estimated from rates of benthic oxygen consumption (e.g. Canfield et al. 1993) Because of the high permeability of the sediments and the low stirring speeds used in our chamber incubations, however, oxygen consumption rates in this study were likely underestimated. Previous *in situ* chamber incubations at the Hausstrand in summer 2004 yielded oxygen consumption rates of 25 mmol C m^{-2} d^{-1} and 56 mmol C m^{-2} d^{-1}, when measured at stirring speeds of 20 and 160 rpm, respectively, indicating that oxygen consumption rates under advective conditions may exceed those under diffusive conditions by a factor of 2.3 (Cook et al. 2007b). Applying this factor to the rates measured here (≤21 mmol C m^{-2} d^{-1}) would yield values close to estimates by Cook et al. (2007b); Heip et al. (1990) and Cramer (1991). The latter authors suggested that meiofauna and macrofauna are only responsible for 1-15% of the total benthic respiration in North Sea sediments. Nevertheless, we found a considerable discrepancy between total benthic respiration and our estimate of bacterial respiration (30-180 mmol C m^{-2} d^{-1}), assuming a 1:1 ratio between bacterial carbon production and respiration (i.e. carbon assimilation efficiency of 50%). The BCP rates of 0.4-2.3 mmol C L^{-1} d^{-1} (for the upper 0-5 cm) measured at *Hausstrand* are well within the range of rates measured in other, more organic-rich areas of the North Sea (van Duyl and Kop 1990, van Duyl et al. 1993, van Duyl & Kop 1994) and in the productive Great Barrier Reef sands (Alongi 1992). Previous measurements at *Hausstrand* yielded DIC fluxes that were about a factor of 2 higher than the oxygen consumption rates measured here (Hedtkamp, pers. comm.). These findings may indicate that a substantial fraction of bacterial respiration in the subtidal sands is anaerobic.

3.4.2 Contribution of sedimentary carbohydrates to benthic metabolism

To estimate the potential turnover of total and EDTA-extractable carbohydrates by extracellular hydrolysis, carbohydrate concentrations were divided by the sum of potential activities of α-glucosidase, β-glucosidase, chitobiase and laminarinase in the 0-5, 5-10, and 10-15 cm depth intervals. As shown in Fig 6A, in July in the 0-5 cm depth interval, 5-15% of the total carbohydrate pool, and almost the entire EDTA-extractable carbohydrate pool could have been hydrolyzed by these enzymes on a daily basis. Even in the 10-15 cm depth interval, a complete hydrolysis of the EDTA-extractable pool would be possible on the order

of 5 days. Potential hydrolysis efficiency was considerably lower in March (I) than in July, but still ranged from 4-12% of the EDTA-extractable pool on a daily basis (Fig. 6B).

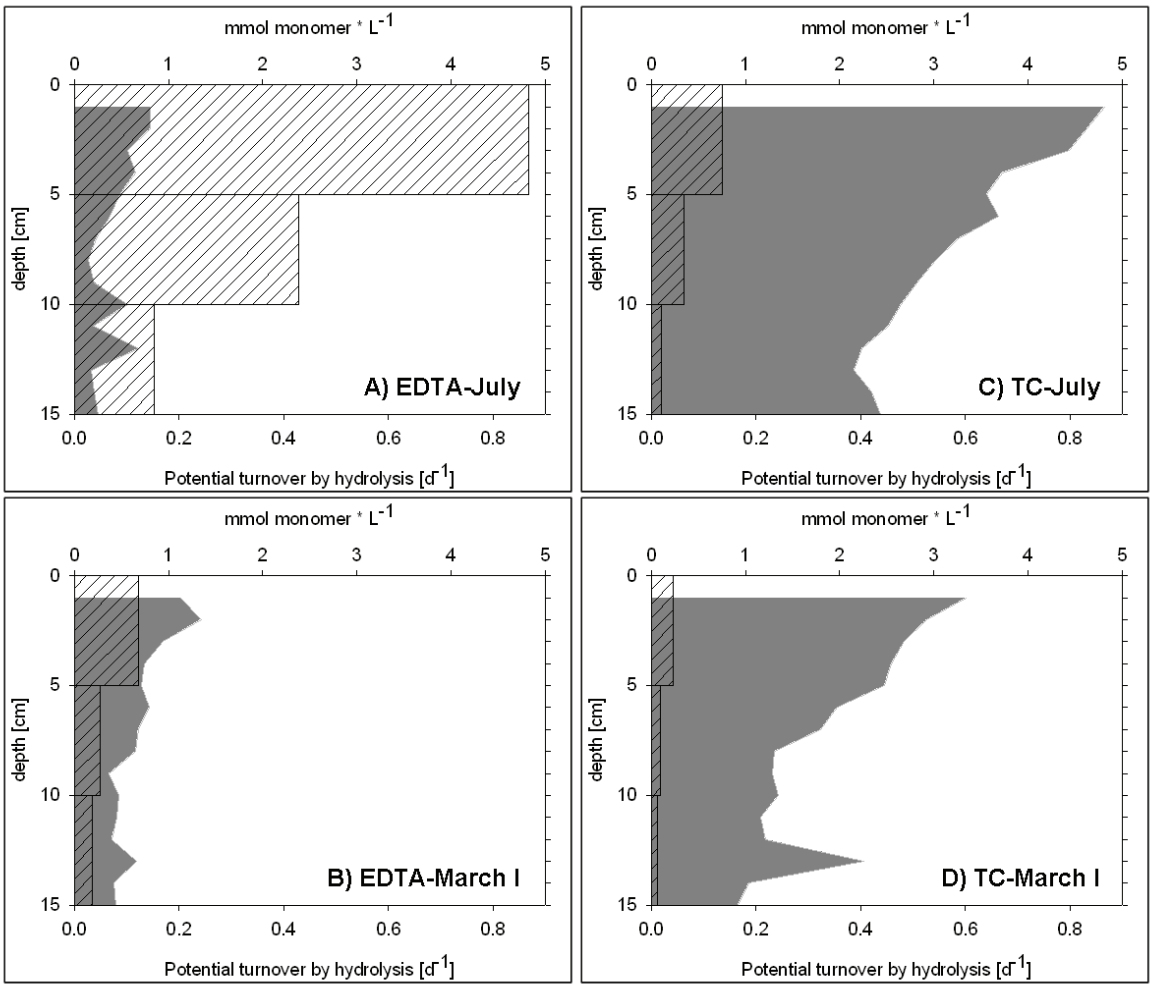

Fig. 6: Potential turnover of A) EDTA-extractable carbohydrates in July 2005 B) EDTA-extractable carbohydrates in March I 2006 C) total carbohydrates in July 2005 and D) total carbohydrates in March I 2006 in sediment from the *Hausstrand*. Average carbohydrate concentrations are shown as shaded area; bars represent turnover times based on the sum of laminarinase, α-glucosidase, β-glucosidase and chitobiase activities.

These rapid turnover rates indicate a high flux of EDTA-extractable carbohydrates throughout the year (Table 2). The seasonal variations are most likely explained by the higher hydrolytic activities in summer. Turnover time of total carbohydrates at the *Hausstrand* is rapid compared to sandy beach sediments of the Ligurian Sea (~3-619 days, Misic & Fabiano 2005). Potential hydrolytic activities of extracellular exoenzymes measured in the present study were as high as those observed in muddy, organic-rich sediments of the Kiel Bight (Meyer-Reil 1986) and Ems-Dollard estuary (van Duyl et al. 1999). Laminarinase activities, although potentially underestimated in the surface layer due to substrate competition (see

below), are comparable to rates measured in microbially active, organic-rich sediments in a range of other environments, including the Skagerrak, Cape Lookout Bight NC, and Svalbard (Hoppe et al. 2002, Arnosti & Holmer 2003, Arnosti & Jørgensen 2006). A high fraction of the total added FLA-polysaccharides were hydrolyzed within 24 h, resulting in an underestimation of hydrolysis rates, particularly in the surface and mid-depths, especially during the July sampling period. Furthermore, differences in assay method played a role, since a comparison of hydrolysis for a range of FLA-labeled polymers demonstrated that rates were 2-6 times more rapid in homogenized sediments compared to intact cores (Arnosti & Holmer 2003).

Table 3: Temporal changes in total bacterial carbon demand and carbon turnover by the benthic bacterial community at the *Hausstrand.* Areal estimates are based on integration of the top 10 cm.

	8-Feb-05	8-Apr-05	2-Jul-05	9-Nov-05	1-Mar-06	27-Mar-06
MPB Biomass [mmol C m^{-2}]	1045	1309	1211	1249	796	1499
Bacterial biomass [mmol C m^{-2}]	308	283	408	333	208	292
Total carbohydrates [mmol C m^{-2}]	2889	2090	2195	2071	1302	1472
EDTA extractable carbohydrates [mmol C m^{-2}]	586	556	292	309	472	227
Bacterial carbon production [mmol C m^{-2} d^{-1}]	50	40	180	30	37	38
Total bacterial C demand [mmol C m^{-2} d^{-1}][a)]	100	79	360	61	73	76
Total potential C generated from hydrolysis of carbohydrates [mmol C m^{-2} d^{-1}][b)]	98	91	277	130	57	59
Coverage of total bacterial C demand by C hydrolysis of carbohydrates [%][a)]	99	115	77	215	77	78
Total carbohydrate C fraction potentially consumed by bacterial community [% d^{-1}][a)]	3	4	16	3	6	5
EDTA extractable carbohydrate C fraction potentially consumed by bacterial community [% d^{-1}][a)]	17	14	124	20	15	34
MPB biomass fraction potentially consumed by bacterial community [% d^{-1}][a)]	10	6	30	5	9	5

[a] Growth yield 50% (Moriarty et al. 1985)
[b] Sum of α-glucosidase, β-glucosidase, chitobiase and laminarinase

Turnover of different constituents of organic matter ultimately derived from MPB photosynthetic production can also be estimated and related to carbon demand by bacteria and by the whole benthic community. Figure 2 and Table 2 show a comparison of the major carbon inventories in the sands. Assuming a gross phototrophic primary production of 100 mmol C m^{-2} d^{-1} in the top 10 cm of sand by adding up net benthic photosynthesis (*Hausstrand* data from May 2003, Wenzhöfer et al., unpublished data) and average respiration rates (assuming a bacterial growth yield of 50%), the MPB biomass (on average

1200 mmol C m^{-2} d^{-1}) could be turned over in 12 days (neglecting a potential input of pelagic phytodetritus of max. a third of MPB during spring bloom situations; de Beer et al. 2005). On average during the investigated period, in the 0-10 cm interval, bacterial biomass was 300 mmol C m^{-2}. Based on a gross bacterial carbon production (BCP) of 60 mmol C m^{-2} d^{-1}; turnover time of the bacterial biomass was 5-6 days. The MPB: bacterial C ratio was approximately 3:1 which is consistent with Evrard (2007), who estimated for the *Hausstrand* site that living MPB account on average for about 30% of the total organic carbon pool, while bacteria, meio- and macrofauna contribute 6, 2 and 0.5% of the total organic carbon pool, respectively. Total carbohydrate concentrations were on average 2000 mmol C m^{-2}, of which ca. 20% were EDTA-extractable carbohydrates. Summing up MPB biomass, bacterial biomass and total carbohydrates (taking into account that up to ca. 30% of algal biomass consist of carbohydrates that are already included in the total carbohydrate pool), and neglecting faunal biomass and organic matter other than carbohydrates, yields a carbon pool of around 3200 mmol C m^{-2},

Assuming a bacterial growth yield (carbon assimilation efficiency) of 50%, as is commonly used for heterotrophic benthic bacteria in surface sediments (Moriarty et al. 1985), the average total bacterial C demand is 120 mmol C m^{-2} d^{-1} (peaking in summer with up to 360 mmol C m^{-2} d^{-1}). On average, this demand could be covered by a daily turnover of 30% of the EDTA extractable carbohydrates (in summer up to 125%), 6% of the total carbohydrates or 11% of the MPB biomass. The sum of the measured potential hydrolytic activities seems to meet this carbon demand. On average, potential extracellular enzymatic hydrolysis of carbohydrates could supply approximately 110% of the total bacterial C demand, as calculated from the combined action of chitobiase, α-glucosidase, β-glucosidase and laminarinase acitivities (Table 2). Since laminarinase activities in the surface layer were most likely underestimated, hydrolysis of carbohydrates could even provide more C than estimated here. Hence, our calculations support the general assumption that EPS could provide most of the substrate required for microbial metabolism (MacIntyre et al. 1996, Underwood & Kromkamp 1999), but other substrate sources are also available (e.g. DOC, proteinaceous materials), and the activities of enzymes not measured in this study may also play a critical role in microbial nutrition.

3.4.3 Vertical zonation of bacterial abundances and activities

The strong hydrodynamic forces at the *Hausstrand* lead to frequent lateral transport and vertical mixing of the upper 5 cm of sediment (Hedtkamp 2005). In addition, bioirrigation/bioturbation by benthic fauna can mix the upper 10 cm of sediment (Huettel et al. 2003). Therefore, we can assume that the top 5 cm are constantly flushed with oxygen-rich water and include considerable amounts of diatom-derived labile organic carbon, while the 5-10 cm layer is less frequently mixed, and the 10-15 cm layer remains relatively undisturbed and is dominated by diffusive solute transport. This picture is supported by the physical characteristics of the sediment. The upper 5 cm of sediment were extremely coarse and yellowish, indicating a zone of strong mixing and oxygenation. The middle layer was greyish and more compact, indicating a transition zone between this oxygen-rich top layer and the diffusion-dominated, deepest layer that was blackish, smelled faintly of sulfide, and contained also small-grained sediments.

Seasonal and short-term changes in wind stress have been shown to influence sediment permeability (Hedtkamp 2005); thus storm impacts include increased horizontal and vertical sediment mixing. Despite these active physical dynamics of tides and wave forces at the field site, at all sampling dates (including April and November, with strong storm events) distinct depth trends were evident in MPB biomass, chlorophyll *a*, and phaeopigments, total carbohydrates and EDTA extractable carbohydrates, as well as in bacterial abundances, growth rates, and extracellular enzymatic activities. Concurrent decreases with depth in hydrolytic activities, bacterial abundances, and bacterial carbon production rates suggest a vertical shift in the availability and nutritional quality of organic matter in the sediment.

Bacterial abundances were statistically correlated to hydrolytic activities of α-glucosidase, β-glucosidase, phosphatase and aminopeptidase (r between 0.84 and 0.94, $p < 0.00024$; Table 2), partially explaining the decrease in hydrolytic activities with depth. But even cell-specific hydrolytic enzyme activities showed a distinct decrease with depth, presumably due to the reduced availability and quality of organic substrates. This is also consistent with the lower turnover of carbohydrates with depth (Fig. 6), and the depth-related decrease in BCP. Similar depth trends have been reported from muddy sediment sites, where particulate organic matter mixing into the sediment depends entirely on bioturbation (e.g. Mayer 1989, Arnosti 1995). However, distinct depth trends in highly dynamic sandy environments are unusual. In Middle Atlantic Bight shelf sediments, a site quite comparable

to *Hausstrand*, Rusch and coworkers (2003) investigated depth-related and seasonal patterns in bacterial and algal cell abundances, exoenzymatic activities and carbon inventory. The bacterial community was found to be highly active, with aminopeptidase and β-glucosidase activities comparable to those measured in the present study; however depth-related patterns were only found during the winter sampling date. Likewise, an investigation of aminopeptidase, β-glucosidase, alkaline phosphatase activities, their natural substrates, bacterial abundances and biomass in two adjacent areas of a sandy beach over one year showed a lack of vertical patterns within the top 15 cm (Misic & Harriague 2007). An intertidal sandy area close to our sampling site (Musat et al. 2006) also did not reveal the pronounced depth-related changes in bacterial abundances that we found in the subtidal sediments. We assume that the observed depth gradient is mainly due to the distribution of the MPB and the biofilm-like association with the bacterial community. This is supported by the tight correlation between bacterial abundances and benthic chlorophyll *a* ($r = 0.83$, $p < 0.00024$). At *Hausstrand*, most of the benthic diatom biomass is found in the top 0-5 cm layer, but moderately high concentrations could be observed down to a depth of -8 cm, corresponding to the maximal depth of advective transport. At these depths, diatoms are non-active, but able to survive several months without loosing their photosynthetic capacity (Sundbäck & Granéli 1988, Nelson et al. 1999).

3.4.4 Temperature-related temporal patterns in bacterial activities

Water temperature followed a typical seasonal pattern over the sampling period (Fig. 2A), with winter temperatures close to the freezing point and summer temperatures of up to 20°C. The spring bloom in the water column of Sylt occurs generally in April, but even at the peak of phytoplankton biomass, the integrated carbon concentrations were <7% of the microphytobenthic standing stock in the sands. Although the temporal variation of the MPB and bacterial standing stock was negligible, the entire benthic community was more active at higher temperatures as indicated by the pattern of total benthic oxygen consumption (Fig. 2B). Extracellular enzymatic activities and bacterial carbon production clearly also followed the temperature trend (Fig. 2C, D) and reached maximum values in July. The lack of temporal variation in MPB and bacterial biomass (Fig. 2C) contrasted with the changes in activity patterns, and may be due to higher grazing pressure in the summer. Likewise, the lack of a temperature-related pattern in carbohydrate concentrations (Fig. 4) could reflect

higher bacterial consumption during times of higher MPB production, since carbohydrate concentrations reflect the net balance between production and consumption.

Higher enzymatic hydrolysis rates may be the result of faster enzyme function and/or of enhanced bacterial enzyme synthesis at higher temperatures. High BCP rates and high benthic oxygen consumption in July provide evidence for a generally enhanced metabolism in summer. Investigating apparent temperature effects on hydrolytic exoenzyme activities at the *Hausstrand* experimentally yielded Q_{10} values between 1.4 and 1.7 (Table 4), indicating that temperature effects could cause an increase in the activity of enzymes only by less than a factor of 2 when temperature rises by 10°C. In the field, a much higher increase of enzyme acivities was observed between winter and summer, suggesting that enzyme synthesis by the benthic bacteria may have changed, possibly due to induction by increased substrate availability in the summer months. Moreover, temperature between July and November dropped by only 7°C while EEA decreased by a factor of up to 5. Temperature has also been found to be an important but not the only influence affecting seasonal variations in extracellular proteolytic enzyme activity in an intertidal mudflat (Mayer 1989). Since bacterial abundances do not necessarily reflect the state of activity of the microbial community (Créach et al. 2003) and no information is available on variations in the fraction of the community producing specific enzymes, changes in bacterial community structure and composition may also have influenced seasonal changes in hydrolytic rates.

Table 4: Q_{10}-values of exoacting extracellular enzymes as determined experimentally with *Hausstrand* sediment. Hydrolysis rates of the respective MUF- and MCA-substrates were measured at 4°C, 16°C and 25°C. Values represent the increase in enzymatic activity when temperature rises by 10°C.

Enzyme	Q_{10}-value
β-Glucosidase	1.7
Lipase	N.A.
Aminopeptidase	1.4
Phosphatase	1.4
α-Glucosidase	1.7
Chitobiase	1.6

The microphytobenthic standing stock – as derived from chlorophyll *a* - was generally stable over the entire sampling period, except for minor peaks in spring, in accordance with observations in other shallow sediment ecosystems (Varela & Peñas 1985, Goto et al. 1998, Goto et al. 2000). The significant temporal changes in microbial activities thus can best be

explained by a combination of the effects of temperature and substrate availability. The minor temporal variations in microbial and MPB biomass are typical for biofilm-type communities; they could result from light and space limitation as well as from extensive grazing pressure. Previous investigations suggested that especially the nano- and meiofauna plays an important role for the removal of bacterial and MPB biomass in permeable sands (Epstein 1997, Cahoon 1999, Middelburg et al. 2000, Urban-Malinga et al. 2006, Evrard 2007).

3.5 Acknowledgements

Susanne Menger is gratefully acknowledged for assistance in the field and with sample processing. Stefanie I.C. Hedtkamp kindly provided 2005 benthic respiration data. Sherif Gobrial is thanked for the GPC analysis of potential endoenzymatic activities and Gabriele Schüßler is recognized for assistance with ^{3}H-thymidine incorporation measurements. Hannelore Halliger and Reimer Magens supported our work on Sylt logistically and technically. The scientific divers Christian Buschbaum and Patrick Polte are gratefully acknowledged for recovery of sediments samples in April 2005 and November 2005. Aerial photo material was kindly provided by the Wadden Sea National Park Office. This study was financed by the Max Planck society. CA received funding from the National Science Foundation (OCE-0323975), as well as support from the Hanse Institute for Advanced Study and the Alfred Wegener Institute for Polar and Marine Research while the manuscript was being written.

3.6 References

Alderkamp AC, van Rijssel M, Bolhuis H (2006) Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. FEMS Microbiol Ecol 59(1):108-117

Alongi DM (1992) Vertical profiles of bacterial abundance, productivity and growth rates in coastal sediments of the central Great Barrier Reef lagoon Mar Biol 112:657-663

Arnosti C (1995) Measurement of depth- and site-related differences in polysaccharide hydrolysis rates in marine sediments. Geochim Cosmochim Acta 59(20):4247-4257

Arnosti C, Holmer M (2003) Carbon cycling in a continental margin sediment: contrasts between organic matter characteristics and remineralization rates and pathways. Est Coast Shelf Sci 58:197-208

Arnosti C (2003) Fluorescent Derivatization of Polysaccharides and Carbohydrate-Containing Biopolymers for Measurement of Enzyme Activities in Complex Media. J. Chromatogr B 793:181-191

Arnosti C, Jørgensen BB (2006) Organic carbon degradation in Arctic marine sediments, Svalbard: A comparison of initial and terminal steps. Geomicrobiol. J. 23:551-563

Asmus RM, Jensen MH, Murphy D, Doerffer R (1998) Primary Production of Microphytobenthos, Phytoplankton and the Annual Yield of Macrophytic Biomass in the Sylt-Rømø Wadden Sea. In: Gätje C, Reise K. (eds.) Ökosystem Wattenmeer. Springer Verlag. Berlin, Germany

Billerbeck M, Røy H, Bosselmann K, Huettel M (2007) Benthic photosynthesis in submerged Wadden Sea intertidal flats. Est Coast Shelf Sci 71(3-4):704-716

Boetius A (1995) Microbial hydrolytic enzyme activities in deep-sea sediments. Helgol Meeresunters 49:177-187

Boetius A, Lochte K (1996a) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. Mar Ecol Prog Ser 140:239-250

Boetius A, Lochte K (1996b) High proteolytic activities of deep-sea bacteria from oligotrophic polar sediments. Arch Hydrobiol Spec Issues Advanc Limnol 48:269-276

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, Nielsen P, Sansone P, Taghon G, Van Raaphorst W, Webster I, Weslawski JM, Wiberg P, Sundby B (2001) Permeable marine sediments: Overturning an old paradigm. EOS 82:135-136

Cahoon LB (1999) The role of benthic microalgae in neritic ecosystems In: Ansell AD, Gibson RN, Barnes M (eds.) Oceanogr Mar Biol Ann Rev, Vol 37. Taylor & Francis. London, UK. pp 47-86

Canfield DE, Jørgensen BB, Fossing H, Glud R, Gundersen J, Ramsing NB, Thamdrup B, Hansen JW, Nielsen LP, Hall POJ (1993) Pathways of organic carbon oxidation in three continental margin sediments. Mar Geol 113:27-40

Cook PLM, Veuger B, Böer S, Middelburg JJ (2007a) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat Microb Ecol 40:165-180

Cook PLM, Wenzhöfer F, Glud RN, Janssen F, Huettel M (2007b) Benthic solute exchange and carbon mineralization in two shallow subtidal sandy sediments: Effect of advective pore-water exchange Limnol Oceanogr 52(5):1943-1963

Cramer A (1990) Seasonal variation in benthic metabolic activity in a frontal system in the North Sea. In: Barnes M & Gibson RN (eds.), Trophic relationships in the marine environment 1990, Proc. 24th Europ. Mar. Biol. Symp. University Press. Aberdeen, UK. pp 54-76

Créach V, Baudoux AC, Bertru G, Le Rouczic B (2003) Direct estimate of active bacteria: CTC use and limitations. J Microbiol Methods 52(1):19-28

D'Andrea AF, Aller RC, Lopez GR (2002) Organic matter flux and reactivity on a South Carolina sandflat: The impacts of porewater advection and macrobiological structures. Limnol Oceanogr 47:1056-1070

D'Andrea AFD, Lopez GR, Aller RC (2004) Rapid physical and biological particle mixing on an intertidal sandflat. J Mar Res 62:67-92

de Beer D, Wenzhöfer F, Ferdelman TG, Boehme SE, Huettel M, van Beusekom JEE, Böttcher ME, Musat N, Dubilier N (2005) Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnol Oceanogr 50(1):113-127

de Brouwer JFC, Stal LJ (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar Ecol Prog Ser 218:33-44

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356

Emery KO (1968) Relict sediments on continental shelves of world. AAPG Bulletin 52:445-464

Epstein SS (1997) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities Microb Ecol 34:188-198

Evrard VPE (2007) Assessing the fate of organic matter in subtidal sandy sediments using carbon and nitrogen stable isotopes as deliberate tracers. Dissertation thesis. Universiteit Utrecht, The Netherlands

Findley SEG, Meyer JL, Edwards RT (1984) Measuring bacterial production via rate of incorporation of [^{3}H]thymidine into DNA. J Microbiol Methods 2:57-72

Findley SEG (1993) Thymidine incorporation into DNA as an estimate of sediment bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds.) Handbook of methods in aquatic microbial ecology. Lewis publishers. Baton Rouge, USA. pp 505-508

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109-120

Gätje C, Reise K (eds.): Ökosystem Wattenmeer: Austausch, Transport und Stoffwandlungsprozesse. Springer Verlag. Berlin/Heidelberg, Germany

Gattuso JP, Gentili B, Duarte CM, Kleypas JA, Middelburg JJ, Antoine D (2006) Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. Biogeosciences 3:489-513

Glabe CG, Harty PK, Rosen SD (1983) Preparation and properties of fluorescent polysaccharides. Anal Biochem 130:287-294

Goto N, Mitamura O, Terai H, Ishida N (1998) Physicochemical features and primary production of microphytobenthos and phytoplankton at Wakaura tidal flat in Japan. Jpn J Limnol 59:391-408

Goto N, Mitamura O, Terai H (2000) Seasonal variation in primary production of microphytobenthos at the Isshiki intertidal flat in Mikawa Bay. Jpn J Limnol 1:133-138

Goto N, Mitamura O, Terai H (2001) Bidegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J Exp Mar Biol Ecol 257:73-86

Grasshoff K, Kremling K, Ehrhardt M (1983) Methods of seawater analysis. Wiley-VHC. Berlin, Germany

Hall SJ (2002) The continental shelf benthic ecosystem: current status, agents for change and future prospects. Environ Conserv 29:350-374

Hedtkamp SIC (2005) Shallow subtidal sand: Permeability, nutrient dynamics, microphytobenthos and organic matter. Dissertation thesis. Christian-Albrechts-Universität. Kiel, Germany

Heip C, Huys R, Vincx M, Vanreusel A, Smol N, Herman R et al. (1990) Composition, distribution, biomass and production of North Sea meiofauna. Neth. J. Sea Res. 26(2-4):333-342

Herbert D, Phipps PJ, Strange RE (1971) Chemical analysis of microbial cells. In: Norris JR, Ribbons DW (eds.) Methods in microbiology. Academic Press. London, UK. pp 209-344

Hoagland KD, Rosowski JR, Gretz MR, Roerner SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J Phycol 29(5):537-566

Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33(5):1225-1228

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar Ecol Prog Ser 11:299-308

Hoppe HG, Kim SJ, Gocke K (1988) Microbial decomposition in aqutic environments: combined process of extracellular enzyme activity and substrate uptake. Appl Environ Microbiol 54:784-790

Hoppe HG, Arnosti C, Herndl GF (2002) Ecological Significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP, Templeton DM (eds.) Enzymes in the environment: activity, ecology, and applications. CRC Press. pp 73-108

Huettel M, Røy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 49:231-236

Jahnke RA (2004) Transport processes and organic matter cycling in coastal sediments. In: Robinson AR, Brink KH (eds.) The Sea. Volume 13.

Köster R, Müller A (1998) Erosion, Sedimentation und Schwebstofftransport im Lister Tidebecken: Ein Überblick. In: Gätje C, Reise K, (eds.) Ökosystem Wattenmeer – Austausch-, Transport- und Stoffumwandlungsprozesse. Springer-Verlag. Berlin, Germany

Lee RE (1980) Phycology. Cambridge University Press. Cambridge

Loebl M, Dolch T, van Beusekom JEE (2007) Annual dynamics of pelagic primary production and respiration in a shallow coastal basin. J Sea Res 58:269-282

Lorenzen CJ (1967) Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol Oceanogr 12(2):343-346

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. 1. Distribution, abundance and primary production. Estuaries 19:186-201

Mayer LM (1989) Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat, Limnol Oceanogr 34(6):973-981

Meyer-Reil LA (1986) Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. Mar Ecol Prog Ser 31:143-149

Meyer-Reil LA (1987) Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. Appl Environ Microbiol 53(8):1748-1755

Michel PH, Bloem J (1993) Conversion factors for estimation of cell production rates for soil bacteria from [^{3}H]thymidine and [^{3}H]leucine incorporation. Soil Biol Biochem 25(7):943-950

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMT, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr 46(6):1224-1234

Misic C, Fabiano M (2005) Enzymatic activity on sandy beaches of the Ligurian Sea (NW Mediterranean). Microb Ecol 49:513–522

Misic C, Harriague AC (2007) Enzymatic activity and organic substrates on a sandy beach of the Ligurian Sea (NW Mediterranean) influenced by anthropogenic pressure. Aquat Microb Ecol 47:239-251

Moriarty DJW, Pollard PC, Hunt WG, Moriarty CM, Wassengerg T (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians on a coral reef flat. Mar Biol 85:293–300

Moriarty DJW (1990) Techniques for estimating bacterial growth rates and production of biomass in aquatic environments. In: Grigorova R, Norris JK (eds.) Methods in microbiology, Vol. 22. Academic Press. London, UK. pp 211-134

Moriarty DJW, Pollard PC (1990) Effects of radioactive labeling of macromolecules, disturbance of bacteria and adsorption of thymidine to sediment on the determination of bacterial growth rates in sediment with tritiated thymidine. J Microbiol Methods 11:127-139

Musat N, Werner U, Knittel K, Kolb S, Dodenhof T, van Beusekom JEE, de Beer D, Dubilier N, Amann R (2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. Syst Appl Microbiol 29:333-348

Nelson JR, Eckmann JE, Robertson CY, Marinelli RL, Jahnke RA (1999) Benthic microalgal biomass and irradiance at the sea floor on the continental shelf of the South Atlantic Bight: Spatial and temporal variability and storm effects. Cont Shelf Res 19:477-505

Ortega-Calvo JJ, Stal LJ (1994) Sulphate-limited growth in the unicellular cyanobacterium *Gloethece* (Nageli) sp. PCC 6909. New Phytol 128(2):273-281

Painter TJ (1983) Algal Polysaccharides. In: Aspinall GO (eds.), The polysaccharides. Academic Press. pp 195-285

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62(2):142-160

Rooney-Varga JN, Giewat MW, Savin MC, Sood S, LeGresley M, Martin JL (2005) Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb Ecol 49:163-175

Rusch A, Huettel M, Reimers CE, Taghon GL, Fuller CM (2003) Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sand. FEMS Microbiol Ecol 44:89-100

Rusch A, Huettel M, Wild C, Reimers CE (2006) Benthic oxygen consumption ond organic matter turnover in organic-poor, permeable shelf sands. Aquat Geochem 12:1-19

Smith DJ, Underwood GJC (1998) Exopolymer production by intertidal epipelic diatoms. Limnol Oceanogr 43:1578-1591

Staats N, de Deckere E, Kornman B, van der Lee W, Termaat R, Terwindt J, de Winder B (2001a) Oberservations on suspended particulate matter (SPM) and microalgae in the Dollard estuary, the Netherlands: Importance of late winter ice cover of the intertidal flats. Est Coast Shelf Sci 53:297-306

Staats N, de Deckere EMGT, de Winder B, Stal LJ (2001b) Spatial patterns of benthic diatoms, carbohydrates and mud on a tidal flat in the Ems-Dollard estuary. Hydrobiologia 448:107-115

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis, 2nd ed. Bulletin of Fisheries Research Board of Canada. p 167

Sundbäck K, Granéli W (1988) Influence of microphytobenthos on the nutrient flux between sediment and water: a laboratory study. Mar Ecol Prog Ser 43:63-69

ter Braak CJF, Šmilauer P (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canocical Community Ordination, Version 4.5

Thorbergsdóttir IM, Gíslason SR, Ingvason HR, Einarsson Á (2004) Benthic oxygen flux in the highly productive subarctic Lake Myvatn Iceland: *in situ* benthic flux chamber study. Aquat Ecol 38:177-189

Underwood GJC, Paterson DM, Parkes RJ (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol Oceanogr 40(7):1243-1253

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Nedwell DB, Raffaelli D (eds.), Advances in Ecological Research, Vol 29. Academic Press Inc. San Diego, USA. pp 93-153

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms In: Callow JA (eds.) Advances in Botanical Research, Vol. 40, Advances in Botanical Research Incorporating Advances in Plant Pathology

Urban-Malinga B, Hedtkamp SIC, van Beusekom JEE, Wiktor J, Weslawski JM (2006) Comparison of nematode communities in Baltic and North Sea sublittoral, permeable sands - Diversity and environmental control. Est Coast Shelf Sci 70:224-238

van Beusekom JEE, Reise K (2008) Editorial: Long-term ecological change in the northern Wadden Sea. Helgol Mar Res 62:1-2

van Duyl FC, Kop AJ (1990) Seasonal patterns of bacterial production and biomass in intertidal sediments of the western Dutch Wadden Sea. Mar Ecol Prog Ser 59:249-261

van Duyl FC, Bak RPM, Kop AJ, Nieuwland G, Berghuis EM, Kok A(1992) Mesocosm experiments: mimicking seasonal developments of microbial variables in North Sea sediment. Hydrobiologia 235/236:267-281

van Duyl FC, van Raaphorst W, Kop AJ (1993) Benthic bacterial production and nutrient sediment-water exchange in sandy North Sea sediments. Mar Ecol Prog Ser 100:85-95

van Duyl FC, Kop AJ (1994) Bacterial production in North Sea sediments: clues to seasonal and spatial variations. Mar Biol 120:323-337

van Duyl FC, de Winder B, Kop AJ, Wollenzien U (1999) Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar Ecol Prog Ser 191:19-32

van Oevelen D, Soetaert K, Middelburg JJ, Herman PMJ, Moodley L, Hamels I, Moens T, Heip CHR (2006) Carbon flows through a benthic food web: integrating biomass, isotope and tracer date. J Mar Res 64:453-482

Varela M, Penas E (1985) Primary production of benthic microalgae in an intertidal sand flat of the Ria de Arosa, NW Spain. Mar Ecol Prog Ser 25:111-119

Warren RAJ (1996) Microbial hydrolysis of polysaccharides. Annu Rev Microbiol 50:183-212

Winkler LW (1888) Determination of dissolved oxygen in water. Berlin Deut. Chem. Ges.

Wollast R Continental margins - review of geochemical settlings. In: Wefer G, Billet D, Hebbeln D, Jørgensen BB, Schlüter M, van Weering TCE (eds.) Ocean margin systems. Springer Verlag.

Chapter 4

Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sandy sediments

S.I. Böer, S.I.C. Hedtkamp, J.E.E. van Beusekom, J.A. Fuhrman, A. Boetius and A. Ramette

submitted to *The ISME Journal*

Abstract

The spatial and temporal variability of microbial communities in subtidal sands are poorly understood, despite their ecological and economic importance as catalysts of organic matter degradation and remineralization. Here, bacterial diversity, community structure and microbial activity were determined together with a large number of contextual environmental parameters over two years in subtidal sandy sediments of the Northern German coast (List on Sylt) in order to identify the main factors shaping microbial community structure and activity. Seasonal temperature changes directly affected bacterial activities and total community respiration, but did not explain variations in community structure. Despite the strong physical forces from tides and wind-induced waves at the field site, strong sediment depth related, spatial patterns were observed for bacterial abundances, carbon production rates and extracellular enzymatic activities. Bacterial community structure also showed a clear vertical variation with higher richness at 10-15 cm depth than in the top 10 cm, probably due to the decreasing disturbance in deeper sediment layers by hydrodynamic forces and bioturbation. Time was the most important factor affecting microbial community structure with a high OTU replacement of up to 47% over two years. However, principal ecosystem functions such as benthic oxygen consumption and extracellular hydrolysis of organic matter were maintained at a high level at all times, indicating that the bacterial community in general has a high functional redundancy. A multivariate analysis applied to the bacterial community data and a range of contextual environmental parameters showed that time, sediment depth, chlorophyll *a*, NO_3, wind speed, and bacterial abundance altogether explained more than 80% of the overall variation in bacterial community structure.

4.1 Introduction

Coastal seas represent a large and economically valuable ecosystem directly affected by global change including overfishing and other types of resource exploitation, eutrophication, pollution, coastal development and introduction of alien species (Huettel et al. 2003a). While increasing scientific effort concentrates on the analysis of natural and man-made changes in biodiversity of animals and plants of coastal seas, still very little is known about temporal and spatial variations in microbial communities which act as a bioreactor for the processing of huge amounts of deposited organic matter. Important questions remain as to the link between environmental disturbances and patterns in microbial diversity and activity, and their effect on ecosystem function.

Several studies have been conducted to determine the link between bacterial community structure and composition with environmental parameters in benthic habitats. Sediment depth-related patterns in bacterial community structure were, for instance, detected for a variety of benthic habitats such as cold seep sediments (Inagaki et al. 2002), the warm deep Mediterranean sea (Luna et al., 2004), the western Pacific coast (Urakawa et al. 2000), Antarctic continental shelves (Bowman et al. 2003, Bowman & McCuaig 2003), coral reef sediments (Hewson & Fuhrman 2006), as well as for continental shelf sediments of the southern North Sea (Franco et al. 2007). Environmental parameters such as wave impacts (Hewson & Fuhrman 2006), organic carbon content and chlorophyll *a* in the sediment (Polymenakou et al. 2005), sediment type (Franco et al. 2007), enzyme (alkaline phosphatase) activities and sediment water content (Hewson et al. 2007), eutrophication associated with fish farms (Bissett et al. 2007, Vezzulli et al. 2002) and inorganic nutrients enrichment in sediment-water mesocosms (Hewson et al. 2003) have been correlated to shifts in benthic bacterial community structure. All benthic studies to date have, however, included a comparably low number of environmental parameters and generally did not consider temporal dynamics of the highly complex coastal ecosystem. To our knowledge, only one recent study has treated seasonal changes in bacteria community composition in coastal sandy sediments (Musat et al. 2006). This approach was based on phylogenetic approaches and did not aim at linking community structure to ecosystem dynamics.

Here we undertook an intensive field study over two years on a shallow subtidal sand flat in the North Frisian Wadden Sea ("*Hausstrand"*, List on Sylt), characterized by strong hydrodynamic forces of waves and tides, strong storm impacts, considerable seasonal changes

in temperature and light availability and two consecutive phytoplankton spring blooms. Previously, we had investigated the temporal and vertical variations in microbial activities and carbon turnover at the *Hausstrand* field site (Böer et al. 2008). Here, we determined the bacterial community structure by automated ribosomal intergenic spacer analysis (ARISA). We have analyzed these diversity data using 22 different contextual environmental parameters, to answer the following questions: 1) Are there stable depth-related patterns in microbial activities and community structure despite the strong physical forces (e.g. tidal currents, wind induced waves), and do microbial activities and community structure generally follow the same depth-related patterns? 2) Are the strong seasonal dynamics in temperature and productivity reflected in the same manner in microbial activities and community structure? 3) Which are the environmental parameters that best explain depth-related patterns and temporal changes in microbial community structure?

4.2 Materials and methods

4.2.1 Study site and sample collection

The study was conducted on a shallow subtidal sand flat in the Sylt-Rømø Basin in the North Frisian Wadden Sea close to the German-Danish border. The so-called "*Hausstrand*" field site is located directly south of the harbor of List/Sylt (55° 00´47.7´´N, 008° 25´59.3´´E) (Fig. 1).

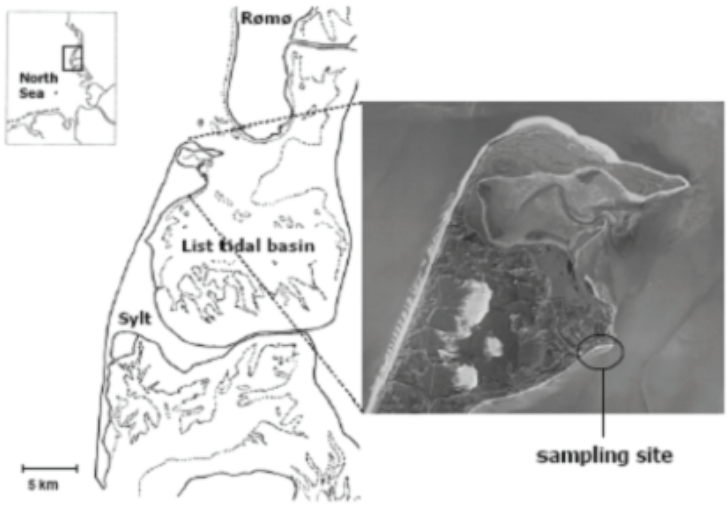

Fig. 1: Location of the sampling site in the List tidal basin close to the island of Sylt in the North Frisian Wadden Sea, Germany (picture obtained from the Wadden Sea National Park Office Schleswig-Holstein).

The tidal amplitude at the site is ~2 m with a water depth ranging from 0.5-2.5 m, depending on the tidal phase. Sediments consist of well to moderately well sorted sands with a medium particle size of 350 µm. Push cores (ten cores per sampling time; 3.6 cm inner diameter, 15-20 cm length) were collected on August 5 2004 (August 2004), October 16 2004 (October 2004), February 8 2005 (February 2005), April 8 2005 (April 2005), July 2 2005 (July 2005), November 11 2005 (November 2005), March 1 2006 (March I 2006) and March 27 2006 (March II 2006) during low tide. These sampling dates covered water temperatures between 1.5°C and 20.4°C (Fig. 2A). Additionally, four bigger cores ("chambers", 8.4 cm inner diameter, 30 cm length) were collected to determine benthic oxygen consumption rates. On April 8 2005 and November 11 2005, sampling was performed by scuba-diving, due to stormy weather and high water level. No "chambers" could be recovered on November 11 2005.

Immediately after collection, sediment cores were sectioned in 1-cm intervals down to 15 cm depth, porewater was extracted, and sediment slices of the same depth were pooled and homogenized with a sterile spatula to obtain sufficient volumes of sediment subsequent measurements. Sub-samples for the measurement of photopigments and carbohydrates were frozen immediately and stored at -20°C. The rest of the homogenized sediment was pooled in 5-cm intervals (0-5, 5-10, and 10-15 cm) and was subjected to automated ribosomal intergenic spacer analysis (ARISA), bacterial cell counts, measurements of extracellular enzymatic activities (EEA), and bacterial carbon production (BCP). Sub-samples for ARISA were immediately frozen in sterile plastic containers and stored at -20°C until further analysis. Sub-samples for total cell number estimations were fixed in 2% formaldehyde in seawater. Samples for the measurement of EEA and BCP were instantly processed. "Chambers" were covered with water from the field site, vented with a bubbling stone and pre-incubated overnight at near *in situ* temperature.

Except of the ARISA fingerprinting data and the analysis of interstitial porewaters, all contextual environmental variables obtained between 2005 and 2006 are presented in Böer et al. (2008). Additional contextual data are shown here for the 2 sampling dates in 2004. Water column data consisting of pelagic chlorophyll a, pH and water temperature was obtained from the Sylt time series (Dr. Justus van Beusekom, long-term ecological time series of the Alfred Wegener Institute for Polar and Marine Research in the North Sea, Germany). Data on wind speed was obtained from the Deutsche Wetterdienst (German Weather Bureau) and derived from the Weather station in List on Sylt.

4.2.2 Biogeochemical analyses

For nutrient analyses, pore water was blown out with nitrogen as described by Billerbeck et al. (2006). All pore water samples were passed through 0.45 µm nylon syringe filters and stored at 4°C until dissolved silicate and salinity analyses were performed, or kept at -20°C until ammonium, phosphate, nitrate and nitrite analyses were performed. Concentrations of dissolved silicate were photometrically measured according to Grasshoff et al. (1983). Ammonium, nitrate, nitrite and phosphate were spectrophotometrically measured with a continuous-flow analyzer (Bran & Lübbe GmbH, Norderstedt, Germany) using a variant of the method of Grasshoff et al. (1983). Pore water salinity was measured with a hand refractometer (Reichert Scientific Instruments Co., Depew, NY, USA).

Chlorophyll *a* and phaeophytine concentrations were measured photometrically in lyophilized samples and calculated as described by Lorenzen (1967). Total carbohydrates and EDTA extractable carbohydrates were spectrophotometrically quantified based on the phenol-sulfuric acid assay (Dubois et al., 1956) following the procedure of Underwood et al. (1995). A detailed description of the procedures is given in Böer et al. (2008).

4.2.3 Bacterial cell counts and activities, benthic oxygen consumption

Bacterial abundances were estimated by epifluorescence microscopy (x1000) after staining with Acridine Orange (AO) using a modification of the method of Hobbie et al. (1977).

Bacterial carbon production was estimated by measuring the rate of [Methyl-^{3}H] thymidine (TdR) incorporation into DNA (Fuhrman and Azam 1982, Moriarty and Pollard 1990), following the extraction procedures of Findley et al. (1984) and Michel & Bloem (1993) with some modifications. Rates were calculated using a conversion factor of $2x10^6$ cells produced per pmol thymidine incorporated (Findley 1993), and a carbon conversion factor of 20 fg C cell^{-1} (Fuhrman and Azam 1982).

Potential extracellular enzymatic activities (α-glucosidase, β-glucosidase, chitobiase, lipase and phosphatase) were measured fluorometrically using 4-methylumbelliferone (MUF) labeled substrate proxies (Hoppe 1983, Hoppe et al. 1988, Boetius & Lochte 1994).

Oxygen concentrations in samples withdrawn from incubated intact chambers over a 5-point timecourse were determined by duplicate Winkler titrations (Winkler 1888, Grasshoff et al. 1983) according to the procedure of Strickland & Parsons (1972). Gross benthic oxygen

consumption rates were calculated from the averages of oxygen decrease over time according to Thorbergsdóttir et al. (2004).

A detailed description of all procedures is given in Böer et al. (2008).

4.2.4 Community structure analysis by Automated rRNA intergenic spacer analysis (ARISA)

DNA was extracted from 1 g of homogenized sediment sample using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions with the following modifications: bead beating was performed twice in a FastPrep Instrument (Q·BIO gene, Morgan Irvine, CA, USA) for 45 s at maximum speed.

Changes in bacterial community structure were estimated by ARISA (Fisher and Triplett 1999). PCR reactions (50 µl) were conducted in triplicate and contained 1×PCR buffer (Promega, Madison, WI, USA), 2.5 mM $MgCl_2$ (Promega), 0.25 mM of dNTP mix (Promega), bovine serum albumine (3 µg μl^{-1}, final concentration), 25 ng extracted DNA, 400 nM each of universal primer ITSF (5′-GTCGTAACAAGGTAGCCGTA-3′) and eubacterial ITSReub (5′-GCCAAGGCATCCACC-3′; Cardinale et al., 2004) labeled with the phosphoramidite dye HEX, and 0.05 units GoTaq polymerase (Promega). PCR was carried out in an Eppendorf MasterCycler (Eppendorf, Hamburg, Germany) with an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 90 s, and a final extension at 72°C for 5 min. The PCR products were purified utilizing Sephadex G-50 Superfine (Sigma Aldrich) and a standardized amount of DNA (150 ng DNA) was added to 0.5 µl of internal size standard Map Marker®1000 ROX (50-1000 bp) (BioVentures Inc., Washington, DC, USA), 0.5 µl of tracking dye (BioVentures) and 14 µl of deionized Hi-Di –formamide (Applied Biosystems, Foster City, CA, USA). Discrimination of the PCR-amplified fragments via capillary electrophoresis was carried out on an ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems) and the ARISA profiles were analyzed using the GeneMapper Software v 3.7 (Applied Biosystems). To account for run-to-run variations in signal intensity, the total peak area per sample was normalized to one (Yannarell & Triplett, 2005). To include the maximum number of peaks while excluding background fluorescence, only fragments above a threshold of 50 fluorescence units and between 100-1000 bp length were taken into consideration. The GeneMapper output file was reformatted using custom

Perl scripts and further analyzed by custom R (version 2.4.0; The R foundation for Statistical Computing) scripts. A "fixed window" binning strategy with a bin size of 2 bp was applied to the ARISA generated data to account for size calling imprecision according to Hewson & Fuhrman (2005). The binning frame that offered the highest pairwise similarities among samples was then further subjected to multivariate analyses.

4.2.5 Statistical analysis

To improve ARISA profile reliability, replicate profiles obtained from a given sample were combined in such a way that only OTUs occurring in at least 2 out of 2-3 replicates were kept in a consensus profile. The consensus ARISA table (samples by OTUs) was then used to calculate pairwise similarities among samples based on the Bray-Curtis similarity index (Bray-Curtis 1957). The resulting matrix was used to examine seasonal and spatial (depth) patterns in bacterial community composition via non-metric multidimensional scaling (NMDS) as implemented in the R package. NMDS places the samples in a two-dimensional coordinate system so that the ranked dissimilarities between the samples are preserved. A stress function assesses the goodness-of-fit of the ordination compared to the original sample ranking (Ramette 2007). Analysis of similarity (ANOSIM) was performed to test whether differences between various *a posteriori* groupings of the samples in the NMDS ordinations were statistically significant. The resulting test statistic *R* indicates the degree of separation, with a score of 1 standing for complete separation, a score of 0 representing no separation and negative values signalizing a higher degree of similarity between groups than within groups (Clarke 1993).

Correlations between all environmental and biological parameters were calculated by using the Pearson's correlation coefficient for the complete dataset (n=24). P values were corrected for multiple testing using Bonferroni correction (Ramette 2007). To incorporate the main patterns of diversity variation in the correlation analyses, the ARISA table was subjected to principal component analysis (PCA) to extract uncorrelated axes of variation. The first four axes of PCA explained approximately 80% of the overall variation and were included in the pairwise comparisons. Sample scores on the first four PCA axes were normalized to zero mean and a standard deviation of 1 prior to computation of the Pearson's correlation coefficients.

The relationship between environmental variables and patterns in bacterial community structure was studied in more detail by multivariate statistics using the software packages CANOCO for Windows 4.5 (terBraak & Smilauer 1998) and R. In CANOCO, detrended correspondence analysis (DCA, Hill & Gauch 1980) was performed to determine whether linear or unimodal species models better fitted the current ARISA dataset (Ramette 2007). Canonical redundancy analysis (RDA) was used to further investigate the significance of environmental parameters in explaining the variation in bacterial community structure. In RDA models, sampling dates were set as nominal variables and most of the data (except depth, pH, water temperature, wind speed and salinity) were $\log_{10}$-transformed prior to the analysis in order to normalize their distribution. Significant variables for the analysis were pre-selected from groups of variables (sampling time, depth, nutrients, photopigments, chemical parameters, physical parameters, water column data, enzymatic activities, bacterial abundances and growth) by forward model selection. The significance of the RDA models and of the selected variables were tested by Monte Carlo permutation tests (999 permutations) at $P<0.05$ for each group.

The respective effects of variables or groups of variables on the variation in bacterial community composition were further investigated by canonical variation partitioning (Legendre & Legendre 1998, Ramette & Tiedje 2007). Using the software R, the variation and co-variation of significant explanatory variables were partitioned into the effects of each factor alone (i.e. when removing the effects of all other factors in the model, which is also called "partialling out") and of their covariation (Borcard et al. 1992). Statistical significances of the respective fractions were calculated by 999 Monte Carlo permutations under the full multivariate model.

4.3 Results

4.3.1 Seasonal variation in the water column and benthos at *Hausstrand*

The daily average water temperature showed clear seasonal patterns with the highest values in summer (21°C) and lowest values at the end of winter (-2°C), spanning a temperature range of more than 20°C (Fig. 2A). Daily average wind speed ranged from 1.9 m s^{-1} to 16.5 m s^{-1} and was generally higher in winter compared to summer (data not shown). The spring blooms followed the first temperature increases in March. Daily average chlorophyll *a* concentrations

in the water column reached 22 µg L^{-1} during the diatom spring bloom of March 2005, followed by a *Phaeocystis* bloom in May/June with concentrations of up to 21 µg L^{-1}; a succession that is generally observed for the List tidal basin. In March and April 2006, a maximum of 33 µg L^{-1} was observed during the diatom spring bloom, while chlorophyll *a* concentrations during the rest of the year were on average 4.2 µg L^{-1}. No distinct autumn blooms were observed during the period of observation and no uncommon weather events occurred.

Fig. 2 summarizes the main temporal trends in the investigated environmental parameters. The variation in total integrated benthic chlorophyll *a* (Fig 2B) was much less than that of pelagic chlorophyll *a*. Maximum values were reached during times of the plankton spring bloom with 6.8 g L^{-1} in April 2005 and 7.5 g L^{-1} in March II 2006. Lowest chlorophyll *a* concentrations were detected in August 2004 and March I 2006 (4.5 g L^{-1}). In contrast, benthic total carbohydrate inventory did not show a seasonal variation but seemed to decline between August 2004 and March 2006 from a maximum of 1180 g gluc. equiv. L^{-1} to ~530 g gluc. equiv. L^{-1}. Total community respiration was most closely related to the seasonal temperature variations and varied between a high of 29 in August 2004 and 21 in July 2005, to a low of ~5.5 in February 2005 and March I 2006.

Interstitial nitrate concentrations were highest just before and during the pelagic spring bloom with values of 43-57 µM on average in the top 0-5 cm. Nitrate was depleted during the summer and autumn months. Interstitial phosphate concentrations were quite variable but showed higher concentrations in summer time and lows in winter and during the spring blooms.

Temporal variations in extracellular enzymatic activities and bacterial carbon production were strong and mostly related to temperature (Fig. 2C-E; Table A2). Bacterial secondary production reached a maximum in August 2004 and July 2005 with values of 27-28 mg C L^{-1} d^{-1} in the top 5 cm and a low of 2 mg C L^{-1} d^{-1} in October 2004, while in all other months rates were ~ 5-7 mg C L^{-1} d^{-1} (0-5 cm). The hydrolytic activities of the extracellular enzymes acting on carbohydrates (α-, β-glucosidase (Fig. 2C), chitobiase, laminarinase) also varied 3-6-fold between summer and winter months (0-5 cm). Only a very low temporal variation was detected in total bacterial abundance (Fig. 2C). This temporal variation caused by temperature change was visible not only in the top 0-5 cm layer, but also down to 10-15 cm sediment depth in the extracellular hydrolytic activities as well as in the bacterial production.

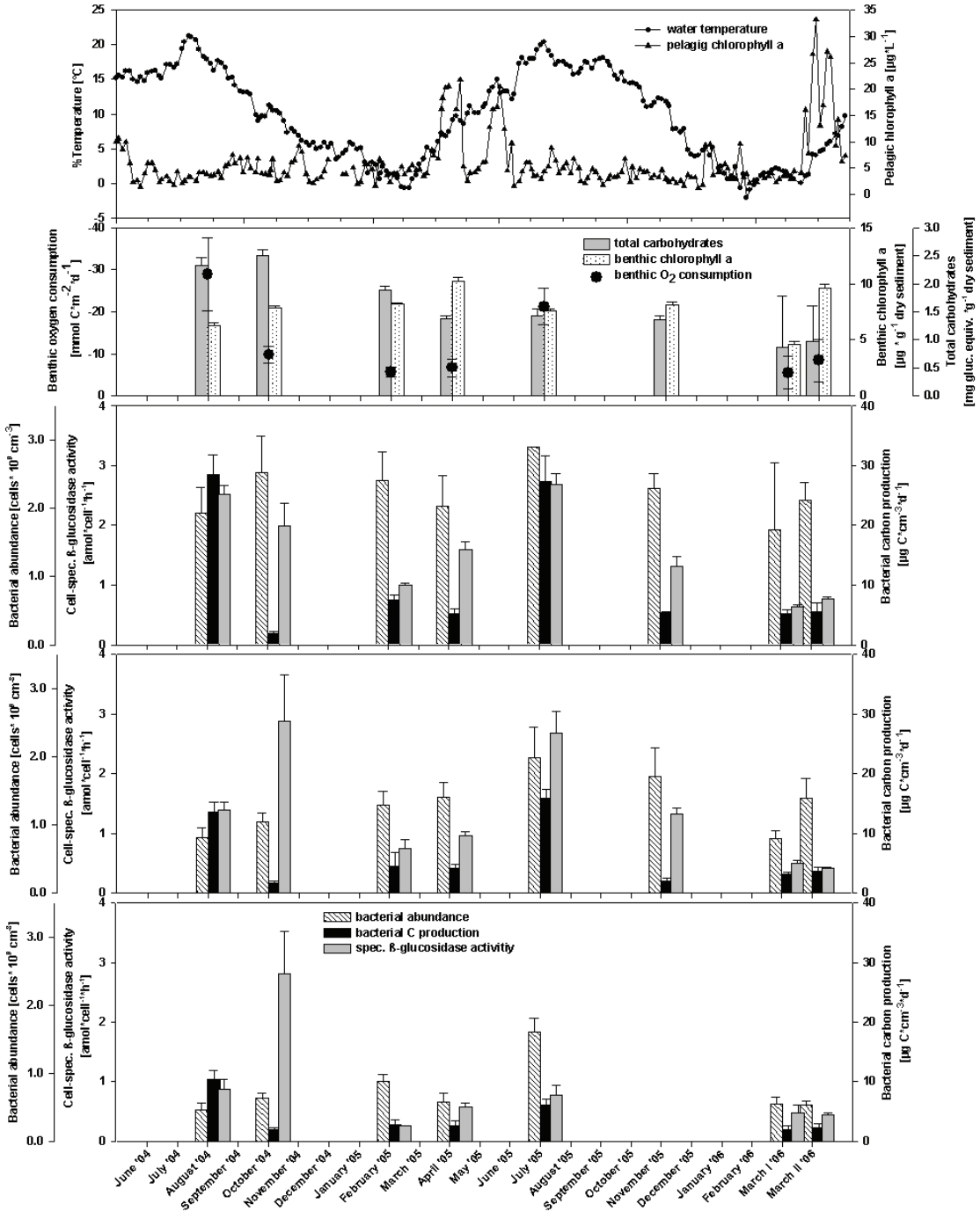

Fig. 2: Seasonal patterns of major environmental variables at the sampling site. Error bars represent standard deviations. **A**) Pelagic chlorophyll a concentrations (triangles) and water temperature (circles). **B**) Mean benthic chlorophyll a concentrations integrated over the upper 5 cm of sediment (dotted bars) and benthic oxygen consumption rates (circles, n = 4). Bacterial abundances (black bars, n = 6), bacterial carbon production rates as measured via thymidine incorporation (striped bars, n = 4-5) and cell- specific α-glucosidase activities (grey bars, n = 5) for sediment horizons 0-5 cm (**C**), 5-10 cm (**D**) and 10-15 cm (**E**). Fig. 2A and B were adapted from Böer et al. (2008).

4.3.2 Sediment-depth related variation in environmental parameters

Depth-related patterns of pore water nutrient concentrations reflected mixing and oxygenation of the sediment (Table A1). Nitrate concentrations were highest in the 0-5 cm sediment depth layer and decreased rapidly below (Fig. 3A). Phosphate (Fig. 3B) and nitrite followed comparable depth-patterns with highest concentrations of 13.7 µM and 4.5 µM in the upper -3 to -7 cm of sediment. Ammonium concentrations reached a peak of 38-62 µM on average at -6 cm sediment without any apparent seasonal trend.

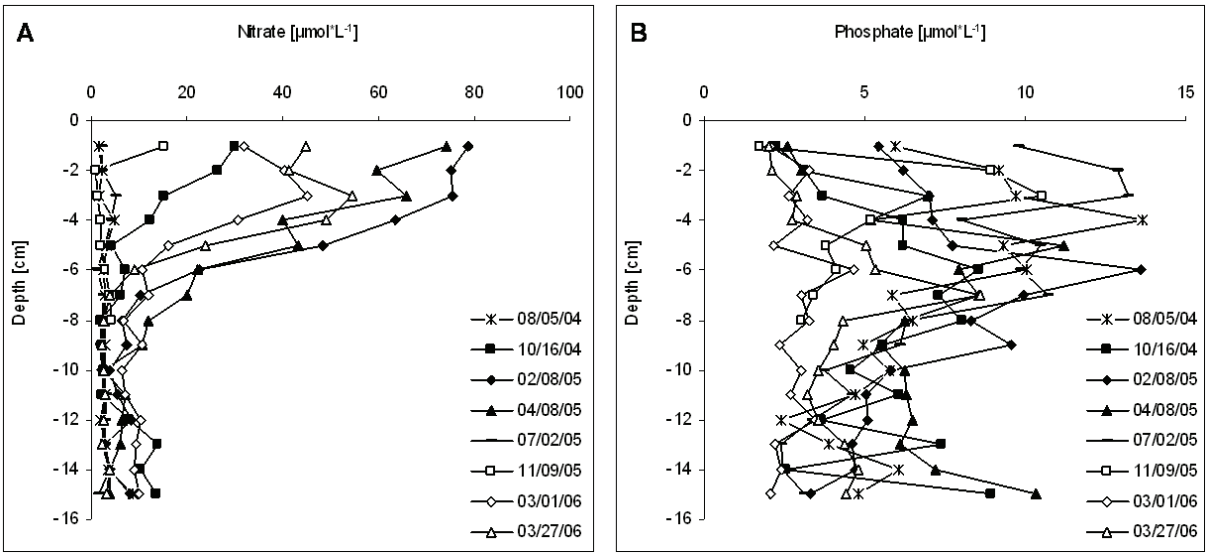

Fig. 3: Seasonal and vertical variation in interstitial nitrate and phosphate concentrations at *Hausstrand.*

Briefly, the sediment showed persistent vertical gradients in bacterial abundances, bacterial carbon production and extracellular enzymatic activities at all times (Fig. 2C-E; Table A2). On average, values decreased 1.4-1.8 fold from the 0-5 cm layer to the 5-10 cm layer, and by 2.3-3.8 fold compared to the 10-15 cm layer (except for lipase activity that did not decrease). In some cases, especially for lipase and chitobiase, highest enzymatic activities were recorded in the 5-10 cm layer.

4.3.3 Variation in bacterial richness and composition

A total of 451 operational taxonomic units (OTUs; here number of binned ARISA peaks) were detected for the entire range of sampling months and depths. Highest OTU richness was

obtained in August 2004 with 302 OTUs, whereof 65 were unique to this month. Lowest OTU richness occurred in fall (195 and 178 OTUs for October 2004 and November 2005, respectively; Fig. 4). All other months showed no or only a small number of unique OTUs (i.e. 3-8). Ninety-eight OTUs (~22 % of all OTUs) were discovered at all times. Thereof, 21 OTUs (~5 % of all OTUs) were common to the entire depth range. Pair-wise comparison of presence/absence of OTUs between sampling months revealed between 53% to 95% shared OTUs between two sampling dates (Table A3). Thereby, an average of 57% of OTUs discovered in August 2004 could be recovered in any of the other sampling months and an average of 80% of OTUs when any other sampling dates were compared. Strongest bacterial community dynamics were observed between August 2004, October 2004 and February 2005 with a loss of 47% between August and October 2004, and a loss of 38% between October 2004 and February 2005. All other pairs of sampling dates had at least 76% of OTUs in common (Fig. 4).

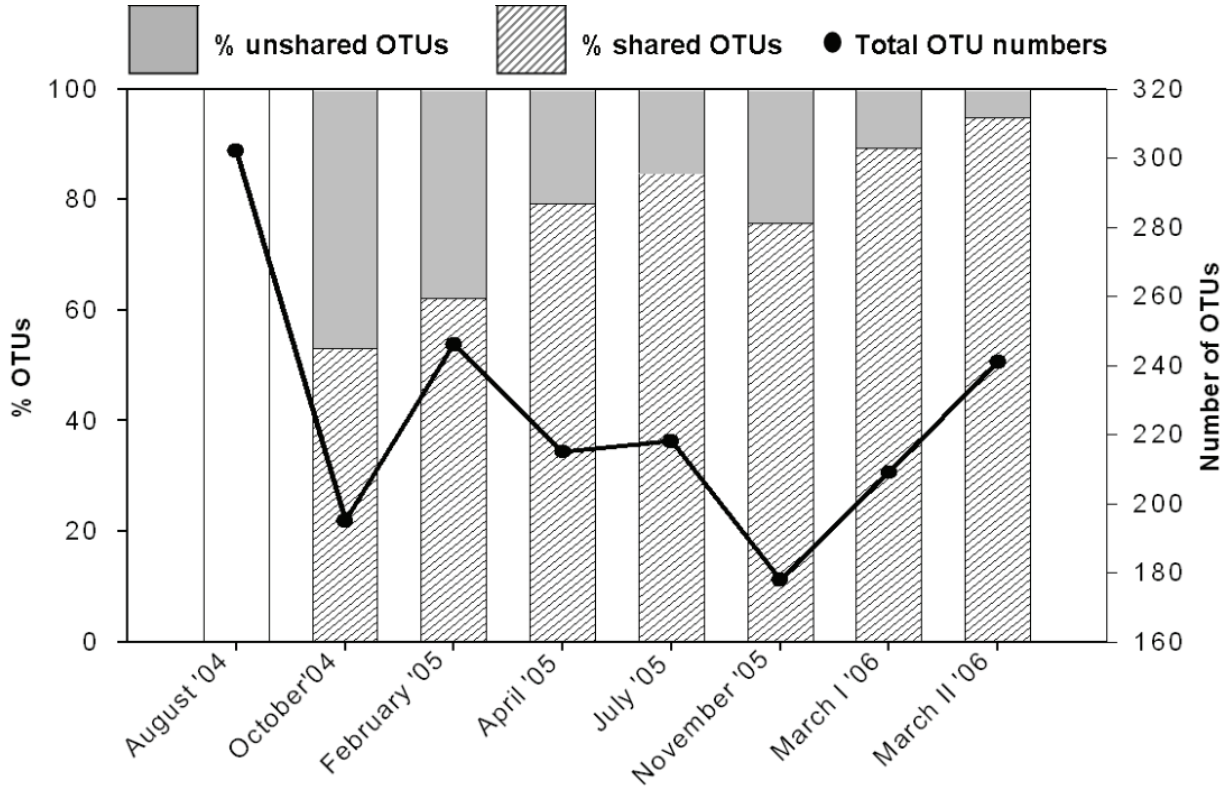

Fig. 4: OTU turnover over successive sampling times. The solid line represents the total number of OTUs obtained from ARISA profiles from all three sediment horizons combined. Bars show which percentages of OTUs in any of the investigated months are shared (striped) or not shared (grey) with the corresponding previous sampling date.

From the "pool" of 451 OTUs that were detected over the 2-year sampling period, each different time point comprised between 38-68% in the top 15 cm of sediment. Almost 37% of the entire OTU pool, was exclusively detected within the first three sampling months, while community structure in general was temporally more stable between April 2005 and March II 2006. Half of the 284 OTUs that were detected between April 2005 and March II 2006 were found throughout these 5 sampling dates.

OTU richness varied over sampling depth, with the highest number of ARISA bands in the 10-15 cm sediment layer (178 ± 28.7) compared to the 0-5 cm (145 ± 45.7) or the 5-10 cm sediment layer (144 ± 28.8; Fig.5). The difference in OTU richness between the 0-5 cm layer and the 10-15 cm layer was marginally significant ($p = 0.05$; *Student's t test*). If each month was regarded separately, an average of 40% of OTUs present in a respective month were common to the entire depth range, ~13% were unique to the 0-5 cm layer, ~3% were unique to the 5-10 cm layer and ~20% were unique to the 10-15 cm layer, whereas ~7% and ~12% overlapped between the medium and upper layer and between the medium and deepest layer, respectively. If all seasons were considered, twenty-two OTUs (~5% of all OTUs) remained to be exclusively found in the 0-5 cm layer, 8 OTUs (~2% of all OTUs) were unique to the 5-10 cm layer and 29 OTUs (~6% of all OTUs) were unique to the 10-15 cm layer. All other OTUs showed partial overlap between layers at two or more of the sampling dates.

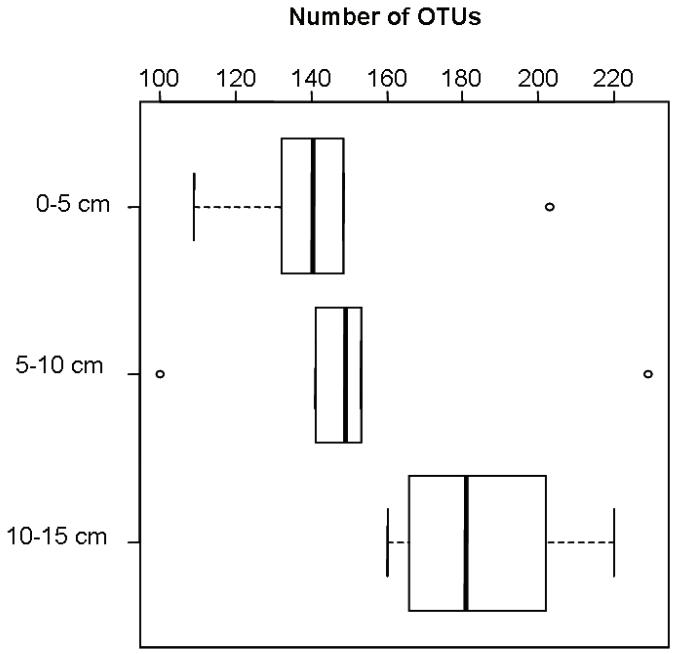

Fig. 5: Boxplot of OTU richness (number of ARISA peaks) vs. sampling depth integrating all time points.

4.3.4 Main correlations between environmental parameters and bacterial diversity

When all samples were considered (n=24), the environmental variables consisted of sampling dates, sampling depth, average wind speed on the day before sampling, benthic chlorophyll a, benthic chlorophyll a: phaeophytine ratio, sedimentary SiO_2, PO_4, NO_2, NO_3 and NH_4, salinity, water pH and temperature, bacterial cell abundance, bacterial carbon production as well as all enzymatic activities. The results of the correlation analyses are shown in detail in Table A4 Part I and II. Briefly, amongst the environmental parameters, NH_4, bacterial abundance, benthic chlorophyll *a* and the chlorophyll *a*: phaeophytine ratio exhibited a negative relationship with sediment depth ($r = 0.71$, $r = -0.85$, $r = -0.97$ and $r = -0.75$, respectively) while the latter three were positively linked to each other ($r \geq 0.71$). Sampling time was negatively correlated with pH, salinity and PO_4 ($r = -0.77$, $r = -0.82$ and $r = -0.85$, respectively). Bacterial carbon production was significantly correlated with phosphatase and chitobiase activity ($r = 0.78$ and 0.74, respectively). The model was recalculated for samples from which total carbohydrates and EDTA-extractable carbohydrates were measured (n = 20). There were no significant correlations between EDTA extractable carbohydrates and total carbohydrates with any of the other variables, following Bonferroni corrections of the significance level. At a significance level of $P < 0.01$, EDTA-extractable carbohydrates and total carbohydrates showed strong correlations with sediment depth and the bacterial community data. In addition, EDTA extractable carbohydrates were correlated with chlorophyll *a* and sedimentary NO_3, while total carbohydrates were correlated with bacterial abundance (data not shown).

We used Principal Component Analysis (PCA) to extract the main patterns of variation in bacterial community composition. The four major PCA axes explained 38, 29, 8 and 4% of the total variation in ARISA data, respectively, and were all included in the correlation analyses (Fig. 6; Table A4 Part I and II).

Variation in bacterial community structure was significantly correlated with sampling time ($r = 0.76$), bacterial abundance ($r = 0.75$), benthic chlorophyll *a* ($r = 0.78$), chitobiase activity ($r = 0.74$), α-glucosidase activity ($r = 0.77$) and phosphatase activity ($r = 0.74$).

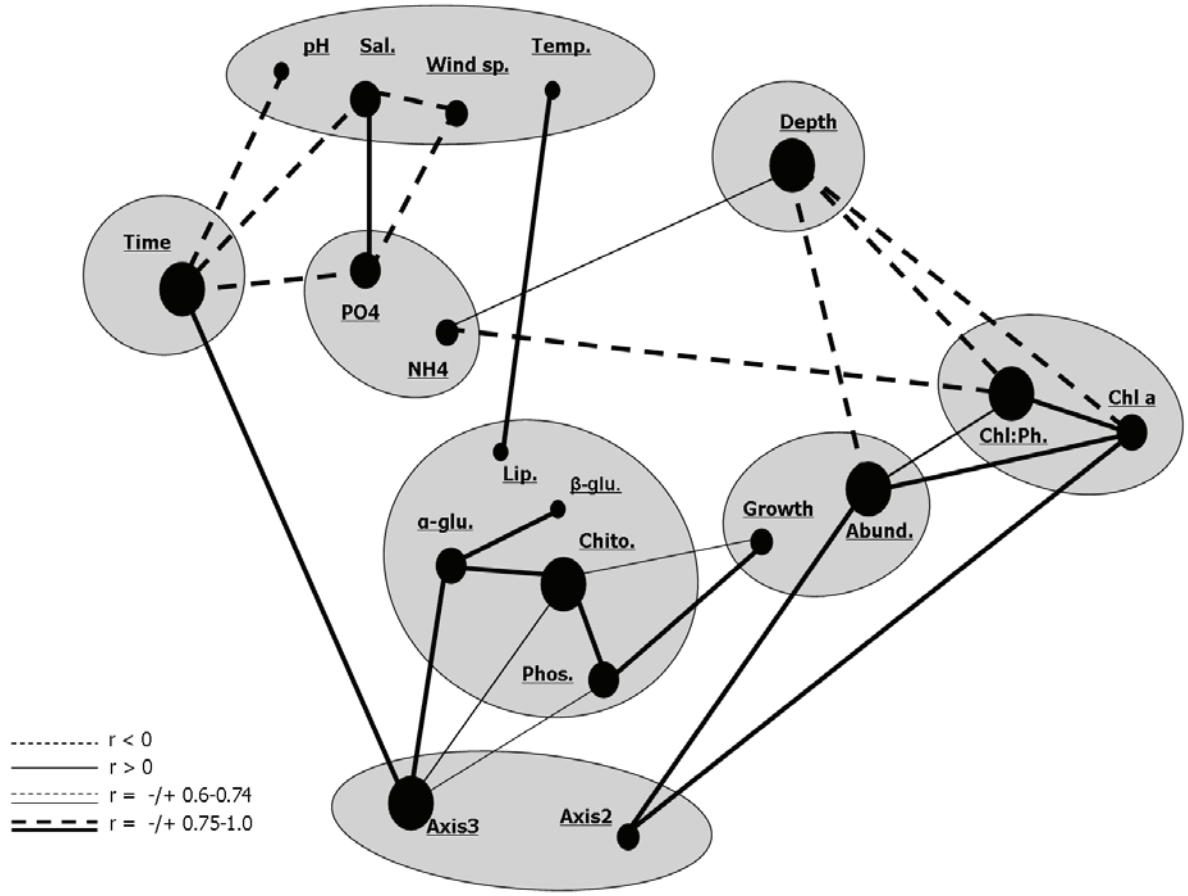

Fig. 6: Correlations between temporal, spatial, environmental and microbial variables measured at all eight sampling dates between August 2004 and March 2006. Only significant Pearson correlations at the Bonferroni- corrected level $P \leq 0.0001$ (0.05/ 465) are depicted. Thin and large connecting lines represent significant correlations with coefficients <0.75 and ≥0.75, respectively, while continuous and dashed lines represent positive and negative coefficients, respectively. The size of the dots is proportional to the number of significant correlations a respective variable has with other parameters. The variables include: Time (all sampling months), wind speed (Wind sp), salinity (Sal), temperature (Temp), lipase (Lip), β-glucosidase (β-glu), phosphatase (Phos), chitobiase (Chito), α-glucosidase (α-glu; all enzymatic activities refer to cell-specific enzymatic activities), bacterial abundance (Abund), bacterial carbon production (Growth), benthic chlorophyll *a* (Chl a), chlorophyll *a*:phaeophytine ratio in the sediment (Chl:Ph), and two of the three major axes of the principal component analysis that explain variation in the bacterial community structure (Axis 2 and 3). Variables that were not significantly linked to any other variables were removed from the plot.

To only retain significant environmental variables for further analyses of their ecological relevance in explaining changes in bacterial community structure, the variables were grouped into categories. Within each category, only the variables that significantly explained variation in ARISA data were kept using a forward selection process. Those categories corresponded to sampling date, depth, chlorophyll *a*, NO_3, wind speed, and bacterial abundance (Table 1). A redundancy analysis (RDA) applied to those datasets yielded

two main axes that explained 36% and 27%, respectively, of the total variance in bacterial community data (Fig. 7), with the selected environmental variables accounting for 83% of the total variation in bacterial community structure.

The strongest relationship with changes in bacterial community structure was explained by time and wind speed on the first RDA axis. Axis 2 was mainly associated to depth-related patterns in organic carbon availability and sediment chemistry as reflected by moderate correlations with benthic chlorophyll *a*, bacterial abundance, and NO_3 (Fig. 7).

Table 1: Conditional effects of forwardly selected environmental parameters as determined by RDA. Horizontal lines mark groupings for forward selection.

Environmental Variable	Lambda A	P-value	F-factor
August	0.17	0.005*	4.35
October	0.16	0.002*	5.25
February	0.13	0.001*	4.90
April	0.03	0.320	1.10
November	0.03	0.350	1.00
July	0.01	0.876	0.44
March I	0.01	0.976	0.28
Depth	0.23	0.001*	6.60
NO_3	0.14	0.007*	3.56
SiO_2	0.07	0.107	1.90
NH_4	0.07	0.076	2.07
PO_4	0.05	0.234	1.38
NO_2	0.02	0.889	0.39
Chlorophyll a	0.26	0.001*	7.63
Chl a/phaeophytine ratio	0.02	0.735	0.56
Wind speed	0.1	0.032*	2.57
pH	0.07	0.135	1.66
Salinity	0.08	0.088	2.02
Temperature	0.04	0.282	1.23
β-glucosidase	0.09	0.064	2.28
Lipase	0.06	0.140	1.71
Phosphatase	0.06	0.116	1.77
α-glucosidase	0.1	0.064	2.32
Chitobiase	0.04	0.363	1.04
Bacterial abundance	0.21	0.001*	5.9
Bacterial carbon production	0.05	0.216	1.28

* represents environmental variables that significantly explain variation in bacterial community structure

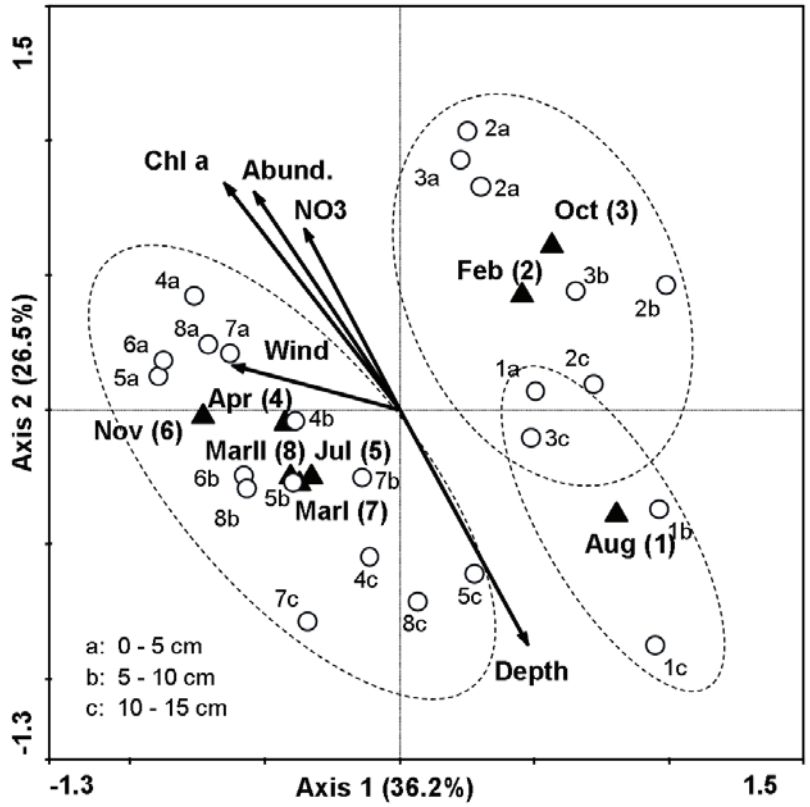

Fig. 7: RDA biplot of bacterial diversity and contextual parameters. Open circles represent consensus ARISA profiles for all 8 sampling dates and the three sediment horizons analyzed (n = 24). Numbers next to the circles indicate the sampling dates August 04 (1), October 04 (2), February 05 (3), April 05 (4), July 05 (5), November 05 (6), March I 06 (7), and March II 06 (8). Letters associated with the numbers correspond to sampling depth 0–5 cm (a), 5–10 cm (b), and 10–15 cm (c). Sampling months were set as nominal variables in the analyses and are represented as filled triangles. Environmental variables that significantly explained variability in microbial community structure in the forward selection are shown as vectors.

Noticeably, the grouping of samples as a function of depth (0-5 cm, 5-10 cm, 10-15 cm) observed in the RDA biplot was confirmed by *a posteriori* grouping in NMDS analysis (Fig. 8A) and further non-parametric tests that indicated that the separations were indeed significant (ANOSIM R = 0.373, $P < 0.001$). The RDA biplot indicated that samples in the 0-5 cm layers were associated with high chlorophyll *a* and NO_3 concentrations as well as with high cell abundances in contrast to samples from deeper layers for which decreasing values of these environmental parameters were predicted.

Grouping of samples in the RDA plot also indicated the existence of three groups according to sampling dates (Fig. 7). Group 1 contained all samples deriving from August 2004, group 2 contained all samples deriving from October 2004 and February 2005 and group 3 comprised the samples from all remaining sampling dates. The significance of the groupings was independently confirmed by NMDS analysis combined with ANOSIM

significance testing (Fig. 8B). The groups were highly separated ($r = 0.78$, $p < 0.001$), however sampling months within group 2 and 3 turned out to be quite similar (r-values equal or close to zero).

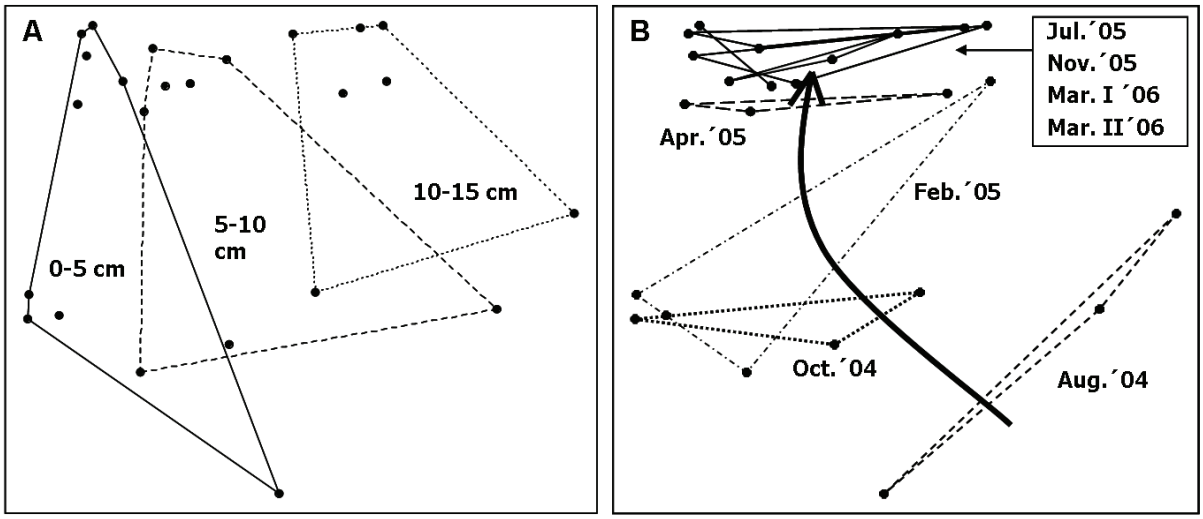

Fig. 8: Non-metric multi-dimensional scaling (NMDS) ordination. Each dot represents the consensus of 2-3 replicate ARISA profiles for the 24 samples with *a posteriori* grouping according to A) sampling depth and B) sampling date. In A) the sediment horizons are represented by a solid line (0 – 5 cm), dashed line (5 – 10 cm) and a dotted line (10 – 15 cm), respectively; in B) the sampling dates are represented by dashed line (August 2004), dotted line (October 2004), dotted-dashed line (February 2005), long-dashed line (April 2005), solid lines (all remaining sampling months from July 2005 until March II 2006). The error indicates the trend in temporal succession. The stress value for the 2-dimensional ordination was 0.085 according to Kruskal's stress formula.

Overall, there was no apparent cyclic seasonal pattern in the succession of bacterial community structure (i.e. summer samples resembling each other, winter samples resembling each other etc.) but rather a unidirectional succession from a more dissimilar bacterial assemblage in 2004 and early 2005 to a more similar bacterial community in 2005 and 2006.

A second RDA was performed on a subset of samples (n=20; four samples from the deepest horizon lacked measures of total carbohydrates and EDTA-extractable carbohydrates). Following a stepwise model selection, wind speed was not significant and was excluded from the model. Instead, total and EDTA-extractable carbohydrates were included as explanatory variables. The two main RDA axes explained 42% and 26% of the total variance in bacterial community data, respectively. All first four axes explained up to 81% with selected environmental variables in this model accounting for 92% of the total variation in bacterial community structure. The overall ordination of samples and

environmental variables were mostly maintained in the new analysis with RDA axis 1 mainly correlating with sampling date and total carbohydrates and axis 2 mainly explained by sediment depth, chlorophyll *a*, NO_3, EDTA extractable carbohydrates and bacterial abundance (data not shown).

In order to disentangle the respective effects of each factor on the variation of community patterns, a variation partitioning analysis was performed (Fig. 9). The model based on the complete dataset (n = 24) included the following sets of explanatory variables: time (all seasons), sediment depth, sediment chemistry (chlorophyll *a*, NO_3) and bacterial parameters (bacterial abundance). The amount of variation explained by all pure and covarying factors was 67%. The pure fractions (i.e. the fraction explained by a factor when removing the effects of all other factors or variables in the model) contributed 34% (time, $P < 0.001$), 1% (depth, non-significant), 6% (sediment chemistry, $P = 0.032$) and 2% (bacterial abundance, non-significant) of the variation in bacterial community structure. The combination of sediment depth, bacterial abundance and sediment chemistry had the largest covarying effect and accounted for 27% of the variation in the community data.

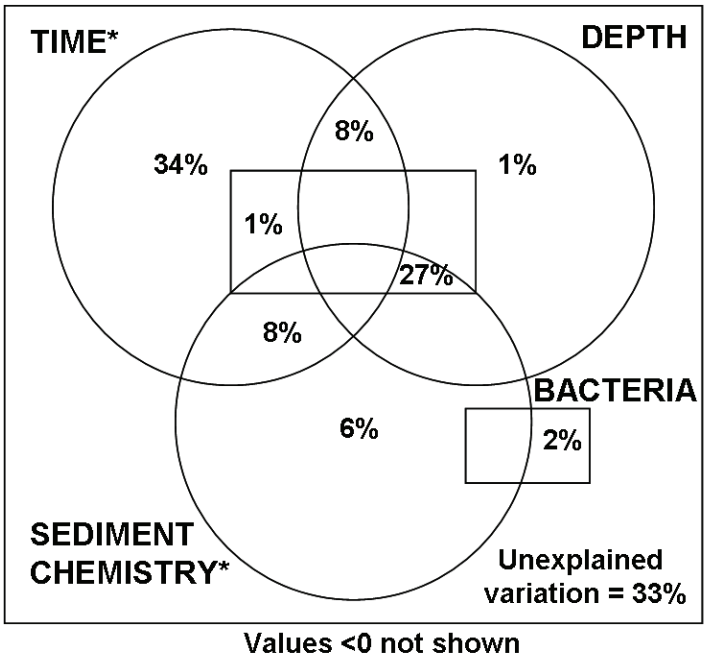

Fig. 9: Partitioning of the microbial variation into the relative effects of significant contextual parameters alone or in combination. A statistically significant contribution of pure fractions to variation is indicated by * (P <0.001 and <0.032 for time and sediment chemistry, respectively), as determined by 999 Monte Carlo permutations under the full multivariate model.

4.4 Discussion

In this investigation we have analyzed whether spatial and temporal variations in the physicochemical condition of the environment affect bacterial diversity and community structure. A widely accepted hypothesis on the biodiversity of microorganisms is that these are everywhere, but under a strong selection by the environment (Baas-Becking 1934). However, recently, with the increasing use of high resolution-high throughput molecular fingerprinting methods, evidence has been collected that microbial communities display strong temporal and spatial structures (Martiny et al. 2006, and literature therein). In the following, we discuss whether and how the strong spatial and temporal variations in environmental parameters typical for temperate sandy coasts control bacterial diversity and community structure.

Despite their constant exposure to strong hydrodynamic forces such as tidal currents and wind-induced wave surge, the sandy sediments of the *Hausstrand* site (List, Sylt) show strong vertical and temporal gradients in bacterial abundances, bacterial carbon production and extracellular enzymatic activities at all times (Böer et al. 2008). The vertical distribution of these parameters was found to be tightly coupled to that of the microphytobenthos, causing the strong vertical shift in both availability and nutritional quality of degradable organic matter in the sediment, reflected in the decrease in chlorophyll *a*, total and EDTA extractable carbohydrates with sediment depth (Böer et al. 2008). Another important factor in the spatial structuring of the sandy sediments is the advective transport of water through the sand pores by currents and wave surge. The nutrient data show that the zone of 0-5 cm depth is flushed with bottom water and hence at least temporarily oxygenated, in contrast to the 5-10 and 10-15 cm depth layers, which represent relatively stable anoxic environments (Fig. 3).

The analysis of temporal change in extracellular hydrolytic enzyme activities, bacterial carbon production and benthic community respiration showed strong (factor of 4-5) variations with highest activities in summer and lowest in winter (Böer et al. 2008; Fig. 2). These variations were mostly related to seasonal temperature change, but also to changes in substrate availability. A close coupling between temperature and benthic oxygen consumption rates has been described earlier for North Sea sediments (Cramer 1990, van Duyl & Kop 1990). According to Heip et al. (1990) and Cramer (1991) meiofauna and macrofauna are only responsible for 1-15% of the total benthic respiration in North Sea sediments. We can therefore assume that the enhanced benthic oxygen consumption is the result of increased

remineralization rates of benthic bacteria at higher temperatures. Interestingly, the temporal patterns in activity were barely reflected in microphytobenthic or bacterial biomass. Other factors than temperature, for example substrate availability (Sander & Kalff 1993, van Duyl & Kop 1994), grazing (Epstein 1997) and viral lysis (Fuhrman 1999) may also add to the dynamics of the microbial community of sandy sediments.

In the following we have tested whether 1) bacterial species richness and community structure shows stable depth-related patterns that can be explained by patterns in contextual environmental parameters; 2) temporal changes in microbial activity and biomass can be linked to variations in bacterial species richness and community structure; 3) some environmental parameters can explain depth-related patterns and temporal changes in microbial community structure better than others.

4.4.1 Effects on OTU richness

It is generally assumed that the number of ecological niches and food resources is higher at the surface seafloor, as a result of the presence of meio- and macrofauna and the availability of a large variety of different organic resources (labile and refractory, of floral, faunal, microbial and terrestrial origin) and electron acceptors. However, in the *Haustrand* sands, OTU richness was higher in the deeper layer (10-15 cm) as compared with the mid (5-10 cm) and upper (0-5 cm) layers (Fig. 5). Hence, we can conclude that bacterial species richness shows spatial structuring on the scale of centimeters, as it has been observed for the contextual environmental parameters (Böer et al. 2008). An increased OTU richness with depth has been described earlier for coastal marine sediments of the western Pacific, the southern North Sea and the Namibian, but based on other molecular techniques than ARISA, e.g. by T-RFLP, quinone profiling, DGGE and16S rRNA gene sequencing (Urakawa et al. 2000. Franco et al. 2007, Julies et al., unpublished data). However, at *Hausstrand* and possibly also other coastal sites, bacterial species richness seems to be linked negatively to microphytobenthic biomass, and bacterial abundance and activity, at least for the three depth zones investigated.

The relationship between disturbance and species diversity, which has been shown for a variety of organisms (Connell 1978, McCabe & Gotelli 2000, Death & Winterbourn 1995, Townsend et al. 1997), may also serve as an explanation for the apparent link between sediment depth and bacterial OTUs at the *Hausstrand* site. However, to our knowledge, this

hypothesis has never been tested before on benthic microorganisms. Indeed, the strong hydrodynamic pressure at the field site leads to a constant vertical and horizontal mixing of the upper five sediment centimeters (Hedtkamp 2005), probably restricting this habitat for bacteria that are able to cope with occasional resuspension, physical abrasion generated by moving sediment particles, grazing, fluctuating concentrations of oxygen and of organic and inorganic nutrients (Table A1; Fig. 2; Fig. 3). The high sediment permeability observed at the field site ($1.0\text{-}3.4\times10^{-11}$ m^2; Hedtkamp 2005), suggests that advection, and bioturbation are the main transport processes in the upper 10 cm of sediment (Huettel et al. 2003b). In contrast, the deepest sediment layer may provide a relatively stable anoxic habitat dominated by diffusive solute transport. We thus hypothesize that the decreasing environmental variation and disturbance towards the deeper sediment layer is a main factor in the development of a more diverse bacterial community. As an alternative explanation, a few previous investigations have found that high nutrient availability lead to less bacterial diversity (Hewson et al. 2003). For instance, Torsvik et al. (1998) observed a much higher prokaryotic diversity in pristine aquatic sediments than in sediments below fish farms which receive a substantial input of nutrients via fish feed. These results match with findings by Abrams (1995) who stated that an increased productivity increases competitive exclusion and results in lower species diversity. Accordingly, the high availability of inorganic nutrients, and of labile organic matter produced by the microphytobenthic algae, in the upper sediment layers at our study site may have favored growth of presumably oxygen-tolerant, microaerophilic generalists. In the deepest sediment layer, lack of mixing may support a more heterogeneous microbial community of potentially slow-growing, anaerobic taxa with specific metabolic capabilities.

As to the effect of vertical space on dynamics in community composition, it is interesting to note that only about forty percent of all OTUs were found to inhabit the entire depth range. These OTUs probably represent bacterial taxa that are able to utilize a wide range of organic substrates and electron acceptors for active growth and may tolerate aerobic as well as anaerobic conditions. In contrast, on average per sampling time, 13%, 3% and 20% of OTUs were uniquely found in the upper, medium and deepest layer, respectively. The medium and upper layer shared 7% and the medium and deepest layer shared 12% of OTUs. These OTUs most likely represented bacteria that were restricted to the specific biochemical and physical conditions prevailing in these sediment horizons. Integrated over the complete sampling cycle, only 5%, 2% and 6% of the entire pool of OTUs were unique to the upper,

medium and deepest layer, respectively. This reveals a highly dynamical bacterial community over time, most likely influenced by sediment mixing and subsequent shifts in oxygen and chemical gradients.

As to the temporal variation of bacterial diversity, OTU numbers generally varied between 178 and 246 per sampling date, and only a small number (0-8) of OTUs were unique to any of the dates (with the exception in August 2004 when 65 OTUs were unique to this time). Compared to other ARISA studies, these numbers match well the range of shallow water benthic bacterial diversity (~236 OTUs; Hewson et al. 2003) and are higher than those in coral reef surface sediments (51-148 OTUs; Hewson & Fuhrman 2006). Out of the 451 OTUs identified over the entire 2-year sampling period, between 38-68% of OTUs were detected at individual sampling dates, revealing substantial dynamics in OTU richness in the top 15 cm of sediment (Fig. 4). Only 22% of all OTUs were temporally stable and found at all sampling months.

The variable part of the bacterial community was neither gradually lost or gained over time, nor did OTU occurrence generally followed seasonal patterns, so that specific OTUs could be exclusively associated with either summer or winter sampling months. This result is different from ARISA studies of temporal variation of bacterioplankton diversity, which found a predictable combination of bacterial OTUs at specific times of the year (Fuhrman et al. 2006). In contrast, in the subtidal sands, OTUs tended to disappear at one point and reappear again at a later time point without any apparent predictable patterns. Community dynamics were, however, found to be stronger between the first three sampling dates than towards the end of the experiment, where 50% of the bacterial community was found to be temporally stable. Regarding these results, it seems that the bacterial community underwent a development from a more dynamic towards a more stable bacterial community between August 2004 and March II 2006. The reason for this development remains unknown and is not clearly reflected in the variations of any of the contextual parameters. In conclusion, we could not find evidence for a strong link between temporal variation in microbial activity and biomass, and bacterial diversity or community structure.

4.4.2 Environmental factors explaining depth- and time-related changes in bacterial community structure

We have used multivariate analysis to further analyze the main factors controlling the detected spatial and temporal variations in bacterial community structure. Both RDA and variation partitioning mainly assigned the depth-related patterns in bacterial community structure to changes in organic carbon availability and redox potentials over depth (Fig. 7; Fig. 9). Accordingly, benthic chlorophyll *a* and bacterial abundances were strongly negatively correlated with depth. Also NO_3 showed a distinct vertical decrease. NO_3 can be seen as an indicator of the redox state of the habitat, and represents the depth of downward mixing of bottom water. Flushing of the sediments may represent a main disturbance, influencing not only OTU richness, but also bacterial community structure directly by selecting for aerobic or at least oxygen-tolerant species. As a second factor, the distribution of microphytobenthic biomass and activity has a strong effect on the spatial structuring of the bacterial community in the subtidal sands. Microphytobenthic algae have been shown to provide a significant source of organic matter and oxygen for benthic communities via photosynthesis (Underwood & Kromkamp 1999, Köster et al. 2005), by channeling a major fraction of the total primary production into the synthesis of extracellular polymeric substances which may serve as a growth substrate for benthic bacteria (Smith & Underwood 1998, Smith & Underwood 2000, de Brouwer & Stal 2001). Accordingly, Middelburg et al. (2000) showed by *in situ* ^{13}C-labeling that benthic autotrophic and heterotrophic processes were tightly coupled particularly in dynamic, sandy areas such as our study site. Depth-related decreases in chlorophyll *a* concentrations, bacterial abundances, growth rates and extracellular enzymatic activities indicate vertical shifts in both the quality and the nutritional quality of organic matter in the sediment (Böer et al. 2008). Both the quantity and quality of organic matter have been shown to influence bacterial community structure previously (Muylaert 2002, Crump 2003, Lebaron 1999) and our results suggest that the vertical shifts in organic carbon availability could be an important factor in the depth-related variation of the bacterial community structure.

Strongly significant temporal changes in bacterial community structure were observed over the 2-year period (Fig. 4; Fig. 6; Fig. 7). Multivariate analysis showed that time alone significantly explained variation in the bacterial community structure with twenty percent of the variation (Fig. 9). However, we could not identify a single other contextual parameter, or a combination thereof, explaining the observed temporal variation. The temporal changes in

community structure did not exhibit cyclical patterns as could have been predicted from the substantial seasonal changes in temperature and substrate availability at *Hausstrand*. Rather, a community shift was observed from a more dissimilar bacterial community at the beginning of the study towards a more similar bacterial community at the end of the study (Fig. 4). One could therefore assume that dynamic interactions between the members of the bacterial community themselves lead to changes in bacterial community structure which are independent from external environmental parameters. Such independent dynamics have been observed previously in chemostat-bioreactors (Fernández et al. 1999, Fernández et al. 2000). However, we cannot exclude that other important ecological factors were overlooked. For instance, grazing pressure and viral lysis are temporally changing factors that have been shown to influence bacterial community structure in aquatic ecosystems, but were not investigated here (Weinbauer & Rassoulzadegan 1998, Jürgens et al. 1999, van Hannen et al. 1999, Hahn & Höfle 2001). High bacterial carbon production rates and high benthic oxygen consumption versus comparably low bacterial abundances and low MPB biomass in August 2004 at the beginning of this study suggest that grazing may have indeed played a role at the study site. The temporal changes in the bacterial community structure were also linked to changes in carbohydrate concentrations both in the RDA and the variation partitioning (data not shown). Again, these variables were strongly related to the first three sampling dates of the study, when the strongest community dynamics occurred. MPB-related carbohydrate concentrations, however, exhibit strong short-term variations in marine sediments (de Brouwer & Stal 2001), thus the causes for the strong community dynamics at the beginning of the study remain uncertain.

In conclusion, the data suggest that unusual physicochemical and biological conditions may lead to strong dynamics in the bacterial community structure in subtidal sediments, here indicated by a replacement of >25% of the OTUs within <3 months, followed by a long phase of restabilization. Interestingly, despite the high dynamics in diversity and composition of the bacterial community observed here, general ecosystem functions mediated by bacteria such as organic matter remineralization (oxygen consumption, enzymatic activity) and productivity (bacterial growth) were not affected, indicating high functional redundancy. In conclusion, this study shows that the ecological modeling of high-resolution fingerprinting patterns together with their environmental context is a successful approach to test hypotheses on the causes of changes in microbial diversity and community structure. Future work is needed to

address the composition of the microbial communities in subtidal sandy sediments and the taxonomic levels that are the targets of environmental controls.

4.5 Acknowledgements

Susanne Menger is gratefully acknowledged for excellent assistance in the field and with sample processing. Tatyana Romanova and Gabriele Schüßler are thanked for assistance with nutrient analyses and ^{3}H-thymidine incorporation measurements, respectively. Hannelore Halliger and Reimer Magens supported our work on Sylt logistically and technically. The scientific divers Christian Buschbaum and Patrick Polte are gratefully recognized for providing us with samples when Sylt showed its stormy nature. Aerial photo material was kindly provided by the Wadden Sea National Park Office. This study was financed by the Max Planck Society (MPG).

4.6 References

Abrams PA (1995) Monotonic or unimodal diversity-productivity gradients: what does the competition theory predict? Ecology 76:2019-2027

Baas-Becking LGM (1934) Geobiologie of Inleiding Tot de Milieukunde. Van Stockkum & Zoon. The Hague

Billerbeck M, Werner U, Polerecky L, Walpersdorf E, de Beer D, Huettel M (2006) Surficial and deep pore water circulation governs spatial and temporal scales of nutrient recycling in intertidal sand flat sediment. Mar Ecol Prog Ser 326:61-76

Bissett A, Burke C, Cook PLM, Bowman JP (2007) Bacterial community shifts in organically perturbed sediments. Environ Microbiol 9(1):46-60

Böer SI, Arnosti C, van Beusekom JEE, Boetius A (2008) Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments. Biogeosciences Discussions 5:4271-4313 (Chapter 3)

Boetius A, Lochte K (1994) Regulation of microbial enzymatic degradation of OM in deep-sea sediments. Mar Ecol Prog Ser 104:299-307

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73(3):1045-1055

Bowman JP, McCammon SA, Gibson JAE, Robertson L, Nichols PD (2003) Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Appl Environ Microbiol 69(5):2448-2462

Bowman JP, McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. Appl Environ Microbiol 69(5):2463-2483

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27(4):325-349

Cardinale M, Brusetti L, Quatrini P, Borin S, Puglia AM, Rizzi A et al. (2004) Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Appl Environ Microbiol 70(10):6147-6156

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199(4335):1302-1310

Cramer A (1990) Seasonal variation in benthic metabolic activity in a frontal system in the North Sea. In: Barnes M & Gibson RN (eds.) Trophic relationships in the marine environment 1990. Proc. 24th Europ. Mar. Biol. Symp. University Press Aberdeen, UK. pp 54-76

Cramer A (1991) Activity of benthic macrofauna at a frontal system in the southern North Sea. In: Benthic metabolic activity at frontal systems in the North Sea. Dissertation thesis. University of Amsterdam, The Netherlands

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an Antarctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69(4):2253-2268

Death RG, Winterbourn MJ (1995) Diversity patterns in stream benthic invertebrate communities: The influence of habitat stability. Ecology 76:1446-1460

de Brouwer JFC, Stal LJ (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. Mar Ecol Prog Ser 218:33-44

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356

Epstein SS (1997) Microbial Food Webs in Marine Sediments. I. Trophic interactions and Grazing rates in two tidal flat communities. Microb Ecol 34(3):188-198

Fernández A, Huang S, Seston S, Xing J, Hickey RF, Criddle C et al. (1999) How stable is stable? Function versus community stability. Appl Environ Microbiol 65 (8):3697-3704

Fernández AS, Hashsham SA, Dollhopf SL, Raskin L, Glagoleva O, Dazzo FB et al. (2000) Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. Appl Environ Microbiol 66(9):4058-4067

Findley SEG, Meyer JL, Edwards RT (1984) Measuring bacterial production via rate of incorporation of [^{3}H]thymidine into DNA. J Microbiol Methods 2:57-72

Findley, SEG (1993) Thymidine incorporation into DNA as an estimate of sediment bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds.) Handbook of methods in aquatic microbial ecology. Lewis publishers. Baton Rouge, USA. pp 505-508

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65(10):4630-4636

Franco MA, de Mesel I, Demba Diallo M, van der Gucht K, van Gansbeke D, van Rijswijk P et al. (2007) Effect of phytoplankton bloom deposits on benthic bacterial communities in two contrasting sediments in the southern North Sea. Aquat Microb Ecol 48:241-254

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109-120

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature. 399:541-548

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. PNAS 103(35):13104-13109

Grasshoff K, Kremling K, Ehrhardt M (1983) Methods of seawater analysis. Wiley-VHC. Berlin, Germany

Hedtkamp SIC (2005) Shallow subtidal sand: Permeability, nutrient dynamics, microphytobenthos and organic matter. Dissertation thesis. Christian-Albrechts-Universität Kiel, Germany

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113-121

Heip C, Huys R, Vincx M, Vanreusel A, Smol N, Herman R et al. (1990) Composition, distribution, biomass and production of North Sea meiofauna. Neth. J. Sea Res. 26(2-4):333-342

Hewson I, Vargo GA, Fuhrman JA (2003) Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: effects of virus infection, containment, and nutrient enrichment. Microb Ecol 46:322-336

Hewson I, Fuhrman JA (2005) Improved strategy for comparing microbial assemblage fingerprints. Microb Ecol 51:147-153

Hewson I, Fuhrman JA (2006) Spatial and vertical biogeography of coral reef sediment bacterial and diazotroph communities. Mar Ecol Prog Ser 306:79-86

Hewson I, Jacobson/Meyers ME, Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ Microbiol 9(4):923-933

Hill MO, Gauch HG jr. (1980) Detrended correspondence analysis: an improved ordination technique. Plant Ecol 42(1-3):47-58

Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33(5):1225-1228

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar Ecol Prog Ser 11:299-308

Hoppe HG, Kim SJ, Gocke K (1988) Microbial decomposition in aqutic environments: combined process of extracellular enzyme activity and substrate uptake. Appl Environ Microbiol 54:784-790

Huettel M, Weslawski JM, Massel S, Middelburg JJ, van Beusekom J, Glud R et al. (2003a) Coastal sands as biocatalytical filters (COSA). Coastline 12:8-12

Huettel M, Røy H, Precht E, Ehrenhauss S (2003b) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231-236

Inagaki F, Yuni S, Inoue A, Kato C, Horikoshi K (2002) Molecular phylogenetic analyses of reverse-transcribed bacterial rRNA obtained from deep-sea cold seep sediments. Environ Microbiol 4(5):277-286

Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65(3):1241-1250

Köster M, Dahlke S, Meyer-Reil L-A (2005) Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). Aquat Microb Ecol 39:69-83

Lebaron P, Servais P, Troussellier M, Courties C, Vives-Rego J, Muyzer G et al. (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat Microb Ecol 19:255-267

Legendre L, Legendre P (1998) Numerical Ecology. 2nd English Edition. Elsevier Science BV. Amsterdam, The Netherlands

Lorenzen CJ (1967) Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol Oceanogr 12(2):343-346

Luna GM, Dell'Anno A, Giuliano L, Danovaro R (2004) Bacterial diversity in deep Mediterranean sediments: relationship with the active bacterial fraction and substrate availability. Environ Microbiol 6(7):745-753

Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumings JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102-12.

McCabe DJ, Gotelli NJ (2000) Effects of disturbance frequency, intensity, and area on assemblages of stream macroinvertebrates. Oecologia 124:270-279

Michel PH, Bloem J (1993) Conversion factors for estimation of cell production rates for soil bacteria from [^{3}H]thymidine and [^{3}H]leucine incorporation. Soil Biol Biochem 25(7):943-950

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMT, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr 46(6):1224-1234

Moriarty DJW, Pollard PC (1990) Effects of radioactive labeling of macromolecules, disturbance of bacteria and adsorption of thymidine to sediment on the determination of bacterial growth rates in sediment with tritiated thymidine. J Microbiol Methods 11:127-139

Musat N, Werner U, Knittel K, Kolb S, Dodenhof T, van Beusekom JEE et al.(2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. Syst Appl Microbiol 29:333-348

Muylaert K, van der Gucht K, Vloemans N, de Meester L, Gillis M, Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68(10):4740-4750

Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Links between geographic location, environmental factors, and microbial community composition in sediments of the eastern Mediterranean Sea. Microb Ecol 49:367-378

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62(2):142-160

Ramette A, Tiedje JM (2007) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. PNAS 104(8):2761-2766

Sander BC, Kalff J (1993) Factors controlling bacterial production in marine and freshwater sediments. Microb Ecol 26(2):79-99

Smith DJ, Underwoood GJC (1998) Exopolymer production by intertidal epipelic diatoms. Limnol Oceanogr 43:1578-1591

Smith DJ, Underwood GJC (2000) The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. J Phycol 36(2):321-333

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis. 2nd ed. Bulletin of Fisheries Research Board of Canada. p 167

ter Braak CJF, Šmilauer P (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canocical Community Ordination, Version 4.5.

Thorbergsdóttir IM, Gíslason SR, Ingvason HR, Einarsson Á (2004) Benthic oxygen flux in the highly productive subarctic Lake Myvatn Iceland: in situ benthic flux chamber study. Aquat Ecol 38:177-189

Torsvik V, Daae FL, Sandaa R-A, Øvreås (1998) Novel techniques for analyzing microbial diversity in natural and perturbed environments. J Biotechnol 64(1):53-62

Townsend CR, Scarsbrook MR, Dolédec S (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. Limnol Oceanogr 42:938-949

Underwood GJC, Paterson DM, Parkes RJ (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol Oceanogr 40(7):1243-1253

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Nedwell DB, Raffaelli D (eds.) Advances in Ecological Research, Vol 29. Academic Press Inc. San Diego, USA. pp 93-153

Urakawa H, Yoshida T, Nishimura M, Ohwada K (2000) Characterization of depth-related population variation in microbial communities of a coastal marine sediment using 16S rDNA-based approaches and quinine profiling. Environ Microbiol 2(5):542-554

van Duyl FC, Kop AJ (1990) Seasonal patterns of bacterial production and biomass in intertidal sediments of the western Dutch Wadden Sea. Mar Ecol Prog Ser 59:249-261

van Duyl FC, Kop.AJ (1994) Bacterial production in North Sea sediments: clues to seasonal and spatial variations. Mar Biol 120:323-337

van Hannen EJ, Zwart G, van Agterveld MP, Gons HJ, Ebert J, et al. (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl Environ Microbiol 65:795-801

Vezzulli L, Chelossi E, Ricardi G, Fabiano M (2002) Bacterial community structure and activity in fish farm sediments of the Ligurian sea (Western Mediterranean). Aquac Int 10:123-141

Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? Environ Microbiol 6(1):1-11

Winkler LW (1888) Determination of dissolved oxygen in water. Berlin Deut. Chem. Ges.

Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. Appl Environ Microbiol 71(1):227-239

Chapter 5

Spatial and temporal variations in bacterial activities and community structure associated with faunal exclusion experiments in intertidal sands of the North Sea (Sylt)

S.I. Böer, A.M. Al-Raei, M.E. Böttcher, N. Volkenborn, A. Ramette

in preparation for submission to *Environmental Microbiology*

Abstract

The present study revealed strong effects of space, depth and time on bacterial activities and bacterial community structure in a large-scale lugworm exclusion experiment on an intertidal sand flat in the Königshafen Bay (German Wadden Sea), as determined by variation partitioning analyses. We suggest that these effects are mainly mediated by shifts in the abundance and activity of the microphytobenthos (MPB) and thus by the availability of MPB-associated labile organic carbon in the sediment. Bacterial distribution and activities were strongly correlated to the distribution of the MPB and MPB-derived carbohydrates. A vertical decrease in bacterial activities was accompanied by a vertical shift in bacterial community structure. The bacterial community structure in the oxic, MPB-rich surface layer was significantly different from that in the anoxic zone while the physico-chemical transition zone (1-2 cm) was characterized by a bacterial community that shared features of both the oxic surface and the anoxic subsurface sediment. A richer MPB biofilm developed in fine-grained sediments compared to medium-sized sands which supported a more active bacterial community. These differences in sediment characteristics and MPB-associated carbon content were responsible for a large part of the spatial variations in bacterial community structure observed in this study. A temperature-related shift in MPB and bacterial activities between autumn and spring was accompanied by a significant shift in bacterial community structure. Also, bacterial abundances and activities responded to an enhanced fine particle content and MPB-associated carbon supply in ambient sediments on experimental *Arenicola marina* exclusion plots, whereas bacterial community structure was not affected. As the impact of lugworms on sediment properties was limited to the sediment surface, where MPB biomass and productivity was generally highest, the magnitude of the lugworm-mediated modification in organic carbon availability was presumably too small to cause noticeable bacterial community shifts.

5.1 Introduction

Despite their small size, microbes constitute an essential part of the earth's biota. They undertake unique and indispensable biochemical transformation processes and as such have a strong global impact on aquatic and terrestrial carbon and nutrient cycles and on the gaseous composition of the atmosphere (Whitman et al. 1998), however the biodiversity of microbes and their distribution in space and time is still not well understood. Traditional microscopy and cultivation techniques missed a tremendous part of microbial diversity. The breakthrough of molecular tools such as ribosomal RNA sequencing greatly expanded out knowledge of microbial phylogenetic diversity (Donachie et al. 2007) and modern community fingerprinting techniques such as DGGE, TRFLP and ARISA allow to explore whole microbial communities within a relatively short time. This has created a new interest in the field of prokaryotic biogeography which aims to examine patterns of microbes at local, regional and continental scales, in time, and along environmental gradients (Ramette & Tiedge 2007a, Green et al. 2008). Still, the exploration of microbial biogeograpic patterns by means of molecular approaches is still in its infancy and our knowledge of these patterns is limited.

In the present study we focus on spatial, depth-related and temporal patterns in bacterial activities and community structure in an intertidal sand flat in the Königshafen area, a sheltered bay on the island of Sylt in the German Wadden Sea (North Sea) which is typically densely populated by the lugworm *Arenicola marina*. The study was embedded in the framework of a large-scale *in situ* lugworm exclusion experiment which was set up and described by Volkenborn & Reise (2006). They observed that the experimental removal of *Arenicola marina* caused a restructuring of the macrofaunal community. While *Nereis diversicolor* profited from the greater sediment stability and competitive release on lugworm exclusion plots and invaded these in high numbers, *Scoloplos* cf. *armiger* was negatively affected by lugworm exclusion (Volkenborn & Reise 2006). Bioturbation and bioirrigation by lugworms were found to affect sediment and porewater characteristics well beyond the vicinity of the burrows including the prevention of sediment clogging with fine particles and associated organic material and thus maintaining a high sediment permeability and low nutrient concentrations in the porewater (Volkenborn et al. 2007a). The impact of the lugworm on chemical and biological sediment properties, however, was spatially and temporally variable, whereby the spatial effects could mainly be related to the prerequisites of the sediment in terms of sediment type and hydrodynamic exposition (Volkenborn et al.

2007b), thus confirming small-scale studies which focused directly on worm burrows and showed that the impact of polychaetes on solute fluxes (Kristensen & Hansen 1999, Meysman et al. 2006) and benthic respiration (Banta et al. 1999), but also on microbial community composition (Papaspyrou et al. 2006) depends on the composition and/or organic matter content of the sediment. Sediment characteristics (porosity, density, grain size distribution, organic matter content) have long been known to drastically impact biogeochemical processes in marine sediment and have also been shown to influence bacterial community composition (Franco et al. 2007); however the sole impact of sediment properties on microbial community composition and structure is not well constrained and difficult to separate from pure spatial or other environmental effects (Kristensen & Kostka 2004).

In the present study we chose a multivariate statistical approach to disentangle the respective effects of space, depth, time and large-scale effects of lugworm presence/absence on variations in bacterial community structure, bacterial activities and sediment biochemistry in intertidal sands. Primary production by the microphytobenthos is believed to be the main source of organic carbon to the bacterial community in light-exposed coastal sands (MacIntyre et al. 1996, Underwood & Kromkamp 1999, Böer et al. 2008a) and we hypothesized that time, depth as well as differences in sediment characteristics (grain size, organic matter content) caused by the spatial setting of the experimental blocks and/or by large-scale effects of *Arenicola marina* would strongly affect microphytobenthic biomass and production (reflected in the production of carbohydrates). Our main questions were 1) to which extent these temporal, vertical and spatial shifts in MPB-associated organic carbon availability affect bacterial activities (extracellular enzymatic activities, sulfate reduction) and 2) whether variations in bacterial activities are accompanied by shifts in microbial community structure. Aim of this study was to explore which are the environmental factors that govern bacterial community structure in this highly complex and dynamic bioadvective coastal ecosystem and to gain a better understanding of the relationship between bacterial community structure and general bacterial community functions.

5.2 Materials and Methods

5.2.1 Site description and design of large-scale *Arenicola marina* exclusion experiment

The study was conducted on an intertidal sandflat in the Königshafen area, an unvegetated embayment at the northern tip of the island of Sylt in the German Wadden Sea (55°02′ N;

8°26´E; Fig. 1). The sampling site is characterized by medium to fine sand of low organic carbon content (< 1%) and densely populated by the lugworm *Arenicola marina*. The mean tidal range is 1.8 m. For a detailed geological and biological description of the Königshafen area see Reise (1985) and Austen (1994).

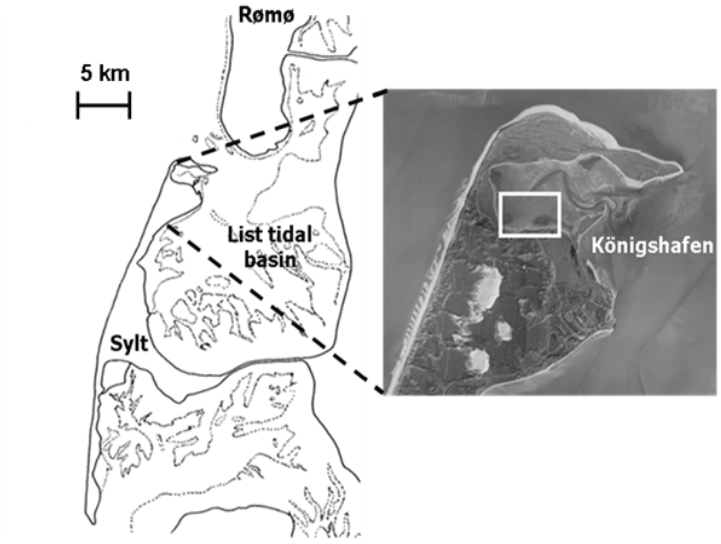

Fig. 1: Location of the study area in the Königshafen bay, a tidal flat at the northern tip of the island of Sylt in the North Frisian Wadden Sea, Germany (picture obtained from the Wadden Sea National Park Office Schleswig-Holstein).

Sampling was performed on large-scale experimental lugworm exclusion and corresponding control plots created and first described by Volkenborn & Reise (2006). Briefly, the experimental set-up consisted of 6 experimental blocks that were arranged in a two-factorial (3 x 2 levels) nested block design (Fig. 2A). Three blocks (block 1, 2 and 3) were situated in the low intertidal (3-4 h emersion per tide), characterized by fine grained sand (200-220 µm median grain size) and three blocks (block 4, 5 and 6) were situated in the mid intertidal (6-7 h emersion per tide), characterized by medium sized sand (330-340 µm median grain size). Each block consisted of three plots, each of them 20 x 20 m = 400 m^2 in area (exclusion, control and ambient control). On lugworm exclusion plots, a 1-mm meshed polyethylene net was inserted at 10 cm depth into the sediment in spring 2002 which led to a continual removal of *Arenicola marina* (Fig. 2B). Sediment control plots were likewise disturbed with a backhoe as had been necessary for exclusion plots while ambient control plots were left undisturbed.

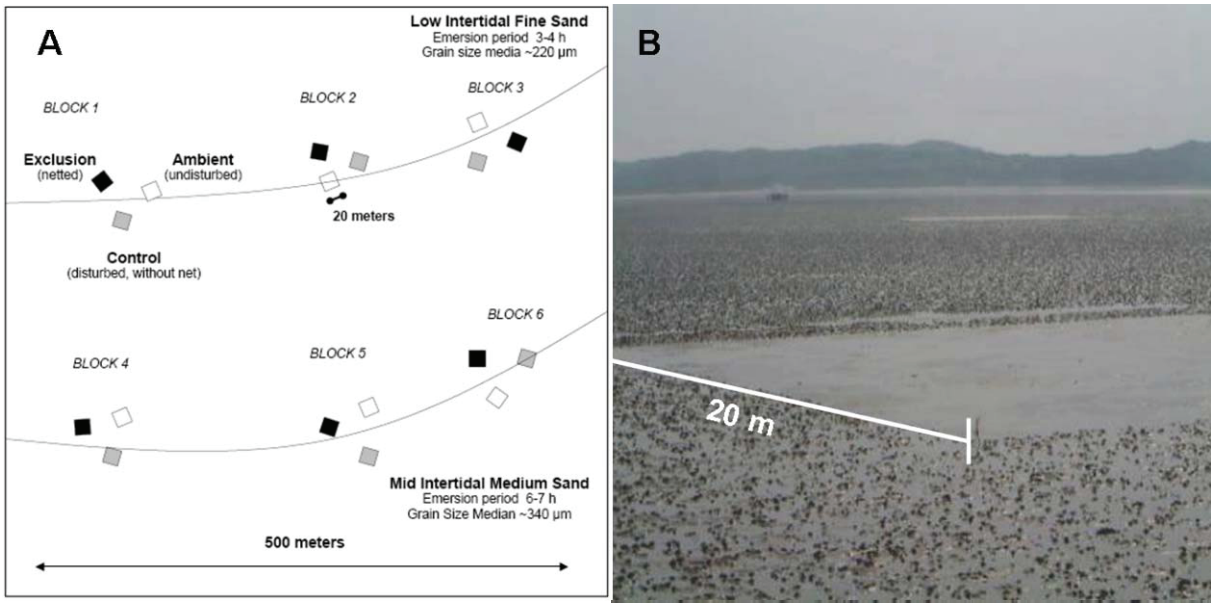

Fig. 2: Experimental set-up of the large-scale lugworm exclusion experiment. A) Six experimental blocks were nested in a 2-factorial design so that 3 blocks were situated in the low intertidal, characterized by fine sand and an emersion period of 3-4 hours, and 3 blocks were situated in the mid intertidal, characterized by medium-sized sand and an emersion period of 6-7 hours. Each block comprised 3 lugworm treatments: on exclusion plots *Arenicola marina* was excluded by the burial of a 1mm-meshed net at 10 cm depth, control plots were disturbed, but left without a net, and ambient controls represented a natural, undisturbed area. B) Picture of one of the lugworm exclusion plots. *Arenicola marina* was consistently and effectively excluded.

5.2.2 Sample collection and sample processing

The first field campaign was conducted in September 2005, the second one in March 2006. Water temperatures in September and March were ~18°C and ~3°C, respectively. Since previous studies on sediment properties and the macrobenthic community on the experimental plots showed no perceptible differences between control and ambient control (e.g. Volkenborn et al., 2007b; Volkenborn & Reise, 2007), only lugworm exclusion and control plots were compared in this study.

A low-vertical-resolution study was performed on all 6 blocks, focusing on the 0-2 cm, 2-5 cm and 5-8 cm sediment layer, with the goal to study the respective effects of time, space, depth and lugworm exclusion on variations in bacterial community structure, bacterial abundances, chitobiase activity, total carbohydrates, EDTA extractable carbohydrates, chlorophyll *a* and phaeophytine. In a second higher-vertical-resolution study which focused on block 1 and 4 only, 1-cm depth intervals were chosen to examine the effect of these factors on a wider range of parameters in more detail. In addition to the parameters mentioned above, β-glucosidase activity, sulfate reduction rates, porosity, DIC, sulfate, sulfide, silicate,

phosphate, nitrite+nitrate and ammonium concentrations were measured and included in the analysis.

Sediment samples were collected in September 2005 and March 2006 by the use of 60-ml cut-off syringes. On each of the 6 blocks, 10 sediment samples were collected on lugworm exclusion plots and 10 samples on control plots. Directly in the field, the samples were sectioned at intervals of 0-2 cm, 2-5 cm and 5-8 cm for block 2, 3, 5 and 6. For block 1 and 4, samples were sectioned into 1-cm intervals down to -8 cm depth. For each plot, sediment slices of the same depth were pooled in sterile Petri dishes to obtain representative samples and sufficient volumes for all measurements. Sub-samples of homogenized sediment were immediately frozen in the lab for the measurement of chloroplastic pigments and carbohydrates as well as for automated ribosomal intergenic spacer analysis (ARISA) in separate sterile plastic containers and stored at -20°C in the dark until further analysis. Sub-samples for total cell number estimations were fixed in 2% formaldehyde in seawater. Samples for the measurement of extracellular enzymatic activities were instantly processed.

On block 1 and 4, pore waters from -1, -3, -5 and -8 cm depth were collected during low tide shortly after exposure of the study sites with pore water lances made of PEEK connected to a plastic syringe. Aliquots of pore waters were immediately passed through 0.45 µm disposable membrane filters into different pre-conditioned vials: sub-samples for sulphate and sulfide were collected in plastic centrifuge tubes containing 5 % Zn acetate solution, sub-samples for DIC were collected in acid pre-cleaned glass vacutainers containing saturated mercury chloride solution, and nutrient samples were collected in tubes without any further addition. Samples were either stored frozen (nutrients) or in the dark at 4°C until further analysis. To determine porosity, 1 additional “syringe core” per plot was taken on block 1 and 4, sectioned into 1-cm intervals and samples stored frozen at -20°C. Two replicate push cores (inner diameter 2.6 cm) with silicon-covered injection holes were collected for sulphate reduction measurements. The incubation was started immediately after sampling in the nearby laboratory.

5.2.3 Biochemical analyses

Sulfate in the pore water was analyzed by ion chromatography using a Waters® anion-exchange column, calibrated with a sodium sulphate solution (Jørgensen 2001). Sulfide was spectrophotometrically analyzed according to Cline (1969) using a UV-160A Shimadzu spectrophotometer. Analyses of silicate, phosphate and ammonium were performed

spectrophotometrically with a Skalar continuous-flow-analyzer according to methods described by Grasshoff et al. (1999). DIC was determined by flow injection analysis (Hall & Aller 1992). Data were extrapolated to obtain profiles for the entire sediment range of 8 cm.

Porosity was calculated based on the water content of the sediment samples which was measured gravimetrically during lyophilization.

Total carbohydrates and EDTA extractable carbohydrates were spectrophotometrically quantified in lyophilized sediment samples based on the phenol-sulfuric acid assay (Dubois et al., 1956) following the procedure of Underwood et al. (1995). All measurements were done in triplicates.

For chlorophyll *a* and phaeophytine quantification, triplicate samples of approximately 1 g of lyophilized sediment from each sediment layer were mixed with 7.5 ml of 90% acetone and extracted in the dark at 4°C for 24 hours. Samples were centrifuged at 4000 rpm for 10 min and the supernatant was transferred to quarz kuvettes to measure extinction at 665 and 750 nm before and after acidification with 2 drops of 10% HCl. Chlorophyll a and phaeophytine concentrations were calculated as described in Lorenzen (1967).

5.2.4 Bacterial cell counts

Bacterial cell counts on fixed sediment samples were performed within 3 weeks after sampling following a modified protocol of the method used by Hobbie et al. (1977). Briefly, bacterial cells were removed from the sediment by sonification (240 s with an ultrasonic disintegrator, power: 72/D, cycle: 30%) while keeping the vial on ice. Sub-samples of the diluted cell suspensions were filtered onto 0.2 µm black polycarbonate filters and stained with Acridine Orange solution (final concentration 0.01%) for 3 min. Filters were rinsed with citrate buffer and bacteria enumerated by epifluorescence microscopy. Final counts represent the averages of 6 filters (2 filters from 3 replicate field samples), with 10-20 grids counted on each filter with at least 1000 cells per filter.

5.2.5 Bacterial activities

Potential extracellular enzymatic activities of the hydrolases β-glucosidase and chitobiase were measured fluorometrically using the 4-methylumbelliferone (MUF) labeled substrate analogs MUF-β-D-glucopyranoside and MUF-N-acetyl-β-glucosamine (both Sigma-Aldrich, Munich, Germany), respectively (Hoppe, 1983; Boetius & Lochte, 1994). For each depth

interval, five 0.5-ml replicate subsamples of the homogenized sediment were diluted 1:10 with sterile filtered seawater. Pre-experiments were conducted prior to the start of the study to confirm that the final concentrations of MUF and MCA substrates generally represented substrate saturation levels and yielded maximum velocities (Boetius & Lochte, 1996). Substrates were added at a final concentration of 2.5 mM and the samples agitated at near *in situ* temperature in the dark for the duration of the experiment. Enzymatic reactions in sub-samples were terminated by the use of a borate buffer (pH 10) after 1 hour and again after 2-4 hours of incubation and the fluorescence of the enzymatically released MUF was measured at 445 nm with 365 nm excitation (Hitachi F-2000 spectrophotometer, San Jose, CA, USA). Relative fluorescence units were calculated from the increase of fluorescence over time. These were converted into molar amounts of fluorochrome released by calibration via a standard curve of MUF (Sigma).

Microbial sulphate reduction rates were measured using the whole-core incubation technique as suggested by Fossing & Jørgensen (1989). Carrier-free 35S labelled sulphate was injected in 1-cm intervals into the sediment through the silicon-covered injection holes and the cores were incubated in a dark thermo-constant cold-room at near *in situ* temperature. Reduced sulphur species (total reducible inorganic sulphur) were extracted according to the hot one step method (Fossing & Jørgensen, 1989) with further distillation according to Kallmeyer et al. (2004). Sulfide was measured spectrometrically (Cline, 1969). Activities were determined using a Packard scintillation counter.

5.2.6 Community structure analysis by Automated rRNA intergenic spacer analysis (ARISA)

DNA was extracted from 1 g of homogenized sediment sample using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions with the following modifications: bead beating was performed twice in a FastPrep Instrument (Q·BIO gene, Morgan Irvine, CA, USA) for 45 s at maximum speed.

Changes in bacterial community structure were estimated by ARISA (Fisher and Triplett, 1999). PCR reactions (50 µl) were conducted in triplicate and contained 1×PCR buffer (Promega, Madison, WI, USA), 2.5 mM $MgCl_2$ (Promega), 0.25 mM of dNTP mix (Promega), bovine serum albumine (3 µg μl^{-1}, final concentration), 25 ng extracted DNA, 400 nM each of universal primer ITSF (5′-GTCGTAACAAGGTAGCCGTA-3′) and

eubacterial ITSReub (5′-GCCAAGGCATCCACC-3′; Cardinale et al., 2004) labeled with the phosphoramidite dye HEX, and 0.05 units GoTaq polymerase (Promega). PCR was carried out in an Eppendorf MasterCycler (Eppendorf, Hamburg, Germany) with an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 90 s, and a final extension at 72°C for 5 min. The PCR products were purified utilizing Sephadex G-50 Superfine (Sigma Aldrich) and a standardized amount of DNA (150 ng DNA) was added to 0.5 µl of internal size standard Map Marker®1000 ROX (50-1000 bp) (BioVentures Inc., Washington, DC, USA), 0.5 µl of tracking dye (BioVentures) and 14 µl of deionized Hi-Di –formamide (Applied Biosystems, Foster City, CA, USA). Discrimination of the PCR-amplified fragments via capillary electrophoresis was carried out on an ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems) and the ARISA profiles were analyzed using the GeneMapper Software v 3.7 (Applied Biosystems). To account for run-to-run variations in signal intensity, the total peak area per sample was normalized to one (Yannarell & Triplett, 2005). To include the maximum number of peaks while excluding background fluorescence, only fragments above a threshold of 50 fluorescence units and between 100-1000 bp length were taken into consideration. The GeneMapper output file was reformatted using custom Perl scripts and further analyzed by custom R (version 2.4.0; The R foundation for Statistical Computing) scripts. A “fixed window” binning strategy with a bin size of 2 bp was applied to the ARISA generated data to account for size calling imprecision according to Hewson & Fuhrman (2005). The binning frame that offered the highest pairwise similarities among samples was then further subjected to multivariate analyses.

5.2.7 Statistical analysis

Prior to performing statistical analyses, the ARISA data were further processed in order to improve the reliability of ARISA profiles. Replicate profiles obtained from a given sample were combined in such a way that only operational taxonomic units (OTUs) occurring more than once in a sample were kept in a consensus profile. For the high-vertical-resolution study on block 1 and 4, consensus profiles were obtained by combining replicate samples from the single 1-cm sections. To comply with the requirements of the low-vertical-resolution study, samples from block 1 and 4 were treated in such a way that the sections between 0 and 2 cm depth, between 2 and 5 cm depth, and between 5-8 cm depth were regarded as one sample and all replicate profiles in these sections were combined in one consensus profile. In accordance, the environmental data from block 1 and 4 were adapted to the low-vertical-resolution study

by averaging all values within these respective sections. For both studies, the consensus ARISA tables (samples by OTUs) were used to calculate pairwise similarities among samples based on the Bray-Curtis similarity index (Bray-Curtis, 1957).

The resulting matrices were then explored for seasonal, spatial, vertical or lugworm-related patterns in bacterial community structure via non-metric multidimensional scaling (NMDS) as implemented in the R package. NMDS arranges the OTU profiles in a two-dimensional coordinate system so that the ranked dissimilarities between the samples are preserved. The goodness-of-fit of the ordination compared to the original sample ranking is determined by Shephard's stress function. A stress value <0.1 indicates that the ordination is almost perfect while a stress value <0.2 indicates that the ordination varies from the original sample ranking but is still useful for interpretation (Clarke & Warwick, 2001). Analysis of similarity (ANOSIM) was performed to test whether differences between various *a posteriori* groupings of the samples in the NMDS ordinations were statistically significant. The resulting test statistic *R* indicates the degree of separation between groups, with a score of R>0.75 indicating a good separation, a score of R>0.5 indicating that the groups are overlapping but clearly different, a score of R<0.25 representing no separation and negative values signalizing a higher degree of similarity between groups than within groups (Clarke, 1993; Clarke & Warwick, 2001).

In order to facilitate the environmental interpretation of the ARISA data, correlations between all environmental and biological parameters in the low-vertical-resolution study were calculated by using the Pearson's correlation coefficient for the complete dataset (n=15). P values were corrected for multiple testing using Bonferroni correction (Ramette, 2007). The environmental and bacteria-related parameters were $\log_{10}$-transformed prior to the analysis in order to normalize their distribution. To incorporate the main patterns of diversity variation in the correlation analyses, the main patterns in bacterial community structure were extracted by NMDS. The two major NMDS axes were then used for the subsequent correlation analyses. Sample scores on the first two NMDS axes were normalized to zero mean and a standard deviation of 1 prior to computation of the Pearson's correlation coefficients.

The contribution of all environmental and bacteria-related parameters to patterns in bacterial community structure was investigated by multivariate statistics using the software packages CANOCO for Windows 4.5 (terBraak & Smilauer, 1998) and R. In CANOCO, detrended correspondence analysis (DCA, Hill & Gauch, 1980) was performed to determine whether linear or unimodal species models better fitted the current ARISA datasets (Ramette, 2007). Canonical redundancy analysis (RDA) was then used to pre-select significant variables

from pre-defined categories of variables by forward model selection. The significance of the variables was tested by Monte Carlo permutation tests (999 permutations) at $p < 0.05$ for each group and only significant variables were included in the subsequent RDA to test the amount of variation in bacterial community structure that was explained by all parameters included in the low-vertical- and high-vertical-resolution study.

The respective effects of time, space, depth and the lugworm's presence/absence on the variation in bacterial community structure were further explored by canonical variation partitioning (Legendre & Legendre, 1998; Ramette & Tiedje, 2007b). Using the software R, the variation and co-variation of significant explanatory variables were partitioned into the effects of each factor alone (i.e. when removing the effects of all other factors in the model, which is also called "partialling out") and of their covariation (Borcard et al., 1992). Statistical significances of the respective fractions were calculated by 999 Monte Carlo permutations under the full multivariate model. The same procedure was applied to determine the respective effects of time, space, depth and *Arenicola marina* on variations in the environmental data.

5.3 Results

5.3.1 Temporal and spatial patterns in biochemical sediment parameters, MPB, bacterial abundances and bacterial activities

Chlorophyll a and phaeophytine concentrations as well as total and EDTA extractable carbohydrate concentrations were generally higher in the fine sands of the low intertidal than in the medium-sized sands of the mid intertidal (Table A5, Part I and 2; Table A6, Part I a and Part IIa). Bacterial abundances and bacterial activities (chitobiase and β-glucosidase activities, sulfate reduction rates) followed this general trend of the MPB and tended to be slightly enhanced in the low intertidal (Table A5, Part I and II; Table A6, Part I a/b and Part II a/b).

Distinct vertical chlorophyll *a* gradients were observed on all experimental plots, while for phaeophytine these gradients occurred only in the low intertidal. Total and EDTA extractable carbohydrates usually showed a maximum in the top 2 cm, corresponding to the surface peak of the MPB (as represented by chlorophyll *a*); however the concentrations below -2 cm stayed more or less invariable (Table A5, Part I and II; Table A6, Part I a and Part II a). Correspondingly, bacterial abundances and bacterial activities were generally highest in the top 2 cm of sediment. While bacterial abundances and β-glucosidase activity peaked at the

surface and did not vary considerably below, and as such reflected the vertical distribution of the carbohydrate inventory, chitobiase activities and sulfate reduction rates showed a more gradual decrease, thus coinciding with the chlorophyll *a* profiles (Table A5, Part I and II; Table A6, Part I a/b and Part II a/b). While the surface peak in bacterial abundances was well pronounced in March, the vertical gradients in September were generally weak. Also, chitobiase activities on block 2 and 3 were highest at 2-5 cm depth, corresponding to a slight peak of total and EDTA extractable carbohydrates in this depth layer in September.

Ammonium, silicate, phosphate and DIC concentrations in September generally peaked at -5 cm on lugworm exclusion plots, while on lugworm populated control plots, metabolite profiles were generally very flat and only in a few cases showed at peak at -3 cm. Sulfide tended to accumulate with depth on all plots. In March, ammonium, silicate, phosphate, sulfide and DIC generally peaked at -3 cm depth; however on the block 1 lugworm exclusion plot, all these metabolites strongly accumulated with depth.

Total and EDTA extractable carbohydrate concentrations as well as bacterial activities (extracellular enzymatic activities, sulfate reduction rates) showed a distinct temporal variation and were in general substantially higher in September (~18°C) than in March (~3°C; Table A5, Part I and II; Table A6 Part I a and Part II a). These temperature-related seasonal patterns were not reflected in bacterial abundances and MPB biomass (as represented by chlorophyll *a*). While they exhibited almost no temporal variation below -2 cm (chlorophyll a concentrations were only slightly enhanced in March; bacterial abundances were only slightly enhanced in September), MPB biomass and especially bacterial biomass were considerably higher in March than in September at the sediment surface. This discrepancy between microalgal activities (as represented by a higher production of carbohydrates) and MPB biomass and between bacterial activities and bacterial biomass, respectively, is most likely the result of extensive grazing and thus an overall strong enhancement of benthic metabolism at higher temperatures. In agreement with this, DIC, silicate, phosphate and sulfide concentrations were higher in autumn compared to spring (Table A6, Part I b and Part II b); however ammonium concentrations did not show any clear seasonal trend. In agreement with the overall higher production of carbohydrates (and presumably higher excretion of EPS) and higher benthic metabolism in autumn, sediment porosity was slightly enhanced in September compared to spring.

Exclusion of the lugworm *Arenicola marina* resulted in an increase of MPB biomass and associated carbohydrates and to a stimulation of bacterial activities in the top layers of the sediment. In the absence of lugworms, chlorophyll a concentrations and β-glucosidase

activities were generally higher in the upper 2 cm, while phaeophytine concentrations, total and EDTA extractable carbohydrate concentrations and chitobiase activities were generally enhanced down to a depth of -5 cm when compared to the lugworm populated sites. Below these depths, there were either no significant differences between lugworm exclusion and control plots or values on control plots were slightly enhanced (Table A5, Part I and Part II; Table A6, Part I a and Part II a). Bacterial abundances and sulfate reduction rates did not follow any consistent pattern regarding lugworm presence or absence (Table A5, Part I and Part II; Table A6, Part I b and Part II b). In September, metabolite concentrations generally reflected the overall trend towards higher microbial biomass and activities in the absence of *Arenicola marina*: ammonium, silicate, phosphate and DIC concentrations were generally higher on lugworm exclusion plots than on lugworm control plots; however sulfide concentration did not show any clear pattern. In March, only DIC was slightly higher on exclusion plots than on control plots, while ammonium, phosphate and sulfide tended to be higher when the lugworm was present. Silicate concentrations did not show any clear pattern (Table A6, Part I b and Part II b).

5.3.2 Respective effects of time, space and lugworm exclusion on environmental parameters, bacterial abundances and activities

In order to disentangle the pure effects of time, space, depth, and lugworm presence/absence on variations in the environmental and bacteria-related data, variation partitioning analyses were performed for each of the measured parameters (Table 1). Briefly, the presence/absence of the lugworm, depth, time and space were included as potential explanatory variables in the models. The combined effects of these four factors were able to significantly ($p < 0.001$) explain 55% of the variation in bacterial abundances, 57% of the variation in chitobiase activity, 94% of the variation in benthic chlorophyll *a*, 42% of the variation in benthic phaeophytine, 59% of the variation in EDTA extractable carbohydrates and 79% of the variation in total carbohydrates, respectively. Depth as a pure factor, i.e. when all other factors in the models were excluded, significantly explained a substantial part of the variation in all environmental and bacteria-related parameters ($p < 0.001$). As an outstanding example, ninety-one percent of the variation in chlorophyll *a* data could be explained by the pure effect of depth, thus depth mainly serves as a proxy for the availability of labile organic matter within the sediment in this study. Also the variation in phaeophytine concentrations and bacterial abundances were mainly linked to the pure effect of depth alone, with 26% and 41%

contribution to the overall variation, respectively. This supports our general observations and our results from the correlation analysis (see below), namely that bacterial distributions and bacterial activities are strongly linked to the availability of MPB-associated organic matter.

Space and time, in addition, were of considerable importance for all parameters. When regarded as a pure factor, time significantly explained a large part of the variation in all MPB-related and bacteria-related parameters ($p < 0.001$ except for bacterial abundances and total carbohydrates with $p = 0.038$ and 0.035, respectively). phaeophytine with $p = 0.002$). Particularly chitobiase activity was affected by time alone (31%), supporting our above findings that bacterial activities were largely influenced by temperature and thus higher in September.

Space alone also contributed to part of the variation in all parameters ($p < 0.001$, except for phaeophytine with $p = 0.002$), thus supporting our previous observations which suggested that these parameters varied with the tidal range. The greatest effect of space thereby was on total and EDTA extractable carbohydrate concentrations (30% and 25%, respectively).

The analyses further revealed that *Arenicola marina* had neither a significant effect on variation in bacterial abundances and enzymatic activities, nor on variation in chloroplastic pigments. The pure effect of the lugworm, however, significantly explained part of the variation in EDTA extractable carbohydrates (3%, $p = 0.015$) and total carbohydrates (12%, $p < 0.001$), underlining our general observations that carbohydrates were considerably enhanced in the upper layers of lugworm exclusion plots. In addition, 1% of the variation in total carbohydrates was explained by the covarying effect of the lugworm and space.

Table 1: Partitioning of the microbial variation into the relative effects of contextual parameters alone or in combination. Analysis refers to all 6 experimental blocks. The data represents the variation in the ARISA data as a function of W, D, T and S which indicate worm presence/ absence, depth, time and space, respectively. Values represent the percentage of microbial variation that is explained by the respective variable or by the covarying effect of more than one variable (indicated by +). A statistically significant contribution of pure fractions to variation is indicated by p-values < 0.05 and marked with a *.

	explained variation	p-value model	unexplained variation	W	p-value W	D	p-value D	T	p-value T	S	p-value S	W+S | D+T
Bacterial abundance	55	< 0.001*	45		0.433	41	< 0.001*	2	0.038*	15	< 0.001*	
Chitobiase activity	57	< 0.001*	43		0.925	13	< 0.001*	31	< 0.001*	17	< 0.001*	
Chlorophyll a	94	< 0.001*	6		0.378	91	< 0.001*	2	< 0.001*	6	< 0.001*	
Phaeophytine	42	< 0.001*	58		0.315	26	< 0.001*	9	< 0.001*	9	0.002*	
EDTA colloidal carbohydrates	59	< 0.001*	41	3	0.015*	13	< 0.001*	20	< 0.001*	25	< 0.001*	
Total carboydrates	70	< 0.001*	30	12	< 0.001*	28	< 0.001*	2	0.035*	30	< 0.001*	1

Variation partitioning analyses on samples obtained at a high vertical resolution on block 1 and 4 only largely confirmed our previous findings (Table 2). The combined effects of lugworm presence/absence, depth, space and time significantly explained between 27% and 90% of the variations in all parameters ($p < 0.001$). Again, variations in MPB-related parameters (chloroplastic pigments, carbohydrates) were mostly explained by depth and also major parts of the variation in bacterial abundance and bacterial activities were explained by depth alone, reflecting the dependency of the bacterial community on MPB-related labile organic matter we suggested above. Accordingly, part of the variation in sulfide, silicate and DIC concentrations was explained by the pure effect of depth. However, the main explanatory factor for variations in β-glucosidase activity (48%), sulfate reduction rates (76%), phosphate (59%), sulfide (67), silicate (51%) and porosity (50%) was time ($p < 0.001$), which reflects our observations that benthic metabolism was strongly influenced by temperature. Bacterial and algal activities and metabolic products were generally higher in September than in March. The overall strong effect of time on benthic activities was not reflected to the same extent by bacterial abundances and MPB biomass (as represented by chlorophyll *a*), which were only explained to minor extents by the pure effect of time, thus supporting our interpretation that bacterial and MPB abundances are extensively influenced by grazing.

Except for phaeophytine, space as a pure factor again significantly explained variation in all parameters that were already included in the variation partitioning analyses on all experimental blocks, chitobiase even to a major extent (63%). Variation of all additionally incorporated parameters (except phosphate) was partly explained by the pure effect of space as well.

The pure effect of lugworm presence in this high-vertical-resolution study did not significantly explain variation in any of the parameters, however strong covarying effects between lugworm presence/absence and the spatial setting of the blocks were responsible for variations in bacterial abundances (1%), ammonium and sulfide (2%), phaeophytine (6%), EDTA extractable carbohydrates (8%), DIC (11%), silicate and total carbohydrates (24%), indicating that the effect of the lugworm on bacterial and environmental parameters was spatially variable.

Table 2: Partitioning of the microbial variation into the relative effects of significant contextual parameters alone or in combination. Results refer to the high-vertical resolution study on experimental blocks 1 and 4. The data represents the variation in the ARISA data as a function of W, D, T and S which indicate worm presence/ absence, depth, time and space, respectively. Values represent the percentage of microbial variation that is explained by the respective variable or by the covarying effect of more than one variable (indicated by +). A statistically significant contribution of pure fractions to variation is indicated by p-values < 0.05 and marked with a *.

	explained variation	p-value model	unexplained variation	W	p-value W	D	p-value D	T	p-value T	S	p-value S	W+S | D+T
Bacterial abundance	65	< 0.001*	35			51	< 0.001*	12	< 0.001*	4	0.008*	1
Beta-glucosidase activity	81	< 0.001*	19			34	< 0.001*	48	< 0.001*	2	0.006*	
Chitobiase activity	84	< 0.001*	16			24	< 0.001*	1	0.076	63	< 0.001*	
Sulfate reduction rates	83	< 0.001*	17			10	< 0.001*	76	< 0.001*		0.326	
Chlorophyll a	90	< 0.001*	10			87	< 0.001*	3	< 0.001*	4	< 0.001*	
Phaeophytine	28	< 0.001*	72			23	< 0.001*		0.25		0.25	6
EDTA colloidal carbohydrates	27	< 0.001*	73			17	< 0.001*		0.36	4	0.046*	8
Total carbohydrates	60	< 0.001*	40			21	< 0.001*		0.478	18	< 0.001*	24
Ammonium	41	< 0.001*	59			1	0.154	1	0.176	38	< 0.001*	2
Nitrate+Nitrite	29	< 0.001*	71			2	0.105	27	< 0.001*		0.819	2
Phosphate	56	< 0.001*	44			1	0.152	59	< 0.001*		0.298	
Sulfide	65	< 0.001*	25			9	< 0.001*	67	< 0.001*	1	0.12	2
Silicate	66	< 0.001*	34			2	0.024*	51	< 0.001*	4	0.006*	12
DIC	45	< 0.001*	55			33	< 0.001*	1	0.113	2	0.065	11
Porosity	49	< 0.001*	51				0.609	50	< 0.001*	2	0.059	

5.3.3 Temporal and spatial patterns of variation in bacterial community structure

The differences in ARISA consensus profiles were depicted by non-metric multidimensional scaling (NMDS) and visual separations of groups were tested for significance by analysis of similarity (ANOSIM).

Space and particularly the tidal setting played an important role for variations in bacterial community structure. When samples were grouped according to the experimental blocks they originated from, samples from different tidal regimes separated visually, except for block 1 which exhibited similarities with all other blocks (Fig. 3A).

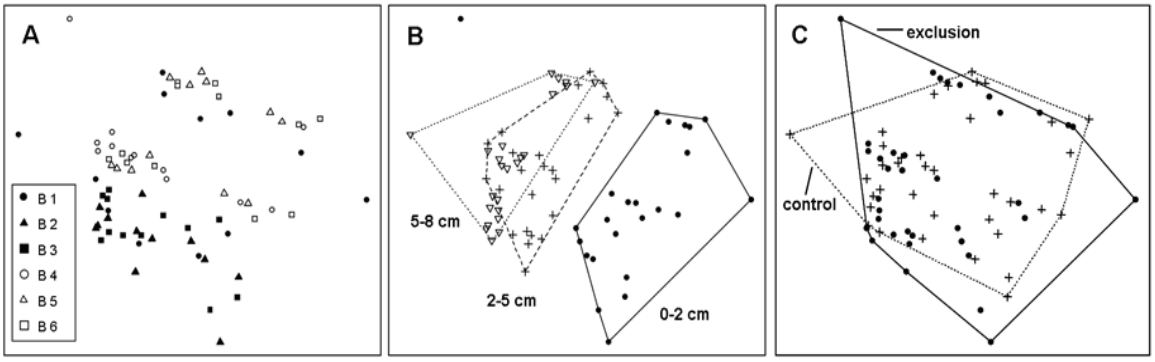

Fig. 3: Non-metric multi-dimensional scaling (NMDS) ordination of ARISA data from all 6 experimental blocks. Each dot represents the consensus profile of 3-9 replicate ARISA samples. Samples were grouped *a posteriori* according to A) experimental blocks, B) depth and C) lugworm presence and lugworm absence, whereby A, B and C refer to the same ordination. Symbols in each figure are only valid for the respective figure. The stress value for the 2-dimensional ordination was 0.19 according to Kruskal's stress formula.

Indeed, block 2 and 3 were significantly separated from all blocks in the mid intertidal (R between 0.4 and 0.51; $p \leq 0.0033$), while block 1 was only weakly separated from block 4 ($R = 0.34$; $p \leq 0.0033$). Blocks within the respective tidal zones were not separated at all. Samples from the block 1 exclusion plot were more similar to other samples from the low intertidal, while samples from the block 1 control plot were more similar to samples from the mid intertidal, however this pattern was only marginally significant. The spatial separation exhibited some seasonal dependency (Table 3). Overall, blocks were stronger separated in March than in September. While separation in September generally occurred between blocks in different tidal regimes, there was a stronger block-to-block variation between almost all of the blocks in March (except between block 2 and 3 and block 5 and 6, respectively). Also, the bacterial community structure on all blocks, except for block 4, differed between March and

September. Temporal separations were only marginally significant, however a limited statistical replication (n = 6 per group) was available for this comparison.

Table 3: Analysis of similarity (ANOSIM) of microbial communities between all 6 experimental blocks and different sampling dates.

	Block 2 September	Block 3 March	Block 3 September	Block 5 March	Block 5 September	Block 6 March	Block 6 September	Block 1 March	Block 1 September	Block 4 March	Block 4 September
Block 2 March	**0.44***	**0.00**	**0.32***	**0.93*****	**0.51****	**0.90*****	**0.54*****	**0.76*****	**0.30***	**0.44***	**0.57****
Block 2 September		**0.42***	**0.04**	**1.00*****	**0.62****	**0.99*****	**0.55*****	**0.79*****	**0.05**	**0.58***	**0.70****
Block 3 March			**0.45***	**0.94*****	**0.66****	**0.89*****	**0.64*****	**0.59****	**0.32***	**0.57***	**0.70****
Block 3 September				**0.99*****	**0.33***	**0.95*****	**0.23**	**0.79*****	**-0.04**	**0.33***	**0.45***
Block 5 March					**0.82*****	**-0.11**	**0.74****	**0.33***	**0.87*****	**0.59****	**0.78*****
Block 5 September						**0.77*****	**-0.05**	**0.78*****	**0.31***	**0.12**	**0.18**
Block 6 March							**0.64****	**0.25***	**0.86*****	**0.51****	**0.75*****
Block 6 September								**0.74*****	**0.24**	**0.15**	**0.20**
Block 1 March									**0.60****	**0.62****	**0.79*****
Block 1 September										**0.34***	**0.32***
Block 4 March											**0.23**

R-values are presented in bold font in the upper part of the table. Stars indicate separation of groups at $p < 0.05$. No star = $R < 0.25$, indicates that the groups are not separated; * = R between 0.25 and 0.5, indicates that groups are different but strongly overlapping; ** = $R > 0.5$, indicates that groups are overlapping but clearly different; *** = $R > 0.75$, indicates that groups are well separated. The separations were only marginally significant (p between 0.002 and 0.05).

Depth was another important factor for variations in the ARISA data. The bacterial community structure in the top 2 cm differed significantly from the bacterial community structure at 2-5 cm and 5-8 cm depth ($p \leq 0.017$, Bonferroni-corrected; Fig. 3B). Thereby the separation was much stronger between the upper and deepest layer (R = 0.64) than between the upper and middle layer (R = 0.38), while the middle and deepest layer were rather similar (R = 0.07). Similar findings were obtained in the high-vertical-resolution study on block 1 and 4, however depth trends could be further resolved. While the bacterial community structure in the surface layer was not different from that in the 1-2 cm layer, the separation between the sediment surface (0-1 cm) and all other sediment layers was significant and increased with increasing sediment depth (R = 0.3-0.56, $p < 0.01$; Table 4). The bacterial community structure in the 1-2 cm layer differed significantly only from that in the 6-7 cm and 7-8 cm layer; however with substantial OTU overlaps.

No significant differences existed between the bacterial community structure on lugworm exclusion and lugworm control plots (R = 0.007, p = 0.28; Fig. 3C). When the bacterial community structure was explored at a higher vertical resolution (block 1 and 4), a very weak separation between lugworm exclusion and control plots on block 1 was observed when seasons were regarded separately (R = 0.36 in September and R = 0.29 in March, respectively), however this separation was not statistically significant.

Table 4: Analysis of similarity (ANOSIM) of microbial communities at different sediment depths. Results refer to the high-vertical-resolution study based on block 1 and 4.

	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	7-8 cm
0-1 cm	**0.06**	**0.30***	**0.39***	**0.51****	**0.39***	**0.50****	**0.56****
1-2 cm		**-0.04**	**0.10**	**0.19**	**0.19**	**0.25***	**0.29***
2-3 cm			**-0.10**	**-0.06**	**0.01**	**0.03**	**0.06**
3-4 cm				**-0.09**	**-0.06**	**-0.05**	**0.00**
4-5 cm					**-0.10**	**-0.08**	**-0.04**
5-6 cm						**-0.10**	**-0.07**
6-7 cm							**-0.08**

R-values are presented in bold font in the upper part of the table. Stars indicate statistically significant separation of groups at $p < 0.018$ (Bonferroni-corrected). No star = $R < 0.25$, indicates that the groups are not separated; * = R between 0.25 and 0.5, indicates that groups are different but strongly overlapping; ** = $R > 0.5$, indicates that groups are overlapping but clearly different; *** = $R > 0.75$, indicates that groups are well separated.

5.3.4 Overall correlations between biological and environmental parameters

In order to better understand how bacterial community structure, abundances and activities and MPB-related environmental variables were linked on all experimental blocks, significant correlations ($p \leq 0.00055$, Bonferroni-corrected) among environmental and bacteria-related parameters and between these parameters and variation in bacterial community structure were determined in a correlation analysis (Table 5, Fig. 4). The two major NMDS axes used for ordination (Fig. 3 A-C) were included in this correlation analysis.

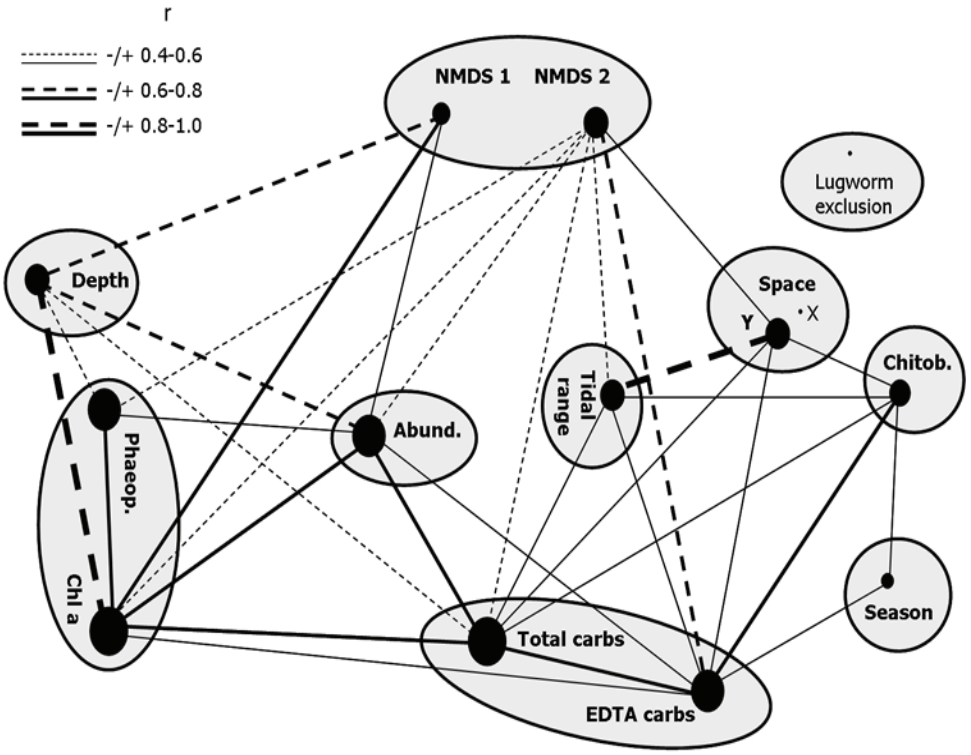

Fig. 4: Correlations between temporal, spatial, environmental and microbial variables measured on all 6 experimental blocks. Only significant Pearson correlations at the Bonferroni- corrected level $P \leq 0.00055$ (0.05/ 91 comparisons) are depicted. Thin, medium and large connecting lines represent significant correlations with coefficients 0.4-0.6, 0.6-0.8 and 0.8-1.0, respectively, while continuous and dashed lines represent positive and negative coefficients, respectively. The size of the dots is proportional to the number of significant correlations associated with a respective variable. The variables included: Seasons (September ’05, March `06), space (x and y coordinates), tidal range, chitobiase (Chitob.), total carbohydrates (total carbs), EDTA extractable carbohydrates (EDTA carbs), bacterial abundance (Abund.), benthic chlorophyll a (Chl a), phaeophytine (Phaeop.), depth and the 2 major axes of the NMDS ordination (Fig. 3) of bacterial community structure (NMDS 1, NMDS2).

Table 5: Pearson correlation coefficients at the Bonferroni-corrected level. Analysis comprises all 6 experimental blocks.

	x coord.	y coord.	Worm pres./abs.	Tidal range	Depth	Time	Chl a	Phaeoph.	EDTA coll. Carbohyd.	Total Carbohyd.	Chitob. activity	Bact. abund.	NMDS axis 1	NMDS axis 2
x coordinate		0.951	0.499	1.000	1.000	1.000	0.570	0.818	0.265	0.840	0.971	0.038	0.710	0.133
y coordinate	**-0.007**		0.612	0.000	1.000	1.000	0.040	0.006	0.000	0.000	0.000	0.009	0.179	0.000
Worm presence/absence	**-0.081**	**0.061**		1.000	1.000	1.000	0.767	0.342	0.085	0.002	0.786	0.586	0.685	0.733
Tidal range	**0.000**	***-0.993**	**0.000**		1.000	1.000	0.040	0.009	0.000	0.000	0.000	0.015	0.161	0.000
Depth	**0.000**	**0.000**	**0.000**	**0.000**		1.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.020
Time	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		0.275	0.009	0.000	0.219	0.000	0.156	0.001	0.083
Chlorophyll a	**0.068**	**0.242**	**0.035**	**0.243**	***-0.927**	**-0.130**		0.000	0.000	0.000	0.002	0.000	0.000	0.000
Phaeophytine	**-0.028**	**0.323**	**0.114**	**0.305**	***-0.505**	**-0.308**	***0.606**		0.132	0.001	0.642	0.000	0.001	0.000
EDTA colloidal carbohydrates	**0.133**	***0.488**	**0.205**	***0.479**	**-0.363**	***0.437**	***0.472**	**0.179**		0.000	0.000	0.000	0.507	0.000
Total carbohydrates	**0.024**	***0.565**	**0.366**	**0.532**	***-0.518**	***0.147**	***0.646**	**0.375**	***0.764**		0.000	0.000	0.030	0.000
Chitobiase acitivity	**-0.004**	***0.417**	**0.033**	***0.410**	**-0.358**	***0.550**	**0.358**	**0.056**	***0.601**	***0.499**		0.052	0.548	0.013
Bacterial abundance	**0.246**	**0.307**	**0.065**	**0.285**	***-0.626**	**-0.169**	***0.711**	***0.553**	***0.498**	***0.624**	**0.230**		0.000	0.000
NMDS axis 1	**0.045**	**-0.160**	**-0.049**	**-0.167**	***-0.726**	**-0.368**	***0.698**	**0.386**	**0.079**	**0.256**	**0.072**	***0.540**		0.983
NMDS axis 2	**-0.179**	***-0.579**	**-0.041**	***-0.581**	**0.274**	**-0.206**	***-0.434**	***-0.495**	***-0.654**	***-0.508**	**-0.292**	***-0.455**	**0.003**	

Pearson correlation coefficients calculated on standardized data are shown in bold font in the bottom part of the table. P-values of correlations are shown in normal font in the upper part of the table. *denotes significant coefficients at the Bonferroni-corrected level ($p < 0.05/91 = 0.00055$). X coordinate and y coordinate = coordinates of the experimental plots, NMDS axis 1 and 2 = major axes of non-metric multidimensional scaling that best explain variation in bacterial community structure.

NMDS axis 1 was significantly correlated with sediment depth (r = -0.726), benthic chlorophyll *a* (R = 0.698) and bacterial abundances (r = 0.54). NMDS axis 2 was significantly correlated with benthic chlorophyll *a* (R = -0.434), benthic phaeophytine (R = -0.495), bacterial abundances (R = -0.455), EDTA extractable carbohydrates (R = -0.654), total carbohydrates (R = -0.508), the tidal range (R = 0.581) and the y axis of the plot coordinates (R = -0.579). None of the NMDS axes was significantly correlated with the presence/absence of *Arenicola marina*. Time and chitobiase activity were weakly correlated to variation in bacterial community structure, however only at a level of $p < 0.05$ with r = 0.368 (NMDS axis 1) and r = -0.292 (NMDS axis 2), respectively.

The importance of those variables that were significantly correlated to ARISA patterns were further confirmed by redundancy analysis (RDA). The total set of environmental variables was thus grouped into different categories and a forward selection process was applied to these categories in order to identify those variables that significantly explained variation in bacterial community structure in RDA. The presence/absence of *Arenicola marina*, the x coordinate of the plot setting and the tidal range were non-significant. All other variables significantly explained variation in bacterial community structure (Table 6). The RDA applied only to the significant environmental variables yielded four main axes that explained 20%, 17%, 10% and 2% of the total variance in bacterial community structure, respectively, whereby the selected environmental variables accounted for 55% of the total variation in the ARISA data.

Detailed information about significant correlations among environmental and bacteria-related parameters is presented in Table 5. Briefly, bacterial abundances, benthic photopigments and the total carbohydrate inventory were negatively correlated with sediment depth, while being positively correlated with each other (except for phaeophytine that was not correlated with total carbohydrates). Bacterial abundances and benthic chlorophyll *a* were also positively correlated with EDTA extractable carbohydrates. Chitobiase activity, total and EDTA extractable carbohydrates were positively correlated with each other and with the y-axis of the plot coordinates, but negatively with tidal range. Tidal range and the y-axis of the plot coordinates were negatively related. Chitobiase activity and EDTA extractable carbohydrates, in addition, exhibited a negative relationship with time. None of the bacteria-related and environmental parameters exhibited a statistically significant correlation with lugworm presence/absence. Only total carbohydrates were weakly correlated with the lugworm presence/absence (R = 0.366) at a significance level of $p < 0.01$.

Table 6: Conditional effects of forwardly selected environmental parameters as determined by RDA. Analysis comprises all 6 experimental blocks.

Environmental Variable	Lambda A	P-value	F-factor
Exclusion/Control	0.020	0.222	1.31
block setting - y coordinate	0.130	0.001*	10.78
block setting - x coordinate	0.020	0.120	1.64
Tidal range (mid intertidal, low intertidal)	0.020	0.275	1.20
Depth	0.170	0.001*	14.36
March 2006 / September 2005	0.120	0.001*	9.91
Chlorophyll a	0.170	0.001*	13.91
Phaeophytine	0.040	0.001*	4.34
EDTA-extractable carbohydrates	0.080	0.001*	7.31
Water-extractable carbohydrates	0.040	0.004*	4.04
Chitobiase activity	0.040	0.001*	3.62

* represents environmental variables that significantly explain variation in bacterial community structure

5.3.5 Respective effects of time, space and lugworm exclusion on variation in bacterial community structure

Non-parametric analysis of similarities (ANOSIM) showed that space and depth and potentially also time affected bacterial community patterns. In order to disentangle the respective effects of each of these factors on variation in bacterial community structure, a variation partitioning analysis was performed (Fig. A1), whereby the model was based on the ARISA data as a function of lugworm presence/absence, depth, time and space (plot coordinates). The amount of variation explained by all these factors was 42% ($p < 0.001$). The pure fractions (i.e. the fraction explained by a factor when removing the effects of all other factors in the model) were responsible for 1% (lugworm presence/absence), 17% (depth), 12% (time) and 14% (space), respectively, of the variation in the bacterial community structure. Thereby, the effect of the lugworm was only marginally significant ($p = 0.05$), while the effect of all other factors was highly significant ($p < 0.001$; Fig. A1).

Comparable results were obtained when a variation partitioning analysis was performed only on data from block 1 and 4 with high vertical resolution (Fig. A2). In this case, the lugworm presence/absence, depth, time and space were able to explain 0%, 5%, 9% and 11%, respectively, of the variation in the ARISA data, whereby the latter 3 were statistically significant ($p < 0.001$). Furthermore, the covarying effect of space with the

lugworm explained additional 2% of the overall structural variation on these two blocks. Although the important role of depth, time and space could be confirmed in this high-resolution study, 74% of the variation in bacterial community structure remained unexplained; only 26% of the overall variation could be explained by this model ($p < 0.001$).

In order to test if any of the bacteria-related or environmental parameters we measured in the high-vertical-resolution study were linked to variation in bacterial community structure, an RDA was performed. In a forward selection procedure applied to different categories of variables, only DIC did not significantly contribute to variation in the bacterial community structure (Table 7).

Table 7: Conditional effects of forwardly selected environmental parameters as determined by RDA. Analysis comprises experimental blocks 1 and 4 as measured at a high vertical resolution.

Environmental Variable	Lambda A	P-value	F-factor
Exclusion/Control	0.05	0.011*	2.97
x coordinate	0.18	0.001*	13.49
y coordinate	0.03	0.017*	2.64
Depth	0.09	0.001*	5.94
Porosity	0.06	0.002*	3.78
March 2006 / September 2005	0.10	0.001*	7.04
Chlorophyll a	0.10	0.001*	6.99
Phaeophytine	0.05	0.001*	4.11
EDTA-extractable carbohydrates	0.05	0.001*	3.86
Water-extractable carbohydrates	0.02	0.020*	2.58
Sulfide	0.06	0.003*	5.11
Dissolved inorganic carbon	0.02	0.106	1.75
NH_4	0.10	0.001*	7.65
NO_3+NO_2	0.00	0.800	0.57
PO_4	0.03	0.037*	2.27
SiO_2	0.03	0.009*	3.32
Chitobiase activity	0.10	0.001*	6.55
β-glucosidase activity	0.05	0.002*	3.63
Sulfate reduction	0.11	0.001*	8.93
Bacterial abundance	0.07	0.001*	4.89

* represents environmental variables that significantly explain variation in bacterial community structure

An RDA applied to the remaining significant variables yielded four main axes that explained 28%, 12%, 4% and 4% of the total variance in the bacterial community data, respectively. Thereby, the selected bacteria-related and environmental variables accounted for 56% of the total variation in bacterial community structure on block 1 and 4.

5.4 Discussion

Multivariate analyses revealed strong effects of space, depth and time on bacterial activities and bacterial community structure on an intertidal sand flat in the present study and we suggest that these effects are mainly mediated by shifts in the abundance and activity of the MPB and thus by the availability of MPB-associated labile organic carbon in the sediment. Coastal sandy sediments often harbor a dense microbial biofilm at the light-exposed, photosynthetically active sediment surface, where diatoms and bacteria are closely associated (Huettel et al. 2003) and where the heterotrophic bacteria rely mainly on the primary production by the MPB. Thereby the carbon flow from microalgae to microbes is believed to be mainly mediated by extracellular polymeric substances (EPS) that can be secreted in substantial amounts by the MPB to promote migration, adhesion to sediment particles, and sediment cohesion (Middelburg et al. 2000, Hoagland et al. 1993, Underwood & Paterson 2003). MPB-derived carbon has been suggested previously to be the main nutrient source for benthic heterotrophic bacteria in coastal sandy sediments (MacIntyre et al. 1996, Underwood & Kromkamp 1999) and one of our previous studies showed that products of primary productivity by the MPB may indeed cover most of the bacterial carbon demand in coastal sands (Böer et al. 2008a).

It was thus not surprising that the strong vertical decrease in MPB biomass (represented by chlorophyll *a*) and MPB-derived carbon (represented by total and EDTA extractable carbohydrates) due to the lack of light (and thus primary productivity) and the decline of advective forcing with increasing sediment depth (as suggested by vertical profiles of MPB-related parameters) in the present study caused a vertical decrease in bacterial abundances and activities. Chitobiase activities were significantly correlated with the carbohydrate inventory. Also, bacterial abundances, chlorophyll *a*, total and EDTA extractable carbohydrates were significantly correlated with each other in the sediment (Table 5; Fig. 4), emphasizing the strong dependency of the bacterial community on primary productivity by the MPB and thus supporting previous findings in Sylt sandy sediments (Böer et al. 2008a). This decrease in MPB-related carbon and bacterial activities was accompanied

by a vertical shift in bacterial community structure. The bacterial community structure in the 0-2 cm layer was significantly different from the bacterial community structure in the layers below. The high-vertical-resolution study on block 1 and 4 thereby revealed that the bacterial community in the 1-2 cm layer differed significantly only from the bacterial community at 6-8 cm depth and showed strong similarities with both the top sediment layer and the adjacent deeper sediment layers, thus reflecting the transition zone of the physico-chemical conditions between surface and subsurface. While the sediment surface is frequently disturbed by wind-induced waves and tidal currents, the deeper sediment layers remain mainly undisturbed from these physical forces. Oxygen was found to penetrate 9-13 mm deep under calm conditions at the sampling site; however enhanced advection in stormier periods was suggested to cause physical advection and deeper oxygen penetration into the sediment (Volkenborn et al. 2007a). The 1-2 cm layer is thus characterized by discontinuous redox potentials and marks the transition zone between the aerobic surface and the anaerobic subsurface. In addition, the bacterial community data was significantly correlated to total carbohydrates and chlorophyll *a* which strongly decreased with depth and were highest concentrated in the top 1-2 cm of the sediment. This vertical shift in the quantity of labile organic matter on the one hand and the bioavailability (presence of reactive electron acceptors) in the sediment on the other hand apparently leads to the development of a bacterial community in the oxic surface layer that is fundamentally different from the bacterial community in the anoxic subsurface layer. We observed such vertical patterns in bacterial community structure before in Sylt subtidal sandy sediments, whereby the observed shifts were more gradual and took place in deeper sediment depths (Böer et al. 2008b). Due to the strong hydrodynamics prevailing at the previous study site, however, MPB-related carbon intruded deeper into the sediment and the nitrate profiles suggested a greater oxygen penetration depth. Multivariate analyses indeed revealed that the depth-related community patterns could mainly be assigned to the depth-related changes in MPB-related organic carbon availability and redox potentials over depth, thus supporting our present findings. Depth-related shifts in bacterial community structure thus seem to be a common feature of coastal permeable sediments densely populated by diatoms, and these shifts in general seem to be governed by MPB-dependent nutrient supply.

Furthermore, our results suggest that sediment characteristics (grain size, MPB-associated carbon) may be responsible for spatial variations in bacterial activities, but also for spatial variations in bacterial community structure. As Kristensen & Kostka (2004) commented, sediment characteristics (porosity, density, grain size distribution, organic matter content) are known to drastically affect biogeochemical processes in marine sediments,

however the impact of sediment properties on microbial community composition is not well explored. We found that the bacterial community structure varied significantly with space on a total area of approximately 500 m x 500 m on the investigated intertidal sand flat. Although in March some block-to-block variation occurred within the tidal zones, across-shore variation (variation between different tidal ranges) was found to be higher than along-shore variation. We thus propose that a large part of the spatial bacterial community variation is a result of differences in sediment characteristics and the therewith associated differences in MPB-associated labile organic carbon content between the different tidal regimes (as represented by chlorophyll *a*, total carbohydrates and EDTA extractable carbohydrates). MPB biomass, MPB-related carbohydrates, and to a lesser extent bacterial abundances and activities were higher in the low intertidal than in the mid intertidal (Table A5 Part I and II, Table 6 Part I a/b and Part II a/b) which can most likely be related to the higher surface-area to volume ratio of the finer grained sediments in the low intertidal compared to the more medium-sized sands in the mid intertidal. These differences in grain size apparently permit the development of a richer microphytobenthic biofilm in low intertidal sediments that in lockstep can support a more active microbial community, especially in the diatom-dominated surface sediments. Variation in bacterial community structure was significantly linked to variations in MPB biomass, carbohydrates, bacterial abundances and extracellular enzymatic activities (Fig. 4, Table 5). We suggest that at least part of the variation in bacterial community structure is based on selective processes related to spatial variation in MBP-associated carbon availability. This is consistent with findings of previous studies that have shown that bacterial communities in marine sediments are influenced by chlorophyll *a* contents (Polymenakou et al. 2005), sediment type (Franco et al. 2007), and sediment water content (Hewson et al. 2007) and emphasizes the necessity of considering sediment characteristics when describing spatial patterns in bacterial communities on a local scale.

Our results also illustrate the importance of considering temporal community shifts when describing spatial patterns of bacterial communities. Time had a strong effect on bacterial activities in this study, with overall higher activities (higher extracellular enzymatic activities and sulfate reduction rates, higher metabolite concentrations) in autumn compared to spring. We believe that this was a direct effect of enhanced primary productivity by the MPB at higher temperatures (as indicated by overall higher total and EDTA extractable carbohydrate concentrations) and the therewith associated increased availability of labile organic carbon to the bacterial community. These increased activities were not reflected in MPB and bacterial biomass which we interpret to be the result of an overall enhanced benthic

metabolism and extensive grazing on diatoms and bacteria during summer. The temporal shifts in microphytobenthic and bacterial activities were accompanied by a significant overall shift in the bacterial community structure on all experimental blocks. While in September spatial differences in bacterial community structure mainly occurred between blocks in different tidal regimes, the bacterial community in March exhibited a stronger block-to-block variation. This suggests that under overall organic-rich conditions at warmer temperatures, spatial variation in MPB-associated carbon availability in intertidal sands becomes less pronounced and accordingly space becomes a less important determinant of bacterial community structure, at least on a local scale. Seasonal differences in bacterial community composition have been described previously (Franco et al. 2007), and were found to be stronger in fine sediments than in coarse sediments, indicating that time may be of general importance for bacterial communities in temperate coastal sediments, whereby the impact of time may differ between sediment types. However, if time affects bacterial community structure (or composition) on shorter temporal scales (seasons) and on local spatial scales already, this rises new questions when describing spatial patterns of bacteria on larger (e.g. continental) scales and proposes that large-scale bacterial community data has to be interpreted with care, especially when it is known that samples were taken under different seasonal conditions or when the seasonal conditions are unknown. Description of large-scale community patterns including repetitive sampling over time could lead to new insights into this interesting aspect of microbial ecology.

Worm burrows have been shown previously to support the development of a microbial community that is fundamentally different from the nearby surface and subsurface sediments (Steward et al. 1996, Matsui et al. 2004, Papaspyrou et al. 2007), whereby these differences could be directly related to burrow maintenance and feeding activities. Volkenborn et al. (2007a) showed that the effects of *Arenicola marina* on sediment properties, such as grain size and permeability, however, may reach well beyond the vicinity of the burrows. We were thus interested in the large-scale effects on bacterial activities and community structure by *Arenicola marina* in order to see whether the large-scale changes in sediment properties affect the bacterial community. Previous investigations on the Königshafen experimental plots showed that *Arenicola marina* through its bioturbation and grazing reduces microphytobenthic biomass and prevents a clogging of the sediment with fine particles and associated organic material. Permeability thus remained high in the presence of the worms, facilitating advective exchange of overlying and porewater and enlarged the oxic, metabolically most active layer. The sediment was much more stable in the absence of the

worm, as was reflected in a smaller median grain size and a higher silt fraction. Consequently, enhanced particulate organic carbon and chlorophyll contents were found in the surface layer of exclusion plots compared to disturbed controls (Volkenborn et al. 2007b). Our results suggests that the development of a dense microphytobenthic biofilm in the fine-grained surface layer of lugworm exclusion plots indicated by higher extracellular enzymatic activities and DIC concentrations in the present study fuels organic matter mineralization by the benthic microbes. High amounts of EDTA extractable carbohydrates in the upper sediment layers of lugworm exclusion plots indeed suggest that EPS production was higher on non-bioturbated plots, where it potentially stimulated microbial growth. This phenomenon of highly reactive zones in the surface layer of undisturbed sediments confirm earlier studies by Papaspyrou et al. (2007) and Kristensen et al. (2000). Bacterial abundances were not affected by the lugworm, contrasting with findings by Goñi-Urriza et al. (1999) who found bacterial abundances to be approximately one order of magnitude higher on non-bioturbated sites compared to bioturbated controls. Grazing (Epstein 1997) and viral lysis (Fuhrman 1999) however, may have masked the effect of the lugworm on bacterial abundances. Although MPB biomass and MPB-associated carbohydrates in surface sediments were higher on lugworm exclusion plots than on control plots, presumably due to the higher surface-area to volume ratio of fine grains accumulating in the absence of the worm, and although bacterial activities responded to this enhanced carbon supply and were higher in the top layer of exclusion plots than on control plots, bacterial community structure did not reflect this overall pattern and was not significantly affected by lugworms in any of the depth layers. Possibly, the strong invasion of *Nereis diversicolor* on lugworm exclusion plots (Volkenborn & Reise 2006) maintained general habitat properties and prevented a severe bacterial community shift. However, the impact of *Arenicola marina* on sediment properties was limited to the sediment surface, where MPB biomass and productivity is generally highest. Presumably, the magnitude of the lugworm-mediated modification in organic carbon and oxygen availability was basically too small to cause noticeable bacterial community shifts. Still, we were only able to resolve qualitative changes in the bacterial community. Although community structure was not affected by the lugworm, single functional groups may have been stimulated in either the presence or absence of *Arenicola marina*.

In conclusion, we were able to show that temporal, vertical and spatial shifts in MPB-associated organic carbon availability affect bacterial activities and distribution in intertidal sands and that these variations in general bacterial community functions are accompanied by shifts in the bacterial community structure. Future work is needed to look

closer at the impact of these factors on bacterial community composition in sandy sediments and to identify the taxonomic levels at which environmental control takes place in order to gain a better understanding of community-function relationships.

5.5 Acknowledgements

Susanne Menger is gratefully acknowledged for assistance in the field and with sample processing. Hannelore Halliger and Reimer Magens supported our work on Sylt logistically and technically. Justus van Beusekom is gratefully recognized for providing lab space during our field campaigns on Sylt. Oleg Dmytrenko is thanked for performing microscopic cell counts. Aerial photo material was kindly provided by the Wadden Sea National Park Office. This study was financed by the Max Planck society.

5.6 References

Austen I (1994) The surficial sediments of Königshafen – variations over the past 50 years. Helgol Meeresunters 48:163-171

Banta GT, Holmer M, Jensen MH, Kristensen E (1999) Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment. Aquat Microb Ecol 19:189-204

Böer SI, Arnosti C, van Beusekom JEE, Boetius A (2008a) Temporal variations in microbial activities and carbon turnover in permeable sandy sediments. Biogeosciences Discussions 5: 4271-4313 (Chapter 3)

Böer SI, Hedtkamp SIC, van Beusekom JEE, Fuhrman JA, Boetius A, Ramette A (2008b) Temporal and depth variations of bacterial activities and community structure in coastal sandy sediments. submitted to The ISME Journal (Chapter 4)

Boetius A, Lochte K (1994) Regulation of microbial enzymatic degradation of OM in deep-sea sediments. Mar Ecol Prog Ser 104:299-307

Boetius A, Lochte K (1996) Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments. Mar Ecol Prog Ser 140:239-250

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73(3):1045-1055

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27(4):325-349

Cardinale M, Brusetti L, Quatrini P, Borin S, Puglia AM, Rizzi A et al. (2004). Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Appl Environ Microbiol 70(10) 6147-6156

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143

Clarke KR, Warwick RM (2001) Change in Marine Communities. An Approach to Statistical Analysis and Interpretation. 2nd edition: PRIMER-E. Plymouth. UK

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454-458

Donachie SP, Foster JS, Brown MV (2007) Culture clash: challenging the dogma of microbial diversity. The ISME Journal 1:97-99

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356

Epstein SS (1997) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb Ecol 34:188-198

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65(10):4630-4636

Fossing H, Jørgensen BB (1989) Measurement of Bacterial Sulfate Reduction in Sediments - Evaluation of a Single-Step Chromium Reduction Method. Biogeochemistry 8:205-222

Franco MA, de Mesel I, Demba Diallo M, van der Gucht K, van Gansbeke D, van Rijswijk P et al. (2007). Effect of phytoplankton bloom deposits on benthic bacterial communities in two contrasting sediments in the southern North Sea. Aquat Microb Ecol 48:241-254

Fuhrman JA (1999). Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548

Goñi-Urriza M, de Montaudouin X, Guyoneaud R, Bachelet G, de Wit R (1999) Effect of macrofaunal bioturbation on bacterial distribution in marine sandy sediments, with special reference to sulphur-oxidising bacteria. J Sea Res 41:269-279

Grasshoff K, Kremling K, Ehrhardt M (1999) Methods of seawater analysis. Wiley-VCH Verlag. Berlin, Germany

Green J, Bohannan BJM, Whitaker RJ (2008) Microbial biogeography: From taxonomy to traits. Science 320(5879):1039-1043

Hall POJ, Aller RC (1992) Rapid, small-volume, flow injection analysis for ΣCO2 and NH4+ in marine and freshwaters. Limnol Oceanogr 37:1113-1119

Hewson I, Fuhrman JA (2005) Improved strategy for comparing microbial assemblage fingerprints. Microb Ecol 51:147-153

Hewson I, Jacobson/Meyers ME, Fuhrman JA (2007). Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ Microbiol 9(4): 923-933

Hill MO, Gauch HG jr. (1980) Detrended correspondence analysis: an improved ordination technique. Plant Ecol 42(1-3):47-58

Hoagland KD, Rosowski JR, Gretz MR, Roerner SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J Phycol 29(5):537-566

Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33(5):1225-1228

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar Ecol Prog Ser 11:299-308

Huettel M, Røy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia 494:231-236

Jørgensen, B.B. 2001. Life in the diffusive boundary layer, p. 144-179. *In* B. P. Boudreau and B. B. Jørgensen, [eds.]. The benthic boundary layer: transport processes and biogeochemistry. Oxford Univ. Press.

Kallmeyer J, Ferdelman TG, Weber A, Fossing H, Jørgensen BB (2004) A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements. Limnol Oceanogr Methods 2:171-180

Kristensen K, Hansen K (1999) Transport of carbon dioxide and ammonium in bioturbated (*Nereis diversicolor*) coastal, marine sediments. Biogeochemistry 45:147-168

Kristensen K, Andersen FO, Holmboe N, Holmer M, Thongtham N (2000) Carbon and nitrogen mineralization in sediments of the Bangrong mangrove area, Phuket, Thailand. Aquat Microb Ecol 22:199-213

Kristensen E, Kostka (2004) Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions. In: The Ecogeomorphology of Tidal Marshes. Coastal and Estuarine Studies 59. American Geophysical Union

Lawton JH (1994) What do species do in ecosystems? Oikos 71:367–374

Legendre L, Legendre P (1998) Numerical Ecology. 2nd English Edition. Elsevier Science BV. Amsterdam, The Netherlands

Lorenzen CJ (1967) Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol Oceanogr 12(2):343-346

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. 1. Distribution, abundance and primary production. Estuaries 19:186-201

Matsui GY, Ringelberg DB, Lovell CR (2004) Sulfate reducing bacteria in tubes constructed by the marine infaunal polychaete Diopatra cuprea. Appl Environ Microbiol 70:7053-7065

Meysman FJR, Galaktionov OS, Gribsholt B, Middelburg JJ (2006) Bioirrigation in permeable sediments: Advective pore-water transport induced by burrow ventilation. Limnol Oceanogr 51(1):142-156

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMT, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr 46(6):1224-1234

Papaspyrou S, Kristensen E, Christensen B (2007) *Arenicola marina* (Polychaeta) and organic matter mineralization in sandy marine sediments: In situ and microcosm comparison. Est Coast Shelf Sci 72:213-222

Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005). Links between geographic location, environmental factors, and microbial community composition in sediments of the eastern Mediterranean Sea. Microb Ecol 49: 367-378

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62(2):142-160

Ramette A, Tiedje JM (2007a) Biogeography: An emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. Microb Ecol 53(2): 197-207

Ramette A, Tiedje JM (2007b) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. PNAS 104(8):2761-2766

Reise K (1985) Tidal Flat Ecology. Springer. Berlin. p. 191

Steward CC, Nold SC, Ringelberg DB, White DC, Lovell CR (1996) Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments. Mar Ecol Prog Ser 133:149-165

ter Braak CJF, Šmilauer P (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canocical Community Ordination, Version 4.5

Underwood GJC, Paterson DM, Parkes RJ (1995) The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnol Oceanogr 40(7):1243-1253

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries. In: Nedwell DB, Raffaelli D (ed) Advances in Ecological Research. Vol 29. Academic Press Inc. San Diego, USA. pp 93-153

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. In: Callow JA (eds.) Advances in Botanical Research, Vol. 40. Advances in Botanical Research Incorporating Advances in Plant Pathology

Volkenborn N, Reise K (2006) Lugworm exclusion experiment: Responses by deposit feeding worms to biogenic habitat transformations. J Exp Mar Biol Ecol 330:169-179

Volkenborn, N., Reise, K. (2007) Effects of *Arenicola marina* on polychaete functional diversity revealed by large-scale experimental lugworm exclusion. J Sea Res 57:78-88.

Volkenborn N, Polerecky L, Hedtkamp SIC, van Beusekom JEE, de Beer D (2007a) Bioturbation and bioirrigation extend the open exchange regions in permeable sediments. Limnol Oceanogr 52(5):1898-1909

Volkenborn, N., Hedtkamp, SIC., Beusekom, JEE van, Reise, K (2007b) Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. Est Coast Shelf Sci 74(1/2):331-343.

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. PNAS 95:6578-6583

Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. Appl Environ Microbiol 71(1):227-239

Chapter 6

Summary and Outlook

Summary

Within the last two decades, the importance of coastal sandy sediments for oceanic and global carbon and nutrient cycles has been increasingly recognized. The microphytobenthos and diverse heterotrophic bacteria are the key players in this ecosystem, however temporal and spatial patterns in microbial carbon turnover and the link to dynamics in community structure are not well constrained.

In the first part of this thesis (Chapter 2 and Chapter 3), the role of the microphytobenthos (MPB) as a carbon and energy source for the bacterial community was explored, and it was investigated to which extent the spatial and temporal distribution of the MPB controls bacterial distribution and activity. The second part (Chapter 4 and Chapter 5) focused on the temporal and spatial variations in bacterial community structure and was aimed at identifying the main environmental drivers of these dynamics.

Part I: The role of the MPB as a carbon and energy source for the bacterial community in coastal sands

Main objectives:

- To explore the role of the microphytobenthos as a carbon and energy source for the bacterial community in coastal sandy sediments.
- To study vertical and seasonal changes in microbial activities in coastal sands.
- To determine the main environmental drivers for temporal and spatial variations in microbial activities in this ecosystem.

Summary Part I

Previous studies showed that advective interfacial flows in coastal sandflats may carry suspended planktonic and benthic diatoms as well as bacteria into permeable sediments, indicating that advective particle transport may play an important role for the carbon budget of sandy shelf sediments (Rusch & Huettel 2000, Huettel & Rusch 2000, Rusch et al. 2001, Ehrenhauss & Huettel 2004). Phytoplankton bloom simulation experiments with non-labeled and ^{13}C-labeled diatoms proposed that settling phytoplankton may indeed be rapidly remineralized by benthic microbes (Ehrenhauss et al. 2004, Bühring et al. 2006). Variations of the organic matter load in the water column has been frequently shown to influence benthic mineralization rates in Wadden Sea sediments (Kristensen et al. 1997), and a range of *in situ*

studies confirmed that the deposition of phytoplankton detritus stimulates microbial activities in coastal sands (Meyer-Reil 1987, van Duyl et al. 1992, Rooney-Varga et al. 2005). Investigations on a subtidal sandflat in the present study (the so-called *Hausstrand*) showed, however, that even during spring bloom situations, the input of carbon via pelagic phytodetritus would be less than a third of the microphytobenthic (MPB) standing stock (Chapter 3; de Beer et al. 2005). Estimations by Werner et al. (2005) suggested that approximately 50% of the organic carbon that is remineralized within *Hausstrand* sediments may be provided by benthic photosynthesis. Others suggested that primary production by the MPB is the major organic carbon source for benthic heterotrophic bacteria in light-exposed sandy sediments (MacIntyre et al. 1996, Underwood & Kromkamp 1999). Fluxes of carbon are generally believed to be promoted by the excretion of extracellular polymeric substances (EPS) by the MPB. EPS consists mainly of carbohydrates and is primarily produced for migration or adhesion purposes (Hoagland et al. 1993, Underwood & Paterson 2003). In addition, increased amounts of carbohydrates may be released by the MPB in order to maintain cell-nutrient balances upon nutrient-depletion (Ortega-Calvo & Stal 1994, Smith & Underwood 1998).

The first study of this thesis (Chapter 2) indeed confirmed that fixed carbon excreted by the MPB is rapidly utilized by bacteria. The uptake of ^{13}C by bacteria and microphytobenthos (MPB) was traced by analysis of ^{13}C incorporation into hydrolysable amino acids after addition of a $H^{13}CO_3^-$ pulse to sediment mesocosms incubated under nutrient-replete and nutrient-limited conditions. A significant and rapid transfer of ^{13}C from the MPB to bacterial biomass was observed in both treatments within 24 h of label addition, revealing that fixed carbon excreted by the MPB was rapidly utilized by bacteria. Under nutrient deplete conditions, high rates of photosynthesis were sustained, however cellular growth stopped (as defined by the synthesis of chlorophyll *a* and amino acids) and the carbon fixed consisted predominantly of carbohydrates produced through “overflow metabolism” in order to maintain cell-nutrient balances. Under nutrient-replete conditions, algal growth was stimulated and label incorporation was more balanced, with carbohydrates accounting for a much smaller fraction of newly fixed organic carbon. The ratio of bacterial production to primary production was generally twice as high under nutrient-limited conditions as under nutrient-replete conditions, indicating a stronger coupling between the MPB and the bacteria through the increased exudation of dissolved organic carbon (DOC) by the MPB. Although faunal grazing may lead to cell lysis and thus a release of DOC, representing an additional mechanism through which carbon fixed by photoautotrophs can be transferred to

heterotrophic bacteria, a lack of macrofauna and low phaeophytine concentrations in the mesocosms suggested that grazing was most likely negligible in the experiment. A close agreement between net carbon fixation based on O_2 fluxes and the increase of particulate organic carbon in the sediment under both nutrient-limited and nutrient-replete conditions further suggested that very little fixed carbon was lost to the water column as DOC which was consistent with direct measurements of DOC release using ^{14}C. Although resuspension and advection may play an important role in disaggregating and dissolving EPS under natural *in situ* conditions and stimulate DOC release from the sediment, the results of the present mesocosm study are still valuable to indicate a very tight coupling between algal and bacterial production. Even under nutrient-replete conditions, the MPB exuded a large fraction of the carbon fixed which subsequently served as a growth substrate for the heterotrophic bacterial community.

This tight coupling between microphytobenthic and bacterial production was not restricted to the artificial conditions imposed in the mesocosm experiment, but could be confirmed in the second *in situ* study on the *Hausstrand*, a subtidal sand flat at the coast of Sylt (Chapter3). Despite the strong hydrodynamic forces of tides and wind-induced waves, the sandy sediments at the sampling site showed persistent vertical gradients in bacterial abundances, bacterial carbon production and extracellular enzymatic activities at all times. Although vertical gradients in bacterial biomass and bacterial growth have been observed in sandy sediments at various sites in the North Sea (20 to 350 m water depth; van Duyl & Kop 1994), such depth-patterns are rather untypical for surge-exposed coastal stations (e.g. Rusch et al. 2003, Musat et al. 2006, Misic & Harriague 2007, Franco et al. 2007). At *Hausstrand*, these vertical gradients were significantly and tightly correlated to the benthic chlorophyll *a* concentrations which serve as a proxy for the availability of labile organic carbon (Boon & Duineveld 1996). The observed vertical gradients are thus most likely the result of a biofilm-like association of the MPB with the benthic bacteria (Huettel et al. 2003) and the vertical distribution of the MPB-associated labile organic carbon at the field site. There was no tight correlation between MPB-derived carbohydrates (total and EDTA extractable carbohydrates) and bacterial biomass or activities in *Hausstrand* sediments, however previous studies showed dramatic diurnal dynamics in EPS concentrations in Wadden Sea sediments and as such implied a rapid production of EPS by the MPB and a subsequent rapid consumption by the bacteria (van Duyl et al. 1999). Therefore a high production of EPS is not necessarily reflected in high EPS standing stocks.

Despite the low organic carbon content typical for surge-exposed sandy sediments, high extracellular enzymatic activities and bacterial carbon production rates indicated a very active heterotrophic bacterial community, with a gross secondary productivity of 30-180 mmol C m^{-2} d^{-1}, and a biomass turnover time of 2-18 days. This corresponds to previous findings which demonstrated that the low organic matter content at sandy sediment sites reflects high microbial turnover rather than sediment inactivity (Huettel et al. 1998, Boudreau et al. 2001, Dauwe et al. 2001, de Beer et al. 2005). The present data suggest that these high turnover rates are generated by the rapid flux of carbohydrates from primary productivity by the MPB to the benthic bacteria. Assuming a bacterial growth yield (carbon assimilation efficiency) of 50%, as is commonly used for heterotrophic benthic bacteria in surface sediments (Moriarty et al., 1985), the average total bacterial carbon demand was 120 mmol C m^{-2} d^{-1} (peaking in summer with up to 360 mmol C m^{-2} d^{-1}). On average, this demand could be covered by a daily turnover of 30% of the EDTA extractable carbohydrate pool (in summer up to 125%), 6% of the total carbohydrate pool or 11% of the MPB biomass. On average, potential extracellular enzymatic hydrolysis of carbohydrates could supply approximately 110% of the total bacterial carbon demand, as calculated from the combined action of chitobiase, α-glucosidase, β-glucosidase and laminarinase activities. Of course other substrate sources than carbohydrates may be utilized by the bacterial community, such as dissolved organic carbon and proteinaceous materials released upon grazing, and the activities of enzymes not measured in this study may play a critical role for bacterial nutrition. Nethertheless, these turnover rates estimates support the general assumption that EPS is an important growth substrate for benthic bacteria in light-exposed sandy sediments (MacIntyre et al., 1996; Underwood and Kromkamp, 1999) and comply with the results of the mesocosm experiment.

Bacterial carbon production rates and extracellular enzymatic activities exhibited strong seasonal variations and were strongly enhanced in summer. The benthic oxygen consumption rates of roughly 5-21 mmol C m^{-2} d^{-1} measured in the present study (although most likely underestimated due to the experimental set-up), followed the same seasonal trend, suggesting that the major part of oxygen consumption was due to bacterial respiration. Seasonal patterns in benthic oxygen consumption rates have frequently been observed for sandy sediment sites. For example, Werner et al. (2005) found potential oxygen consumption rates to be approximately 10-20 times higher in summer than in winter. Comparable results were obtained by Rusch & Huettel (2000) who obtained seasonally varying rates between 2 and 50 mmol C m^{-2} d^{-1} in natural sands of the Königshafen bay in the north of Sylt. Although

higher temperatures may directly stimulate biological and chemical processes, the enhanced bacterial activities in this study are presumably in large parts the result of a more productive MPB community in summer. This is supported by previous studies at *Hausstrand* which showed that benthic photosynthesis varies seasonally with higher rates in summer compared to autumn or spring (Billerbeck, Wenzhöfer, unpublished data). As a further line of evidence, *in situ* Q_{10}-values derived from the relations between extracellular enzymatic activities and temperature at the *Hausstrand* well exceeded the ranges in Q_{10}-values that were determined for *Hausstrand* sediments in a temperature experiment, suggesting that an increased substrate availability in summer stimulated extracellular enzymatic activities. A lack of temporal variation in MPB and bacterial biomass contrasted with these changes in microphytobenthic and bacterial activity patterns. This could be either caused by light and space limitation in the biofilm-type community or by extensive grazing on the bacterial and MPB assemblages. Nano- and meiofauna have been found in high abundances in permeable coastal sediments (Epstein 1997, Cahoon 1999, Middelburg et al. 2000, Urban-Malinga et al. 2006) and Evrard (2007) showed in a radiolabeled tracer study that indeed part of the MPB at *Hausstrand* is grazed upon by the benthic meiofauna. These results suggest that grazers may control bacterial and MPB standing stocks at *Hausstrand* and that these standing stocks do not necessarily reflect productivities.

In conclusion, it could be demonstrated that microbial activities in coastal sandy sediments may exhibit strong temporal and depth-related variations and that these variations are tightly linked to the primary productivity of the MPB. Carbohydrates excreted by the microphytobenthic algae were found to be rapidly utilized by the heterotrophic microbial community in a radiolabeled tracer experiment, and data from a long-term *in situ* study confirmed that the MPB indeed plays an important role as a carbon and energy source for the bacterial community in coastal sands.

Part II: Temporal and spatial variations in bacterial community structure in coastal sands and their causes

Main objectives:

- To gain an understanding of variations in bacterial diversity in coastal sandy sediments.

- To investigate vertical patterns in bacterial community structure in coastal sands and their causes.
- To study temporal dynamics in bacterial community structure and to explore whether they are linked to seasonal changes in microbial activities.
- To study spatial variability in bacterial community structure and activity in relation to macrofauna distribution
- To determine the main environmental drivers for temporal and spatial variations in bacterial community structure in coastal sandy sediments.

Summary Part II

The vertical and temporal changes in microbial activities at *Hausstrand* were accompanied by significant shifts in bacterial community structure as determined by automated ribosomal intergenic tracer analysis (ARISA) and demonstrated in the 3rd study of this thesis (Chapter 4). This study included the six sampling dates examined already in the 2nd *Hausstrand* study dealing with temporal changes in bacterial carbon turnover, plus two earlier sampling dates. Twenty-two contextual parameters were included in the analyses to determine the environmental drivers of these changes.

The bacterial community structures at 0-5 cm, 5-10 cm and 10-15 cm sediment depth differed significantly, although there were substantial operational taxonomic unit (OTU) overlaps between these three layers. The main part of the bacterial community was found to inhabit the entire depth range: 40% percent of OTUs were present on average in all three depth layers on a monthly basis. These OTUs probably represent bacterial taxa that are able to utilize a wide range of organic substrates and electron acceptors for active growth and may tolerate aerobic as well as anaerobic conditions. While the medium and upper layer shared 7% and the medium and deepest layer shared 12% of OTUs, an average of 13%, 3% and 20% of OTUs were uniquely found in the upper, medium and deepest layer, respectively, most likely representing bacteria that were restricted to the specific biochemical and physical conditions prevailing in these sediment horizons. When considering the complete sampling cycle, the OTUs showed more frequent overlaps between depth layers, thus revealing vertical dynamics of the bacterial community over time, most likely influenced by sediment mixing and the involved shifts in oxygen and chemical gradients. Accordingly, benthic chlorophyll *a,* bacterial abundances, extracellular enzymatic activities and NO_3 exhibited strong and persistent vertical gradients. NO_3 can be seen as an indicator of the redox state of the sediment, and represents the depth of downward mixing of oxygen-rich bottom water.

Flushing of the sediments may influence bacterial community structure directly by selecting for aerobic or at least oxygen-tolerant species. Second, the microphytobenthic algae have been shown to play an important role as a carbon and energy source for the benthic bacteria inhabiting coastal sands in Part I of this thesis (Chapter 2 and Chapter 3) and elsewhere (Underwood & Kromkamp 1999, Köster et al. 2005). Depth-related decreases in chlorophyll *a* concentrations, bacterial abundances, growth rates and extracellular enzymatic activities indicate vertical shifts in both the quality and the nutritional quality of organic matter in the sediment. Both the quantity and quality of organic matter, however, have been shown to influence bacterial community structure previously (Muylaert, 2002; Crump, 2003; Lebaron, 1999) and the results of the present study suggest that the vertical shifts in MPB biomass and thus in MPB-associated organic carbon have, besides the redox state of the sediment, a strong effect on the spatial structuring of the bacterial community in subtidal sands. Consistently, a variation partitioning analysis demonstrated that 27% of the total variation in bacterial community structure at *Hausstrand* could be explained by the covarying effect of depth, bacteria (bacterial abundance) and sediment chemistry (including NO_3, chlorophyll *a*) while the independent effects of these 3 factors contributed with 1%, 2% and 6% to the overall variation in bacterial community structure (however only sediment chemistry significantly).

Although it can be generally assumed that the number of ecological niches and food resources is higher in the top layers of sediment, as a result of the presence of meio- and macrofauna and the availability of a large variety of different organic resources (labile and refractory, of floral, faunal, microbial and terrestrial origin) and electron acceptors, OTU richness was significantly higher in the deeper layer (10-15 cm) as compared with the top 10 cm, which is presumably a result of the relative stability of deeper layers less subjected to hydrodynamic forces and bioturbation and indicates that bacterial species richness may be spatially structured on the scale of centimeters. Strong hydrodynamic pressures predominating at *Hausstrand* lead to a constant vertical and horizontal mixing of the upper 5 cm of sediment (Hedtkamp 2005), probably selecting for bacteria that are able to cope with occasional resuspension, physical abrasion generated by moving sediment particles, grazing, fluctuating concentrations of oxygen and of organic and inorganic nutrients. Sediment permeabilities between 1 and 3.4 $\times 10^{-11}$ m^2 (Hedtkamp, 2005) suggest that advection and bioirrigation/bioturbation caused by the activity of the benthic fauna are the main transport processes occurring in the upper 10 cm of sediment (Huettel et al. 2003). The medium layer may thus still be constantly disturbed and homogenized by mixing and flushing, while the deepest layer may provide a temporally undisturbed, heterogeneous habitat dominated by

diffusive solute transport. This temporal stability potentially allows for the development of a more diverse bacterial community. These findings are consistent with general beliefs in ecology, namely that ecosystem diversity is reduced by strong disturbance (Connell 1978, McCabe & Gotelli 2000, Death & Winterbourn 1995, Townsend et al. 1997). An increased OTU richness with sediment depth has been found previously in other coastal areas around the globe (Urakawa et al. 2000, Franco et al. 2007, Julies et al. unpublished data), thus supporting the present findings.

Bacterial community structure also underwent strong temporal changes over the 2-year study period. Out of a total pool of 451 OTUs identified over the entire 2-year sampling period, between 38% and 68% of OTUs were detected at individual sampling dates, revealing substantial temporal variation in OTU numbers in the top 15 cm of sediment. Only 22% of all OTUs could be detected at all sampling dates. OTU numbers generally varied between 178 and 246, with only a small number of OTUs (0-8) that were unique to any of the sampling dates (with the exception of August 2004 where 65 OTUs were unique to the sampling date). These numbers resembled OTU numbers previously detected by ARISA in the shallow oligotrophic benthos (~236 OTUs; Hewson et al. 2003), however were higher than those in coral reef surface sediments (51-148 OTUs; Hewson & Fuhrman 2006). Unlike microbial activities, temporal changes in bacterial diversity did not show any cyclical patterns as could have been envisioned from the substantial seasonal changes in temperature and substrate availability at the field site. The variable part of the bacterial community was neither gradually lost or gained over time, nor did OTU occurrence generally follow seasonal patterns, so that specific OTUs could be exclusively associated with either summer or winter sampling months. This result is different from ARISA studies of temporal variation of bacterioplankton diversity, which found a predictable combination of bacterial OTUs at specific times of the year (Fuhrman et al. 2006). Community dynamics were, however, found to be stronger between the first three sampling dates than towards the end of the experiment. Almost 37% of the entire OTU pool was exclusively detected within the first three sampling months (August and October 2004, February 2005), while community structure between April 2005 and March II 2006 was in general temporally more stable with 50% of OTUs being constantly present within these 5 months and between 76% and 95% of common OTUs. Indeed, the eight sampling months formed three distinct groups that varied significantly in their bacterial community structure. While group 1 contained only samples from August 2004, group 2 contained all samples deriving from October 2004 and February 2005 and group 3 contained all remaining sampling months (April 2005, July 2005, November 2005, March I

and II 2006). While these groups were highly separated concerning their bacterial community structure, sampling months within group 2 and 3 were rather similar. Regarding these results, it seems that the bacterial community underwent a development from a more dynamic towards a more stable bacterial community between August 2004 and March II 2006. The reason for this development remains unknown and is not clearly reflected in the variations of any of the contextual parameters. A multivariate analysis showed that time alone significantly explained variation in the bacterial community structure with twenty percent of the variation. However, no single other contextual parameter, or a combination thereof, could be identified, explaining the observed temporal variation. Unfortunately, the strong dynamics at the beginning of the study cannot be clarified in retrospect. The data set as present implies, that it was dynamic interactions between the members of the bacterial community themselves that led to temporal changes in bacterial community structure which were independent from external environmental parameters. Such independent dynamics have been observed previously in chemostat-bioreactors. For instance, succession in methanogenic populations was observed in a functionally stable, well-mixed methanogenic reactor (Fernández et al., 1999). Such a stable performance implies a steady-state production and consumption of metabolites along the trophic chain, thus changes in populations are presumably caused by variations in particular metabolites, their rates in production and consumption and the therewith associated interspecific competitions between the community members, suggesting similar dynamics for natural ecosystems. Interestingly, despite the high non-cyclical dynamics in bacterial community structure observed here in the subtidal sands, general ecosystem functions mediated by bacteria such as organic matter remineralization (oxygen consumption, enzymatic activity) and productivity (bacterial growth) were not affected, but followed clear seasonal patterns, thus indicating a high functional redundancy. Consistently, previous studies found aggregated functions (e.g. community biomass and respiration) to be insensitive to community composition (Langenheder et al. 2006), reflecting the patterns observed here. Nethertheless, it cannot be excluded that important factors other than those measured in this study were overlooked. Biological factors such as grazing pressure and viral lysis have been observed previously to influence bacterial community structure in aquatic ecosystems (Weinbauer & Rassoulzadegan, 1998, Jürgens et al., 1999; van Hannen et al., 1999; Hahn & Höfle, 2001) and in the marine benthos (Hewson et al. 2003), but were not measured here. The relatively weak temporal changes in MPB and bacterial biomass at *Hausstrand* in comparison to the strong seasonal changes in activities (see Part I) suggest that grazing and/or viral lysis may indeed play a role at the study site. Furthermore, bacterioplankton community

structure has been shown to change relatively fast in response to the seasonal phytoplankton succession, indicating that dissolved organic matter from different algal sources may select for different bacteria (Pinhassi et al. 2004, Abell & Bowman 2005). The strong relationship between the bacterial community and the MPB observed in the present thesis suggests that a relationship like this might also be possible for the benthic ecosystem at *Hausstrand*. The impact of biotic factors on bacterial community structure, however, was well beyond the scope of this study. Still, biotic factors seem to be an interesting aspect to keep in mind for future research plans on bacterial diversity in coastal sands.

The results of the *Hausstrand* studies were largely confirmed by the 4th study of this thesis (Chapter 5) which was performed in the framework of a large-scale *Arenicola marina* exclusion experiment on an intertidal sandflat in the *Königshafen* bay at the northern tip of Sylt. This study again revealed strong effects depth and time, but additionally also of space on bacterial activities and bacterial community structure. Overall, depth, time and space explained variation in bacterial community structure to comparable extends with 17%, 12% and 14%, respectively, as revealed by variation partitioning analyses. These effects were mainly mediated by shifts in the abundance and activity of the MPB and thus by the availability of MPB-associated labile organic carbon in the sediment. Like observed for the *Hausstrand* site, bacterial distribution and activities were strongly correlated to the distribution of the MPB and MPB-derived carbohydrates and the vertical decrease in bacterial activities was accompanied by a vertical shift in bacterial community structure. The bacterial community structure in the oxic, MPB-rich surface layer was significantly different from that in the anoxic zone while the physico-chemical transition zone (1-2 cm) was characterized by a bacterial community that shared features of both the oxic surface and the anoxic subsurface. This generally reflects the patterns observed also at *Hausstrand* where the transition, however, was more gradual and occurred in deeper sediment layers, most likely as a result of the very strong hydrodynamics prevailing at this site. Also, temperature-related changes in bacterial activities between autumn and spring were accompanied by a temporal shift in bacterial community structure, and the data suggests that this shift can be at least partly explained by a temperature-related shift in MPB productivity. As a new aspect of the *Königshafen* study, bacterial activities and bacterial community structure appeared to be strongly influenced by spatial variations in sediment composition. The development of a richer MPB biofilm was observed in fine-grained sediments of the low intertidal compared to medium-sized sands in the mid intertidal and this richer MPB biofilm supported a more active bacterial community. The differences in sediment characteristics and MPB-associated carbon content were also

responsible for a large part of the spatial variations in bacterial community structure observed in this study (250000 m^2 total area). This is consistent with findings of previous studies that showed that bacterial communities in marine sediments are influenced by chlorophyll *a* contents (Polymenakou et al. 2005), sediment type (Franco et al. 2007), and sediment water content (Hewson et al. 2007), thus emphasizing the necessity of considering sediment characteristics when describing spatial patterns in bacterial communities on a local scale. A comparison of ambient sediment on natural and *Arenicola marina* exclusion plots further showed that bacterial abundances and activities responded to an enhanced fine particle content and MPB-associated carbon supply as it was caused by the experimental exclusion of the lugworm on a large scale, however bacterial community structure was not affected. As the impact of the lugworm on sediment properties was limited to the sediment surface, where MPB biomass and productivity is generally highest, the magnitude of the lugworm-mediated modification in organic carbon availability was presumably too small to cause noticeable bacterial community shifts.

In conclusion, a high bacterial diversity was found in coastal sandy sediments. The bacterial community structure at both *Hausstrand* and on the *Königshafen* experimental plots showed strong vertical variations which could mainly be assigned to the depth-related changes in MPB distribution and productivity and to the redox state of the sediment. Although at *Hausstrand* 40% of the bacterial community members were found to inhabit the entire depth range (15 cm), particular OTUs were found exclusively in individual sediment layers implying that these taxa were restricted to the specific biochemical and physical conditions prevailing in these sediment horizons. Additionally, bacterial diversity significantly increased with depth, potentially as a result of the strong physical, chemical and biological disturbances at the sediment surface, indicating that bacterial diversity in subtidal sands may vary on a scale of centimeters.

The bacterial community structure at both field sites showed strong temporal dynamics. In the intertidal sands of *Königshafen*, this temporal variation was at least partly a result of seasonal variations in MPB productivity. At Hausstrand, temporal changes did not follow any cyclical patterns as could have been envisioned from the substantial seasonal changes in temperature and substrate availability at the field site. Only 22% of OTUs could be detected throughout the entire 2-year study period. The variable part of the bacterial community was neither gradually lost or gained over time, nor could specific OTUs could be exclusively associated with either summer or winter sampling months.

Rather, the bacterial community underwent a structural change from a more diverse community at the beginning of the study (with an OTU replacement of >25% within 3 months) towards a more stable bacterial community at the end. The reason for this development remains unknown and was not clearly reflected in the variations of any of the contextual parameters. The data suggests that dynamic interactions between the members of the bacterial community themselves led to these strong temporal changes in bacterial community structure, presumably based on trophic interactions between the different community members. Despite the strong unpredictable dynamics in bacterial community structure, general ecosystem functions mediated by bacteria such as organic matter remineralization (oxygen consumption, enzymatic activity) and productivity (bacterial growth) were not affected, indicating a high functional redundancy.

On the *Königshafen* experimental plots, microbial activities and bacterial community structure showed strong spatial variations on a scale of hundreds of meters. The variations could mainly be linked to spatial variations in sediment composition related to the tidal range and the therewith associated differences in MPB distribution. Bioturbation by *Arenicola marina* altered the sediment properties beyond the vicinity of the burrows and thereby influenced microbial activities in the upper layers of ambient sediment. These effects, however, did not cause significant variations in bacterial community structure.

Outlook

It was demonstrated in this thesis that ecological modeling of high-resolution fingerprinting patterns with their environmental context can successfully provide new insights about the functioning and changes in microbial communities and their activities in a complex natural ecosystem like coastal sandy sediments. Future work will need to quantitatively address the composition of the microbial communities in this habitat and the taxonomic levels that are the targets of environmental controls. Previous phylogenetic studies in the marine benthos showed that indeed the composition of dominating bacterial community members may change with depth and time in coastal sediments (Llobet-Brossa 2000, Musat et al. 2006); however these studies utilized 16S rDNA sequence based methods and were not able to demonstrate the strong community dynamics shown here by ARISA fingerprinting. Recent studies revealed that 16S rDNA sequences may not sufficiently resolve physiological diversity. As an example, cultured isolates of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*

vary widely in their pigment compositions and growth responses to light and nutrients, yet show greater than 96% identity in their 16S rDNA sequences. Sequencing of the 16S-23S rDNA internal transcribed spacer (ITS) region was found to be much more suitable to divide these isolates into genetically and physiologically distinct groups, so-called "ecotypes" (Rocap et al. 2002). Similarly, different *Brevundimonas alba* strains with identical 16S rRNA gene sequences were investigated for their ITS sequences and physiological diversities, and the data indicated a considerable diversification and niche differentiation in terms of morphotypes and utilization of different carbon sources in these strains despite their identical phylotypes (Jaspers & Overmann 2004). For pathogenic bacteria it is well established that even phylogenetically identical strains or species can represent distinct "ecotypes" based on virulence properties or host ranges (Jaspers & Overmann 2004), however 16S-23S rDNA ITS length polymorphism was found to be a useful tool to distinguish for example between phylogentically identical *Corynebacterium* species (Aubel et al. 1997). Furthermore, environmental shotgun sequencing projects have identified extensive genomic heterogeneity among co-occurring lineages with nearly identical or identical 16S rDNA sequences (Tyson et al. 2004, Venter et al. 2004). For the present study, which found strong dynamics in bacterial community structure based on the analysis of length heterogeneities in the 16S-23S rDNA ITS region, these findings suggest that phenotypic levels rather than phylogenetic levels of sediment bacteria are targeted by environmental controls in sandy coastal ecosystems. This might imply ecophysiological properties such as the specificity towards different carbon sources, tolerances towards temperature and others, thus reflecting the physiological adaptation to certain environmental niches that may be missed by pure 16S rDNA based phylogenetic approaches. The coupling of ARISA with 16S-ITS rDNA libraries, a method recently developed (Brown et al. 2005) seems to be a promising tool to track microbial populations through temporal, spatial and biochemical gradients at the community (ARISA patterns), species (16S rDNA linked to ARISA) and ecotype level (ITS sequence analysis) alone or in combination. Furthermore, high-throughput methods such as shotgun sequencing or 454 tag sequencing may give new insights into marine bacterial diversity. Investigations using these tools could greatly enlarge our understanding of microbial community dynamics in highly complex natural ecosystems and, when this community information is coupled to the respective environmental backgrounds, help us to better understand relationships between bacterial diversity and ecosystem functioning.

References

Abell GCJ, Bowman JP (2005) Colonization and community dynamics of class *Flavobacteria* in diatom detritus in experimental mesocosms based on Southern Ocean seawater. FEMS Microbiol Ecol 53:379-391

Aubel D, Renaud FNR, Freney J (1997) Genomic diversity of several *Corynebacterium* species identified by amplification of the 16S-23S rRNA gene spacer regions. Int. J. Syst. Bact. 47(3):767-772

Boon AR, Duineveld GCA (1996) Phytopigments and fatty acids as molecular markers for the quality of near-bottom particulate organic matter in the North Sea. J Sea Res 35(4):279-291

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, Nielsen P, Sansone F, Taghon G, van Raaphorst WR, Webster, I, Weslawski JM, Wiberg P, Sundby B (2001) Permeable marine sediments: overturning an old paradigm. EOS Trans. Am. Geophys. Union 82:133-136

Brown MV, Schwalbach MS, Hewson I, Fuhrman JA (2005) Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. Environ Microb 7(9):1466-1479

Bühring SI, Ehrenhauss S, Kamp A, Moodley L, Witte U (2006) Enhanced benthic activity in sandy sublittoral sediments: evidence from 13C tracer experiments. Mar Biol Res 2(2):120-129

Cahoon LB (1999) The role of benthic microalgae in neritic ecosystems. In: Ansell AD, Gibson RN, Barnes M (eds) Oceanogr Mar Biol Ann Rev, Vol 37, Taylor & Francis, London, pp 47-86

Connell JH (1978). Diversity in tropical rain forests and coral reefs. Science 199(4335):1302-1310

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an Antarctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69(4):2253-2268

Dauwe B, Middelburg JJ, Herman PMJ (2001) Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Mar Ecol Prog Ser 215:13-22

Death RG, Winterbourn MJ (1995) Diversity patterns in stream benthic invertebrate communities: The influence of habitat stability. Ecology 76:1446-1460

de Beer D, Wenzhöfer F, Ferdelman TG, Boehme SE, Huettel M, van Beusekom JEE, Böttcher ME, Musat N, Dubilier N (2005) Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Rømø Basin, Wadden Sea. Limnol Oceanogr 50(1):113-127

Ehrenhauss S, Witte U, Janssen F, Huettel M (2004) Decomposition of diatoms and nutrient dynamics in permeable North Sea sediments. Cont Shelf Res 24(6):721-737

Ehrenhauss S, Huettel M (2004) Advective transport and decomposition of chain-forming planktonic diatoms in permeable sediments. J Sea Res 52(3):179-197

Epstein SS(1997) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb Ecol 34:188-198

Evrard VPE (2007) Assessing the fate of organic matter in subtidal sandy sediments using carbon and nitrogen stable isotopes as deliberate tracers, Dissertation thesis, Universiteit Utrecht

Fernández A, Huang S, Seston S, Xing J, Hickey RF, Criddle C et al. (1999) How stable is stable? Function versus community stability. Appl Environ Microbiol 65 (8):3697-3704

Franco MA, de Mesel I, Demba Diallo M, van der Gucht K, van Gansbeke D, van Rijswijk P, Costa MJ, Vincx M, Vanaverbeke J (2007) Effect of phytoplankton bloom deposition on benthic bacterial communities in two contrasting sediments in the southern North Sea. Aquat Microb Ecol 48:241-254

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. PNAS 103(35):13104-13109

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS* Microbiol Ecol 35:113-121

Hedtkamp SIC (2005) Shallow subtidal sand: Permeability, nutrient dynamics, microphytobenthos and organic matter. Dissertation thesis. Christian-Albrechts-Universität Kiel, Germany

Hewson I, Vargo GA, Fuhrman JA (2003) Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: effects of virus infection, containment, and nutrient enrichment. Microb Ecol 46:322-336

Hewson I, Fuhrman JA (2006) Spatial and vertical biogeography of coral reef sediment bacterial and diazotroph communities. Mar Ecol Prog Ser 306 79-86

Hewson I, Jacobson/Meyers ME, Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ Microbiol 9(4):923-933

Hoagland KD, Rosowski JR, Gretz MR, Roerner SC (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry and physiology. J Phycol, 29(5):537-566

Huettel M, Ziebis W, Forster S, Luther GW (1998) Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochim Cosmochim Acta. 62: 613-631

Huettel M, Rusch A (2000) Transport and degradation of phytoplankton in permeable sediment. Limnol Oceanogr 45(3):534-549

Huettel M, Røy H, Precht E, Ehrenhauss S (2003) Hydrodynamical impact on biogeochemical processes in aquatic sediments. Hydrobiologia. 49:231-236

Jaspers E, Overmann J (2004) Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Appl Environ Microb 70:8):4831-4839

Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65(3):1241-1250

Köster M, Dahlke S, Meyer-Reil L-A (2005) Microbial colonization and activity in relation to organic carbon in sediments of hypertrophic coastal waters (Nordrügensche Bodden, Southern Baltic Sea). Aquat Microb Ecol 39:69-83

Kristensen E, Jensen MH, Jensen KM (1997) Temporal variations in microbenthic metabolism and inorganic nitrogen fluxes in sandy and muddy sediments of a tidally dominated bay in the northern Wadden Sea. Helgoländer Meeresuntersuchungen 51:295-320

Langenheder S, Lindström ES, Tranvik LJ (2006) Structure and function of bacterial communities emerging from different sources under identical conditions. Appl Environ Microb 72(1):212-220

Lebaron P, Servais P, Troussellier M, Courties C, Vives-Rego J, Muyzer G et al. (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat Microb Ecol 19:255-267

Llobet-Brossa E, Rossellò-Mora R, Amann R (1998) Microbial community composition of Wadden Sea sediments as revealed by Fluorescence-*in-situ*-Hybridization. Appl Environ Microbiol 64:2691−2696

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. 1. Distribution, abundance and primary production. Estuaries 19:186-201

McCabe DJ, Gotelli NJ (2000) Effects of disturbance frequency, intensity, and area on assemblages of stream macroinvertebrates. Oecologia 124:270-279

Meyer-Reil LA (1987) Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments, Appl Environ Microbiol 53(8):1748-1755

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMT, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol Oceanogr 46(6):1224-1234

Misic C, Harriague AC (2007) Enzymatic activity and organic substrates on a sandy beach of the Ligurian Sea (NW Mediterranean) influenced by anthropogenic pressure. Aquat Microb Ecol 47:239-251

Moriarty DJW, Pollard PC, Hunt WG, Moriarty CM, Wassengerg T (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians on a coral reef flat. Mar Biol 85:293–300

Musat N, Werner U, Knittel K, Kolb S, Dodenhof T, van Beusekom JEE, de Beer D, Dubilier N, Amann R (2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. Syst Appl Microbiol 29:333-348

Muylaert K, van der Gucht K, Vloemans N, de Meester L, Gillis M, Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68(10) 4740-4750

Ortega-Calvo JJ, Stal LJ (1994) Sulphate-limited growth in the unicellular cyanobacterium *Gloethece* (Nageli) sp. PCC 6909. New Phytol 128(2):273-281

Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol O, Malits A, Marrasé C (2004) Changes in bacterioplankton composition under different phytoplankton regimens. Appl Environ Microbiol 70:6753-6766

Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Links between geographic location, environmental factors, and microbial community composition in sediments of the eastern Mediterranean Sea. Microb Ecol 49:367-378

Rocap G, Distel DL, Waterbury JB, Chisholm SW (2002) Resolution of Prochlorococcus and Synechococcus ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl Environ Microb 68(3):1180-1191

Rooney-Varga JN, Giewat MW, Savin MC, Sood S, LeGresley M, Martin JL (2005) Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb Ecol 49:163-175

Rusch A, Huettel M (2000) Advective particle transport into permeable sediments-evidence from experiments in an intertidal sandflat. Limnol Oceanogr 45(3):525-533

Rusch A, Forster S, Huettel M (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry 55(1):1-27

Rusch A, Huettel M, Reimers CE, Taghon GL, Fuller CM (2003) Activity and distribution of bacterial populations in Middle Atlantic Bight shelf sand. FEMS Microbiol Ecol 44:89-100

Smith DJ, Underwood GJC (1998) Exopolymer production by intertidal epipelic diatoms. Limnol Oceanogr 43:1578-1591

Townsend CR, Scarsbrook MR, Dolédec S (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. Limnol Oceanogr 42:938-949

Tyson GW, Chapman J, Hugenholtz J, Allen EE, Ram RJ, Richaredson PM et al. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37-43

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries, In: Nedwell DB, Raffaelli D (eds), Advances in Ecological Research, Vol 29, Academic Press Inc, San Diego, pp 93-153

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms, In: Callow JA (eds), Advances in Botanical Research, Vol. 40, Advances in Botanical Research Incorporating Advances in Plant Pathology

Urakawa H, Yoshida T, Nishimura M, Ohwada K (2000) Characterization of depth-related population variation in microbial communities of a coastal marine sediment using 16S rDNA-based approaches and quinine profiling. Environ Microbiol 2(5):542-554

Urban-Malinga B, Hedtkamp SIC, van Beusekom JEE, Wiktor J, Weslawski JM (2006) Comparison of nematode communities in Baltic and North Sea sublittoral, permeable sands - Diversity and environmental control. Est Coast Shelf Sci 70:224-238

van Duyl FC, Bak RPM, Kop AJ, Nieuwland G, Berghuis EM, Kok A (1992) Mesocosm experiments: mimicking seasonal developments of microbial variables in North Sea sediment. Hydrobiologia 235/236:267-281

van Duyl FC, Kop AJ (1994) Bacterial production in North Sea sediments: clues to seasonal and spatial variations. Marine Biology 120:323-337

van Duyl FC, de Winder B, Kop AJ, Wollenzien U (1999) Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar Ecol Prog Ser 191:19-32

van Hannen EJ, Zwart G, van Agterveld MP, Gons HJ, Ebert J, et al. (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl Environ Microbiol 65:795-801

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA et al. (2004) Environmental genome shotgun sequencing of the Sargoaso Sea. Science 304:66-74

Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? *Environ* Microbiol 6(1):1-11

Werner U, Billerbeck M, Polerecky L, Franke U, Huettel M, van Beusekom J, de Beer D (2005) Spatial and temporal patterns of mineralization rates and oxygen distribution in a permeable intertidal sand flat. In: Werner U. Microbial activity and transport processes in near-shore, permeable sediments. Dissertation thesis. Universität Bremen. Bremen, Germany

Appendix

Table A1: Average values of environmental parameters for 5-cm sediment intervals at *Hausstrand* (Chapter 4).

	Water column				Sediment										
Sampling date	Temp. [°C]	pH	Wind speed [m × s^{-1}]	Benthic O_2 consumpt. [mmol × m^{-2} × d^{-1}]	Sediment layer [cm]	Salinity [°/°°]	Benthic Chl. *a* [µg × g^{-1}]	Phaeophyt. [µg × g^{-1}]	EDTA-extr. carbs [µg × g^{-1}]	Total carbs [µg × g^{-1}]	SiO_2 [µM]	PO_4 [µM]	NO_2 [µM]	NO_3 [µM]	NH_4 [µM]
8-Aug-04	20.7	8.16	3.58	-37.5	0-5	32.0	10.2	2.4	358	2659	28.5	9.6	2.3	2.8	25.5
					5-10	31.7	2.3	1.2	145	1977	52.0	6.6	2.1	2.8	46.4
					10-15	30.4	0.6	0.6	N.A.	N.A.	87.3	4.4	2.4	4.0	51.1
16-Oct-04	9.5	8.09	7.00	-12.7	0-2	28.0	17.0	0.6	280	2278	3.9	2.7	1.0	28.2	10.0
					2-4	28.0	12.1	0.8	226	2743	12.5	5.0	1.0	13.8	23.7
					6-9	27.8	2.7	1.1	91	2311	36.9	7.0	1.2	3.4	53.4
					9-12	27.1	1.6	0.7	N.A.	N.A.	49.6	4.8	0.8	4.3	51.5
8-Feb-05	1.5	8.00	6.67	-7.4	0-5	25.4	13.0	1.6	482	2198	67.4	6.7	1.6	68.3	23.4
					5-10	27.1	3.3	1.2	287	1574	60.0	9.5	2.6	10.0	55.5
					10-15	26.1	1.4	0.8	N.A.	N.A.	71.4	4.6	1.8	5.7	48.0
8-Apr-05	6.9	8.04	11.02	-8.7	0-5	23.2	15.3	1.2	443	1670	66.4	5.8	2.2	56.6	22.2
					5-10	20.3	5.1	0.9	285	1065	143.1	6.9	3.5	13.5	59.6
					10-15	19.8	0.9	0.6	N.A.	N.A.	170.7	7.3	2.6	5.6	61.8
2-Jul-05	18.0	8.03	4.73	-27.5	0-5	30.0	14.4	0.9	269	489	15.0	10.9	0.9	3.3	37.6
					5-10	30.0	4.5	0.7	115	1155	21.2	7.3	0.7	2.0	55.0
					10-15	30.0	1.0	0.5	113	858	19.4	3.2	0.6	2.9	44.9
9-Nov-05	10.8	8.08	9.50	N.A.	0-5	29.3	13.9	1.1	272	1615	9.7	6.0	0.4	4.2	31.7
					5-10	30.0	5.5	1.1	135	1092	17.9	3.1	0.8	4.0	67.8
1-Mar-06	1.7	7.90	5.65	-7.1	0-5	27.8	10.2	1.3	397	1134	36.1	2.7	1.6	28.5	21.6
					5-10	28.2	2.2	0.9	222	577	48.2	3.3	1.6	8.6	33.6
					10-15	28.0	0.9	0.4	175	485	55.4	2.6	1.7	9.6	41.8
27-Mar-06	4.1	8.13	8.82	-10.9	0-5	26.4	17.0	1.2	193	1267	8.2	3.0	1.9	42.7	10.7
					5-10	28.0	6.3	1.1	105	667	25.9	5.2	2.1	4.1	46.7
					10-15	27.8	0.8	0.3	79	181	34.1	4.1	2.6	3.0	38.8

Table A2: Cell-specific extracellular enzymatic activities, bacterial abundances and bacterial carbon production rates in the sediment at *Hausstrand* (Chapter 4).

Sampling date	Sed. layer [cm]	Chitobiase specific activity [amol × h^{-1} × cell]	α-Glucosidase specific activity [amol × h^{-1} × cell]	β-Glucosidase specific activity [amol × h^{-1} × cell]	Lipase specific activity [amol × h^{-1} × cell]	Phosphatase specific activity [amol × h^{-1} × cell]	Bacterial abundance cells × 10^9 × cm^{-3}	Bacterial C production [mg C × L^{-1} × d^{-1}]
8-Aug-04	0-5	4.5	2.0	2.5	0.2	18	1.9	28.4
	5-10	3.1	1.3	1.4	0.3	15	0.8	13.6
	10-15	1.6	0.6	0.9	0.1	10	0.5	10.5
16-Oct-04	0-4	1.0	1.0	2.0	0.1	4	2.5	1.9
	6-9	3.7	1.0	2.9	0.2	9	1.0	1.7
	9-12	0.5	0.4	2.8	0.1	3	0.6	1.9
8-Feb-05	0-5	0.8	0.6	1.0	0.0	7	2.4	7.4
	5-10	2.2	0.4	0.8	0.1	6	1.3	4.5
	10-15	0.3	0.2	0.3	0.0	2	0.9	2.8
8-Apr-05	0-5	0.9	0.8	1.6	0.0	7	2.0	5.3
	5-10	0.9	0.5	1.0	0.0	5	1.4	4.2
	10-15	0.5	0.3	0.6	0.1	3	0.6	2.7
2-Jul-05	0-5	3.4	1.6	2.7	0.3	14	2.9	27.4
	5-10	1.2	0.8	2.4	0.3	9	2.0	15.8
	10-15	0.4	0.2	0.8	0.2	4	1.6	6.0
9-Nov-05	0-5	1.5	0.9	1.3	0.1	10	2.3	5.3
	5-10	1.2	0.5	1.3	0.1	8	1.7	1.9
1-Mar-06	0-5	1.3	0.4	0.6	0.1	6	1.7	5.2
	5-10	0.9	0.3	0.5	0.1	7	0.8	3.1
	10-15	0.4	0.2	0.5	0.0	4	0.6	2.0
27-Mar-06	0-5	0.5	0.5	0.8	0.0	7	2.1	5.5
	5-10	0.9	0.2	0.4	0.0	4	1.4	3.6
	10-15	0.4	0.1	0.4	0.0	3	0.5	2.3

Table A3: Percentages of recoverable operational taxonomic units between sampling months integrated over the top 15 cm at *Hausstrand* (Chapter 4).

	August 2004	October 2004*	February 2005	April 2005	July 2005	November 2005*	MarchI 2006
October 2004*	53						
February 2005	65	62					
April 2005	56	79	79				
July 2005	59	80	78	85			
November 2005*	47	69	64	71	76		
MarchI 2006	56	78	72	83	85	89	
MarchII 2006	63	82	81	88	93	95	95

* Samples did not cover the entire depth range of 15 cm, therefore percentages are most likely underestimated.

Table A4: Pearson correlation coefficients at the Bonferroni-corrected level (Chapter 4). Part I.

	Aug	Oct	Feb	Apr	Jul	Nov	Mar I	Mar II	Dep.	Tem.	Wind	SiO2	Chl a	Chl:Ph	Sal.	PO4	NO2	NO3	NH4	pH	Chito	α-Glu	β-Glu	Lip	Pep	Phos	Abd	Bac. gr.	Axis 1	Axis 2	Axis 3	Axis 4
Aug		0.43	0.51	0.51	0.51	0.60	0.51	0.51	0.81	0.00	0.00	0.42	0.43	0.21	0.02	0.03	0.14	0.13	0.77	0.01	0.00	0.01	0.21	0.05	0.00	0.00	0.21	0.00	0.02	0.10	0.00	0.58
Oct	**-0.17**		0.43	0.43	0.43	0.53	0.43	0.43	0.45	0.95	0.98	0.12	0.62	0.12	0.93	0.85	0.07	0.81	0.33	0.34	0.57	0.59	0.45	0.81	0.01	0.14	0.52	0.02	0.03	0.03	0.40	0.61
Feb	**-0.14**	**-0.17**		0.51	0.51	0.60	0.51	0.51	0.81	0.03	0.79	0.17	0.89	0.43	0.41	0.49	0.36	0.20	0.74	0.22	0.73	0.38	0.22	0.22	0.14	0.39	0.88	0.88	0.14	0.22	0.22	0.97
Apr	**-0.14**	**-0.17**	**-0.14**		0.51	0.60	0.51	0.51	0.81	0.50	0.00	0.01	0.96	1.00	0.00	0.00	0.02	0.19	0.52	0.67	0.37	0.82	0.96	0.25	0.51	0.57	0.72	0.65	0.22	0.97	0.56	0.00
Jul	**-0.14**	**-0.17**	**-0.14**	**-0.14**		0.60	0.51	0.51	0.81	0.03	0.07	0.21	0.98	0.63	0.15	0.17	0.02	0.08	0.43	0.62	0.55	0.48	0.05	0.00	0.64	0.40	0.14	0.01	0.31	0.50	0.52	0.78
Nov	**-0.11**	**-0.13**	**-0.11**	**-0.11**	**-0.11**		0.60	0.60	0.49	0.10	0.12	0.13	0.35	0.74	0.34	0.35	0.03	0.39	0.48	0.62	0.69	0.73	0.57	0.93	0.73	0.32	0.31	0.52	0.09	0.87	0.52	0.50
Mar I	**-0.14**	**-0.17**	**-0.14**	**-0.14**	**-0.14**	**-0.11**		0.51	0.81	0.04	0.29	0.55	0.52	0.43	0.82	0.76	0.87	0.36	0.60	0.00	0.56	0.16	0.10	0.35	0.06	0.67	0.22	0.40	0.25	0.46	0.41	0.81
Mar II	**-0.14**	**-0.17**	**-0.14**	**-0.14**	**-0.14**	**-0.11**	**-0.14**		0.81	0.16	0.16	0.26	0.84	0.72	0.90	0.98	0.18	0.89	0.30	0.06	0.15	0.12	0.10	0.15	0.25	0.35	0.69	0.52	0.21	0.50	0.92	0.09
Dep.	**0.05**	**-0.16**	**0.05**	**0.05**	**0.05**	**-0.15**	**0.05**	**0.05**		0.79	0.80	0.01	0.00	0.00	0.66	0.62	0.36	0.01	0.00	0.79	0.03	0.00	0.00	1.00	0.12	0.03	0.00	0.16	0.18	0.00	0.03	0.86
Tem.	**0.59**	**-0.01**	**-0.43**	**-0.15**	**0.44**	**0.35**	**-0.43**	**-0.30**	**-0.06**		0.06	0.19	0.91	0.89	0.00	0.01	0.06	0.00	0.34	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.60	0.28	0.00	0.90
Wind	**-0.56**	**0.00**	**-0.06**	**0.66**	**-0.38**	**0.33**	**-0.22**	**0.30**	**-0.06**	**-0.39**		0.58	0.37	0.40	0.00	0.00	0.35	0.11	1.00	0.92	0.02	0.12	0.25	0.00	0.09	0.07	0.73	0.01	0.03	0.35	0.14	0.41
SiO2	**0.17**	**-0.33**	**0.29**	**0.54**	**-0.26**	**-0.32**	**0.13**	**-0.24**	**0.54**	**-0.28**	**0.12**		0.00	0.00	0.01	0.01	0.00	0.92	0.00	0.22	0.58	0.09	0.02	0.51	0.29	0.66	0.00	0.87	0.33	0.08	0.33	0.10
Chl a	**-0.17**	**0.11**	**-0.03**	**0.01**	**-0.01**	**0.20**	**-0.14**	**0.04**	***-0.97**	**0.02**	**0.19**	**-0.57**		0.00	0.97	0.92	0.32	0.00	0.00	0.72	0.12	0.00	0.00	0.71	0.30	0.11	0.00	0.24	0.04	0.00	0.06	0.76
Chl: Ph.	**-0.27**	**0.33**	**-0.17**	**0.00**	**0.10**	**0.07**	**-0.17**	**0.08**	***-0.75**	**0.03**	**0.18**	**-0.71**	***0.80**		0.94	0.99	0.15	0.01	0.00	0.61	0.68	0.02	0.00	0.51	0.21	0.91	0.00	0.85	0.12	0.00	0.38	0.96
Sal.	**0.48**	**0.02**	**-0.18**	***-0.82**	**0.30**	**0.21**	**0.05**	**-0.03**	**-0.10**	**0.59**	***-0.76**	**-0.53**	**0.01**	**-0.02**		0.00	0.02	0.02	0.87	0.19	0.01	0.07	0.10	0.00	0.08	0.02	0.60	0.04	0.33	0.47	0.03	0.05
PO4	**0.44**	**0.04**	**-0.15**	***-0.85**	**0.29**	**0.20**	**0.07**	**0.00**	**-0.11**	**0.54**	***-0.75**	**-0.55**	**0.02**	**0.00**	***0.99**		0.02	0.03	0.77	0.24	0.01	0.10	0.13	0.01	0.10	0.03	0.57	0.06	0.34	0.55	0.06	0.03
NO2	**0.31**	**-0.38**	**0.19**	**0.47**	**-0.49**	**-0.45**	**0.04**	**0.28**	**0.20**	**-0.39**	**0.20**	**0.64**	**-0.21**	**-0.30**	**-0.46**	**-0.48**		0.31	0.73	0.76	0.89	0.47	0.12	0.10	0.58	0.93	0.03	0.86	0.52	0.41	0.52	0.17
NO3	**-0.31**	**0.05**	**0.27**	**0.28**	**-0.36**	**-0.18**	**0.20**	**0.03**	**-0.53**	**-0.58**	**0.33**	**-0.02**	**0.56**	**0.50**	**-0.49**	**-0.45**	**0.22**		0.00	0.12	0.21	0.98	0.92	0.00	0.57	0.41	0.08	0.33	0.33	0.00	0.59	0.18
NH4	**0.06**	**-0.21**	**0.07**	**0.14**	**0.17**	**0.15**	**-0.11**	**-0.22**	***0.71**	**0.20**	**0.00**	**0.58**	**-0.69**	***-0.77**	**-0.04**	**-0.06**	**0.08**	**-0.69**		0.87	0.96	0.09	0.15	0.12	0.35	0.98	0.01	0.85	0.49	0.01	0.20	0.81
pH	**0.52**	**0.20**	**-0.26**	**-0.09**	**-0.11**	**0.11**	***-0.77**	**0.39**	**-0.06**	**0.55**	**0.02**	**-0.26**	**0.08**	**0.11**	**0.27**	**0.25**	**0.07**	**-0.32**	**-0.03**		0.20	0.07	0.16	0.30	0.00	0.21	0.86	0.32	0.16	0.77	0.01	0.61
Chito	**0.63**	**-0.12**	**-0.08**	**-0.19**	**0.13**	**0.09**	**-0.12**	**-0.30**	**-0.45**	**0.58**	**-0.49**	**-0.12**	**0.33**	**0.09**	**0.53**	**0.49**	**0.03**	**-0.27**	**0.01**	**0.27**		0.00	0.00	0.00	0.01	0.00	0.17	0.00	0.44	0.39	0.00	0.71
α-Glu	**0.51**	**0.12**	**-0.19**	**-0.05**	**0.15**	**0.07**	**-0.30**	**-0.32**	**-0.67**	**0.61**	**-0.32**	**-0.35**	**0.59**	**0.49**	**0.37**	**0.34**	**-0.15**	**-0.01**	**-0.35**	**0.38**	***0.81**		0.00	0.02	0.00	0.00	0.01	0.00	0.74	0.07	0.00	0.50
β-Glu	**0.26**	**0.16**	**-0.26**	**-0.01**	**0.40**	**0.12**	**-0.34**	**-0.34**	**-0.64**	**0.64**	**-0.24**	**-0.48**	**0.60**	**0.59**	**0.35**	**0.32**	**-0.33**	**-0.02**	**-0.30**	**0.29**	**0.65**	***0.90**		0.01	0.00	0.00	0.00	0.00	0.86	0.03	0.01	0.37
Lip	**0.41**	**-0.05**	**-0.26**	**-0.24**	**0.67**	**-0.02**	**-0.20**	**-0.30**	**0.00**	***0.75**	**-0.60**	**-0.14**	**-0.08**	**-0.14**	**0.57**	**0.53**	**-0.34**	**-0.60**	**0.33**	**0.22**	**0.58**	**0.46**	**0.52**		0.09	0.00	0.46	0.00	0.49	0.35	0.13	0.69
Pep	**0.63**	**0.54**	**-0.31**	**-0.14**	**-0.10**	**-0.08**	**-0.39**	**-0.24**	**-0.32**	**0.60**	**-0.36**	**-0.23**	**0.22**	**0.27**	**0.37**	**0.34**	**-0.12**	**-0.12**	**-0.20**	**0.64**	**0.55**	***0.72**	**0.60**	**0.36**		0.03	0.49	0.13	0.02	0.28	0.00	0.85
Phos	**0.59**	**-0.31**	**-0.19**	**-0.12**	**0.18**	**0.21**	**-0.09**	**-0.20**	**-0.44**	**0.62**	**-0.38**	**-0.09**	**0.34**	**0.03**	**0.48**	**0.44**	**-0.02**	**-0.18**	**-0.01**	**0.27**	***0.85**	**0.71**	**0.60**	**0.63**	**0.43**		0.16	0.00	0.78	0.69	0.00	0.57
Abd.	**-0.26**	**0.14**	**0.03**	**-0.08**	**0.31**	**0.22**	**-0.26**	**-0.09**	***-0.85**	**0.17**	**0.07**	**-0.64**	***0.90**	***0.71**	**0.11**	**0.12**	**-0.43**	**0.37**	**-0.52**	**0.04**	**0.29**	**0.54**	**0.66**	**0.16**	**0.15**	**0.30**		0.17	0.11	0.00	0.32	0.77

Table A4: Pearson correlation coefficients at the Bonferroni-corrected level (Chapter 4). Part II.

	Aug	Oct	Feb	Apr	Jul	Nov	Mar I	Mar II	Depth	Temp	Wind	SiO2	Chl a	Chl:Ph	Sal	PO4	NO2	NO3	NH4	pH	Chito	α-Glu	β-Glu	Lip	Pep	Phos	Abd	Bac. gr.	Axis 1	Axis 2	Axis 3	Axis 4
Bac. gr.	**0.58**	**-0.46**	**-0.03**	**-0.10**	**0.51**	**-0.14**	**-0.18**	**-0.14**	**-0.30**	**0.58**	**-0.54**	**-0.03**	**0.25**	**0.04**	**0.42**	**0.38**	**0.04**	**-0.21**	**-0.04**	**0.21**	***0.74**	**0.67**	**0.59**	**0.65**	**0.32**	***0.78**	**0.29**		0.09	0.22	0.69	0.93
Axis 1	**0.46**	**0.45**	**0.31**	**-0.26**	**-0.21**	**-0.36**	**-0.24**	**-0.26**	**0.29**	**0.11**	**-0.45**	**0.21**	**-0.42**	**-0.33**	**0.21**	**0.20**	**0.14**	**-0.21**	**0.15**	**0.30**	**0.16**	**0.07**	**-0.04**	**0.15**	**0.49**	**-0.06**	**-0.34**	**-0.35**		1.00	1.00	1.00
Axis 2	**-0.34**	**0.45**	**0.26**	**-0.01**	**-0.14**	**0.04**	**-0.16**	**-0.14**	***-0.79**	**-0.23**	**0.20**	**-0.37**	***0.78**	**0.64**	**-0.16**	**-0.13**	**-0.18**	**0.61**	**-0.51**	**-0.06**	**0.19**	**0.38**	**0.44**	**-0.20**	**0.23**	**0.08**	***0.75**	**0.26**	**0.00**		1.00	1.00
Axis 3	***0.76**	**-0.18**	**-0.26**	**-0.12**	**-0.14**	**0.14**	**-0.18**	**0.02**	**-0.45**	**0.56**	**-0.31**	**-0.21**	**0.39**	**0.19**	**0.43**	**0.40**	**0.14**	**-0.12**	**-0.27**	**0.54**	***0.74**	***0.77**	**0.51**	**0.32**	**0.61**	***0.74**	**0.21**	**0.09**	**0.00**	**0.00**		1.00
Axis 4	**0.12**	**-0.11**	**0.01**	**0.59**	**-0.06**	**-0.14**	**-0.05**	**-0.36**	**-0.04**	**0.03**	**0.17**	**0.35**	**0.07**	**0.01**	**-0.40**	**-0.43**	**0.29**	**0.28**	**-0.05**	**-0.11**	**-0.08**	**0.14**	**0.19**	**-0.09**	**0.04**	**-0.12**	**0.06**	**0.02**	**0.00**	**0.00**	**0.00**	

Pearson correlation coefficients calculated on standardized data are shown in bold font in the bottom part of the table. P-values of correlations are shown in normal font in the upper part of the table; *denotes significant coefficients at the Bonferroni-corrected level ($p < 0.05/435 = 0.0001149$). Abbreviations: Aug=August, Oct=October, Feb=February, Apr=April, Jul=July, Nov=November, Mar I=March I, Mar II=March II, Tem=temperature, Dep=Depth, Wind=wind speed, Chl a=benthic chlorophyll a, Chl:Ph=benthic chlorophyll a: phaeophytine ratio, Sal=salinity, Chito=chitobiase activity, Phos=Phosphatase (all enzymatic activities refer to cell-specific activities), Abund.=Bacterial abundance, Bac. gr. =Bacterial carbon production, Axis 1, 2, 3, 4=major axes of the principal compontent analysis that explain variation in bacterial community structure.

Table A5/ Part I – March 2006. Overview over environmental and bacterial data measured at the field site.

	Sampling date	Depth	y coord.	x coord.	Tidal range	Temp.	Chlorophyll a [µg g^{-1} dry sed.]	Phaeophytine [µg g^{-1} dry sed.]	EDTA extr. carbs [µg g^{-1} dry sed.]	Total carbs [µg g^{-1} dry sed.]	Chitobiase act. [µmol L^{-1} h^{-1}]	Bact. Abund. [cells cm^{-3}]
Block 2 excl.	March 2006	0-2 cm	150	280	low intertidal	2.85	28.5	6.4	900	3554	2.59	5.98E+09
	March 2006	2-5 cm	150	280	low intertidal	2.85	8.1	3.9	567	1616	1.95	1.67E+09
	March 2006	5-8 cm	150	280	low intertidal	2.85	2.5	3.4	524	1625	0.87	1.69E+09
Block 2 cont.	March 2006	0-2 cm	180	280	low intertidal	2.85	26.1	7.0	715	2967	2.46	7.36E+09
	March 2006	2-5 cm	180	280	low intertidal	2.85	7.4	2.4	510	1639	5.61	1.23E+09
	March 2006	5-8 cm	180	280	low intertidal	2.85	2.1	1.8	376	965	1.47	1.31E+09
Block 3 excl.	March 2006	0-2 cm	330	250	low intertidal	2.85	34.3	9.7	1035	3653	3.33	6.22E+09
	March 2006	2-5 cm	330	250	low intertidal	2.85	11.5	3.8	430	1481	4.99	1.29E+09
	March 2006	5-8 cm	330	250	low intertidal	2.85	2.8	2.0	435	1210	1.03	7.63E+08
Block 3 cont.	March 2006	0-2 cm	300	250	low intertidal	2.85	26.7	5.3	926	2577	2.42	4.90E+09
	March 2006	2-5 cm	300	250	low intertidal	2.85	8.2	2.4	369	1154	7.22	1.16E+09
	March 2006	5-8 cm	300	250	low intertidal	2.85	2.7	2.0	415	1081	2.74	9.41E+08
Block 5 excl.	March 2006	0-2 cm	150	30	mid intertidal	2.85	13.2	2.4	339	1441	2.44	3.10E+09
	March 2006	2-5 cm	150	30	mid intertidal	2.85	4.1	1.7	285	908	2.69	1.03E+09
	March 2006	5-8 cm	150	30	mid intertidal	2.85	1.5	0.9	211	837	0.61	8.96E+08
Block 5 cont.	March 2006	0-2 cm	180	0	mid intertidal	2.85	11.2	2.9	326	1133	1.13	5.03E+09
	March 2006	2-5 cm	180	0	mid intertidal	2.85	4.5	1.6	213	1234	1.66	9.92E+08
	March 2006	5-8 cm	180	0	mid intertidal	2.85	2.2	1.1	235	815	2.03	7.05E+08
Block 6 excl.	March 2006	0-2 cm	300	30	mid intertidal	2.85	15.5	3.4	339	1956	2.31	5.37E+09
	March 2006	2-5 cm	300	30	mid intertidal	2.85	5.6	2.0	285	808	2.15	1.32E+09
	March 2006	5-8 cm	300	30	mid intertidal	2.85	1.8	1.5	211	757	0.71	9.66E+08
Block 6 cont.	March 2006	0-2 cm	330	30	mid intertidal	2.85	10.6	3.2	326	1247	1.19	4.31E+09
	March 2006	2-5 cm	330	30	mid intertidal	2.85	6.0	2.1	213	880	2.14	7.99E+08
	March 2006	5-8 cm	330	30	mid intertidal	2.85	2.4	1.7	235	849	0.77	6.79E+08
Block 1 excl.	March 2006	0-2 cm	0	280	low intertidal	2.85	33.0	2.0	1055	5138	27.72	3.32E+09
	March 2006	2-5 cm	0	280	low intertidal	2.85	7.0	1.5	612	1964	18.25	2.29E+09
	March 2006	5-8 cm	0	280	low intertidal	2.85	3.0	1.0	615	1700	4.49	1.40E+09
Block 1 cont.	March 2006	0-2 cm	30	250	low intertidal	2.85	16.8	2.2	531	2133	23.35	1.70E+09
	March 2006	2-5 cm	30	250	low intertidal	2.85	7.0	1.3	378	1238	11.00	1.45E+09
	March 2006	5-8 cm	30	250	low intertidal	2.85	3.0	0.9	461	1121	6.94	8.07E+08
Block 4 excl.	March 2006	0-2 cm	0	30	mid intertidal	2.85	15.3	12.6	429	1032	1.94	1.32E+09
	March 2006	2-5 cm	0	30	mid intertidal	2.85	6.2	3.4	350	934	2.78	1.56E+09
	March 2006	5-8 cm	0	30	mid intertidal	2.85	1.9	2.5	294	832	0.80	6.57E+08
Block 4 cont.	March 2006	0-2 cm	30	0	mid intertidal	2.85	9.4	3.7	360	896	1.34	1.34E+09
	March 2006	2-5 cm	30	0	mid intertidal	2.85	5.9	2.1	329	659	1.21	1.39E+09
	March 2006	5-8 cm	30	0	mid intertidal	2.85	2.3	1.6	318	653	0.65	7.60E+08

Table A5/ Part II – September 2005. Overview over environmental and bacterial data measured at the field site.

	Sampling date	Depth	y coord.	x coord.	Tidal range	Temp.	Chlorophyll a [µg g^{-1} dry sed.]	Phaeophytine [µg g^{-1} dry sed.]	EDTA extr. carbs [µg g^{-1} dry sed.]	Total carbs [µg g^{-1} dry sed.]	Chitobiase act. [µmol L^{-1} h^{-1}]	Bact. Abund. [cells cm^{-3}]
2 excl.	Sept. 2005	0-2 cm	150	280	low intertidal	17.96	15	3.1	908	3045	11.71	4.33E+09
	Sept. 2005	2-5 cm	150	280	low intertidal	17.96	5.4	2.4	1131	3158	9.54	1.79E+09
	Sept. 2005	5-8 cm	150	280	low intertidal	17.96	2.4	2.1	792	2109	5.55	1.37E+09
2 cont.	Sept. 2005	0-2 cm	180	280	low intertidal	17.96	16	3.4	895	2120	20.32	7.26E+09
	Sept. 2005	2-5 cm	180	280	low intertidal	17.96	7.5	2.2	847	2104	44.64	2.64E+09
	Sept. 2005	5-8 cm	180	280	low intertidal	17.96	2.4	1.8	743	1387	12.41	1.88E+09
3 excl.	Sept. 2005	0-2 cm	330	250	low intertidal	17.96	22.8	0.1	1324	2186	16.6	1.10E+09
	Sept. 2005	2-5 cm	330	250	low intertidal	17.96	7.7	2.4	1433	2207	21.68	2.64E+09
	Sept. 2005	5-8 cm	330	250	low intertidal	17.96	2.2	1.4	773	1810	7.95	1.31E+09
3 cont.	Sept. 2005	0-2 cm	300	250	low intertidal	17.96	15.8	3	699	1941	14.02	2.54E+09
	Sept. 2005	2-5 cm	300	250	low intertidal	17.96	5.8	1.6	594	1327	26.07	1.67E+09
	Sept. 2005	5-8 cm	300	250	low intertidal	17.96	2.2	1.5	632	1253	9.34	1.49E+09
5 excl.	Sept. 2005	0-2 cm	150	30	mid intertidal	17.96	10.7	1.7	866	1540	31.94	1.33E+09
	Sept. 2005	2-5 cm	150	30	mid intertidal	17.96	4	1.1	681	1401	21.42	1.76E+09
	Sept. 2005	5-8 cm	150	30	mid intertidal	17.96	2	0.7	643	1544	5.69	1.45E+09
5 cont.	Sept. 2005	0-2 cm	180	0	mid intertidal	17.96	9.7	2	608	1261	13.45	1.36E+09
	Sept. 2005	2-5 cm	180	0	mid intertidal	17.96	4.2	1.4	595	1130	7.76	1.10E+09
	Sept. 2005	5-8 cm	180	0	mid intertidal	17.96	1.4	0.9	614	916	4.23	1.07E+09
6 excl.	Sept. 2005	0-2 cm	300	30	mid intertidal	17.96	12.7	2.4	750	2072	31.57	1.59E+09
	Sept. 2005	2-5 cm	300	30	mid intertidal	17.96	4.6	1.1	612	1376	15.82	1.29E+09
	Sept. 2005	5-8 cm	300	30	mid intertidal	17.96	2.4	0.8	367	1103	4.86	1.56E+09
6 cont.	Sept. 2005	0-2 cm	330	30	mid intertidal	17.96	12.9	2.2	653	1309	14.44	1.42E+09
	Sept. 2005	2-5 cm	330	30	mid intertidal	17.96	4.4	1.3	461	1044	10.08	1.01E+09
	Sept. 2005	5-8 cm	330	30	mid intertidal	17.96	2.7	0.8	577	918	3.8	1.03E+09
1 excl.	Sept. 2005	0-2 cm	0	280	low intertidal	17.96	17.7	12.6	764	2218	74.51	2.10E+09
	Sept. 2005	2-5 cm	0	280	low intertidal	17.96	5.2	3.4	446	1826	11.91	1.25E+09
	Sept. 2005	5-8 cm	0	280	low intertidal	17.96	1.9	2.5	312	1171	3.98	4.33E+08
1 cont.	Sept. 2005	0-2 cm	30	250	low intertidal	17.96	14.9	3.7	647	1720	27.9	1.25E+09
	Sept. 2005	2-5 cm	30	250	low intertidal	17.96	5.7	2.1	272	963	33.58	1.08E+09
	Sept. 2005	5-8 cm	30	250	low intertidal	17.96	2.5	1.6	260	976	8.45	3.63E+08
4 excl.	Sept. 2005	0-2 cm	0	30	mid intertidal	17.96	9.7	1.5	566	3065	10.1	9.20E+08
	Sept. 2005	2-5 cm	0	30	mid intertidal	17.96	3.7	0.8	451	2252	3.15	7.02E+08
	Sept. 2005	5-8 cm	0	30	mid intertidal	17.96	1.6	0.7	400	1217	0.97	3.73E+08
4 cont.	Sept. 2005	0-2 cm	30	0	mid intertidal	17.96	9	1.1	467	1197	2.13	1.35E+09
	Sept. 2005	2-5 cm	30	0	mid intertidal	17.96	4.3	0.7	349	657	0.94	8.25E+08
	Sept. 2005	5-8 cm	30	0	mid intertidal	17.96	2.4	0.7	436	728	0.33	3.54E+08

Table A6/Part I – March 2006 a. Overview over environmental and bacterial data measured at the field site.

	Sampl. date	Depth	y coord.	x coord.	Tidal range	Temp.	Chl. a [µg g^{-1} dry sed.]	Phaeophytine [µg g^{-1} dry sed.]	EDTA extr. carbs [µg g^{-1} dry sed.]	Total carbs [µg g^{-1} dry sed.]	Chitobiase act. [µmol L^{-1} h^{-1}]	Beta-gluc. [µmol L^{-1} h^{-1}]
Block 1 excl.	Mar.´06	0-1 cm	0	280	low intertidal	2.85	44.9	2.4	1271	7479	7.95	38.30
	Mar.´06	1-2 cm	0	280	low intertidal	2.85	21.1	1.6	838	2796	4.01	17.15
	Mar.´06	2-3 cm	0	280	low intertidal	2.85	9.7	1.7	528	1976	1.03	23.37
	Mar.´06	3-4 cm	0	280	low intertidal	2.85	5.9	1.5	657	1709	0.77	19.70
	Mar.´06	4-5 cm	0	280	low intertidal	2.85	5.3	1.3	651	2207	0.81	11.68
	Mar.´06	5-6 cm	0	280	low intertidal	2.85	4.1	1.2	936	2094	0.79	6.31
	Mar.´06	6-7 cm	0	280	low intertidal	2.85	2.9	1.0	539	1787	0.69	4.21
	Mar.´06	7-8 cm	0	280	low intertidal	2.85	2.1	0.9	370	1218	0.76	2.94
Block 1 cont	Mar.´06	0-1 cm	30	250	low intertidal	2.85	19.8	2.4	697	2573	4.52	22.62
	Mar.´06	1-2 cm	30	250	low intertidal	2.85	13.8	1.9	365	1694	4.84	24.07
	Mar.´06	2-3 cm	30	250	low intertidal	2.85	10.4	0.9	331	1142	2.22	13.89
	Mar.´06	3-4 cm	30	250	low intertidal	2.85	5.7	1.3	388	1347	0.89	12.31
	Mar.´06	4-5 cm	30	250	low intertidal	2.85	4.9	1.6	414	1226	0.78	6.79
	Mar.´06	5-6 cm	30	250	low intertidal	2.85	3.6	1.0	426	1292	0.63	8.46
	Mar.´06	6-7 cm	30	250	low intertidal	2.85	2.8	1.0	521	1057	0.60	7.67
	Mar.´06	7-8 cm	30	250	low intertidal	2.85	2.5	0.9	435	1013	0.62	4.69
Block 4 excl.	Mar.´06	0-1 cm	0	30	mid intertidal	2.85	17.5	19.3	536	1176	4.11	2.29
	Mar.´06	1-2 cm	0	30	mid intertidal	2.85	13.0	5.8	322	889	2.42	1.58
	Mar.´06	2-3 cm	0	30	mid intertidal	2.85	9.8	3.9	343	921	1.55	3.29
	Mar.´06	3-4 cm	0	30	mid intertidal	2.85	5.2	3.1	298	820	1.10	3.32
	Mar.´06	4-5 cm	0	30	mid intertidal	2.85	3.7	3.3	409	1062	0.88	1.73
	Mar.´06	5-6 cm	0	30	mid intertidal	2.85	2.4	3.1	344	967	0.79	1.01
	Mar.´06	6-7 cm	0	30	mid intertidal	2.85	1.7	2.4	267	814	0.68	0.77
	Mar.´06	7-8 cm	0	30	mid intertidal	2.85	1.5	2.1	270	716	0.59	0.63
Block 4 cont.	Mar.´06	0-1 cm	30	0	mid intertidal	2.85	9.6	4.6	411	1021	2.11	1.26
	Mar.´06	1-2 cm	30	0	mid intertidal	2.85	9.1	2.8	309	771	2.71	1.43
	Mar.´06	2-3 cm	30	0	mid intertidal	2.85	8.7	1.9	330	680	1.64	1.27
	Mar.´06	3-4 cm	30	0	mid intertidal	2.85	5.2	2.3	304	590	0.85	1.43
	Mar.´06	4-5 cm	30	0	mid intertidal	2.85	3.8	2.0	353	707	0.64	0.95
	Mar.´06	5-6 cm	30	0	mid intertidal	2.85	2.8	2.0	369	765	0.55	0.72
	Mar.´06	6-7 cm	30	0	mid intertidal	2.85	2.3	1.5	301	575	0.58	0.71
	Mar.´06	7-8 cm	30	0	mid intertidal	2.85	1.8	1.4	284	617	0.59	0.51

Table A6/Part I – March 2006 b. Overview over environmental and bacterial data measured at the field site.

	Sampl. date	Depth	Porosity [ml cm^{-3}]	Bact. Abund. [cells cm^{-3}]	Silikat [µmol L^{-1}]	Phosphat [µmol L^{-1}]	Ammonium [µmol L^{-1}]	Nitrit_Nitrat [µmol L^{-1}]	DIC [mmol L^{-1}l]	SRR [nmol cc^{-1} d^{-1}]	Sulfide
Block 1 excl.	Mar.´06	0-1 cm	0.59	4.14E+09	32.00	0.45	33.50	3.00	1.64	11.04	0.98
	Mar.´06	1-2 cm	0.44	2.50E+09	58.00	0.45	47.50	2.55	2.32	52.96	1.50
	Mar.´06	2-3 cm	0.57	2.60E+09	85.13	0.40	60.31	2.10	2.97	12.00	2.06
	Mar.´06	3-4 cm	0.64	2.54E+09	101.00	1.80	62.50	1.00	2.91	12.50	1.95
	Mar.´06	4-5 cm	0.61	1.75E+09	115.11	3.06	64.22	0.00	2.84	13.65	1.61
	Mar.´06	5-6 cm	0.60	2.03E+09	117.00	5.85	72.50	0.58	2.78	9.50	28.00
	Mar.´06	6-7 cm	0.67	1.09E+09	117.00	8.65	80.50	1.25	2.71	6.06	51.00
	Mar.´06	7-8 cm	0.66	1.09E+09	117.82	11.74	90.09	1.90	2.65	6.00	76.71
Block 1 cont	Mar.´06	0-1 cm	0.45	2.12E+09	14.00	1.20	40.00	0.20	1.56	8.00	10.90
	Mar.´06	1-2 cm	0.55	1.29E+09	22.00	2.40	66.50	0.18	1.92	0.00	20.75
	Mar.´06	2-3 cm	0.68	1.32E+09	29.41	3.62	92.07	0.16	2.27	0.00	30.66
	Mar.´06	3-4 cm	0.68	1.53E+09	27.00	2.70	83.50	1.00	2.22	11.00	27.75
	Mar.´06	4-5 cm	0.60	1.51E+09	24.82	1.87	74.84	1.87	2.23	13.00	25.09
	Mar.´06	5-6 cm	0.54	1.78E+09	24.00	1.70	70.50	1.73	2.25	6.00	22.00
	Mar.´06	6-7 cm	0.62	1.88E+08	24.00	1.60	65.50	1.55	2.30	0.00	19.00
	Mar.´06	7-8 cm	0.62	4.58E+08	23.85	1.53	59.22	1.38	2.35	5.00	16.62
Block 4 excl.	Mar.´06	0-1 cm	0.39	1.33E+09	12.00	0.70	12.00	0.84	1.56	7.40	2.50
	Mar.´06	1-2 cm	0.51	1.32E+09	18.00	1.05	19.00	1.10	1.82	14.00	4.75
	Mar.´06	2-3 cm	0.58	1.36E+09	25.08	1.42	25.89	1.38	2.00	19.87	6.97
	Mar.´06	3-4 cm	0.56	1.98E+09	21.00	0.90	23.50	1.00	2.00	14.00	6.25
	Mar.´06	4-5 cm	0.63	1.33E+09	16.04	0.41	21.44	0.57	2.01	8.77	5.42
	Mar.´06	5-6 cm	0.63	1.45E+09	15.00	0.45	20.00	0.55	1.96	9.30	4.60
	Mar.´06	6-7 cm	0.64	2.21E+08	15.00	0.35	18.00	0.48	1.98	9.38	3.25
	Mar.´06	7-8 cm	0.32	3.01E+08	15.41	0.31	15.59	0.45	1.92	7.00	2.46
Block 4 cont.	Mar.´06	0-1 cm	0.70	1.36E+09	14.00	1.70	16.00	3.43	1.50	4.15	3.50
	Mar.´06	1-2 cm	0.45	1.31E+09	24.00	3.00	29.50	4.50	1.64	4.42	7.60
	Mar.´06	2-3 cm	0.56	1.26E+09	34.38	4.40	41.37	5.69	1.88	4.63	11.09
	Mar.´06	3-4 cm	0.49	1.62E+09	38.00	3.95	27.50	2.90	1.92	3.50	10.25
	Mar.´06	4-5 cm	0.70	1.28E+09	38.98	3.51	14.37	0.14	1.96	2.91	9.89
	Mar.´06	5-6 cm	0.63	1.59E+09	39.00	3.80	17.00	1.15	2.00	5.50	9.50
	Mar.´06	6-7 cm	0.53	2.63E+08	36.00	4.10	19.00	2.18	2.20	8.18	9.25
	Mar.´06	7-8 cm	0.52	4.29E+08	34.85	4.51	21.48	3.41	1.98	7.00	9.09

Values in red font represents extrapolated data.

Table A6/Part II – September 2005 a. Overview over environmental and bacterial data measured at the field site.

	Sampl. date	Depth	y coord.	x coord.	Tidal range	Temp.	Chl. a [µg g^{-1} dry sed.]	Phaeophytine [µg g^{-1} dry sed.]	EDTA extr. carbs [µg g^{-1} dry sed.]	Total carbs [µg g^{-1} dry sed.]	Chitobiase act. [µmol L^{-1} h^{-1}]	Beta-gluc. [µmol L^{-1} h^{-1}]
Block 1 excl.	Sept.´05	0-1 cm	0	280	low intertidal	18	24.2	19.3	1178	3023	68.05	83.20
	Sept.´05	1-2 cm	0	280	low intertidal	18	11.3	5.8	351	1414	25.22	65.82
	Sept.´05	2-3 cm	0	280	low intertidal	18	6.7	3.9	464	1737	10.30	19.18
	Sept.´05	3-4 cm	0	280	low intertidal	18	5.2	3.1	509	1747	11.12	10.12
	Sept.´05	4-5 cm	0	280	low intertidal	18	3.6	3.3	367	1993	6.02	6.44
	Sept.´05	5-6 cm	0	280	low intertidal	18	2.5	3.1	377	1477	4.41	6.01
	Sept.´05	6-7 cm	0	280	low intertidal	18	1.7	2.4	297	1125	3.56	2.88
	Sept.´05	7-8 cm	0	280	low intertidal	18	1.5	2.1	261	911	3.61	3.06
Block 1 cont	Sept.´05	0-1 cm	30	250	low intertidal	18	19.3	4.6	1015	2546	28.92	32.23
	Sept.´05	1-2 cm	30	250	low intertidal	18	10.4	2.8	280	894	34.26	23.56
	Sept.´05	2-3 cm	30	250	low intertidal	18	7.6	1.9	302	845	5.25	30.62
	Sept.´05	3-4 cm	30	250	low intertidal	18	5.3	2.3	252	1009	3.85	59.14
	Sept.´05	4-5 cm	30	250	low intertidal	18	4.2	2.0	261	1035	3.24	10.98
	Sept.´05	5-6 cm	30	250	low intertidal	18	3.0	2.0	251	1026	3.32	14.13
	Sept.´05	6-7 cm	30	250	low intertidal	18	2.3	1.5	278	973	2.79	6.40
	Sept.´05	7-8 cm	30	250	low intertidal	18	2.2	1.4	251	930	2.82	4.81
Block 4 excl.	Sept.´05	0-1 cm	0	30	mid intertidal	18	14.5	2.0	629	3191	17.77	9.56
	Sept.´05	1-2 cm	0	30	mid intertidal	18	4.8	1.0	503	2939	5.66	10.63
	Sept.´05	2-3 cm	0	30	mid intertidal	18	4.1	1.4	424	2997	5.67	5.20
	Sept.´05	3-4 cm	0	30	mid intertidal	18	3.9	0.7	437	1891	4.83	2.59
	Sept.´05	4-5 cm	0	30	mid intertidal	18	3.0	0.5	493	1868	4.40	1.66
	Sept.´05	5-6 cm	0	30	mid intertidal	18	2.1	0.5	437	1700	3.46	0.46
	Sept.´05	6-7 cm	0	30	mid intertidal	18	1.7	0.9	408	940	3.31	1.69
	Sept.´05	7-8 cm	0	30	mid intertidal	18	1.1	0.6	356	1010	3.19	0.77
Block 4 cont.	Sept.´05	0-1 cm	30	0	mid intertidal	18	10.8	1.7	579	1559	12.63	1.91
	Sept.´05	1-2 cm	30	0	mid intertidal	18	7.1	0.5	354	836	7.68	2.34
	Sept.´05	2-3 cm	30	0	mid intertidal	18	5.0	0.9	328	712	5.77	1.64
	Sept.´05	3-4 cm	30	0	mid intertidal	18	4.1	1.0	371	664	5.12	0.76
	Sept.´05	4-5 cm	30	0	mid intertidal	18	3.7	0.1	346	594	2.63	0.43
	Sept.´05	5-6 cm	30	0	mid intertidal	18	2.8	0.6	407	752	4.22	0.11
	Sept.´05	6-7 cm	30	0	mid intertidal	18	2.3	0.6	454	754	3.70	0.40
	Sept.´05	7-8 cm	30	0	mid intertidal	18	2.0	0.8	448	676	3.17	0.48

Table A6/Part II – September 2005 b. Overview over environmental and bacterial data measured at the field site.

	Sampl. date	Depth	Porosity [ml cm^{-3}]	Bact. Abund. [cells cm^{-3}]	Silikat [µmol L^{-1}]	Phosphat [µmol L^{-1}]	Ammonium [µmol L^{-1}]	Nitrit_Nitrat [µmol L^{-1}]	DIC [mmol L^{-1}l]	SRR [nmol cc^{-1} d^{-1}]	Sulfide
Block 1 excl.	Sept.´05	0-1 cm	0.52	2.71E+09	72.2	10.1	25.2	1.1	1.72	106.32	10.87
	Sept.´05	1-2 cm	0.54	1.49E+09	100.0	10.2	31.5	0.7	1.74	76.00	44.00
	Sept.´05	2-3 cm	0.48	1.47E+09	121.8	10.2	36.3	0.3	1.78	43.59	72.86
	Sept.´05	3-4 cm	0.44	1.33E+09	188.0	12.4	52.5	0.4	2.00	39.00	125.00
	Sept.´05	4-5 cm	0.41	9.52E+08	252.6	15.0	68.3	0.5	2.72	35.50	170.46
	Sept.´05	5-6 cm	0.48	8.52E+08	225.0	13.6	65.5	0.5	2.37	32.50	214.00
	Sept.´05	6-7 cm	0.42	1.58E+08	177.0	12.0	61.0	0.4	2.55	30.00	254.00
	Sept.´05	7-8 cm	0.30	2.89E+08	141.9	10.3	57.4	0.3	2.78	27.43	294.70
Block 1 cont	Sept.´05	0-1 cm	0.41	1.27E+09	67.8	7.4	23.3	1.6	1.68	217.00	32.71
	Sept.´05	1-2 cm	0.43	1.24E+09	66.0	6.7	26.0	1.4	1.86	180.92	40.00
	Sept.´05	2-3 cm	0.45	1.16E+09	65.7	6.0	28.0	1.0	2.03	146.50	49.03
	Sept.´05	3-4 cm	0.45	1.18E+09	68.0	5.4	26.0	0.6	2.10	108.50	57.00
	Sept.´05	4-5 cm	0.30	8.93E+08	72.1	4.9	23.6	0.2	2.18	71.06	63.14
	Sept.´05	5-6 cm	0.55	6.81E+08	68.0	4.8	23.0	0.3	2.20	50.00	72.00
	Sept.´05	6-7 cm	0.46	1.95E+08	66.0	4.6	22.5	0.3	2.22	28.82	80.00
	Sept.´05	7-8 cm	0.43	2.14E+08	65.6	4.6	22.0	0.3	2.23	28.00	87.91
Block 4 excl.	Sept.´05	0-1 cm	0.37	1.10E+09	119.1	7.9	37.4	0.4	2.28	188.81	26.47
	Sept.´05	1-2 cm	0.42	7.43E+08	126.0	8.0	34.0	0.5	2.38	144.00	39.00
	Sept.´05	2-3 cm	0.45	7.13E+08	133.7	8.0	29.9	0.5	2.46	95.30	50.94
	Sept.´05	3-4 cm	0.46	6.33E+08	150.0	9.2	35.0	0.3	2.56	87.00	75.00
	Sept.´05	4-5 cm	0.43	7.61E+08	167.8	10.3	39.6	0.1	2.70	75.19	100.38
	Sept.´05	5-6 cm	0.46	7.37E+08	158.0	10.0	39.0	0.3	2.72	66.00	98.00
	Sept.´05	6-7 cm	0.42	1.71E+08	145.0	9.6	38.0	0.5	2.74	74.00	96.00
	Sept.´05	7-8 cm	0.33	2.09E+08	133.5	9.2	36.7	0.6	2.72	55.43	94.66
Block 4 cont.	Sept.´05	0-1 cm	0.36	1.67E+09	40.7	5.7	15.4	0.7	1.35	137.82	79.74
	Sept.´05	1-2 cm	0.40	1.03E+09	62.0	6.6	24.5	0.5	1.80	105.00	84.00
	Sept.´05	2-3 cm	0.41	1.01E+09	82.8	7.5	32.1	0.3	2.20	67.85	89.65
	Sept.´05	3-4 cm	0.40	7.77E+08	79.0	6.8	31.5	0.3	2.24	61.00	112.00
	Sept.´05	4-5 cm	0.41	6.85E+08	74.3	6.0	30.3	0.3	2.28	53.45	131.07
	Sept.´05	5-6 cm	0.42	5.79E+08	73.0	5.8	24.5	0.4	2.34	38.00	128.00
	Sept.´05	6-7 cm	0.42	2.05E+08	70.0	5.4	23.5	0.4	2.38	23.98	125.00
	Sept.´05	7-8 cm	0.43	2.79E+08	69.8	5.1	20.5	0.3	2.35	24.00	119.70

Values in red font represents extrapolated data.

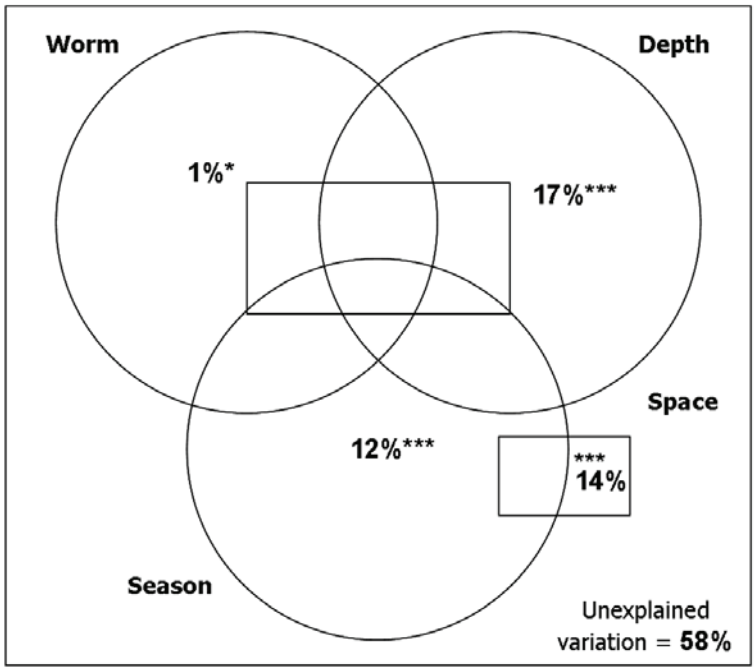

Fig. A1: Partitioning of the microbial community structure variation on all experimental blocks into the relative effects of lugworm presence/absence, depth, time and space and covariations thereof. A statistically significant contribution of pure fractions to variation is indicated by *** ($P < 0.001$), a marginally significant contribution by * ($p < 0.05$), respectively, as determined by 999 Monte Carlo permutations under the full multivariate model.

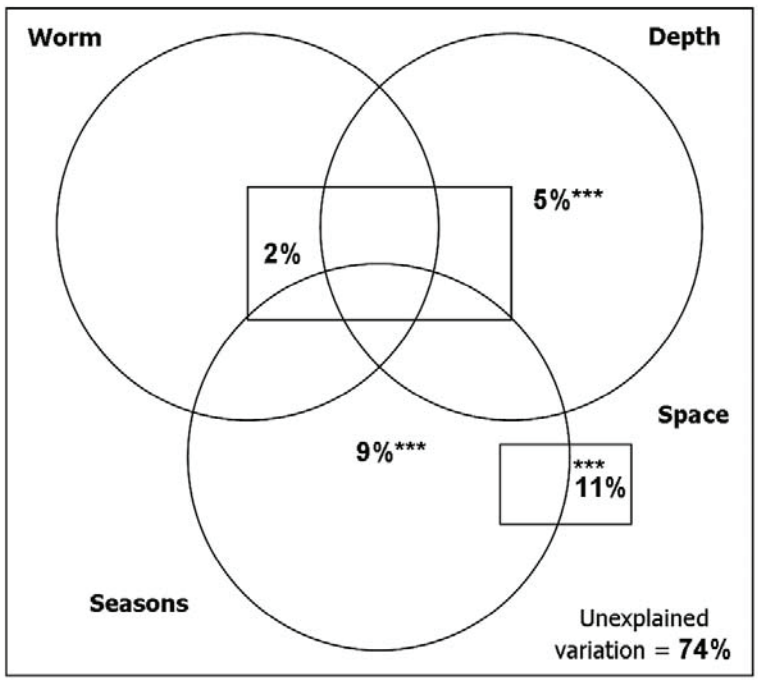

Fig. A2: Partitioning of the microbial variation on experimental blocks 1 and 4, as measured at a high vertical resolution, into the relative effects of lugworm presence/absence, depth, time and space. A statistically significant contribution of pure fractions to variation is indicated by *** ($P < 0.001$), as determined by 999 Monte Carlo permutations under the full multivariate model.

Erklärung gemäß §6 Abs. 5 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche

Hiermit versichere ich, dass ich die vorliegende Dissertation mit dem Titel „*Investigation of the distribution and activity of benthic microorganisms in coastal habitats*“ selbstständig verfasst und keine weiteren als die angegebenen Quellen und Hilfsmittel benutzt, sowie Zitate gekennzeichnet habe.

Bremen,