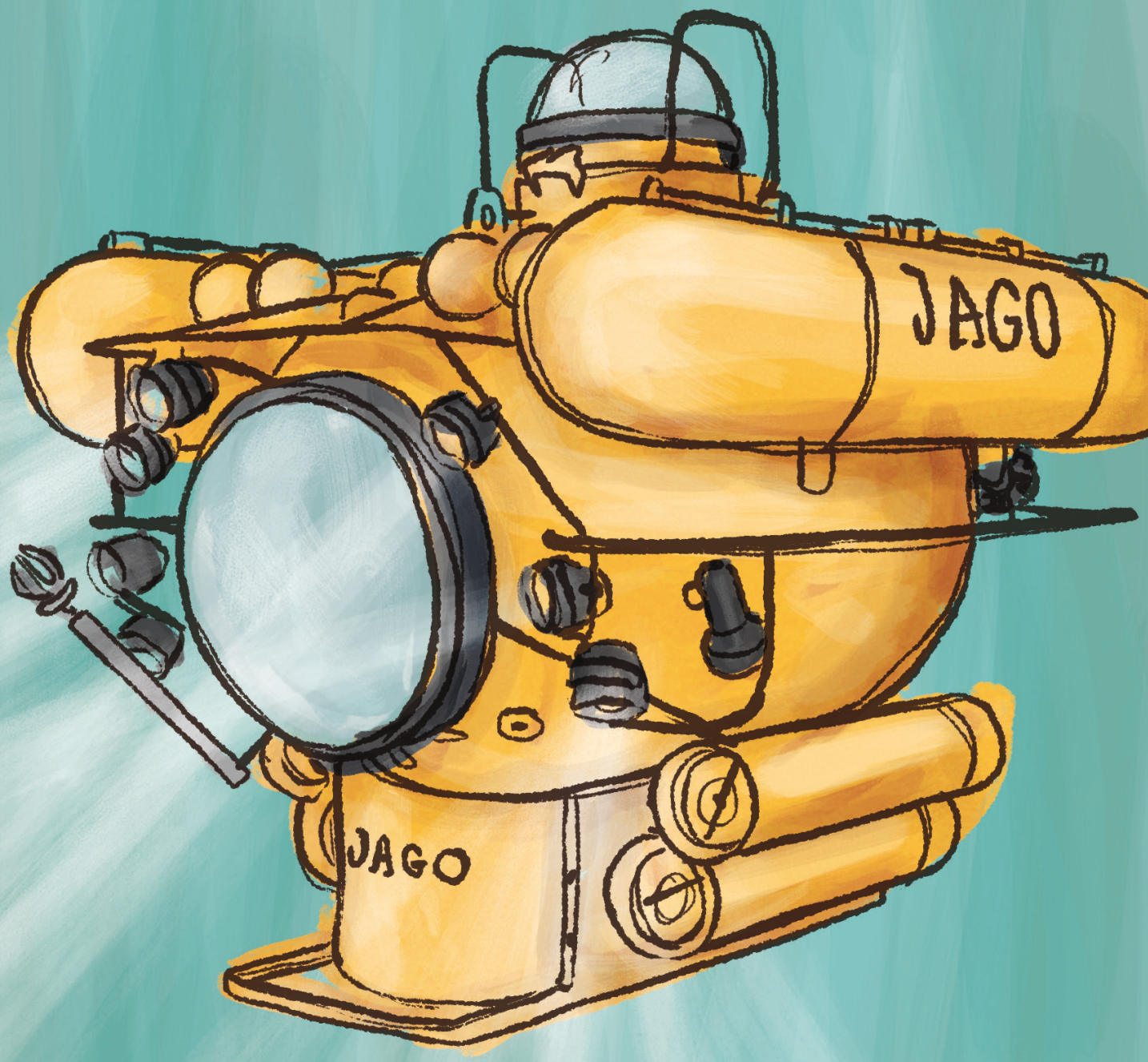
Structural and functional changes in

MARINE MICROBIAL COMMUNITIES

associated with

OXYGEN DYNAMICS

Gerdhard L. Jessen Reyes



Structural and functional changes in marine microbial communities associated with oxygen dynamics

Dissertation

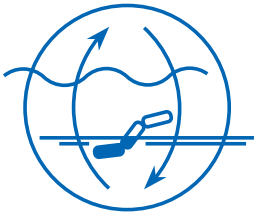
zur Erlangung des Doktorgrades
der Naturwissenschaften
- Dr. rer. nat. -

dem Fachbereich Biologie/Chemie
der Universität Bremen
vorgelegt von

Gerdhard L. Jessen Reyes

May 2015

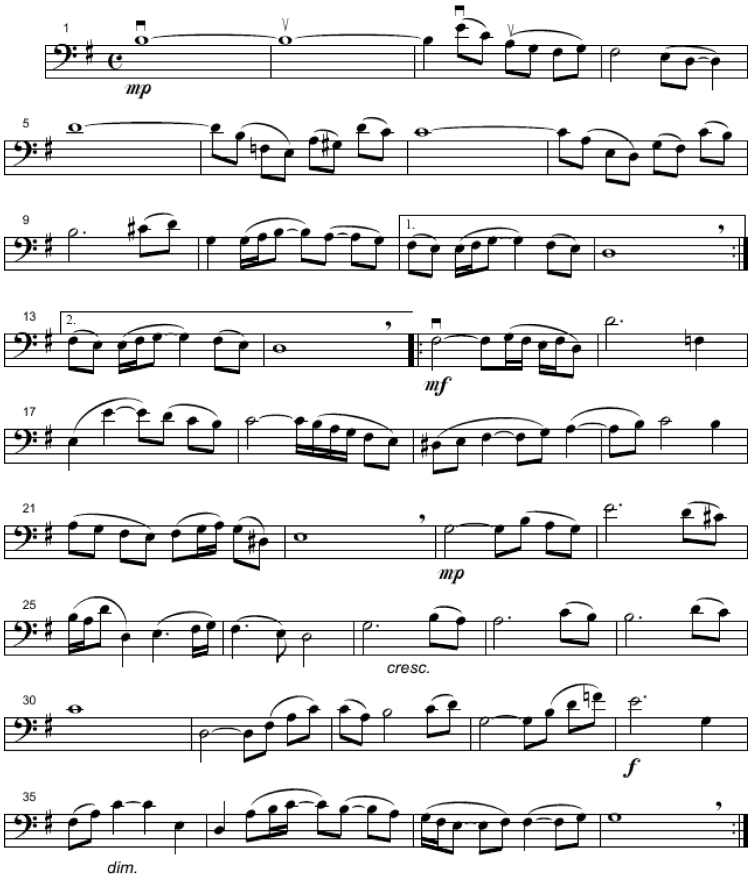
Die vorliegende Doktorarbeit wurde in Zeit von Januar 2011 bis Mai 2015 am Max Planck Institut für Marine Mikrobiologie angefertig.



Gutachterin: Prof. Dr. Antje Boetius
Gutachter : Prof. Dr. Ulrich Fischer

Prüfer : Prof. Dr. Silvio Pantoja
Prüfer : Prof. Dr. Hans-O. Pörtner

Datum des Promotionskolloquiums: 26. Juni 2015



J.S. Bach

Summary

Global warming and eutrophication drive enhanced levels of hypoxia in aquatic systems that, if persistent, induce fauna to emigrate or die. Under such circumstances the microbial size spectrum dominates benthic organic matter degradation and energy fluxes, constituting a shift in the benthic community composition that would likely impact diversity and functions of the entire ecosystem. However, the interrelations between hypoxia, organic matter reactivity and benthic community structure are poorly understood. This PhD study investigated how spatial and temporal changes in oxygen availability affect the benthic community structure and organic matter degradation on the outer Western Crimean Shelf (Black Sea). The selected area of study is an ecosystem naturally exposed to oxygen fluctuations and thus represents a natural laboratory for investigating the complex interconnection between hypoxic conditions and the characteristics of the associated organic matter and benthic community.

In Chapter 2, respiration rates, organic matter remineralization and the distribution of benthic organisms were investigated using an array of in situ and ex situ approaches. We show that with the onset of hypoxia the benthic community oxygen uptake rate decreased, and organic matter degradation pathways shifted from aerobic to anaerobic. Furthermore a shift in macro- and meiobenthos community structure was observed, together with a decrease in abundances of both faunal size classes. Overall, faunal remineralization rates were more important than microbial and geochemical oxidation processes in this system, but faunal remineralization was impacted by fluctuating oxygen concentrations.

In Chapter 3, sediment microbial communities were analyzed using high-throughput sequencing techniques. In parallel, sediment biogeochemical parameters and the lability of organic matter were measured to assess the effects of varying oxygen conditions on organic matter reactivity and on the structure and function of benthic microbial communities. It was observed that surface sediments accumulate more organic matter under hypoxic and anoxic conditions, accompanied by a decrease in faunal activity. However, microbial diversity increased towards anoxic conditions and was accompanied by an increase of microbial activity and a dominance of microbial organic matter degradation.

Where the Black Sea chemocline meets the seabed (between 150-170 m), the seafloor morphology has led to the accumulation of labile organic matter and to a release of sulfide. This environment harbored distinct thiotrophic mat-forming bacteria previously unnoticed in the Black Sea that, from our estimates, can potentially cover up to thousands of square kilometers (Chapter 4). The anaerobic community has the potential to degrade the deposited material under anoxic conditions and with increasing sediment

depth (i.e degradation time) (Chapter 3). However, a fraction escapes remineralization even up to millennial time scales. With the absence of bioturbation the biogeochemical activity of these organisms increases sulfide production that could eventually decrease the degradability of otherwise fresh organic matter. Accordingly, anoxic conditions presented three-fold more unique sulfur-bearing compounds, suggesting that sulfurization could protect organic matter from being degraded (Chapter 5).

Zusammenfassung

Die globale Erwärmung und Eutrophierung verstärken einen Sauerstoffmangel (Hypoxie) in Gewässern, der langfristig dazu führt, dass die vorhandene Fauna abwandert oder ausstirbt. Unter solchen Umständen sind Kleinstlebewesen maßgeblich für die Energieflüsse und den Abbau von organischem Material im Sediment verantwortlich. Dies verändert deren Lebensgemeinschaften, was voraussichtlich die Vielfalt und Funktion des gesamten Ökosystems beeinflusst. Allerdings sind die Wechselwirkungen zwischen Hypoxie, der Abbaubarkeit des organischen Materials und den benthischen Lebensgemeinschaften kaum bekannt. Im Zuge dieser Dissertation wurde daher untersucht, wie sich die räumlichen und zeitlichen Schwankungen der Sauerstoffverfügbarkeit auf die Lebensgemeinschaften und den Abbau organischer Stoffe im Sediment des westlichen Krim-Schelfs (Schwarzes Meer) auswirkt. Das ausgewählte Gebiet ist ein Ökosystem, das natürlichen Sauerstoffschwankungen ausgesetzt ist und sich daher eignet die komplexen Zusammenhänge zwischen Hypoxie, organischen Stoffen und den Lebensgemeinschaften zu erforschen.

Im zweiten Kapitel wurden die Respiration, die Remineralisierung organischen Materials sowie die Verteilung der Organismen mittels verschiedener in situ und ex situ Methoden untersucht. Wir konnten zeigen, dass sich mit einsetzender Hypoxie die Sauerstoffaufnahmerate der benthischen Lebensgemeinschaften verringerte und sich der Abbau organischer Stoffe auf anaerobe Stoffwechselwege verlagerte. Überdies beobachteten wir, dass sich die Makro- und Meiofauna veränderte und in ihrer Häufigkeit abnahm. Insgesamt war die Remineralisierungsrate der Makro- und Meiofauna zwar grösser als jene, die durch mikrobielle und geochemische Prozesse hervorgerufen wurden, jedoch wurde sie auch stärker vom schwankenden Sauerstoffgehalt beeinflusst.

Im dritten Kapitel wurden die mikrobiellen Lebensgemeinschaften im Sediment mittels Hochdurchsatz-Sequenzierungsmethoden analysiert. Zusätzlich wurden biogeochemische Parameter und die Labilität des organischen Materials gemessen, um die Auswirkungen verschiedener Sauerstoffbedingungen auf den Stoffabbau und auf die Struktur und Funktion der mikrobiellen Gemeinschaften zu untersuchen. Oberflächensedimente akkumulierten mehr organisches Material bei hypoxischen und anoxischen Bedingungen, unter gleichzeitiger Abnahme tierischer Aktivität. Die mikrobielle Vielfalt und Aktivität jedoch nahmen unter diesen anoxischen Bedingungen zu.

Dort wo die Chemokline, also der Übergang zwischen sauerstoffhaltigem und sauerstofffreiem Wasser (zwischen 150 - 170 m Wassertiefe), auf den Meeresboden trifft sammelte sich labiles organisches Material und es wurde Sulfid freigesetzt. Diese Umgebung enthielt spezielle thiotrophe Bakterienmatten, die bisher im Schwarzen Meer nicht

beschrieben wurden. Nach unseren Schätzungen könnten diese Matten möglicherweise tausende Quadratkilometer des Meeresbodens bedecken (Kapitel 4). Die anaeroben Mikroorganismen bauen sehr wahrscheinlich die abgelagerten Stoffe unter anoxischen Bedingungen und mit zunehmender Sedimenttiefe ab (Kapitel 3), wobei ein kleiner Teil der Stoffe jedoch nicht remineralisiert wird und sehr lange überdauert. Ohne Bioturbation führt die mikrobielle Aktivität zu einer erhöhten Sulfid-Produktion, die letztlich den Abbau von frischem organischem Material verlangsamt. Tatsächlich konnten wir zeigen, dass anoxische Sedimente viel mehr unterschiedliche Schwefelverbindungen enthielten, als oxische Sedimente, was darauf hindeutet, dass der Abbau von organischem Material durch Verschwefelung verhindert wird (Kapitel 5).

Contents

Abbreviations

454 MPTS	**454 M**assive **P**arallel **T**ag **S**equencing
AODC	**A**cridine **O**range **D**irect **C**ount
ARISA	**A**utomated **R**ibosomal **I**ntergenic **S**pacer **A**nalysis
BMU	**B**enthos **M**ediated oxygen **U**ptake
Chl *a*	**Chl**rophyll ***a***
$\mathbf{C}_{org}$	**o**rganic **C**arbon
CTD	**C**onductivity **T**emperature **D**epth profiler
DI	**D**egradation **I**ndex
DNA	**D**eoxyribo**N**ucleic **A**cid
DOC	**D**issolved **O**rganic **C**arbon
DOM	**D**issolved **O**rganic **M**atter
DOU	**D**iffusive **O**xygen **U**ptake
FT ICR MS	**F**ourier **T**ransform **I**on **C**yclotron **R**esonance **M**ass **S**pectrometer
NMDS	**N**on **M**etric **M**ultidimensional **S**caling
OM	**O**rganic **M**atter
$\mathbf{OTU}_{0.03}$	**O**perational **T**axonomic **U**nit at 3% dissimilarity
OTU	**O**perational **T**axonomic **U**nit
PUC	**PU**sh **C**orer
RNA	**R**ibo**N**ucleic **A**cid
$\mathbf{SSO}_{abs}$	**ab**solute **s**ingletons
$\mathbf{SSO}_{rel}$	**rel**atibe singletons
TDN	**T**otal **D**issolved **N**itrogen
THAA	**T**otal **H**ydrolyzable **A**mino **A**cids
TOU	**T**otal **O**xygen **U**ptake
TV MUC	**T**ele**V**ision guided **MU**lti**C**orer

Chapter 1

Introduction

Since oxygen became available on Earth, its dynamics over geological time scales have produced profound effects on the evolution of life and biogeochemical cycles, being a key electron acceptor for eukaryotes and many bacteria and archaea. Today's rapid global warming of oceans, their enhanced stratification and increased levels of eutrophication foster an imbalance between biological oxygen demand below the oxycline and its supply from surface waters. Together with several climate change related issues, the scientific community and policy-makers are debating how much of the ocean deoxygenation can be attributed to natural cycles or anthropogenic global warming. As a matter of fact, oxygen minimum zones in the oceans are expanding and shoaling, and hypoxic/anoxic conditions are being more often observed in regions previously unaffected by such conditions. Either at regional or global scales these phenomena are transforming the seas as we know them, their functions and therefore biodiversity across the entire range of ecosystem levels. This PhD study focused on the effects of spatial and temporal changes in oxygen availability on organic matter reactivity and benthic communities in the Black Sea. The Black Sea is the largest anoxic water body in the world. Its organic matter-rich shelves and benthic communities are exposed to a wide range of oxygenation regimes; from permanent oxic to fluctuating hypoxic, and permanent anoxic/sulfidic conditions. Therefore, this basin presents a natural laboratory to study the effects of oxygen availability on benthic ecosystems.

The following introduction sets a general overview on the consequences of hypoxia on marine ecosystems in the context of global climate change. In order to provide a comprehensive background, the key biogeochemical processes in marine sediments and concepts of organic matter degradation are reviewed. Furthermore, ecosystem responses to hypoxia are summarized, followed by an introduction of natural laboratories as means

to study the effects of hypoxia on organic matter preservation and biological communities. The objectives give an introduction to the following chapters represented by the enclosed manuscripts. Finally a description of the study area and proxies used to characterize the organic matter as well as the microbial community is presented.

Due to the complexity, remoteness and spatio-temporal variability of the seafloor, the relationships between tightly coupled biological, physical and geochemical processes are still a matter of study and foster the development of new technologies, especially in order to achieve accurate carbon budgets.

1.1 Organic matter in marine sediments

The great majority of organic matter that reaches marine sediments can be traced back to photosynthesis from marine or terrestrial ecosystems, where the latter origin evidently implies a previous transport to and deposition in the marine realm. Primary production in the oceans is estimated to reach up to 470 g C m^2 y^{-1} (Wollast 1998), a load of organic matter that during its journey through the water column, is exposed to a variety of degradation and remineralization processes that modify its composition while releasing inorganic nutrients to the water column. Only a small fraction of the primary produced organic matter reaches the seafloor and is either remineralized there or buried on longer timescales, a process termed the biological carbon pump. On geological timescales the burial of organic matter in sediments causes a net removal of CO_2 from the atmosphere, therefore playing a fundamental role in the global carbon cycle (e.g. Arthur et al., 1988; Berner and Canfield, 1989; Berner, 1990; Siegenthaler and Sarmiento, 1993; Archer and Maier-Reimer, 1994; Mackenzie, 2004; Ridgwell and Zeebe, 2005; Burdige, 2007; Ridgwell and Hargreaves, 2007).

On a global scale, less than ∼0.5% of the organic matter produced in the oceans or transported through riverine runoff escapes remineralization, being finally buried in the sediments (Hedges and Keil, 1995). The burial and diagenesis of this fraction has led to the formation of fossil fuels that are used by mankind since the industrial revolution. Changes in the portion of the buried organic matter can certainly lead to considerable differences in sedimentary carbon reservoir. Considering that marine primary production builds up $30 - 60 \times 10^{15}$ gram of carbon per year (Duarte and Cebrían, 1996) this may also lead to rapid change in the carbon budget of the atmosphere.

Notably, the percentage of the buried organic matter fraction is higher at continental margins (up to 3%), due to high sedimentation rates and shorter sinking times (Berner 1989; Canfield, 1994). Also, about one fifth of the total marine primary production

occur above continental shelves (0-200 m), although these regions cover less than 10% of the global seafloor (Wollast 2002). The high productivity coupled to a short organic matter settling time in the water column, leads to sedimentation rates in the order of 14.1×10^{15} g yr^{-1} (the global accumulation rate of sediments at the modern seafloor amounts to about 19.6×10^{15} g yr^{-1}) (Baturin, 2007; Wallman 2012). So more than 80% of carbon burial occur on the continental shelves, at depths shallower than 1000 m (Berger, 1989). In these regions the estimated organic carbon consumption rate of 0.7×10^{14} g yr^{-1} corresponds to ∼50% of the burial flux, implying that the remaining fraction is further decomposed below the deep biosphere via chemical processes yielding thermogenic gas, oil, kerogen and other products of high-temperature (>100°C) thermal maturation (Wallmann et al., 2012).

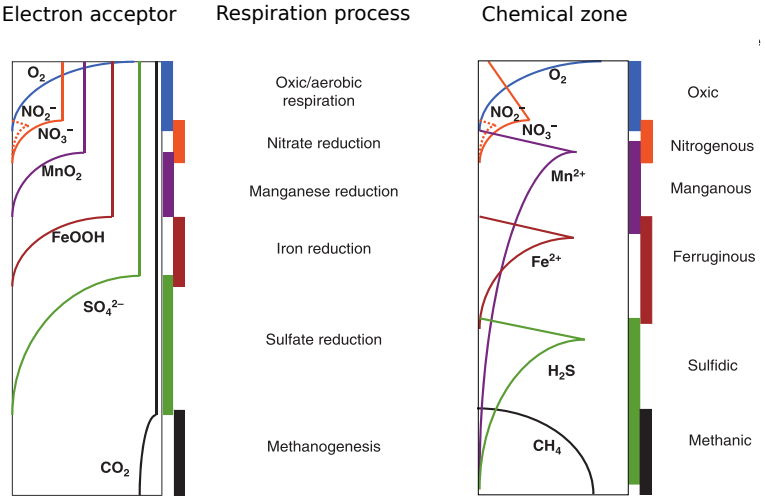
The seafloor is in fact "the ultimate sediment trap" (Herman et al., 2001) and despite the different sources and ongoing transformations its detrital particles are characterized by similar compositions (Burdige, 2007). However, slightly different molecular associations, or ratios between its compounds define major changes in organic matter degradation and consequently the degree of diagenesis. Diagenetic processes comprise the sum of physicochemical and biological alterations of organic matter after its deposition (Berner, 1980). Besides the abiotic factors that promote this complex process such as the availability of electron acceptors (Section 1.1.3), the presented work focuses mainly on the small (but numerous) living organisms that contribute to the diagenesis of organic matter: the microbial community.

1.1.1 Organic matter degradation: partitioning between metabolic pathways

The degradation of benthic organic matter is the result of the combined activity of the whole benthic community of animals and microorganisms, and depends on the composition and concentration of substrates, intermediate compounds, oxidants and reciprocal biological interactions (Berner, 1980; Middelburg, 1989; Hedges and Keil, 1995; Meysman et al., 2006; Burdige, 2007; Arndt et al., 2013; Middelburg, 2015). While benthic deposit feeders are able to take up and consume comparatively large particles by their intra-and extracellular digestive systems (Lopez and Levinton, 1987), only the small dissolved fraction (i.e. <600 daltons) can pass through microbial membranes (Weiss et al., 1991). Therefore, an essential step for metazoans and microorganisms is the depolymerization/hydrolysis of organic matter to intermediate forms with a lower molecular weight. This is possible by the enzymatic cleavage of molecular bonds, where hydrolysis (i.e. using water) is the dominant degradation process, either taking place inside metazoan digestive systems, or in case of microorganisms outside the cells (i.e.

extracellular hydrolysis; Arnosti et al., 2013). Under oxic conditions a single organism can fully mineralize the hydrolytic products to carbon dioxide. This kind of metabolism is usually confined to the sediment surface, defining the oxic zone. In absence of oxygen, organic matter breakdown is performed by a consortium of anaerobes. In parallel to the aerobic and anaerobic respiratory processes, an onset of fermentative metabolisms may co-occur, where organic compounds serve as both electron donors and acceptors inside the cell (Megonigal et al., 2003 and references therein). Thus, hydrolytic products undergo fermentation to alcohols and fatty acids and finally to acetate or hydrogen, i.e. the substrate for respiratory organisms performing the terminal organic matter degradation step. Indeed, many terminal degradation processes such as sulfate reduction or methanogenesis depend completely on fermentation products (Muyzer and Stams, 2008). The use of these substrates follows, with some overlap, a progression of respiration processes as the more thermodynamically favorable terminal electron acceptors become depleted during organic matter degradation (Fig. 1.1). Hence, as oxygen below the subsurface is depleted faster than it is supplied by diffusion, nitrate, manganese, iron, sulfate and carbonate are successively used as oxidants in respiration processes (Froelich et al., 1979; Canfield and Thamdrup, 2009). The spatial dimensions at which the switching between electron acceptors occurs depends on organic matter supply. Moreover, aerobic respiration dominates at lower deposition rates ($<$0.01 cm yr^{-1}) whereas anaerobic processes dominate at higher deposition rates (Canfield 1994, Burdige, 2007).

Different metabolic zones and associated microbial communities, although spatially separated in the sediment, are connected by the use of reduced compounds. For example, the sulfide released from subsurface sulfate reducers forms a concentration gradient which diffuses upwards eventually reaching the oxygenated surface sediments where it can be reoxidized by sulfide oxidizers using free oxygen (e.g. Chapters 2 and 4). Thus, on the long term, molecular oxygen serves as the final oxidative agent also for anaerobic organic matter degradation. In other words, under steady state conditions the benthic oxygen uptake reflects the metabolisms and activity of the whole community of aerobes and anaerobes (Thamdrup et al., 2000; Canfield et al., 2005; Glud, 2008). Benthic respiration rates scale according to organic matter supply, ranging orders of magnitude from the continental shelf to the deep ocean surface sediments. Thus, oxygen uptake in the oligotrophic deep seafloor is in the order of 50 mmol m^{-2} yr^{-1} whereas highly productive areas reach up to 6000 mmol m^{-2} yr^{-1} (e.g. Devol and Christensen 1993; Glud et al., 1994; Berelson et al., 1994; Hammond et al., 1996; Wenzhöfer and Glud 2002).



Reaction	kJ per reaction ΔG (acetate)
$O_2 + 1/2\,C_2H_2O_2^- \rightarrow HCO_3^- + 1/2\,H^+$	- 402
$4/5\,NO_3^- + 3/5H^+ + 1/2\,C_2H_3O_2^- \rightarrow 2/5\,N_2 + HCO_3^- + 1/5\,H_2O$	- 359
$7/2\,H^+ + 2MnO_2 + 1/2\,C_2H_3O_2^- \rightarrow 2MnO_2^+ + HCO_3^- + 2H_2O$	- 385
$15/2H^+ + 4FeOOH + 1/2\,C_2H_3O_2^- \rightarrow HCO_3^- + 4Fe^{2+} + 6H_2O$	- 241
$1/2H^+ + 1/2SO_4^{2-} + 1/2\,C_2H_3O_2^- \rightarrow 1/2H_2S + HCO_3$	- 43.8
$1/2H_2O + 1/2\,C_2H_3O_2^- \rightarrow 1/2CH_4 + HCO_3$	- 19.9

FIGURE 1.1: Upper panel, a scheme representing the depth distribution of common terminal electron acceptors and associated respiration processes and chemical zones. Lower panel, standard Gibbs free energy calculated for the aforementioned respiratory pathways (25°C, pH 7), with acetate as an electron donor (modified after Canfield and Thamdrup, 2009).

1.1.2 Controlling factors in organic matter degradation

Benthic mineralization and (bio-)availability of organic carbon are tightly coupled (Berner, 1980). However, several factors are involved in this strong correlation (Wenzhöfer and Glud, 2002; Glud, 2008; Fischer et al., 2009), controlling remineralization rates and burial of organic matter.

The controlling factors can be grouped according to (i) the time of exposition to degradation (ii) the degradation context (e.g. aerobic or anaerobic) and bioavailability of the organic matter and (iii) the type of degradation (microbial or animal) (Berner, 1980; Lopez and Levinton, 1987; Emerson and Hedges, 1988; Lee, 1992; Canfield et al., 2005; Burdige, 2007; Arndt et al., 2013).

Although several factors can co-occur, degradation is mainly related with water depth and sinking velocities of the particles that rain to the seafloor (Suess, 1980; Armstrong et al., 2001). Once the organic material (and minerals) has settled on the seafloor, the available time for degradation mainly depends on sediment deposition rates and particle reworking (i.e. bioturbation; Aller, 1994; Canfield, 1994). On the other hand, the rate and extent of organic matter degradation is controlled by an array of factors such as: its quality (i.e. bioavailability) (Berner, 1980; Emerson and Hedges, 1988; Niggemann et al., 2007), aggregation via cross-linking and hydrophobic interactions (Nguyen and Harvey, 2001), geopolymerization into organo-sulfur compounds or sulfurization (Adam et al., 2000), metabolite inhibition (e.g. sulfide, Okabe et al., 1995), physical protection inside the detrital pores (Mayer, 1994) grazing and viral lysis (Lee, 1992; Fuhrman, 1999), supply of oxidation agents, (e.g.oxygen, Canfield, 1994) and especially by the microbial community structure and function (Finlay et al., 1997; Arnosti, 2011).

Terms like organic matter quality, reactivity, freshness, lability and bioavailability, although not necessarily synonymous, are extremely related and in some cases used indistinctly (Berner, 1980; Emerson and Hedges, 1988; Middelburg, 1989; Cowie and Hedges, 1992; Canfield et al., 2005; Schubert et al., 2005; Burdige, 2007; Arndt et al., 2013). Indeed, the reactivity and quality of organic matter decreases with increasing degree of diagenesis, becoming aged, less fresh and in some cases less bioavailable. This is because not all of the organic matter constituents are degraded at the same rate and extent or by the same members of the microbial community (Section 1.2, 1.7). Thus, more labile material such as amino acids, carbohydrates, fatty acids, and pigments are degraded first, hence the quality of organic matter decreases with time and sediment depth (Westrich and Berner, 1984; Cowie and Hedges, 1992).

1.1.3 Oxygen availability and microbial organic matter degradation

Microbial degradation/mineralization and organic matter quality are tightly coupled. Indeed, microbial oxygen consumption is higher in coastal areas where organic matter availability is higher compared to the deep ocean seafloor (Wenzhöfer and Glud, 2002; Glud, 2008; Fischer et al., 2009). Similarly, microbial sulfate reduction and production of ammonium increase with the quality of organic matter (Pantoja and Lee, 2003; Schubert et al., 2005; Niggemann et al., 2007), a pattern that supports the often observed response of benthic microbial communities to the availability of fresh organic material (Moodley et al., 2002; Böer et al., 2009; Bienhold et al., 2012; Jacob et al., 2013). However, certain pre- and-post depositional conditions predominant in hypoxic ecosystems (e.g. sulfurization, absence of bioturbation), result in a decreased bioavailability even of high-quality organic matter (Moodley et al., 2011; Koho et al., 2013) that thus escapes rapid

biological degradation (Chapter 2 and 4). Under those circumstances the availability of oxygen plays a crucial role, not only by supplying an efficient electron acceptor, but also by indirectly influencing other organic matter degradation controlling factors, that are listed below.

Assessing the link between oxygen availability and the rate and efficiency of aerobic and anaerobic decomposition of sediment organic carbon has been a matter of geochemical studies for decades (e.g. Demaison and Moore, 1980; Emerson and Hedges, 1988; Pedersen and Calvert, 1990; Lee, 1992; Canfield, 1994; Kristensen et al., 1995; Burdige, 2007; Schmidt et al., 2011; Arndt et al., 2013). Although the answer is not yet clear, the widely accepted hypothesis is that after the degradation of labile compounds (e.g. proteins and carbohydrates) has occurred, the remaining refractory matter accumulates, in the absence of oxygen, due to the slow and inefficient bacterial depolymerization of complex molecules (e.g. complex lipids and lignin) (Kristensen et al., 1995). Even though microbial extracellular enzymatic degradation of organic matter does not require oxygen, some non-hydrolyzable bonds can only be cleaved through highly reactive peroxide groups (i.e. oxygen-oxygen single bond) or by oxygenases and peroxidases, therefore requiring oxygen as enzymatic co-factor (Canfield, 1994; Burdige, 2007 and references therein). Therefore, the presence of burrowing fauna may have strong effects on the efficiency of organic matter degradation, because by reworking surface sediments they can actively supply oxygen to deeper anoxic layers (Aller, 1994; Meysman et al., 2006, Burdige, 2007). Additionally, the accumulation of toxic compounds such as sulfide (Okabe et al., 1995), can be attenuated with the exchange of interstitial water. Moreover, benthic macrofauna can directly ingest up to 15% of detritus in sediments (Lopez and Levinton, 1987) and, through its digestive systems, break down complex molecules, making them available for the whole microbial community (Lee, 1992; Witte, et al., 2003) (Chapter 2 and 3).

It has been shown that microbial communities inhabiting oligotrophic deep ocean surface sediments, can rapidly react to the input of fresh organic matter: (i) at a functional level, by boosting its degradation rates (Moodley et al., 2002; Witte, et al., 2003) and (ii) at a community level, by changing its composition (Bienhold et al., 2012; Jacob et al., 2013). However, the response of microbial communities is less understood, when oxygen rather than the supply of organic matter, is the limiting factor, which is the case for most of the coastal systems subject to episodic or permanent eutrophication and/or hypoxia.

1.2 Hypoxia in marine ecosystems

High rates of benthic respiration and re-oxidation of reduced compounds can deplete oxygen in bottom waters to levels that may restrict (or limit) further aerobic activity. For unicellular organisms and small metazoa, oxygen enters the cell by diffusion. Larger metazoans have evolved gas exchange organs, transport systems and respiratory proteins to overcome the volume to surface ratio limitation of increasing body size (Calder, 1984). Yet, under high energy demands or severe environmental hypoxia metazoans shift to an anaerobic metabolism (Piiper et al., 1982; Pörtner et al., 1985; Grieshaber et al., 1994). However, most metazoans including burrowing fauna and deposit-feeders, cannot tolerate oxygen concentrations <63 μM for longer timescales, albeit the response and threshold may vary across different taxa, body sizes and life stages (Piiper, 1982; Levin et al., 1991; Levin, 2003; Vaquer-Sunyer and Duarte, 2008). Even though there is not one tolerance threshold for hypoxia that is representative for all organisms (Levin et al., 2009), a threshold of 63 μM is widely accepted for defining hypoxia also at an ecosystem level (IPCC 2007; Diaz and Rosenberg, 2008; Middelburg and Levin, 2009).

Dissolved oxygen concentration is typically expressed as mg O_2 L^{-1}, mL O_2 L^{-1} or micromolar oxygen (μM O_2 $\sim$ μmol O_2 L^{-1}). From here on, oxygen concentrations will be expressed as molar concentrations and the hypoxia threshold as concentrations of dissolved oxygen < 63 μM (i.e. 63 μM O_2 = 2 mg O_2 L^{-1} = 1.4 mL O_2 L^{-1}; e.g. IPCC 2007, Middelburg and Levin, 2009). Additionally, since most of the non-sandy coastal sediments are oxic only at the top few millimeters (e.g. Jørgensen, 1982; Revsbech, 1989; Glud, 2008), from here on, the term "oxic", "hypoxic", "anoxic" and "sulfidic" will refer to the bottom water oxygenation (Fig. 1.2).

1.2.1 Ecosystem response to hypoxia

Environmental hypoxia may progressively develop from episodic or periodic events to become persistent and even permanent in broad regions of the oceans, from oxygen minimum zones to small, enclosed basins and fjords with restricted circulation (Diaz and Rosenberg, 2008; Rabalais et al., 2009). In coastal areas, eutrophication and hypoxia are closely related due to the factors mentioned above (Section 1.1.3 and 1.1.4). Indeed, a shift from oxic to anoxic degradation pathways may occur in ecosystems experiencing high organic matter loading or exposed to hypoxic conditions.

High rates of organic matter deposition lead to increased microbial activity and thus increased oxygen consumption. If consumption exceeds oxygen supply sulfate respiration may replace oxic respiration (Section 1.1.3). As the sulfide concentration gradient

increases, the redoxcline compresses the oxic horizon to the sediment water interface together with the habitat of the infauna, which eventually migrate or die. Finally, if sulfide reaches the surface sediments, the development of euxinia (i.e. spread of sulfidic conditions into the water column) can take place (Levin et al., 2009; Middelburg and Levin, 2009). The development of thiotrophic microbial mats is perhaps the most evident response of the microbial community to eutrophication and hypoxia. Thiotrophic mats are dense accumulations of microorganisms which use reduced forms of sulfur as electron donors at the sediment redox interface (Jørgensen, 2010 and references therein). Such microbial mats can cover large portions of the seabed in areas of organic rich sediments, coastal upwelling regions and ecosystems subject to eutrophication and hypoxia. In these regions sulfate reduction occurs at such high rates that free sulfide can reach the oxidized sediment surface (Jørgensen, 1977; Williams and Reimers, 1983; Schulz and Jørgensen, 2001; Mußmann et al., 2003). Such favorable conditions for mat-forming bacteria are met at the continental margins off Perú, Chile, Pakistan, Oman, Namibia, the Baltic Sea and some Scandinavian fjords (Gallardo, 1977; Jørgensen, 1977; Schulz et al., 1999; Jørgensen and Gallardo, 1999; Brüchert et al., 2003; Heyl et al., 2010; Salman et al., 2011). In contrast, not all organic-rich sediments exposed to hypoxic conditions harbor microbial mats. That is for example the case for the inner West Indian shelf, where low sulfate reduction rates, probably associated to low bioavailability of organic matter (Moodley et al., 2011), constrain the release of free sulfide hampering the development of thiotrophic mats (Levin et al., 2009). However, little is known about the microbial community associated with the mats in such ecosystems, which may provide key functions as part of the thiotrophic community (Chapter 4).

1.2.2 Effect of hypoxia on faunal and microbial communities

The ecosystem response to hypoxia depends on the duration, predictability, and intensity of oxygen depletion and on eventual formation of hydrogen sulfide (Levin et al., 2009). However, hypoxia is expected to be accompanied by loss in biodiversity, ecosystem function and mortality of benthic organisms, followed by some level of renewed colonization with the return of normal oxygen conditions (Diaz and Rosenberg, 2008).

Adaptation of metazoans to oxygen depletion has been relatively well studied and is associated with biochemical, physiological and behavioral changes (Levin, 2003). Various taxa exhibit different tolerance levels to low oxygen conditions. Among large metazoans the groups most tolerant to hypoxia are generally considered to be cnidarians and annelids. In contrast, crustaceans, followed by fishes and molluscs represent the taxa most sensitive to low oxygen (Vaquer-Sunyer and Duarte, 2008, Levin et al., 2009). Episodes of massive death of crustacean and fishes along the shore are indeed often an indicator for

hypoxic events (Chan et al., 2008). Under persistent hypoxic conditions, macrobenthos and nekton emigrate or die, and species composition shifts, with subsequent changes in ecosystem functioning (Scheffer, 2010), e.g. production, organic matter cycling. The microbial size spectrum then often dominates benthic energy fluxes (Fig. 1.2; Diaz and Rosenberg, 2008). Therefore, any change in the microbial community structure may have repercussions on the flux of energy and matter through the ecosystem (Chapter 3 and 4). However, little is known about how benthic microbial community structure varies in space and time (Chapter 2) in zones where oxygen concentrations fluctuate around the hypoxic threshold (63 μM).

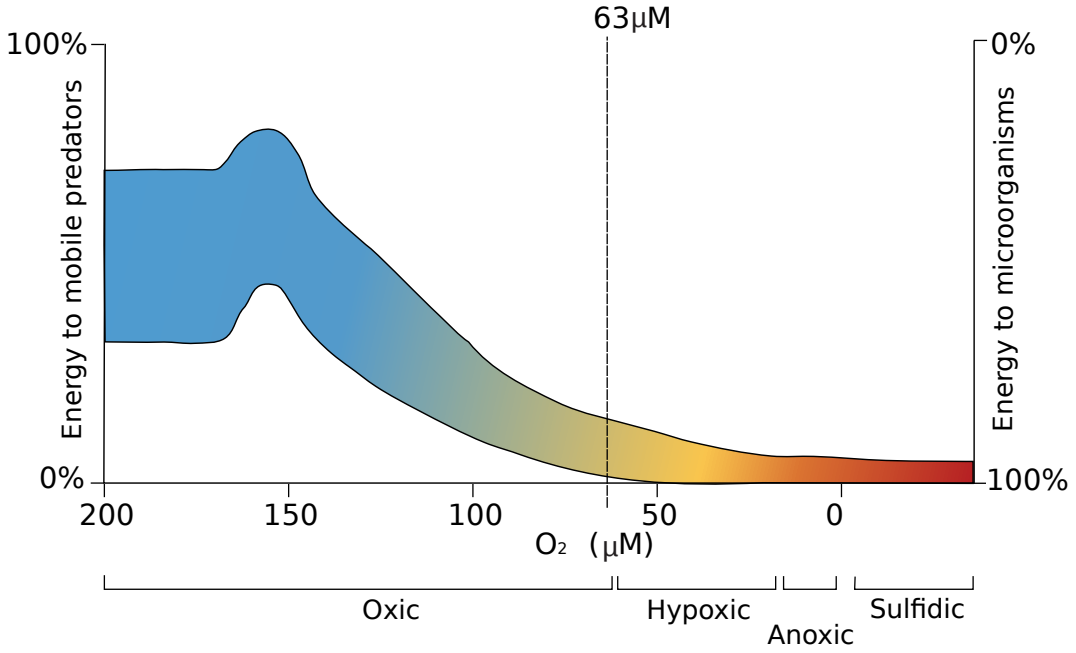


FIGURE 1.2: Conceptual view of how hypoxia alters ecosystem energy flow, showing the range and flux of energy transferred under different stages of hxpoxia (modified after Diaz and Rosenberg, 2008).

Compared to the pelagic realm, less is known about benthic microbial community composition along oxygen gradients. Apart from the differences in spatial scales, pelagic and benthic microbial communities that populate these gradients appear to have a similar ecology (Ulloa et al., 2013 and references therein), probably due to the fact that the water column oxycline is biogeochemically similar to the sediment redox zone (Fenchel and Finlay, 2008).

Typical microbial communities in oxygen-deficient waters are affiliated to *Proteobacteria*, *Bacteroidetes* and candidate division Marine Group A, followed in abundance by *Cyanobacteria*, *Firmicutes*, *Verrucomicrobia*, *Gemmatimonadetes*, *Lentisphaerae*, and *Chloroflexi* (Madrid et al., 2001; Stevens and Ulloa, 2008; Grote et al., 2008; Herlemann

et al., 2011; Zinger et al., 2011; Stewart et al., 2012; Ulloa et al., 2012; Wright et al., 2012; Ulloa et al., 2013). Their distribution is not homogeneous along the redox-gradient, but organized according to oxygen availability (Ulloa et al., 2012; Wright et al., 2012; Ulloa et al., 2013). Accordingly, *Alphaproteobacteria* (SAR11 and *Rhodobacterales*), *Gammaproteobacteria* (SAR86 for oxic, and Arctic96B together with agg47 for hypoxic waters) and *Bacteroidetes* (*Flavobacteriales*) are present from surface oxic waters down to hypoxic conditions, whereas *Deltaproteobacteria* (SAR324 cluster and genus *Nitrospina*) are confined to less oxygenated waters. On the other hand, anoxic and sulfidic conditions are dominated by SUP05 (*Gammaproteobacteria*), together with taxa affiliated with sulfur oxidizers and reducers such as *Arcobacteraceae* (*Epsilonproteobacteria*) and *Desulfobacteraceae* (*Deltaproteobacteria*). Within the Archaea, the most abundant phylotypes are affiliated with *Thaumarchaeota* (Marine Group I), and *Euryarchaetoa* (Marine Group II and III), which are dominant under hypoxic and anoxic conditions (Madrid et al., 2001; Vetriani et al., 2003; Coolen et al., 2007; Woebken et al., 2007; Jeon et al., 2008; Labrenz et al., 2010; Belmar et al., 2011; Ulloa et al., 2013). In case of benthic microbial communities exposed to hypoxic, anoxic or sulfidic bottom waters, *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* are by far the most abundant bacterial clades (Julies et al., 2010; Quaiser et al., 2011; Köchling et al., 2011; Zinger et al., 2011; Julies et al., 2012; Liu et al., 2012; Reese et al., 2013; Sinkko et al., 2013). In general, *Gammaproteobacteria* (*Altermonadales*) decrease in abundance with sediment depth (and towards anoxic conditions), while *Deltaproteobacteria* (*Desulfobacterales*, *Desulfuromonadales*, and *Syntrophobacterales*) and *Chloroflexi* increase. On the other hand, *Bacteroidetes* (*Flavobacteria* and *Sphingobacteria*) are abundant at surface hypoxic sediments and also present in deeper horizons and anoxic bottom water conditions.

The Black Sea microbiological communities associated with hydrocarbon-rich benthic ecosystems are relatively well studied. However, particularly the diversity of Black Sea benthic microbial communities remains less understood (Chapter 3 and 4).

The main groups inhabiting the upper Black Sea hypoxic waters are affiliated with *Alphaproteobacteria* (mainly SAR11), whereas *Gammaproteobacteria* (SUP05, *Methylococaceae*), *Deltaproteobacteria* and Marine Group A become more abundant towards anoxic conditions (Vetriani et al., 2003; Schubert et al., 2006; Lam et al., 2007; Fuchsman et al., 2011). On the other hand, *Chlorobiaceae*, *Epsilonproteobacteria* and *Planctomycetes*, although less abundant, may play an important role as autotrophs associated with the chemocline (Kuypers et al., 2003; Manske et al., 2005; Marschall et al., 2010). Regarding the archaeal community,*Methanomicrobia* and *Crenarchaeota* are the dominant groups under hypoxic conditions (Lin et al., 2006; Schubert et al., 2006; Lam et al., 2007). The far less characterized benthic microbial community comprises taxa affiliated

with *Chloroflexi* (*Anaerolineae* and *Caldilineae*), candidate division JS1 and *Deltaproteobacteria* (*Desulfobacterales*), which dominate surface and subsurface sediments (Ince et al., 2007; Leloup et al., 2007; Tanase et al., 2009; Schippers et al., 2012), whereas *Euryarchaeota* and *Crenarchaeota* present the higher archaeal abundances (Schippers et al., 2012). It is important to notice that all of the above-mentioned studies are based on CARD-FISH or quantitative real-time PCR (qPCR), meaning that only selected groups were screened. Moreover, most of the sediments were retrieved at water depths greater than 400 m, therefore exposed to permanent euxinia.

1.3 Global climate change and ocean deoxygenation

Increasing levels of greenhous gases in the atmosphere are producing a heat excess that is promptly absorbed by the oceans. Higher temperatures lead to a lower oxygen solubility and consolidate the stratification of the water column. At the same time, higher temperatures enhance metabolic rates and boost productivity, increasing the biological oxygen demand, fueling a positive feedback that is in fact deoxygenating the oceans (Keeling et al., 2010; Hoegh-Guldberg and Bruno, 2010; Falkowski et al., 2011; Rhein et al., 2013; Pörtner et al., 2014). Worldwide, oxygen minimum zones are expanding and shoaling, leading to a reduction of available habitats for animals with high oxygen requirements (Stramma et al., 2011; Gilly et al., 2013). Indeed, paleoceanographic evidence has shown major disturbances on continental shelf ecosystems in response to ancient episodes of hypoxia (18 to 11 ka), where ecological recovery spanned up to millennia (Moffitt et al., 2015).

Coastal areas are more susceptible to hypoxic conditions because nutrient loading is either naturally reinforced by coastal upwelling or by surface runoff. The impact of the latter process is largely aggravated by human activities. As a result, a stronger rate of oxygen-decrease is observed in continental margins compared to the open oceans (Gilbert et al., 2010). Indeed, sporadic to periodic hypoxic conditions (i.e. dead zones) in coastal areas have increased in size and number since the last century (Fig. 1.3) (Diaz and Rosenberg, 2008). A further extension of these phenomena would have strong, and potentially irreversible, negative effects on marine ecosystems and their biogeochemical cycles (Pörtner et al., 2014; Rabalais et al., 2014).

Low oxygen concentration areas in the oceans have received some attention since the seminal works of Sverdrup (1938) and Wyrtki (1962). However, the number of scientific studies related to ocean deoxygenation and hypoxia increased exponentially over the last decade (Rabalais et al., 2014). This is mainly due to the expanding and shoaling of oxygen minimum zones and the dramatic increase of hypoxic and anoxic events, or

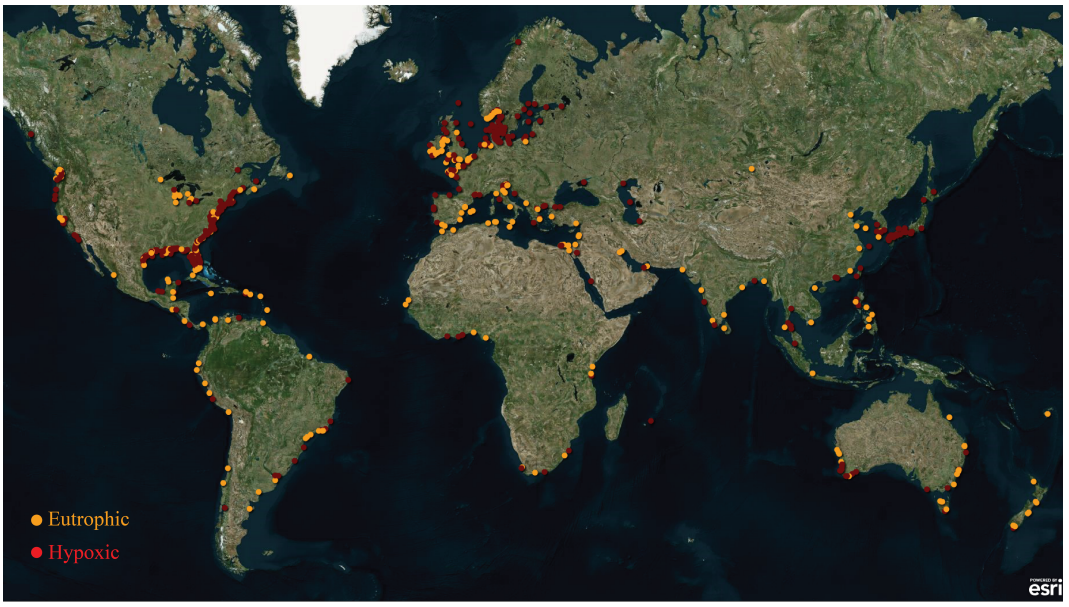


FIGURE 1.3: Global distribution of coastal areas impacted by eutrophication and/or hypoxia (time frame: 1850-2010). Note that distribution of eutrophication and hypoxia matches areas of higher population density and, in many cases, eutrophic and hypoxic conditions occur in the same area (modified after Diaz et al., 2011).

so called dead zones (Diaz and Rosenberg, 2008; Keeling et al., 2010; Rabalais et al., 2014). In contrast to dead zones (Diaz and Rosenberg, 2008), naturally occurring oxygen minimum zones are not associated with eutrophication in coastal waters, but to highly productive areas of the ocean (Keeling et al., 2010; Gilly et al., 2013). Already long ago it was understood that a high oxygen uptake rate coupled to poor ventilation may lead to low oxygen concentrations at intermediate water column depths (Sverdrup, 1938; Wyrtki, 1962). Usually associated with depths between ∼100-1000 m, the limits of these oxygen minimum zones are defined by dissolved oxygen concentrations of about 20 μM O_2 as for the Pacific and the Indian ocean, although a higher threshold (∼45 μM) is applied to the Eastern Atlantic oxygen minimum zone (Karstensen et al., 2008; Keeling et al., 2010 and references therein). The rather stable character of these systems permitted the pelagic and benthic biota to evolve a series of adaptations in their physiology, structure and behavior, in order to cope with the persisting hypoxic conditions (Levin, 2003 and references therein). In case of microorganisms, a shift towards taxa affiliated to anaerobic metabolisms such as sulfate reduction has been shown for oxygen minimum zones (Canfield et al., 2010; Stewart et al., 2012; Ulloa et al., 2012; Wright et al., 2012). Contrarily, besides the relatively well characterized mat-forming bacterial communities, little is known about the structure of benthic microbial communities exposed to hypoxic conditions. However, swifts in function have been shown for the pelagic counterpart (Section 1.2.2), as anaerobic metabolic pathways dominate microbial organic matter degradation towards hypoxic conditions (e.g. Thamdrup and

Canfield, 1996; Niggemann et al., 2007).

Irrespective of whether ocean deoxygenation responds to natural cycles, anthropogenic global warming or a combination of both, models predict that, with continuation of present carbon emissions, oceans may lose up to 7% of oxygen by the year 2100 (Shaffer et al., 2009). Furthermore, open ocean areas experienced an oxygen loss of ~0.5 μM since the second part of last century (Stramma et al., 2008). Thus, it is predicted that by the end of the century dissolved oxygen concentrations may decrease by a range of 20-200 μM in the intermediate water masses of the North Pacific, North Atlantic and Southern ocean (Hoegh-Guldberg et al., 2014), with an expansion of hypoxic zones by 30% (Bopp et al., 2013).

Overall, the predicted ocean deoxygenation and spreading hypoxic conditions will most likely select for highly adapted fauna where the microbial size spectrum would dominate matter and energy fluxes (Fig. 1.2 and 1.4; Pörtner et al., 2014). Comparing how microbial communities differ between oxic, hypoxic and anoxic regimes will help to better assess responses in community structure and function to changing oxygen conditions. In the future this may enable better predictions of how microbial communities and ecosystem functioning may change with the projected spread of hypoxia and dead zones.

As presented in Section 1.2, it is hard to discriminate whether high organic matter deposition, hypoxia or both may cause shifts in the microbial community structure. In this regard, the continental shelf of the Black Sea, with its oxygen dynamics and homogeneous particle flux on its continental shelf (Section 1.6), appears as a natural laboratory for studying the effect of different oxygen conditions on microbial communities and on organic matter degradation (Chapter 2, 3, 4 and 5).

1.4 Natural laboratories to study environmental hypoxia

Oxygen minimum zones appear as natural laboratories for assessing the effect of environmental hypoxia on the ecosystem. Indeed, most of the knowledge regarding biogeochemical cycles, animal adaptations and microbial ecology under hypoxia has been generated from studying oxygen minimum zones. Although most of the research focused on the pelagic realm, oxygen minimum zones impinge upon large portions of the seafloor, covering an area in the order of 1,000,000 km^2 worldwide. More than half of this extent belongs to the Indian Ocean (Arabian Sea and Bay of Bengal) whereas ~30% and 10% are found in the eastern Pacific and the southeastern Atlantic, respectively (Helly and Levin, 2004). However, due to the oceanic features of the oxygen minimum zones, most of the exposed seafloor corresponds to the slope (>300 m water depth) and only

seasonally to shallower sediments. This seasonality makes it difficult to assess long-term effects on benthic communities and sediment geochemistry.

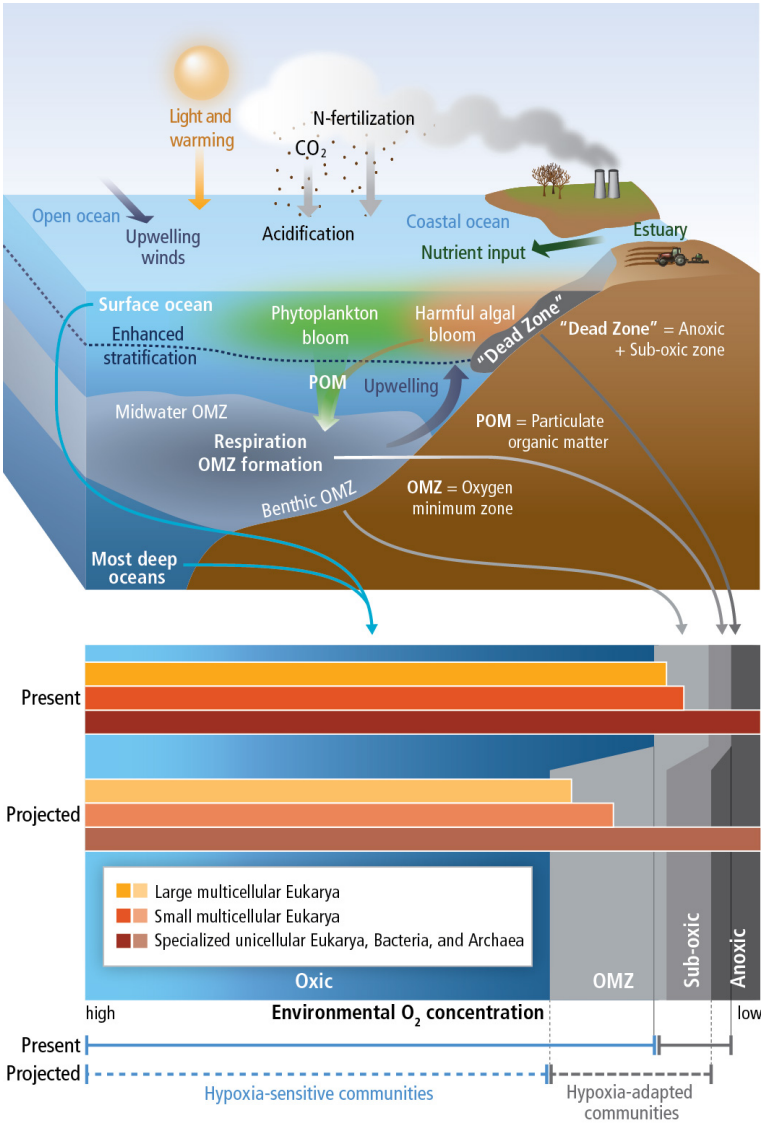


FIGURE 1.4: Upper panel, schematic view of the mechanisms behind the formation of hypoxic conditions on coastal areas and open ocean, including biological, geochemical and physical factors involved (see text for details). Lower panel, present and projected effect of hypoxia on the distribution of marine organisms, from microbes to metazoans (after Pörtner et al., 2014).

Contrarily, the Black Sea presents unique water column characteristics of stable oxic, variable hypoxic and anoxic/euxinic conditions, above, within and below the chemocline, respectively (Section 1.6). These features expose organic-rich sediments of the shelf and shelf-break (~130 to 170 m water depth) to unique environmental conditions, making the Black Sea a natural laboratory for studying the effects of hypoxia on a benthic ecosystem. Thus, the work presented here focused on Black Sea sediments, precisely

from the northwestern shelf and shelf-break of the Peninsula Crimea (Fig. 1.5). The sediments were sampled with the aim of isolating the effects of oxygen availability from the input of organic matter.

1.5 Objectives

Global warming and eutrophication promote hypoxia in aquatic systems with repercussions on fluxes of energy and matter, and consequences on ecosystem diversity and functioning. Under persistent hypoxic conditions, macrobenthos and nekton emigrate or die, and the microbial size spectrum dominates benthic energy fluxes (Diaz and Rosenberg, 2008). Shifts in the structure of benthic communities will likely result in changes of energy and matter flux and overall ecosystem functioning. However, the interrelations between hypoxia, organic matter reactivity and benthic community structure are poorly understood and are the major focus of this thesis.

The overall aim of this thesis was to determine how spatial and temporal changes in oxygen availability affect the diversity, composition and abundance of (i) benthic communities: from macrofauna to microorganisms and (ii) organic matter reactivity: from the bulk to the dissolved fraction.

To specifically test for the effects of hypoxia on organic matter degradation and on natural benthic communities, the Black Sea was used as a natural laboratory, because it offers unique characteristics, with stable oxic, variable hypoxic and anoxic/euxinic conditions meeting organic-rich sediments of the shelf and shelf-break.

The main questions addressed within our studies were:

-How does oxygen supply influence the quality and preservation of organic matter (from the bulk to the dissolved fraction) and its degradation by the benthic community? **(Chapter 2, 3, 4 and 5)**

-To what extent does hypoxia affect rates of aerobic and anaerobic organic matter remineralization? **(Chapter 2 and 3)**

-How do hypoxia influence the structure and function of benthic communities, and how do different benthic size classes respond (i.e. macrofauna and microorganisms); does hypoxia select for certain taxa? **(Chapters 2, 3, and 4)**

1.6 Oceanographic characterization of the Black Sea

The Black Sea is a semi-enclosed inland sea situated between western Asia and eastern Europe. Surrounded by Ukraine, Russia, Georgia, Turkey, Bulgaria and Romania, it is the largest natural anoxic water body in the world. The basin was formed in the Cretaceous by northward drift of the Arabian plate which cause the closure of the Tethys ocean (Brunet and Cloetingh, 2003; Nikishin et al., 2003). Since the Miocene the Marmara Gateway (Bosphorus Strait, Marmara Sea and Dardanelles Strait) has been the only connection of the Black Sea to other oceans (Çağatay et al., 2006). This junction opened at least 12 times over the past 670 ka (Badertscher et al., 2011), acting as a gate not only for water exchange, but also for migration of marine organisms from freshwater to brackish-water Paratethyan environments (Nicholas and Chivas, 2014). Another consequence of this restricted circulation is the development of permanent euxinic (sulfidic) conditions below the chemocline (Fig. 1.6), which converted the Black Sea into a unique ecosystem. Since the end of the ice age the water level in the Black Sea raised gradually over 10 ka until its present status (Yanko-Hombach et al., 2007), The development of euxinic conditions started about 5.3 ka ago and the chemocline oscillation reached the present position about 2.6 ka later (Eckert et al., 2013), acting as a physicochemical barrier for ventilation and constraining the distribution of organisms.

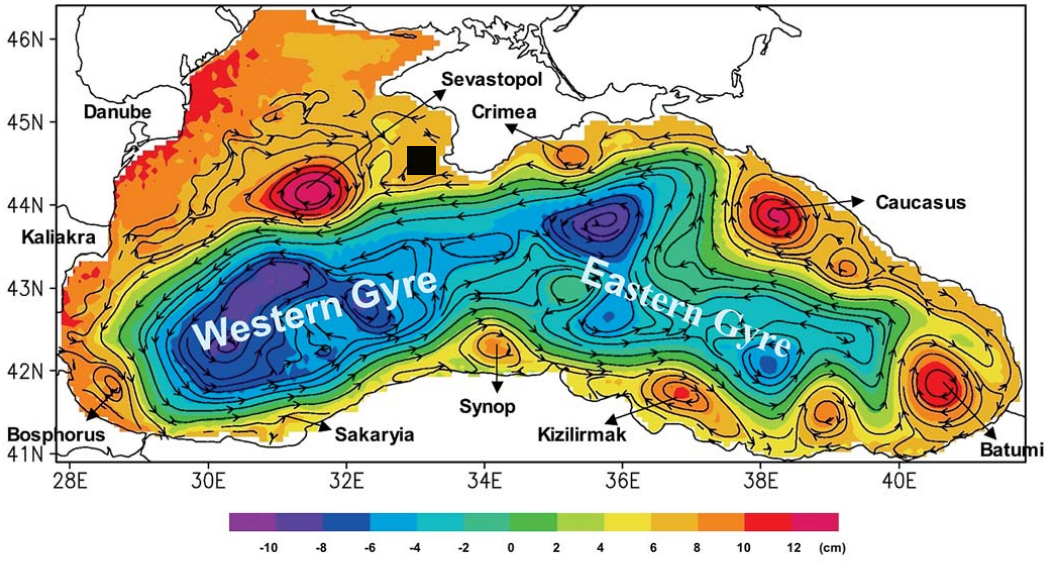


FIGURE 1.5: Sea level (cm) and surface streamlines simulated for the Black Sea, depicting the eastern and western gyres, and the major sub-basin gyres (Batumi and Sevastopol eddies). The name of the major coastal eddies are also given (after Stanev, 2005). The black square in the map depicts the study area.

The Black Sea continental shelf averages about 40 km, from the coast to the shelf break at 100 to 150 m depth (Nicholas and Chivas, 2014). Downslope the basin reaches its maximum depth of 2,206 m at the Abyssal plain (Ross and Degens, 1974).

The continental shelf is represented by seven regions: east and west Anatolian shelves, Caucasian Shelf, Kerch-Taman and southern Crimean Shelf, and South and Northwestern Shelf (western Crimea). The latter, which is the most extensive (190 km), covers more than 90% of the total area of the Black Sea shelf (Panin and Jipa, 2002). The Northwestern Shelf also receives most of the 3×10^2 km^3 river runoff through the Danube, Dniester, Bug, and Dnieper Rivers. This massive fluvial discharge appears as the main driver of eutrophication and hypoxia on the Northwestern Shelf (Capet et al., 2013). The Black Sea is therefore characterized by estuarine features as the result of the asymmetric water exchange trough the Marmara Gateway (the outflowing surface current is two times larger than the inflowing Mediterranean deep counter-current). Indeed, the basin surface salinity (S=17-19) is about half that of the Mediterranean Sea (S=36-38) producing an estuarine type circulation, where the denser Mediterranean water flows at depth through the Bosphorus Strait, mixing with local bottom waters (Stanev, 1990), and flowing out at the surface (Fig. 1.6)(Murray et al., 2007).

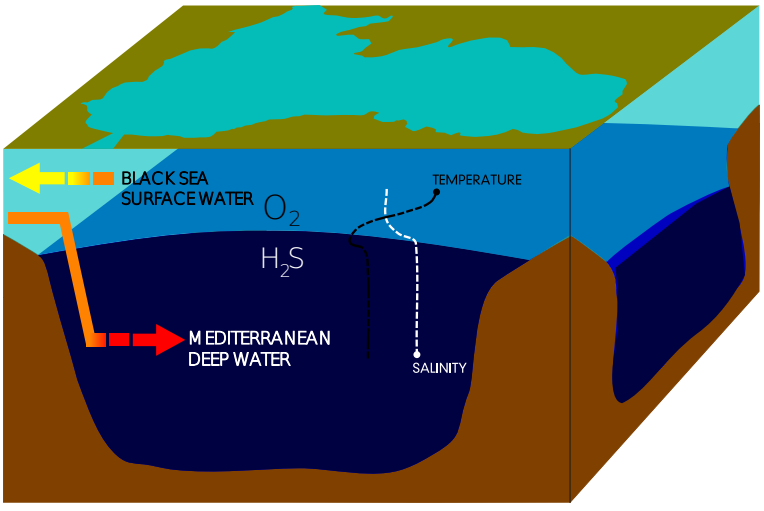


FIGURE 1.6: Schematic view of the water exchange trough the Bosphorus and hydrographic properties of the water column (modified after Oguz et al., 2004; Canfield and Thamdrup, 2009).

However, the Black Sea is also characterized by typical features of the open oceans: wind-driven circulation, gyres and eddies, thermohaline circulation and shallower ventilation into its thermocline (Murray et al., 2007). Water masses of the same density

stratify the water column constraining vertical mixing (Stanev, 2005). Indeed, the combination of a strong thermocline and halocline creates a pycnocline (100-150 m depth), which greatly reduces the mixing of surface oxygenated waters with anoxic waters below the pycnocline (Stanev, 1990). At the interface, a chemocline forms, were oxygen and sulfide may co-exist in dynamic equilibrium (Sorokin, 1972; Jørgensen et al., 1991).

The position of the chemocline is not uniform in the entire water body but dome-shaped, being shallower in the central basin compared to the shelves (Stanev et al., 2014). Along the shelf the chemocline encounters the seafloor, exposing its sediments to a dynamic range of oxygenation regimes from permanent oxic to variable and anoxic/sulfidic conditions. As a result, the Black Sea shelves are exposed to oxic conditions, hypoxic conditions dominate on the shelf breaks, while anoxic and sulfidic conditions prevail from the upper slopes to the abyssal plain (Murray et al., 2007). The extension of this feature appears to be more evident in the Northwestern region of the Black Sea (Crimea), where the shelf reaches its maximum area. Here, even small variations of the chemocline's depth produce drastic changes in bottom-water oxygen availability at the seafloor within timescales of days to hours (Friedrich et al., 2014).

1.7 Proxies to assess organic matter quantity and quality

The amount and quality of available biogenic organic matter in an ecosystem is the result of its production and selective degradation (Fig. 1.7). A series of complementary methods were used in this study to asses organic matter quantity and quality and degradation, from organic carbon (C_{org}) and total nitrogen to Chlorophyll *a* and non-protein amino acids. Hence, the distribution of bulk organic matter such as organic carbon and nitrogen, pigments and total hydrolyzable amino acids were measured to assess the lability of organic matter and to determine the connections between oxygen supply and organic matter reactivity as described in the next subsections.

1.7.1 Organic carbon and total nitrogen content

In the past organic matter has been measured by loss on ignition (Ball, 1964). This method is performed by oxidizing the organic matter to carbon dioxide at temperatures between ~200 and 500°C. The determined weight loss may be correlated to the biogenic material suitable for degradation. Nowadays, instruments such as elemental analyzers, using combustion, can accurately measure the abundance of the organic material from a sediment sample, where carbon and nitrogen are the main components of the biogenic organic matter. Indeed, sediments can be characterized by the concentration (or

percentage) of organic carbon (C_{org}), and classified as "organic-rich" or "organic-poor" sediments (Berner, 1969). Thus, surface marine sediments present values that span over two orders of magnitude: from ~0.2% up to 20% C_{org} for deep ocean seafloor to coastal upwelling regions, respectively (Reimers and Suess, 1983; Emerson et al., 1987). Looking at different profiles of organic carbon content versus sediment depth (Fig. 1.7) it is evident that, even though the concentrations are different, the shape of the profile is somehow preserved, following similar reactivity patterns with depth (i.e. time), as organic matter quality and bioavailability decrease with ongoing degradation.

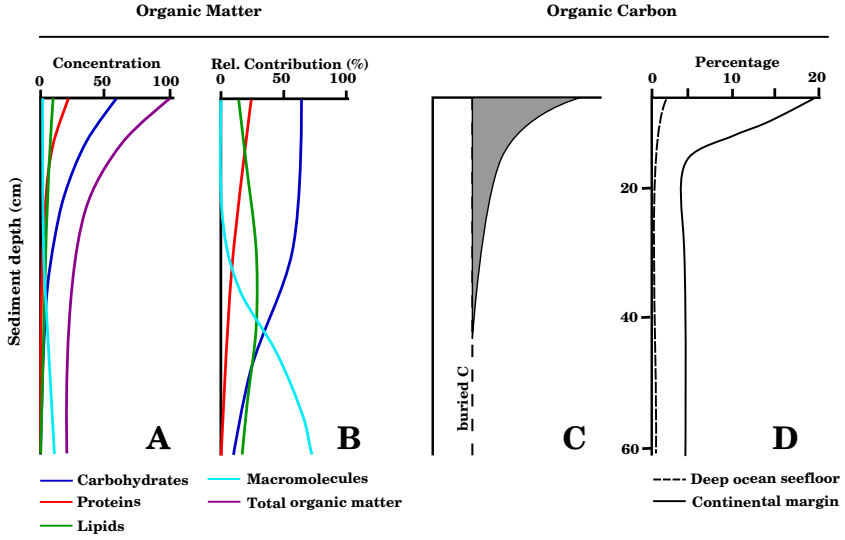


FIGURE 1.7: A and B, Scheme depicting organic matter composition and changes in relative contributions with depth. C, Conceptual model illustrating the reactive and buried fraction of C_{org}. D, Depth distribution of organic carbon (C_{org}%) from coastal areas (continuous line) to deep ocean seafloor (dashed line) (modified after Berner, 1980; Reimers and Suess, 1983; Middelburg, 1989; Burdige, 2007; Arndt et al., 20013).

As mentioned in section 1.3, the rates of organic matter decomposition decrease along with the degradation process itself due to changes in the amount and the lability (i.e. quality) of the organic compounds (Middelburg, 1989). In this context, C_{org} to nitrogen molar ratios (C_{org}/N) can be used as proxies for determining the source and quality of organic matter (Meyers, 1994). In general C_{org}/N ratios of fresh (protein-rich) marine organic matter are around 6 whereas ratios of around 12 are typical for more degraded material. This is because nitrogen is depleted during microbial degradation (Emerson and Hedges, 1988). In contrast, terrestrial organic matter, which is dominated by nitrogen-poor lignin and cellulose, is characterized by relatively high C_{org}/N ratios of >20 (e.g. Meyers, 1994).

1.7.2 Pigments

Chlorins, that include Chlorophylls and their phaeopigment derivatives (Sachs and Repeta, 2000), represent a widely used proxy for assessing organic matter quantity and quality in sediments. These algal biomarkers allow a relatively easy, fast and precise quantification and are unique to photoautotrophic activity, thus can be used to link the production of biomass in photic surface waters to the organic matter deposited onto the seafloor (Pfannkuche, 1993). In fact, with the exception of chemosynthesis-based ecosystems, benthic biota rely on the input of detrital material. The Black Sea is not an exception, and most of the organic matter input that reaches the seafloor originates from photosynthetic organisms (Sorokin, 1964; Karl and Knauer, 1991; King, 1995; Grégoire and Soetaert, 2010). Although photosynthetic organisms can use a wide spectrum of pigments to harvest photons, Chlorophyll *a* is the main pigment for light-dependent primary producers of the Black Sea (Chu et al., 2005; Nesterova et al., 2008) and is thus used here as a proxy for (fresh) photosynthetic material. Phytoplankton pigments can be conveniently expressed as total chloroplastic pigment equivalents (CPE), i.e. the sum of Chlorophyll *a* and its degradation products (laboratory acidified or digested), i.e. phaeopigments. Similar to the carbon and nitrogen ratio, the proportion of chlorophyll a to CPE or Chlorophyll *a* to its acidified extract, i.e. the Chlorin Index (CI) have been used to assess the quality of organic matter (Pfannkuche, 1985, Schubert et al., 2005).

1.7.3 Amino acids

More than half of the organic matter reaching the seafloor is comprised of protein amino acids, decreasing about four fold from surface sediments to deeper layers with ongoing degradation (Wakeham et al., 1997). The analytical measurement of these compounds requires a strong acid hydrolysis, which provides a quantification of total hydrolyzable amino acids (THAA). This procedure may release geopolymerized amino acids, therefore reflecting proteins not necessarily bioavailable (Pantoja and Lee, 2003 and references therein). However, measuring THAA decay rates appeared as a valid proxy for diagenetic processes in marine sediments (e.g. Lee and Cronin, 1984; Cowie and Hedges, 1992; Kinneret et al., 1998).

As mentioned before, the composition of organic matter can be used to infer its origin. The relative abundances of amino acids change across the organic matter spectrum. For instance, cell plasma is enriched in tyrosine, phenylalanine, and glutamic acid, whereas glycine, serine, and threonine are more abundant in structural material such as bacterial cell walls and planktonic chitinous material (Dauwe and Middelburg, 1998 and references

therein). Moreover, as different components of the bulk organic matter are characterized by different degrees of degradability, the amino acid pool changes in its relative concentrations, either by being completely remineralized or by forming new degradation products. Thus microbial degradation of aspartic acid (ASP) and glutamic acid (GLU) forms β-alanine (β-ala) and γ-aminobutyric acid (γ-aba), while ornithine accumulates when arginine is decomposed (Lee and Cronin, 1984). These compositional differences and diagenetic alterations of the amino acid pool are the basis for the Degradation Index (DI) developed by Dauwe and Middelburg (1998) and Dauwe et al., (1999). Dauwe et al., (1999) compiled the mol% values of the 14 most common protein amino acids (THR, ARG, ASP, GLY, VAL, ALA, SER, GLU, MET, PHE, ILE, HIS, LEU and TYR) from a wide variety of environments and matrices ranging from fresh phytoplankton to old sapropelic sediments. The method summarizes the variance in a multivariate scatter of points using principal components analysis (PCA) and derives the principal components, giving the relation between the first PCA axis and the original variables (mole% of protein amino acids). To compare a given dataset, THAA are standardized to the values compiled by Dauwe et al. (1999) and the PCA factor coefficients according to equation 1.1

$$DI = \sum_i [\frac{var_i - AVGvar_i}{STDvar_i}] \times fac.coef_i \tag{1.1}$$

where vari is the original (non-standardized) mole percentage of a given amino acid (i), AVG var_i and STD var_i are its mean and standard deviation from the reference data set, and fac.coef._i the factor coefficient for amino acid i. Thus, fresh phytoplanktonic organic matter scores positive values (+1 or higher) while negative values (towards -2) corresponds to extensively degraded organic material from deep ocean seafloor sediments (Dauwe et al., 1999; Vandewiele et al., 2009).

1.8 Methods to assess microbial diversity and composition

Microbiologists have been developing different techniques to assess microbial diversity and function. From the pioneering research of the Dutch microbiology school during the beginning of last century to the revolution of Sanger et al., (1977) and Woese (Woese, 1987; Woese et al., 1990), and the modern culture-independent massive sequencing methods (Margulies et al., 2005; Bentley et al., 2008), the data acquisition (i.e. resolution) and laboratory time have been scaling inversely.

In this study, automated ribosomal intergenic spacer analysis (ARISA) and 454 massively parallel tag sequencing (454 MPTS) were combined as complementary fingerprinting techniques (Gobet et al., 2013) to reveal the microbial community diversity and structure.

1.8.1 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA is a rapid, low cost, fingerprinting technique for assessing microbial community diversity and structure. This method targets the differences in length of the highly heterogeneous intergenic transcribed spacer region (ITS region) between the small (16S) and large (23S) subunit rRNA genes in the rRNA operon (Fisher and Triplett, 1999).

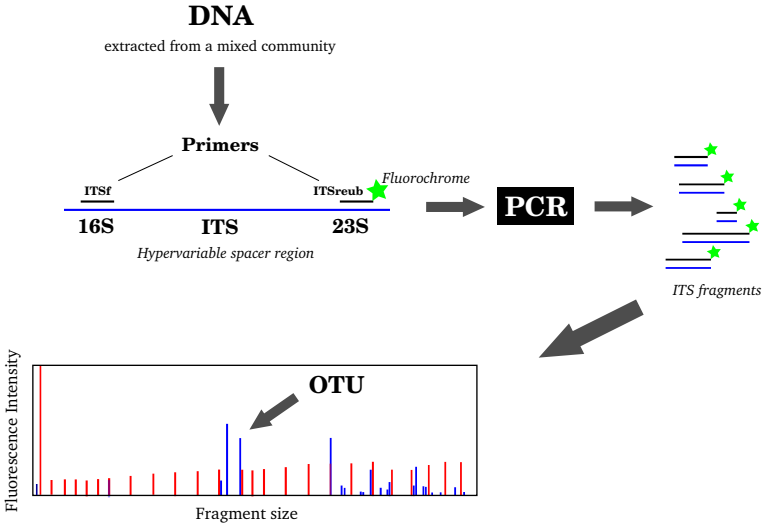


FIGURE 1.8: ARISA workflow (modified from Böer, 2008), see text for details.

Briefly, PCR reactions from standardized amounts of DNA from each sample are amplified targeting conserved regions in the 16S and 23S rRNA genes, one of the primers being fluorescently tagged (Fig. 1.8). After amplification and cleaning of the PCR products, the fragments are analyzed via capillary electrophoresis and measured using an internal base standard. Each peak of the ARISA electropherogram is analyzed, including the binning into ARISA operational taxonomic units (ARISA OTUs) and "relative ARISA OTU abundances", i.e. the ratio between individual peak areas and the total area of peaks in a given sample profile. Based on the derived sample x ARISA OTU tables, bacterial community structure can be further analyzed and interpreted in its ecological context (Böer et al., 2009; Cardinale et al., 2004; Ramette, 2009).

Although the ARISA fingerprinting approach does not allow for an assignment of taxonomic groups, it is a robust and highly reproducible method for the assessment of

bacterial community structure (Fisher and Triplett, 1999; Ramette, 2009; Böer et al., 2009), furthermore overall patterns of community variations are comparable to MPTS-derived OTU defined at the order level (Gobet et al., 2013).

1.8.2 454 Massively Parallel Tag Sequencing (454 MPTS)

454 massively parallel tag sequencing was used for the identification and taxonomic classification of microorganisms. This high-throughput technique combines the sequencing of a significantly great amount of bases, in a short time and for low cost. Aliquots from the same extracted genomic DNA used to perform the ARISA were used for 454 MPTS analyses with specific primers targeting the V4-V6 region of the 16S rRNA (Fig.1.9).

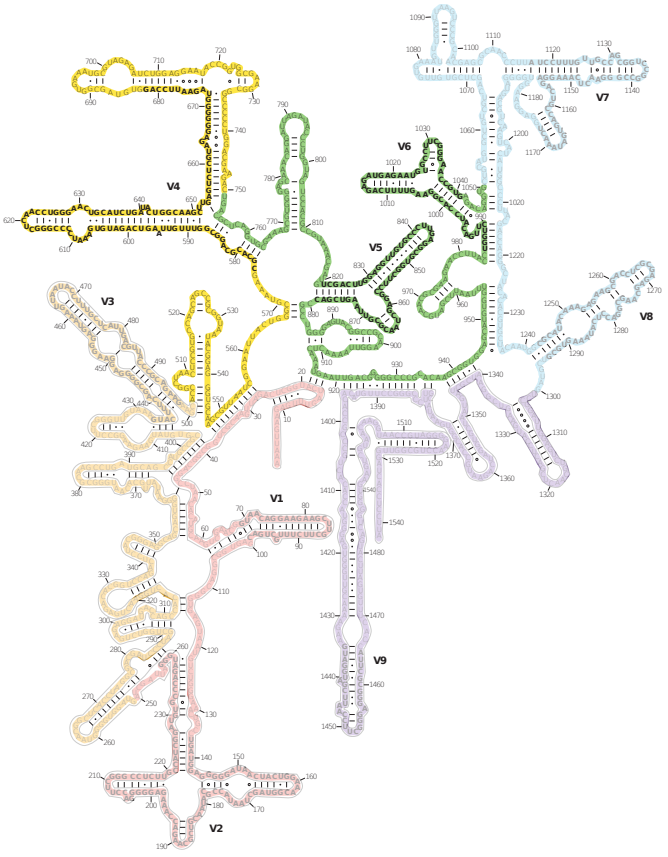


FIGURE 1.9: Secondary structure of the 16S rRNA of *Escherichia coli* depicting variable regions 4-6 of the 16S ribosomal RNA (modified after Yarza et al., 2014).

Fragments were sequenced following the 454 pyrosequencing protocol (Margulies et al., 2005) and Titanium reagent chemistry. Briefly, the 454 life sciences adapters A

and B are ligated to the primers and target the 16S rRNA V4-V6 region (FIG 1.10A). Adapter B enables the immobilization of single-stranded DNA-fragments onto a bead (Figure 1.10C), where an emulsion PCR will yield approximately ten million clonally amplified DNA fragments. Beads are deposited in a Pico Titer plate, and smaller beads with immobilized enzymes are added (Figure 1.10D). The beads are then exposed to sequencing reagents, which are flowed across the Pico Titer plate (Figure 1.10F), with the sequential addition of each base (A, T, G, C). Light is emitted in a chemiluminescent reaction once the nucleotides are incorporated, which is recorded by a charge-coupled device camera. The light intensity varies proportionally with the consecutive number of complementary nucleotides being analyzed, in other words, if there are four consecutive G's in the single-stranded fragment, the amount of light generated would be four fold that of a single G.

FIGURE 1.10: 454 MPTS workflow (after Margulies et al., 2005), see text for details.

The downstream workflow was conducted with "mothur" following the standard operating procedure (Schloss et al., 2009, 2011; including the implemented denoising algorithm). Taxonomic assignments were carried out using the SILVA release 115 (Pruesse et al., 2007; downloaded from http://www.mothur.org in September 2013) and clustered at a 3% sequence difference into operational taxonomic units ($OTU_{0.03}$). The dataset was normalized by the total amount of sequences per sample to get relative abundances. Singletons were treated according to Gobet et al., (2012) as follows; (i) absolute singletons (SSO_{abs}) are $OTU_{0.03}$ that occur with only one sequence in the whole denoised dataset and (ii) a relative singletons (SSO_{rel}) are a $OTU_{0.03}$ with only one sequence in at least one sample, thus the total number of sequences for any SSO_{rel} was larger than one.

1.9 Overview of enclosed manuscripts

Effects of fluctuating hypoxia on benthic oxygen consumption in the Black Sea (Crimean Shelf) (Chapter 2)

Anna Lichtschlag, Daphne Donis, Felix Janssen , Gerdhard L. Jessen, Moritz Holtappels, Frank Wenzhöfer, Sonia Mazulmyan, Nelly Sergeeva, Christoph Waldmann and Antje Boetius.

Biogeosciences Discussions, 12, 6445-6488, 2015 doi:10.5194/bgd-12-6445-2015.

Bottom water oxygen concentrations fluctuating between hypoxia and anoxia affect faunal composition and abundance, therefore controlling sediment-water exchange rates as well as benthic biogeochemical cycles (e.g. Glud, 2008). Previous investigations on the consequences of hypoxia on benthic community structure in the Black Sea have reported a very strong relationship between hypoxia/euxinia and faunal ecology and therefore with organic matter remineralization (Sergeeva et al., 2012; Sergeeva and Zaika, 2013).

For the presented work, respiration rates, organic matter remineralization and the distribution of benthic organisms were investigated on the outer Western Crimean Shelf using an array of in situ and ex situ approaches. The questions addressed were related to what extent the variability in oxygen concentration affects (i) the remineralization rates, (ii) the proportion of microbial vs. fauna-mediated respiration, (iii) the community structure and (iv) the share of anaerobic vs. aerobic microbial respiration pathways.

The study was initiated and planned by AB, AL and FW, the field campaign was conducted by all coauthors. Meio-and macrofaunal data were provided by SM and NS, statistical analyses and corresponding visualization and interpretation were carried out by GLJ. The manuscript was written by AL with support and input from AB, GLJ and all co-authors.

Benthic microbial communities and organic matter preservation associated with hypoxia (Chapter 3)

Gerdhard L. Jessen, Anna Lichtschlag, Silvio Pantoja, Alban Ramette, Carsten J. Schubert, Ulrich Struck, Frank Wenzhöfer and Antje Boetius.

(29.May.2015-in preparation for *Biogeosciences*)

Although the effect of bottom oxygen concentrations on the burial and efficiency of organic matter degradation has been considerably investigated (e.g. Lee, 1992; Aller,

1994; Canfield, 1994; Sun et al., 2002, Arndt et al., 2013), the effect on structure of microbial communities remains poorly understood.

To assess the effects of varying oxygen conditions concurrently on organic matter reactivity and on the structure and function of benthic microbial communities, we examined an oceanographic transect with oxygen concentrations that varied between 0 (sulfidic) and 140 μM O_2 along the outer Western Crimean shelf of the Black Sea. Sediment microbial communities were analyzed using high-throughput sequencing techniques. In parallel, sediment biogeochemical parameters and the lability of organic matter were measured to observe whether the distribution of these properties was related to (i) oxygen supply, (ii) benthic microbial community structure and (iii) organic matter reactivity.

The study was initiated and planed by AB, the field campaign was conducted by GLJ, AL, FW and AB. Molecular analyses were performed by GLJ, part of the analytical procedures were done by GLJ with help of SP. The data assimilation and visualization was done by GLJ. Statistical analyses were carried out by GLJ with help from AR. The manuscript was written by GLJ with support and input from AB and all co-authors.

Distribution and composition of thiotrophic mats in the hypoxic zone of the Black Sea (150-170m water depth, Crimea margin) (Chapter 4)

Gerdhard L. Jessen, Anna Lichtschlag, Ulrich Struck and Antje Boetius.

(29.May.2015-in preparation for *Environmental Microbiology*)

Eutrophication and hypoxic conditions, although inhospitable to most metazoans favor the development of mat-forming filamentous sulfide-oxidizing bacteria. The oxic-anoxic interface, where organic rich sediments are in contact with the Black Sea chemocline, combines ideal conditions for the development of thiotrophic mats. Moreover, just below the chemocline, sulfate reduction becomes the dominant remineralization pathway (Jørgensen et al., 2001; Weber et al., 2001), presumably creating favorable conditions for mat-forming filamentous sulfide-oxidizing bacteria, as soon as oxygen becomes available. However, their emergence, distribution and composition have never been investigated in detail in the Black Sea.

Submarine surveys performed with high-resolution geochemical techniques were combined with microbiological analyses to test the hypothesis that thiotrophic mats are abundant in the hypoxic areas of the Black Sea margin. Key questions were: (i) what

are the dominant factors governing the development of microbial mats? (ii) which microbial types are the key mat formers in the Black Sea? and (iii) does the presence of mats influence the diversity and activity of the associated microbiota?

The study was initiated and planned by AB and GLJ, the submersible sampling was conducted by GLJ. Microsensor measurements were performed and analyzed by AL and C org measurements by US. Molecular and part of the analytical analyses together with data assimilation and visualization were performed by GLJ. The manuscript was written by GLJ with input from AB and all co-authors.

Characterization of organic matter deposited under contrasting oxygen regimes (Chapter 5)

Current authors: Gerdhard L. Jessen and Pamela E. Rossel

(Further input to this study in works will be provided by Jutta Niggemann, Thorsten Dittmar and Antje Boetius)

(29.May.2015 - *in preparation*)

Many prokaryotes require dissolved organic matter (DOM) as carbon and energy source. However, the dissolved organic matter in the ocean's deep waters persists, almost unchanged, since centuries (Dittmar and Stubbins, 2014 and references therein). For the water column it is known that organic matter degradation depends on the concentration and composition of its dissolved form, on the oxygen availability and on the amount of time that DOM has been "exposed" to degradation (Jannasch, 1967; Arrieta et al., 2015; Middelburg, 2015). So far, in this context, little attention has been given to the microbial community, which is responsible for the upstream degradation of organic matter (Arndt et al., 2013 and references therein). Moreover, the few existing studies were carried out in oxygenated water columns (e.g. Osterholz et al., 2014). Therefore potential mechanisms sustaining organic matter preservation, that are commonly found in anoxic/euxinic environments (e.g. sulfur-bonding geopolymerization; Schmidt et al., 2014), where so far never investigated in relation to microbial community structure.

To explore the effect of hypoxia on the composition of dissolved organic matter and potential relationships with microbial community structure, porewater DOM and microbial communities were characterized from sediments exposed to permanent oxic and anoxic/sulfidic conditions at the Crimean Shelf using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and 454 Massively Tag Sequencing (MPTS). The key questions addressed were: (i) Are there differences in the molecular composition of pore water DOM under oxic and anoxic conditions? (ii) Are there variations

in the composition of DOM between oxic and anoxic sites, and can they be related to differences in microbial composition/function?

The study was initiated and planned by AB and GLJ. JN and PR performed DOM characterization and interpretation. The molecular analyses and data assimilation and visualization were performed by GLJ. The manuscript was written by GLJ with input from PR.

This PhD thesis was part of the 7^{th} EU FP project HYPOX (In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies, Grant agreement no.: 226213; Monitoring and observing oxygen depletion throughout the different Earth system components). The overall aim was to develop a global observation system continuously monitoring oxygen at high resolution to better understand global changes in oxygen depletion, including assessment of the role of the seafloor in controlling the sensitivity of aquatic systems to and recovery from hypoxia.

Chapter 2

Effects of fluctuating hypoxia on benthic oxygen consumption in the Black Sea (Crimean Shelf)

A. Lichtschlag[1,⋆], D. Donis[1,⋆⋆], F. Janssen[1,2], G. L. Jessen[1], M. Holtappels[1,3], F. Wenzhöfer[1,2], S. Mazulmyan[4], N. Sergeeva[4], C. Waldmann[3] , and A. Boetius[1,2]

[1]Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany.

[2] HGF MPG Research Group for Deep Sea Ecology and Technology, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen, 27515 Bremerhaven, Germany.

[3]MARUM – Center for Marine Environmental Sciences, University of Bremen, 28334 Bremen, Germany.

[4]A.O. Kovalevsky Institute of Biology of Southern Seas, 2, Nakhimov ave., Sevastopol, 299011, Crimea.

⋆ current address: National Oceanography Centre, University of Southampton Waterfront Campus, European Way, SO14 3ZH, Southampton, UK.

⋆⋆ current address: F.-A. Forel Institute, University of Geneva, Batelle, Bat. D, 7 Route de Drize 1227 Carouge, Geneva, Switzerland.

Received: 6 April 2015 - Accepted: 7 April 2015 - Published: 30 April 2015.

Correspondence to: A. Lichtschlag (alic@noc.ac.uk).

Abstract

The outer Western Crimean Shelf of the Black Sea is a natural laboratory to investigate effects of stable oxic vs. varying hypoxic conditions on seafloor biogeochemical processes and benthic community structure. Bottom water oxygen concentrations varied between normoxic (175 μmol O_2L^{-1}) and hypoxic (<63 μmol O_2L^{-1}) or even anoxic/sulfidic conditions within a few kilometres distance. Variations in oxygen concentrations between 160 and 10 μmol L^{-1} even occurred within hours close to the chemocline at 134 m water depth. Total oxygen uptake, including diffusive as well as fauna-mediated oxygen consumption, decreased from >15 mmol m^{-2} d^{-1} in the oxic zone to <9 mmol m d in the hypoxic zone, correlating with changes in macrobenthos composition. Benthic diffusive oxygen uptake rates, comprising microbial respiration plus reoxidation of inorganic products, were around 4.5 mmol m^{-2} d^{-1}, but declined to 1.3 mmol m^{-2} d^{-1} at oxygen concentrations below 20 μmol L^{-1}. Measurements and modelling of pore water profiles indicated that reoxidation of reduced compounds played only a minor role in the diffusive oxygen uptake, leaving the major fraction to aerobic degradation of organic carbon. Remineralization efficiency decreased from 100% in the oxic zone, to 50% in the oxic-hypoxic, to 10% in the hypoxic-anoxic zone. Overall the faunal remineralization rate was more important, but also more influenced by fluctuating oxygen concentrations than microbial and geochemical oxidation processes.

2.1 Introduction

Hypoxia describes a state of aquatic ecosystems in which low oxygen concentrations affect the physiology, composition and abundance of fauna, consequently altering ecosystem functions including biogeochemical processes and sediment-water exchange rates (Middelburg and Levin, 2009). Coastal hypoxic zones often show reduced faunal abundances, biodiversity, and loss of habitat diversity below a threshold of 63 μmol $O_2 L^{-1}$ (Diaz, 2001; Levin et al., 2009). In dynamic coastal hypoxic zones with fluctuating conditions as the Kattegat (Diaz, 2001), off the coast of New York/New Jersey (Boesch and Rabalais, 1991), or the Romanian Shelf of the Black Sea (Friedrich et al., 2014), mass mortality has been reported when oxygen concentrations drop below 22 μmol L^{-1} (0.5 mL L^{-1}) (Levin, 2003; Levin et al., 2009). In contrast, in regions under stable low-oxygen conditions faunal communities can be adapted to such physiologically challenging conditions, for example in long-term oxygen minimum zones in the SE-Pacific, tropical E-Atlantic and N-Indian Ocean (Levin et al., 2009). Here, the thresholds for faunal activity can reach much lower oxygen concentrations than in regions, which are facing periodic hypoxia.

Low faunal bioturbation rates in hypoxic zones limit sediment ventilation (Glud, 2008), decreasing oxygen availability for aerobic respiration. Hence, sediments underlying a low oxygen water column often show oxygen penetration depths of only a few millimeters (Archer and Devol, 1992; Glud et al., 2003; Rasmussen and Jørgensen, 1992). This increases the contribution of anaerobic microbial metabolism to organic matter remineralization at the expense of aerobic degradation by microbes and fauna as reported from the Romanian Shelf area of the Black Sea (Thamdrup et al., 2000; Weber et al., 2001), the Neuse River Estuary (Baird et al., 2004), and the Kattegat (Pearson and Rosenberg, 1992). Consequently, oxygen is channeled into the reoxidation of reduced substances produced during anaerobic degradation of organic matter. Even temporarily reduced bottom water oxygen concentrations can repress seafloor oxygen uptake that should become enhanced by algae blooms and temperature increases (Rasmussen and Jørgensen, 1992). However, depending on frequency and duration of oxygen oscillations, oxygen consumption following an anoxic event can also be significantly increased (Abril et al., 2010). Thus, overall not only the degree of oxygenation plays an important role in oxygen uptake, but also the frequency and persistency of the low oxygen conditions can shape faunal activity, biogeochemical processes, and the functioning of the ecosystem as a whole.

In the Black Sea, the depth of the oxic-anoxic interface increases from about 70–100 m in open waters (Friedrich et al., 2014) to depths of >150 m above the shelf break (Stanev et al., 2013). This interface is stabilized by a halocline that separates the upper layer of

brackish, oxic water (salinity <17) from the saline, anoxic and sulfidic deep waters below (Tolmazin, 1985). Due to mixing processes by internal waves and eddies, the location of this interface zone is more dynamic along the margins of the Black Sea compared to the open sea. In the shelf region, hypoxic waters with oxygen concentrations <63 μmol L^{-1} oscillate over >70 m in water depth on time scales of hours to months (Stanev et al., 2013). On the outer Western Crimean Shelf, such strong vertical fluctuations affect a 40 km wide area of the slope (Friedrich et al., 2014; Luth et al., 1998).

Previous investigations on the consequences of hypoxia on benthic community structure on the Black Sea shelf focused on seasonally hypoxic coastal areas with water stagnation and a high organic carbon accumulation (Zaika et al., 2011), on shallow, active methane seeps with detrital-microbial mats (Zaika and Gulin, 2011) and on the effects of the basin-wide chemocline below the permanently hypoxic water column (Kolesnikova et al., 2014; Sergeeva et al., 2012, 2013; Zaika and Sergeeva, 2012). Sergeeva and Zaika (2013) reported that under permanently hypoxic conditions, some protozoa (*Gromiida* and *Foraminifera*) and some metazoa (*Harpacticoida*, *Polychaeta*,*Nematoda*) can complete full life cycles, depending on the water depth and the distance from the sulfidic zone. Some meiobenthos species even seem to prefer hypoxic conditions (Sergeeva and Anikeeva, 2014; Sergeeva et al., 2013). Here we investigated processes on the outer Western Crimean Shelf to assess how natural fluctuations in bottom water oxygen concentrations influence biogeochemical processes such as respiration, organic matter remineralization and the distribution of benthic organisms. The questions addressed are to what extent the variability in oxygen concentration has an effect on (1) the remineralization rates, (2) the proportion of microbial vs. fauna-mediated respiration, (3) the community structure and (4) the share of anaerobic vs. aerobic microbial respiration pathways.

2.2 Methods

2.2.1 Study site on the outer Western Crimean Shelf

Investigations of bottom water oxygen concentrations and biogeochemistry of the underlying seafloor of the outer Western Crimean Shelf were carried out over a time period of 2 weeks (20 April-7 May 2010) during leg MSM 15/1 of R/V Maria S. Merian. The selected area on the outer shelf has a gentle slope and a maximum width of around 60 km until the shelf break at approx. 200 m water depth. The sediment and the water column were sampled along a transect from 95 to 207 m water depth within an area of about 100 km (Fig. 2.1). Detailed information of all stations in the working area is given in Table 2.1.

All biogeochemical data are deposited in the Earth System database www.PANGAEA.de and are available at http://doi.pangaea.de/10.1594/PANGAEA.844879.

2.2.2 Water column CTD and oxygen measurements

Bottom water oxygen concentrations were recorded repeatedly between 95 to 206 m water depth at different spatial and temporal scales with various sensors, which were all calibrated by Winkler titration (Winkler, 1888). A total of 26 casts were performed with a CTD/Rosette equipped with a SBE 43 oxygen sensor (Seabird Electronics, Bellevue, WA, USA). A mooring was deployed at 135 m water depth, equipped with a Seaguard current meter with CTD and a type 4330 oxygen optode (Aanderaa Data Instruments, Bergen, Norway) recording at 60 s intervals at a distance of 1.5 m above the sediment from the 30 April to the 7 May 2010. A second mooring was deployed for the same time period at 100 m water depth, with a CTD attached at 1.5 m above the sediment (type SBE 16, Seabird Electronics) to record density, salinity and temperature. CTD water column casts and the mooring at 135 m showed that oxygen concentrations strongly correlate with density (r^2=0.997). Hence, oxygen concentrations at the 100 m mooring site were calculated from the density recordings at this site using a density-oxygen relationship (4th order polynomial fit) based on the compiled mooring/CTD data. Additionally, bottom water oxygen concentration was measured at the seafloor by oxygen optodes mounted on the manned submersible JAGO (GEOMAR, Kiel; optode type 3830), and to a Benthic Boundary Layer-Profiler (Holtappels et al., 2011) (type 4330). Furthermore, microprofilers equipped with oxygen microsensors were mounted on a lander and a crawler (see Sect. 2.5.1). For consistency with other hypoxia studies, we use the oxygen threshold of 63 μmol L^{-1} as upper boundary for hypoxia (Diaz, 2001). Sulfide concentrations were determined in bottom water collected with Niskin bottles during CTD casts and JAGO dives at 13 different locations between 135 and 218 m water depth. For all water column oxygen and sulfide concentrations a limit of 2 μmol L^{-1} was defined, below which concentrations were assumed to be zero.

2.2.3 Visual seafloor observations and micro-topography scans

To observe organisms, their traces of life, and the resulting micro-topography at the surface of the different seafloor habitats, a laser scanning device (LS) and the highresolution camera MEGACAM were used on the benthic crawler MOVE (MARUM, Bremen). The LS consisted of a linear drive that moved a downward looking line laser together with a monochrome digital camera horizontally along a 700 mm long stretch of the seafloor. The position of the approx. 200 mm wide laser line in image-series recorded by

the camera from an angle of 45°the 3-D micro-topography of the scanned area was determined on a 1 × 1 mm^2 horizontal grid at sub-mm accuracy (for a detailed description see Cook et al., 2007). The roughness of the sediment surface was quantified in three 700 mm long profiles extracted from the sides and along the center line of 7, 2, 6, and 2 micro-topographies scanned at 104, 138, 155, and 206 m water depth, respectively. Roughness was determined for different length scales by calculating mean absolute vertical differences to the same profile previously smoothed by applying moving average with 3 to 300 mm averaging window size.

The downward-looking MEGACAM was either attached directly to MOVE or added to the horizontal drive of the LS; the latter configuration facilitating imaging of larger sediment stretches by photo-mosaicking. In addition, visual seafloor observations were carried out before pushcore sampling by JAGO. Dive videos were recorded with a type HVR-V1E HDV Camcorder (SONY, Tokyo, Japan) mounted in the center of JAGO's large front viewport during 19 dives. During each dive, video still images were captured by video-grabber from the running camera.

2.2.4 Faunal analyses

Meiofauna organisms were counted in 5 cm sediment horizons of 2–4 cores per station, with each core covering an area of 70.9 cm^2 (TVMUC) and 41.8 cm^2 (for JAGO pushcore) (Table 2.1, Fig. 2.1). The abundances were extrapolated to m^2. Sediments were washed with distilled water through sieves with mesh sizes of 1 mm and 63 μm, and preserved in 75% alcohol to conserve the morphological structures of the meiofauna. Subsequently, samples were stained with Rose Bengal, to separate living and dead/decaying organisms (Grego et al., 2013), and sorted in water under a microscope. Only organisms that strongly stained with Rose Bengal and showed no signs of morphological damage were considered as being alive at the time of sampling. All of the isolated organisms were counted and identified to higher taxa. In addition, macrofauna distribution was qualitatively assessed sieving sediments from several multicorer cores (area of 70.9 cm^2) and JAGO pushcores (area of 41.8 cm^2) (Table 2.1, Fig. 2.1) with a 2 mm size mesh. Statistical analyses of the similarity of fauna communities were conducted using the R package vegan (Oksanen et al., 2010) and performed in R (v. 3.0.1; http://www.R-project.org). Richness was calculated from species (taxa) presence/absence. A matrix based on Bray–Curtis dissimilarities was constructed from the Hellinger-transformed abundances for meiofauna taxa. The non-parametric Analysis of Similarity (ANOSIM) was carried out to test whether the communities (based on different bottom-oxygen zones) were significantly different (Clarke, 1993).

2.2.5 Benthic exchange rates

2.2.5.1 In situ microsensor measurements

Vertical solute distributions were measured in situ at high resolution in sediment pore waters and the overlying waters with microsensors mounted on microprofiler units (Boetius and Wenzhöfer, 2009). In particular, Clark-type O_2 microsensors (Revsbech, 1989) and H_2S microsensors (Jeroschewski et al., 1996) were used as well as microsensors for pH–either LIX-type (de Beer et al., 1997) or needle-type (type MI 408, Microelectrodes Inc., Bedford, NH, USA). A two-point oxygen sensor calibration was done in situ, using water column oxygen concentrations obtained from simultaneous oxygen recordings and zero readings in anoxic sediment layers. The H_2S sensors were calibrated at in situ temperature on board at stepwise increasing H_2S concentrations by adding aliquots of a 0.1 mol L Na_2S solution to acidified seawater (pH<2). pH sensors were calibrated with commercial laboratory buffers and corrected with pH obtained from water samples taken with Niskin bottles operated by JAGO.

Profiler units were mounted either on the benthic crawler MOVE (Waldmann and Bergenthal, 2010) or on a benthic lander (Wenzhöfer and Glud, 2002). The MOVE vehicle was connected to the ship via a fiber optic cable that allowed continuous access to video and sensor data. The maneuverability of the vehicle allowed to target spots of interest on the seafloor in the cm range. The profiler units were equipped with 3–4 O_2 microsensors, 2 H_2S microsensors, and 1–2 pH sensors. Microprofiles across the sediment-water interface were performed at a vertical resolution of 100 μm and had a total length of up to 18 cm. During each deployment of the lander the microsensor array performed up to three sets of vertical profiles at different horizontal positions, each 26 cm apart.

From the obtained oxygen profiles the diffusive oxygen uptake (DOU) was calculated based on the gradients in the diffusive boundary layer (DBL) according to Fick's first law of diffusion,

$$J = \frac{dc}{dx} \times D_0 \tag{2.1}$$

where J is the oxygen flux, dc/dx is the concentration gradient, and D_0 is the diffusion coefficient of oxygen in water (D_0O_2=1.22×10^{4}m^{2}d^{-1}, Broecker and Peng, 1974) at the ambient temperature (8°C) and salinity (18-20). For each station, selected oxygen profiles were fitted using the software PROFILE (Berg et al., 1998) to determine oxygen consumption from the shape of the pore water gradient and to identify depth intervals of similar oxygen consumption based on statistical F testing.

2.2.5.2 In situ benthic chamber incubations

Total oxygen uptake (TOU) of sediments was measured by in situ benthic chamber incubations using 2 platforms: (1) two benthic chambers, each integrating an area of 0.2×0.2 m (Witte and Pfannkuche, 2000) mounted to the same benthic lander frame used for microprofiler measurements (Wenzhöfer and Glud, 2002) and (2) a circular chamber (r=0.095 m, area=0.029 m^2) attached to the benthic crawler MOVE for video-guided chamber incubations. After positioning MOVE at the target area the chamber was lowered into the sediment, controlled by the video camera of MOVE and operated online through the MOVE-electronics. Both systems were equipped with a stirrer and syringe samplers that took up to 6 successive samples (V=50 mL) from the 0.1–0.15 m high overlying bottom water. Benthic exchange rates were determined from the linear regression of oxygen solute concentration over time inside the enclosed water body that was continuously monitored for a period of 2 to 4 h by 1 or 2 oxygen optodes mounted in the chamber lid. The optodes were calibrated with a zero reading at in situ temperature on board and with bottom water samples, in which concentrations were determined either by Winkler titration (Winkler, 1888) or with a calibrated Aanderaa optode attached to the outside of the chamber. Oxygen concentrations in the chamber was the same as in in situ bottom water concentrations. During deployments in the hypoxic-anoxic zone, oxygen concentrations in the chambers were higher than in the surrounding bottom water, due to enclosure of oxygen-rich water during descent. These measurements were used to estimate potential TOU rates at intermittently higher oxygen concentration. To estimate the in situ ratio of TOU/DOU for the hypoxic-anoxic zone, we modeled the DOU based on the volumetric rate and the DBL thickness determined by the in situ microsensor profile.

2.3 Geochemical analyses of the sediments and sulfate reduction rates

Sediments for geochemical analyses were sampled with a video-guided multicorer (TV-MUC) at 4 stations between 104 and 207m (Table 2.1). Pore water was extracted from sediment cores within 3 h after retrieval in 1 cm (upper 5 cm) or 2 cm (>5 cm) intervals with Rhizons (type: CSS, Rhizosphere Research Products, pore size <0.2 μm) at in situ temperature (8°C) in a temperature-controlled room, and fixed for Fe(II), Mn(II), sulfide and sulfate analyses as described in Lichtschlag et al. (2010). For ammonium analyses 3 mL of the samples were frozen at −20°C. In addition, one sediment core from each station was sliced in 1 cm intervals (upper 10 cm) and 2 cm intervals (>10 cm depth) for solid phase analyses. Aliquots were stored at 4°C for porosity

analyses and frozen at −20°C for ^{210}Pb and solid phase iron, manganese and elemental sulfur analyses. Pore water constituents were analyzed by the following procedures: Dissolved Mn(II) and Fe(II) were measured with a Perkin Elmer 3110 flame atomic absorption spectrophotometer (AAS) with a detection limit of 5 μmol L^{-1} for iron and manganese. Total sulfide concentrations (H_2S+HS^-+S^{2-}) were determined with the diamine complexation method (Cline, 1969). A Skalar Continuous-Flow Analyzer was used for ammonium analyses following the procedures described in Grasshoff (1983), with a detection limit of 1 μmol L^{-1}. Sulfate concentrations in pore water were determined by non-suppressed anion exchange chromatography (Metrohm 761 Compact IC) after filtration and dilution. To determine fluxes of iron, manganese, sulfide and ammonium the pore water profiles were fitted using the software PROFILE (Berg et al., 1998).

Total zero-valent sulfur in sediments was extracted with methanol from sediment preserved in ZnAc (Zopfi et al., 2004) and analyzed by HPLC. Concentrations of acid volatile sulfide (AVS=Fe_3S_4, FeS) and chromium reducible sulfur (CRS = FeS_2, some S^0, remaining Fe_3S_4) were determined on frozen sediment aliquots by the two-step Cr II distillation method (Fossing and Jørgensen, 1989). Solid phase reactive iron and manganese were extracted from frozen sediments after the procedure of Poulton and Canfield (2005) using sequentially Na-acetate, hydoxylamine-HCl, dithionite and oxalate. Manganese and iron concentrations were measured as described above.

Sulfate reduction rates were determined with the whole core incubation method described in Jørgensen (1978). On board 10 μL aliquots of an aqueous $^{35}SO_4^-$ tracer solution (activity 11.5 kBq μL) were injected into the sediments in 1 cm intervals and samples were incubated for up to 24 h at in situ temperature, until the sediments were sliced into 20 mL 20% ZnAc. Tracer turnover rates were determined with the single-step cold distillation method (Kallmeyer et al., 2004). Three replicates were measured per station and results were integrated over the upper 10 cm of the sediment.

Porosity and solid-phase density were determined by drying a wet sediment aliquot of known volume at 105°C until constant weight and weighing before and after. Sedimentation rates were determined from excess ^{210}Pb activity ($^{210}Pb_{xs}$) in frozen sediment aliquots of the upper 10 cm that were freeze-dried and homogenized by grind- ing. Activities of ^{210}Pb, ^{214}Pb, and ^{214}Bi were determined on 5-30 g aliquots by non-destructive gamma spectrometry using an ultra-low-level germanium gamma detector (EURISYS coaxial type N, Canberra Industries, Meriden, CT, USA). Sediment accumulation rates (g cm^{-2}yr^{-1}) were calculated from the undisturbed part of the sediments from the change of the unsupported $^{210}Pb_{xs}$ activity with sediment accumulation, expressed as

cumulative dry weight (gcm^{-2}) as described by Niggemann et al. (2007). This calculation is based on the assumption that the $^{210}Pb_{xs}$ flux and sedimentation were constant over time.

2.4 Results

2.4.1 Oxygen regime of the Outer Western Crimean Shelf

Recordings of bottom water oxygen concentrations (n=85) along the transect from 95 to 206 m water depth served to differentiate four zones of different bottom water oxygenation within a distance of more than 30 km (Table 2.1; Figs. 2.1 and 2.2).

The "oxic zone" at water depths of 95 to 130 m had oxygen concentrations of on average 116±29 μmol L^{-1} (31% air saturation at ambient conditions; 8°C, salinity of 19), and remained above the threshold for hypoxia (63 μmol L^{-1}) throughout the period of our observations. Recordings from the mooring at 100 m water depth showed some fluctuations (Fig. 2.8a in the Supplement), with oxygen concentrations varying between 100-160 μmol L^{-1} within 6 days. In this oxic zone, sediment surface color was brownish, and the seafloor looked rather homogenous, without ripple structures, but with faunal traces (Fig. 2.9a). The top 5 cm of the sediment comprised some shell debris of 2-6 mm diameter encrusted with a bright orange layer of up to 3 mm thickness, which most probably consisted of iron-oxides (Fig. 2.9b). During JAGO dives and MOVE deployments we recorded living fauna in the oxic zone such as clams, ascidians, phoronids, cerianthids, porifera and many fish. Traces of recent faunal activity at the seafloor included trails, worm borrows and feces (Fig. 2.9c). During our sampling campaign the horizontal distance to the oxic-anoxic interface (chemocline) was on average 13 km. The oxic zone served as reference for further comparisons of hypoxic effects on biogeochemical processes and faunal community composition.

In the "oxic-hypoxic zone" at water depths between 130 to 142 m, average bottom water oxygen concentrations were 94±56 μmol L^{-1} (approx. 25% air saturation at ambient conditions; 8°C, salinity of 20). However, we observed strong variations in oxygen concentrations with maxima of up to 176 μmol L^{-1} and minima of 9 μmol L^{-1}, respectively. Hypoxic conditions prevailed for 30% of the observation period of 7 days, as recorded by the stationary mooring at 135 m water depth (Fig. 2.8b). Constantly rising oxygen concentrations over days were interspersed by a substantial drop from fully oxic to almost anoxic conditions within <3 h (Fig. 2.8b). Horizontal distance to the oxic-anoxic interface was on average 7 km during our expedition. In the oxic-hypoxic

zone, only few fishes were observed, and video-observations of the seafloor showed a clear reduction of epibenthos abundance and their traces compared to those in the oxic zone.

In the "hypoxic-anoxic" zone between 142 and 167 m water depth sediments showed fluctuating hypoxic conditions between 0–63 μmol L^{-1} (average 11±16 μmol L^{-1} ; 3% air saturation at ambient conditions; 8°C, salinity of 20). Unexpectedly, during a short period at these water depths, some fish (the sprattus Sprattus phalericus at 145 and 163 m water depth, and the whiting Merlangius merlangus euxinus at 145 m water depth, Zaika and Gulin, 2011) were observed when oxygen concentrations were as low as 20 μmol L^{-1} (Fig. 2.9f). The seafloor was covered with fluffy greenish-brownish material and sediments showed a fine lamination (Fig. 2.9e). No epibenthic life was observed, nor borrows or other traces of bottom dwelling fauna.

Below 167 m, the bottom water was permanently anoxic during the time period of our campaign. Below 180 m sulfide was constantly present in the bottom water, with concentrations ranging between 5-23 μmol L^{-1} . In this "anoxic-sulfidic" zone sediments were dark green-blackish. Neither macrofauna, nor traces of bottom-dwelling infauna were observed.

2.4.2 Meiofauna composition and abundance

Abundance and composition of meiobenthos and macrobenthos >2 mm as retrieved from the top 5 cm of pooled core samples were compared across the different zones of oxygen availability in Fig. 2.6 and Tables 2.5 and 2.6 in the Supplement. The macrobenthos abundances presented here are not quantitative for the entire size class, due to the limit in sample size available; they might represent mostly small types and juvenile stages. These decreased by more than one order of magnitude from the oxic zone (21×10^3 individuals m^{-2}) to the hypoxic-anoxic zone (1×10^3 individuals m^{-2}) (Table 2.5). In the oxic zone, cnidaria dominated the benthic community next to oligochaetes and polychaetes, also bivalves and gastropods were present. A peak in macrobenthos abundances in both the oxic and the oxic-hypoxic zone at around 129-138 m was related to an accumulation of cnidarians with abundances of up to 54×10^3 individuals m^{-2} (Table 2.5). Also the two hypoxic zones were dominated by cnidaria. In accordance with the results from sampling, no larger macrofauna was documented during JAGO dives in these zones.

Meiobenthos was composed of similar groups and abundances in the oxic and oxic-hypoxic zone with densities of around 200×10^4 individuals m^{-2}(Fig. 2.6, Table 2.6). A substantial decrease to 50×10^4 individuals m^{-2} was observed between these two zones and the hypoxic-anoxic zone. The meiofaunal community structure changed according

to the oxygenation regime (Fig. 2.7), showing significant differences between oxic and hypoxic-anoxic zones (ANOSIM-R=0.7, Bonferroni corrected P value <0.05) together with the highest dissimilarities (up to 50%, Table 2.7). Nematodes dominated meiofauna composition in all oxic and hypoxic zones (Table 2.6). In the oxic zone ostracodes were the 2nd most abundant species. These were replaced by benthic foraminifera in the oxic-hypoxic and the hypoxic-anoxic zone. Altogether meiofaunal richness (taxa count, average±SD) was similar in the oxic zone and oxic-hypoxic zone (15±2 and 15±1) and dropped to 9±1 in the hypoxic-anoxic zone.

2.4.3 Benthic oxygen fluxes and respiration rates

A total of 33 oxygen microprofiles were measured during seven deployments of the benthic crawler MOVE and the lander at water depths between 104 and 155 m. Oxygen penetration depths and dissolved oxygen uptake rates are summarized in Table 2.2. The shape of the profiles and the differences in oxygen penetration depth as shown in Fig. 2.3 reflect the spatial variations of oxygen bottom water concentrations and oxygen consumption rates. In the shallowest, oxic zone (104 m) clear signs of bioturbation were visible from the irregular shape of about 25% of the profiles, occasionally increasing the oxygen penetration depth up to approximately 10 mm. Bioturbation activity was in accordance with a significant bioturbated surface layer and more pronounced roughness elements at the sediment surface at the shallowest site as compared to deeper waters (see Sect. 3.5). In contrast, the shape of the oxygen profiles obtained in the oxic-hypoxic and the hypoxic-anoxic zone showed no signs of bioturbation. Small-scale spatial heterogeneity was low between parallel sensor measurements and within one deployment (area of 176 cm^2 sampled). However, strong temporal variations occurred in response to the fluctuations in bottom water oxygen concentration. For example, in the oxic-hypoxic zone a clear relation between oxygen penetration depth and bottom water oxygen concentration was detectable, with increased bottom water oxygen concentration leading to deeper oxygen penetration depth (Fig. 2.3a-c). Except where bioturbation led to slightly deeper penetration, oxygen was depleted within the first 0.4-3 mm of the surface layer (Fig. 2.3, Table 2.2).

Diffusive oxygen uptake (DOU) varied within an order of magnitude between all zones (Table 2.2). The highest DOU of 8.1 mmol m^{-2} d^{-1} was calculated from a profile obtained at 104 m water depth in the oxic zone, but the averages of all oxygen fluxes measured in the oxic and oxic-hypoxic zones were similar (averages of 4.6±1.8 and 4.4±1.9 mmol m^{-2} d^{-1}, respectively, Table 2.2). The higher variability within the oxic-hypoxic zone, spanning from 0.6 to 8 mmol m^{-2} d^{-1} between measurements, matches the higher variability in bottom water oxygen concentrations observed for this zone (Fig.

2.3b). Diffusive oxygen uptake in that zone was lowest after a nearly anoxic event (∼10 μmol O_2 L^{-1}; Fig. 2.8b). Highest fluxes in the oxic-hypoxic zone, however, were not recorded during a "normoxic event" (149 μmol O_2 L^{-1}), but at the typical intermediate bottom water oxygen concentration of approx. 90 μmol L^{-1} (Fig. 2.4b and c, Fig. 2.8b). In the hypoxic-anoxic zone DOU was only 25% of that in the oxic and oxic-hypoxic zones (average: 1.3±0.5 mmol $m^{-2}d^{-1}$).

In bottom waters of the hypoxic-anoxic zone high resolution measurements of pH indicated a pH of around 7.8, decreasing to values between 7.2-7.4 in the sediment. With the H_2S microsensors no free sulfide was detected in the pore waters of the oxic, oxic-hypoxic or hypoxic-anoxic zones. In the anoxic-sulfidic zone the microsensor measurements failed. Bottom water sulfide concentrations were >5 μmol L^{-1}, and the pore water analyses indicated high concentrations of sulfide of up to 1000 μmol L^{-1} in the sediment (see Sect. 3.4).

Total oxygen uptake (TOU) including the faunal respiration, was generally higher than DOU (Table 2.2). Individual measurements varied from 20.6 to 3.2 mmol $m^{-2}d^{-1}$ across all zones. Average TOU showed a clear reduction from the oxic zone (average: 14.9±5.1 mmol m d) to the oxic-hypoxic zone (average: 7.3±3.5 mmol $m^{-2}d^{-1}$). TOU at the oxic-hypoxic station compare well with a TOU of 6.0 and 4.2 mmol $m^{-2}d^{-1}$ determined by simultaneous eddy correlation measurements averaged over a time period of 14 h (Holtappels et al., 2013).

Accidental trapping of oxygen-enriched waters in the chambers during deployments carried out at the hypoxic-anoxic zone led to higher initial oxygen concentrations in the enclosed water as compared to ambient bottom waters. Therefore, we could only obtain potential TOU rates at elevated bottom water concentrations of 70 μmol L^{-1}. A potential TOU of 7 mmol $m^{-2}d^{-1}$ was measured and a potential DOU of 5.6±0.5 was modeled from the volumetric rates and DBL thickness obtained by the microsensor profiles. The contribution of DOU was lowest in the oxic zone (30%), and increased with decreasing TOU towards the oxic-hypoxic (60%) and hypoxic-anoxic zone (80%) (Table 2.2).

2.4.4 Sediment geochemistry

Cores from all sites had the typical vertical zonation of modern Black Sea sediments with a brown/black fluffy layer (oxic and hypoxic zones, Fig. 2.9d), or dark/grey fluffy layer (anoxic-sulfidic zone), covering beige-grey, homogenous, fine-grained mud. Substantial differences in the concentration profiles and fluxes of dissolved iron, dissolved manganese, sulfide, and ammonium were found in pore waters from surface sediments

sampled from the four different oxygen regimes (Fig. 2.5). In the oxic zone, dissolved iron and manganese were present in the pore water with maximal concentrations of 217 μmol L^{-1} (Fig. 2.5a) and 30 μmol L^{-1} (Fig. 2.5b), respectively, and no free sulfide was detected (Fig. 2.5c). In the oxic-hypoxic zone, concentrations of dissolved iron were reduced (max. 89 μmol L^{-1} , Fig. 2.5h), manganese concentrations were below detection (Fig. 2.5i), but free sulfide was still not present in the pore waters (Fig. 2.5j). In the hypoxic-anoxic zone dissolved iron and sulfide concentrations were below or close to detection limit (Fig. 2.5o and q), and some dissolved manganese was present in the lower part of the sediment (Fig. 2.5p). The station in the anoxic-sulfidic zone had no dissolved iron and manganese, but pore water concentrations of sulfide increased to up to 1000 μmol L^{-1} at 30 cm sediment depth (Fig. 2.5v-x).

In solid phase extractions, reactive iron was elevated in the 0-1 cm interval of the oxic zone and iron oxides were present throughout the upper 30 cm of surface sediments (Fig. 2.5e). In contrast, concentrations of iron-oxides in the upper 10 cm of the oxic-hypoxic zone were clearly reduced and dropped to background concentrations below 10 cm. The same trend was observed in sediments of the hypoxic-anoxic and the anoxic-sulfidic zone (Fig. 2.5l, s, and z). Solid phase manganese concentration was only clearly elevated in the 0-1 cm interval of the oxic zone (Fig. 2.5f) and at or close to background concentration below 1 cm, as in all other zones (Fig. 2.5m, t and aa).

Although pore water concentrations of sulfide were below detection limit in the oxic to hypoxic-anoxic zones, the presence of reduced solid sulfide phases (AVS, CRS and S^0, Fig. 2.5g, n, u, and a b) and measured sulfate reduction rates indicate that sulfate reduction takes place below the oxygenated sediment. Sulfate reduction rates, integrated over the upper 10 cm of the sediment, represent gross sulfide production and compare well to net sulfide fluxes calculated from the pore water profiles in Table 2.3. Altogether, seafloor sulfate reduction rates were increasing nearly 40 fold from <0.1 mmol m^{-2} d^{-1} in the oxic zone to 3.7 mmol m^{-2} d^{-1} in the anoxic-sulfidic zone. In all cores sulfate concentrations were constant with 16 mmol L^{-1} over the upper 30 cm of the sediment and methane concentrations were close to or below detection limit (data not shown).

2.4.5 Sediment accumulation and bioturbation

Sediment porosity was similar across all sites with 0.9±0.03 in the top cm and 0.8±0.07 averaged over the top 10 cm. Sediment accumulation rates, calculated from the decrease of $^{210}Pb_{xs}$ with depth and cumulative dry weight, varied around 1±0.5 mm yr^{-1} for the upper 10 cm of the oxic-hypoxic and the hypoxic-sulfidic zone. Nearly constant $\ln^{210}Pb_{xs}$

values in the upper 2 cm of the oxic zone indicate active sediment mixing by bioturbation. In all other zones, the linear decrease starting right below the sediment surface indicates a continuous decay and, hence, the absence of sediment mixing processes. A stronger bioturbation at the oxic site as compared to the oxic-hypoxic and hypoxic-anoxic site matches the micro-topographies observed at the different sites. Average absolute roughness heights at a water depth of 104 m were generally ∼1.8, ∼3.2, and ∼3.9 times larger than at 138, 155, and 206 m depth, respectively, at all investigated length scales (i.e., averaging windows). At an averaging window of 50 mm, a horizontal scale that covers many biogenic roughness elements, e.g., fecal mounds or funnels of burrows, average absolute deviations from the smoothed surface were 0.42±0.16 mm at 104 m, 0.23±0.03 mm at 138 m, 0.15±0.03 mm at 155 m, and 0.13±0.01 mm at 206 m water depth. Fig. 2.10 shows example 3-D micro-topographies and extracted profiles (original and smoothed at 155 mm window size).

2.5 Discussion

2.5.1 Effect of oxygen availability on remineralization rates and reoxidation processes

Rates of benthic oxygen consumption are governed by a variety of factors including primary production, particle export, quality of organic matter, bottom water oxygen concentrations, and faunal biomass (Jahnke et al., 1990; Middelburg and Levin, 2009; Wenzhöfer and Glud, 2002). Here we investigated the effects of variable hypoxic conditions, with bottom water oxygen concentrations oscillating between 180-0 μmol L^{-1} within one region of similar productivity and particle flux. On the outer Western Crimean Shelf rapid and frequent variations of oxygen concentrations included strong drops in oxygen concentrations within hours, lasting for up to a few days (Fig. 2.8b). Such events are likely connected to the special hydrological system of the area, including the strongly variable Sevastopol Eddy (Murray and Yakushev, 2006), that is known to be of importance for the ventilation of the Crimean Shelf (Stanev et al., 2002), possibly in combination with internal waves (Luth et al., 1998; Staneva et al., 2001).

Assuming an annual surface primary productivity of 220 g C m^{-2} yr^{-1}, and a particulate organic carbon (POC) export flux of around 30% (Grégoire and Friedrich, 2004), about 15 mmol C m^{-2} d^{-1} is expected to reach the seafloor in the investigated area. With a respiratory quotient of 1.0 (i.e., one mole of oxygen consumed per one mole of CO_2 produced, Canfield et al., 1993a), the average TOU observed in the oxic zone would be sufficient to remineralize nearly all of the organic carbon exported to the

seafloor (Table 2.2), with oxygen fluxes measured in this study being similar to those previously reported from the same area (Table 2.4, including references; Grégoire and Friedrich, 2004). This suggests that within the oxic zone, most deposited carbon is directly remineralized and little carbon is escaping benthic consumption. However, already in the oxic-hypoxic zone, total benthic respiration decreases by 50%, and by 90% in the hypoxic-anoxic zone along with decreases in the abundance and composition of macrofauna (Table 2.5). By bioturbation and aeration of sediments, macrofauna plays a key role in enhancing total as well as microbially-driven remineralization rates. Absence of macrofauna and low bioturbation activity in areas with temporary hypoxia will affect biogeochemical processes (Levin et al., 2009, and discussion below). Macrofauna abundance estimates, visual observations, as well as radiotracer and roughness assessments show that already under oxic-hypoxic conditions sediment aeration by fauna drops rapidly. Consequently, at the onset of hypoxia, substantial amounts of organic matter are not remineralized rapidly, but accumulate in the sediments. Another effect of variable hypoxic conditions on organic matter remineralization rates is the reduced exposure time to oxygen during organic matter degradation (oxygen exposure time: oxygen penetration depth/sediment accumulation). At a sediment deposition rate of 1 mm yr^{-1}, as estimated from ^{210}Pb measurements, particles deposited at the oxic site, are exposed much longer to aerobic mineralization processes (>5 yr) compared to the other zones (0.4–1.6 yr). Earlier studies showed that oxygen availability can be a key factor in the degradability of organic carbon and some compounds such as chlorophyll (King, 1995) and amino acids (Vandewiele et al., 2009) will favorably accumulate in the sediments exposed to hypoxic conditions.

To evaluate the contribution of chemical reoxidation to TOU at the outer Western Crimean Shelf, we fitted measured pore water profiles of dissolved manganese, iron, ammonium, and sulfide with 1-D models to quantify upward directed fluxes (Berg et al., 1998, Table 2.3, Fig. 2.5). Taking the stoichiometries of the reaction of oxygen with the reduced species into account, the maximal oxygen demand for the reoxidation of reduced pore water species was less than 8% (Table 2.3). This is less than in other studies in eutrophic shelf sediments, where the chemical and microbial reoxidation of reduced compounds, such as sulfide, dominated and the heterotrophic respiration by fauna contributed around 25% to total oxygen consumption (Glud, 2008; Heip et al., 1995; Jørgensen, 1982; Konovalov et al., 2007; Soetaert et al., 1996).

2.5.2 Effect of bottom water fluctuations on faunal respiration and diffusive oxygen uptake

Comparing total remineralization rates across all zones, including the oxygen demand by anaerobic microbial processes (Table 2.3), the capacity of the benthic communities to remineralize the incoming particle flux decreased from the oxic zone, to the oxic-hypoxic, hypoxic-anoxic and the anoxic zone. Total remineralization rates were similar in the hypoxic-anoxic and stable anoxic zone, but in the latter, anaerobic processes dominated over aerobic processes, most likely due to the decline in macrofauna abundance. Total oxygen uptake (TOU), as measured in situ with benthic chambers, represents an integrated measure of diffusive microbial respiration, as well as oxygen uptake by benthic fauna. The diffusive oxygen uptake (DOU), as calculated from microsensor profiles, represents mainly aerobic respiration of microorganisms or – although not relevant in our area (see above) - chemical reoxidation (Glud, 2008). In general, the DOU of the outer Western Crimean Shelf sediments was lower than in other shelf zones with seasonally hypoxic water columns (e.g., Glud et al., 2003), but in the same range as fluxes reported in other Black Sea studies (Table 2.4). Average DOU was similar in the oxic and oxic-hypoxic zone and only clearly reduced when oxygen concentrations were close to zero (20 μmol L^{-1}). To test if lower fluxes at reduced bottom water oxygen concentrations rather reflect lowered efficiency of oxygen consumption processes (i.e., rate limitation), or decreased diffusional uptake (i.e., transport limitation), we calculated the highest possible oxygen fluxes in relation to bottom water oxygen concentration. For this we assumed complete consumption of oxygen at the sediment surface (i.e., oxygen penetration depth approaches zero and volumetric rates approaches infinity), and calculated the flux from measured O_2 concentrations in the bottom water and the observed diffusive boundary layer thickness of 500 μm using Ficks' first law of diffusion (Eq. 1). Maximum theoretical fluxes were 4.3 to 36.4 mmol m^{-2} d^{-1} for the oxic-hypoxic zone and 2.7 to 4.6 mmol m^{-2} d^{-1} for the hypoxic-anoxic zone (for oxygen concentrations see Table 2.4). Thus, while fluxes are generally not transport limited, the benthic uptake of oxygen approaches its potential maximum when bottom water oxygenation decreases.

TOU at the oxic-hypoxic zone was substantially lower as compared to the oxic zone despite bottom water oxygen concentrations mostly above the common threshold for hypoxia of 63 μmol L^{-1} (Figs. 2.2 and 2.3). This indicates that total oxygen uptake is more sensitive to varying bottom water oxygen concentrations than diffusive uptake mediated by microorganisms. To quantify the extent to which benthos-mediated oxygen uptake (BMU) is affected by dynamic oxygen conditions, BMU was calculated from the difference between TOU and DOU (Glud, 2008; Wenzhöfer and Glud, 2004). BMU includes not only oxygen demand of the fauna itself but also oxygen consumption that

is related to the increase in oxygen-exposed sediment area due to sediment ventilation and reworking by faunal activity. Based on these calculations we assume that up to 70% of the total oxygen uptake in the oxic zone, 40% in the oxic-hypoxic zone and 20% in the hypoxic-anoxic zone is due to benthos-mediated oxygen uptake. The remaining share (30, 60, 80%, respectively) will mainly be channeled directly into the aerobic degradation of organic carbon by microbes (and potentially also some meiofauna). A BMU of 70% (10.3 mmol m^{-2} d^{-1}) in the oxic zone was considerably higher than values of 15–60% reported from shelf sediments underlying both normoxic (Glud et al., 1998; Heip et al., 2001; Moodley et al., 1998; Piepenburg et al., 1995) and hypoxic water columns (Archer and Devol, 1992; Wenzhöfer et al., 2002). A BMU of 40% in the oxic-hypoxic zone was still well within the ranges of some normoxic water columns (Glud et al., 1998; Heip et al., 2001; Moodley et al., 1998; Piepenburg et al., 1995).

It has previously been shown that sediment-water exchange rates can be altered due to changes in fauna composition in response to different bottom water oxygenation (Dale et al., 2013; Rossi et al., 2008). Also in the outer Western Crimean Shelf area the overall reduction of BMU from the oxic zone to the oxic-hypoxic zone relates well with changes in macrobenthos composition. In the oxic zone the higher fauna-mediated uptake was probably partly caused by irrigation and bioturbation by polychaetes, bivalves, and gastropods (Table 2.5). Ventilation of the upper sediment layer is indicated by the presence of oxidized Fe and Mn solid phase minerals in the oxic zone and in the upper 10 cm of the oxic-hypoxic zone (Fig. 2.5). Decreased bioturbation in the other zones is due to reduced abundances of sediment infauna. Loss of sediment ventilation also explains changes in sediment biogeochemistry, in particular the ceasing of the iron and manganese cycle upon lower bottom water oxygen concentrations (Fig. 2.5). This is in accordance with previous studies that have shown that reoxidation of reduced iron and manganese is mainly stimulated by bioturbation, and thus recycling efficiency of the metals primarily depends on bottom-water oxygen levels and rates of bioturbation (Canfield et al., 1993b; Thamdrup et al., 2000; Wijsman et al., 2001).

The restriction of bivalves and gastropods to the upper oxic-hypoxic zone is surprising, as representatives of these groups are known to be able to maintain their respiration rate at hypoxic oxygen concentrations (Bayne, 1971; Taylor and Brand, 1975). Oxygen concentrations on the outer Western Crimean Shelf (Fig. 2.2) were mostly well above reported oxygen thresholds, e.g., 50 μmol L^{-1} for bivalves and 25 μmol L^{-1} for gastropods (Keeling et al., 2010; Vaquer-Sunyer and Duarte, 2008). While mollusc distribution indicated low hypoxia-tolerance for the species found in the area, fish were observed in the hypoxic-anoxic zone at oxygen concentrations as low as $<$20 μmol L^{-1}, which although beyond previously-reported tolerance thresholds (Gray et al., 2002; Pihl et al.,

1991; Vaquer-Sunyer and Duarte, 2008), is consistent with the adaptations of some fish species of the Black Sea (Silkin and Silkina, 2005).

The overall role of meiobenthos in oxygen consumption is difficult to assess as it can add to both BMU and DOU by bio-irrigating the sediment as well as enhancing diffusional fluxes (Aller and Aller, 1992; Berg et al., 2001; Rysgaard et al., 2000; Wenzhöfer et al., 2002). Altogether, different distribution patterns were found for meiofauna as compared to macrofauna. Meiobenthos abundances were similar in the oxic and oxic-hypoxic zone, and only sharply decreased in the hypoxic-anoxic zone. As shown previously (Levin et al., 2009) nematodes and foraminifera dominate meiofauna in hypoxic zones due to their ability to adapt to low oxygen concentrations. In particular, nematodes are known to tolerate hypoxic, suboxic, anoxic or even sulfidic conditions (Sergeeva et al., 2012; Steyaert et al., 2007; Van Gaever et al., 2006). The relatively high abundance of apparently living foraminifera in the hypoxic zone, including low abundances also in the anoxic zone, might be related to the ability of some species to respire nitrate under anoxic conditions (Risgaard-Petersen et al., 2006).

Regarding the validation of the traditionally-used hypoxia threshold for impact on fauna (63 μmol O_2 L^{-1}, e.g., Diaz, 2001), our results support previous studies where significant changes in community structure were reported already at the onset of hypoxia (Gray et al., 2002; Steckbauer et al., 2011; Vaquer-Sunyer and Duarte, 2008). Our results indicate that fauna-mediated oxygen uptake and biogeochemical fluxes are strongly reduced already at periodical hypoxic conditions, as caused by transport of low-oxygen waters via internal waves or eddies close to the shelf break (Fig. 2.8b). Depending on hydrographic conditions, ecosystem functioning could thus be negatively impacted in much larger areas adjacent to hypoxic ecosystems.

The Supplement related to this article is available online at doi:10.5194/bgd-12-6445-2015-supplement.

2.6 Acknowledgements

We thank the Captain and shipboard crew of the RV Maria S. Merian, the JAGO team (GEOMAR, Kiel) and shipboard scientists of the cruise MSM 15/1 for their excellent work at sea. We are grateful for technical assistance from Rafael Stiens, Martina Alisch, Erika Weiz, and Kirsten Neumann. We thank the Sea-Tech technicians of the HGF MPG Joint Research Group for Deep-Sea Ecology and Technology (MPI-AWI) for the construction and maintenance of the in situ devices and the technicians of the Microsensor Group for the construction of microsensors. We thank Tim Ferdelman and Gail Lee

Arnold for help with the sedimentation rate measurements. This project was financed by the EU 7th FP project HYPOX (In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies) EC Grant 226213.

The article processing charges for this open-access publication were covered by the Max Planck Society.

2.7 References

Abril, G., Commarieu, M.-V., Etcheber, H., Deborde, J., Deflandre, B., Živainović, M. K., Chaillou, G., and Anschutz, P.: In vitro simulation of oxic/suboxic diagenesis in an estuarine fluid mud subjected to redox oscillations, Estuar. Coast. Shelf S., 88, 279-291, 2010.

Aller, R. and Aller, J.: Meiofauna and solute transport in marine muds, Limnol. Oceanogr., 37, 1018-1033, 1992.

Archer, D. and Devol, A.: Benthic oxygen fluxes on the Washington shelf and slope: a comparison of in situ microelectrode and chamber flux measurements, Limnol. Oceanogr., 37, 614-629, 1992.

Baird, D., Christian, R. R., Peterson, C. H., and Johnson, G. A.: Consequences of hypoxia on estuarine ecosystem function: energy diversion from consumers to microbes, Ecol. Appl., 14, 805-822, 2004.

Bayne, B. L.: Oxygen consumption by three species of lamellibranch mollusc in declining am- bient oxygen tension, Comp. Biochem. Phys. A, 40, 955-970, 1971.

Berg, P., Risgaard-Petersen, N., and Rysgaard, S.: Interpretation of measured concentration profiles in sediment pore water, Limnol. Oceanogr., 43, 1500-1510, 1998.

Berg, P., Rysgaard, S., Funch, P., and Sejr, M.: Effects of bioturbation on solutes and solids in marine sediments, Aquat. Microb. Ecol., 26, 81-94, 2001.

Boesch, D. F. and Rabalais, N. N.: Effects of hypoxia on continental shelf benthos: comparisons between the New York Bight and the Northern Gulf of Mexico, in: Modern and Ancient Continental Shelf Anoxia, edited by: Tyson, R. V. and Pearson, T. H., Geological Society Special Publication 58, 27-34, Geological Soc., London, 1991.

Boetius, A. and Wenzhöfer, F.: In situ technologies for studying deep-sea hotspot ecosystems, Oceanography, 22, 177, doi:10.5670/oceanog.2009.17, 2009.

Broecker, W. S. and Peng, T. H.: Gas exchange rates between air and sea, Tellus, 26, 21-35, 1974.

Canfield, D. E., Jørgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., Thamdrup, B., Hansen, J. W., Nielsen, L. P., and Hall, P. O. J.: Pathways of organic carbon oxidation in three continental margin sediments, Mar. Geol., 113, 27-40, 1993a.

Canfield, D. E., Thamdrup, B., and Hansen, J. W.: The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction, Geochim. Cosmochim. Ac., 57, 3867-3883, 1993b.

Clarke, K.-R.: Non-parametric multivariate analyses of changes in community structure, Australian J. Ecol., 18, 117-143, 1993.

Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters, Limnol. Oceanogr., 14, 454-458, 1969.

Cook, P. L. M., Wenzhöfer, F., Glud, R. N., Janssen, F., and Huettel, M.: Benthic solute exchange and carbon mineralization in two shallow subtidal sandy sediments: effect of advective porewater exchange, Limnol. Oceanogr., 1943-1963, 2007.

Dale, A. W., Bertics, V. J., Treude, T., Sommer, S., and Wallmann, K.: Modeling benthic– pelagic nutrient exchange processes and porewater distributions in a seasonally hypoxic sediment: evidence for massive phosphate release by Beggiatoa?, Biogeosciences, 10, 629– 651, doi:10.5194/bg-10-629-2013, 2013.

de Beer, D., Glud, A., Epping, E., and Kuhl, M.: A fast-responding CO_2 microelectrode for profiling sediments, microbial mats, and biofilms, Limnol. Oceanogr., 42, 1590-1600, 1997.

Diaz, R. J.: Overview of hypoxia around the world, J. Environ. Qual., 30, 275-281, 2001.

Fossing, H. and Jørgensen, B. B.: Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method, Biogeochemistry, 8, 205-222, 1989.

Friedl, G., Dinkel, C., and Wehrli, B.: Benthic fluxes of nutrients in the northwestern Black Sea, Mar. Chem., 62, 77-88, 1998.

Friedrich, J., Janssen, F., Aleynik, D., Bange, H. W., Boltacheva, N., Çağatay, M. N., Dale, A. W., Etiope, G., Erdem, Z., Geraga, M., Gilli, A., Gomoiu, M. T., Hall, P. O. J., Hansson, D., He, Y., Holtappels, M., Kirf, M. K., Kononets, M., Konovalov, S., Lichtschlag, A., Livingstone, D. M., Marinaro, G., Mazlumyan, S., Naeher, S., North, R. P., Papatheodorou, G., Pfannkuche, O., Prien, R., Rehder, G., Schubert, C. J., Soltwedel, T., Sommer, S., Stahl, H., Stanev, E. V., Teaca, A., Tengberg, A., Waldmann, C., Wehrli, B., and Wenzhöfer, F.: Investigating hypoxia in aquatic environments: diverse approaches to addressing a complex phenomenon, Bio-geosciences, 11, 1215-1259, doi:10.5194/bg-11-1215-2014, 2014.

Glud, R. N.: Oxygen dynamics of marine sediments, Mar. Biol. Res., 4, 243-289, 2008.

Glud, R. N., Holby, O., Hoffmann, F., and Canfield, D. E.: Benthic mineralization and exchange in Arctic sediments (Svalbard, Norway), Mar. Ecol.Prog. Ser., 173, 237-251, 1998.

Glud, R. N., Gundersen, J. K., Røy, H., and Jørgensen, B. B.: Seasonal dynamics of benthic O_2 uptake in a semienclosed bay: importance of diffusion and faunal activity, Limnol. Oceanogr., 48, 1265-1276, 2003.

Grasshoff, K.: Methods of Seawater Analysis, Verlag Chemie, Weinheim, 1983.

Gray, J. S., Wu, R. S.-S., and Or, Y. Y.: Effects of hypoxia and organic enrichment on the coastal marine environment, Mar. Ecol.-Prog. Ser., 238, 249-279, 2002.

Grego, M., Stachowitsch, M., De Troch, M., and Riedel, B.: CellTracker Green labelling vs. rose bengal staining: CTG wins by points in distinguishing living from dead anoxia-impacted copepods and nematodes, Biogeosciences, 10, 4565-4575, doi:10.5194/bg-10-4565-2013,2013.

Grégoire, M. and Friedrich, J.: Nitrogen budget of the north-western Black Sea shelf as inferred from modeling studies and in-situ benthic measurements, Mar. Ecol.-Prog. Ser., 270, 15-39, 2004.

Heip, C. H. R., Goosen, N. K., Herman, P. M. J., Kromkamp, J., Middelburg, J. J., and Soetaert, K.: Production and consumption of biological particles in temperate tidal estuaries, Ann. Rev. Ocean. Mar. Biol, 33, 1-150, 1995.

Heip, C. H. R., Duineveld, G., Flach, E., Graf, G., Helder, W., Herman, P. M. J., Lavaleye, M., Middelburg, J. J., Pfannkuche, O., Soetaert, K., Soltwedel, T., de Stigter, H., Thomsen, L., Vanaverbeke, J., and de Wilde, P.: The role of the benthic biota in sedimentary metabolism and sediment-water exchange processes in the Goban Spur area (NE Atlantic), Deep-Sea Res. Pt. II, 48, 3223-3243, 2001.

Holtappels, M., Kuypers, M. M., Schlüter, M., and Brüchert, V.: Measurement and interpretation of solute concentration gradients in the benthic boundary layer, Limnol. Oceanogr.-Meth., 9, 1-13, 2011.

Holtappels, M., Glud, R. N., Donis, D., Liu, B., Hume, A., Wenzhöfer, F., and Kuypers, M. M. M.: Effects of transient bottom water currents and oxygen concentrations on benthic exchange rates as assessed by eddy correlation measurements, J. Geophys. Res.-Oceans, 118, 1157-1169, 2013.

Jahnke, R. A., Reimers, C. E., and Craven, D. B.: Intensification of recycling of organic matter at the sea floor near ocean margins, Nature, 348, 50-54, 1990.

Jeroschewski, P., Steuckart, C., and Kühl, M.: An amperometric microsensor for the determination of H_2S in aquatic environments, Anal. Chem., 68, 4351-4357, 1996.

Jørgensen, B. B.: A comparison of methods for the quantification of bacterial sulfate reductionin coastal marine sediments, Geomicrobiol. J., 1, 11-27, 1978.

Jørgensen, B. B.: Mineralization of organic matter in the sea bed-the role of sulphate reduction, Nature, 643-645, 1982.

Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., and Jørgensen, B. B.: A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements, Limnol. Oceanogr.-Meth., 2, 171-180, 2004.

Keeling, R. F., Kortzinger, A., and Gruber, N.: Ocean deoxygenation in a warming world, Annu. Rev. Marine Sci., 2, 199-229, 2010.

King, L. L.: A mass balance of chlorophyll degradation product accumulation in Black Sea sediments, Deep-Sea Res. Pt. I, 42, 919-942, 1995.

Kolesnikova, E., Zaika, V., Sergeeva, N., and Lichtschlag, A.: Harpacticoida (Copepoda) under hypoxia conditions in the Black Sea, in: Copepods: Diversity, Habitat and Behavior, edited by: Seuront, L., Nova Science, New York, 5176, 2014.

Konovalov, S. K., Luther III, G. W., and Yücel, M.: Porewater redox species and processes in the Black Sea sediments, Chem. Geol., 245, 254-274, 2007.

Levin, L. A.: Oxygen minimum zone benthos: adaptation and community response to hypoxia, Oceanogr. Mar. Biol., 41, 1-45, 2003.

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., Neira, C., Rabalais, N. N., and Zhang, J.: Effects of natural and human-induced hypoxia on coastal benthos, Biogeosciences, 6, 2063-2098, doi:10.5194/bg-6-2063-2009, 2009.

Lichtschlag, A., Felden, J., Wenzhöfer, F., Schubotz, F., Ertefai, T. F., Boetius, A., and de Beer, D.: Methane and sulfide fluxes in permanent anoxia: in situ studies at the Dvurechenskii mud volcano (Sorokin Trough, Black Sea), Geochim. Cosmochim. Ac., 74, 5002-5018, 2010.

Luth, U., Luth, C., Stokozov, N. A., and Gulin, M. B.: The chemocline rise effect on the northwestern slope of the Black Sea, in: Methane Gas Seep Explorations in the Black Sea MEGASEEBS), Project Report. Ber. Zentrum Meeres- u. Klimaforsch., edited by: Luth, U., Luth, C., and Thiel, H., Univ. Hamburg, Reihe E, 14, 59-77, 1998.

Middelburg, J. J. and Levin, L. A.: Coastal hypoxia and sediment biogeochemistry, Biogeosciences, 6, 1273-1293, doi:10.5194/bg-6-1273-2009, 2009.

Moodley, L., Schaub, B. E. M., Zwaan, G. J. v. d., and Herman, P. M. J.: Tolerance of benthic foraminifera (Protista: Sarcodina) to hydrogen sulphide, Ecology Progress Series, 169, 77-86, 1998.

Murray, J. W. and Yakushev, E.: The suboxic transition zone in the Black Sea, in: Past and Present Marine Water Column Anoxia, edited by: Neretin, L., NATO Science Series IV: Earth and Environmental Sciences, 64, Springer, Dordrecht, 105-138, 2006.

Niggemann, J., Ferdelman, T. G., Lomstein, B. A., Kallmeyer, J., and Schubert, C. J.: How depositional conditions control input, composition, and degradation of organic matter in sediments from the Chilean coastal upwelling region, Geochim. Cosmochim. Ac., 71, 1513-1527, 2007.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Wagner, H.: vegan: Community Ecology Package, R package version of The Comprehensive R Archive Network, 1.17-3, 2010.

Pearson, T. H. and Rosenberg, R.: Energy flow through the SE Kattegat: a comparative examination of the eutrophication of a coastal marine ecosystem, Neth. J. Sea Res., 28, 317-334, 1992.

Piepenburg, D., Blackburn, H. T., v. Dorrien, F. C., Gutt, J., Hall, J. P. O., Hulth, S., Kendall, A. M., Opalinski, W. K., Rachor, E., and Schmid, K. M.: Partitioning of benthic community respiration in the Arctic (northwestern Barents Sea), Mar. Ecol.-Prog. Ser., 118, 199-213, 1995.

Pihl, L., Baden, S. P., and Diaz, R. J.: Effects of periodic hypoxia on distribution of demersal fish and crustaceans, Mar. Biol., 108, 349-360, 1991.

Poulton, S. W. and Canfield, D. E.: Development of a sequential extraction procedure for iron: implications for iron partitioning in continentally derived particulates, Chem. Geol., 214, 209-221, 2005.

Rasmussen, H. and Jørgensen, B. B.: Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion, Mar. Ecol.-Prog. Ser., 81, 289-303, 1992.

Revsbech, N. P.: An oxygen microsensor with a guard cathode, Limnol. Oceanogr.,34, 474-478, doi:10.4319/lo.1989.34.2.0474, 1989.

Risgaard-Petersen, N., Langezaal, A. M., Ingvardsen, S., Schmid, M. C., Jetten, M. S. M., Opden Camp, H. J. M., Derksen, J. W. M., Pina-Ochoa, E., Eriksson, S. P., Peter Nielsen, L., Revsbech, N. P., Cedhagen, T., and van der Zwaan, G. J.: Evidence for complete denitrification in a benthic foraminifera, Nature, 443, 93-96, 2006.

Rossi, F., Gribsholt, B., Middelburg, J. J., and Heip, C.: Context-dependent effects of suspension feeding on intertidal ecosystem functioning, Mar. Ecol.-Prog. Ser., 354, 47-57, 2008.

Rysgaard, S., Christensen, P., Sørensen, M., Funch, P., and Berg, P.: Marine meiofauna, carbon and nitrogen mineralization in sandy and soft sediments of Disko Bay, West Greenland, Aquat. Microb. Ecol., 21, 59-71, 2000.

Sergeeva, N. G. and Anikeeva, O. V.: Soft-walled foraminifera under normoxia/hypoxia conditions in the shallow areas of the Black Sea, in: Foraminifera. Aspects of Classification, Stratigraphy, Ecology and Evolution, edited by: Georgescu, M. D., Nova Publ., New York, 227-247, 2014.

Sergeeva, N. G. and Zaika, V. E.: The Black Sea meiobenthos in permanently hypoxic habitat, Acta Zool. Bulgar., 65, 139-150, 2013.

Sergeeva, N., Gooday, A. J., Mazlumyan, S. A., Kolesnikova, E. A., Lichtschlag, A., Koshelva, T. N., and Anikeeva, O. V.: Meiobenthos of the oxic/anoxic interface in the south western region of the Black Sea: abundance and taxonomic composition, in: ANOXIA: Evidence for Eukaryote Survival and Paleontological Strategies, edited by: Altenbach, A. V., Bernhard, J. M., and Seckbach, J., Springer, Dordrecht, 369-401, doi:10.1007/978-94-007-1896-8_20, 2012.

Sergeeva, N. G., Mazlumyan, S. A., Çağatay, N., and Lichtschlag, A.: Hypoxic meiobenthic communities of the Istanbul Strait's (Bosporus) outlet area of the Black Sea, Turk. J. Fish. Aquat. Sc., 13, 33-41, 2013.

Silkin, Y. A. and Silkina, E. N.: Effect of hypoxia on physiological-biochemical blood parameters in some marine fish, J. Evol. Biochem. Phys., 41, 527-532, 2005.

Soetaert, K., Herman, P. M. J., and Middelburg, J. J.: A model of early diagenetic processes from the shelf to abyssal depths, Geochim. Cosmochim. Ac., 60, 1019-1040, 1996.

Stanev, E. V., Beckers, J. M., Lancelot, C., Staneva, J. V., Le Traon, P. Y., Peneva, E. L., and Gregoire, M.: Coastal-open ocean exchange in the Black Sea: observations and modelling, Estuar. Coast. Shelf S., 54, 601-620, 2002.

Stanev, E. V., He, Y., Grayek, S., and Boetius, A.: Oxygen dynamics in the Black Sea as seen by Argo profiling floats, Geophys. Res. Lett., 40, 3085-3090, 2013.

Staneva, J. V., Dietrich, D. E., Stanev, E. V., and Bowman, M. J.: Rim current and coastal eddy mechanisms in an eddy-resolving Black Sea general circulation model, J. Marine Syst., 31, 137-157, 2001.

Steckbauer, A., Duarte, C. M., Carstensen, J., Vaquer-Sunyer, R., and Conley, D. J.: Ecosystem impacts of hypoxia: thresholds of hypoxia and pathways to recovery, Environ. Res. Lett., 6, 025003, doi:10.1088/1748-9326/6/2/025003, 2011.

Steyaert, M., Moodley, L., Nadong, T., Moens, T., Soetaert, K., and Vincx, M.: Responses of intertidal nematodes to short-term anoxic events, J. Exp. Mar. Biol. Ecol., 345, 175-184, 2007.

Taylor, A. C. and Brand, A. R.: A comparative study of the respiratory responses of the bivalves Arctica islandica (L.) and Mytilus edulis L. to declining oxygen tension, P. Roy. Soc. Lond. B Bio., 190, 443-456, 1975.

Thamdrup, B., Rosselló-Mora, R., and Amann, R.: Microbial manganese and sulfate reduction in Black Sea shelf sediments, Appl. Environ. Microb., 66, 2888-2897, 2000.

Tolmazin, D.: Changing coastal oceanography of the Black Sea. Part 1: Northwestern shelf, Prog. Oceanogr., 15, 217-276, 1985.

Vandewiele, S., Cowie, G., Soetaert, K., and Middelburg, J. J.: Amino acid biogeochemistry and organic matter degradation state across the Pakistan margin oxygen minimum zone, Deep-Sea Res. Pt. II, 56, 376-392, 2009.

Van Gaever, S., Moodley, L., de Beer, D., and Vanreusel, A.: Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments, Mar. Ecol.-Prog. Ser., 321, 143-155, 2006.

Vaquer-Sunyer, R. and Duarte, C. M.: Thresholds of hypoxia for marine biodiversity, P. Natl. Acad. Sci. USA, 105, 15452-15457, 2008.

Waldmann, C. and Bergenthal, M.: CMOVE-a versatile underwater vehicle for seafloor studies, OCEANS 2010, IEEE conference, 20-23 September, Seattle, WA, USA, doi:10.1109/OCEANS.2010.5664261, 2010.

Weber, A., Riess, W., Wenzhöefer, F., and Jørgensen, B. B.: Sulfate reduction in Black Sea sediments: in situ and laboratory radiotracer measurements from the shelf to 2000 m depth, Deep-Sea Res. Pt. I, 48, 2073-2096, 2001.

Wenzhöfer, F. and Glud, R. N.: Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade, Deep-Sea Res. Pt. I, 49, 1255-1279, 2002.

Wenzhöfer, F. and Glud, R. N.: Small-scale spatial and temporal variability in coastal benthic O_2 dynamics: effects of fauna activity, Limnol. Oceanogr., 49, 1471-1481, 2004.

Wenzhöfer, F., Riess, W., and Luth, U.: In situ macrofaunal respiration rates and their importance for benthic carbon mineralization on the northwestern Black Sea shelf, Ophelia, 56, 87-100, 2002.

Wijsman, J. W. M., Middelburg, J. J., and Heip, C. H. R.: Reactive iron in Black Sea sediments: implications for iron cycling, Mar. Geol., 172, 167-180, 2001.

Winkler, L.: The determination of dissolved oxygen in water, Ber. Dtsch. Chem. Ges., 21, 2843-2857, 1888.

Witte, U. and Pfannkuche, O.: High rates of benthic carbon remineralisation in the abyssal Arabian Sea, Deep-Sea Res. Pt. II, 47, 2785-2804, 2000.

Zaika, V. E. and Gulin, M. B.: The maximum depths of fish inhabitation in the Black Sea and features of their trophic strategy nearly of oxic/anoxic interface, Mar. Ecol. J., 10, 39-47, 2011, (in Russian).

Zaika, V. E. and Sergeeva, N. G.: Deep-water benthic Polychaetes (Vigtorniella zaikai and Protodrilus sp.) in the Black Sea as indicators of the hydrogen sulfide zone boundary, Vestn. Zool., 46, 19-27, 2012.

Zaika, V. E., Konovalov, S. K., and Sergeeva, N.: The events of local and seasonal hypoxia at the bottom of Sevastopol bays and their influence on macrobenthos, Mar. Ecol. J., 10, 15-25, 2011.

Zopfi, J., Ferdelman, T. G., and Fossing, H.: Distribution and fate of sulfur intermediates -sulfite tetrathionate, thiosulfate, and elemental sulfur-in marine sediments, in: The Biogeochemistry of Sulfur, GSA Special Paper, edited by: Amend, J., Edwards, K., and Lyons, T., Geol. Soc. America, London, 97-116, 2004.

2.8 Tables and Figures

TABLE 2.1: Measurements and samples (including PANGAEA event labels) taken in zones with different oxygen regime. PUC=JAGO push-cores, MOVE=benthic crawler move (in situ microsensor measurements and/or benthic chamber deployment), TVMUC=video-guided multicorer, KAMM=lander (in situ microsensor measurements and/or benthic chamber deployment).

Zone	Water depth (m)	Station/PANGAEA event label	Position		Date	Device	Method
	101	MSM15/1_482_PUC 1, 3, 5, 6	44°49.00'N	33°09.37'E	3 May 2010	PUC	Macro- and meiobenthos
	104	MSM15/1_484-1	44°49.49'N	33°09.32'E	3 May 2010	MOVE	Benthic oxygen uptake
oxic zone	104	MSM15/1_464-1	44°49.45'N	33°09.26'E	2 May 2010	TVMUC	Macro- and meiobenthos
< 130 m	104	MSM15/1_462-1	44°49.45'N	33°09.26'E	2 May 2010	TVMUC	Geochemistry
	106	MSM15/1_469-1	44°49.46'N	33°09.67'E	2 May 2010	KAMM	Benthic oxygen uptake
bottom water	105	MSM15/1_444_PUC 1	44°49.32'N	33°09.46'E	1 May 2010	PUC	Macro- and meiobenthos
oxygen conc.	117	MSM15/1_440_PUC 5, 6	44°40.49'N	33°05.53'E	1 May 2010	PUC	Macro- and meiobenthos
> 63 µmol L^{-1}	120	MSM15/1_459-1, 2	44°40.48'N	33°05.53'E	2 May 2010	TVMUC	Macro- and meiobenthos
	129	MSM15/1_486_PUC 1, 7	44°39.13'N	33°01.78'E	4 May 2010	PUC	Macro- and meiobenthos
oxic-hypoxic	136	MSM15/1_487-1	44°38.78'N	33°00.25'E	4 May 2010	TVMUC	Geochemistry
(130–142 m)	137	MSM15/1_434-1	44°38.93'N	32°59.98'E	1 May 2010	KAMM	Benthic oxygen uptake
	137	MSM15/1_455-1	44°38.92'N	32°59.97'E	2 May 2010	MOVE	Benthic oxygen uptake
bottom water	138	MSM15/1_460_PUC-1	44°39.26'N	33°01.12'E	2 May 2010	PUC	Macro- and meiobenthos
oxygen conc.	138	MSM15/1_489- 1, 2	44°38.79'N	33°00.25'E	4 May 2010	TVMUC	Macro- and meiobenthos
> 63 to > 0 µmol L^{-1}	140	MSM15/1_499-1	44°38.80'N	33°00.26'E	5 May 2010	KAMM	Benthic oxygen uptake
	145	MSM15/1_512-3	44°37.39'N	32°56.21'E	5 May 2010	PUC	Macro- and meiobenthos
hypoxic-anoxic	151	MSM15/1_372_PUC 1	44°37.46'N	32°54.91'E	25 Apr 2010	PUC	Macro- and meiobenthos
(142–167 m)	154	MSM15/1_383-1	44°37.74'N	32°54.92'E	26 Apr 2010	KAMM	Benthic oxygen uptake
	155	MSM15/1_379-1	44°37.55'N	32°54.97'E	26 Apr 2010	TVMUC	Macro- and meiobenthos
bottom water	156	MSM15/1_386-1	44°37.58'N	32°54.97'E	26 Apr 2010	MOVE	Benthic oxygen uptake
oxygen conc.	162	MSM15/1_374-1	44°37.07'N	32°53.49'E	25 Apr 2010	PUC	Macro- and meiobenthos
63–0 µmol L^{-1}	163	MSM15/1_425-1	44°47.09'N	31°58.05'E	30 Apr 2010	TVMUC	Macro- and meiobenthos
	164	MSM15/1_393-1	44°37.08'N	32°53.48'E	27 Apr 2010	TVMUC	Geochemistry
anoxic-sulfidic zone (> 167 m) sulfide present in anoxic bottom water	207	MSM15/1_448-1	44°35.84'N	32°49.03'E	1 May 2010	TVMUC	Geochemistry

TABLE 2.2: Diffusive oxygen uptake (DOU) rates, total oxygen uptake (TOU) rates and oxygen penetration depth under different oxygen regimes at the outer Western Crimean Shelf. Chamber measurements in the hypoxic-anoxic zone represent potential rates, scaled to a bottom water oxygen concentration of 20 μmol O_2 L^{-1} (instead of 70 μmol O_2 L^{-1}).

Zone	DOU J_{O_2} ± SD ($mmol\,m^{-2}\,d^{-1}$)	TOU J_{O_2} ± SD ($mmol\,m^{-2}\,d^{-1}$)	DOU : TOU ration (%)	Oxygen penetration depth ± SD (mm)
oxic zone < 130 m bottom water oxygen conc. > 63 µmol L^{-1}	4.6 ± 1.8 range: 2.4 to 8.1, *n* = 15	14.9 ± 5.1 range: 9 to 20.6, *n* = 5	30 : 70	5.3 ± 2.5
oxic-hypoxic (130–142 m) bottom water oxygen conc. > 63 to > 0 µmol L^{-1}	4.4 ± 1.9 range: 0.6 to 8.0, *n* = 12	7.3 ± 3.5 range: 3.2 to 9.4, *n* = 3	60 : 40	1.6 ± 1.2
hypoxic-anoxic (142–167 m) bottom water oxygen conc. 63–0 µmol L^{-1}	1.3 ± 0.5 range: 0.8 to 2.1, *n* = 5 (potential rate: 5.6)	1.6 ± 0.5 **modeled**	80 : 20 (**modeled from potential rates**)	0.4 ± 0.1

TABLE 2.3: Diffusive oxygen uptake compared to fluxes of reduced species, calculated from the modeled profiles (Fig. 5) or measured directly (SRR=Sulfate reduction rates). The sum in oxygen equivalents is calculated from the stoichiometry of the oxidation processes (respective formulas are displayed at the lower end of the table), and oxygen available for direct aerobic respiration is calculated by subtracting the potential oxygen demand from the available oxygen flux.

	Oxygen flux ($mmol\,m^{-2}\,d^{-1}$) DOU (J_{O_2}) see Table 2	Reduced species fluxes ($mmol\,m^{-2}\,d^{-1}$) $J_{Fe^{2+}}$	$J_{Mn^{2+}}$	$J_{sulfide/SRR}$	$J_{NH_4^+}$	SUM in oxygen equivalents	Diffusive oxygen consumption (direct aerobic mineralization : re-oxidation) in $mmol\,m^{-2}\,d^{-1}$ and %
oxic zone < 130 m, bottom water oxygen conc. > 63 µmol L^{-1}	−4.6	0.1	< 0.1	0[a]/< 0.1	0.1	0.23	4.38 : 0.23 95% : 5%
oxic-hypoxic 130–142 m, bottom water oxygen conc. > 63 to > 0 µmol L^{-1}	−4.4	0.1	0	0[a]/0.4	< 0.1	< 0.1	4.36 : < 0.1 > 98% : < 2%
hypoxic-anoxic 142–167 m, bottom water oxygen conc. 63–0 µmol L^{-1}	−1.3	0	0	0[a]/0.2	< 0.1	< 0.1	1.3 : < 0.1 > 92% : < 8%
anoxic-sulfidic zone > 167 m, sulfide present in anoxic bottom water	0	0	0	0.5/3.7	0.1	1.1	0 : 1.1[b] 0% : 100%

Negative numbers denote downward flux, positive numbers upward flux.
[a] Bottom water sulfide was zero.
[b] Potential oxygen demand is higher than oxygen availability, thus reducing components are emitted.
$OM + O_2 \rightarrow CO_2 + H_2O$ ratio 1 : 1
$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$ ratio 1 : 2
$4Fe^{2+} + O_2 + 6H_20 \rightarrow 4FeOOH + 8H^+$ ratio 4 : 1
$2Mn^{2+} + O_2 + 2H_20 \rightarrow 2MnO_2 + 4H^+$ ratio 2 : 1
$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_20 + 2H^+$ ratio 1 : 2

TABLE 2.4: Oxygen consumption in hypoxic areas of the Black Sea, n.d. = not determined.

Area	Water depth (m)	Oxygen concentration ($\mu mol\,L^{-1}$)	TOU ($mmol\,m^{-2}\,d^{-1}$)	DOU ($mmol\,m^{-2}\,d^{-1}$)	Method	Fauna	Reference
Bay of Varna	24	230	33.3		in situ chamber (TOU)	living organisms	Fridel et al. (1998)
Danube delta front	26	160	25.9			living organisms	
Danube prodelta	27	0	5.7			living organisms	
shelf edge	134	40				no living organisms	
shelf edge	142	30				living organisms	
Romanian Shelf	62	211	39.8	11.9	in situ chamber (TOU)/ microsensors (DOU)	*Mytilus galloprovinciales*	Wenzhöfer et al. (2002)
	77	213	11.1	5.8		*Modiolus phaseolinus*	
	100	75	4.3	2.3		*Modiolus phaseolinus*	
	180	8	0	0		no macrofauna	
NW Shelf	52	285	13.5, 10, 11.6		ex situ core incubations (TOU)	n.d.	Wijsman et al. (2001)
	54	314	11, 6.1				
	57	243	3.7				
	72	284					
	120	126					
	137	190					
Crimean Shelf	135	95	4.2–6		Eddy correlation		Holtappels et al. (2013)
Crimean Shelf	104	110–134	11.6	4.6	in situ chamber (TOU)/ microsensors (DOU)	living organisms	this study
	135	18–149	6.7	4.4		living organisms	
	155	19–11	n.d.	1.3		living organisms, including fish	

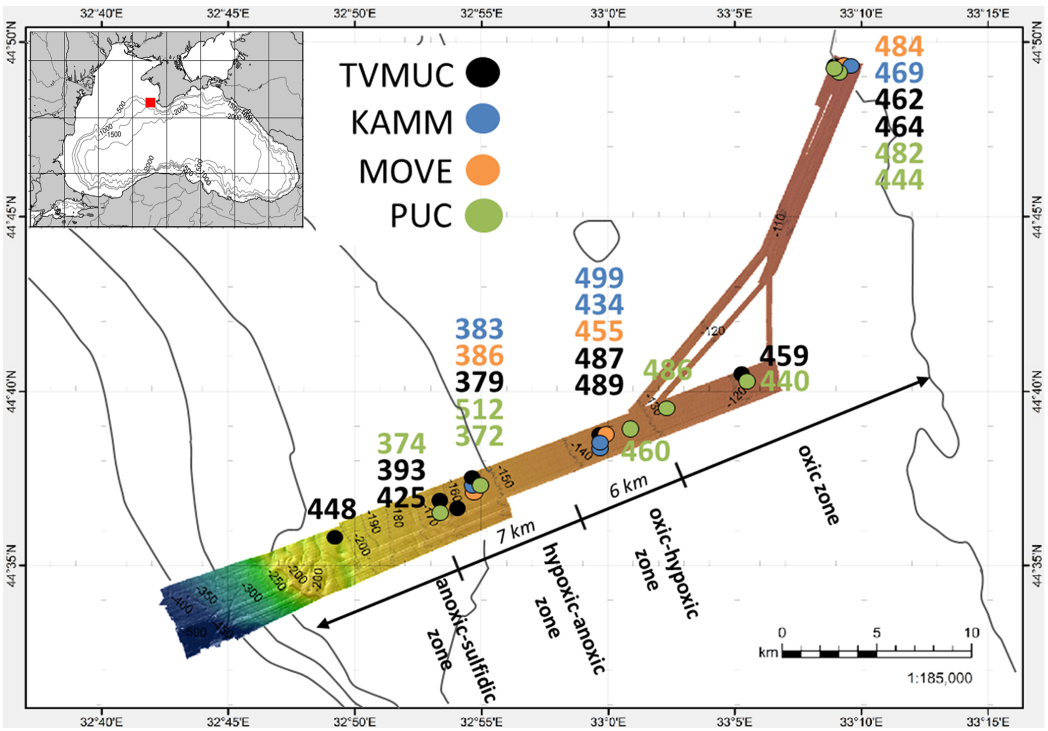


FIGURE 2.1 Sediment sampling locations (TVMUC=video-guided multicorer, PUC=JAGO pushcores) and deployment sites of benthic chamber and microprofiler with MOVE and lander (KAMM) along the transect from shallower (101 m) to deeper (207 m) water depth. Inset: working area on the outer western Crimean shelf (red square) in the Black Sea.

Figure 1. Sediment sampling locations (TVMUC represents the video-guided multicorer, PUC represents the JAGO pushcores) and deployment sites of benthic chamber and microprofiler with MOVE and lander (KAMM) along the transect from shallower (101 m) to deeper (207 m) water depth. Inset: working area on the outer western Crimean shelf (red square) in the Black Sea.

gen threshold of 63 $\mu mol\,L^{-1}$ as an upper boundary for hypoxia (Diaz, 2001). Sulfide concentrations were determined in bottom water collected with Niskin bottles during CTD casts and JAGO dives at 13 different locations between 135 and 218 m water depth. For all water-column oxygen and sulfide concentrations a limit of 2 $\mu mol\,L^{-1}$ was defined, below which concentrations were assumed to be zero.

2.3 Visual seafloor observations and microtopography

attached direc
the LS; the la
sediment stret
seafloor obser
pling by JAG
V1E HDV ca
center of JAG
ing each dive,
grabber from

2.4 Faunal

Meiofauna or
ment horizons
covering an ar
pushcore; Tab
to m^2. Sedime
through sieves
served in 75 %
tures of the m
with rose beng
isms (Grego e
lar (x 90 mag
using differen
isms that were
no signs of m
ing alive at th
isms were cou
cores we ana
and that from
thos. Also thi

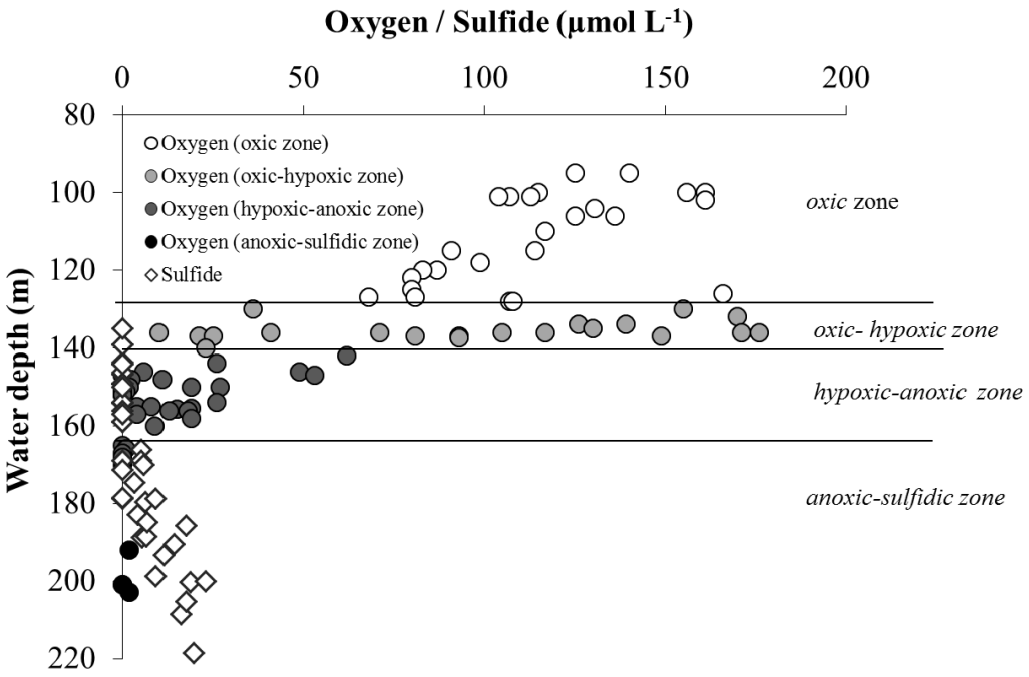


FIGURE 2.2: Synthesis of oxygen concentrations in bottom water (circles) measured during the 2 weeks of the cruise (n=85). For continuously measuring instruments (BBL profiler, optode on JAGO, benthic lander, moorings) only an average value per deployment, dive or day was included. Maximum depth above the sediment was 12 m (CTD), minimum depth above the sediment was about 5 cm (Clark-type oxygen microelectrodes). Additionally, sulfide distribution in bottom waters during the same sampling period are shown (white diamonds, n=43). From depth distribution of oxygen and sulfide the distribution in (i) oxic, (ii) oxic-hypoxic, (iii) hypoxic-anoxic and (iv) anoxic-sulfidic zone was deduced.

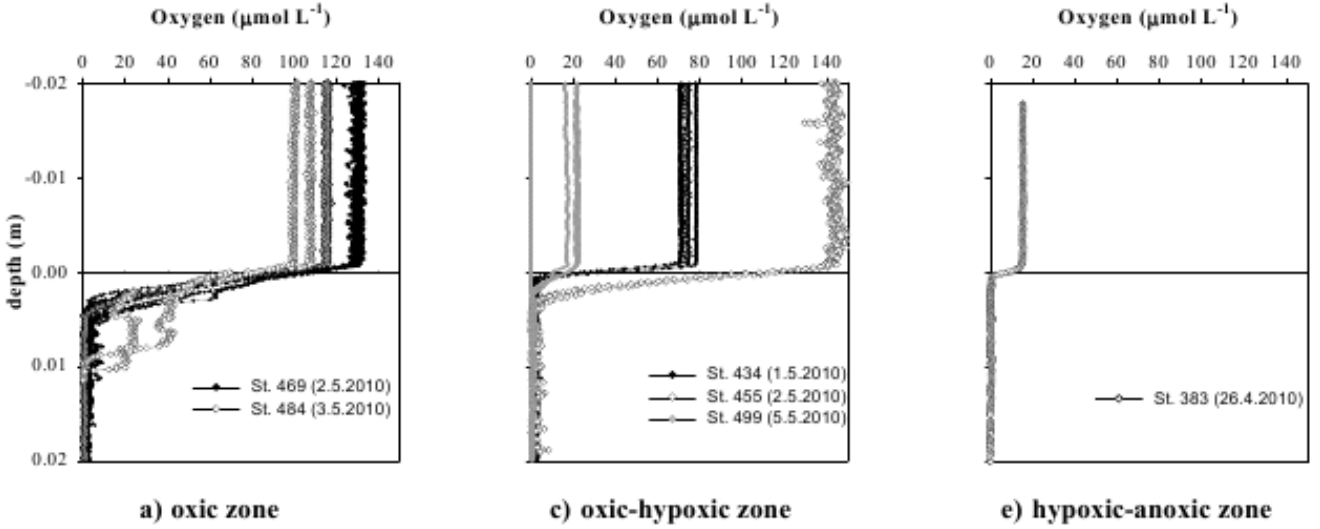


FIGURE 2.3: Examples of high-resolution oxygen profiles under different oxygen regimes. Differences in bottom water oxygen concentrations (reflected in profile shape and oxygen penetration depth) are clearly visible between sites and deployments.

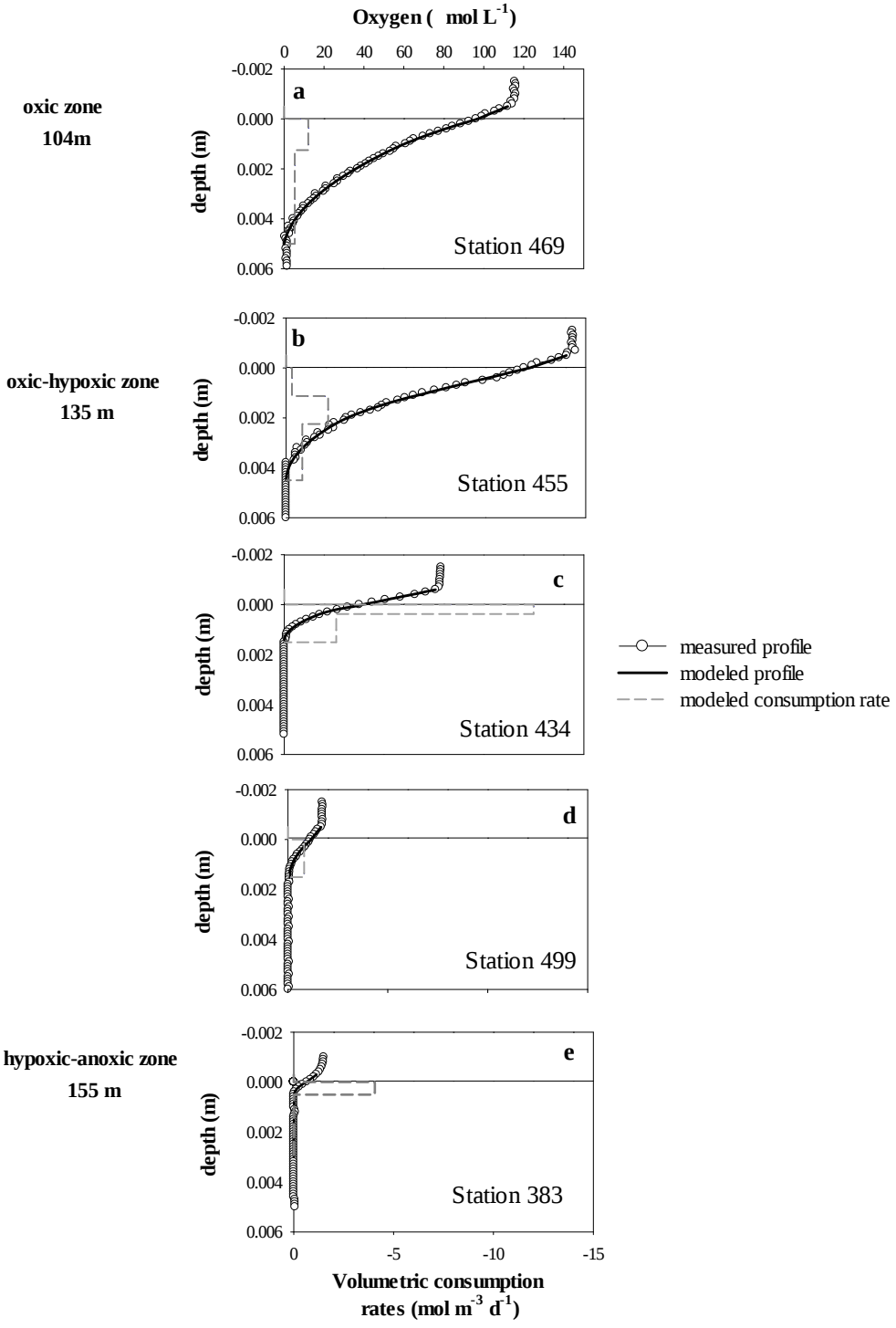


FIGURE 2.4: Examples of individual oxygen profiles measured in the sediment (white circles) and modeled with PROFILER (black lines). Volumetric rates are combined in discrete layers (dashed line) and exhibit different depths and degrees of oxygen consumption rates in different zones and under different bottom water oxygenation.

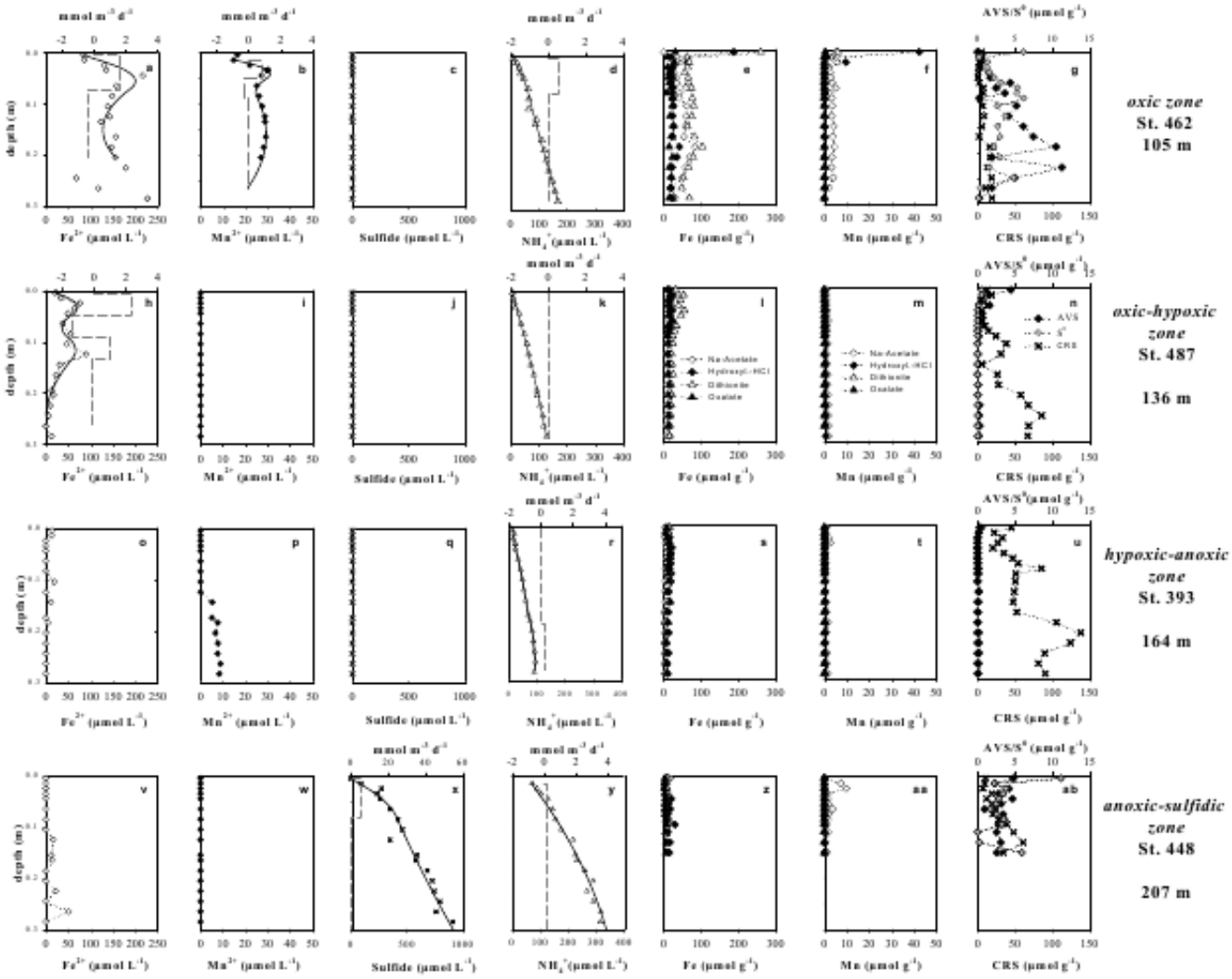


FIGURE 2.5: Distribution of reduced pore water species and oxidized and solid phase iron and sulfur species along the depth transect in the upper 30 cm of the sediment (symbols with dotted lines). Solid lines are the model results and dashed lines represent production and consumption rates.

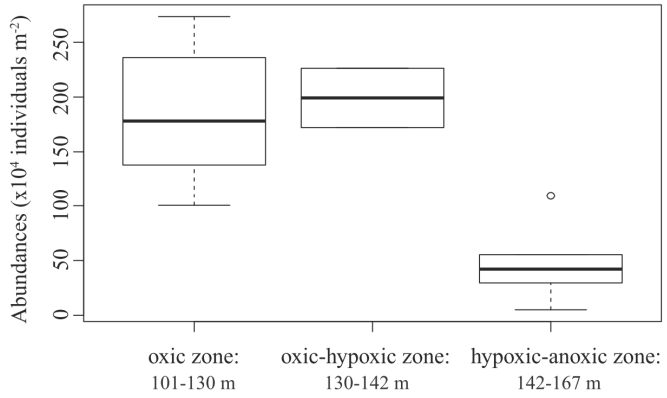


FIGURE 2.6: Abundance of meiobenthos in the upper five centimeter of the sediment under different oxygen regimes. The middle line in each box depicts the median, while both whiskers and outliers indicate the distribution of remaining data points.

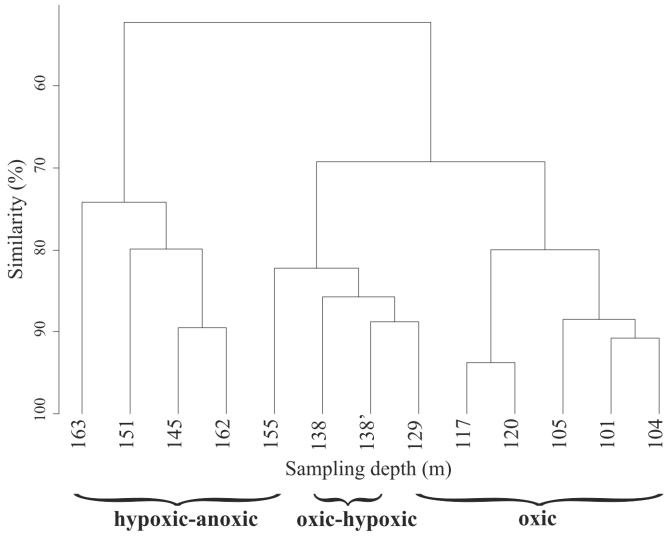


FIGURE 2.7: Cluster dendrogram of meiofauna abundances for different station depths based on the inverse of Bray–Curtis dissimilarity. Apostrophes denote stations with same depth.

Supplementary Tables and Figures

TABLE 2.5: Macrofauna composition and abundance per sampling depth (m) at the outer Western Crimean Shelf. Results were grouped according to bottom water oxygenation zones and integrated over the upper 5 cm. Apostrophe denotes replicate station within the same depth.

Taxa	101	104	105	117	120	129	138	138'	145	151	155	162	163
Ascidiacea	0.2	0	0	0	0	0	0	0	0	0	0	0	0
Bivalvia	0.1	0.4	0	0	0	0	0	0	0	0	0	0	0
Cnidaria	2.1	0.4	1.4	7.5	50.1	53.1	35.4	23.4	1.7	0	1.4	1	0
Gastropoda	0	0.4	0	0	0	0	0	0	0	0	0	0.2	0
Nemertini	0	0	0.5	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0.8	0.3	3.1	1.6	0.6	0.7	14.3	2.6	0.7	0	0	0	0.1
Polychaeta	1.6	0.8	1.7	0.7	0.1	0	1.7	0.3	0	0	0	0	0
Porifera	0.2	0	0	0	0	0	0	0	0	0	0	0	0
Σ	5	1.9	6.7	9.8	50.8	53.8	51.4	26.3	2.4	0	1.4	1.2	0.1
x10^3 individuals m^{-2} (average ±SD)				**21.3 ±24.1 oxic**			**38.9 ±17 oxic-hypoxic**				**1 ±1 hypoxic-anoxic**		

TABLE 2.6: Meiofauna composition and abundance (x10^4 individuals m^{-2}) per sampling depths (m) at the outer Western Crimean Shelf. Results were grouped according to bottom water oxygenation zones and integrated over the upper 5 cm. Apostrophe denotes replicate station within the same depth.

Taxa	101	104	105	117	120	129	138	138'	145	151	155	162	163
Acari	0	0.26	0	0	0.01	0.04	0.05	0	0	0	0	0	0
Amphipoda	0	0	0	0.02	0	0	0	0	0	0	0	0	0
Bivalvia	0.45	0.2	0.74	0.17	0.16	0.08	0.29	0.23	0.88	0	0.04	0.31	0.06
Ciliophora	1.52	0.16	3.08	0.81	1.52	4.91	0.79	3.55	3.49	12.41	1.02	3.32	0.97
Cnidaria	0.59	0.03	0.29	0.07	1.14	0.63	0.02	3.8	0.98	0.14	0	1.36	0.04
Forams hard shelled	0.25	0.09	0.05	0.05	0.16	7.39	13.39	15.42	2.49	0.24	0.23	1.05	0.01
Forams soft shelled	1.05	0.37	2.8	2.22	3.56	7.17	13.27	7.68	17.88	10.45	1.28	3.83	0.19
Gastropoda	0.01	0.03	0	0.02	0.01	0.04	0	0.01	0	0	0.01	0	0
Gromia	1.13	0.66	0.53	2.36	3.32	2.01	4.57	2.28	0.6	1.24	1.41	0.81	0
Harpacticoida	1.7	1.3	3.99	0.66	0.76	1.45	7.91	3.46	0.14	0	0.27	0.02	0.19
Kinorhyncha	0.41	0.12	0.62	0.11	0.32	0.05	0.17	0.01	0	0	0	0	0
Nauplia Decapoda	0.93	0.26	0.12	0	0	0.02	0	0.43	0	0	0	0	0
Nematoda	221.5	128.75	248.78	91.98	183.06	131.62	183.82	134.44	82.78	30.66	25.03	31.23	3.36
Nemertini	0	0.01	0	0	0	0	0	0.01	0	0	0	0	0
Oligochaeta juvenile	0.02	0.32	0	0	0.01	0.38	0.98	0.04	0	0	0	0	0
Ostracoda	2.88	2.8	6.74	0.05	0	0	0.14	0.01	0	0	0	0	0
Polychaeta	1.12	0.66	1.86	0.23	0.58	0.92	0.74	0.42	0.22	0.12	0.16	0.19	0.18
Tardigrada	1.11	0.57	1.55	0	0	0	0	0	0	0	0	0	0
Turbellaria	0.69	1.38	2.18	1.66	4.33	0.07	0.12	0.33	0.02	0.02	0.04	0	0
Others	0.65	0	0.05	0	0.51	0	0	0.04	0.02	0	0.03	0	0
Σ	**236.0**	**138.0**	**273.4**	**100.4**	**199.5**	**156.8**	**226.3**	**172.2**	**109.5**	**55.3**	**29.5**	**42.1**	**5.0**
x10^4 individuals m^{-2} (average ±SD)				**oxic**		**184 ±65**	**oxic-hypoxic**	**199 ±38**			**hypoxic-anoxic**		**48 ±39**

TABLE 2.7: Meiofauna community dissimilarity per sampling depths. Upper triangle: dissimilarity (based on Bray-Curtis), values closer to 1 represent high dissimilarity. Lower triangle: percentage of shared taxa. Colors depict oxygenation regimes, oxic (blue), oxic-hypoxic (pink), hypoxic-anoxic (red). Apostrophe denotes station within the same depth.

	101	104	105	117	120	129	138	138'	145	151	155	162	163
101		0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.2	0.3	0.4
104	89		0.1	0.2	0.2	0.3	0.3	0.3	0.4	0.5	0.3	0.4	0.4
105	88	78		0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.3
117	76	68	75		0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3
120	76	78	65	75		0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3
129	82	83	71	71	93		0.1	0.1	0.2	0.3	0.1	0.2	0.3
138	76	78	75	75	87	81		0.1	0.2	0.3	0.2	0.2	0.3
138'	88	89	76	76	76	82	76		0.2	0.3	0.2	0.2	0.3
145	62	56	71	71	71	67	71	62		0.2	0.2	0.1	0.3
151	50	44	57	57	57	53	57	50	80		0.3	0.2	0.3
155	62	56	60	71	71	67	60	62	82	64		0.2	0.3
162	56	50	64	64	64	60	64	56	90	70	73		0.2
163	50	44	57	57	57	53	57	50	80	60	64	89	

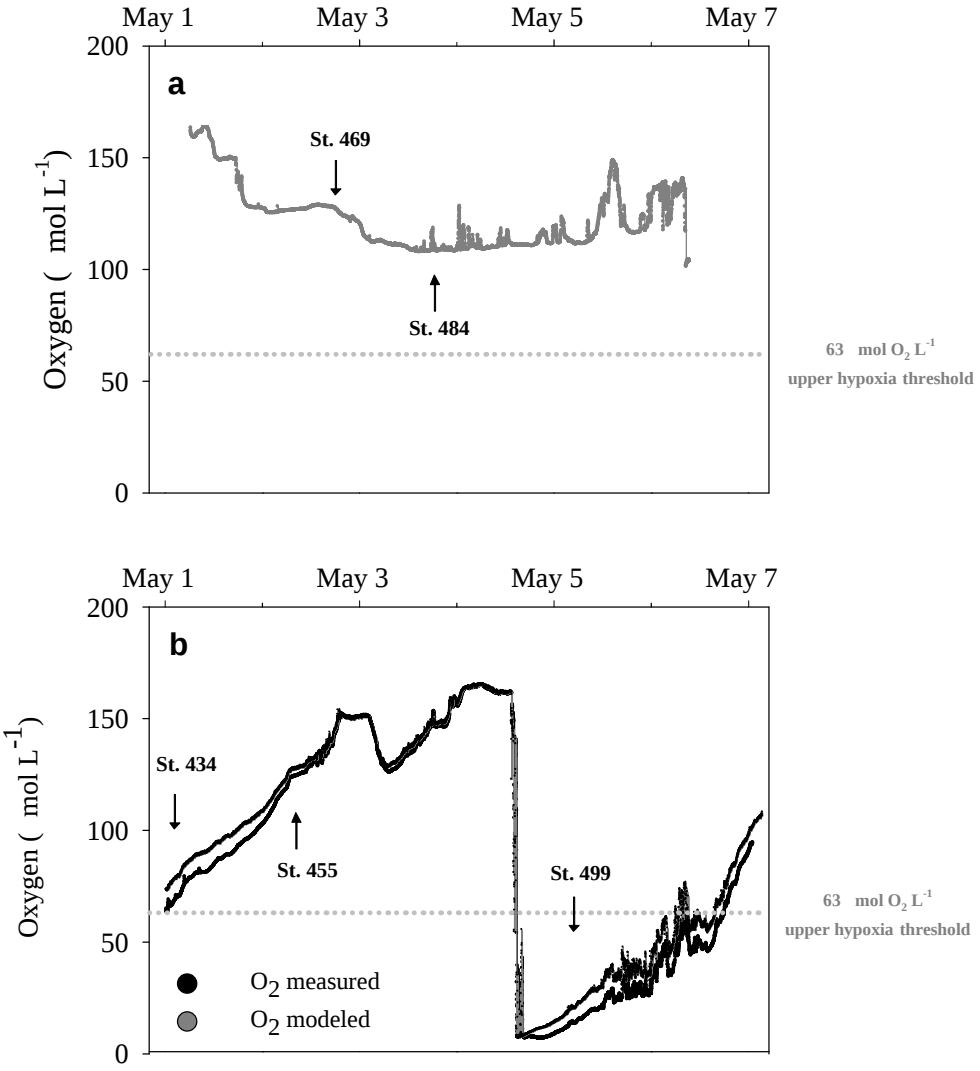


FIGURE 2.8: Stationary moorings with sensors measuring one meter above the sediment over a time period of 7 days; a) the oxygen concentration from the mooring at 100 m was modeled from recorded density data; bottom water was always oxic during the measurements, still strong variations (up to 60 μmol O_2 L^{-1}) were visible during the deployment time; b) at the mooring at 135 m water depth, measuring 1.5 m above the sediment, the water column oxygen concentration strongly varied between oxic and hypoxic conditions, dropping to nearly anoxic conditions on May 5^{th}. Time points where oxygen consumption was measured at these two water depths are indicated. The horizontal line indicates the conventional hypoxia threshold concentration of 63 μmol O_2 L^{-1}.

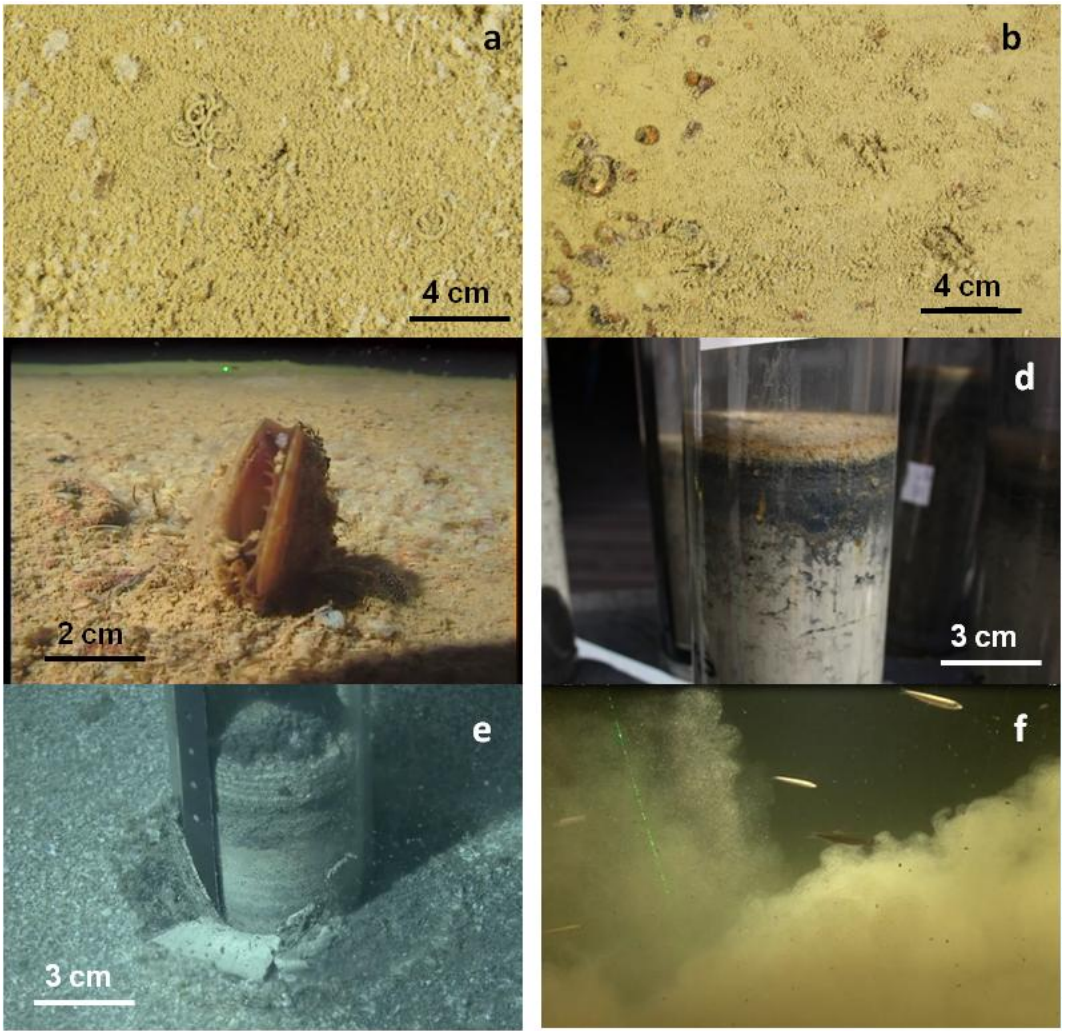


FIGURE 2.9: Images of seafloor and sediments; a) fecal structures on top of the sediment in the oxic zone, b) brown iron-encrusted shells in the oxic zone, c) living bivalve on top of the sediment in the oxic zone; d) vertical layering of the sediment with oxygenated sediment on top in the oxic zone; e) vertical layering of the sediment during coring, f) fish at >153 m and O_2 concentrations below 25 μmol L^{-1}; photographs are copyright JAGO-Team GEOMAR.

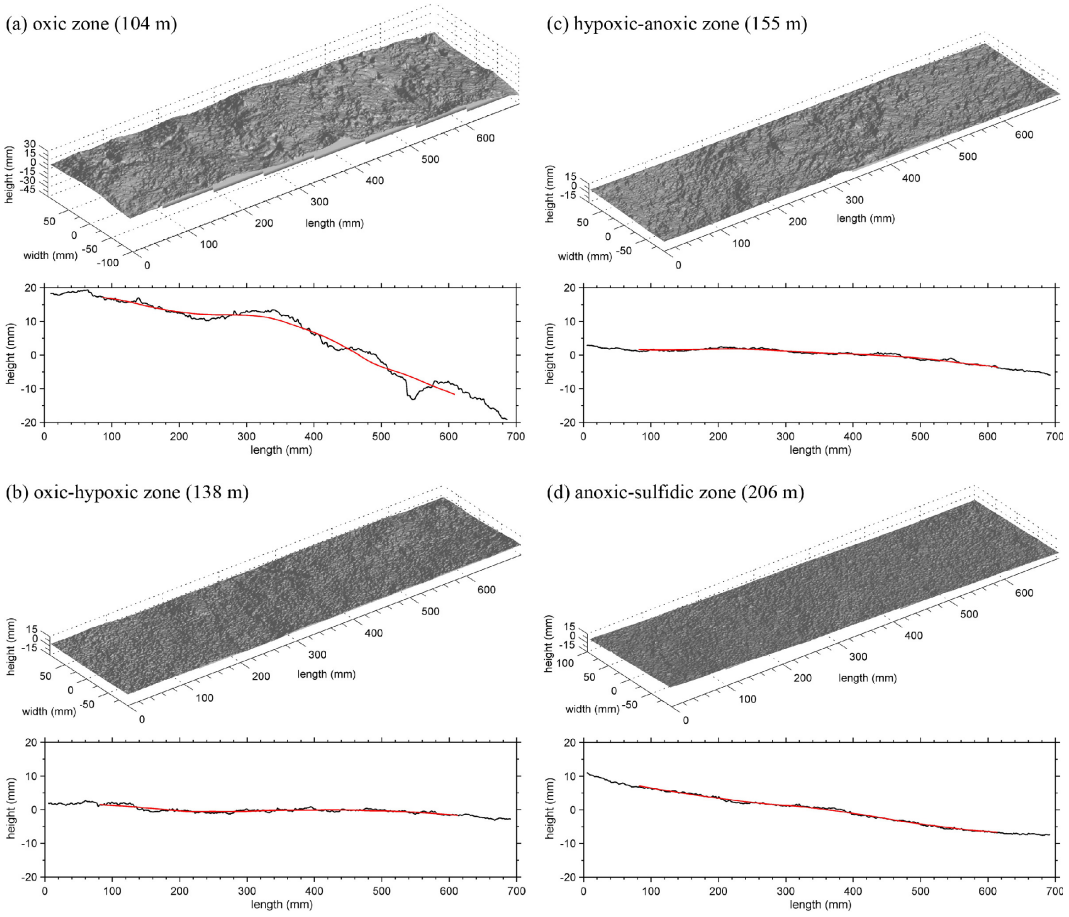


FIGURE 2.10: Shaded 3D surfaces showing examples of micro-topography measurements obtained at (a) 104, (b) 138, (c) 155, and (d) 206 m water depth. The 2D plots show topography profiles extracted along the center line of the respective surfaces (black line). The red line shows the running average of the same profile (155 mm averaging window). Deviations of the profile from profiles smoothed at different window sizes were used to compare roughness between stations (see section 2.3 and 3.5 for details)

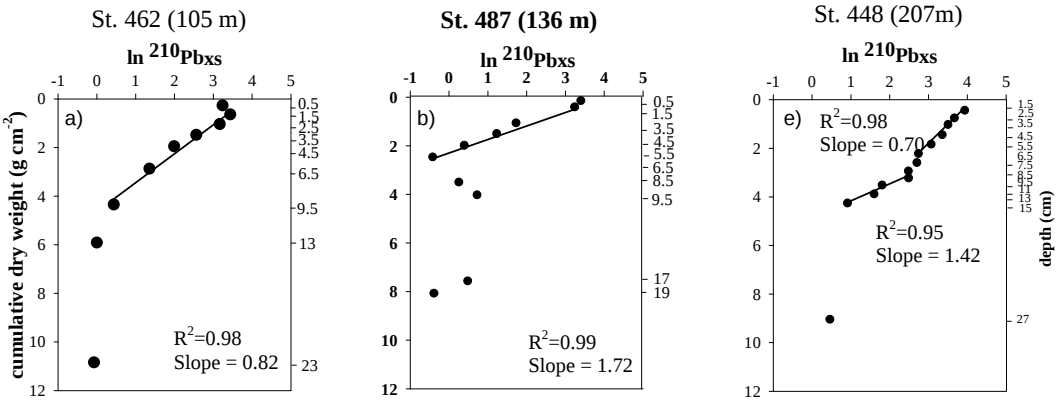


FIGURE 2.11: Profiles of excess ^{210}Pb activity, cumulative dry weight (left y-axis) and depth (right y-axis). Regression lines are plotted for data that was included in calculations of sedimentation rates.

Chapter 3

Benthic microbial communities and organic matter preservation associated with hypoxia

Gerdhard L. Jessen[1], Anna Lichtschlag[1,⋆], Silvio Pantoja[2], Alban Ramette[1,⋆⋆], Carsten J. Schubert[3], Ulrich Struck[4], Frank Wenzhöfer[1,5] and Antje Boetius[1,5].

[1] HGF MPG Research Group for Deep Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany.

[2] Department of Oceanography and COPAS Sur-Austral, University of Concepción, Concepción, Chile

[3] Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

[4] Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung Berlin, Germany

[5] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen, Bremerhaven, Germany

⋆ current address: National Oceanography Centre, Southampton, United Kingdom

⋆⋆ current address: Institute of Social and Preventive Medicine (ISPM) University of Bern

To be submitted to *Biogeosciences*

Abstract

The effect of varying oxygen conditions on organic matter reactivity and marine benthic microbial communities was investigated at the outer Western Crimean Shelf (Black Sea). Sampling was performed along an oceanographic transect subjected to oxygen concentrations between oxic (150 μM), variable (3-60 μM O_2) and anoxic to sulfidic conditions. Overall, more organic matter is degraded in surface sediments under permanent oxic regimes where bioturbation is highest. In contrast, variable hypoxic conditions already contribute to carbon accumulation by ca. 50% over the same sediment depth (time) interval, consistent with remineralization rates measured for the area. Towards anoxic conditions and in absence of bioturbation, the community of anaerobes requires ca. threefold the time to degrade the deposited material and reach similar background organic matter concentrations. However, a fraction escapes remineralization even up to millennial time scale, apparently, due to assemblages of fermenters and sulfate reducers which increase sulfide production and eventually decreasing the degradability of otherwise fresh organic matter. Our results suggest that variations in oxygen supply even on short time scales cause strong differences in the preservation of organic matter, because of (i) direct effects on benthic fauna composition and function, and (ii) indirect effects on bacterial community composition.

3.1 Introduction

Marine sediments preserve only <1% of primary produced organic matter due to remineralization by benthic fauna and microorganisms. However, globally and over geological time scales, this burial rate effects the global carbon and oxygen cycle. To better understand the factors governing preservation and degradation of organic matter in marine sediments is one of the key junctures between organic geochemistry and microbial ecology. One important factor apparently controlling burial and efficiency of organic carbon degradation is bottom water oxygen concentration (Emerson and Hedges, 1988; Canfield, 1994; Burdige, 2007; Arndt et al., 2013). It has been hypothesized that after the degradation of labile compounds occurs, in absence of oxygen, the remaining refractory matter accumulates, due to the slow and inefficient bacterial depolymerization of complex molecules (e.g. Kristensen et al., 1995). Even though microbial extracellular enzymatic degradation of organic matter does not require oxygen, some nonhydrolyzable bonds can be only cleaved trough highly reactive peroxide groups, or by oxygenases and peroxidases, therefore requiring oxygen as enzymatic co-factor (Canfield, 1994; Burdige, 2007 and references therein).

Previous investigations have compared different scenarios for the effects of oxygen limitation on degradation rates-oxic versus anoxic conditions or oscillations of both (e.g. Lee, 1992; Aller, 1994; Canfield, 1994; Sun et al., 2002). Several abiotic and biotic factors can cause oscillatory redox conditions in sediments. For example, variations in water coverage can alter oxygenation of intertidal sediments, events of high organic matter deposition rates can cause temporary oxygen depletion, and hydrographic transport phenomena can reduce the supply of oxygenated waters or cause the advection of low-oxygen waters (Aller, 1994; Moore, 2010). On the other hand, a preponderant effect on organic matter degradation efficiency is attributed to the presence of burrowing fauna that, by reworking surface sediments, can actively supply oxygen to anoxic sub layers (Aller, 1994; Meysman et al., 2006, Burdige, 2007).

Although the role of oxygen in carbon preservation and the relation between redox conditions and organic matter decomposition has been addressed in many previous studies, most have focused on effects of the geochemical composition and structure of buried matter, or on fauna. As a consequence of oxygen depletion, benthic fauna will emigrate or die, so that the microbial size spectrum dominates benthic energy fluxes (Diaz & Rosenberg, 2008). However, little is known if and in which ways organic matter degradation efficiencies depend on the structure and composition of the microbial community. In the case of the Black Sea, the presence or absence of oxygen (or hydrogen sulfide) has been proposed as main driver for the changes in microbial community structure and

function (Nikitine, 1925; Sorokin, 1964; Ross et al., 1970; Karl, 1978; Thamdrup et al., 2000).

The aim of this study was to assess the effects of varying oxygen conditions synchronously on organic matter reactivity and on structure and function of the benthic microbial communities at the Crimean shelf in the Black Sea. We examined an oceanographic transect subjected to varying oxygen concentrations between 0-140 μM along the outer Western Crimean shelf of the Black Sea (Holtappels et al., 2013). To test the hypothesis that variations in oxygen supply cause shifts in structure and function of microbial communities, sediment microbial communities were analyzed using high-throughput sequencing techniques. The distribution of sediment biogeochemical parameters and hydrolysable amino acids were measured to assess lability of organic matter and to determine links among oxygen supply, benthic microbial community structure, and organic matter reactivity.

3.2 Materials and methods

3.2.1 Study area

The Black Sea is a semi enclosed inland sea situated between western Asia and eastern Europe, it is the largest natural anoxic water body in the world. The permanent stratification of the water column define a permanent anoxic/sulfidic deep water mass and a ventilated oxic surface layer divided by a chemocline at ca. 100 m depth (Ross et al., 1970; Murray et al., 2007). The position of the chemocline is dome-shaped, being shallower in the central basin compared to the shelves (Stanev et al., 2014). Along the shelf the chemocline encounters the seafloor exposing its sediments to a dynamic range of oxygenation regimes from permanent oxic to variable and anoxic/sulfidic conditions (Fig. 3.1). In the outer Western Crimea shelf, even small variations in depth of the chemocline produce drastic changes in bottom-water oxygen availability at the seafloor, in the scale of days to hours (Friedrich et al., 2014).

3.2.2 Sampling

Sediments were collected by a video-guided multiple corer (TV-MUC, 96 mm inner diameter core tubes) along a ca. 40 lineal km oceanographic transect on the Crimean Shelf, during the MSM 15-1 expedition to the Black Sea (R/V Maria S. Merian, Crimean Leg; 25 April - 7 May 2010; Fig. 3.1). Seven stations were sampled across the Crimean

shelf (105 to 207-m water column depth). Before each TV-MUC deployment, a CTD-cast (SBE 911plus, with additional sensors for oxygen -SBE43- and fluorescence -Wetlab ECO-AFL/FL-) was obtained to characterize the water column. Additionally, bottom-water oxygen concentration was complemented with data from a benthic boundary layer profiler and submarine JAGO (Lichtschlag et al., 2015). Oxygen measurements from the CTD were calibrated by the Winkler titration (Winkler, 1888). Sediments with no visible signs of disturbance from recovery were processed for further analysis at in situ temperature of about 8°C. Sampling sites and respective labels are summarized in Table 3.1 and Fig 3.1.

3.2.3 Biogeochemical characterization of the sediments

Although a down core analysis was conducted, most of the following methodology and discussion is focused on the top centimeter with references to deeper horizons.

3.2.3.1 Bulk-sediment analysis

Samples for organic carbon and nitrogen, pigments (chlorophyll *a* and its degradation products; chloroplastic pigment equivalents, CPE) and total hydrolyzable amino acids (THAA) were measured in triplicate from freeze-dried and homogenized sediment retrieved from 3 different cores at stations 462, 487, 393 and 448 (Table 3.1) and from single cores at the remaining stations.

Sediment organic carbon and nitrogen were measured using a Leco CS 200 elemental analyzer (LECO, St. Joseph, MI, USA). For organic carbon determination samples were pre-treated with 12.5% HCl to remove carbonates. Chlorophyll *a* and CPE were measured spectrophotometrically according to Schubert et al. (2005). Total hydrolyzable amino acids (THAA) were measured after Pantoja and Lee (2003) from ca. 100 mg sediment samples. Briefly, after hydrolysis (6 N HCl at 105 °C, for 21 h under N_{2gas}), supernatant was removed and neutralized (6-0.1 N KOH). Amino acid identification and quantification were conducted by HPLC after pre-column derivatization with o-phthaldialdehyde and 2-mercaptoethanol (Lindroth and Mopper, 1979; Pantoja and Lee, 1999). Degradation index (DI) of organic matter, depicting selective diagenetic alteration of sedimentary amino acids, was used as a proxy of lability of organic matter (Dauwe and Middelburg, 1998; Dauwe et al., 1999).

3.2.4 Microbial community characterization

3.2.4.1 Cell counts

Number of cells in the sediments were determined in stations 462, 487, 393 and 448 (Table 3.1, Fig. 3.1) by the Acridine Orange Direct Count (AODC) method. Subsampled sediment were poisoned with 2% formaldehyde in seawater, stored at 4 °C and treated according to Velji and Albright (1986) and Boetius and Lochte (1996). Total cell numbers were determined by randomly counting at least 30 grids per filter (for two replicate filters).

3.2.4.2 DNA extraction

Total community DNA was assessed from 3 different cores at each station. On board, sediment was subsampled and stored at -20 °C for further analysis. Total DNA was extracted from ca. 1 g wet sediment using UltraClean Soil DNA Isolation Kits (Mo-Bio Laboratories Inc., Carlsbad, CA). Extracted DNA was quantified with a microplate spectrometer (Infinite® 200 PRO NanoQuant, TECAN Ltd, Switzerland) and its concentration adjusted for each step of the subsequent molecular protocol.

3.2.4.3 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

The bacterial community structure was determined by the ARISA fingerprinting method according to Fisher and Triplett (1999). Triplicate PCR reactions from standardized amounts of DNA (10 ng) from each sample were amplified using the bacteria forward FAM-labelled ITSF and reverse IT-SReub specific primers (Cardinale et al., 2004). All the following molecular protocol including the binning into operational taxonomic units (OTU) and data formatting was carried out as described previously (Ramette, 2009).

3.2.4.4 454 Massively Parallel Tag Sequencing (MPTS)

Extracted DNA was amplified and sequenced by the Research and Testing Laboratory (Lubbock Texas, USA). The V4-V6 region of the 16S rRNA genes were amplified using the bacterial primers 341F and 907R according to Klindworth et al., (2013). Fragments were sequenced following the 454 pyrosequencing protocol (Margulies et al., 2005) and Titanium reagent chemistry. The raw sequences and all the upstream workflow were conducted with “mothur” following the standard operating procedure (Schloss et al., 2009,

2011; including the implemented denoising algorithm). Taxonomic assignments were carried out using the SILVA reference file for bacteria (Pruesse et al., 2007; downloaded from http://www.mothur.org in September 2013) and clustered at a 97% identity level into operational taxonomic units ($OTU_{0.03}$). The dataset was normalized by the total amount of sequences per sample to get relative abundances. Singletons were treated according to Gobet et al., (2012) as follows; (i) an absolute singletons (SSO_{abs}) is an $OTU_{0.03}$ that occurred with only one sequence in the whole denoised dataset and (ii) a relative singletons (SSO_{rel}) is an $OTU_{0.03}$ with only one sequence in at least one sample, thus the total number of sequences for any SSO_{rel} was larger than one.

3.2.5 Statistical analysis

All the statistical analysis were conducted following Ramette (2007) others if necessary using the R package vegan (Oksanen et al., 2010) and performed in R (v. 3.0.1; http://www.R-project.org) using vegan and custom R scripts. All the diversity indexes for the 454 MPTS data were obtained with "mothur" (Schloss et al., 2009).

3.3 Results

3.3.1 Geochemical gradients related to oxygen content of bottom water

A continuous decrease in bottom water oxygen content was observed with increasing water depth below 120 m along a distance of ca. 40 km, sampled at 7 sites (Table 3.1). Bottom-water oxygen ranged from ca. 130 μM at 100 m to <1 μM (detection limit) deeper than 170 m. At this depth, the bottom-waters contained 3-10 μM H_2S (Table 3.1, Fig 3.1). Accordingly, we defined 4 different regions in terms of oxygen supply; (i) permanent oxic, (ii) variable oxic/hypoxic, (iii) variable anoxic/hypoxic and (iv) permanent anoxic with sulfide in bottom waters. The entire hypoxic zone comprised the range of 60-6 μM oxygen and spanned a distance of 10 km (Fig. 3.1).

The entire study area is characterized by a rather stable primary productivity of 220 g C m^{-2} yr^{-1} as estimated from ocean color algorithms (Grégoire and Friedrich, 2004). Assessing satellite data variation in chl a content of surface waters over 10 years, sea surface concentration of chl a fluctuated both spatially and temporally only within a concentration of ca. 0.4±0.1 mg m^{-3} (Fig. 3.4) letting us assume no major changes in organic matter input along the transect. Previous investigations show that approximately 1% of the primary produced OM is buried at the seafloor (Calvert et

al., 1991), with 1±0.5 mm yr^{-1} (Table 3.1), i.e. the top 1 cm of sediment represents a processing of matter over approximately 10 years (Lichtschlag et al., 2015).

Along the transect sampled, all sediments were of fine-grain muddy composition, and retrieved with the presence of a greenish fluff layer on top (∼0.5 cm). The porosity varied similarly along the transect, averaging 0.9±0.03 and 0.8±0.07 for the top cm and the uppermost 10 cm respectively (Lichtschlag et al., 2015). A clear change in coloration in surface sediments was observed from beige-brownish in the oxic zone, to dark grey in the hypoxic zone, and blackish towards anoxic conditions. The chemocline, as the boundary between oxidized and reduced bottom water and surficial sediments was detected visually at 150 m depth by the change in faunal traces and sediment color (Fig. 3.2). Oxygen penetration depth measured by microsensors reached up to 10 mm in sediments exposed to permanent oxic conditions decreasing to <0.5 mm towards hypoxic stations (Lichtschlag et al., 2015).

3.3.1.1 Pigments

Pigment contents in sediments increased from oxic to anoxic conditions (Table 3.1, Fig. 3.5). In surface sediments (top 1 cm) Chlorophyll *a* contents were ca. 10 μg g dw^{-1} at the shallowest oxic station, but more than 40 μg g dw^{-1} at the deepest anoxic site. When including the detrital phaeopigments (chloroplastic pigment equivalents CPE; Chl *a* + phaeopigments), a similar trend was observed, with the highest concentrations found under anoxic conditions (more than 90 μg gdw^{-1}). Chlorophyll *a* averaged 43±7.2% of CPE, with the highest percentage of 50% both at the oxic end member of the transect.

3.3.1.2 Other organic compounds and amino acid-based degradation index

Surface sedimentary organic carbon ranged between 2.6 and 5.3% dry weight in the top cm. The lowest content was found at the oxic site increasing toward anoxic conditions (Table 3.1, Fig. 3.2). Comparing oxygenation regimes, background C_{org} of ∼2% was reached at ca. 4 cm under permanent oxic to hypoxic conditions, whereas toward anoxic conditions this was already reached at ca. 3 cm (Fig. 3.2). At the chemocline (150 m water depth), values of more than 5% were found. C_{org}:N ratio showed low variability between the different zones, averaging 9.8±2.6. However, a substantial variation was detected in total hydrolysable amino acids (THAA), with the highest concentration under variable hypoxic conditions with >30 μmol THAA g dw^{-1}, and also increased values under anoxic conditions compared to the stable oxic stations.

The degradation index (DI) based on protein amino acids (Dauwe and Middelburg, 1998; Dauwe et al., 1999) averaged 1.4±0.4, and ranged from 0.3 to 1.5, with a steep increase along the transect from oxic to anoxic conditions (Table 3.1). The most degraded material (the lowest scores) was found at the sites exposed to oxygen, whereas in general the index of sites under anoxia indicated less degraded material.

3.3.2 Microbial community characterization

3.3.2.1 Total cell abundance

Cell abundance averaged $2.2\pm0.3\times10^9$ cells cm^{-3} sediment, ranging from 1.9 to 2.5×10^9 cells cm^{-3} (Table 3.1) and presenting slightly higher abundances in sub-surface sediments exposed to oxic compared to anoxic conditions (2-4 cm; 3 and 3.5×10^9 cells cm^{-3} respectively, data not shown).

3.3.2.2 Bacterial community structure based on ARISA fingerprinting

The total number of operational taxonomic units (OTUs) detected across all samples (sediment depth 0-1 cm) were 326, ranging between 172 and 247 along the transect (Table 3.2). Comparing the number of observed OTUs at different bottom water oxygenation conditions, the number of OTUs ranged from 161±24 to 186±9 (Table 3.3), and no significant difference in richness was found (ANOVA, P=0.25). Overall, ca. 60% of OTUs detected were present at all sites, and less than 13% OTU occurred only within one zone. The permanently oxic zone (Station 462) had no unique OTUs. Comparing all zones pairwise, >50% of the OTUs were shared, ranging between 51% and 76% (Table 3.2). In general, sites exposed to oxic conditions showed higher percentages of shared OTUs, with decreasing similarities towards low-no oxygen regimes (Table 3.3). When relative abundance of the OTUs detected was taken into consideration, the non-metric multidimensional scaling (NMDS) ordination plot (based on Bray-Curtis distance matrix) showed differences in the bacterial community structure, with the stations grouped according to the different oxygenation regimes (Figure 3.6). The analysis of similarity (ANOSIM) of the communities exposed to permanent oxic conditions showed significantly different community structure, compared to those at variable and stable anoxic conditions (Bonferroni corrected p value < 0.05, Table 3.4). Moreover, almost no overlap was observed between end member conditions (r value = 0.9, Table 3.4). Variation partitioning analysis performed showed that the bottom water oxygenation regime (11%, p<0.001) and total hydrolysable amino acids (11%, p<0.001) explained most of the community variation, whereas 3 and 5% accounted for organic carbon and the combined

effect of the three factors respectively. This suggest both oxygen and the availability of labile organic matter has an effect not only on the bacterial alpha diversity (OTU richness), but also on its community structure in response to hypoxia.

3.3.2.3 Bacterial community structure based on 454 MPTS

In total, 45,238 reads were retrieved from the 454 MPTS analysis, while 35783 were retrieved of surface sediments from selected sites representing each one oxygenation regime (stations 462, 487, 393 and 448, Table 3.5). These comprised 4670 individual OTUs (97% clustering, in the following referred to as $OTUs_{0.03}$) with ca. 50% singletons, the latter representing less than 6% of the total tag sequences and hence not further discussed here.

Two phyla clearly dominated the dataset: Bacteroidetes and Proteobacteria contained ca. 43 and 27% of the tags, respectively (Fig. 3.3). Of the 4,670 individual $OTUs_{0.03}$, 135 $OTUs_{0.03}$ were shared between all oxygenation regimes, depicting only ca. 3% of the individual $OTUs_{0.03}$, but representing more than 50% of all tags. From this subset of $OTUs_{0.03}$ more than 80% of the reads belonged to Bacteroidetes and Proteobacteria (41 and 42% respectively). At class level, *Deltaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria* were by far the most abundant bacterial classes representing ca. 60% of the total sequences, and up to 40% of those occurring in all zones.

As already indicated by ARISA; most $OTU_{0.03}$ occurring in the oxygenated zone also occurred in the other zones. Of the taxa dominating the oxygenated zone, but decreasing in sequence abundance with decreasing oxygen availability were *Caldilineae* and *Anaerolineae* (Fig. 3.3, Table 3.6).

Overall, the observed $OTUs_{0.03}$ number increased with decreasing concentration of oxygen in bottom waters (Table 3.5). This trend was explained by around 15% of the $OTU_{0.03}$ which increased with an $r>0.8$ in relative abundance towards anoxic conditions (Table 3.8). These comprised for example the *Deltaproteobacteria*, which increased from (15%) under permanent oxic conditions to 23-25% of the sequences under variable to anoxic conditions. Other bacterial taxa affiliated to *Flavobacteria* decreased from >28% under permanent oxic and anoxic conditions to <18% under variable oxygenation regimes.

Other groups reached the highest sequence abundances under variable hypoxic conditions, decreasing again towards stable anoxic conditions (Table 3.5). These included *Gammaproteobacteria* which relative abundances increased from <9% in case of permanent oxic and anoxic regimes, to 27% under variable hypoxic conditions. Its most abundant families were affiliated to JTB255 marine benthic group and *Alteromonadaceae*.

Generally, an increase in evenness was detected for permanent oxic and anoxic conditions (Inverse Simpson index; 1/D = 19 to 1/D=42), with an up to six-fold difference under variable hypoxic regimes (Inverse Simpson index; 1/D=116-128). Interestingly this trend was not only limited to the surficial sediments along the gradient between oxic and hypoxic regimes, but similar differences in richness and evenness were detected for the bacterial community inhabiting the top layer compared to deeper horizons of the sediment column (Table 3.5). Moreover when rare types were taken into consideration (based on Chao1 diversity index), the increase in richness with decrease in oxygen availability becomes more evident, indicating that rare members can take advantage under changing oxygenation regimes which is consistent with the less even distribution under such conditions.

3.4 Discussion

The key question addressed here is the relation between bacterial community structure and function to organic matter degradability in sediments under varying oxygen availability. Microbial degradation/mineralization and organic matter quality are tightly coupled. Its availability boost microbial oxygen consumption (Wenzhöfer and Glud, 2002; Glud, 2008; Fischer et al., 2009), sulfate reduction and production of ammonium (Pantoja and Lee, 2003; Schubert et al., 2005; Niggemann et al., 2007). Consistent with the often observed response of benthic microbial communities to the availability of fresh organic material (Moodley et al., 2002; Böer et al., 2009; Bienhold et al., 2012; Jacob et al., 2013). However, certain pre-and-post depositional conditions predominant in hypoxic ecosystems (e.g. sulfurization, absence of bioturbation), result in a decreased bioavailability even of high-quality organic matter (Moodley et al., 2011; Koho et al., 2013) that thus escapes rapid biological degradation. Under those circumstances the availability of oxygen plays a crucial role, not only by supplying an efficient electron acceptor or enzymatic co-factor, but also by indirectly influencing other organic matter degradation controlling factors.

The top centimeter of deposited sediment investigated here represents ca. 10 years of sedimentation (years 2000-2010; Lichtschlag et al. 2015), during which different microbial communities and diagenetic processes could have developed under contrasting redox conditions, but with a comparable input of fresh organic matter. Previous investigations allowed to separate the stations sampled into distinct zone of oxygen exposure, with the permanent oxic zone showing oxygen penetration depths down to ca. 10 mm, with clear signs of bioturbation, and the permanent anoxic zone showing a presence of reduced solid sulfide phases, active sulfate reduction, and no oxygen exposure (Lichtschlag et

al. 2015). Within the hypoxic zone, fluctuations in oxygen concentration and penetration depth were observed (120-30 μM, 4-2 mm), with substantial effects on both OM degradability and microbial community composition.

3.4.1 Hypoxia and organic matter preservation

Surface sediment organic carbon along the 40 km transect fell within the range described for surficial sediments of the Black Sea (Calvert et al., 1991; Cowie & Hedges, 1991; Weber et al., 2001) and other hypoxic ecosystem at similar water depths and sedimentation rates (140 m depth, Pakistan margin Oxygen Minimum Zone; Vandewiele et al., 2009). Contents of total hydrolysable amino acid (THAA; Table 3.1) agrees with data reported for surficial sediments of the Black Sea (e.g. Mopper et al., 1978) and the Oxygen Minimum Zone in the Chilean upwelling coast (Pantoja and Lee, 2003) at similar water depths. High values under variable or permanent hypoxia have been reported for the Pakistan margin Oxygen Minimum Zone (Vandewiele et al., 2009) and the Arabian Sea (Koho et al., 2013), interpreted as enhanced preservation of amino acids in sediments exposed to hypoxic conditions (Vandewiele et al., 2009). Surprisingly, surficial bulk organic carbon showed a difference only between the oxic stations compared to the other sites, indicating that even variable hypoxic conditions - i.e. phases of a few days of hypoxia - already contribute to carbon accumulation (Table 3.1, Fig. 3.2), here by ca. 50% over the same sediment depth (time) interval. Likewise, a previous study of oxygen consumption rates in the same area indicate that OM remineralization is slowed by 50-90% along the same transect, with an almost complete remineralization in the oxic zone ($\sim$15 mmol C m^{-2} d^{-1}), decreasing towards the hypoxic zone to $\sim$7 mmol C m^{-2} d^{-1}. In the anoxic zone, OM degradation depends on sulfate reduction rates in the absence of oxygen and nitrate and reached $\sim$7 mmol C m^{-2} d^{-1}. Clearly, the presence of fauna at the oxic zone accelerates OM degradation, being responsible for about 70% of total OM remineralization (Lichtschlag et al., 2015). Therefore, the presence of burrowing fauna appeared to have strong effects on the efficiency of organic matter degradation. Indeed, together with the oxygen influx pumped down to anoxic layers (Aller, 1994; Meysman et al., 2006, Burdige, 2007), benthic macrofauna can directly ingest up to 15% of detritus in sediments (Lopez and Levinton, 1987) and, through its digestive systems, break down complex molecules, making them available for the whole microbial community (Lee, 1992; Witte, et al., 2003).

Interestingly, comparing the decrease in C_{org} with sediment depth, background C_{org} values of 2% are reached at 4 cm sediment depth under oxic conditions, an at 3 cm sediment depth under permanent bottom-water anoxia (equivalent to 30 years of degradation) (Fig 3.2). This indicates that with time, and especially when stable anaerobic

conditions develop, the community of anaerobes has the potential to degrade the deposited material. However, oxic conditions exhibit lower background concentrations compared to sediments exposed to anoxic/sulfidic conditions (∼1.6 and ∼2.2% respectively). This suggests that a fraction escapes remineralization, even down to 70 cm sediment depth (Fig. 3.8), i.e. not decadal but millennial time scale. To further test the hypothesis of an influence of oxygen availability and potentially microbial community structure on the degradation rates and composition of OM, we assessed not only the bulk, but the pool of hydrolyzable amino acid of the organic matter and its preferential loss or accumulation due to remineralization. Following Dauwe et al., (1999), we calculate the degradation index (DI) in order to link biochemical composition of biogenic material and its degradation. As expected, the DI values along the transect showed the most degraded material (lowest scores) at the sites exposed to oxic conditions, whereas the index for sites under lower oxygenation regimes indicated in general less degraded material (Table 3.1), in agreement with other marine environments subjected to permanent or seasonal hypoxia and anoxia (e.g. Vandewiele et al., 2009; Koho et al., 2013). Although comparatively lower values have been reported for those areas (mostly negative scores), our results fell in the range described for coastal sediments (Dauwe et al., 1999 and references therein) and coastal areas exposed to seasonal oxygen minimum conditions at comparable depths (Pantoja and Lee, 2003). Additionally, from the amino acid dataset of Mopper et al., (1978) for Black Sea sediments exposed to permanent anoxic conditions, we calculated positive DI scores of 1.2 in agreement with our results.

Similarly, the pigments content ranged according to the oxygen minimum zone in the Chilean upwelling system (Gutiérrez et al., 2000) and the Pakistan margin (Woulds and Cowie, 2009), with a similar trend of lower concentrations under oxic regimes compared to anoxic ones. Thus under different oxygenation regimes, we found ca. two-fold increase in accumulation of chloroplastic compounds under variable compared to oxic conditions and up to three-fold increase comparing the oxic vs anoxic end members. In contrast to C_{org} (Fig. 3.1, Fig. 3.5, 3.7 and 3.8), a fraction of the THAA and chlorophyll pool apparently escapes from microbial degradation under permanent anoxic conditions. It has been previously suggested that chlorophyll and its degradation products will favorably accumulate in the sediments compared to oxic conditions above the chemocline (King, 1995). This can be explained by pre-depositional transformation of the organic matter (Moodley et al., 2011) not evidenced with the proxies here addressed. On this regard, the sulfurization (also referred as vulcanization) of lipids and carbohydrates through inorganic sulfur incorporation (Burdige, 2007 and references therein) have been proposed for carbon preservation in euxinic marine sediments. Among the sources for sulfur in the organic sulfur compounds are most likely pore water sulfide, polysulfides, and elemental sulfur (Hebting et al., 2006; Werne et al., 2008). The relatively high sulfate

reduction rates but low pore water sulfide measured (Table 3.1) suggest that reduced sulfur compounds are being sequestered into pyrite and/or to the organic matter pool (e.g. Burdige, 2007). Wakeham et al. (1995) previously showed for the Black Sea that this process can occur during very early stages of sedimentary diagenesis and even at the sediment-water interface.

The rather uniform microbial abundances along the transect (Table 3.1), suggest that the changes in organic matter degradation are not related to the abundance of microorganisms, but to its diversity and function which may be directly affected by bottom water oxygen concentration. Here we tested the hypothesis that bottom water hypoxia and anoxia affect bacterial community structure in surface sediments, and that this may affect OM degradability.

3.4.2 Role of benthic bacterial communities in OM degradation under varying oxygen availability

Shifts in benthic microbial community structure in margin sediments as a response to environmental conditions have been previously reported in the literature, mostly pointing to the availability of phytodetritus (Bienhold et al., 2012; Jacob et al. 2014) as a key factor controlling the structure of microbial communities. Here we compared patterns under the same productivity and deposition regime, but under varying availability of oxygen in bottom and pore waters.

Compared to the pelagic realm, less is known about benthic microbial community composition along oxygen gradients. However, pelagic and benthic microbial communities that populate these gradients appear to have a similar ecology (Fenchel and Finlay, 2008, Wright et al., 2012; Ulloa et al., 2013). Benthic microbial communities exposed to hypoxic, anoxic or sulfidic bottom waters, *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* are by far the most abundant bacterial clades (Julies et al., 2010; Quaiser et al., 2011; Köchling et al., 2011; Zinger et al., 2011; Julies et al., 2012; Liu et al., 2012; Reese et al., 2013; Sinkko et al., 2013). In general, *Gammaproteobacteria* (*Altermonadales*) decrease in abundance with sediment depth (and towards anoxic conditions), while *Deltaproteobacteria* (*Desulfobacterales*, *Desulfuromonadales*, and *Syntrophobacterales*) and *Chloroflexi* increase. On the other hand, *Bacteroidetes* (*Flavobacteria* and *Sphingobacteria*) are abundant at surface hypoxic sediments and also present in deeper horizons and anoxic bottom water conditions.

Regarding post-depositional organic matter processes, it has been proposed that the presence of benthic bottom dwelling fauna (bioturbation) enhances organic matter degradation, as an indirect effect of oxygen availability (e.g. Hunter et al., 2012). In our study

area, the effect of bioturbation/ventilation was evident in the overall higher OM degradation rate (Lichtschlag et al. 2015). Moreover, the degradation of chlorophyll *a* reached background concentrations already at the surface, confirming the positive effect of the mechanical disturbance of the sediments and ventilation (exchange) of metabolites and enzymes (Lee, 1992 and references therein). For the Black Sea, Cowie and Hedges (1991) highlighted the important role played by bioturbation in organic matter preservation. In the same fashion, Koho et al. (2013) proposed the lack of a complex macrofaunal community as a factor to consider for the decoupling between quality of organic matter and bioavailability in the sediments of Arabian Sea oxygen minimum zone.

Total cell counts did not show a change with varying bottom water oxygenation, however, the patterns of alpha (community richness) and beta diversity (e.g. how different components of the community change in response to environmental conditions) (Whittaker, 1972) differed substantially along the transect (Table 3.3, Table 3.6). A large fraction of taxa overlapped between all sites, and more than 80% of the total sequences retrieved belonged to *Bacteroidetes* and *Proteobacteria* (41 and 42% respectively), confirming their relevance and resilience to varying oxygen conditions.

Flavobacteria were by far the most abundant bacterial classes representing ca. 60% of the sequences (Fig 3.3), and showed a rather constant relative abundance throughout the transect. About 10% of the individual *Flavobacteria* $\text{OTUs}_{0.03}$ were shared among all oxygenation regimes, making up 70% of all *Flavobacteria* sequences, suggesting that this group might perform both fermentation and aerobic degradation of organic matter when oxygen is available. This is consistent with its global distribution, dominance in benthic coastal ecosystems (Zinger et al., 2011) and copiotrophic characteristics (Fierer et al., 2007), as well as the metabolic versatility of the clade (Bernardet et al., 2002 and references therein) and its use in sequential wastewater treatment process that uses anaerobic, anoxic, and oxic chambers (Kim et al., 2013). Moreover, members of this clade showed high abundances on organic rich sediments exposed to oxic and hypoxic conditions in the Baltic Sea (Sinkko et al., 2013) and Namibian Shelf (*Bacteroidetes*; Julies et al., 2012) representing in this ecosystem an important fraction also in anoxic sediments. In experimental setups and cultivation approaches members of this clade appeared able to switch from fermentative to aerobic degradation of organic carbon (Köpke et al., 2005; McKew et al., 2013).

Members of the *Flavobacteria* clade are typically heterotrophic bacteria with hydrolytic and fermentative properties, able to conduct the breakage of a wide range of complex polymers, including proteins and chitin to more refractory terrestrial material like cellulose (Cottrell and Kirchman, 2000; Bernardet et al., 2002 and references therein; Lydell

et al., 2004; Julies et al., 2010). *Gammaproteobacteria* which represent the most abundant class under variable oxic conditions, its relative abundances decreased from 27 to 15% compared to variable anoxic conditions and <9% in case of permanent oxic and anoxic regimes. Its most abundant families along the transect were affiliated to JTB255 marine benthic group and *Alteromonadaceae*. Their cultivated strains are typically chemo-organotrophic bacteria (Ivanova and Mikhailov, 2001 and references therein; Zhou et al., 2008; Losey et al., 2013; Tindall, 2014). In case of JTB255 marine benthic group, Vandieken et al., (2012) reported this group as ubiquitously distributed among manganese oxide-rich sediments. For *Alteromonadaceae* most of the sequences were grouped within the NOR5/OM60 clade, which members can perform anoxygenic phototrophy and have a potential for thiosulfate oxidation (Fuchs et al., 2007; Huggett and Rappé, 2012 and references therein).

Of the abundant bacterial groups, none declined significantly in sequence abundance from the oxic-hypoxic zones towards the anoxic zone. On the contrary, the group of sulfate reducing bacteria increased (Table 3.8), and potentially have a main role in OM degradation under permanent anoxia. Among the Deltaproteobacteria the most abundant families were affiliated to the sulfate/sulfur reducers *Desulfobacteraceae* and *Desulfobulbaceae* (Muyzer and Stams, 2008). Although cultivated strains affiliated to *Desulfobulbaceae* can grow chemolithoautotrophically exclusively by the disproportionation of inorganic sulfur compounds (e.g. Finster et al., 1998), it has been shown that these two groups can dominate the degradation of fermentation products through sulfate reduction in anoxic incubations (McKew et al., 2013). Indeed, a substantial increase in richness and evenness was observed towards anoxic conditions, both along the transect and downcore (Table 3.5) followed by high abundances of sequences affiliated to *Desulfobacterales* (Fig. 3.3). It has been shown in long term experiments on the microbial degradation of refractory organic matter, that the most abundant *Deltaproteobacteria* sequences were affiliated to *Desulfobacteraceae* and *Desulfobulbaceae* (Reimers et al., 2013). For these experimental conditions was proposed that these groups may profit from fermenting bacteria of the *Clostridia* and/or *Bacteroidetes*, both abundant clades in our dataset (Fig 3.3). Similar trends have been reported in coastal sediments exposed to oxic and hypoxic conditions (Reese et al., 2013; Sinkko et al., 2013) and also after long term microbial fuel cell experiments under elevated and oscillatory redox conditions (Reimers et al., 2013). This pattern can be explained by the obligate anaerobic nature of the clade composed mainly by sulfate/sulfur reducers (Muyzer and Stams, 2008), consistent with the dominance of these families of sulfate/sulfur reducers; *Desulfobacteraceae* and *Desulfobulbaceae*, corresponding to the highest rates of sulfur reduction here detected (Table 3.1). Thus, towards anoxic conditions sulfate reduction become

the dominant mineralization process (Table 3.1), in agreement with previous investigations at the Black Sea (Thamdrup et al., 2000; Jørgensen et al., 2001; Weber et al., 2001) and organic rich coastal sediments subjected to hypoxia (e.g. Thamdrup and Canfield, 1996). It was previously speculated that the higher organic matter preservation under anoxic conditions, results from an inhibition of the hydrolytic capacities towards anoxic/sulfidic conditions (Hoppe et al., 1990) potentially related to pre-depositional transformation of the organic matter, or to a switch in bacterial communities with altered hydrolytic potential. Schmidt et al., (2014), showed that sulfurization of organic matter also occurs in its dissolve fraction and may play a role in the preservation of organic matter towards hypoxic/sulfidic conditions. We did not observe a substantial decline of any group of bacteria towards hypoxic or anoxic conditions, but an increase of sulfide-production by sulfate reducing bacteria, potentially affecting the degradability of at least some fractions of the deposited OM, confirming the hypothesis of Schmidt et al., (2014).

3.5 Conclusions

A key question is as to the effects of deoxygenation and the expanding OMZs on microbial community structure and function (Beman and Carolan, 2013). To explain the changes in diversity of bacteria across varying redox conditions, concurrent hypothesis emerging from pelagic (Madrid et al., 2001; Ulloa et al., 2012; Wright et al., 2012) and benthic (Fenchel and Finlay, 2008; Lasher et al., 2009) ecosystems, agree that higher microbial diversity in step redox gradients and anaerobic environments scale with the dynamic presence of several electron donors/acceptors and metabolic pathways, enhancing the number of niches. Bacterial taxa such as *Flavobacteria* are able to adapt to varying oxygen availability. However, periods of hypoxia and anoxia repress bioturbation and favor assemblages of fermenters and sulfate reducers which increase sulfide production, eventually decreasing the degradability of organic matter. Hence, our results show that variations in oxygen supply even on short time scales cause strong differences in the preservation of organic matter, because of direct effects on benthic fauna composition and function, and indirect effects on bacterial community composition.

3.6 Acknowledgments

We thank the officers, crew and shipboard scientific party of RV Maria S. Merian for support at sea during the expedition MSM 15-1. We would also like to acknowledge Axel

Nordhausen, Daphne Donis, Wiebke and Rafael Stiens, Martina Alisch, Erika Weiz-Bersch and Mirja Meiners for their assistance on board and in laboratory. Lilian Nuñez and Paulina Concha are acknowledge for their help on amino acid analysis and COPAS Sur-Austral (PFB-31) for additional funding on this regard. This study was financially supported by the EU 7^{th} FP project HYPOX (In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies) EC Grant 226213 and the Leibniz program of the DFG to AB, as well as the Max Planck Society for additional support.

3.7 References

Aller, R. C.: Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation, Chem. Geol., 114(3-4), 331-345, doi:10.1016/0009-2541(94)90062-0, 1994.

Arndt, S., Jørgensen, B. B., LaRowe, D. E., Middelburg, J. J., Pancost, R. D. and Regnier, P.: Quantifying the degradation of organic matter in marine sediments: A review and synthesis, Earth-Science Rev., 123, 53-86, doi:10.1016/j.earscirev.2013.02.008, 2013.

Beman, J. M. and Carolan, M. T.: Deoxygenation alters bacterial diversity and community composition in the ocean's largest oxygen minimum zone., Nat. Commun., 4, 2705, doi:10.1038/ncomms3705, 2013.

Bernardet, J., Nakagawa, Y. and Holmes, B.: Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family, Int. J. Syst. Evol. Microbiol., 52(3), 1049-1070, doi:10.1099/ijs.0.02136-0, 2002.

Bienhold, C., Boetius, A. and Ramette, A.: The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments., ISME J., 6(4), 724-32, doi:10.1038/ismej.2011.140, 2012.

Böer, S. I., Hedtkamp, S. I. C., van Beusekom, J. E. E., Fuhrman, J. a, Boetius, A. and Ramette, A.: Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sands., ISME J., 3(7), 780-91, doi:10.1038/ismej.2009.29, 2009.

Boetius, A. and Lochte, K.: Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea sediments, Mar. Ecol. Prog. Ser., 140, 239-250, doi:10.3354/meps140239, 1996.

Burdige, D. J.: Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets?, Chem. Rev., 107(2), 467-85, doi:10.1021/cr050347q, 2007.

Calvert, S. E., Karlin, R. E., Toolin, L. J., Donahue, D. J., Southon, J. R. and Vogel, J. S.: Low organic carbon accumulation rates in Black Sea sediments, Nature, 350(6320), 692-695, doi:10.1038/350692a0, 1991.

Canfield, D. E.: Factors influencing organic carbon preservation in marine sediments., Chem. Geol., 114(93), 315-29, 1994.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A. M., Rizzi, A., Sorlini, C., Corselli, C., Zanardini, E. and Daffonchio, D.: Comparison of Different Primer Sets for

Use in Automated Ribosomal Intergenic Spacer Analysis of Complex Bacterial Communities Comparison of Different Primer Sets for Use in Automated Ribosomal Intergenic Spacer Analysis of Complex Bacterial Communities, , doi:10.1128/AEM.70.10.6147, 2004.

Cottrell, M. T. and Kirchman, D. L.: Natural Assemblages of Marine Proteobacteria and Members of the Cytophaga-Flavobacter Cluster Consuming Low- and High-Molecular-Weight Dissolved Organic Matter, Appl. Environ. Microbiol., 66(4), 1692-1697, doi:10.1128/AEM.66.4.1692-1697.2000, 2000.

Cowie, G. L. and Hedges, J. I.: Organic carbon and nitrogen geochemistry of Black Sea surface sediments from stations spanning the oxic:anoxic boundary, in Black Sea Oceanography, edited by E. İzdar and J. W. Murray, pp. 343-359, Springer Netherlands., 1991.

Dauwe, B. and Middelburg, J.J. (1998) Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol. Oceanogr. 43: 782–798.

Dauwe, B., Middelburg, J. J., Herman, P. M. J. and Heip, C. H. R.: Linking diagenetic alteration of amino acids and bulk organic matter reactivity, Limnol. Oceanogr., 44(7), 1809-1814, doi:10.4319/lo.1999.44.7.1809, 1999.

Diaz, R. J. and Rosenberg, R.: Spreading dead zones and consequences for marine ecosystems., Science, 321(5891), 926-929, doi:10.1126/science.1156401, 2008.

Emerson, S. and Hedges, J. I.: Processes controlling the organic carbon content of open ocean sediments, Paleoceanography, 3(5), 621-634, doi:10.1029/PA003i005p00621, 1988.

Fenchel, T. M. and Finlay, B. J.: Oxygen and the spatial structure of microbial communities., Biol. Rev. Camb. Philos. Soc., 83(4), 553-69, doi:10.1111/j.1469-185X.2008.00054.x, 2008.

Fierer, N., Bradford, M. a and Jackson, R. B.: Toward an ecological classification of soil bacteria., Ecology, 88(6), 1354-64, 2007.

Finster, K., Liesack, W. and Thamdrup, B. O.: Elemental Sulfur and Thiosulfate Disproportionation by Desulfocapsa sulfoexigens sp. nov ., a New Anaerobic Bacterium Isolated from Marine Surface Sediment Elemental Sulfur and Thiosulfate Disproportionation by Desulfocapsa sulfoexigens sp . nov ., a New, Appl. Environ. Microbiol., 64(1), 119-125, 1998.

Fischer, J. P., Ferdelman, T. G., D'Hondt, S., Røy, H. and Wenzhöfer, F.: Oxygen penetration deep into the sediment of the South Pacific gyre, Biogeosciences, 6(8), 1467-1478, doi:10.5194/bg-6-1467-2009, 2009.

Fisher, M.M. and Triplett, E.W.: Automated Approach for Ribosomal Intergenic Spacer Analysis of Microbial Diversity and Its Application to Freshwater Bacterial Communities. Appl. Environ. Microbiol. 65: 4630–4636, 1999.

Friedrich, J., Janssen, F., Aleynik, D., Bange, H. W., Boltacheva, N., Çagatay, M. N., Dale, a. W., Etiope, G., Erdem, Z., Geraga, M., Gilli, A., Gomoiu, M. T., Hall, P. O. J., Hansson, D., He, Y., Holtappels, M., Kirf, M. K., Kononets, M., Konovalov, S., Lichtschlag, A., Livingstone, D. M., Marinaro, G., Mazlumyan, S., Naeher, S., North, R. P., Papatheodorou, G., Pfannkuche, O., Prien, R., Rehder, G., Schubert, C. J., Soltwedel, T., Sommer, S., Stahl, H., Stanev, E. V., Teaca, A., Tengberg, A., Waldmann, C., Wehrli, B. and Wenzhöfer, F.: Investigating hypoxia in aquatic environments: diverse approaches to addressing a complex phenomenon, Biogeosciences, 11(4), 1215-1259, doi:10.5194/bg-11-1215-2014, 2014.

Fuchs, B. M., Spring, S., Teeling, H., Quast, C., Wulf, J., Schattenhofer, M., Yan, S., Ferriera, S., Johnson, J., Glöckner, F. O. and Amann, R.: Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis., Proc. Natl. Acad. Sci. U. S. A., 104(8), 2891-6, doi:10.1073/pnas.0608046104, 2007.

Gobet, A., Böer, S. I., Huse, S. M., van Beusekom, J. E. E., Quince, C., Sogin, M. L., Boetius, A. and Ramette, A.: Diversity and dynamics of rare and of resident bacterial populations in coastal sands., ISME J., 6(3), 542-53, doi:10.1038/ismej.2011.132, 2012.

Grégoire, M. and Friedrich, J.: Nitrogen budget of the northwestern Black Sea shelf inferred from modeling studies and in situ benthic measurements, Mar. Ecol. Prog. Ser., 270, 15-39, doi:10.3354/meps270015, 2004.

Grote, J., Jost, G., Labrenz, M., Herndl, G. J. and Jürgens, K.: Epsilonproteobacteria represent the major portion of chemoautotrophic bacteria in sulfidic waters of pelagic redoxclines of the Baltic and Black Seas., Appl. Environ. Microbiol., 74(24), 7546-51, doi:10.1128/AEM.01186-08, 2008.

Gutiérrez, D., Gallardo, V. A., Mayor, S., Neira, C., Vásquez, C., Sellanes, J., Rivas, M., Soto, A., Carrasco, F. and Baltazar, M.: Effects of dissolved oxygen and fresh organic matter on the bioturbation potential of macrofauna in sublittoral sediments off Central Chile during the 1997 / 1998 El Niño, Mar. Ecol. Prog. Ser., 202(Smith 1992), 81-99, 2000.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J. and Andersson, A. F.: Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea., ISME J., 5(10), 1571-9, doi:10.1038/ismej.2011.41, 2011.

Holtappels, M., Glud, R. N., Donis, D., Liu, B., Hume, A., Wenzhöfer, F. and Kuypers, M. M. M.: Effects of transient bottom water currents and oxygen concentrations on benthic exchange rates as assessed by eddy correlation measurements, J. Geophys. Res. Ocean., 118(3), 1157-1169, doi:10.1002/jgrc.20112, 2013.

Hoppe, H. G., Gocke, K. and Kuparinen, J.: Effect of H2S on heterotrophic substrate uptake, extracellular enzyme activity and growth of brackish water bacteria, Mar. Ecol. Prog. Ser., 64, 157-167, 1990.

Huggett, M. J. and Rappé, M. S.: Genome sequence of strain HIMB55, a novel marine gammaproteobacterium of the OM60/NOR5 clade., J. Bacteriol., 194(9), 2393-4, doi:10.1128/JB.00171-12, 2012.

Hunter, W. R., Veuger, B. and Witte, U.: Macrofauna regulate heterotrophic bacterial carbon and nitrogen incorporation in low-oxygen sediments., ISME J., 6(11), 2140-51, doi:10.1038/ismej.2012.44, 2012.

Ivanova, E. P. and Mikhailov, V. V: A New Family, Alteromonadaceae fam. nov., Including Marine Proteobacteria of the Genera Alteromonas, Pseudoalteromonas , Idiomarina, and Colwellia, Microbiology, 70(1), 10-17, 2001.

Jacob, M., Soltwedel, T., Boetius, A. and Ramette, A.: Biogeography of Deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic)., PLoS One, 8(9), e72779, doi:10.1371/journal.pone.0072779, 2013.

Jøørgensen, B. B., Weber, A. and Zop, J.: Sulfate reduction and anaerobic methane oxidation in Black Sea sediments, Deep Sea Res. Part I Oceanogr. Res. Pap., 48, 2097-2120, 2001.

Julies, E. M., Fuchs, B. M., Arnosti, C. and Brüchert, V.: Organic Carbon Degradation in Anoxic Organic-Rich Shelf Sediments: Biogeochemical Rates and Microbial Abundance, Geomicrobiol. J., 27(4), 303-314, doi:10.1080/01490450903410464, 2010.

Julies EM, Brüchert V, Fuchs BM, Planck M, Microbiology M. (2012). Vertical shifts in the microbial community structure of organic-rich Namibian shelf sediments. African J Microbiol Res 6:3887-3897.

Karl, D. M.: Distribution, abundance, and metabolic states of microorganisms in the water column and sediments of the Black Sea, Limnol. Oceanogr., 23(5), 936-949, doi:10.4319/lo.1978.23.5.0936, 1978.

Kim, B.-C., Kim, S., Shin, T., Kim, H. and Sang, B.-I.: Comparison of the bacterial communities in anaerobic, anoxic, and oxic chambers of a pilot A(2)O process using pyrosequencing analysis., Curr. Microbiol., 66(6), 555-65, doi:10.1007/s00284-013-0311-z, 2013.

King, L. L.: A mass balance of chlorophyll degradation product accumulation in Black Sea sediments, Deep Sea Res. Part I Oceanogr. Res. Pap., 42(6), 919-942, doi:10.1016/0967-0637(95)00029-6, 1995.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F. O.: Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies., Nucleic Acids Res., 41(1), e1, doi:10.1093/nar/gks808, 2013.

Köchling, T., Lara-martín, P., González-mazo, E., Amils, R. and Sanz, J. L.: Microbial community composition of anoxic marine sediments in the Bay of Cádiz (Spain), Int. Microbiol., 14, 143-154, doi:10.2436/20.1501.01.143, 2011.

Koho, K. a., Nierop, K. G. J., Moodley, L., Middelburg, J. J., Pozzato, L., Soetaert, K., van der Plicht, J. and Reichart, G.-J.: Microbial bioavailability regulates organic matter preservation in marine sediments, Biogeosciences, 10(2), 1131-1141, doi:10.5194/bg-10-1131-2013, 2013.

Köpke, B., Wilms, R., Engelen, B., Sass, H. and Cypionka, H.: Microbial Diversity in Coastal Subsurface Sediments: a Cultivation Approach Using Various Electron Acceptors and Substrate Gradients, , doi:10.1128/AEM.71.12.7819, 2005.

Kristensen, E., Ahmed, S. I. and Devol, A. H.: Aerobic and Anaerobic Decomposition of Organic Matter in Marine Sediment: Which is Fastest?, Limnol. Oceanogr., 40(8), 1430-1437, 1995.

Lasher, C., Dyszynski, G., Everett, K., Edmonds, J., Ye, W., Sheldon, W., Wang, S., Joye, S. B., Moran, M. A. and Whitman, W. B.: The diverse bacterial community in intertidal, anaerobic sediments at Sapelo Island, Georgia., Microb. Ecol., 58(2), 244-61, doi:10.1007/s00248-008-9481-9, 2009.

Lee, C.: Controls on organic carbon preservation: The use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems, Geochim. Cosmochim. Acta, 56, 3323-3335, 1992.

Lichtschlag, A., Donis, D., Janssen, F., Jessen, G. L., Holtappels, M., Wenzhöfer, F., Mazulmyan, S., Sergeeva, N., Waldmann, C. and Boetius, A.: Effects of fluctuating hypoxia on benthic oxygen consumption in the Black Sea (Crimean Shelf), Biogeosciences Discuss., 12(8), 6445-6488, doi:10.5194/bgd-12-6445-2015, 2015.

Lindroth, P. and Mopper, K.: High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde, Anal. Chem., 51, 1667-1674, 1979.

Liu, M., Xiao, T., Wu, Y., Zhou, F., Huang, H., Bao, S. and Zhang, W.: Temporal distribution of bacterial community structure in the Changjiang Estuary hypoxia area and the adjacent East China Sea, Environ. Res. Lett., 7(2), 025001, doi:10.1088/1748-9326/7/2/025001, 2012.

Lopez, G. R. and Levinton, J. S.: Ecoloay of denosit-feeding animals in marine sediments, Q. Rev. Biol., 62, 235-260, 1987.

Losey, N. a, Stevenson, B. S., Verbarg, S., Rudd, S., Moore, E. R. B. and Lawson, P. a: Fontimonas thermophila gen. nov., sp. nov., a moderately thermophilic bacterium isolated from a freshwater hot spring, and proposal of Solimonadaceae fam. nov. to replace Sinobacteraceae Zhou et al. 2008., Int. J. Syst. Evol. Microbiol., 63(Pt 1), 254-9, doi:10.1099/ijs.0.037127-0, 2013.

Lydell, C., Dowell, L., Sikaroodi, M., Gillevet, P. and Emerson, D.: A population survey of members of the phylum Bacteroidetes isolated from salt marsh sediments along the east coast of the United States., Microb. Ecol., 48(2), 263-73, doi:10.1007/s00248-003-1068-x, 2004.

Madrid, V. M., Taylor, G. T., Scranton, M. I. and Chistoserdov, A. Y.: Phylogenetic Diversity of Bacterial and Archaeal Communities in the Anoxic Zone of the Cariaco Basin Phylogenetic Diversity of Bacterial and Archaeal Communities in the Anoxic Zone of the Cariaco Basin † Downloaded from http://aem.asm.org/ on February 19 , , doi:10.1128/AEM.67.4.1663, 2001.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. a, Berka, J., Braverman, M. S., Chen, Y.-J., Chen, Z., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V, Godwin, B. C., He, W., Helgesen, S., Ho, C. H., Ho, C. H., Irzyk, G. P., Jando, S. C., Alenquer, M. L. I., Jarvie, T. P., Jirage, K. B., Kim, J.-B., Knight, J. R., Lanza, J. R., Leamon, J. H., Lefkowitz, S. M., Lei, M., Li, J., Lohman, K. L., Lu, H., Makhijani, V. B., McDade, K. E., McKenna, M. P., Myers, E. W., Nickerson, E., Nobile, J. R., Plant, R., Puc, B. P., Ronan, M. T., Roth, G. T., Sarkis, G. J., Simons, J. F., Simpson, J. W., Srinivasan, M., Tartaro, K. R., Tomasz, A., Vogt, K.

a, Volkmer, G. a, Wang, S. H., Wang, Y., Weiner, M. P., Yu, P., Begley, R. F. and Rothberg, J. M.: Genome sequencing in microfabricated high-density picolitre reactors., Nature, 437(7057), 376-80, doi:10.1038/nature03959, 2005.

McKew, B. a, Dumbrell, A. J., Taylor, J. D., McGenity, T. J. and Underwood, G. J. C.: Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments., FEMS Microbiol. Ecol., 84(3), 495-509, doi:10.1111/1574-6941.12077, 2013.

Meysman, F. J. R., Middelburg, J. J. and Heip, C. H. R.: Bioturbation: a fresh look at Darwin's last idea, Trends Ecol. Evol., 21(12), 688-695, doi:10.1016/j.tree.2006.08.002, 2006.

Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M. J. and Heip, C. H. R.: Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus, Mar. Ecol. Prog. Ser., 236(23-29), 2002.

Moodley, L., Nigam, R., Ingole, B., Prakash Babu, C., Panchang, R., Nanajkar, M., Sivadas, S., van Breugel, P., van Ijzerloo, L., Rutgers, R., Heip, C. H. R., Soetaert, K. and Middelburg, J. J.: Oxygen minimum seafloor ecological (mal) functioning, J. Exp. Mar. Bio. Ecol., 398(1-2), 91-100, doi:10.1016/j.jembe.2010.12.015, 2011.

Moore, W. S.: The Effect of Submarine Groundwater Discharge on the Ocean, Ann. Rev. Mar. Sci., 2(1), 59-88, doi:10.1146/annurev-marine-120308-081019, 2010.

Mopper, K., Michaelis, W., Garrasi, C. and Degens, E. T.: Sugars, Amino Acids, and Hydrocarbons in Black Sea Sediment from DSDP Leg 42B Cores, in Leg 42, Part 2, of the cruises of the drilling vessel Glomar Challenger; Istanbul, Turkey, to Istanbul, Turkey, May-June 1975, edited by J. L. Usher, pp. 697-705., 1978.

Murray, J. W., Stewart, K., Kassakian, S. and Krynytzky, M.: Oxic, suboxic, and anoxic anoxic conditions in the Black Sea, 1-21, 2007.

Muyzer, G. and Stams, A. J. M.: The ecology and biotechnology of sulphate-reducing bacteria., Nat. Rev. Microbiol., 6(6), 441-54, doi:10.1038/nrmicro1892, 2008.

Niggemann, J., Ferdelman, T. G., Lomstein, B. A., Kallmeyer, J. and Schubert, C. J.: How depositional conditions control input, composition, and degradation of organic matter in sediments from the Chilean coastal upwelling region, Geochim. Cosmochim. Acta, 71(6), 1513-1527, doi:10.1016/j.gca.2006.12.012, 2007.

Nikitine, B.: The Biological Conditions of the Black Sea observed in 1923-25, Nature, 116(2928), 863-863, doi:10.1038/116863b0, 1925.

Pantoja, S. and Lee, C.: Peptide decomposition by extracellular hydrolysis in coastal seawater and salt marsh sediment, Mar. Chem., 63(3-4), 273-291, doi:10.1016/S0304-4203(98)00067-X, 1999.

Pantoja, S. and Lee, C.: Amino acid remineralization and organic matter lability in Chilean coastal sediments, Org. Geochem., 34(8), 1047-1056, doi:10.1016/S0146-6380(03)00085-8, 2003.

Quaiser, A., Zivanovic, Y., Moreira, D. and López-García, P.: Comparative metagenomics of bathypelagic plankton and bottom sediment from the Sea of Marmara., ISME J., 5(2), 285-304, doi:10.1038/ismej.2010.113, 2011.

Ramette, A.: Multivariate analyses in microbial ecology., FEMS Microbiol. Ecol., 62(2), 142-160, doi:10.1111/j.1574-6941.2007.00375.x, 2007.

Ramette, A.: Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities., Appl. Environ. Microbiol., 75(8), 2495-505, doi:10.1128/AEM.02409-08, 2009.

Reese, B. K., Mills, H. J., Dowd, S. E. and Morse, J. W.: Linking Molecular Microbial Ecology to Geochemistry in a Coastal Hypoxic Zone, Geomicrobiol. J., 30(2), 160-172, doi:10.1080/01490451.2012.659331, 2013.

Reimers, C. E., Alleau, Y., Bauer, J. E., Delaney, J., Girguis, P. R., Schrader, P. S. and Stecher, H. a.: Redox effects on the microbial degradation of refractory organic matter in marine sediments, Geochim. Cosmochim. Acta, 121, 582-598, doi:10.1016/j.gca.2013.08.004, 2013.

Ross, D. a, Degens, E. T. and MacIlvaine, J.: Black Sea: Recent Sedimentary History, Science (80-.)., 170(3954), 163-165, doi:10.1126/science.170.3954.163, 1970.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. a, Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J. and Weber, C. F.: Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities., Appl. Environ. Microbiol., 75(23), 7537-41, doi:10.1128/AEM.01541-09, 2009.

Schloss, P. D., Gevers, D. and Westcott, S. L.: Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies., PLoS One, 6(12), e27310, doi:10.1371/journal.pone.0027310, 2011.

Schmidt, F., Koch, B. P., Witt, M. and Hinrichs, K.-U.: Extending the analytical window for water-soluble organic matter in sediments by aqueous Soxhlet extraction, Geochim. Cosmochim. Acta, doi:10.1016/j.gca.2014.06.009, 2014.

Schubert, C. J., Niggemann, J., Klockgether, G. and Ferdelman, T. G.: Chlorin Index: A new parameter for organic matter freshness in sediments, Geochemistry, Geophys. Geosystems, 6(3), n/a-n/a, doi:10.1029/2004GC000837, 2005.

Sinkko, H., Lukkari, K., Sihvonen, L. M., Sivonen, K., Leivuori, M., Rantanen, M., Paulin, L. and Lyra, C.: Bacteria contribute to sediment nutrient release and reflect progressed eutrophication-driven hypoxia in an organic-rich continental sea., PLoS One, 8(6), e67061, doi:10.1371/journal.pone.0067061, 2013.

Sorokin, J. I.: On the Primary Production and Bacterial Activities in the Black Sea, ICES J. Mar. Sci., 29(1), 41-60, doi:10.1093/icesjms/29.1.41, 1964.

Stanev, E. V., He, Y., Staneva, J. and Yakushev, E.: The Black Sea biogeochemistry: focus on temporal and spatial variability of oxygen, Biogeosciences Discuss., 11(1), 281-336, doi:10.5194/bgd-11-281-2014, 2014.

Stevens, H. and Ulloa, O.: Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific., Environ. Microbiol., 10(5), 1244-59, doi:10.1111/j.1462-2920.2007.01539.x, 2008.

Stewart, F. J., Ulloa, O. and DeLong, E. F.: Microbial metatranscriptomics in a permanent marine oxygen minimum zone., Environ. Microbiol., 14(1), 23-40, doi:10.1111/j.1462-2920.2010.02400.x, 2012.

Sun, M.-Y., Aller, R. C., Lee, C. and Wakeham, S. G.: Effects of oxygen and redox oscillation on degradation of cell-associated lipids in surficial marine sediments, Geochim. Cosmochim. Acta, 66(11), 2003-2012, doi:10.1016/S0016-7037(02)00830-X, 2002.

Thamdrup, B. O. and Canfield, D. E.: Pathways of carbon oxidation in continental margin sediments off central Chile., Limnol. Oceanogr., 41(8), 1629-1650 [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/11540503, 1996.

Thamdrup, B. O., Rosselló-Mora, R. A. and Amann, R.: Microbial Manganese and Sulfate Reduction in Black Sea Shelf Sediments, Appl. Environ. Microbiol., 66(7), 2888-2897, doi:10.1128/AEM.66.7.2888-2897.2000, 2000.

Tindall, B. J.: The family name Solimonadaceae Losey et al. 2013 is illegitimate, proposals to create the names "Sinobacter soli" comb. nov. and "Sinobacter variicoloris" contravene the Code, the family name Xanthomonadaceae Saddler and Bradbury 2005 and the order name X, Int. J. Syst. Evol. Microbiol., 64(Pt 1), 293-7, doi:10.1099/ijs.0.057158-0, 2014.

Ulloa, O., Wright, J. J., Belmar, L. and Hallam, S. J.: The Prokaryotes, in The Prokaryotes - Prokaryotic Communities and Ecophysiology, edited by E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, Springer Berlin Heidelberg, Berlin, Heidelberg., 2013. Vandewiele, S., Cowie, G. L., Soetaert, K. and Middelburg, J. J.: Amino acid biogeochemistry and organic matter degradation state across the Pakistan margin oxygen minimum zone, Deep Sea Res. Part II Top. Stud. Oceanogr., 56(6-7), 376-392, doi:10.1016/j.dsr2.2008.05.035, 2009.

Vandieken, V., Pester, M., Finke, N., Hyun, J.-H., Friedrich, M. W., Loy, A. and Thamdrup, B. O.: Three manganese oxide-rich marine sediments harbor similar communities of acetate-oxidizing manganese-reducing bacteria., ISME J., 6(11), 2078-90, doi:10.1038/ismej.2012.41, 2012.

Velji, M. I. and Albright, L. J.: Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments, Can. J. Microbiol., 32(2), 121-126, doi:10.1139/m86-024, 1986.

Wakeham, S. G., Sinninghe Damsté, J. S., Kohnen, M. E. L. and De Leeuw, J. W.: Organic sulfur compounds formed during early diagenesis in Black Sea sediments, Geochim. Cosmochim. Acta, 59(3), 521-533, doi:10.1016/0016-7037(94)00361-O, 1995.

Weber, A., Riess, W., Wenzho, F. and Jørgensen, B. B.: Sulfate reduction in Black Sea sediments: in situ and laboratory radiotracer measurements from the shelf to 2000 m depth, Deep Sea Res. Part I Oceanogr. Res. Pap., 48, 2073-2096, 2001.

Wenzhöfer, F. and Glud, R. N.: Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade, Deep Sea Res. Part I Oceanogr. Res. Pap., 49(7), 1255-1279, doi:10.1016/S0967-0637(02)00025-0, 2002.

Whittaker, R. H.: Evolution and Measurement of Species Diversity, Taxon, 21, 213-251, 1972. Winkler, L. W.: Die Bestimmung des im Wasser gelösten Sauerstoffes, Berichte der Dtsch. Chem. Gesellschaft, 21(2), 2843-2854, doi:10.1002/cber.188802102122, 1888.

Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.-R., Jørgensen, B. B. and Pfannkuche, O.: In situ experimental

evidence of the fate of a phytodetritus pulse at the abyssal sea floor., Nature, 424(6950), 763-6, doi:10.1038/nature01799, 2003.

Woulds, C. and Cowie, G. L.: Sedimentary pigments on the Pakistan margin: Controlling factors and organic matter dynamics, Deep Sea Res. Part II Top. Stud. Oceanogr., 56(6-7), 347-357, doi:10.1016/j.dsr2.2008.05.033, 2009.

Wright, J. J., Konwar, K. M. and Hallam, S. J.: Microbial ecology of expanding oxygen minimum zones., Nat. Rev. Microbiol., 10(6), 381-94, doi:10.1038/nrmicro2778, 2012.

Zhou, Y., Zhang, Y.-Q., Zhi, X.-Y., Wang, X., Dong, J., Chen, Y., Lai, R. and Li, W.-J.: Description of Sinobacter flavus gen. nov., sp. nov., and proposal of Sinobacteraceae fam. nov., Int. J. Syst. Evol. Microbiol., 58(Pt 1), 184-9, doi:10.1099/ijs.0.65244-0, 2008.

Zinger, L., Amaral-Zettler, L. a, Fuhrman, J. a, Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., Martiny, J. B. H., Sogin, M., Boetius, A. and Ramette, A.: Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems., PLoS One, 6(9), e24570, doi:10.1371/journal.pone.0024570, 2011.

3.8 Tables and Figures

TABLE 3.1: Surficial sediments (0-1 cm) characterization at 7 stations along the oceanographic transect on the Crimean Shelf (Black Sea): Organic carbon (C_{org}), molar organic carbon to nitrogen ratio (C_{org}:N), chlorophyll *a* (Chl *a*), chloroplastic pigment equivalents (CPE), total hydrolyzable amino acids (THAA), degradation index (DI), sulfate reduction rate (SRR), total cell counts based on acridine orange direct count (AODC). Values represent average and standard deviation (±) when available; or standard deviation of counting in case of AODC, - : no data available, <d.l.: value below detection limit. (*) Data from Lichtschlag et al., (2015).

Station	Location	Water depth	Bottom- water O_2 or H_2S	C_{org}	C_{org}:N	Chl *a*	CPE	THAA	DI	*NO_3^-	*Fe_2^+	*SO_4^{2-}	*H_2S	*PO_4^{3-}	*CH_4	*SRR	AODC
	(Lat/Long)	(m)	(µM)	(%)	(mol/mol)	(µg gdw^{-1})	(µg gdw^{-1})	(µmol gdw^{-1})		(µM)	(µM)	(mM)	(mM)	(µM)	(µM)	(nmol $mL^{-1}d^{-1}$)	(10^9cells cm^{-3} sed)
462	44° 49.45'N 33° 9.26' E	105	116±31 O_2	2.6 ± 1.7	10.6±1.9	11 ± 4	19	16.0±4.2	0.6±0.1	< d.l.	83.8	15.6	< d.l	2.8	0.8	< d.l.	2.5 ± 0.2
459	44° 40.48'N 33° 5.53' E	120	100±18 O_2	2.9	9.4	12	37	15.7	0.3	-	-	-	-	-	-	-	-
487	44° 38.78'N 33° 0.25' E	136	60±53 O_2	4.6 ±0.9	9.3	26 ± 9	64±19	23.5±9.5	1.1±0.3	< d.l.	20.7	13.2	< d.l	5.1	1.0	14±8	2.2 ± 0.0
513	44° 37.87' N 32° 57.22' E	147	26 O_2	4.2	9.7	34	82	13.7	0.8	-	-	-	-	-	-	-	-
393	44° 37.08' N 32° 53.48' E	164	4 O_2	5.3 ± 0.5	10.4	32 ± 2	86±4	34.1±11.7	1.3±0.1	< d.l.	13.6	17.2	< d.l	6.5	1.7	15±10	1.9 ± 0.1
506	44° 36.38' N 32° 52.72' E	171	3 H_2S	3.8	9.3	29	66	23.4	0.7	-	-	-	-	-	-	-	-
448	44° 35.84' N 32° 49.03' E	207	19 H_2S	4.9 ± 1.1	11.4±1.8	41 ± 9	94±16	24.9±3.4	1.5±0.5	< d.l.	< d.l.	16.5	< d.l	2.8	1.4	83±45	2.4 ± 0.4

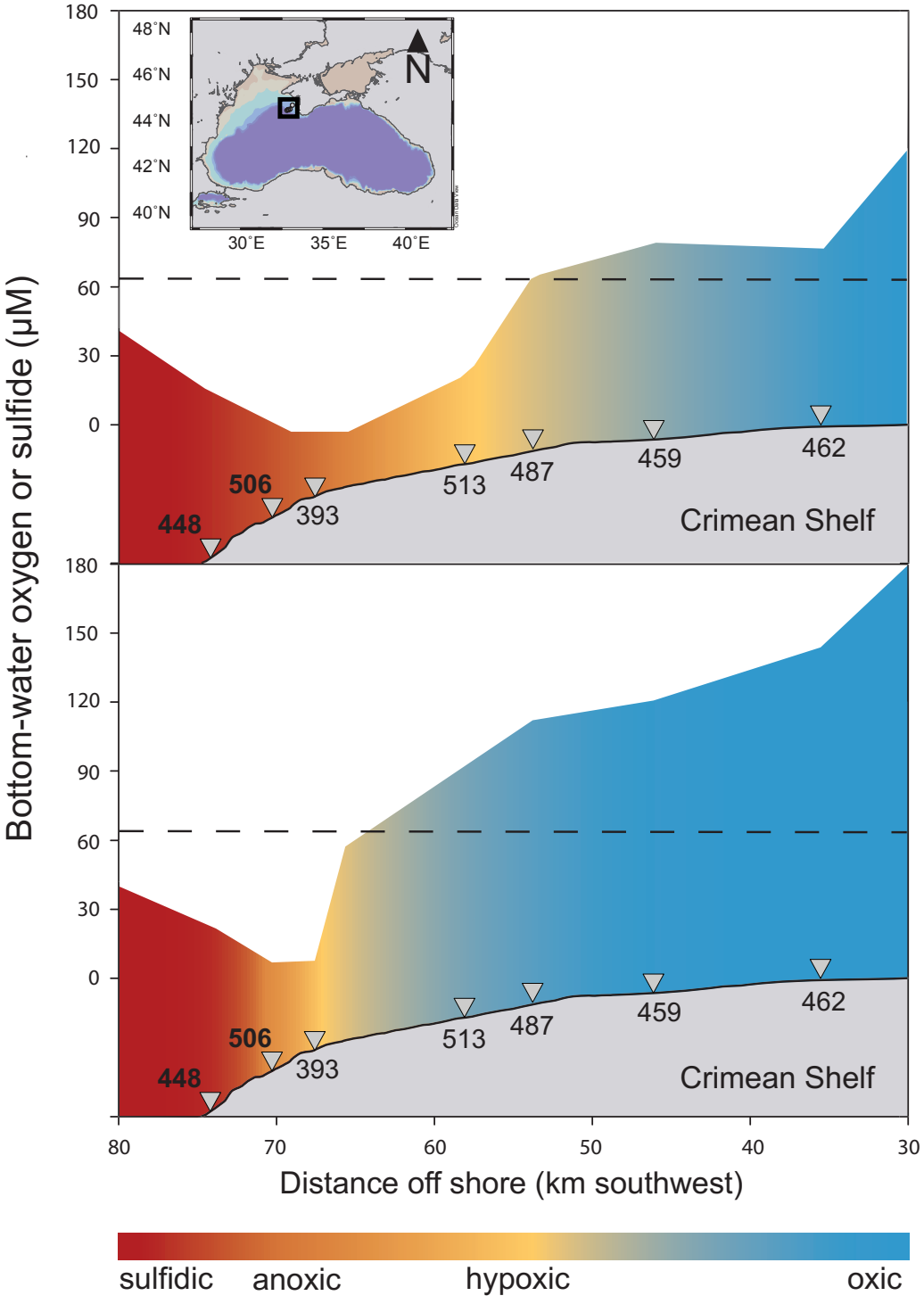


FIGURE 3.1: Section of the outer Western Crimean Shelf showing the position of sampling sites (inverted triangles). The lower and upper bottom-water oxygen or sulfide concentration range impinged on the sediments over a month is represented by the filled area; from oxic (blue) to hypoxic (yellow) and anoxic/sulfidic conditions (orange). Dashed line depicts the hypoxia threshold (63 μM oxygen).

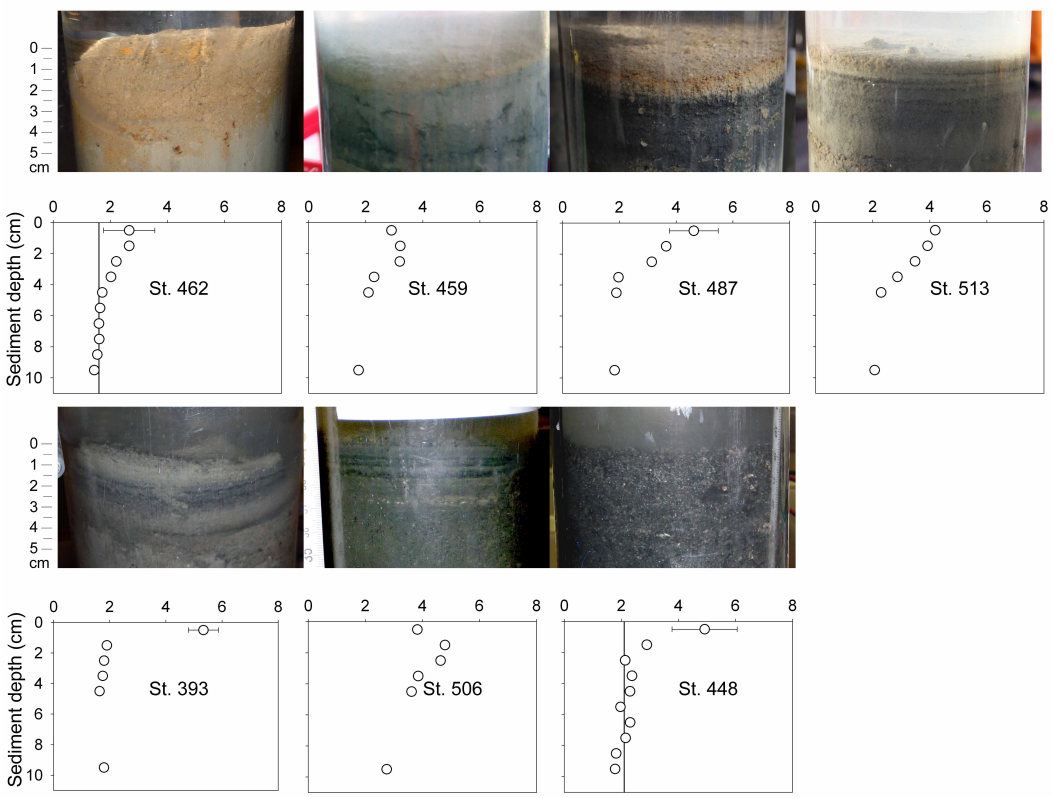


FIGURE 3.2: Surface sedsampled by coring and respective percentage of organic carbon content downcore (x axis is % C_{org}). The vertical line at ~2% C_{org} depicts the background characteristic for subsurface sediments (3-30 cm) on the western Black Sea continental shelf (Weber et al., 2001).

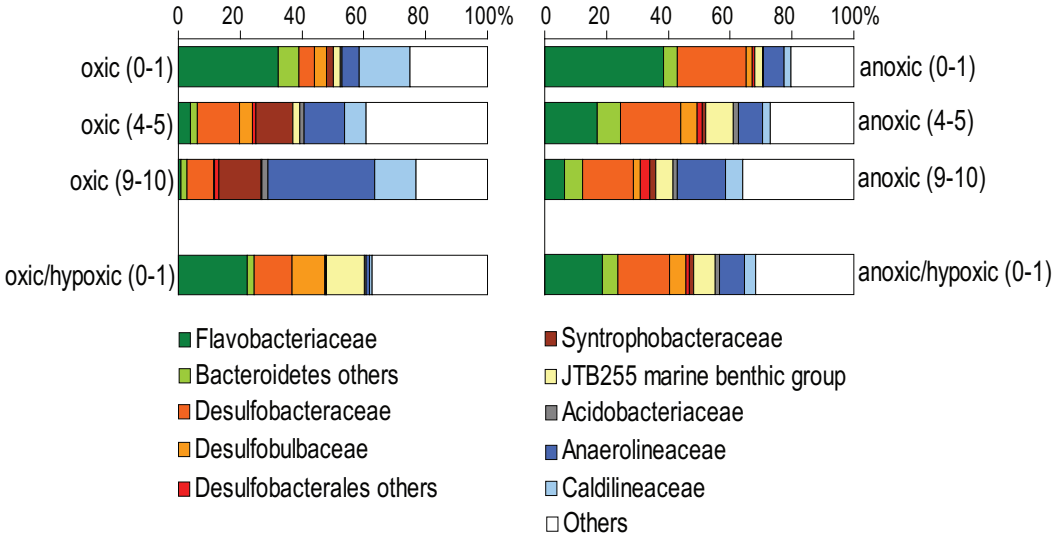


FIGURE 3.3: Family level representation of the most abundant bacterial taxa comparing different sediment horizons (1-10 cm) and oxygenation conditions. The term "oxic" for deeper horizons refers to the bottom-water oxygen concentration and not to the actual oxygen penetration.

Supplementary Tables and Figures

TABLE 3.2: Percentage of shared and total number of OTUs (based on ARISA profiles) between stations.

Station	**462**	**459**	**487**	**513**	**393**	**506**	**OTU number**	**Oxygenation regime**
462							172	Stable oxic
459	63						229	Stable oxic
487	61	72					243	Oxic/hypoxic
513	61	74	75				209	Oxic/hypoxic
393	58	62	68	66			247	Anoxic/hypoxic
506	57	60	67	69	58		182	Stable anoxic
448	58	67	76	66	66	67	247	Stable anoxic

TABLE 3.3: Pairwise comparisons of shared and unique OTU numbers (based on ARISA profiles) between oxygenation regimes. Upper triangle contains number of unique OTUs and the lower triangle the percentage of shared OTUs.

	Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable Anoxic	OTU number
Stable oxic		56	83	79	246
Oxic/hypoxic	80		57	61	258
Anoxic/hypoxic	72	80		58	257
Stable anoxic	73	79	80		257

TABLE 3.4: Analysis of similarity (ANOSIM) based on Bray-Curtis distance matrix between oxygenation conditions (based on ARISA profiles). Upper triangle contains R values and lower triangle P-values (after Bonferroni correction).

	Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable anoxic
Stable oxic		0.3	0.5	0.9
Oxic/hypoxic	< 0.05		0.3	0.5
Anoxic/hypoxic	< 0.05	0.19		0.1
Stable anoxic	< 0.05	0.10	1.00	

TABLE 3.5: Diversity index for Bacteria based on 454 MPTS data for the different oxygenation regimes and 3 sediment horizons (subscripts) for end member conditionsReciprocal of Simpson index (1/D).

Station	**Number of $OTU_{0.03}$**	**Number of sequences**	**Number of $OTU_{0.03}$ singletons**	**% of $OTU_{0.03}$ singletons**	**Chao1**	**1/D**
Stable oxic $_{(0\text{-}1\ cm)}$	701	5719	178	25	1177	19
Stable oxic $_{(4\text{-}5\ cm)}$	1256	6068	483	38	2223	61
Stable oxic $_{(9\text{-}10\ cm)}$	758	7292	210	28	1084	49
Oxic/hypoxic $_{(0\text{-}1\ cm)}$	2080	9999	822	40	3754	116
Anoxic/hypoxic $_{(0\text{-}1\ cm)}$	2268	13219	736	32	3891	128
Stable anoxic $_{(0\text{-}1\ cm)}$	1218	6846	341	28	2248	42
Stable anoxic $_{(4\text{-}5\ cm)}$	1809	7416	611	34	3418	166
Stable anoxic $_{(9\text{-}10\ cm)}$	1185	4179	377	32	2159	190

TABLE 3.6: Ranking of most abundant bacterial classes in decreasing order of their relative sequence number between oxygenation regimes. The percentage of sequence abundance is given in parentheses.

Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable anoxic
Flavobacteria (28)	Gammaproteobacteria (27)	Deltaproteobacteria (23)	Flavobacteria (32)
Deltaproteobacteria (15)	Deltaproteobacteria (25)	Gammaproteobacteria (15)	Deltaproteobacteria (23)
Caldilineae (14)	Flavobacteria (18)	Flavobacteria (14)	Gammaproteobacteria (9)
Sphingobacteria (7)	Sphingobacteria (5)	Anaerolineae (6)	Anaerolineae (6)
Gammaproteobacteria (6)	Alphaproteobacteria (4)	Sphingobacteria (5)	Sphingobacteria (5)
Anaerolineae (5)	Actinobacteria (2)	Alphaproteobacteria (3)	Alphaproteobacteria (2)
Alphaproteobacteria (2)	Clostridia (2)	Actinobacteria (3)	Caldilineae (2)
Actinobacteria (1)	Anaerolineae (1)	Caldilineae (3)	Clostridia (2)
Verrucomicrobiae (1)	Caldilineae (1)	Clostridia (2)	Verrucomicrobiae (1)
Clostridia (1)	Verrucomicrobiae (<1)	Verrucomicrobiae (1)	Actinobacteria (1)

TABLE 3.7: Pairwise comparisons between oxygenation regimes (at $OTU_{0.03}$ level) for the three key bacterial groups, *Deltaproteobacteria* (up), *Gammaproteobacteria* (center) and *Flavobacteria* (down). Upper triangle dennotes β -diversity (calculated as Bray-Curtis dissimilarity) and the lower triangle the percentage of shared $OTU_{0.03}$.

Deltaproteobacteria	Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable anoxic
Stable oxic		0.6	0.6	0.8
Oxic/hypoxic	20		0.6	0.6
Anoxic/hypoxic	20	30		0.8
Stable anoxic	17	32	25	

Gammaproteobacteria	Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable anoxic
Stable oxic		0.8	0.7	0.4
Oxic/hypoxic	17		0.5	0.7
Anoxic/hypoxic	16	26		0.7
Stable anoxic	21	26	23	

Flavobacteria	Stable oxic	Oxic/hypoxic	Anoxic/hypoxic	Stable anoxic
Stable oxic		0.6	0.7	0.4
Oxic/hypoxic	35		0.3	0.4
Anoxic/hypoxic	28	31		0.4
Stable anoxic	30	53	28	

Table 3.8: Bacterial taxa that showed a linear increase (+) or decrease (-) in number of MPTS sequences (>0.1%) with anoxia.

Class	Order	Family	Genus	Correlation
Bacteroidetes;	Bacteroidia;	Bacteroidales;	Marinilabiaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Flavobacteriaceae;	+
Bacteroidetes;	Flavobacteria;	Flavobacteriales;	Cryomorphaceae;	+
Bacteroidetes;	Sphingobacteria;	Sphingobacteriales;	vadinHA17	+
Bacteroidetes;	Sphingobacteria;	Sphingobacteriales;	BD2-2;	+
Bacteroidetes;	Sphingobacteria;	Sphingobacteriales;	BD2-2;	+
Bacteroidetes;	Sphingobacteria;	Sphingobacteriales;	PHOS-HE51;	+
Bacteroidetes;	Sphingobacteria;	Sphingobacteriales;	KD3-93;	+
Proteobacteria;	Alphaproteobacteria;	Caulobacterales;	Hyphomonadaceae;	+
Proteobacteria;	Alphaproteobacteria;	Rhodobacterales;	Rhodobacteraceae;	+
Proteobacteria;	Betaproteobacteria;	TRA3-20;	unclassified;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+

Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfarculales;	Desulfarculaceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfarculales;	Desulfarculaceae;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Deltaproteobacteria;	Sh765B-TzT-29;	unclassified;	+
Proteobacteria;	Deltaproteobacteria;	Sh765B-TzT-29;	unclassified;	+
Proteobacteria;	Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	+
Proteobacteria;	Gammaproteobacteria;	Alteromonadales;	Alteromonadaceae;	+
Proteobacteria;	Gammaproteobacteria;	endosymbionts;	unclassified;	+
Proteobacteria;	Gammaproteobacteria;	Legionellales;	Legionellaceae;	+
Proteobacteria;	Gammaproteobacteria;	unclassified;	unclassified;	+
Proteobacteria;	Gammaproteobacteria;	Oceanospirillales;	Oceanospirillaceae;	+
Proteobacteria;	Gammaproteobacteria;	unclassified;	unclassified;	+
Proteobacteria;	Gammaproteobacteria;	Thiotrichales;	Piscirickettsiaceae;	+
Proteobacteria;	Gammaproteobacteria;	NKB5;	unclassified;	+
Proteobacteria;	Gammaproteobacteria;	Oceanospirillales;	OM182;	+
Proteobacteria;	Gammaproteobacteria;	Sedimenticola;	endosymbionts;	+
Proteobacteria;	JTB23;	unclassified;	unclassified;	+
Proteobacteria;	Milano-WF1B-44;	unclassified;	unclassified;	+
Chloroflexi;	Anaerolineae;	Anaerolineales;	Anaerolineaceae;	+
Chloroflexi;	Anaerolineae;	Anaerolineales;	Anaerolineaceae;	+
Chloroflexi;	Anaerolineae;	Anaerolineales;	Anaerolineaceae;	+
Chloroflexi;	Anaerolineae;	Anaerolineales;	Anaerolineaceae;	+
Chloroflexi;	GIF3;	unclassified;	unclassified;	+
Chloroflexi;	vadinBA26;	unclassified;	unclassified;	+
Chloroflexi;	vadinBA26;	unclassified;	unclassified;	+
Actinobacteria;	Actinobacteria;	Actinobacteridae;	Actinomycetales;	+
Actinobacteria;	Actinobacteria;	Acidimicrobidae;	Acidimicrobiales;	+
Actinobacteria;	Actinobacteria;	Actinobacteridae;	Actinomycetales;	+
Verrucomicrobia;	Opitutae;	Puniceicoccales;	Puniceicoccaceae;	+
Verrucomicrobia;	Verrucomicrobiae;	Verrucomicrobiales;	unclassified;	+
Verrucomicrobia;	Opitutae;	Puniceicoccales;	Puniceicoccaceae;	+
Verrucomicrobia;	Verrucomicrobiae;	Verrucomicrobiales;	unclassified;	+
Firmicutes;	Clostridia;	Clostridiales;	Peptococcaceae	+

Firmicutes;	Clostridia;	Clostridiales;	Lachnospiraceae	+
Firmicutes;	Clostridia;	Clostridiales;	Gracilibacteraceae;	+
BHI80-139;	unclassified;	unclassified;	unclassified;	+
Candidate_division_OD1;	unclassified;	unclassified;	unclassified;	+
Candidate_division_TM6;	unclassified;	unclassified;	unclassified;	+
Chlamydiae;	Chlamydiae;	Chlamydiales;	Simkaniaceae;	+
Cyanobacteria;	Chloroplast;	unclassified;	unclassified;	+
Cyanobacteria;	Chloroplast;	unclassified;	unclassified;	+
Cyanobacteria;	SHA-109;	unclassified;	unclassified;	+
Cyanobacteria;	SHA-109;	unclassified;	unclassified;	+
Cyanobacteria;	SHA-109;	unclassified;	unclassified;	+
Cyanobacteria;	SubsectionI;	Synechococcus;	unclassified;	+
Cyanobacteria;	SubsectionIII;	Halomicronema;	unclassified;	+
Deferribacteres;	Deferribacterales;	LCP-89;	unclassified;	+
Hyd24-12;	unclassified;	unclassified;	unclassified;	+
Planctomycetes;	Phycisphaerae;	Phycisphaerales;	Phycisphaeraceae;	+
Planctomycetes;	Planctomycetacia;	Planctomycetales;	Planctomycetaceae;	+
Spirochaetes;	Spirochaetes;	Spirochaetales;	Leptospiraceae;	+

Class	**Order**	**Family**	**Genus**	**Correlation**
Sphingobacteria;	Sphingobacteriales;	Flammeovirgaceae;	Flexithrix;	-
Sphingobacteria;	Sphingobacteriales;	SB-5;	unclassified;;	-
Sphingobacteria;	Sphingobacteriales;	WCHB1-69;	unclassified;;	-
Alphaproteobacteria;	Rhodospirillales;	Rhodospirillaceae;	Pelagibius;	-
Alphaproteobacteria;	Rhizobiales;	unclassified;	unclassified;	-
Alphaproteobacteria;	Rhodobacterales;	Rhodobacteraceae;	Roseobacter_clade	-
Deltaproteobacteria;	Syntrophobacterales;	Syntrophobacteraceae;	uncultured;	-
Deltaproteobacteria;	Myxococcales;	JG37-AG-15;	unclassified;;	-
Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	uncultured;	-
Deltaproteobacteria;	Sva0485;	unclassified;;	unclassified;;	-
Deltaproteobacteria;	Desulfobacterales;	Desulfobacteraceae;	uncultured;	-
Deltaproteobacteria;	Sh765B-TzT-29;	unclassified;;	unclassified;;	-
Deltaproteobacteria;	Desulfobacterales;	Nitrospinaceae;	uncultured;	-
Deltaproteobacteria;	Desulfarculales;	Desulfarculaceae;	uncultured;	-
Gammaproteobacteria;	Legionellales;	Legionellaceae;	uncultured;	-

Anaerolineae;	Anaerolineales;	Anaerolineaceae;	uncultured;	-
Anaerolineae;	Anaerolineales;	Anaerolineaceae;	uncultured;	-
Anaerolineae;	Anaerolineales;	Anaerolineaceae;	uncultured;	-
Anaerolineae;	Anaerolineales;	Anaerolineaceae;	uncultured;	-
Anaerolineae;	Anaerolineales;	Anaerolineaceae;	uncultured;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	uncultured;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	uncultured;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	uncultured;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	Caldilinea;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	uncultured;	-
Caldilineae;	Caldilineales;	Caldilineaceae;	uncultured;	-
Actinobacteria;	Acidimicrobidae;	Acidimicrobiales;	Acidimicrobineae;	-
Actinobacteria;	Actinobacteridae;	Actinomycetales;	Streptosporangineae;	-
Actinobacteria;	Acidimicrobidae;	Acidimicrobiales;	Acidimicrobineae;	-
Actinobacteria;	Rubrobacteridae;	Solirubrobacterales;	Conexibacteraceae;	-
Verrucomicrobiae;	Verrucomicrobiales;	Verrucomicrobiaceae;	uncultured;	-
Verrucomicrobiae;	Verrucomicrobiales;	Verrucomicrobiaceae;	Luteolibacter;	-
Clostridia;	Clostridiales;	Veillonellaceae;	Schwartzia;	-
Clostridia;	Clostridiales;	Ruminococcaceae(96)	uncultured	-
Actinobacteria;	Rubrobacteridae;	Solirubrobacterales;	Conexibacteraceae;	-
Actinobacteria;	Acidimicrobidae;	Acidimicrobiales;	Acidimicrobineae;	-
Actinobacteria;	Actinobacteridae;	Actinomycetales;	Streptosporangineae;	-
Actinobacteria;	Acidimicrobidae;	Acidimicrobiales;	Acidimicrobineae;	-
unclassified;;	unclassified;;	unclassified;;	unclassified;;	-
unclassified;;	unclassified;;	unclassified;;	unclassified;;	-
Lentisphaeria;	WCHB1-41;	unclassified;;	unclassified;;	-
unclassified;	unclassified;	unclassified;	unclassified;	-
SHA-109;	unclassified;;	unclassified;;	unclassified;;	-

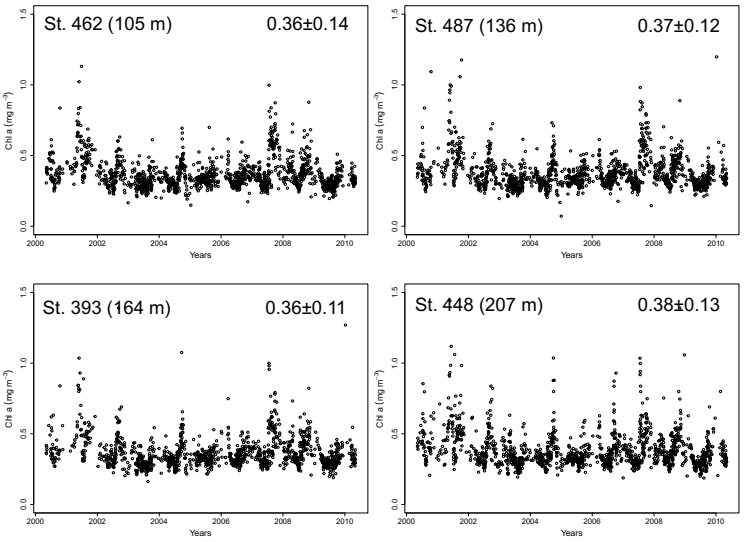


FIGURE 3.4: Satellite-based surface Chlorophyll *a* concentration (generated using *MyOcean Products*) over ten years (05 May 2000-05 May 2010). Values represent average and standard deviation (±).

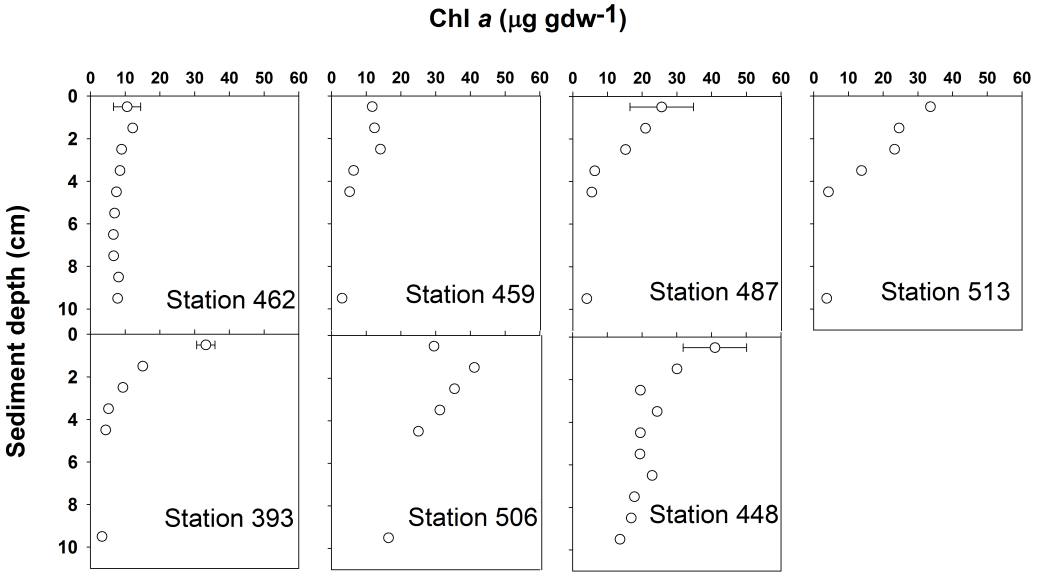


FIGURE 3.5: Downcore Chl *a* concentrations (μg dw^{-1}) from the sampling sites on the Crimean Shelf.

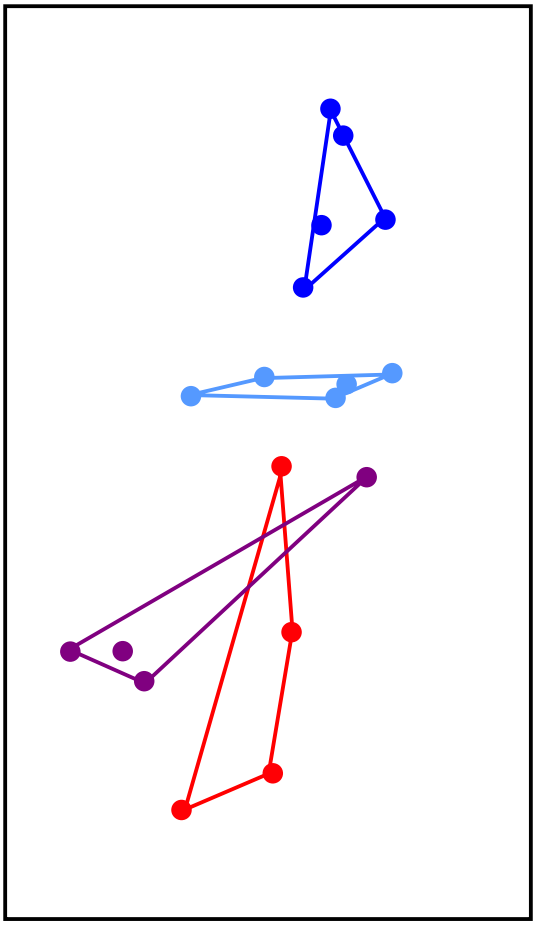


FIGURE 3.6: NMDS. Non-metric multidimensional scaling (NMDS) ordination plot (Bray- Curtis distance matrix) of ARISA profiles for the sampling stations for surface sediments (0-1 cm b.s.f.). Different colors represents different oxygenation regimes; stable oxic (blue), variable oxic/hypoxic (light blue), variable anoxic/hypoxic (purple) and stable anoxic (red). Stress=0.12.

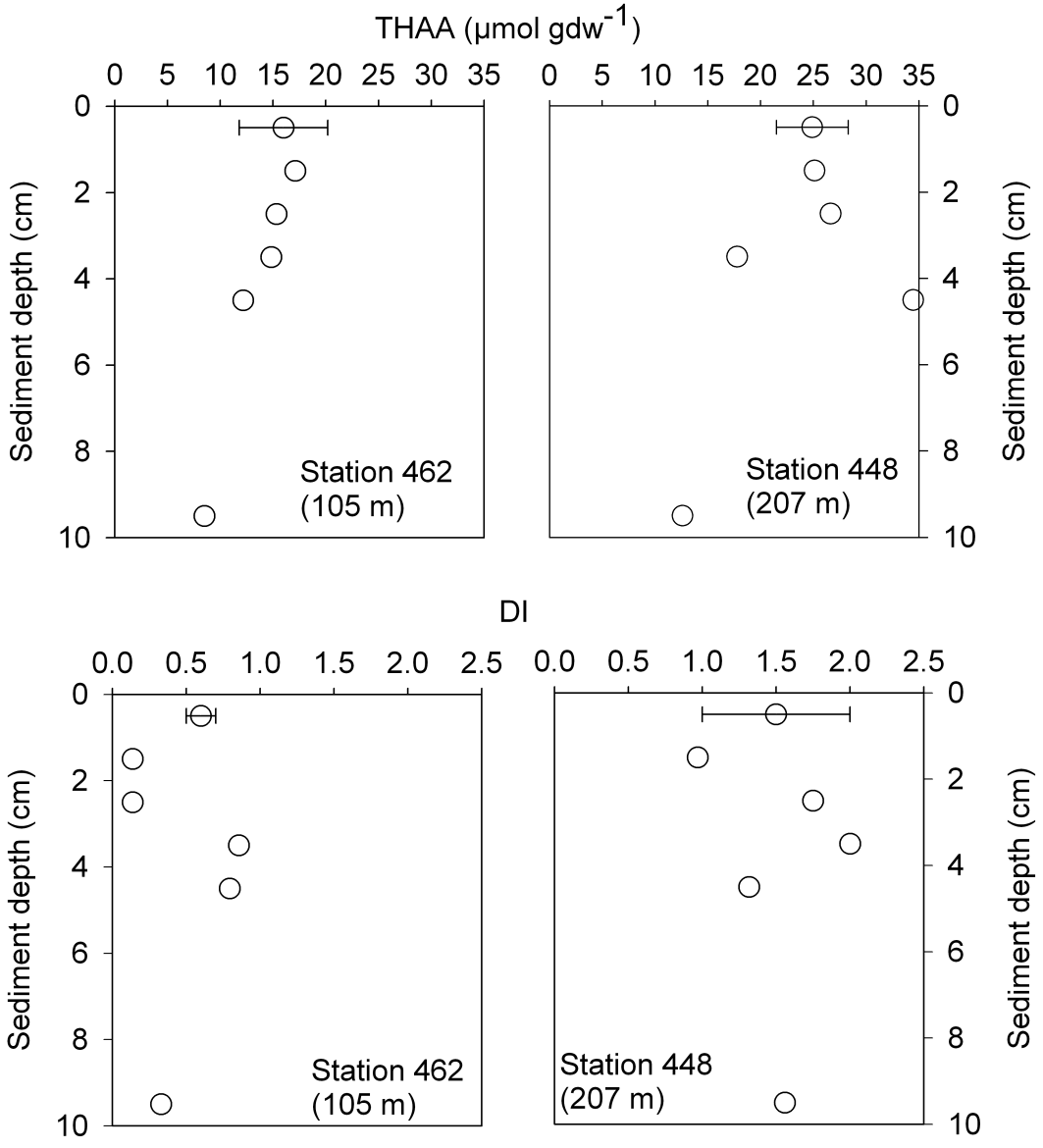


FIGURE 3.7: Downcore total hydrolyzable amino acids concentrations (THAA; μmol gdw^{-1}) and degradation index (DI) comparing permanent oxic (station 462, 105 m) and anoxic (Station 448, 207 m) conditions on the Crimean Shelf.

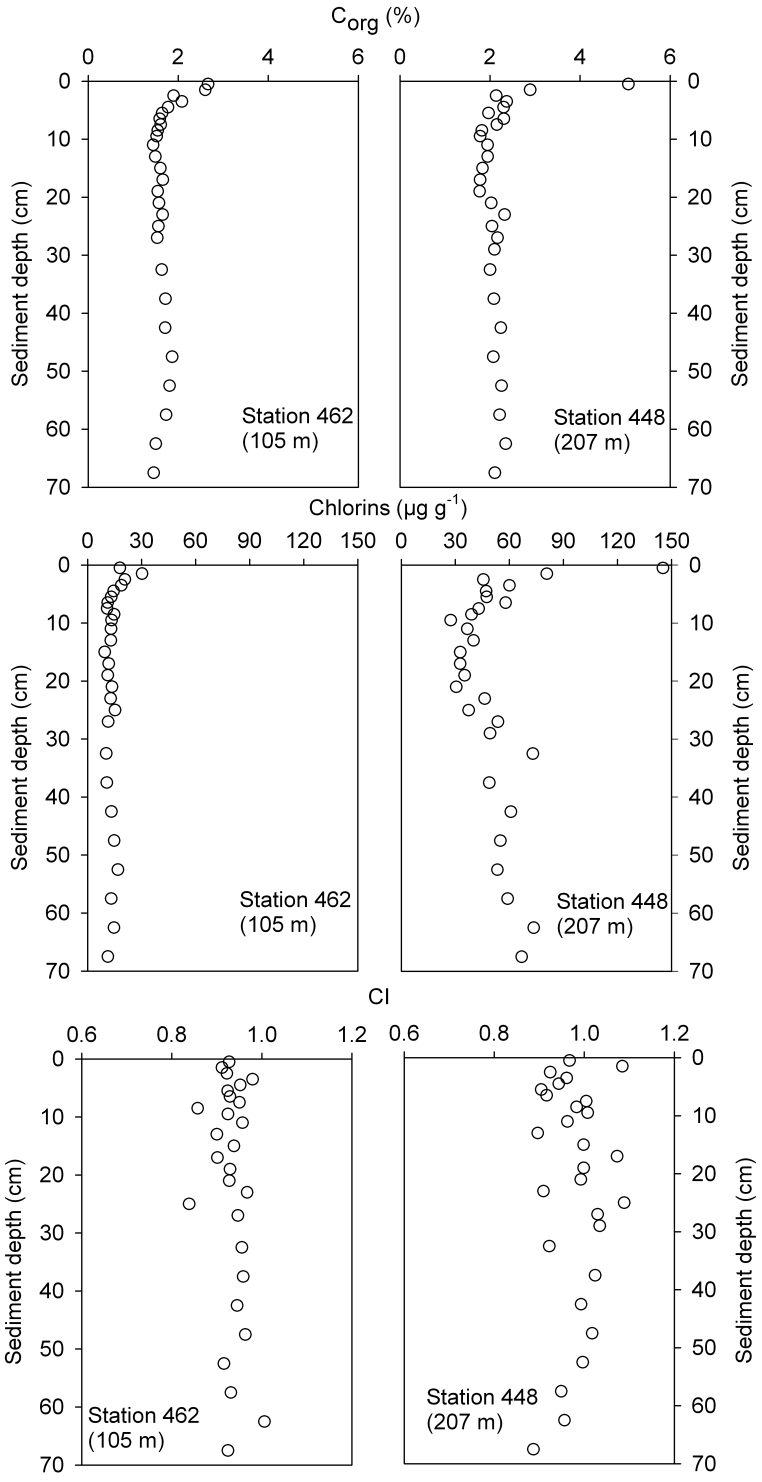


FIGURE 3.8: Downcore organic carbon (C_{org}%), chlorins (μg g^{-1}) and chlorins index (CI) in the upper 70 cm comparing permanent oxic (station 462, 105 m) and anoxic (Station 448, 207 m) conditions on the Crimean Shelf. Chlorins and CI were analyzed according to Schubert et al., (2005).

Chapter 4

Distribution and composition of thiotrophic mats in the hypoxic zone of the Black Sea (150-170m water depth, Crimea margin)

Gerdhard L. Jessen[1], Anna Lichtschlag[1,⋆], Ulrich Struck[2], Antje Boetius[1,3]

In preparation for *Environmental Microbiology*

[1]HGF-MPG Group for Deep Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany.

[2]Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung Berlin, Germany.

[3]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany.

⋆ current address: National Oceanography Center, University of Southampton Waterfront Campus, Southampton, UK.

Keywords: microbial mats, labile organic matter accumulation, bacterial and archaeal community, sulfide oxidation, sulfate reduction

Abstract

Distribution and composition of thiotrophic mats were analyzed using high-resolution biogeochemical and community fingerprinting at the outer shelf of the Northwestern Black Sea. Dives with the submersible JAGO showed accumulations of organic matter forming dark patches on the seafloor, which were covered by mat-forming whitish microbial filaments. Where the Black Sea chemocline met the seabed (between 150-170 m), these mats covered between 25 to 55% of the seafloor, and appeared to form a belt of 3 km width. The mats were composed of Beggiatoa-like large filamentous sulfur bacteria based on 16S rRNA sequences from the mat. The microbial community under the mats was enriched with taxa affiliated with polymer degrading, fermenting and sulfate reducing microorganisms. Under the mats, higher organic matter accumulation, as well as higher remineralization and sulfate reduction rates were measured compared to outside the mat. Mat-covered and mat-free sediments showed similar degradability of the bulk organic matter pool, suggesting that the higher sulfide fluxes and subsequent development of the thiotrophic mats in the patches are consequences of the accumulation of organic matter rather than its qualitative composition. Our observations suggest that the key factors for the distribution of thiotrophic mat-forming communities near to the Crimean shelf break are (i) hypoxic conditions repressing grazers and favoring thiotrophic bacteria and (ii) the specific seafloor topography that enhances the accumulation of labile organic matter.

4.1 Introduction

Thiotrophic microbial mats are dense, visible accumulations of microorganisms, dominated by functional groups that gain their energy by using reduced forms of sulfur as electron donors, enabling chemoautotrophic biomass production. They are found at the oxic-anoxic interface of sediments and rocks characterized by high fluxes of sulfide (Jørgensen, 2010 and references therein). Typical marine mat-forming thiotrophs on shelf sediments are *Candidatus* Maribeggiatoa spp., *Candidatus* Marithioploca spp.; *Thiomargarita* spp.(Schulz, 2006; nomenclature from Salman et al., 2011); *Candidatus* Marithrix spp.; *Thiobacterium* spp (Grünke et al., 2011; nomenclature from Salman et al., 2011) and representatives of the family *Thiovulgaceae* (Campbell et al., 2006), as well as some types of *Arcobacter* (Wirsen et al., 2002). Thiotrophic mats can cover large areas of shelf seas and upper continental margins in areas of seafloor oxygen depletion from high deposition rates of organic matter, such as coastal upwelling regions, oxygen minimum zones and ecosystems subjected to eutrophication (Jørgensen, 1977; Williams and Reimers, 1983; Schulz and Jørgensen, 2001; Levin, 2003; Mußmann et al., 2003). In these systems, the rapid depletion of oxygen by organic matter remineralization at the seafloor favors high rates of sulfate reduction - and hence sulfide production which supports the growth of thiotrophs into dense accumulations (Neira and Rackemann, 1996; Freitag et al., 2003; Lehto et al., 2014). Thiotrophic mats are also prominent features of cold seep ecosystems (Grünke et al., 2012), where the anaerobic oxidation of hydrocarbons via sulfate reduction fuels high sulfide fluxes (Boetius et al., 2000; Joye et al. 2004), at sulfide-emitting hydrothermal vents (Jannasch et al., 1989; Meyer et al., 2013; Urich et al., 2014), as well as in caves, profiting from sulfide-rich streams (Macalady et al., 2006). Among the factors controlling the distribution and thickness of mats in shelf sediments are the fluxes of hydrogen sulfide from the subsurface, and of the electron acceptors oxygen or nitrate from the overlying bottom water (Møller et al., 1985; Schulz et al., 1999; Mußmann et al., 2003; Kamp et al., 2006; Preisler et al., 2007; Macalady et al., 2008; Lichtschlag et al., 2010; Grünke et al., 2011).

Such favorable conditions are met at the oxic-anoxic interface where the surface seafloor is in contact with the Black Sea chemocline. The permanent stratification of the water column by a gradient in salinity separates the fresher, oxic surface layer from the permanently anoxic and sulfidic deep water mass (Ross et al., 1970; Murray et al., 2007). At the interface, a chemocline forms, were oxygen and sulfide may co-exist in dynamic equilibrium (Sorokin, 1972; Karl, 1978; Jørgensen et al., 1991). This chemocline persists at about 100 m in open waters of the Black Sea, and deepens towards the margin to depths of 150-160 m (Stanev et al., 2014). Where the chemocline meets the seafloor, it exposes the organic rich sediments to variable hypoxic to anoxic conditions (Friedrich

et al., 2014). Just below the chemocline, sulfate reduction becomes the dominant remineralization pathway (Jørgensen et al., 2001) increasing from 50% to up o 100% of the total mineralization of organic matter (Weber et al., 2001). However, their distribution and composition has not been investigated in detail before in the Black Sea.

Here we studied the outer Western Crimean shelf by combining submersible surveys with high-resolution geochemical and microbiological analyses, to test the hypothesis that thiotrophic mats are abundant in the hypoxic areas of the Black Sea margin. Key questions addressed: (i) the dominant factors that govern the development of microbial mats, (ii) the microbial types forming mats in the Black Sea, and (iii) the microbial diversity and activity of the mats.

4.2 Results and discussion

Dives with the submersible JAGO confirmed our hypothesis that thiotrophic microbial mats are associated with the hypoxic zone of the outer Black Sea, above the shelf break. We defined the hypoxic zone according to a common ecological threshold (Diaz, 2001) as the area marked by bottom water oxygen concentrations below 63 μmol L^{-1} O_2 as detected by oxygen optodes mounted to JAGO. This zone ranged from ∼140-170 m water depth, covering a distance of ca. 6 km perpendicular to the slope (Fig. 4.1A). No larger benthic fauna was observed to graze the seafloor within this zone, and the seafloor did not show signs of animal traces such as burrows. Within this area, mats were associated with the middle of the chemocline, i.e. the interface between oxic and anoxic, sulfidic bottom waters. The chemocline was positioned on average at 160 m water depth during our investigations in late April-early May 2010. However, in situ observations indicate that this zone is dynamic and fluctuates due to internal waves and eddies (Friedrich et al., 2014; Stanev et al., 2014). The observed oxygen concentrations in bottom waters of the mat area ranged from a few micromoles per Liter (detection limit of sensor 1 μmol L^{-1}) at mats 1-3 to ca. 50 μmol L^{-1} at mat 4 as measured by JAGO or CTD casts. These conditions are inhospitable to most metazoans (e.g. Levin, 2003 and references therein), but favor mat-forming filamentous sulfide-oxidizing bacteria (Schulz and Jørgensen, 2001).

Thiotrophic mats associate with the Black Sea chemocline

The submersible surveys close to the position of the chemocline showed a color change of the seafloor from beige-brownish to dark-grey with increasing water depth. The seafloor was covered with a fluffy layer of greenish detritus particles. Because of the low oxygen

concentrations close to the chemocline, no benthic fauna was observed to dwell the seafloor. Darker patches of organic matter accumulations in seafloor depressions, ranging from few decimeters to >3 m in diameter, were observed in this area. These patches were covered partially to fully by mat-forming, whitish microbial filaments (Fig 4.1 B). The average mat diameter was 0.6±0.3 m (n=1091). The detritus accumulations formed a slightly elevated dome within the depressions. Cutting into the mat-covered patches with the JAGO manipulator or with push cores showed laminated, almost gelatinous layers of particle deposits of around 4 cm thickness (Fig 4.1C). In contrast, the particle layer outside the mats was around 1 cm thickness.

No mats were observed below the chemocline within oxygen-free sulfidic waters > 170 m water depth, or above the 150 m isobath where the seafloor depressions got rare (Fig. 4.1). From the dimension and distribution of the patches, we estimate that the mats covered an area in the range of 25 to 55% of the explored seafloor around the chemocline. The middle of the chemocline to its lower border varied between 150-170 m water depth, covering a downslope distance of 3.25 km. Assuming a belt of mat patches between these two isobaths around the Black Sea, this means that of the 2,156 km^2 of seafloor area exposed to this conditions, the mats could potentially cover between 500 and 1,200 km^2 of Black Sea seafloor (Supplement Fig. 4.5).

Accumulation of organic matter prompts sulfide production for mat development

Biogeochemical analyses of sediment samples from the mat-covered vs. mat-free seafloor confirmed the enhanced accumulation of organic matter underlying the mats compared to the surrounding sediments (Fig 4.2). The pore waters from sediments with mats contained sulfide concentrations up to 300 μmol L^{-1}, and microsensor measurements showed fluxes of 0.7-3.2 mmol sulfide m^{-2} d^{-1} (average 1.9 mmol sulfide m^{-2} d^{-1}), consistent with sulfate reduction rate measurements and porewater analysis (Supplement Fig. 4.6). Outside the mats, sulfide concentrations and fluxes were below detection limit whether measured with sulfate reduction rates, with the microsensor profiler (upper 4 cm, Fig. 4.3) or from the extracted porewater (down to 30 cm, Supplement Fig. 4.6). This indicates that the occurrence of thiotrophic mats was limited to locations with sulfide fluxes high enough to reach the oxygenated surface seafloor. Indeed, the steep gradient of the sulfide microprofiles within the top few mm is consistent with its complete removal by thiotrophic mats (Lichtschlag et al., 2010). Previous sulfate reduction rates from the Romanian shelf also detected a similarly enhanced sulfate reduction rate associated with the chemocline at 180 m, but did not report on the presence of microbial mats (Weber et al., 2001). Even at sulfate reduction rates of 2.2 mmol m^{-2} d^{-1} , relatively much organic matter will accumulate over time in the area of the chemocline.

The carbon rain rate from sedimentation is around 15 mmol C m^{-2} d^{-1} in the area (Grégoire and Becker, 2004), and the total microbial organic carbon remineralization was around 100 mmol C m^{-2} d^{-1} in the top 4 cm below the mat (Fig. 4.3), equivalent to a remineralization rate of 7 mmol m^{-2} d^{-1} at a sediment accumulation rate of 1 mm yr^{-1} (Lichtschlag et al., 2015).

Next we investigated the reason for these enhanced sulfide fluxes in the seafloor depressions. The organic carbon (C_{org}) content of the surface sediment samples (top 1 cm) from the mat patches and outside of the mats fell within the range described for surficial sediments of the Black Sea of 1-5% (e.g. Cowie and Hedges, 1991; Jørgensen et al., 2004). The patches consistently showed a higher C_{org} compared to the reference sites, averaging 5.2±1.2% versus 3.0±1.4% respectively, resulting in ca. two-fold higher C_{org} values within the top 4 cm (Fig. 4.2A). This enrichment was not reflected in the percentage of total nitrogen integrated over the same section, and isotopic composition was the same inside and outside the mat (Table 4.1). However, both chloroplastic pigments from algal detritus and total hydrolyzable amino acids, showed higher concentrations under the mat compared to the reference sites (Fig. 4.2B and C). We also tested for differences in the degradability of the bulk organic matter pool available to the mat communities by a number of proxies based on the above concentration measurements, such as the C/N molar ratio (Meyers, 1994), the amino acid based degradation index (Dauwe and Middelburg, 1998; Dauwe et al., 1999) and the chlorophyll a to total pigment equivalent (CPE) ratio (Pfannkuche, 1993). The respective values were comparable between mat-covered sediments and the reference sites (Table 4.1), suggesting that local differences in the accumulation of matter, and not the quality and composition of the material, was responsible for the higher sulfide fluxes and subsequent development of microbial mats. Apparently, the depressions in the seafloor act as detritus traps and accumulate matter. From the shape of the sulfide and ammonium profiles these appeared as rather recent deposits not yet in steady state (Supplement Fig. 4.7). We can only speculate about the origin of the abundant depressions observed close to the chemocline at the shelf break but not above or below. It may be possible that these are formed by the impact of internal waves and eddies in the region (Trembanis et al., 2011; Stanev et al., 2014).

Composition and diversity of the thiotrophic mats on the Crimea margin

The mats consisted of motile, colorless filaments of cells ranging from 10-40 μm diameter forming chains of up to a few mm length. The filaments contained light refracting granules of elemental sulfur (Fig. 4.1D) explaining the whitish appearance of the mats, and resembled the morphology of *Candidatus* Maribeggiatoa spp. (Schulz and Jørgensen,

2001; nomenclature from Salman et al., 2011). Moreover, comparatively high concentrations of phosphate were found in sediments under the mats (Supplement Fig. 4.6), consistent with the known physiologic response of Beggiatoa strains to anoxic/sulfidic conditions (Brock and Schulz-Vogt, 2011). Accordingly, 16S rRNA sequences from the mat included relatives of the *Candidatus* Isobeggiatoa divolgata (Salman et al., 2011; Supplement Fig. 4.5), together with other deeply branched *Thiotrichaceae* (Supplement Fig. 4.5). Representatives of the *Isobeggiatoa* clade (marine and brackish types; Salman et al., 2011 and references therein) share morphological characteristics with the filaments sampled from the Black Sea mats, such as disc-shaped cells of multicelullar filaments with sulfur inclusions. The presence of such sequences in deeper horizons of the mat-free reference site 2 (Supplement Fig. 4.5) indicates that the thiotrophs could be wide-spread as members of the rare biosphere in Black Sea sediments, but only accumulate to denser mats when they meet favorable conditions (Jørgensen, 2010).

We used cell counts and community fingerprinting (ARISA and 454-MPTS) to assess the distribution and composition of the bacterial and archaeal communities associated with the thiotrophic mats. The mat-associated communities showed threefold higher cell abundances in the upper sediment horizons (0-4 cm b.s.f.) compared to the surrounding sediments (Fig. 4.2). Significant differences in the bacterial community structure were evident based on the ARISA data comparing mat and reference sites (Bonferroni corrected p-value <0.001; Analysis of Similarity based on Bray-Curtis R-value$=0.7$)(Fig. 4.4A), consistent with the variations in the composition of bacterial and archaeal taxa detected by 454-MPTS (upper 2 cm, Fig. 4.4B). Within the same horizon, only $45\pm6\%$ of the dominant bacterial types detected by ARISA overlapped between bacterial mats and surrounding reference sediments (FIG. 4.4A , Supplement Table 4.2), and the thiotrophic mats hosted 11% unique sequences.

The 454-MPTS analysis of the composition of bacterial sequences in mat-covered and surrounding sediments indicated that *Flavobacteriaceae* dominated up to 50% of the individual taxonomic units (97% clustering, in the following referred to as $OTU_{0.03}$) at the surface of the patches (Fig. 4.4B). *Flavobacteria* are relevant marine polymer degraders with hydrolytic and fermentative capacities (Cottrell and Kirchman, 2000; Bernardet et al., 2002 and references therein; Lydell et al., 2004; Julies et al., 2010). Their sequence abundance were two-to fourfold in mat-covered sediments compared to the surrounding sediments, and generally decreased with sediment depth and with decreasing organic matter content, as observed already in other marine habitats (Bissett et al., 2008). Probably profiting from the products of fermentation, the families of sulfate/sulfur reducers *Desulfobacteraceae* and *Desulfobulbaceae* (Muyzer and Stams, 2008) were more abundant in the mat-covered sediments compared to the surrounding sediments. These groups are typical for reduced sediments (Reese et al., 2013; Sinkko

et al., 2013), and were previously found associated to *Beggiatoa* spp. mats (Lloyd et al., 2010). Cultivated strains affiliated to *Desulfobulbaceae* can disproportionate inorganic sulfur compounds and grow chemolithoautotrophically (e.g. Finster et al., 1998), and they are known to use fermentation products from algal matter for sulfate reduction in anoxic incubations (McKew et al., 2013). Two fractions of the bacterial community did not show differences between mat and mat-free sediments, the families *Anaerolineaceae* and *Caldilineaceae* involved in fermentation (Yamada et al., 2006).

Also the archaeal community showed differences between mat-covered sediments and the surrounding seafloor (Fig. 4.4B). Thermoplasmata were typical for the mat-covered sediments, but almost absent from the surrounding sediments. They are known as facultative anaerobes performing sulfur respiration (Huber and Stetter, 2006 and refrences therein), suggesting a contribution to the porewater sulfide flux (Fig. 4.3).

Thaumarcheota sequence abundances were low in the sulfidic mat-covered sediments, but were the most abundant archaeal sequence elsewhere. Many of the archaeal $OTU_{0.03}$ were affiliated to the Marine Group I. This clade dominated subsurface sediments at the mat-covered sediments and also the surface and subsurface of the references sites (Fig. 4.4B). Cultivated strains affiliated to this group are described as aerobic ammonia oxidizers (Könneke et al., 2005). In agreement with our results they can inhabit oxic and anoxic environments, but little is known as to which electron acceptors they could use in the absence of oxygen (La Cono et al., 2013). In addition, we detected several types associated with the thiotrophic mats belonging to "extremophile" groups (Fig. 4.4B), including *Thermoprotei* and *Halobacteria* considered thermophilic and halophilic correspondingly (Huber et al., 2006; Oren, 2006 and references therein). Although most of the representatives of the *Halobacteria* group are halophiles, uncultured members have been reported in less saline environments such as estuaries (e.g. Singh et al., 2010). *Halobacteria* were more abundant in the mat-covered sediments; they are known to reduce sulfur compounds in low-salt environments (Elshahed et al., 2004), however the Black Sea has a $S = \sim 22$ in the bottom waters.

In conclusion, we could confirm our hypothesis of an association of thiotrophic mats with the hypoxic areas of the Black Sea margin, specifically the zone where the chemocline met the seafloor. The key factor for their distribution appeared to be the proximity to both oxic and sulfidic bottom waters at the chemocline reducing pressure by grazing due to the absence of fauna, but also the specific hydrographic conditions near to the shelf break, causing a seafloor topography that enhances the accumulation of relatively labile organic matter. A high abundance of seafloor depressions were observed which served as particle traps, accumulating marine organic matter and enhancing sulfate reduction rates. Low oxygen concentrations and a relatively high sulfide flux favored the

development of thiotrophic microbial mats in this zone. Due to the dynamic positioning of this interface zone by water mass movement, we estimate that the thiotrophic mat zone forms a belt around the Black Sea outer shelf, covering thousand square kilometers. The thiotrophic mats were formed by filamentous large sulfur bacteria related to the *Beggiatoa* clade. The mat habitat enriched for polymer degrading, fermenting and sulfate reducing microorganisms, which provide key functions to support a thiotrophic community.

4.3 Experimental Procedures

Study area and seafloor sampling

The study area (44.57°-44.70°N and 32.80°-33.15°E, 110-200 m depth) is located on the Crimean shelf break of the Northwestern Black Sea. Sampling was performed during the MSM 15-1 expedition (R/V Maria S. Merian, 12 April - 8 May 2010; Fig. 4.1A). In order to assess presence and distribution of microbial mats, video transects were carried out with the submersible JAGO, covering the region between 110-200 m depth, and an area of 20 km^2 (Fig. 4.1 A). Mats were identified visually by their whitish color, and their dimensions were measured by the laser pointers of JAGO. Mat and underlying sediments were retrieved either by push coring (PUC) with the manipulator of the submersible "JAGO" (inner diameter of 72 mm), or a video-controlled multiple corer (TV-MUC, inner diameter of 96 mm). The sediment samples analyzed in this study comprise cores from 4 different microbial mats and 3 reference sites a few meters away from mats (Table 4.1). An additional sediment core was taken as reference for porewater geochemistry (station ref 4). All sediments were sampled on 4^{th} May 2010 with push cores except "mat 4" and "ref 4", sampled with a video-controlled multiple corer (TV-MUC) on 26^{th} and 27^{th} April 2010 respectively. To assess oxygen concentrations in the bottom waters, a CTD-cast profile (SBE 911plus system, with additional sensors for oxygen-SBE43) was taken before TV-MUC sampling. In case of the JAGO dives, bottom water oxygen concentration was continuously recorded by an oxygen optode (4330 Mk2 AANDERAA optode, detection limit 1 μM) fixed to the frame of the submersible. The optodes were calibrated with water samples in which the oxygen concentrations were determined by Winkler titration (Winkler, 1888).

Sampling points and respective labels are summarized in Table 4.1 and Figure 4.1.

Microsensor measurements

High-resolution geochemical gradients of H_2S and pH were measured ex-situ with microsensors (Jeroschewski et al., 1996; de Beer et al., 1997) in undisturbed mat-covered sediments, and in sediments without mat. The H_2S sensors were calibrated by stepwise increasing H_2S concentrations by adding aliquots of a 0.1 mol L^{-1} Na_2S solution to acidified seawater (pH <2). pH sensors were calibrated with commercial laboratory buffers. The total sulfide (H_2S+HS^-+S_2^-) was calculated from the H_2S concentrations and the local pH using equilibrium constants. The sensors were mounted on a motor-driven micromanipulator and lowered into the water column and the sediment in increments of 100 μm. The data acquisition was performed using a DAQ-PAD 6015 (National Instruments Corporation, Austin, TX, USA) and a computer and the flux calculations were performed according to Lichtschlag et al. (2010).

Pore water geochemistry

Pore water from a mat-covered sediment and a reference site was extracted with Rhizons (Rhizosphere Research Products, particle retention down to 0.1 μm) according to Seeberg-Elverfeldt et al., (2005) (station mat 4 and ref 4; Fig. 4.1A). The initial 0.5 mL was discarded and less than 4 mL were extracted per Rhizon to prevent mixing between horizons. The retrieved pore water was immediately sub-sampled and fixed in 20% Zn acetate (total H_2S), or frozen at -20°C (NH_4^+ and PO_4^{3-}) for further analysis. NH_4^+, PO_4^{3-}, and total H_2S were measured spectrophotometrically using the method of Grasshoff et al., (1983) for nutrients and Cline (1969) to assess total H_2S (methylene blue).

Bulk-sediment analyses

Immediately after retrieval, sediments were sliced into 1-cm-thick subsamples in a cold room set to in situ temperature (8°C), and processed for further analyses as explained in the next sub-sections.

Samples for organic carbon (C_{org}) and total nitrogen, pigments (chlorophyll *a* and its degradation products; chloroplastic pigment equivalents, CPE) and total hydrolyzable amino acids (THAA) were measured in triplicate from freeze dried and homogenized sediment.

Pigments (chlorophyll *a* and CPE) were measured spectrophotometrically according to Schubert et al., (2005). Total hydrolyzable amino acids (THAA) were measured after Pantoja and Lee, (2003) from ca. 100 mg sediment. Briefly, after hydrolysis (6 N HCl at 105 °C, for 21 h under N_{2gas}), the supernatant was removed and neutralized (0.1

N KOH). The amino acid identification and quantification was conducted by HPLC after pre-column derivatization with o-phthaldialdehyde and 2-mercaptoethanol (Lindroth and Mopper, 1979; Pantoja and Lee, 1999). The results obtained were used to assess the degradation index (DI) of the organic matter, which represents the selective diagenetic alteration of the sedimentary amino acids as a proxy of lability of the organic matter (Dauwe and Middelburg, 1998; Dauwe et al., 1999). Porosity was determined as the loss of water content in the sediment before and after drying at 60 °C until constant weight.

Stable isotope analysis and concentration measurements of nitrogen and carbon were performed simultaneously with a THERMO Delta V isotope ratio mass spectrometer, coupled to a THERMO Flash EA 1112 elemental analyzer via a THERMO Conflo IV-interface. Inorganic carbon was removed by acidification (12.5% HCl solution). Stable isotope ratios are expressed in the conventional delta notation (δ ^{13}C per mill) relative to VPDB (Vienna PeeDee Belemnite standard). Standard deviation for repeated stable isotope measurements of lab standard material (peptone) is generally better than 0.15 per mill for carbon. Standard deviations of concentration measurements of replicates of our lab standard are <3% of the concentration analyzed.

Sulfate reduction rates

Rates of sulfate reduction (SR) were measured by the whole core injection method (Jørgensen, 1978) at mat 3. Here sediments were vertically sub-sampled with subcore liners (250 mm inner diameter) in triplicate and injected with $^{35}SO_4^{2-}$ radiotracer. After 12 h incubation in the dark at in situ temperature, the reactions were stopped by transferring the core slice (1 cm) into 20% Zn acetate. Sulfate reduction rates were measured according to Kallmeyer et al., (2004).

Microbial community characterization

The total number of cells in the sediments were assessed in the home laboratory by the Acridine Orange Direct Count (AODC) method. Subsampled sediments were fixed in 2% formaldehyde/seawater, stored at 4°C and treated according to Velji and Albright (1986) and Boetius and Lochte, (1996). Single cell numbers were determined by randomly counting at least 30 grids per filter (for two replicate filters).

Total DNA was extracted from ca. 1 g wet sediment stored at -20°C using UltraClean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, CA). Extracted DNA was quantified with a microplate spectrometer (Infinite® 200 PRO NanoQuant, TECAN Ltd, Switzerland) and its concentration adjusted for each step of the subsequent molecular protocol. The bacterial community structure was determined by the automated ribosomal intergenic spacer analysis (ARISA) fingerprinting method according to Fisher and Triplett (1999). Triplicate PCR reactions from standardized amounts of DNA (10 ng)

from each sample were amplified using the bacteria forward FAM-labelled ITSF and reverse IT-SReub specific primers (Cardinale et al., 2004). All the following molecular protocol including the binning into operational taxonomic units (OTU) and data formatting was carried out as described previously (Ramette, 2009).

Extracted DNA was amplified and sequenced by the Research and Testing Laboratory (Lubbock Texas, USA) via 454 Massively Parallel Tag Sequencing (454-MPTS). The V4–V6 region of the 16SrRNA genes were amplified using the bacterial primers 341F and 907R according to Klindworth et al., (2013). Fragments were sequenced following the 454-MPTS protocol (Margulies et al., 2005) and Titanium reagent chemistry. The raw sequences and all the upstream workflow were conducted with "mothur" following the standard operating procedure (Schloss et al., 2009, 2011; including the implemented denoising algorithm). Taxonomic assignments were carried out using the SILVA reference file for bacteria (Pruesse et al., 2007; downloaded from http://www.mothur.org in September 2013) and clustered at a 97% identity level into operational taxonomic units ($OTU_{0.03}$). The dataset was normalized by the total amount of sequences per sample to get relative abundances. Singletons were treated according to Gobet et al., (2012). All statistical analyses were conducted following Ramette (2007 and references therein) using the R package vegan (Oksanen et al., 2010) and performed in R (v. 3.0.1; http://www.R-project.org) using vegan and custom R scripts. The diversity indexes for the 454-MPTS data were obtained with "mothur" (Schloss et al., 2009).

4.4 Acknowledgments

We thank the officers, crew and shipboard scientific party of R/V Maria S. Merian, and the JAGO team (GEOMAR, Kiel) for support at sea during the expedition MSM 15-1. We would also like to acknowledge Alban Ramette for his help on the sequencing workflow, and Pier Luigi Buttigieg, Stefanie Meyer, Rafael Laso-Pérez and Viola Krukenberg for helpful discussions. Silvio Pantoja is thanked for access to analytical facilities of the Organic Geochemistry Lab, University of Concepción - COPAS Sur-Austral (PFB-31), and Lilian Nuñez and Paulina Concha for laboratory assistance. Daphne Donis, Wiebke and Rafael Stiens, Martina Alisch, Erika Weiz-Bersch and Mirja Meiners are gratefully acknowledged for their assistance on board and in laboratory, and the technicians of the Microsensor Group (MPI) for the construction of microsensors. This study was financially supported by the EU 7^{th} FP project HYPOX (In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies) EC Grant 226213 and the Leibniz program of the DFG to AB, as well as the Max Planck Society for additional support.

4.5 References

Ahmad, A., Barry, J., and Nelson, D. (1999) Phylogenetic Affinity of a Wide, Vacuolate, Nitrate-AccumulatingBeggiatoa sp. from Monterey Canyon, California, withThioploca spp. Appl. Environ. Microbiol. 65: 270–277.

De Beer, D., Schramm, A., Santegoeds, C.M., and Kuhl, M. (1997) A Nitrite Microsensor for Profiling Environmental Biofilms. Appl. Environ. Microbiol. 63: 973–977.

Bernardet, J., Nakagawa, Y., and Holmes, B. (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. Int. J. Syst. Evol. Microbiol. 52: 1049–1070.

Bissett, a, Bowman, J., and Burke, C. (2008) Flavobacterial response to organic pollution. Aquat. Microb. Ecol. 51: 31–43.

Boetius, A. and 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.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, a, et al. (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623–6.

Brock, J. and Schulz-Vogt, H.N. (2011) Sulfide induces phosphate release from polyphosphate in cultures of a marine Beggiatoa strain. ISME J. 5: 497–506.

Campbell, B.J., Engel, A.S., Porter, M.L., and Takai, K. (2006) The versatile epsilonproteobacteria: key players in sulphidic habitats. Nat. Rev. Microbiol. 4: 458–68.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., 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: 6147–56.

Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458.

La Cono, V., La Spada, G., Arcadi, E., Placenti, F., Smedile, F., Ruggeri, G., et al. (2013) Partaking of Archaea to biogeochemical cycling in oxygen-deficient zones of meromictic saline Lake Faro (Messina, Italy). Environ. Microbiol. 15: 1717–33.

Cottrell, M.T. and Kirchman, D.L. (2000) Natural Assemblages of Marine Proteobacteria and Members of the Cytophaga-Flavobacter Cluster Consuming Low- and High-Molecular-Weight Dissolved Organic Matter. Appl. Environ. Microbiol. 66: 1692–1697.

Cowie, G.L. and Hedges, J.I. (1991) Organic carbon and nitrogen geochemistry of Black Sea surface sediments from stations spanning the oxic:anoxic boundary. In, İzdar,E. and Murray,J.W. (eds), Black Sea Oceanography. Springer Netherlands, pp. 343–359.

Dauwe, B. and Middelburg, J.J. (1998) Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol. Oceanogr. 43: 782–798.

Dauwe, B., Middelburg, J.J., Herman, P.M.J., and Heip, C.H.R. (1999) Linking diagenetic alteration of amino acids and bulk organic matter reactivity. Limnol. Oceanogr. 44: 1809–1814. Diaz, R.J. (2001) Overview of hypoxia around the world. J. Environ. Qual. 30: 275–281.

Elshahed, M.S., Najar, F.Z., Roe, B.A., Oren, A., Dewers, T.A., and Krumholz, L.R. (2004) Survey of Archaeal Diversity Reveals an Abundance of Halophilic Archaea in a Low-Salt, Sulfide- and Sulfur-Rich Spring. Appl. Environ. Microbiol. 70: 2230–2239.

Finster, K., Liesack, W., and Thamdrup, B. (1998) Elemental Sulfur and Thiosulfate Disproportionation by Desulfocapsa sulfoexigens sp . nov ., a New Anaerobic Bacterium Isolated from Marine Surface Sediment Elemental Sulfur and Thiosulfate Disproportionation by Desulfocapsa sulfoexigens sp . nov ., a New. Appl. Environ. Microbiol. 64: 119–125.

Fisher, M.M. and Triplett, E.W. (1999) Automated Approach for Ribosomal Intergenic Spacer Analysis of Microbial Diversity and Its Application to Freshwater Bacterial Communities. Appl. Environ. Microbiol. 65: 4630–4636.

Freitag, T.E., Klenke, T., Krumbein, W.E., Gerdes, G., and Prosser, J.I. (2003) Effect of anoxia and high sulphide concentrations on heterotrophic microbial communities in reduced surface sediments (Black Spots) in sandy intertidal flats of the German Wadden Sea. FEMS Microbiol. Ecol. 44: 291–301.

Friedrich, J., Janssen, F., Aleynik, D., Bange, H.W., Boltacheva, N., Çagatay, M.N., et al. (2014) Investigating hypoxia in aquatic environments: diverse approaches to addressing a complex phenomenon. Biogeosciences 11: 1215–1259.

Gallardo, V.A. (1977) Large benthic microbial communities in sulphide biota under Peru–Chile Subsurface Countercurrent. Nature 268: 331–332.

Gobet, A., Böer, S.I., Huse, S.M., van Beusekom, J.E.E., Quince, C., Sogin, M.L., et al. (2012) Diversity and dynamics of rare and of resident bacterial populations in coastal sands. ISME J. 6: 542–53.

Grasshoff, K., Kremling, K., and Ehrhardt, M. (1983) Methods of Seawater Analysis Wiley-VHC, Berlin.

Grégoire, M. and Becker, J.W. (2004) Modeling the nitrogen fluxes in the Black Sea using a 3D coupledhydrodynamical-biogeochemical model: transport versus biogeochemical-processes, exchanges across the shelf break and comparison of the shelf anddeep sea ecodynamics. Biogeosciences 1: 33–61.

Grünke, S., Felden, J., Liesack, W., Girnth, a-C., de Beer, D., Wenzhöfer, F., and Boetius, A. (2011) Niche differentiation among mat-forming, sulfide-oxidizing bacteria at cold seeps of the Nile Deep Sea Fan (Eastern Mediterranean Sea). Geobiology 9: 330–48.

Grünke, S., Lichtschlag, A., de Beer, D., Felden, J., Salman, V., Ramette, a., et al. (2012) Mats of psychrophilic thiotrophic bacteria associated with cold seeps of the Barents Sea. Biogeosciences 9: 2947–2960.

Huber, H., Huber, R., and Stetter, K.O. (2006) Thermoproteales. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), The Prokaryotes. Springer New York, New York, NY, pp. 10–22.

Huber, H. and Stetter, K.O. (2006) Thermoplasmatales. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), The Prokaryotes. Springer New York, New York, NY, pp. 101–112.

Jannasch, H.W., Nelson, D.C., and Wirsen, C.O. (1989) Massive natural occurrence of unusually large bacteria (Beggiatoa sp.) at a hydrothermal deep-sea vent site. Nature 342: 834–836.

Jeroschewski, P., Steuckart, C., and Kühl, M. (1996) An Amperometric Microsensor for the Determination of H 2 S in Aquatic Environments. Anal. Chem. 68: 4351–4357.

Jørgensen, B.B. (1978) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments: 1. Measurements with radiotracer techniques. Geomicrobiol. J. 1: 11–27.

Jørgensen, B.B. (2010) Big sulfur bacteria. ISME J. 4: 1083–4.

Jørgensen, B.B. (1977) Distribution of colorless sulfur bacteria (*Beggiatoa* spp.) in a coastal marine sediment. Mar. Biol. 41: 19–28.

Jørgensen, B.B., Böttcher, M.E., Lüschen, H., Neretin, L.N., and Volkov, I.I. (2004) Anaerobic methane oxidation and a deep H_2S sink generate isotopically heavy sulfides in Black Sea sediments. Geochim. Cosmochim. Acta 68: 2095–2118.

Jørgensen, B.B., Fossing, H., Wirsen, C.O., and Jannasch, H.W. (1991) Sulfide oxidation in the anoxic Black Sea chemocline. Deep Sea Res. Part A. Oceanogr. Res. Pap. 38: S1083–S1103.

Jørgensen, B.B., Weber, A., and Zop, J. (2001) Sulfate reduction and anaerobic methane oxidation in Black Sea sediments. Deep Sea Res. Part I Oceanogr. Res. Pap. 48: 2097–2120.

Julies, E.M., Fuchs, B.M., Arnosti, C., and Brüchert, V. (2010) Organic Carbon Degradation in Anoxic Organic-Rich Shelf Sediments: Biogeochemical Rates and Microbial Abundance. Geomicrobiol. J. 27: 303–314.

Kallmeyer, J., Ferdelman, T.G., Weber, A., Fossing, H., and Jørgensen, B.B. (2004) A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements. Limnol. Oceanogr. Methods 2: 171–180.

Kamp, A., Stief, P., and Schulz-Vogt, H.N. (2006) Anaerobic sulfide oxidation with nitrate by a freshwater Beggiatoa enrichment culture. Appl. Environ. Microbiol. 72: 4755–4760.

Karl, D.M. (1978) Distribution, abundance, and metabolic states of microorganisms in the water column and sediments of the Black Sea. Limnol. Oceanogr. 23: 936–949.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F.O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41: e1.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D. a (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543–6.

Lehto, N., Glud, R.N., a Noroi, G., Zhang, H., and Davison, W. (2014) Anoxic microniches in marine sediments induced by aggregate settlement: biogeochemical dynamics and implications. Biogeochemistry 119: 307–327.

Levin, L.A. (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanogr. Mar. Biol. an Annu. Rev. 41: 1–45.

Lichtschlag, A., Felden, J., and Bru, V. (2010) Geochemical processes and chemosynthetic primary production in different thiotrophic mats of the Håkon Mosby Mud Volcano (Barents Sea). Limnol. Oceanogr. 55: 931–949.

Lichtschlag, A., Donis, D., Janssen, F., Jessen, G. L., Holtappels, M., Wenzhöfer, F., Mazulmyan, S., Sergeeva, N., Waldmann, C. and Boetius, A. (2015) Effects of fluctuating hypoxia on benthic oxygen consumption in the Black Sea (Crimean Shelf), Biogeosciences Discuss., 12(8), 6445-6488.

Lindroth, P. and Mopper, K. (1979) High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Anal. Chem. 51: 1667–1674.

Lloyd, K.G., Albert, D.B., Biddle, J.F., Chanton, J.P., Pizarro, O., and Teske, A. (2010) Spatial structure and activity of sedimentary microbial communities underlying a Beggiatoa spp. mat in a Gulf of Mexico hydrocarbon seep. PLoS One 5: e8738.

Lydell, C., Dowell, L., Sikaroodi, M., Gillevet, P., and Emerson, D. (2004) A population survey of members of the phylum Bacteroidetes isolated from salt marsh sediments along the east coast of the United States. Microb. Ecol. 48: 263–73.

Macalady, J.L., Dattagupta, S., Schaperdoth, I., Jones, D.S., Druschel, G.K., and Eastman, D. (2008) Niche differentiation among sulfur-oxidizing bacterial populations in cave waters. ISME J. 2: 590–601.

Macalady, J.L., Lyon, E.H., Koffman, B., Albertson, L.K., Meyer, K., Galdenzi, S., and Mariani, S. (2006) Dominant microbial populations in limestone-corroding stream biofilms, Frasassi cave system, Italy. Appl. Environ. Microbiol. 72: 5596–609.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L. a, et al. (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437: 376–80.

McKew, B. a, Dumbrell, A.J., Taylor, J.D., McGenity, T.J., and Underwood, G.J.C. (2013) Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. FEMS Microbiol. Ecol. 84: 495–509.

Meyer, S., Wegener, G., Lloyd, K.G., Teske, A., Boetius, A., and Ramette, A. (2013) Microbial habitat connectivity across spatial scales and hydrothermal temperature gradients at Guaymas Basin. Front. Microbiol. 4: 207.

Meyers, P.A. (1994) Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. 114: 289–302.

Møller, M.M., Nielsen, L.P., and Jørgensen, B.B. (1985) Oxygen Responses and Mat Formation by Beggiatoa spp. Appl. Environ. Microbiol. 50: 373–82.

Murray, J.W., Stewart, K., Kassakian, S., Krynytzky, M., and DiJulio, D. (2007) OXIC , SUBOXIC , AND ANOXIC CONDITIONS IN THE BLACK SEA. In, Yanko-Hombach,V., Gilbert,A.S., Panin,N., and Dolukhanov,P.M. (eds), The Black Sea Flood Question: Changes in Coastline, Climate, and Human Settlement. Springer Netherlands, pp. 1–21.

Mußmann, M., Schulz, H.N., Strotmann, B., Kjaer, T., Nielsen, L.P., Rosselló-Mora, R. a, et al. (2003) Phylogeny and distribution of nitrate-storing Beggiatoa spp. in coastal marine sediments. Environ. Microbiol. 5: 523–33.

Muyzer, G. and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat. Rev. Microbiol. 6: 441–54.

Neira, C. and Rackemann, M. (1996) Black spots produced by buried macroalgae in intertidal sandy sediments of the Wadden Sea: Effects on the meiobenthos. J. Sea Res. 36: 153–170.

Oksanen, J., Blanchet, G., Kindt, R., Legendre, P., and O'Hara, B. (2010) vegan: Community Ecology Package.

Oren, A. (2006) The Order Halobacteriales. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), The Prokaryotes. Springer New York, New York, NY, pp. 113–164.

Pantoja, S. and Lee, C. (2003) Amino acid remineralization and organic matter lability in Chilean coastal sediments. Org. Geochem. 34: 1047–1056.

Pantoja, S. and Lee, C. (1999) Peptide decomposition by extracellular hydrolysis in coastal seawater and salt marsh sediment. Mar. Chem. 63: 273–291.

Pfannkuche, O. (1993) Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. Deep Sea Res. Part II Top. Stud. Oceanogr. 40: 135–149.

Preisler, A., de Beer, D., Lichtschlag, A., Lavik, G., Boetius, A., and Jørgensen, B.B. (2007) Biological and chemical sulfide oxidation in a Beggiatoa inhabited marine sediment. ISME J. 1: 341–53.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35: 7188–96.

Ramette, A. (2007) Multivariate analyses in microbial ecology. FEMS Microbiol. Ecol. 62: 142–60.

Ramette, A. (2009) Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Appl. Environ. Microbiol. 75: 2495–505.

Reese, B.K., Mills, H.J., Dowd, S.E., and Morse, J.W. (2013) Linking Molecular Microbial Ecology to Geochemistry in a Coastal Hypoxic Zone. Geomicrobiol. J. 30: 160–172.

Ross, D. a, Degens, E.T., and MacIlvaine, J. (1970) Black Sea: Recent Sedimentary History. Science (80-.). 170: 163–165.

Salman, V., Amann, R., Girnth, A.-C., Polerecky, L., Bailey, J. V, Høgslund, S., et al. (2011) A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria. Syst. Appl. Microbiol. 34: 243–59.

Schloss, P.D., Gevers, D., and Westcott, S.L. (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One 6: e27310.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75: 7537–41.

Schubert, C.J., Niggemann, J., Klockgether, G., and Ferdelman, T.G. (2005) Chlorin Index: A new parameter for organic matter freshness in sediments. Geochemistry, Geophys. Geosystems 6: n/a–n/a.

Schulz, H.N. (2006) The genus Thiomargarita. In, Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds), The prokaryotes. Springer New York, pp. 1156–1163.

Schulz, H.N., Brinkhoff, T., Ferdelman, T.G., Mariné, M.H., Teske, A., and Jørgensen, B.B. (1999) Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments. Science (80-.). 284: 493–495.

Schulz, H.N. and Jørgensen, B.B. (2001) Big bacteria. Annu. Rev. Microbiol. 55: 105–37.

Seeberg-Elverfeldt, J., Schlüter, M., Feseker, T., and Kölling, M. (2005) Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. Limnol. Oceanogr. Methods 3: 361–371.

Singh, S.K., Verma, P., Ramaiah, N., Chandrashekar, A. a, and Shouche, Y.S. (2010) Phylogenetic diversity of archaeal 16S rRNA and ammonia monooxygenase genes from

tropical estuarine sediments on the central west coast of India. Res. Microbiol. 161: 177–86.

Sinkko, H., Lukkari, K., Sihvonen, L.M., Sivonen, K., Leivuori, M., Rantanen, M., et al. (2013) Bacteria contribute to sediment nutrient release and reflect progressed eutrophication-driven hypoxia in an organic-rich continental sea. PLoS One 8: e67061.

Sorokin, Y.I. (1972) The Bacterial Population and the Processes of Hydrogen Sulphide Oxidation in the Black Sea. ICES J. Mar. Sci. 34: 423–454.

Stanev, E.V., He, Y., Staneva, J., and Yakushev, E. (2014) The Black Sea biogeochemistry: focus on temporal and spatial variability of oxygen. Biogeosciences Discuss. 11: 281–336.

Teske, A. and Nelson, D.C. (2006) The Prokaryotes Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K.-H., and Stackebrandt,E. (eds) Springer New York, New York, NY.

Trembanis, A.C., Nebel, S., Skarke, A., Coleman, D.F., Ballard, R.D., Yankovsky, A., et al. (2011) Bedforms, coastal-trapped waves, and scour process observations from the continental shelf of the northern Black Sea. In, Buynevich, Yanko-Hombach, Gilbert, and Martin (eds), Geology and Geoarchaelogy of the Black Sea Region: Beyond the Flood Hypothesis. Geological Society of America Special Paper 473. Geological Society of America, pp. 165–178.

Urich, T., Lanzén, A., Stokke, R., Pedersen, R.B., Bayer, C., Thorseth, I.H., et al. (2014) Microbial community structure and functioning in marine sediments associated with diffuse hydrothermal venting assessed by integrated meta-omics. Environ. Microbiol. 16: 2699–710.

Velji, M.I. and Albright, L.J. (1986) Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. Can. J. Microbiol. 32: 121–126.

Weber, A., Riess, W., Wenzho, F., and Jørgensen, B.B. (2001) Sulfate reduction in Black Sea sediments: in situ and laboratory radiotracer measurements from the shelf to 2000 m depth. Deep Sea Res. Part I Oceanogr. Res. Pap. 48: 2073–2096.

Williams, L.A. and Reimers, C. (1983) Role of bacterial mats in oxygen-deficient marine basins and coastal upwelling regimes: Preliminary report. Geology 11: 267.

Winkler, L.W. (1888) Die Bestimmung des im Wasser gelösten Sauerstoffes. Berichte der Dtsch. Chem. Gesellschaft 21: 2843–2854.

Wirsen, C.O., Sievert, S.M., Cavanaugh, C.M., Molyneaux, S.J., Ahmad, A., Taylor, L.T., et al. (2002) Characterization of an Autotrophic Sulfide-Oxidizing Marine Arcobacter sp. That Produces Filamentous Sulfur. Appl. Environ. Microbiol. 68: 316–325.

Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., and Kamagata, Y. (2006) Anaerolinea thermolimosa sp. nov., Levilinea saccharolytica gen. nov., sp. nov. and Leptolinea tardivitalis gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes Anaerolineae classis nov. and Caldilineae classis nov. in the . Int. J. Syst. Evol. Microbiol. 56: 1331–40.

4.6 Tables and Figures

TABLE 4.1: Biogeochemical composition of mat-covered sediments (mat) and mat-free reference sites. Organic carbon (C_{org}% dw), nitrogen (TN% dw), carbon stable isotopic composition (δ^{13}C), molar organic carbon to nitrogen ratio (C_{org}:N), Chlorophyll *a* to chloroplastic pigment equivalents ratio (Chl *a*: CPE) and quantitative amino-acid degradation index (DI). Values represent average and standard deviation (±) when available, -: no data.

Sampling site ID	Location (Lat/Long)	Depth (m)	C_{org}(%dw)	TN (%dw)	δ^{13}C	C_{org}:N	Chl *a*: CPE	DI
mat 1	44.62696667° N 32.91266333° E	152	4.4±0.5	0.6±0.2	-24.6	8.3±0.9	0.4	1.2
mat 2	44.62710167° N 32.912615° E	152	2.9±0.8	0.3±0.2	-24.4	9.4±1.3	0.4	1.5
mat 3	44.62740167° N 32.91257167° E	152	4.1±.0.0	0.5±0.0	-24.6	8.7±0.2	0.4	-
mat 4	44.62583333° N 32.91633333° E	156	5.9±0.9	0.6±0.3	-24.2	9.6±0.2	0.5	1.2
ref 1	44.62696667° N 32.91266667° E	152	5.0±0.1	0.6±0.0	-24.2	9.9±0.4	0.4	1.6
ref 2	44.62711667° N 32.912615° E	152	1.7±0.4	0.2±0.0	-	8.0±0.4	0.3	1.1
ref 3	44.62718167° N 32.912615° E	152	3.8±0.2	0.3±0.0	-24.7	8.8±0.6	0.5	1.3

Figures

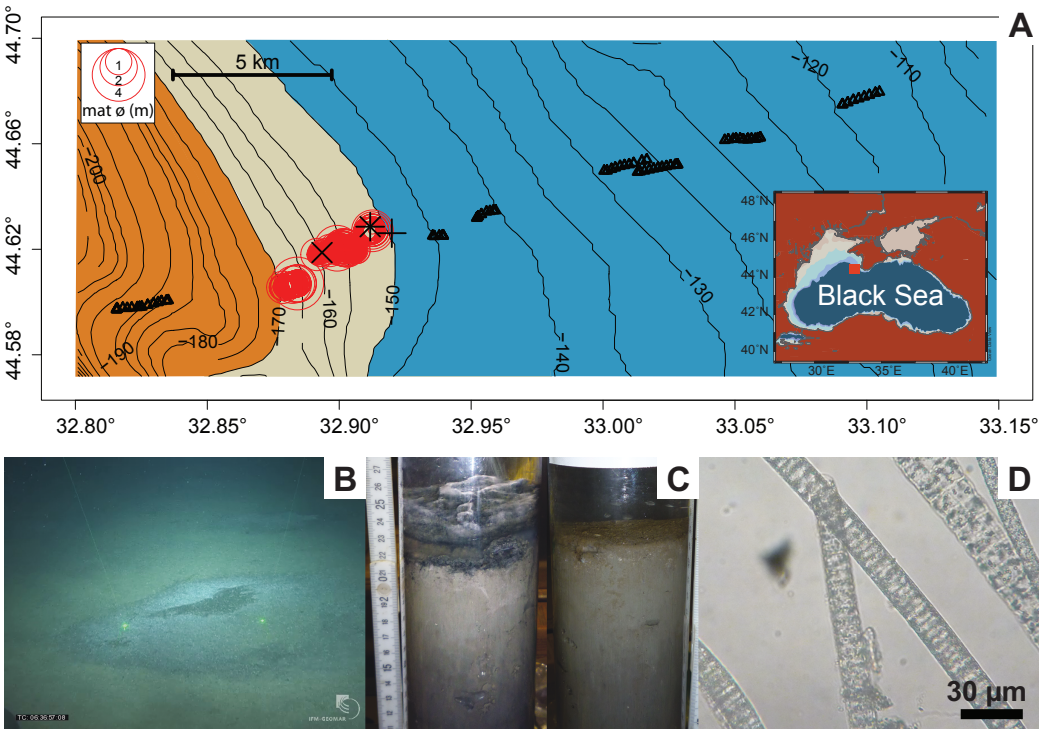


FIGURE 4.1: A; Study area depicting the zone where the middle of the chemocline impinged the sediments (beige belt between 150-170 m depth) between the more oxic (blue) and anoxic/euxinic conditions (brownish). Submersible dive tracks are represented by black triangles while the occurrence of microbial mats and its diameter (ø m) by the red circles. Mat and reference sites 1-3 are represented by the black star, while black cross and black "x" depict position of mat and reference 4, respectively. B: JAGO-based image of accumulated organic matter covered by microbial mats (copyright IFM-GEOMAR), the distance between the two laser pointers is 50 cm C: Push cores from mat-covered sediments (left) and reference site (right). D: light microscopy image of the mat-forming filaments.

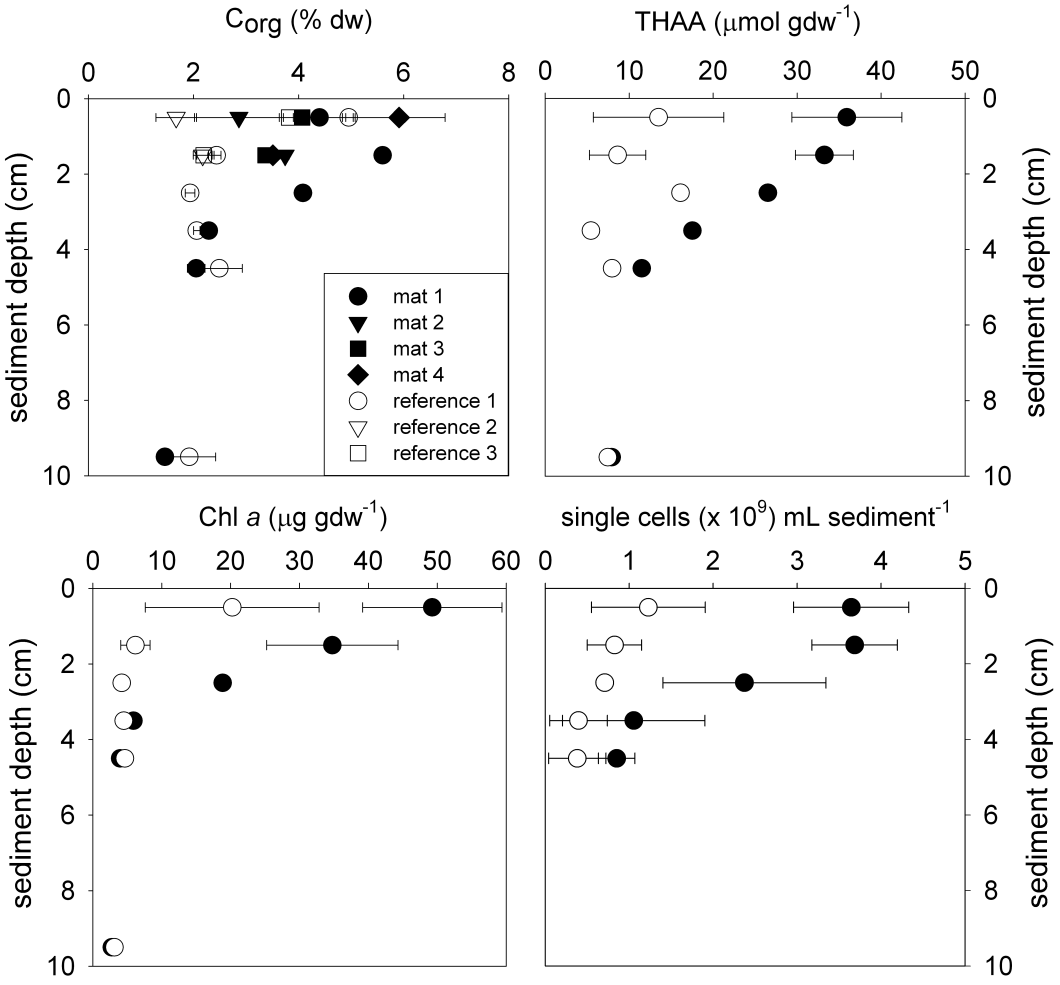


FIGURE 4.2: Bulk analysis of the sediments comparing mat-covered (black symbols) and references sites (white symbols). Total hydrolyzable amino acids (THAA).

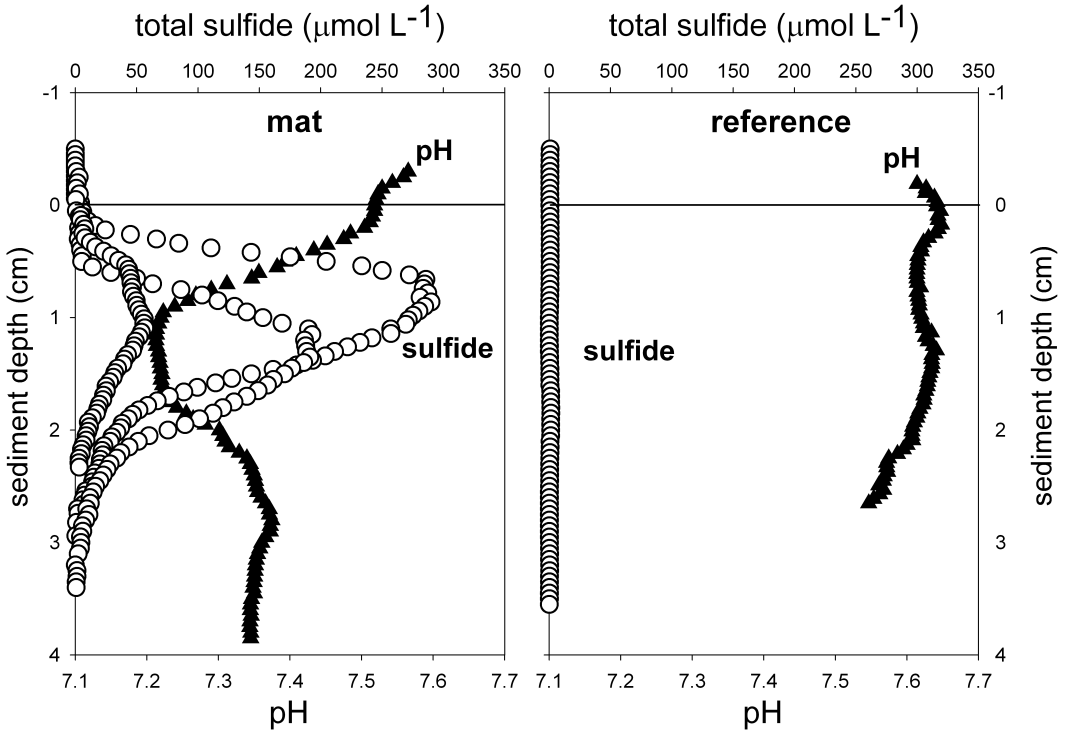


FIGURE 4.3: Ex situ microprofiles of total sulfide and pH measured in sediment with microbial mats (left) and in close-by sediments without mat (right).

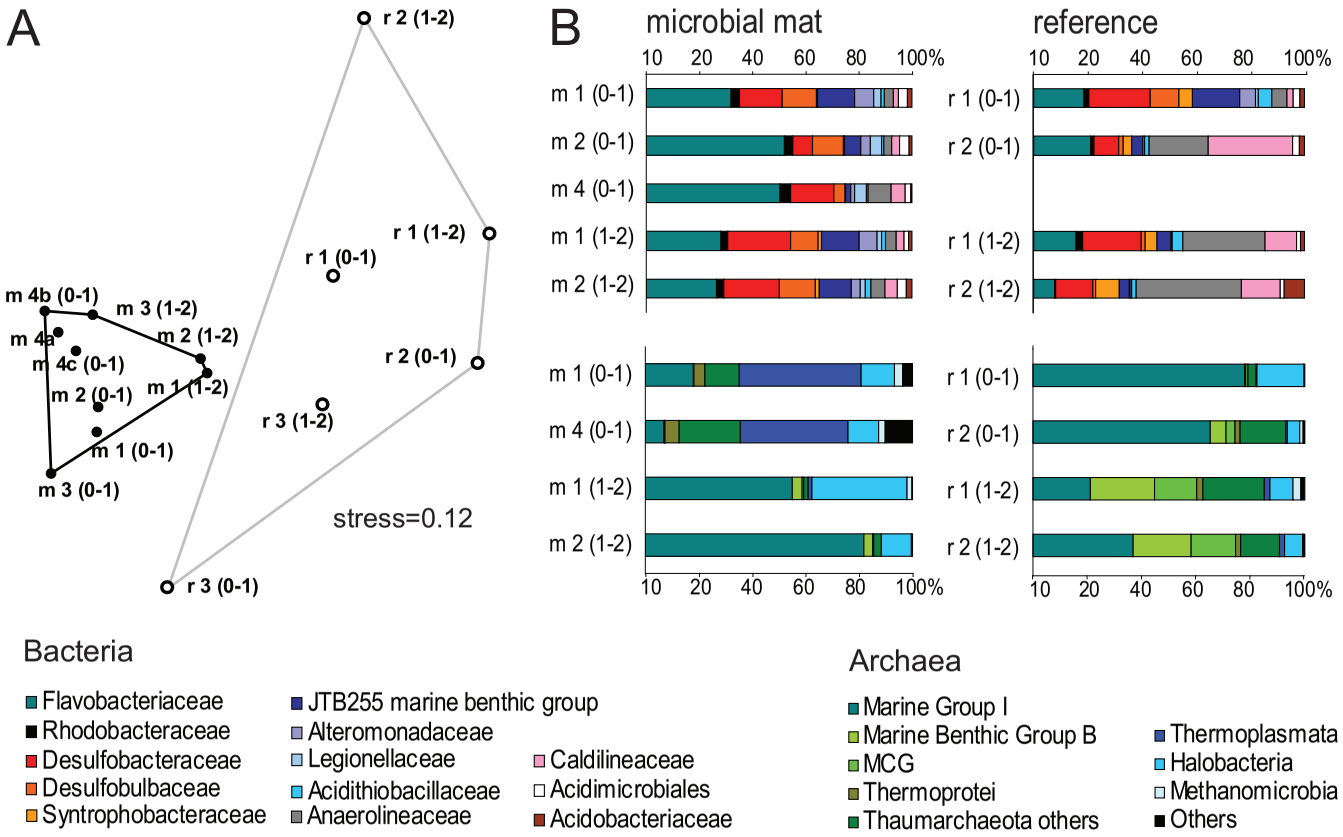


FIGURE 4.4: A; Nonmetric Multi Dimensional Scaling (NMDS) ordination plot based on Bray-Curtis distance matrix (from ARISA profiles). Convex hulls depict significant differences between groups of mat-covered sediments (black circles) and references sites (white circles). Bonferroni corrected p-value <0.001; Analysis of Similarity (ANOSIM) based on Bray-Curtis R-value=0.7. B; Relative abundance of bacterial (upper panel) and archaeal (lower panel) sequences from the upper 2 cm (0-1 and 1-2 cm) obtained by 454-MPTS comparing mat-covered sediments (left) and references sites (right). MCG-Miscellaneous Crenarchaeotal Group.

Supplementary Tables and Figures

TABLE 4.2: Percentage of shared OTU (from ARISA profiles) between mat-covered sediments and reference sites

	mat 1 (0-1)	mat 1 (1-2)	mat 2 (0-1)	mat 2 (1-2)	mat 3 (0-1)	mat 3 (1-2)	mat 4a (0-1)	mat 4b (0-1)	mat 4c (0-1)	ref 1 (0-1)	ref 1 (1-2)	ref 2 (0-1)	ref 2 (1-2)	ref 3 (0-1)
mat 1 (0-1)														
mat 1 (1-2)	62													
mat 2 (0-1)	70	64												
mat 2 (1-2)	64	66	65											
mat 3 (0-1)	69	55	73	56										
mat 3 (1-2)	62	60	70	62	64									
mat 4a (0-1)	68	61	71	60	63	64								
mat 4b (0-1)	63	57	69	60	62	68	76							
mat 4c (0-1)	62	58	59	57	59	60	71	67						
ref 1 (0-1)	49	64	51	59	44	50	48	47	47					
ref 1 (1-2)	36	43	38	48	37	41	36	38	40	56				
ref 2 (0-1)	38	43	42	52	37	41	38	40	40	53	56			
ref 2 (1-2)	39	41	41	44	40	39	39	41	40	44	46	47		
ref 3 (0-1)	54	50	52	49	54	47	50	49	52	41	36	39	37	
ref 3 (1-2)	48	53	52	57	47	48	46	47	47	56	55	59	53	47

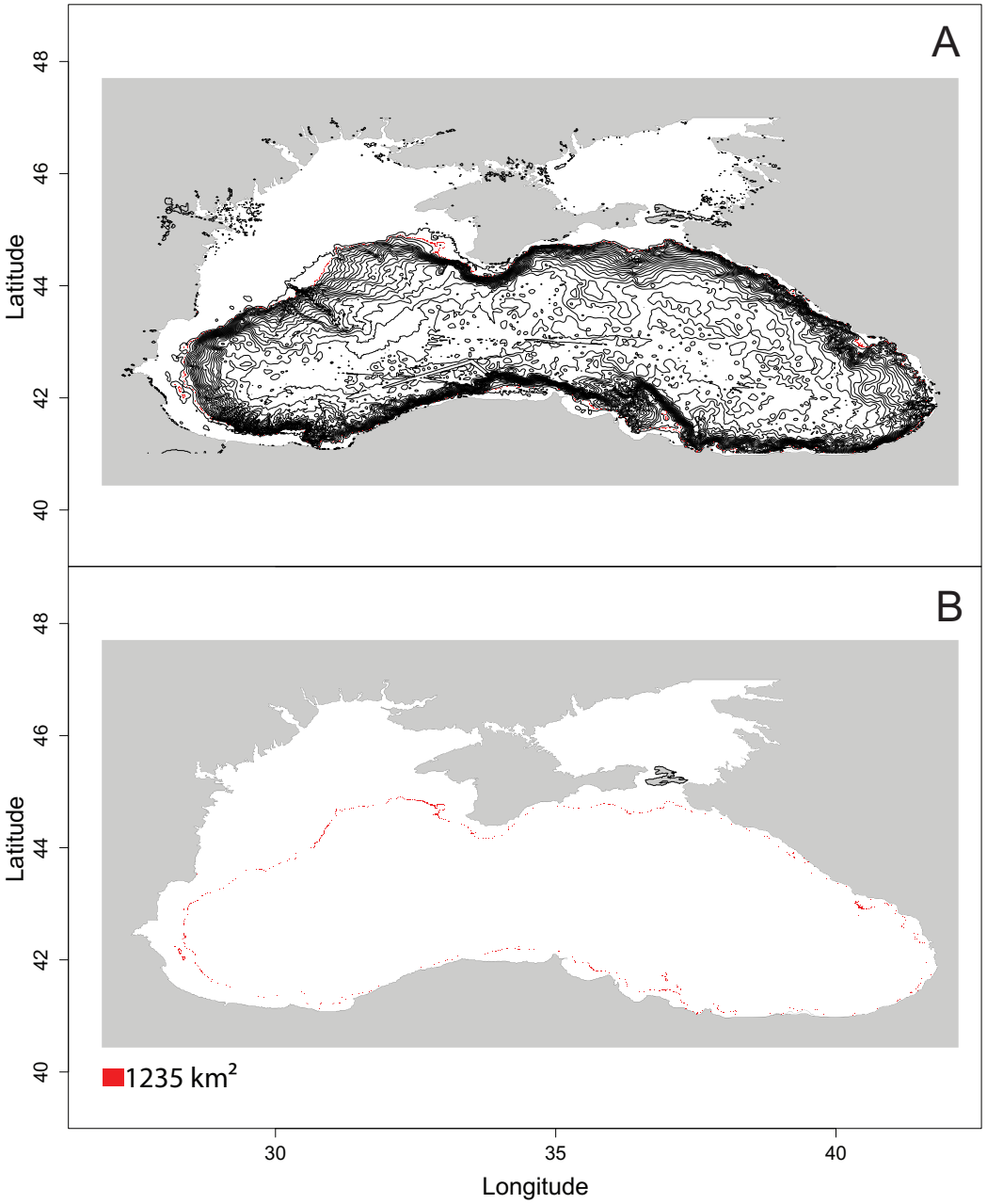


FIGURE 4.5: Hypothesized belt of thiotrophic mats surrounded the Black Sea margin between 150-170 m depth (A). B; isobaths were removed for better visualization of the estimated area of coverage. Coverage area calculated and visualized with the R package "MarMap" (Pante and Simon-Bouhet, 2013).

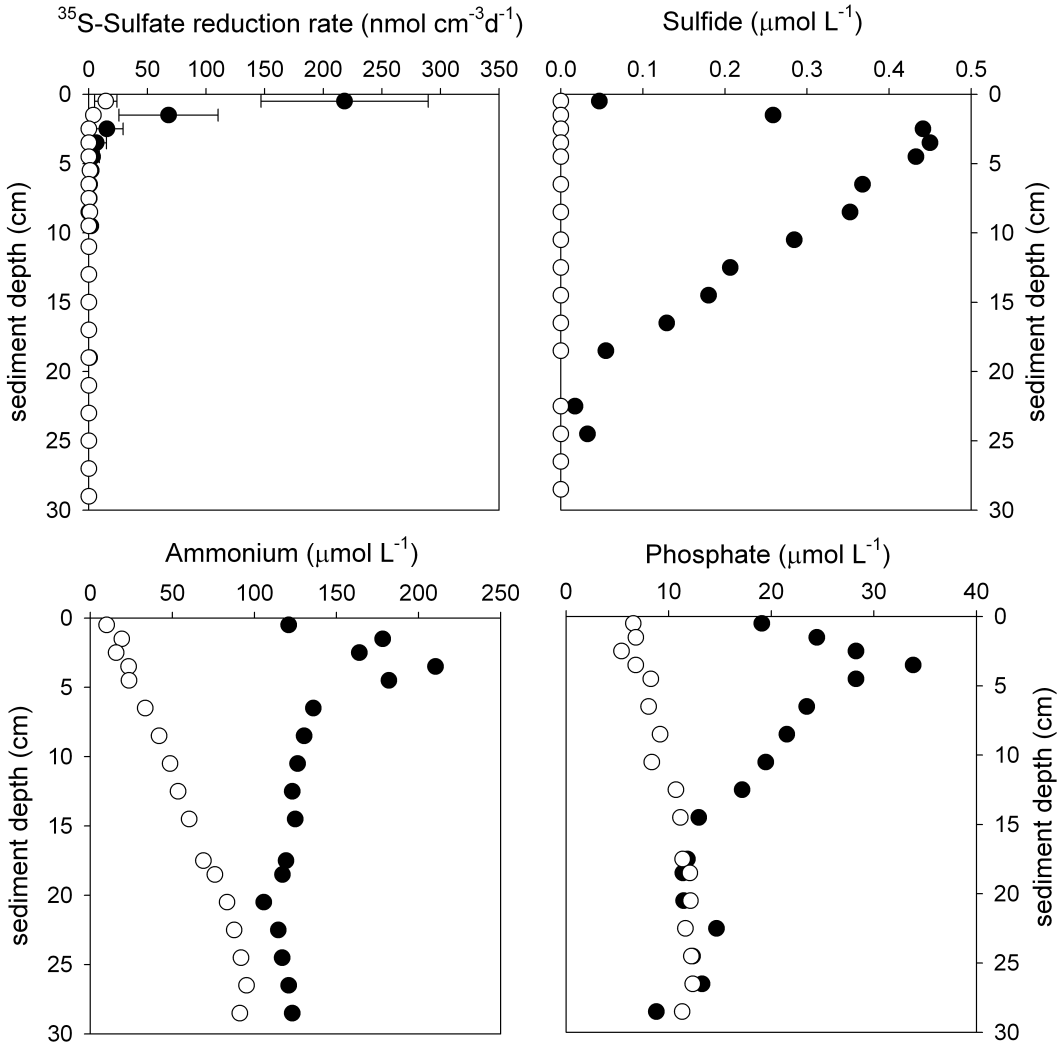


FIGURE 4.6: Depth distributions of ^{35}S-Sulfate reduction rates and dissolved species from the mat-covered site "mat 4" (black circles) and "ref 4" (white circles; reference, 164 m depth).

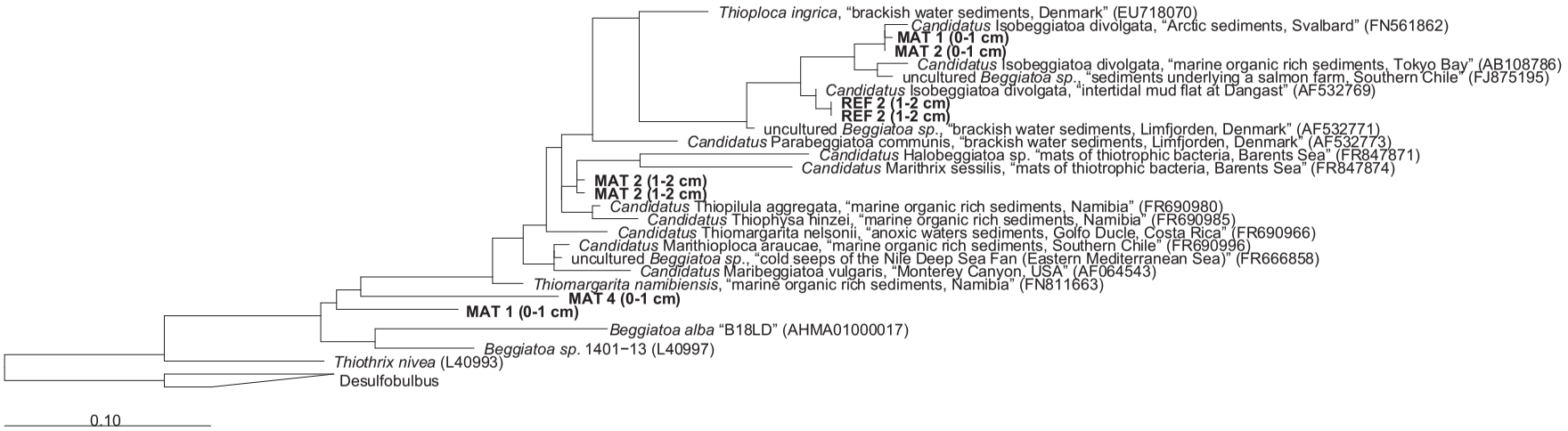


FIGURE 4.7: Phylogenetic 16S rRNA gene based tree showing the affiliation of sequences extracted from mat-covered and reference sediments of the Crimea margin with other filamentous sulphur-oxidizing bacteria. Initial tree reconstruction was conducted with nearly full length sequences. Partial sequences were subsequently inserted into the reconstructed consensus tree by applying the parsimony criteria. Only selected sequences are shown. The scale bar corresponds to 10% estimated sequence divergence. Phylogenetic classification was carried out with the software package ARB (Ludwig et al., 2004) based on the SILVA database (SSURef v119, release date: 24 July 2014). Phylogenetic tree was calculated with the maximum likelihoodt algorithm PHYML (100 bootstraps) as implemented in ARB using a positional variabiliy filter.

Chapter 5

Characterization of organic matter deposited under contrasting oxygen regimes

Current authors: Gerdhard L. Jessen[1], Pamela E. Rossel[1,2,3]

(Further input to this study in works will be provided by Jutta Niggemann, Thorsten Dittmar and Antje Boetius)

Short report on additional results obtained in this thesis

(29.May.2015 - *in preparation*)

[1]HGF MPG Research Group for Deep Sea Ecology and Technology, Max Planck Institute for Marine Microbiology, Bremen, Germany.

[2] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen, Bremerhaven, Germany.

[3]Research Group for Marine Geochemistry, University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment (ICBM), Oldenburg, Germany

Many prokaryotes require dissolved organic matter (DOM) as carbon and energy source. However, DOM in the ocean's deep waters persists, almost unchanged, since centuries (Dittmar and Stubbins, 2014 and references therein). For the water column it is known that organic matter degradation depends on the concentration and composition of its dissolved form, on the oxygen availability and on the amount of time that DOM has been "exposed" to degradation (Jannasch, 1967; Arrieta et al., 2015; Middelburg, 2015). During my thesis research, we discovered that periods of hypoxia and anoxia repress bioturbation and favor assemblages of fermenters and sulfate reducers which increase sulfide production, eventually decreasing the degradability of the bulk organic matter. However, the proxies we used to assess organic matter quality (pigments and amino acids) lack resolution to resolve its bioavailability. With this in mind, we further investigated the potential mechanisms sustaining organic matter preservation, characterizing the dissolved fraction of the organic matter, in relation to microbial community structure and function.

To explore the effect of hypoxia on the composition of dissolved organic matter and potential relationships with microbial community structure, porewater DOM and microbial communities were characterized from sediments exposed to permanent oxic and anoxic/sulfidic conditions at the Crimean Shelf (Stations 462 and 448, Chapter 3) using a 15 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and 454 Massively Tag Sequencing (MPTS). The key questions addressed were: (i) are there differences in the molecular composition of pore water DOM under oxic and anoxic conditions? (ii) are there variations in the composition of DOM between oxic and anoxic sites, that can be related to differences in microbial composition/function? and (iii) How are changes in DOM related to sedimentary organic matter.

A detailed description of the study area is given in Chapters 2 and 3. Briefly, porewater from sediments exposed to permanent oxic (station 464, 105 m water depth) and anoxic/sulfidic (station 448, 207 m water depth) overlying waters, were extracted and analyzed as described below. In parallel, the bacterial community was sampled from three different sediment horizons (0-1, 4-5 and 9-10 cm), and characterized as described in Chapter 3. Obtained operational taxonomic units at 3% identity level ($OTU_{0.03}$) were pooled in order to make them comparable with the dissolved organic matter characterization (0-10 cm sediment depth).

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations

DOM analysis was performed combining porewater from 0-10 0 cm. DOC and TDN concentrations were analyzed via catalytic oxidation at high temperature on

a TOC-V Shimadzu instrument (Stubbins and Dittmar, 2012) on both the original porewater samples and the solid phase extracted samples, the latter determined on the methanol extracts after evaporation overnight and further dissolution in ultrapure water at pH 2. The accuracy of the analysis was corroborated by analyzing deep seawater from the Consensus Reference Material Project (http://yyy.rsmas.miami.edu/groups/biogeochem/Table1.htm).

Solid phase extraction and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) analysis

Porewater samples were solid phase extracted (SPE) using 1g styrene divinyl benzene polymer column (Varian PPL) prior to mass spectrometric analysis according to Dittmar et al. (2008). Extraction was performed on porewater samples previously acidified at pH 2 with HCl and later were passed through a PPL column, previously rinsed with methanol. The cartridges were cleaned several times with ultrapure water at pH 2 to remove the salts from the columns and were then dried under a stream of ultrapure nitrogen. SPE-DOM were eluted with methanol and an aliquot was diluted in a 1:1 mixture of methanol: ultrapure water to a final DOC concentration of 10-15 mg C L^{-1}. Molecular analyses were performed by direct infusion into an ultra-high resolution 15 Tesla FT-ICR-MS (Bruker Solarix) equipped with an electrospray ionization (ESI; Apollo II Bruker Daltonik GmbH, Bremen, Germany) source, in negative ion mode. Samples were infused at 120 μL h^{-1} and mass spectral data were obtained using an ion accumulation of 0.25 s and capillary voltage of 4kV and 500 individual broadband scans. Molecular formula assignments were performed with Matlab (2010) routine developed in the Research group for Marine Geochemistry Lab (Oldenburg, Germany), that searches for all potential combinations of C, H, O, N, S, P elements with an error < 0.5 ppm. Formula assignments were accomplished for mass peaks with a signal to noise ratio >4 and based on previously reported criteria (e.g., Rossel et al., 2013).

Molecular information was evaluated based on contribution of CHO, CHON, CHOS, CHOP and CHONS molecular formulae in each sample and by the intensity weighted average (wa) of the elemental ratios H/Cwa and O/Cwa, as well as the Double Bond Equivalents (DBEwa) and Aromaticity Index (AImodwa) (McLafferty and Turecek, 1993; Koch and Dittmar, 2006). Furthermore, molecular formulae were organized into different molecular groups, which have been shown recently to provide a good overview on the molecular composition of the samples (Seidel et al., 2014). Nevertheless, it is necessary to keep in mind that if a specific molecular formula has the same formula as a known molecular group this does not necessarily indicate the presence of a specific molecular structure (Seidel et al., 2014). Molecular groups considered are described in the

Supplementary methods. Finally, another molecular group evaluated were carboxylic-rich alicyclic molecules (CRAM), which are considered to be an abundant refractory component of marine DOM (Hertkorn et al., 2006).

To discuss the DOM molecular variations, data analysis was performed under the assumption that most of the microbial activity occurs in surface sediment. Indeed, bulk organic carbon (% C_{org} $\sim$ 3 (oxic) and $\sim$ 5 (anoxic)) reach background concentrations at $\sim$3 cm sediment depth for both oxygen regimes ($\sim$2%) (Chapter 3, Fig. 3.2). Consequently, differences in DOM molecular composition of surface sediment porewater are better represented by molecular formulae occurring exclusively in each condition. On the other hand, common formulae rather represent the background DOM signal.

DOC concentrations in porewater sediments are summarized in Table 5.1. DOC were in the range of previous results from the Black Sea (Schmidt et al., 2011). Oxic and anoxic conditions have similar number of identified formulae, most of which were common ($\sim$89%) (and $\sim$11% were exclusive). Regarding the bacterial community, three families clearly dominated the dataset: *Flavobacteriaceae*, *Desulfobacteraceae* and *Anaerolineaceae*, containing 25, 20% and 19% of the tags, respectively (Fig. 5.1).

Higher DOC concentrations in porewater were observed in oxic (387μM) compared to anoxic (275 μM) conditions (Table 5.1), suggesting higher degradation of particulate organic carbon in sediments exposed to oxic overlying waters compared to anoxic conditions. This is consistent with lower preservation of the bulk C_{org} (Chapter 3) observed in sediments exposed to oxic compared to anoxic conditions (Chapter 2), suggesting an accumulation of hydrolytic products after degradation of the particulate organic matter (Arnosti, 2011). This first degradation step may be performed by *Flavobacteriaceae* (Fig. 5.1), clade abundant in the dataset and characterized by their hydrolytic and fermentative capacities (Bernardet et al., 2002 and references therein).

TABLE 5.1: Comparison of porewater composition from oxic and anoxic conditions. Dissolved organic carbon (DOC), and integrated (1-10 cm) sulfate reduction rate (SRR). ($\star$) Data from Chapter 2.

	Oxic	Anoxic
DOC (μM)	387	275
Extraction efficiency (%)	51	80
SRR (mmol m^{-2} d^{-1})$\star$	0.1	3.7

Additionally, the extraction efficiency of DOC was higher from anoxic conditions (80%) compared to the oxic environment (51%) (Table 5.1). This suggest that the fraction of

DOC that escapes from our analytical window is either of (i) low molecular weight (e.g., monomers or colloidal material and volatiles not extracted by PPL) or (ii), a new fraction in the DOM pool produced increase the extraction efficiency under anoxic conditions (Table 5.1). Assuming the first scenario, low molecular weight compounds can result from microbial fermentation of hydrolytic products (Middelburg et al., 1993). Indeed, bacteria involved in anaerobic fermentation of carbohydrates assimilate 10% of the substrate, excreting the remaining as monomers (Clark 1989; Arnosti and Repeta 1994). Although fermentation is a common feature among bacteria (e.g. Megonigal et al., 2003 and references therein), some taxa typically perform such metabolic pathways. For instance, members of *Anaerolineaceae* are characterized by their fermentative metabolism (Yamada et al., 2006). Thus, the accumulation of low molecular weight compounds appeared linked to its high abundances under oxic conditions (Fig. 5.1), suggesting this taxa may be also performing fermentation in deeper sediment layers. However, still intriguing is its accumulation in sediments exposed to oxic conditions.

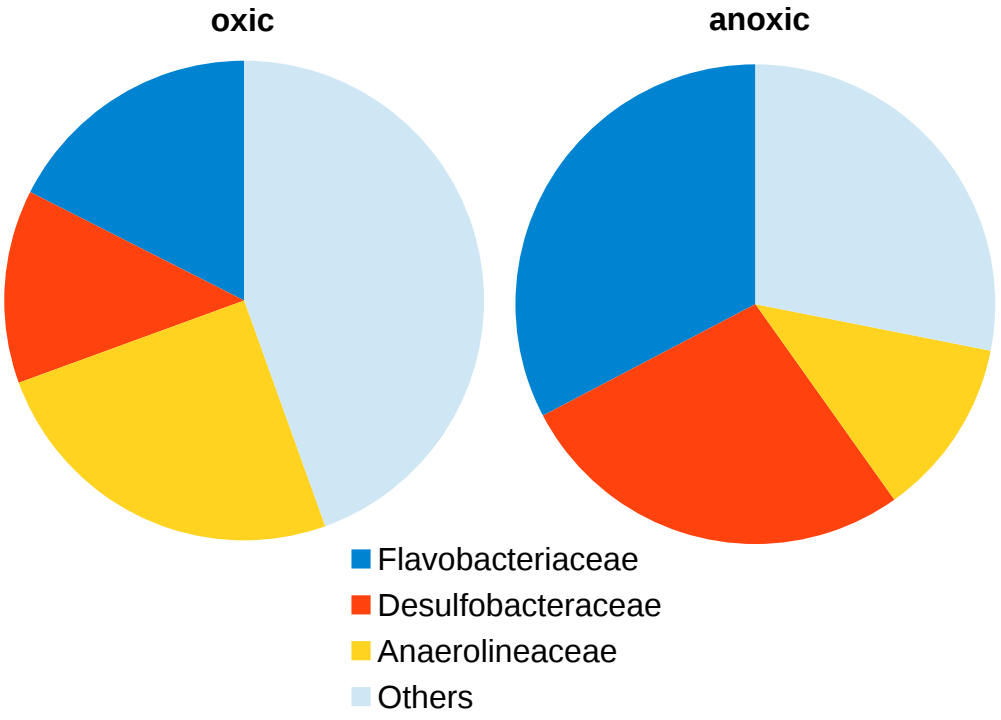


FIGURE 5.1: Family level representation of the three most abundant bacterial taxa (pooled data from 0-1, 4-5 and 9-10 cm) comparing sediments exposed to permanent oxic and anoxic/sulfidic bottom water oxygenation conditions.

On the other hand, an active community of sulfate reducers (Table 5.1) appeared to uptake the available fermentation and hydrolytic products to perform complete remineralization of organic matter under anoxic conditions. This is consistent with high abundances of *Desulfobacteraceae* under anoxic conditions (Fig. 5.1) and its dominance in the degradation of fermentation products through sulfate reduction (e.g. McKew et al., 2013).

Regarding DOM composition, nitrogen-containing compounds (CHON), represented 45% of the exclusive formulae compared to 27% in anoxic conditions (Fig. 5.2). Moreover, higher contribution of formulae related to peptides (13 and 3%) and unsaturated aliphatic compounds (15.9 and 12.3%) were detected in oxic compared to anoxic conditions (Supplementary Table 5.2), suggesting higher contribution of fresh material in oxic compared to anoxic conditions. Furthermore, the exclusive formulae in the anoxic environment were characterized by higher aromaticity (AImodwa = 0.32 vs. 0.23 for oxic conditions) and double bond equivalents (DBEwa = 9.30 vs. 8.78 for oxic conditions), and higher contribution of CRAM (44 vs. 39% for oxic conditions), all indicators of more decomposed/refractory DOM (Hertkorn et al., 2006)(Supplementary Table 5.1). Thus, the DOM in anoxic conditions appeared as a product of a more extensive degradation of the available dissolved material, resulting in a lower DOC concentrations and more refractory DOM signal left behind in anoxic porewaters.

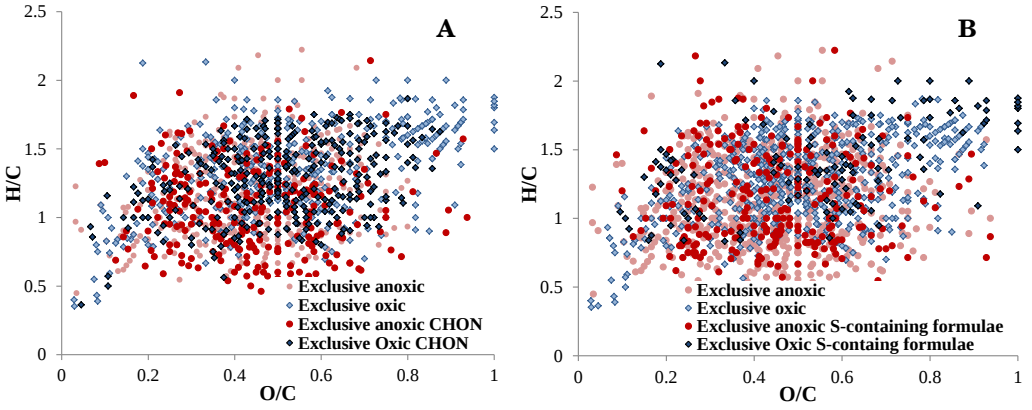


FIGURE 5.2: Van Krevelen Diagrams of H/C and O/C ratios with all exclusive molecular formulae detected under oxic and anoxic conditions. Highlighting A) CHON formulae and B) S-containing compounds for both oxic (dark blue) and anoxic conditions (dark red).

As mentioned in Chapter 3, a fraction of the particulate organic matter escapes microbial degradation under anoxic conditions. Thus, a threefold higher bulk pigment

concentration was found in permanent anoxic sediments compared to sediments exposed to oxic overlying waters (Chapter 3). Potential explanations for this contradiction between refractory DOM in porewater vs. high pigment content in sediments under anoxic conditions may be related to its sulfurization (Adam et al., 2000; Schmidt et al., 2014). Accordingly, anoxic conditions presented three-fold more sulfur-bearing compounds (CHOS, CHONS and CHOSP) compared to conditions (57 and 19%, Fig. 5.2B), suggesting that sulfurization could protect organic matter from being degraded. Thus, we calculated the sulfurization ratio to assess the extent of organic matter sulfurization in the DOM (Schmidt et al., 2014; Supplementary Equation 5.1). Our results showed higher ratios under anoxic conditions (data not shown), indicating the incorporation of reduced sulfur compounds (e.g. sulfide) into the DOM fraction.

Our results revealed differences in the molecular composition of porewater DOM and concentration of DOC under oxic and anoxic conditions. Under oxic conditions, high DOC concentrations and low extraction efficiency suggested contribution of low molecular weight compounds, apparently mediated by bacteria affiliated with taxa capable of hydrolysis and fermentative degradation. Under anoxic conditions, these compounds are less abundant suggesting their uptake by an active community of sulfate reducers, resulting in lower DOC concentrations and a more refractory DOM signal left behind in anoxic porewaters. The changes in bacterial composition and function support the hypothesis that microbial activity plays a role not only in the degradation and remineralization of organic matter, but also indirectly on its preservation. Overall these differences were strong enough to remain detectable besides the lack of vertical sediment resolution, however, further research is needed to better resolve in which phase of diagenesis this occur.

Supplementary methods

$$\frac{(C_cH_{h+2}O_{o-1}S_1 + C_cH_{h+4}O_{o-2}S_2)}{C_cH_hO_o} \tag{5.1}$$

Were $C_cH_hO_o$ is the potential precursor of the sulfurization reaction, in which for the incorporation of 1 S atom 1 oxygen atom is removed and 2 H are added (Schmidt et al., 2014).

Molecular groups considered on DOM characterization were; 1) formulae of unsaturated aliphatics (1.5< H/C <2, O/C <0.9, N=0); 2) Peptide molecular formulae (1.5< H/C <2, O/C<0.9 and N>0); 3) saturated fatty acids (H/C>2, O/C<0.9), without (saturated FA) or with heteroatoms (N, S or P) (saturated FA-CHOx); 4) formulae of sugars (O/C>0.9), without (sugars) and with heteroatoms (sugars-CHOx); 5) highly unsaturated compounds (AImod<0.5 and H/C<1.5); 6) polyphenols (0.67 $\geq$ AImod>0.5) and 7) condensed aromatics or dissolved black carbon (DBC, AImod $\geq$ 0.67), represented by 3 subgroups with either <15 (DBC-<15C), $\geq$ 15 (DBC- $\geq$ 15C) C atoms both without heteroatoms and with heteroatoms (DBC-CHOx). Additionally, unsaturated aliphatics, highly unsaturated and polyphenol formulae were separated into oxygen-rich compounds (O-rich, O/C ratio was >0.5) or oxygen-poor (O-poor, O/C ratios <0.5).

TABLE 5.2: Molecular characteristics of common and exclusive formulae.

Group formulae	No. formulae	MW_{wa}	H/C_{wa}	O/C_{wa}	AImo d_{wa}	DBE_{wa}	Average $formula_{wa}$	Total CHO	Total CHON	Total S-bearing compounds	Total CHOP	Total CRAM	Highly unsaturated O-		DBC			Poly-O-		Unsaturated Aliph. O-		Peptides
													Rich	Poor	< C15	> C15	CHOx	Rich	Poor	Rich	Poor	
Common formulae	5287	364.03	1.27	0.48	0.25	7.43	$C_{17.10}H_{21.68}O_{8.10}N_{0.33}S_{0.10}P_{0.00}$	37.1	39.2	22.6	0.5	49.2	28.0	28.0	0.7	0.6	2.9	3.0	12.1	28.0	34.4	5.2
Exclusive Oxic	678	450.39	1.33	0.51	0.23	8.78	$C_{20.72}H_{26.80}O_{10.18}N_{0.33}S_{0.10}P_{0.00}$	32.6	44.8	17.4	28	38.6	26.3	28.8	0.3	0.9	3.4	1.6	6.9	10.0	5.9	12.8
Exclusive Anoxic	630	403.43	1.16	0.44	0.32	9.30	$C_{18.60}H_{21.42}O_{7.89}N_{0.60}S_{0.76}P_{0.04}$	14.2	27.0	56.9	12	44.2	20.8	32.7	0.2	2.6	5.0	4.5	18.1	3.5	8.8	2.9

5.1 References

Adam, P., Schneckenburger, P., Schaeffer, P. and Albrecht, P.: Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules, Geochim. Cosmochim. Acta, 64(20), 3485–3503, doi:10.1016/S0016-7037(00)00443-9, 2000.

Arnosti, C.: Microbial Extracellular Enzymes and the Marine Carbon Cycle, Ann. Rev. Mar. Sci., 3(1), 401–425, doi:10.1146/annurev-marine-120709-142731, 2011.

Arnosti, C. and Repeta, D.: Extracellular enzyme activity in anaerobic bacterial cultures: evidence of pullulanase activity among mesophilic marine bacteria., Appl. Environ. Microbiol., 60(3), 1994.

Arrieta, J. M., Mayol, E., Hansman, R. L., Herndl, G. J., Dittmar, T. and Duarte, C. M.: Dilution limits dissolved organic carbon utilization in the deep ocean, Science (80-.)., 348(6232), 331–333, doi:10.1126/science.1258955, 2015.

Bernardet, J., Nakagawa, Y. and Holmes, B.: Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family, Int. J. Syst. Evol. Microbiol., 52(3), 1049–1070, doi:10.1099/ijs.0.02136-0, 2002.

Clark, D.: The fermentation pathways of *Escherichia coli*, FEMS Microbiol. Rev., 63, 223–234, 1989.

Dittmar, T. and Stubbins, A.: Dissolved Organic Matter in Aquatic Systems, in Treatise on Geochemistry, vol. 12, pp. 125–156, Elsevier Ltd., 2014.

Dittmar, T., Koch, B., Hertkorn, N. and Kattner, G.: A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater, Limnol. Oceanogr. Methods, 6(6), 230–235, doi:10.4319/lom.2008.6.230, 2008.

Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., Kettrup, A. and Hedges, J. I.: Characterization of a major refractory component of marine dissolved organic matter, Geochim. Cosmochim. Acta, 70(12), 2990–3010, doi:10.1016/j.gca.2006.03.021, 2006.

Jannasch, H. W.: Growth of marine bacteria at limiting concentrations of organic carbon in seawater, Limnol. Oceanogr., 12(2), 264–271, doi:10.4319/lo.1967.12.2.0264, 1967.

Koch, B. P. and Dittmar, T.: From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter, Rapid Commun. Mass Spectrom., 20(5), 926–932, doi:10.1002/rcm.2386, 2006.

McKew, B. a, Dumbrell, A. J., Taylor, J. D., McGenity, T. J. and Underwood, G. J. C.: Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments., FEMS Microbiol. Ecol., 84(3), 495–509, doi:10.1111/1574-6941.12077, 2013.

McLafferty, F. W. and Turecek, F.: Interpretation of mass spectra, Fourth edi., edited by F. T. F. W. McLafferty, University Science Books,, Mill Valley, California., 1993.

Megonigal, J. P., Hines, M. E. and Visscher, P. T.: Treatise on Geochemistry, Elsevier., 2003. Middelburg, J. J.: Oceanography. Escape by dilution., Science, 348(6232), 290, doi:10.1126/science.aaa9852, 2015.

Middelburg, J. J., Vlug, T., Jaco, F. and van der Nat, W. A.: Organic matter mineralization in marine systems, Glob. Planet. Change, 8(1-2), 47–58, doi:10.1016/0921-8181(93)90062-S, 1993.

Rossel, P. E., Vähätalo, A. V., Witt, M. and Dittmar, T.: Molecular composition of dissolved organic matter from a wetland plant (Juncus effusus) after photochemical and microbial decomposition (1.25 yr): Common features with deep sea dissolved organic matter, Org. Geochem., 60, 62–71, doi:10.1016/j.orggeochem.2013.04.013, 2013.

Schmidt, F., Koch, B. P., Elvert, M., Schmidt, G., Witt, M. and Hinrichs, K.-U.: Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea., Environ. Sci. Technol., 45(12), 5223–9, doi:10.1021/es2003414, 2011.

Schmidt, F., Koch, B. P., Witt, M. and Hinrichs, K.-U.: Extending the analytical window for water-soluble organic matter in sediments by aqueous Soxhlet extraction, Geochim. Cosmochim. Acta, 141, 83–96, doi:10.1016/j.gca.2014.06.009, 2014.

Seidel, M., Beck, M., Riedel, T., Waska, H., Suryaputra, I. G. N. a., Schnetger, B., Niggemann, J., Simon, M. and Dittmar, T.: Biogeochemistry of dissolved organic matter in an anoxic intertidal creek bank, Geochim. Cosmochim. Acta, 140, 418–434, doi:10.1016/j.gca.2014.05.038, 2014.

Stubbins, A. and Dittmar, T.: Low volume quantification of dissolved organic carbon and dissolved nitrogen, Limnol. Oceanogr. Methods, 10, 347–352, doi:10.4319/lom.2012.10.347, 2012.

Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H. and Kamagata, Y.: *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and

description of the new classes Anaerolineae classis nov. and Caldilineae classis nov. in the , Int. J. Syst. Evol. Microbiol., 56(Pt 6), 1331–40, doi:10.1099/ijs.0.64169-0, 2006.

Chapter 6

Discussion and Perspectives

Global warming and eutrophication promote hypoxia in aquatic systems, with projected decreases in ocean oxygenation and changes at all levels of biological organization. This scenario may have repercussions on fluxes of energy and matter and consequences for ecosystem diversity and functioning (Rabalais et al., 2014). Hypoxic areas in the world's oceans are increasing in number and periodicity (Diaz et al., 2008). At the same time, the expansion of oxygen minimum zones is reducing habitats of important fisheries resources and may negatively affect key species, with unknown consequences on overall ecosystem functioning. Ecosystem recovery after deoxygenation events is slow and the return to pre-perturbation conditions can take centuries (Moffit et al., 2015). The Crimean Shelf in the Black Sea, is naturally exposed to oxygen fluctuations, including projected future oxygenation regimes under the influence of global environmental changes (Pörtner et al., 2014). Moreover, its sediments experience the whole range of oxygen dynamics, from permanent oxic to variable hypoxic and anoxic/sulfidic conditions. Aerobic and anaerobic conditions are separated by the chemocline. Sediments impacted by the dynamic chemocline (e.g. changes between >63 - 0 μM oxygen) harbour a community that seems to be adapted to changes in oxygenation. The Black Sea thus represents a suited natural laboratory to study the interrelations between hypoxia, organic matter reactivity and benthic community structure, which was the overall aim of this PhD thesis. It comprises geochemical and molecular approaches to assess benthic functions and community structure. The presented studies emphasize the need for a characterization of ecosystems including all size classes of the community. At the same time, the characterization of the composition of organic matter, including the dissolved and particulate fractions is an important step toward a better characterization of links between benthic community structure and organic matter composition and degradation.

6.1 Quality of organic matter and its degradation, importance of timescales

Within this PhD my collaborators and I had the opportunity to assess the relation between organic matter quality and oxygen availability, from the bulk to the dissolved fraction (Chapters 3, 4 and 5). The sampled cores integrate current geochemical conditions but are also a window to past events. In well ventilated environments different sediment layers may be well mixed as a result of bioturbation by benthic fauna. But hypoxia often causes a decline/absence of macrofauna, preventing bioturbation and resulting in a distinct layering of sediment horizons. Differences in organic matter concentration and composition across different layers can be used to assess degradation and organic matter reactivity (e.g. Middelburg 1989); whereas the changes in microbial communities between sediment horizons give indications for its turnover, i.e. how many bacterial groups are shared or unique between two layers.

Comparing surface sediments along the Crimean Shelf, we observed a lower preservation of organic matter under oxic conditions, whereas under hypoxic and anoxic conditions more organic matter was preserved. These differences were strongly related to changes in the benthic community structure and function (Chapter 2 and 3). Under oxic conditions, the benthic community is capable to remineralize most of the organic carbon input ($\sim$15 mmol C m^{-2} d^{-1}; Chapter 2), explaining the differences in chlorophyll a concentration measured down-core, where background values (i.e. no change in concentration) were reached already at the surface (Chapter 3). Total oxygen uptake (diffusive and fauna-mediated oxygen consumption) decreased by 50% towards hypoxic conditions (Chapter 2), indicating that even variable hypoxic conditions (i.e. periods of a few days of hypoxia) already contribute to a decrease in mineralization, hence causing an increased carbon accumulation (Chapter 3). This is consistent with the higher contributions of surficial bulk organic carbon, pigments and total hydrolyzable amino acids (0-1 cm $\sim$ 10 years of deposition; Chapter 2 and 3) towards hypoxic and anoxic conditions. Total benthic oxygen uptake even decreased by 90% where hypoxic conditions were combined with anoxic episodes (Chapter 2). However, surficial organic carbon content did not show this gradual difference in preservation, but rather a steep increase between the permanent oxic stations and the hypoxic and anoxic sites (Chapter 3). Moreover, the lower degradation rates at low oxygen conditions were accompanied by a lack of bioturbation, shifts in macro-and meiobenthos composition and diversity, and an overall decrease in abundances of both faunal size classes (Chapter 2). Prokaryotic cell numbers on the other hand remained rather stable, as discussed below (Chapter 3). At the same time, the exposure time of organic matter to oxygen (oxygen penetration/sediment accumulation; Chapter 2) strongly decreased from >5 years in oxic conditions to

a couple of months at the onset of hypoxia, hampering aerobic degradation. Bioturbation by animals plays a key role in controlling organic matter degradation and sediment biogeochemistry. On the Crimean Shelf this coupling appeared very tight, having also an indirect effect on organic matter bioavailability (Chapter 3 and 5). Indeed, a fraction of the organic matter escaped remineralization, as its bioavailability seemed to be low under euxinic conditions, even at millennial time scales scale ($\sim$ 70 cm sediment depth, Chapter 3), suggested by proxies indicating high organic matter quality (amino acid-based degradation index, pigments and total amino acids) even at large depths. Differences in organic matter preservation were observed in surficial sediments between different oxygenation regimes, however, background values of ca. 2% Corg were reached at similar sediment depths ($\sim$ 3 cm; equivalent to 30 years of degradation) under stable oxic and anoxic/sulfidic conditions. This indicates that with time, the community of anaerobes has the potential to degrade the deposited material. Hence, highlighting the importance of taking into account the relevant timescales in assessments of organic matter degradation and the evaluation of hypoxia effects on ecosystems.

During this study we used a series of proxies to characterize organic matter quantity and quality. As mentioned before, under anoxic conditions and with increasing sediment depth (i.e. degradation time) the community of anaerobes has the potential to degrade the deposited material (Chapter 3). Indeed, we found the highest sulfate reduction rates associated with highest organic matter quality (Chapters 2, 3 and 4). However, a fraction of the organic matter escapes degradation under anoxic conditions and is buried despite its apparent high quality (i.e. high chlorophyll *a* concentrations, high DI scores). It is well known that complex organic matter constituents such as lignins are extremely resistant to anaerobic degradation (Section 1.7). In contrast, amino acids and pigments are highly labile and similar degradation rates have been observed under aerobic or anaerobic conditions (Section 1.1.4). Even though our proxies to assess the quality of bulk organic matter lack resolution to resolve its potential geopolymerization (Chapter 3), the dissolved fraction provided evidence for a decrease in bioavailability, apparently related to the absence of bioturbation at stations with low oxygen availability (Chapter 2, 3 and 5). Indeed, by using fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS; Chapter 5), we expand our analytical window to the dissolved fraction of the organic matter. Besides sampling limitations and coarse resolution of the sediment (0-10 cm sediment horizons were merged), contrasting oxygen conditions imprint their signature in the dissolved organic matter. Accordingly, anoxic conditions presented three-fold more sulfur-bearing compounds, suggesting that sulfurization could protect organic matter from being degraded. Interestingly, the dissolved organic matter composition under anoxic conditions appeared as the product of a more extensive microbial degradation (Chapter 2, 3, and 5). Thus, the availability of oxygen

plays a crucial role, not only by supplying an efficient electron acceptor or enzymatic co-factor (Section 1.1.4), but also its absence indirectly influences other organic matter degradation controlling factors (e.g. bioturbation).

6.1.1 Hypoxia and changes in ecosystem functioning

With the onset of hypoxia we observed that the benthic community decreased its oxygen uptake and changed from aerobic to anaerobic degradation pathways (Chapter 2 and 4). Fauna- and microbial-mediated oxygen uptake presented opposite trends, with a decreasing contribution of faunal respiration at lower oxygen concentrations. Under oxic conditions 70% of the total oxygen uptake was fauna-mediated, decreasing to 40% and 20% towards hypoxic and variable hypoxic/anoxic conditions. The microbial community complemented the remaining percentages and their contribution to total oxygen uptake increased from 30% at aerobic conditions to 60% and 80% at variable hypoxic / anoxic conditions (Chapter 2), indicating a shift from fauna-mediated to microbial-mediated degradation. Finally, under anoxic conditions, organic matter degradation was solely performed by microorganisms, and sulfate reduction accounted for 100% of organic matter remineralization. These effects observed under oxygen depletion and/or organic matter accumulation scenarios are in line with studies from coastal ecosystems that experienced hypoxia, such as the Gulf of Mexico, Chesapeake Bay and the Danish coast (Kemp et al., 2005; Conley et al., 2007; Turner et al., 2008), validating the present approach to study the effects of hypoxia on benthic ecosystems. The comparison of benthic communities between oxic, hypoxic and anoxic regimes helps to better assess responses in community structure and function to changing oxygen conditions. This knowledge sets the basis for better predictions of how the biota may change with the projected spread of hypoxia and dead zones. Moreover, the shift of degradation pathways from aerobic to anaerobic and from fauna to microbes was previously only inferred by models (Baird and Ulanowicz, 1989; Pearson and Rosenberg, 1992; Baird and Peterson, 2004). Considering the projected ocean deoxygenation and spreading hypoxic conditions, this thesis presents empiric evidence that will contribute toward improving our predictions of ecosystem responses to hypoxia (Diaz et al., 2008; Pörtner et al., 2014).

6.1.2 Changes in benthic community structure, from microorganisms to macrofauna

Hypoxia triggers a switch from aerobic to anaerobic degradation of organic matter in the benthic ecosystem. The system largely shifts towards a dominance of microbial activity, whereas the other functional classes (e.g. deposit feeders and burrowers) are

suppressed and the community is dominated by microbial processes. The studies within this thesis confirmed this prediction for all benthic size classes using different approaches (Chapter 2, 3 and 4). Microbial abundances were rather constant among different oxygen regimes (Chapter 3), suggesting that changes in organic matter degradation are not related to the abundance of microorganisms, but rather to their community composition, diversity and function. A special case was found in areas of the Black Sea, where labile organic matter accumulated in seafloor depressions, and the chemocline meets the sediments (Chapter 4). At those sites, the accumulated organic matter harbored two-fold higher microbial abundances and distinct thiotrophic mat-forming bacteria previously unnoticed in the Black Sea, potentially covering up to thousands of square kilometers. Interestingly, the associated microbial community of sulfate reducers and fermenters resembled communities from other sediments analyzed for this study that were exposed to anoxia and high organic matter loads (∼200 m water depth, Chapter 3), consistent with the dominance of anaerobic degradation under high deposition rates (e.g. Canfield 1994 and references therein).

The abundance and richness of macro-and meiofauna sharply decreased towards anoxic conditions (Chapter 2). Although not all taxa responded to hypoxia in the same way, no larger fauna was visualized under hypoxic conditions, probably due to the rather high oxygen requirements of bigger size classes (Vaquer-Sunyer and Duarte, 2008). Moreover, the decrease in macrofauna abundance was accompanied by a decrease in body size and taxonomic richness, thus the better adapted taxa represented by small annelids and cnidaria became dominant towards hypoxic conditions (Levin, 2003; Vaquer-Sunyer and Duarte, 2008). However, despite the fact that stable conditions allow to develop metabolic adaptations, no macrofauna was observed under anoxic conditions. The smaller fauna size class represented by meiofauna followed a similar trend, it was however less sensitive to hypoxia. Thus, fauna well adapted to hypoxia such as nematodes and foraminifera dominate meiofauna in hypoxic zones (Levin et al., 2009). Contrarily to the changes in community structure of benthic fauna (Chapter 2), microbial diversity and evenness increased towards anoxic conditions. This increase was observed both horizontally, i.e. along the oxygen gradient from the coast to off-shore stations, and vertically, i.e. from the sediment surface to deeper layers (Chapter 3). Our results agree with hypotheses emerging from pelagic and benthic ecosystems (Madrid et al., 2001; Fenchel and Finlay, 2008; Lasher et al., 2009; Ulloa et al., 2012; Wright et al., 2012), that suggest that higher microbial diversity in steep redox gradients and anaerobic environments scale with the dynamic presence of several electron donors/acceptors and metabolic pathways, increasing the number of niches. Furthermore, this study showed that with an increase in microbial diversity also microbial activity increased, resulting in

a dominance of microbial organic matter degradation towards anoxic conditions (Chapter 2, 3 and 4). This is consistent with the fact that, under oxic conditions a single organism can fully mineralize the hydrolytic products to carbon dioxide, whereas in the absence of oxygen, organic matter breakdown is performed by consortia of anaerobes (Canfield et at., 2005; Arndt et al., 2013; Arnosti et al., 2013).

The number of rare types (i.e. sequences/bacterial groups occurring once or twice in the dataset) increased toward anoxic conditions, indicating that rare members of the community may play an important role for ecosystem functioning at low oxygen concentrations (Chapter 3). Regarding bacterial composition, *Bacteroidetes* and *Proteobacteria* widely dominated in sequence number at all stations, hence confirming their relevance and resilience to varying oxygenation (e.g. Zinger et al., 2011). As mentioned before, the benthic community of fauna and microbes showed opposite responses to hypoxia. Thus, from the most abundant bacterial groups at class level represented by *Deltaproteobacteria* and *Flavobacteria*, none declined in sequence abundance towards anoxic conditions (Chapter 3). On the contrary, the group of sulfate reducing bacteria affiliated with *Desulfobacteraceae* and *Desulfobulbaceae* (*Deltaproteobacteria*) increased under anoxic conditions, corresponding to high rates of sulfur reduction at these sites (Chapter 2, 3 and 4). Hence, indicating a tight coupling between changes in community structure and function towards anoxic conditions. Sulfate reduction became the dominant mineralization process with increasing hypoxia, in agreement with previous investigations in the Black Sea and with predicted changes in ecosystem function with hypoxia. Other winners under hypoxia were affiliated with *Flavobacteriaceae* (*Flavobacteria*), by far the most abundant bacterial family in this study with ca. 50% of all MPTS counts (Chapter 3 and 4). The high overlap of $OTU_{0.03}$ affiliated with *Flavobacteria* between different oxygenation regimes, suggests that this group might perform both fermentation and aerobic degradation of organic matter when oxygen is available, consistent with the metabolic versatility of this clade (Bernardet et al., 2002; Kp̈ke et al., 2005; McKew et al., 2013). Indeed, high relative abundances of *Flavobacteriaceae* were associated with the availability of organic matter rather than oxygen at surface sediments (Chapter 3), reaching highest values where labile organic matter accumulated (Chapter 4). This is consistent with the copiotrophic characteristics of the clade (Fierer et al., 2007) and its dominance in organic rich sediments exposed to oxic, hypoxic and anoxic conditions (e.g. Julies et al., 2012 ; Sinkko et al., 2013). Thus, sulfate-reducing *Deltaproteobacteria*, such as *Desulfobacteraceae* and *Desulfobulbaceae*, and taxa with hydrolytic/fermentative capabilities such as *Flavobacteriaceae* present key community members at anoxic/hypoxic and eutrophic conditions, respectively. They may thus be referred to as part of a typical hypoxic microbiome, as it has been proposed for pelagic oxygen minimum zones (e.g. Ulloa et al., 2013). In this regard, a global approach focusing on sediments exposed to

hypoxic/anoxic conditions, and including comparisons to other benthic systems, would be needed to confirm the presence of such a hypoxic microbiome, and further define its composition / specific members.

6.2 Concluding remarks

The Black Sea is a natural laboratory that can be used to assess ecosystem changes in response to increasing hypoxia and anoxia, which are projected for the world's oceans. The results of this study confirm that with the onset of hypoxia, benthic communities experienced a loss of faunal abundance and activity to a final dominance of the microbial size spectrum. The changes in community structure were followed by changes in function and partitioning of metabolic pathways, from aerobic to anaerobic with the onset of hypoxic conditions. Moreover, the response was even visible in the form of thiotrophic microbial mats, located in places where the seafloor morphology allowed for the accumulation of labile organic matter and a release of reduced compounds such as sulfide.

Overall, with the studies presented in this PhD thesis, we were able to contribute with empirical evidence to a better evaluation of the effects of hypoxia (>63 μM oxygen) on ecosystem functions. However, further studies are needed in order to assess the effects of increasing hypoxia in a temporal context, e.g. how fast can changes in the oxygenation regime occur for adaptations to take place, and how long does it take to establish new communities at previously undisturbed sites, and if communities are able to re-establish their original state after temporary hypoxic or anoxic events.

6.3 Perspectives

The response of benthic fauna to hypoxic events and its recovery afterwards have been assessed in environmental studies (Vaquer-Sunyer and Duarte, 2008 and references therein), and also experimentally by monitoring changes in community structure and function, after hypoxia and anoxia had been induced (Stachowitsch et al., 2007; Riedel et al., 2014). However, similar approaches for microbial communities are scarce, mainly due to potential bottle effects (Stewart et al., 2012), and nano-aerophilic capabilities of some bacteria (Stolper et al., 2010). Indeed, due to these limitations, Stewart and coworkers, (2012) were not able to isolate the effect of oxygen from other factors on gene expression of pelagic microbial communities inhabiting OMZs. However, they were able to demonstrate the fast responses (day timescale) of microbial communities to environmental stressors and the applicability of metatranscriptomic approaches to assess

ecological questions regarding hypoxia. For benthic communities, Reimers and coworkers, (2013) used an elegant set up based on anodes and cathodes, and during a 2 year experiment the authors were able to change sediment redox conditions with minimum perturbation. However, no clear changes were reported for microbial community structure (based on 454 MPTS) or organic matter degradation. As a result, the authors concluded that molecular oxygen may be required to effectively catalyze the degradation of refractory organic matter and the resulting elevated redox potential alone is not sufficient. It is challenging to overcome issues related to the artificial manipulation of the supply of oxygen to the sediment without disturbing the sediment layer structure. In this regard a "thin layer incubation approach" - where a thin sediment layer (∼1.5 mm) is exposed to the overlying water - would allow to assess microbial processes under natural diffusive oxygen limitation, minimizing sediment perturbation (Kristensen et al., 1995). In addition, a next step will be the use of RNA-based approaches such as metatranscriptomics (Gilbert et al., 2008) to reveal the active fraction of the microbial community and its functional adaptations and responses to changes in oxygenation. This can be complemented by stable isotope-probing methods based on specific 16S rRNA analyses (Mag-SIP; Miyatake et al., 2013) to assess differences in substrate utilization by the proposed key microbial groups under hypoxia and eutrophication (e.g. *Desulfobacteraceae* and *Flavobacteriaceae*). These approaches coupled with the high resolution characterization of DOM (e.g. by FT-ICR-MS) will help to better resolve how organic matter is transformed under hypoxic conditions, and whether DOM composition may be linked to microbial community composition. Thus, either using natural or artificial laboratories, more research is needed to better understand ecosystem responses to oxygen depletion.

Bibliography

Adam P, Schneckenburger P, Schaeffer P, Albrecht P. (2000). Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. Geochim Cosmochim Acta 64:3485-3503.

Aller RC. (1994). Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chem Geol 114:331-345.

Archer D, Maier-Reimer E. (1994). Effect of deep-sea sedimentary calcite preservation on atmospheric CO_2 concentration. Nature 367:260-263.

Armstrong RA, Lee C, Hedges JI, Honjo S, Wakeham SG. (2001). A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. Deep Sea Res Part II Top Stud Oceanogr 49:219-236.

Arndt S, Jørgensen BB, LaRowe DE, Middelburg JJ, Pancost RD, Regnier P. (2013). Quantifying the degradation of organic matter in marine sediments: A review and synthesis. Earth-Science Rev 123:53-86.

Arnosti C. (2011). Microbial Extracellular Enzymes and the Marine Carbon Cycle. Ann Rev Mar Sci 3:401-425.

Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen a. D, Stromberger M, et al. (2013). Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. Biogeochemistry 117:5-21.

Arrieta JM, Mayol E, Hansman RL, Herndl GJ, Dittmar T, Duarte CM. (2015). Dilution limits dissolved organic carbon utilization in the deep ocean. Science (80) 348:331-333.

Arthur MA, Dean WE, Pratt LM. (1988). Geochemical and climatic effects of increased marine organic carbon burial at the Cenomanian/Turonian boundary. Nature 335:714-717.

Badertscher S, Fleitmann D, Cheng H, Edwards RL, Göktürk OM, Zumbühl a., et al. (2011). Pleistocene water intrusions from the Mediterranean and Caspian seas into the Black Sea. Nat Geosci 4:236-239.

Baird D, Christian RR, Peterson CH, Johnson GA. (2004). Consequences of hypoxia on estuarine ecosystem function: energy diversion from consumers to microbes. Ecol Appl 14:805-822.

Baird D, Ulanowicz RE. (1989). The seasonal dynamics of the Chesapeake Bay ecosystem. Ecol Monogr 59.

Ball DF. (1964). Loss-on-ignition as an estimate of organic matter and organic carbon in non-calcareous soils. J Soil Sci 15:84-92.

Baturin GN. (2007). Issue of the relationship between primary productivity of organic carbon in ocean and phosphate accumulation (Holocene-Late Jurassic). Lithol Miner Resour 42:318-348.

Belmar L, Molina V, Ulloa O. (2011). Abundance and phylogenetic identity of archaeoplankton in the permanent oxygen minimum zone of the eastern tropical South Pacific. FEMS Microbiol Ecol 78:314-26.

Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53-9.

Berelson WM, Hammond DE, McManus J, Kilgore TE. (1994). Dissolution kinetics of calcium carbonate in equatorial Pacific sediments. Global Biogeochem Cycles 8:219-235.

Bernardet J, Nakagawa Y, Holmes B. (2002). Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int J Syst Evol Microbiol 52:1049-1070.

Berner RA. (1990). Atmospheric carbon dioxide levels over phanerozoic time. Science 249:1382-6.

Berner RA. (1989). Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over phanerozoic time. Palaeogeogr Palaeoclimatol Palaeoecol 75:97-122.

Berner RA. (1980). Early Diagenesis: A Theoretical Approach. First edit. Princeton University Press: Princeton, N.J.

Berner RA. (1969). Migration of iron and sulfur within anaerobic sediments during early diagenesis. Am J Sci 267:19-42.

Berner RA, Canfield DE. (1989). A new model for atmospheric oxygen over Phanerozoic time. Am J Sci 289:333-61.

Böer SI. (2008). Investigation of the distribution and activity of the benthic microorganisms in coastal habitats. University of Bremen.

Böer SI, Hedtkamp SIC, van Beusekom JEE, Fuhrman J a, Boetius A, Ramette A. (2009). Time- and sediment depth-related variations in bacterial diversity and community structure in subtidal sands. ISME J 3:780-91.

Boetius A, Albrecht S, Bakker K, Bienhold C, Felden J, Fernández-Méndez M, et al. (2013). Export of algal biomass from the melting Arctic sea ice. Science 339:1430-2.

Bopp L, Resplandy L, Orr JC, Doney SC, Dunne JP, Gehlen M, et al. (2013). Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10:6225-6245.

Brüchert V, Jørgensen BB, Neumann K, Riechmann D, Schlösser M, Schulz H. (2003). Regulation of bacterial sulfate reduction and hydrogen sulfide fluxes in the central namibian coastal upwelling zone. Geochim Cosmochim Acta 67:4505-4518.

Brunet M-F, Cloetingh S. (2003). Integrated Peri-Tethyan Basins studies (Peri-Tethys Programme). Sediment Geol 156:1-10.

Çağatay MN, Görür N, Flecker R, Sakınç M, Tünoglu C, Ellam R, et al. (2006). Paratethyan-Mediterranean connectivity in the Sea of Marmara region (NW Turkey) during the Messinian. Sediment Geol 188-189:171-187.

Calder W. (1984). Size, function and life history. Harvard University Press.: Cambridge, MA.

Canfield DE. (1994). Factors influencing organic carbon preservation in marine sediments. Chem Geol 114:315-29.

Canfield DE, Kristensen E, Thamdrup BO. (2005). Aquatic geomicrobiology. Adv Mar Biol 48:1-599.

Canfield DE, Stewart FJ, Thamdrup BO, De Brabandere L, Dalsgaard T, Delong EF, et al. (2010). A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast. Science 330:1375-8.

Canfield DE, Thamdrup BO. (2009). Towards a consistent classification scheme for geochemical environments, or, why we wish the term “suboxic” would go away. Geobiology 7:385-392.

Capet a., Becker JW, Grégoire M. (2013). Drivers, mechanisms and long-term variability of seasonal hypoxia on the Black Sea northwestern shelf - is there any recovery after eutrophication? Biogeosciences 10:3943-3962.

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 70:6147-56.

Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, Peterson WT, et al. (2008). Emergence of anoxia in the California current large marine ecosystem. Science 319:920.

Chu PC, Ivanov LM, Margolina TM. (2005). Seasonal variability of the Black Sea chlorophyll-a concentration. J Mar Syst 56:243-261.

Conley DJ, Carstensen J, AErtebjerg G, Christensen PB, Dalsgaard T, Hansen JLS, et al. (2007). Long-term changes and impacts of hypoxia in Danish coastal waters. Ecol Appl 17:S165-S184.

Coolen MJL, Abbas B, van Bleijswijk J, Hopmans EC, Kuypers MMM, Wakeham SG, et al. (2007). Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. Environ Microbiol 9:1001-16.

Cowie GL, Hedges JI, Calvert SE. (1992). Sources and relative reactivities of amino acids, neutral sugars, and lignin in an intermittently anoxic marine environment. Geochim Cosmochim Acta 56:1963-1978.

Dauwe B, Middelburg JJ. (1998). Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol Oceanogr 43:782-798.

Demaison GJ, Moore GT. (1980). Anoxic environments and oil source bed genesis. Org Geochem 2:9-31.

Devol AH, Christensen JP. (1993). Benthic fluxes and nitrogen cycling in sediments of the continental margin of the eastern North Pacific. J Mar Res 51:345-372.

Diaz RJ, Rosenberg R. (2008). Spreading dead zones and consequences for marine ecosystems. Science 321:926-9.

Diaz RJ, Selman M, Chique. C. (2011). Global Eutrophic and Hypoxic Coastal Systems. World resources Institute. Eutrophication and Hypoxia: Nutrient Pollution in Coastal Waters.

Dittmar T, Stubbins A. (2014). Dissolved Organic Matter in Aquatic Systems. In:Treatise on Geochemistry, Vol. 12, Elsevier Ltd., pp. 125-156.

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

Eckert S, Brumsack H-J, Severmann S, Schnetger B, Marz C, Frollje H. (2013). Establishment of euxinic conditions in the Holocene Black Sea. Geology 41:431-434.

Emerson S, Hedges JI. (1988). Processes controlling the organic carbon content of open ocean sediments. Paleoceanography 3:621-634.

Emerson S, Stump C, Grootes PM, Stuiver M, Farwell GW, Schmidt FH. (1987). Estimates of degradable organic carbon in deep-sea surface sediments from 14C concentrations. Nature 329:51-53.

Falkowski PG, Algeo T, Codispoti L, Deutsch C, Emerson S, Hales B, et al. (2011). Ocean deoxygenation: Past, present, and future. Eos, Trans Am Geophys Union 92:409.

Fenchel TM, Finlay BJ. (2008). Oxygen and the spatial structure of microbial communities. Biol Rev Camb Philos Soc 83:553-69.

Fierer N, Bradford M a, Jackson RB. (2007). Toward an ecological classification of soil bacteria. Ecology 88:1354-64.

Finlay BJ, Maberly SC, Cooper JI, Copeland A, Microbial JI. (1997). Microbial Diversity and Ecosystem Function. Oikos 80:209-213.

Fischer JP, Ferdelman TG, D'Hondt S, Røy H, Wenzhöfer F. (2009). Oxygen penetration deep into the sediment of the South Pacific gyre. Biogeosciences 6:1467-1478.

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 65:4630-4636.

Friedrich J, Janssen F, Aleynik D, Bange HW, Boltacheva N, Çagatay MN, et al. (2014). Investigating hypoxia in aquatic environments: diverse approaches to addressing a complex phenomenon. Biogeosciences 11:1215-1259.

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, et al. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim Cosmochim Acta 43:1075-1090.

Fuchsman C A, Kirkpatrick JB, Brazelton WJ, Murray JW, Staley JT. (2011). Metabolic strategies of free-living and aggregate-associated bacterial communities inferred from biologic and chemical profiles in the Black Sea suboxic zone. FEMS Microbiol Ecol 78:586-603.

Fuhrman J, (1999), Marine viruses and their biogeochemical and ecological effects, Nature 399:541-8.

Gallardo VA, (1977), Large benthic microbial communities in sulphide biota under Peru-Chile Subsurface Countercurrent, Nature 268:331-332.

Gilbert D, Rabalais NN, Díaz RJ, Zhang J. (2010). Evidence for greater oxygen decline rates in the coastal ocean than in the open ocean. Biogeosciences 7:2283-2296.

Gilly WF, Beman JM, Litvin SY, Robison BH. (2013). Oceanographic and biological effects of shoaling of the oxygen minimum zone. Ann Rev Mar Sci 5:393-420.

Glud R, Gundersen JK, Barker Jørgensen B, Revsbech NP, Schulz HD. (1994). Diffusive and total oxygen uptake of deep-sea sediments in the eastern South Atlantic Ocean:in situ and laboratory measurements. Deep Sea Res Part I Oceanogr Res Pap 41:1767-1788.

Glud RN, (2008), Oxygen dynamics of marine sediments, Mar Biol Res 4:243-289.

Gobet A, Böer SI, Huse SM, van Beusekom JEE, Quince C, Sogin ML, et al. (2012). Diversity and dynamics of rare and of resident bacterial populations in coastal sands. ISME J 6:542-53.

Gobet A, Boetius A, Ramette A. (2013). Ecological coherence of diversity patterns derived from classical fingerprinting and Next Generation Sequencing techniques. Environ Microbiol.

Grégoire M, Soetaert K. (2010). Carbon, nitrogen, oxygen and sulfide budgets in the Black Sea: A biogeochemical model of the whole water column coupling the oxic and anoxic parts. Ecol Modell 221:2287-2301.

Grieshaber M, Hardewig I, Kreutzer U, Pörtner H-O. (1994). Physiological and metabolic responses to hypoxia in invertebrates. Rev Physiol Biochem Pharmacol 125:43-147.

Grote J, Jost G, Labrenz M, Herndl GJ, Jürgens K. (2008). Epsilonproteobacteria represent the major portion of chemoautotrophic bacteria in sulfidic waters of pelagic redoxclines of the Baltic and Black Seas. Appl Environ Microbiol 74:7546-51.

Hammond DE, McManus J, Berelson WM, Kilgore TE, Pope RH. (1996). Early diagenesis of organic material in equatorial Pacific sediments: stpichiometry and kinetics. Deep Sea Res Part II Top Stud Oceanogr 43:1365-1412.

Hedges JI, Keil RG. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar Chem 49:81-115.

Helly JJ, Levin LA. (2004). Global distribution of naturally occurring marine hypoxia on continental margins. Deep Sea Res Part I Oceanogr Res Pap 51:1159-1168.

Herlemann DP, Labrenz M, Jürgens K, bertilsson S, Waniek JJ, Anderson AF. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571-9.

Herman PM., Soetaert K, Middelburg JJ, Heip C, Lohse L, Epping E, et al. (2001). The seafloor as the ultimate sediment trap—using sediment properties to constrain benthic-pelagic exchange processes at the Goban Spur. Deep Sea Res Part II Top Stud Oceanogr 48:3245-3264.

Heyl K, Woelfel J, Schumann R, Karsten U. (2010). Microbial Mats from Wind Flats of the Southern Baltic Sea. In:Microbial Mats, Seckbach, J and Oren, A (eds) Cellular Origin, Life in Extreme Habitats and Astrobiology Vol. 14. Springer Netherlands: Dordrecht, pp. 301-319.

Hoegh-Guldberg O, Bruno JF. (2010). The impact of climate change on the world's marine ecosystems. Science 328:1523-8.

Ince BK, Usenti I, Eyigor A, Oz NA, Kolukirik M, Ince O. (2007). Analysis of Methanogenic Archaeal and Sulphate Reducing Bacterial Populations in Deep Sediments of the Black Sea. Geomicrobiol J 23:285-292.

IPCC (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. Cambridge, UK.

Jacob M, Soltwedel T, Boetius A, Ramette A. (2013). Biogeography of Deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic). PLoS One 8:e72779.

Jannasch HW. (1967). Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol Oceanogr 12:264-271.

Jeon S-O, Ahn T-S, Hong S-H. (2008). A novel archaeal group in the phylum Crenarchaeota found unexpectedly in an eukaryotic survey in the Cariaco Basin. J Microbiol 46:34-9.

Jørgensen BB. (1977a). Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Mar Biol 41:7-17.

Jørgensen BB. (2010). Big sulfur bacteria. ISME J 4:1083-4.

Jørgensen BB. (1982). Mineralization of organic matter in the sea bed—the role of sulphate reduction. Nature 296:643-645.

Jørgensen BB. (1977b). The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). 22:814-832.

Jørgensen BB, Fossing H, Wirsen CO, Jannasch HW. (1991). Sulfide oxidation in the anoxic Black Sea chemocline. Deep Sea Res Part A Oceanogr Res Pap 38:S1083-S1103.

Jørgensen BB, Gallardo VA. (1999). Thioploca spp.: filamentous sulfur bacteria with nitrate vacuoles. FEMS Microbiol Ecol 28:301-313.

Jørgensen BB, Weber A, Zop J. (2001). Sulfate reduction and anaerobic methane oxidation in Black Sea sediments. Deep Sea Res Part I Oceanogr Res Pap 48:2097-2120.

Julies EM, Brüchert V, Fuchs BM, Planck M, Microbiology M. (2012). Vertical shifts in the microbial community structure of organic-rich Namibian shelf sediments. African J Microbiol Res 6:3887-3897.

Julies EM, Fuchs BM, Arnosti C, Brüchert V. (2010). Organic Carbon Degradation in Anoxic Organic-Rich Shelf Sediments: Biogeochemical Rates and Microbial Abundance. Geomicrobiol J 27:303-314.

Karl DM, Knauer G, (1991), Microbial production and particle flux in the upper 350 m of the Black Sea. Deep Sea Res Part A Oceanogr Res Pap 38:S921-S942.

Karstensen J, Stramma L, Visbeck M. (2008). Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. Prog Oceanogr 77:331-350.

Keeling RF, Körtzinger A, Gruber N. (2010). Ocean Deoxygenation in a Warming World. Ann Rev Mar Sci 2:199-229.

Kemp WM, Boynton WR, Adolf JE, Boesch DF, Boicourt WC, Brush G, et al. (2005). Eutrophication of Chesapeake Bay: historical trends and ecological interactions . Mar Ecol Prog Ser 303:1-29.

King L, (1995), A mass balance of chlorophyll degradation product accumulation in Black Sea sediments. Deep Sea Res Part I Oceanogr Res Pap 42:919-942.

Kinneret L, Biol F, Fish J, Geol J, Planet E, Res DS, et al. (1998). Linking diagenetic alteration of amino acids and bulk organic matter reactivity.

Köchling T, Lara-Martín P, González-Mazo E, Amils R, Sanz JL. (2011). Microbial community composition of anoxic marine sediments in the Bay of Cádiz (Spain). Int Microbiol 14:143-154.

Koho K, Nierop KGJ, Moodley L, Middelburg JJ, Pozzato L, Soetaert K, et al. (2013). Microbial bioavailability regulates organic matter preservation in marine sediments. Biogeosciences 10:1131-1141.

Köpke B, Wilms R, Engelen B, Cypionka H, Sass H. (2005), Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. Appl Environ Microbiol 71:7819-30.

Kristensen E, Ahmed SI, Devol AH. (1995). Aerobic and Anaerobic Decomposition of Organic Matter in Marine Sediment: Which is Fastest? Limnol Oceanogr 40:1430-1437.

Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jørgensen BB, Kuenen JG, et al. (2003). Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422:608-11.

Labrenz M, Sintes E, Toetzke F, Zumsteg A, Herndl GJ, Seidler M, et al. (2010). Relevance of a crenarchaeotal subcluster related to Candidatus Nitrosopumilus maritimus to ammonia oxidation in the suboxic zone of the central Baltic Sea. ISME J 4:1496-508.

Lam P, Jensen MM, Lavik G, McGinnis DF, Müller B, Schubert CJ, et al. (2007). Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc Natl Acad Sci USA 104:7104-9.

Lee C, (1992), Controls on organic carbon preservation: The use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. Geochim Cosmochim Acta 56:3323-3335.

Lee C, Cronin C, (1984). Particulate amino acids in the sea: Effects of primary productivity and biological decomposition. J Mar Res 42:1075-1097.

Leloup J, Loy A, Knab NJ, Borowski C, Wagner M, Jørgensen BB, (2007). Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment. Black Sea. Environ Microbiol 9:131-42.

Levin LA, (2003), Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanogr Mar Biol an Annu Rev 41:1-45.

Levin LA, Ekau W, Gooday AJ, Jorissen F, Middelburg JJ, Naqvi SWA, et al, (2009). Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences 6:2063-2098.

Levin LA, Huggett CL, Wishner KF, (1991). Control of deep-sea benthic community structure by oxygen and organic-matter gradients in the eastern Pacific Ocean. J Mar Res 49:763-800.

Lin X, Wakeham SG, Putnam IF, Astor YM, Scranton MI, Chistoserdov AY, et al, (2006). Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence in situ hybridization. Appl Environ Microbiol 72:2679-90.

Liu M, Xiao T, Wu Y, Zhou F, Huang H, Bao S, et al, (2012). Temporal distribution of bacterial community structure in the Changjiang Estuary hypoxia area and the adjacent East China Sea, Environ Res Lett 7:025001.

Lopez GR, Levinton JS, (1987). Ecoloay of denosit-feeding animals in marine sediments. Q Rev Biol 62:235-260.

Mackenzie FT, Lerman A, Andersson AJ, (2004). Past and present of sediment and carbon biogeochemical cycling models. Biogeosciences 1:11-32.

Madrid VM, Taylor GT, Scranton MI, Chistoserdov AY, (2001). Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the Cariaco Basin. Appl Environ Microbiol 67:1663-74.

Manske AK, Glaeser J, Kuypers MMM, (2005). Physiology and Phylogeny of Green Sulfur Bacteria Forming a Monospecific Phototrophic Assemblage at a Depth of 100 Meters in the Black Sea Physiology and Phylogeny of Green Sulfur Bacteria Forming a Monospecific Phototrophic Assemblage at a Depth of 100 m.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben L a, et al. (2005). Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376-80.

Marschall E, Jogler M, Hessge U, Overmann J, (2010). Large-scale distribution and activity patterns of an extremely low-light-adapted population of green sulfur bacteria in the Black Sea. Environ Microbiol 12:1348-62.

Mayer LM, (1994), Surface area control of organic carbon accumulation in continental shelf sediments. Geochim Cosmochim Acta 58:1271-1284.

McKew B a, Dumbrell AJ, Taylor JD, McGenity TJ, Underwood GJC, (2013). Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. FEMS Microbiol Ecol 84:495-509.

Megonigal JP, Hines ME, Visscher PT, (2003), Treatise on Geochemistry, Elsevier. Meyers PA, (1994). Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem Geol 114:289-302.

Meysman FJR, Middelburg JJ, Heip CHR, (2006). Bioturbation: a fresh look at Darwin's last idea. Trends Ecol Evol 21:688-695.

Middelburg JJ, (1989). A simple rate model for organic matter decomposition in marine sediments, Geochim Cosmochim Acta 53:1577-1581.

Middelburg JJ, (2015). Oceanography. Escape by dilution. Science 348:290.

Middelburg JJ, Levin LA, (2009), Coastal hypoxia and sediment biogeochemistry. Biogeosciences 6:1273-1293.

Miyatake T, Macgregor BJ, Boschker HTS, (2013). Depth-related differences in organic substrate utilization by major microbial groups in intertidal marine sediment. Appl Environ Microbiol 79:389-92.

Moffitt SE, Hill TM, Roopnarine PD, Kennett JP, (2015). Response of seafloor ecosystems to abrupt global climate change. Proc Natl Acad Sci U S A. doi:10.1073/pnas.1417130112.

Moodley L, Middelburg JJ, Boschker HTS, Duineveld GCA, Pel R, Herman PMJ, et al. (2002). Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus, Mar Ecol Prog Ser 236.

Moodley L, Nigam R, Ingole B, Prakash Babu C, Panchang R, Nanajkar M, et al. (2011). Oxygen minimum seafloor ecological (mal) functioning. J Exp Mar Bio Ecol 398:91-100.

Murray JW, Stewart K, Kassakian S, Krynytzky M, DiJulio D. (2007). Oxic, Suboxic, and Anoxic Conditions in The Black Sea. In: The Black Sea Flood Question: Changes in Coastline, Climate, and Human Settlement, Yanko-Hombach, V, Gilbert, AS, Panin, N, and Dolukhanov, PM (eds), Springer Netherlands, pp. 1-21.

Mußmann M, Schulz HN, Strotmann B, Kjaer T, Nielsen LP, Rosselló-Mora RA, et al. (2003). Phylogeny and distribution of nitrate-storing Beggiatoa spp. in coastal marine sediments. Environ Microbiol 5:523-33.

Muyzer G, Stams AJM. (2008). The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441-54.

Nesterova D, Moncheva S, Mikaelyan A, Vershinin A, Akatov V, Shirshov P, et al. (2008). The state of phytoplankton. In:State of the Environment of the Black Sea (2001 - 2006/7), Oguz, T (ed), Publications of the Commission on the Protection of the Black Sea Against Pollution (BSC) 2008-3, Istanbul, Turkey, p. 448.

Nguyen RT, Harvey HR. (2001). Preservation of protein in marine systems: Hydrophobic and other noncovalent associations as major stabilizing forces. Geochim Cosmochim Acta 65:1467-1480.

Nicholas WA, Chivas AR. (2014). Chapter 14 Late Quaternary sea-level change on the Black Sea shelves. Geol Soc London, Mem 41:199-212.

Niggemann J, Ferdelman TG, Lomstein BA, Kallmeyer J, Schubert CJ. (2007). How depositional conditions control input, composition, and degradation of organic matter in sediments from the Chilean coastal upwelling region. Geochim Cosmochim Acta 71:1513-1527.

Nikishin AM, Korotaev M V, Ershov A V, Brunet M-F. (2003). The Black Sea basin: tectonic history and Neogene-Quaternary rapid subsidence modelling. Sediment Geol 156:149-168.

Oguz T, Tugrul S, Kideys AE, Ediger V, Kubilay N. (2004). Physical and biogeochemical characteristics of the Black Sea. In:The Sea, Volume 14B: The Global Coastal Ocean: Interdisciplinary Regional Studies and Synthesis, Robinson, AR and Kenneth, HB (eds), Harvard University Press, Cambridge, MA, pp. 1131-1369.

Okabe S, Nielsen PH, Jones WL, Characklis WG. (1995). Sulfide product inhibition of Desulfovibrio desulfuricans in batch and continuous cultures. Water Res 29:571-578.

Osterholz H, Dittmar T, Niggemann J. (2014). Molecular evidence for rapid dissolved organic matter turnover in Arctic fjords. Mar Chem 160:1-10.

Panin N, Jipa D. (2002). Danube River Sediment Input and its Interaction with the North-western Black Sea. Estuar Coast Shelf Sci 54:551-562.

Pantoja S, Lee C. (2003). Amino acid remineralization and organic matter lability in Chilean coastal sediments. Org Geochem 34:1047-1056.

Pearson T, Rosenberg R. (1992). Energy flow through the SE Kattegat: a comparative examination of the eutrophication of a coastal marine ecosystem. Netherlands J Sea Res 28:317-334.

Pedersen T, Calvert SE. (1990). Anoxia vs. Productivity: What Controls the Formation of Organic-Carbon-Rich Sediments and Sedimentary Rocks? Am Assoc Pet Geol Bull 74:454-466.

Pfannkuche O. (1993). Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47°N, 20°W. Deep Sea Res Part II Top Stud Oceanogr 40:135-149.

Pfannkuche O. (1985). The deep-sea meiofauna of the Porcupine Seabight and abyssal plain (NE Atlantic): population structure, distribution, standing stocks. Oceanol Acta 8:343-353.

Piiper J. (1982). Respiratory gas exchange at lungs, gills and tissues: mechanisms and adjustments. J Exp Biol 100:5-22.

Pörtner H-O, Karl D, Boyd PW, Cheung W, Lluch- Cota SE, Nojiri Y, et al. (2014). Ocean sytems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change, C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. Maccracken, PRM and LLW (ed), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Pörtner H-O, Heisler N, Grieshaber MK. (1985). Oxygen consumption and mode of energy production in the intertidal worm Sipunculus nudus L.: definition and characterization of the critical PO_2 for an oxyconformer. Physiol Neurobiol 59:361-377.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188-96.

Quaiser A, Zivanovic Y, Moreira D, López-García P. (2011). Comparative metagenomics of bathypelagic plankton and bottom sediment from the Sea of Marmara. ISME J 5:285-304.

Rabalais NN, Cai W-J, Carstensen J, Conley D, Fry B, Hu X, et al. (2014). Eutrophication-Driven Deoxygenation in the Coastal Ocean. Oceanography 27:172-183.

Rabalais NN, Turner RE, Diaz RJ, Justic D. (2009). Global change and eutrophication of coastal waters. ICES J Mar Sci 66:1528-1537.

Ramette A. (2009). Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Appl Environ Microbiol 75:2495-505.

Reese, B. K., Mills, H. J., Dowd, S. E. and Morse, J. W.: Linking Molecular Microbial Ecology to Geochemistry in a Coastal Hypoxic Zone, Geomicrobiol. J., 30(2), 160-172.

Reimers CE, Alleau Y, Bauer JE, Delaney J, Girguis PR, Schrader PS, et al. (2013). Redox effects on the microbial degradation of refractory organic matter in marine sediments. Geochim Cosmochim Acta 121:582-598.

Reimers CE, Suess E. (1983). The partitioning of organic carbon fluxes and sedimentary organic matter decomposition rates in the ocean. Mar Chem 13:141-168.

Revsbech NP. (1989). An oxygen microsensor with a guard cathode. Limnol Oceanogr 34:474-478.

Rhein M, Rintoul SR, Aoki S, Campos E, Chambers D, Feely RA, et al. (2013). Observations: Ocean. In:Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, VB and PMM (ed), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., pp. 255-316.

Ridgwell A, Hargreaves JC. (2007). Regulation of atmospheric CO2 by deep-sea sediments in an Earth system model. Global Biogeochem Cycles 21.

Ridgwell A, Zeebe RE. (2005). The role of the global carbonate cycle in the regulation and evolution of the Earth system. Earth Planet Sci Lett 234:299-315.

Riedel B, Pados T, Pretterebner K, Schiemer L, Steckbauer A, Haselmair A, et al. (2014). Effect of hypoxia and anoxia on invertebrate behaviour: ecological perspectives from species to community level. Biogeosciences 11:1491-1518.

Ross DA, Degens ET. (1974). Recent sediments of the Black Sea. In:The Black Sea Geology, Chemistry and Biology, Degens, ET and Ross, DA (eds), American Association of Petroleum Geologists, pp. 183-199.

Sachs JP, Repeta DJ. (2000). The purification of chlorins from marine particles and sediments for nitrogen and carbon isotopic analysis. Org Geochem 31:317-329.

Salman V, Amann R, Girnth A-C, Polerecky L, Bailey J V, Høgslund S, et al. (2011). A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria. Syst Appl Microbiol 34:243-59.

Sanger F, Nicklen S, Coulson AR. (1977). DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463-7.

Scheffer M. (2010). Complex systems: Foreseeing tipping points. Nature 467:411-2.

Schippers A, Kock D, Höft C, Köweker G, Siegert M. (2012). Quantification of Microbial Communities in Subsurface Marine Sediments of the Black Sea and off Namibia. Front Microbiol 3:16.

Schloss PD, Gevers D, Westcott SL. (2011). Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One 6:e27310.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-41.

Schmidt F, Koch BP, Elvert M, Schmidt G, Witt M, Hinrichs K-U. (2011). Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea. Environ Sci Technol 45:5223-9.

Schmidt F, Koch BP, Witt M, Hinrichs K-U. (2014). Extending the analytical window for water-soluble organic matter in sediments by aqueous Soxhlet extraction. Geochim Cosmochim Acta 141:83-96.

Schubert CJ, Coolen MJL, Neretin LN, Schippers A, Abbas B, Durisch-Kaiser E, et al. (2006). Aerobic and anaerobic methanotrophs in the Black Sea water column. Environ Microbiol 8:1844-56.

Schubert CJ, Niggemann J, Klockgether G, Ferdelman TG. (2005). Chlorin Index: A new parameter for organic matter freshness in sediments. Geochemistry, Geophys Geosystems 6:Q03005.

Schulz HN, Brinkhoff T, Ferdelman TG, Mariné MH, Teske A, Jørgensen BB. (1999). Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments. Science (80) 284:493-495.

Schulz HN, Jørgensen BB. (2001). Big bacteria. Annu Rev Microbiol 55:105-37.

Sergeeva NG, Gooday AJ, Mazlumyan SA, Elena A, Lichtschlag A, Kosheleva TN, et al. (2012). Meiobenthos of the Oxic/Anoxic Interface in the South- western Region of the Black Sea: Abundance and Taxonomic Composition. In:Anoxia: Evidence for Eukaryote Survival and Paleontological Strategies, Cellular Origin, Life in Extreme Habitats and Astrobiology, Altenbach, A V., Bernhard, JM, and Seckbach, J (eds) Cellular Origin, Life in Extreme Habitats and Astrobiology Vol. 21, Springer Netherlands: Dordrecht, pp. 369-401.

Sergeeva NG, Zaika V. (2013). The Black Sea Meiobenthos in Permanently Hypoxic Habitat. Acta zoolica Bulg 65:139-150.

Shaffer G, Olsen SM, Pedersen JOP. (2009). Long-term ocean oxygen depletion in response to carbon dioxide emissions from fossil fuels. Nat Geosci 2:105-109.

Siegenthaler U, Sarmiento JL. (1993). Atmospheric carbon dioxide and the ocean. Nature 365:119-125.

Sinkko H, Lukkari K, Sihvonen LM, Sivonen K, Leivuori M, Rantanen M, et al. (2013). Bacteria contribute to sediment nutrient release and reflect progressed eutrophication-driven hypoxia in an organic-rich continental sea. PLoS One 8:e67061.

Sorokin JI. (1964). On the Primary Production and Bacterial Activities in the Black Sea. ICES J Mar Sci 29:41-60.

Sorokin YI. (1972). The Bacterial Population and the Processes of Hydrogen Sulphide Oxidation in the Black Sea. ICES J Mar Sci 34:423-454.

Stachowitsch M, Riedel B, Zuschin M, Machan R. (2007). Oxygen depletion and benthic mortalities: the first in situ experimental approach to documenting an elusive phenomenon. Limnol Oceanogr Methods 5:344-352.

Stanev EV. (1990). On the mechanisms of the Black Sea circulation. Earth-Science Rev 28:285-319.

Stanev EV. (2005). Understanding Black Sea Dynamics: Overview of Recent Numerical Modeling. Oceanography 18:56-75.

Stanev EV, He Y, Staneva J, Yakushev E. (2014). The Black Sea biogeochemistry: focus on temporal and spatial variability of oxygen. Biogeosciences Discuss 11:281-336.

Stevens H, Ulloa O. (2008). Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol 10:1244-59.

Stewart FJ, Dalsgaard T, Young CR, Thamdrup B, Revsbech NP, Ulloa O, et al. (2012). Experimental incubations elicit profound changes in community transcription in OMZ bacterioplankton. PLoS One 7:e37118.

Stewart FJ, Ulloa O, DeLong EF. (2012). Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ Microbiol 14:23-40.

Stolper D a, Revsbech NP, Canfield DE. (2010). Aerobic growth at nanomolar oxygen concentrations. Proc Natl Acad Sci U S A 107:18755-60.

Stramma L, Prince ED, Schmidtko S, Luo J, Hoolihan JP, Visbeck M, et al. (2011). Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. Nat Clim Chang 2:33-37.

Suess E. (1980). Particulate organic carbon flux in the oceans—surface productivity and oxygen utilization. Nature 288:260-263.

Sun M-Y, Aller RC, Lee C, Wakeham SG. (2002). Effects of oxygen and redox oscillation on degradation of cell-associated lipids in surficial marine sediments. Geochim Cosmochim Acta 66:2003-2012.

Sverdrup HU. (1938). On the Explanation of the Oxygen Minima and Maxima in the Oceans. ICES J Mar Sci 13:163-172.

Tanase A, Vassu T, Trasca C. (2009). In Situ Visualization of Natural Microbial Communities in Black Sea Coastal Shelf Sediments. Rom Biotechnol Lett 14:4187-4193.

Thamdrup BO, Canfield DE. (1996). Pathways of carbon oxidation in continental margin sediments off central Chile. Limnol Oceanogr 41:1629-1650.

Thamdrup BO, Rosselló-Mora RA, Amann R. (2000). Microbial Manganese and Sulfate Reduction in Black Sea Shelf Sediments. Appl Environ Microbiol 66:2888-2897.

Turner RE, Rabalais NN, Justic. D. (2008). Gulf of Mexico hypoxia: alternate states and a legacy. Environ Sci and Technol 42.

Ulloa O, Canfield DE, DeLong EF, Letelier RM, Stewart FJ. (2012). Microbial oceanography of anoxic oxygen minimum zones. Proc Natl Acad Sci U S A 109:15996-16003.

Ulloa O, Wright JJ, Belmar L, Hallam SJ. (2013). The Prokaryotes. In:The Prokaryotes-Prokaryotic Communities and Ecophysiology, Rosenberg, E, DeLong, EF, Lory, S, Stackebrandt, E, and Thompson, F (eds), Springer Berlin Heidelberg: Berlin, Heidelberg.

Vandewiele S, Cowie GL, Soetaert K, Middelburg JJ. (2009). Amino acid biogeochemistry and organic matter degradation state across the Pakistan margin oxygen minimum zone. Deep Sea Res Part II Top Stud Oceanogr 56:376-392.

Vaquer-Sunyer R, Duarte CM. (2008). Thresholds of hypoxia for marine biodiversity. Proc Natl Acad Sci USA 105:15452-7.

Vetriani C, Tran H V, Kerkhof LJ. (2003). Fingerprinting Microbial Assemblages from the Oxic/Anoxic Chemocline of the Black Sea. 69. doi:10.1128/AEM.69.11.6481.

Wakeham SG, Lee C, Hedges JI, Hernes PJ, Peterson MJ. (1997). Molecular indicators of diagenetic status in marine organic matter. Geochim Cosmochim Acta 61:5363-5369.

Wallmann K, Pinero E, Burwicz E, Haeckel M, Hensen C, Dale A, et al. (2012). The Global Inventory of Methane Hydrate in Marine Sediments: A Theoretical Approach. Energies 5:2449-2498.

Weber A, Riess W, Wenzho F, Jørgensen BB. (2001). Sulfate reduction in Black Sea sediments: in situ and laboratory radiotracer measurements from the shelf to 2000 m depth. Deep Sea Res Part I Oceanogr Res Pap 48:2073-2096.

Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz G. (1991). Molecular architecture and electrostatic properties of a bacterial porin. Science (80) 254:1627-1630.

Wenzhöfer F, Glud RN. (2002). Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. Deep Sea Res Part I Oceanogr Res Pap 49:1255-1279.

Westrich JT, Berner RA. (1984). The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol Oceanogr 29:236-249.

Williams LA, Reimers CE. (1983). Role of bacterial mats in oxygen-deficient marine basins and coastal upwelling regimes: Preliminary report. Geology 11:267.

Witte U, Wenzhöfer F, Sommer S, Boetius A, Heinz P, Aberle N, et al. (2003). In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature 424:763-6.

Woebken D, Fuchs BM, Kuypers MMM, Amann R. (2007). Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl Environ Microbiol 73:4648-57.

Woese CR. (1987). Bacterial evolution. Microbiol Rev 51:221-71.

Woese CR, Kandlert O, Wheelis ML. (1990). Towards a natural system of organisms: Proposal for the domains. Proc Natl Acad Sci 87:4576-4579.

Wollast R. (2002). Continental margins: review of geochemical settings. In:Ocean margin systems, Wefer, G et al. (ed), Springer: Berlin, pp. 15-31.

Wollast R. (1998). Evaluation and comparison of the global carbon cycle in the coastal zone and in the open ocean. In:The Sea, Volume 10, KH, B and AR, R (eds), Wiley: NY, USA, pp. 213-252.

Wright JJ, Konwar KM, Hallam SJ. (2012). Microbial ecology of expanding oxygen minimum zones. Nat Rev Microbiol 10:381-94.

Wyrtki K. (1962). The oxygen minima in relation to ocean circulation. Deep Sea Res 9:11-23.

Yanko-Hombach V, Gilbert AS, Dolukhanov P. (2007). Controversy over the great flood hypotheses in the Black Sea in light of geological, paleontological, and archaeological evidence. Quat Int 167-168:91-113.

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer K-H, et al. (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635-645.

Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, Welch DBM, et al. (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS One 6:e24570.

Acknowledgements

During the hard days of writing I found peace thinking about all the people who directly or indirectly were involved to make this possible. Thank you all.

Thank you Antje, for rescuing from the ruins of my "earthquaked" hometown to explore the Black Sea. For your guidance and support, thank you.

Silvio Pantoja and Alban Ramette, thank you for introducing me into geochemistry and multivariate statistics, advice and also the friendly relationship.

Prof. Ulrich Fischer for agreeing to review my thesis and Prof. Hans-O. Pörtner, Katy Hoffmann and Anastasia Resteu for being part of my defense committee. Also to Dr. Alban Ramette for being almost part as well.

CONICYT Becas Chile and The Max Planck Society for the funding.

Bernd Stickford, for finding the unfindable literature, Christiane Glöckner, Anita Tingberg and Ulrike Tietjen for your help since the very beginning. Kim, Joe and Burak for contributing to my thesis during their lab rotations

All TA's from the HGF MPG Joint Research Group for Deep-Sea Ecology and Technology (formerly known as HABITAT Group), in special to Erika, Wiebke and Rafael, Martina, Mirja and of course Axel and Volker for your help in the lab, the sea and the land (and with my bikes). Lily and Paulina, thanks for your help en Conce. Of course, all my MPI fellows; Mari I thank you in my thesis not like you, Ulrike for the mate tea that keep me awake during the last weeks, Anna for the feedback, Felix and Felix, ciao Massi, ciao Stefano!, zdravo Petra, Christiane for your help with all those R questions. Pier and Gunter, for your precise answers to my imprecise questions, Gunter again for the last comments. Emil, for the nice times playing music and science. Josi, Katy, Rafa, you guys really made my day so many times, thanks a lot for your help on the final rush. Viola, for all our office to office shouting chats and mutual support praying the mantra "we are going to make it", yes, we are going to make it.

Antonio, for your help on everything related to make computers understand what one want to do and of course, todas esas buenas birras tío. Also, Lisa for your help with la computación. Ana and Sven, our time in Bremen will be forever.

My band Verbena, for balance my life with music. Same to my MPI music fellows, Alban, Diana, Zwiebel, Basti, Clara, Dima, Emiliano, Emil, Ivo, was great to play with you guys. Alban, I'll never forget our gig with the Bremen musicians.

Dani, I will be all my life in debt with you.

Christina, for all your time hearing and reading me, specially when I need it more, for all the laughs and great moments during my years here, thank you. I am sure our lives will cross again as spokes in a bike.

Pamela, I am so lucky to have you close as scientist and friend, gracias de verdad. Xavi gracias amigazo, Gema, Asturias es España y lo sabemos. Claudia, Sven, Alex, all those never ending weekends really keep me away from getting crazy (getting crazy). Gonzalo, Pedro, Oscar, Pablo, Oscar, gracias por todas esas visitas salvadoras, Finuras!. Oh Natalie, I though it would be impossible to find someone with my sense of humor and also love for drums, great moments every moment. Thank you for the cover design and of course Shortriver for the beautiful illustration.

Daphne, our friendship cannot be described with the known words, reason why from el empieezo we ended up using a weird language...nilaiam saicrag infiniDas amiguiDa linda.

Mamá, Papá, Vero, Gonzo, mucho de lo que soy es por y para por ustedes.

Paula, vacío vivo sin ti.

Appendix

Cruise participations

R/V M.S.Merian MSM 15/1, Black Sea (7 April-8 May 2010)

Additional studies not included as manuscripts

A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria

Verena Salman[a], Rudolf Amann[a], Anne-Christin Girnth[a], Lubos Polerecky[a],Jake V. Bailey[b], Signe Høgslund[c], Gerdhard Jessen[a], Silvio Pantoja[d], Heide N. Schulz-Vogt[a]

[a] Max Planck Institute for Marine Microbiology, Celsiusstraße 1, D-28359 Bremen, Germany

[b] Department of Geology and Geophysics, University of Minnesota - Twin Cities, 310 Pillsbury Drive SE, MN 55455, USA

[c] Department of Biological Sciences, Aarhus University, Ny Munkegade 114, DK-8000 Aarhus, Denmark

[d] Department of Oceanography and Center for Oceanographic Research in the Eastern South Pacific, University of Concepción, Casilla 160-C, Concepción, Chile

Systematic and Applied Microbiology **2011, 34(4); 243-259**

Abstract

The colorless, large sulfur bacteria are well known because of their intriguing appearance, size and abundance in sulfidic settings. Since their discovery in 1803 these bacteria have been classified according to their conspicuous morphology. However, in microbiology the use of morphological criteria alone to predict phylogenetic relatedness has frequently proven to be misleading. Recent sequencing of a number of 16S rRNA genes of large sulfur bacteria revealed frequent inconsistencies between the morphologically determined taxonomy of genera and the genetically derived classification. Nevertheless, newly described bacteria were classified based on their morphological properties, leading to polyphyletic taxa. We performed sequencing of 16S rRNA genes and internal transcribed spacer (ITS) regions, together with detailed morphological analysis of hand-picked individuals of novel non-filamentous as well as known filamentous large sulfur bacteria, including the hitherto only partially sequenced species *Thiomargarita namibiensis*, *Thioploca araucae* and *Thioploca chileae*. Based on 128 nearly full-length 16S rRNA-ITS sequences, we propose the retention of the family Beggiatoaceae for the genera closely related to Beggiatoa, as opposed to the recently suggested fusion of all colorless sulfur bacteria into one family, the *Thiotrichaceae*. Furthermore, we propose the addition of nine *Candidatus* species along with seven new *Candidatus* genera to the family *Beggiatoaceae*. The extended family *Beggiatoaceae* thus remains monophyletic and is phylogenetically clearly separated from other related families.

Workshops and Conferences

May 2011, Annual HYPOX meeting, Horw, Switzerland (Oral presentation)

August 2011, Goldschmidt Conference, Prague, Czech Republic. (Poster)

March 2012, HYPOX Final annual meeting, Rome, Italy

April 2012 European Geosciences Union General Assembly 2012 (EGU), Vienna, Austria. (Oral presentation)

September 2013 SAME; Aquatic Microbial Ecology 2013, Stressa, Italy. (Poster)

May 2014 GHER Colloquium, Marine Environmental Monitoring, Modelling and Prediction, Liège, Belgium. (Oral presentation)

Erklärung

Hiermit erkläre ich, Gerdhard L. Jessen Reyes, dass ich

1. die Arbeit selbstständig verfasst und geschrieben habe,

2. keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet habe und

3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe und

4. die 3 vorgelegten Exemplare dieser Arbeit in identischer Ausführung abgegeben habe.

Ort, Datum Unterschrift

2015

Cover illustration by Shortriver
Cover design by Natalie de Gregorio