

**BIOGEOCHEMICAL CONTROLS ON
CARBON AND SULFUR CYCLING
IN BALTIC SEA SEDIMENTS**

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BIOGEOCHEMICAL CONTROLS ON CARBON AND SULFUR CYCLING IN BALTIC SEA SEDIMENTS

Dissertation

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“What we know is a drop, what we don't know is an ocean”

Isaac Newton

“Success begins the new challenges”

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SUMMARY

The Baltic Sea is the largest brackish inland sea in the world with the salinity gradient decrease from 27‰ in Danish Strain to < 5‰ in the Bothnia. Over the last 100 years, the Baltic Sea has received a large amount of nutrients from surrounding watersheds (e.g domestic waste, agriculture) that has increased the primary production, and eutrophication in the water column. Thus, the seafloor of Baltic Sea has received large amounts of organic matter that lead to the enhancement of gas bearing sediments (H₂S, CH₄, etc). In turn, the gas release from sediments may impact ecosystems and climate. The biogeochemical and geophysical controls representing the formation of shallow gas-bearing sediments were the major goal of EU project - BONUS - Baltic Gas project. An important aspect was to study the link between organic carbon delivery into the Baltic Sea floor and S, CH₄ and C cycling as function of salinity gradients and sediment accumulation rate.

Several sampling campaigns throughout the Baltic Sea took place in 2009 and 2010 from the Baltic Sea – North Sea transition zone to the Northern Baltic Sea (Aarhus Bay, Merkenburg, Bornholm Basin, Gdansk Basin, Gotland Deep, Himmerfjärden estuary, Bothnia Sea and Bothnia Bay) to test hypotheses covering the factors that impact biogeochemistry of carbon and sulfur cycling in the Baltic Sea sediments. Overall, wet and solid geochemistry of C, and S were used to delineate the distribution of C and S species in the sediments. The radiotracer methods (¹⁴C, ³⁵S) were also used to determine the turnover rate of C and S cycling. The abundance of radionuclides of ²¹⁰Pb, ¹³⁷Cs were used to calculate the sediment accumulation rate, whereas the natural stable isotopes of ³⁴S, ³²S were used as an indicator of S cycling. Additionally, one dimensional modeling and calculations were also used to estimate rates.

Several outcomes of my study could clarify the biogeochemical controls on C and S cycling in the Baltic Sea. I examined: 1) The impact of sediment and organic matter fluxes on methane and sulfur cycling in Himmerfjärden estuary sediments; 2) The role of sulfate penetration depth on carbon preservation and sulfur burial in the sediments of in the Baltic Sea; and 3) The impacts of reactive Fe reactivity on sulfurization of organic matter and oxidative sulfur cycling in Gdansk Basin sediments.

Himmerfjärden (Swedish coast) represents a littoral of the central Baltic Sea. High sediment accumulation rates (0.65-0.95 cm a⁻¹) is resulted from high primary production and

Summary

sediment delivery from the surrounding watershed. Likewise, the low concentration of sulfate of the overlying water (4.3 - 4.8 mM) and rapid depletion in top 20 cm depth leads to an increase of methane concentration ($>2 \text{ mmol L}^{-1}$) within in the top 20 cm sediment and a steep gradient through the sulfate zone. Although the rate of bicarbonate methanogenesis integrated over 1 m depth were low (0.96 -1.09), sulfate reduction rates in the upper 14 cm depth were also low ($1.46 -1.92 \text{ mmol m}^{-2} \text{ a}^{-1}$). Additionally, bioirrigation due to the invasive polychaete *Marenzelleria* enhances flux of methane to the sediment-water interface ($0.32 -0.78 \text{ mmol m}^{-2} \text{ a}^{-1}$). High sediment accumulation rates also limited organic matter exposure time in the sulfate reduction and led to high rates of organic carbon and reduced sulfur preservation. These high rates of sediment accumulation and organic carbon burial distinguish the littoral Himmerfjärden sediments from the central Baltic Sea and typical continental margin sediments. The Himmerfjärden littoral sediments, therefore, are distinguished from central basin Baltic Sea sediments of the Baltic and typical continental shelf margin sediments.

The brackish conditions of the Baltic Sea (3-21 mM sulfate concentration at the sediment –water interface) and high sediment accumulation rates ($0.01 - 0.95 \text{ cm}^{-1}$) tend to limit sulfate penetration depletion (typically 0.17 -1.47 mbsf). Due to high sediment accumulation rates throughout the Baltic Sea (most $> 0.1 \text{ g cm}^{-1} \text{ a}^{-1}$), anaerobic processes such as microbially mediated sulfate reduction contribute significantly to C_{org} degradation in organic rich sediments. We tested the idea that sulfate penetration depth impacts organic carbon preservation (15-93%). A transport - reaction model was applied to fit dissolved inorganic carbon and sulfate pore water profiles to estimate total organic carbon degradation rates. Combined with organic carbon accumulation rates, we estimated the efficiency of organic carbon preservation. We calculated high efficiencies of C_{org} preservation in the Baltic Sea. Overall, we conclude that it is the exposure time of the C_{org} rich sediments to sulfate (sulfate exposure time), that is a good predictor of C_{org} preservation.

In contrast, variations of local sulfate inputs from bottom seawater, groundwater discharge and deeper ice lake sediments have established a unique environment in the Gdansk sediments that potentially sustain both reductive and oxidative sulfur cycling. In addition to sulfate profiles, the geochemistry of carbon and Fe was highly variable in Gdansk Basin sediments. Reactive iron was shown to be the major possible factor to control sulfurization of organic matter due to FeOOH reduction coupled sulfide oxidation and oxidative sulfur cycling via enhancement of sulfide oxidation. These additional sulfide oxidation pathways apparently play an important role in isotope fractionation of S cycling in the deeper sediments.

Summary

In the Baltic Sea, low sulfate penetration, and high rate of sediment accumulation rates, are the master variables that control the biogeochemistry of carbon and sulfur cycling; in particular, they may significantly allow for high organic carbon preservation and eventual methane release into the water column and in the Baltic Sea.

ZUSAMMENFASSUNG

Die Ostsee ist das größte offene Brackwasser der Welt mit einem ungewöhnlich großen Salinitätsgradienten, der von 27‰ in der Dänischen Meerstrasse, bis zu weniger als 5‰ im Bottnischen Meerbusen reicht. In den letzten 100 Jahren flossen von den umliegenden Wassereinzugsgebieten beträchtliche Mengen Nährstoffe in die Ostsee (Abfallstoffe von Landwirtschaft und grösseren Städten). Als Konsequenz beobachtete man in der Wassersäule eine Zunahme der Primärproduktion und zunehmend auch Eutrophierung. Dadurch lagerte sich eine große Menge an organischem Material am Meeresboden der Ostsee ab, das den Gasgehalt im Sediment erhöhte (H_2S , CH_4 , etc.). Man vermutet, dass der zunehmende Gasgehalt in den Sedimenten das bestehende Ökosystem ändern und das Klima beeinflussen könnte. Diese Effekte zu analysieren waren die wichtigsten Ziele des EU Projektes BONUS – Baltic Gas. Von besonderem Interesse war es die Beziehung zwischen dem Fluss organischen Kohlenstoffs an den Meeresboden der Ostsee und den Kreislauf von Schwefel (S) und Kohlenstoff (C) als Funktion des Salinitätsgradienten und der Sedimentierungsrate zu bestimmen.

In den Jahren 2009 und 2010 fanden mehrere Kampagnen statt, bei denen ich Proben am Übergang von Ostsee zur Nordsee bis in den Nordteil der Ostsee (Bucht von Aarhus, Mecklenburg, Bornholm Becken, Danziger Becken, Gotlandtief, Himmerfjärden Mündung und Bottnischer Meerbusen) nahm. Ziel war es die Hypothese zu testen, ob die zunehmende Ablagerung von organischem Material in der Ostsee den Schwefel- und Kohlenstoffkreislauf in den Sedimenten beeinflusst. Ich untersuchte die Verbindungen von C und S im Porenwasser und in Sedimenten um die Verteilung der spezifischen Verbindungen zu bestimmen. Mit Hilfe von radioaktivem ^{14}C und ^{35}S wurden die Umsatzraten von C und S bestimmt. Das Vorkommen der stabilen Isotope ^{210}Pb und ^{137}Cs nutzte ich um die Sedimentierungsrate zu berechnen, währenddessen die stabilen Isotope ^{34}S und ^{32}S zur Identifizierung von Prozessen des S-Kreislaufs verwendet wurden. Zusätzlich konnte ich mit Hilfe von eindimensionalen Modellierungen und Massenbilanzen die geschätzten Umsatzraten bestätigen.

Die Resultate meiner Studie konnten die biogeochemischen Kontrollfaktoren für den S- und C-Kreislauf in der Ostsee erkenntlich machen. Diese Kontrollfaktoren werden in den einzelnen Teilen meiner Arbeit wiedergegeben: 1) Der Einfluss der Sedimentierung und des

Summary

Fluxes von organischem Material auf Methan und auf den Schwefelkreislauf in den Sedimenten der Ostsee (Himmerfjärden, Sweden) (“The impact of sediment and organic matter fluxes on methane and sulfur cycling in littoral Baltic Sea sediments (Himmerfjärden, Sweden)“); 2) die Rolle der Sulfat Expositionszeit auf die Einlagerung von Kohlenstoff und Schwefel in diversen Untersuchungsgebieten der Ostsee (“The role of sulfate exposure time on carbon preservation and sulfur burial in Baltic Sea sediment“); und 3) Der Einfluss der reaktiven Eisen auf die Schwefelung von organischem Kohlenstoff und der oxidative Schwefelkreislauf in den Sedimenten des Danziger Becken (“The impact of reactive iron on sulfurization of organic matter and oxidative sulfur cycling in Gdansk Basin sediments“)

Die Himmerfjärdenmündung liegt an der Schwedischen Küste im mittleren Bereich der Ostsee. Der grosse Partikeleintrag aus dem anliegenden Einzugsgebiet und die große Primärproduktion führen zu einer ausgesprochen hohen Sedimentierungsrate (0.65-0.95 cm J⁻¹). Vermutlich trägt der hohe Partikeleintrag auch zur geringen Sulfatkonzentration im lokalen Meerwasser bei (4.3 – 4.8 mM), die sehr schnell in den obersten 20 cm des Sediments aufgebraucht wird und den hohen Methangehalt erklärt, der unterhalb 20 cm Sedimenttiefe über 2 mM beträgt und in der Sulfatzone rasch abnimmt. Die über einen Meter Tiefe integrierte Bikarbonat-Methanogenese (0.96 – 1.09 mmol m⁻² J⁻¹) war ähnlich gering wie die Sulfat-Reduktionsrate in den oberen 14 cm (1.46 – 1.92 mmol m⁻² J⁻¹). Dazu kommt, dass durch die Bioturbation der invasive Polychaete *Marenzelleria* Methan aus dem Sediment in die Wassersäule verloren geht (0.32 - 0.78 mmol m⁻² J⁻¹). Die hohe Sedimentierungsrate limitiert auch die Zeit in der das organische Material durch Sulfatreduktion verbraucht wird und führt zu hohen Einbettungsraten von organischem C und reduziertem S im Sediment. Die zuvor genannten Charakteristiken unterscheiden die Sedimente in der Himmerfjärden Mündung von den Sedimenten des zentralen Beckens der Ostsee und den Rändern des flachen Schelfmeeres.

Die Brackwasserbedingungen (3 – 21 mM Sulfatkonzentration an der Sediment-Wasser-Grenze) und die hohen Sedimentierungsraten (0.01 - 0.95 cm⁻¹) limitieren die Eindringtiefe des Sulfats in das Sediment (0.17 – 1.47 mbsf). Ich untersuchte die Möglichkeit, dass die Eindringtiefe von Sulfat für die hohe Konservierung von organischem Kohlenstoff (15 – 93%) verantwortlich ist. Die hohe Sedimentierungsrate, die man überall in der Ostsee antrifft (meistens über 0.1 g cm⁻¹ a⁻¹), und die anaeroben Prozesse wie die Reduktion von Sulfat durch Mikroorganismen steuern zum Abbau von organischem C in den organisch reichen Sedimenten bei. Ein Transport – Reaktion Model wurde angewandt um mit den

Summary

Profilen vom gelösten anorganischen C (DIC) und von der Sulfatkonzentration im Porenwasser die Gesamtrate des Abbaus von organischem C zu schätzen. In Kombination mit der Akkumulierungsrate von organischem C konnte ich die Effizienz der Erhaltung von organischem C in den Sedimenten der Ostsee berechnen. Insgesamt kann ich folgern dass die Expositionszeit von organischen C im Sediment zu Sulfat (Sulfat Expositionszeit) eine gute Voraussage der Erhaltung von organischem C erlaubt.

Einen großen Kontrast dazu findet man im Danziger Becken. Die Variationen der lokalen Sulfateinträge aus tiefem Meerwasser, Grundwasser, und aus tiefen Gletscherseesedimente erschaffen ein einzigartiges Milieu in den Sedimenten des Danziger Beckens, das gleichzeitig reduktive und oxidative Schwefelprozesse ermöglicht. Daneben spielen auch Variationen der geochemischen C und Eisen (Fe) Profile eine große Rolle. Der hohe Gehalt an reaktiven Fe ist vermutlich der wichtigste Faktor, der den oxidativen Teil des S-Kreislaufs kontrolliert, da er die Oxidierung von Sulfid erhöht. Die Prozesse von die Oxidierung von Sulfid haben großen Einfluss auf die Fraktionierung von Schwefelisotopen im S-Kreislauf in den tieferen Sedimenten.

In meiner Arbeit in den Sedimenten der Ostsee konnte ich zeigen, dass die geringe Eindringtiefe von Sulfat, die hohen Sedimentierungsraten und die Eutrophierung die wichtigsten Variablen der Biogeochemie des C- und S-Kreislaufs sind. Die Variablen ermöglichen besonders die Erhaltung von organischem C im Sediment und beeinflussen dabei den Methanflux in die Wassersäule der Ostsee.

Chapter 1
General introduction

Gassy sediments (N_2 , CO_2 , CH_4 , etc.) are present in river deltas, estuaries, coasts, and even in deeper waters on continental shelf, where large amount of organic matter from natural or anthropogenic sources are deposited. The organic matter is easily converted to methane, one of the most important greenhouse gases, after undergoing a series of organic matter degradation processes. Gassy sediments can be hazards to ecosystem, industrial construction, agriculture, and aquaculture activities (Best et al., 2006). Scientists predict also the high levels of methane in seafloor will impact to the climate change and sea level rise within the next 100 years (Best et al., 2006). Nevertheless, controls on gas release from shallow sediments and their impact on the water column, and the atmosphere remain inconclusive.

The *BONUS – Baltic Gas* Project (www.balticgas.au.dk) is a multiple-study, focused on shallow gassy sediments of the Baltic Sea (Jørgensen & Fossing, 2011). Annual eutrophication enhances organic matter setting to seafloor. *Baltic Gas* aims to clarify how long-term eutrophication and climate change affect the accumulation of shallow gas and the release of methane and hydrogen sulfide from the seafloor to the water column and atmosphere. Gassy sediments occur widespread in the Baltic Sea sediments. The goals of the *BONUS – Baltic Gas* Project were to quantify and define the formation and consumption conditions, magnitude of gas storage, and their potential instability. Eventually the plan was to model the dynamics of Baltic Sea methane distributions in the past, present, and future. Using seismo-acoustic and biogeochemical approaches it also explored the hot-spots of shallow gas enhance ebullition, and emission of greenhouse gas and toxic hydrogen sulfide. Fifteen expeditions were made in 10 focus areas from Arhus Bay in the Baltic Sea – North Sea transition to the Bothnia Bay in the North (Jørgensen & Fossing, 2011).

My doctoral project analyzed controls on the relevant key biogeochemical processes including sulfur and carbon cycling involved in the Baltic Gas project. Organic matter delivery into sediments, the degradation and preservation organic matter in the sediment, the formation and consumption of methane into the sediments upon biogeochemical conditions are major aspects of this research. Fluxes of methane in some areas in the Baltic Sea were also studied. My research is divided into four major parts as chapters. Firstly, I introduce the general information connecting the organic matter delivery and general biogeochemical process in the marine sediments; methane distribution - sulfur cycling, and their link between the methane and sulfur cycling (Chapter 1). Research areas and my objectives are also presented in this chapter. Chapter 2 determines the effects of sediment and organic carbon fluxes on the biogeochemistry of methane and sulfur in littoral Himmerfjärden sediments. The

role of sulfate exposure time on carbon preservation is examined in Chapter 3. Chapter 4 consists of an abstract of a manuscript on assessing microbial activity with stable isotope in Himmerfjärden estuary sediments. The last manuscript, Chapter 5, concerns the sulfur biogeochemistry in Gdansk Basin sediments.

1. CARBON CYCLING IN MARINE ENVIRONMENT

1.1. Primary Productivity

The biological carbon cycle begins with photosynthesis of CO_2 and the fixation into organic matter (CH_2O) or (C_{org}) using chlorophyll α as a catalyst (Eq.1). The autotrophic process occurs in continental, oceanic environment and even within Weddell Sea ice in Antarctic spring and summer time (Meiners et al., 2009). In ocean, almost all photosynthesis is carried out in near the surface water column by the photosynthetic plankton that accounts for 95% of the global oceanic primary production (Killops and Killips. 1993).

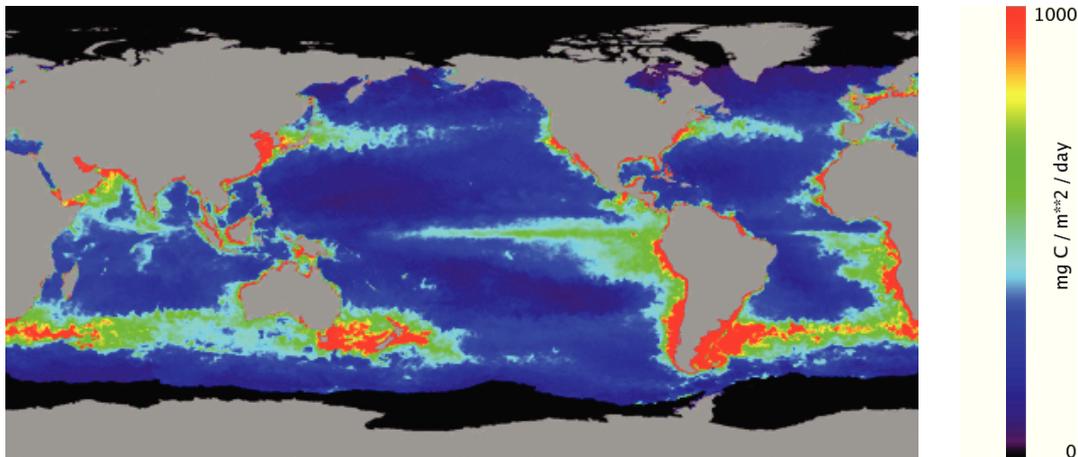


Figure 1: The estimated global oceanic primary production flux based on chlorophyll α distribution

Sources: <http://www.science.oregonstate.edu/ocean.productivity/> (2005)

An estimated $30\text{-}50 \times 10^9$ tons of C_{org} per year is attributed to annual primary production in the global ocean. However, the rate of primary production is not everywhere the same in the global ocean (Fig.1). To support this photosynthesis, dissolved nutrients (N, P) are considered as essential limited nutrients for the C_{org} production efficiency. Supply due to upwelling system, oceanic volcanic activity, or anthropogenic activities (agriculture,

aquaculture waste and domestic waste water) contribute to large organic carbon fixation rates and fluxes (Berger et al., 1989; Hedges & Keil, 1995). For instance, the upwelling systems off continental margin (e.g. Namibia, Peru) or coastal areas, or inland sea such as the Baltic Sea are areas of high C_{org} deposition (Fig 1.).

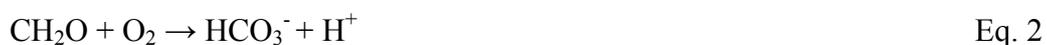
Figure 1 presents the primary production in the global ocean. The primary production rate is highly various from low content up to $1000 \text{ mgC m}^{-2} \text{ day}^{-1}$. The low production occurs in deep ocean such as the Atlantic, Pacific, Oceanic, and Arctic. The primary production in the coastal, continental margin, and inland sea is in a range of medium to high efficiency. The primary production in the Baltic Sea is high (Fig. 1; Conley et al., 2009, 2011).

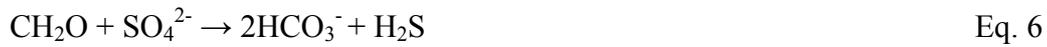
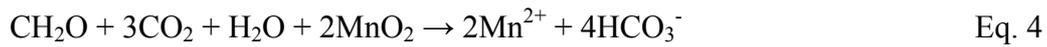
1.2. Organic matter deposition

In the open oceans, most of the primary production is already mineralized in the water column, whereas 10-50% of primary production can land in margin sediments (Jørgensen, 1982, Canfield 2005). Although the margin areas comprise small marine surface areas (7.5%, Wollast, 2002), the margin sediment is considered as the important potential sink for the C_{org} deposition due to a large primary production (18-33% of the global organic matter production, Wollast, 2002). In inland sea system, C_{org} present in the sediments is derived both from primary production and surrounding fluvial watershed deposition. In the margin and inland seas deposition of C_{org} on the seafloor sediments is high compared to the pelagic areas due to incomplete mineralization in the water column. For instance, the annual C_{org} deposition in Baltic Sea is up to $3.5 \pm 2.9 \cdot 10^6 \text{ tones a}^{-1}$ (Leipe et al., 2011).

1.3. Organic matter mineralization

C_{org} is mostly deposited in continental margin and shelf sediments (Wollast, 1998). Only a small amount of this organic matter in the marine sediments is buried in deep sediment before they passed the mineralization (Hedges & Keil, 1995). C_{org} undergoes extensive mineralization within the surface sediments (Hedges & Keil 1995). The degradation processes can be divided into two groups: aerobic respiration with oxygen as an electron acceptor and anaerobic respiration with nitrate, metal (Mn and Fe oxides), or sulfate as alternative electron acceptors, as shown in the following equations (Eq. 2 – Eq. 7):





The distribution of electron acceptors for C_{org} degradation in marine sediments is shown in Figure 5. Oxygen is known to be a major electron acceptor in the water column, and the surface sediments. In the sediments oxygen is generally depleted within millimeters or centimeters. Oxygen may also be consumed during nitrification, sulfide oxidation, and metal oxidation (Jørgensen & Kasten 2006). Oxygen respiration can still be accounted for 50% of the total C_{org} mineralization or even more (Canfield 2005).

The mineralization of the high and low molecular weight C_{org} via fermentation involves various microorganism that can degraded by various bacteria in large time scales from hours to millions of years (Canfield 2005). High molecular weight organic matter is broken into low molecular weight compounds such as volatile fatty acids, alcohols, amino acid, and simple sugar that can be easily attacked by the microbial activity (Fenchel et al. 1998) (Fig. 3). These compounds can be degraded to CO_2 and eventually CH_4 or carbonate by terminal electron processes.

Due to the limited nitrate distribution and low reactivity of metal oxides in sediments at the continental shelf, sulfate reduction is the major anaerobic pathway of C_{org} degradation and can be accounted for 25 - 50% of C_{org} mineralization (Jørgensen 1982). The role of sulfate reduction is variable across various marine environments. For instance, sulfate reduction can contribute up to 100% as electron acceptor for C_{org} degradation in some euxinic environments, where completely depleted oxygen and presence of sulfide occur in the bottom water column such as in the Black Sea (Jørgensen et al. 2004a), whereas in offshore Namibia upwelling system, a small but significant fraction of C_{org} is degraded via sulfate reduction (Ferdelman et al. 1999). Below the sulfate zone, C_{org} is still mineralized to methane or forms with other C_{org} fossils fuels (petroleum or natural gas).

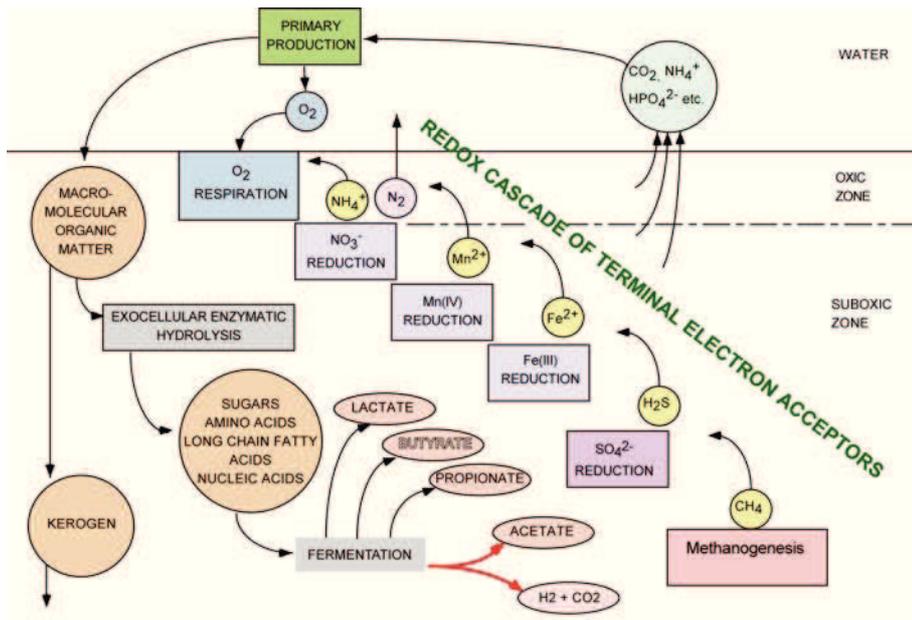


Figure 2: The distribution of electron acceptors and products in C_{org} mineralization process in marine sediments (Jørgensen & Kasten 2006)

Figure 2 describes the general depth sequence of oxidant and reductant used in the mineralization of C_{org} . Based on the decrease of the redox potential of oxidants, $O_2 \rightarrow NO_3^- \rightarrow MnO_2 \rightarrow FeOOH \rightarrow SO_4^{2-} \rightarrow$ Methanogenesis lead to a decrease of free energy availability and biogeochemical processes in the sediments. The last process of C_{org} mineralization is methane production.

2. METHANE

Methane (CH_4) is a hydrocarbon compound, composed of one C and four H atoms. CH_4 is present in the atmosphere, the hydrosphere, the lithosphere and the biosphere. It is nowadays one of the most important energy sources for human activity. Methane was one of dominant components of the early atmosphere 2.7 billion years ago. Over time, the presence of oxygen in the atmosphere changed the air composition, and CH_4 became a trace gas (0.8 ppmv). Nevertheless, due to the human activities associated with industry, agriculture and human waste water, methane concentration has increased to 1.7 ppmv over the past two decades and is expected to increase in the future (NOAA data) (Fig. 3).

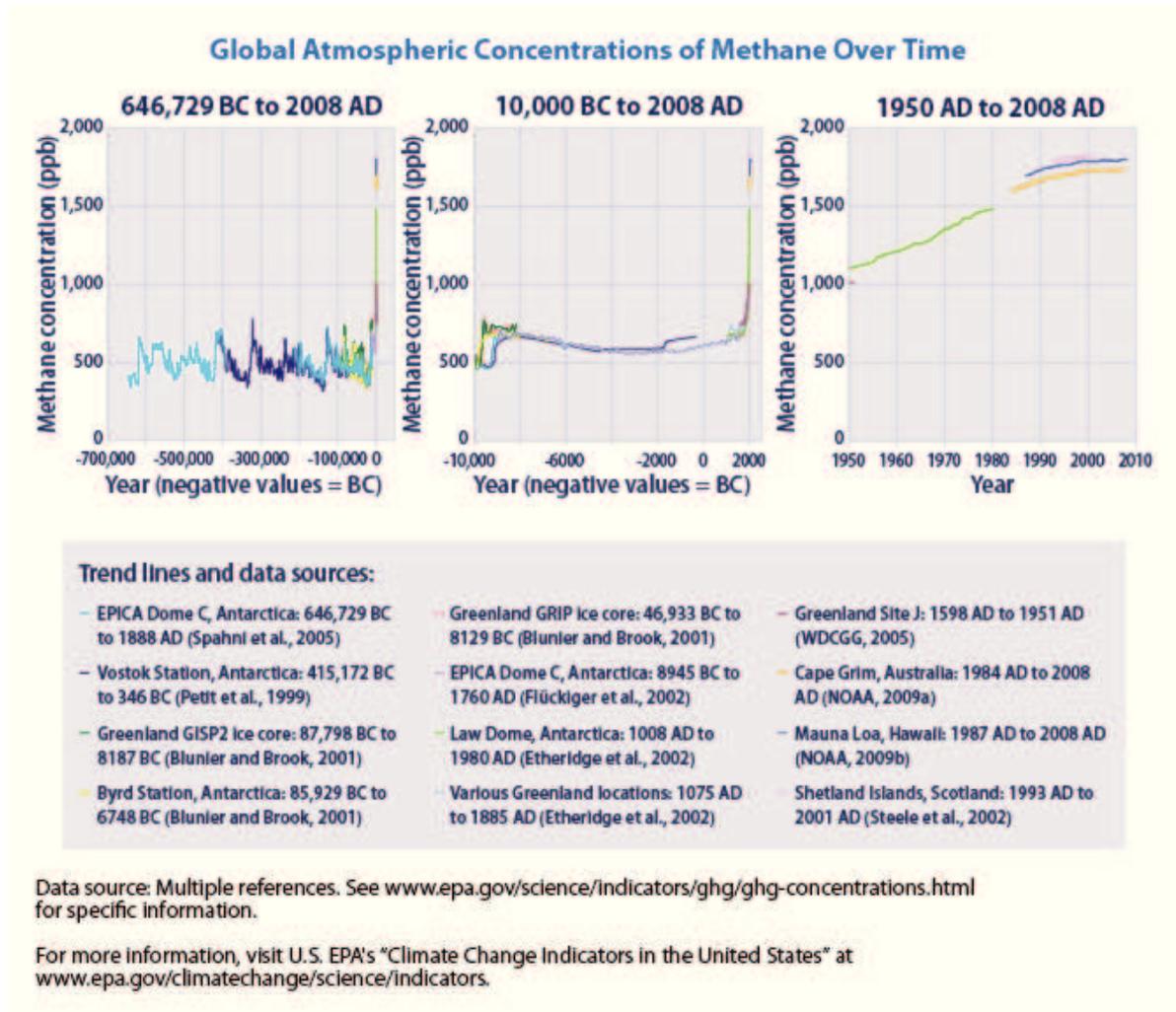


Figure 3: Methane concentration in the atmosphere recently

Although the low methane concentration in the atmosphere, methane is still considered as one of the most important greenhouse gases. Methane is more efficient in trapping infrared radiation in the atmosphere compared to CO₂ (Lilieveld et al. 1993). Thus, methane could significantly influence global climate change (Lilieveld et al. 1993). Through experiments, Lilieveld et al. (1993) have demonstrated that one CH₄ mole is 26 times more effective in trapping infrared radiation than one CO₂ mole does. The increase of methane from both the natural and the anthropogenic sources to the atmosphere has been remarkable. Oceanic sediments comprise a major reservoir of the global methane budget, which is mostly derived from microbial activity (Claypool & Kvenvolden 1983). Nonetheless, the marine environment contributes only 20 Tg a⁻¹ methane release into the atmosphere (Judd 2002) and is counted a minor source (Fig. 5).

The discovery that in the Arctic sediments (Shakhova et al. 2010) a vast amount of methane (up to 7 Tg a⁻¹ from small areas of seabed (2.1 x10⁶ km²)) vents to the atmosphere,

could lead to a change of the global methane budget. Likewise, the extensive eutrophication and the anthropogenic activities in inland sea system as Baltic Sea or coastal areas probably also enhance the release of methane into atmosphere (Meybeck et al. 1989; Smith et al. 2010; Borges & Abril 2012; Thang et al. 2013). This was a major reason to examine controls on methane distribution and fluxes in the *Baltic Gas* project.

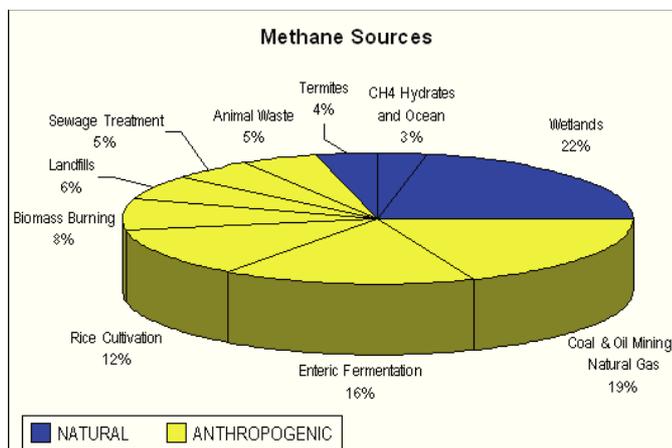


Figure 4: Estimated atmosphere sources of methane (<http://icp.giss.nasa.gov/education/methane/intro/cycle.html>)

Methane production is one of principal biogeochemical process of C_{org} mineralization. More than 10% of total global C_{org} is converted to methane in sediment (Clayton 1992). Acetoclastic methanogenesis and bicarbonate reduction methanogenesis (Gunsalus & Wolfe 1978; Deppenmeier et al. 1996; Diekert et al. 1998; Ferry 1999) are the major processes (Eq.8 & 9):



In nature, most methane production is formed via acetoclastic fermentation and bicarbonate reduction. Other substrates (methanol, methylamine, formate) may contribute to methanogenesis also (Eq. 10) (Oremland & Polcin 1982; Ferdelman et al. 1997).



In the brackish and the fresh sediments, where sulfate supply is limited, methane may be still generated in the sulfate reduction zone due to non-competitive methylated substrates and methane is the main product of organic carbon mineralization (Parkes et al. 2008; Knab et al. 2008; Yoshioka et al. 2009; Jørgensen & Parkes 2010). Overall, that the production and

consumption of marine methane is very closely linked to the extensive sulfur cycling operation in marine sediments (Marsten & Berner 1974).

3. GLOBAL MARINE SULFUR CYCLING IN SEDIMENTS

3.1. Reductive sulfur in marine sediments

Sulfur cycling in marine sediment begins with diffusive and advective transport of sulfate from the water column into the sediments, where it is reduced to H₂S by sulfate reducing bacteria. Dissimilatory sulfate reduction is a widespread anaerobic mineralization process in high deposition, coastal sediment, and euxinic bottom waters (Canfield 2005). Beside oxygen respiration, sulfate reduction is the major pathway of reductive sulfur cycling dealing with C_{org} degradation, which accounts for ~ 55% total mineralization of carbon in marine sediments (Canfield 2005). Sulfate reduction occurs in anoxic water and the lake sediments, and also occurs a wide range of temperatures from below 0°C to over 100°C, Jørgensen et al. 1992; Sagemann et al. 1998; Koska et al. 1999) and salinities up to 100‰, Al-Najjar et al. (unpublished data). The role of sulfate reduction in C_{org} degradation varies from insignificant in the abyssal plain, continental slope (< 1%) to prominently significant in the euxinic basin as in the Black Sea (~ 100%) (Jahnke 1996; D'Hondt et al. 2004; Jørgensen et al. 2004b).

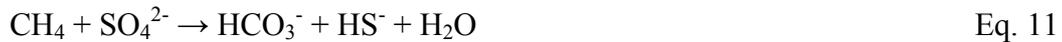
There are two types of general sulfate reduction: organoclastic sulfate reduction (Eq. 6) and methanotrophic sulfate reduction (Eq. 7) (Fig 5). In continental margins, 90% of sulfate reduction occurs in top 30 cm of sediments as organoclastic sulfate reduction (Fossing et al. 2000; Jørgensen & Parkes 2010), whereas sulfate reduction associated with anaerobic oxidation of methane occurs in the bottom of the sulfate zone and consumes most methane production in marine sediments (Fossing et al. 2000).

3.2. Sulfate reduction associated anaerobic oxidation of methane (AOM)

Ocean sediments contain more than 80% of the total amount of methane in the global methane budget. However, in the ocean, most methane is consumed due to anaerobic oxidation of methane (AOM) coupled sulfate reduction (SR) (Hanson & Hanson 1996) (Eq. 11). Methane is consumed via this process before it reaches into the water column and further to the atmosphere. The consumption of methane in the sediments has been estimated to

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account for 80% of upward diffusive methane and counted to 300 Tg a^{-1} (Reeburg 1996; Hinrichs & Boetius 2002) (Fig. 5).



In the system, where sulfate concentrations are low, methanogenesis may be the main pathway of anaerobic C_{org} mineralization (Canfield 2005). The sulfate reduction associated AOM has also been confirmed by clear identification of methanotrophic archaea ANME-1, ANME-2 or ANME-3 in AOM sediments that are related to the group of Methanosarcinales (Boetius et al. 2000; Knittel et al. 2002; Knittel et al. 2005; Niemann et al. 2006). Due to slow growth of methane oxidizing consortia (Nauhaus et al. 2007), Regnier et al. (2011) have demonstrated that typically 80 years are needed in response to achieving steady state of methane and sulfate gradients in the pore water in marine sediment.

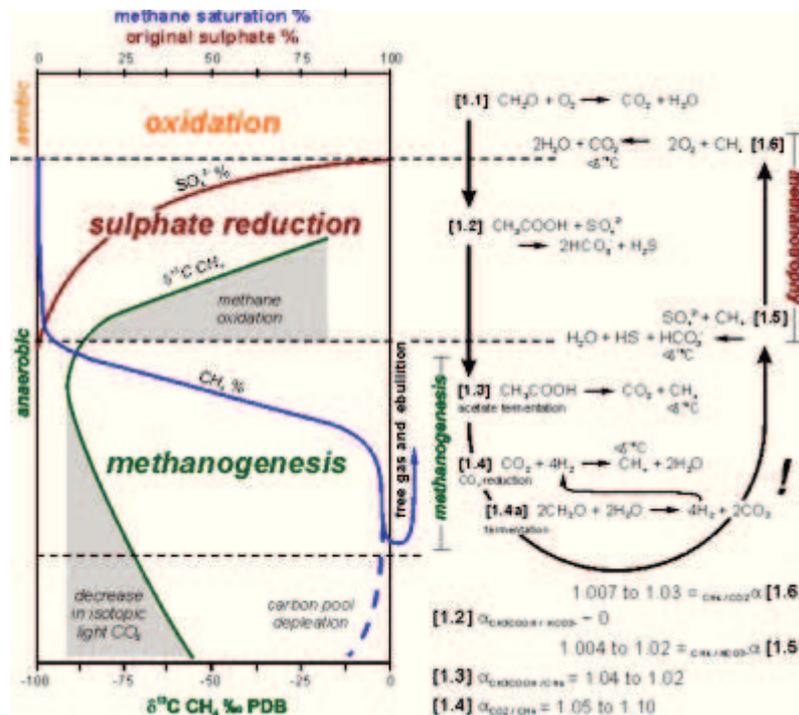


Figure 5: Methane and sulfur cycling in marine sediment (Greinert, 1999)

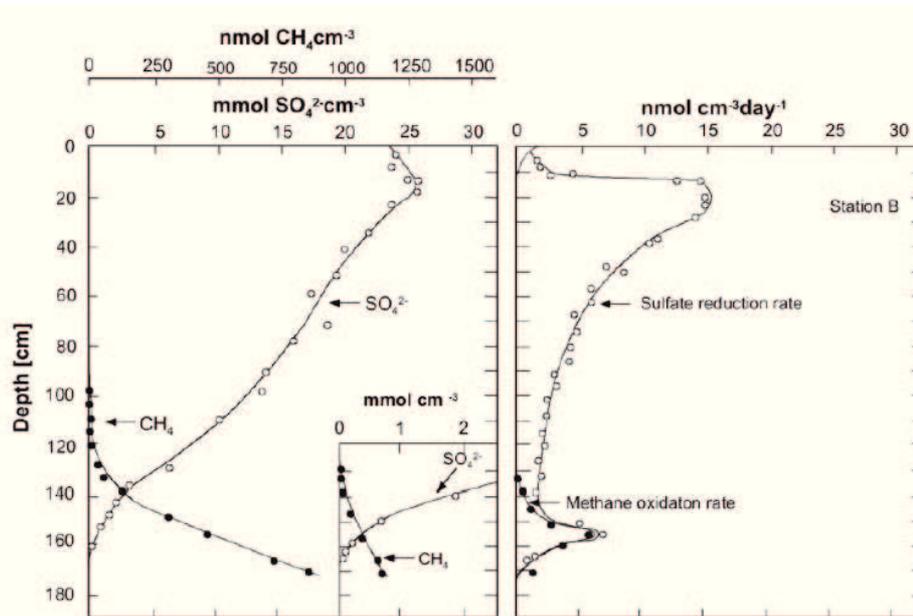
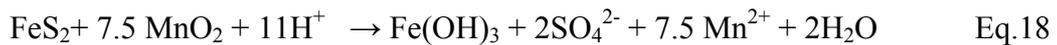
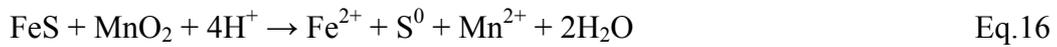
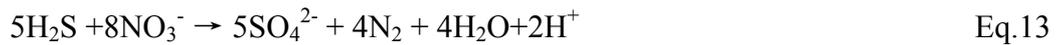


Figure 6: Typical profiles of pore water methane, sulfate concentration and sulfate reduction rate (SRR) and anaerobic oxidation of methane (AOM) in marine sediments from the Baltic Sea – North Sea transition (Iversen and Jørgensen, 1985)

Figure 6 presents an example of a typical sulfate–methane transition zone of both sulfate–methane gradient and radioactive tracer of sulfate reduction coupled AOM in a continental shelf sediments from the Kattegate (Denmark) (Iversen & Jørgensen 1985).

3.3. Oxidative sulfur cycling in sediment

In marine sediments, sulfide is generated via sulfate reduction in both organoclastic and methanotrophic sulfate reduction. A small fraction of free sulfide is trapped and buried as acid volatile of sulfur (FeS , Fe_3S_4), pyrite, other metal sulfides, and organic sulfur; the remainder could exist in pore water in free sulfide form (H_2S) or reoxidize to elemental S or sulfate (Bernier 1984). Typically 90% of the sulfide or more, is reoxidized again to sulfate or various intermediates as elemental S (S^0), polysulfide (S_n^-) thiosulfate ($\text{S}_2\text{O}_3^{2-}$), and sulfite (SO_3^{2-}) (Jørgensen B.B. 1982; Canfield 1993, Zopfi et al. 2004). This process occurs at all depth of the sediments; predominantly, however it is more rapidly in upper anoxic and oxic zone, and also in the deeper and sulfidic sediments (Jørgensen 2006, Riedinger et al. 2010). Sulfides (FeS_2 , FeS , H_2S) can be oxidized by oxygen (O_2), nitrate (NO_3^-), and metals oxides (MnO_2 , and FeOOH) back to sulfate via a series of intermediates (Canfield 1989; Thamdrup et al. 1993; Schippers & Jørgensen 2002 Jørgensen & Nelson 2004, Riedinger et al. 2010) as in following equations:



Depending on various oxidants, sulfides can be oxidized to S^0 or to sulfate (Eq. 12 – Eq. 18). S^0 produced by sulfide oxidation with metal oxides can be disproportionated to sulfide and sulfate (Eq. 19-Eq. 21) (Thamdrup et al. 1993; Canfield & Thamdrup 1994; Habicht & Canfield 2001; Jørgensen & Nelson 2004). The sulfide oxidation and S^0 disproportionation that also occurs during oxidative sulfur cycling are controlled by bacteria and the availability of metal oxides (Jørgensen 1977; Riedinger et al. 2005, 2010).



Recently, Milucka et al. (2012) have proposed another pathway of disulfide disproportionation in sulfate reduction associated anaerobic oxidation of methane in culture (Eq. 22).



3.3. Stable isotope biogeochemistry of sulfur

The bacteria involved in both reductive and oxidative sulfur transformation in marine sediments fractionate sulfur isotopes during the metabolism of sulfur compounds. Both in pure cultures and natural settings the isotope fractionation has been observed during sulfate reduction, sulfide oxidation, and sulfur intermediates disproportionation. The stable isotope ratios (^{32}S , ^{34}S , ^{33}S and ^{36}S) of the different sulfur species can be utilized as indicators of natural biogeochemical processes, and to reconstruct the paleoenvironment in which these compounds were formed (Canfield 2001; Brüchert 2004; Riedinger et al. 2010; Arnold et al.

2012; Strauss et al. 2012). The $^{34}\text{S}/^{32}\text{S}$ ($\delta^{34}\text{S}$) is more commonly used than other ratios ($^{33}\text{S}/^{34}\text{S}$; $^{36}\text{S}/^{32}\text{S}$). Thus, isotope signatures are also used to investigate the formation and generation of sulfur compounds such as sulfate, sulfide, AVS, pyrite and organic sulfur, to decipher pathways of diagenetic organic matter sulfurization (Brüchert & Pratt 1996; Böttcher & Lepland 2000; Ruy et al. 2006; Sinnginhe Damsté et al. 2007) and to reconstruct the reductive - oxidative sulfur cycling in the marine sediments (Canfield & Thamdrup 1994; Habicht & Canfield 2001; Brüchert 2004; Brunner & Bernasconi 2005; Riedinger et al. 2010; Sim et al. 2011; Strauss et al. 2012).

4. LINK BETWEEN CARBON AND SULFUR CYCLINGS IN MARINE SEDIMENT

4.1. Carbon preservation

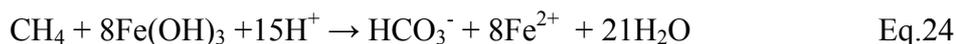
Only a small fraction of C_{org} delivered to the sediment surface is buried in deep sediment layers. Oxygen respiration in the surficial sediments, and further anaerobic processes such as denitrification, metal oxide reduction, sulfate reduction, and finally methanogenesis, generally allow $< 1\%$ of the C_{org} flux to the sediment to reach the deep sediment biosphere (Hedges & Keil 1995). Nevertheless, this small leak (i.e. carbon preservation) in the carbon cycle over geological time scales leads to the formation of enormous reservoirs of reduced carbon buried in marine sediments (Hedges & Keil 1995), which has implications for the chemistry of the ocean and the redox balance of the Earth's surface (Berner & Raiswell 1983). In some marine sediment regions on the Earth and throughout Earth' evolution, a large amount of C_{org} is still well-preserved in the deep biosphere (Hedges et al. 1988; Canfield 1994; Wortmann & Chernyavsky 2007). The real mechanistic controls on carbon preservation remain controversial (Canfield 2005), and include of physical, chemical, and biological mechanisms (Canfield 1994; Hedges & Oades 1997; Burdige 2007; Zonneveld et al. 2010). Not surprisingly, the degree of C_{org} preservation also reflects depositional scenarios in the paleoenvironment, e.g. chemical, physical, and biological effects, as well the amount and quality of C_{org} .

In modern sediments there is a positive relationship between sulfur and carbon burial but during the Cretaceous period of geologic time these rates have been inversely related (Paytan et al. 2004). During the Early Cretaceous, Wortmann & Chernyavsky (2007) proposed that extensive evaporite deposition, especially gypsum, depleted the ocean sulfate

reservoir. This resulted in a decoupling of organic carbon burial and sulfur burial rates during the Early Cretaceous. The authors implied, based on isotope relationships between C_{org} and reduced S species that the sulfate depletion led to enhanced preservation of organic carbon.

4.2. The link between carbon, sulfur, and iron cycling

Sulfide produced from organoclastic and methanotrophic sulfate reduction (Eq.6, Eq.11) is oxidized to sulfate or titrated by ferrous iron and then buried in deep sediments as inorganic sulfur (FeS or FeS_2) (Jørgensen & Kasten 2006). Bioturbation, bioirrigation, and sulfurization control the cycling of iron, C_{org} and sulfur (Fig. 8). Bacterial sulfate and iron reduction are the main C_{org} degradation driving forces affecting the S and Fe cycles (Lovely 1991; Böttcher & Lepland 2000; Neretin et al. 2004). Sulfides (FeS , H_2S) and ferrous iron oxidation may also be involved in C_{org} carbon assimilation (Kuennen 1979, Shipper et al. 2004). Recently, Beal et al. (2009) has proposed other pathways of anaerobic oxidation of methane coupled Mn and Fe reduction (Eq.23, 24). Although rates of anaerobic oxidation of methane coupling manganese – iron reduction are lower than sulfate – associated with anaerobic oxidation of methane rates, due to large amount of manganese and iron from continental input to global ocean, manganese and iron dependent AOM potentially contributes as global sink of methane (Beal et al. 2009).



Sulfide oxidation combined with iron reduction could contribute to CO_2 fixation by sulfur bacteria (Jørgensen & Nelson 2004). This process produces sulfur intermediates (elemental S, polysulfides, thiosulfate, tetrathionate) that return to sulfate and sulfide via disproportionation by autotrophic and heterotrophic bacteria in sediments. In particular, ferric iron - rich sediments play a significant role during the oxidation of sulfur species and sulfur cycling in the deep sediments (Eq. 14, 15, 16, 17) (Holmkvist et al. 2011, Tarpgaard et al. 2011).

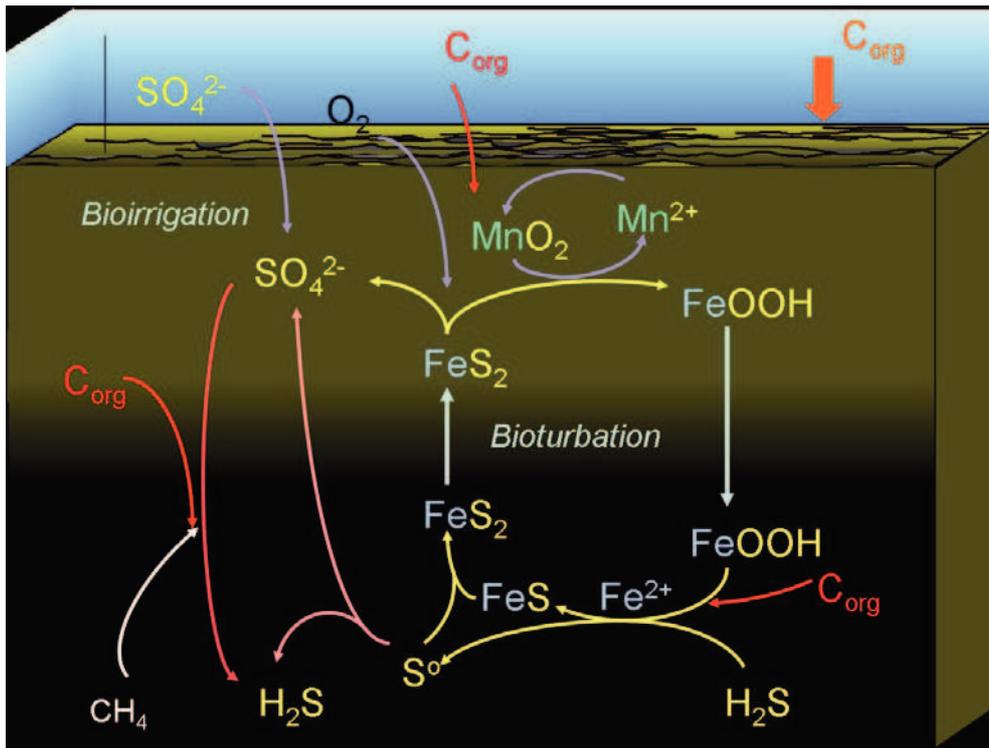


Figure 7: The principal link of sulfur, carbon and iron cycling in marine sediments
(modified from Jørgensen & Nelson, 2004)

5. STUDY AREAS

The Baltic Sea is an inland Sea and located in Northern Europe, is bounded by the Scandinavian Peninsula (Lithuania, Latvia, and Estonia) in the East, the mainland of Europe (Germany and Poland) in the South, and Danish Island (Denmark, Sweden) in the West. With more than 377,000 km² of surface area and average depth of 46 m, the Baltic Sea is connected with the North Sea and the Atlantic Ocean via Skagerrak, Kattegat Bay, and Belt Sea. The Baltic Sea can be divided into several major parts, such as the Bothnia Sea and Bay in the North, on its northeastern edge by the Gulf of Finland, the Baltic Proper, Gotland Deep and Orland Deep (the Centre of Baltic Sea), further in the South locates Bornholm Basin, Gdansk Basin, Arkona and Mecklenburg Basin. Additionally, various small bays can also be considered important parts of the Baltic Sea with its more than 8000 km of coastline.



Figure 8: Satellite image of *Cyanobacteria* bloom in the Baltic Sea (Sources: NASA, 2011)

The Baltic Sea is one of the largest brackish water bodies due to a low rate of exchange of water with the ocean and receives large amounts of freshwater from the surrounding watershed. Over its more than 1000 km length, the salinity gradient decreases from 27‰ in the Kattegat Bay to 10 -15‰ in Mecklenburg, Gdansk Basin, to the central basin, 6 - 8‰, to end in the North, 3 -5‰. The Baltic Sea annually receives about 500 km³ of river runoff with a large inorganic and organic nutrients from agriculture, and domestic waste that increased the primary production. Thus, over the past 60 years, the Baltic Sea has experienced a period of eutrophication in large areas (Larsson et al. 1985; Conley et al. 2009, 2011). Areas that were considered in the BONUS – Baltic Gas project and this study are shown in Figure 9 and described below.

Aarhus Bay is located at the transition of the North Sea and the Baltic Sea, eastern coast of Denmark. The Holocene sediments mainly contain fine grained sediments, with variable sulfate penetration depths ranging from 0.5 to > 4m depth.

Mecklenburg Bay is dominated by interaction between the North Sea and the Baltic Sea and strong salinity gradients, and estuarine impact. Oxygen depletion present in coastal areas but not problem in open water. Organic carbon contents in the sediments are moderate

(1-4%, Leipe et al. 2012). A high population of benthic fauna is generally found in these areas (Leipe et al. 2012).

Bornholm Basin is one of the major basins in the southern Baltic Sea. Organic rich sediments and hypoxic to anoxic bottom water environment are predominant characteristics of this basin. Bornholm sea floor is characterized by flat bottom and silty sediments (Leipe et al. 2010) and is strongly impacted by currents that drive spatial differences in the thickness of Holocene mud sediments and C_{org} distribution (Christoffersen et al. 2007).

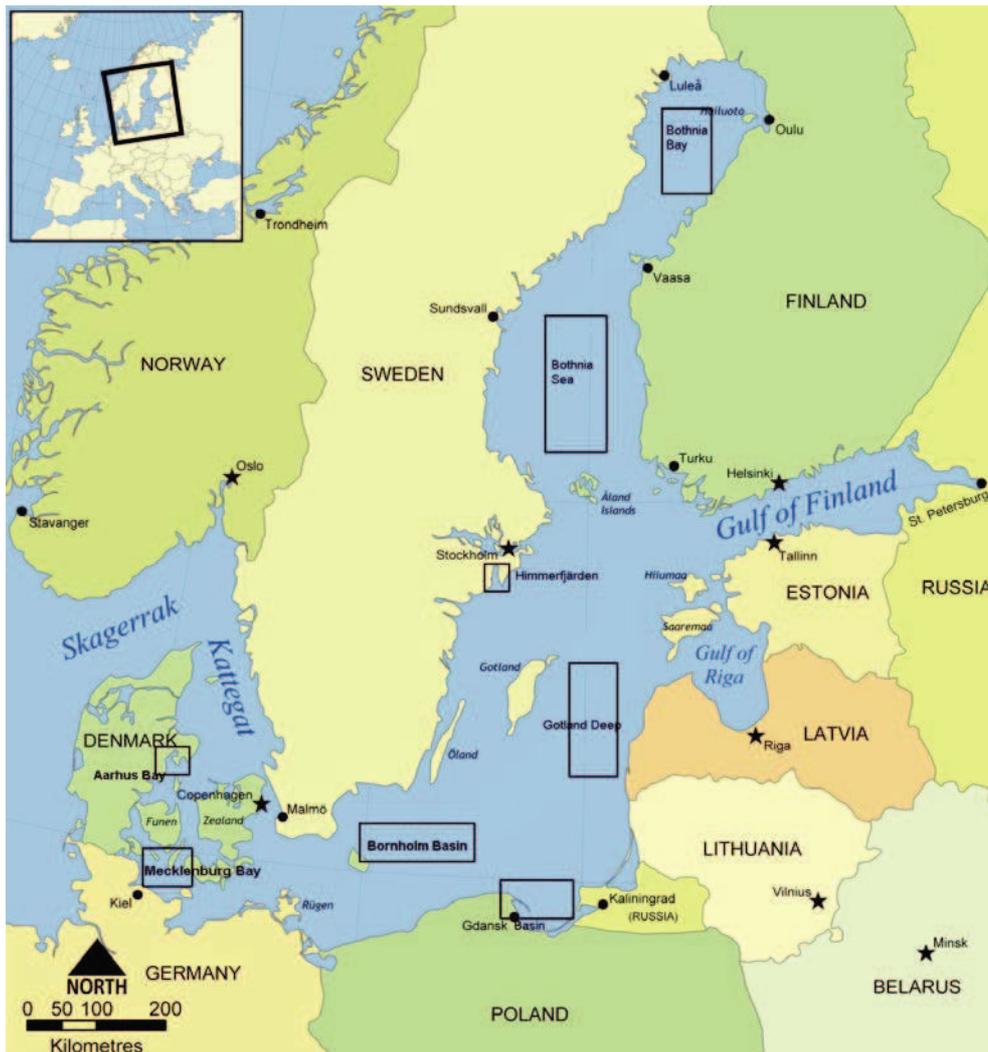


Figure 9: Map of study sites in the Baltic Sea that were focused in this thesis
(modified from Wikipedia)

Gdansk Basin is located in the southern Baltic Sea, off Poland. The Gdansk Basin covers an area of approximately 25600 km² and includes the Gdansk Bay (inner and outer Puck Bay) and the Gdansk Deep. The Gdansk Basin is one of major basins of the Baltic Sea, with brackish water conditions (6 – 14 ‰). Similar to other parts of the Baltic Sea, the

Gdansk Basin experienced a period of eutrophication in large areas of the coastal water body due to increased primary production in the 20th century (Stigebrandt 1991). The study area is characterized by the high primary productivity ($\approx 190 \text{ g C m}^{-2} \text{ a}^{-1}$) with high sedimentary rate (1.1-3.6 mm a^{-1} , Suplińska & Pietrzak-Flis 2008), and moderate to high particulate organic carbon accumulation rates (4 - 8% organic carbon content, Leipe et al. 2010). The Gdansk Basin received a large amount C_{org} and nutrient from Vistula River.

Gotland Deep is located in the central Baltic Sea and is the deepest basin of the Baltic Sea. Low sedimentation rates, but high C_{org} contents are major characteristics of this basin. Likewise, this area is dominated by anoxic condition in the bottom water. This is the euxinic part in the Baltic Sea.

Himmerfjärden estuary is one of the large estuarine systems along the Swedish coast. It receives a large amount of freshwater discharge from the watershed and a waste water treatment plant, and agriculture runoff. This had led to eutrophication of the Himmerfjärden estuary for the past 60 years and a very high sediment accumulation (Bianchi et al. 2003; Reuss et al. 2005; Thang et al. 2012).

Bothnia Sea and Bay are located further the northmost of the Baltic Sea, where the salinity is the lowest in the Baltic Sea. The productivity is lower in comparison to other areas in the Baltic Sea due to the lowest temperature and the low nutrient concentration that has led to the C_{org} deposition in sediments and predominant oxygen concentration in the bottom water (Leipe et al. 2010).

6. OBJECTIVES OF RESEARCH

This PhD project was conducted as a part of the BONUS – Baltic Gas project that addressed methane in the Baltic Sea and its mutual coupling to climate change and eutrophication. The Baltic Gas Project employed seismo-acoustic technique and geochemical approaches to map shallow gas, and hot-spots of accumulation gas in the seabed (Jørgensen & Fossing 2011).

This thesis mainly focused on the geochemistry of carbon and sulfur species in the pore water and the solid phase. Reaction transport model was also applied to fit some profiles and used to investigate fluxes of methane, sulfate and dissolved inorganic carbon. Some

radiochemical approaches were carried out to calculate some microbial processes (methane production, methane consumption, and sulfate reduction). Additionally, natural abundance isotope studies (S stable isotopes) and (^{210}Pb and ^{137}Cs) were used to study sulfur biogeochemistry and sediment accumulation rate, respectively.

The main objectives of this work were to:

- Examine low salinity, salinity gradient and sulfate penetration depth influence on carbon and sulfur biogeochemistry;
- Determine the effect of sediment accumulation rate on biogeochemical carbon and sulfur cycling;
- Examine methane distribution, its fluxes, and methane production and consumption in the Baltic Sea sediments;
- Explore reductive sulfur and oxidative sulfur cycling in the Baltic Sea sediment.

The results of this project are presented as the following manuscripts that represent chapters within this thesis.

Overview of Manuscripts

The manuscripts that are provided in this thesis describe the biogeochemical controls on carbon and sulfur cycling in Baltic Sea sediments in key research areas including Himmerfjärden estuary and Gdansk Basin.

Chapter 2: The impact of sediment and carbon fluxes on the biogeochemistry of methane and sulfur in littoral Baltic Sea sediments (Himmerfjärden, Sweden).

Nguyen Manh Thang, Volker Brüchert, Michael Formolo, Gunter Wegener, Livija Ginters, Bo Barker Jørgensen, Timothy G. Ferdelman

The sampling at Marine Research Center of Stockholm University on Askö Island of the *R/V Limanda* to Himmerfjärden estuary was performed by V. Brüchert, G. Wegener, L. Ginters, T. G. Ferdelman and myself. Methane was analyzed by V. Brüchert. Methanogenesis

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was determined and evaluated by L. Ginters and me. Pore water sulfide and ferrous iron were measured by T. G. Ferdelman and myself. Other data such as SRR, AOM, pore water sulfate, DIC, ^{210}Pb and ^{137}Cs , solid phase geochemistry (C_{org} , TS, C: N ratios, reactive iron, chlorin) were measured by myself with some help to evaluate the data from M. Formolo, G. Wegener, T.G. Ferdelman. The manuscript was written by me, with support and input from V. Brüchert, M. Formolo, B. B. Jørgensen, and T.G. Ferdelman. This manuscript has been published in *Estuaries and Coasts*. I was the corresponding author.

Chapter 3: The role of sulfate exposure time on carbon preservation and sulfur burial in Baltic Sea sediments.

Nguyen Manh Thang, Michael Formolo, Laura Lapham, Henrik Fossing, Sabine Flury, Bo Barker Jørgensen, Timothy G. Ferdelman

Several sampling campaigns were carried out throughout the Baltic Sea including two above cruises. L. Lapham provided some sulfate, DIC data from the *Maria S. Merian* cruise - MSM.16 - (Bornholm, Gotland Deep, Bothnia Sea and Bay) while H. Fossing and T.G. Ferdelman contributed some pore water DIC, sulfate, and some solid chemistry in *Poseidon* cruise - Poseidon 372 - (Bornholm Basin). S. Flury contributed from Gdansk Basin (DIC) and Aarhus Bay (sulfate, DIC). Almost all solid phase geochemistry and radiochemistry (^{210}Pb and ^{137}Cs) were measured by myself. The reaction – transport model, data evaluation, and writing the manuscript were done by myself with help from M. Formolo, L. Lapham, B.B. Jørgensen, S. Flury and T.G. Ferdelman.

The manuscript is written in a short form style suitable for a journal as *Nature Geoscience* or *Geology*. Therefore materials, methods, and the details are provided a supplementary material section within this chapter.

Chapter 4: Assessing subsurface microbial activity by stable isotope probing with deuterated water

Gunter Wegener, Marlene Bausch, Thomas Holler, Nguyen Manh Thang, Xavier Prieto Mollar, Matthias Y. Kellermann, Kai-Uwe Hinrichs and Antje Boetius. *Environmental*

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Microbiology, 2012. In this research I contributed during the field sampling, analytical measurement of pore water chemistry, and co-authorship. Only the abstract is presented.

Chapter 5: The impact of reactive iron on early sulfurization of organic matter and oxidative sulfur cycling in sulfidic sediments from the Gdansk Basin (Baltic Sea)

Nguyen Manh Thang, Timothy G. Ferdelman, Sabine Flury, Benjamin Brunner, Bo Barker Jørgensen, Michael Formolo

The sampling on board of the *R/V Oceania* cruise to Gdansk Basin was performed by S. Flury and myself with the help of Thomas Max. Pore water concentrations of sulfate, sulfide, geochemical solid phase (C, S, CRS, AVS ...) and isotope composition were evaluated by me, while M. Formolo helped to measure elemental S. The manuscript was written by me, with support of M. Formolo, S. Flury, B. Brunner, B.B. Jørgensen and T.G. Ferdelman. This manuscript is in preparation for *Journal of Sea Research* or *Chemical Geology*.

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Chapter 2

The impact of sediment and carbon fluxes on the biogeochemistry of methane and sulfur in littoral Baltic Sea sediments (Himmerfjärden, Sweden)

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ABSTRACT

Three sediment stations in Himmerfjärden estuary (Baltic Sea, Sweden) were sampled in May 2009 and June 2010 to test how low salinity (5 – 7 ‰), high primary productivity partially induced by nutrient input from an upstream waste water treatment plant, and high overall sedimentation rates impact the sedimentary cycling of methane and sulfur. Rates of sediment accumulation determined using $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs were very high (0.65 – 0.95 cm year⁻¹), as were the corresponding rates of organic matter accumulation (8.9 – 9.5 mol C m⁻² year⁻¹) at all three sites. Dissolved sulfate penetrated < 20 cm below the sediment surface. Although measured rates of bicarbonate methanogenesis integrated over 1 m depth were low (0.96 – 1.09 mol m⁻² year⁻¹), methane concentrations increased to >2 mmol L⁻¹ below the sulfate-methane transition. A steep gradient of methane through the entire sulfate zone led to upward (diffusive and bio-irrigative) fluxes of 0.32 to 0.78 mol m⁻² year⁻¹ methane to the sediment-water interface. Areal rates of sulfate reduction (1.46 – 1.92 mol m⁻² year⁻¹) integrated over the upper 0-14 cm of sediment appeared to be limited by the restricted diffusive supply of sulfate, low bio-irrigation ($\alpha = 2.8 - 3.1 \text{ year}^{-1}$), and limited residence time of the sedimentary organic carbon in the sulfate zone. A large fraction of reduced sulfur as pyrite and organic-bound sulfur was buried and thus escaped reoxidation in the surface sediment. The presence of ferrous iron in the pore water (with concentrations up to 110 μM) suggests that iron reduction plays an important role in surface sediments, as well as in sediment layers deep below the sulfate-methane transition. We conclude that high rates of sediment accumulation and shallow sulfate penetration are the master variables for biogeochemistry of methane and sulfur cycling; in particular, they may significantly allow for release of methane into the water column in the Himmerfjärden estuary.

Key words: Sediment accumulation, methane flux, methanogenesis, sulfate reduction, sulfide, Baltic Sea

1. INTRODUCTION

1.1. Introduction

Biogenic methane produced in marine sediments is one of the largest reservoirs of methane on Earth (Claypool and Kvenvolden 1983). In most marine systems, very little of this methane is released into the seawater and atmosphere, because it is efficiently oxidized by sulfate reduction coupled to the anaerobic oxidation of methane (AOM) within the sulfate-methane transition (SMT) (Reeburgh 1975; Boetius et al. 2000; Orphan et al. 2001; Treude et al. 2003). The amount of methane that escapes from continental margin sediments through the water column and into the atmosphere accounts for only 2% of global methane emission (Judd et al. 2002).

The largest part of the marine methane emission, about 75%, is probably released from near-shore coastal environments (Bange et al. 1994). This is because coastal regions (estuaries, bays, and other shallow areas) are often characterized by high rates of organic matter deposition due to large amounts of terrestrial and riverine runoff, high primary production in the water column, and the discharge of anthropogenic waste, e.g. from sewage treatment plants (Meybeck et al. 1989; Smith et al. 2010; Borges and Abril 2012). These factors support high rates of carbon mineralization in the sediment by oxygen respiration, denitrification, metal oxide reduction, sulfate reduction and, ultimately, methanogenesis (Borges and Abril 2012). The depletion of sulfate allows methanogenesis to occur at shallow depths. In addition to a high organic carbon load that drives high rates of organic carbon mineralization, rates of sulfate reduction and methanogenesis depend on the season, the sediment temperature, the salinity, and the sulfate concentrations in the water column (Marten and Klump 1980a; Kipphut and Martens 1982; Heyer and Berger 2000; Valentine 2002). In coastal systems with low salinity, sulfate depletion often occurs in the topmost tens of centimeters; methane is less efficiently oxidized and a significant amount of methane can escape as bubbles (Chanton et al. 1989; Heyer and Berger 2000).

The purpose of this study is to understand how low salinity, high organic matter input and high overall sedimentation rates impact carbon mineralization rates and the turnover of methane and sulfur in eutrophic, littoral Baltic Sea sediments. Himmerfjärden, a large estuarine system on the Swedish Baltic coast connecting Lake Mälaren with the central Baltic Sea is ideal for this study due to its point – source anthropogenic loading, high sediment accumulation rates, and low salinity.

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1.2. Study site

The Baltic Sea is the largest brackish water body in the world. Salinity decreases from 25‰ in the Danish Straits to 2‰ in the Gulf of Bothnia. Over the past 50 years, the Baltic Sea has experienced eutrophication with increased primary production supported by increased discharge of inorganic and organic nutrients (Bartnicki and Valiyaveetil 2008; Lasson et al. 1985; Rosenberg et al. 1990; Stigebrandt 1991), which has led to an expansion of coastal and open-water anoxia (Conley et al. 2009, 2011).

Table 1: Location and seasonal range of characteristics of Station H5, H3 and H2 in Himmerfjärden estuary

Site	Location	Water depth (m)	Salinity (‰)	Oxygen content (mM)	Chlorophyll α (mg m ⁻³)
H5	N 59° 02' 19, E 17° 43' 40	25	5 – 6.0	0.06 -0.44	1 -12.5
H3	N 58° 50' 40, E 17° 47' 42	50	5.7 -6.5	0.12 -0.41	1 – 8
H2	N 58° 56'04, E 17°43' 81	30	5.5-6.7	0.06-0.44	1-6.5

Source <http://www2.ecology.su.se/dbHFJ/index.htm>

Himmerfjärden covers an area of 174 km² (Engqvist and Omstedt 1992). The estuary has a mean water depth of 18 m and a maximum depth of 52 m (Fig. 2.1). It consists of multiple silled sub-basins with limited water exchange (Engqvist and Omstedt 1992). The system receives freshwater discharge from surface runoff, Lake Mälaren, and a local sewage treatment plant, which treats the sewage of approximately 250,000 people within the greater Stockholm metropolitan region. This has led to eutrophication of the estuary for the past 60 years (Savage et al. 2010). The total nitrogen discharge from the sewage treatment plant has been shown to have a direct effect on plankton productivity in the estuary (Bianchi et al. 2002; Larsson et al.1985).

Three stations, H2, H3, and H5, were selected for this study (Fig. 1). They belong to a suite of long-term monitoring stations (<http://www2.ecology.su.se/dbHFJ/index.htm>) and are located in water depths of 25 – 50 m. Salinity in the bottom water at the stations varies between 5.0 - 6.7‰, bottom water oxygen concentrations are in the range of 0.06 – 0.44 mM, and surface chlorophyll α concentrations vary between 1 and 12.5 mg m⁻³ (Table 1). Station H5 is closest to the waste water treatment plant and is characterized by the lowest salinity (6-6.7 ‰) and highest chlorophyll α content (1-12.5 mg m⁻³) (Table 1). Oxygen concentrations at 25 m depth vary between 0.12 mM in the summer to 0.41 mM in the winter when the

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stratified bottom waters mix (Table 1). Station H3 is in 50 m depth and generally has oxygenated bottom water year round. Station H2 is furthest downstream and is the least affected by the waste water treatment plant as indicated by the lowest chlorophyll α content ($1 - 6.5 \text{ mg m}^{-3}$). Bottom water oxygen concentrations at station H2 range from 0.06 to 0.44 mM year round (Table 1).

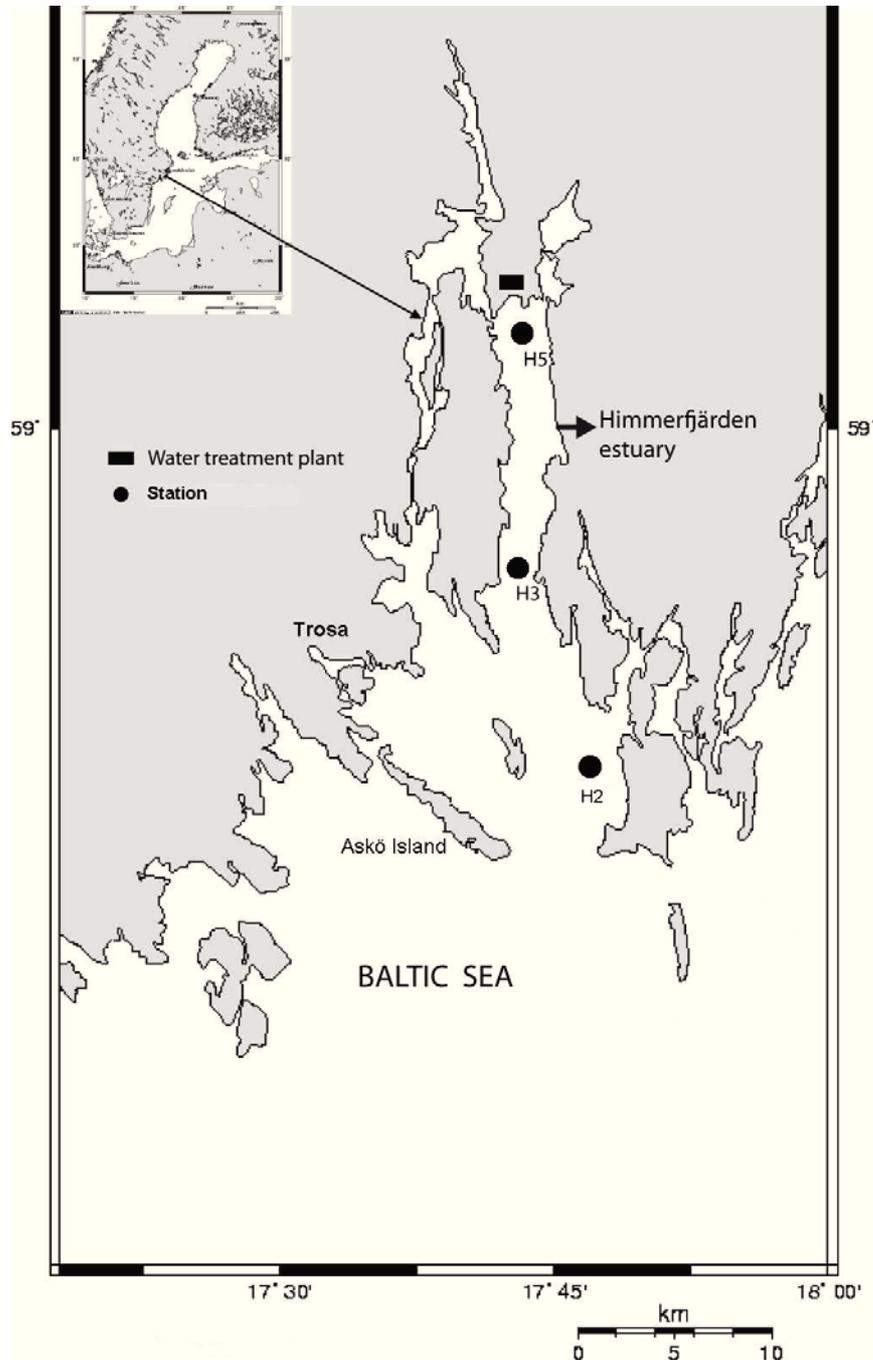


Figure. 1: Location of Stations H5, H3 and H2 in Himmerfjärden estuary, Sweden

The sediment at the three stations consists of organic-rich clays with a one to several centimeter thick brown iron oxyhydroxide-rich layer at the top. The Holocene organic-rich

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mud overlays a compact layer of clay below 120 centimeter below sea floor (cmbfsf) at station H2 that probably represents the upper boundary of the brackish glacial lake deposits (8500 - 7500 BP) (Heinsalu et al. 2000). Additionally, at station H5, laminated intervals occur in the topmost 20 cm suggesting historical anoxic bottom water conditions.

2. MATERIALS AND METHODS

2.1. Sediment sampling

Sediments from the three stations were sampled during two campaigns with the research vessel R/V *Limanda* in May 2009 and June 2010. The sediments were collected with a multicorer and a small gravity corer (Rumohrlot corer) with lengths of approximately 40 cm and 140 cm, respectively. Methane samples were immediately collected and fixed on board as described below. Otherwise, the cores were capped with rubber stoppers, transported to the Marine Research Center of Stockholm University on Askö Island and kept cold (+ 4°C) until utilized for experiments and sampling.

Subsamples for methane concentration measurements were taken using 3 cm³ cutoff syringes that were immediately inserted into predrilled holes in gravity cores on board the R/V *Limanda*. The sediments were transferred to serum 20 ml vials containing 5 mL of 5 M NaCl. Pore water was directly extracted using Rhizons connected to 10 mL syringes (Elverfeldt et al. 1984) at a resolution of 1 cm for the Multicorer and 5 cm for the Rumohrlot cores. The total volume of pore water extracted was 8 to 10 mL, of which the first 1 mL was discarded to clean the syringe and remove air trapped in the syringe, and the Rhizon. Sediment samples were collected for total carbon (TC), total inorganic carbon (TIC), total sulfur (TS), total nitrogen (TN), reactive iron, density, and porosity. Samples for ²¹⁰Pb and ¹³⁷Cs analysis were retrieved from multicores by slicing the core at a resolution of 1 to 2 cm.

³⁵S – sulfate reduction rates (SRR) were measured in intact subcores (28 mm diameter) at a resolution of 1 to 2 cm. Samples for determination of ¹⁴C- methane for measurements of AOM and of ¹⁴C - bicarbonate methanogenesis were taken with 5 mL cutoff syringes, plugged with butyl rubber stoppers, and stored in N₂ – filled plastic bags before injection of the radiotracer.

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2.2. Analytical procedures

2.2.2. Methane measurement

Methane concentration was measured in the headspace by gas chromatography with a flame ionization detector using a Shimadzu GC-8a gas chromatograph. Nitrogen was used as carrier gas at a flow rate of 15 mL min⁻¹ at 40°C. The methane concentration was calculated per volume sediment corrected for the sediment porosity according to the following equation:

$$[CH_4] = \frac{V_{head} \times A \times \alpha}{24.1 \times V_{sed} \times \phi} \quad (\text{nmol cm}^{-3}) \quad \text{Eq. 1}$$

where, V_{head} is the volume of the headspace in the sample vial (cm³), ϕ is the sediment porosity, A is the peak area of methane eluted at 0.8 minutes, α is the slope of the standard curve (ppmV), and V_{sed} is the volume of the sediment sample (cm³). The molar volume of methane at 20°C (24.1 L mol⁻¹) was used to convert from partial volume CH₄ to mole CH₄.

2.2.3. Pore water analyses

The extracted pore water was subsampled by preserving 1 mL with 100 µL ZnCl₂ (5%) for hydrogen sulfide and sulfate analyses, and collecting 2 mL headspace-free pore water for dissolved inorganic carbon (DIC) and chloride measurements.

Sulfate (10 to 20 fold dilution) and chloride (200 fold dilution) samples were measured by ion chromatography (761 Compact IC, Ω Metrohm using 838 Advanced Sample Processor Ω Metrohm) with 3.2 mM Na₂CO₃ and 1 mM NaHCO₃ as eluent. The detection limit of sulfate in pore waters was 50 µM. Sulfide was determined using the photometric methylene blue method after Cline (1969) (Shimadzu UV120 spectrophotometer, 2 µM limited detection).

Dissolved inorganic carbon concentrations were determined by flow injection analysis (Hall and Aller 1992). Due to the high sulfide concentrations in the samples, 100 µL of 0.5 M NaMoO₄·2H₂O solution were added to trap H₂S (Lustwerk and Burdige 1995). The detection limit is 0.1 mM for these measurements.

Dissolved iron (Fe²⁺) in the pore water was measured using the Ferrozine method (Viollier et al. 2000) and determined on a Pharmacia LKB Ultraspec III spectrophotometer, 0.3 µM detection limit.

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2.2.4. Solid-phase analyses

Total nitrogen, total sulfur, and organic carbon (C_{org}) concentrations in the sediment were determined with an elemental analyzer (GC - FISON 1500) at 1052°C. Total inorganic carbon was measured on a CM 5012 CO₂ Coulometer (UIC) after acidification with H₃PO₄. Organic carbon was calculated as the difference between TC and TIC. The C: N molar ratio was also calculated. Based on organic carbon profiles and sedimentation rates, organic carbon accumulation ($J_{C_{org}}$) rates were calculated as:

$$J_{C_{org}} = \frac{C_{org}}{100} \times \frac{1}{12} \times \omega \times \rho \quad (\text{mol m}^{-2} \text{ year}^{-1}) \quad \text{Eq. 2}$$

where $J_{C_{org}}$ is the organic carbon accumulation rate, C_{org} content, ω and ρ are the organic carbon content (% dry weight), sedimentation rate (cm year⁻¹), and density of dry bulk sediment (g cm⁻³).

Sediments for porosity measurements were taken using 5 cm³ cut off syringes. A 3 cm³ subsample of wet sediment was weighed, the density was determined from the wet mass per cm³, and the sediments were dried at 60°C until they reached a constant mass. The difference between wet and dried mass was used to calculate the porosity.

For chlorin measurement, 10 – 20 mg of freeze-dried sediment was extracted three times in the dark with 5 mL 100% acetone in an ice-bath according to Schubert et al. (2005). The extracted solution was immediately analyzed with a Hitachi F-2000 fluorometer at 428 nm. Chlorophyll α was used as calibration standard. The extracted solutions then were acidified and re-measured. The Chlorin Index (CI) was calculated based on the ratio of the fluorescence intensity (FI) of non-acidified to acidified extracts (Schubert et al. 2005).

Total reactive iron in the sediment was extracted by a two-step ascorbate – dithionite extraction under anoxic conditions according to März et al. (2008). The reactive ascorbate extractable iron (Fe_{asc}) and reactive dithionite extractable iron (Fe_{dithio}) extractions were measured for total dissolved iron (Fe^{2+} and Fe^{3+}) by the atomic absorption spectroscopy (AAS), Thermo Scientific iCE 3000 series using the ASX-520 Autosampler. The extractions of Fe_{asc} and Fe_{dithio} represent of reactive amorphous iron and crystalline iron (oxyhydro)oxides, respectively (März et al. 2008).

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2.2.5. Process rate measurements

Sulfate reduction rates were determined by injecting ^{35}S -sulfate tracer (50 kBq into the retrieved subcores followed by 6 to 8 hours of incubation. The incubation was stopped by transferring the sediment into 50 mL plastic centrifuge tubes containing 20 mL zinc acetate (20%, v/v). The total amount of ^{35}S -labeled reduced inorganic sulfur was determined using the single step cold distillation method of Kallmeyer et al. (2004) by counting on a Tricarb 2500 liquid scintillation counter. Sulfate reduction rates ($\text{nmol cm}^{-3} \text{ day}^{-1}$) were calculated using the following equation (Jørgensen 1978):

$$SRR = \frac{TRI^{35}S}{^{35}SO_4^{2-} + TRI^{35}S} \times \frac{\{SO_4^{2-}\}}{t} \times 1.06 \text{ (nmol cm}^{-3} \text{ day}^{-1}) \quad \text{Eq. 3}$$

where $\{SO_4^{2-}\}$ is the pore water sulfate concentration corrected for porosity (ϕ) ($\text{nmol cm}^{-3}_{\text{wet sed}}$), $TRI^{35}S$ and $^{35}SO_4^{2-}$ are the radioactivities (Bq) of sulfate and total reduced sulfur species, respectively, and t is the incubation time in days. The factor 1.06 is the estimated fractionation factor between ^{35}S and the natural isotope ^{32}S (Jørgensen and Fenchel 1974). SRR were determined in three parallel cores for all depth intervals and the values reported are the median values of the triplicates.

Bicarbonate methanogenesis rates were measured by injecting $^{14}\text{C-HCO}_3^-$ tracer (20 kBq) into the sediment in the 5 cm^3 cutoff syringes taken from the Rumohrlot cores and incubating for 16 hours at *in situ* temperatures (the same temperature at the coring time, 4°C). The incubations were stopped by transferring the sediment into 25 mL of 2.5% NaOH in glass jars (50 mL). In the laboratory, the headspace gas was flushed by a carrier gas of 79% N_2 : 21% O_2 at 30 mL/min for 30 min through 850°C copper oxide columns as catalyst to oxidize $^{14}\text{CH}_4$ to $^{14}\text{CO}_2$. The CO_2 was trapped in a series of two scintillation vials containing 10 mL of Carbosorb solution (Optiphase Hisafe -3 plus β -phenylethylamine: in vol/vol ratio of 4:1). The radioactivity was measured on a liquid scintillation counter (Tricarb 2500). The methanogenesis rate (ME) was calculated using the following equation:

$$ME = \frac{^{14}\text{CH}_4}{H^{14}\text{CO}_3^- + ^{14}\text{CH}_4} \times \frac{\{DIC\}}{t} \text{ (nmol cm}^{-3} \text{ day}^{-1}) \quad \text{Eq. 4}$$

where $\{DIC\}$ is the concentration of dissolved inorganic carbon per cm^{-3} sediment corrected for porosity ($\{DIC\} = \text{DIC} \times \phi$) in the pore water, $^{14}\text{CH}_4$ and $H^{14}\text{CO}_3^-$ are the activities (kBq)

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of labeled methane and bicarbonate, respectively, and t is the incubation time in days. The bicarbonate methanogenesis rates were measured on three parallel samples and the values presented here are the median rates of the triplicates.

Rates of anaerobic oxidation of methane were determined by injecting ^{14}C -methane tracer (10 kBq) directly into 3 cm^{-3} sediment samples in cutoff syringes and incubating the sediment for 14 hours at in situ temperature. After the incubation, the microbial activity was stopped by transferring the sediments to 50 mL glass tubes containing 20 mL NaOH (2.5%). AOM rates ($\text{nmol cm}^{-3}\text{ day}^{-1}$) were calculated based on the ratio of ^{14}C -bicarbonate and ^{14}C -methane using the methane concentration in each sample (Treude et al. 2003) according to the following equation:

$$AOM = \frac{{}^{14}\text{CO}_2}{{}^{14}\text{CH}_4 + {}^{14}\text{CO}_2} \times \frac{\{\text{CH}_4\}}{t} \quad (\text{nmol cm}^{-3}\text{ day}^{-1}) \quad \text{Eq. 5}$$

where $\{\text{CH}_4\}$ is the porosity-corrected concentration of methane at the beginning of the incubation. $^{14}\text{CO}_2$ is the activity (Bq) of carbon dioxide, $^{14}\text{CH}_4$ is the activity of methane also trapped as $^{14}\text{CO}_2$, and t is the incubation time (day). The rate of anaerobic oxidation of methane is calculated as $\text{nmol cm}^{-3}\text{ day}^{-1}$. Similar to the SRR, AOM rates were measured in three parallel cores and the values presented here are the median values.

2.2.6. ^{210}Pb and ^{137}Cs analyses and calculation of sedimentation rates

Dry and ground sediment samples for radiochemical measurement ($^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs activities) were sealed in polysulfone vials and equilibrated for at least three weeks. Activities of the radionuclides were determined using ultra – low level gamma spectroscopy on a closed-end coaxial well detector (Ge Coaxial Type N gamma detector) for 1 to 3 days. The total ^{210}Pb radioactivity was determined directly by measuring the ^{210}Pb at 46.5 KeV gamma peak and ^{224}Ra that was indirectly determined by measuring the gamma activity of ^{214}Pb (at 295 and 352 KeV) and ^{214}Bi (609 KeV). The $^{210}\text{Pb}_{\text{excess}}$ was determined by the total ^{210}Pb minus the supported ^{210}Pb that derives from ^{226}Ra . Self-absorption corrections were made on each sample following the technique of Cutshell et al. (1983).

^{137}Cs activities were determined by measurement of the 662 KeV gamma peak intensity. Elevated ^{137}Cs is retained as an artificial tracer (produced from nuclear bomb testing) introduced to the Baltic Sea environment in the 1950s and in 1963. Additionally, the study area received a large amount of ^{137}Cs as a result of the Chernobyl catastrophe in 1986.

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Sediment accumulation rates were assumed to be constant overtime. A geochronology was established using the down-core distribution of $^{210}\text{Pb}_{\text{excess}}$ activities ($\lambda = 22.3$ a half-life) using a constant initial concentration model (Appleby and Oldfield 1983):

$$t = \frac{1}{\lambda} \ln \frac{C_{(0)}}{C} \quad \text{Eq. 6}$$

where $C_{(0)}$ is the unsupported ^{210}Pb activity at the sediment surface, C is the activity at the depth of age determination, and λ is the ^{210}Pb decay constant. In addition, a date of 1986 was assigned to the peak ^{137}Cs activity as an independent chronostratigraphic marker.

3. RESULTS

3.1 Pore water chemistry

Pore water chloride concentrations were between 95 and 105 mM and remained constant with depth at all stations (data not shown).

At stations H2 and H5, methane concentrations increased from 0.1 mmol L⁻¹ at 1 cm sediment depth to the saturation concentration of 1.95 mmol L⁻¹ (1 atm, 10°C) at a depth of 20 cm and continued to increase linearly with depth to the bottom of the core (Fig. 2a). Similarly, methane concentrations increased in almost linear fashion at station H2, but concentrations > 2 mmol L⁻¹ at station H2 were reached below 30 cm depth, whereas these concentrations at station H5 were found below 10 cm depth (Fig. 2a). By contrast, at station H3, methane concentrations remained stable below 57 cm depth and slightly varied between 4.3 and 4.8 mmol L⁻¹ (Fig. 2a). Calculation of the saturation concentration of methane at the respective water depths for the three stations indicated that all methane concentrations remained below the solubility limit at the in situ pressures. At all three stations, the sulfate-methane transition was very broad and not marked by a distinct decrease in methane concentrations at the depth of sulfate depletion (Fig. 2a).

Sulfate concentrations in the surface sediments increased from 4.3 mM at H5, to 4.5 mM at H3 to 4.8 at H2. At all three stations sulfate concentrations decreased in a nearly linear fashion to concentrations of 0.1 mM at 17 – 20 cm depth. Although the penetration depth (defined as the depth at which sulfate concentrations reached 0.1 mM) at station H5 was shallowest, 17 cm, sulfate gradients were steepest at station H2. The pore water gradients

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were very sensitive to surface sulfate concentrations. This is also reflected in the calculated sulfate fluxes (see Section 3.5). Below 20 cm, sulfate concentrations remained < 0.1 mM down to the bottom of the cores (ca. 120 cm depth) (Fig. 2a).

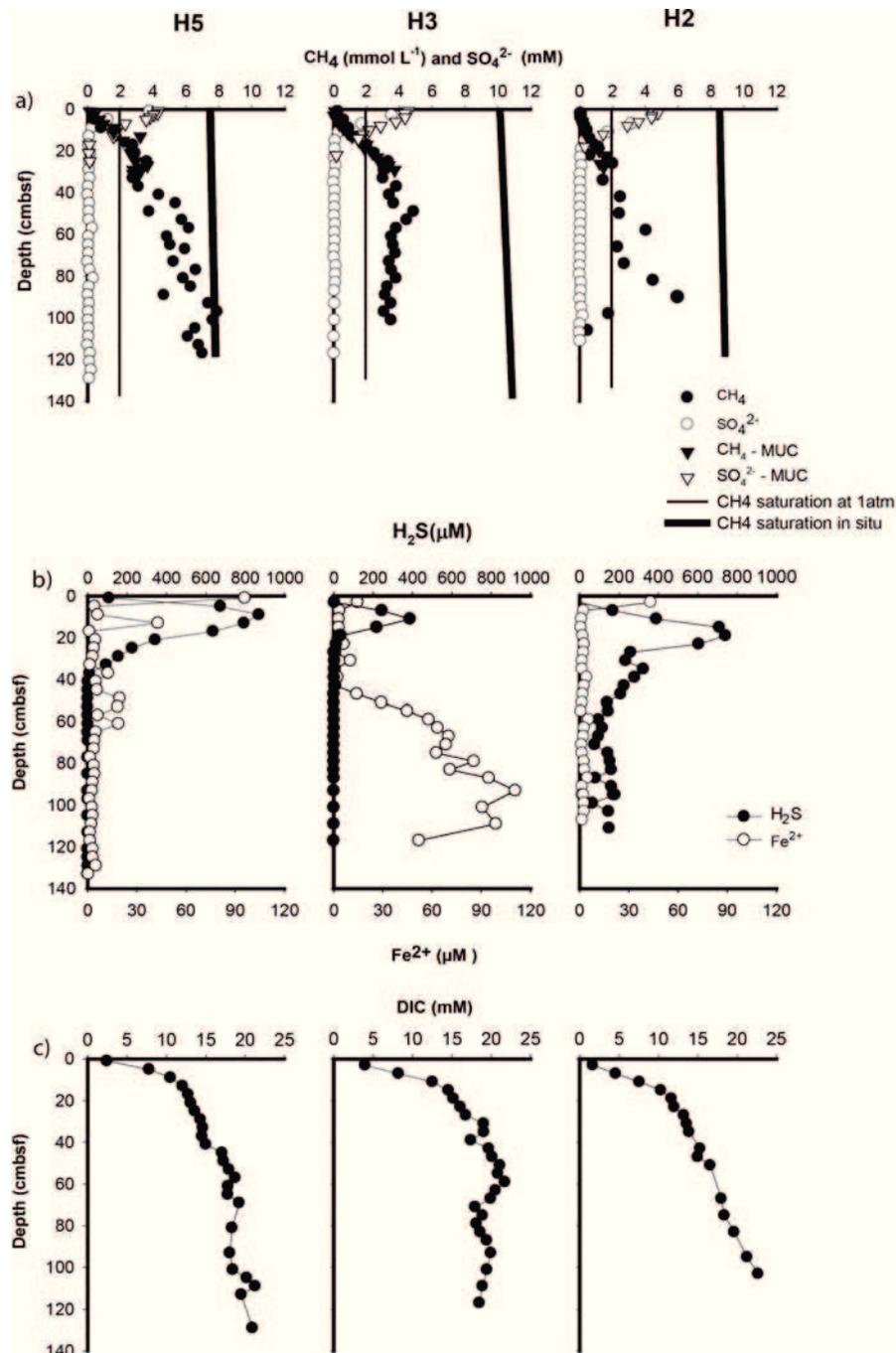


Figure 2: a) Concentration of methane and sulfate, b) concentrations of sulfide and iron (II), and concentrations of dissolved inorganic carbon (DIC) in pore water

The highest dissolved sulfide concentrations were measured at Station H5 (871 μ M) and the lowest at station H3 (387 μ M). At all three stations, dissolved sulfide concentrations showed a maximum between 9 and 19 cm before decreasing with depth. Sulfide

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concentrations decreased to very low values near the detection limit of 1 μM at stations H3 and H5, whereas they remained between 65 and 180 μM at below the SMT at station H2 (Fig. 2b).

Dissolved iron concentrations followed the general pattern of elevated or very high concentration in the topmost centimeter decreasing to near detection in the sulfidic zone. In the sulfide-free zone at depths below 47 cm, dissolved iron increased again at station H3, whereas no increase was observed at station H5 and H2. At station H5, the dissolved iron concentrations were the highest of all three stations in the topmost centimeter (Fig. 2b).

Dissolved inorganic carbon concentrations increased with depth reaching values between 21.3 and 22.7 mM. The steepest increase occurred in the upper 20 cm in the sulfate reduction zone, whereas in the methanogenesis zone below, DIC concentration remained more or less constant (station H3), or increased only very gradually (stations H5 and H2) (Fig. 2c).

3.2. Solid phase geochemistry

The TIC content at all stations was very low (0.01 – 0.02 dry wt. %) and close to detection. We therefore assume that TC is almost entirely comprised of C_{org} and only present the C_{org} data (Fig. 3a). The C_{org} content at station H5 steadily decreased from 2.7 dry wt. % at the top of the sediment core to 1.9 dry wt. % at the bottom of the core. At station H3, the C_{org} content decreased down core from 3.2 to 2.5 dry wt. % (7.5 to 107.5 cm depth). At station H2, the C_{org} content decreased with depth from 3.7 to 2.6 dry wt. % (Fig. 3a). Sedimentary C: N ratios showed a low variation between 9.6 and 10.6 throughout the core (Fig. 3a).

Total sulfur (TS) contents at station H5 ranged between 0.5 and 1.2 dry wt. % with a pronounced minimum between 30 and 50 cm depth. The TS content at H3 was almost constant throughout the core (0.5 -0.6 dry wt. %), whereas at station H2, it gradually increased from 0.4 dry wt. % at the surface to 1.5 dry wt. % at 45 cm depth. Further below, the contents slightly decreased to 1.1 dry wt. % at 75 cm depth (Fig. 3a).

In general, the chlorin content gradually decreased with depth at all stations (Fig. 3b). At station H5, the chlorin content decreased from 9.5 to 3.0 $\mu\text{g g}^{-1}$ (dry wt.) between 37.5 and 88.5 cm depth. At station H3, the concentration decreased from 15.2 to 5.0 $\mu\text{g g}^{-1}$ between 7.5 and 97.5 cm depth, with the exception at the depth of 37.5 cm, where the chlorin concentration was 40.5 $\mu\text{g g}^{-1}$. At station H2, concentrations decreased from 9.8 to 3

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$\mu\text{g g}^{-1}$ between 12.5 and 72.5 cm depth (Fig. 3b). The calculated chlorin index (CI) varied between 0.65 and 0.99 with values close to 1 indicating refractory material at all stations, except at 37.5 cm depth at station H3, where the CI value was 0.54.

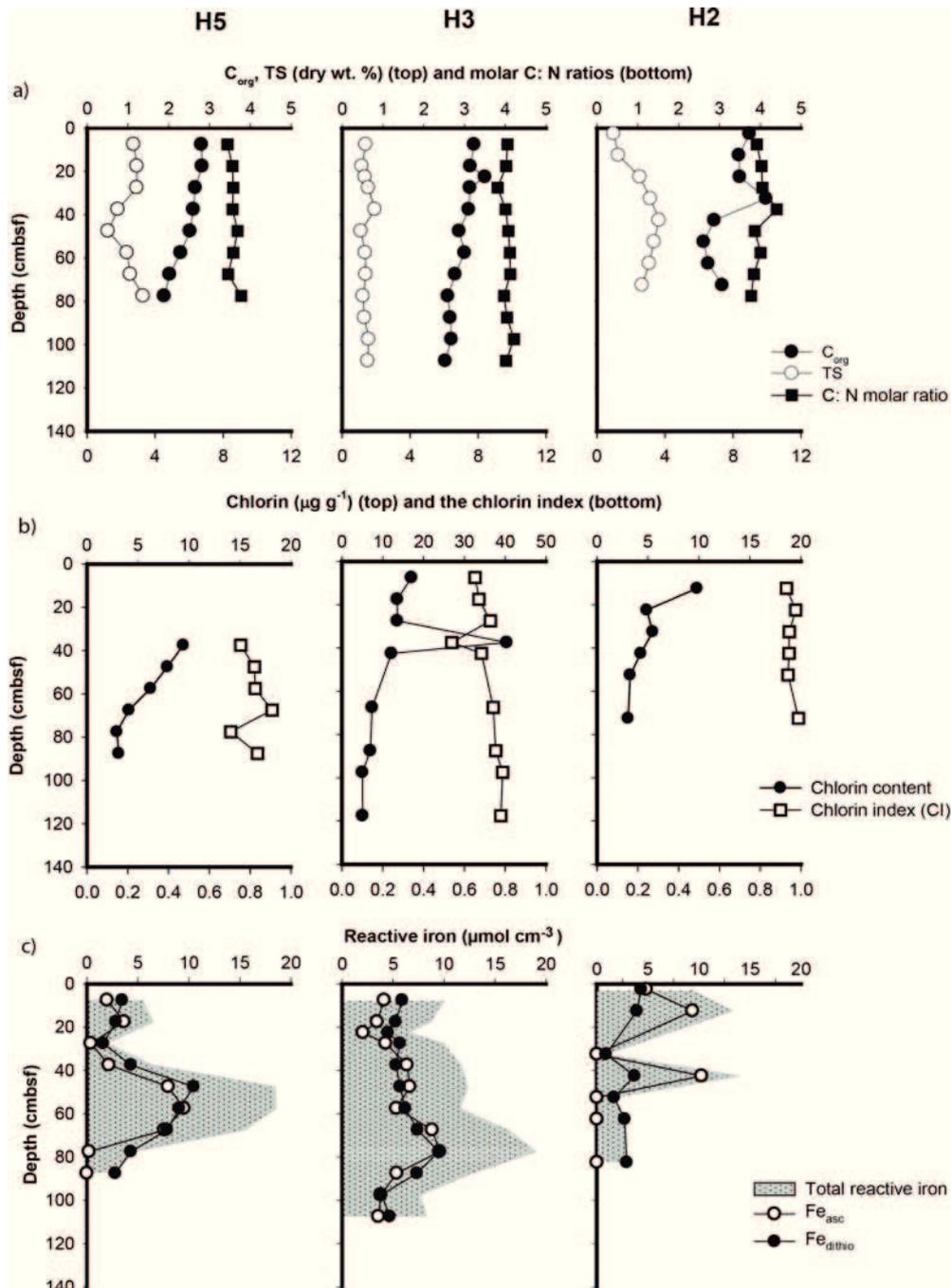


Figure 3: a) Solid phase TOC, TS, and C/N ratios, b) chlorin content and index (CI), and c) reactive iron in Himmerfjärden sediments

High concentrations of reactive Fe were observed throughout the cores at all three sites (Fig. 2.3c). Total reactive iron, as defined by $\text{Fe}_{\text{asc}} + \text{Fe}_{\text{dithio}}$, was much greater at Stations

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H3 and H5 compared to station H2, where total Fe reactive was $< 10 \mu\text{mol cm}^{-3}$ (Fig. 3c). Concentrations of total reactive iron showed a distinct peak of $> 18 \mu\text{mol cm}^{-3}$ at 45 cmbsf at station H5. In general, concentrations of reactive Fe were $> 10 \mu\text{mol cm}^{-3}$ at both stations H3 and H5. Stations H3 and H5 were also similar in that the reactive Fe was almost equally divided between the dithionite and ascorbate reducible fractions. At station H2, easily reducible Fe_{asc} was only significant in the upper 0-5 cm of the sediment. The low concentrations of Fe_{asc} correlated with the presence of high dissolved H_2S concentrations.

3.3. Rates of sulfate reduction, anaerobic oxidation of methane, and methanogenesis

We could not calculate accurate specific activities necessary for precisely determining sulfate reduction rates below 14 cm, due to the very low sulfate concentrations (expectedly $< 50 \mu\text{M}$). At station H5 peak SRR ($46 \text{ nmol cm}^{-3} \text{ day}^{-1}$) in the upper part were found within the surface 0-1 cm, whereas peak rates in the upper layer of sediment varied from $25 \text{ nmol cm}^{-3} \text{ day}^{-1}$ (station H3 3.5 cm) to $29 \text{ nmol cm}^{-3} \text{ day}^{-1}$ (station H2 5.5 cm). At all three sites, a second distinct SRR peak rate was observed near the bottom of the sulfate penetration. These rates varied from $33 \text{ nmol cm}^{-3} \text{ day}^{-1}$ at station H2 to $76 \text{ nmol cm}^{-3} \text{ day}^{-1}$ at Stations H3 and H5. This deep peak of SRR is consistent with the peak of AOM measured at H3 ($16 \text{ nmol cm}^{-3} \text{ day}^{-1}$) at 14 cm depth (Fig. 4a). Integrated SRRs over 14 cm were nearly similar at all three sites and ranged from $1.46 \text{ mol m}^{-2} \text{ year}^{-1}$ (station H3) to $1.92 \text{ mol m}^{-2} \text{ year}^{-1}$ (station H2) (Table 2).

Table 2: Organic matter accumulation rate ($J_{\text{OM} - \text{C}}$), depth-integrated bicarbonate methanogenesis rate (Bi-ME), and gross sulfate reduction in Himmerfjärden sediments

Station	J_{Corg} ($\text{mol m}^{-2} \text{ year}^{-1}$)	Bi – ME ($\text{mol m}^{-2} \text{ year}^{-1}$)	GSR* ($\text{mol m}^{-2} \text{ year}^{-1}$)	$J_{\text{S burial}}$ ($\text{mol m}^{-2} \text{ year}^{-1}$)	$J_{\text{S burial}} / \text{GRS}$ (%)
H5	9.5	1.09	1.52	1.23	81
H3	9.3		1.46	0.60	41
H2	8.9	0.96	1.92	0.95	49

Rates of bicarbonate methanogenesis varied between 0.2 to $1.2 \text{ nmol cm}^{-3} \text{ day}^{-1}$ and 0.1 to $3.2 \text{ nmol cm}^{-3} \text{ day}^{-1}$ at station H2 and H5, respectively. Methanogenesis rates increased below the sulfate zone. At station H5 methanogenesis rates peaked immediately right below the SMT and decreased again further below (Fig. 4b). Integrated bicarbonate methanogenesis

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rates over 100 cm depths were variable between $1.09 \text{ mol m}^{-2} \text{ year}^{-1}$ at station H5 and $0.96 \text{ mol m}^{-2} \text{ year}^{-1}$ at station H2 (Table 2).

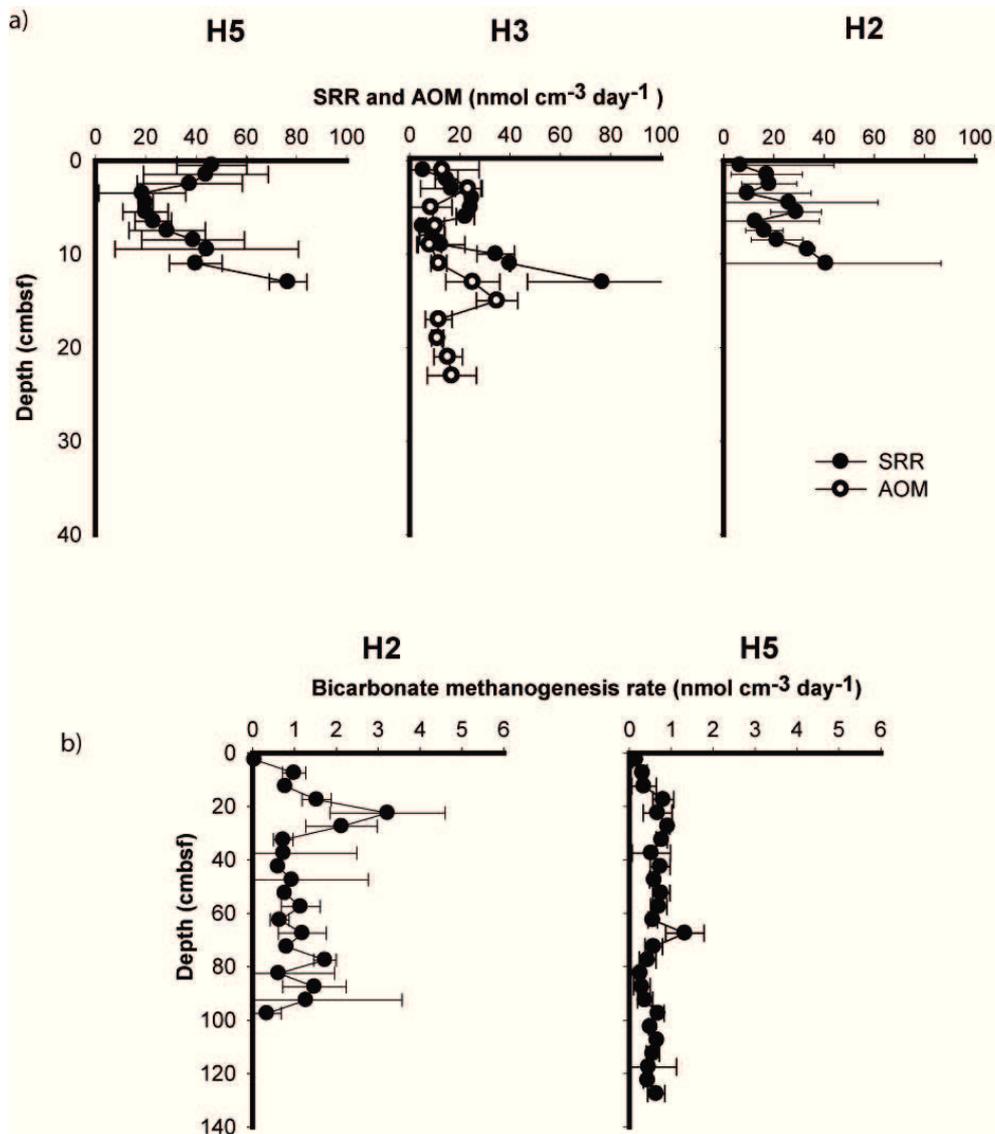


Figure 4: a) Sulfate reduction rate (SRR) and rate of anaerobic oxidation of methane (AOM; St. H3 only), and b) bicarbonate – methanogenesis rate

3.4. Sedimentary $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs distribution, and sedimentation rates

At all stations, $^{210}\text{Pb}_{\text{excess}}$ decreased with depth, but did not reach zero levels in the top 27 cm. Based on the distribution of $^{210}\text{Pb}_{\text{excess}}$, and assuming steady-state input of ^{210}Pb and minimal sediment mixing, we calculated sedimentation rates of $0.98 \text{ cm year}^{-1}$ at station H5, $0.82 \text{ cm year}^{-1}$ at station H3 and, $0.77 \text{ cm year}^{-1}$ at station H2 (Table 3).

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Table 3: Sedimentation rates of the three stations in Himmerfjärden estuary based on ^{210}Pb excess and ^{137}Cs profiles

Station	Mean rate (cm year ⁻¹)				
	$^{210}\text{Pb}_{\text{ex}}$	^{137}Cs	$^{210}\text{Pb}_{\text{ex}}$	^{137}Cs	$^{210}\text{Pb}\&^{137}\text{Cs}$
H5	0.98	0.91	1.36 ^a	1.32 ^a	
H3	0.82	0.82			0.89 ^b
H2	0.77	0.65			

a: Data from Bianchi et al. (2002) for Station H5,

b: Data from Reuss et al. (2005) from average rates in Himmerfjärden estuary.

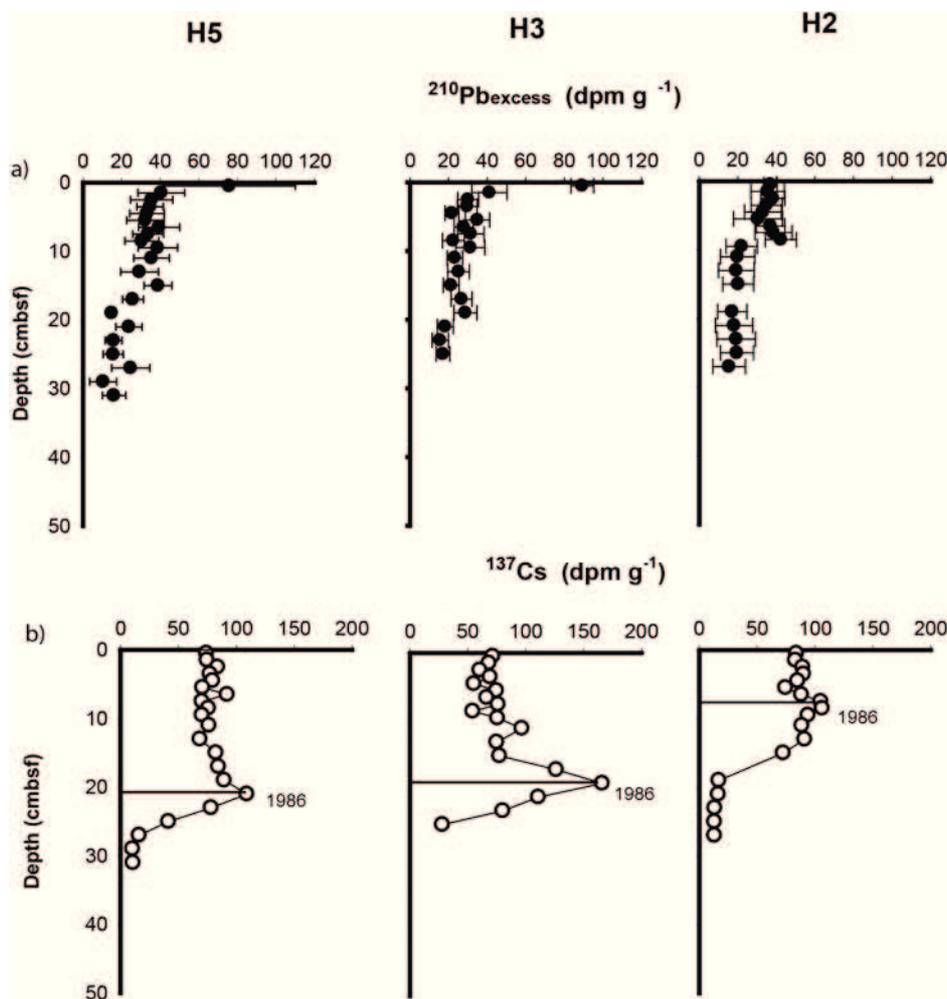


Figure 5: a) ^{210}Pb excess and b) ^{137}Cs profiles in Himmerfjärden estuary sediments

3.5. Methane and sulfate fluxes based on reaction-transport modeling

Utilizing the methane, sulfate, and DIC profiles, the reaction-transport model of Wang et al. (2008) was applied to calculate the fluxes between the water column and underlying

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sediments, using diffusion coefficients for 5°C obtained from Schulz and Zabel (2006). Due to the relatively high rates of sediment accumulation, advective pore water transport was also considered. Average sedimentation rates obtained from the ^{210}Pb and ^{137}Cs approaches for each station were used. A value of 0.05 for significance level was employed for the fitting program. The results indicate that methane fluxes (in $\text{mol m}^{-2} \text{ year}^{-1}$) from the sediment to the sediment-water interface were $0.37 \text{ mol m}^{-2} \text{ year}^{-1}$ for H5, $0.25 \text{ mol m}^{-2} \text{ year}^{-1}$ for H3, and $0.11 \text{ mol m}^{-2} \text{ year}^{-1}$ for H2. The sulfate fluxes from water column into the sediment were 0.34, 0.30, and $0.45 \text{ mol m}^{-2} \text{ year}^{-1}$ at stations H5, H3, and H2, respectively (Table 4, Fig. 6). The DIC fluxes in the sulfate reduction zone were 1.18, 1.15, and $0.89 \text{ mol m}^{-2} \text{ year}^{-1}$ for station H5, H3, and H2, respectively (Table 4).

Table 4: The upward advective - diffusive flux ($J_{\text{ad-di}}$) and advective – diffusive-bioirrigative ($J_{\text{ad-di-bio}}$) flux of methane to the sediment - water interface (J_{CH_4}), downward sulfate flux into the sediment ($J_{\text{SO}_4^{2-}}$), and dissolved inorganic carbon (DIC) flux in the sulfate reduction zone (J_{DIC}) based on reaction-transport and bioirrigation flux models.

Station	J_{CH_4} ($\text{mol m}^{-2} \text{ year}^{-1}$)		$J_{\text{SO}_4^{2-}}$ ($\text{mol m}^{-2} \text{ year}^{-1}$)		J_{DIC} ($\text{mol m}^{-2} \text{ year}^{-1}$)	
	$J_{\text{ad-di}}$	$J_{\text{ad-di-bio}}$	$J_{\text{ad-di}}$	$J_{\text{ad-di-bio}}$	$J_{\text{ad-di}}$	$J_{\text{ad-di-bio}}$
H5	0.37	0.78	-0.25	-1.66	1.18	5.70
H3	0.25	0.46	-0.30	-1.59	1.15	5.28
H2	0.11	0.32	-0.45	-2.08	0.89	4.65

4. DISCUSSION

4.1. Sediment accumulation in Himmerfjärden

To understand methane and sulfur biogeochemistry of Himmerfjärden it is important to appreciate the high rates of sediment accumulation, and ultimately, the delivery of organic carbon from the water column to the underlying sediment. $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs distributions indicate that Himmerfjärden sediments accumulate at very high rates from $0.98 \text{ cm year}^{-1}$ at station H5 in the upper estuary to $0.65 \text{ cm year}^{-1}$ at the lower end (Table 2). These sediment accumulation rates agree well with those calculated by Reuss et al. (2005) ($0.89 \text{ cm year}^{-1}$ based on $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs profiles). Our rates are slightly lower than rates determined by Bianchi et al. (2002), ($1.32 \text{ cm year}^{-1}$ at station H5 based on $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs profiles) and

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Meili et al. (1998) who estimated a rate of 1 cm year⁻¹ in Himmerfjärden archipelago based on ¹³⁷Cs profiles. The uppermost sediment layers may now be accumulating at a lower rate, based on the ²¹⁰Pb_{excess} distributions, within 0.15 – 0.17 cm year⁻¹ in the top 5 cm at all stations. Nevertheless, sediment accumulation rates in Himmerfjärden estuary are still 1.5 – 3.5 fold higher than in the open Baltic Sea basins, for example the Bothnian Sea, Bothnian Bay, Finland Bay, and Baltic Proper (0.26 – 0.62 cm year⁻¹, Mittila et al. 2006).

Table 5: Inventory of ¹³⁷Cs in the Baltic Sea sediment due to Chernobyl disaster

Areas	Inventory (Bq g ⁻¹ dry)	References
Poland Coastal Areas (Baltic Sea)	<0.57	Callaway et al. 1996
The western European coastal areas (the Nederland, England coastal areas)	<0.2	Callaway et al. 1996; Walling and He. 1996
Gävle Bay (Swedish coastal)	<14.9	Holby and Evans. 1996
Himmerfjärden (Swedish coastal)	0.3 – 2	Meili et al. 1998
Finland lakes	1.5 – 46	Ilus and Saxén. 2005
Bothnian Bay	<0.5	Ilus et al. 2007
Bothnian Sea	<1.5	Ilus et al. 2007
Gulf of Finland	<2.3	Ilus et al. 2007
Baltic Proper	<0.3	Ilus et al. 2007
Himmerfjärden	0.2 - 2.8	This study

The ¹³⁷Cs profiles represent a pulsed input from the Chernobyl catastrophe in 1986 and earlier inputs from above-ground atomic bomb tests, with a fallout peak in 1963. We assigned the peak in the ¹³⁷Cs distributions to the Chernobyl event (see Fig. 5b). The overall distribution of ¹³⁷Cs can also be affected by sediment mixing, which would broaden the peak. Sediment mixing does occur at Station H2 as indicated by the broadening of the ¹³⁷Cs peak as compared to the sharp peaks at Station H3 and H5 (Fig. 2.5b). Input of allochthonous post-Chernobyl ¹³⁷Cs-bearing particles also affects the distribution of ¹³⁷Cs. The elevated ¹³⁷Cs activities above the putative 1986 Chernobyl peak in Figure 2.5b are likely derived from ¹³⁷Cs containing particles from the Himmerfjärden watershed. Satellite imagery shows that agricultural activities dominate the watershed land-use. ¹³⁷Cs activities measured in Himmerfjärden (1-2.8 Bq g⁻¹ dry) fall into the range given for Chernobyl impacted soils and sediments in Finland and Scandinavia (0.3 to 46 Bq g⁻¹ dry) (Table 2.5). The enhanced ¹³⁷Cs activities above the Chernobyl (1986) peak indicate that the sedimentation patterns in

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Himmerfjärden are dominated by resuspended sediments and sediment delivered from upstream and soils in the Himmerfjärden watershed.

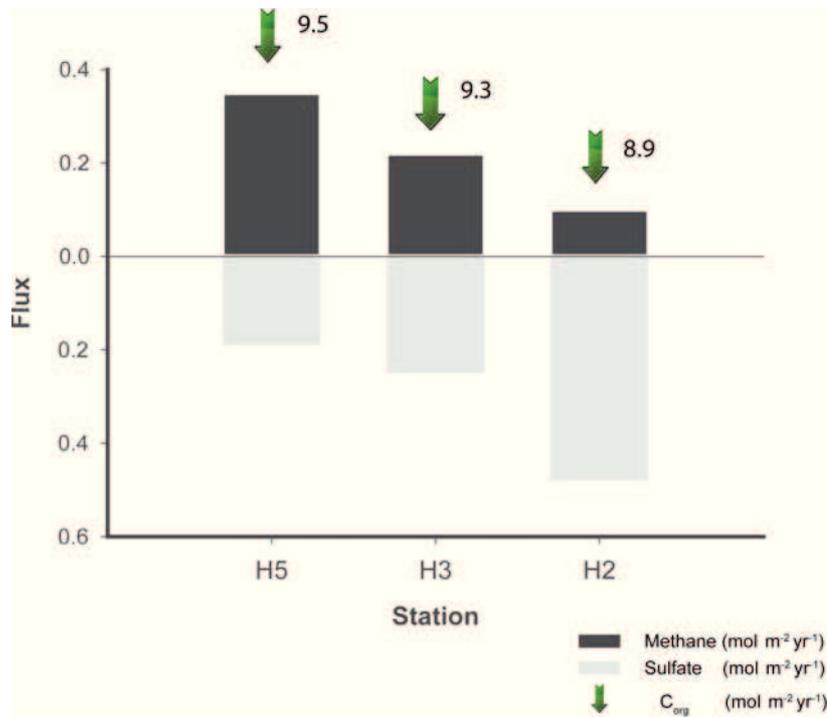


Figure 6: Upward flux of methane to the sediment - water interface and downward flux of sulfate based on the reaction-transport model, and deposition of organic matter (OM) in Himmerfjärden estuary sediments.

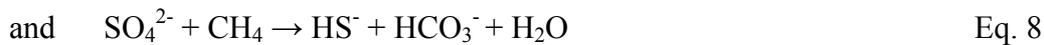
Sediment accumulation rates are greatest in the inner Himmerfjärden at Station H5. Bianchi et al. (2002) attributed high sediment accumulation rates at station H5 to the presence of the nearby sewage treatment plant. By implication, one would expect the highest rates of C_{org} accumulation at station H5, which our data confirm (Table 2, Fig. 6). In contrast, the highest C_{org} contents in the core top were measured at station H2 (3.8%), compared to 3.2% at Stations H3, and 2.8% at station H5. Although the station H2 sediments have the highest C_{org} contents, this organic carbon exhibits the greatest degree of chlorin degradation, as indicated by the chlorin index value near unity (Fig. 3b). This is consistent with lower sedimentation rates and possibly sediment mixing due to bioturbation. We observe that the sediment accumulation rates along Himmerfjärden decrease by 30% from station H5 to station H2. Thus, the combination of decreasing sedimentation rates from the head (station H5) to the mouth (station H2), and greater C_{org} contents towards lower end of the estuary result in estimated C_{org} burial rates greatest at station H5, and correspondingly lowest station H2 (Table 2, Fig. 6). The relatively high sediment accumulation rates and organic carbon burial

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fluxes have, as will be discussed below, important implications for the biogeochemistry of sulfur and methane throughout the Himmerfjärden estuary.

4.2. Sulfate reduction and sulfur fluxes in Himmerfjärden

Sulfate reduction is usually the dominant anaerobic pathway of organic carbon decomposition in organic-rich, marine sediments (Jørgensen and Kasten 2006). The downward flux of sulfate from the sediment surface to bottom sulfate zone drives the organoclastic sulfate reduction (Eq.7) and sulfate dependent AOM (Eq.8):



Organoclastic sulfate reduction (Eq. 7) usually exceeds methanotrophic sulfate reduction (Eq. 8) driven by upward diffusing methane (Martens and Klump 1984). In spite of the high sedimentation rates and organic carbon burial rates, sulfate reduction rates only varied between 1.46 – 1.92 mol m⁻² year⁻¹ in the upper 14 cm, with the greatest areal rates at station H2 and the least at station H3 (Table 2). Integrated sulfate reduction rates in Himmerfjärden estuary sediments are similar to those of Bornholm Basin and Gotland Deep sediments in the Baltic Sea (Lapham, Brüchert unpublished data). The rates of sulfate reduction, however, are lower than those measured in shallow, high deposition sedimentary environments (13.0 mol m⁻² year⁻¹) and in estuaries and embayments (2.6 mol m⁻² year⁻¹) (Canfield et al. 2005) (Table 6). We attribute the relatively low rates of integrated sulfate reduction to the shallow sulfate penetration depth. Salinity in Himmerfjärden is only 6 to 6.5‰ leading to surface sulfate concentrations that are also low (< 4.7 mM). Additionally, and perhaps more importantly, the high sediment burial rates result in a limited residence time of the C_{org} in the sulfate reduction zone (20 to 30 years).

In Himmerfjärden, organoclastic sulfate reduction and AOM appear to overlap within the upper 20 cm of the sediment (Fig. 4a). In the low sulfate environment (<5 mM), the thermodynamics of both the organoclastic and the methanotrophic sulfate reduction are favorable (Jørgensen, 2006; Knab et al., 2008). At station H3, we measured AOM rates throughout the sulfate zone and the SMT at 20 cm depth (Fig. 4a). Based on the integrated rates of AOM over the upper 14 cm (0.3 mol m⁻² year⁻¹), 20% of the overall sulfate reduction can be attributed to AOM (Eq. 8). Overall, in the sulfate reduction zone, 25% of the organic carbon was degraded via organoclastic sulfate reduction, which is consistent with the decrease

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of C_{org} (16%) in solid phase (3.7% in the 0 – 5 cm sediment interval to 3.1% below the SMT and the chlorin concentration decline (25%), ($17.6 \mu\text{g g}^{-1}$ at 5 cm depth, to $13.5 \mu\text{g g}^{-1}$ at 22.5 cm depth). Therefore, at station H3, 75% of C_{org} that reached the surface sediment was buried into the methanogenic zone. At stations H2 and H5, organic matter burial below the sulfate reduction zone was similar to station H3, which is a very high proportion for marine sediments (Hartnett et al. 1998).

Table 6: Sulfate reduction rates in coastal marine sediments

Areas	Sulfate reduction rate ($\text{mol m}^{-2} \text{ year}^{-1}$)	References
Kattegat Bay	8.8 -12.4	Iversen and Jørgensen 1985
Brackish coastal Aarhus Bay	16.1 – 45.8	Thode – Andersen and Jørgensen 1989
Shallow, high deposition (average)	13	Canfield et al. 2005
Estuaries and Embayments (average)	2.6	Canfield et al. 2005
Bornholm Basin and Gotland Deep (Central Baltic Sea)	1.4 - 4.4	Lapham and Brüchert, unpublished data
Himmerfjärden	1.5 -1.9	This study

Sulfate fluxes into the sediment based on diffusion and pore fluid advection due to burial are inversely proportional to the organic carbon burial and methane fluxes (Fig. 6). They are also significantly lower than the total sulfate reduction rates estimated for the upper 14 cm of sediment; the modeled diffusion – advection flux is only about 16 - 23% of the total gross sulfate reduction. This suggests that another transport mechanism of sulfate into the sediments must exist. We have not accounted for sulfate transported into the upper 10 -15 cm by bio-irrigating organisms. The sediments of Himmerfjärden are populated by *Marenzelleria*, a widely distributed invasive polychaete (Kautsky 2008; Blank et al. 2008). Hedman et al. (2011) have demonstrated that *Marenzelleria* enables solute transport down to more than 15 cm depth.

The impact of bioirrigation on pore fluid exchange can be estimated by the use of a simple, one-dimensional, non-local exchange model. In this case the sediment interval of 0 to 14 cm is considered as a discrete layer. The irrigation coefficient, α , is used the Eq. 9 (Fossing

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et al. 2000), which describes the fraction of pore fluid exchanged with the surface per unit time:

$$\alpha = \frac{SRR_{meas} - SRR_{diff}}{\varphi \times (C_0 - C_{14cm})} \quad \text{Eq. 9}$$

where SRR_{meas} = the GSR (the integrated gross sulfate reduction rate; Table 2) times the sulfate reduction layer (14 cm) and SRR_{diff} = calculated flux from the fitting model (Table 4). C_0 is the sulfate concentration of the overlying surface sediment, C_{14cm} is the concentration at 14 cm depth (mol m^{-3}), and φ (porosity) was set to 0.9 (the average porosity between surface sediment (0.93) and 14 cm interval (0.86)). We calculate values of α between 2.85 and 3.12 year^{-1} . These values are at the lower range of values estimated for coastal regions (12 - 180 year^{-1}) in the top 20 cm depth (Boudreau 1997) and suggest that, on average, the pore fluids are exchanged with the overlying surface water three times per year. Albeit not vigorous, bioirrigation may serve to maintain the 10 to 15 cm-deep sulfate penetration depth observed throughout Himmerfjärden.

While *Marenzelleria* probably do not mix the sediment much (Hedman et al. 2011), they will significantly enhance the overall flux of not only sulfate, but also other dissolved components such as DIC and methane (Table 4). We use the value for α to estimate the flux of other dissolved constituents using the relationship (Boudreau 1997, p. 143):

$$F_l = \alpha \times L_l \times (C_{14cm} - C_{0cm}) \quad \text{Eq. 2.10}$$

where F_l is the flux to the sediment-water interface, assuming constant pore water irrigation (constant α) over the zone L_l (in our case 14 cm). This is a very simple approach to a complex phenomenon, but the resulting fluxes are significantly greater than the diffusive/burial advection fluxes calculated for methane (about 2-fold) and DIC (about 4-fold). Moreover, these DIC fluxes out of the sediment are more consistent with measured carbon turnover rates (sulfate reduction and methanogenesis) than those determined without bioirrigation. Although bioirrigation enhances dissolved fluxes, the presence of laminated sediment in the top 20 cm at station H5 does suggest that local and seasonal hypoxia in Himmerfjärden limit sediment mixing and to a certain degree bio-irrigation (i.e. the α values are not very large). As a consequence, regeneration of sulfate by reoxidation of sulfide and the advection supply of sulfate are limited in these sediments. This is also reflected in the relatively large fraction of sulfur that is buried as reduced solid phase (Table 2).

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The accumulated reduced sulfur in pyrite and organic sulfur below the SMT corresponds to 41 - 81% of the gross sulfate reduction rate (Table 2). The large sediment accumulation rates at stations H5 and H3 play a decisive role. Although free dissolved sulfide is present in the sulfate reduction zone, the reduced sulfide is completely scavenged from the pore waters (Fig. 2b). Essentially, the rapid burial rate removes a significant fraction of sulfur out of the surface layers.

At both stations, H5 and H3, high amounts of reactive iron are available below the sulfate reduction zone (Fig. 3c) and ferrous iron is released into the pore water. In contrast, at station H2, where the burial flux of reactive iron is significantly lower, free sulfide is not only present as a peak in the sulfate reduction zone, but is never completely titrated by reactive iron in the lower 100 cm of the core (70 – 240 μM) (Fig. 2b). The Fe_{asc} may exist in part as FeS, which is very likely to be dissolved under the strong Fe complexing conditions used in the ascorbate-citrate treatment. At stations H3 and H5, sulfide below the SMT is very efficiently scavenged and a large inventory of reactive iron, both Fe_{asc} and $\text{Fe}_{\text{dithio}}$, remains below the sulfate zone. This may be related to the lower sedimentation rates at station H2, and ultimately the rate of reactive iron delivery relative to the sulfate reduction rate. In contrast, high rates of reactive iron delivery below the sulfate zone, result in an effective scavenging of sulfide from the deeper pore waters, and potentially allow for continued organic carbon degradation via iron reduction.

The presence of Fe^{2+} in pore water below the sulfate zone suggests that iron reduction occurs, and may be a result of the interaction between dissolved sulfides (H_2S , FeS) and FeOOH species leading to ferrous iron production and the formation of elemental S (Riedinger et al. 2010; Holmkvist et al. 2011; Tarpard et al. 2011). Dissolved reduced iron may result from the direct coupling of anaerobic oxidation of methane (AOM) to iron reduction (Beal et al. 2009). The flux ratios of DIC and SO_4^{2-} range from 2.3 (station H2) to 3.3 (stations H3 and H5), thus exceeding the maximum ratio of 2 expected for organoclastic sulfate reduction (Jørgensen and Parkes 2010; Burdige and Komada 2011). This also suggests an additional terminal electron acceptor process, e.g. iron reduction, in the Himmerfjärden sediments.

4.3. Controls on methane fluxes

In the Himmerfjärden sediments the methane-bearing, sulfate-free (methanogenic) zone is within the upper 20 cm of the sediment column due to both high organic matter

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accumulation rates and low sulfate concentrations (4.3 - 4.8 mM). In spite of the presence of sulfate at all three sites, methane concentrations exhibit linear gradients extending to the sediment-water interface. As shown in Figure 6, the calculated flux of methane by diffusive transport to the sediment-water interface is greatest at station H5 ($0.37 \text{ mol m}^{-2} \text{ year}^{-1}$) and lowest at station H2 ($0.11 \text{ mol m}^{-2} \text{ year}^{-1}$), consistent with the organic carbon burial rates. These upward methane fluxes to the sediment-water interface are in the range of previously calculated methane release rates from other brackish sediments ($0 - 2.5 \text{ mol m}^{-2} \text{ year}^{-1}$) (Hariss and Sebacher 1982; Lyimo et al. 2002; Middelburg et al. 2002), from the northern Baltic Sea ($0 - 0.2 \text{ mol m}^{-2} \text{ year}^{-1}$, Gotland Deep, Bothnian Sea and Bothnian Bay, Brüchert et al. unpublished data), as well as from the coastal southern Baltic Sea ($0.02 - 133 \text{ mol m}^{-2} \text{ year}^{-1}$) (Heyer and Berger, 2000). Due to bio-irrigation, the actual methane fluxes ($0.32 - 0.78 \text{ mol m}^{-2} \text{ year}^{-1}$) may be greater than the estimated advective-diffusive methane fluxes to the sediment-water interface (Table 4). These values are much lower than values for methane fluxes from sediments from freshwater or wetland ecosystems ($1.25 - 3.75 \text{ mol m}^{-2} \text{ year}^{-1}$) (Crill et al. 1988; Miller and Oremland 1988; Purvaja and Ramesh 2001; Nakik and Mitsh 2011). Nevertheless, the brackish Himmerfjärden estuary sediments have the potential for significant release of methane to the sediment-water interface, as in other low salinity coastal regions.

Interestingly, the rates of bicarbonate methanogenesis in the sulfate-depleted sediments are less than $3.2 \text{ nmol cm}^{-3} \text{ day}^{-1}$, and generally less than $1 \text{ nmol cm}^{-3} \text{ day}^{-1}$ in the methanic zone. Although only measured at two stations (H5 and H2), rates of bicarbonate reduction to methane are also slightly higher at the upstream station H5. In comparison to methanogenesis rates previously determined for coastal brackish sediments in the southern Baltic Sea ($55 - 200 \text{ nmol cm}^{-2} \text{ day}^{-1}$, Heyer et al. 1990), Gotland Deep ($0.3 - 2.8 \text{ nmol cm}^{-2} \text{ day}^{-1}$ down to 20 cm depth, Piker et al. 1998), and Eckernförde Bay (up to $37 \text{ nmol cm}^{-3} \text{ day}^{-1}$; Treude et al. 2005), the methanogenesis rates in Himmerfjärden sediments are low. If data are integrated over the cored depth interval, however, the resulting flux of bicarbonate methanogenesis (0.96 and $1.09 \text{ mol m}^{-2} \text{ year}^{-1}$) fit well to the calculated upward fluxes of methane to the sediment-water interface (0.32 and $0.78 \text{ mol m}^{-2} \text{ year}^{-1}$) (Table 2 & 4).

Methanogenesis can also occur in the sulfate zone if sufficient non-competitive substrates are available for both sulfate reduction and methane production processes (Lovley and Klug 1982; Oremland and Polcin 1982). This has been observed in marine sediments of the Skagerrak (Parkes et al. 2007; Knab et al. 2008) and Limfjorden where sulfate was < 5

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mM (Jørgensen and Parkes 2010). Indeed, we measured low rates of bicarbonate methanogenesis ($0.2 - 0.8$, $0.1 - 1 \text{ nmol cm}^{-3} \text{ day}^{-1}$) in the active sulfate reduction zones at stations H5 and H2, respectively. This suggests that in addition to the methane flux from below, methane is being produced in the surface sediment. This methane is either transported out of the sediment or may be, albeit slowly, oxidized. However, measured rates of bicarbonate methanogenesis may in part represent a back reaction of tracer of up to 5% during anaerobic oxidation of methane (Holler et al. 2011). Methanogenesis within the surface sulfate-bearing zone needs further detailed investigation.

At all three stations, methane concentrations linearly decrease from the methanic sediments through the sulfate-bearing zone to the sediment-water interface. Although AOM was measured, this overlap of methane and sulfate suggests that the oxidation of methane is rather “sluggish”, as also observed in Black Sea sediments (Knab et al. 2009), and that a substantial methane flux to the water column may occur. An intriguing possibility is that the methane-oxidizing community may not be able to keep pace with the high rates of sediment accumulation. Methane oxidizing consortia are notoriously slow growing (Nauhaus et al. 2007). Regnier et al. (2011) have demonstrated that the microbial response to rapid changes in the pore water methane and sulfate gradients, e.g. due to rapid burial or ebullition events, can be delayed over years (typically 80 years for a sudden gas ebullition event). Due to the high sedimentation rates in Himmerfjärden estuary, the residence time of a microbial community in the sulfate zone is only 20 to 30 years. Thus, the broad overlapping zones of organoclastic and methanotrophic sulfate reduction in the sulfate zone in Himmerfjärden may occur as a consequence of a small, inefficient AOM community at the bottom of the sulfate zone.

CONCLUSIONS

Himmerfjärden is a littoral benthic ecosystem typical for the brackish coastal waters of the central Baltic Sea. Eutrophication and low concentrations of sulfate in the overlying water impact methane production, consumption, and release from the sediment to the water column. The unusual controlling variable for sulfur and methane biogeochemistry in Himmerfjärden, however, is the high rate of sediment accumulation. We propose that the depth of the sulfate zone (ca. 15 cm) is controlled by the irrigating activity of the invasive polychaete *Marenzelleria*. High rates of sediment accumulation as they occur in the Himmerfjärden estuary shorten the residence time of the sediment in the sulfate reduction zone. Such a short

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residence time (ca. 20 to 30 years) has important consequences for sulfur, iron and carbon biogeochemistry. Thus, the Himmerfjärden sediments have certain characteristics that distinguish them from central basin sediments of the Baltic and continental shelf margins. Reduced sulfur produced within the sulfate reduction zone escapes to a large extent by burial, which reduces the re-oxidation of sulfide in the surface sediments. In addition, unusually large amounts of reactive iron become buried into the methanic zone. The role of this reactive iron in organic carbon and methane at depth is unclear.

Although methane production rates are not unusually high when compared to other marine sediments, the narrow sulfate zone allows for a significant upward flux of methane to the sediment-water interface. This methane flux from the methanogenic zone to the sediment-water interface correlates with the burial flux of organic carbon, and may be enhanced by bioirrigation. The oxidation of methane in the sulfate zone appears to be sluggish. The slow growing methane oxidizing communities that are responsible for methane oxidation may not be able to keep up with the fast sediment accumulation. Therefore, the Himmerfjärden estuary (Sweden) is regarded as a model area for study the early stages diagenesis of biogeochemical processes, is certainly a prime candidate to achieve this goal.

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Chapter 3

The role of sulfate exposure time on carbon preservation and sulfur burial in Baltic Sea sediments

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ABSTRACT

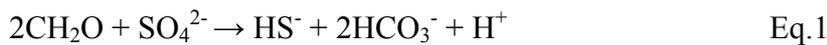
The fraction of organic carbon (C_{org}) that reaches the seafloor but escapes mineralization and is buried in deep marine sediments over longer geological periods has implications for the redox balance on the Earth's surface. Aerobic degradation is known to be a factor controlling C_{org} preservation; however, anaerobic processes such as microbially mediated sulfate reduction (SR) and methanogenesis contribute significantly to C_{org} degradation in organic-rich sediments. Recently it has been suggested that limitations on SR due to depleted levels of sulfate can lead to enhanced rates of C_{org} preservation. We tested this hypothesis by examining the extent of C_{org} preservation along a salinity gradient in Baltic Sea sediments. A simple model was applied to fit dissolved inorganic carbon pore water profiles to estimate total C_{org} degradation rates and, combined with C_{org} accumulation rates, to estimate C_{org} preservation. Our results indicate that C_{org} preservation only weakly correlates with sulfate concentrations of overlying water, sediment accumulation rate, and total C_{org} flux. Moreover, in spite of low sulfate concentrations, carbon and sulfur are not decoupled from one another as apparently was the case in the Late Cretaceous. The major determinant of C_{org} preservation appeared to be the sulfate penetration depth. Overall, it is the exposure time of the C_{org} rich sediments to sulfate (sulfate exposure time), which is influenced by sediment accumulation rate, that is a strong predictor of C_{org} preservation.

Keywords: carbon preservation, sediment accumulation rate, sulfate reduction, initial sulfate concentration, sulfate penetration depth, sulfur burial, Baltic Sea

INTRODUCTION

Only a small fraction of organic carbon (C_{org}) delivered to the sediment surface is buried in deep sediment layers. Generally $< 1\%$ of the C_{org} flux to the sediment reaches the deep sediment biosphere (Hedges and Keil, 1995). Oxygen respiration in surficial sediments and further anaerobic processes, such as denitrification, metal oxide reduction, sulfate reduction (SR), and methanogenesis, are responsible for the degradation of C_{org} . Nevertheless, this small leak (i.e. carbon preservation) in the carbon cycle over geological time scales leads to the formation of enormous reservoirs of reduced carbon buried in marine sediments ($0.16 \times 10^{15} \text{ gC a}^{-1}$, Hedges and Keil, 1995), and has implications for the chemistry of the ocean and the redox balance of the Earth's surface (Berner and Raiswell, 1983).

Sulfate reduction is a dominant anaerobic pathway of C_{org} (CH_2O) decomposition in organic-rich, marine sediments (Jørgensen and Kasten, 2006), and can be represented by the following equation (Eq. 1):



The subsequent formation of sulfide from sulfate reduction and burial of sulfide as pyrite (FeS_2) in deep sediments is also a major sink of reducing power (Berner, 1984). Over the majority of the Phanerozoic the extent of pyrite generation in marine sediments has been limited by organic-matter availability or iron oxide availability. The availability of sulfate is usually not considered to be limiting (Berner and Raiswell, 1983).

Thus, in modern sediments there is a positive relationship between sulfur and carbon burial. During the Cretaceous period, however, the relationship has been inversely correlated (Paytan et al., 2004). Wortmann and Chernyavsky (2007) proposed that extensive evaporite deposition, specifically gypsum, depleted the global ocean sulfate reservoir during the Early Cretaceous. This resulted in a decoupling of organic carbon burial and sulfur burial rates during the Early Cretaceous. The authors proposed, based on isotope relationships between reduced C and S species that near complete removal of sulfate from the world's ocean led to enhanced preservation of organic carbon.

Studies have indicated that the absence of oxygen and the consequent absence of aerobic respiration lead to increased carbon preservation (Hartnett et al., 1998). Anaerobic processes are considered to not be as effective in oxidizing complex organic carbon (Canfield, 1993). Whether there is a similar effect on carbon preservation with or without sulfate as

implied by Wortmann and Chernyavsky (2007) is not at all apparent and is difficult to test experimentally.

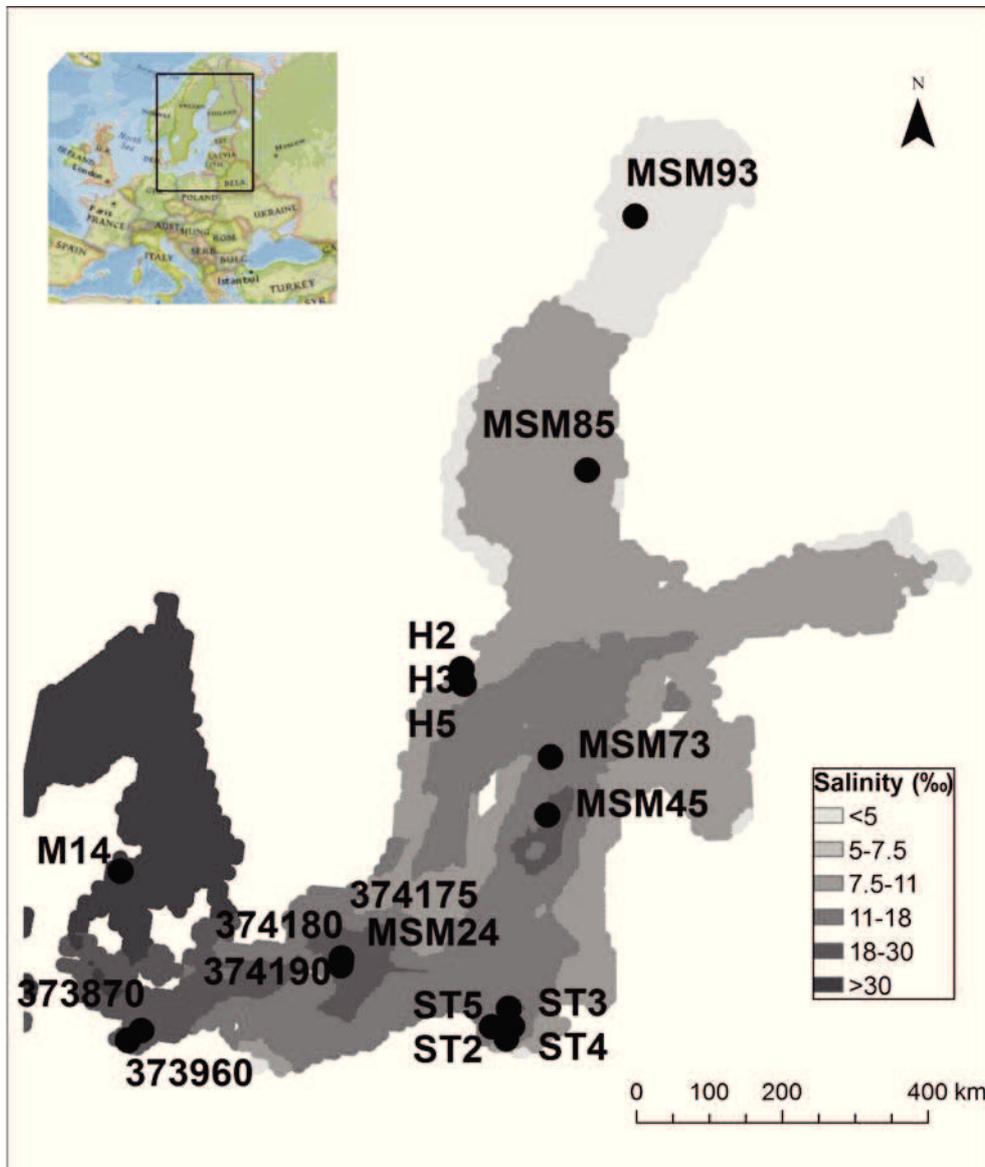


Figure 1: Location of sampling in the salinity in the Baltic Sea

The modern Baltic Sea provides a natural analog to past times in Earth's history when oceanic sulfate concentrations were depleted but organic carbon burial rates remained elevated. The Baltic Sea is the largest brackish water body in the world, with an overall length exceeding 1000 km and a salinity gradient from 27‰ in the Baltic Sea – North Sea transition to 3 - 5‰ in the Bothnian Bay (Fig. 1). Surface sulfate concentrations correspondingly range from 22 to < 3 mM. Over its recent history, the Baltic Sea has experienced a period of eutrophication in large areas due to increased nutrient discharge (Rahm et al., 1995; Stigebrandt, 1991). Eutrophication after the marine incursion ca. 8000 yr BP has led to an

increase of sediment and organic carbon accumulation (Conley et al., 2009). Therefore, the Baltic Sea sediments provide a natural test site for how variable sediment accumulation rates (SAR) and salinity gradients influence carbon preservation and sulfur burial rate in modern low-sulfate marine systems.

Carbon preservation can be defined as that fraction of C_{org} flux that escapes microbial mineralization and becomes buried in a deep sediment layer (Canfield, 2005). As shown in equation 2, C_{pre} is the ratio of the C_{org} burial flux (J_{burial}) to the total C_{org} accumulation flux (J_{acc}) to the sediment.

$$C_{pre} = \left(\frac{J_{burial}}{J_{acc}} \right) \times 100\% = \left(\frac{J_{burial}}{J_{burial} + J_{min}} \right) \times 100(\%) \quad \text{Eq.2}$$

We use Martens et al. (1993) approach to determine the C_{pre} based on fluxes of C_{org} burial to the deep sediment, carbon mineralization (J_{min}) in the sulfate reduction zone, and total C_{org} accumulation flux ($J_{acc} = J_{burial} + J_{min}$) into the surface sediment (Eq.2, Fig. 2). Any C_{org} escaping the sulfate zone, which is defined as the zone where sulfate concentrations decrease to < 0.1 mM (See Supplementary), is considered to represent buried C_{org} .

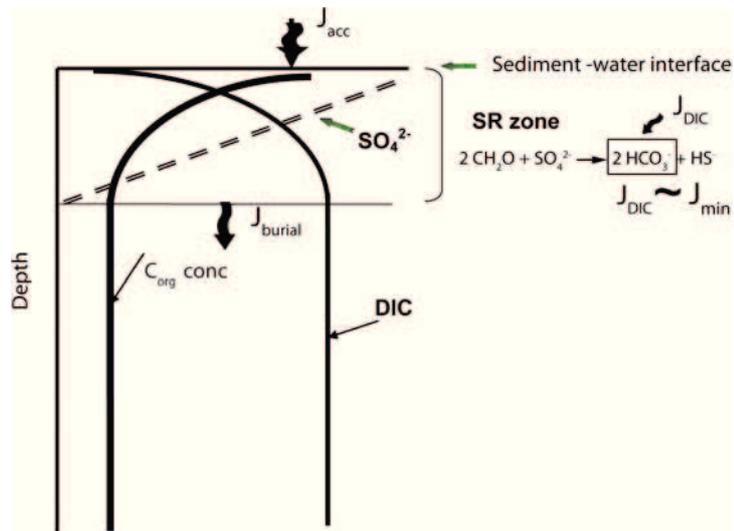
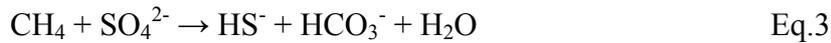


Figure 2: A simple model of organic carbon preservation in organic –rich marine sediment

We assume that the system is steady-state with respect to mineralization and sediment accumulation rate. We calculate the J_{burial} from the sediment accumulation rate (SAR) (cm a^{-1}) determined from $^{210}\text{Pb}_{excess}$ and ^{137}Cs profiles or obtained from the literature (Table 1), multiplied by the amount of C_{org} burial. J_{min} ($J_{min} = J_{DIC} + J_{TIC}$) is the flux of total carbon mineralization including flux of dissolved inorganic carbon (DIC) (J_{DIC}) ($\text{mmol cm}^{-2} \text{ a}^{-1}$) in pore water of the upper sulfate zone (Fig. 2) whereas J_{TIC} is defined as the burial flux of solid inorganic carbon into the deeper sediments (below the sulfate zone). J_{DIC} represents the flux

from organoclastic SR (Eq.1) and SR associated with the anaerobic oxidation of methane at the sulfate methane transition (Eq.3).



J_{DIC} was calculated from pore water DIC data obtained from several Baltic Sea expeditions (2009 - 2010) (Fig.1, Table 2). J_{TIC} values were calculated from the TIC content multiplied by the SAR. J_{TIC} values tend to be very small in the Baltic Sea sediments that we investigated.

RESULTS AND DISCUSSIONS

Over the course of all sampling campaigns (Table 1; See Supplementary Information) eighteen sites throughout the entire extent of the Baltic Sea were visited; with salinities ranging from 25‰ (21 mM sulfate) in Aarhus Bay to 3.5‰ (2.9 mM sulfate) in the Bothnian Bay (Table 1). Sediment accumulation rates ranged from 0.02 to 0.95 cm a⁻¹. The calculated contributions of J_{TIC} were minimal (<0.01 to 0.07 mmol cm⁻² a⁻¹) (Table 1), whereas J_{DIC} was variable between 0.01 and 0.29 mmol cm⁻² a⁻¹ (Table 2). Total J_{acc} varied between 0.05 and 0.95 mmol cm⁻² a⁻¹ (Table 1).

Controls on organic carbon preservation

Organic carbon preservation, C_{pre} , in the Baltic Sea sediments varied in a range of 24 to 93% (Table 2). The Baltic Sea C_{pre} values are very high when compared to global marine sediments (< 1%, Hedge and Keil, 1994), even higher than the maximum recorded C_{pre} of 78% (Canfield, 1994). Our C_{pre} estimates may be high due to the underestimates of DIC mineralization due to SR obtained from the pore water modelling.

There are two problems with an estimate of DIC production from C_{org} mineralization: 1) SR is not the only process to mineralize C_{org} , although it is the main pathway to C_{org} mineralization, and 2) the impact of bioirrigation has not been taken into account. Concerning the role of aerobic versus anaerobic respiration for C_{org} degradation, Thamdrup et al (1994) showed that Mn^{2+} , Fe^{2+} , and HS^- were the main reactants that were oxidized with dissolved oxygen in the narrow oxic zone (0-5 mm) in the coastal marine sediments in the west Baltic Sea (Aarhus Bay). Furthermore, the authors also demonstrated that SR was the predominant pathway of C_{org} mineralization. Large areas of the Baltic Sea near bottom waters are also depleted oxygen (Conley et al., 2009, 2011). Thus, in the Baltic Sea sediments, the aerobic

respiration directly coupled to C_{org} mineralization is expected to be insignificant relative to anaerobic respiration (organoclastic sulphate reduction) in the sediments.

Bioirrigation may impact carbon mineralization, particularly within the coastal regions of the Baltic Sea. Pore water modelling provides a net estimate of DIC production related to C_{org} mineralization. We have likely underestimated the C_{org} mineralization by assuming a purely diffusive model. Within these shallow water depths *Marenzelleria* (red-gilled mud worm) are common and actively exchange water into and out of the sediments (Blank et al., 2008; Hedman et al., 2011). To address the impact of bioirrigation on the exchange of DIC between the bottom water and sediment pore waters (α factor – i.e, frequency of pore fluid exchange per year, Boudreau, 1997), we applied a diagenetic model that incorporated bioirrigation into our DIC curve fitting procedure (Wang et al., 2008) and calculated the flux of DIC combined bioirrigation ($J_{\text{DIC-BIO}}$) in two scenarios: 1) bioirrigation effects that are similar to those in Himmerfjärden ($\alpha = 3$) (Thang et al., 2013) and 2) bioirrigation effects $\alpha = 3$ for sites < 90 m and $\alpha = 1.5$ for sites > 90 m water depth (See Supplementary Information). Thamdrup et al. (1994) proposed a value of $\alpha = 5.3$ in the top 10 cm of the sediments at 16 m of the water column depth in Aarhus Bay. The application of $\alpha = 3$ or 1.5 for the top 15 cm of the sediment to estimate the bioirrigation effects are more likely values due to the deeper water column and even hypoxic to euxinic environment in our research sites.

The resulting $J_{\text{DIC-BIO}}$ in the calculation is greater than the purely diffusive model, approximately by 1.5 to 10 fold (Table 2). Our model results indicate that changing the bioirrigation in the top 15 cm depth leads to different calculated efficiencies of C_{pre} (Table 2). Nevertheless, the overall trends remain the same. Furthermore, under both bioirrigation scenarios, the results still indicate a high degree of carbon preservation up to 68% and are consistent with previous results in the same region (9 - 50%, Canfield, 1994; Jørgensen and Parkes, 2010). The C_{pre} with bioirrigation estimates (15 - 68%) is also in agreement with other sulfide-rich marine systems, such as Black Sea, Cariaco Trench, Saanich Inlet, and Skan Bay (13 - 78%) (Canfield, 1994). Despite the different efficiencies of C_{pre} with the different α values, the trends in both model scenarios are the same.

Chapter 3

Table 1: Site specific data corresponding to the cores in this study

Site	Location	Expeditions	Areas	¹ C. Length m	² W.Depth m	Salinity ‰	³ SO ₄ ²⁻ mM	SPD mbsf	S. rate cm a ⁻¹	⁴ SO ₄ ²⁻ ex. year	J _{acc} mmol cm ⁻² a ⁻¹	J _{TIC} mmol cm ⁻² a ⁻¹	TS Burial μmol cm ⁻² a ⁻¹	S _{inorg} Burial μmol cm ⁻² a ⁻¹
M14	N:56° 06'14 E: 10° 26'18	R/V Susanna	Aarhus Bay	<6m	20.1	25	21.0	1.47	0.06 ⁺	7350	0.21	0.05	nd	⁵ nd
373870	N:54° 11'06 E: 11° 22'13	R/V Poseidon	Mecklenburg Bay	<1	23		14.6	0.82	0.21	390	0.34	0.00	51.3±8	nd
373960	N:54° 02'49 E: 11° 07'25			<1	25.5		13.4	0.84	0.13	646	0.34	0.00	33.0±6	nd
374175	N 55° 21' 04 E 15° 26' 37			<1.2	93		12.2	0.43	0.13	330	0.25	0.01	23.6±4	nd
374180	N 55° 20' 12 E 15° 26' 12			<1.2	92.8		12.5	0.38	0.22	172	0.26	0.02	16.9± 6	nd
374190	N 55° 17' 39 E 15° 26' 16			<1.2	91.2		10.8	0.38	0.39	98	0.49	0.04	54.5±1	nd
MSM24	N 55° 15' 01 E 15° 26' 08	R/V M. Merian	Bornholm Basin	<12m	93.7	16.5	12.0	0.43	0.22	195	0.53	0.00	47.2±9	nd
ST2	N:54° 27'28 E: 19° 03'22			<1	62	9.6	8.8	1.29	0.02	6450	0.05	0.00	5.8±3	4.4±2
ST3	N:54° 36'39 E: 19° 10'14	R/V Oceania	Gdansk Basin	<1.2	83	7.2	8.5	0.53	0.22	241	0.60	0.01	46.8±8	17.0±7
ST4	N:54° 50'04 E: 19° 04'54	R/V Oceania	Gdansk Basin	<1.4	103	11.8	9.8	⁶ cnd	0.28	nd	0.66	0.00	69.4±13	31.0±10
ST5	N:54° 35'44 E: 19°43'51			<1.2	51	11	7.2	0.19	0.48	40	0.79	0.02	73.6±11	28.3±14
MSM45	N 57° 15' 00 E 19° 50' 00	R/V M. Merian	Gotland Deep	<12m	210	12.6	11.5	cnd	<0.01 ⁺⁺	nd	0.04	0.01	4.4±0.4	nd
MSM73	N 57° 58' 45 E 20° 53' 19	R/V M. Merian	Gotland Deep	<12m	193.5	11.9	8.9	0.43	0.36	119	0.51	0.07	100.1±7	nd
H5	N 59° 02' 19 E 17° 43' 40	R/V Limanda	Himmerfjärden estuary	< 1	20	6.5	4.3	0.18	0.95	19	0.95	0.00	122.7±33	80.9±39
H3	N 58° 50' 40 E 17° 47' 42	R/V Limanda	Himmerfjärden estuary	<1.4	52	6.5	4.5	0.24	0.82	29	0.93	0.00	60.1±11	28.4±10
H2	N 58° 56'04 E 17°43' 81	R/V Limanda	Himmerfjärden estuary	<1	30	6.5	4.8	0.29	0.65	45	0.89	0.00	89.7±28	47.9±25
MSM85	N 61° 32' 17 E 20° 42' 20	R/V M. Merian	Bothnian Sea	<6	85	6.5	5.1	0.65	0.23	282	0.15	0.00	35.9±18	nd
MSM93	N 64° 41' 06 E 22° 37' 44	R/V M. Merian	Bothnian Bay	<6	93	3.5	2.9	0.41	0.15	273	0.14	0.00	22.6±14	nd

¹Core length, ²water depth, ³sulfate concentration at the sediment –water interface, ⁴ sulfate exposure time, ⁵not determine, ⁶can not determine, ⁺Fossing et al., 2004 ⁺⁺Mattila et al., 2006.

Table 2: Efficiency of carbon preservation in the Baltic Sea sediments responding to the DIC flux and bioirrigation consideration

Sites	Carbon preservation (%)					
	Without bioirrigation consideration			Bioirrigation consideration		
	$\alpha=0$		$\alpha=3$	$\alpha=1.5^1, 3$		
	J_{DIC} (mmol cm ⁻² a ⁻¹)	C_{pre} (%)	$J_{DIC-BIO}$ (mmol cm ⁻² a ⁻¹)	C_{pre} (%)	$J_{DIC-BIO}$ (mmol cm ⁻² a ⁻¹)	C_{pre} (%)
M14	0.29	57±3	0.46	19±2	0.37	22±2
373870	0.07	78±1	0.59	28±3	0.59	28±3
373960	0.10	66±1	0.50	48±5	0.50	48±1
374175	0.06	73±9	0.37	37±1	0.22	49±5
374180	0.06	75±2	0.37	31±2	0.21	43±3
374190	0.11	77±5	0.78	38±6	0.45	51±7
MSM24	0.13	75±3	0.68	36±3	0.41	48±3
ST2	0.04	24±13	0.23	15±5	0.23	15±5
ST3	0.16	71±4	0.43	48±4	0.43	48±4
ST4	0.03	93±1	0.24	nd	0.24	Nd
ST5	0.02	90±2	0.23	68±4	0.23	65±5
MSM45	0.01	48±16	nd	48±16	nd	48±16
MSM73	0.05	76±9	0.35	44±17	0.22	52±17
H5	0.12	90±2	0.57	65±4	0.57	65±4
H3	0.12	89±2	0.53	63±4	0.53	63±4
H2	0.09	88±1	0.46	60±3	0.46	60±3
MSM85	0.01	93±1	0.07	68±3	0.07	68±3
MSM93	0.03	80±0	0.19	38±1	0.11	51±1

Although, the actual mechanistic controls on carbon preservation remain controversial (Canfield, 2005), we can examine the roles initial surface sulfate concentration at the sediment-water interface (initial sulfate concentration), sediment accumulation rate (SAR), and sulfate penetration depth (SPD) on C_{org} preservation. When considering the sulfate concentration of the seawater, we see that the correlation of C_{pre} with the initial sulfate is poor and biased by one value ($R^2 = 0.21$, $p < 0.0001$; $n = 18$ with diffusion model and $R^2 = 0.4$, $p < 0.0001$; $n = 18$ with bioirrigation model) (Fig. 3A). This suggests that bottom water sulfate

¹ The $\alpha = 1.5$ was applied for sites with the water column depth over 90 m

concentrations are overall not limiting, even low and near freshwater concentrations of sulfate in surface sediment appear to provide enough sulfate for extensive C_{org} mineralization.

The high SAR observed in the Baltic Sea (mostly $> 0.1 \text{ cm a}^{-1}$) could act to rapidly transport reactive C_{org} quickly through the zone of active SR (Canfield, 2005). Nevertheless, as shown in figure 3B, only a weak correlation exists between C_{pre} and SAR in both diffusion ($R^2 = 0.41, p = 0.0040; n = 18$) and bioirrigation models ($R^2 = 0.48, p = 0.0015; n = 18$) (Fig. 3B).

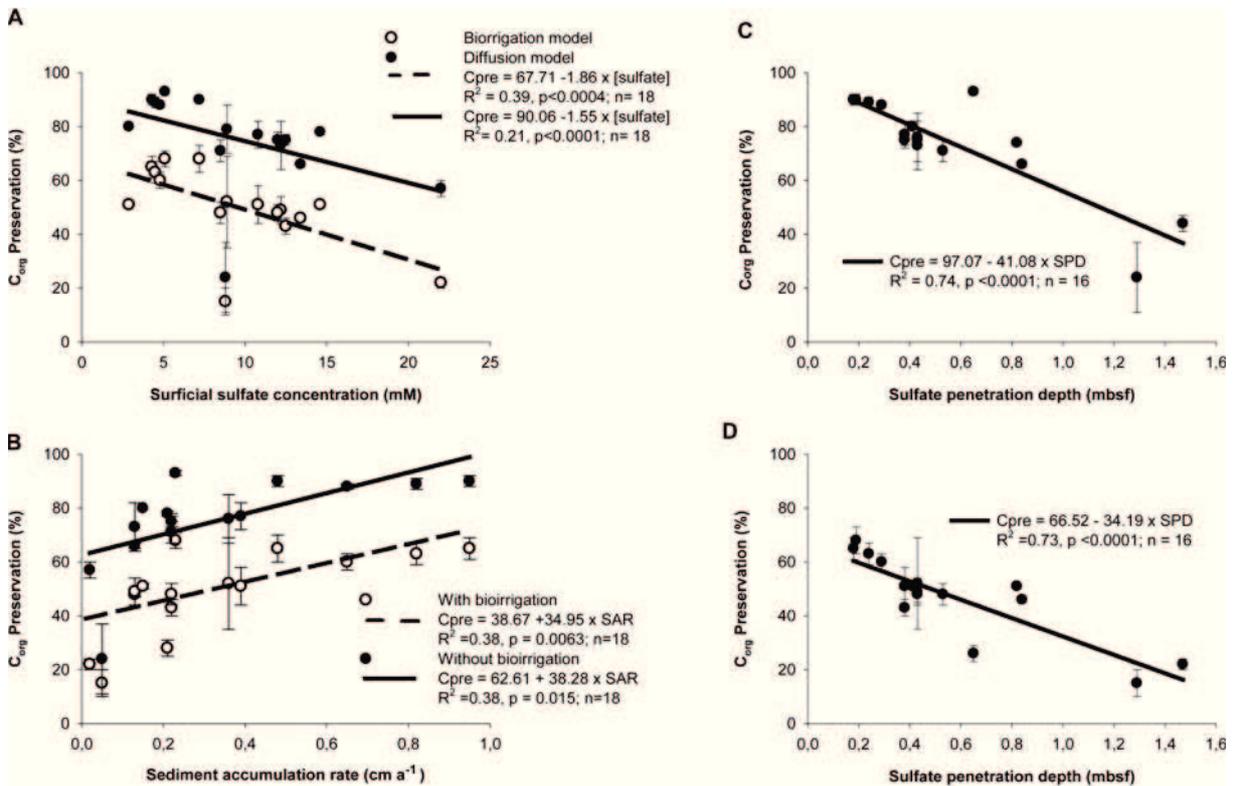


Figure 3: The correlation between organic carbon preservation and surficial sulfate concentration (A), sediment accumulation rate (B), and sulfate penetration depth: C) without bioirrigation; D) with bioirrigation with $\alpha = 1.5$ at the sites $> 90 \text{ m}$ of water column and $\alpha = 3$ at sites $< 90 \text{ m}$.

Most interestingly, C_{pre} appears to depend on the SPD as shown in figures 3C and 3D (with the exception of two sites, MSM45 and ST4, where we were unable to determine the depth of sulfate penetration; See Supplementary Information, Fig. S3). The C_{pre} exhibits a significantly statistical greater dependence on SPD in both diffusive and bioirrigation models ($R^2 = 0.78, p < 0.001; n = 16$) than on other factors.

What controls SPD in the Baltic Sea sediments? Firstly, sulfate penetration depths are not a simple function of the decrease in surface sulfate concentration along the salinity gradient from Danish Straits (25‰) to the Gulf of Bothnia (3.5‰) (Table 1, See Supplementary Information Fig. S1). The SPD also does not correlate with total carbon flux

(J_{acc}) ($R^2 = 0.40$, $p=0.0395$, $p=0.0098$; $n = 16$) (Fig. 4A) or with initial sulfate concentration ($R^2 = 0.43$, $p=0.0059$; $n = 16$) (Fig. 4B). Although the transport of solutes as sulfate is a complex function of molecular diffusion, SAR, bioirrigation, and biogeochemical reaction rates (Dale et al., 2009), the most significant statistical relationship is between SPD and SAR ($R^2 = 0.84$, $p < 0.0001$; $n = 16$), (Fig. 4C).

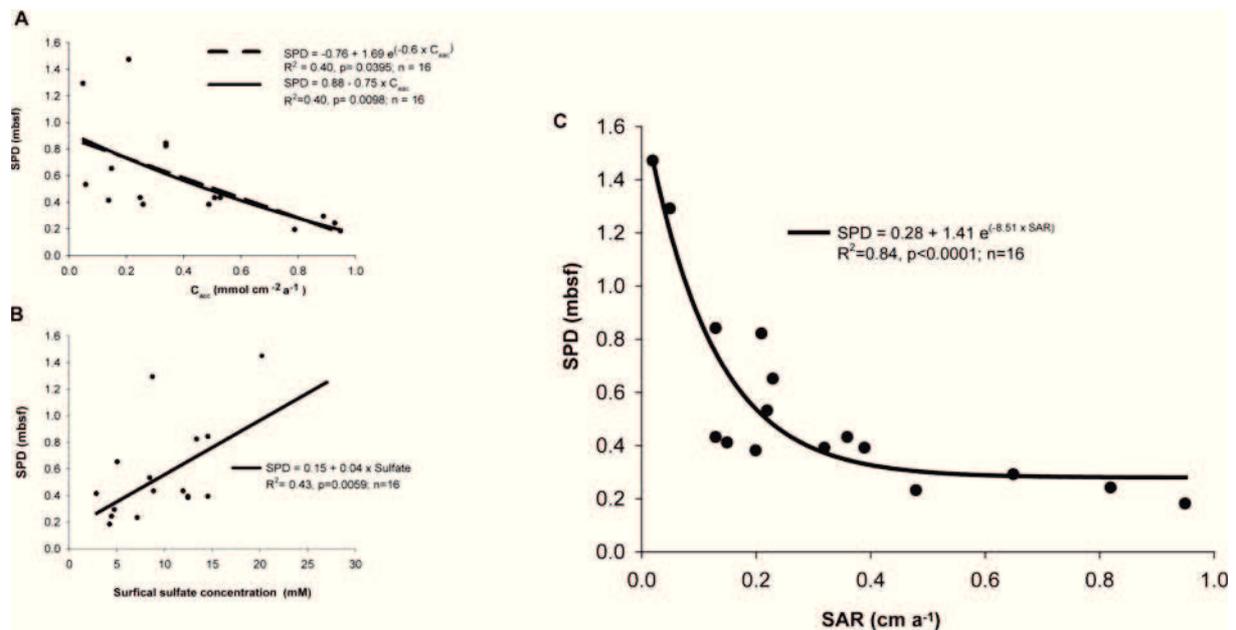


Figure 4: The relationship between: A) sulfate penetration depth and organic carbon accumulation (dashed line is present for e curve and solid line is present for linear curve), B) sulfate penetration depth and initial sulfate concentration, and C) sulfate penetration depth and sediment accumulation rate in the Baltic Sea sediments.

A possible link between SAR and SPD concerns the feedback between SARs, C_{org} reactivity, and SPD (i.e. the depth of the sulfate-methane transition). Meister et al. (2013) suggest that under certain circumstances the delivery of C_{org} of lower reactivity can counter-intuitively lead to greater methane production and a shallower SPD. This would be consistent with our observations of shallow SPD correlated with high SAR. Flury et al (in preparation) have also proposed that a shallow SPD in Aarhus Bay could be linked to high rates of organoclastic SR and a large flux of methane to the sulfate zone. A simple explanation may be that high sediment loads (high SAR) deliver greater contents of less reactive C_{org} (thus higher potential to be preserved) and therefore, the C_{pre} values increases.

The variability of SPD in the Baltic Sea may influence the temporal and spatial window of opportunity in which organoclastic SR occurs. Most SR occurs in the top 30 centimeters (Fossing et al., 2000; Jørgensen & Parkes, 2010), the bacterial SR community also depends on the presence of sulfate, quality of C_{org} , favorable physical conditions such as

temperature, salinity, and time to build up microbial communities (Canfield, 1994; Jørgensen and Kasten, 2006; Regnier et al., 2011). The upstream mineralization processes, in particular the hydrolysis of macromolecular organic matter, are rate limiting steps in the rate of C_{org} mineralization (Arnosti, 2004). If these rates are low relative to residence time of the C_{org} in the sulfate-bearing sediment, a significant fraction of C_{org} can escape into the deeper methanogenic layers. To assess the impact of C_{org} transport time in a mineralized zone (sulfate zone), we define the sulfate-exposure time (τ), as the time that accumulated C_{org} remains in the sulfate zone (Eq. 4):

$$\tau = \frac{SPD}{SAR} \times 100 \quad \text{Eq. 4}$$

where τ is the transport time of C_{org} through the sulfate zone (years), SPD (m) is the depth of sulfate penetration that was determined based on Wang et al (2008), and SAR is the sediment accumulation rate (cm a^{-1}). The SARs were assumed to be constant throughout most of the Holocene and that marine conditions existed in the Baltic Sea throughout this period of time.

For the Baltic Sea sediments investigated, we calculate values of τ that range from 19 to 7350 years, although the system was not in steady state when the Holocene mud first started to accumulate (Mogollón et al., 2012). Aarhus Bay with a τ value approximate 7350 years probably represents a minimum exposure time for this area whereas most of the calculated τ for the other areas may be a reasonable approximation. The relationship between exposure time of C_{org} in the sulfate zone and efficiency of C_{pre} is significant in both diffusion and bioirrigation models ($R^2=0.78$, $p<0.0001$; $n=16$; Fig. 5). This relationship suggests that the exposure time to sulfate may be the primary control on C_{pre} . Limited exposure of C_{org} to sulfate appears to be a good predictor for the preservation of organic matter in deep sediments in the Baltic Sea. When compared to other mechanisms proposed to impact C_{pre} such as sedimentation rates, oxygen exposure, mineral adsorption etc (Canfield, 1991, 1994; Hartnett et al., 1998; Burdige, 2007; Zonneveld et al., 2010), we would predict that sulfate exposure time is also an important fraction control on C_{pre} . This also supports Wortman and Chernyavsky hypothesis (2007) that SPD may be the primary control on C_{pre} and a simple factor in shallow marine environments, specifically in marine environments characterized by sulfate concentrations lower than modern seawater, high sediment accumulation rates, and high C_{org} deposition.

A causal mechanism for the establishment of the correlation between sulfate exposure time and C_{pre} can not be derived from these data alone. The presence or absence of sulfate as

an electron acceptor may not play the same biochemical role that oxygen does with regards to organic carbon degradation. Indeed, SR continues below the sulfate-methane transition at the Aarhus site, as demonstrated by Holmkvist et al. (2011). The authors suggest that the depth distribution of SR, including SR below the sulfate-methane transition zone, is primarily controlled by C_{org} degradation. That methanogenesis is less effective C_{org} mineralization than SR has thus far never been demonstrated, and remains to be tested. Alternatively, enhanced SR in a narrow zone will lead to the formation of sulfide and subsequently to the formation of organic sulfur compounds. Sulfurization may reduce the susceptibility of organic matter towards microbial degradation through cross-linking (Ferdelman et al., 1991; Passier et al., 1997; Werne et al., 2003). The Baltic Sea sediments studied contain a significant fraction of organic sulfur (Thang et al, in preparation), and there is a strong correlation between buried rates of S and C_{org} (see discussion below).

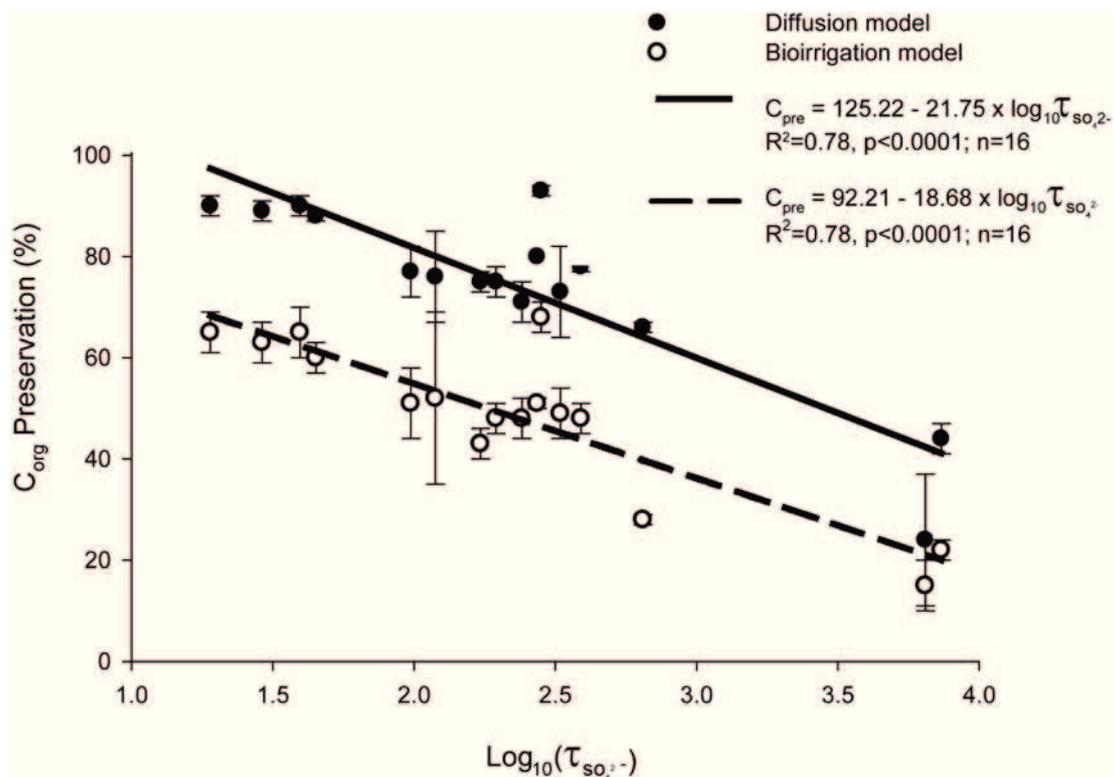


Figure 5: The correlation between sulfate exposure time and C_{pre} with diffusion model (open circles and dashed line) and C_{pre} with bioirrigation model (filled circles and solid line) in the Baltic Sea sediments.

Sulfate penetration influences sulfur burial

In modern marine systems there is a positive correlation between sulfur and carbon accumulation rates. As previously mentioned this relationship does not hold true throughout

Earth's history (Paytan et al., 2004). One hypothesis for a past decoupling between sulfur and carbon is the extensive removal of sulfur from the ocean in evaporite deposits (Wortmann and Chernyavsky, 2007). The increase in organic carbon accumulation rates under these low sulfate conditions suggests that the presence of sulfate is a primary control on C_{org} preservation. The modern Baltic Sea provides the framework to test this. Sulfide is the product of both organoclastic SR and methane driven SR. The majority of this sulfide is reoxidized to sulfate. Only a small fraction is captured as pyrite and organic sulfur (Jørgensen and Nelson., 2004). We found a strong positive correlation between buried total S (S_{burial}) and C_{org} ($R^2 = 0.65$, $p=0.0004$; $n=16$, Fig. 6). This suggests that buried S and C_{org} are positively coupled in the Baltic Sea in spite of the low sulfate in the brackish water. Although, consistent with the previous studies in the Baltic Sea (See Supplementary Information, Fig. S4, R&D2), this is in contrast to periods in Earth's history where S and C_{org} were decoupled, such as during the Cretaceous (Paytan et al., 2004). Indeed, sulfur accumulation rates in the Baltic Sea are elevated compared to modern marine sediments and similar to sediments deposited under euxinic conditions (Berner, 1984).

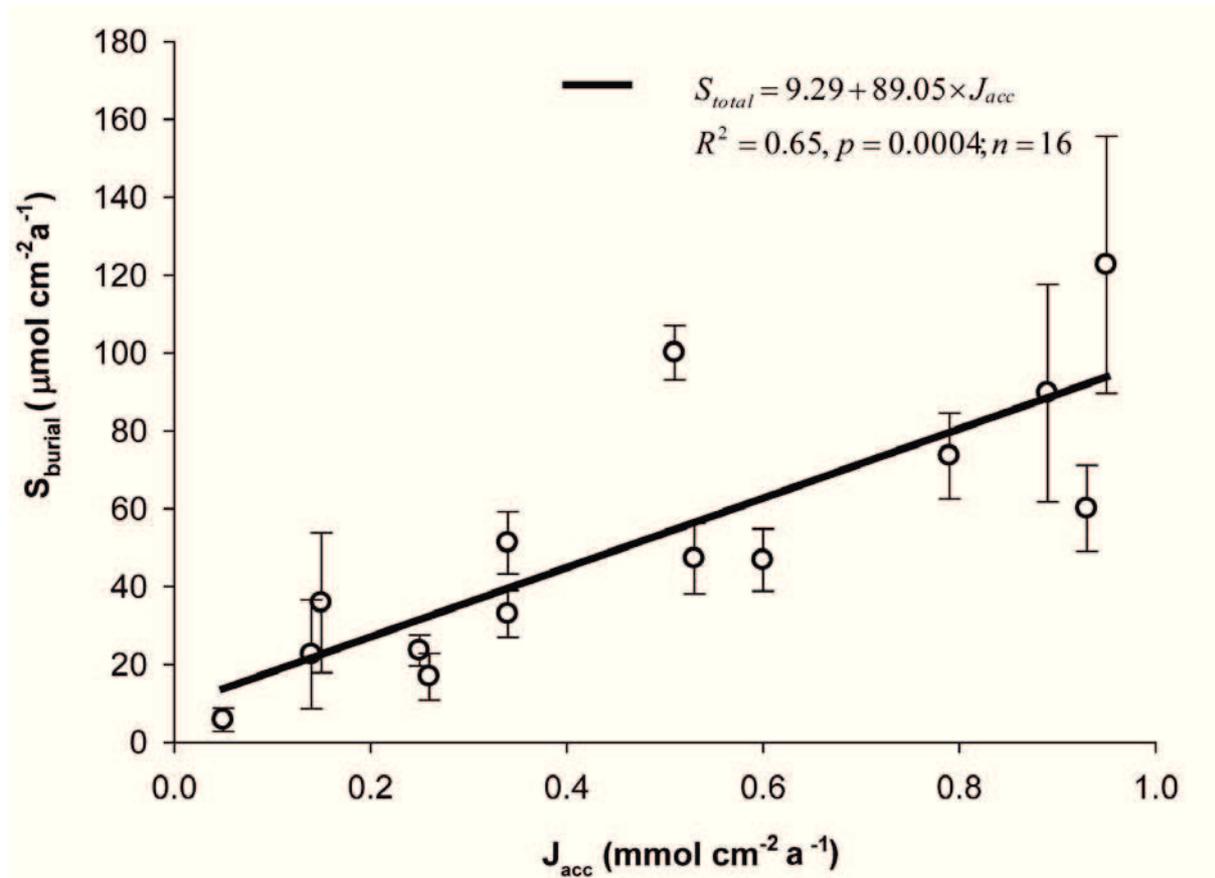


Figure. 6: The correlation between C_{org} and S burial in the Baltic Sea sediments

Interestingly, pyrite concentrations in Himmerfjärden estuary and Gdansk Basin sediments (0.17 – 1.05%) (See Supplementary information, Fig. S4C) are much lower than the total sulfur inventory (0.5 - 3%) and general Baltic Sea sediments (< 7%) (See Supplementary information, Fig. S4A, B), which suggests that there is a large pool of organic sulfur in these sediments. Our results suggest that the lowered water column sulfate concentrations do not affect sulfur accumulation rates in the Baltic Sea. In this sense then, the modern Baltic Sea may not a good model for a putative low sulfate Cretaceous Ocean. Instead, other factors such as the availability of reactive iron and the presence of C_{org} susceptible to sulfurization, the sulfur recycling in the sediments could be a more dominant control than overlying water-column sulfate concentrations. The actual underlying mechanisms for sulfur burial in the Baltic Sea sediments require further study.

CONCLUSIONS

The Baltic Sea is the largest brackish water body in the world and as such could provide a model for sulfur cycling in low sulfate ocean environment. Our results suggest that the Baltic Sea's detailed history of expansion of eutrophication, and high rates of sediment accumulation have created areas where sulfate penetration is shallow and appears to enhance organic carbon preservation. Nevertheless, sulfate penetration depths correlate statistically with the sediment accumulation rate rather than the initial sulfate concentration at the sediment-water interface or the flux of organic carbon to the sea floor. Similar to the idea that of oxygen-exposure time controls organic carbon degradation at the sediment surface, the sulfate-exposure time appears to be a first order predictor of the degradation and preservation of organic carbon. Ultimately, it is not the concentration of sulfate in the overlying water, but rather the rapid transport of organic carbon, due to high sediment accumulation rates, through the zone of SR into the methanogenic zone that may promote the long-term preservation of organic carbon. Moreover, the correlation of buried S and C_{org} in the Baltic Sea suggests that the background sulfate supply may not control sulfur accumulation rates in shallow marine systems. In contrast with the proposed depletion of sulfate in the Early Cretaceous conditions (Wortmann and Chernyavsky, 2007), we observe that it is the sulfate exposure time of organic matter that is a decisive factor rather than the starting sulfate concentrations that impact carbon preservation and sulfur burial in the Baltic Sea.

SAMPLES AND METHODS

Cores and samples for pore water analysis and solid phase geochemistry were obtained from 18 sites over several sampling campaigns throughout the Baltic Sea (2009-2010) (Fig. 1, Table 1; See Supplementary Information – Materials and Methods). Pore water DIC and sulfate, ^{210}Pb and ^{137}Cs distributions, and solid phase C and S contents are the input for the reaction transport model, and for the calculation of carbon preservation and sulfur burial.

Reaction-transport model: Utilizing the sulfate and DIC concentration profiles with diffusion coefficients for 5°C (Schulz and Zabel, 2006), a reaction-transport fitting program (Wang et al., 2008) was applied to fit the concentration profiles. Based on the fitting program, we determined sulfate penetration depth (m) and calculated J_{DIC} . Advective pore water transport and a significance level of 0.05 were also considered in the model.

Carbon burial, sulfur burial, and carbon preservation model:

Carbon and sulfur burial rates were calculated using total carbon (TC), total inorganic carbon (TIC), and total sulfur (TS) content, along with sediment accumulation rates, by the following equations:

$$J_{\text{burial}} = \frac{TC}{100} \times \frac{1}{12} \times \omega \times d \quad (\text{mmol cm}^{-2} \text{ a}^{-1})$$

$$J_{\text{TIC}} = \frac{TIC}{100} \times \frac{1}{12} \times \omega \times d \quad (\text{mmol cm}^{-2} \text{ a}^{-1})$$

$$S_{\text{burial}} = \frac{TS}{100} \times \frac{1}{32} \times \omega \times d \quad (\text{mmol cm}^{-2} \text{ a}^{-1})$$

where J_{burial} , J_{TIC} and S_{burial} are the fluxes of total carbon (TC), total inorganic carbon (TIC) and total sulfur (TS) burial rates, respectively. The mass content of C and S was converted to mmol content by dividing the mass content by the individual molecular weight 12 and 32, respectively. TC, TIC, TS, ω and d are the carbon content, inorganic carbon content, total sulfur content (% dry weight), sedimentation rate (cm a^{-1}), and density of dry bulk sediment (g cm^{-3}). The average sedimentation rates for the sites M14, MSM45, and MSM24 were taken from literature values close to these sites whereas for all other sites, sediment accumulation rate obtained from the $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs approaches.

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SUPPLEMENTARY INFORMATION

The Supplementary Information file includes

- Supplementary Figures S1-S4
- Supplementary Methods
- Supplementary Results and Discussion R&D1-R&D2
- References

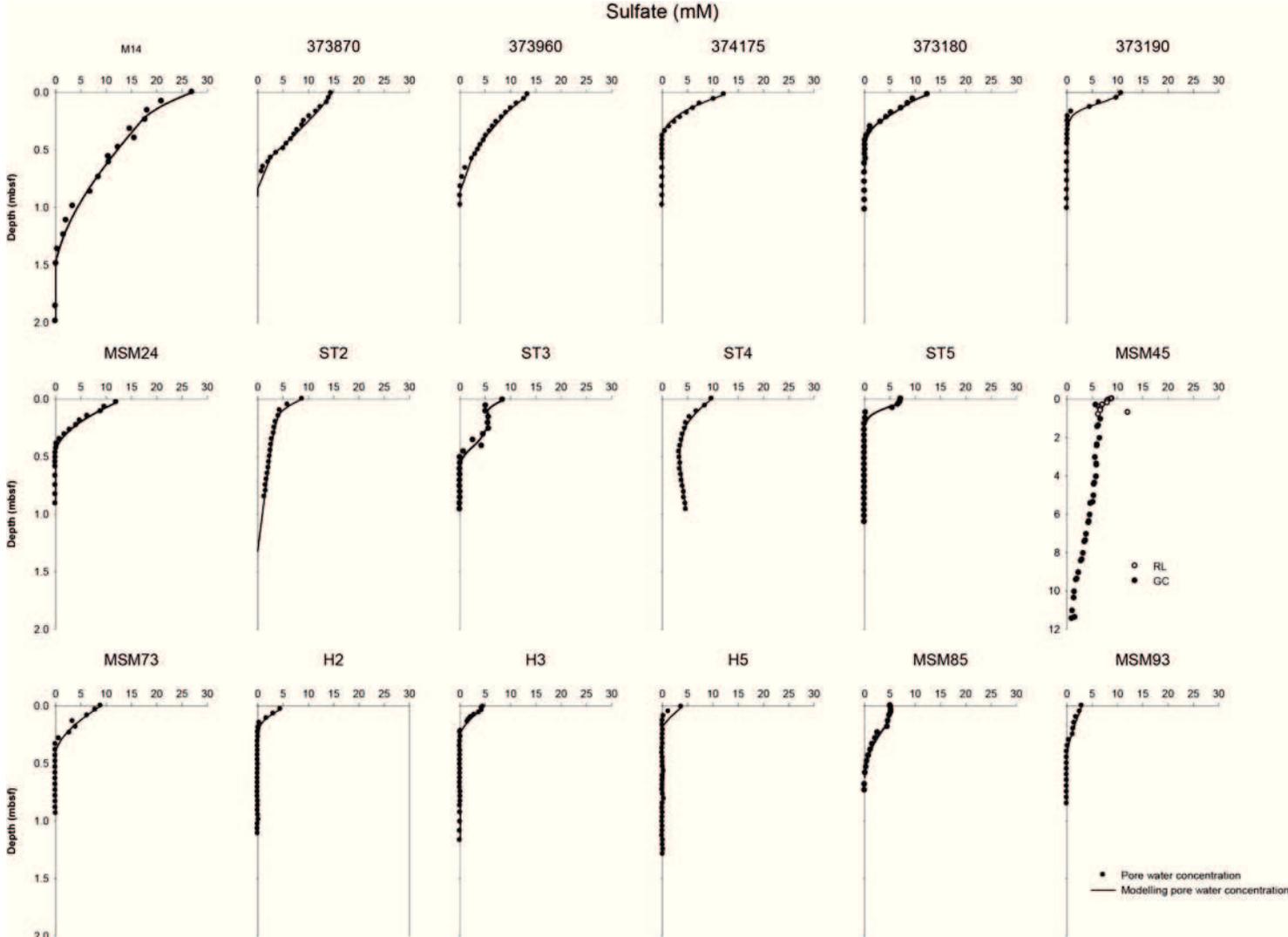


Figure S1: Sulfate concentration and reaction transport model results. The sulfate concentration from analytical measurement (circles) and the sulfate concentration from reaction transport model (solid lines) for each site.

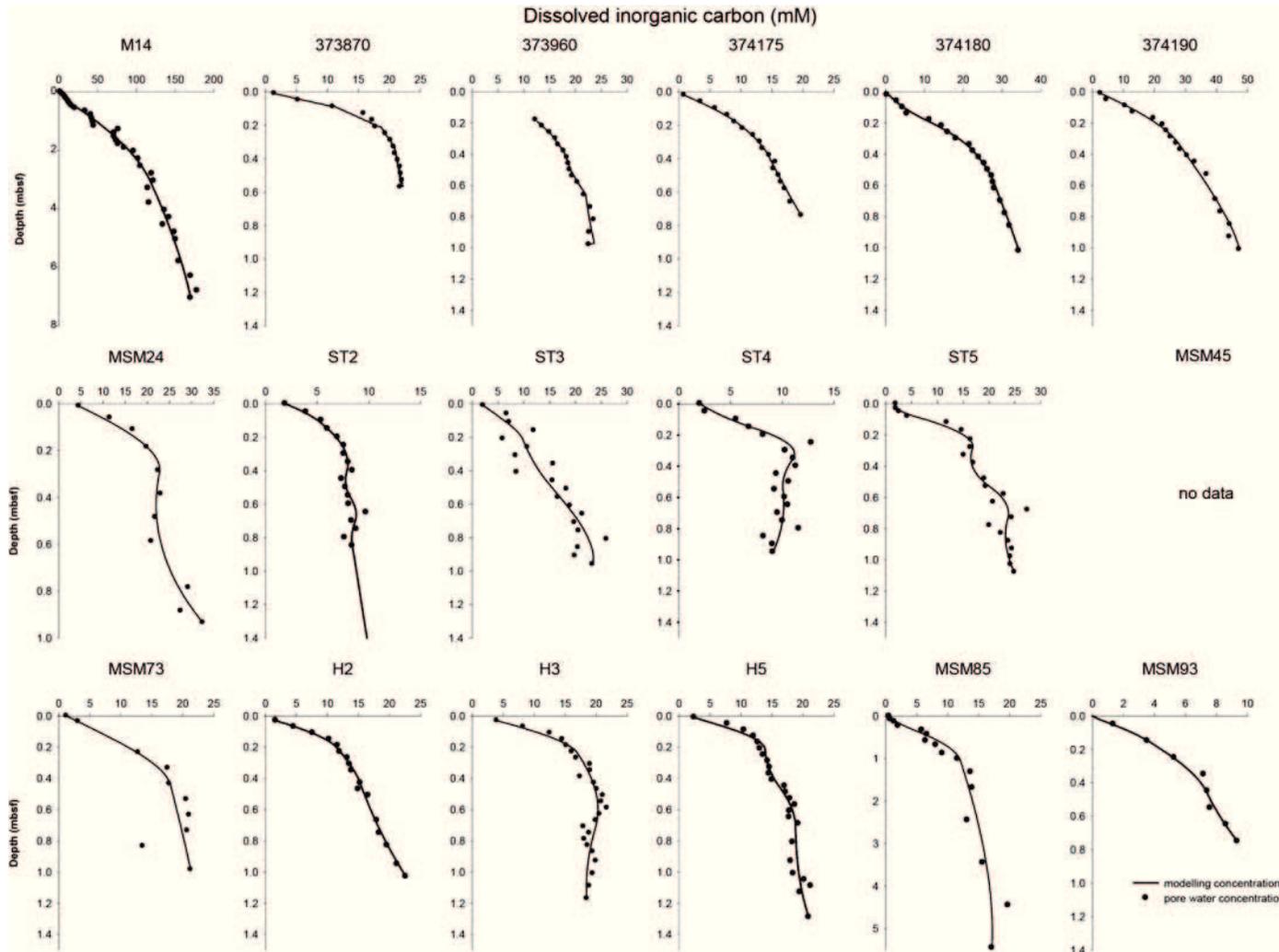


Figure S2: Dissolved inorganic carbon (DIC) concentration and reaction transport model results. The DIC concentration from analytical measurement (circles) and the DIC concentration from reaction transport model (solid lines) for each site.

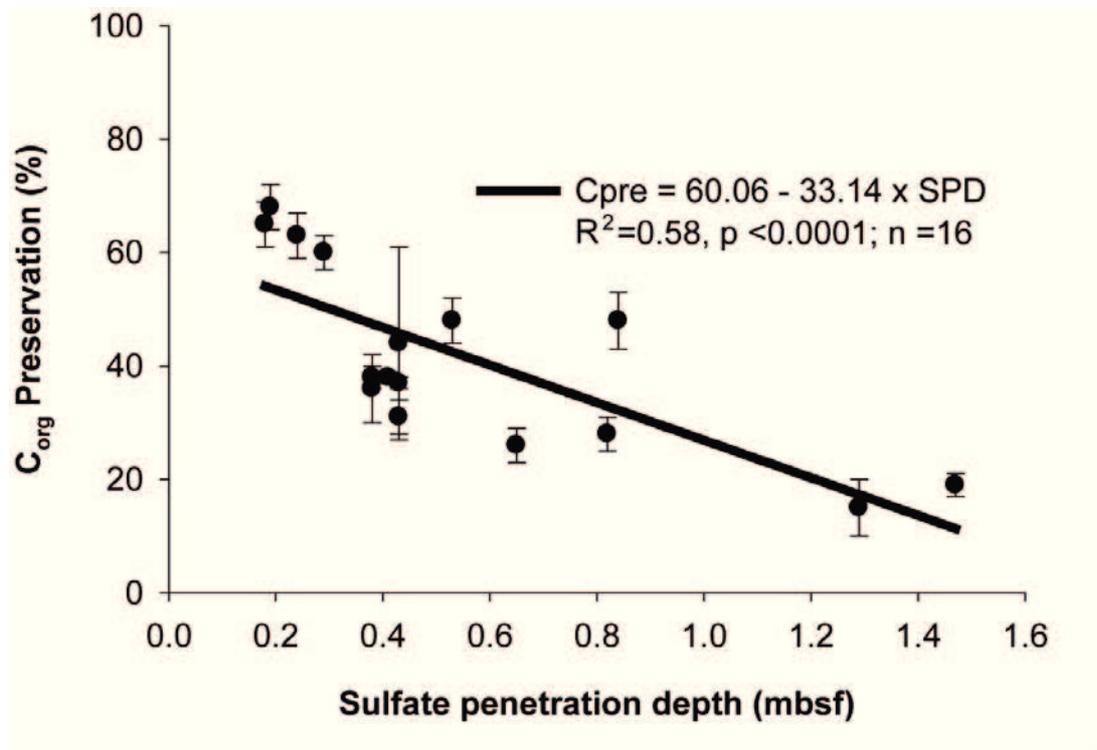


Figure S3: The correlation between organic carbon preservation and sulfate penetration depth with bioirrigation ($\alpha = 3$).

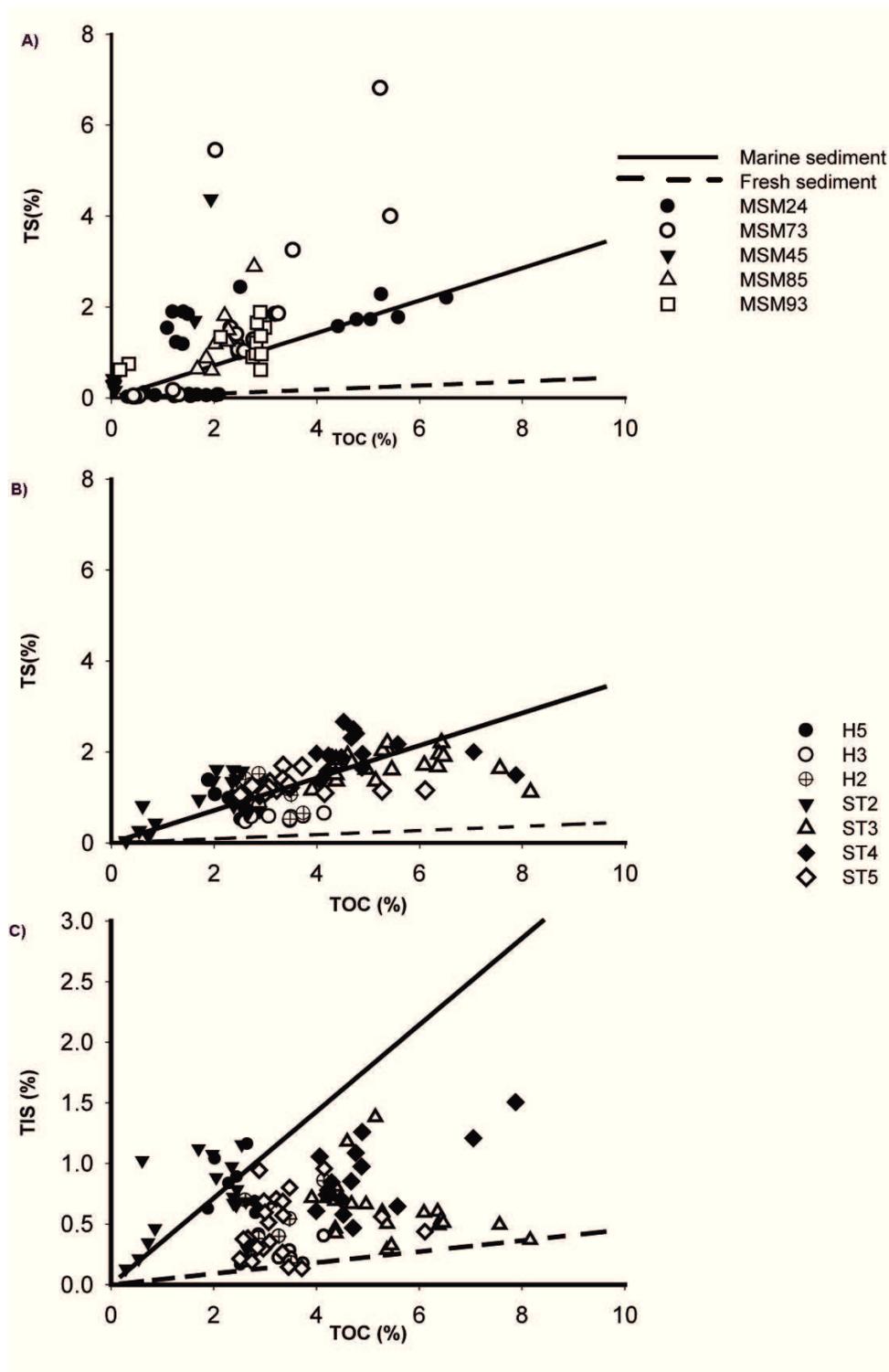


Figure S4: C: S ratios in the Baltic Sea: A) deep Baltic Sea sediments, B) shallow Baltic Sea sediments, and C: TIS ratios in shallow Baltic Sea sediments.

2. MATERIALS AND METHODS

2.1. Sediment sampling

Corers were collected from 18 sites during 2009 and 2010 over several sampling campaigns (the R/V Limanda (May, 2009) to Himmerfjärden estuary, R/V Oceania (November, 2009) to Gdansk Basin; R/V Poseidon (December, 2009) to Mecklenburg Bay and Bornholm Basin, R/V Susanna (May, 2010) to Arhus Bay, and R/V Maria S. Merian (August – September, 2010) to almost all parts of the Baltic Sea (from Bornholm Basin, to Gotland Deep, Bothnian Sea, and Bothnian Bay). A detailed description of these cores is provided in Table 1 (main text). The cores penetrated between 0.4 and 16 meter below sea floor (mbsf). The pore water and solid phase samples were collected from gravity corers at a resolution of 3 to 25 cm. Samples for ^{210}Pb and ^{137}Cs analysis were retrieved from multicores or gravity cores by slicing the core at a resolution of 1 to 2 cm. The pore water was subsampled for sulfate, and dissolved inorganic carbon (DIC). For DIC measurements 2 ml of pore water were preserved in headspace-free in 2.5 ml glass vials (Zinsser), and sulfate samples were preserved by adding 100 μl of 5% ZnCl_2 to 1 ml pore water.

2.2. Methods

Pore water analyses: Sulfate samples were measured on 10 to 20 fold–diluted samples, by ion chromatography (761 Compact IC, Ω Metrohm). With dilution, the detection limit was 50 μM for the sulfate measurements. Dissolved inorganic carbon (DIC) concentrations were determined by flow injection analysis (Hall and Aller, 1992). The detectable limitation was 0.1 mM for DIC measurements.

Solid-phase analyses: Total sulfur (TS) and total carbon (TC) concentrations in the sediment were determined from freeze-dried sediment using an elemental analyzer (GC - FISIONS 1500) at 1052 $^{\circ}\text{C}$. Total inorganic carbon (TIC) was measured on a CM 5012 CO_2 Coulometer (UIC) after acidification with H_3PO_4 (35%). Total organic carbon (TOC) was calculated as the difference between TC and TIC. Chromium reducible sulfur (CRS) ($\text{FeS}_2 + \text{S}^0$) was separately distilled in two steps with hot HCl combined Cr(II) solution according to Fossing and Jørgensen (1989). The separated sulfide was trapped by 5 ml 10% $\text{Zn}(\text{CH}_3\text{COO})_2$. A sub-sample from the ZnS trap was used to determine sulfide concentrations according to the Cline method (1969) and then back-calculated for CRS content.

Sedimentation rates: Dry and ground sediment samples were measured for $^{210}\text{Pb}_{\text{total}}$, and ^{137}Cs concentration at 46.5 and 662 KeV. Activities of the radionuclides were determined using ultra – low level gamma spectroscopy on a closed-end coaxial well detector (Ge Coaxial

Type N gamma detector) for 1 to 3 days. $^{210}\text{Pb}_{\text{excess}}$ was calculated by subtracting ^{226}Ra background. ^{226}Ra was calculated from daughters ^{214}Bi , ^{214}Pb (295, 352, 609 KeV) assuming secular equilibrium.

A geochronology was established using the down-core distribution of excess ^{210}Pb activities ($\lambda = 22.3$ a half-life) using a constant initial concentration model (Appleby and Oldfield, 1983) and by assigning a date of 1986 to the latest ^{137}Cs peak.

3. RESULTS AND DISCUSSION

R&D1

Fluxes of dissolved constituents affected by bioirrigation were calculated using the following equation (Boudreau 1997, p. 143):

$$J_I = \alpha \times L_I \times (C_{15\text{cm}} - C_{0\text{cm}}) \quad \text{Eq. 1}$$

where J_I is the flux to the sediment-water interface, assuming constant pore water irrigation (constant α) over the zone of bioirrigation (L_I). In Himmerfjärden, *Marenzelleria* has the highest abundance in Himmerfjärden compared to the rest of the Baltic Sea coast (Blank et al. 2008). Furthermore, Thang et al., (in press) suggested that *Marenzelleria* can only exchange pore water within the upper 14 cm of sediment ($L=15\text{cm}$) was therefore used for all other scenarios. For the different bioirrigation scenarios we applied: 1) $\alpha = 3$ in first scenario with bioirrigation effects similar to Himmerfjärden (Thang et al., 2012) and 2) $\alpha = 3$ for bioirrigation effects at sites $< 90\text{m}$ because *Marenzelleria* were only found with water depth $< 90\text{ m}$ (Olenin. 2006) and $\alpha = 1.5$ at sites $> 90\text{m}$. The different α value affected the DIC fluxes (Table 2, main text).

The sulfate penetration depth was defined as the depth where pore water sulfate had decreased to 0.1 mM based on the reaction transport model (Wang et al., 2008). The detection of sulfate measurement was 0.05 mM and sulfate still remained around 0.1 mM in deep pore water Baltic Sea sediments (Fig. S1).

R&D 2

In the Baltic Sea sediments, the C:S ratios are variable but close to the straight line with the slope of 2.8 which is indicative of normal marine sediment. In particular, sediment from the Bornholm and Gottland Deep were enriched in S (Fig. S4 A, B). The C: S ratios are

good agreement with previous researches in the Baltic Sea sediments (Andrén et al., 2000; Böttcher and Lapland, 2000).

In initial Littorina Sea sediments of the Bornholm Basin and Gotland Deep and in sandy sediment at ST2 in Gdansk Basin the content of both S and C was low. However, for shallow sediments in the Himmerfjärden Estuary and the Gdansk Basin, the ratios between C: S and C: TIS ratios are strikingly different (Fig. S4B, C).

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Chapter 4

Assessing subsurface microbial activity by stable isotope probing with deuterated water

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ABSTRACT

Sub-seafloor sediments are populated by large numbers of microbial cells but not much is known about their metabolic activities, growth rates and carbon assimilation pathways. Here we introduce a new method enabling the sensitive detection of microbial lipid production and the distinction of auto- and heterotrophic carbon assimilation. Application of this approach to anoxic sediments from a Swedish fjord allowed to compare the activity of different functional groups, the growth and turnover times of the bacterial and archaeal communities. The assay involves dual stable isotope probing (SIP) with deuterated water (D_2O) and $^{13}C_{DIC}$ (dissolved inorganic carbon). Culture experiments confirmed that the D content in newly synthesized lipids is in equilibrium with the D content in labelled water, independent on whether the culture grew hetero- or autotrophically. The ratio of $^{13}C_{DIC}$ to D_2O incorporation enables distinction between these two carbon pathways in studies of microbial cultures and in environmental communities. Furthermore, D_2O -SIP is sufficiently sensitive to detect the formation of few hundred cells per day in a gram of sediment. In anoxic sediments from a Swedish fjord, we found that > 99% of newly formed lipids were attributed to predominantly heterotrophic bacteria. The production rate of bacterial lipids was highest in the top 5 cm and decreased 60-fold below this depth while the production rate of archaeal lipids was rather low throughout the top meter of seabed. The contrasting patterns in the rates of archaeal and bacterial lipid formation indicate that the factors controlling the presence of these two lipid groups must differ fundamentally.

Chapter 5

The impact of reactive iron on the early sulfurization of organic matter and oxidative sulfur cycling in sulfidic sediments from the Gdansk Basin (Baltic Sea)

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ABSTRACT

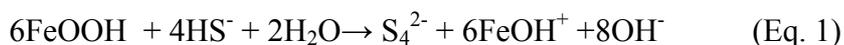
The biogeochemistry of sulfur, iron and carbon are important factors for reconstructing paleo-environment deposits (i.e. post-glacial) of the Baltic Sea, such as the impact of low salinity (9–11‰), high sediment accumulation rates, and elevated organic matter deposition. The influence of reactive iron content on the concentration distributions of buried pyrite sulfur and organic sulfur pools and their respective sulfur isotope compositions were studied at four sites within the Gdansk Basin (Baltic Sea). Variability in the deposition and hydrologic environments were manifested in the large inter-station variability of down-core sediment distributions of pore water sulfur (sulfate, sulfide, and their $\delta^{34}\text{S}$ composition) and solid phase sulfur (FeS_2 , acid of volatile sulfur (AVS), elemental sulfur, and their $\delta^{34}\text{S}$) and Fe (Fe_{asc} and Fe_{ditho}). The two dominant sinks for reduced sulfur, organic matter and FeS_2 , however, shared similar features in their respective concentration ($7.4 - 272 \mu\text{mol cm}^{-3}$ and $3.0 - 73.3 \mu\text{mol cm}^{-3}$, respectively) and S-isotope profiles ($\delta^{34}\text{S}_{[\text{OS}]}$: -42.6‰ to -0.6‰ and $\delta^{34}\text{S}_{[\text{FeS}_2]}$: -46.3‰ to -0.8‰), which suggested a co-genesis of early diagenetic organic sulfur compounds and pyrite. Nevertheless, differences between the organic sulfur and pyrite sulfur, and sulfur isotope signatures could be attributed to the mechanism and timing of sulfurization of organic matter sulfurization versus pyrite formation, and could be linked to the reactive iron content of the sediments. The presence of reactive iron oxides led to enhanced sulfide oxidation that leaves a distinct isotopic signal in the pyrite and organic sulfur pools buried in the Gdansk sediments.

Key words: sulfur cycling, organic sulfur, oxidative sulfur, $\delta^{34}\text{S}$, reactive iron, Gdansk Basin.

1. INTRODUCTION

The biogeochemical sulfur cycle is one of the most important element cycles in the marine environment, and in particular, is fundamentally important for the anaerobic oxidation of organic carbon (Jørgensen. 1982; Jørgensen et al., 1990; Jørgensen & Kasten. 2006). Sulfur species include in both inorganic and organic forms, with sulfate and hydrogen sulfide species forming the major dissolved forms of inorganic sulfur. Sulfide produced during sulfate reduction may be trapped and buried as iron monosulfides (FeS), pyrite (FeS₂), and organic sulfur in marine sediments (Kaplan et al., 1963, Berner. 1984). In early diagenesis, the sulfurization processes involve sedimentary iron and organic sulfurization, as well as sulfur intermediates such as elemental sulfur, polysulfur, polysulfides, thiosulfate, tetrathionate and sulfite (Zopfi et al., 2004). Sedimentary pyrite formation results from the reaction of reactive iron minerals and sulfide (Berner. 1984), while organic sulfur is produced from the reaction of organic matter with reduced inorganic sulfur species, such as sulfide and polysulfides (Brassell et al., 1986; Wakeham et al., 1995; Werne et al., 2004; Sinninghe Damsté et al., 2007).

Organic-sulfur rich sediments are often associated with reactive iron depleted systems where organic matter is abundant (Gransch & Posthuma. 1974; Brassell et al., 1986; Wakeham et al., 1995; Sinninghe Damsté et al., 2007). Reactive iron is considered to be iron component in sediments, principally in the form of ferric oxides and hydroxides, that rapidly react with dissolved sulfide to form metal sulfides such as pyrite (Canfield. 1989). Reactive iron is thought to act rapidly with dissolved sulfide, thus removing sulfide as a potential sulfurization agent. Fe reactivity (iron hydroxides e.g. FeOOH), however has not only been tied to pyrite sulfur formation and oxidative sulfur cycling (Canfield. 1989; Thamdrup et al., 1993; Jørgensen & Nelson. 2004; Riedinger et al., 2005, 2010), but also paradoxically related to sulfurization of organic matter (Filley et al., 2002). For instance, there exist examples of Fe-rich sediments that actively promote organic matter sulfurization during the earliest stage where FeOOH continuously reacted with sulfides to generate polysulfides (Filley et al., 2002), (Eq. 1):



According to this scenario, reactions of oxidized Fe with sulfide derived from sulfate reduction will provide polysulfides such as S₄²⁻ for organic sulfur formation and ferrous iron FeOH⁺ for further sulfurization to pyrite

The subsequent formation and burial of sulfide as pyrite (FeS_2) and organic sulfur in deep sediments is also a major sink of reducing power (Brüchert & Pratt. 1996; Sternbeck & Sohlennuis. 1996; Filley et al., 2001; Jørgensen & Kasten. 2006; Ryu et al., 2006, Strauss et al., 2012, Thang et al., 2013). Strauss et al. (2012) and Thang et al. (2013) have shown that in sulfidic sediments of the Baltic Sea, organic sulfur is one of the important buried sulfur pools. Organically bound sulfur appears to be formed in the Holocene muds deposited during periods of high organic matter deposition (Conley et al., 2009, 2011; Leipe et al., 2010).

Stable isotope composition of sulfurization end products, such as pyrite or organic sulfur, may be utilized to understand biogeochemical processes and to reconstruct the paleoenvironment conditions (Canfield. 2001; Brüchert. 2004; Paytan et al., 2004; Strauss. 2004; Jørgensen et al., 2004; Wortmann & Chernyvkyy. 2007; Arnold et al., 2012). Previous studies have used sulfur isotope signatures to investigate the formation and degradation of reduced sulfur compounds such as pyrite and organic sulfur, and to decipher pathways of pyrite formation and diagenetic organic matter sulfurization (Brüchert & Pratt. 1996; Sternbeck & Sohlennuis. 1996; Wakeham et al., 1995; Böttcher & Lepland. 2000; Jørgensen et al., 2004; Werne et al., 2004; Ryu et al., 2006; Sinnginhe Damsté et al., 2007).

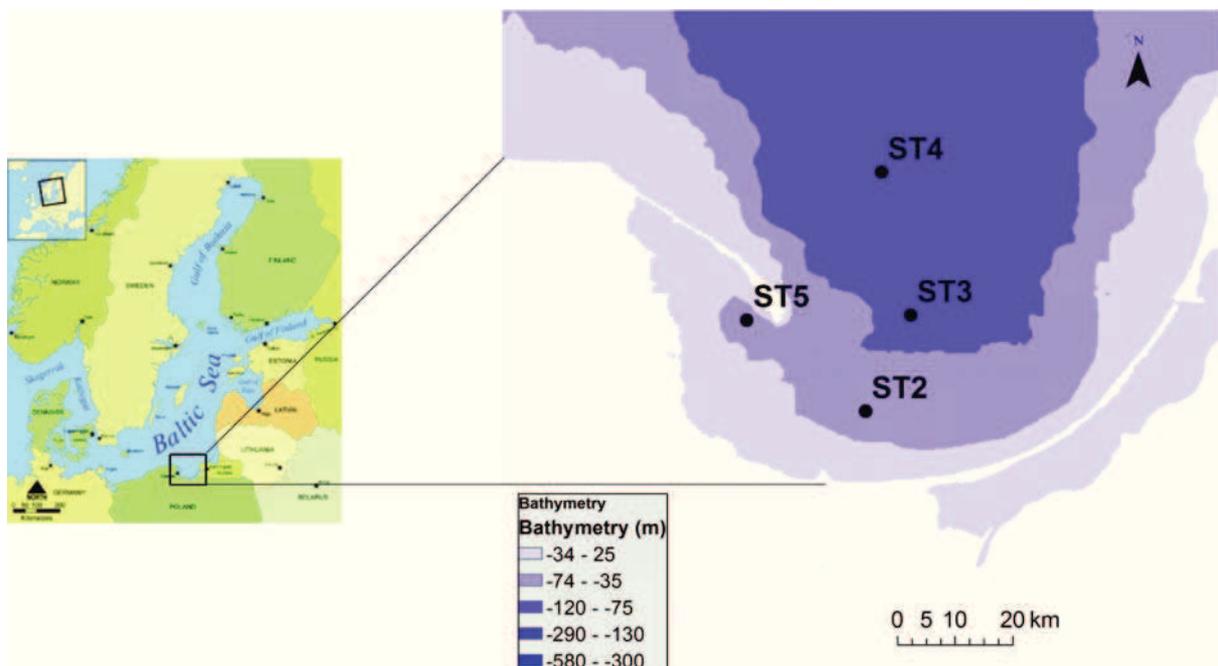


Figure 1: Study location sites (ST2, ST3, ST4, ST5) in Gdansk Basin, Baltic Sea

Gdansk Basin (Poland), located in the southern Baltic Sea, provides an excellent setting to study the sulfurization of organic matter and the potential effects of oxidative sulfur cycling. The Gdansk Basin sediments are characterized by brackish water (9–11‰), high

sediment accumulation rates, and elevated organic matter deposition. Periodic hypoxic conditions in the shallow overlying water (50 – 100 m depth) and submarine groundwater discharge (Pempkowiak et al., 2010) also impact sulfur cycling. Therefore, to explain the impact of iron on organic matter sulfurization, diagenetic sulfide mineral formation in low sulfate and organic-rich sediments, and oxidative sulfur cycling, both geochemical and isotope evidence of sulfur species provide insights into the mechanisms and timing of these processes. This study employs geochemical and isotope approaches, including a mass balance isotope model to decipher how the local geochemical conditions, including reactive iron content, impact the sulfurization of organic matter and iron in the low-sulfate Baltic Sea sediments of the Gdansk Basin.

2. MATERIALS AND METHODS

2.1. Study site

Gdansk Basin is located in the southern Baltic Sea, off Poland (Fig. 1). The Gdansk Basin is one of major basins of the Baltic Sea, with brackish water conditions (9 – 11‰) that depend on water column depth, season and temperature (Cyberska. 1990). The Gdansk Basin covers an area of approximately 25600 km² (Ehlin et al., 1974) and includes the Gdansk Bay (inner and outer Puck Bay) and the Gdansk Deep. Due to the inflow of the Vistula River, near shore areas have a lower salinity compared to open areas of Gdansk Bay and Gdansk Deep (Graca et al., 2006). Like other parts of the Baltic Sea, the Gdansk Basin has experienced extensive eutrophication in the 20th century (Stigebrandt. 1991). The study area is characterized by high primary productivity ($\approx 190 \text{ g C m}^{-2} \text{ a}^{-1}$), with high sediment accumulation rate (1.1-3.6 mm a⁻¹, Suplińska & Pietrzak-Flis. 2008), and moderate to high particulate organic carbon accumulation rates (Leipe et al., 2010). Large parts of the Gdansk Bay are characterized by hypoxic conditions in the bottom water due to eutrophication (Witek et al., 2003).

In this study, Stations ST2, ST3 and ST5 are located in the Gdansk Bay, and Station ST4 is located in the Gdansk Deep (Fig. 1). As shown in Table 1, in November 2009 salinity varied between 7.2 and 9.6‰; temperatures were in the range of 6.2 to 8.5°C (November, 2009); and bottom water oxygen concentrations were in the hypoxic to oxic range of 69 to 110 $\mu\text{mol L}^{-1}$ (Table. 1).

Table 1: Description of sediment sampling station

Site	Coordinate	Water depth (m)	Salinity (‰)	Temperature (°C)	Oxygen content ($\mu\text{mol L}^{-1}$)
ST2	N:54° 27.479 E: 19° 03.377	62	9.6	7.5	69
ST3	N:54° 36.656 E: 19° 10.245	83	7.2	7.5	78
ST4	N:54° 50.072 E: 19° 04.903	103	11.8	8.5	94
ST5	N:54° 35.742 E: 19° 43.861	51	11	6.2	103

Gdansk Basin sediments represent muddy to sandy types. Sandy sediments occur at the coastal area, while further offshore muddy sediments predominate. At Station ST2 surface sediment has little sand content, but becomes sandy at depths between 60 and 80 cm depth. At Station ST3 and ST5, all sediment is fine texture; whereas at Station ST4 the clay is the dominant face below the top 10 cm muddy sediment.

2.2. Sampling

Sediments from the four stations were collected by a small gravity corer (Rumohrlot corer) down to a depth of 75 cm and 130 cm during a research expedition with the *R/V Oceania* in November 2009. Three cores per station were collected for pore-water and solid phase sampling. Pore-waters were immediately extracted using Rhizons[®] (Seeberg-Elverfeldt et al., 2005) connected to 10 mL syringes. Eight to ten mL of pore-water was extracted after 2 to 4 hours and fixed by 0.5 mL $\text{Zn}(\text{CH}_3\text{COO})_2$ (20% w/v) for sulfate and sulfide concentration and sulfur and oxygen isotope composition measurements ($[\text{H}_2\text{S}]$, $\delta^{34}\text{S}_{\text{sulfide}}$; $[\text{SO}_4^{2-}]$, $\delta^{34}\text{S}_{\text{sulfate}}$, $\delta^{18}\text{O}_{\text{sulfate}}$). Solid phase samples were collected using 10 ml cutoff syringes and frozen immediately for measurements for total carbon (TC), total inorganic carbon (TIC), total sulfur (TS), reactive iron content, AVS, CRS, S^0 , and their sulfur isotope compositions. Pieces of frozen solid sediments were freeze-dried for measurement of TC, TIC, and TS. Otherwise, fresh samples were thawed immediately before analysis under anoxic conditions. The pore-water sampling resolution interval is 3 cm in top 10 cm depth and 5 cm below this depth. Solid phase sampling resolution is 5 cm throughout the core.

2.3 Methods

2.3.1 Sulfate and sulfide

Sulfate is measured on 20 – 50 fold diluted samples by ion chromatography (761 Compact IC, Ω Metrohm) with 3.2 mM Na_2CO_3 and 1 mM NaHCO_3 as eluent. The detection

limit for pore water sulfate is 50 μ M. Sulfide was determined by using the photometric methylene blue method according to Cline (1969) and measured on a Shimadzu UV120 spectrophotometer. The detection limit for sulfide is 2 μ M.

2.3.2 Solid phase samples

Acid volatile sulfur ($\text{H}_2\text{S} + \text{FeS}$) and CRS ($\text{FeS}_2 + \text{S}^0$) were sequentially distilled by first using cold 6M HCl to liberate the AVS, followed by conversion of CRS to H_2S with hot HCl-Cr(II) solution according to Fossing and Jørgensen (1989). The evolved sulfide of each step was trapped as ZnS by 10 ml 10% ZnAc solution. A sub-sample of the ZnS was used to determine the sulfide concentration in the trap (Cline, 1969) and then back-calculated for AVS and CRS content. The remained ZnS was then used for isotope composition.

Elemental sulfur was extracted from thawed sediment using 10 mL of pure methanol (MeOH) in 15 mL capped plastic centrifuge vials (Falcon) with 24 hours of constant shaking. The elemental sulfur content was determined on filtered extracts by HPLC using a Supelco Discovery C18 column, a Dionex GP50 Gradient Pump, and a Dianies UVD340S Diode Array Detector. Methanol served as the eluant carrier. The detection limit was in a range of 200 – 400 nM S^0 . The remaining solution from the S^0 extraction was converted to H_2S by hot HCl-Cr(II) solution according to Fossing & Jørgensen (1989). The sulfide was then trapped by 10 ml 10% ZnAc to procedure ZnS. The ZnS sampled were used to measure the isotopic composition of elemental sulfur.

Total sulfur and total carbon concentrations of freeze-dried sediment were determined with an elemental analyzer (GC – Carlo Reba NA – 1500 CNS analyzer). Vanadium oxide (V_2O_5) was used as catalyst by adding approximately 10 mg to each sample to ensure complete combustion of the sulfur compounds. Additionally, total inorganic carbon was measured on the CM 5012 CO_2 Coulometer (UIC) after H_3PO_4 acidification. The total organic carbon (TOC) was calculated as the difference between TC and TIC.

Reactive iron in the sediment was extracted with ascorbate – dithionite under anoxic conditions according to März et al. (2008). Approximately, 200 mg fresh sediment was used for extraction from frozen sediment. Iron concentrations in the ascorbate extractable reactive iron (Fe_{asc}) – FeS, Fe_3S_4 and dithionite extractable reactive iron ($\text{Fe}_{\text{dithio}}$) - FeOOH fractions were measured using atomic absorption spectroscopy (AAS; Thermo Scientific iCE 3000 series).

Pyrite S content was determined as the difference between CRS and S⁰ content, whereas total organic sulfur (OS) was calculated as the difference between TS and total inorganic sulfur forms (sulfate, AVS, and CRS).

2.3.3 Isotope composition measurements

Sulfur and oxygen isotope composition were determined using continuous flow – isotope ratio mass spectrometry (CF – IRMS), coupled to either an elemental analyzer (EURO EA Elemental Analyzer) or a high temperature carbothermic reduction device (ThermoFinnigan TC/EA), respectively.

To prepare the pore-water samples, the ZnS containing solution was first filtered off (0.2 μm) and the filtrate collected. Barium chloride solution (~1.3 M in 1 M HCl) was added to the filtrate to precipitate the sulfate as barium sulfate (BaSO₄) for the analysis of both the sulfur and oxygen isotope composition of sulfate. Barium sulfate was separated from the supernatant, washed three times with 18 MΩ water and dried overnight in a 50° C oven. For the measurement of sulfur isotope composition of sulfide species (AVS, CRS), elemental sulfur, a 5% AgNO₃ solution was added to the ZnS precipitate to convert the sample to Ag₂S. The Ag₂S was separated from the supernatant by centrifugation and subsequently dried overnight in a 50°C oven. To determine δ³⁴S – total S, 10 mg of dry sediment was analyzed. . For all sulfur isotope determinations, V₂O₅ was added to obtain the complete combustion of sulfur compounds.

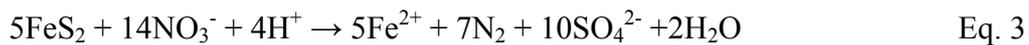
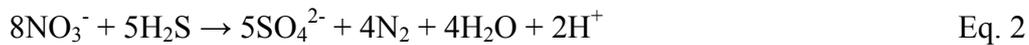
The isotopic composition is reported in delta notation as δ³⁴S relative to Vienna Canon Diablo Troilite (VCDT) with a standard error (1σ) of ~0.2‰. The oxygen isotopic compositions is reported relative to Vienna Standard Mean Ocean Water (VSMOW) with a standard error (1σ) ~0.8‰.

3. RESULTS AND DISCUSSION

3.1. Variable sulfur and iron geochemistry of the Gdansk sediments

The four sites studied in Gdansk Bay exhibit a wide range of sulfate concentration profiles and sulfate penetration depths, although all sites have similar bottom water sulfate concentrations (8.7-9.1 mM). We attribute this variability to differences in hydrologic and microbial processes. At Stations ST2 and ST5 sulfate concentrations decrease with depth and penetrate from 20 to > 100 cmbsf, whereas the δ³⁴S_[SO₄2-], δ¹⁸O_[SO₄2-] values become enriched with decreasing sulfate concentrations from +20.5 to +65.1‰ and +9.6‰ to +19.6‰ in

between 1 and 85 cmbsf, respectively (Fig. 2). This enrichment in both S and O isotopes suggests that the isotope composition of sulfate at Stations ST2 and ST5 are controlled by microbial sulfate reducing processes, and are supplied by diffusion of sea water sulfate (Brunner et al., 2005). In contrast, at Station ST3, there is a small fluctuation between 16 and 26 cmbsf where the sulfate concentration slightly increases from 5.2 to 5.7 mM at 36 and 41 cmbsf. Concurrently, there is a depletion in both $\delta^{34}\text{S}_{[\text{SO}_4^{2-}]}$ (+36.2‰ to +20.2‰) and $\delta^{18}\text{O}_{[\text{SO}_4^{2-}]}$ (+12.1‰ to +6.3‰) between 16 and 36 cmbsf depth values, which indicate another subsurface source of sulfate or sulfide oxidation in these depths. We propose that ground water discharge in the region (Falkowska & Piekarek-Jankowska. 1999; Pempkowiak et al., 2010) could provide the electron acceptor such as NO_3^- , which would enhance H_2S oxidation to sulfate (Jørgensen & Nelson. 2004) (Eq. 2), and also eventual FeS_2 oxidation (Eq. 3) (Appelo & Postma. 2005):



As consequence of these processes, $\delta^{34}\text{S}_{[\text{SO}_4^{2-}]}$ and $\delta^{18}\text{O}_{[\text{SO}_4^{2-}]}$ are depleted compared to seawater. Additionally, the dominant H_2S concentration in pore water and the depleted $\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$ values in top 20 cmbsf compared to below this depth may suggest that the electron donor could be H_2S rather than FeS_2 .

At Station ST4, the sulfate concentrations exhibit an increase below 46 cmbsf (Fig 2a) and $\delta^{34}\text{S}_{[\text{SO}_4^{2-}]}$ profile showed an enrichment with ^{34}S with values from +20.3 to +67.4‰ between 1 and 46 cm depth. Below 46 cmbsf the $\delta^{34}\text{S}$ composition slightly decreases to +55.3‰ at 96 cm depth, which suggests that there is another subsurface source of sulfate. Most likely this may be a result of the diffusive migration of sulfate from the deeper ice sea-lake sediments, similar to the model proposed for the deep Arkona and Bornholm Basin sediments (Holmkvist et al., 2009; Mogollón et al., 2012; Fossing & Ferdelman, unpublished data).

Although sulfate profiles differ from site to site, the sulfide profiles are consistent with the SO_4^{2-} profiles. At Station ST2 sulfide is close to the limit of detection (4.5-50 μM), while all other stations showed a clear peak of sulfide at depth (1.5, 0.87, and 0.71 mM at Stations ST3, ST4, and ST5, respectively; Fig. 2a). Stations ST3 and ST5 have profiles that correlate with the production of sulfide and removal of sulfate via bacterial sulfate reduction, and the sulfide concentrations remain high down to 96 cmbsf at Station ST3 (Fig. 2a). Core ST4 has an increase in sulfide in the upper portion of the core that decreases throughout the bottom of

the core as an external supply of sulfate is diffusing into the core (Fig. 2). The $\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$ values are consistent with microbial sulfate reducing processes acting to remove sulfate; thus, $\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$ values are enriched to compared to $\delta^{34}\text{S}_{[\text{SO}_4^{2-}]}$ at Stations ST4, ST5 and in topmost 20 cm at Station ST3. An exception is the interval between 20 and 40 cmbsf at Station ST3 where $\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$ values are similar to $\delta^{34}\text{S}_{[\text{SO}_4^{2-}]}$.

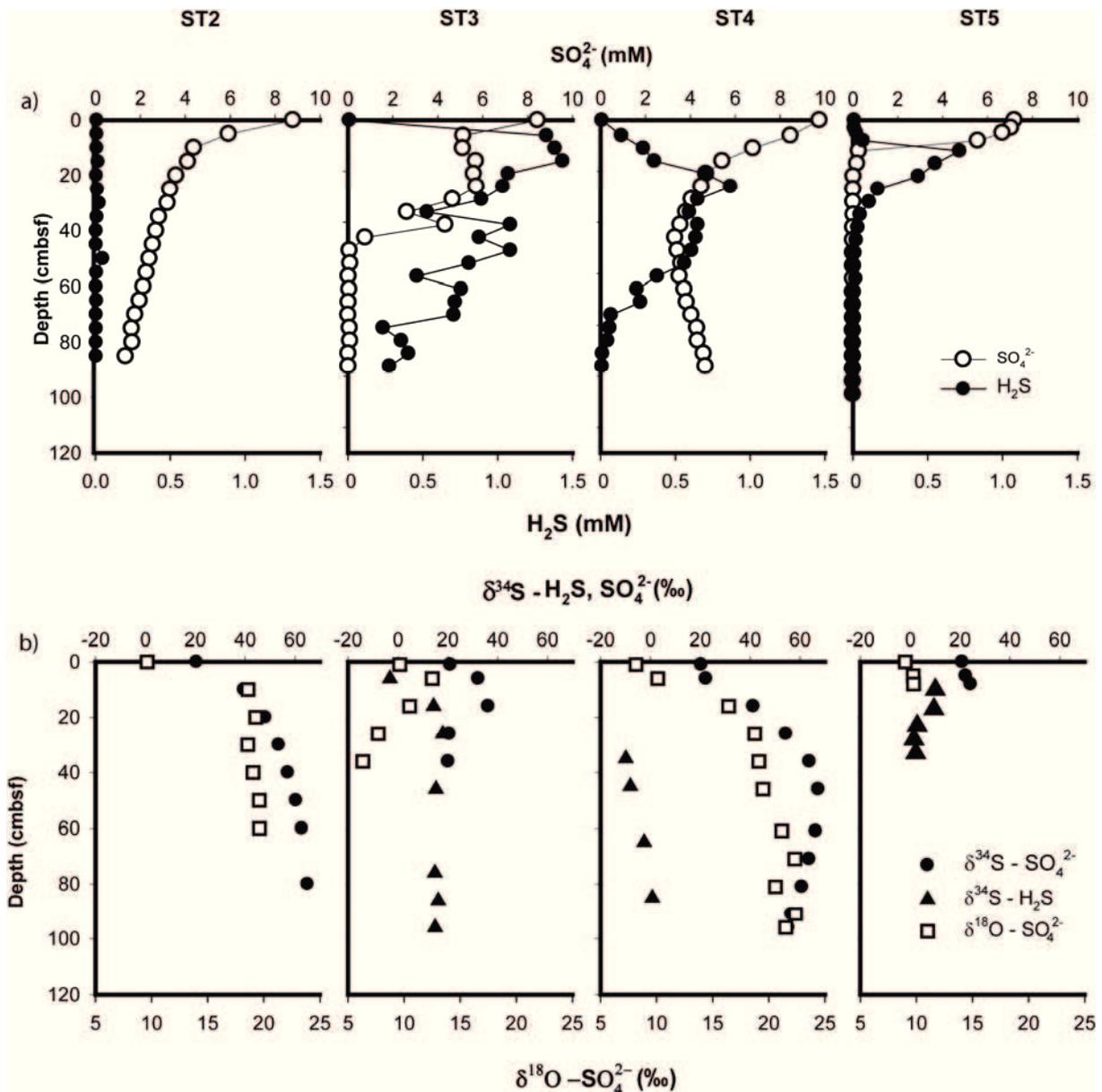


Figure 2: Pore water sulfate and sulfide geochemistry in the Gdansk Bay sediments

The variations of sulfur biogeochemistry are observed not only in pore water but also in the solid geochemistry. For instance, at Station ST2, AVS concentration decreases with depth with a slight increase between 60 and 80 cmbsf. In contrast, FeS_2 concentrations exhibit a mirror image of AVS and reach maximum concentrations of $81.7 \mu\text{mol cm}^{-3}$ at 42.5 cmbsf. At Station ST3, AVS content was dominant distribution in top 30 cmbsf while FeS_2 content

increased with depth. At Station ST4 FeS_2 concentration was more or less constant with depth whereas AVS content decreased from the surface sediment to 57.5 cm depth, and below this depth, it was very low content at Station ST4. In complete contrast to the AVS and FeS_2 at the other three stations, both AVS and FeS_2 have similar concentrations that scatter between 25 and $75 \mu\text{mol cm}^{-3}$ in the upper 80 cmbsf.

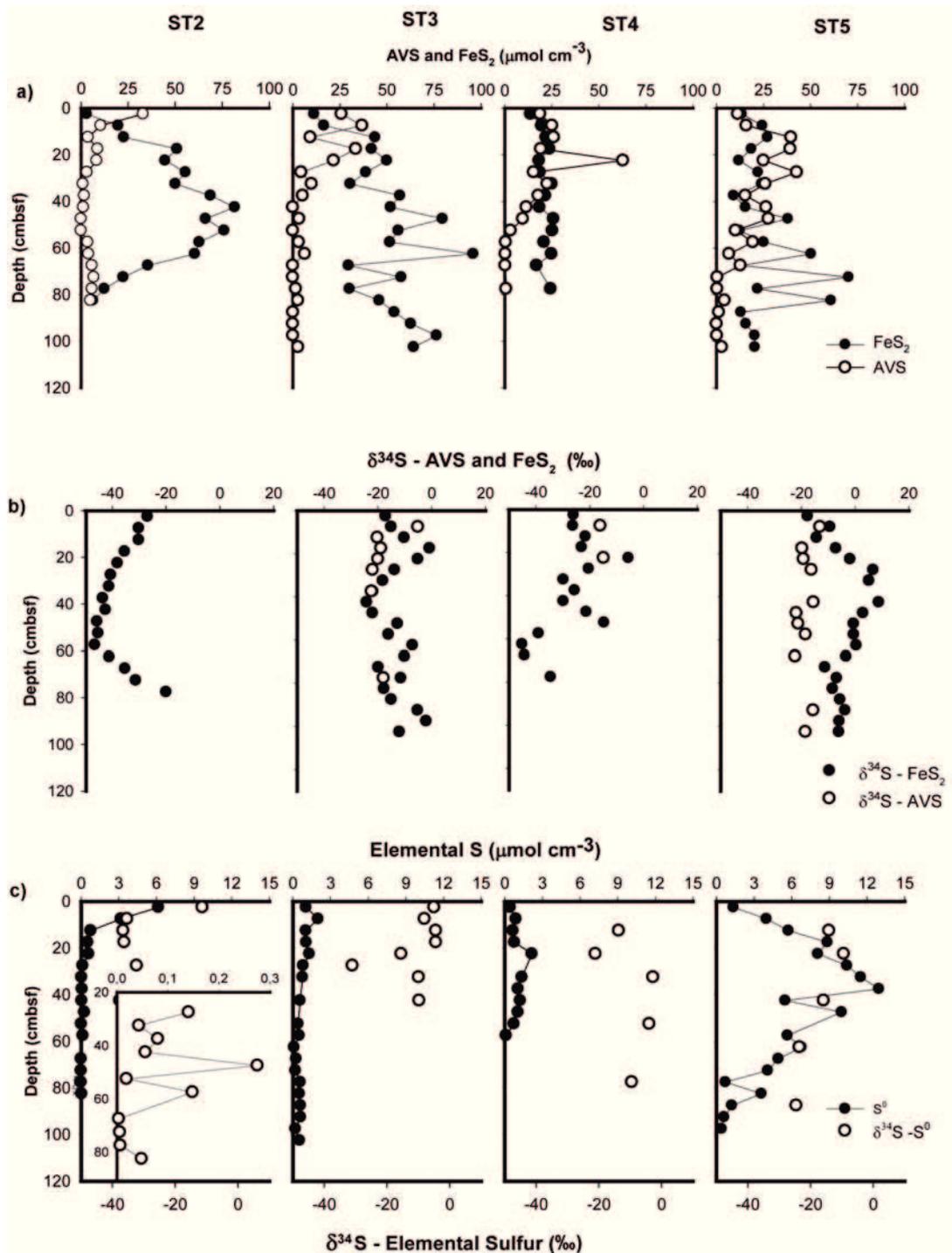


Figure 3: a) Solid phase content of AVS and FeS_2 , b) $\delta^{34}\text{S}_{[\text{AVS and FeS}_2]}$ compositions, and c) elemental S content and $\delta^{34}\text{S}_{[\text{S}^0]}$ compositions in the Gdansk Bay sediments

The variable AVS and FeS₂ contents in Gdansk Basin sediment are also mirrored in the $\delta^{34}\text{S}_{[\text{AVS}]}$, $\delta^{34}\text{S}_{[\text{FeS}_2]}$ values. Likewise, S⁰ geochemistry differs from core to core consistent with expected variation of biogeochemical process (see discussion below in 4.3).

We have also observed elevated concentrations of calculated organic sulfur (OS), similar to other Baltic Sea sediments (Strauss et al., 2012, Thang et al., 2013). The calculated OS content at Station ST2 varies between 16.6 – 170.4 $\mu\text{mol cm}^{-3}$. From the surface sediment to 62.7 cmbsf, the OS content increases to 170.4 $\mu\text{mol cm}^{-3}$. Between 67.5 and 82.5 cmbsf, the OS content decreases to 16.6 (Fig. 5a). At Stations ST3, ST4 and ST5, the OS content increases between the surface and bottom sediment. The organic sulfur content is in the range of 7.4 – 272, 53 – 210, 22.6 – 224 $\mu\text{mol cm}^{-3}$ at Stations ST3, ST4, and ST5, respectively (Fig.5a). Interestingly, the concentration profiles of organic S and pyrite (Fig. 3a & Fig. 5a) are similar, which implies that a co-genesis of FeS₂ and OS during early diagenesis.

When compared to ST3 and ST4 the ten-fold larger concentrations of Fe_{asc} (20 - 70 $\mu\text{mol cm}^{-3}$) and Fe_{dithio} (15 - 34.0 $\mu\text{mol cm}^{-3}$) at Station ST5 and Fe_{asc}-Fe_{dithio} (15 - 22.1 $\mu\text{mol cm}^{-3}$) in the topmost 20 cm of Stations ST2 are striking (Fig. 3b). The relative abundance of Fe_{asc} over Fe_{dithio} at Station ST5 and topmost 20 cmbsf of ST2 suggest that this reactive iron pool has been freshly formed. In contrast, we observe that the Stations ST3 and ST4 are poor in reactive Fe; any reactive fraction of iron has been reacted to form pyrite.

Although the total S contents are similar between stations (Fig. 3a) extreme differences in the S speciation may be linked to the Fe geochemistry in the Gdansk Basin sediments. This is also reflected not only in the geochemical profile but also in the isotopic composition of sulfur species. Below, we discuss how these differences in iron geochemistry affect the early diagenetic process of sulfurization and oxidative sulfur cycling which we discuss below (below discussions, 4.2 & 4.3).

4.2. Co-genesis of sulfurized organic matter and pyrite

Elevated organic matter contents of the sediments in the Gdansk Basin (4 – 8%) result from high rates of sediment accumulation (1.1 – 3.6 mm a^{-1} , Suplińska & Pietrzak-Flis, 2008; 0.02 – 4.8 mm a^{-1} and this study data not shown), high primary production (Witek et al., 1997, 2003) and annual summer algal blooms (Conley et al., 2011). In the brackish Gdansk Basin the sulfate concentrations are lower (8.7 - 9.1 mM) than typical marine conditions (27 mM), however, sulfate reduction is still the dominant organic matter mineralization process (Chapter 3).

Overall, concentrations of organic sulfur and FeS₂ increase with increasing depth; however, OS concentrations are greater than the FeS₂ concentrations. At Station ST3, ST4, the persistence of pore water H₂S and an absence of reactive iron promote organic matter sulfurization. Hence, pyrite and organic sulfur signatures in concentration patterns and $\delta^{34}\text{S}_{[\text{FeS}_2]}$ values imply that major reduced source of sulfur for FeS₂ is controlled by bacterial organoclastic sulfate reduction (Strauss et al., 2012). Previous studies have demonstrated that the sulfur isotope composition of organic sulfur can be used to decipher the pathway of organic formation from sulfurization of organic matter in marine sediments (Mossmann et al., 1991; Brüchert & Pratt. 1996; Anderson & Pratt. 1998; Passier et al., 1997, Canfield et al., 1998; Werne et al., 2004, 2008; Lojen et al., 2005; Amrani et al., 2008, Strauss et al., 2012). Sulfur isotope fractionation during organic matter sulfurization is generally minimal (0-2.7‰) (Fry et al., 1986, 1988). Thus, the organic sulfur isotope composition is mainly dependent on, and records, the sulfur sources, including sulfate, sulfide and reactive intermediates such as elemental sulfur, polysulfide and thiosulfate (Pyzik & Sommer. 1981; Canfield. 2001). To address the input of these various sources, and the impact on the resulting $\delta^{34}\text{S}$ signatures of OS to explain the sources of OS existence in the sediments, an isotope mass balance calculation following the method of Fry et al. (1984, 2008) is applied to the Gdansk Basin sulfur geochemical results. In general, in the environment one element is present in different species, thus the overall isotope composition of this element is the result of mixing of the various sources with different isotope compositions (Eq. 4).

$$\delta_{total} A_{total} = \delta_1 A_1 + \delta_2 A_2 + \dots + \delta_n A_n \quad (\text{Eq. 4})$$

where, A_n is mass concentration of species A, δ_n is the isotope composition of species A, δ_{total} and A_{total} represent the total isotope composition and concentration of this element. In marine sediments, sulfur species are present as sulfate, AVS, FeS₂, S⁰, OS forms and sulfur intermediates such as thiosulfate and sulfite. Sulfur intermediate contents, however, are quite low and only present in the pore water. We thus assume, that the isotope composition of total sulfur and its concentration in sediments can be defined by mixing of sulfate, AVS, S⁰, FeS₂, and OS (Eq. 5)

$$\delta_{total} S_{total} = \delta_{\text{SO}_4^{2-}} [\text{SO}_4^{2-}] + \delta_{\text{AVS}} [\text{AVS}] + \delta_{\text{S}^0} [\text{S}^0] + \delta_{\text{FeS}_2} [\text{FeS}_2] \quad (\text{Eq. 5})$$

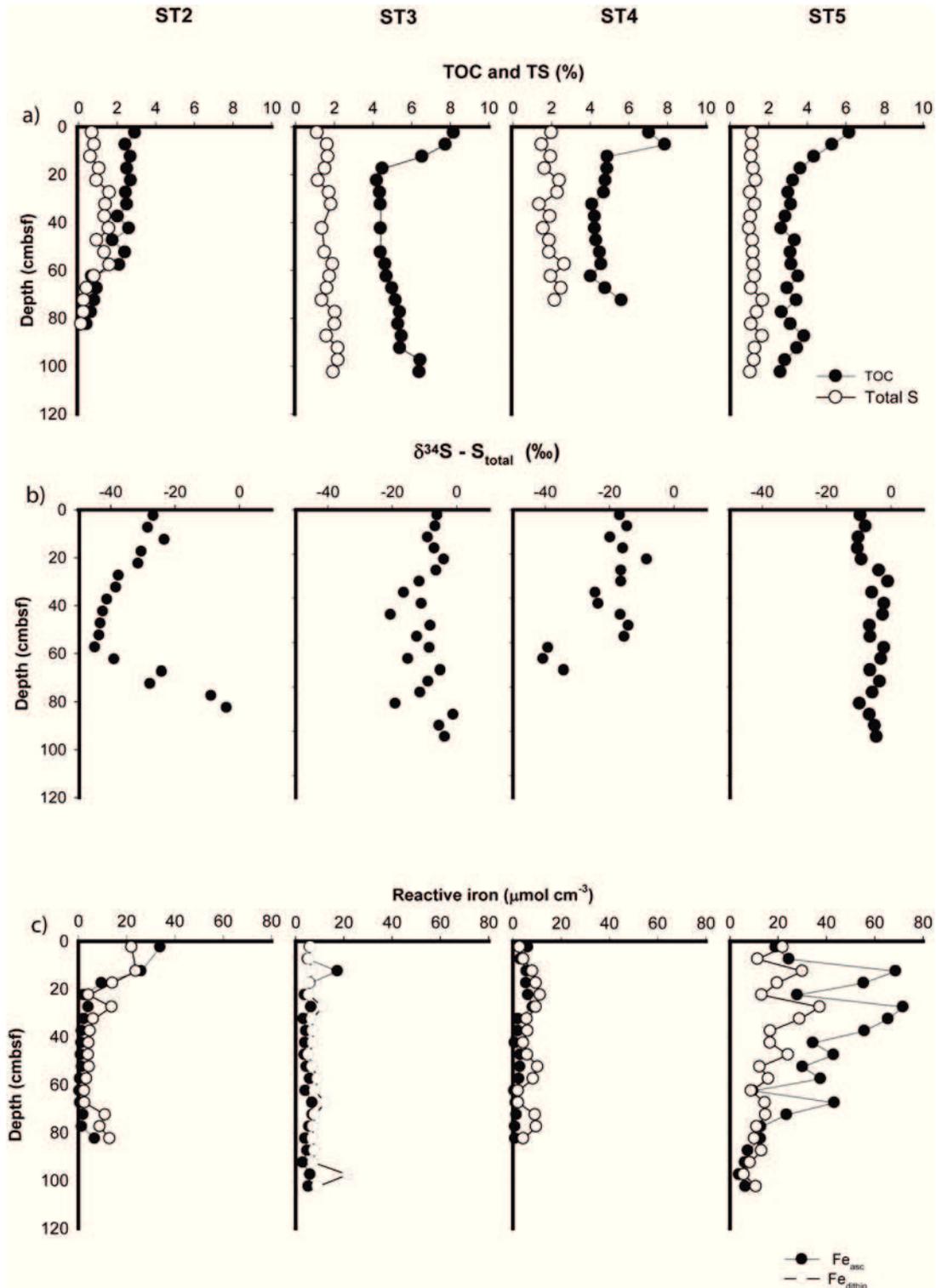


Figure 4: a) Solid phase content of TOC and TS; b) $\delta^{34}\text{S}_{[\text{total S}]}$ composition; and c) ascorbate extractable iron (Fe_{asc}) and dithionite extractable iron ($\text{Fe}_{\text{dithio}}$) in solid phase in the Gdansk Basin sediments

These concentrations of total sulfur, sulfate, AVS , S^0 , and FeS_2 and their $\delta^{34}\text{S}$ values were determined by direct analysis, the organic sulfur concentration was calculated as the difference between total sulfur and total inorganic sulfur (sulfate, AVS , FeS_2 , and S^0) contents. Therefore, the $\delta^{34}\text{S}_{[\text{OS}]}$ value is given by following equation (Eq. 6).

$$\delta_{OS} = \frac{\delta_{total S_{total}} - (\delta_{SO_4^{2-}} [SO_4^{2-}] + \delta_{AVS} [AVS] + \delta_{S^0} [S^0] + \delta_{FeS_2} [FeS_2])}{[OS]} \quad (\text{Eq.6})$$

The sulfur isotope composition of the pore water sulfate, AVS, FeS₂, S⁰, and total S were measured. The results of the model on the sulfur isotope profiles of SO₄²⁻, total S, AVS, CRS, and OS are presented in Fig. 6. At Station ST2, the profile of δ³⁴S_[OS] values (-44.9‰ to -3.0‰) is similar to δ³⁴S_[FeS₂] and δ³⁴S_[total S]. However, δ³⁴S_[OS] are more depleted δ³⁴S compared with δ³⁴S_[FeS₂] and δ³⁴S_[total S] in top 20 cm depth, however in other depths, δ³⁴S_[OS] is more enrichment than δ³⁴S_[FeS₂]. At Station ST3, δ³⁴S_[OS] values are variable between -19.5‰ and +1.5‰; these values are enriched δ³⁴S compared to FeS₂ and AVS. The δ³⁴S_[OS] composition varies between -40.3 to -8.2‰ and is also depleted in the δ³⁴S to compared to the FeS₂ at Station ST4. At Station ST5, δ³⁴S of organic sulfur is variable from -40.3‰ to -8.2‰ and δ³⁴S of organic sulfur is enriched and depleted compared to δ³⁴S_[AVS] and δ³⁴S_[AVS], respectively (Fig. 4c).

The δ³⁴S_[OS] values are in the range of δ³⁴S values measured for specific organic sulfur compound (fulvic, humic acids and polar bitumen sulfur) and total organic sulfur (-30.6 to +8‰) in previous studies (Francois. 1987; Brüchert & Pratt. 1996; Werne et al., 2004; Lojen. 2005; Strauss et al., 2012). In contrast, these values are very different to δ³⁴S values of primary organic matter of marine plankton (+20 – +60‰, Fry et al., 2000) and the Baltic Sea plankton (10.3 – 18‰; Alling et al., 2008), and δ³⁴S_[SO₄²⁻] values in pore water sediments (+20.3 to +67.5‰). This suggests that the organic sulfur in the Gdansk sediments does not originate from the input of primary “bio” sulfur such as plankton or assimilatory sulfate reduction, but records the incorporation of the organic matter and the reduced sulfur during early diagenesis (Strauss et al., 2012). Likewise, Filley et al. (2002) proposed that the pathway of the sulfurization of organic matter significantly relate to iron reduction coupled sulfide oxidation where H₂S reacts to of polysulfur (S_n⁻) or directly incorporate with polysulfide (HS₂⁻) (Eq. 1). The geochemical profiles provide evidence of co-diagenesis of pyrite and OS, however the difference of Fe reactivity in the Gdansk sediments probably impacts the mechanism and timing OS formation.

Interestingly, at ST5 and top 20 cm of ST2 the δ³⁴S_[OS] values do show an enrichment compared to δ³⁴S_[FeS₂] (Fig. 4c). This suggests that OS is formed before FeS₂. The δ³⁴S_[OS] and δ³⁴S_[S⁰] values are similar due to the cooperation of organic matter and polysulfur (S_n⁻) produced from sulfide oxidation coupled to iron reduction. The generation of pyrite results from the reaction of the remaining HS⁻ with FeOH⁺ to produce FeS (FeOH⁺ + HS⁻ → FeS +

H₂O, and further to pyrite (Filley et al., 2002)). This explains why $\delta^{34}\text{S}_{[\text{FeS}]}$ composition appears to be enriched when compared to $\delta^{34}\text{S}_{[\text{FeS}_2]}$. Fe reactivity enhances the ability of sulfide capture in the sediments of Stations ST2 and ST5. In contrast, at Stations ST3 and ST4 and deeper 20 cm depth at Station ST2, the calculated $\delta^{34}\text{S}$ of OS is enriched compared to FeS₂. This reflects first iron sulfurization to pyrite and then sulfurization of organic matter to form OS. Thus organic matter continuously reacts with remaining free sulfide or polysulfide to form OS.

Combining both the geochemical evidence of the similar features in concentration profiles and the similar isotope signatures of organic sulfur and FeS₂ implies that there is a co genesis of organic sulfur compounds and pyrite during early diagenesis. The presence of reactive iron appears to be the decisive mechanism and timing of the sulfurization of organic matter. Although the generations of pyrite and OS must be significantly related to HS⁻ and S_n⁻, the $\delta^{34}\text{S}_{[\text{FeS}_2]}$, and the calculated $\delta^{34}\text{S}_{[\text{OS}]}$ in the Gdansk sediments do exhibit a large a range of values. Variations of isotopic signals may also be linked to variations in the oxidative pathways in the sediments. Furthermore, oxidative sulfur cycling may be linked to the presence or absence of reactive Fe.

4.3. Controls on oxidative S cycling

Vast amounts of sulfide are formed through sulfate reduction in coastal environments. This sulfide can be oxidized within the sediment via S⁰ and other intermediates (e.g., polysulfides, thiosulfate) to sulfate (Elsgaard & Jørgensen. 1992; Jørgensen & Nelson. 2004). Among the intermediates, S⁰ is the main product of sulfides oxidation by MnO₂ and FeOOH when oxygen and nitrate are absent to oxidized dissolved sulfides and FeS to S⁰ instead of to sulfate (Moses et al., 1987; Jørgensen et al, 1990b; Podgorsek & Imhoff. 1999; Shippers & Jørgensen. 2001; Zopfi et al., 2004; Jørgensen & Nelson. 2004, Riedinger et al., 2005, 2010 Kamyshny et al., 2010; Holmkvist et al., 2011). In the Gdansk Bay sediments, the S⁰ content ranges from 0.2 to 13 $\mu\text{mol cm}^{-3}$ (Fig. 2b), which is lower compared to the in Aarhus Bay (10 – 17 $\mu\text{mol cm}^{-3}$, Troelsen & Jørgensen. 1982), and greater than the 0.34 – 6.4 $\mu\text{mol cm}^{-3}$ observed in other typical marine and euxinic sediment (Thode-Andersen & Jørgensen. 1989; Thamdrup et al., 1994; Podgorsek & Imhoff. 1999).

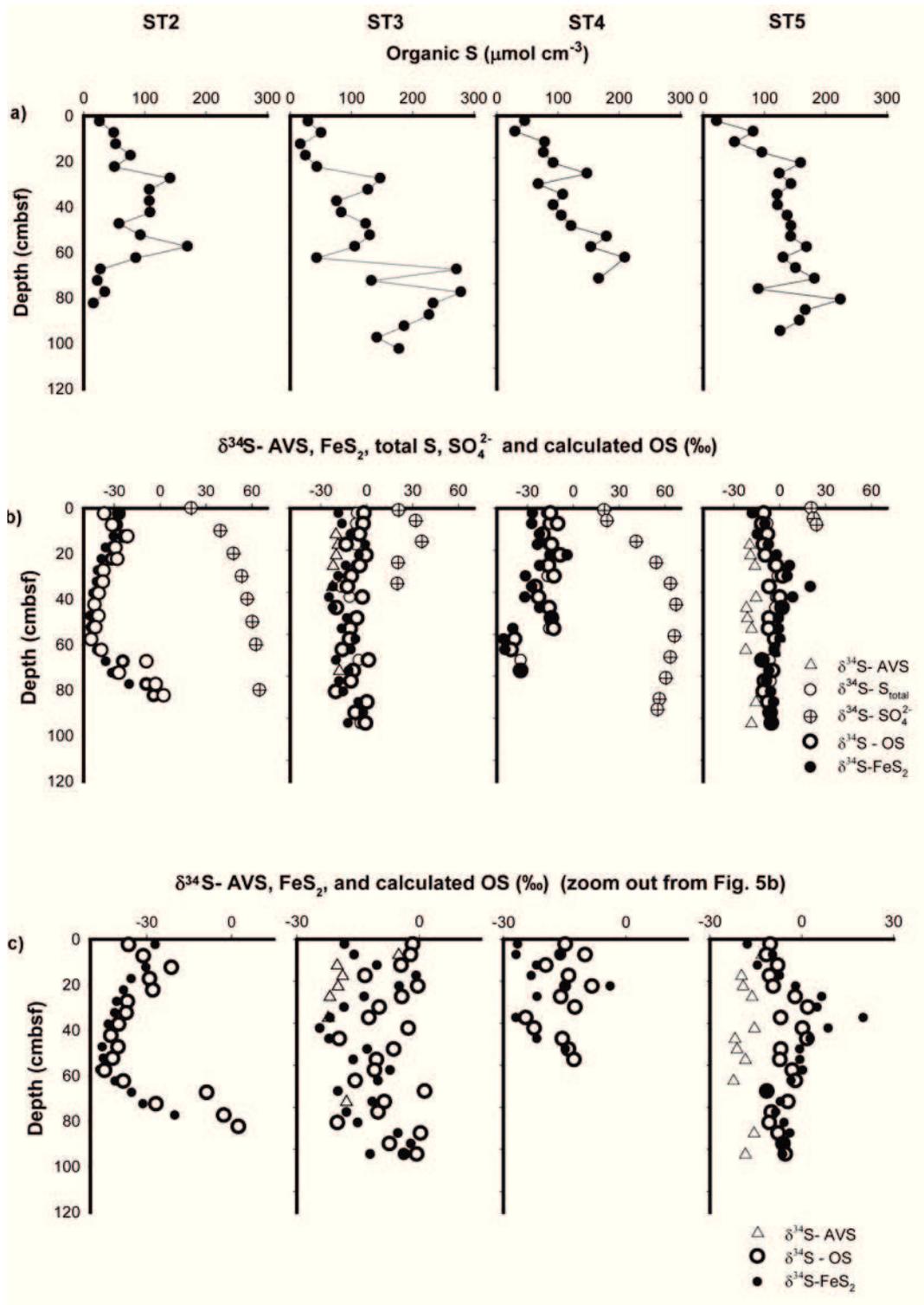


Figure 6: a) Total calculated organic sulfur; b) $\delta^{34}\text{S}$ - AVS, CRS, TS, SO_4^{2-} and calculated OS, and c) $\delta^{34}\text{S}$ - AVS, CRS, TS, and calculated OS (zoom out from Fig. 6b) in the Gdansk Bay sediments.

The reaction involved in the formation and consumption of S^0 are numerous in the Gdansk Basin sediments. For instance, at Station ST2, the higher S^0 content is associated with elevated concentrations of reactive iron, both as extractable Fe_{asc} and $\text{Fe}_{\text{dithio}}$. The sulfide concentration is low ($< 50 \mu\text{M}$), however, the covariance of AVS with S^0 is striking. This

suggests that S^0 may be the main reaction product of FeS oxidation (Moses et al., 1987; Shippers & Jorgensen, 2001, 2002) for instance. Low abundance of such Mn- species are related to the low bulk concentration of Mn in the Gdansk sediments, however, the sediment – surface is not anoxic enough to allow the formation of Mn-bicarbonate (Belmans et al., 1993). Additionally, a common observed polychaete (*Marenzelleria*) may enable O_2 transport down more than 15 cm depth in Baltic Sea sediments (Kautsky. 2008; Blank et al., 2008; Hedman et al., 2011). This could allow oxidation of dissolved Mn^{2+} to form MnO_2 , which would then oxidize FeS (Lovely et al., 1993; Eq. 7):



At Station ST5, the isotope composition of S^0 is similar to AVS and is enriched compared to H_2S (Fig. 2b & 3b). Given the large concentrations of reactive Fe at the station, sulfide may be coupled to inorganic, sulfide-driven iron reduction, in which FeS and S^0 are produced (Jørgensen & Nelson. 2004) (Eq.8).



Moreover, the sulfur isotope offset between sulfide, AVS and S^0 (Fig 2b, 3b, 3c) indicates a lack of isotopic exchange between isotope compositions of H_2S , AVS and S^0 (Canfield et al., 1998) at Station ST5. This isotope disequilibrium is the result of the production and consumption of S^0 in the absence of isotope fractionation under varying concentrations of potential oxidants.

At Station ST3 a different oxidative cycle may be operating. The low reactive Fe pool, where there may be an entrainment of NO_3^- via groundwater discharge (Pempkowiak et al., 2010), sulfide and eventual pyrite oxidation (Eq.2 & 3) may result in sulfate that is depleted in $\delta^{34}S$ and cause a lighter $\delta^{34}S_{[SO_4^{2-}]}$ with depth as in other stations whereas $\delta^{34}S_{[H_2S]}$ is enriched (Fig 2b). Furthermore, the low concentrations of reactive Fe could lead to high free H_2S in pore water and limit H_2S capture to produce AVS and further pyrite (Fig 2a). Additionally, the depleted $\delta^{34}S_{[FeS_2]}$ compared to $\delta^{34}S_{[AVS]}$ between 20 – 40 cm depth suggests that pyrite is formed due to initial sulfate reduction near surface sediments while AVS is produced later based on further sulfate reduction due to the groundwater discharge. This is consistent with a distinct peak in measured sulfate reduction at these depths at this station (Flury. unpublished data).

At Station ST4, low concentrations of reactive iron do not lead to sulfide oxidation, in spite of the presence of high concentrations of sulfide in pore water. At this site, similar $\delta^{34}S$ compositions of H_2S , AVS, and S^0 (Fig. 2b & 3b) do suggest the presence of substantial

isotope exchange between H_2S and S^0 ($\delta^{34}\text{S}_{[\text{H}_2\text{S}]} \leftrightarrow \delta^{34}\text{S}_{[\text{S}^0]} \leftrightarrow \delta^{34}\text{S}_{[\text{AVS}]}$) (Fossing et al., 1995). Therefore, the equilibrium of S^0 and sulfide leads to isotope exchange between these pools and results in small differences of isotope composition ($\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$, $\delta^{34}\text{S}_{[\text{S}^0]}$, $\delta^{34}\text{S}_{[\text{AVS}]}$) (Fossing et al., 1995).

Thus, we can conclude that the presence and reactivity of detrital iron minerals are one of the major drivers of sulfide oxidation and S^0 disproportionation processes in the sulfidic portion of coastal sedimentary systems in the Baltic Sea (Böttcher & Lepland, 2000; Holmkvist, 2009). This Fe reactivity also leads to the isotopic systematic of sulfur pools in Baltic Sea sediments.

CONCLUSIONS

The present study shows that the presence of reactive iron and potentially external supplies of SO_4^{2-} impact early diagenetic sulfurization of reduced iron and organic matter in the Gdansk Bay sediments. This is particularly interesting given the lower background sulfate in the overlying water. Moreover, the variation of local sulfate inputs from bottom seawater, groundwater discharge, and limnic sediments contribute to an environment that has the potential to sustain both reductive and oxidative sulfur cycling. The discharge of ground water may provide the electron acceptor for enhanced sulfide oxidation and supply external sulfate in between 20-40 cm depth of some areas in the Gdansk sediments.

Additionally, the variability of Fe pools in the system also impacts the sulfurization of organic matter and pyrite and the oxidative sulfur cycling. Firstly, pyrite and organic sulfur are formed contemporaneously during early diagenesis as reduced products of sulfate reduction. Depending on the availability of reactive iron pools in the sediments, the sulfurization process is enhanced by the formation of polysulfides due to sulfide oxidation associated to the reaction of FeOOH (Filley et al., 2002). The $\delta^{34}\text{S}$ composition of OS provides evidences for the mechanism and timing of sulfurization of organic matter and pyrite. In organic-rich sediments as the Baltic Sea, consequently, FeS_2 and OS pool is one of the important pools of sulfur burial in the sediments (chapter 3).

Secondly, presence or absence of a reactive iron pool may be the major factor to control oxidative sulfur cycling. The iron-rich pool improves sulfide oxidation and results in FeS and S^0 coincided production and similar isotope composition. In contrast, a sediment with low amounts of reactive iron may exhibit little sulfide oxidation. The long term existence of

H₂S, FeS and S⁰ in sediments and consequent isotope exchange between $\delta^{34}\text{S}_{[\text{H}_2\text{S}]}$ \leftrightarrow $\delta^{34}\text{S}_{[\text{S}_0]}$ \leftrightarrow $\delta^{34}\text{S}_{[\text{AVS}]}$ will lead to similar isotope signatures in the pyrite and organic sulfur fractions.

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PERSPECTIVES

The Baltic Sea is regarded as a model area to study the early stage diagenesis of biogeochemical processes. Due to high sediment accumulation rates, a salinity gradient from seawater concentrations to near freshwater concentrations, high primary productivity, regions of low oxygen concentration, the Baltic Sea sediments form an ideal system to examine the biogeochemistry of carbon, sulfur, and iron cycling. The Baltic Sea is also an interesting place to test or reconstruct the paleoenvironment on a geological time scale related to local (European) and global anthropogenic changes. The major goal of the present project was to understand whether salinity gradient and high rates of sediment accumulation could control the carbon preservation, methane, and sulfur cycling in the Baltic Sea sediments. In order to reach this goal, biogeochemical approaches such as modeling of pore water chemistry, experimental tracer techniques, and isotope chemistry of sulfur species, were employed. The biogeochemistry of iron, sulfur, and carbon was examined in relation to salinity gradients, depositional environments, and organic matter delivery to sediments, such as Himmerfjärden estuary (Sweden), Gdansk Basin (Poland), Aarhus Bay (Denmark), Mecklenburg Bay (Germany), Bornholm Basin (Denmark), Gotland Deep (Sweden), Bothnia Sea and Bothnia Bay (Sweden). Generally, we could show that the rate of sediment delivery and depositional environment plays a major role in the subsequent biogeochemistry of iron, sulfur, and methane, nevertheless, major questions remain to be tested

Methanogenesis and Carbon Preservation

From the beginning of the project, it was planned to test the salinity gradient influence on methanogenesis and organic carbon preservation. As we have seen salinity exerts a major control on the sulfate gradient and sulfate penetration depth. Interestingly, in the Himmerfjärden sediments, we found that the bicarbonate methanogenesis activity still occurs in the low sulfate concentration (<5 mM) sediments (Chapter 2). It would be interesting to test the at what sulfate concentration the methanogenesis becomes quantitatively important. Bicarbonate and acetolastic methanogenesis are the major pathways to produce methane in the sediments. However, methylated compounds such as methylamine and methanol may also provide an excellent source of methane in rapidly depositing sediments. The existence of high

acetate concentrations in the pore water of Bornholm Basin could in addition to drive methanogenesis, or suggests the existence of a cryptic methane cycle in rich organic matter sediments. Euxinic environments present in the bottom water of the Gotland Deep and Bornholm Basin may also serve as a site of methane production.

In most places of the Baltic Sea, a large fraction of C_{org} is delivered to the sediments, is buried in the methanogenic zone (Chapter 2 & 3). An open question is whether the large amounts of organic sulfur in the Baltic Sea sediments lead to enhanced organic carbon preservation. The speciation of the organic sulfur compounds need to be established. Additionally, roles of S^0 in deep sediments associated with anaerobic oxidation of methane and in reacting with organic matter needs to be examined.

A second major question is whether methanogenesis is as efficient as sulfate reduction for C_{org} mineralization or is C_{org} preferentially preserved in methanic zone. There may be a thermodynamic control on the sulfate reduction, and especially methanogenesis as opposed to the kinetic control represented by the standard model of organic carbon degradation (Knab et al, 2008¹; Bethke et al, 2011²). This is especially likely in the deeper layers where the terminal electron accepting processes operate close to thermodynamic equilibrium. Indeed, the continued presence of sulfate reduction in methanogenic zones and the low rates of methanogenesis that we measured in our study at Himmerfjärden Bay, suggest that methanogenesis is inefficient compared to sulfate reduction. Nevertheless, thick deposits of methanogenic sediment lead to the build-up of methane saturated sediments.

The fluxes of methane into the sediment-water interface, however, do not appear to depend on salinity and overlying water sulfate concentration. However, the sulfate penetration depth could control on the methane fluxes into the sediment-water interface. The high sedimentation rate coupled to the shallow sulfate penetration depth could control the level of anaerobic oxidation of methane via build up a microbial community statement that may be reasonable for methane fluxes into the sediment-water interface, and further into the atmosphere.

¹ Knab, N. J., A. D. Dale, K. Lettmann, H. Fossing and B. B. Jørgensen. 2008. Thermodynamic and kinetic control on anaerobic oxidation of methane in marine sediments. *Geochimica et Cosmochimica Acta* 72: 3746–3757.

² Bethke, C.M., R.A. Sanford, M.F. Kirk, Q. Jin, and T.M. Flynn, 2011. The thermodynamic ladder in geomicrobiology. *American Journal of Science* 311: 183–210.

Deep iron reduction

Interestingly, the presence of ferrous iron in pore water sediments in the Himmerfjärden estuary (Chapter 2) or deep Bornholm Basin (Fossing & Ferdelman, unpublished data) suggests another pathway of iron reduction coupled anaerobic oxidation of methane ($\text{CH}_4 + 8\text{Fe}(\text{OH})_3 + 15\text{H}^+ \rightarrow \text{HCO}_3^- + 8\text{Fe}^{2+} + 2\text{H}_2\text{O}$, Beal et al., 2010³) or organoclastic iron reduction in high organic carbon preservation ($\text{CH}_2\text{O} + 7\text{CO}_2 + 4\text{Fe}(\text{OH})_3 \rightarrow 4\text{Fe}^{2+} + 8\text{HCO}_3^- + 3\text{H}_2\text{O}$), or coupling to cryptic sulfur cycling ($\text{FeS} + 2\text{FeOOH} + 6\text{H}^+ \rightarrow 3\text{Fe}^{2+} + \text{S}^0 + 4\text{H}_2\text{O}$, $2\text{S}^0 + 4\text{H}_2\text{O} \rightarrow \text{H}_2\text{S} + \text{SO}_4^{2-} + 2\text{H}^+$, $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$ etc, Holmkvist et al., 2011⁴) must exist. Testing these hypotheses would provide a clear link between Fe-C-S in brackish environment as the Baltic Sea sediments. Future studies also need to couple rate measurements, molecular incubation and distribution of microorganism to obtain a more complete picture of the anaerobic carbon degradation process including anaerobic oxidation of methane coupled iron reduction.

³ Beal, J. H., H.C. House, and J. V. Orphan. 2009. Manganese–and iron dependent marine methane oxidation. *Science* 325: 184 -187.

⁴ Holmkvist, L., T.G. Ferdelman, and B. B. Jørgensen. 2011. A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochimica et Cosmochimica Acta* 75(12): 3581-3599

Curriculum Vitae

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- 2009 -** PhD candidate at Max Planck Institute for Marine Microbiology – University of Bremen
- 2012** “Biogeochemical controls on carbon and sulfur cycling in Baltic Sea sediments”.
Supervisor: Dr Timothy G. Ferdelman, Dr. Micheal J. Formolo, and Prof. Dr. Bo Barker Jørgensen
- 2006** InWent training, “Sustainable Using coastal and marine environment and resources”, Germany
- 2005** Vietnam National University – Master of Geochemistry
“The biogeochemistry of N, P cycling in water environment of Catba bay (Vietnam)”.
Supervisor: Dr Nguyen Duc Cu
- 2003** Vietnam National Univeristy – Bachelor of Geology
“Geoenvironmental characters of coastal wetland in Tienhai, Thaibinh, Vietnam”
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Expeditions:

<i>R/V M. Merian</i>	Baltic Sea (2010)
<i>R/V Susanne</i>	Aarhus Bay, Denmark (2010)
<i>R/V Limanda</i>	Himmerfjärden estuary, Sweden. (2009)
<i>R/V Aranda</i>	Baltic Sea (2009)
<i>R/V Oceana</i>	Gdansk Basin (Baltic Sea) (2009)

Course and workshops (2009-2012):

- April-June, 2009* Several subjects of Marine Geosciences Master courses: Biogeochemistry, Organic Geochemistry, and Modeling process in Marine Biogeochemistry
- March, 2010* Scientific writing for Natural Scientists (course), MARUM/GLOMAR
- Sep. 2010* Stable isotopes in Stockholm University (course)

Conferences (2009 -2012):

- 2011 Nguyen M Thang**, Volker Brüchert, Gunter Wegener, Michael J Formolo, Livija Ginters, Bo Barker Jørgensen, Timothy G. Ferdelman. 2011. Biogeochemistry of methane and sulfate in Himmerfjärden estuary sediments, Sweden. Poster presentation. European Geoscience Union – General Assembly 2011, Vienna, Austria. 03rd -08th, April, 2011.
- Nguyen M Thang**, Michael J. Formolo, Sabine Flurry, Bo Barker Jørgensen, Timothy G. Ferdelman, 2011. Biogeochemistry of sulfur cycling in Gdansk Bay sediments, Baltic Sea. Poster presentation. European Geoscience Union – General Assembly 2011, Vienna, Austria. 03rd - 08th, April, 2011.
- Laura Lapham, Sabine Flury, Henrik Fossing, Volker Bruchert, Tim Ferdelman, **Thang Manh Nguyen**, Livija Ginter, and Bo Barker Jørgensen. Using stable carbon isotope ratios of CH₄ and CO₂ to follow the production and consumption of CH₄ along the salinity gradient in the Baltic Sea. Poster presentation. Baltic Sea Science Congress. St, Petersburg, Russian. 22nd – 26th, August, 2011.
- Volker Brüchert, Livija Ginters, David Bastviken, **Thang Manh Nguyen**, Timothy G. Ferdelman. Sediment-water and sea-air fluxes of methane in the Himmerfjärden area of the Baltic – an embayment with a pronounced salinity and eutrophication gradient in the central Baltic, Sweden. Baltic Sea Science Congress. St, Petersburg, Russian. 22nd – 26th, August, 2011.
- 2010 Nguyen M Thang**, Timothy G. Ferdelman, Volker Brüchert, Gunter Wegener, Michael J Formolo, Maja Reinholdsson, Bo Barker Jørgensen. 2010. Biogeochemistry of methane and its potential oxidants in Himmerfjärden estuary sediments, Sweden. Poster presentation. annual BONUS conference. 19th – 21st, January; Vilnius, Lithuania.
- Volker Brüchert, **Thang M Nguyen**, André Deutschmann, Michael Böttcher, Tim Ferdelman, 2010. Bacterial sulfate reduction and methanogenesis in brackish, oligotrophic northern Baltic Sea sediments. Oral presentation. EGU meeting. 2nd – 7th May, 2100, Vienna, Austria.
- Gregor Rehder, Henrik Fossing, Laura Lapham, Rudi Ender, Volkhard Spiess, Volker Brüchert, **Thang M Nguyen**, Wanda Gülzow, Jens Schneider von Deimling, Dan Conley, Bo Barker Jørgensen. 2010. Methane fluxes and their controlling process in the Baltic Sea. Poster Presentation. American Geophysical Union – Fall Meeting, San Fransisco, California, USA. 13rd -17th, December, 2010.
- 2008 Nguyen Manh Thang**, Tran Dinh Lan, Nguyen Duc Cu, 2008. Impacts of Haiphong port activities to some

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Publications:

2012 Nguyen Manh Thang, Volker Brüchert, Gunter Wegener, Michael J Formolo, Livija Ginters, Bo Barker Jørgensen, Timothy G. Ferdelman. 2012. The Impact of Sediment and Carbon Fluxes on the Biogeochemistry of Methane and Sulfur in Littoral Baltic Sea Sediments (Himmerfjärden, Sweden). *Estuaries and Coasts*. (in press)

Gunter Wegener, Marlene Bausch, Thomas Holler, **Nguyen Manh Thang**, Xavier Prieto, Mollar Matthias, Y. Kellermann, Kai-Uwe Hinrichs, Antje Boetius. 2012. Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and ¹³C-bicarbonate. *Environmental Microbiology* 14(6), 1517-1527.

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2010 Publications in Vietnamese related to coastal research not listed.

Manuscripts in preparation:

Nguyen Manh Thang, Micheal J. Fomolo, Laura Lapham, Bo Barker Jørgensen, Henrik Fossing, Sabine Flurry, Timothy G. Ferdelman. The role of sulfate exposure time on carbon preservation and sulfur burial in Baltic Sea sediments.

Nguyen Manh Thang, Michael Fomolo, Sabine Flurry, Benjamin Brunner, Bo Barker Jørgensen, Timothy G. Ferdelman. The impact of reactive iron on early sulfurization of organic matter and oxidative sulfur cycling in sulfidic sediments from the Gdansk Basin (Baltic Sea).

Sabine Flury, **Nguyen Manh Thang**, Micheal J. Fomolo, Timothy G. Ferdelman, Andy Dale, Laura Lapham. CH₄ cycling in Gdansk Bay (Baltic Sea).

Laura Lapham, Sabine Flury, Henrik Fossing, Bo Barker Jørgensen, Timothy G. Ferdelman, **Nguyen Manh Thang**, Volker Brüchert, Michael Schlüter, Torben Gentz. Controls on surface methane fluxes in Baltic Sea sediments

Volker Brüchert, **Nguyen Manh Thang**, Laura Lapham, Henrik Fossing, Michael Böttcher, Timothy G. Ferdelman. Bacterial sulfate reduction and methanogenesis in brackish, oligotrophic northern Baltic Sea sediments.

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