Mineralization pathways and thermophilic sulfate reduction in Arctic sediments, Svalbard

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Summary

This study extends the small database about degradation of organic material in arctic open shelf sediments. It emphasizes the importance of microbial Mn(IV) and Fe(III) reduction for carbon cycling in sediments of the Barents Sea. Furthermore, it increases our, to date limited, knowledge and the understanding of the regulation of anaerobic carbon mineralization in these permanently cold sediments.

5 stations were investigated in the northern Barents Sea between 75° and 81° north with different water depths (208-503 m). The sites were characterized by low bottom water temperatures (0.2-3.3°C) and were, except for the most southern station, still covered with ice or had been covered with ice during the last year. The water columns of the stations were dominated by different water regimes of Atlantic and/or Arctic Water. Total oxygen uptake rates and sulfate reduction rates (SRR) ranged between 1.5-3.7 mmol m^{-2} d^{-1} and <0.1-0.22 mmol m^{-2} d^{-1} for O_2 and for SRR, respectively. Total oxygen uptake rates were similar or lower compared to previous investigations of the northern Barents Sea, whereas SRR present new data.

Anaerobic carbon oxidation and mineralization rates of the different electron accepting pathways (SSR, Mn(IV)- and Fe(III)reduction) were studied in sediment bag incubations to quantify the dominating respiratory pathways. These were then compared to pore water and solid phase constituents in the sediment in order to reveal the depth distribution of the different mineralization processes. Lower oxygen consumption rates, areal SRR as well as lower rates of anaerobic carbon mineralization were found in open shelf sediments compared to fjord sediments from the west coast of Svalbard. This is probably caused by limited carbon supply to sediments due to lower primary production. The extended presence of ice cover in the northern Barents Sea has a reducing effect on primary production since ice reduces the availability of light in the water column. Arctic fjords on the west coast of Svalbard are not so deep and higher deposition rates of organic material weare found. The differences in ice conditions between the Barents Sea and fjords on the west coast of Svalbard depend on the different water masses, which influence each region. The West Spitsbergen Current that streams along the west coast transports relatively warm Atlantic Water, thereby keeping the fjords often ice-free during the whole year. The Barents Sea, however, is

influenced by two different water masses. From the South relatively warm Atlantic Water enters the Barents Sea with the West Spitsbergen Current, whereas from the North cold Arctic Water flows in from the Polar Ocean and the Kara Sea. Thus, great parts of the northern Barents Sea are ice-covered every winter and the benthic community seems to be mainly limited by carbon availability in this area.

On the northern stations high concentrations of particulate Fe(III) (≥108 µmol cm^{-3}) and particulate Mn (≥60 µmol cm^{-3}) were measured. On the southern stations concentrations of particulate Fe(III) were lower but still relatively high (84 and 37 µmol cm^{-3}), whereas concentrations of Mn(IV) were significantly lower (≤30 and ≤2 µmol cm^{-3}).

As a result, Fe and Mn reduction played an important role in anaerobic carbon oxidation of all 5 stations and were even the dominantanaerobic processes in 3 of the 5 stations (≥87-98%). SRR were extremely low or below the detection limit in these three sediments, whereas the other two sediments exhibited the typical depth distribution of the different electron accepting processes with sulfate reduction as the dominating pathway.

In one of the two sediments, the southermost of the 3 northern stations, the following sequence of microbial respiratory pathways was found: Mn(IV)reduction from 0-3 cm, followed by concurrent Fe(III)- and sulfate reduction from 3-5 cm, and sulfate reduction being the only important process from 5-10 cm. In the other sediment with a typical zonation, which was the most southern station studied, Mn-, Fe(III)- and sulfate reduction occurred simultaneously in the top 4 cm whereas below sulfate reduction was the sole electron accepting process responsible for carbon oxidation. On this last station gross-SRR were probably underestimated due to reoxidation processes of the radioactive tracer. Due to the low SRR tracer incubation periods had to be increased. Long time incubations, however, bear the problem that reoxiation of the tracer cannot be excluded. As a consequence, the contribution of sulfate reduction to anaerobic carbon mineralization can only be an estimate (≥80%) and present a minimum value.

As a conclusion, the relative contribution of microbial Mn(IV)- and Fe(III)reduction to anaerobic carbon mineralization were regulated by high concentrations of particulate Mn(IV) and Fe(III), low overall carbon mineralization rates, bioturbation and the low organic carbon content due to the extended presence of ice cover in the northern Barents Sea.

In an additional set of experiments the temperature regulation of sulfate reduction was investigated in sediments of an Arctic fjord on the west coast of Svalbard. The psychrotolerant community showed highest sulfate reduction rates at 21°C and a second optimum was found at 54°C. Pasteurization of the sediment did not affect thermophilic sulfate reduction whereas sulfate reduction below 35°C ceased. We concluded that the sulfate reducing bacteria (SRB) responsible were most likely spore-formers. At in situ temperatures they were probably present as metabolically inactive and protected spores but germinated at the incubation temperature of 50°C. The instantaneous increase in the concentrations of volatile fatty acids after sediment heating to 50°C indicated that thermophilic fermentative bacteria, most likely spore formers, were also present. We have not presented unambiguous proof that they are spores. However, to date no bacterium is known that is active at 50°C, survives pasteurisation and grows or survives at 2°C.

The delay of thermophilic sulfate reduction of 16-24 h after the sediment was heated to 50°C may represent the induction time needed for the SRB to germinate and become metabolically active. Fermentation started without delay, which indicates that the fermentative bacteria were faster to respond to temperature changes. It seems unlikely that these thermophilic bacteria grow in the cold sediments in which they are found. However, the source of the spores in Smeerenburgfjord remains unknown.

Zusammenfassung

In der vorliegende Studie wurde die Bedeutung der mikrobiellen Mangan (Mn(IV)) und Eisenreduktion (Fe(III)) für den Abbau von organischem Material in arktischen Schelfsedimenten der Barents See untersucht. Sie erweitert unser bisher noch eingeschränktes Wissen und Verständnis über die Regulation der mikrobiellen Kohlenstoffmineralisierung in diesen kalten Sedimenten.

5 Stationen mit unterschiedliche Wassertiefen (208-503 m) wurden in der nördlichen Barents See untersucht. Die Stationen waren durch niedrige Bodenwasser-temperaturen (0.2-3.3°C) charakterisiert und sie waren, mit Ausnahme der südlichsten Station, entweder noch von einer Eisschicht bedeckt oder während des vergangenen Winters eisbedeckt gewesen. Die Wassersäulen der Stationen wurden von kaltem Arktischen Wasser oder von wärmeren Atlantischem Wasser beeinflußt. Sauerstoffzehrungsraten und Sulfatreduktionraten (SRR) betrugen 1.5-3.7 mmol m^{-2} d^{-1} für O_2 und <0.1-0.22 mmol m^{-2} d^{-1} für SRR. Sauerstoffzehrungsraten waren ähnlich oder niedriger als Raten, die in früheren Studien gemessen wurden. SRR hingegen sind in dieser Region bisher noch nicht gemessen worden und daher neu.

Die anaerobe Kohlenstoffmineralisierung und Raten der unterschiedlichen anaeroben mikrobiellen Abbauprozesse (SSR, Mn (IV) und Fe(III)reduktion) wurden in Sedimenttüteninkubationen untersucht, um die dominanten Stoffwechselwege zu quantifizieren und wurden mit der Zusammensetzung des Porenwassers und der Festphase verglichen, um eine Tiefenverteilung der verschiedenen Prozesse bestimmen zu können Sowohl Sauerstoffzehrungsraten, flächenintegrierte SRR als auch Raten der anaeroben Kohlenstoffmineralisierung waren niedriger in den Schelfsedimenten der nördlichen Barents See als in arktischen Fjordsedimenten der Westküste Svalbards. Dies ist wahrscheinlich darauf zurückzuführen, dass der Eintrag von organischem Kohlenstoff ins Sediment der Barents See geringer ist, was auch in den niedrigeren Gehalten an organischem Kohlenstoff in den Sedimenten zu sehen war. Die Primärproduktion durch Phytplankton, die das organische Material bildet welches dem Benthos zur Verfügung steht, wird in arktischen Breiten durch saisonale Eisbedeckung stark beeinflußt und limitiert. Arktische Fjorde sind nicht so tief und werden oft das ganze Jahr durch den Westspitzbergenstroms, der relative warmes Atlantisches

Wasser an der Westküste Svalbards entlang transportiert, eisfrei gehalten. Hingegen wird die Barents See von zwei, sich sehr unterscheidenden, Wassermassen beeinflußt. Von Süden bringen die Ausläufer des Westspitzbergenstroms warmes, nährstoffreiches Atlantisches Wasser in die Barents See, während von Norden kaltes Arktisches Wasser vom Nordpolarmeer und der Kara See eindringt. Große Teile der Barents See sind daher im Winter regelmäßig von einer Eisschicht bedeckt. Daher scheint es, daß die mikrobiellen Gemeinschaften des Benthos in der nördlichen Barents See durch geringere Verfügbarkeit an organischem Kohlenstoff limitiert sind.

An den nördlichen Stationen der nördlichen Barents See wurden hohe Konzentrationen an partikulärem Fe(III) (≥108 μmol cm^{-3}) und partikulärem Mn(IV) (≥60 μmol cm^{-3}) gemessen. Auf den südlichen Stationen hingegen waren die Fe-konzentrationen geringer, aber auch noch relativ hoch (84 und 37 μmol cm^{-3}), während die Mn-konzentration wesentlich niedriger waren (≤30 und ≤2 μmol cm^{-3})

Die Mn und Fe-reduktion spielte an allen 5 Stationen eine wichtige Rolle und war in 3 von 5 Sedimenten der vorherrschende Abbauweg der anaeroben Kohlenstoff-mineralisierung (≥87-98%). SRR waren extrem niedrig oder sogar unter der Detektionsgrenze in diesen 3 Sedimenten, während auf den anderen 2 Stationen die typische Tiefenverteilung der unterschiedlichen elektronenakzeptierenden Abbauwege gefunden wurden und Sulfatreduktion dominierte.

An der einen dieser zwei Stationen, welches die südlichste Station der 3 nördlich gelegenen Stationen war, dominierte Mn(IV)reduktion von 0-3 cm, gefolgt von gleichzeitiger Fe(III)- und Sulfatreduktion von 3-5 cm und von 5-10 cm fand ausschließlich Sulfatreduktion statt. An der anderen Station, die südlichste die überhaupt untersucht wurde, fanden in den obersten 4 cm des Sediments Mn(IV)-, Fe(III)- und Sulfatreduktion gleichzeitig statt, während darunter nur noch Sulfatreduktion ablief. Auf der letztgenannten Station wurden die Netto-Sulfatreduktion höchstwahrscheinlich durch gleichzeitig ablaufende Reoxidations-prozesse unterschätzt. Wegen der geringen SRR mußte die Tracerinkubation verlängert werden, so daß eine Regenierung des Tracer während der Inkubation durch Reoxidation nicht ausgeschlossen werden kann. Daher kann der Anteil der SRR an der Gesamtmineralisierung von organischem Kohlenstoff nur als Näherungswert angegeben (≥80%) werden und bezeichnet den Mindestanteil.

Insgesamt werden die relative Bedeutung der mikrobiellen Mangan und Eisenreduktion durch relativ hohe Konzentrationen an partikulärem Mangan und Eisen, durch niedrige Gesamtumsatzraten, durch Bioturbation und durch den geringen organischen Gehalt in den beschriebenen Sedimenten reguliert.

In weiteren Untersuchungen wurde die Temperaturregulation der Sulfatreduktion in Sedimenten eines arktischen Fjordes auf Svalbard untersucht. Die psychrotolerante Bakteriengemeinschaft zeigte die höchsten Sulfatreduktionsraten bei 21°C und ein zweites Optimum bei 54°C. Pasteurisierung des Sediments beeinflußte diese thermophile Sulfatreduktion nicht, tötete jedoch die psychrotolerante Bakteriengemeinschaft ab. Dies führte zu der Annahme, daß die beteiligten thermophilen Bakterien wahrscheinlich Sporenbildner sind. Bei den in situ Temperaturen von 2.3°C liegen diese Bakterien wahrscheinlich als Sporen im Sediment vor und keimen erst bei Temperaturen von 50°C aus. Die Konzentration kurzkettiger Fettsäuren, Ausscheidungsprodukte des fermentativen Stoffwechsels, stieg nach Erhitzen des Sedimentes auf 50°C stark an, was darauf hindeutet, daß ebenfalls thermophile fermentative Bakterien im Sediment vorhanden sind. Mit großer Wahrscheinlichkeit liegen auch diese als Sporen in den kalten Sedimenten vor. Leider konnte bisher kein letzter, sicherer Beweis für die Existenz von Sporen in den kalten arktischen Sedimenten geliefert werden. Andererseits ist bisher noch kein Bakterium bekannt, welches bei 50°C kontinuierlich aktiv ist, Pasteurisierung überlebt und gleichzeitig bei 2°C wachsen und leben kann.

Die thermophile Sulfatreduktion startete erst 16-24 h nachdem das Sediment auf 50°C erhitzt worden war, was die These von sporenbildenden Bakterien unterstützt, welche erst auskeimen müßen, um aktiv zu werden. Die Fermentation hingegen begann sofort nach Erhitzen, was darauf hinweist, daß die fermentativen Bakterien schneller auf einen Temperaturwechsel reagieren. Es ist unwahrscheinlich, daß diese thermophilen Bakterien in den permanent kalten Sedimenten der Arktis wachsen, in denen sie gefunden wurden. Allerdings ist ihr Ursprungsort bisher noch unbekannt.

1. Introduction

1.1. Organic carbon mineralization in Arctic marine sediments

The Arctic Ocean covers the area around the North Pole. It differs from all other oceans by the combination of sea ice, large volumes of runoff from land, and its enormous continental shelves (Chen et al., 2003). It is characterized by large annual changes in light intensity, with >120 days of midnight sun and >107 days of polar night (Hisdal, 1998), sea ice formation and ice melting. Ice cover comprises a permanent pack ice in the oceans interior with the marginal seas exhibiting a seasonal ice cover from approximately October to June (Jakobsson et al., 2004). All these factors influence the formation of organic material in the water column of the Arctic Ocean, the ultimate source of the organic carbon in arctic marine sediments.

1.1.1. Production of organic material in the water column

One of the major driving forces influencing the composition and activity of benthic assemblages is the carbon flux from the photic zone to the seafloor. The organic matter produced in the Arctic Ocean is primarily derived by phytoplankton in the water column and microalgae associated with ice (Falk-Petersen et al., 2000). The productivity of plankton depends on the partial or complete retreat of ice from the marginal seas allowing light to become available for photosynthesis. When the ice melts in spring or summer a stratified water column with a nutrient rich euphotic zone develops, due to the input of low-salinity meltwater and suppressed wind mixing (Wassmann and Slagstad, 1993). This supports the development of intensive phytoplankton blooms that follow the receding ice edge and hence, greatest primary production is found along the marginal ice zone (Sakshaug and Slagstad, 1991). Estimates of annual primary production in the high Arctic range from 25 (White Sea) to 300 Tg C yr^{-1} (Chukchi Sea) (Grebmeier and McRoy, 1989). Only a fraction of about ~2% of this primary production reaches the seafloor at water depth below 1000 m (Schlüter et al., 2001).

1.1.2. Degradation of organic material by benthic microorganisms

The organic material that is deposited on sediments consists of macromolecular compounds such as structural carbohydrates, proteins, nucleic acids and lipid complexes. Bacteria are not

able to take up molecules with a molecular size of ≥600 Dalton through their cell wall (Weiss et al., 1991) therefore polymeric substances have to be degraded first.

This depolymerization is mediated by exoenzymes that are either released by specific bacteria into the environment or stay attached to their cell wall. The resulting monomeric compounds are rapidly assimilated and metabolized. The complete oxidation of organic material by benthic microorganisms is mediated through a consecutive sequence of respiratory processes with different inorganic electron acceptors (O_2, NO_3^-, Mn(IV), Fe(III) and SO_4^{2-}) (Froelich et al., 1979). The general depth sequence of these oxidants corresponds to a gradual decrease in their redox potential. As a result and also because the availability of most electron acceptors is limited, a vertical zonation of the pathways is observed in sediments (Jørgensen, 2000).

The oxic zone in shelf sediments is usually only mm-to-cm thick, whereas in slope or deep-sea sediments it can expand to several cm or dm (Jørgensen, 2000). Much of the organic mineralization thus takes place within the anoxic sediment. Furthermore, oxygen consumption is not only coupled to organic matter mineralization but is also used for the reoxidation of reduced compounds, like Fe(II), Mn(II) and H_2S that are produced in deeper layers and transported upwards by diffusion, advection, or bioturbation (Canfield, 1993).

The process that yields nearly as much energy as aerobic respiration is denitrification, followed by Mn(IV)- and Fe(III) reduction. Bacteria that respire with one of these electron acceptors can utilize a wide range of organic substances and often respire them completely to CO_2. Sulfate reducers, on the contrary, have a much narrower substrate spectrum and depend on fermentation products like volatile fatty acids (e.g. lactate, formate, acetate, butyrate, propionate) and H_2 (Sørensen et al., 1981) but can also use a few sugars, phenyl-substituted acids and some other aromatic compounds (Widdel and Bak, 1992). Methanogenic bacteria, mainly archaea, are also limited in their substrate spectrum and primarily use H_2, CO_2, acetate and C1 compounds like methanols and methylamines ((Capone and Kiene, 1988), (Ferry, 1999)).

The quantitative importance of the various carbon mineralization pathways in sediments has been studied intensely. In shelf sediments aerobic respiration and sulfate reduction are generally the most important metabolic pathways (Jørgensen, 1982). Nitrate reduction is considered to be of minor importance, contributing ≤6% to total carbon oxidation ((Canfield et al., 1993a), (Kostka et al., 1999), (Glud et al., 2000)). Microbial Mn reduction dominates carbon oxidation over Fe reduction and sulfate reduction in marine sediments that are rich in Mn oxides, such as the Panama basin and deep parts of Skagerrak ((Aller, 1990),

(Canfield et al., 1993b)). However, these Mn-rich sites are unusual and in most marine sediments Mn reduction plays an insignificant role in carbon oxidation ((Thamdrup and Canfield, 1996), (Kostka et al., 1999)). The relative contribution of Fe reduction is highly variable ranging from below detection to 75% with an average of 17% for a wide range of continental sediments ((Thamdrup, 2000), (Jensen et al., 2003)).

1.1.3. Regulation of carbon oxidation in Arctic sediments

It is well known that chemical processes and bacterial metabolism generally slow down with decreasing temperature. The Arctic Ocean as well as its counterpart in Antarctica is characterized by permanently low temperatures. However, biological processes do not appear to be slower or less efficient in polar regions compared to temperate environments. Overall carbon mineralization rates from Arctic fjord sediments were well within the range of rates reported for temperate shelf sediments ((Archer and Devol, 1992), (Kostka et al., 1999)). Comparison of potential nitrification rates between sediments from Svalbard, an archipelago in the Arctic Ocean, and temperate sediments showed that the catalytic efficiency of the nitrifying communities were comparable despite a 20°C difference in temperature ((Henriksen et al., 1981), (Thamdrup and Fleischer, 1998)). Furthermore, sulfate reduction rates (Sagemann et al., 1998) and potential hydrolysis rates for polysaccharides (Arnosti et al., 1998) that were measured in Arctic fjord sediments were also of magnitudes comparable with rates in sediments of more moderate climate ((Moeslund et al., 1994), (Arnosti, 1995)). Benthic respiration rates in Antarctic coastal sediments showed an immediate stimulation by increased settling of fresh organic material due to sedimentation of ice algae biomass onto the bottom (Nedwell et al., 1993). The same has been reported for sediments from a Greenland fjord (Rysgaard et al., 1998). After breaking of the sea ice in July bacterial activity - measured as sediment O_2 uptake, fluxes of dissolved organic carbon, as well as denitrification and sulfate reduction - increased following the input of fresh organic material. Thus, it appears that benthic microbial communities are not limited by low temperatures but rather by the availability of organic carbon.

1.2. Arctic fjords and open shelf sediments around Svalbard

The Arctic Ocean confines a deep central basin surrounded by the continental shelves of the Barents, Kara, Laptev, East Siberian, Chukchi, Beaufort, and Lincoln Seas (Weber, 1989) and

by the low continental shelves of Russia, Alaska, Canada and Greenland. The only break in this ring of continental crust is the Fram Strait between northeast Greenland and northwest Svalbard.

Svalbard is an archipelago in the Arctic Ocean with the main islands Spitsbergen, Nordaustlandet, Edgeøya and Barentsøya and is approximately 1000 km away from the norwegian mainland. The islands are situated in a transition zone between different water masses of Arctic and Atlantic origin (Gerdes and Schauer, 1997). The south and west coast of Svalbard is influenced by the "West Spitsbergen Current", a branch of the "North Atlantic Current" that transports warm, nutrient rich Atlantic Water and provides for a relatively mild climate, which keeps the fjords ice-free for most of the year (Andruleit et al., 1996).

The area north and east of Svalbard is part of the Barents Sea, a shelf sea with an average depth of 230 m and an area of 1.4 million km^2. The two main water masses entering the Barents Sea are cold Arctic Water from northeast and warmer Atlantic Water from southwest (Sakshaug, 1997). After crossing the Polar front, which separates Arctic from Atlantic Water, the Atlantic Water subducts beneath the lighter and colder Arctic Water (Loeng, 1991). The Arctic Water is periodically ice-covered and the maximum extension of ice is close to the Polar front in the western and central part of the Barents Sea, while ice cover in the eastern part is more extensive (Loeng, 1991).

Light intensities largely determine the level of primary production due to the limiting effect of an extended presence of ice cover. Even though fjords on the west coast of Svalbard often remain ice-free during winter primary production was comparable with production levels for the seasonally ice-covered Barents Sea (Sakshaug et al., 1994), (Hegseth, 1998). Estimates of the annual primary production in fjords of Svalbard and in the Barents Sea range from 25 to 150 g C m^{-2} yr^{-1} ((Eilertsen et al., 1989), (Hop et al., 2002)).

1.3. Extremophiles - Bacterial activity at high temperatures

Living organisms are ubiquitous and observed in almost every ecological niche on earth. It is now well recognized that may parts of the world would be considered by many to be extreme such as geothermal environments, polar regions, acidic and alkaline springs and the cold pressurized depths of the oceans. However, they are colonized by microorganisms, which are often specifically adapted to these exceptional environments and that are called "Extremophiles" (MacElroy, 1974). Extremophiles are organisms with optimal growth

conditions found outside the range of "normal" environments, with "normal" being a temperature between 4°C and 40°C, pH between 5 and 8.5, and with a salinity between that of freshwater and that of seawater (Kristjánnsson and Hreggvidsson, 1995). Apart from temperature, pH and salinity extreme conditions include physical (pressure, radiation) or geochemical (desiccation, oxygen species and redox potentials) parameters and microorganisms have adapted over evolutionary time to overcome the principal stresses encountered in their preferred habitat. In the following some examples of extreme lifestyles and corresponding bacteria will be given.

Pressure: A strain DB21MT-2, highly similar to *Shewanella benthica* and close relatives was isolated from the world's deepest sediment the Mariana Trench, Challenger Deep, at a depth of 10,898 m (Kato et al., 1998). The optimal pressure conditions for growth were at 80 MPa and no growth was detected at pressures of less than 50 MPa. A major effect of increased pressure lies on lipid membranes, which pack tighter resulting in decreased membrane fluidity (Pledger et al., 1994). To regain the fluidity needed for biological activity bacteria increase the proportion of unsaturated fatty acids in their membranes (Bartlett and Bidle, 1999), as it was also reported for DB21MT-2.

pH: *Leptospirillum ferrooxidans* is another example of extreme adaptation. It thrives at pH 1.5 and is thereby one of the most acidophilic organisms known to date (Maeda et al., 1999). For organisms to live at extreme pH values it is crucial to maintain their cytoplasm at neutral pH, thus avoiding the need for evolution of altered internal physiology (Matin, 1990). Mechanisms of acidophiles to deal with high external H^+ concentrations are high internal buffer capacity, overexpression of H^+ export enzymes and unique transporters (Pick, 1999). *Bacillus alcalophilus* thrives in the other end of the pH scale. It can keep its internal pH around 9.2 even when the external pH is as high as pH 11 (Guffanti et al., 1979). Mechanisms of alkaliphiles to overcome low pH conditions include negatively charged cell-wall polymers among others (Krulwich et al., 1998).

Salinity: *Ectothiorhodospira* is known as the so far most extreme halophilic organisms (Inhoff and Trüper, 1977). Optimal salt concentrations for growth were 14-27 M with growth observed in the range of 10-37 M. Many microorganisms respond to increases in osmolarity by accumulating osmotica in their cytosol, which protects them from cytoplasmic dehydration and desiccation (Yancey et al., 1982).

Temperature: It is one of the most important environmental factors influencing bacterial activity. As the temperature rises, rates of chemical and enzymatic reactions in the cell increase. However, this works only up to certain temperatures above which proteins, nucleic acids, and other cellular compounds become irreversibly damaged. On the other hand, it is well known that bacterial metabolism is slowed down by low temperatures. Yet, metabolic rates do not appear to be less efficient in polar regions compared to temperate environments. In each temperature range, from the freezing point (Bakermanns et al., 2003) to an upper limit of 113°C (Blöchl et al., 1997) there appear to be prokaryotes that are well adapted and even thrive at that temperature.

Besides isolation of microorganisms that thrive at extremely high or low temperatures bacteria have quite frequently been isolated from environments that do not support their growth and that are not even within their temperature range for metabolic activity. (Barnes et al., 1998) isolated mesophilic spore-forming sulfate reducing bacteria (SRB) that showed highest sulfide production at 34°C from permanently cold deep-sea sediments (in-situ temperature 3°C). Another mesophilic SRB strain was isolated from permafrost soil (in-situ temperature -5°C) that showed highest biomass accumulation and highest sulfide production at 28°C (Vainshtein et al., 1995). Since there were no indications for growth or metabolic activity of these strains under in-situ conditions the authors concluded that these bacteria were most likely present as spores in the sediment. 16S rDNA analysis and comparison of morphological, physiological and biochemical properties indicated that these strains belonged to the genus *Desulfotomaculum*.

The genus *Desulfotomaculum* includes meso- and thermophilic species, which have mainly been isolated from thermal sites ((Daumas et al., 1988), (Min and Zinder, 1990), (Karnauchow et al., 1992), (Love et al., 1993)). The characteristic feature of *Desulfotomaculum* species is the formation of heat-resistant endospores, which distinguish them from all other SRB.

However, not only mesophilic members of the genus *Desulfotomaculum* have been found in cold sediments. Recently a thermophilic *Desulfotomaculum* strain was isolated from sediments (0-15°C annual temperature range) in temperate environments (Isaksen et al., 1994). The strain showed optimal growth at 63°C and was designated *Desulfotomaculum kuznetsovii*. The special feature of these bacteria is that they occur as spores in sediments, which are unlikely the place where they have grown originally. The run-off water from a

waste water treatment plant was considered as a possible source for *Desulfotomaculum kuznetsovii*. Other possible sources for thermophilic spore-forming bacteria are warm environments such as deep sub-seafloor sediments or hydrothermal systems of mid-oceanic spreading ridges, from where these spores could have been transported. Such spores might then occur almost worldwide, which leads to the third manuscript (Chapter 2.3) of this study that deals with the presence of thermophilic sulfate reducing bacteria in Arctic marine sediment.

1.4. Aim of the present study

The quantitative importance of the various carbon mineralization pathways in Arctic sediment has been studied intensely ((Glud et al., 1998), (Rysgaard et al., 1998), (Kostka et al., 1999), (Glud et al., 2000)). However, most studies have focused on fjord sediments and much less data is available from open shelf sediments like the Barents Sea. This study is part of the international CABANERA project (Carbon flux and ecosystem feed back in the northern Barents Sea in an era of climate change). Within the project a multi-disciplinary international team of scientists is involved to address two major questions:

A. How will the distinct changes in extension and duration of ice cover affect the dissolution and biological C pump on the Nordic shelves fringing the Polar Ocean?
B. What consequence has warming on the atmospheric-ocean CO_2 exchange, C sequestration, food-web responses, food availability of pelagic fish and the pelagic-benthic coupling?

Our task within the project and the aims of the present study were
1. to determine carbon turnover rates in sediments of the northern Barents Sea
2. To identify the most important electron accepting pathways.
3. To investigate the response of the benthic community to food supply and the efficiency of benthic mineralization in seasonally ice-covered regions.

Beside the quantification of carbon oxidation pathways by psychrotolerant bacteria the temperature regulation of sulfate reduction in an Arctic fjord sediment should be determined from 0-80°C. During fieldwork on Svalbard in 1999 and 2000, Bo B. Jørgensen (unpublished data) studied the temperature regulation of sulfate reduction from 0-40°C. He noted that

sulfate reduction had an optimum at 20°C and no activity at 30-35°C. Surprisingly, however, sulfate reduction increased again above 40°C indicating that another community of sulfate reducing bacteria might exist next to the psychrotolerant community.

The question addressed in the present study were

1. Can thermophilic bacterial activity be found in high Arctic sediments?
2. If thermophilic activity can be detected, what will the optimum temperature for growth and metabolic activity be?
3. Does this high Arctic sediment carry only spores of sulfate reducing bacteria or, which appears more reasonable, does a whole diverse community of substrate producing and substrate consuming organisms exist? This could include fermentative and hydrolytic bacteria that might also survive in some kind of resistant form, like spores.

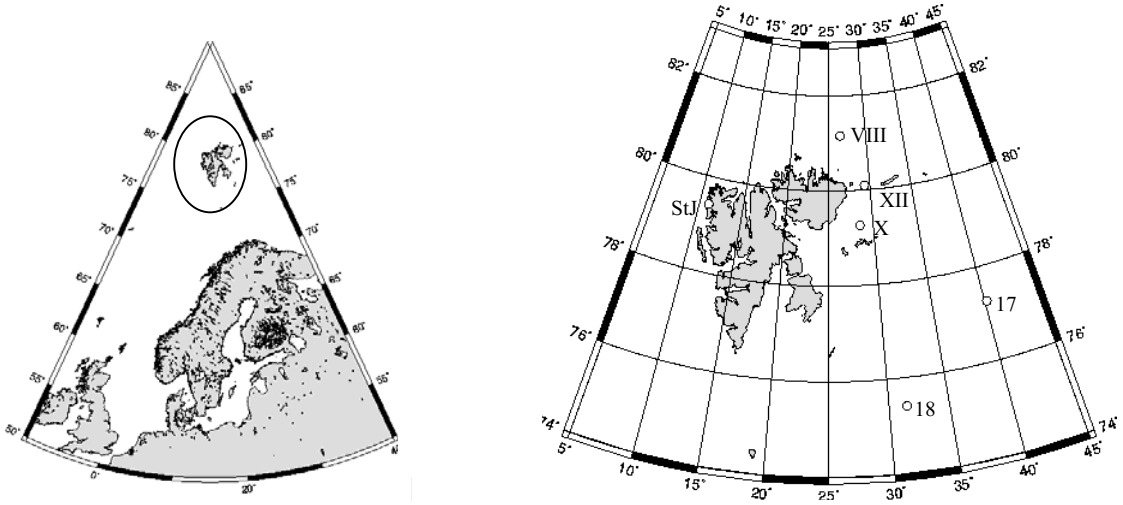

Fig. 1 Map of Scandinavia (left) with Svalbard encircled and sampling sites of Svalbard (right)

1.5. Area of investigation

Sediment was sampled on 5 different stations in the northern Barents Sea to determine rates and pathways of benthic carbon mineralization. The stations were characterized by different water regimes (Arctic or Atlantic Water dominated) and different ice conditions (ice cover ranged from 0-70%). In 2004 the stations VIII, X, and XII were sampled (Chapter 2.1), while in 2005 the stations XVII and XVIII were sampled (Chapter 2.2).

Sediment for the investigation of thermophilic bacterial activity (Chapter 2.3) was sampled in Smeerenburgfjord (Station J) (Fig.1) from several HAPS cores during cruises with MS "FARM" in 2003 and 2005. The bottom water temperature at Station J was +2.3°C and the organic carbon content (C_{org}) was 1.6 (% dry weight).

1.6. References

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2. Manuscripts

Overview of manuscripts

2.1 Carbon mineralization in Arctic sediments northeast of Svalbard: Mn(IV) and Fe(III) reduction as principal anaerobic respiratory pathways

Verona Vandieken, Maren Nickel &Bo Barker Jørgensen

2.2 Ice cover and organic carbon deposition as regulating factors for microbial Mn(IV) and Fe(III) reduction in the northern Barents Sea

Maren Nickel, Verona Vandieken, Volker Brüchert & Bo Barker Jørgensen

2.3 Thermophilic bacterial activity in an Arctic sediment, Svalbard

Maren Nickel, Volker Brüchert & Bo Barker Jørgensen

Carbon mineralization in Arctic sediments northeast of Svalbard: Mn(IV) and Fe(III) reduction as principal anaerobic respiratory pathways

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KEY WORDS: Microbial Mn(IV), Fe(III) reduction, Carbon mineralization, Arctic sediments, Barents Sea, Sediment incubations

ABSTRACT

Carbon oxidation rates and pathways were determined in 3 sediments at 79° to 81° N in the Barents Sea, where the ice cover restricts primary production to a few months of the year. Oxygen uptake (1.5 to 3.5 mmol m^{-2} d^{-1}) and sulfate reduction (<0.1 to 0.22 mmol m^{-2} d^{-1} over 0 to 10 cm depth) rates were measured by whole core incubation. Pathways of anaerobic carbon oxidation were determined by combining results of anoxic sediment bag incubations with pore water and solid phase analyses of the sediments. In accordance with the high contents of solid Mn (≥60 µmol cm^{-3}) and Fe(III) (≥108 µmol cm^{-3}), dissimilatory Mn(IV) and Fe(III) reduction contributed between 69 and ≥90% to anaerobic carbon mineralization in the upper 10 cm of the sediments. At 2 of the 3 stations, sulfate reduction rates were below our detection limit of 1 nmol cm^{-3} d^{-1}. Solid Mn and Fe(III) were abundant from the surface to 10 cm sediment depth and were apparently the only important anaerobic electron acceptors. At the third station, vertical zonation of anaerobic mineralization was observed, with Mn(IV) reduction at 0 to 3 cm followed by concurrent Fe(III) and sulfate reduction at 3 to 5 cm and sulfate reduction at 5 to 10 cm. Rates of microbial carbon oxidation were low compared to those in fjords of the west and south coast of Svalbard. This is in accordance with the limited organic carbon supply by primary and secondary productivity caused by long periods of ice coverage.

INTRODUCTION

Benthic microbial communities in sediments around Svalbard in the Arctic Ocean experience permanently low temperature, whereas the flux of organic matter to the sea floor varies seasonally. Phytoplankton productivity depends on the short ice-free periods in the summer when light is available for photosynthesis ((Hebbeln and Wefer, 1991), (Wassmann and Slagstad, 1993)). The west and south coast of Svalbard are influenced by the relatively warm Atlantic water of the West Spitsbergen Current that flows northwards and keeps the coast ice-free most of the year. The north and east coast, where this study was done, are characterized

by cold polar water from the Arctic Ocean, resulting in a later seasonal thawing of the sea ice. Correspondingly, primary production is significantly lower in areas influenced by polar waters and long periods of ice coverage than in areas influenced by Atlantic water ((Wassmann and Slagstad, 1993), (Sakshaug, 1997)). The differences in primary production are reflected in higher organic carbon contents and oxygen uptake rates of sediments southwest of Svalbard compared to sediments off the northeast coast (Hulth, Hall et al., 1996).

As the fjord sediments along the south and west coast of Svalbard are characterized by relatively high organic carbon deposition (Eilertsen, Taasen et al., 1989) mineralization rates are as high as in comparable temperate environments ((Arnosti, Jørgensen et al., 1998), (Arnosti, Finke et al., 2005), (Sagemann, Jørgensen et al., 1998), (Thamdrup and Fleischer, 1998), (Kostka, Thamdrup et al., 1999)). In these Arctic sediments, as well as in sediments along the east coast of Greenland, dissimilatory Fe(III) and sulfate reduction were found to be important terminal, electron accepting pathways for anaerobic carbon oxidation ((Rysgaard, Thamdrup et al., 1998), (Kostka, Thamdrup et al., 1999), (Glud, Risgaard-Petersen et al., 2000)). Studies on a wide range of coastal marine sediments have shown that Fe reduction is important for carbon oxidation, whereas microbial Mn reduction is in general insignificant due to low Mn oxide concentrations and to shallow depth distribution of Mn(IV) (Thamdrup, 2000). As an exception, dissimilatory Mn reduction was found to be quantitatively important for benthic carbon mineralization in sediments of the Skagerrak, the Panama Basin, and the Black Sea due to high Mn oxide contents and high rates of bioturbation ((Aller, 1990), (Canfield, Jørgensen et al., 1993), (Canfield, Thamdrup et al., 1993), (Thamdrup, Rosselló-Mora et al., 2000)).

This study was part of the Norwegian CABANERA (carbon flux and ecosystem feed back in the northern Barents Sea in an era of climate change) project with the primary goal of understanding how productivity and carbon flux in the Arctic Ocean may change as a result of

global warming. Models predict that, within 50 to 60 yr, much of the Arctic Ocean will become ice-free during summer (ACIA 2004), resulting in dramatic shifts in productivity, communities and carbon flux ((McGowan, Cayan et al., 1998), (Anderson and Piatt, 1999)). Little is known about how Arctic benthic ecosystems will respond to increased organic fluxes and how this might affect the balance between the main mineralization processes. A comparative study of sediment processes in the Arctic controlled by short ice-free summers northeast of Svalbard and longer ice-free summers along the southwest coast of Svalbard was therefore initiated. The southwest areas were studied during earlier research cruises by Kostka, Thamdrup et al. (1999) and Vandieken, Finke et al. (2006).

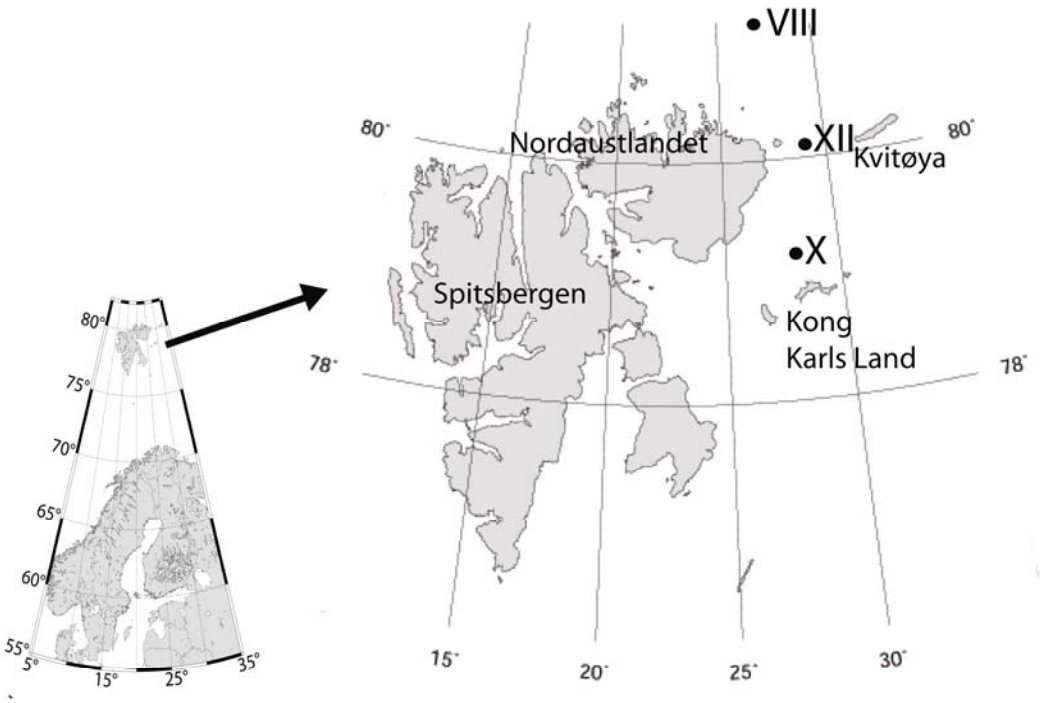

Fig. 1. Sampling stations NE of Svalbard (see Table 1 for details)

We present here, for the first time data on pathways of microbial respiration in sediments northeast of Svalbard. The sediments have high concentrations of particulate Mn and Fe(III) and low organic carbon deposition. Rates of Mn, Fe and sulfate reduction were determined in

relation to the distribution Mn and Fe in the solid phase. The present study shows that, besides aerobic respiration, Mn(IV) and Fe(III) reduction are most important for carbon mineralization in the sediments.

MATERIAL AND METHODS

Sampling sites. Sediments were sampled during the second CABANERA cruise from 20 July to 3 August 2004 on board RV 'Jan Mayen' off the northeast coast of Nordaustlandet. An overview of the location and characteristics of the benthic stations are given in Fig. 1 & Table 1. Stns VIII and XII were located in trenches and were ice-free at the time of sampling. Stn X was positioned in an area with melting ice floes and was coupled to a pelagic sampling station in the CABANERA project. The sediments of stations VIII and X were sampled with a multiple corer that retrieved up to 4 cores with 10 cm inner diameter. At Stn XII, sediment was subsampled from a 50 cm x 50 cm box core into the cores of the multiple corer.

Table 1. Sample site information and sediment characteristics at 3 stations (VIII, X, XII) from 20 July to 3 August 2004. Data are for whole core incubations. O_2: O_2 consumption rate; SRR: sulfate reduction rate (0-10 cm).

Description	**VIII** Northern Kvitøya trench	**X** North of Kong Karls Land	**XII** Central Kvitøya Trench
Latitude	81°16.65' N	79°26.50' N	80°09' N
Longitude	26°51.18' E	28°48.43' E	29°36' E
Water depth (m)	503	303	286
Bottom water temperature (°C)	2.9	2.5	2.3
Total organic carbon (%)	1.46	1.41	1.45
Total organic nitrogen (%)	0.17	0.17	0.18
O_2 (mmol m^{-2} d^{-1})	2.1 ± 0.7	1.5 ± 0.4	3.5 ± 1.6
SRR (mmol m^{-2} d^{-1})	<0.2	<0.2	0.46 ± 0.13

Anoxic bag incubations. Sediment from the upper 10 cm of the cores was sliced into the following depth intervals: Stn VIII 0-3 and 6-12 cm, Stns X and XII 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, and 7-10 cm. Sediment from the same depth interval of several cores was filled under a constant stream of N_2 into gastight plastic bags outdoors at an air temperature around 0°C. The incubation bags were closed (without gas phase) and incubated near *in situ* temperature at 0°C inside larger, N_2-filled plastic bags to ensure anoxia. Over a period of 55 to 60 d incubation, subsamples were withdrawn 10 times from each bag, in a cold room (4°C).

Pore water and solid phase sampling. Pore water of whole cores and pore water from the bags was retrieved by a pore water press under N_2 through GF/F filters. Pore water was filtered directly into Ferrozine-solution to measure Fe^{2+} (see later subsection). We collected 1.8 ml aliquots for dissolved inorganic carbon (DIC) and alkalinity analyses in glass vials without headspace and capped with Viton septa; these were fixed with $HgCl_2$, and stored at 4°C until analysis. We froze 1.5 ml of pore water for NH_4^+, NO_3^-, and NO_2^- analysis. For Mn^{2+} and Ca^{2+} determination, 0.5 ml was acidified with 6 M HCl and stored at 4°C. Pore water for sulfate and sulfide analyses was preserved with Zn acetate or $ZnCl_2$. Bottom water of the stations was stored frozen for determination of NH_4^+, NO_3^-, and NO_2^- and (acidified with HCl) for Mn^{2+} analysis.

For the extraction of solid phase Fe and Mn with dithionite and for the determination of total organic C and N, subsamples were stored frozen at –21°C. For the analysis of elemental sulfur, a subsample of 0.5 to 2 g sediment was mixed with 2 ml 20% Zn acetate and stored frozen at –21°C.

Pore water analyses. DIC was analyzed by flow injection with conductivity detection (Hall and Aller, 1992). Fe(II) was measured spectrophotometrically with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) at 562 nm (Dr. Lange LP2W) (Stookey, 1970). NH_4^+ was determined spectrophotometrically at 630 nm (Shimadzu UV 1202) (Grasshoff, Kremling et al., 1999). NO_3^- and NO_2^- were measured using a NO_x-Analyzer (Thermo Environmental

Instruments) (Braman and Hendrix, 1989). Ca^{2+} and Mn^{2+} in pore water were measured by inductively coupled plasma atomic-emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was measured by non-suppressed ion chromatography (Waters, column IC-Pak™, 50 x 4.6 mm). Sulfide was determined by the methylene blue spectrophotometric method at 670 nm (Shimadzu, UV 1202) (Cline, 1969). Alkalinity was determined by Gran titration using 0.02 M HCl.

Solid phase analyses. Fe was extracted by HCl (0.5 M HCl for 1 h) and the extract analyzed for Fe(II) with Ferrozine and for total Fe with Ferrozine plus 1% (w/v) hydroxylamine hydrochloride. Fe(III) concentrations were calculated by subtracting concentrations of Fe(II) from total Fe concentrations. Solid Mn in the sediment was quantified after freeze drying and extraction with dithionite-citrate-acetic acid (Canfield, 1989) by flame atomic absorption spectrometry (Perkin Elmer, Atomic Absorption Spectrometer 3110). Freeze dried samples for determination of total organic C and N contents were pretreated with HCl, dried again, and analyzed using a CNS analyzer (Fisons™ Na1500 elemental analyzer). For elemental sulfur analysis a subsample of the sediment frozen in Zn acetate was extracted with 5 ml methanol (Zopfi, Ferdelman et al., 2004). With a Zorbax ODS column (125 x 4 mm, 5 μm; Knauer), using methanol as eluent, the sulfur was determined by HPLC from absorption at 265 nm (detection limit 1 μM).

Sulfate reduction rates. Sulfate reduction rates were measured in 3 parallel cores of 3 cm diameter each using the $^{35}SO_4^{2-}$ whole core injection technique (Jørgensen, 1978). Sulfate reduction in the anoxic bags was determined at each sampling time point in subsamples incubated with 100 kBq $^{35}SO_4^{2-}$ radiotracer in 5 ml glass tubes. After 6 h, the incubations were stopped with 20% Zn acetate. Total reduced inorganic sulfur was analyzed by a cold chromium distillation (Kallmeyer, Ferdelman et al., 2004).

Oxygen consumption rates. Sediment cores with an inner diameter of 54 mm were closed (without gas phase) with rubber stoppers. The cores were incubated in the dark at 0°C

with continuous stirring of the water column by a magnetic stirring bar at the top of the water column. Oxygen consumption of the sediment was measured during the whole incubation with a micro-optode (Holst, Glud et al., 1997). The volume of the water column was determined by addition of a NaBr solution. Concentrations of NaBr were analyzed by anion chromatography (Dionex DX500, eluent: 9 mM $NaCO_3$, precolumn: AG9 HC, column: AS9 HC). Total oxygen consumption rates were calculated from duplicate cores for Stn VIII, and triplicates for Stns X and XII.

Calculations. The precipitation of $CaCO_3$ during bag incubation was calculated according to (Thamdrup, Rosselló-Mora et al., 2000) from decreasing Ca^{2+} concentrations: $\Delta CaCO_3 = \Delta[Ca^{2+}]_{sol} (1 + K_{Ca})$, where K_{Ca} is the adsorption constant for Ca^{2+} ($K_{Ca} = 1.6$) (Li and Gregory, 1974). The production of DIC was calculated as DIC production = DIC accumulation + $CaCO_3$ precipitation.

The saturation of pore waters by rhodocrocite was calculated with the program PHREEQC using the thermodynamic constants of the database (Parkhurst, 1995). Measured alkalinity and concentrations of DIC, Ca^{2+}, and Mn^{2+} were included in the calculations.

The penetration depth of oxygen, h, was estimated according to Revsbech et al. (1980): $h = 2\ D_S\ C_0\ \Phi / J$, where D_S is the diffusion coefficient in the sediment, C_0 is the oxygen concentration at the sediment surface, ϕ is the porosity, and J is the oxygen uptake rate. D_S was calculated according to (Iversen and Jørgensen, 1993): $D_S = D_0 / [1 + 3 (1 - \phi)]$, where D_0, the diffusion coefficient in seawater (taken from (Schulz and Zabel, 2000)): $D_0(O_2) = 1.25\ 10^{-9}\ m^2\ s^{-1}$ (at 0°C).

The depth-integrated rates of Stn VIII in Table 2 were calculated from sediment incubations of the depth intervals 0 to 3 and 6 to 12 cm.

RESULTS

Pore water and solid phase chemistry

The depth distribution of DIC, NH_4^+, NO_3^-, Mn^{2+} and Fe^{2+} in the pore water as well as solid phase Mn and Fe are shown in Fig. 2 for the 3 stations. DIC and NH_4^+ concentrations increased with increasing depth from the overlying sea water into the sediment at all stations (Fig. 2A,D,G). Nitrate and nitrite concentrations in the sea water at all 3 stations were ≤17 and ≤0.7 µM, respectively (Fig. 2B,E,H). Highest concentrations of nitrate were measured at 0 to 1 cm sediment depth at the 3 stations and concentrations decreased below a background level of <2 µM below 6 cm at Stn VIII and 3 cm at Stns X and XII. At the depths where nitrate concentrations decreased, soluble Mn^{2+} concentrations started to increase to maximum concentrations of >75 µM (Fig. 2B,E,H). Fe^{2+} in the pore water was mostly below detection at Stn X (Fig. 2E). Fe^{2+} concentrations at Stns VIII and XII were low (<20 µM) and its distribution similar to that of Mn^{2+} (Fig. 2B,H). The extractable Mn and Fe(III) contents of the sediments were high, with concentrations of ≥60 µmol cm^{-3} Mn and ≥108 µmol cm^{-3} Fe(III) at all stations (Fig. 2C,F,I). Mn and Fe(III) were enriched at the surface at Stns X and XII, whereas at Stn VIII highest concentrations occurred between 4 and 8 cm depth. At Stn VIII, Fe(III) was depleted at 12 cm and Mn was present from the surface to >20 cm depth. In contrast, at Stns X and XII, Mn was depleted at shallower depths in the sediment compared to Fe(III) which reached down to 8 and 6 cm, respectively. At all stations the sulfide concentration in the pore water was below the detection limit of 1 µM and the sulfate concentration stayed constant over 0 to 20 cm depth. The elemental sulfur content at Stn XII peaked (0.26 µmol cm^{-3}) at 12 cm (Fig. 3A), whereas it was below detection at the other 2 stations. The total organic nitrogen and carbon contents of the sediments were similar for all 3 stations, with 0.17 to 0.18% organic nitrogen and 1.41 to 1.46% organic carbon (Table 1).

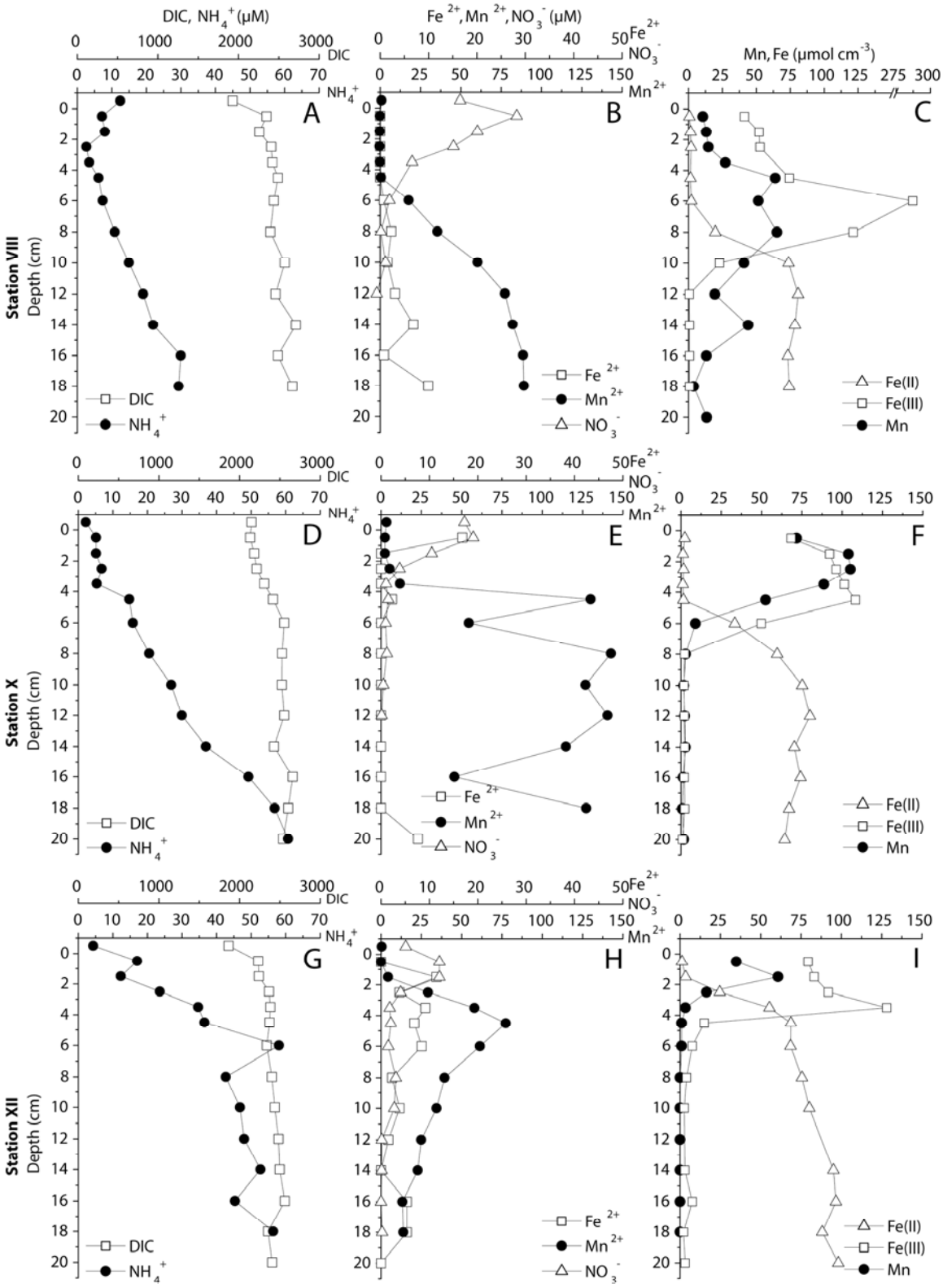

Fig. 2. Concentrations of the pore water dissolved inorganic carbon (DIC), NH_4^+, NO_3^-, Fe^{2+} and Mn^{2+} and solid phase Mn, Fe(III), and Fe(II) at Stns VIII, X, XII. Note change in scale in abscissa of (C).

Oxygen consumption and sulfate reduction rates of whole cores

The oxygen uptake rates of the sediments were lowest at Stn X and highest at Stn XII (Table 1). Based on these rates the oxygen penetration depths were calculated according to (Revsbech, Jørgensen et al., 1980) to be 1.8, 2.4, and 1.1 cm for Stns VIII, X, and XII, respectively. Sulfate reduction rates were below our detection limit of 1 nmol cm^{-3} d^{-1} at 0 to 20 cm depth in the cores of Stns VIII and X. At Stn XII, sulfate reduction was below detection in the top 3 cm and increased to a maximum at 4 to 15 cm depth (Fig. 3B).

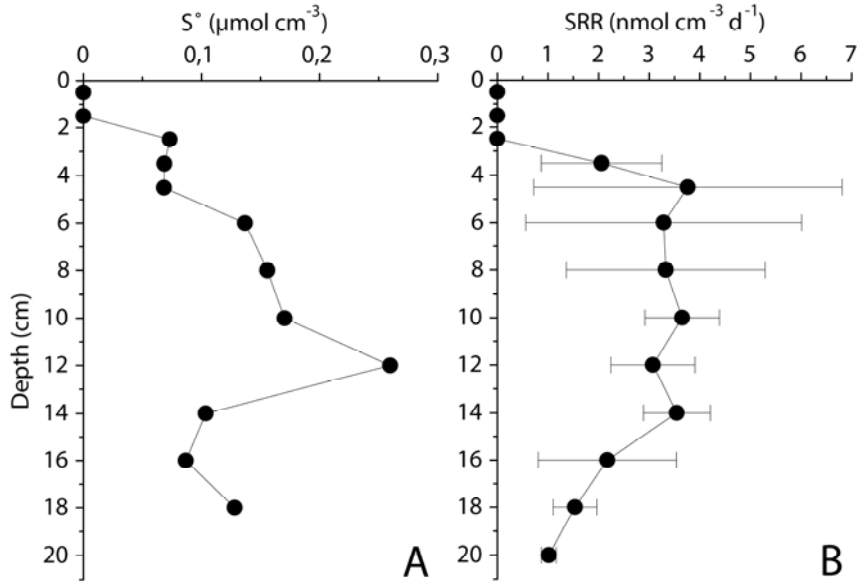

Fig. 3. (A) Solid phase S° and (B) sulfate reduction rates (SRR) at Stn XII. Data are mean ±SD of triplicate measurements. S° concentrations and SRR were below detection at the other stations.

Anoxic bag incubations

Carbonate precipitation. DIC concentrations in bag incubations are expected to increase linearly during constant degradation of organic carbon. In this study, linear increases of DIC concurrent with constant Ca^{2+} concentrations were measured only in the pore water of the 2 bags for Stn X from 0 to 1 and 1 to 2 cm depth, in the bag from 6 to 12 cm for Stn VIII, and in the 4 bags from 3 to 10 cm for Stn XII (data not shown). Decreasing or constant DIC concentrations, either right from the beginning or after an initial increase, were measured in

all other bags (Stn VIII 0 to 3 cm, Stn X between 2 and 10 cm, Stn XII between 0 and 3 cm). As an example, data for Stn XII are shown in Fig. 4A. Non-linear changes in DIC concentration were related to decreasing Ca^{2+} concentrations over time in the respective bags (Fig. 4B) indicating a precipitation of $CaCO_3$. DIC accumulation rates were corrected for $CaCO_3$ precipitation according to (Thamdrup, Rosselló-Mora et al., 2000). $CaCO_3$ precipitation, however, did not seem to account for the complete carbonate precipitation. Additional precipitation of carbonate with Mn^{2+} was indicated by supersaturation of pore waters with rhodocrocite in most bags (ion activity products generally exceeded the solubility constant >2-fold) and decreasing Mn^{2+} accumulation rates towards the end of the incubations in the bags for Stn XII from 0 to 3 cm (Fig. 4C). Correction for $MnCO_3$ precipitation was not done, as precipitation is dependent on the Mn^{2+} concentrations, which increased due to Mn reduction. Therefore, the corrected DIC production rates represent minimum rates (Thamdrup, Rosselló-Mora et al., 2000).

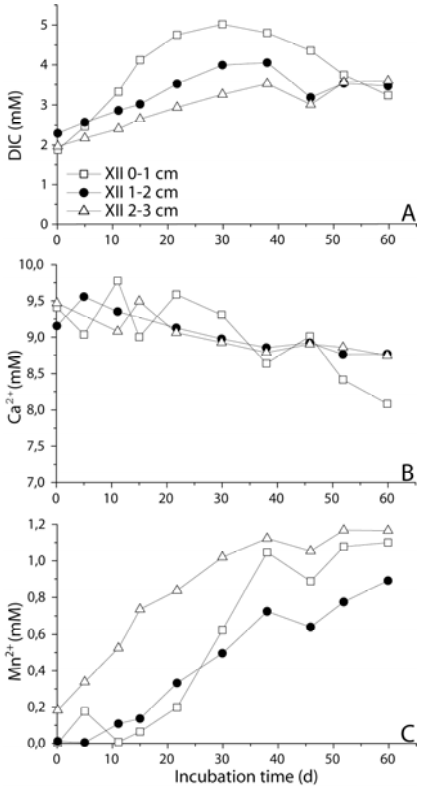

Fig. 4. Changes in pore water concentrations of DIC, Ca^{2+} and Mn^{2+} in sediment bags from 0-1, 1-2, and 2-3 cm depth at Stn XII during 2 mo anoxic incubation.

DIC and NH_4^+ accumulation and sulfate reduction rates. In general, rates of DIC and NH_4^+ accumulation in the pore water during anoxic sediment incubations decreased with depth (Fig. 5). The depth-integrated rates were similar at Stns VIII and X, while rates were 2- to 4-fold higher at Stn XII (Table 2). Sulfate reduction rates were below the detection limit at Stns VIII and X, similar to the whole core incubations. Sulfate reduction rates were also below detection between 0 and 3 cm at Stn XII, but increased with increasing depth below 3

cm (Fig. 6). Sulfate reduction rates were almost constant during the bag incubations, so average rates were used (data not shown). The depth-integrated sulfate reduction rate for bag incubations of Stn XII (Table 2) was 3-fold higher than in whole core incubations for the same depth interval (Table 1), probably due to a stimulation of carbon degradability by mixing of the sediment ((Kostka, Thamdrup et al., 1999), (Arnosti, Finke et al., 2005)). Assuming a stoichiometry of 2:1 mol of carbon oxidized (= DIC produced) to sulfate reduced (Thamdrup and Canfield, 1996), there was good agreement between DIC production based on sulfate reduction rates and the measured DIC production rates at 5 to 10 cm (Fig. 6). At 0 to 5 cm measured DIC production rates exceeded DIC production based on sulfate reduction. Thus, we conclude that electron acceptors other than sulfate were utilized for microbial carbon oxidation at Stn XII from the sediment surface down to 5 cm depth, and also at Stns VIII and X from 0 to 10 cm, where no contribution of sulfate reduction to anaerobic carbon oxidation was detected.

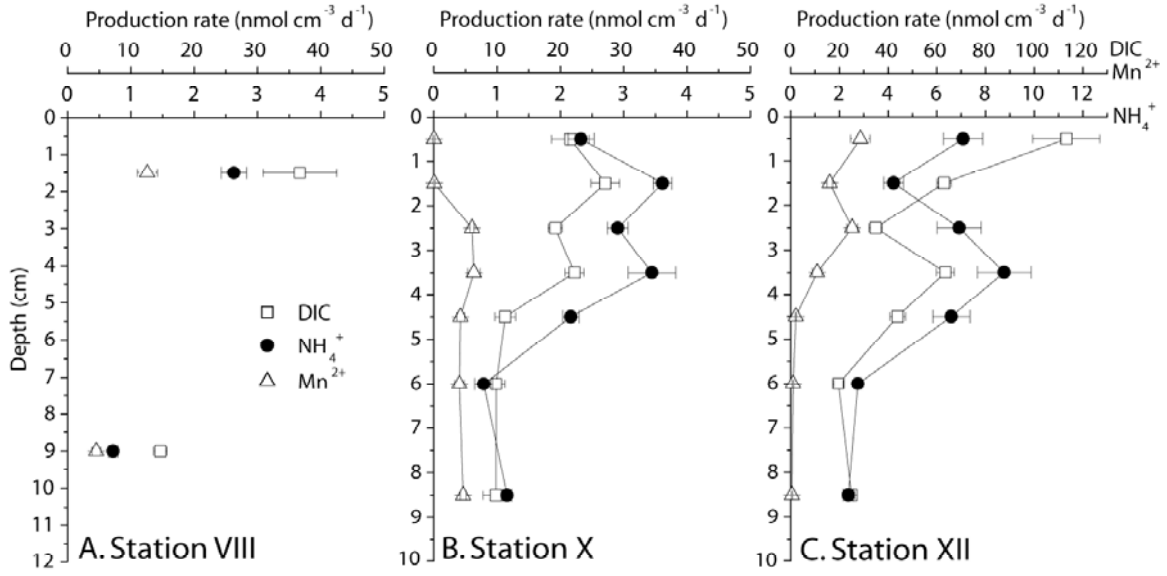

Fig. 5. Anaerobic DIC and NH_4^+ mineralization rates and soluble Mn^{2+} accumulation rates at Stns VIII, X, XII. Data are mean ±SE of linear regression of concentration over time. Note different production rates scales (abscissas)

Mn^{2+} and Fe^{2+} accumulation. Indications that microbial Mn and Fe reduction was an important respiratory processes in the sediments were given by the accumulation of Mn^{2+} and Fe^{2+} in the pore water during bag incubations. Mn^{2+} accumulation in the bags scaled with solid Mn distribution at Stns VIII and XII, where rates were highest near the surface (Fig. 5A,C). In contrast, at Stn X, Mn^{2+} concentrations at 0 to 1 and 1 to 2 cm sediment depth did not increase at all throughout incubation (Fig. 5B), although solid Mn was present. At 2 to 3 cm depth, Mn^{2+} started to accumulate after 15 d (data not shown), and in the bags from 3 to 10 cm the concentrations increased linearly throughout the incubation. Fe^{2+} concentrations remained below background level (≤16 μM) throughout incubation in all bags at Stns VIII and X. At Stn XII in the bag from 3 to 4 cm sediment depth, Fe^{2+} started to accumulate in the pore water halfway through the incubation period, and Mn^{2+} no longer accumulated (Fig. 7). Fe^{2+} concentrations also increased in the 2 bags from 4 to 5 and 5 to 7 cm towards the very end of incubation (Fig. 7), whereas Fe^{2+} concentrations remained low in the bags from 0 to 3 and 7 to 10 cm depth.

DICUSSION

Terminal electron accepting pathways in Arctic sediments

Aerobic respiration. Total oxygen uptake rates were 1.5 to 3.5 mmol m^{-2} d^{-1} at the 3 stations northeast of Svalbard (Table 1). Similar rates of 1.9 to 4.1 mmol m^{-2} d^{-1} have been measured north and northeast of Svalbard, whereas higher rates (3.6 to 11.2 mmol m^{-2} d^{-1}) have been determined in sediments along the south and west coast ((Hulth, Blackburn et al., 1994), (Glud, Holby et al., 1998), (Vandieken, Finke et al., 2006)). The difference has been suggested to result from different water masses of Atlantic and Arctic origin determining the duration of the annual ice cover and, thus, the phytoplankton production (Hulth, Blackburn et al., 1994). Benthic oxygen consumption can also vary interannually as a response to the

settling of fresh organic material from the water column due to ice melting ((Rysgaard, Thamdrup et al., 1998), (Glud, Gundersen et al., 2003)).

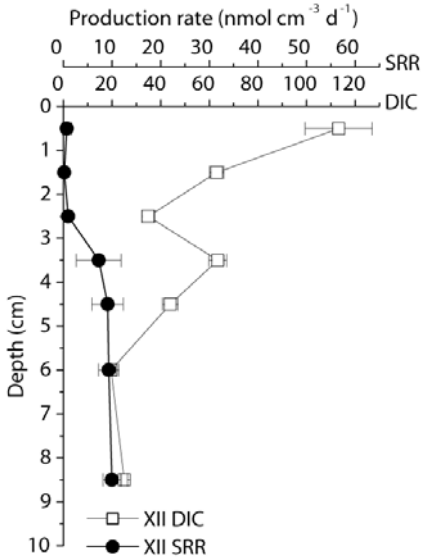

Fig. 6. Vertical profiles of anaerobic carbon mineralization at Stn XII, showing sulfate reduction rates (SRR) and DIC production rates. Data are mean ±SE of linear regression of DIC production and SD of SRR. Production rates plotted at ratio of 2:1 for DIC production to SRR.

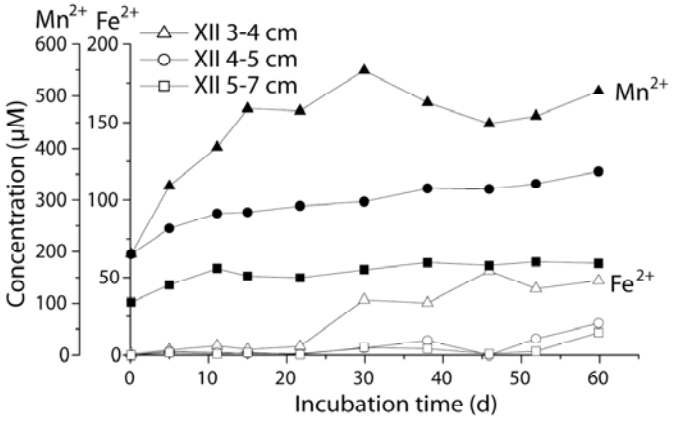

Fig. 7. Accumulation of Mn^{2+} (filled symbols) and Fe^{2+} (open symbols) in pore water during anoxic incubation of 3 sediment bags at 3-4, 4-5 and 5-7 cm of Stn XII.

Hulth, Blackburn et al. (1994) measured oxygen penetration depths of 1.7 to ≥5.9 cm in sediments off the north and northeast coast of Svalbard. Based on oxygen uptake rates, we calculated oxygen penetration depths of 1.1 to 2.4 cm for the sediments studied. The presence of oxygen in the surface sediments was also indicated by the pore water chemistry. Maximum concentrations of nitrate below the surface indicated a zone of nitrification that requires the presence of oxygen (Fig. 2B,E,H). The depletion of oxygen in the sediments was indicated by decreasing concentrations of nitrate, probably due to denitrification, and increasing concentrations of Mn^{2+} with depth (Fig. 2B,E,H). The calculated oxygen penetration depths of 2.4 cm for Stn X and 1.1 cm for Stn XII were in accordance with the depletion of nitrate and the accumulation of Mn^{2+} below 2 and 1 cm depth, respectively. For Stn VIII, the distribution

of nitrate and Mn^{2+} in the pore water indicated the deepest penetration of oxygen of ~4 cm, whereas the calculated oxygen penetration depth was only 1.8 cm. A deeper penetration of oxygen might result from active bioirrigation by polychaetes, which introduce oxygen into deeper sediment layers (Jørgensen, Glud et al., 2005) and might cause spatial heterogeneity of porewater constituents. Because nitrate reduction is in general of minor importance for the degradation of organic matter and accounted for only 2-3% of total carbon oxidation in fjord sediments of Svalbard (Kostka, Thamdrup et al., 1999), oxic respiration was probably the most important process in the surface sediments of the 3 stations. We conclude that, although the anoxic bag incubations revealed a high potential for anaerobic respiration even in the surface sediments, aerobic respiration and to a lesser extent, denitrification were important for organic carbon oxidation in surface-sediment intervals of 1 to 4 cm at all 3 stations.

Sulfate reduction. At Stn XII, the contribution of sulfate reduction to anaerobic carbon oxidation during sediment bag incubations for 0 to 10 cm depth was 0.67 mmol m^{-2} d^{-1} or 31% (assuming a stoichiometry of 2:1 DIC produced to sulfate reduced) (Table 2). Although at Stns VIII and X sulfate reduction rates were below the detection limit, we assume that sulfate reduction occurs at these stations. As anaerobic carbon oxidation rates were low (Fig. 5A,B, Table 2), either the incubation time with the $^{35}SO_4^{2-}$ tracer might have been too short or too little tracer had been injected to detect sulfate reduction. However, based on the detection limit, we calculated the maximum contribution of sulfate reduction to anaerobic carbon oxidation integrated over 0 to 10 cm sediment depth to be <0.1 mmol m^{-2} d^{-1} or <10% for Stn VIII, and <0.1 mmol m^{-2} d^{-1} or <13% for Stn X (Tables 1 & 2).

A higher contribution of sulfate reduction might have been masked by biologically catalyzed re-oxidation of sulfide to SO_4^{2-} concomitant with Mn reduction ((Aller and Rude, 1988), (King, 1990), (Thamdrup, Finster et al., 1993)). Thamdrup et al. (1993) suggested that in such experiments the chemical oxidation of HS^- to S° by Mn(IV) or Fe(III) is accompanied

by microbial disproportionation of S° to SO_4^{2-} and HS^-. However, the abundance of easily reducible Mn and Fe oxides should allow Mn- and Fe-reducing bacteria to exercise their thermodynamic advantage over sulfate reducers in competition for common substrates (Lovley and Phillips, 1987). We conclude that electron acceptors other than sulfate, most likely Mn(IV) and Fe(III), were the most important for anaerobic carbon oxidation at 0 to 10 cm sediment depth at Stns VIII and X and at 0 to 5 cm at Stn XII.

Mn and Fe reduction. The importance of Mn and Fe as microbial electron acceptors was supported by high contents of solid Mn and Fe(III) in the surface sediments of all stations from 5 to 10 cm depth (Fig. 2C,F,I). Additionally, high concentrations of Mn^{2+} in the pore water indicated zones of Mn reduction and corresponded to increasing Mn^{2+} concentrations in most bags during sediment incubations (Figs. 2B,E,H, & 5). Solid Mn was present from the sediment surface to a depth of 20 cm at Stn VIII (Fig. 2C) and dissolved Mn^{2+} accumulated in both bags of 0 to 3 and 6 to 12 cm depth during the incubation (Fig. 5A). Similarly, the accumulation of Mn^{2+} in the bags at 0 to 3 cm depth at Stn XII (Fig. 5C) was in agreement with solid Mn concentrations of 16 to 61 μmol cm^{-3} (Fig. 2I). At Stn X, a correlation between solid Mn contents and accumulation rates of soluble Mn^{2+} was only determined for the 2 to 6 cm depth interval (Figs. 2F & 5C).

Despite high concentrations of solid Mn, Mn^{2+} did not accumulate in the pore water of the top 2 cm. High rates of DIC and NH_4^+ production indicated active microbial carbon oxidation (Fig. 5B), yet it was not clear which alternative electron acceptors other than Fe and Mn could have been used. Based on the oxygen consumption rate, the oxygen penetration depth, and the bottom water concentration of oxygen (334 μM), oxygen should have been depleted within less than 3 d incubation. Equivalent nitrate (17 μM) and nitrite concentrations (0.4 μM) were low, and probably were depleted fast. The lack of another obvious electron acceptor responsible for the continuous DIC and NH_4^+ production leads us to conclude that

Mn reduction was also active at 0 to 2 cm but that the Mn^{2+} produced was totally adsorbed. Similar results were found in Skagerrak sediments of Denmark, where the lack of Mn^{2+} liberation in bag incubations of surface sediments was observed for 2 stations, whereas at a third station Mn^{2+} accumulated in the top sediment with high rates (Canfield, Thamdrup et al., 1993). Adsorption experiments showed that dissolved Mn^{2+} could be completely adsorbed onto sediments containing high concentrations of Mn oxide with oxidation levels of 3.6 to 3.8 ((Murray, Balistrieri et al., 1984), (Canfield, Thamdrup et al., 1993)). It was assumed that Mn^{2+} adsorbed on surface sites of Mn oxides and could only accumulate after these sites had been saturated. Based on such experiments, (Canfield, Thamdrup et al., 1993) suggested that the surface sediment where Mn^{2+} did not accumulate differed in capacity for adsorption of Mn^{2+} from the sediment of the third station, where Mn^{2+} accumulated and where the oxidation level of Mn oxides was probably less than 3.6 to 3.8. Correspondingly, we propose that the surface sediment from 0 to 2 cm depth at Stn X contained oxidized Mn oxides, whose surface completely adsorbed the Mn^{2+} produced during the incubation. Initial complete adsorption was also indicated for the bag from the underlying sediment (2 to 3 cm), in which Mn^{2+} started to accumulate after 15 d incubation (data not shown).

At 6 to 10 cm depth, Mn^{2+} accumulated in the pore water, even though the Mn content was low (<10 μmol cm^{-3}) (Fig. 2F). In contrast to the profile in Figure 2F, the Mn content of the bags from 6 to 10 cm depth at Stn X was ≥20 μmol cm^{-3} (data not shown). This difference is probably due to local heterogeneity and the fact that sediment from 10 cores was pooled for the bag incubations. In Black Sea sediments, Mn concentrations above ~10 μmol cm^{-3} led to inhibition of sulfate reduction by microbial Mn reduction (Thamdrup, Rosselló-Mora et al., 2000). Thus, with Mn concentrations of ≥20 μmol cm^{-3} between 0 to 10 cm depth at Stn X, Mn was probably the most important electron acceptor.

Fe(III) was present at all stations and, except at Stn VIII, penetrated deeper into the sediment than Mn (Fig. 2C,F,I). However, Fe^{2+} did not accumulate during incubations in most

bags. Heterotrophic Fe(III) reduction in the presence of Mn oxide might be masked by the reoxidation of produced Fe^{2+} by Mn(IV) ((Lovley and Phillips, 1988), (Myers and Nealson, 1988)). Correspondingly, we could not exclude simultaneous microbial Fe and Mn reduction in the presence of high concentrations of solid Mn at the 3 stations.

At Stn XII at 3 to 4 cm, high Fe(III) concentrations of 128 μmol cm^{-3} and low Mn concentrations of 3 μmol cm^{-3} probably limited microbial Mn reduction and favored Fe reduction in this zone. We suggest that produced Fe^{2+} first was abiotically oxidized by Mn(IV), which resulted in Mn^{2+} accumulation during the first half of the incubation (Fig. 7). After complete Mn(IV) reduction by Fe(II), Fe^{2+} could accumulate during the second half of incubation. Similar observations have been found in pure-cultures experiments (Myers and Nealson, 1988). In the 2 bags of 4 to 5 and 5 to 7 cm, the accumulation of Fe^{2+} was also detected towards the very end of incubation (Fig. 7) but, with decreasing Fe(III) concentration, sulfate reduction became the dominating respiration pathway (Fig. 6). Altogether, Mn was probably the sole anaerobic terminal electron acceptor in the surface sediment at St XII, whereas below 3 cm a transition from microbial Mn to Fe reduction occurred. Fe and sulfate reduction concurred at 3 to 5 cm and at 5 to 10 cm sulfate was the sole important electron acceptor.

Mn^{2+} and Fe^{2+} liberation rates might indicate these to be the dominating respiration pathways, yet, as other reactions such as precipitation, adsorption, and chemical oxidation occur simultaneously, metal reduction rates are usually underestimated ((Canfield, Thamdrup et al., 1993), (Thamdrup and Canfield, 1996), (Glud, Risgaard-Petersen et al., 2000), (Thamdrup, 2000), (Jensen, Thamdrup et al., 2003)). The rates of dissimilatory Mn and Fe reduction can be calculated by subtraction of DIC production based on from the total DIC production, where the excess of carbon oxidation can be attributed to Mn and/or Fe reduction (Thamdrup, 2000). Accordingly, at Stn VIII we attributed >90%, at Stn X >87%, and at Stn

XII 69% of anaerobic carbon oxidation to microbial Mn and Fe reduction for an interval of 0 to 10 cm (Table 2).

It is likely that *in situ* Mn and Fe reduction in the surface sediments is suppressed through the presence of oxygen and nitrate. The abundance of oxygen and nitrate in surface sediments is important for the Mn- and Fe-cycles, as they reoxidize the reduced Mn and Fe. Calculations for Danish coastal sediments showed that Mn and Fe atoms are recycled 100 to 300 times before their ultimate burial ((Canfield, Thamdrup et al., 1993), (Thamdrup, Glud et al., 1994)). The maintenance of Mn and Fe reduction in the suboxic zone is dependent on mixing processes ((Aller, 1990), (Canfield, Thamdrup et al., 1993), (Thamdrup, Glud et al., 1994)). Bioturbation enables the downward transport of organic matter and Mn and Fe oxides as well as upward transport of Fe(II) and Mn(II) for the re-oxidation by oxygen, nitrate, and, in the case of Fe(II), by Mn(IV) and the formation of new oxides. We observed the highest abundance of polychaetes and polychaete tubes at Stn XII, indicating bioturbation, which correlates with highest rates of carbon oxidation and fastest turnover of Mn and Fe at 0 to 5 cm.

In the zone where oxygen and nitrate are depleted, high concentrations of Mn and Fe(III) were present in the 3 sediments so that microbial Mn and Fe reduction *in situ* were the dominating respiratory pathways. The zone of Mn and Fe reduction was indicated by pore water concentrations of Mn^{2+} and Fe^{2+} (Fig. 2). In most marine shelf sediments, dissimilatory Mn reduction is insignificant for the oxidation of carbon because of low Mn oxide contents and a shallow depth distribution (Thamdrup, 2000). Mn concentrations were low (<4 μmol cm^{-3}) or intermediate (~18 μmol cm^{-3}) in sediments of Van Mijenfjorden and Smeerenburgfjorden on the west coast and in Storfjorden at the southeast coast of Svalbard ((Kostka, Thamdrup et al., 1999), (Vandieken, Finke et al., 2006)). Microbial Mn reduction was therefore assumed to be insignificant for carbon oxidation. Sediments with very high Mn oxide concentrations (≥100 μmol cm^{-3}) are restricted to small areas of the world ocean such as

the Panama Basin, where Mn is of hydrothermal origin, or the deep parts of the Skagerrak, where Mn released from more reducing sediments is trapped. At these sites, microbial Mn reduction was shown to account for more than 90% of the mineralization and could be detected as deep as 10 cm in the sediment ((Aller, 1990), (Canfield, Jørgensen et al., 1993), (Canfield, Thamdrup et al., 1993)). The relatively high Mn contents of ≥60 μmol cm^{-3} and Fe(III) contents of ≥108 μmol cm^{-3} in sediments of the 3 stations off the northeast coast of Svalbard were related to the significance of dissimilatory Mn and Fe reduction for carbon oxidation (Table 2).

Our study supports the importance of metal reduction for carbon mineralization in permanently cold sediments. Previous studies determined that Fe reduction accounted for 0 to 26% of the total carbon oxidation in fjord sediments of the west coast of Svalbard ((Kostka, Thamdrup et al., 1999), (Vandieken, Finke et al., 2006)) and for 21 to 26% in sediments of east Greenland ((Rysgaard, Thamdrup et al., 1998), (Glud, Risgaard-Petersen et al., 2000)). This is in agreement with contributions of 0 to >50% of Fe reduction to total carbon mineralization found in a wide selection of coastal sediments (Thamdrup, 2000).

Table 2. Depth-integrated rates of dissolved inorganic carbon (DIC) and NH_4^+ production and sulfate reduction (mmol m^{-2} d^{-1}) and percentage Mn/Fe reduction in anoxic bag incubations at 0-10 cm Stns VIII, X and XII. Mn/Fe reduction calculated from sulfate-independent DIC production rates as percentage anaerobic carbon oxidation.

Rate measured	VIII	X	XII
DIC production	2.0	1.5	4.3
NH_4^+ production	0.12	0.20	0.46
Sulfate reduction	<0.1	<0.1	0.67
Mn/Fe reduction (%)	>90	>87	69

Rates and pathways of carbon oxidation in Arctic sediments

Aerobic and anaerobic rates of carbon oxidation were highest for the middle station, Stn XII and lowest at the southernmost Stn X (Tables 1 & 2), indicationg that the sediment of Stn XII is supplied annually with more organic carbon. A possible explanation is the semi-permanent polynya observed west and southwest of Kvitøya ((Vinje and Kvambekk, 1991), (Falk-Petersen, Hop et al., 2000)). This region is ice-free earlier than the surrounding area and probably has an earlier spring bloom and enhanced biomass production over the whole year ((Strass and Nöthig, 1996), (Falk-Petersen, Hop et al., 2000)).

The retreat of the sea ice during late spring in general proceeds from 2 sides towards the northeast of Svalbard: along the east coast northwards and along the north coast eastwards (Falk-Petersen, Hop et al., 2000). Although Stn VIII was the deepest and northernmost station (Fig. 1 & Table 1), higher aerobic and anaerobic carbon oxidation rates were measured compared to the southernmost station, X. As Stn VIII was situated in a trench that extends along the east coast of Nordaustlandet, we suggest that sediment might be transported from the surrounding slopes and northwards into the trench. This was also indicated by the sedimentation rates which were twice as high at Stn VIII (1.3 mm y^{-1}) compared to Stn X (0.6 mm y^{-1}) (Zaborska, unpubl. data). Observations and satellite images covering several years have shown that in some years the area around Kong Karls Land (Stn X) is ice-covered longer than the area further north; this may restrict primary production. Thus, Stn X may be supplied with less organic material than the other 2 stations. In conclusion, primary production is probably higher at Stn VIII than at Stn X, and additional organic material may be transported to Stn VIII, becoming available for benthic oxidation and supporting higher mineralization rates in the north. However, the difference in carbon oxidation rates between the 2 stations was not very distinct. A variety of environmental differences e.g. ice coverage, currents, and bottom topography make it difficult to define a single underlying cause.

Most studies of anaerobic benthic mineralization around Svalbard have been carried out in fjord sediments on the south and west coast ((Glud, Holby et al., 1998), (Rysgaard, Thamdrup et al., 1998), (Sagemann, Jørgensen et al., 1998), (Thamdrup and Fleischer, 1998), (Kostka, Thamdrup et al., 1999), (Finke, 2003), (Arnosti, Finke et al., 2005), (Vandieken, Finke et al., 2006)). Anaerobic carbon oxidation rates of sediment incubations (11 to 24 mmol m^{-2} d^{-1}) and sulfate reduction rates (0.9 to 4.2 mmol m^{-2} d^{-1}) in those fjord sediments are considerably higher than the rates measured in the sediments in this study (Tables 1 & 2) ((Sagemann, Jørgensen et al., 1998), (Kostka, Thamdrup et al., 1999), (Finke, 2003), (Vandieken, Finke et al., 2006)). We suggest that temperature does not account for these differences, since the *in situ* temperature in sediments around Svalbard varies only from –1 to +3°C. Water depths of ≥300 m and the annually low primary production on the northeast coast ((Wassmann and Slagstad, 1993), (Sakshaug, 1997)) result in less organic carbon settling to the sea floor and may be responsible for the low rates of carbon oxidation in these sediments. In contrast, fjord sediments on the west coast receive a relatively large flux of organic carbon through primary productivity in the water column ((Eilertsen, Taasen et al., 1989), (Hop, Peason et al., 2002)). Therefore, we suggest that the supply and availability of organic carbon, and not low temperature, limits benthic activity on the northeast coast off Svalbard.

The organic carbon deposition to the sediment is an important factor regulating the importance of microbial Mn and Fe reduction (Thamdrup, 2000). High carbon deposition favors sulfate reduction. The hydrogen sulfide produced reacts with Mn and Fe, leaving less for microbial reduction. Intermediate organic carbon deposition increases the relative importance of suboxic respiration pathways (nitrate, Mn and Fe reduction) ((Wang and Van Cappellen, 1996), (Wijsman, Herman et al., 2002)). Accordingly, microbial Fe reduction in the fjord sediments of Svalbard and Greenland, with relatively high primary production, contributed 0 to 26% to carbon oxidation ((Rysgaard, Thamdrup et al., 1998), (Kostka,

Thamdrup et al., 1999), (Glud, Risgaard-Petersen et al., 2000), (Vandieken, Finke et al., 2006)). In sediments of the open Barents Sea with lower plankton production due to cold currents and long periods of ice coverage, we found that microbial Mn and Fe reduction were the most important anaerobic terminal electron accepting pathways (69 to >90% of anaerobic carbon oxidation).

In conclusion, the terminal electron accepting pathways in these Arctic sediments are regulated by carbon deposition to the sea floor, which is highly influenced by the duration of ice coverage. In sediments from shallow water depths and with long ice-free periods, such as fjord sediments at the south and west coast of Svalbard, sulfate reduction will be the most important pathway for carbon mineralization. However, large parts of the Arctic Ocean are deeper and covered with ice during most or all of the year. Here, the suboxic pathways of Mn and Fe reduction will be important terminal electron accepting pathways.

A gradual retreat of the sea ice cover due to global warming will expectedly result in a preferential stimulation of sulfate reduction at the expense of metal reduction and a shallower suboxic zone. This will enhance the mobilization of Mn from the sediment and loss of Mn^{2+} to the water column. In the long term, this could result in a re-allocation of Mn in Arctic sediments from the continental shelf into deeper waters.

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Ice cover and organic carbon deposition as regulating factors for microbial Mn(IV) and Fe(III) reduction in the northern Barents Sea

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Carbon oxidation rates and pathways were determined in two sediments at latitude 75° and 78° in the northern Barents Sea. Seasonal ice cover restricts primary production to few months a year, which determines the sedimentation rate of organic material to the seafloor. Lower organic carbon content and lower sedimentation rates were found in sediments with an extended presence of ice cover. These factors accompanied by relatively high concentrations of reactive Mn(IV) and Fe(III) oxides favored dissimilatory Mn(IV) and Fe(III) reduction (>98% of anaerobic carbon oxidation) over sulfate reduction from 0-12 cm. In contrast, sediments that had not been ice-covered for the last 12 month contained more organic carbon and showed higher sedimentation rates. A vertical zonation of anaerobic mineralization was observed, with Fe(III) reduction at 0-3 cm followed by concurrent Fe(III) and sulfate reduction at 3-4.5 cm and sulfate reduction at 4.5-12 cm. Oxygen consumption rates (1.9 and 3.7 mmol m^{-2} d^{-1}) and anaerobic CO_2 production rates (1.3 and 6.4 mmol m^{-2} d^{-1}) were comparable to rates from open ocean sediments further north in the Barents Sea but lower compared to those in fjords.

INTRODUCTION

Benthic microbial communities depend on sedimentation of organic carbon from the water column to the sediment. In Arctic environments the organic material can derive from primary production by phytoplankton from the surface water or sea-ice, and additionally be released from melting sea-ice (Schubert and Calvert, 2001) and glaciers. In the northern Barents Sea the productivity of plankton depends on the partial or complete retreat of ice from the marginal seas allowing light to become available for photosynthesis. When the ice melts in spring or summer a stratified water column with a nutrient rich euphotic zone develops, due to the input of low-salinity meltwater and suppressed wind mixing (Wassmann and Slagstad, 1993). This supports the development of intensive phytoplankton blooms that follow the receding ice edge. Greatest primary production is found along the marginal ice zone (Sakshaug and Slagstad, 1991). The open water season in the northern Barents Sea is relatively

short with a maximum ice extent during late winter / spring and a minimum in late autumn (Falk-Petersen et al., 2000) restricting primary production to a short period. Recent studies showed that the organic carbon (C_{org}) content and sedimentation rates in Arctic sediments around Svalbard were related to the sea-ice conditions in the surface waters ((Hulth et al., 1996), (Vandieken et al., 2006b)). The longer an area had been ice-free the more organic material settled to the sea floor.

The complete oxidation of this deposited material by benthic organisms is mediated through a consecutive sequence of respiratory processes with different inorganic electron acceptors (O_2, NO_3^-, Mn(IV), Fe(III) and SO_4^{2-}). The general depth sequence of these oxidants corresponds to a gradual decrease in their redox potential and as a result, a vertical zonation of the pathways is observed in sediments (Jørgensen, 2000). The different electron accepting pathways can thereby spatially overlap with each other, depending on substrate availability or substrate reactivity ((Canfield et al., 1993b), (Kostka et al., 1999)).

The quantitative importance of the various carbon mineralization pathways has been studied intensely and it is generally found that oxygen and sulfate play the major role in shelf sediments (Jørgensen, 2000). An important factor controlling the relative significance of each microbial process is the availability of organic carbon. Oxic mineralization is the dominant degradation pathway at low carbon fluxes (Wijsman et al., 2002). With increasing carbon deposition rates oxygen is rapidly consumed in the surface sediments and anaerobic degradation processes become more important. 25-50% of C_{org} might then be mineralized by sulfate reducing bacteria (Jørgensen, 1982). The relative importance of suboxic respiration pathways like NO_3^--reduction, Mn- and Fe-reduction increase when the carbon flux is intermediate ((Wang and Van Cappellen, 1996), (Wijsman et al., 2002)).

Many studies have focused on the quantification of benthic processes in Arctic environments. Most studies, however, were conducted in fjord sediments and much less data is available from open ocean areas. 3 stations in the northern Barents Sea, northeast of Svalbard, were investigated the preceding year where carbon deposition rates were intermediate concomitantly with high concentrations of particulate Mn and Fe(III) (Vandieken et al., 2006b). These conditions together with only short ice-free periods during the year favored microbial Mn and Fe reduction, which accounted for 69% to >90% of total anaerobic carbon mineralization. Significantly lower contributions (0-26%) were found in Arctic fjord sediments that often remain ice-free

throughout the year ((Rysgaard et al., 1998), (Glud et al., 1998), (Kostka et al., 1999), (Vandieken et al., 2006a)). The aim of the present study was to quantify the different pathways of microbial respiration in marine sediments off the south east coast of Svalbard with particular focus on microbial Mn and Fe reduction. The relationship between ice cover, sedimentation rates and benthic microbial processes should be further investigated to extend the so far small database from Arctic open ocean areas. The Barents Sea offers good opportunities for this kind of studies, because the extent and duration of ice cover vary significantly from south to north. Two stations were chosen, one being still ice-covered in late May and thereby being comparable to the ice-covered station further north (Vandieken et al., 2006b) and the other one having been ice-free during the last year.

MATERIAL AND METHODS

Sampling sites. Sediments were sampled during the third and last CABANERA (Carbon flux and ecosystem feed back in the northern Barents Sea in an era of climate change) cruise from 18 May to 5 June 2005 on board RV "Jan Mayen". An overview of the location and characteristics of the benthic stations are given in Fig. 1 and Table 1. Station 17 was located in the northern Barents Sea, east of Svalbard on the east side of Storbanken. Up to 70% of the area was ice-covered during sampling. Station C was positioned in the Hopen trench, southeast of Svalbard and was free of ice. Both stations were coupled to pelagic sampling stations within the CABANERA project. The sampling stations were continuously numbered with Latin numbers to simplify the possibility for comparison between datasets from different investigators. However, since the present study focused on two stations with numbers that can easily be mixed when written in Latin (XVII and XVIII), we chose to use the corresponding roman numbers instead. All sediments were subsampled from a 50 cm x 50 cm box core into multiple coring tubes with an inner diameter of 10 cm.

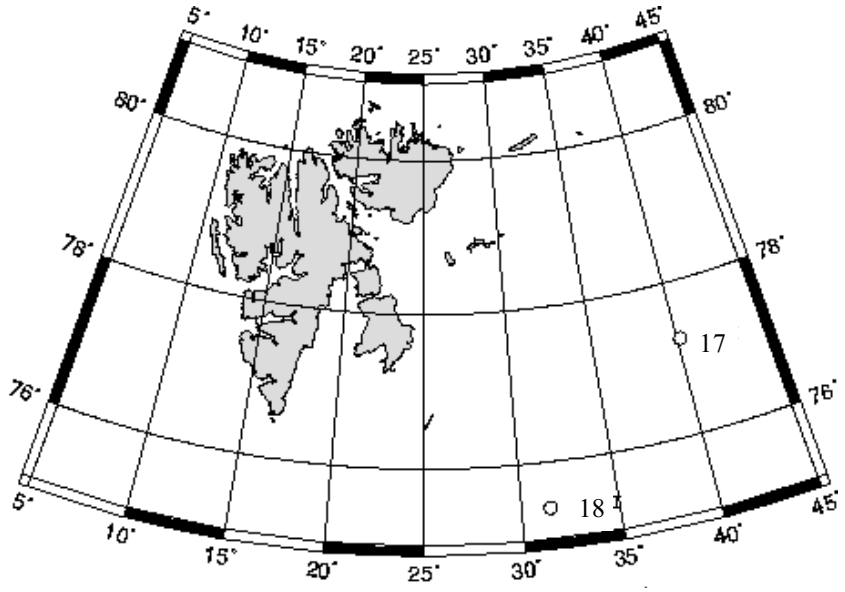

Fig. 1 The Svalbard archipelago with the sampling stations indicated.

Anoxic bag incubations. Sediment from the upper 12 cm of several cores was sliced into the following depth intervals: 0-1.5, 1.5-3, 3-4.5, 4.5-6, 6-8, 8-10, and 10-12 cm. The specific depth intervals were pooled from 15 cores and filled into gastight plastic bags (Hansen et al., 2000) under a constant stream of N_2. The bag preparations were performed on deck at an air temperature around 0°C. The incubation bags were closed without air space and incubated near the in situ temperature at 0°C inside larger plastic bags that were also filled with N_2 to ensure oxygen-free conditions. Ten subsamples were withdrawn over an incubation period of 29 and 45 days for station 18 and 17, respectively, in a cold room at 4°C. After each subsampling the outer bags were flushed and filled with N_2.

Pore water and solid phase sampling. Separate cores with an inner diameter of 36 mm were taken out of the box core for sampling of pore water and sediment. Pore water of whole cores and pore water from the bags were obtained with a pore water press under N_2 pressure and passed directly through GF/F filters. Pore water was directly filtered into a Ferrozine-solution to determine the concentration of Fe^{2+} (see below). 1.8 ml aliquots for dissolved inorganic carbon (DIC) was collected in glass vials, fixed with 20 µl of saturated $HgCl_2$ solution, capped with Viton septa without headspace, and stored at 4°C until later analysis. 1.5 ml of pore water was frozen for the analysis of NH_4^+, NO_3^-, and NO_2^-. For the analysis of Mn^{2+} and Ca^{2+} 0.5 ml was

acidified with 20 µl of 6 M HCl and stored at 4°C. Pore water for sulfate (450µL) and sulfide (500µL) analyses was preserved with 2% Zn acetate (50µL for sulfate) or 2% $ZnCl_2$ (500µL for sulfide). Bottom water from the sampling stations was stored frozen for later determination of NH_4^+, NO_3^-, and NO_2^- and acidified with 6 M HCl for Mn^{2+} analysis.

For the extraction of solid phase Fe and Mn as well as for the determination of total organic C and N, subsamples were stored frozen at –21°C.

Table 1. Sample site information and sediment characteristics at 2 stations (17 and 18) from 18 May to 5 June 2005. Data are for whole core incubations. O_2: O_2 comsumption rate; SRR: sulfate reduction rate (0-20 cm).

Stationnumber	**17**	**18**
Description	Central Barents Sea, East Storbanken	Hopen Trench
Latitude	77° 25.64 N	75° 40.80 N
Longitude	40° 18.31 E	31° 48.70 E
Water depth (m)	208	340
Sediment temperature (°C)	0.0	2.2
Total organic carbon (% dry weight)	1.42	2.55
Total organic nitrogen (% dry weight)	0.19	0.32
O_2 (mmol m^{-2} d^{-1})	1.9 ± 0.9	3.7 ± 1.2
SRR (mmol m^{-2} d^{-1})	0.03 ± 0.02	0.14 ± 0.08

Pore water analyses. DIC was analyzed by flow injection with conductivity detection (Hall and Aller, 1992). Fe was measured spectrophotometrically with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) at 562 nm using a Dr. Lange LP2W Spectrophotometer (Stookey, 1970). NH_4^+ was determined spectrophotometrically at 630 nm with a Shimadzu UV 1202 spectrophotometer (Grasshoff et al., 1999). NO_3^- and NO_2^- were measured using a NOx-Analyzer (Thermo Environmental Instruments, Franklin, USA) after (Braman and Hendrix, 1989). Ca^{2+} and Mn^{2+} in pore water were measured by inductively coupled plasma atomic emission spectrometry on a Perkin Elmer Optima 3300 RL. Sulfate was measured by non-suppressed ion chromatography (Waters, column IC-PakTM, 50 x 4.6 mm) and conductivity detection after (Ferdelman et al., 1997). Sulfide was determined by the methylene blue method at 670 nm with a Shimadzu, UV 1202 spectrophotometer after (Cline, 1969).

Solid phase analyses. Reactive Fe was extracted from frozen sediment with HCl (0.5 M HCl for 1 h at RT) and the extract was analyzed for Fe(II) with Ferrozine and for total Fe with Ferrozine plus 1% (w/v) hydroxylamine hydrochloride (Kostka and Luther III, 1994). Fe(III) concentrations were calculated by subtracting concentrations of Fe(II) from total Fe concentrations. Reactive Mn in the sediment was quantified after freeze-drying and extraction with dithionite-citrate-acetic acid (Canfield, 1989) by flame atomic absorption spectrometry (Perkin Elmer, Atomic Absorption Spectrometer 3110). For determination of total organic C and N contents, freeze dried samples were pretreated with HCl, dried again, and analyzed using a CNS analyzer (FisonsTM Na1500 elemental analyzer).

Sulfate reduction rates. Sulfate reduction rates were measured on three parallel cores of 28 mm diameter using the $^{35}SO_4^{2-}$ whole core injection technique (Jørgensen, 1978). Sulfate reduction rates in the anoxic bags were determined at every time point by subsampling sediment into 5 ml glass tubes, which were incubated with 100 kBq of $^{35}SO_4^{2-}$ radiotracer. The incubations were stopped after 12-24 h with 20% Zn acetate. Total reduced inorganic sulfur was extracted by the cold chromium distillation (Kallmeyer et al., 2004). Rates were calculated following the procedures described in (Jørgensen, 1978).

Sediment oxygen uptake rates. 5-7 sediment cores with an inner diameter of 54 mm were taken from each station and submerged into a water bath that was filled with bottom seawater and incubated in the dark at 0°C. The sediment cores remained open for 7-96 h before they were closed with rubber stoppers without gas phase. The water column overlying the sediment was continuously stirred by a magnetic bar, which was placed about 10 cm above the sediment surface. Oxygen concentrations in the water were measured continuously with a micro-optode (Holst et al., 1997) that was immersed into the water overlying the sediment. The micro-optode was calibrated with bottom water that had been flushed with N_2 or O_2 for 1 h, for 0% and 100% oxygen saturation.

RESULTS

Pore water and solid phase chemistry of Station 17 and 18. The total organic carbon and total organic nitrogen content of the sediment were higher at station 18 than at station 17 (Table 1). Dissolved inorganic carbon (DIC) concentrations and ammonium concentrations increased with depth (Fig. 2A and 2D). Sulfate concentrations stayed constant over depth, and were 26 mM at both stations (data not shown). Sulfide concentrations in the pore water were below the detection limit of 1 μM. Dissolved Mn^{2+} concentrations in the pore water of the northwestern station 17 increased below 3 cm to a maximum of 29 μM at 10 cm depth, whereas they never exceeded 2.5 μM at station 18 over the entire core length (Fig. 2B and 2E). At station 18 dissolved Fe^{2+} concentrations of the pore water stayed below 4 μM over the whole core length (Fig. 2E). Dissolved Fe^{2+} was below the detection limit of 1 μM at station 17. Bottom water concentrations of nitrate were 9 and 12 μM at station 17 and 18 and the penetration depth were 8 and 2 cm, respectively, below which a stable background level of 2 μM was reached (Fig. 2B and 2E). Nitrite concentrations were <1μM at station 17 and station 18.

Station 17 had high concentrations of solid phase Mn (IV) and Fe(III) in the upper 10 cm and 14 cm, respectively (Fig. 2C). Fe(II) increased below 6 cm. At station 18 solid Mn (IV) was only found in the uppermost cm of the sediment (2.2 μmol cm^{-3}), below which depth concentrations remained ≤0.3 μmol cm^{-3}. Fe(III) was detectable to 4 cm depth, concomitant with an increase in Fe(II) from the surface to 3 cm depth, and a more gradual increase below this depth (Fig. 2F).

Oxygen consumption and sulfate reduction rates of whole cores. Sediment oxygen uptake rates were twice as high at station 18 as at station 17 (Table 1). Sulfate reduction rates were very low at station 17 with a maximum of 0.6 nmol cm^{-3} d^{-1} at 3 cm depth (Fig. 3A). They were higher at station 18 and increased with depth to a maximum of 1.8 nmol cm^{-3} d^{-1} at 18 cm depth (Fig. 3B). Integrated rates were 5 times the rates from station 17.

Anaerobic carbon oxidation in bag incubations. DIC concentrations increased linearly during the bag incubation at both stations, which indicated constant

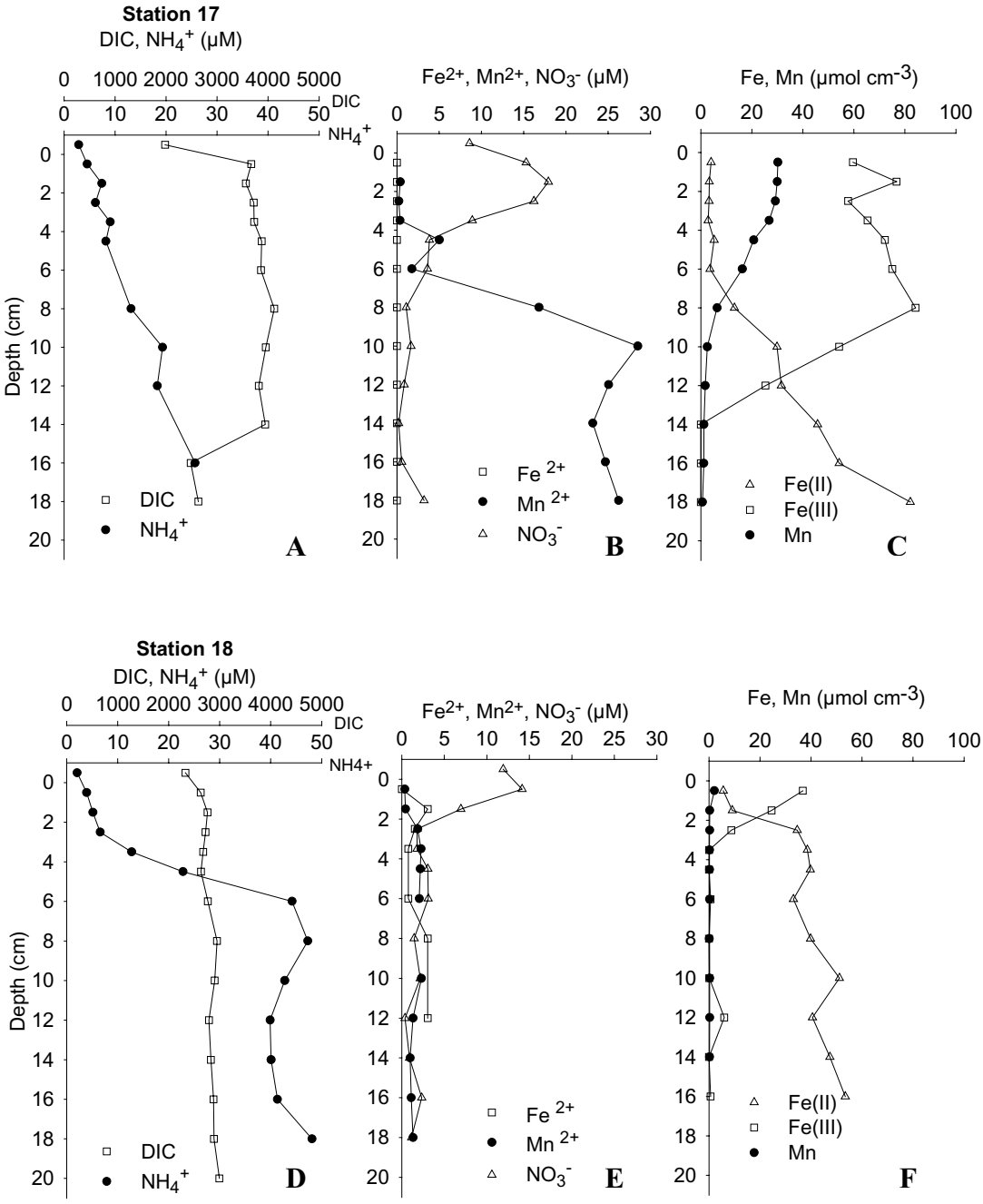

Fig. 2 Concentrations of pore water constituents (DIC, NH_4^+, Fe^{2+}, Mn^{2+}, and NO_3^-) and solid phase (Mn, Fe(II) and Fe(III)) from station 17 (A-C) and station 18 (D-F).

degradation of organic matter (data not shown). This was supported by constant Ca^{2+} concentrations, which showed that $CaCO_3$ did not precipitate (data not shown).

Station 17

DIC and NH_4^+ production rates in the pore water decreased with sediment depth (Fig. 4A). Sulfate reduction rates were below 0.4 nmol cm^{-3} d^{-1}, similar to the whole core

incubations (Fig. 4A and Fig. 3A). Pore water sulfide was not detected. Mn^{2+} accumulated in the pore water of all bag incubations and rates were highest in the 3-4.5 cm depth interval (6.9 nmol cm^{-3} d^{-1}) (Fig. 4A). In the two bags containing the upper 3 cm of the sediment, Mn^{2+} concentrations in the pore water increased only after 16 days (data not shown), even though solid phase Mn was present (Fig. 2C). Fe^{2+} concentrations in all bags were low throughout the incubation.

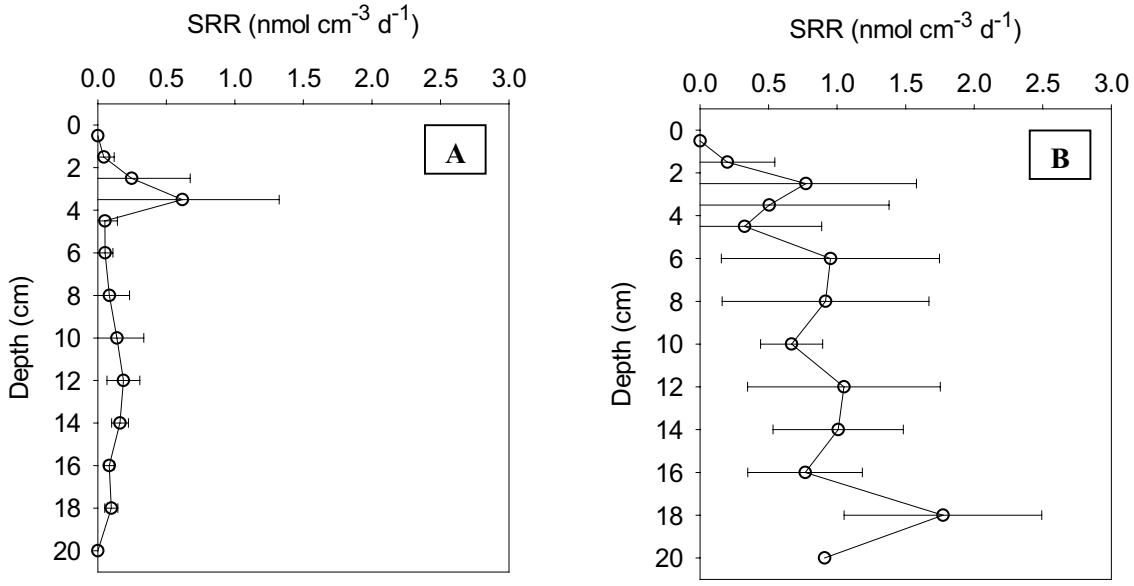

Fig. 3 Sulfate reduction rates (SRR) at station 17 (A) and 18 (B) from whole-core incubations. Error bars indicate standard deviation from triplicate measurements.

Station 18

The rates of DIC production in the pore water increased with depth while NH_4^+ production rates were relatively invariable with depth (Fig. 4B). Sulfate reduction rates over time were very low in the top 1.5 cm (<2 nmol cm^{-3} d^{-1}) and stayed constant from 1.5-3 cm (<8 nmol cm^{-3} d^{-1}) (data not shown). Below 3 cm rates increased with time. Highest sulfate reduction rates were found at 8-10 cm, yielding 40 nmol cm^{-3} d^{-1} after 29 days (Fig. 4B). Sulfide was not detected in the pore water. Mn^{2+} only accumulated in the pore water in the top 3 cm where the accumulation rate was highest, 7.5 nmol cm^{-3} d^{-1} in the top 1.5 cm (Fig. 4B). This corresponded to the distribution of solid phase Mn, which also showed highest concentrations in the top 1.5 cm, but decreased steeply below. Reactive Fe(III) was available in the upper 3 cm of the sediment and Fe^{2+} accumulated in the pore water with low rates down to 8 cm (data not shown). The steepest increase in the Fe^{2+} concentrations was found in the

bag containing sediment from 1.5-3cm depths after the Mn^{2+} concentrations stayed constant.

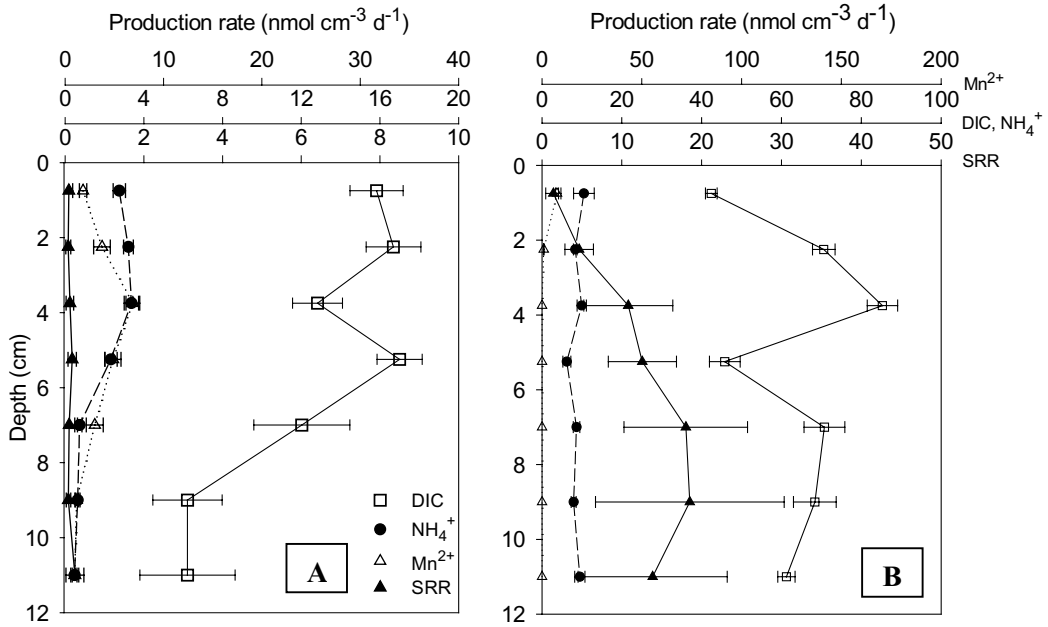

Fig. 4 Anaerobic carbon mineralization rates (DIC, NH_4^+ and Mn^{2+}) and sulfate reduction rates (SRR) from bag incubations at station 17 (A) and station 18 (B). Error bars indicate standard errors from linear regression of concentration over time, in case of DIC, NH_4^+ and Mn^{2+}, and standard deviation of SRR over time.

DISCUSSION

Anaerobic carbon oxidation at station 17 and station 18. Many biological and chemical reactions are involved in the chemistry and recycling of Fe and Mn in the marine sediment. Bacteria reduce Fe(III) or Mn(IV) to oxidize organic matter but often compete with abiotic reactions because the oxidized and reduced forms of the metals react spontaneously with a variety of compounds. The most significant reductant for Fe(III) in sediments is hydrogen sulfide (Canfield et al., 2005a) produced by sulfate reducing bacteria. These can be responsible for up to 50% of the total carbon mineralization in coastal sediments (Jørgensen, 1982). Mn oxides can be reduced by H_2S or Fe(II). Because of these re-oxidation processes with Mn and Fe(III) it is difficult to quantify dissimilatory Mn and Fe reduction and distinguish them from abiotic reduction. So far, no assays are available to determine Fe and Mn reduction separately (Thamdrup and Canfield, 2000). A useful, but indirect method for quantifying dissimilatory Fe and Mn reduction rates in marine sediments is to

determine CO_2 accumulation rates in anaerobic bag incubations and to subtract the contribution of sulfate reduction (Canfield et al., 1993b). To compare sulfate reduction with DIC production rates sulfate reduction rates are commonly multiplied by 2 assuming a stoichiometry of 2:1 moles of carbon oxidized (= DIC produced) to sulfate reduced (Thamdrup and Canfield, 1996). Since sediment is incubated anaerobically, the supply of oxygen and nitrate from the water column is cut off. Thus, the excess of carbon oxidation above that explained from sulfate reduction can be attributed to dissimilatory metal reduction.

Station 17

DIC production at station 17 exceeded sulfate reduction at all depth indicating that electron acceptors other than sulfate were used for organic matter mineralization. Relatively high contents of solid Mn and solid Fe(III) reached down to 8 and 12 cm, respectively, with solid Fe(III) concentrations being approximately two-fold higher (up to 84 µmol cm^{-3}) than Mn(IV) (up to 30 µmol cm^{-3}) (Fig. 2C). Additionally, Mn^{2+} accumulated in the pore waters of all bags, which indicated active Mn reduction (Fig. 4A). In contrast, Fe^{2+} concentrations in the pore water remained low in all bags throughout the incubation. We assume that microbial Fe reduction indeed took place but could not be detected due to rapid chemical re-oxidation of Fe^{2+} by Mn(IV). It has been shown that sulfate reduction can be inhibited by Fe reduction (Lovley and Phillips, 1987) and/or by high contents of Mn oxides in marine sediments (Canfield et al., 1993b). The inhibitory effect is presumably competition for common substrates of sulfate-, Fe, and Mn reducing bacteria. Based on the Mn and Fe oxide content of the sediment and the accumulation rates of Mn^{2+} we infer that the excess DIC production over SRR at station 17 from 0-12 cm is attributable to microbial Mn and Fe reduction. Due to the reoxidation processes it was not possible to discriminate between Mn and Fe reduction and as a result we could not determine whether dissimilatory Fe or Mn reduction dominated. The contribution of dissimilatory metal reduction to anaerobic carbon oxidation was calculated to be >98%, while SRR accounted for <2% at station 17 from 0-12 cm.

Station 18

At station 18 DIC production also exceeded sulfate reduction in the upper 12 cm of the sediment. Concentrations of solid Mn and Fe were however, in contrast to station

17, only detected down to 1 and 4 cm, respectively (Fig. 2F), so they could not support dissimilatory metal reduction below 4 cm. The Mn^{2+} accumulation rate in the pore water was high in the upper 1.5 cm (7.5 nmol cm^{-3} d^{-1}), while Fe^{2+} concentrations stayed low (≤7 nmol cm^{-3}) (Fig. 4B and Fig. 2E). Rapid depletion of reactive Mn was supported by the low Mn content of the solid phase (<2.2 µmol cm^{-3}) suggesting that all Mn^{2+} production in this depth was coupled to the chemical re-oxidation of Fe and sulfide, rather than being due to microbial respiration (Canfield et al., 1993a). The high Mn^{2+} accumulation rate indicates that actual Fe reduction rates were much higher than those measured from the accumulation of Fe^{2+} in the pore water. In the bag with mixed sediment from 1.5-3 cm depth interval, Fe^{2+} accumulated at a high rate in the pore water (4.9 nmol cm^{-3} d^{-1}) while the Mn^{2+} concentration stayed low. Thus, we conclude that dissimilatory Fe reduction was the predominant process of anaerobic carbon mineralization in the upper 3 cm of the sediment. Below 3 cm, Fe^{2+} production rates were low (0.6 nmol cm^{-3} d^{-1}), which was in accordance with decreasing Fe(III) concentrations (Fig. 2F), while sulfate reduction rates increased (Fig. 4B). We therefore conclude that from 3-4.5 cm Fe reduction and sulfate reduction occurred simultaneously in the sediment.

From 3-12 cm neither Mn nor Fe(III) were detected by HCl extraction (Fig. 2F) and sulfate reduction rates were too low to account for all of the DIC production (Fig. 4B). Thus, we assume that the experimentally measured sulfate reduction rates underestimated the in-situ sulfate reduction, since no other electron acceptors were available below 4 cm.

With the method of determining sulfate reduction rates by means of incubating sediment with radiolabeled SO_4^{2-} tracer some considerations have to be taken into account. Rates are calculated based on the reduction of $^{35}SO_4^{2-}$ to $H_2{}^{35}S$. In such an experiment $H_2{}^{35}S$ is only the first product being formed, whereas secondary radiolabeled products like $^{35}S^0$, $Fe^{35}S$ and $Fe^{35}S_2$ will evolve from $H_2{}^{35}S$ via chemical reactions or by isotopic exchange (Fossing, 1995). $H_2{}^{35}S$ can also be re-oxidized to $^{35}SO_4^{2-}$ and rate determinations can therefore greatly underestimate true sulfate reduction rates. (Fossing, 1995) observed that the linear relation between the incubation time for determining sulfate reduction rates and the radioactivity of the reduced sulfur pool depended on the re-oxidation rate. A high gross sulfate reduction rate was found at the very beginning of the experiment with marine surface sediment from Skagerrak, Denmark. However, with time, rates deviated from the gross sulfate

reduction rate to approach a net sulfate reduction rate that was 67% lower. Similar observations were made by (Moeslund et al., 1994) and were in both cases explained by re-oxidation of reduced ^{35}S-compounds, particularly of $H_2{}^{35}S$, to $^{35}SO_4{}^{2-}$. From that the authors concluded that the incubation length had to be short enough to ensure a linear relationship between time and decrease in radioactivity.

Short term incubations (2-6h) with radiotracer are therefore usually performed to determine SRR ((Canfield et al., 1993b), (Arnosti et al., 1998), (Kostka et al., 1999)). From our former experience with Arctic sediments (Vandieken et al., 2006b), incubation times of 12-24h were chosen in order to be able to detect sulfate reduction in these sediments with low carbon oxidation rates. Nevertheless, within 24 h some re-oxidation probably has taken place so that the measured sulfate reduction rates most likely underestimated the gross rates. Hence to be able to calculate to what extent sulfate reduction contributed to anaerobic carbon oxidation we assume that below the zone where Fe(III) and Mn was detected (≤4 cm), sulfate reduction was the only important anaerobic carbon oxidation pathway. Metal reduction was, accordingly, only important in the uppermost 4 cm of the sediment. Since sulfate reduction in this part of the sediment was probably also underestimated we calculated a maximum contribution of metal reduction for 0-12 cm to be <20% of the anaerobic carbon mineralization, while sulfate reduction contributed the remaining 80%.

Fe and Mn reduction in Arctic sediments. The quantitative importance of the different terminal electron accepting pathways for carbon oxidation has been studied by many authors. Aerobic respiration and sulfate reduction are generally considered the most important metabolic pathways in shelf sediments. Sulfate reduction typically accounts for 25-50% of the total carbon oxidation in coastal sediments (Jørgensen, 1982). Nitrate reduction has been shown to be of minor importance for organic matter oxidation in marine sediments (≤6% of total carbon oxidation) ((Canfield et al., 1993a), (Kostka et al., 1999), (Glud et al., 2000)). Microbial Mn(IV) reduction is suggested to be insignificant because the Mn oxide content in marine shelf sediments is generally low. However, there are continental margin areas with high Mn deposits, such as the Panama Basin and deep parts of Skagerrak, where dissimilatory Mn reduction contributed >90% to carbon mineralization ((Aller, 1990), (Canfield et al., 1993b)). Microbial Fe(III) reduction was found to contribute 17% in average to total

carbon mineralization in a broad selection of continental shelf sediments (Thamdrup, 2000).

Table 2. Comparison of station characteristics and carbon mineralization rates from selected Arctic environments. nd not determined

Description	This study St. 17	This study St. 18	Barents Sea northeast of Svalbard (St.VIII, X, XII)	Northern Barents Sea, south east of Svalbard	Southern Fjords of Svalbard	Smeeren burg-fjord, North Svalbard	Young Sound, Green-land
Water depth (m)	208	340	503 303 286	191 240 188	155 115 175	212	36
C_{org} (%)	1.4	2.6	1.5 1.4 1.5	1.4	1.5 1.8 2.4	nd	1.4
Sediment accumulation rate (mm y^{-1})	0.7	1.4	1.3 0.6 -[a]	nd	nd	nd	2.3
Oxygen uptake rate (mmol m^{-2} d^{-1})	1.9	3.7	2.1 1.5 3.5[b]	4.3 6.7 3.9	16.4 13.1 9.0[c]	3.8[d]	5-13[e]
Anaerobic C oxidation[f] (mmol m^{-2} d^{-1})	1.3	6.4	2.2 1.5 4.3	nd	24 12 11	10	12
Fe and Mn reduction in % of C oxidation[g]	98	≤23	>90 >87 69	nd	0 26 10	13	25
Ref. [h]	This study	This study	(1)	(2)	(3)	(4)	(5)

[a] A. Zaborska, unpublished data

[b] Sediment oxygen uptake rate measured in cores

[c] Oxygen uptake rates determined with benthic flux chambers

[d] Uptake was determined from the difference between anaerobic DIC production and DIC release in whole core incubations (0-5cm)

[e] 5 mmol m^{-2} d^{-1} when fjord was ice-covered, 13 mmol m^{-2} d^{-1} without ice cover

[f] calculated from $\sum CO_2$ accumulation rate in bag incubations: Ref. (1), (3), (5), and this study 0-10 cm; Ref. (2) 0-5 cm

[g] Ref. (2), (3), and (5): Contribution of Fe and Mn reduction to total C oxidation. Ref. (1) and this study: Contribution of Fe and Mn reduction to anaerobic C oxidation, calculated from sulfate reduction independent DIC production rates

[h] References: (1) (Vandieken et al., accepted), (2) (Hulth et al., 1994), (3) (Glud et al., 1998), (Kostka et al., 1999), (4) (Vandieken et al., 2006a), (5) (Rysgaard et al., 1998)

Three basic conditions must be fulfilled for microbial Mn or Fe reduction to contribute significantly to carbon oxidation in marine sediments: high contents of reactive Mn and Fe oxides, high reactivity of the metal oxides and intermediate carbon loading. Concentration of Mn oxides have to be >20 μmole cm^{-3} since sediments where Mn reduction is only of minor importance have typically lower value (Thamdrup, 2000). Compilation of data from different marine sediments show that dissimilatory iron reduction approximates Michaelis-Menten-type kinetics with respect to Fe(III) (Canfield et al., 2005b). The half-saturation concentration, meaning the minimum concentration of Fe(III) that is needed for Fe reduction to contribute 50% of anaerobic carbon oxidation, was ~10 μmol cm^{-3} (Canfield et al., 2005b).
The deposition rate of reactive Mn and Fe to sediments is usually low compared to that of organic carbon. Accordingly, active mixing of the sediment by infauna is necessary to replenish metal compounds that are reduced in deeper layers (Thamdrup, 2000). Another factor is the availability of organic carbon. With high deposition rates of C_{org}, oxygen is rapidly consumed in the surface sediments and, consequently, anaerobic degradation prevails. However, with high sulfate reduction rates most of the Mn(IV) or Fe(III) will be consumed by hydrogen sulfide, leaving less Mn and Fe for organic matter oxidation (Wijsman et al., 2002). In sediments with low carbon deposition, such as deep-sea sediments, organic matter mineralization is mainly mediated by aerobic respiration (Glud et al., 1994). Consequently, Mn and Fe reduction contribute the most to C_{org} oxidation when the carbon input is intermediate and SRR low (Kostka et al., 1999).

The organic carbon content of the sediments examined in this study (Table 1) was low to intermediate for shelf sediments and agreed well with data from other Arctic sediments (Table 2). (Vandieken et al., 2006b) measured C_{org} concentrations from 1.41-1.46% in the Barents Sea northeast of Svalbard. The area between station 17 and station 18 was investigated by (Hulth et al., 1994) and C_{org} was determined to be 1.4%. Somewhat higher concentrations, 1.5-2.4% were found in fjords along the south coast of Svalbard (Glud et al., 1998). The highest concentration of 3.2% was found in Storfjorden, a semi-enclosed bay situated southeast in the Svalbard archipelago (A.Ahke, unpublished data). Storfjorden is supplied with Arctic Water but is also influenced by nutrient-rich Atlantic Water through the West Spitsbergen current

(Skogseth et al., 2005). C_{org} concentrations comparable to station 18 (2.6%) were found in Hopen Trench (2.4%) and Hopen Bank (2.4%) (A. Ahke, unpublished data). By including data from other CABANERA cruises ((A.Ahke, unpublished data), (Vandieken et al., 2006b)) we could not find a correlation between C_{org} and water depth, which confirmed observations by (Stein et al., 1994) who investigated the distribution of organic carbon in eastern central Arctic Ocean sediments. However, a relationship could be established between carbon concentrations, sedimentation rates, and extension of ice coverage, as described by (Hulth et al., 1996). Sea ice generally retreats northwards along the east coast of Svalbard during late spring (Falk-Petersen et al., 2000). With ice melting and concomitant blooming of phytoplankton, organic material is produced in the water column and will eventually settle down to the sea floor. Thereby, regions that are ice-free during longer periods receive stronger pulses of organic matter. In fact, highest C_{org} values were detected southeast of Svalbard (A. Ahke, unpublished data) while concentrations further north and east were in average ~1% lower. This was also observed for station 17 and 18. Sedimentation rates and C_{org} were twice as high at station 18 (1.4 mm y^{-1}, A. Zaborska unpublished data), which was ice-free at the time of sampling and had been ice-free during the last year (taken from ice charts from the Sea Ice Service from the Norwegian Meteorological Institute, http://met.no) (Table 2). About 70% of the area around station 17 was still covered by ice at the end of May. The sedimentation rate was 0.7 mm y^{-1} (A. Zaborska unpublished data). A similar relationship between ice-cover and C_{org} was found in sediments northeast of Svalbard (Vandieken et al., 2006b) (Table 2). Station VIII, positioned in the Kvitøya trench, became ice-free earlier in the year, due to the northwards retreating sea ice along the east coast of Svalbard. The sedimentation rate (1.3 mm y-1) was twofold higher compared to station X (0.6 mm y-1) even though station VIII was much deeper (Table 2). The much lower sedimentation rate was attributed to the annually longer period of ice coverage at Station X, which was situated further south than station VIII (Vandieken et al., 2006b).

Recently Arctic marine sediments were discovered to also harbor Mn and Fe concentrations sufficient for high rates of dissimilatory metal reduction ((Kostka et al., 1999), (Vandieken et al., 2006a), (Vandieken et al., 2006b)). The organic C deposition in these areas varies as a function of the seasonal light availability and the ice coverage. Microbial metal reduction may account for 0-26% of the carbon mineralization in different fjord sediments of Svalbard (0-10 cm) ((Kostka et al.,

1999), (Vandieken et al., 2006a)). Similar values were also obtained for a fjord system in Northeast Greenland (25% in 0-15 cm) (Rysgaard et al., 1998) (Table 2). A much higher contribution was found in the Barents Sea, northeast of Svalbard (Vandieken et al., 2006b). High concentrations of reactive Mn and Fe(III) accompanied by extremely low SRR and low organic carbon contents in the surface sediments (~1.5%) supported dissimilatory Fe and Mn reduction, which made up >87% of anaerobic carbon mineralization (Table 2). The data presented in this study further substantiate the importance of microbial metal reduction in higher latitudes with low to intermediate carbon supply. Fe and Mn reduction accounted for 98% and ≤23% (integrated over the upper 10 cm of the sediment) of anaerobic carbon mineralization at station 17 and 18, respectively (Table 2).

We propose that the predominance of metal reduction at station 17 is the result of annually longer periods of ice coverage, lower sedimentation rates and lower C_{org} contents in the sediment compared to station 18 (Table 2). This is supported by the high concentrations of Mn and Fe down to 6 and 12 cm depth, respectively, and low rates of sulfate reduction (Fig. 2C and 3A).

Apart from differences in ice cover and sedimentation rate the stations were also distinguished by the bottom water temperature. Station 17 was dominated by a cold overlying water column, where temperatures increased from -1.7°C at the surface to +0.2°C just above the sediment, while bottom water at station 18 was considerably warmer with temperatures increasing from +1.4°C at the surface to +3.3°C at the bottom (Sundfjord et al., submitted).

Carbon oxidation in Arctic marine sediments. Total sediment oxygen uptake rates are often used to estimate overall carbon mineralization in marine sediments. This benthic oxygen consumption integrates aerobic mineralization and the amount of oxygen used to oxidize reduced compounds that are produced by anaerobic processes. The oxygen uptake rates measured in this study of 1.9 and 3.7 mmol m^{-2} d^{-1} (Table 1) were comparable to previous measurements obtained for the central Barents Sea east and northeast of Svalbard by (Vandieken et al., 2006b) (1.5-3.5 mmol m^{-2} d^{-1}) (Table 2). Somewhat higher values (3.9-6.7 mmol m^{-2} d^{-1}) were determined by (Hulth et al., 1994) for the region around station 18 in the southern Barents Sea. We assume that, as for the relative importance of the different terminal electron accepting pathways, the annual duration of ice cover controls the overall microbial activity in Arctic

sediments. This is supported by higher sediment oxygen consumption rates in Arctic fjords that often remain ice-free even during winter compared to open waters. (Kostka et al., 1999) measured O_2 uptake rates of 13.1 and 16.4 mmol m^{-2} d^{-1} in fjord sediments on the west coast of Svalbard (Table 2). This region is influenced by the Western Spitsbergen current with relatively warm North Atlantic Water that flows north along the coast and keeps the fjords ice-free for most of the year (Andruleit et al., 1996). Furthermore, when the oxygen consumption was measured before and after ice melting in a Greenland fjord, (Rysgaard et al., 1998) found that rates almost tripled, from 5 mmol m^{-2} d^{-1} to 13 mmol m^{-2} d^{-1} (Table 2).

The same pattern was reflected if only anaerobic processes were considered. Instead of using the total oxygen consumption of the sediment, the production of CO_2 was used as a measure of the total anaerobic carbon mineralization. Anaerobic DIC production rates, integrated over the upper 10 cm of the sediment, at the ice-free station 18 (6.4 mmol m^{-2} d^{-1}) were fivefold higher than at station 17 (1.3 mmol m^{-2} d^{-1}). Rates within this range were also found further north (1.5-4.3 mmol m^{-2} d^{-1}) by (Vandieken et al., 2006b) (Table 2). The relatively high DIC production rate of 4.3 mmol m^{-2} d^{-1} was explained by a semi permanent polynya west and south west of Kvitøya. Outside this polynya, anaerobic mineralization rates decreased to values comparable to station 17, likewise with an extended ice-cover during the year.

As for oxygen consumption, considerably higher rates of anaerobic carbon mineralization were found in fjords compared to the open ocean (Table 2). These rates were two- to tenfold higher (10-24 mmol m^{-2} d^{-1}), even including the fjord studied on Greenland, which was only ice-free for 2 months per year (Rysgaard et al., 1998).

CONCLUSIONS

Pathways of anaerobic carbon mineralization processes in sediments from the Barents Sea are controlled by the input of organic material from the water column. In high Arctic environments this depends on how fast the sea ice melts in spring to give rise to primary production. Where the water column experiences only short ice-free periods during the year sediments are characterized by lower organic carbon deposition. These conditions in combination with relatively high concentration of reactive Mn and Fe were found to favor microbial Mn and Fe reduction in the Barents Sea.

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Thermophilic bacterial activity in an Arctic sediment, Svalbard

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ABSTRACT

Sulfate reduction was measured in sediment slurries from Smeerenburgfjord, Svalbard in a temperature profile from 0-80°C. Two optima of sulfate reduction rates were observed at 21°C and 54°C with a significant low between the two temperatures. The large temperature gap of 30°C between the two optima indicated that two communities of sulfate reducing bacteria were present, a psychrotolerant and a thermophilic community. After pasteurization only thermophilic sulfate reduction was detected and rates were comparable to the non-pasteurized sediment, which indicated that the sulfate reducing bacteria (SRB) were most likely spore-forming species. Time course experiments at 50°C showed that thermophilic SRB needed 16-24 h to germinate. After that SRR increased exponentially until the SRB were limited by substrate or electron acceptor availability or both. The presence of fermentative bacteria was indicated by increasing concentrations of volatile fatty acids immediately after heating. Thus, the fermentative bacteria appeared to germinate faster than the SRB. Substrate producing and consuming organisms were found after permanently cold sediment with an in situ temperature of 2.3°C was heated to 50°C. It seems unlikely, given the large difference of 50°C that the thermophilic bacteria originate from Arctic sediments. However, the source of these bacteria is still unknown.

INTRODUCTION

Thermophilic aerobic and anaerobic spore-forming bacteria with temperature optima for growth at 50-65°C have been discovered in permanently cold marine environments that do not support growth of these organisms ((Isaksen et al., 1994), (Bartholomew and Paik, 1966)). Aerobic spore-forming *Bacillus*-strains were isolated from ocean basin sediment about 2.5 m below the sediment surface with an in-situ temperature of 4°C (Bartholomew and Paik, 1966). All of their isolates grew at 65°C

but not at 28°C. (Isaksen et al., 1994) discovered that activity of thermophilic sulfate reducing bacteria could be induced in cold sediments of Denmark, a temperate environment.

It is unlikely that these thermophilic bacteria could have grown in the cold sediments in which they were found and different sources have been suggested by the authors. (Bartholomew and Paik, 1966) presented as a possible explanation for the origin of thermophilic spores in deep-sea sediment cores at about 175 cm core depth that these spores might be living fossils that were deposited at the same time as the sediment. Waste water was considered as a possible origin for *Desulfotomaculum kuznetsovii*, which was isolated from Århus bight, Denmark where temperatures never exceed 15°C (Isaksen et al., 1994). Due to the isolation of Bacillus strains from geothermal areas, from environments with constantly low temperature and additionally from air samples (Bonjour et al., 1988) suggested that spores could be transported by wind from the place they originate to other environments. Additionally, the authors concluded that spores would need high and constant humidity to remain viable for a long time, because changing conditions would probably interrupt their dormancy, shortening their survival.

The aim of the present study was to search for thermophilic sulfate reducing bacteria in a pristine environment of the opposite temperature range. If spores can be transported by wind or water one might be able to find them in remote places far away from where they originate. Marine sediments of high latitudes are characterized by low temperatures and seasonal to permanent ice cover. These conditions make them the least likely spot to find thermophilic bacteria. The following questions will be addressed with this study: 1) Can thermophilic bacterial activity be found in Arctic marine sediments, 2) if activity can be detect at elevated temperatures, how fast do these bacteria germinate and grow, and 3) does a complex bacterial community exist, adapted to high temperature, when the in-situ temperature is only 4°C? Furthermore, the diversity of biogeochemical processes at elevated temperature was to be investigated.

MATERIAL AND METHODS

Location and Sampling. Marine sediment was sampled from Station J in Smeerenburgfjord (79°42.006' N, 11°05.199' E) with a HAPS corer on board R/V

Farm in August 2003 and July 2005. Smeerenburgfjord is situated on the northwest coast of Spitsbergen, the main island of the archipelago Svalbard. The water depth was 212 m and the bottom water temperature was +2.3°C. Sediment from two depth intervals (0-3cm and 3-9cm) was filled into gas-tight plastic bags (Hansen et al., 2000) on board the ship. The bags were placed inside larger plastic bags that were filled with N_2 to ensure oxygen-free conditions and stored at 4°C in the dark.

Sulfate reduction in whole cores. Subcores (ø 28 mm) were taken from intact sediment cores immediately after core retrieval. Along the sides of the tubes were silicone rubber ports at intervals of 1 cm through which 10µL of a $^{35}SO_4^{2-}$-tracer solution (10 kBq μL^{-1}) were injected without disturbing the sediment. The cores were incubated in the dark for 8h at in-situ temperature. To stop bacterial sulfate reduction the sediment was cut into slices of 1-2 cm thickness, mixed with 20 mL of zinc acetate (20% w/v) and frozen for transport. Reduced ^{35}S was analyzed by the cold chromium distillation after (Kallmeyer et al., 2004). Sulfate reduction rates were calculated per cm^3 sediment or per cm^3 sediment slurry (see below) as described by (Jørgensen, 1978).

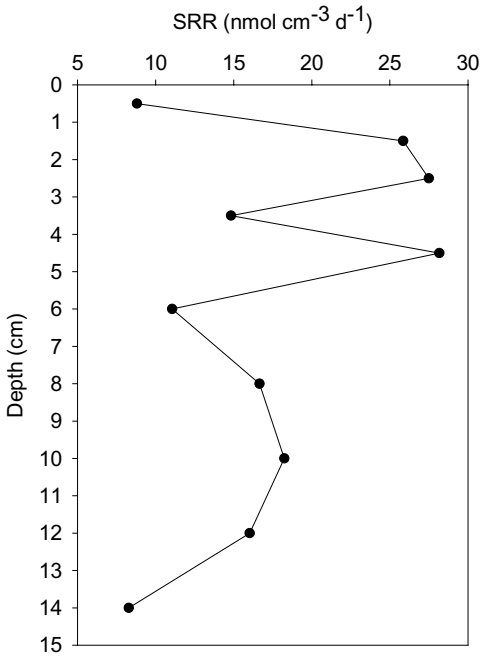

Fig. 1 Depth profile of sulfate reduction rates in the sediment from Station J, Smeerenburgfjord (2003). Data show the mean of two cores.

Temperature gradient block experiments. Slurries were prepared by mixing sediment (3-9 cm depth interval, August 2003) 1:1 (v/v) with anoxic surface seawater. 10 mL of this homogenized slurry were transferred anoxic into 15 mL Hungate tubes that were sealed with butyl rubber stoppers. The sediment was mixed and allowed to settle for 4 days before the tubes were transferred into the temperature gradient block (TGB). This was in order to minimize the transient increase in volatile fatty acids (VFA) that generally occurs after homogenizing sediment (Finke, 1999). The TGB

consisted of a 200 cm x 15 cm x 15 cm insulated aluminum block that was cooled on one end and heated on the other. The temperature range was set from 0°C to 81°C with 2.5°C temperature increments.

After 2 days of preincubation in the TGB 100 µL of a $^{35}SO_4^{2-}$-tracer solution (1 kBq μL^{-1}) were injected and mixed into each tube. Sulfate reduction was terminated after 8 h by transferring the slurry into 20 mL of a zinc acetate solution (20% w/v) and subsequent freezing. All samples were stored frozen until analysis. Pasteurization of sediment samples was carried out in Hungate tubes in a water bath at 85°C for 1 h. Pasteurized samples were immediately transferred to the TGB and preincubated for 24 h before $^{35}SO_4^{2-}$-tracer was added. All experiments in the TGB were performed in duplicates.

Time-course experiment at 4°C and 50°C. Two bags containing sediment from the depth intervals 0-3 cm and 3-9cm were incubated at 4°C and two bags at 50°C to conduct a time-course experiment (July 2005). Subsamples for pore water analyses and sulfate reduction rate measurements were withdrawn after 0, 8 and 16 h, 1, 2, 3, 4, and 6 days. Rates and concentrations at T_0 always correspond to an incubation temperature of 4°C. After subsamples were withdrawn at T_0 the corresponding bags were immediately transferred to 50°C.

Pore water sampling and analyses. Pore water was obtained with a pore water press under N_2 pressure and directly passed through GF/F filters. 1.8 ml aliquots were collected in glass vials for the determination of dissolved inorganic carbon (DIC), fixed with 20 µL of a saturated $HgCl_2$ solution, capped with Viton septa without headspace, and stored at 4°C. DIC was analyzed by flow injection with conductivity detection (Hall and Aller, 1992). 1.5 ml of pore water was frozen for the analysis of NH_4^+ and later spectrophotometrically determined at 630 nm with a Shimadzu UV 1202 Spectrophotometer with the Indophenol-method after (Grasshoff et al., 1999). 0.5 ml pore water was acidified with 20 µL of 6 M HCl and stored at 4°C for the analysis of Ca^{2+}, which was measured by inductively coupled plasma atomic emission spectrometry on a Perkin Elmer Optima 3300 RL. Pore water for sulfate analysis was preserved with Zn acetate (2% w/v) and measured by non-suppressed ion chromatography (Waters, column IC-PakTM, 50 x 4.6 mm) and conductivity detection after (Ferdelman et al., 1997).

For the analysis of volatile fatty acids, sediment was extruded into Spinex (Phenomenex) filter columns, centrifuged (2000g, 10 min) and 1 mL pore water was frozen immediately in precombusted glass vials. Samples were analyzed following (Albert and Martens, 1997) with slight differences in the mobile phase: the concentrations of butanol and tetrabutylammonium hydroxide in solvent A were 1.25% and 1 mmol L^{-1}, respectively. In solvent B, the concentration of tetradecyltrimethylammonium bromide was reduced to 25 mmol L^{-1}. The flow rate was reduced to 1 mL min^{-1}.

Sulfate reduction rate measurements in bags. To determine sulfate reduction rates (SRR) sediment was pressed out of the bags directly into 10 cm long glass tubes (ø 1 cm), two tubes for each time point. The tubes were capped with butyl rubber stoppers at one end. The other end was stoppered with a plastic syringe for easy discharge of the sediment into zinc acetate when bacterial activity should be stopped. The tubes were incubated inside a N_2 flushed gas-tight plastic bag at 50°C or 4°C. 10 µL of a $^{35}SO_4^{2-}$-tracer solution (10 kBq μL^{-1}) was injected into duplicate glass tubes. Bacterial sulfate reduction was terminated after 4 h by transferring the sediment into 10 mL zinc acetate (20% w/v). All samples were stored frozen until analysis.

RESULTS

Sulfate reduction in whole cores. The depth profile of sulfate reduction showed rates in the range of 4-43 nmol cm^{-3} d^{-1} with highest rates at 1-3 cm and 4-5 cm depth (Fig. 1). Previous studies showed that highest rates of sulfate reduction generally occurred in the 3-9 cm depth horizon (B.B. Jørgensen, unpublished results). Therefore this interval was chosen for determining temperature profiles of sulfate reduction. For the bag incubation studies a second interval, the 0-3 cm horizon, was included.

Temperature regulation of sulfate reduction. Sulfate reduction in anoxic sediment slurries was measured in a temperature gradient block. After 2 days of incubation in the TGB sulfate reduction was detected from 0°C to 66°C (Fig. 2A). SRR increased 8-fold with increasing temperature from 0°C to the optimum at 21°C, from 42 to 344 nmol cm^{-3} slurry d^{-1}. A second peak of sulfate reduction was found at 54°C with a 10-

fold increase from 32°C to 54°C, from 16 to 180 nmol cm^{-3} slurry d^{-1} (Fig. 2A). This demonstrates, to our knowledge, for the first time sulfate reduction in the thermophilic range in permanently cold Arctic sediment. Above 21°C and 54°C rates decreased steeply. These two peaks with 30°C difference demonstrate the presence of two physiologically distinct populations of sulfate reducing bacteria.

When the sediment slurry was pasteurized for 1h at 85°C prior to incubation for 24h in the TGB, sulfate reduction was detected from 35°C to 60°C (Fig. 2B). The main peak of sulfate reduction (120 nmol cm^{-3} slurry d^{-1}) was found at 59°C.

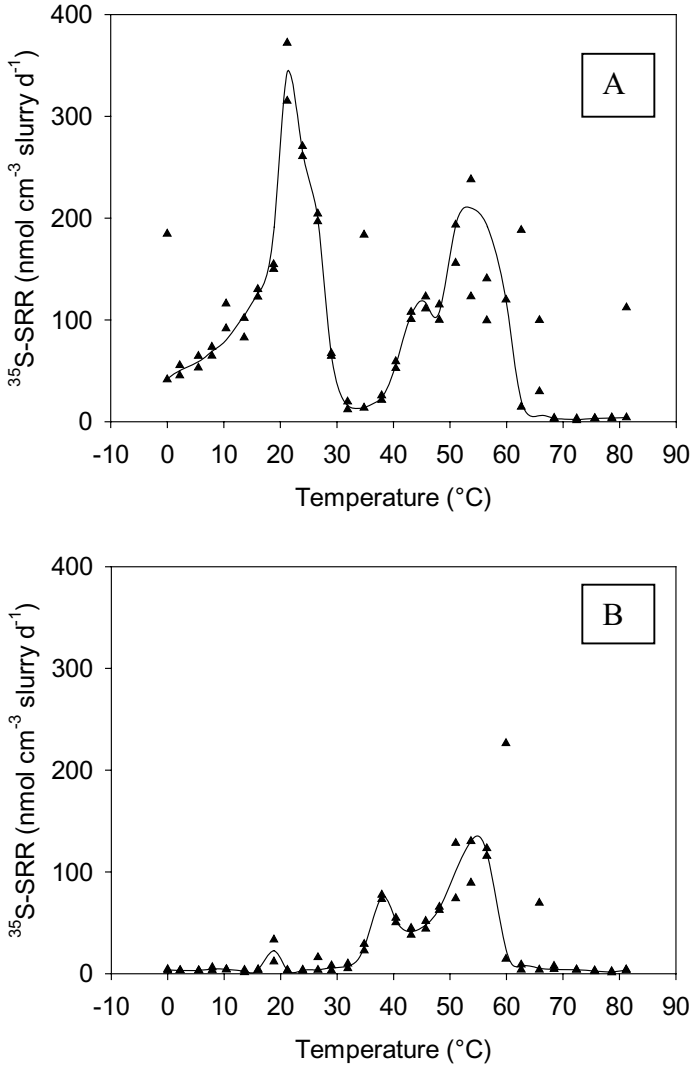

Fig. 2 Sulfate reduction rates from temperature gradient incubations in a (A) non-pasteurized sediment slurry and (B) pasteurized sediment slurry (for 1 h at 85°C). Sediment was pooled from 3-9 cm depth.

Anaerobic organic carbon oxidation at 4°C and 50°C. Concentrations of dissolved inorganic carbon (DIC) in the pore water increased linearly over time at 4°C (Fig. 3A+B) whereas an exponential increase was seen in the bags at 50°C (Fig. 3A+B).

The mean DIC accumulation rate per day was 4 and 6 times higher in the bags at 50°C compared to 4°C in the 0-3 cm and 3-9 cm interval, respectively. Concentrations of Ca^{2+} stayed constant in all incubations, which indicated that CO_2 did not precipitate as $CaCO_3$ (data not shown). NH_4^+ concentrations increased in all incubations mostly during the first 24 h after which the increase was slower (Fig. 3C+D). Concentrations of DIC and NH_4^+ were always lower in the bags from the 3-9 cm depth interval compared to the bags from the top 3 cm, even though the DIC and NH_4^+ concentrations at Station J increased with depth indicating increasing carbon mineralization rates (Vandieken et al., 2006a). This could be explained by the 17 days storage between sediment sampling and actual start of experiments. At in-situ conditions CO_2 and NH_4^+ escape from the sediment surface into the water column but this loss is prevented when the sediment is enclosed in bags. The concentrations in the bags with sediment from 0-3 cm depth were therefore overestimated, which resulted in higher DIC and NH_4^+ concentrations compared to the sediment from 3-9 cm.

Sulfate reduction.

The concentrations of sulfate stayed constant around 25 mM at 4°C (Fig. 3E+F). At 50°C sulfate concentrations were only constant during the first 2 days, after which they decreased (Fig. 3E+F). After 6 days sulfate was almost depleted in the 0-3 cm depth interval (1.4 mM) whereas the concentration in the 3-9 cm depth was considerably higher (14.4 mM). Sulfate reduction rates (SRR) showed a linear increase at 4°C in the top 3 cm, whereas rates stayed constant from 3-9 cm indicating a constant population of bacteria (Fig. 3G+H). Sulfate reduction rates decreased after transfer of the sediment bags to 50°C. After 0.3 days at 50°C SRR were still detectable but lower compared to SRR at 4°C, indicating that not all psychrotolerant SRB were killed yet (Fig. 3G+H). After 0.6 days at 50°C SRR had dropped to below the detection limit in both bags. This was followed by an exponential increase over 4 days, after which rates decreased again.

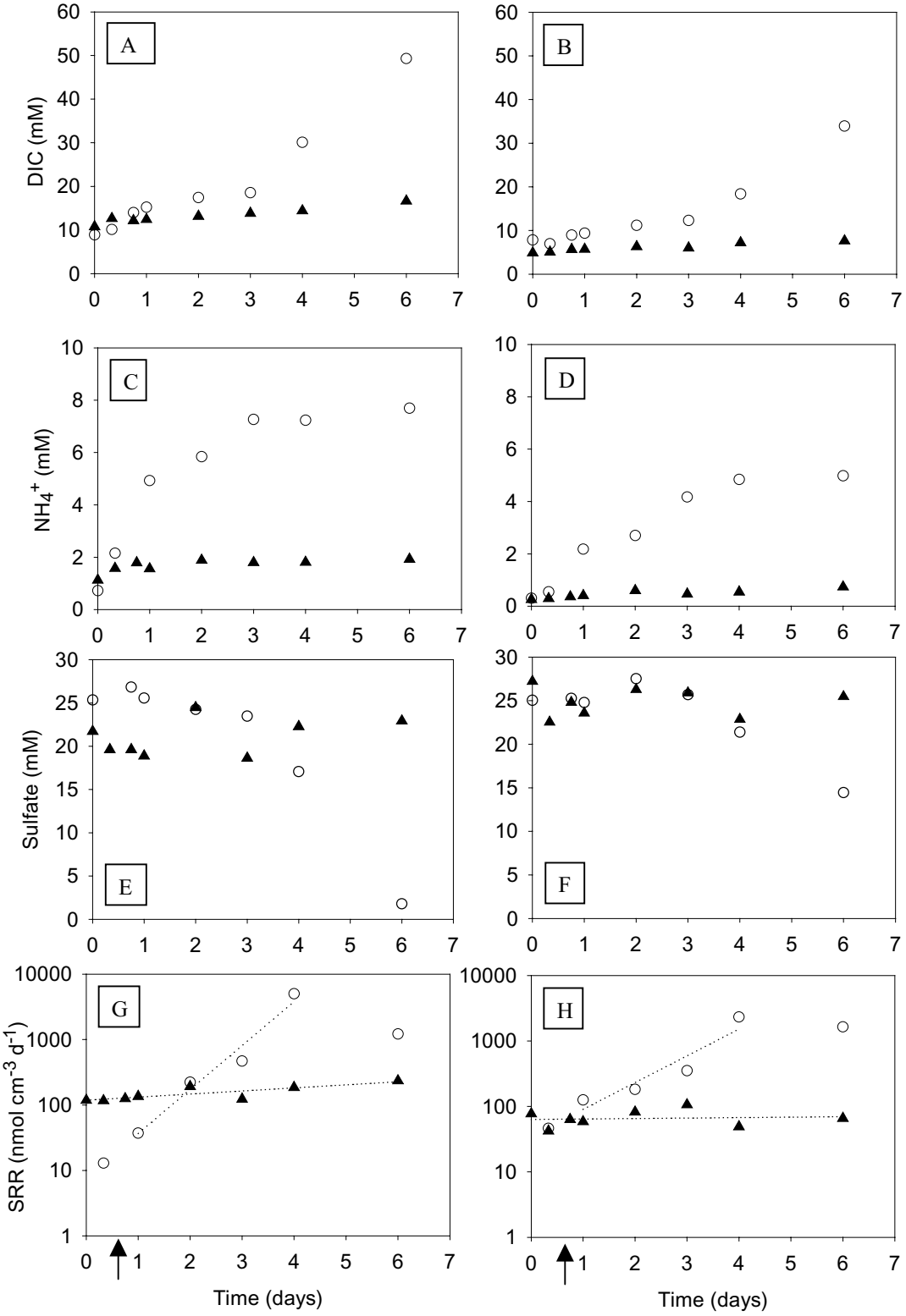

Fig. 3 Evolution of pore water concentrations of DIC (A,B), NH_4^+ (C,D), sulfate (E,F) and SRR (G,H) at 4°C (▲) and 50°C (◯) in bag incubations over 6 days. A, C, E, and G show incubations with pooled sediment from 0-3 cm depth, while B, D, F, and H show pooled sediment from 3-9 cm depth. Note the log scale for SRR (G,H). The arrow in plot G+H indicates that SRR at 50°C were below detection after 16 h.

Volatile fatty acids (VFAs).

The evolution of VFAs at 4°C and 50°C was monitored to analyze the fermentative metabolism. Acetate concentrations were in average nine times more abundant than the sum of all other VFAs in the bags from the top 3 cm at 4°C and 50°C (Fig. 4A+B), while they were three and five times more abundant in the deeper sediment layer (Fig. 4C+D). Acetate concentrations stayed constant around 150-200 µM in the upper 3 cm at 4°C, and decreased after 4 days (Fig. 4A). Formate, propionate, isobutyrate and butyrate concentrations were all below 20 µM and decreased during the incubation. In the bag with sediment from 3-9 cm depth acetate accumulated during the first 2 days reaching a maximum of 25µM, where after the concentrations decreased (Fig. 4C). Formate, propionate and lactate concentrations were all below 5µM. Isobutyrate and butyrate were not detected. At 50°C all VFA concentrations increased during the first 2 to 4 days, followed by a decrease (Fig. 4B+D). The only exceptions were formate in the 0-3 cm depth and lactate in the 3-9 cm depth, which were used up within 0.6 days. Maximum concentrations of acetate, propionate, isobutyrate, and butyrate were 19900 µM (11530), 1690 µM (1000), 450 µM (350), 580 µM (290) for 0-3 cm (3-9 cm), respectively. The next VFA after formate and lactate to drop was butyrate after 2 days, followed by propionate and isobutyrate after 3 days. The last VFA to decline was acetate.

DISCUSSION

Temperature profile of sulfate reduction in an Arctic marine sediment. A peak of sulfate reduction in the mesophilic range (21°C) (Fig. 2A) has been found in previous studies from permanently cold sediment from the Arctic (Sagemann et al., 1998), (Finke, 2003) and Antarctic (Nedwell, 1989). While in situ temperatures ranged between –1,7°C (Storfjord, Arctic) and +2,6°C (Hornsund, Arctic), optimum temperatures for sulfate reduction were generally between 21-28°C. (Finke, 2003) showed that the optimum temperature in such TGB incubations depends on the preincubation time before sulfate reduction was determined. The optimum was 27°C after 8 h incubation and decreased to 18°C after 8,5 days. The reason for that could be that short time incubations show the potential respiratory activity of the

psychrotolerant community, whereas long time incubations offer time for growth, which has a lower temperature optimum. This was supported by pure culture studies by (Isaksen and Jørgensen, 1996) who found that the cardinal temperatures for growth and metabolic activity could differ substantially. For instance the optimum temperature for growth of the psychrotolerant SRB strain ltk10 was 10°C lower than its optimum temperature for sulfate reduction (Isaksen and Jørgensen, 1996).

Temperature profiles measured in those studies ((Nedwell, 1989), (Sagemann et al., 1998), (Finke, 2003)) never exceeded 40°C, which appears reasonable since most of the world's seafloor is permanently below 5°C and sulfate reduction rates decreased sharply at temperatures higher than 30°C (Sagemann et al., 1998). The second maximum of sulfate reduction at 54°C found in our study (Fig. 2A) indicates that another bacterial community must exist in addition to the psychrotolerant community that lives and grows under in situ conditions. Bacteria, which have their optimal respiratory activity at 54°C, are defined as being thermophilic, a group that is characterized by growing at temperatures between 40°C and 75°C. So far no bacterium is known with such a high temperature optimum, which at the same time is able to grow at 0°C.

Pasteurization of the sediment prior to incubation inhibited SRR below 35°C, which indicated that the psychrotolerant community of SRB was killed at such high temperatures. However, pasteurization did not significantly affect sulfate reduction rates between 35°C and 57°C (Fig. 2B) and we conclude that the bacteria surviving the pasteurization are most likely spore-forming sulfate reducing bacteria. Under in situ conditions at low temperatures these bacteria will probably exist as endospores and only germinate and start to grow when the temperature is elevated. It is well known that such spores are resistant towards heat and drought (Widdel, 1992) and a moderately thermophilic endospore-forming SRB, *Desulfotomaculum arcticum*, was indeed isolated from a fjord in close vicinity to our sampling station (Vandieken et al., 2006b). The temperature optimum of this strain was 44°C and it could not grow at in situ temperature.

Activation of spores and their depth distribution. SRR increased linearly at 4°C in the top 3 cm (Fig. 3G) indicating a slow growing population of psychrotolerant SRB. This was further reflected in a linear increase of DIC concentrations of that depth (Fig. 3A). The concentration changes of DIC over time at 4°C were comparable in the

upper and deeper sediment layer, but a steeper increase was found in the surface sediment. This could be due to decreasing cell numbers with depth or limited substrate availability in the deeper sediment layers. Quantification of total cell numbers from station J showed indeed a decrease of bacteria with depth (Ravenschlag et al., 2000). Additionally, concentrations of VFAs were lower in the deeper sediment layer (Fig.4B+D).

SRR after 8 h at 50°C (Fig. 3G+H) resulted most likely only from psychrotolerant SRB, because it might be possible that not all sediment in the bags had reached 50°C yet. Hence some SRB were still alive and active and responded to an increase in temperature, as also seen in the TGB experiment where SRR only dropped above 30°C (Fig. 2A). The decrease of SRR in both sediment bags (0-3 cm and 3-9 cm) after transfer to 50°C showed that the psychrotolerant community of SRB was killed within 16h. Consequently the SRR after 1 day at 50°C must be completely attributed to thermophilic SRB, which shows that spores needed at least 16-24h for germination. The subsequent exponential increase of SRR indicated that either more spores germinated after 24h, or that the thermophilic community of SRB grew, or both.

The decrease of SRR after 6 days at 50°C (Fig. 3G+H) could be due to different factors. The SRB might have become limited by substrate and/or electron acceptor availability. Major substrates for sulfate reduction in marine sediments are volatile fatty acids and hydrogen (Sørensen et al., 1981). The VFAs measured in this study were used up in the sequence formate/lactate, butyrate, isobutyrate, and propionate. Only acetate was still present after 6 days (Fig. 4B+D). Previous studies showed that acetate accounted only for 10% (0-2 cm) and 40% (5-9 cm) of SRR in Smeerenburgfjord under in situ conditions (Finke et al., accepted). It is possible that acetate was also the least favored substrate for sulfate reduction at 50°C and the decrease of SRR was due to progressing lack of energetically more favorable electron donors. This is further supported by the substrate spectrum of the thermophilic spore-forming *Desulfotomaculum arcticum*, isolated from a close-by fjord (Vandieken et al., 2006b), which does not utilize acetate as an electron acceptor but rather formate, lactate, propionate and butyrate. However, as sulfate concentrations also decreased after 6 days, we assume that a combination of decreasing electron donor and acceptor availability was responsible for the decline of sulfate reduction after 6 days.

The response of SRR over time at 50°C was comparable in the top (0-3 cm) and in the deeper layer of the sediment (3-9 cm) (Fig. 3G+H), but the exponential increase of SRR was steeper in the surface sediment (0-3 cm). This could have several reasons. The total spore number of SRB was lower at 3-9 cm. To verify this assumption quantification of the spores would be necessary. As this was not done in the present study we cannot exclude this possibility. However, differences between depths could also be related to the availability of substrate, as it was noted at 4°C. Concentrations of VFAs were indeed lower in the 3-9 cm depth compared to the top 3 cm.

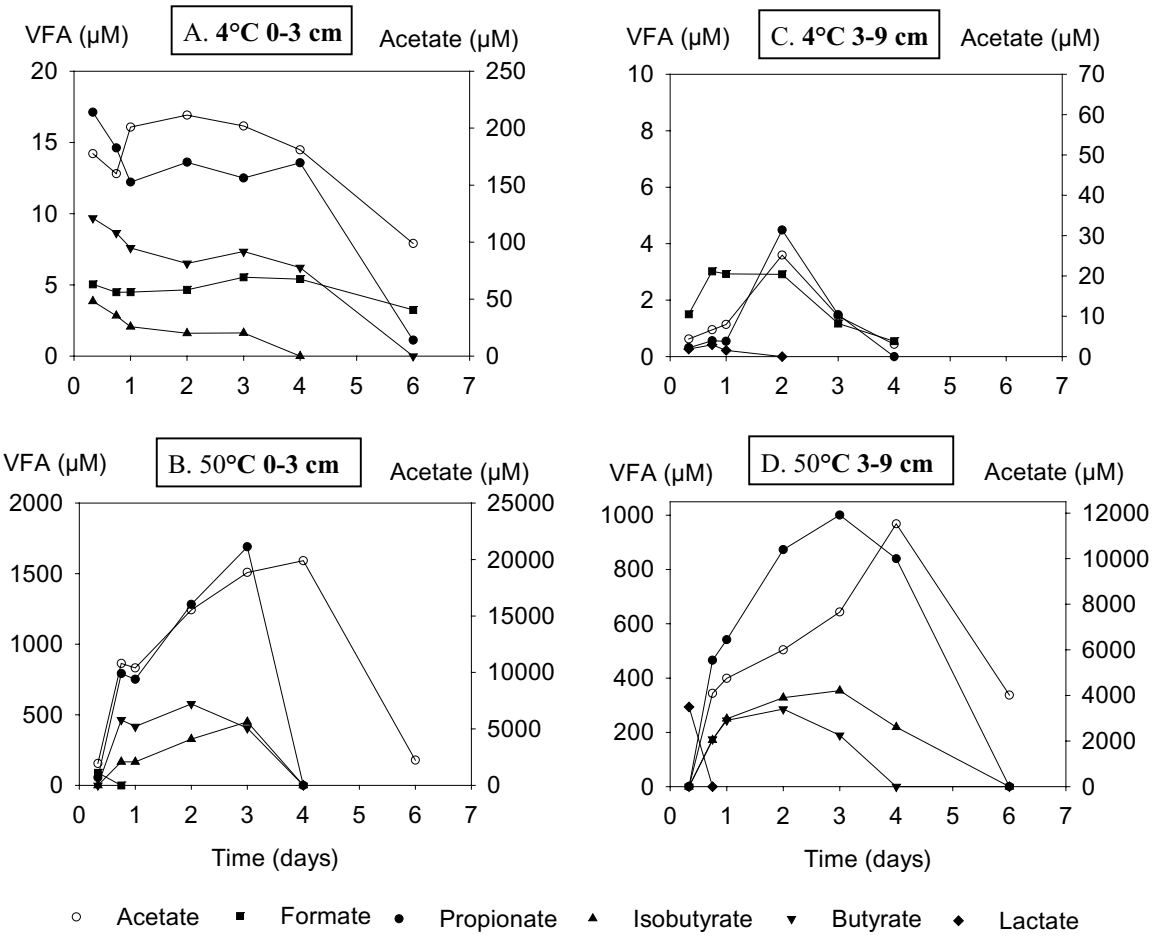

Fig. 4 Evolution of different volatile fatty acids over time at 4°C (A,C) and 50°C (B,D). A and B show incubations with pooled sediment from 0-3 cm depth, while C and D show pooled sediment from 3-9 cm depth. Note different scales.

Volatile fatty acids and fermentation. The constant or decreasing concentrations of VFAs at 4°C (Fig.4A+C) indicated that fermentation and sulfate reduction were closely coupled at in situ temperature. The increase in concentrations of acetate and propionate at 3-9 cm depth during the first two days (Fig. 4C) resulted most likely

from a stimulation of fermentation due to kneading of the sediment bags. This accumulation was not reflected in SRR and we assume that these overall changes rather reflect the balance between production and consumption in very dynamic sediment.

The evolution of volatile fatty acids at 50°C (Fig. 4B+D) indicated that also thermophilic fermentative bacteria were present in the sediment. The accumulation of all VFAs in the beginning of the incubation (except for formate and lactate) indicated that these thermophilic fermentative bacteria germinated earlier compared to thermophilic SRB. But once the SRB community was established and most likely started growing, the rate of VFA consumption accelerated and their concentrations decreased again. Lactate (3-9 cm) and formate (0-3 cm) were depleted within 8h at 50°C. They have probably been used up by the psychrotolerant SRB, since the thermophilic SRB only germinated after 16-24h. Butyrate was found to be the favored VFA for the SRB followed by isobutyrate and propionate, according to their order of disappearance. Acetate reached the highest concentration and was the last VFA to drop. This could indicate that acetate was only used by SRB because their favored substrates were no longer available. It could also indicate that various SRB communities exist in the sediment. The order of disappearance of VFAs would then indicate a shift from a SRB community of non-acetate-users to an acetate-using community. However, without detailed characterization of these thermophilic SRB we cannot distinguish among which SRB communities were present.

The rapid accumulation of high acetate concentrations (20 mM and 12 mM from 0-3 cm and 3-9 cm) seemed surprisingly at first, but was supported by other studies. (Wellsbury et al., 1997) performed heating experiments with coastal surface sediment and found maximum acetate concentrations of 24 mM after 7 days at about 50°C. They considered the production to be of bacterial origin, as little acetate was produced at >60°C.

NH_4^+ release. NH_4^+ is released during organic matter decomposition and is a measure of overall microbial mineralization of organic material in sediments similar to DIC production. The main increase occurred in all incubations during the first 24h (Fig. 3C+D), which might be due to the extensive kneading of the bags every 8h when subsamples were taken. The physical mixing of sediment might have brought the bacteria and possible substrates closer to each other, thereby enhancing the

degradation of organic material. After 24h subsamples were withdrawn only once a day and the increase in NH_4^+ production slowed down. However, this possibly enhanced mineralization was not reflected in an increased DIC production. The initial release of NH_4^+ at 50°C might also be due to a heat effect. Thus, NH_4^+ might desorb from minerals until a balance between adsorption and accumulation of NH_4^+ in the pore water is reached.

CONCLUSION

In conclusion, we found thermophilic bacterial activity of sulfate reducing and fermentative bacteria in permanently cold sediments from Svalbard, Arctic Ocean.
We assume that these bacteria are present as spores at in situ conditions because pasteurization of the sediment did not affect their activity, whereas it killed the psychrotolerant community. This is further supported by the isolation of a spore-forming sulfate reducing bacterium in the vicinity to our sampling station. The low temperature of the polar environment does not offer conditions for germination of these spores or growth under natural conditions. The origin of these thermophilic spore-forming bacteria remains unknown.

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3. Outlook

Anaerobic carbon mineralization in sediments of the northern Barents Sea

This study extends the small database quantifying anaerobic carbon mineralization pathways in Arctic marine sediments. Data available to date have mostly been conducted in Arctic fjords ((Thamdrup and Fleischer, 1998); (Kostka et al., 1999); (Arnosti and Jørgensen, 2003)) where carbon mineralization rates were comparable to sediments from temperate environment and sulfate reduction was the dominant anaerobic respiration pathway. Studies from open shelf sediments are rare. In contrast to the fjords, dissimilatory Fe and Mn reduction were the dominating respiration pathways in these sediments due to relatively high abundances of reactive Fe and Mn oxides, low organic carbon contents, low sedimentation rates as well as long periods with ice cover. However, conclusions about the general importance of dissimilatory Fe and Mn reduction in open shelf sediments cannot be drawn, because the number of investigated stations (5) was too small. More studies are needed to adequately assess if microbial Fe and Mn reduction are of global importance in C_{org}-poor sediments. In addition, the nitrogen turnover will have to be included in further studies to determine its significance for microbial respiration in Arctic open shelf sediments.

Thermophilic bacteria in marine sediments

Activity of thermophilic bacteria was found when Arctic sediment with an in situ temperature of 2°C was incubated at 50°C. Since pasteurization did not affect this activity, whereas it killed all psychrotolerant bacteria, we concluded that the responsible bacteria were most likely present as spores in the sediment. The origin of the spores is still unknown. A subsequent project, initiated by V. Brüchert, will look for spores in water samples. If the spores enter the sediment from above, it should be possible to detect thermophilic bacterial activity in the water column. Furthermore it needs to be elucidated whether the occurrence of thermophilic bacteria is a characteristic for marine sediments in general. This year different fjords on the west and north coast of Svalbard were sampled and will be analyzed for potential

thermophilic sulfate reduction by C. Hubert (MPI), which will give new insights into the geographic distribution of spores.

An important question that remains is how many spores are present in the sediments. Isolation and quantification of thermophilic sulfate reducing bacteria will be performed by an improved most-probable-number method (Vester and Ingvorsen, 1998). This method is based on the use of a natural media that consists of anaerobically prepared sterilized sediment slurries obtained from the sampling site. The advantage of this method is that is uses the natural environment of the sulfate reducing bacteria as growth medium, providing natural conditions.

The microbial community composition at 50°C will be determined with molecular methods including DGGE (denaturing gradient gel electrophoresis) and clone libraries. Additionally, more information about spores in other sediments, including isolation, characterization, and quantification, is needed for geographic comparison. With that the global and local distribution of spores as well as possible sources can be elucidated.

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