TEMPERATURE CONTROL OF BACTERIAL CARBON MINERALIZATION PROCESSES IN MARINE SEDIMENTS

Dissertation
Zur Erlangung des Grades eines
Doktors der Naturwissenschaften
-Dr. rer. Nat.-

am Fachbereich Biologie/Chemie der
Universität Bremen
vorgelegt von

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Bremen 2009

Die vorliegende Arbeit wurde am Max Planck Institut für Marine Mikrobiologie in Bremen angefertigt.

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Tag des Promotionskolloquiums: 18. September 2009

Este trabajo esta dedicado a mi familia,
especialmente a Marian y José Miguel, gracias.

SUMMARY

The present work analyzes the potential impact of anticipated global warming on the bacterial carbon cycling in marine shelf sediments. Current changes of the marine biological carbon cycle in response to climate warming in different regions of the world ocean are closely coupled to the response of bacteria to environmental temperatures.

We investigated the correlation between ambient temperatures and the physiological adaptations, in terms of energy metabolism, of sulfate-reducing bacteria (SRB) in polar, temperate and tropical sediments. In short-term sediment incubations in a temperature gradient block, sulfate-reduction rates (SRR) were measured using ^{35}S-sulfate. Resulting temperature response profiles were used to examine the competitiveness of SRB, in terms of relative SRR of maximal potential rates, the temperature dependence for energy metabolism of SRB and the correlation of cardinal temperatures of sulfate reduction and sediment temperatures. We observed that SRB in polar sediments are more competitive than their counterparts in warmer habitats at similar low temperatures. Although metabolic rates in warmer latitudes exhibited higher temperature dependence below 8-18°C, optimal temperature conditions for sulfate reduction in these environments are closer to their ambient temperatures resulting in a higher competitiveness at in situ conditions. Together, these observations imply that biography and, consequently, environmental temperature variability play an important role in the physiological selection and divergence of microbiota in different latitudes.

Over a long-term (2 year) temperature incubation experiment, we measured ^{35}S-SRR in a temperature gradient block and used CARD-FISH of sulfate-reducing bacteria to describe the temperature control of carbon mineralization rates via sulfate reduction in Arctic marine

sediments in comparison to a temperate habitat. This study is innovative in that we examine the consequences of temperature shifts by investigating the activity and the population dynamics of sulfate-reducing bacteria. We found that the investigated Arctic sediment hosts a sulfate-reducing bacterial community that changes rapidly and is not capable of accommodating long-term temperature upshifts as high as 20 °C. Lower bulk sulfate reduction rates at 20°C compared to 0°C and 10°C in arctic sediments are indicative of the strong temperature effect on the active SRB community. In contrast, the community in a temperate habitat appears to be largely insensitive to temperature changes, whether down or up, and appears to contain abundant psychrotolerant/mesophilic bacteria that outcompete specialized psychrophiles even during the cold season.

We also investigated rates and temperature optima of extracellular enzymatic hydrolysis as well as the dynamics of key intermediates in anoxic carbon degradation pathways, and their relationship to sulfate reduction, the terminal step in carbon cycling. Following 24 months incubation at 0°C, 10°C, and 20°C, we observed increasing concentrations of dissolved organic carbon (DOC) and total dissolved carbohydrates, particularly at higher temperatures, as well as limitation in sulfate reduction rates. Together, our results showed increasing decoupling between hydrolysis and terminal oxidation of organic matter via sulfate reduction. The decline of sulfate reduction rates, particularly in the Arctic sediments, suggests an inability of the fermentative community to transform refractory DOC to substrates suitable for sulfate reducers.

ZUSAMMENFASSUNG

Die vorliegende Arbeit beschreibt die potentiellen Auswirkungen einer globalen Erwärmung auf den bakteriellen Kohlenstoffumsatz in marinen Schelf-Sedimenten. Die durch die globale Erwärmung verursachten Veränderungen des marinen biologischen Kohlenstoffkreislaufs sind eng an die Temperaturabhängigkeit bakterieller Prozesse gekoppelt.

Wir untersuchten den Zusammenhang zwischen Umgebungstemperaturen und den Adaptionen des Energiemetabolismus von Sulfat reduzierenden Bakterien (SRB), welche aus den verschiedensten Sedimenten der polaren, gemäßigten und tropischen Breiten stammten. Während kurzer Sedimentinkubationen bei unterschiedlichen Temperaturen wurden Sulfatumsatzraten mit Hilfe von ^{35}S-Sulfat Isotopen ermittelt. Die gemessenen Sulfatreduktionsraten als Funktion der Temperatur geben Aufschluß über die Fitness Sulfat reduzierender Bakterien hinsichtlich der Temperaturabhängigkeit des Energiestoffwechsels und der maximalen potentiellen Raten. Wir konnten zeigen, dass SRB's aus arktischen Sedimenten bei niedrigen Temperaturen konkurrenzfähiger sind als SRB's aus wärmeren Gebieten. Die Umsatzraten in warmen Breiten zeigten eine höhere Temperaturabhängigkeit bei Temperaturen unter 8-18°C. Dagegen lag das Temperaturoptimum der Sulfatreduzierer aus diesen Breiten in der Nähe der Umgebungstemperaturen und erhöhte deren Wettbewerbsfähigkeit unter in situ Bedingungen. Aus diesen Beobachtungen ließ sich schließen, dass die Geographie und die Variabilität der Umgebungstemperaturen eine entscheidende Rolle bei der Selektion und Divergenz mikrobieller Gemeinschaften spielt.

Während eines zwei jährigen Inkubationsexperiments wurde die Temperaturabhängigkeit von Sulfatreduktionsraten gemessen und

parallel mittels CARD-FISH die Populationen Sulfat reduzierender Bakterien bestimmt. Damit konnten wir erstmalig die Auswirkungen von Temperaturänderungen auf die Aktivität und die Populationsdynamik Sulfat reduzierender Bakterien beschreiben. Wir konnten zeigen, dass die Gemeinschaft Sulfat reduzierender Bakterien in den untersuchten arktischen Sedimenten sich einerseits schnell verändert, sich aber nicht dauerhaft an Temperaturerhöhungen auf 20°C anpassen kann. Die verminderten Sulfatreduktionsraten bei 20°C, verglichen mit denen bei 0°C und 10°C, verdeutlichten die starke Temperaturabhängigkeit der aktiven Sulfat reduzierender Bakterien in den arktischen Sedimenten. Dagegen zeigten die Sulfat reduzierenden Bakterien aus den gemäßigten Breiten eine hohe Anpassungsfähigkeit an erhöhte und erniedrigte Temperaturen. Diese Sedimente enthielten psychrotolerante/mesophile Bakterien, welche in der Lage waren, die spezialisierten psychrophilen Bakterien selbst in den kalten Jahreszeiten zu verdrängen.

Weiterhin untersuchten wir die Raten und das Temperaturoptimum der enzymatischen extrazellulären Hydrolyse, sowie die Kopplung der wichtigsten Zwischenprodukte des anoxischen Kohlenstoffabbaus mit der Sulfatreduktion. Nach einjähriger Inkubation der Sedimente bei 0°C, 10°C, und 20°C beobachteten wir steigende Konzentrationen von gelöstem organischem Kohlenstoff und gelösten Kohlenhydraten, besonders bei erhöhten Temperaturen, sowie eine Limitierung der Sulfatreduktionsraten. Zusammenfassend zeigen unsere Ergebnisse eine erhöhte Entkopplung zwischen Hydrolyse und der Oxidation organischen Materials mittels Sulfatreduktion. Die Abnahme der Sulfatreduktionsraten, besonders in den arktischen Sedimenten, läßt sich mit verringerten Fermentationsraten von zunehmend refraktärem gelöstem Kohlenstoff erklären, wodurch weniger Substrat für Sulfat reduzierende Bakterien zur Verfügung steht.

ACKNOWLEDGMENTS

I owe gratitude to Volker Brüchert, who gave me the opportunity to join this research project. I am greatly thankful to Bo Barker Jørgensen for his encouragement and inspiring discussions. Tim Ferdelman is acknowledged for his great support at the Department of Biogeochemistry. Alex Loy is also acknowledged for his great commitment despite my constant delays. I am deeply indebted to Drew Steen and Carol Arnosti for their support and for the long talks and discussions. I am also especially thankful to Drew Steen for the good Bourbon.

I would also like to express my gratitude to Professors Ignacio Moriyón and José L. Vizmanos at the Universidad de Navarra for their inspiring courses and initial support in my scientific career.

I would like to thank the captain Stig Henningsen and John Mortensen of MS FARM as well as the scientific party 2005 and 2006 for the exciting expeditions.

I would like to express my gratitude to the great support received from people at the Max Planck Institute for Marine Microbiology, at the MarMic International Research School and especially at the Biogeochemistry Department. This study was funded by the German Research Foundation through the Priority Program 1162 'The impact of climate variability on aquatic ecosystems (Aquashift)', and by the Max Planck Society.

I was fortunate to have the company and support of so many friends, this thesis could not have been written without them. I am also thankful to my 'german family' for making me feel like at home.

I wish to thank my girlfriend Sabine for being so close in the distance.

CONTENTS

OVERVIEW OF MANUSCRIPTS

MANUSCRIPT 1

Author Contributions Original idea by B.B. Jørgensen; this study was designed by A. Robador, V. Brüchert and B.B. Jørgensen; sediment incubation experiments and estimation of SRR were conducted by A. Robador; the paper was written by A. Robador with input from other co-authors.

MANUSCRIPT 2

Author Contributions This study was designed by A. Robador, V. Brüchert and B.B. Jørgensen; sediment sampling was performed by A. Robador, V. Brüchert and B.B. Jørgensen; sediment incubation experiments, estimation of SRR and CARD-

FISH analysis were conducted by A. Robador; the paper was written by A. Robador with input from other co-authors.

MANUSCRIPT 3

Author Contributions This study was designed by A. Robador and A.D. Steen with input from other co-authors; sediment samples were collected by A. Robador, V. Brüchert and A.D. Steen; sediment incubation experiments were conducted by A. Robador; enzymatic hydrolysis and total dissolved carbohydrates were measured and analyzed by A.D. Steen and C. Arnosti; DOC and VFA's concentrations were measured and analyzed by A. Robador, the paper was written by A. Robador with input from other co-authors.

GENERAL INTRODUCTION

CLIMATE CHANGE AND THE MARINE CARBON CYCLE

There is compelling evidence that the global carbon budget has changed from pre-industrial levels due to human activity (Fig. 1) and it is very likely that this has exerted a substantial warming influence in climate which exceeds that due to natural process. There is very high confidence that many natural systems are being affected by regional climate changes, particularly temperature increases, as a consequence of anthropogenic greenhouse gas emissions (IPCC 2007; Rosenzweig et al., 2008). In this context, understanding the temperature response of carbon production, recycling, and preservation is important for prediction of the effects of climate change on the global carbon cycle.

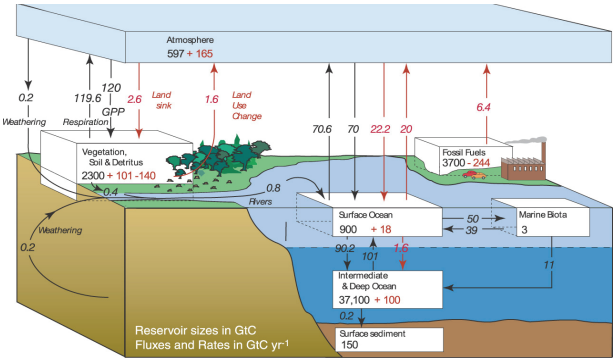

Figure 1. The global carbon cycle for the 1990s, showing the main annual fluxes in GtC yr^{-1} with changes in pool sizes: pre-industrial 'natural' fluxes in black and 'anthropogenic' fluxes in red (from Denman et al., 2007).

The world ocean constitutes the largest carbon reservoir (Fig. 1) and small feedback mechanisms of the marine carbon cycle to climate may combine to induce a significant change in the global carbon cycle (Denman et al., 2007). The ocean may contribute to the atmospheric carbon dioxide (CO_2) through different mechanisms; changes in solubility of gaseous CO_2 ('solubility pump'), changes in the CO_2 fixation into organic matter and subsequent cycling of this carbon which can be respired and returned to the atmosphere or exported into deeper water layers ('organic carbon pump'), and changes in the release of CO_2 during the planktonic formation of calcium carbonate ('$CaCO_3$ counter pump'). Alterations in the marine carbon pump therefore, in combination with the global ocean mixing, can induce important changes in the concentration of atmospheric CO_2 (Wohlers et al., 2009).

Couplings between oceanic climate change and marine biogeochemical carbon cycle processes

The physical properties of the world ocean have changed in response to ocean surface conditions and the spatial distribution of these changes is consistent with the large-scale ocean circulation (Fig. 2). This alteration of the physical characteristics of the ocean in conjunction with the reported increasing concentrations of atmospheric CO_2 (Forster et al., 2007) may affect the biological activity, with further consequences for the biogeochemical cycles.

Current warming of the ocean together with changes in salinity is affecting the solubility and chemical equilibration of gases, i.e. CO_2 and dissolved oxygen (O_2), with consequences for the inorganic carbon chemistry in seawater as well as the biological production and cycling of organic carbon. As atmospheric CO_2 increases, additional CO_2 dissolves in the ocean causing the acidification of the surface (Fig. 2) and resulting in changes of seawater buffering and the biological production, and dissolution in sediments, of $CaCO_3$. Changes in the circulation of oceanic water masses reduce the upward supply of carbon and nutrients to the surface, the ventilation of oxygen-depleted waters and the transport of anthropogenic carbon to deeper layers, which are also major factors controlling the biological production, export, and cycling of organic carbon.

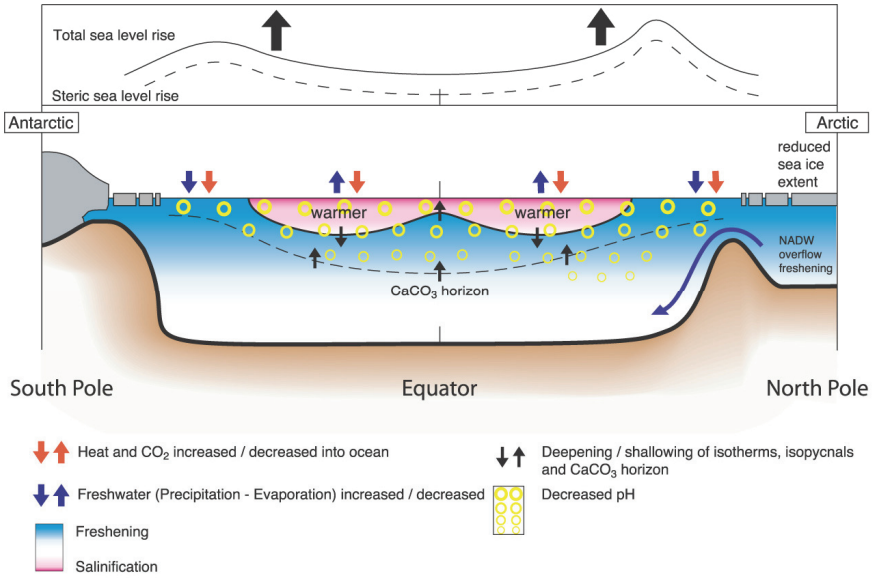

Figure 2. Schematic of the observed changes in the ocean state. The legend identifies the direction of the changes in these variables (from Bindoff et al., 2007).

THE ROLE OF BACTERIA IN THE MARINE BIOGEOCHEMICAL CARBON CYCLE

Primary production and the export of organic matter to the seafloor

The current biogeochemical cycles are the result of the co-evolution between the microbial metabolic and geochemical processes (Falkowski et al., 2008). The marine carbon cycle in fact is driven largely by bacteria, which dominate the abundance, diversity and metabolic activity of the ocean (Azam, 1998; 2007). The fixation of CO_2 by photosynthetic bacteria, plants, or algae, or chemosynthetic microorganisms, occurs in the photic zone of the ocean. Most of this primary production enters the biological food web in these surface waters and is subsequently respired or used for new heterotrophic biomass production. A large fraction of

this newly formed organic matter is exclusively available to heterotrophic bacteria and is mineralized rapidly at the lower boundary of the photic zone (Fig. 3). Below the photic zone however, the biological and chemical degradation of the organic matter decreases rapidly (Fig. 3) and the quantity of remaining organic matter that reaches the ocean seafloor is directly dependent on the water depth and sinking velocity. Shallow coastal and shelve sediments therefore receive considerably higher amounts of organic carbon than deep-water regions away from the continents, and are quantitatively important regions for the overall mineralization of organic matter. Upon reaching the benthic boundary layer, close to the sediment/water interface, the consumption of organic matter is enhanced. This degradation of organic material is eventually further extended deeper into in the sediment where bacteria play a major role (Fig. 3).

Sedimentary mineralization of organic matter

The organic carbon exported from the euphotic zone and reaching the seafloor is degraded with increasing depth, mostly by microbial mineralization, into CO_2, dissolved organic carbon, and nutrients, which are largely released back into the water column and thus escape burial (Fig. 5; Fenchel and Jørgensen, 1977; Berger et al., 1989). Upon reaching the sediment, particulate organic matter is first degraded by a series of microbially mediated hydrolytic and fermentative processes. Prokaryotes can not transport high molecular weight organic compounds and only produced dissolved intermediates of about 600 Da (Benz and Bauer, 1988) are oxidized by heterotrophic microorganisms (Fig. 5). The organic carbon may be mineralized completely to CO_2 with depth in a complex sequence of oxidants. Much of this heterotrophic oxidation of organic carbon in coastal and shelve sediments takes place through anaerobic pathways (Canfield, 1993), of which bacterial sulfate reduction is a biogeochemical main process (Jørgensen, 1982). Because of the intense recycling of organic matter by bacteria, any variations of their cycling patterns in response to global temperature change could alter the overall carbon flux in the ocean.

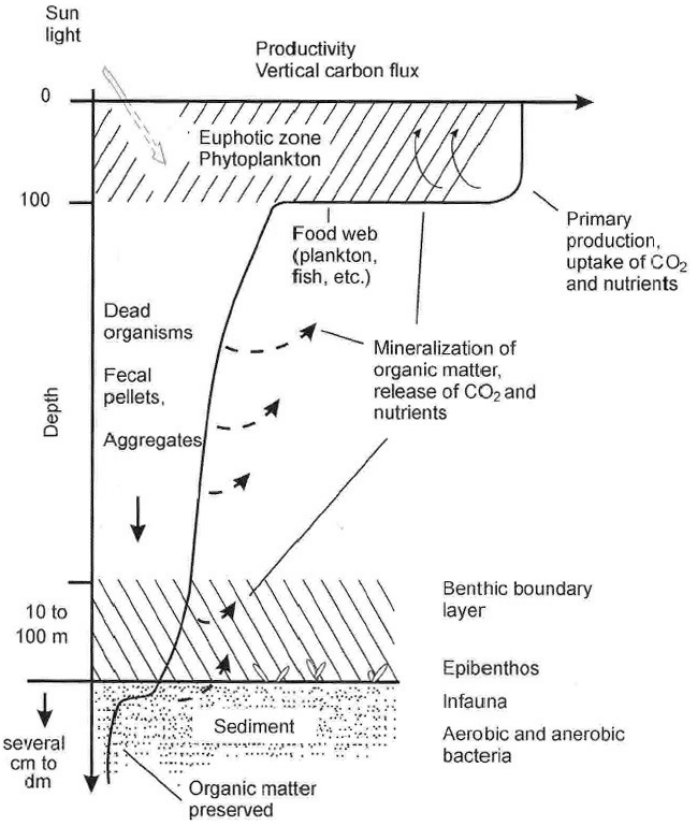

Figure 3. Schematic representation of the organic matter flux to the ocean seafloor (modified after Rullkötter, 2006).

Global warming and the carbon mineralization in costal and continental shelve sediments: The importance of the Arctic Ocean.

About 90% of marine sediments have temperatures below 3°C and 50% of shelf sediments are subjected to temperatures below 1°C (Fig. 4). Substantial changes in mean temperatures have been reported in the 0- to 1000-meter layer of each ocean (Levitus et al., 2005; 2009) and, since

the mineralization of sedimentary organic carbon in the seabed is concentrated primarily on this upper 1000 m water depth of the ocean margins (Jørgensen and Kasten, 2006), small but permanent temperature increases at the scale of predicted climate change are likely to have an important effect on these ecosystems and induce changes in the microbial carbon cycling patterns.

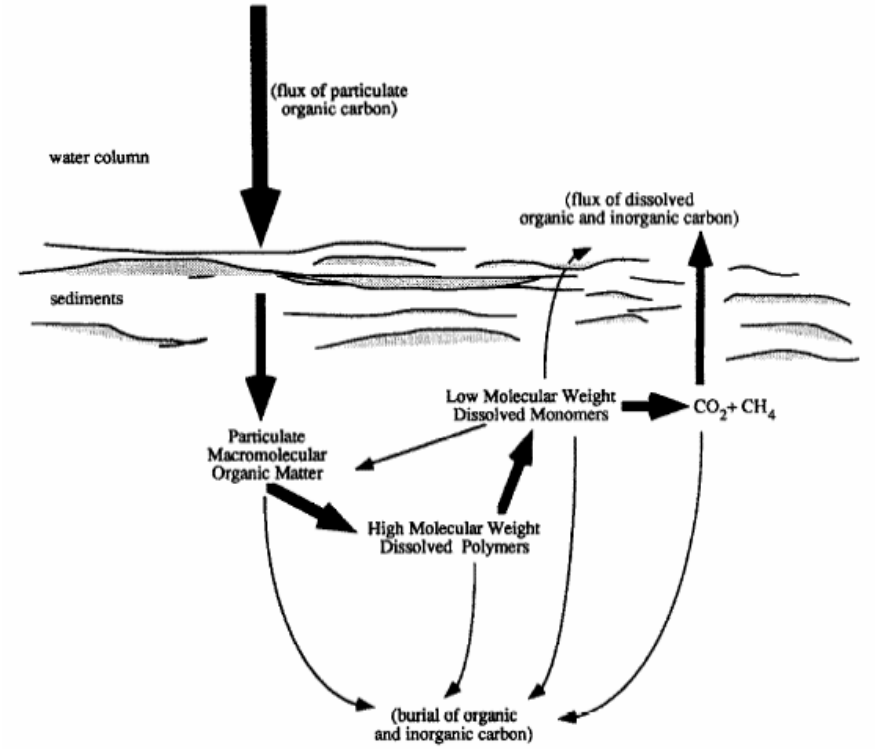

Figure 5. A schematic model of organic matter transformations in sediments. Arrows represent only the major organic matter transformation pathways (after Arnosti et al., 1994).

Particularly sensitive to increasing temperatures are the Arctic regions, which are observed and predicted to warm more rapidly than the global average (Anisimov et al., 2007). The Arctic Ocean accounts for 20% of the world's continental shelves and these receive, transport, and

store organic carbon to an extent significant at the global scale (Rachold et al., 2005). In fact, burial of organic carbon in the Arctic Ocean may account for ca. 7 to 11% of the global budget (Stein and Macdonald, 2004). The Arctic Ocean therefore plays a very important role in the global carbon cycle and the disturbances resulting from elevated temperatures may be critical for the Earth System.

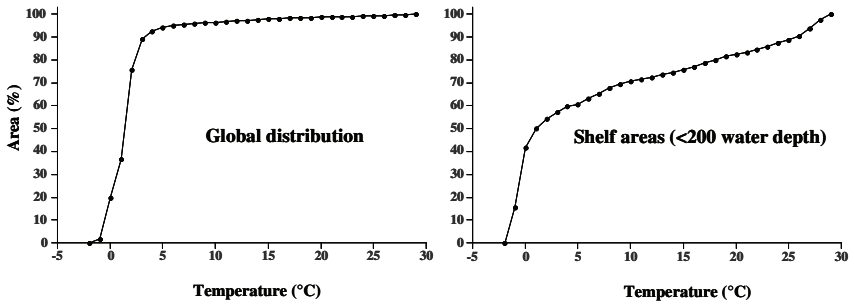

Figure 4. Global bottom water temperatures of marine sediments (data from Levitus and Boyer, 1994).

STATE OF RESEARCH

Bacterial adaptations to environmental temperatures

Temperature is an important factor regulating the rate of biological processes (Nedwell, 1999) and, therefore, is likely to exert a selective pressure in natural environments. The isolation of psychrophilic bacteria from permanently cold Arctic sediments able to grow at -2°C illustrates an adaptation towards low temperatures near the ambient range (Knoblauch et al., 1999a, Vandieken et al., 2006a). In order to cope with reduced chemical reaction rates induced by low temperatures psychrophiles are able to synthesize enzymes with higher catalytic activities in this thermal range (Feller, 2003). This physiological adaptation provides psychrophiles with a greater fitness to natural cold

habitats (Harder and Veldkamp, 1968). Nevertheless, microbial psychrophiles appear to be much less abundant than expected in polar environments (Norkrans and Stehn, 1978; Vincent, 1988; Ray et al., 1998). Many bacteria isolated from arctic regions show a psychrotolerant temperature response with more limited physiological regulation within their ambient temperature range and optimal growth conditions closer to those of temperate habitats (Bakermans et al., 2003, Vandieken et al., 2006b). However, even these organisms show an adaptive fit towards their environment. For instance, psychrotolerant bacteria can acclimate to cold changing the fluidity of the cell membrane by increasing the amounts of unsaturated fatty acids (Könneke and Widdel, 2003). Furthermore, psychrotolerant bacteria may possess enzymes with activity ranges that permit metabolism in the moderately warm to cold temperature range (Rabus et al., 2002). According to Vincent (2000), the observation of these physiological characteristics of microorganisms in cold regions implies an environmental selection for adaptive genotypes and the potential for evolutionary divergence from temperate latitude microbiota.

Short vs. long-term temperature control of sedimentary carbon mineralization rates

The biogeochemical recycling of deposited organic matter in marine sediments does not appear to be less efficient or less complete in polar regions than in temperate or tropical environments (Jørgensen, 2006). Significant temporal variations in sedimentary mineralization rates of organic matter however are commonly observed in temperate environments characterized by a strong seasonality. Annual rates of carbon mineralization are significantly lower during the winter period than during the warmer summer time (Middelburg et al., 1996). In contrast, permanently cold sediments exhibit relatively higher metabolic activities than the temperate sediments at low temperatures near 0°C (Arnosti, 1998; Sagemann et al., 1998).

Studies of the terminal carbon oxidation process via bacterial sulfate reduction in arctic marine sediments have shown the presence of psychrophilic organisms capable of sustaining higher cell specific metabolic rates than their mesophilic counterparts at similar low temperatures (Isaksen and Jørgensen, 1996; Knoblauch and Jørgensen,

1999; Knoblauch et al., 1999b). The temperature dependence of terminal metabolism however also depends on the availability of organic substrates and, therefore, on the preceding carbon transformation processes. There is evidence for a differential temperature response among steps in carbon mineralization pathways (Brüchert and Arnosti, 2002; Arnosti et al., 2005; Finke and Jørgensen, 2008). Together, these observations indicate the different temperature adaptations existing within bacterial communities in arctic and warmer habitats. In order to understand the effect of short-term (i.e. seasonal variations) versus long-term temperature changes (i.e. global warming) on the efficiency of carbon mineralization, it is important to study what populations are present in an ecosystem, in terms of their temperature adaptation, the population size, and how populations interact with eachother.

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MANUSCRIPT 1

ADAPTATION OF SULFATE-REDUCING BACTERIA TO AMBIENT SEDIMENT TEMPERATURES IN POLAR, TEMPERATE, AND TROPICAL MARINE ENVIRONMENTS

For submission to *ISME Journal*

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Running title: Adaptation of sulfate reduction to ambient temperatures

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SUMMARY

Temperature is an important factor regulating the rate of biological processes and, therefore, is likely to exert a selective pressure in the environment. The temperature response of carbon mineralization via bacterial sulfate reduction of polar, temperate and tropical marine sediments was studied in temperature-gradient incubations experiments by measuring sulfate reduction rates (SRR) using ^{35}S-sulfate. Sediment slurries were incubated in a thermal gradient between -10°C and +50°C to cover the physiological temperature range of the active sulfate-reducing bacteria (SRB) and the resulting temperature response profiles were used to characterize the competitiveness of SRB, in terms of relative SRR at in situ temperatures, the temperature dependence for energy metabolism of SRB and the correlation of cardinal temperatures of sulfate reduction and sediment temperatures. In polar regions, only temperatures close to the freezing point of the sediment limit the rates of sulfate reduction. In these environments SRB exhibited high metabolic rates of ca 10-17% of maximal potential rates at the in-situ temperature of 0°C. Similar relative SRR in temperate and tropical sediments were only observed at temperatures around 15-20°C. These observations imply psychrophilic adaptation of polar SRB and the predominance of mesophilic SRB in warmer latitudes. Further examination of the temperature dependency for sulfate reduction using Arrhenius plots in temperate sediments revealed that tropical sediments exhibited a more limited metabolic regulation at temperatures below 8-18°C and optimal temperature conditions for sulfate reduction closer to their ambient temperatures. Together, the inspection of the temperature responses for metabolic activity of SRB in marine sediments showed that temperature adaptations of SRB form a continuum with respect to their environmental latitude, which implies the potential of environmental temperatures for the selection of adaptive physiologies and for evolutionary divergence of microbiota in different latitudes.

INTRODUCTION

Mineralization rates of organic matter in coastal marine sediments frequently show a strong variability associated with seasonal changes at

ambient temperatures (e.g. Middelburg et al., 1996; Arnosti et al., 1998; Rysgaard et al., 1998; Thamdrup and Fleischer, 1998). Among the different processes implicated in the anaerobic benthic degradation of organic carbon, bacterial sulfate reduction has the quantitatively dominant role (Jørgensen, 1982). Consequently, most work on the environmental temperature dependence of sedimentary metabolism has involved seasonal studies of sulfate reduction in coastal sediments (e.g. Jørgensen, 1977; Aller and Yingst, 1980; Moeslund et al, 1994; Kristensen et al., 2000). Coastal environments, however, are highly variable and dynamic systems and the interpretation of the response of sulfate reduction rates to ambient temperatures is not straightforward. Westrich and Berner (1988) observed that the temperature dependence of SRR is more pronounced in sediments with lower benthic mineralization rates, and suggested that the temperature response of sulfate reduction may depend on the reactivity of accessible organic carbon. In fact, although rates of sulfate reduction are generally considered to be limited by the quantity and quality of available organic substrates (Arnosti et al., 1998; Sagemann, 1998), the physiological adaptations of the active SRB to ambient temperatures may have an important role in regulating the benthic terminal oxidation of organic matter.

The examination of the temperature characteristics for sulfate reduction of bacteria isolated from marine sediments has shown the coexistence of SRB populations with different temperature adaptations. Isaksen and Teske (1996) isolated a moderately psychrophilic sulfate-reducing bacterium from temperate sediments able to grow and sustain a higher catalytic activity at lower temperatures when compared to a mesophilic strain isolated from the same sediments (Isaksen and Jørgensen, 1996). Laboratory studies on the temperature dependence of sulfate reduction in Arctic and Antarctic marine sediments (i.e. Sagemann et al., 1998; Isaksen and Jørgensen, 1996) showed that the bacterial community is predominantly psychrophilic, while in temperate sediments the SRB were mostly mesophilic (Isaksen et al., 1994). When similar permanently cold and temperate sediments were compared over long-term temperature incubation experiments, Robador et al. (2009) observed that the response of bacterial sulfate reduction to warming was closely related to the physiological characteristics of the active SRB community. Together, these observations suggest the strong influence of

environmental temperatures on microbial selection and, therefore, on the rates of carbon degradation.

The aim of the present study was to investigate the physiological adaptation to environmental sediment temperatures, in terms of sulfate reduction rates (SRR), as an important mechanism controlling competition and other microbial interactions and, ultimately, the efficiency of carbon cycling. In order to understand the effect that ambient temperatures may have on the microbial carbon cycling in marine sediments, we compared the temperature dependence of the SRB community in sediments from different latitudes using temperature gradient incubation experiments.

MATERIAL AND METHODS

Sampling sites

Study sites for the present work were selected extending from polar regions to temperate and tropical latitudes in order to obtain a representative range of sediments exposed to different environmental temperatures. A detailed description of the study sites is provided in Table 1. Samples were obtained from the upper 10 cm depth of sediment from each site.

Temperature-gradient experiments

The temperature dependence of microbial energy metabolism was evaluated in temperature-gradient incubation experiments (Battley, 1964). Sediment slurries were incubated in Hungate tubes in an aluminum temperature-gradient block heated electrically at one end and cooled at the other end with a refrigerated and thermostated water bath. The temperature span was from 0° to +50°C to cover a large potential physiological temperature range of the active organisms, which was well above the optimal conditions for growth in some of the studied sediments. Additionally, the incubation temperature gradient for sites 1 to 5 (Table 1) was extended to -10°C in order to explore the physiological

limits of microorganisms at temperatures below the freezing point. Sediments slurries were prepared by dilution 1:1 with anoxic artificial seawater. Anoxic artificial seawater was prepared as described by Widdel and Bak (1992). Sediment slurries were made anoxic by bubbling with N_2 and 5 ml of slurry were transferred into each Hungate tube. Hungate tubes were flushed with N_2 according to the Hungate technique (Bryant, 1972) and sealed with butyl rubber stoppers. The Hungate tubes were immediately placed in a temperature-gradient block and preincubated for at least 5 hours to allow them to reach thermal equilibrium. Measurements of bacterial sulfate reduction were performed using ^{35}S-sulfate according to Kallmeyer et al. (2004). In order to minimize bacterial growth during the experiment the incubation time with the radiotracer was only 24 hours. For each temperature-gradient incubation experiment, average deviations of SRR were calculated based on four triplicate samples in the gradient block.

Temperature dependence

Activation energy and Q_{10} values were calculated from the slope of the linear range in Arrhenius plots to characterize the temperature dependence of metabolic activity. The Arrhenius curves were obtained from temperature-gradient incubations and represent the variation of metabolic rate as a function of temperature as follows:

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{R} \cdot \frac{1}{T} \right)$$

where E_a is the activation energy (J mol^{-1}), k is the rate of sulfate reduction (nmol cm^{-3} day^{-1}), A is the Arrhenius constant, R is the gas constant (8.314 J K^{-1} mol^{-1}), and T is the absolute temperature (K).

Q_{10} values between 20°C and 30°C were calculated according to the following equation:

$$Q_{10} = \exp \left[\frac{E_a \cdot 10}{RT(T + 10)} \right]$$

Table 1. Sampling site description

	Study sites	Coordinates	Sampling date	Sampling device	Water depth [m]	Average environmental temperature [°C]	Salinity [‰]	Sediment description
1	Arctic Ocean (Smeerenburgfjord, Svalbard)	79° 42´ N 11° 05´ E	ago-07	HAPS core	215	0[a]	33-34[a]	Permanently cold sediment with abundant worm burrows, soft brown colored in surface grading to clayey mottled dark grey-black over depth
2	Arctic Ocean (Isfjord, Svalbard)	78° 16´ N 14° 02´ E	jul-05	Push core	Subtidal	0[a]	27-30[a]	Seasonally freezing-thawing sediment located at the tidal-dominated fringe of a glacier moraine and consisting of black colored coarse-grained sand with abundant dead macrophytes
3	Southern Ocean (Weddell Sea)	65° 26´ S 61° 26´ W	sep-07	Multi core	850	0[a]	34[a]	Permanently cold sediment situated in the proximity of a methane-venting cold seep, consisting of light-grey colored stiff clay
4	Wadden Sea (German Bight, North Sea)	53° 27´ N 08° 07´ E	May-07	Push core	Intertidal	12[b]	22-30[b]	Estuary system subjected to strong seasonal temperature changes with abundant meio- and macrofauna, sediment consisting of light-brown sandy mud changing to black mud over depth
5	Baltic Sea (Arkona Basin)	54° 46´ N 13° 48´ E	jun-07	Multi core	9	12[b]	8-9[b]	Sediment subjected to seasonal temperature changes consisting of dark-brown and black colored mud
6	Arabian Sea (off the coast of Goa, India)	15° 6´ N 73° 24´ E	abr-07	Multi core	60	26[a]	34-35[a]	Permanently warm sediment from an upwelling system consisting of green colored soft fine-grained and watery mud
7	Arabian Sea (Sadeyat Island, United Arab Emirates)	24° 31´ N 54° 26´ E	sep-07	Push core	Intertidal	30[a]	200[a]	Permanently warm hypersaline sediment covered by a 0.5 cm-thick microbial mat and consisting of yellow with grey-black streaks fine-grained sand
8	Andaman Sea (Phuket Island, Thailand)	08° 03´ N 98° 25´ E	ago-07	Push core	Intertidal	28[a]	28-34[a]	Permanently warm tide-dominated mangrove forest, sediment consisting of brown colored coarse-grained sand
9	South China Sea (Hainan Island, China)	19° 35´ N 110° 48´ E	sep-07	Push core	Intertidal	30[a]	15-25[a]	Permanently warm sediment with abundant worm burrows, consisting of dark-brown colored dry sand

[a] *In-situ* measurements
[b] Measurements from closest automated monitoring station

RESULTS AND DISCUSSION

Rates of sulfate reduction and competitiveness of SRB in marine sediments

Studies on the temperature response of initial and terminal steps of organic carbon turnover in marine sediments suggest that absolute rates of sulfate reduction are mainly controlled by the availability of suitable electron donors rather than by temperature (e.g. Arnosti et al., 1998). SRR in marine sediments increase following the amendment with organic carbon compounds which has been interpreted as substrate limitation under in-situ conditions (Sagemann et al., 1998; Arnosti et al.,

2005). Therefore, absolute SRR measured in the present study (Fig. 1) likely reflect variations in the content and quality of organic matter in the different sediments studies here. In fact, the lowest SRR were measured in an oligotrophic environment, (Fig. 1.3 and Table 1) while the highest rates were observed in a habitat characterized by the high content of organic matter derived from decomposing macrophytes (Fig. 1.2 and Table 1). The examination of the competitiveness of SRB, understood in terms of relative metabolic rates at in situ temperatures, may be more useful to understand the overall response of sulfate reduction to ambient sediment temperatures.

The SRR measured in the Arctic and Antarctic study sites at 0 °C relative to those measured at the T_{opt}, 10-17 % (Fig. 1.1, 1.2 and 1.3), are in the range previously described for psychrophilic SRB communities in similar polar marine sediments (Isaksen and Jørgensen, 1996; Robador et al., 2009). A relatively high metabolic rate at low temperatures represents one of the main physiological adaptations of microorganisms to cold habitats (Harder and Veldkamp, 1968). Among the three polar environments, the Antarctic sediment exhibited the strongest psychrophilic response, with the lowest T_{opt} for sulfate reduction and the highest relative rates at low temperatures. Arctic sediments collected from Smeerenburgfjorden and Isfjorden on the west coast of Svalbard are influenced by slightly warmer water currents than the Weddell Sea, which may be the reason for the broader temperature range of the active SRB community. In permanently, but moderately cold sediments from temperate regions sulfate reduction showed rather a mesophilic temperature response which was comparable to that of other temperate environments (Isaksen and Jørgensen, 1996). These observations together suggest that, in addition to temperature, there are other important geographical factors influencing the temperature adaptations of SRB.

SRR measured at 0°C in polar sediments relative to maximal rates at the T_{opt} were comparable to those of temperate sediments at an approximately 15°C higher temperature, close to their respective in situ temperatures (Fig. 1.4, 1.5). This difference provides further indication for the adaptation of psychrophilic SRB to the permanently cold sediment temperatures in polar regions. Temperate sediments, however, are only seasonally exposed to low temperatures during winter and thus, the relative SRR, at 0 °C were only 3-6 % of the rates at T_{opt}. This is

in agreement with previously published data on the temperature characteristics of SRB communities in similar temperate sediments (Isaksen et al., 1994; Robador et al., 2009) and suggests a suboptimal adaptation to low temperatures in comparison to polar regions.

SRR were only 0.1-3 % of maximal rates at 0°C for the tropical sediments (Fig. 1.6, 1.7, 1.8 and 1.9). The permanently warm conditions in these environments may exert a strong pressure on SRB with low temperature adaptations and instead select for a community best adapted to permanently warm temperatures. In fact, sediments in the intertidal zone of the Arabian Sea can occasionally be exposed to temperatures close to 50°C (Al-Najjar, personal communication). At in situ temperatures at the time of collection, approximately 30 °C, SRR were 23-64 % of maximal rates which suggests that permanently warm sediments are dominated by a mesophilic SRB community with an optimum temperature for metabolism close to the ambient range.

Interestingly, several of the studied sediments, irrespective of their latitudinal position, showed a rapid increase in SRR above the T_{opt} (Fig. 1.1, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8). These rates indicated a thermophilic temperature response. In the present work, none of the study sites were situated in a region supporting in-situ growth at these high temperatures. Only the Antarctic site was located in the vicinity of a methane-venting seep, where thermophilic sulfate reducers could have originated from the deep subsurface through seepage transport to the seawater. Incubation experiments with sediments from temperate zones (Isaksen et al., 1994) also reported SRR in the thermophilic range which were attributed to the germination of SRB endospores. Thermophilic spore-forming bacteria have also been detected in the high Arctic (Hubert et al., submitted), but their activity was only induced after incubations for more than 12-16 hours at 50°C.

Temperature dependency for metabolic activity of SRB in marine sediments

Arrhenius plots are commonly used to examine the temperature dependence of thermally-induced process or reactions. The Arrhenius equation (Arrhenius, 1908) is generally applied to model the temperature dependency of the rate of a chemical reaction. The slope of the linear range obtained from plotting the natural logarithm of the

reaction rate against the reciprocal of the absolute temperature is proportional to the E_a of the reaction. The E_a can be defined as the minimum energy required to start a chemical reaction and small E_a values will therefore result in an increase of the reaction rate. In the context of temperature adaptations, for instance the catalysis of a chemical reaction by an efficient enzyme will yield a low E_a in order to render the appropriate rates at the ambient temperatures (Marx et al., 2007; D'Amico, 2002).

In the present study, chemical reaction rate has been substituted for SRR to examine the temperature response of sulfate reduction. The Arrhenius plots (Fig. 2) presented here derive from the ^{35}S-determinations of sulfate reduction in sediments (Fig. 1) and are characterized by a range of linearity, mostly extending below and above the environmental temperature range. Sulfate reduction occurs via a complex enzymatic system and the E_a estimated from the slope of the linear temperature range are only apparent activation energies. Furthermore, sulfate reduction is the terminal step in the sequential mineralization process of sedimentary organic matter and, therefore, calculated E_a are not necessarily activation energies by the sulfate-reducing community only, but reflect rather the response of a complex mixture of microbial populations. Despite these limitations, Knoblauch and Jørgensen (1999) found that calculated E_a values for pure cultures of SRB were similar to those estimated for whole sulfate-reducing communities in marine sediments. Coincident apparent E_a indicate that the response of sulfate reduction to increasing temperatures in pure cultures and natural sediments is comparable, and consequently, E_a may be a useful parameter to describe and compare the temperature sensitivity of SRB communities between sediments from different climate regimes.

Apparent E_a ranged between 36.1 kJ mol^{-1} and 69.1 kJ mol^{-1} (Q_{10}, 1.6-2.5; Fig. 2). These values are at the lower end of apparent E_a estimated in seasonal studies of coastal marine sediments, 36-132 kJ mol^{-1} (Westrich and Berner, 1988). Westrich and Berner (1988) observed that deeper buried sediments with lower SRR exhibited a more pronounced temperature dependency, i.e., higher E_a values, and attributed this effect to variations in the quantity of organic matter readily amenable to fuel sulfate reduction. However, similar experiments but with a higher temperature resolution showed that apparent E_a in substrate-limited

sediments, 40-75 kJ mol^{-1}, did not change significantly after the addition of organic substrates (Sagemann et al., 1998). It is therefore possible that the variability of apparent E_a previously observed in marine sediments is not due to the reactivity of the organic matter but due to the different physiological adaptations of coexisting SRB populations.

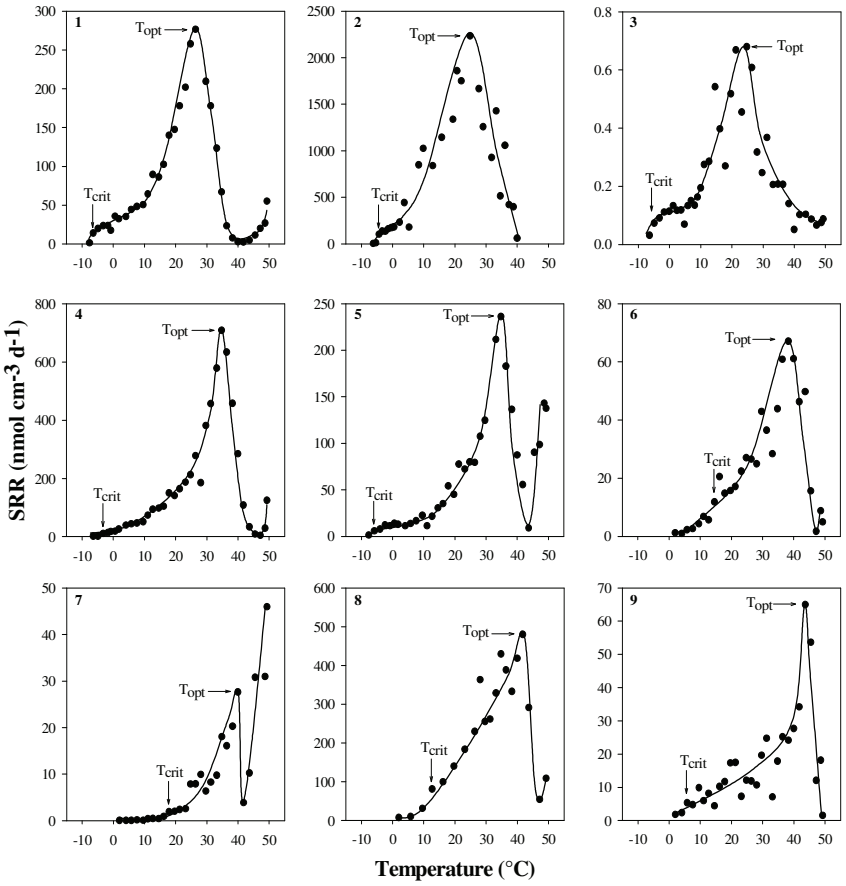

Figure 1. SRR measured in temperature-gradient incubation experiments of sediment slurries from stations: (1) 20 % deviation; (2) 32 % deviation; (3) 13 % deviation; (4) 19 % deviation; (5) 20 % deviation; (6) 15 % deviation; (7) 35 % deviation; (8) 17 % deviation; (9) 25 % deviation.

In the present work, estimated E_a remained constant over a linear range that extended from the T_{opt} down to an apparent transition temperature (Fig. 2). Below this temperature the slope changed sharply demonstrating higher E_a values. The temperature at the intersection of the two slopes is defined as the 'critical temperature', T_{crit} (Lamanna et al., 1973). The T_{crit} has been previously explained as the transition temperature between optimal and sub-optimal domains for bacterial growth (Guillou and Guespin-Michel, 1996). Although the cellular basis for T_{crit} remains uncertain, this temperature is likely the result of the uncoupling of cellular energy metabolism at low temperatures. The existence of T_{crit} has been described for psychrotolerant, mesophilic and thermophilic microorganisms (Harder and Veldkamp, 1968; Mohr and Krawiec, 1980; Reichardt and Morita, 1982). Reports for psychrophilic microorganisms are lacking, probably because growth rates of psychrophiles have not been examined systematically at sufficiently low temperatures (Bakermans and Nealson, 2004). In addition to E_a, T_{crit} may be a functional parameter important to explain the physiological temperature dependency of microorganisms.

T_{crit} has been described for sulfate reduction in SRB isolates (Tarpgaard et al., 2006), although there are no reports in marine sediments. This is probably because most of the studies have investigated the psychrophilic response of SRB in low-temperature environments at minimum temperatures of -3.5°C (e.g. Robador et al., 2009). In the present study, however, the temperature dependency of sulfate reduction in polar and temperate samples was examined down to -10°C. T_{crit} were apparent at temperatures, between -3.4°C and -6.4°C, which is at or below the freezing point of the sediment slurries (Fig. 2.1 to 2.5). Below the T_{crit}, apparent E_a increased abruptly to 328.3-1244.6 kJ mol^{-1} (Q_{10}, 80.7-1.9x10^8). These values are well above those reported previously (e.g. Westrich and Berner, 1988) and likely reflect the physico-chemical constrains (i.e. low water activity, low nutrient content, high salinity) imposed by the freezing process of the sediment rather than the physiological response of the cells to extreme low temperatures. These results clearly indicate that the Arctic sulfate-reducing community is well adapted to tolerate temperatures down or beyond the freezing point of seawater, which may permit survival and recovery even after temporary freeze conditions.

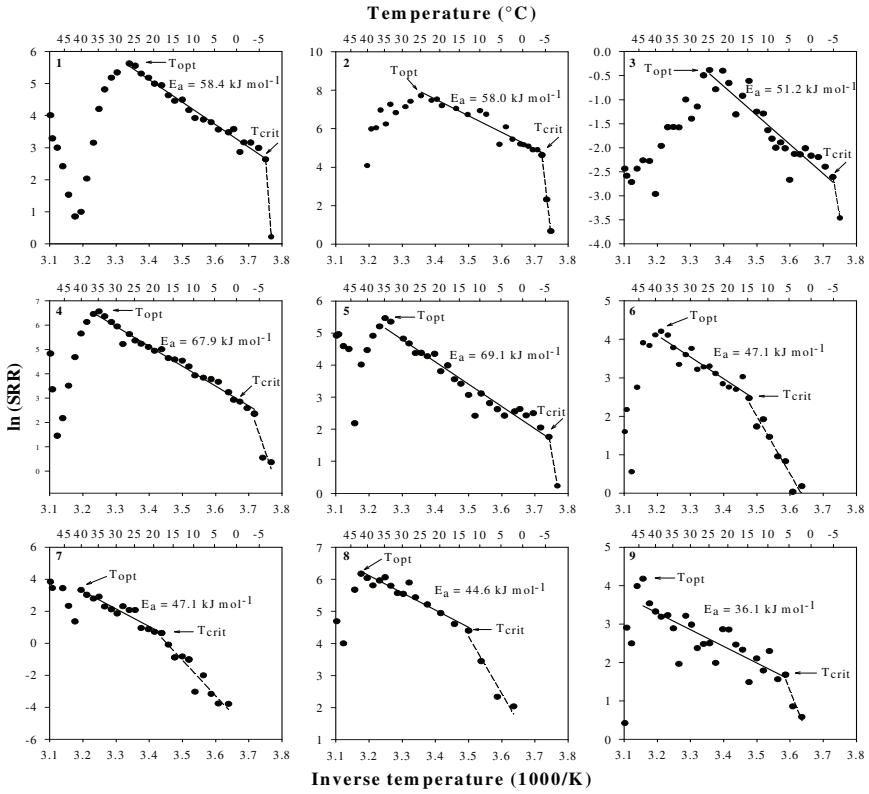

Figure 2. Arrhenius plots of the data in fig. 1.

By contrast, in sediments from tropical latitudes, T_{crit} were between +8°C and +18°C (Fig. 2.6 to 2.9). Below these temperatures apparent E_a values increased to 124.6-184.6 kJ mol^{-1} (Q_{10}, 5.3-11.8), which suggests that sulfate-reducing communities in permanently warm climates, in contrast with the Arctic sediments, have a higher temperature dependence than communities in seasonally changing and permanently cold habitats.

Conclusions - Correlation between environmental temperatures and cardinal temperatures of sulfate reduction

Our results suggest a direct relationship between the ambient environmental temperature and sedimentary bacterial energy metabolism reflected in the temperature of maximal sulfate reduction rates (T_{opt}) (Fig. 3). Although T_{opt} generally exceeds the in situ temperatures experienced by the microbial communities and despite the frequent use in the literature at optimum temperature does not reflect their optimum temperature, the proportional increase with the mean ambient temperatures (Fig. 3A) implies diverse temperature sensitivities of the microbial community in the studied environments. Arctic and Antarctic sediments exhibited T_{opt} for sulfate reduction of 24-26 °C (Fig. 1.1, 1.2 and 1.3), similar to those previously reported for some psychrophilic SRB isolates (Knoblauch et al., 1999). The T_{opt} observed in warmer temperate and tropical sediments, however, are in the range of those reported for nominal mesophiles (Isaksen and Jørgensen, 1996). Sediments from temperate latitudes showed broader thermal ranges than polar sediments and sulfate reduction could be measured from temperatures below 0°C up to the T_{opt} at 35°C (Fig. 1.4, 1.5). Tropical sediments exhibited a shift of the thermal range for sulfate reduction towards higher T_{opt}, 38-44°C (Fig. 1.6, 1.7, 1.8 and 1.9). All SRB share a unique metabolic pathway for the reduction of sulfate defined by a distinct set of enzymes (Madigan et al., 2009). Increasing T_{opt} for sulfate reduction reflect the diverse nature of these enzymes and their temperature adaptations.

The difference between environmental temperatures and T_{opt} of bacterial sulfate reduction, however, varied between the sediments. At in-situ temperatures of 0°C in corresponding polar regions the difference was approximately 27°C, while at in situ temperatures of 30°C in tropical habitats this difference was reduced to 15°C (Fig. 3A). Previous studies (i.e. Isaksen and Jørgensen, 1996; Knoblauch and Jørgensen, 1999) also observed that in pure cultures of psychrophilic bacteria there is

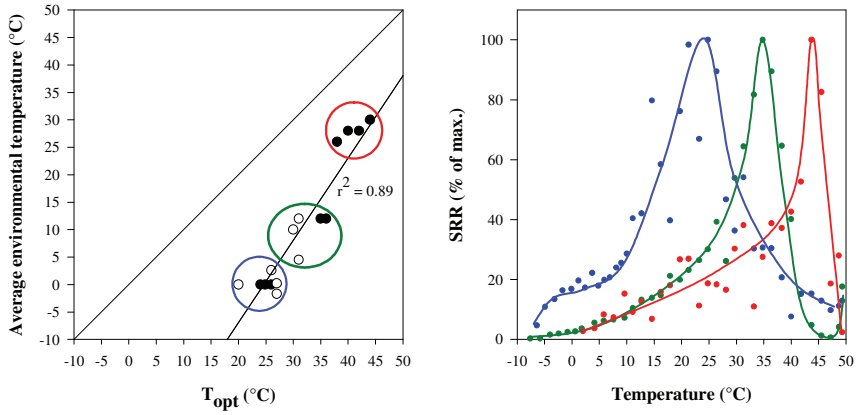

Figure 3. (A) Relations between average environmental temperatures and Topt for sulfate reduction in marine sediments grouped according to sampling latitude: Polar regions, blue line; Temperate regions, green line; Tropical regions, red line. The plot is based on: data presented in this study, full circles; data compiled from Isaksen et al. (1994), Isaksen and Jørgensen (1996), Arnosti et al. (1998) and Sagemann et al. (1998), open circles. The straight line passing through the origin is the theoretical curve if environmental temperatures and Topt for SRR were the same. The regression line indicates the empirical relation between environmental temperatures and Topt for SRR. (B) SRR expressed as percentage of maximum rates, corresponding to data in panels 3, 4 and 9 of figure 1. Profiles were selected to represent the characteristic temperature responses of each group in panel A.

a larger difference between T_{opt} of SRR than in mesophilic bacteria. The explanation for the larger difference in cardinal temperatures of psychrophilic bacteria is unclear, but it is likely due to different adaptation mechanisms of the catalytic enzymes and the membrane structure. The adaptation of enzymes to colder temperatures is presumably achieved through structural changes associated with a low thermal stability, which is evidenced by lower T_{opt} for activity (Feller and Gerday, 1997). Enzymes of psychrophilic microorganisms have reached a state that is close to the lowest possible stability and they cannot be less stable without losing the native and active conformation (cold denaturation) and, consequently, T_{opt} always remain well above the low ambient temperatures (Feller and Gerday, 2003). As a result, the

relative activity of psychrophilic SRB in polar sediments is high at low temperatures, though it is lower than that exhibited by their mesophilic counterparts in tropical latitudes at their environmental temperature, 17% and 34% respectively (Fig. 3B).

In conclusion, the physiological responses described in this study demonstrate that psychrophilic and mesophilic SRB in polar and tropical environments, respectively, have evolved to adapt their energy metabolism to the stenothermal environmental conditions. Ambient temperatures outside the upper or lower limits of their thermal range likely result in functional constraints. In eurythermal habitats, the overall rates of organic carbon mineralization are likely determined by the combined metabolic response of coexisting populations to the wide range of temperatures that characterize these environments. The heterogeneous temperature adaptations of the coexisting SRB in these habitats can explain the broad temperature response described above. However, a higher competitiveness of mesophilic SRB compared to their psychrophilic counterparts at their respective in situ temperatures may explain the predominance of mesophilic microorganisms in these habitats.

The potential significance of environmental temperatures and habitat temperature variability has generally not been taken into account in the study of the temperature response of carbon mineralization in marine environment (Wohlers et al., 2009). Bacterial temperature response is generally assumed to be well described by Q_{10} values between 2 and 3 (Pomeroy and Wiebe, 2001). The present study shows that biogeographic variability, selection of adaptive physiologies, and evolutionary divergence of microbiota in different latitudes need also be considered for an improved quantification of respiration effects in response to ocean warming.

ACKNOWLEDGMENTS

Mohammad Al-Najjar, Naik Hema, Moritz Holtappels, Erik Kristensen, Uwe Krumme, S.W.A. Naqvi and Helge Niemann are gratefully acknowledged for providing sediment samples and for detailed information of the sampling sites. Casey Hubert is also acknowledged for helpful discussions. Kirsten Imhoff, Anna Kaufmann, Susanne Schwarz and Katharina Zyromski are acknowledged for

assistance in the lab. Financial support was provided by the German Research Foundation through the Priority Program 1162 'The impact of climate variability on aquatic ecosystems (Aquashift)', and by the Max Planck Society.

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MANUSCRIPT 2

THE IMPACT OF TEMPERATURE CHANGE ON THE ACTIVITY AND COMMUNITY COMPOSITION OF SULFATE-REDUCING BACTERIA IN ARCTIC VERSUS TEMPERATE MARINE SEDIMENTS

Environmental Microbiology **11**: 1692-1703

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Running title: The impact of temperature change on bacterial sulfate reduction

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Keywords: arctic sediment, temperate sediment, sulfate reduction, temperature adaptation

SUMMARY

Arctic regions may be particularly sensitive to climate warming and, consequently, rates of carbon mineralization in warming marine sediment may also be affected. Using long-term (24 months) incubation experiments at 0°C, 10°C and 20°C, the temperature response of metabolic activity and community composition of sulfate-reducing bacteria were studied in the permanently cold sediment of northwestern Svalbard (Arctic Ocean) and compared to a temperate habitat with seasonally varying temperature (German Bight, North Sea). Short-term ^{35}S-sulfate tracer incubations in a temperature gradient block (between -3.5° and +40°C) were used to assess variations in sulfate reduction rates (SRR) during the course of the experiment. Warming of arctic sediment resulted in a gradual increase of the temperature optima (T_{opt}) for sulfate reduction suggesting a positive selection of psychrotolerant / mesophilic sulfate-reducing bacteria (SRB). However, high rates at *in-situ* temperatures compared to maximum rates showed the predominance of psychrophilic SRB even at high incubation temperatures. Changing apparent activation energies (E_a) showed that increasing temperatures had an initial negative impact on sulfate reduction that was weaker after prolonged incubations, which could imply an acclimatization response rather than a selection process of the SRB community. The microbial community composition was analyzed by targeting the 16S ribosomal RNA using catalyzed reporter deposition fluorescence *in-situ* hybridization (CARD-FISH). The results showed the decline of specific groups of SRB and confirmed a strong impact of increasing temperatures on the microbial community composition of arctic sediment. Conversely, in seasonally changing sediment sulfate reduction rates and sulfate-reducing bacterial abundance changed little in response to changing temperature.

INTRODUCTION

The marine carbon cycle in arctic regions currently occupies a prominent role in the study of climate warming effects as there is evidence from observational and modeling studies for strong seasonal

temperature increases in northern high-latitude environments (Serreze et al., 2000).This is further motivated by the fact that biogeochemical processes react very sensitively to temperature changes (Denman et al., 2007; IPCC 2007). Although primary production and organic sedimentation exert the dominant influence on organic matter oxidation rates in marine sediments (Arnosti et al., 1998; Kostka et al., 1999), the seasonal variation of SRR in temperate habitats shows that temperature has a modulating effect on top of the control by sedimentation (Moeslund et al., 1994, Rysgaard et al., 1998). Pomeroy and Wiebe (2001) considered that low substrate concentrations and low temperatures act together to control the metabolic activity of heterotrophic bacterial communities in marine environments. Yet, the extent to which the amount and quality of organic matter rather than temperature regulate the mineralization of organic matter is often not clear and differs with the temperature regime.

Metabolic activity at permanently low temperatures requires specific adaptations (Panoff et al., 1998; Deming, 2002; Cavicchioli, 2006; Chattopadhyay, 2006). Among the most important is the capacity of cold-adapted bacteria to synthesize more enzymes with high catalytic activities at low temperatures (Feller and Gerday 2003; Hoyoux et al., 2004). Competition experiments carried out by Harder and Veldkamp (1971) showed that, at low temperatures, psychrophiles grow faster than psychrotolerant bacteria. Knoblauch et al. (1999a) showed that psychrophilic sulfate-reducing bacteria (SRB) isolated from Svalbard sediments have higher cell-specific metabolic rates than mesophilic SRB at the same low temperatures. A study of competition between psychrophilic and psychrotolerant bacterial groups in coastal arctic waters suggests that psychrophiles can dominate in waters only recently subjected to permanently cold conditions (Connelly et al., 2006). Lastly, the observation that the preservation of deposited organic carbon in coastal and shelf sediments of the Arctic is not higher than in

comparable temperate sediments provides indirect evidence for active psychrophilic heterotrophic bacteria (Bowman, 2004).

Experimental studies of the ecological importance of psychrophilic bacteria in marine habitats (Helmke and Weyland, 2004) suggest that psychrophiles prevail in Arctic sediments, although temporary and regionally limited intrusions of warm Atlantic water result in a heterogeneous temperature response of the bacterial sediment communities. Norkrans and Stehn (1978) found that only one third of the strains isolated from the deep Norwegian Sea were psychrophilic. We therefore address the question whether SRB communities in permanently cold sediments, and thus the SRR, are particularly sensitive to temperature shifts and regulated differently in sediments from arctic and from temperate environments. The geographic distribution of psychrophiles is not only restricted to permanently cold environments, since psychrophilic bacteria can also be enriched in winter during studies of temperate environments (Poremba et al., 1999; Helmke and Weyland, 2004). Yet, why do rates of microbial processes decrease so strongly during winter in sediments with seasonal temperature changes? We hypothesize that habitats with seasonally changing temperature favor the development of psychrotolerant / mesophilic communities whereas psychrophiles do not grow fast enough to compete and establish a significant community during the cold season. Furthermore, they may not survive the warm season. Experiments were designed to study the functional response of SRB communities to long-term temperature shifts in permanently cold versus seasonally changing marine sediments using ^{35}S-sulfate reduction rate measurements and CARD-FISH analysis of specific SRB abundance.

RESULTS

Characterization of the arctic and temperate study sites

The *in-situ* concentrations of sulfate measured at the Arctic study site decreased slightly with depth from 29 mM to 26 mM. Sulfate reduction rates increased from 4 nmol cm^{-3} day^{-1}at the sediment surface to 22.5 nmol cm^{-3} day^{-1}at 8 cm depth and decreased sharply below this depth (Fig. 1A). Sulfate concentrations along Dangast sediment core were between 12.5 and 15 mM reflecting the influence of fresh water input. The profile of SRR was comparable to the Arctic site and increased from a few nmol cm^{-3} day^{-1} at the sediment surface to 32 nmol cm^{-3} day^{-1} at a depth of 9 cm (Fig. 1B).

Temperature response of sulfate reduction rates

Temperature-gradient incubation experiments with the arctic and temperate sediment were carried out at the beginning of the experiment (initial conditions) and after 2, 6, 12 and 24 months of incubation. Fig. 2 shows the temperature-dependence of SRR determined at initial conditions for the two study sites. A common feature in both environments was an exponential increase of the sulfate reduction rates to the corresponding temperature optimum (T_{opt}), defined as the temperature with the maximum ^{35}S-sulfate reduction rates, followed by a drop above the T_{opt}. The linear range of the Arrhenius plots extended from temperatures below the *in-situ* conditions to the T_{opt} in all experiments. Tables 1 and 2 summarize the sulfate reduction rates at 0°C and T_{opt}, the range of linearity, and the calculated activation energies (E_a) for the sediment from the Arctic and North Sea, respectively. In both sediments, SRR and the average deviation of SRR were slightly elevated at the initial conditions relative to the subsequent time points.

However, the percentage of SRR at *in-situ* temperature relative to T_{opt} and the range of linearity were similar to the conditions after two months of incubation. This effect can likely be attributed to sediment sampling and the bagging of the sediment in the polyvinyl bags, but had no evident effect on the relative temperature response in these sediments.

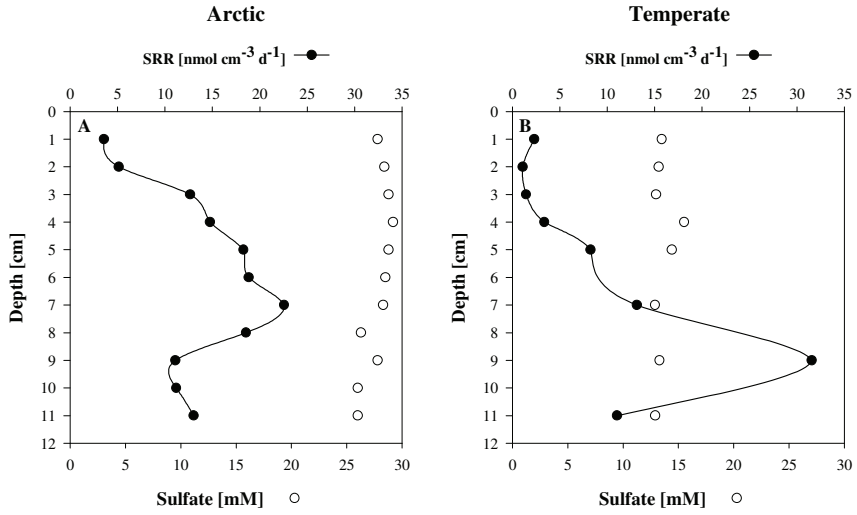

Figure 1. Depth profile of sulfate concentrations and sulfate reduction rates in (A) the arctic and (B) the temperate study sites.

Under initial conditions, in the arctic sediment, SRR increased from the *in-situ* temperature to the T_{opt} of 22°C, where the rate was 546±136 nmol cm^{-3} day^{-1} (Fig. 2A). At the *in-situ* temperature, the SRR was 13% of the maximum rate. In the temperate sediment, the SRR at 0°C was initially only 2% of the maximum rate of 1333±293 nmol cm^{-3} day^{-1}, which was measured at 33°C (Fig. 2B). An E_a of 50 kJ mol^{-1} was determined from the Arctic temperature response profile (Fig. 2C),

which contrasted with an E_a value of 81 kJ mol^{-1} in the sediment from Dangast (Fig. 2D).

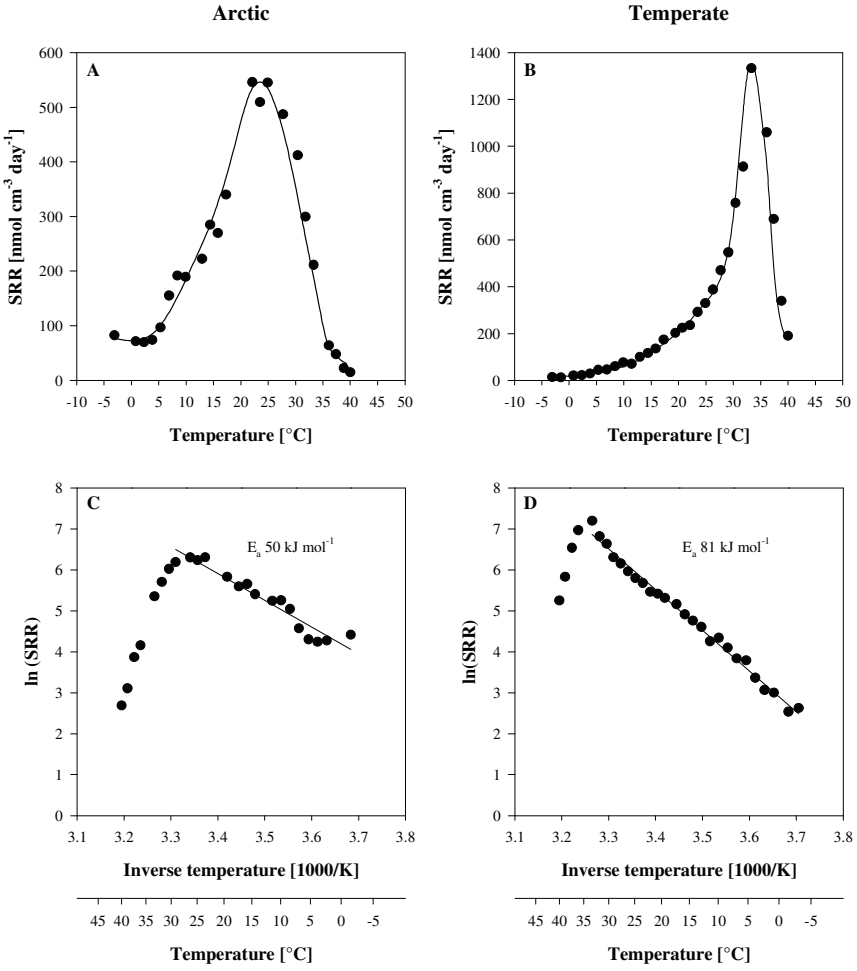

Figure 2. Sulfate reduction rates determined from the temperature-gradient incubation experiment of (A) the arctic and (B) the temperate sediment at initial conditions. (C) and (D) Arrhenius plots of the data in panels (A) and (B), respectively.

In the permanently cold sediment, as the incubation progressed, a gradual increase of the T_{opt} was observed relative to the 0°C incubation experiment (Fig. 3A). The 10°C experiment showed a difference in the T_{opt} of approximately 5°C relative to the 0°C experiment. Incubations at 20°C resulted in a difference of the T_{opt} of 8°C (Fig. 3A). In the temperate sediment the T_{opt} remained constant at 33°C in the 10°C long-term treatment, and was only slightly different in the 0° and 20°C treatments (Fig. 3B). However, these observed changes are likely to reflect the error in the determination of the T_{opt}, often between 1° to 2°C, rather than a biological response to temperature changes.

Table 1. Sulfate reduction activities and Arrhenius parameters determined in the temperature-gradient incubation experiments for arctic sediment

Incubation temperature [°C]	Incubation time [months]	$T_{opt.}$ [°C]	Sulfate reduction rates [nmol cm^{-3} day^{-1}] At incubation temperature	At 0°C	At T_{opt}	% SRR[a]	Range of linearity[b] [°C]	E_a [kJ mol^{-1}]	Q_{10}
0	i.c.	22	72 ± 18	72 ± 18	546 ± 136	13	-3, +22	50	1.9
	12	22	48 ± 3	48 ± 3	377 ± 23	13	-3, +22	54	2.1
	24	24	26 ± 7	26 ± 7	369 ± 96	7	-3, +24	50	1.9
10	2	26	62 ± 9	23 ± 4	344 ± 53	7	-3, +26	74	2.7
	6	24	59 ± 13	26 ± 6	227 ± 50	12	-3, +24	63	2.3
	12	29	66 ± 20	20 ± 6	121 ± 37	17	-3, +29	41	1.7
	24	30	20 ± 5	9 ± 2	48 ± 13	18	-3, +30	41	1.7
20	2	29	208 ± 30	35 ± 5	437 ± 63	8	-3, +29	56	2.1
	6	29	247 ± 109	28 ± 12	148 ± 66	19	-3, +29	53	2.0
	12	31	214 ± 59	45 ± 12	360 ± 99	12	-3, +31	50	1.9
	24	32	61 ± 6	9 ± 1	121 ± 13	8	-3, +32	53	2.0

i.c., initial conditions

[a] % of SRR of 0°C to optimum temperature

[b] The term "Range of linearity" refers to the linear part of the Arrhenius plot

In the arctic sediment, the apparent E_a and Q_{10} values initially increased in the incubations at higher temperatures. However, prolonged incubation at 10° and 20°C resulted in a subsequent gradual decrease (Table 1). In contrast, in the temperate sediment E_a and Q_{10} values varied little over the whole incubation period (Table 2). SRR decreased substantially in the three long-term incubation temperature treatments in the period between 12 and 24 months (Table 2). This effect was more evident at the 10° and 20°C incubation temperatures. In the

temperate sediment, a significant decrease in SRR was also observed after 24 months, but only in the 20°C treatment (Table 3).

Total cell counts and CARD-FISH

Total cell numbers were determined by DAPI staining and different bacterial groups were quantified by CARD-FISH during the time-course experiments. A summary of the total microbial numbers, the percentage of the total DAPI cell counts and the cell numbers detected with each of the probes is shown in Table 3.

Total microbial cell numbers

At the beginning of the experiments, the arctic sediment contained 4.1×10^9 cells cm^{-3}. In the 0°C experiment bag, these numbers remained constant over 24 months. In the 10° and 20°C experimental bags the number of cells had decreased to 3.4×10^9 cells cm^{-3} and 2.2×10^9 cells cm^{-3}, respectively, after 24 months of incubation. The temperate sediment had 5.0×10^9 cells cm^{-3} at the beginning of the experiment. Cell numbers in the 0°C, 10°C and 20°C bag changed insignificantly over the course of the experiment.

Bacteria

In the 0°C experiment bag with arctic sediment 80% of the total DAPI cell counts initially hybridized to the eubacterial EUBI-III probe. The number of *Bacteria* was 3.3×10^9 cells cm^{-3}. After 24 months of incubation at 0° and 10°C, no significant decrease in the cell numbers was observed, as the abundance of *Bacteria* was 79 and 81% of the total DAPI cell counts. The number of *Bacteria* decreased progressively in the 20°C experiment. At the end of this experiment only 1.2×10^9 cells cm^{-3} were

detected and the number of *Bacteria* detected by CARD-FISH accounted for only 56% to the DAPI-stained cells.

Prolonged incubation at 0°C of the temperate sediment resulted in a decrease of the bacterial numbers from 4.2 x 10^9 cells cm^{-3} at the initial conditions to 2.9 x 10^9 cells cm^{-3} after 24 months of incubation. The abundance of *Bacteria* decreased from 84% to 76%, respectively. In the 20°C experiment, a decrease of the abundance of *Bacteria* to 3.7 x 10^9 cells cm^{-3} was observed, which corresponded to a significant decrease in 12% of the DAPI counts. The detection rate with the NON338 nonsense probe remained always below 0.1% of the DAPI cell counts.

Table 2. Sulfate reduction activities and Arrhenius parameters determined in the temperature-gradient incubation experiments for temperate sediment

Incubation temperature	Incubation time	$T_{opt.}$	Sulfate reduction rates [nmol cm^{-3} day^{-1}]			% SRR[a]	Range of linearity[b]	E_a	Q_{10}
			At incubation temperature	At 0°C	At T_{opt}				
[°C]	[months]	[°C]					[°C]	[kJ mol^{-1}]	
0	12	30	15 ± 4	15 ± 4	536 ± 144	3	-3, +30	75	2.73
	24	32	17 ± 2	17 ± 2	696 ± 101	2	-3, +31	75	2.73
10	i.c.	33	76 ± 28	20 ± 7	1333 ± 293	2	-3, +33	81	2.95
	2	33	47 ± 8	18 ± 3	799 ± 129	2	-3, +33	77	2.81
	6	33	96 ± 20	26 ± 5	1174 ± 244	2	-3, +33	81	2.95
	12	33	40 ± 12	14 ± 4	502 ± 153	3	-3, +33	73	2.64
	24	33	35 ± 5	14 ± 2	512 ± 74	3	-3, +33	73	2.64
20	2	35	568 ± 162	21 ± 6	682 ± 194	3	-3, +35	71	2.6
	6	36	300 ± 33	33 ± 4	866 ± 94	4	-3, +36	67	2.46
	12	33	288 ± 49	14 ± 2	413 ± 70	3	-3, +33	72	2.63
	24	35	91 ± 15	9 ± 1	217 ± 35	4	-3, +35	72	2.63

i.c., initial conditions

[a] % of SRR of 0°C to optimum temperature

[b] The term "Range of linearity" refers to the linear part of the Arrhenius plot

Sulfate-reducing bacteria

In the experiments with the arctic sediment cells targeted by the probe DSS658 showed the highest detection rates, 12% of DAPI at initial conditions. The relative abundances of cells hybridized by probes DSR651, DSB985 and Sval428 were 4.1%, 1% and 2.7% of DAPI counts, respectively. With increasing incubation time and temperature the

abundance of groups targeted by these probes decreased gradually. After 24 months of incubation at 10°C, the detection rate of probes DSR651, DSB985, and Sval428 had decreased to 1%, 2.2% and 0.2%, respectively.. At the end of the 20°C incubation experiment no cells were detected with the exception of cells that hybridized with the DSR651 probe, which detected 0.4 % of the DAPI cell counts.

At the initial conditions in the temperate sediment from Dangast, probe DSS658 also targeted the largest number of cells, 9.1 % of total DAPI counts. Probes DSR651, DSB985 and Sval428 targeted 2.9, 1.6 and 1.8%, respectively. In contrast to the arctic sediment from Svalbard, different incubation temperatures and incubation time had no significant influence on the relative abundances of the specific SRB groups.

Table 3. Quantification* of bacteria by CARD-FISH in arctic and temperate sediments

		Arctic												Temperate											
Incubation temperature [°C]		0			10				20					0		10					20				
Incubation time [months]		i.c.	12	24	2	6	12	24	2	6	12	24		12	24	i.c.	2	6	12	24	2	6	12	24	
DAPI	Cell number [10^9 cm^{-3}]	4.1	4.5	4.3	4.3	3.6	4.8	3.4	4.1	3.5	1.8	2.2		3.8	3.8	5.0	5.8	6.1	6.1	4.8	4.6	5.0	5.3	5.1	
Probe EUB 338	Cell number [10^9 cm^{-3}]	3.3	3.7	3.4	3.5	2.9	3.9	2.8	3.4	2.8	1.3	1.2		3.1	2.9	4.2	4.9	4.6	4.8	3.7	3.9	4.3	4.4	3.7	
	% DAPI	80	84	79	81	82	82	81	83	80	72	56		83	76	84	85	76	78	79	84	86	84	72	
Probe DSS 658	Cell number [10^8 cm^{-3}]	4.9	5.2	5.5	4.6	2.9	2.6	n.d.	3.4	2.0	0.4	n.d.		3.8	5.1	4.5	5.7	6.6	6.1	4.5	5.2	4.7	5.9	5.0	
	% DAPI	12	12	13	11	8.2	5.4	n.d.	8.3	5.7	2.1	n.d.		10	14	9.1	9.8	11	9.9	9.5	11	9.4	11	9.7	
Probe DSR 651	Cell number [10^8 cm^{-3}]	2.2	1.2	1.0	1.7	0.8	0.6	0.3	1.7	1.0	0.2	0.1		0.5	1.2	1.4	1.4	0.9	3.6	0.5	1.2	1.3	1.8	0.5	
	% DAPI	5.2	2.7	2.4	4.1	2.3	1.3	1.0	4.2	3.0	0.9	0.4		1.4	3.1	2.9	2.4	1.5	5.9	1.1	2.6	2.5	3.4	1.0	
Probe DSB 985	Cell number [10^8 cm^{-3}]	2.1	1.2	1.5	0.4	0.9	0.9	0.8	0.6	0.6	0.3	n.d.		0.8	0.4	0.8	0.6	0.6	1.3	0.7	0.4	0.9	1.1	0.5	
	% DAPI	5.0	2.7	3.4	1.0	2.5	2.0	2.2	1.4	1.6	1.8	n.d.		2.0	1.1	1.6	1.0	1.0	2.1	1.5	0.9	1.7	2.1	1.1	
Probe Sval 428	Cell number [10^8 cm^{-3}]	1.6	0.8	1.1	1.1	0.8	0.5	0.1	1.1	0.9	0.3	n.d.		0.8	0.6	0.9	0.8	0.4	1.1	0.7	0.7	0.6	1.0	0.2	
	% DAPI	3.9	1.8	2.5	2.7	2.2	1.0	0.2	2.7	2.5	1.9	n.d.		2.2	1.5	1.8	1.4	0.7	1.9	1.5	1.4	1.1	2.0	0.4	

*The results are expressed as the average of duplicate measurements
i.c., initial conditions
n.d., not detected

DISCUSSION

Characterization of the temperature response of sulfate reduction

The depth-profiles of mineralization rates via sulfate reduction were comparable at the two study sites (Fig. 1). Other studies of benthic carbon mineralization rates in coastal sediments have measured similar rates in arctic and temperate regions (Glud et al., 1998). However, our temperature-gradient incubations of both arctic and temperate sediment showed distinct temperature responses for sulfate reduction that suggest different characteristic community responses specific for arctic and for temperate sediment.

In the arctic sediment, the respiration rates increased exponentially with temperature from below *in-situ* temperatures up to 22°C at the T_{opt} (Fig. 2A). This temperature range for sulfate reduction is consistent with results of previous experiments with similar sediments from Svalbard (Sagemann et al., 1998). The arctic sediment incubated at 0°C showed constant T_{opt} values over 24 months of incubation and remained in the range previously described for psychrophilic bacteria (Knoblauch and Jørgensen 1999). However, long-term incubation experiments at 10° and 20°C resulted in an upshift of the T_{opt} for sulfate reduction to approximately 30 and 32°C, respectively (Fig. 3A). The resulting T_{opt} for respiration are comparable to the response observed in temperate sediment (Fig. 2B and 3B). Moreover, these higher values are similar to reported T_{opt} for psychrotolerant and mesophilic SRB (Isaksen and Jørgensen, 1996). These coincident T_{opt} would imply a shift of the community composition, and that, after 24 months of incubation experiments, the arctic sediment does not host a dominant psychrophilic community but a mixed community of psychrophilic, psychrotolerant and mesophilic SRB. It is plausible that a SRB community, which can be considered only "potential" under low *in-situ* temperatures, became active in the mineralization process of organic matter when incubating

the sediment at 10° and 20°C. Temperatures below the optimal growth range of an organism can cause temporary inhibition of its regulatory processes resulting in lower enzyme activity (Ng et al., 1969). Moreover, some bacteria can also resist environmental stress by switching to slow-growing or dormant vegetative forms (Balaban et al., 2007). However, the characterization of the SRB communities on the basis of T_{opt} for SRR may not be ecologically significant, since SRB can have their highest SRR 5° to 10°C above the T_{opt} for growth (Knoblauch and Jørgensen 1999). An examination of the respiration rates at *in-situ* temperatures relative to the rates at T_{opt} may be more informative (Isaksen and Jørgensen, 1996).

The activity at 0°C in the Arctic was sixfold higher relative to T_{opt} than it is in the temperate sediment (Fig. 2). Comparative studies between psychrophilic bacteria and their mesophilic counterparts have shown that high metabolic activity at low temperatures is the main physiological adaptation to cold at the enzymatic level (Feller, 2003). After 24 months of incubation, the rates of sulfate reduction at *in-situ* temperatures (Table 1) were similar to the *in-situ* rates measured in a previous investigation of arctic sediment (Knoblauch et al., 1999a). Moreover, the relative activities at the *in-situ* temperature remained between 7 and 18% of maximal activities after 24 months of incubation (Fig. 3A). Following the definition of Harder and Veldkamp (1968), these high rates of respiration at low temperatures would suggest that a psychrophilic SRB community remained after 24 months of incubation at all three temperatures. Moreover, low Q_{10} values (Table 1) agree with previous studies of Antarctic sediment (Isaksen and Jørgensen, 1996) indicating a relative high activity of SRB at low temperatures. The E_a values calculated for each of the short-term incubation experiments increased with increasing incubation temperatures (Table 1). However, the E_a values decreased gradually after long-term incubation at 10° and 20°C. Deviations from the initial apparent E_a values may be interpreted as the result of deficient temperature acclimation. Shaw (1967) observed that, following transfer of psychrophilic yeast cultures to higher

temperatures, a lag period in growth occurs, which may be explained by temporary damage and repair thereof at the high temperature. This lag period was followed by an increase of the growth rates until the normal rate of growth was achieved at the new temperature. Apparent E_a values are a measure of a response of the whole sulfate-reducing bacterial community to temperature changes. It is therefore possible that the high E_a indicate the transient response of a dominant active psychrophilic population rather than a change in the community composition.

The initial temperature response profile observed at the temperate study site (Fig. 2B) is in good agreement with previously published data for a temperate habitat in the Baltic (Isaksen et al., 1994). The E_a values were always higher in the temperate than in the arctic sediment incubation experiments and suggest that respiration rates in temperate sediment are more affected by low temperatures (Table 1 and 2). Nevertheless, the consistent linear temperature range in the Arrhenius plots from -3° to +35°C (Table 2) implies that the active SRB community in temperate sediment is active at low temperatures for long periods. According to Harder and Veldkamp (1968), these temperature characteristics are representative of nominal psychrotolerant bacteria. Helmke and Weyland (2004) found in a temperate habitat that cold-adapted bacteria were more abundant during winter and argued that at constant cold conditions, if enough substrates are available, psychrophiles can achieve high growth rates and outcompete psychrotolerant bacteria. In our experiments with the North Sea sediment, sulfate reduction rates were only 2% of the rates at T_{opt} after 24 months of incubation at 0°C (Fig. 3B). This low percentage indicates the absence of an abundant specialized cold-adapted community in the temperate environment. Moreover, relatively high Q_{10} values (Table 2) compared with the arctic sediment also expressed the low SRR at low temperatures. It has been shown that psychrotolerant bacteria may have adaptive mechanisms to maximize and maintain a high growth yield (not growth rate) at low temperature (Bakermans and Nealson, 2004).

This growth strategy may explain how psychrotolerant organisms are able to live over a broad temperature range. We conclude that temperate sediments are dominated by a SRB community which, although it has a competitive disadvantage against psychrophiles, dominates also in winter due to the maintenance of a large population.

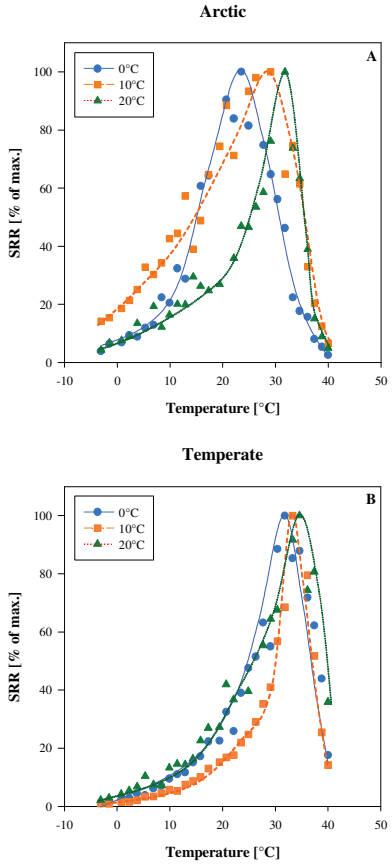

Figure 3. Sulfate reduction rates determined from the temperature-gradient incubation experiment of (A) the arctic and (B) the temperate sediment after twenty-four months of incubation at 0°C, 10°C and 20°C.

Effect of long-term temperature shifts on the microbial community composition

At initial conditions, the total microbial cell numbers (Table 3) determined by DAPI staining were comparable to previous studies with similar arctic and temperate sediment (Ravenschlag et al. 2001 and Llobet-Brossa 2002). However, higher detection of bacterial cells (Table 3) in the present work may be explained by the enhanced sensitivity of CARD-FISH and the additional EUB338-II and EUB338-III probes used in this study. The *Desulfosarcina-Desulfococcus* and *Desulfobulbaceae* groups targeted by probes DSS658 and DSR651, respectively, were dominant among the SRB detected in our study and comparable cell abundances were observed in arctic and temperate sediment (Table 3). Relative numbers of cells targeted by DSB985 and Sval428 probes were higher in the arctic than in the temperate sediment. *Desulfobacter* spp. and *Desulfobacula* spp. were previously not detected in arctic sediment by FISH, and only small amounts of rRNAs of these organisms were found (Ravenschlag et al., 2000). This corroborates the observed enhanced cell detection of the applied CARD-FISH protocol. The *Desulfotalea* cluster targeted by the Sval428 probe was previously characterized as psychrophilic (Sahm et al., 1999). Nevertheless, significant cell numbers belonging to this cluster were also detected in the temperate sediment (Table 3), which indicates the difficulty to discriminate temperature adaptations based on phylogeny. Phylogenetic analysis of several psychrophilic strains by Sahm and coworkers (1999) showed that psychrophiles are closely related to mesophiles and that psychrophily is a polyphyletic property. However, time-course analysis using CARD-FISH showed different temperature effects on the microbial community composition in arctic and temperate sediment (Table 3). In the arctic sediment, the steady decrease of the microbial cells and the relative contribution of *Bacteria* and specific groups of SRB to the total microbial numbers with increasing incubation time and temperature (Table 3) implies that a large fraction of the community was negatively affected by the 10° and 20°C long-term incubation temperatures. This is in good agreement with the observations that arctic sediment is characterized by a specialized SRB community that is sensitive to temperature increases over 10°C. In contrast, despite the decrease in microbial cells numbers in the temperate sediment incubated

at 0°C, the absence of a significant temperature-induced effect in the abundance of *Bacteria* and SRB suggest a seasonally stable community. This finding is also supported by data from Mußmann et al. (2005), who found indications of a seasonally stable community structure responsible for the SRR measured in similar temperate sediments.

Effect of long-term temperature shifts on SRR: Implications for the carbon cycle in marine sediments

All experimental incubation temperatures initially stimulated SRR in arctic and temperate sediment relative to *in-situ* conditions. SRR were initially stimulated two- and fourfold at 10°C and 20°C, but this effect disappeared after 24 months of incubation (Table 1 and 2). Rates of terminal metabolism in permanently cold and temperate habitats generally increase rapidly in response to carbon input (Arnosti et al., 2005; Hee et al., 2001) which suggests that electron donors are naturally limiting in marine sediments. These observations raise the question, whether the measured changes of the SRB community and the SRR are caused by organic substrate limitation rather than temperature limitation. In our experiments, organic substrate was never depleted since the rates of sulfate reduction measured in the different experimental sediment bags (Table 1 and 2) were never below those measured in the sediment cores (Fig. 1). Substrate-limiting conditions, if existent, were equal in all experimental sediment bags since they were not amended with organic material during the course of the incubation experiment.

Sediments of the Arctic shelves contain abundant organic matter (Stein and Macdonald, 2004) and the temperature increases of shelf sediment may lead to increased mobilization and mineralization of previously refractory organic matter. The long-term decrease in SRR provides a first insight to the finite size of the buried organic carbon reservoir in marine sediment that can be mineralized at elevated temperatures. Our experiments on the temperature response of sulfate

reduction in marine coastal sediments are therefore relevant for predicting the effects of seasonal temperature increases that may occur in the high Arctic. Nevertheless, temperature effects on other carbon mineralization processes need to be taken into account to better predict the time scale and rate of additional CO_2 regeneration from warming marine sediment. Further studies of the initial steps of organic matter mineralization in sediments as well as the concentrations of DOC and its constituents would deepen our understanding of the impact of increasing temperatures in the ocean carbon cycle.

EXPERIMENTAL PROCEDURES

Sampling

Samples were taken from two different locations, a permanently cold and a seasonally changing environment. Sediment from a permanently cold area was collected in August 2004 using a Haps corer. The study site was in the central part of Smeerenburgfjord, on the west coast of Svalbard, Arctic Ocean (Station J; 79°42´N, 11°05´E; water depth 215 m). At the time of sampling the temperature was 1.6°C. Sediment was brown-colored in the upper 2 cm, and contained numerous worm burrows and occasional drop stones and brittle stars. Below ca. 3 cm depth, the sediment was clayey and changed to a mottled dark grey-black. Sediment from the seasonally changing area was collected with push cores in May 2005. The sampling site was a brackish intertidal area subjected to strong seasonal temperature variations near Dangast, Wadden Sea, German Bight (53°27´N, 08°07´E). The seasonal temperature variation was from 3°C in winter to 20°C in summer. However, exposure of the sediment at low tide can, occasionally, lead to much higher temperatures during summer and to freezing during winter. At the time of collection the temperature was 10°C. The uppermost 0-1 cm of the sediment consisted of a light brown sandy mud

that changed at depth to a black mud with abundant meio- and macrofauna consisting of abundant polychaetes and sporadic bivalves.

Measurements of sulfate and sulfate reduction rates

Collected cores were closed with rubber stoppers at both ends and transported to a 4°C room. Measurements of sulfate concentrations were carried out using parallel sediment cores sliced at the following depth intervals (cm): 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, 7-9, 9-11 and 11-13. Sediment samples were centrifuged at 3500 rpm in capped centrifuge tubes with nitrogen headspace at 4°C for 15 minutes. Supernatant porewater (1 ml) was preserved with 200µl of 1% (w/v) zinc-acetate (Zn-acetate) solution and stored at –20°C. Dissolved sulfate was analyzed by non-suppressed ion chromatography with a Waters IC-Pak anion exchange column (50 X 4.6 mm), and a Waters 430 conductivity detector (Ferdelman et al 1997). Prior to injection, samples were diluted 100-fold with distilled H_2O. The pump flow rate was 1 ml min^{-1} and 1 mM isophthalic acid in 10% methanol, adjusted to pH 4.5 with saturated sodium borohydrate, was used as eluent.

Bacterial SRR were measured in two parallels using whole-core incubations (Jørgensen, 1978) with the injection of 5µl of carrier-free $^{35}SO_4^{2-}$ -tracer solution in 4% NaCl (~100kBq per injection) at one centimeter intervals to a depth of 13 centimeters. In order to minimize cell growth and re-oxidation of the radiolabeled sulfide, the incubation was terminated after 24h by extruding the sediment into centrifuge tubes containing 20 ml of 20% (w/v) Zn-acetate solution. The ^{35}S incorporated into the Total Reduced Inorganic Sulfur (TRIS) was recovered as zinc sulfide in traps containing 7 ml of 5% (w/v) Zn-acetate solution by an improved single-step distillation method (Kallmeyer et al., 2004). Rates of sulfate reduction in sediments were calculated as described by Kallmeyer et al. (2004).

Experimental setup

Long-term sediment incubations

Upon recovery sediment cores were sub-sampled for long-term incubations from a depth between 3 and 9 cm, which corresponds to the zone of maximal sulfate reduction (Fig. 1). Sediments were transferred into 2 l gas-tight plastic bags (Hansen, 2000) without airspace and stored at *in-situ* temperatures until further processing. These bags allowed the long-term incubation of anoxic sediment for the study of microbial and geochemical processes over time. Homogenization was performed by simple kneading, thus avoiding continuous stirring, introduction of a gaseous headspace, or dilution with seawater. It has been shown that dilution may cause significant changes in the rate of sulfate reduction (Jørgensen 1978). Three experimental incubation bags from each sampling site were permanently relocated to incubators kept at 0°C, 10°C and 20°C for 24 months. Also, in order to maintain anoxic conditions, sediment was subsampled under nitrogen gas using an inflatable polyethylene glove bag (Two-hand AtmosBag™, Aldrich®). In order to avoid the depletion of the electron acceptor for sulfate reduction, prior to every subsampling of sediment, incubation bags were homogenized for 10 minutes by manual kneading and sulfate concentrations in porewater were measured as previously described. Experimental bags contained sediment of a known volume and porosity. Consumption in electron acceptor was balanced by adding equivalent amounts of sulfate. Experimental bags were not replenished with any organic substrates since continuous amendments may result in the enrichment of particular microbial populations over the course of the experiment.

Short-term temperature-gradient incubations

In addition to long-term incubations, the temperature response of sulfate reduction rates was determined in short-term temperature-gradient incubation experiments using an aluminum temperature-gradient block (Battley, 1964), heated electrically at one end and cooled at the other end with a refrigerated and thermostated water bath. The block has 4 parallel rows with 30 wells each and holds up to 120 vials. The temperature increment between wells was 1.5°C and the total temperature range was from -3.5°C to +40°C. Sediment samples were incubated in the temperature gradient block using 5 ml glass test vials. Each vial was filled with approx. 2 ml sediment and sealed with a nitrogen headspace using butyl rubber stoppers. Due to the physical disturbance of the sediment during subsampling, samples were kept for 24 h at the original incubation temperatures prior to the measurement of bacterial SRR which were performed as described above. For each temperature-gradient incubation experiment, the average and the deviation of SRR were calculated using three triplicate samples in the gradient block.

Temperature dependence

The effect of temperature on bacterial sulfate reduction rates has been previously evaluated using the Arrhenius model (Aller & Yingst, 1980; Westrich & Berner, 1988; Isaksen & Jørgensen, 1996). The Arrhenius profiles are obtained from temperature-gradient incubations and represent the variation of metabolic rate as a function of temperature. The activation energy for sulfate reduction is estimated by plotting the natural logarithm of rate versus the inverse of temperature as follows:

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{R} \cdot \frac{1}{T} \right)$$

E_a is the activation energy (J mol^{-1}), k is the reaction rate (nmol cm^{-3} day^{-1}), A is the Arrhenius constant, R is the gas constant (8.314 J K^{-1} mol^{-1}), and T is the absolute temperature (K). Apparent E_a are not activation energies in the chemical sense. Sulfate reduction occurs via a complex enzymatic system and the calculated E_a values do not reflect the cooperative process between structural elements of an enzyme or a rate-limiting chemical step but rather a measure of an ecological response of the whole SRB community to temperature changes. The Arrhenius profiles derive from ^{35}S determinations of sulfate reduction in natural sediments and are characterized by a range of linearity usually below and above the environmental temperature range. Deviations from the linear range express the inability of SRB to maintain their metabolic activity. The apparent E_a are useful values to describe and compare the competitive strategies of microbial populations that determine carbon mineralization rates in both arctic and temperate sediment.

Q_{10} is the factor by which the rate of reaction increases with a temperature increase of 10°C. The selected temperature range was between 2° to 12°C. Q_{10} was calculated using the following equation:

$$Q_{10} = \exp\left[\frac{E_a \cdot 10}{RT(T + 10)} \right]$$

CARD-FISH

For microbial community analyses 0.5 cm^3 of sediment were sampled from incubation bags and fixed in 4% paraformaldehyde (1 part 16% paraformaldehyde and 3 parts 1 x phosphate-buffered saline, PBS) overnight at 4°C. Fixed samples were washed three times with 1 x PBS,

with centrifugation steps at 10,000 rpm for 5 min. between washes, and stored in PBS/Ethanol (2:3) at -20°C until further processing. Samples were then filtered onto polycarbonate membrane filters (type GTTP; pore size, 0.2 µm; diameter, 2.5 mm; Sartorius, Göttingen, Germany) and stored at -20°C. In order to characterize the change in microbial community composition over time, a set of previously described 16S rRNA-targeted oligonucleotide probes was selected (Table 4). *Bacteria* present in the sediment were quantified using the probe EUBI-III, which is a mixture of the eubacterial probe EUB338, *Planctomycetes* probe EUB338P and the *Verrucomicrobium* spp. probe EUB338V (Daims et al., 1999). To discriminate the change of SRB communities against the total number of bacteria the selection included probes for some of the most relevant groups of SRB within the delta subgroup of *Proteobacteria* present in Arctic marine sediments (Knoblauch et al., 1999b; Sahm et al., 1999; Ravenschlag et al., 2000).

Table 4. CARD-FISH probes and hybridization conditions used in this study

Probe name	Specificity	Sequences (5´-3´)	Fa[a](%)	T_h[b] (°C)	Reference
EUB (I-III)	Bacteria	Mixture of the following three probes	35	46	Daims et al., 1999
EUB338	Most Bacteria	GCT GCC TCC CGT AGG AGT			Amann et al., 1990
EUB338P	*Planctomycetes*	GCA GCC ACC CGT AGG TGT			Daims et al., 1999
EUB338V	*Verrucomicrobium* spp.	GCT GCC ACC CGT AGG TGT			Daims et al., 1999
NON338	Antisense of EUB338	ACT CCT ACG GGA GGC AGC	35	46	Wallner et al., 1993
DSR651	*Desulfobulbaceae*	CCC CCT CCA GTA CTC AAG	35	46	Manz et al., 1998
DSS658	*Desulfosarcinales*	TCC ACT TCC CTC TCC CAT	50	46	Manz et al., 1998
DSB985	*Desulfobacter* spp., *Desulfobacula* spp.	CAC AGG ATG TCA TCT TCA AAA	20	46	Manz et al., 1998
Sval428	*Desulfotalea* spp.	CCA TCT GAC AGG ATT TTA C	25	46	Sahm et al., 1999

[a] Formamide concentration in the hybridization buffer

[b] Hybridization temperature

Note that selected SRB probes do not fully cover the targeted groups (Lücker et al., 2007). Thus, the intention is to monitor the SRB in the sediment in response to temperature and not to provide the total number of SRB. The probe NON338 was used as a negative control. Oligonucleotide probes labeled with HRP at the 5´end were purchased from ThermoHybaid (Interactiva Division, Ulm, Germany). Fluorescence *in-situ* hybridization (FISH) was performed on sections from the filters mentioned above using an improved CARD-FISH

protocol (Pernthaler et al., 2002; Sekar et al., 2003; Ishii et al., 2004). Microscopic counting of hybridized and DAPI (4´,6-diamidino-2-phnylindole)-stained cells were performed in duplicate samples as previously described (Snaidr et al., 1997; Llobet-Brossa et al., 1998; Pernthaler et al., 2002). Between 700 and 1000 DAPI-stained cells were counted per sample.

ACKNOWLEDGEMENTS

Special thanks go to Maren Nickel for all the help and fruitful discussions. We thank the captain Stig Henningsen and John Mortensen of MS FARM as well as the scientific party 2005 and 2006 for the successful and exciting expeditions. We also thank the Koldewey Station for support in Ny Alesund, Svalbard. Thanks to Linda Gätjen and Jacqueline Schmidt for technical assistance. The constructive criticism of the anonymous reviewers is gratefully acknowledged. Funding for this work was provided by the Deutsche Forschungsgemeinschaft (DFG) Schwerpunktprogramm "The impact of climate variability on aquatic ecosystems (AQUASHIFT)", and the Max Planck Society.

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MANUSCRIPT 3

TEMPERATURE INDUCED DECOUPLING OF ENZYMATIC HYDROLYSIS AND CARBON REMINERALIZATION IN LONG-TERM INCUBATIONS OF ARCTIC AND TEMPERATE SEDIMENTS

Geochimica et Cosmochimica Acta, In press.

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Running title: Decoupling of carbon cycling in sediments

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ABSTRACT

Extracellular enzymatic hydrolysis of high-molecular weight organic matter is the initial step in sedimentary organic carbon degradation and is often regarded as the rate-limiting step. Temperature effects on enzyme activities may therefore exert an indirect control on carbon mineralization. We explored the temperature sensitivity of enzymatic hydrolysis and its connection to subsequent steps in anoxic organic carbon degradation in long-term incubations of sediments from the Arctic and the North Sea. These sediments were incubated under anaerobic conditions for 24 months at temperatures of 0°C, 10°C, and 20°C. The short-term temperature response of the active microbial community was tested in temperature gradient block incubations. The temperature optimum of extracellular enzymatic hydrolysis, as measured with a polysaccharide (chondroitin sulfate), differed between Arctic and temperate habitats by about 8-13°C in fresh sediments and in sediments incubated for 24 months. In both Arctic and temperate sediments, the temperature response of chondroitin sulfate hydrolysis was initially similar to that of sulfate reduction. After 24 months, however, hydrolysis outpaced sulfate reduction rates, as demonstrated by increased concentrations of dissolved organic carbon (DOC) and total dissolved carbohydrates. This effect was stronger at higher incubation temperatures, particularly in the Arctic sediments. In all experiments, concentrations of volatile fatty acids (VFA) were low, indicating tight coupling between VFA production and consumption. Together, these data indicate that long-term incubation at elevated temperatures led to increased decoupling of hydrolytic DOC production relative to fermentation. Temperature increases in marine sedimentary environments may thus significantly affect the downstream carbon mineralization and lead to the increased formation of refractory DOC.

INTRODUCTION

Recent research on global climate change has shown that some of the most pronounced increases in temperature are expected to occur in the Arctic regions (Anisimov et al., 2007). Over the period 1920-1998, seasonal temperatures in the Arctic archipelago of Svalbard have gradually increased (winter +1°C, spring and summer +0.5°C, autumn 0.0°C; Hanssen-Bauer, 2002). Warming by as much as 3 to 4°C has been predicted for the Arctic surface temperatures over the next century (Moritz et al., 2002), with consequences for enhanced nutrient and carbon runoff, changes in sea ice coverage, air-sea CO_2 exchange, and primary production in Arctic shelves (Loeng et al., 2005; Denman et al., 2007).

Almost 50 % of the Arctic Ocean is in water depths less than 50 m (Jakobsson et al., 2003) so that a large fraction of organic matter exported from the euphotic zone reaches the seafloor. In shelf sediments, anaerobic pathways may comprise between 30 – 90 % of the mineralization of organic matter to CO_2 (Canfield et al. 1993). In sediments, benthic microbial communities enzymatically hydrolyze organic substrates to sizes sufficiently small (ca 600 Dalton; Benz and Bauer, 1988) to permit uptake. The hydrolysis products are subsequently fermented, mainly to low-molecular-weight organic compounds such as volatile fatty acids (VFA) (Capone and Kiene, 1988), and then oxidized to CO_2. Since burial of organic carbon in the Arctic Ocean may account for ca. 7 to 11% of the global budget (Stein and Macdonald, 2004), understanding the temperature response of carbon production, recycling, and preservation in Arctic sediments is crucial for prediction of the effects of climate change on the global carbon cycle (Belicka et al., 2002).

Because benthic microbial communities in Arctic sediments include psychrophilic organisms that are well-adapted to cold conditions (e.g. Knoblauch and Jørgensen, 1999), the response of Arctic sedimentary microbial communities to temperature changes can best be investigated

by comparison with temperate sedimentary communities. Microbial communities that experience a considerable temperature range on an annual basis may respond differently to warming than Arctic communities. Furthermore, long-term changes in temperature may lead to shifts in microbial community composition, such that numerically minor members of a community that are more tolerant to higher temperatures may become dominant given sufficient time, and their carbon cycling capabilities could then dictate the response of the net community (e.g. Robador et al., 2009). In order to investigate such a possibility, long-term rather than short-term experimental temperature shifts are required.

Temperature response profiles of polysaccharide hydrolysis and sulfate reduction in freshly-collected permanently cold and seasonally changing sediments have shown that under *in situ* conditions, there is a close coupling between these two key phases of organic matter degradation (Arnosti et al., 1998). Comparative studies from temperate and Arctic sediments (Arnosti and Holmer, 2003; Brüchert and Arnosti, 2003; Arnosti and Jørgensen, 2006) of the initial and terminal steps of organic carbon degradation suggested that turnover of carbon through the dissolved pool occurs quite rapidly. Moreover, an experimental study of the effect of sudden temperature changes on the robustness of this coupling showed a strong balance over a temperature range that exceeds environmental conditions of both temperate and Arctic marine sediments (Finke and Jørgensen, 2008). An investigation of sulfate reduction in Arctic and temperate sediments carried out concurrently with the present study, however, showed that long-term temperature increases may have a pronounced effect on rates of sulfate reduction as well as on the composition of the sulfate-reducing community, and therefore on the extent to which organic matter is recycled or preserved in marine sediments (Robador et al, 2009).

In the present study, the temperature sensitivity of enzymatic hydrolysis in Arctic and temperate marine sediments was investigated

using a polysaccharide substrate, because carbohydrates are major components of both particulate and dissolved organic carbon in sediments (Hedges et al. 1988; Arnosti and Holmer, 1999). The polysaccharide, chondroitin sulfate, was selected since previous work has strongly suggested that the enzymes hydrolyzing this polysaccharide are induced in Arctic as well as temperate sediments (Arnosti, 2000; Brüchert and Arnosti, 2003; Arnosti, 2004), providing the means of probing the enzyme-producing capabilities of a microbial community after prolonged incubation at different temperatures, as well as the characteristics of the enzyme itself. By measuring activities of an enzyme produced in direct response to substrate addition, it is possible to determine whether the characteristics of the enzymes produced by a microbial community change as a result of long-term incubation. Moreover, the activity of the enzyme hydrolyzing chondroitin sulfate exhibits psychrophilic characteristics, with the lowest temperature optimum reported to date among enzymes active in marine sediments (Arnosti and Jørgensen, 2003).

Measurements of the initial hydrolytic step were compared with concentrations of dissolved organic carbon (DOC), total dissolved carbohydrates, and volatile fatty acids (VFA), key intermediates in carbon degradation pathways, in order to more closely examine steps preceding terminal remineralization, and to determine their responses to long-term temperature shifts. Long-term responses to different temperature regimes may differ from short-term responses, since an incubation of 24 months is equivalent to many microbial generations, providing the opportunity to observe the consequences of any changes in microbial community function and structure over longer timescales.

METHODS

Sampling and experimental set-up

Two sets of sediments were collected, one from a permanently cold region (Svalbard, Arctic Ocean, 79°42´N, 11°05´E; sediment temperature typically around 0 °C) collected in 2004, and sediments from a temperate region (Wadden Sea, German Bight, 53°27´N, 08°07´E; sediment temperature typically +3 °C to +20°C), collected in 2005. Sampling sites are described in further detail in Robador et al. (2009).

Sediment from both sampling sites was maintained anaerobically in gas-tight bags stored at 0 °C, 10 °C, and 20 °C after collection. ^{35}S-sulfate reduction rates were measured in these sediments upon collection, and then periodically during the 24 months of incubation, as reported in Robador et al. (2009). Total prokaryotic cell numbers were counted by 4',6-diamidino-2-phenylindole (DAPI) staining. Bacteria, as well as specific groups of sulfate-reducing bacteria (SRB), were quantified using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH) at the same time intervals, as reported in Robador et al (2009). In order to avoid sulfate limitation of carbon remineralization during the 24-month incubation, sulfate was added to the bags to reconstitute *in situ* concentrations whenever concentrations decreased to 3-5 mM. Extracellular enzymatic hydrolysis rates were measured as a function of temperature after 24 months of sediment incubation. Additionally, fresh sediment was collected in 2007 from both sampling sites and used as a reference for temperature responses of enzymatic hydrolysis. The sampling procedure was similar to that described in Robador et al. (2009). Concentrations of VFA, DOC, and total dissolved carbohydrates were measured in fresh sediments as well as in sediments incubated for 24 months.

Hydrolysis rate measurements

Chondroitin sulfate hydrolysis was measured using fluorescently-labeled chondroitin sulfate (sulfated polymer of N-acetyl galactosamine and glucuronic acid, MW ~200 kDa, Fluka; Arnosti, 1996; 2003). Chondroitin sulfate was labeled by the method of Glabe et al. (1983), as modified by Arnosti (1995; 2003). Sediments from experimental bags were homogenized by manual kneading and diluted 1:1 with anoxic artificial seawater for incubation in a temperature gradient block (see below). Artificial seawater was prepared anaerobically as described by Widdel and Bak (1992). Sediment slurries were purged with N_2 and 5 ml of slurry were transferred into Hungate tubes, which were also gassed with N_2 according to the Hungate technique (Bryant, 1972) and sealed with butyl rubber stoppers. Hungate tubes containing only anoxic artificial seawater were prepared to serve as thermal controls. Hungate tubes were immediately placed in the wells of the temperature-gradient block and pre-incubated for approximately 4 hours to allow them to equilibrate to the block temperatures. The block was cooled to -1.5°C at one end and heated to +37.4°C yielding temperature increments between wells of approximately 1.5°C. After 4 hours, for each experiment chondroitin sulfate (a single concentration of 10-100 µl volume) was added to each tube for a final concentration of 25 to 250 nmol monomer ml^{-1} sediment. In all cases, the added concentration most probably oversaturated the enzymes such that rates are zero-order with respect to substrate concentration (see Arnosti 1995 for further discussion.).

After an incubation period of 63 hours for the Arctic and 6-24 hours for the temperate sediments, the tubes were centrifuged and porewater was filtered through 0.2 µm pore size surfactant-free cellulose acetate syringe filters, then frozen and stored at -20°C until further processing. Incubation times for Arctic sediments were selected based on previous experiments (Arnosti and Jørgensen 2003). Several incubation times

were tested for temperate sediments. In order for activity to be detected, a fraction of the added substrate must be hydrolyzed to molecular weights within the size resolution range of the gel permeation chromatography system. Since enzyme activities vary as a function of temperature, substrate hydrolysis for temperate sediments incubated at 10°C and 20°C was essentially complete at the temperatures bracketing the optimum temperature (T_{opt}) by the time activities were detected at lower temperatures. This phenomenon accounts for the flattened shape of the T_{opt} for temperate sediments incubated at 10°C and 20°C; samples incubated for 10 hours showed a sharper T_{opt} at 30°C, but hydrolysis particularly at temperatures below 15°C was not well resolved. Changes in the size distribution of the fluorescently labeled chondroitin sulfate relative to a killed (autoclaved) control were determined using gel permeation chromatography. Hydrolysis rates were calculated from the changes in size distribution, as described in Arnosti (2003). Triplicate tubes were incubated in parallel wells (at the same temperature) at four points along the temperature gradient block in order to investigate reproducibility of hydrolysis rates. All data (including replicate measurements) are shown in Figs. 1-2.

Carbohydrate concentration measurements

Carbohydrate concentrations in porewater were analyzed using the 2,4,6 tripyridyl-*s*-triazine (TPTZ) method (Myklestad et al., 1997). Total carbohydrates were measured after acid hydrolysis to convert combined carbohydrates to monsaccharides. Following the hydrolysis procedure of Burdige et al. (2000), samples were amended with H_2SO_4 to a final concentration of 1.2 M, heated to 100°C for 3 hr, and neutralized with NaOH. Concentrations were calculated from glucose standards that had been subjected to the same hydrolysis protocol. Carbohydrate concentrations are expressed in μM units of carbon, assuming that one carbohydrate represents 6 C atoms (i.e., all carbohydrates are hexoses).

Volatile fatty acids (VFA)

The low-molecular weight fatty acids glycolate, lactate, acetate, formate, propionate, butyrate, and isobutyrate were measured by HPLC as derivatives of 2-nitrophenylhydrazide after pre-column derivatization (Albert and Martens, 1997). Samples for fatty acid analysis were stored in brown Teflon-capped borosilicate glass vials (cleaned and then combusted at 480°C for 6 hours) and kept frozen until analysis. The fatty acids were derivatized with 2-nitrophenylhydrazine and injected onto an HPLC system consisting of a Sykam 1121 HPLC pump, connected to a 20 x 4 mm ID LiChrosphere RP-8 5 µm guard column and a 250 x 4 mm ID LiChrosphere RP-8 column, both kept at 25°C in a Jetstream Plus column oven. Peaks were detected with a Linear UVIS UV detector set at 400 nm. Concentrations were determined after calibration with standard mixtures containing glycolate, formate, lactate, acetate, propionate, isobutyrate, butyrate, and valerate. A standard was measured after every fifth sample. The detection limit of the method was 200 nM. Integration was performed with a Eurochrom 2000 Integration package.

DOC measurements

DOC concentrations were determined in porewater samples using a Shimadzu TOC 5050A total organic carbon analyzer equipped with an ASI 5000 autosampler (Shimadzu, Kyoto, Japan). In short, diluted samples (1:4) were acidified to pH 2 with 2 M HCl and purged with CO_2-free carrier gas for 5 min at a flow rate of 125 ml min^{-1}, to remove inorganic carbon, and subsequently combusted to CO_2 in the internal furnace, filled with an oxidation catalyst and heated to 680°C. The combustion products were carried into the detection cell by high purity CO_2 free gas through an electronic dehumidifier for removal of water vapor followed by a sub-micron particle filter. CO_2 generated from the

combusted carbon was detected in a non-dispersive infrared gas analyzer. DOC concentrations of the samples are proportional to the peak area count and are calculated using standard solutions of potassium hydrogen phthalate.

RESULTS

In fresh sediments, the T_{opt} of chondroitin sulfate hydrolysis were 22°C and 26°C for the Arctic and temperate sediments, respectively (Table 1, Fig. 1). In the Arctic sediments, the hydrolysis rate decreased abruptly above 22.1°C from 1.4 nmol monomer cm^{-3} h^{-1} to 0.2 - 0.4 nmol monomer cm^{-3} h^{-1}, whereas in the temperate sediments the T_{opt} was broader and the rate at the T_{opt} was only 0.6 nmol monomer cm^{-3} h^{-1}. After 24 months, the T_{opt} of Arctic sediments incubated at 0°C, 10°C , and 20°C were 19.4°C, 17.3°C, and 23.5°C, respectively, little different from the initial conditions (Table 1, Fig. 2). Compared to the fresh sediment, rates of hydrolysis at T_{opt} after 24 months of incubation were similar at 0°C (1.4 and 1.3 nmol monomer cm^{-3} h^{-1}, respectively), but decreased to 0.9 nmol cm^{-3} h^{-1} for sediment incubated at 10°C, and to 0.3 nmol monomer cm^{-3} h^{-1} for sediments incubated at 20°C. The temperature response of chondroitin sulfate hydrolysis for the Arctic sediments incubated for 24 months thus showed a decrease in the maximum rate, but an overall similar temperature profile.

Table 1. Hydrolysis activities determined in the temperature-gradient incubations

Sediment type	Incubation time (months)	Incubation temperature (°C)	Maximum hydolysis rates (nmol monomer cm^{-3} h^{-1})	$T_{opt.}$ [a] (°C)
Arctic	Fresh	0	1.4	22.1
	24 months	0	1.3	19.4
		10	0.9	17.3
		20	0.3	23.5
Temperate	Fresh	10	0.6	31.8
	24 months	0	0.9	30.4
		10	0.7	30.4
		20	0.6	31.8

a. Temperature at which maximum hydrolysis rates were measured

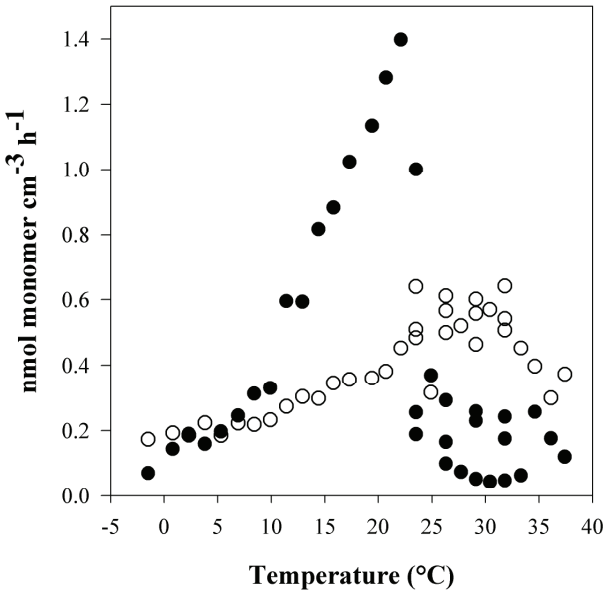

Figure 1. Hydrolysis rates of chondroitin sulfate determined from the temperature-gradient incubation experiment of fresh Arctic and temperate sediments.

For the temperate sediments, higher incubation temperatures did not change the T_{opt}, which remained in the range of 30.4-31.8°C for all treatments (Table 1, Fig. 2). The need for longer incubation times to detect activity at temperatures below 15°C flattened the profile near the T_{opt} for sediments incubated at 10°C and 20°C (see Methods.) After 24 months of incubation at 0°C, hydrolysis rates at the T_{opt} were on average slightly higher compared to the fresh sediment, but remained nearly unchanged in the 10°C and 20°C incubations (Table 1, Fig. 2). Sediments incubated for 24 months at 10°C and 20°C, however, had lower hydrolysis rates in the range 0-15°C compared to sediments that had been incubated at 0°C for 24 months.

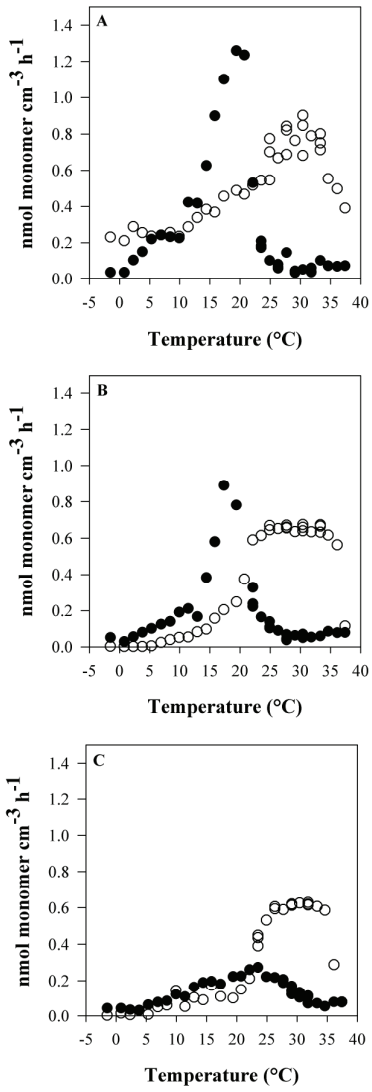

Figure 2. Hydrolysis rates of chondroitin sulfate determined from the temperature-gradient incubation experiment for the Arctic and temperate sediments after 24 months of incubation at (A) 0°C, (B) 10°C and (C) 20°C.

Dissolved porewater constituents

Concentrations of DOC were quite similar in fresh Arctic and temperate sediments, 2701 µM C and 2256 µM C, respectively. After 24 months of incubation, DOC concentrations increased in proportion to incubation temperature (Fig. 3). At 20°C, DOC in Arctic and temperate sediments was more than three times higher than in the fresh sediment, 9490 µM C in Arctic sediments and 7002 µM C in temperate sediments (Fig.3).

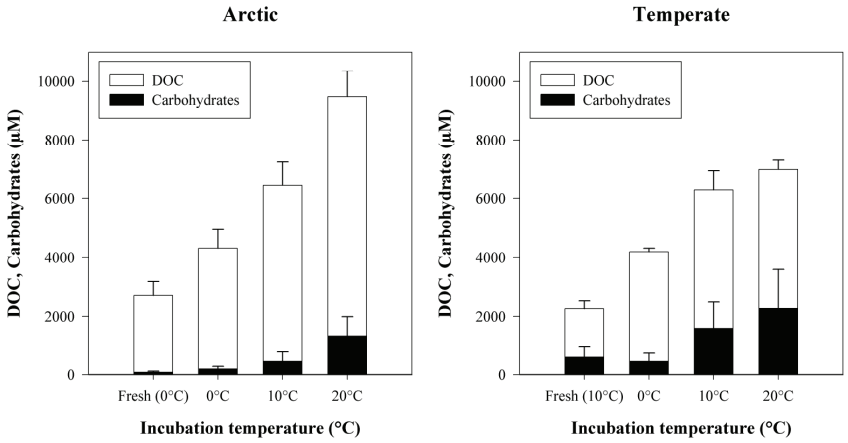

Figure 3. Dissolved organic carbon (DOC) and dissolved carbohydrate concentrations in fresh sediments and in the 0°C, 10°C and 20°C experiment bags after 24 months of incubation of (A) the Arctic and (B) the temperate sediment. Bars show mean values and deviations of duplicate measurements of DOC. Measurements of dissolved carbohydrates were made in triplicate; bars show standard deviations in measurements of the total dissolved carbohydrates.

Contributions of total carbohydrates to DOC differed between the Arctic and temperate sediments (Fig. 3). In Arctic sediment, total carbohydrates initially constituted 2.9 % of DOC, whereas the average initial concentrations of total carbohydrates in temperate sediments

were 28% of DOC. The contribution of carbohydrates to DOC increased systematically with increasing temperature in Arctic and temperate sediments. Total dissolved carbohydrates in Arctic sediments constituted 14 % of DOC after 24 months at 20°C (Fig. 3). In the temperate sediment, the contribution of carbohydrates to DOC was highest at the highest incubation temperature (32%). The dissolved carbohydrate concentrations therefore increased in both absolute and relative terms at higher incubation temperatures.

VFA concentrations were low irrespective of the differences observed with varying temperatures (Fig. 4). Only lactate, acetate and formate were present in quantifiable concentrations (Fig. 4). Propionate, isobutyrate and valerate were also detected but at concentrations too close to the detection limit (200 nM) for accurate quantification. In the Arctic sediment, concentrations of all VFA's including acetate were higher in the fresh sediments than after 24 months (Fig. 4A). In temperate sediment, concentrations of formate in fresh sediments and of acetate after 24 months of incubation at 0°C and 10°C were also relatively higher. VFA's however, represented just a small portion of DOC (between 0.2 % and 3%).

DISCUSSION

Temperature response of enzymatic hydrolysis to long-term incubation

Measurements of hydrolysis rates as a function of temperature can yield basic information about the characteristics of enzymes, including T_{opt}. This approach is a valuable means of probing the role of enzymes in organic carbon degradation, since individual hydrolytic enzymes have so far not been isolated from sediments, thereby precluding specific biochemical measurements. Since the identities of organisms producing extracellular enzymes in sediments are largely unknown, molecular

techniques cannot be used to investigate the relationship between microbial community structure and enzyme activities.

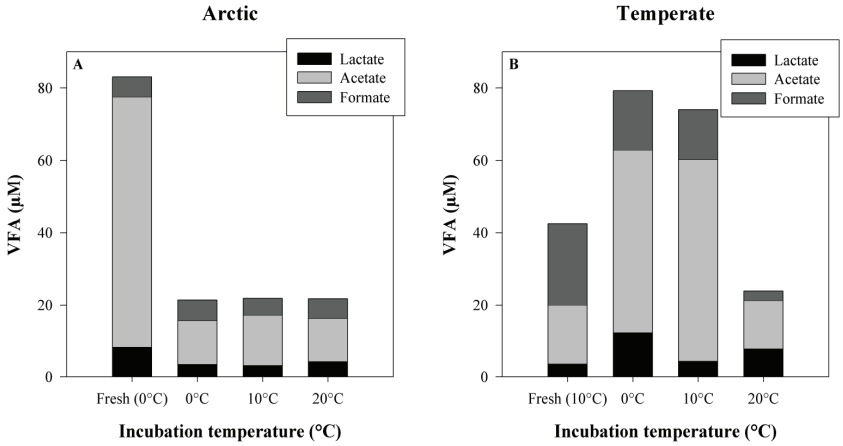

Figure 4. Volatile fatty acid concentrations in the fresh sediments and in the 0°C, 10°C and 20°C experimental bags after 24 months of incubation of (A) the Arctic and (B) the temperate sediment. Error bars show mean values of duplicate measurements.

Although the T_{opt} of some extracellular enzymes exceeds the T_{opt} for growth of the organisms and even the temperature at which the organisms can survive (Burini et al., 1994; Feller et al., 1992), T_{opt} is a useful parameter to characterize enzymes in the environment, since psychrophilic organisms typically express enzymes with lower T_{opt} than do closely-related mesophilic organisms (Feller and Gerday 2003). Moreover, changes in enzyme T_{opt} imply changes in the structural characteristics of enzymes such as thermal stability (D'Amico et al., 2002). After incubation at 0°C, 10°C, and 20°C, the T_{opt} of extracellular enzymatic hydrolysis changed little, with Arctic sediments showing a T_{opt} of 17.3-23.5°C (Table 1, Fig. 2). The T_{opt} range and the shape of the temperature response profile for the Arctic sediments is close to that

reported (ca. 15-18°C) in a previous investigation of chondroitin hydrolysis in Arctic sediments collected from a different fjord in Svalbard (Arnosti and Jørgensen 2003). Temperate sediments showed a considerably higher T_{opt} centered around 30°C (Table 1, Fig. 2). The persistence of the T_{opt} suggests that the temperature characteristics of the extracellular enzymes produced in these sediments in response to the addition of chondroitin sulfate were more closely related to the original temperature of the sediment than to the temperature of sediment incubation, and the nature of this response changed little after 24 months of incubation.

The consistency in temperature response of enzymatic hydrolysis after long-term incubation contrasts with the response observed for sulfate reduction in Arctic sediments. After 24 months of incubation at 10°C and 20°C, the temperature optimum of sulfate reduction increased by 5°C and 8°C in Arctic sediments, respectively, relative to the 0°C incubation (Robador et al. 2009). The temperature response of temperate sediments, however, remained unchanged after 24 months incubation at 0°C, 10°C, and 20°C. The change in temperature response of sulfate reduction for the Arctic sediments was also accompanied by a shift in the community composition of sulfate reducers, as demonstrated by CARD-FISH, whereas the temperate sediments showed no change (Robador et al., 2009).

Arctic sediments incubated for 24 months at 0°C showed little difference in hydrolysis rates at the T_{opt} relative to fresh sediments (Table 1, Fig. 2). At elevated temperatures, however, potential hydrolysis rates measured at T_{opt} declined, suggesting that either the absolute quantity or the catalytic efficiency of enzymes produced in response to substrate addition (and incubation in the temperature gradient block) had changed relative to sediments incubated at 0°C. Such a decline could be due to decreasing numbers of organisms capable of producing suitable extracellular enzymes. Cell counts support this possibility, since the microbial populations of Arctic

sediments incubated at 10°C and 20°C for 24 months declined, 17-46% respectively, relative to initial numbers (Fig. 5).

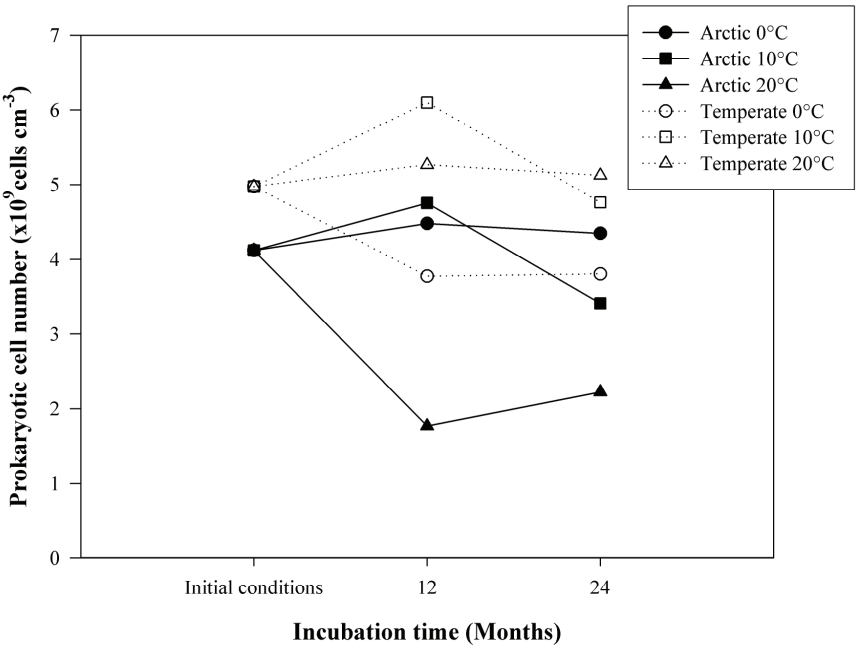

Figure 5. Total prokaryotic cell numbers (DAPI staining) in Arctic and temperate sediments during the 24 month time course of sediment incubation. Data from Robador et al. (2009).

For temperate sediments, hydrolysis rates at the T_{opt} were similar for the fresh sediments and sediments incubated for 24 months (Table 1, Fig. 2). This consistency could be due to the fact that the experimental temperature span was within the natural temperature range of these sediments. Although cell counts did not decline greatly over time in the temperate sediment (Fig. 5), absolute hydrolysis rates at the lower end of the temperature range were affected by prolonged incubation at

higher temperatures. Sediments incubated for 24 months at 10°C and 20°C and then incubated briefly (24 h) in the temperature gradient block at temperatures below 10°C were considerably lower than rates measured in sediments incubated for 24 months at 0°C. This difference was even discernable for the hydrolysis rates up to 20°C, suggesting that prolonged incubation at elevated temperature adversely affected the low-temperature capacity of the microbial community to respond to substrate addition.

Carbon cycling under changed temperature regimes: Evidence of decoupling

Long-term incubation at temperatures of 0°C, 10°C, and 20°C facilitates comparison of the temperature dependency of different steps in carbon remineralization pathways, and their effects on carbon inventories in Arctic and in temperate sediments. Investigations of carbon cycling pathways are limited in part by our inability as a community to measure specific processes with precision, including turnover times of many intermediate pools. Nonetheless, a comparison of the rates of initial and terminal steps in anaerobic carbon cycling with the concentrations of intermediate pools reveals information about possible rate-limiting steps in carbon transformations.

A comparison of DOC concentrations in fresh sediments with the sediments incubated for 24 months shows clear signs of changes in carbon transformation pathways. DOC is a critical intermediate, since DOC concentrations reflect the net balance between solubilization/hydrolysis of particulate organic carbon (POC) and consumption of DOC via transformation (e.g., hydrolysis of high molecular weight DOC to low molecular weight DOC, fermentation of low molecular weight DOC to VFA's) and terminal oxidation (Figure 6). DOC concentrations in fresh sediments are consistent with previously published data on sediment porewater concentrations from muddy

coastal and continental shelf environments (e.g. Arnosti and Holmer, 1999; Alperin et al. 1999; Burdige et al., 2000). The elevated concentrations of DOC in sediments incubated for 24 months, in particular the high concentrations of DOC at elevated incubation temperature (Fig. 4; Fig.6 boxes 2 and 2′ and arrow B′), provide strong evidence of changes in carbon processing in both Arctic and temperate sediments.

Increases in DOC concentrations in response to temperature changes have been reported previously, but these increases have been transitory or were only examined over short periods of time. Seasonally increasing temperatures coincided with accumulation of porewater DOC and depletion of porewater sulfate in organic-rich coastal sediments from a temperate environment (Alperin et al 1994). Within a few weeks, however, the high concentrations (up to 8 mM) of DOC were consumed by the terminal oxidizing community, and DOC concentrations returned to 2-3 mM. In Arctic sediments, substrate addition and/or homogenization also led to transient buildup of DOC constituents such as VFA (Brüchert and Arnosti, 2003; Arnosti et al. 2005), but within days to weeks, the accumulated DOC was consumed again. Short-term experiments with substrate-amended coastal sediments also showed that production and consumption of DOC can become unbalanced over the temperature range typically experienced by temperate sediments (Weston and Joye 2005).

High concentrations of DOC in sediments that have been incubated at stable temperatures for 24 months thus demonstrate that the balance between DOC production and consumption has changed in more than a transient manner (Fig. 6: flow via arrows B and B′ has changed, box 2′ increases in size.) Refractory carbohydrates constitute a significant portion of this DOC increase. Their contribution to DOC increased with increasing temperature in Arctic and in temperate sediments (Fig 3; Fig. 6 box 2′). VFA, in contrast, contributed a very low (0.2-3%) and

relatively constant fraction to DOC in both sediment types (Fig. 4; Fig. 6, box 3).

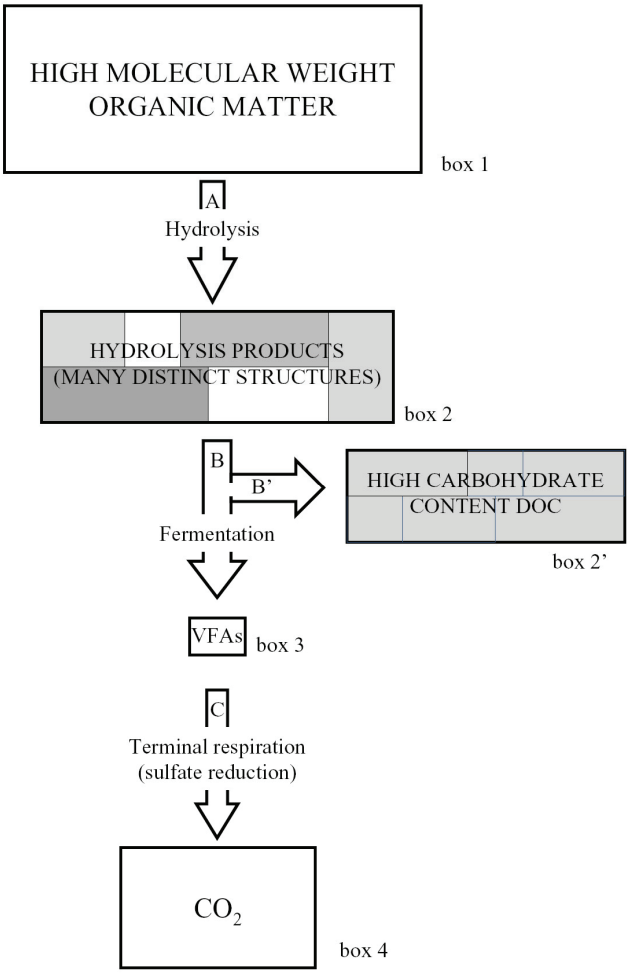

Figure 6. Conceptual model of carbon degradation, illustrating buildup of high carbohydrate-content, fermentation-resistant DOC. Divisions in boxes 2 and 2′ indicate different size classes, different shades indicate compositions.

The accumulation of DOC shows that conversion of POC to DOC continued in these sediments (Fig. 6, arrow A); increasing relative and absolute concentrations of carbohydrates (Fig. 6, boxes 2 and 2′) indicated that substrates broadly suitable for fermentation should have been available to the microbial community. In fact, a comparison of sulfate reduction rates at 0°C, 10°C, and 20°C with inventories of VFA and DOC suggest that downstream carbon processing was increasingly decoupled from the initial hydrolytic step at elevated temperatures. Sulfate reduction rates in the Arctic and temperate sediments incubated for 24 months at 0°C and 10°C showed a two- to four-fold decrease relative to the initial conditions and an even greater decline, with respect to the initial increase, after incubation for 24 months at 20°C (Fig. 6, rate at arrow C decreasing; Fig. 7). The decreased sulfate reduction rates are most likely due to substrate limitation for sulfate-reducing bacteria, since sulfate was not limiting and these rates reflect in part the availability of substrates suitable to fuel sulfate reduction.

An alternative explanation might be that sulfate-reducing bacteria were less able to tolerate long-term exposure to higher temperatures than other members of the microbial food chain. In fact, increasing incubation temperatures had a strong impact in Arctic sediments as demonstrated by the temperature sensitivity exhibited by the microbial SRB community and the decline of specific groups of SRB identified by CARD-FISH (Robador et al., 2009). However, the fact that a similarly sharp decline in sulfate reduction rates was also observed for the temperate sediments (Fig. 7), where neither total prokaryotic cell counts nor CARD-FISH counts for bacteria or sulfate reducers decreased significantly (Fig. 5; Robador et al., 2009) suggest that microbially limited substrate transformation was the overriding cause for the decline in sulfate reduction rates in the Arctic and temperate sediments.

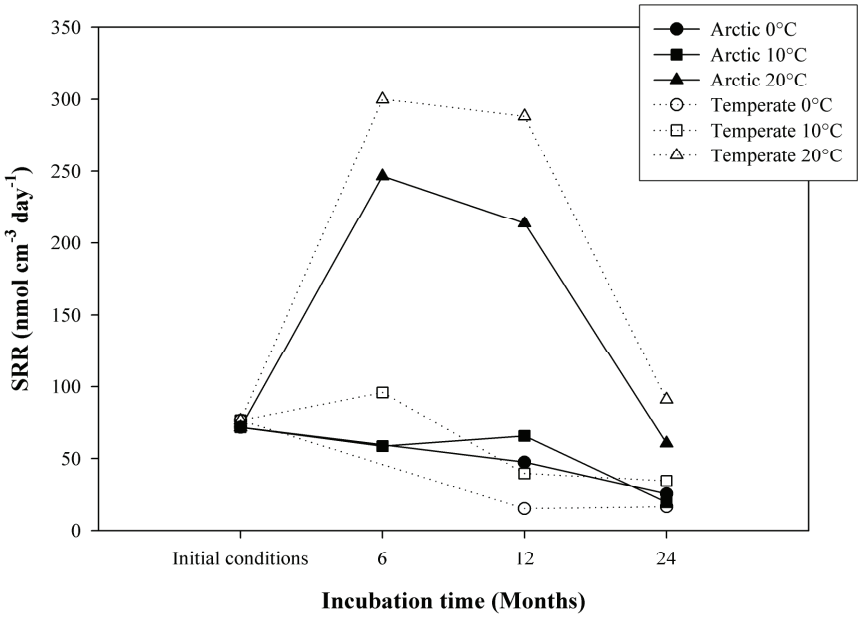

Figure 7. Sulfate reduction rates in Arctic and temperate sediments incubated at 0°C, 10°C, and 20°C for 24 months. Data from Robador et al. (2009).

All the VFA detected in the present study constitute major substrates for sulfate-reducing bacteria (Sørensen et al., 1981; Fukui et al., 1997). Low concentrations (Fig. 4; Fig. 6, small size of box 3) indicate that the balance between production of these intermediates and their consumption was maintained at all temperatures. Specific inhibition experiments of sulfate reduction typically result in increasing VFA concentrations reaching over 1500 μM (Finke et al., 2007), between one and two orders of magnitude greater than the values measured in the present study. If sulfate-reducing bacteria were more intolerant to higher temperatures than fermenting bacteria, VFA concentrations

should have increased well above the 20 to 80 μM concentrations shown in Fig. 4 (Fig. 6, box 3 should have increased in size). In addition, previous investigations with comparable sediments from Svalbard have shown a close coupling between fermentation and sulfate reduction sustained over a broad temperature range of 0 °C to 25-30°C (Finke and Jørgensen, 2008). Together these data imply that carbon limitation of sulfate reduction occurred at or before the fermentative step (Fig. 6, limitation prior to box 3).

The accumulation of DOC resistant to transformation or uptake is also in accordance with observations of DOC generated in short experiments conducted with coastal temperate sediments (Weston & Joye, 2005). In that study, passage of artificial anoxic porewater through bioreactors filled with temperate sediments collected at different seasons and incubated at the *in situ* temperatures of 12-29°C yielded an outflow of DOC from the bioreactors. Less than half of the DOC generated within the bioreactors was remineralized by sulfate reducers, leading Weston and Joye (2005) to conclude that the DOC was refractory, on the 10-day timescale of the experiment. Although the bioreactor experiments could not be used to distinguish between hydrolytic and fermentative steps, the data from our temperate and Arctic sediments suggest that the 'sticking point' might be found within the fermentative community.

An inability to ferment a growing fraction of the DOC pool to VFA's (Fig. 6, flow via arrow B' rather than arrow B) could be due to the production of refractory DOC, or to production of DOC that is intrinsically difficult to transport across the cell membrane, even though it may be within the direct uptake size limit. A rarely-addressed but possibly critical problem may relate to substrate transport: once a substrate is of sufficiently small size, it still needs to pass through a microbial porin (the water-filled inlet channels spanning the outer membrane of gram-negative bacteria). The structure of these porins represents an additional aspect of substrate selectivity. Work with

enrichment cultures of anaerobic marine bacteria has demonstrated that there may be difficulties in uptake by sedimentary microbial communities of selected disaccharides that are within the size limit for direct transport (Arnosti and Repeta, 1994). These difficulties could be related to discrimination among solutes by general uptake porins: solute uptake rates across general uptake porins may vary by an order of magnitude based on solute charge or hydrophobicity (Delcour 2003), and can also vary with specific structural features, as discussed by Arnosti and Repeta (1994).

An additional possibility is the alteration of otherwise bioavailable DOC to DOC that is difficult to hydrolyze further. A model encompassing this idea has been proposed by Burdige and Gardner (1998) and discussed further by Burdige (2001), who suggest that the pore water size/reactivity model can account for the distribution of a relatively unreactive pool of lower molecular weight (below ca 3 kD) DOC in sediments. Specific characteristics and/or mechanisms operating in sediments that contribute to this pool remain elusive, but investigations of DOC in seawater provide possible insights. For example, structural alterations such as methylation of protein have been shown to impede uptake yet allow for hydrolysis (Keil and Kirchman, 1992), and exposure of DOC to microbial remineralization has been found to increase resistance of otherwise labile protein (Keil and Kirchman, 1994). Furthermore, Nagata and Kirchman (1996) hypothesized that adsorption processes of polymeric organic matter to colloids may lead to the formation of a semi-labile DOC pool which can result in the decoupling of DOC production and rapid bacterial mineralization and, therefore, a net accumulation of recalcitrant DOC (Fig. 6, increasing sizes of boxes 2 and 2').

Conclusions

Prolonged incubations, particularly at elevated temperatures, had differential effects on sedimentary microbial communities. The community of organisms producing extracellular enzymes continued to be responsive to additional substrates and substrates present in the sediment, as demonstrated by induced hydrolysis of chondroitin sulfate and increased sedimentary concentrations of DOC and dissolved carbohydrates. Accumulation of DOC demonstrated that the activities of organisms and enzymes responsible for the solubilization/hydrolysis of POC to DOC outpaced DOC consumption by sulfate reducers. Low concentrations of volatile fatty acids and temperature-related decline in sulfate reduction rates demonstrate close coupling between sulfate reduction and the production of volatile fatty acids, such that the sulfate-reducing community became increasingly substrate-limited, despite the presence of abundant DOC. The extent to which this roadblock in carbon transformation might reflect an inability of the fermentative community to grow or whether the intermediate transformation products of DOC could not be transformed further because of structural limitations remains to be determined. Since these effects occurred both in temperate and Arctic sediments, they may reflect a general characteristic of microbial communities responding to long-term temperature changes. As environmental temperature changes in Arctic and in temperate environments, the balance of microbial remineralization processes may also shift. Further study of the long-term stability of the accumulating dissolved organic carbon is important to understand the overall significance of warming sediments in the marine carbon cycle.

ACKNOWLEDGEMENTS

We thank Martina Meyer for the assistance in the lab and captain Stig Henningsen and John Mortensen of MS *FARM* for the successful cruise in Svalbard. We also thank two anonymous reviewers for thoughtful comments. This study was supported by the Max Planck Society and by the German Research Foundation through the Priority Program 1162 'The impact of climate variability on aquatic ecosystems (AQUASHIFT) BR 2174-1-1'. Funding was also provided to C.A. by the National Science Foundation (OCE-0323975; OCE-0848703) and to A.S. by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program.

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APPENDIX

FUNCTIONAL DYNAMICS OF SULFATE-REDUCING BACTERIA COMMUNITIES IN WARMING ARCTIC AND TEMPERATE MARINE SEDIMENTS

GRANT PROPOSAL, AWARDED WITH A FEMS RESEARCH FELLOWSHIP

INTRODUCTION

Arctic regions are known to be particularly vulnerable to the effects of global warming and exposure of these habitats to temperatures above the ecosystem-specific critical thresholds is very likely to result in substantial structural and functional changes. In fact, long-term incubation experiments have showed that elevated temperatures had a strong impact in Arctic sediments as demonstrated by the higher temperature dependence exhibited by the bacterial sulfate reduction, which is an important pathway on the mineralization of organic carbon, and the decline of specific groups of sulfate-reducing bacteria (SRB) identified by CARD-FISH (Robador et al., 2009).

Current trends in ecological research however are also beginning to consider the consequences of biodiversity, understood in terms of diversity of genotypes and functional groups, for the

functioning of the ecosystems. Biodiversity may not just be a passive response variable of extrinsic disturbance factors but also an important element explaining ecosystem properties and process (Gamfeldt and Hillebrand, 2008). The aim of the proposed work is to study the temporal dynamics of SRB communities and evaluate the relationship between the differences in the SRB community composition and the stability of carbon mineralization rates via sulfate reduction in Arctic and temperate marine sediments subjected to increased temperatures.

WORKING HYPOTHESIS

Robador and coworkers (2009) reported a loss of diversity in SRB communities for Arctic sediments, as demonstrated by CARD-FISH, whereas the temperate sediments showed no change during the course of the experiments. The results of CARD-FISH analysis however were limited to specific SRB groups which were only partially covered by the target probes and, therefore, specific dynamics of the SRB community may have been overlooked.

Long-term changes in temperature may lead to a turnover in the SRB community composition, such that numerically minor members of a SRB population that are more tolerant to higher temperatures become dominant given sufficient time, and their metabolic capabilities could then dictate the response of the net community and the stability of the carbon cycling via sulfate reduction. In order to investigate such a possibility, sediment samples taken at regular intervals during long-term incubation experiments (Robador et al., 2009) are available and currently stored at -80°C.

RESEARCH PLAN AND SPECIFIC GOALS

The current work intends to specifically asses changes in the SRB community by means of:

- Sequencing analysis of clone libraries constructed from functional dissimilatory sulfite reductase (dsrAB) and *Desulfobacter*-specific 16S rRNA genes retrieved, prior to the incubation experiment, from the zone of maximal sulfate reduction in the Arctic and temperate natural sediments (Robador et al., 2009). The dsrAB gene encodes a key enzyme of the energy metabolism of sulfate reduction and can be used as a functional marker whereas the *Desulfobacter* group has been previously quantified with CARD-FISH and shown to have a differential temperature response among Arctic and temperate sediments (Robador et al., 2009).
- Time-course assessments of the SRB community using denaturing gradient gel electrophoresis (DGGE) fingerprinting of the amplified 16S rRNA gene products of the specific group *Desulfobacter* as well as terminal restriction fragment length polymorphism (TRFLP) fingerprinting of the dsrAB genes of the entire SRB community.

The proposed study fits into the overall research plan, as outlined below:

GOALS AND RESULTS

1. Construction of dsrAB and 16S RNA gene-based clone libraries.
   Complete. Clone libraries available.
2. Characterization of in situ SRB community.
   Bulk of the 3-month proposed project.
3. Assessment of SRB community composition change in selected time points using fingerprinting methods.
   Bulk of the 3-month proposed project.
4. Correlate dynamics of SRB communities with metabolic response to temperature.
   Outcome of the 3-month proposed project.

ANTICIPATED OUTCOMES

Preliminary results (Fig. 1) support a sulfate-reducing bacterial population turnover in the Arctic sediments subjected to a temperature increase and showed a different response when compared to temperate sediments. The proposed 3-month research project can profit from the considerable time invested in the construction of the clone libraries and implementation of the fingerprinting techniques and the currently available biogeochemical data. The identification of responses to a stressor like temperature change, at the species and community levels, will be highly relevant to project the effects of climate change on Arctic ecosystems.

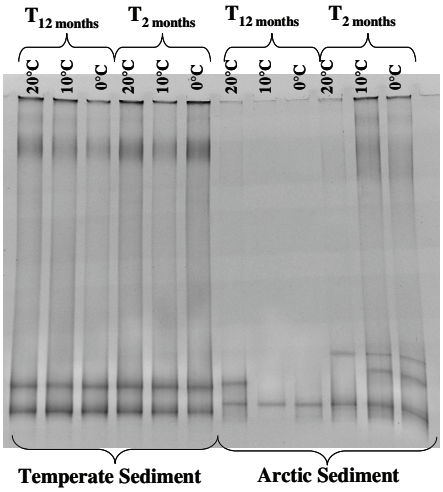

Figure 1. 16S rRNA gene-based DGGE fingerprint of the *Desulfobacter* group in Arctic and temperate sediments after 2 and 12 months of incubation time at 0, 10 and 20°C.

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CONCLUDING REMARKS AND FUTURE PROSPECTS

The bacterial cycling of organic carbon in marine sediments relates closely to ambient sediment temperatures over a wide variety of latitudinal and temporal scales. On a latitudinal scale, the direct relationship between environmental temperatures and the energy metabolism of sedimentary bacteria illustrate the biogeographical selection of adaptative physiologies and the evolutionary divergence of microbiota (see manuscript 2.1.). On a temporal scale, long-term temperature increases in Arctic sediments, in stark contrast with temperate sediments, resulted in the permanent loss of bacterial diversity and functional groups (see manuscript 2.2.). This drastic change on the microbial community composition may be a common feature of polar sediments subjected to increasing temperatures and it would be expected to have important consequences for the efficiency of bacterial carbon cycling. The species turnover in sedimentary bacterial communities in response to temperature shifts however remains one of the most challenging uncertainties in studying the impact of global warming in the functioning of ecosystems (see appendix) and future efforts should be focus on this area of research.

The physiological characteristics of the bacterial community actively involved in the terminal carbon mineralization have been identified as important feedback mechanisms influencing rates of organic matter degradation (see manuscript 2.2.). In addition, temperature may have an indirect effect on the downstream mineralization of organic matter in marine sediments. We observed a long-term decoupling leading to DOC accumulation most likely, due to effects on fermentative organisms involved on intermediate carbon degradation pathways that produce substrates suitable for terminal oxidizers (see manuscript 2.3.). Previous studies (e.g. Alperin et al. 1994; Weston & Joye 2005) have clearly

showed evidence of seasonal or short-term decoupling in dissolved organic carbon production and consumption. In the present work however, this balance changed in more than a transient manner and further study of the long-term stability of the increasing dissolved organic carbon will be important for the quantification of the effects of warming in the oceanic carbon cycle. ^{13}C nuclear magnetic resonance (NMR) spectroscopy, has been a particularly revealing analytical approach for molecular characterization of organic matter (e.g. Arnosti and Repeta, 1995; Hedges et al., 2000) and it could be applied to the discrimination of the structural changes of specific organic components during degradation. Molecular enrichment with ^{13}C stable isotope can be detected with greatly enhanced sensitivity and used for the identification of active organisms linked to the specific carbon degradation process through DNA- or RNA-stable isotope probing (SIP; Whitby et al., 2005; Wagner et al., 2006).

Understanding biological processes regulating carbon exchanges between the land, oceans and atmosphere is one of the biggest scientific challenges in climate change research according to the recently published report of the Intergovernmental Panel on Climate Change (IPCC 2007). The present study has examined the effects of environmental temperatures as well as long-term temperature variations on carbon cycling dynamics in sediments and identified different biological components that may help to recognize climate-ecosystem feedbacks in the Arctic which could, potentially, amplify or dampen regional or global climate change in the scope of a warming Earth.

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