RIJKSUNIVERSITEIT GRONINGEN

Cycling of iron and manganese in freshwater, estuarine and deep sea sediments

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 27 juni 2003
om 14.15 uur

door

Imola Ferro
geboren op 23 november 1972
te Bujumbura, Burundi

voor Corrie

Table of Contents

1. Introduction

1.1. Iron in marine systems

Iron is an important metal for living organisms, as iron is required for respiratory pigments, proteins and many enzymes. Besides the other nutrients iron availability may limit primary production and thereby the food availability for heterotrophic organisms and the oceanic CO_2 uptake from the atmosphere. Iron limitation of phytoplankton has consequently received a lot of interest. In some oceanic regions there is a low biomass despite a high concentration of nutrients (the high nutrient – low chlorophyll-a (HNLC) regions) and since the start of the 20th century research and speculations have been made about the causes (Gran 1931, Heart 1934 in:de Baar et al. 1990, De Baar et al. 1999, Martin 1990, Martin et al. 1994). Because ambient iron concentrations are low, iron limitation of primary production has been tested by bottle incubations as well as large scale *in situ* experiments for a number of the HNLC-regions e.g. the sub-arctic and equatorial Pacific and the Southern Oceans (Pakulski et al. 1996, DeBaar et al. 1995, de Baar et al. 1990, de Baar et al. 1999b, Behrenfeld and Kolber 1999, Hall and Safi 2001). The phytoplankton communities in these areas are not iron starved *per se*, but the low ambient iron concentrations may constrain biomass accumulation (Martin et al. 1994, Coale et al. 1996, Boyd et al. 2000). However, most results indicate that iron is not the only limiting factor for primary productivity. Other factors are probably as important or are affected by iron enrichments, for example a seasonal wind mixing effect on the light regime or regional silica limitation for diatoms in the remote pacific waters of the southern Ocean (de Baar et al. 1999a). The effect of iron enrichment in the HNLC regions on the bacterial community has not yet been investigated in detail. It appears that the bacterial biomass is not limited by iron (alone) (Church et al. 2000, Hall and Safi 2001). However addition of iron had a considerable impact on the microbial components of the food web (Hall and Safi 2001).

The sources of iron for the pelagic community are several. Aeolian input with teregenous matter is a source occurring in a large part of the oceanic waters (Donaghay 1991, Duce and Tindale 1991, Bowie et al. 2002). Riverine input is another source, especially affecting iron concentrations in coastal areas. In deeper water re-mineralization of organic matter can release iron and after upwelling this can be a source of iron. Finally, sediments can be a source of iron if sedimentary

iron oxides are reduced (directly or indirectly) during anaerobic mineralization processes and then escape to the overlying water. This last process couples the iron cycle to the carbon cycle.

1.2.   Organic carbon

Autotrophic organisms fix carbon dioxide during the assimilation processes and form organic carbon molecules. The organic matter produced in pelagic water sinks down together with other settling materials and may arrive at the sediment surface. During sinking the remineralization of organic matter to CO_2 starts. After settling to the sediment surface and mixing into the sediment the organic matter is further degraded by various (micro) organisms. Most of the produced organic carbon is eventually remineralized to CO_2. A small part is of the organic carbon is removed from this short-term biological cycle by burial. Thereby it enters the long-term geological carbon cycle.

1.2.1.   Coastal areas

Marine systems account for about halve of the global primary production. Despite the relatively small surface area of the coastal areas, i.e. 8 % of the global ocean surface, these regions contribute 18-35 % to the global production of the ocean. This high productivity is due the river input of nutrients, upwelling of fertile deep waters, the absence of losses below a compensation depth and the close coupling between benthic and pelagic systems (Wollast 1991).
Organic carbon in marine sediment results form several resources: (1) benthic organisms that form organic tissue and faeces, (2) deposited pelagic organic carbon that reaches the sea floor before it is degraded, e.g. produced by autotrophic organisms in the surface waters fixing carbon dioxide and (3) input of external organic carbon e.g. via wind transported dust or run off of adjacent land via rivers. In shallow ecosystems a large fraction (50%) of the organic carbon produced reaches the sea floor where it drives diagenesis (Heip et al. 1995). In contrast to the deep-sea where most organic carbon (> 90 %) is remineralized before the sediment surface is reached. Due to particle mixing and sediment accumulation the settled organic matter is transported to the deeper sediment layers. This ensures a more uniform depth distribution and enhanced diagenetic activity at large depth. The preservation of organic carbon in the sediments depends on many environmental factors (sedimentation rates, grain size of sediments, faunal activity etc.) and the efficiency of the mineralization pathways.

1.3. Organic matter degradation

The efficiency and degradation rate of organic carbon depends among others on the quality of the organic matter pool. The quality of organic matter pool is a function of availability, the composition of the organic matter and the consumer involved. Simple structures like glucose are easily remineralized whereas complex structures like cellulose are more refractory. Breakdown of highly reactive organic carbon results in a high-energy gain for the bacteria. Therefore these components are preferentially degraded, leaving the more complex structures behind. Thus in sediment profiles the composition of the organic carbon pool usually becomes more refractory in the deeper layers (Middelburg 1989). The decrease in organic matter lability and the decrease in concentrations with depth results in a decrease of degradation rates in these deeper layers (Dauwe et al. 1999).

Table 1. Free energy gain from mineralization reactions from Froelich 1973. Energy gain is presented as kJ mol-1 glucose. Organic carbon is represented by CH_2O.

Process	Reaction	Energy gain (kJ $mole^{-1}$)
Oxic degradation	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-3190
Nitrification	$5\ CH_2O + 4NO_3^- \rightarrow CO_2 + 2N_2 + 4HCO_3^- + 3H_2O$	-2750
Mn-reduction	$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-3090
Fe-reduction	$CH_2O + 7\ CO_2 + 2\ Fe_2O_3 \rightarrow 4\ Fe^{2+} + 8\ HCO_3^-$	-1410
sulphate reduction	$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-380
Methanogenesis	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-350

1.3.1. Oxidation pathways

Mineralization of organic carbon is an oxidation reaction because zero valent carbon in organic matter is oxidized to tetravalent carbon in carbon dioxide. The microbial degradation pathways cover a wide range of aerobic and anaerobic reactions, using different types of electron acceptors. The energy gain of the oxidation-reduction reaction determines the profit for the bacterial community involved and depends on the redox couple. Pathways with a high-energy gain are preferentially used over pathways with a lower gain (Table 1 from Froelich et al. 1978). In sediments the availability of the electron acceptors is limited and preferential use of the most favourable electron acceptor results in zonation of the pathways. This creates a vertical layering, starting with oxygen as the most

favourable electron acceptor, followed by nitrate, Mn-oxide, Fe-oxide, sulphate and finally methane formation (Fig. 1). Due to spatial heterogeneity of dynamic sediments (bioturbation, micro-niches) the vertical separation is not very strict and several processes may occur at the same depth in different patches (Davison et al. 1997, Harper et al. 1999).
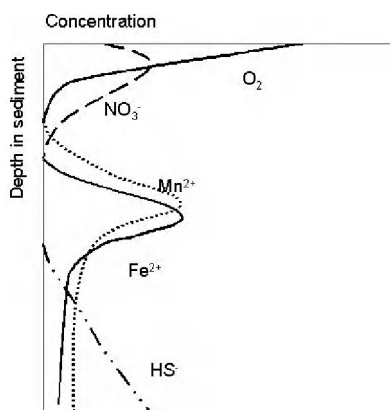

Fig. 1 Redox zonation in marine sediments. First O_2 and NO_3^- are depleted, deeper in the sediment concentration Mn^{2+} and Fe^{2+} increase paralel with the process of metal oxide reduction, followed by an increase in HS^-.

The occurrence or dominance of degradation pathways and electron acceptors depends not only on the energy gain of organic matter oxidation but also on other specific characteristics such as energy gain with respect to re-oxidation of the reduced products, inhibition by products of the other pathways or electron acceptors and chemical reduction processes.

Aerobic organic carbon degradation, using oxygen as electron acceptor, is the pathway with highest energy gain. Diffusion of oxygen into the sediment is usually limited and at high or intermediate organic carbon loads oxygen is depleted within the first 1-2 cm of the profile (Revsbech et al. 1980). At high organic carbon inputs sediment oxygen consumption is not exclusively attributable to organic carbon degradation. The re-oxidation of upward diffusing reduced products also consumes oxygen (Soetaert et al. 1996). The importance of organic carbon degradation via the aerobic pathway differs and is relatively low (~10 % of total organic matter mineralization) in sediment with a high organic carbon input to approximately 100

% of total organic matter mineralization in organic poor sediments like the ones found in the Mediterranean deep-sea areas (this thesis).

Aerobic organic carbon degradation is relatively fast and efficient. There has been and still is much debate about the effect of oxygen on organic matter degradation. Kristensen and Holmer 2001, reviewed the literature and argued that for highly degradable organic matter aerobic and anaerobic degradation rates are similar, whereas the aerobic pathway more efficiently degrades refractory organic matter. Dauwe et al. 2001 proposed that differences between aerobic and anaerobic degradation are mainly related to absolute mineralization rates and do not necessarily depend on the lability of the sedimentary organic matter.

Nitrate is a dissolved electron acceptor that is used after oxygen becomes lower than a few μM. The dissimilatory reduction of nitrate can be coupled to oxidation of organic matter or re-oxidation of Mn (II) or Fe (II) (Straub et al. 1996). Nitrate reduction occurs in a small layer directly after oxygen is depleted. The importance of nitrate for the organic matter degradation depends on the amount of nitrate, temperature, the oxygen concentration and the availability of organic carbon (Middelburg et al. 1996b). Middelburg et al. 1993 estimated the total contribution of nitrate to organic carbon mineralization at 7-11 %. Availability of nitrate as an oxidant is often restricted because it is one of the limiting nutrients for primary production (Ryther and Dunstan 1971). In freshwater sediments, the role of NO_3 in organic matter degradation is usually larger because of the higher concentrations (Knowles et al. 1981).

Manganese oxides are intermediate sub-oxic electron acceptors in particulate form. In marine sediment most manganese oxide is present in the solid phase because of the low solubility of Mn (III) and Mn (IV). Reduced manganese mostly occurs in the porewater as solute, because this form is more soluble. However, reduced manganese can also adsorb onto sediment particles or Mn-oxides thereby disappearing from the porewater solution (Canfield et al. 1993b, Slomp et al. 1997). In general sediment concentrations of manganese-oxide are relatively low compared to two other electron acceptors, iron oxide and sulphate. Reduced manganese (Mn (II)) can re-oxidize with nitrate and oxygen. Manganese-oxides exist in various forms, which have different reactivities. Reactivity of manganese oxides is controlled by surface reactions and therefore by factors like surface area and crystallinity (Burdige et al. 1992) Manganese oxide reduction can occur coupled to the oxidation of organic carbon. At low reduction rates surface area and crystalinity are the controlling factors of microbial reduction (Burdige et al. 1992). The importance of manganese as electron acceptor in the organic carbon mineralization depends on the availability and the reactivity of the oxides present.

In some sediments manganese oxides are present in high concentrations like in the Skagerrak S9 site (Canfield et al. 1993b) or the Panama basin (Aller 1990). Here manganese oxide is an important electron acceptor in the organic carbon degradation (Panama Basin: 100 %, S9: ~ 99% S9). Manganese oxide reduction can also occur coupled to the re-oxidation of Fe (II) (Postma 1985), or pyrite and iron sulphide oxidation (Schippers and Jorgensen 2001). Because of the competitive chemical reduction of Mn-oxides and the low sediment concentration of Mn-oxides bacterial Mn-reduction is often assumed to be zero and neglected in a lot of studies.

Iron oxide is a second intermediate sub-oxic electron acceptor. The iron oxide concentration in sediments is usually much higher (10 times) than that of manganese oxide. Due to low solubility of ferric iron or Fe (III), oxidized iron is mostly in the particulate form. Reduced iron (ferrous iron or Fe (II)) is more soluble and can be found in the porewater solution or adsorbed onto particulate matter (Kostka and Luther 1994). Like for manganese various types of iron oxides exist. These range from amorphous structures to highly crystalline oxide forms. Reduced iron forms can be re-oxidized with oxygen, nitrate and manganese oxides as electron acceptors (Postma 1985). Microbial iron oxide reduction can be coupled to the cycling of organic carbon. The relative importance of the iron oxide reduction pathway is very variable. In freshwater sediments iron oxides are the most important anaerobic electron acceptors (Roden and Wetzel 1996). In saline sediments the contribution of iron oxide reduction tot total mineralization varies from 0.2 % of mineralization in the Mediterranean sediments (this thesis) to 78% in the Skagerrak S4 site (Canfield et al. 1993b). Iron oxide reduction can also be coupled to the re-oxidation of sulphide (Sorensen and Jorgensen 1987). Reducibility of iron oxides is determined by their reactivity which, is controlled by crystal structure (Larsen and Postma 2001). More reactive oxides provide a higher energy gain for iron oxide reducing bacteria. The rate of microbial iron oxide reduction can therefore be controlled by reactivity (Jakobsen and Postma 1999, Postma and Jakobsen 1996) and the available specific reaction sites (Roden and Zachara 1996.)

In marine sediments with a high organic carbon input sulphate reduction is the most important anaerobic degradation pathway. Due to diffusion of sulphate from the overlying water into the sediment and the high concentration of sulphate in seawater, sulphate availability is high and this process can account for a large part of organic matter degradation (Henrichs and Reeburgh 1987). Thus in organic rich coastal sediments sulphate reduction is often the major mineralization pathway (20-90% of total mineralization, Jorgensen 1982, Canfield et al. 1993b, Thamdrup and

Canfield 1996). Sulphide, which is one of the main products of sulphate reduction, can re-oxidize with O_2, NO_3, Mn-oxides and Fe-oxides (Jorgensen 1977, Sorensen and Jorgensen 1987, Moeslund et al. 1994). In freshwater systems sulphate concentrations are low and sulphate reduction is not an important degradation pathway in most anaerobic freshwater sediments.

The final mineralization pathway is methane formation, where organic carbon components are fermented. In marine sediments methanogenesis is only of major importance if sediment organic carbon load is sufficient to fuel all preceding mineralization pathways and deplete all stocks of alternative electron acceptors. In freshwater sediments methanogenesis can be an important degradation pathway (e.g. Roden and Wetzel 1996, Conrad and Klose 1999). Methane is oxidized in presence of oxygen (Bender and Conrad 1992, Canfield 1993, Bodelier and Frenzel 1999). Anaerobic methane oxidation by a consortium of sulphate reducing and methane oxidising bacteria has been reported in marine sediments (Boetius et al. 2000) and is subject of intensive study.

The relative importance of the different degradation pathways are affected by (1) The quantity and degradability of the organic carbon. (2) Availability of the electron acceptors to be used in bacterial degradation pathways. At low fluxes of fresh organic carbon, the rate of materialisation is small and oxygen can account for mineralization of all labile organic carbon. At higher carbon fluxes oxygen is depleted and the anaerobic processes become more important, starting with denitrification as dominant process. At increasing carbon fluxes the degradation process shifts to the other pathways until sulphate reduction is the dominant pathway (Fig. 2, Soetaert et al. 1998, Wijsman et al. 2001a). The absolute carbon input at which the changes occur depends on the availability of the different electron acceptors. The models indicate that the changes in dominant pathway do not always occur gradually but may appear in swaps. These swaps have not been observed yet in field measurements, but may be an explanation for the existence of sulphate or iron-oxide reduction dominated sediments. A number of other factors, like temperature and sediment disturbances, affect the rates and distribution of the pathways.

Understanding the factors and mechanisms affecting organic matter degradation pathways is relevant in furthering our understanding and modelling of ecosystems. Together with changes in degradation pathways other aspects, such as redox state, pH, and concentrations of other constituents, might change as well. This can significantly affect the system. Many trace metals, like cadmium and lead, are sorbed or incorporated within sedimentary manganese or iron oxides. Dissolution of these oxides as a consequence of organic matter degradation may result in
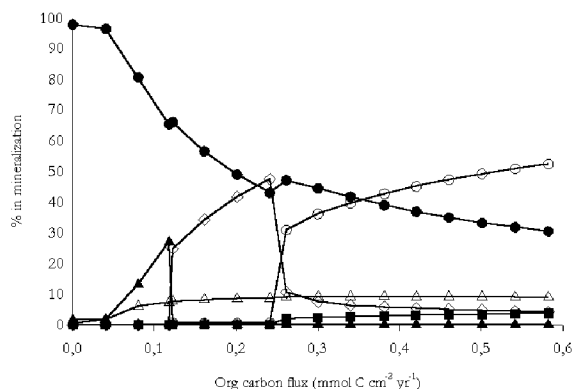
Fig. 2 Effect of the organic matter flux (mmol C m⁻² yr⁻¹) to the sediment on the relative importance (%) of the various pathways as modelled by Wijsman et al. 2002. Oxic materialisation - solid circles, nitrification - open triangles, Mn-oxide reduction - solid triangles, Fe-oxide reduction – open diamonds, Sulfate reduction - open circles, methanogenesis - solid squares.

liberation of these trace metals as well thereby linking the iron and trace metal cycles (Warren and Haack 2001, Zachara et al. 2001), for example Pb, Zn, Cu and Cd co-precipitate with iron oxides (Tessier et al. 1985, Dong et al. 2000, Lee et al. 2002). Studies have shown that the sediment oxygen state affects trace metal concentrations. Cd, Cu, Mo, Re, U and V are enriched in anoxic sediments (see references in Namerhoff et al. 2002). Fein et al. 1999 described the concomitant release of Al, Co, Cr, Cu Ni, Pb Ti and V from rocks by bacterial activity. Besides, some metals are highly dissolved in their oxidized state, whereas their reduced forms may be insoluble sulphides (Warren and Haack 2001, Skei et al. 1988). Therefore changes in sediment redox conditions affect mobility and bioavailability of these components. Morse 1994 described the bioavailability of trace metals, which co-precipitate with iron pyritization. Phosphor, which is an important nutrient, is associated with iron oxides. Dissolution of iron oxides due to changing redox-conditions or dissimilatory iron reduction results in liberation of phosphate as well, eventually resulting in eutrophication of the system (Nixon 1981, Roden and Edmonds 1997). Sulphate reduction results in the production of HS⁻ that is toxic for most benthic organisms (Magni and Montani 1998). In environments containing reactive metal oxides these can act as a buffer of the system against HS⁻ by re-oxidising the sulphide and forming metal sulphides (King 1990, King and

8

Klug 1982). Hereby suitable conditions are maintained for as long as the metal oxide buffer exists.

In rice paddies and wetlands the importance of the different degradation pathways determines the potential methane efflux towards the atmosphere. Because of the large capacity of methane as a global warming gas, and the large area of rice fields needed to feed a growing global population, the process of methane production, effluxes to the atmosphere and limiting factors of these have been a subject of interest for several years (e.g. Kruger et al. 2001, Frenzel et al. 1999, van Bodegom and Scholten 2001).

Recently, interest has grown on the role of anaerobic and metal-oxide reducing microorganisms in soil remediation. Langenhoff et al. 1997 reported that microbial reduction of manganese could be coupled to toluene degradation. Using less crystalline manganese oxides or adding organic ligands can enhance the degradation rates. Dissimilatory iron-oxide reducing organisms have the ability to destroy organic contaminants, including chlorinated contaminants and aromatic hydrocarbons, by oxidising them to CO_2 (Lovley and Anderson 2000). Like for manganese-oxide degradation the use of chelators enhances iron oxide reduction rates (Lovley et al. 1996). Moreover iron oxide reducing bacteria have been found to reduce other metals and convert them to soluble forms that are likely to be immobilised in the subsurface (Lovley et al. 1991, Warren and Haack 2001).

Providing an electron acceptor in the form of an anode enlarges the remediating capacity of iron oxide reducers (Bond et al. 2002). Furthermore, iron-oxide-reducing bacteria in marine sediment are able to conserve energy to support their growth by oxidising organic carbon with an electrode as the sole electron acceptor (Bond et al. 2002). Energy stored in organic matter can be harvested with this pathway by placing an anode in the anoxic sediment zone and a cathode in the oxic zone. This finding provides a potential method to harvest energy from wastewater.

1.4.   Metal oxide reduction

The mineralization pathways involving dissolved constituents, O_2, NO_3, SO_4 and CH_4 have been studied intensively and a lot of knowledge has been gained. Microbes using the metal-reduction pathways have been discovered only about two decades ago (Lovley 1987, Lovley and Phillips 1986, Phillips et al. 1993). Because of the low transport rates of these particulate electron acceptors these mineralization pathways have been considered of no or limited importance for a long time and were therefore not included in studies on mineralization pathways. This traditional view changed with the recognition of intensive sediment metal

recycling, allowing one metal molecule to be used in organic matter degradation a number of times before burial (Fig. 3, Aller 1990). Solid phase metal oxide is reduced in the metal oxide reduction zone in the anoxic sediment. The reduced metal, which is dissolved in the porewater, can re-oxidize after diffusion into an aerobic layer or by reaction with a re-oxidizing agent. A subsequent mixing event, like bioturbation, mixes the newly formed particulate metal oxide into the reducing layer where the metal can be re-used. As long as no metals escape this cycle, metal oxide reduction can continue. If the cycle is not complete this may lead to an efflux of reduced metals to the overlying water or burial of metal-oxides in the sediment. The final effect of cycling on mineralization pathways depends on the recycling rate and the presence of the other electrons. Studies on the role of metal oxide reduction in organic carbon mineralization in marine sediments showed a broad range of rates and relative importance (e.g. Aller 1994b, Canfield et al. 1993b, Thamdrup and Canfield 1996, Glud et al. 1998). In anaerobic freshwater sediments iron oxide can be the most abundant electron acceptor and therefore an important component in anaerobic degradation (Roden and Wetzel 1996).

Nowadays, iron and manganese oxide reduction are known to be potentially important pathways for organic matter degradation and both have been discussed in a lot of studies (e.g. Canfield et al. 1993b, Kostka et al. 2002, Thamdrup and Canfield 1996, Slomp et al. 1997, Wijsman et al. 2001a, Roden and Wetzel 2002). However, the solid phase nature and chemical properties of these oxides do impose some difficulties on the experimental research. Metal oxide reduction rates are often estimated by subtracting other pathways (which can be relatively easily determined) from total organic degradation rates, resulting in a lumped rate for metal-oxide reduction.
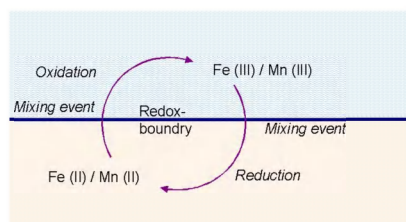

Fig. 3 Metal cycling across a redox boundary. Cycling of metals can occur within the sediment or across the sediment – water interface. The term 'mixing' involves both dissolved and particulate mixing mechanisms, such as bioirrigation and bioturbation respectively.

1.4.1. Reduction rates

Establishing the role of metal oxide reduction in organic carbon degradation at different conditions is an important aspect to model biogeochemical sediment processes, and to gain insight in bio-remediation potentials. However, chemical and kinetic characteristics of the metal oxides are more complex than of the other electron acceptors. Therefore studying metal oxide reduction is rather complex and laborious. Especially side reactions that cause reduction or re-oxidation of the metal (Postma 1985, Canfield et al. 1992, Sorensen and Jorgensen 1987) or precipitation and adsorption on clay particles, cell surfaces or iron oxides (Roden and Zachara 1996, Kostka et al. 1999a) complicate rate measurements. These side reactions should either be measured as well, or prevented by using inhibitors (Oremland and Capone 1988, Canfield et al. 1993b).

1.4.2. Reactivity

Energy gain and preferences of bacteria to use metal oxides depend on the crystal structure of the oxide (Jakobsen and Postma 1999, Postma and Jakobsen 1996) Amorphous metal oxides are very reactive and can easily be used by bacteria at a high rate, whereas more crystalline phases provide lower energy gain, which reduces the competition position of metal oxide reducing bacteria towards sulphate reducers. Sedimentary metal oxide reactivities provide insight on the potential for metal oxide reduction pathways. Traditional methods are based on iron oxide speciation and concentration. However, these are affected by pool size and sediment specific surface area and are not suitable for comparing reactivity between samples with different characteristics. Reactivity of some iron oxides towards dissolution has been studied, indicating that less polymerised or less crystalline oxides dissolve better (Deng and Stumm 1994). This does not allow for a quantification of iron oxide reactivity in sediment containing a mixture of iron oxide species. A decade ago Postma 1993 reported a method to quantify iron oxide reactivity using a reactive continuum approach. Using this method Larsen and Postma 2001 reported the reactivity of pure iron oxides to be independent of pool size and the specific surface area. Recently Van der Zee and van Raaphorst 2002 applied this approach to assess manganese oxide reactivity in North Sea sediments. Applicability of this method for a range of sediments has not been tested yet. Testing a range of sediments is necessary to estimate the usefulness of this method in biogeochemical research. Besides, if reactivity data are to provide insight in the potential microbial reduction of the oxides investigating this link will be necessary as well. As far as we know, the method is not yet linked to data on iron oxide reduction rates.

1.4.3. Bioturbation effect

Aller 1990, Aller 1994b described the effect of bioturbation on the importance of manganese reduction for organic matter degradation. He established the important role for particle mixing as this allows newly formed metal oxides to be mixed from the upper sediment layer into the reducing layer. The overall effect of bioturbation is a faster mixing and faster turnover due to enlargement of the (sub) oxic surface area via the burrow walls and a higher exchange of porewater and overlaying water (Kristensen 2000). The net effect depends on bioturbation intensity and the initial sediment conditions. Bioturbation can be split in two mechanistic components, sediment irrigation and particle mixing. The effects of these two components are likely different for iron and manganese due differences in their basic aquatic chemistry. In a continental slope sediment Van der Zee 2002 revealed particle mixing as the rate limiting factor for the iron cycle, while particle mixing and oxidation kinetics were rate limiting for manganese cycling. The separate effects of particle mixing and irrigation on the metal reduction pathways have not been experimentally investigated. However this type of information is essential to further our understanding and to improve and validate diagenetic models.

1.5. This thesis

To elucidate some of the lacks in our knowledge on sedimentary iron and manganese cycling the programme on Sedimentary Manganese and Iron cycLEs (SMILE) was started (funded by NWO-ALW). In this research programme the aspects of sedimentary iron and manganese were studied along a carbon gradient. The programme was divided in three components. Component I focused on bioturbation and manganese and iron cycling in estuarine sediments and the results are presented in this thesis. Component II focused on the cycling of manganese, iron and phosphorous in sediments from the continental shelf and slope (Royal Netherlands Institute for Sea Research, results in: Van der Zee 2002) and component III comprised the cycling of Fe, Mn related trace elements and sulphur in sediments of the Eastern Mediterranean (University of Utrecht).

1.5.1. Component I

This thesis describes results of the first component of SMILE. The objectives of the study were:

1. To compare iron oxide reactivity within and between marine sediments and establishing the effect of iron oxide reactivity on iron oxide reduction rates.

2. To investigate the rate of iron oxide reduction as a function of organic carbon and reactive iron oxide loading.

3. Examine the effect of bioturbation and its separate mechanistic components on sedimentary iron and manganese oxide reduction.

4. Studying the importance of metal oxide reduction rates for organic matter degradation in different sediments and the effect of side reactions.

Field and experimental studies are performed with three types of sediment covering a gradient of salinity and organic carbon input. A freshwater site, Appels, was situated along the Scheldt River in the intertidal zone and was flooded twice a day. The sediment was very fine and had a high organic carbon content due to a high organic carbon input from benthic algae and particles from the Scheldt river and the adjacent marsh. Two subtidal estuarine sites in Lake Grevelingen (salinity 29) were selected. One, Vliegertje, is a sandy littoral site situated on a plain, the other, Geul, is situated in a gully. Due to focussing of fine material the sediment in the gully was finer than the Vliegertje sediment. However, at both sites sediments were coarser than at the freshwater site Appels. Organic carbon inputs were much lower as well. A deep-sea sediment was sampled in the Eastern Mediterranean. These sediments are hemipelagic muds with a low organic carbon content and small grain sizes.

Chapter 2 describes a method, the reactive continuum approach (Postma 1993) to quantify iron oxide reactivity in sediments. The iron oxide reactivity is described using a gamma distribution. We compare results of this method for samples from the three sites with results of extractions for iron oxide speciation (e.g. concentration of amorphous iron oxides). The effect of iron oxide pools size and sediment specific surface area on reactivity is evaluated and no simple relations were found.

In Chapter 3 the relation between organic matter input, iron oxide concentration and iron oxide reduction rates was investigated using slurry experiments. Iron oxide reduction rates in the freshwater sediment were dependent on iron oxide concentrations, whereas in the estuarine and deep-sea sediment organic carbon availability was the rate-limiting factor. V_{max} (potential maximal iron oxide reduction rate) and Km (affinity of the microbes for the substrate i.e. iron oxides) have been estimated and were consistent with these findings. In organic-rich freshwater sediment V_{max} of iron oxide reduction was independent or organic carbon concentrations, whereas in deep-sea sediments we found increasing V_{max} values with increasing quantities of degradable organic carbon indicating the dependence on organic matter availability.

The effect of bioturbation on metal cycling has been investigated in a mesocosmos experiment (Chapter 4). Bioturbation can be split in two components: sediment irrigation and particle mixing. The sole and combined effect of these processes on metal oxide reduction was investigated. Due to the differences in the basic chemistry of both metals iron-oxide reduction is primarily affected by particle mixing, whereas manganese oxide reduction is affected by both particle mixing and irrigation.

In Chapter 5 the importance of metal oxide reduction in organic carbon degradation has been investigated in anaerobic bag incubations. The results provide a link between our experimental data and field observations. Metal oxide reduction rates were highly different between the sediments. Metal oxide reduction layers can be discerned within the profiles. Furthermore in the estuarine sediment inhibitors were used to discern chemical and microbial metal oxide reduction rates.

The concluding Chapter 6 integrates our findings of the various chapters and provides a discussion of sedimentary iron and manganese cycling.

2. A comparison of iron oxide reactivity within and between aquatic sediments

Imola Ferro, Jack J. Middelburg, Caroline P. Slomp, Ralf R. Haese

2.1. Abstract

The reactivity of sedimentary iron oxide is usually characterised by the size of various operationally defined iron pools. These different iron pools partly overlap, cover a large range of reactivities, and are affected by various sediment properties. We used a kinetic method (Postma 1993) in which iron oxide reactivity is represented as a reactive continuum, and compare results of a freshwater, an estuarine and a deep-sea sediment to traditional, single-step extraction results. Depth profiles were derived using both approaches. The traditional extraction methods have sufficient resolving power to reveal concentration differences of reactive iron oxides between samples from deep-sea, estuarine and freshwater sediments, but result in homogeneous depth profiles for iron oxide composition in the latter two sediments. The reactive continuum approach resulted in heterogeneous depth profiles of initial iron oxide reactivity and reactivity distribution for estuarine and freshwater sediments, thereby revealing differences in iron oxide composition. Due to low concentrations of reactive iron oxides the reactive continuum approach was not applicable in deep-sea sediments. Reactivities were independent of sediment surface area and the load of reactive iron oxides.

2.2. Introduction

In aquatic sediments bacteria degrade organic matter. In the first stage of degradation this is done aerobically, with oxygen as electron acceptor. When all oxygen is depleted anaerobic respiration starts, with mineralization processes occurring in the following order: nitrification, manganese oxide reduction, iron oxide reduction, sulphate reduction and methanogenesis. Both aerobic and anaerobic degradation pathways have intensively been studied (e.g. Canfield et al. 1993a, Canfield et al. 1993b, Kristensen and Holmer 2001, Jorgensen 1977, Thamdrup et al. 1994a, Roden and Edmonds 1997, Thamdrup 2000).

For iron- and manganese oxides, both solid-phase electron acceptors, sediment characteristics such as organic carbon concentration, pool size and the degree of sediment disturbance (e.g. bioturbation by benthic fauna) are important factors determining the relative importance of the corresponding degradation pathways

(Soetaert et al. 1996, Wijsman et al. 2002, Aller 1990, Canfield 1994). Physical or biological reworking may enhance sediment-water exchange and increase recycling of both oxides (Aller 1994b, Aller 1990, Mulsow et al. 1998, Kristensen 2000). Furthermore, particle mixing is important to replenish the electron acceptors at the depth of reduction, which is of limited importance for dissolved electron acceptors such as nitrate or sulfate. Therefore, in sediments with a high bioturbation iron- and manganese oxide reduction can be important mineralization pathways. For example, Canfield et al. 1993a reported a contribution of iron oxide reduction of 71-84 % to the total decomposition of organic matter in some Skagerrak sediments. However, in another Skagerrak sediment the high manganese-oxide concentration and the high biodiffusion coefficient results in organic mater degradation completely performed via dissimilatory manganese reduction. An important role for manganese oxide reduction due to bioturbation has been reported as well for the sediment along the continental margin of Chile (Thamdrup and Canfield 1996) and sediments of Long Island Sound (Aller 1994b). Thamdrup et al. 1994a reported smaller contributions of iron and manganese oxide reduction to total mineralization for Aarhus Bay than for Skagerrak sediment due to the smaller rate of bioturbation.

The relative importance of iron oxide reduction in anaerobic degradation of organic matter depends not only on the above-mentioned factors (organic carbon loading, availability of other electron acceptors), but also on the size and characteristics (i.e. reactivity) of the sedimentary iron pool (Postma and Jakobsen 1996, Jakobsen and Postma 1999). Sedimentary iron oxides form a heterogeneous group of minerals with different structures and reactivities (Canfield 1989, Postma 1993, Burdige 1993). The structures and reactivities range from amorphous and very reactive (e.g. freshly precipitated iron oxides) to crystalline and nearly inert for aged iron oxides.

Sedimentary iron oxides originate from two major sources. They are either external and include detrital and those formed in the water column (hydrogenous iron oxides), or they are newly formed in the sediments (diagenetic). This distinction has consequences for their reactivity (Haese et al. 2000).

The external iron oxide input, i.e. detrital iron oxides, results from settling particles. They may originate either from the adjacent land (recycled from rocks or formed during weathering) or were formed during riverine and marine transport. Moreover, they may also originate from re-suspension of nearby or distal sediments. Because of this variety of possible iron oxide inputs, it is very likely that detrital iron oxides vary in chemical composition, grainsize and crystalinity and therefore also in reactivity.

16

The formation of iron oxides within sediments results in a pool of diagenetic iron oxides. These oxides are formed by (re)-oxidation of sedimentary Fe (II). The freshly formed iron oxides are less crystalline and therefore have a higher initial reactivity than older, more crystalline, oxides (Larsen and Postma 2001). Iron oxide formation and precipitation occurs upon chemical or microbiological oxidation of Fe (II), with O_2, nitrate or Mn-oxides (Postma 1985, Aller 1990, Luther et al. 1997, Sobolev and Roden 2001). The oxidation process may result in an iron-rich layer in the sediment occurring just above the reduced dissolved iron peak, where initial iron oxide reactivity is expected to be higher than in the rest of the profiles.

Iron-enriched layers have been observed in many sediments e.g. Black Sea sediment (Thamdrup et al. 2000, Wijsman et al. 2001c, Wijsman et al. 2001b), Skagerrak sediments (Canfield et al. 1993b), North Sea sediments (Slomp et al. 1996), sediments from the Mediterranean Sea (DeLange et al. 1989, Pruysers et al. 1993), Atlantic sediments (Wilson et al. 1986) and salt marsh sediments (Kostka and Luther 1994, Luther et al. 1992).

The reactivity and distribution of sedimentary iron oxides are usually based on extractions with different leachants (Canfield 1989). Iron-oxide pools can be defined on an operational basis, separating pools e.g. of amorphous iron oxides, AVS, and crystalline Fe (III) oxides. However, application of these extractions to study the reactivity of iron oxides within and between sediments is not straightforward. Leachants do not extract specific minerals. Iron may be released from a variety of phases during one extraction. Moreover, these operationally defined iron extractions result in data which are dependent on the sediment grain size (Munch J.C. and Ottow 1980). Therefore, the operationally defined extractions do not provide a quantification of iron oxide reactivity by means of a rate constant, but a quantity of a potentially reactive fraction of the total sediment. As an alternative, iron oxide reactivity has been proposed to be regarded as a continuum, which leads to an operationally kinetic approach (Postma 1993).

Since the reactivity of iron oxides may determine the rate of iron oxide reduction (Postma and Jakobsen 1996, Jakobsen and Postma 1999), this implies that iron oxide reactivity may have an important influence on the competition of organic matter degradation pathways. Consequently, the determination of rate constants for iron reduction in various environments may help to understand the controls on microbial pathways. Postma 1993 presented an approach to quantify the reactivity of iron oxides and that allows a comparison of iron oxide reactivity within and between sediments. It involves an extraction of iron oxides with an ascorbic acid solution and measurement of the release of iron as a function of time. The iron release with time curve is interpreted with a reactive continuum approach allowing

calculation of the iron oxide reactivity distribution and initial reactivity. A more detailed description of the approach is given below.

Here, we apply the reactive continuum approach to sediments from freshwater, subtidal coastal and deep sea environments differing in grain size, organic carbon and iron oxide concentrations. Iron oxide characterisations based on the traditional extraction approach are compared with those based on the reactive continuum approach.

2.3. Materials en Methods

2.3.1. Sites

Sediment was collected at a tidal freshwater site, two subtidal saline sites and two deep-sea stations (Table 1). Sediments were collected by box coring unless indicated otherwise. Appels, the tidal freshwater site is in the tidal reach of the Scheldt River. Cores were taken manually during low tide. Porewater measurements result from samples taken in October 1998, extraction data result from samples taken in June 2000. No microelectrode results are available. The two subtidal estuarine sites are situated in Lake Grevelingen (The Netherlands): Vliegertje and Geul. These sites were sampled three times: in February 1999 microelectrode and porewater profiles were measured, while cores taken in April 1999 were used for extractions and the kinetic approach as well as those taken in June 2000. The deep-sea sites are located in the Eastern Mediterranean Sea: Florence Rise and Eratosthenes Seamount. Samples were taken by box coring and multi-coring during a cruise with R.V. Logachev in August-September 1999.

2.3.2. Suspended particulate matter

At the intertidal station Appels, sediment suspended and deposited during high tide periods was collected with cylindrical traps dug in the sediment. This material includes the bed load and will therefore be more similar to the sediment than if only suspended particulate matter would have been collected. For sake of simplicity we call this fraction suspended particulate matter. Sediment trapped in cores higher on the flat are presented as −0.75 cm, while the sediment trapped in cores situated in the lower flat are at −0.25 cm in the depth profiles. In Lake Grevelingen suspended particulate matter was collected in July 2000, on Teflon sheets by continuous flow centrifugation (see Middelburg and Nieuwenhuize 1998). The material was divided in size / density fractions that were separated by position on the collection sheet. For our analyses we used three operationally defined fractions, a light fraction which is composed of fine small sized particles, an intermediate

fraction and a heavy fraction which is composed of coarse more sandy material (Vliegertje only middle and light). Differences among the size / density fractions (3 in Geul, 2 in Vliegertje) may give additional qualitative information (e.g. Middelburg and Nieuwenhuize 1998). Hereafter we will refer to the different fractions as fine, middle and coarse for the light, intermediate and heavy fraction respectively.

Table 1. Characteristics and co-ordinates of sample sites.

	Co-ordinates	Depth (m)	Salinity	BET-N2 specific surface area $(m^2 g^{-1})$	Median grain size (μm)	Organic carbon (wt %)	Organic carbon (wt %)
Appels	51.02.43 N 04.05.37 E	0	0.7	4.8	63.5	2.29	2.48 [a]
Vliegertje	51.45.78 N 03.58.47 E	3	28	2.1-0.9	334	0.18	10.5 [a], 12.3 [a]
Geul	51.44.22 N 03.58.13 E	13.5	28	1.4-1.1	210	0.31	6.9 [a], 7.7 [a], 2.9 [a]
Florence Rise	34.47.17 N 31.31.38 E	2300	38	25-40	Na	0.4	2.5 [b]
Eratosthenes Seamount	33.45.04 N 32.50.41 E	970	38	Na	Na	0.27	1.3 [b]

[a] Organic carbon content of suspended particulate matter. At the Appels site this is the sediment collected in the sediment traps. Values at Vliegertje are for the fine and middle fraction respectively, at Geul values are for the fine, middle and coarse fraction.
[b] Organic carbon content of the sapropel S1.
Na = not available

2.3.3.   Sediment characteristics

Total organic carbon was determined on freeze-dried and powdered sediment. Samples were combusted in a Carlo Erba Elemental Analyser NA-1500 after removal of carbonate with HCl in silver cups (Nieuwenhuize et al. 1994). Sediment specific surface area was measured on a surface area analyser (Quantachroom nova 3000 series) according to BET multipoint theory and using N_2 5.0 as adsorbent. Sediment grainsize spectrum was determined using a Malvern Particle sizer 3600-EC with laser diffraction.

2.3.4. Sampling & slicing

Sediments were (sub)sampled with Plexiglas cores. After sampling cores were cut in appropriate slices (0.25 - 4 cm thickness). These were put in gastight plastic bags, flushed with nitrogen, sealed and kept at 4ºC until further handling, i.e. porewater collection or solid phase extraction. Parallel samples were freeze dried for determination of water content and for use in total destruction. Porosity was calculated from water content assuming a sediment dry density of 2.5 g cm^{-3}.

Concentrations of dissolved Fe (II) and dissolved Mn (II) were measured in porewater collected from the sliced profiles. To this end the bags were transferred into an anaerobic chamber (Coy Laboratory Products) filled with > 97 % N_2 and < 3% H_2. Sediment of Appels was transferred to tubes and centrifuged (for 15-30 minutes at ~ 355 G). A modification of the system for sandy sediments (Saager et al. 1990) was used for porewater collection in Vliegertje (15 min, at 355 G), these samples were taken February 1999. No porewater was collected in Geul sediments. After centrifugation, porewater was filtered over a 0.45 µm cellulose-acetate filter and a 2 ml portion was put in headspace vials, capped and acidified with 0.1 ml 20 % H_2SO_4.

Porewater collection of the Mediterranean sediments was as follows. Subcores were sliced at in-situ temperature (13°C) in a nitrogen-filled glovebox immediately after collection. Porewater was collected by centrifugation of sediment in disposable polypropylene tubes (15 min., 4400 G) and subsequent filtration of the overlying water (Acrodisc polysulfone filters, 0.2 µm). All pore water samples were split into several portions under nitrogen. Sub-samples for Fe and Mn analyses were acidified to pH 1 (with 12 M HCl).

2.3.5. Solid-phase extractions

A number of extractions were used to characterise the pools of iron oxides. Ascorbate extractions were performed in an anaerobic chamber (Coy Laboratory Products) filled with > 97 % N_2 and < 3 % H_2.

The pool of easy reducible Fe (III) oxides (Fe$_{asc}$) was extracted using ~ 0.3 g wet sediment and 5 ml of an anaerobic solution of 10 g sodium citrate, 10 g sodium bicarbonate and 4 g ascorbic acid in 200 ml demineralised water (Kostka and Luther 1994). The extraction lasted about 24 hrs and the samples were continuously shaken in the dark. After filtration (0.45 µm cellulose acetate filter) iron concentrations were analysed in the filtrate. The amorphous iron extracted with this method is considered the most reactive part of the total sediment iron pool.

Dithionite extractable iron (Fe$_{dith}$) is known to comprise amorphous iron (III) oxides, crystalline iron (III) oxides and the pool of iron bound to acid volatile sulfides (FeS) (Canfield 1989). Leachant (5 ml, 0.35 M acetic acid/0.2M Na-citrate solution 50 g l^{-1} dithionite) was added to ca. 0.3 gwet sediment. After 2 hours shaking, samples were filtered over a 0.45 µm filter (cellulose-acetate) and the filtrate was acidified with 0.1 ml 6 N HCl / ml filtrate.

The pool of total sediment iron (Fe$_{total}$) was measured in freeze-dried and powdered sediment. The method used HNO$_3$/HCl as destructant, a microwave for high temperature and a pressure digester (Nieuwenhuize et al. 1991).

2.3.6.   Analyses

The ferrozine method (Stookey 1970) was used to determine iron concentrations in ascorbic acid, dithionite and kinetic extractions of Vliegertje 1999 and Geul 1999. In all other samples, Fe, Mn and Al was analysed with an Inductively Coupled Plasma – Optical Electron Spectrometry (ICP-OES) (Perkin-Elmer Optima 330 DV, using axial or radial views depending on concentration levels). Diluted pore water samples (10x) of Mediterranean sediments were analysed for total Fe and Mn in Utrecht using a Perkin Elmer 4100 ZL Zeeman AAS. All samples were analysed in triplicate. Precision was generally better than 5% for Fe and 4% for Mn (Slomp et al. 2002).

Pore water O$_2$ and HS$^-$ were measured simultaneously with a gold amalgam voltametric micro-electrode (Brendel and Luther 1995) inserted in whole cores taken from the Mediterranean Sea and from Lake Grevelingen in February 1999. An elaborate description of this method can be found in Brendel and Luther 1995 and Luther et al. 1998.

2.3.7.   Kinetic approach

All kinetic experiments were also conducted in the anaerobic chamber. A 20 mM anaerobic ascorbate solution (250 ml) was used and stirred continuously. Before and directly after sediment addition a 4 ml slurry sample was taken with a syringe and filtered (0.45 µm filter cellulose-acetate). More than 25 samples were taken during each kinetic experiment lasting for about 4.5 hours, with 5 minute intervals in the first hour and up to 30 minutes during the last hour. Iron concentration was measured by either the ferrozine method or ICP-OES (see: Analyses). Postma 1993 showed that there should be excess ascorbate in order to ensure that all sorption sites of iron oxides are occupied, so slight differences in ascorbate concentration or sample weight are not expected to alter results. The total mass (*m*0) of reactive

iron oxides was determined by dithionite extraction of the same sediment samples. The time evolution, during this extraction, of dissolved iron, normalised over the total mass of reactive iron oxides, is the basis of the reactive continuum approach (see Fig. 1 for an example).

The interpretation of the kinetic experimental data involves fitting the time evolution of the solid phase iron fraction ($m/m0$) with eq. (1)

$$\frac{m}{m0} = \left(\frac{a}{a+t} \right)^{v} \tag{1}$$

Where m is the concentration of reactive iron (mol) at time t, $m0$ is the initial concentration of reactive iron (mol) and a and v are curve fitting parameters that have been derived by non-linear regression using STATISTICA.
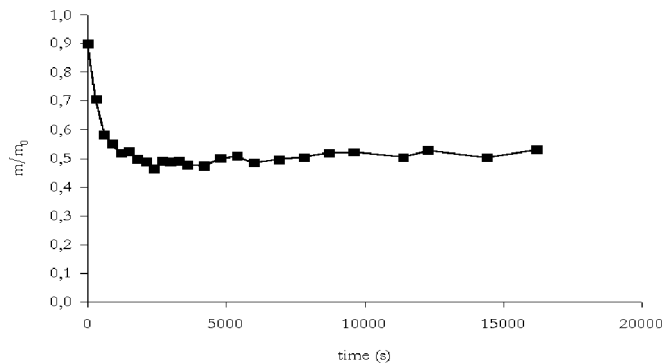

Fig. 1: Time evolution of reactive iron disslution in an estuarine surface sediment (Vliegertje, 0-0.25 cm depth)) during the ascorbate extraction used in the reactive continuum approach. $m/m0$ is the mass of solid phase reactive iron oxides, normalised over the initial pool of solid phase reactive iron oxides, that is left at time, t (seconds) of the extraction period.

Equation 1 is based on a Gamma type reactive continuum distribution (Boudreau and Ruddick 1991) and can be rearranged (Postma 1993) to derive an expression for the rate of iron-oxide reduction (J, mol s^{-1}) normalised to the initial concentration of reactive iron, eq.(2).

$$\frac{J}{m0} = \frac{v}{a}\left(\frac{m}{m0}\right)^{1+\frac{1}{v}} \qquad (2)$$

Where the exponent $(1 + 1/v)$ provides a measure of the degree of heterogeneity of the oxides undergoing reduction and (v/a) is an apparent rate constant for a mixture. Eq. 2 is very similar to the general rate expression for dissolution of minerals under constant solution composition (Christoffersen and Christoffersen 1976), eq. 3.

$$\frac{J}{m0} = k' \cdot \left(\frac{m}{m0}\right)^{y} \qquad (3)$$

Where k' is a pseudo initial rate constant and y is an exponent.

Rate equations based on eq. 2 or 3 can be displayed in terms of $-\log\left(J/m0\right)$ vs. $-\log\left(m/m0\right)$. Such plots will result in straight lines with the slope corresponding to $(1 + 1/v)$ or its equivalent y, and the intercept at $-\log\left(m/m0\right)$ equals the initial rate constant (v/a) or its equivalent k'. This type of plots (Fig. 2) facilitates comparison among sediments (Fig. 2a) and between sediments and pure minerals (Fig. 2b).

2.4. Results

2.4.1. Sediment properties

Sediment characteristics are summarised in Table 1. Appels is a tidal freshwater site flooded twice a day, with fine sediments. The high organic carbon content is due to the high organic carbon input by benthic algae and by particulate organic carbon supplied from the river Scheldt and from the vegetated marsh adjacent to the flat. The material collected in the sediment trap, likely reworked during flooding, had an organic carbon concentration similar to that of the top 2 cm of the sediment profile.

Both subtidal estuarine sites are located in Lake Grevelingen (The Netherlands). Vliegertje is a sandy littoral site situated on a plain. The sediment has a low organic carbon concentration. The BET-N_2 surface area was only high in the thin top layer and decreased to about 1 m^2 g^{-1} at 0.25-0.5 cm. The site is prone to erosion and little sediment accumulation occurs.
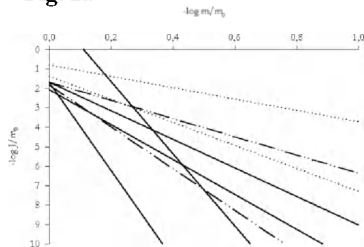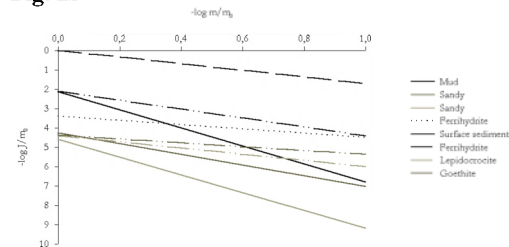
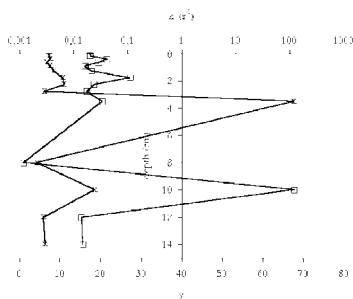## Fig. 2a

Fig. 2b

Fig. 2c-I
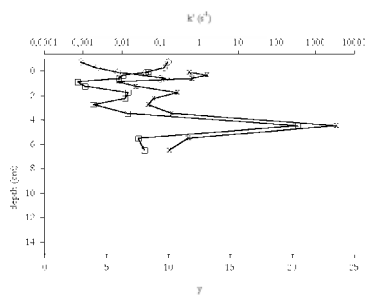

Fig. 2c-II
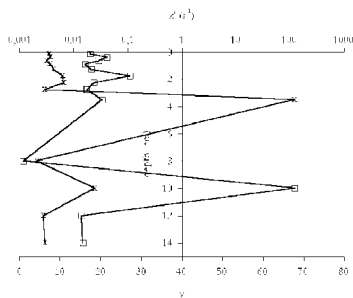

Fig. 2c-III

Fig. 2: $-\log J/m0$ vs. $-\log m/m0$, with values of the x-axis representing the fraction of reactive iron oxides which are not dissolved normalised over the initial pool of reactive iron oxides and the numbers on the y-axis indicate the change in initial reactivity of the dissoluting iron oxide fractions during the extraction. a. samples from Vliegertje (dotted lines), Geul (dashed-dotted lines), Appels (solid lines). b. literature data from Postma (1993, data from two sandy sediments, a mud sediment and ferrihydrite; all solid lines), Larsen & Postma (2001, all other pure iron oxide data; dotted and dashed-dotted lines), Roden (2002, data from a surface sediment; solid line). c. Depth profiles of k' (s^{-1}) and γ, I. Appels:. II. Vliegertje and III. Geul: data 1999: k' - open squares, γ - crosses , data 2000: k' - open circles, γ - open diamonds. Note the logarithmic scale for the k' data.

Geul is situated in a gully. The sediment grain size was significantly (p<0.05) smaller than at Vliegertje due to focussing of fine material. Organic carbon content was higher than for Vliegertje, with highest values upto 0.45 wt.% organic carbon in the upper 0.75 cm. BET-N_2 sediment surface decreased somewhat from the surface layer to 2-2.5 cm depth. Unfortunately due to material shortage, we have no BET-N_2 data for deeper samples.

The suspended particulate collected at Geul and Vliegertje had different organic carbon concentrations. Observations during collection indicated that the fine fraction consisted mostly of algae, the middle fraction contained algae and fine mud and the coarse fraction resembled the sediment.

The eastern Mediterranean sediments are hemi-pelagic muds. The Florence Rise cores contained 40 cm of sediment, and included the most recent sapropel (S1). A sapropel is an anaerobic, organic-rich layer intercalated in organic-poor sediments. Sapropels contain high amounts of relatively undegraded biogenic material that was deposited under conditions of increased surface ocean productivity and/or anoxic bottomwater (e.g. Wehausen and Brumsack 1999, Rossignol-Strick et al. 1982, Calvert et al. 1992). Sapropel S1 was formed between 6000 and 9500 BP (Mercone et al. 2000). After deposition oxygen penetrated the sediment till the top of the sapropel (De Lange et al. 1989, DeLange et al. 1989,Van Santvoort et al. 1996). The reactive surface area of these sediments is much higher than that of the coastal sediments. Organic carbon content was rather low and decreased downwards from the top-layer to 0.2 wt. %. Sapropel S1 started at ca. 30 cm until the end of the sampled profile. The position of the upper manganese oxide peak (profile not shown) and observations during slicing, indicated that a 2-3 cm thick layer of burnt down sapropel S1 (Van Santvoort et al. 1996, Mercone et al. 2000) overlies the sapropel, where the penetrating oxygen has post-depositionally reduced the sapropel organic matter.

The Eratosthenes Seamount site has similar sediment characteristics as those of Florence Rise. The largest difference between the Florence Rise and Eratosthenes Seamount sediment is the depth and thickness of the sapropel, which starts at ca. 25.5 cm in Eratosthenes Seamount and has a thickness of about 2.5 cm. Observations during slicing indicate that a 3.5 cm thick layer of burnt down sapropel S1 preceded the sapropel.

2.4.2.   Porewater and iron extraction profiles

Dissolved porewater Fe (II) concentrations (Fig. 3) of Appels are rather uniform (150-200 μM) throughout the profile, except for the top-layer (ca. 350 μM).

Appels sediment has a high concentration of Fe_{asc} (71 to 186 µmol g^{-1}). A large part of the Fe_{asc} consists of Fe (II) and Fe (III) adsorbed on the clay particles as is indicated by the large amounts of Fe (II) and Fe (III) which are released during a HCl extraction. Fe_{asc} concentrations in the material collected in the sediment trap is only slightly higher than in the profile.

Dithionite extractable iron basically showed the same pattern but with higher values (101-356 µmol g^{-1}). Total iron concentrations were about twice that of Fe_{dith} and again the shape of the Fe_{total} profile is similar to those of Fe_{dith} and Fe_{asc}. Total Fe concentration in the trapped sediment was similar to those in the bulk sediment. Comparison with a total digestion method using HF indicated that about 15 % of the iron in Appels is not extracted with the HCl/HNO$_3$ method. This 15% likely consists of silicate bound iron which is only completely liberated by using acid mixtures including HF (see Middelburg 1991).

Results of Vliegertje and Geul are presented in Fig. 4 and Fig. 5 respectively. Results for particulate matter are presented at depth above the sediment-water interface with - 0.25, - 0.5 and - 0.75 cm corresponding to the coarse, middle and fine material respectively.
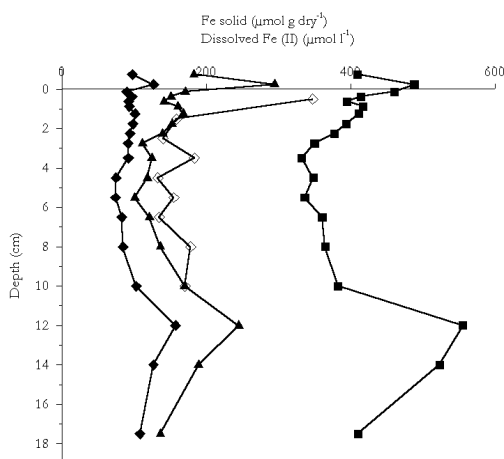

Fig. 3: Appels profiles of dissolved (µmol l^{-1}) and solid phase iron (µmol g^{-1}): dissolved Fe (II), October 1998 - open diamonds Fe_{asc} - solid diamonds, Fe_{dith} - triangles, Fe_{total} - squares
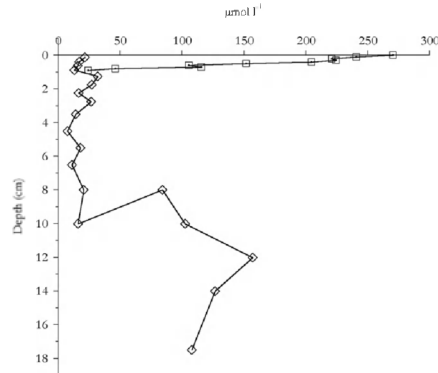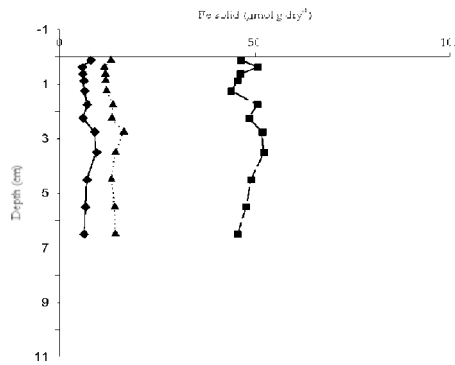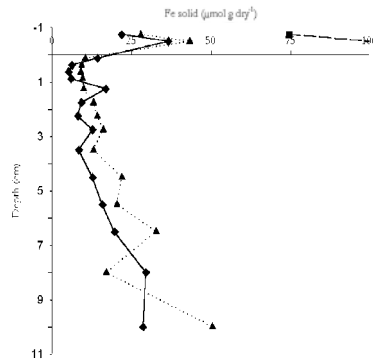
Fig 4a

Fig 4b

Fig 4c

Fig. 4. Vliegertje profiles. a. February 1999: O_2 (μmol l^{-1}) - squares, dissolved Fe (II) - open diamonds (μmol l^{-1}) b. Solid phase iron (μmol g^{-1}) April 1999: Fe$_{asc}$ - solid diamonds, Fe$_{dith}$ - triangles, Fe$_{total}$ - squares (all three: μmol g^{-1}). c. Solid phase iron (μmol g^{-1}) July 2000: Fe$_{asc}$ - solid diamonds, Fe$_{dith}$ - triangles, Fe$_{total}$ - squares

27

Dissolved Fe (II) is only available for Vliegertje and concentrations vary from 7 - 32 μM with a maximum at 1-3 cm. Vliegertje profile shows the effects of advective porewater flow, characterised by the gradual decrease in O_2 in the surface sediment. The O_2 decrease starts after 0.3 cm and O_2 penetrates till ~ 1 cm. O_2 concentrations in the Geul sediment decrease steeply within the first 0.4 cm. Hydrogen sulfide concentrations in both profiles were below the detection limit of the micro-electrode (5 μM) consistent with the presence of dissolved Fe (II) and particulate Fe-oxides.

Ascorbate, dithionite extractable and total iron profiles are rather uniform throughout the whole profile for Vliegertje and Geul. All iron oxide concentrations in Geul sediment are higher than those in Vliegertje. Moreover, at both sites iron oxide concentrations in the suspended particulate matter are much higher than those in the sediments.
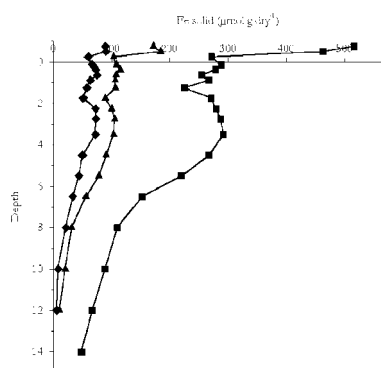
Fig. 5a   **Fig. 5b**
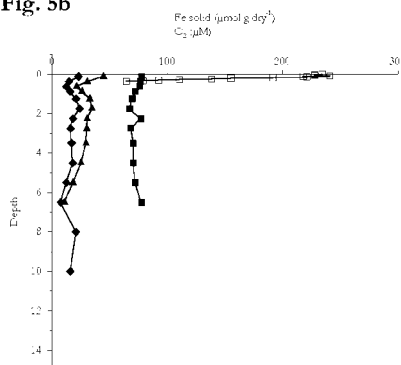
Fig. 5. Geul profiles: a. February 1999 O_2 (μmol l^{-1}) – open squares, solid phase iron (μmol g^{-1}) April 1999: Fe_{asc} - solid diamonds, Fe_{dith} - triangles, Fe_{total} – solid squares (all three: μmol g dry sediment [-1]). b. Solid phase iron (μmol g [-1]) July 2000: Fe_{asc} - solid diamonds, Fe_{dith} - triangles, Fe_{total} - squares (all three: μmol g dry sediment [-1]).

28

Fig. 6a
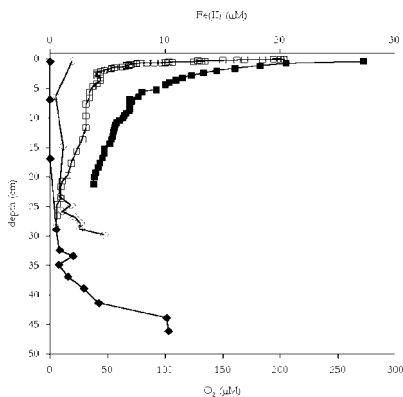
Fig. 6b.
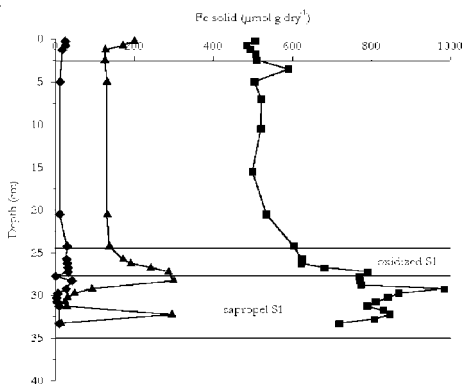
Fig. 6c
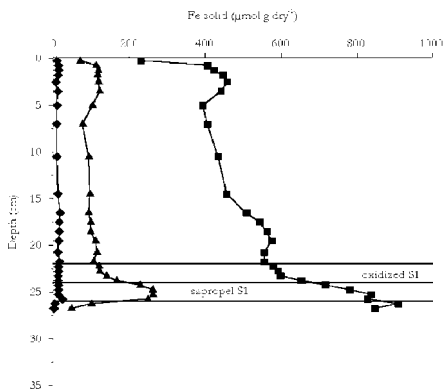
Fig. 6. Mediterranean profiles. a. O_2 (μmol l^{-1}): Florence Rise - solid squares, Erathostenes Seamount - open squares, dissolved Fe (II) (μmol l^{-1}): Florence Rise - solid diamonds, Erathostenes Seamount - open diamonds. b. Solid phase iron (μmol g^{-1}) in Florence Rise: Fe_{asc} - solid diamonds, Fe_{dith} - triangles, Fe_{total} - squares . c. Solid phase iron (μmol g^{-1}) Erathostenes Seamount: Fe_{asc} - solid diamonds, Fe_{dith} - triangles, Fe_{total} - squares.

29

Average Fe_{asc} in the sediment at the time of suspended matter collection is 13 and 51 μmol g^{-1} in Vliegertje and Geul respectively vs. suspended particulate matter values of 29 and 89 μmol g^{-1} (not shown in figure). Likewise Fe_{dith} in the sediments is 17 and 82 μmol g^{-1} and 35 and 178 μmol g^{-1} in the suspended particulate matter of Vliegertje and Geul respectively. The total iron concentrations in the suspended particulate matter are higher for Geul than in Vliegertje as well (75 and 100 μmol g^{-1} for the fine and the middle fraction in Vliegertje and 271-515 μmol g^{-1} for Geul).

In Eastern Mediterranean sediments dissolved Fe (II) is not detectable in the aerobic part of the sediments, i.e. above the sapropel. When all O_2 is depleted dissolved Fe (II) concentrations gradually increase (Fig. 6). Visible signs of oxygen depletion (i.e. the sediment turned grey) were seen at 30 cm depth in Florence Rise and at 25.5 cm depth in Eratosthenes Seamount, i.e. at the top of S1 where it is depleted due to an upward flux of reductants (Van Santvoort et al. 1996).

In the Mediterranean sediments ascorbate extractable iron concentrations are very low (< 10 μmol g^{-1} in most of the aerobic sediment and the anaerobic layers) and only increase in the iron oxide rich layer overlying the sapropel. Dithionite extractable iron concentrations in the aerobic sections of both sites were rather high (Florence Rise: 130 μmol g^{-1}; Eratosthenes 93 μmol g^{-1}) and exhibit clear peaks with nearly doubled values just above sapropel S1 layer. In the reduced sapropel, the Fe_{dith} concentration decreased rapidly to values even lower than those in the oxidized part of the profile. Total iron concentrations show a similar pattern as dithionite iron, but concentrations are higher and the sapropels are not depleted but rather enriched in total Fe.

2.4.3. Iron reactivity

In the Mediterranean sediments, reactivity has been measured in samples from 6 depth-intervals. These were selected based on the characteristic sediment layering in the cores. Mediterranean sediments contain almost no ascorbate extractable iron-oxides (Fig. 6) and the amount of iron dissolved during the kinetic experiment was too small to fit the data to eq. (1). R^2 values of the fits were very low and not significant. A pilot experiment with dissolution times upto 7.5 days did not improve the results. Therefore, this kinetic approach can not be applied to the Mediterranean sediments due to the extremely low iron oxide reactivity.

Fig. 2 provides diagrams for a representative selection of our samples. The intercepts at $-\log\left(m/m0\right) = 0$ reflect the initial reactivities (k') and the slopes of the lines (γ) provide a measure of the heterogeneity or change in dissolution rate during the experiment. Literature data on iron oxide reactivity in other sediments and pure iron oxides are compared in fig. 2b. In general, our sediments have a

higher apparent dissolution rate and the iron-oxide distribution is more heterogeneous than the literature samples. As expected the pure iron oxides have most homogeneous reactivity distributions.

Moreover, differences within our depth profiles (Table 2) are as large as those between sediments and among sediment and suspended particulate matter and have similar ranges. However, more variation in the depth profiles of initial

Table 2 Values of initial reactivity (k') and change in dissolution rate (y) of Fe in samples used in the reactive continuum method. Asterixes indicate data on solid particulate matter in Vliegertje and Geul (both in table 2b) with - 0.25, - 0.5 and - 0.75 cm corresponding to the coarse, middle and fine material respectively. Iin table 2a asterixes indicate data on or reworked sediment of Appels, sediments from higher on the flat – 0.75 cm and lower on the flat – 0.25 cm). [b] for Grevelingen indicates samples taken in 2000.

Table 2a

Depth (cm)	Appels		
	$m0$ (Fe$_{dith}$ µmol g^{-1})	k' (10^{-2} s^{-1})	y
-0.75*	356.1	8.44 10^{-1}	8.97
-0.25*	182.5	1.89	6.67
0.125	170.8	2.06	7.36
0.375	150.9	4.16	7.57
0.625	141.6	2.88	6.70
0.875	160.9	1.62	7.63
1.25	167.7	2.12	8.51
1.75	152.6	1.10 10^{1}	10.70
2,25	139.4	2.37	11.00
2.75	111.4	1.77	6.24
3.5	124.4	3.39	67.57
8	136.4	1.19 10^{-1}	4.33
10	169.9	1.20 10^{+4}	18.56
12	244.9	1.40	5.95
14	189.4	1.49	6.33

reactivity and the distribution (Fig. 2c) is observed than for the iron oxide extractions (Fig. 3-5). The profiles of k' and y appear to be rather capricious, except for Vliegertje where some pattern seems to exist. The trapped sediment values in Appels were similar and resembled sediment values. The suspended particulate matter of Grevelingen had other values than the sediment. However, these showed no simple relation with size-fraction.

Table 2b

Depth (cm)	Vliegertje			Geul (slib -2000, sed -1999)		
	$m0$ (Fe$_{dith}$ μmol g^{-1})	k' (10^{-2} s^{-1})	y	$m0$ (Fe$_{dith}$ μmol g^{-1})	k' (10^{-2} s^{-1})	y
-0.75*	27.9	$1.56 \times 10^{+2}$	2.92	171.4	4.39×10^{-1}	4.30
-0.50*	43.3	$1.22 \times 10^{+2}$	4.21	183.9	1.19	6.28
-0.25*				103.1	6.17×10^{-1}	6.84
0.125[b]	10.5	4.08	5.93	108.4	1.19	6.28
0.625[b]	9.0	9.64	9.76	107.7	1.02	5.70
0.125	13.1	4.74	11.68	44.8	3.86	13.29
0.375	11.4	1.07	13.10	30.7	2.89	13.48
0.625	11.6	8.25×10^{-1}	11.88	21.4	4.36	11.78
0.875	11.7	7.35×10^{-2}	5.94	26.0	2.06	9.29
1.25	12	1.13×10^{-1}	7.37	32.8	2.99	13.28
1.75	13.6	1.47	10.74	34.6	2.03	22.65
2,25	13.3	1.21	8.80	30.4	4.16×10^{-1}	9.12
2.75	16.4	1.84×10^{-1}	8.36	30.2	2.30	10.80
3.5	14.3	1.40	10.27	29.6	1.70	11.80
4.5	13.2	$3.50 \times 10^{+4}$	23.52	25.1	4.79	13.12
5.5	14.1	2.69	11.60	18.2	1.78	9.99
6.5	14.3	3.85	10.07	10.8	1.10	11.14

2.4.4. Mn reactivity

Analyses of samples from the kinetic experiment by ICP-OES allowed parallel measurement of Mn in the kinetic experiments (see Materials & Methods which experiments are analysed with ICP-OES). Therefore we calculated Mn-oxide initial reactivity and reactivity distribution, using the same method as for iron oxide, in a selection of these samples (Table 3).

In most Mediterranean sediments Mn reactivity was too low to provide reasonable fits to eq.1, except for the Mn-oxide rich layer just above the sapropel. The reactivity distribution of Mn-oxide in this layer was very homogeneous ($y = 1.0$) and its initial reactivity ($k' = 8.1 \ 10^{-3}$) was in the range of values reported for North Sea sediments by Van der Zee 2002 ($k' = 3.3 \ 10^{-3}$ to $8.9 \ 10^{-1}$).

As for iron, the initial reactivity of Mn-oxides in suspended particulate matter in Grevelingen is higher and the reactivity distribution is more heterogeneous than in the sediment. At Appels, the reactivity distribution of Mn oxides tends to be more homogeneous than for the iron, though this was not a general trend. Initial reactivity of Mn covers a wide range within and between the sediments ($k' = 1.42 \ 10^{-4}$ to $2.69 \ 10^{2}$, Table 3) and are comparable to those of Van der Zee 2002 for North Sea sediments ($k' = 3.3 \ 10^{-3}$ to $8.9 \ 10^{-1}$). Van der Zee 2002 defined $m0$ by using the ascorbate extractable Mn-oxides whereas we used dithionite extractable Mn for the $m0$ definition. However in the North Sea sediments Mn_{dith} concentrations are also most similar to dithionite extractable manganese oxides, therefore results may be compared. Moreover, they found y values from 1.3 to 8.9 consistent with our observations ($y = 1.1$ to 7.8, Table 3).

Table 3 Values of initial reactivity (k') and change in dissolution rate (y) of Mn in samples used in the reactive continuum method.

Site	Depth (cm)	Mn		
		$m0$ (Mn dith μmol g⁻¹)	k' (s⁻¹)	y
Florence Rise	27.75	149.4	8.11 E-3	1.00
Vliegertje	fine	34.4	1.51 E2	1.07
	medium	44.0	2.22 E 2	6.39
other $m0$	0.125	1.6	2.05 E-3	1.20
	0.625	1	1.42 E-4	1.37 E1
Geul	fine	15.4	3.81 E-2	4.28
	coarse	10.9	6.09	1.57
	0.125	3.6	4.01 E-2	5.70
	0.625	4.0	5.04 E-3	3.91
Appels	-0.75	16.4	2.73 E-2	6.44
	-0.25	29.2	2.69 E2	7.09
	0.125	15.1	1.40 E-2	2.88
	2.25	11.6	4.17 E-1	7.75
	12	18.7	3.12 E-3	1.10

2.5.   Discussion

2.5.1.   Sediment texture and sediment iron pools

Iron-oxide pool sizes are generally linked to sediment particle size (Munch and Ottow 1980). Small sized particles, like clay, have a larger specific surface area and therefore a higher capability to adsorb iron oxides and to support nucleation on their surfaces (Ernstsen et al. 1998). This results in a large iron oxide pool. Furthermore, iron- rich layers with high concentrations of precipitated iron-oxides have smaller particle sizes and larger surface areas than the average sediment due to the large surface area of solid phase iron oxides themselves (e.g. goethite: 153 m⁻² g⁻¹, 2-line Ferrihydrite: 230 m² g⁻¹ (Larsen and Postma 2001), amorphous Fe (III) 600 m² g⁻¹ (Schwertmann and Cornell 1991 in: Roden and Zachara 1996). This effect can be observed in the diagenetically formed iron oxide rich sediment layers just above the sapropel in the Mediterranean sediments (Van Santvoort et al. 1996)

where the sediment surface area (40 m² g⁻¹) is higher than in the overlying Fe-poor sediment (~ 30 m² g⁻¹).

The effect of grainsize on iron oxide pools is also evident when comparing sediments. The fine grained Mediterranean sediments have highest total iron concentrations, whereas the sandy Vliegertje sediments have the lowest values with Appels and Geul data in between. However, Fe_{asc} and Fe_{dith} values are not only affected by grainsize. These pools are also affected by sediment diagenesis. Dithionite and ascorbate extractable iron of Mediterranean sediments, are lower than could have been expected based on their sediment surface area. This is likely due to the aged character of the iron oxides caused by the long duration of steady aerobic conditions and a low input of fresh material (Haese et al. 2000, Haese et al. 1998). The iron-oxide concentrations in Vliegertje (medium grain size 334 μm) and Geul (medium grain size 210 μm) do reflect the effect of grain size, with highest Fe-oxide values in Geul.

The effect of particle size may also account for differences in iron oxide concentrations between suspended particulate matter and sediments. Extractable iron-oxide concentrations are higher in the suspended particulate matter of Vliegertje and Geul than in the sediment. Moreover, in Geul the extractable iron concentrations are higher in the fine than in the coarse fraction of suspended particulate matter. Concentrations in the suspended Appels sediment are higher than within the profile, because smaller particles are more easily resuspended than large particles.

2.5.2.   Reactivity

Initial reactivity and reactivity distributions are highly different in the depth profiles. In Vliegertje both measures have a similar pattern, samples with a high initial reactivity also have a high heterogeneity. This may indicate a source of fresh iron oxides. The general increase in discerned differences in comparison with traditional extractions reflects the potential of the method to detect differences in reactivity within iron oxide pools. Moreover high initial reactivity may indicate the presence of more amorphous iron oxides. As these oxides are more recently formed, the high initial reactivity may indicate (diagenetic) iron oxide formation.

Iron oxide reactivity (k') and heterogeneity of reactivity distribution (y) are not related in a simple way (Fig. 7). This is not surprising as a homogeneous reactivity distribution may either result from an aged sediment where the most reactive iron oxides have been used, leaving a homogeneous pool of iron oxides with low reactivity, or from diagenetic formation of iron oxides, without subsequent mixing, resulting in a homogeneous pool of iron oxides with high reactivity. Most of our samples (Fig. 2a) have higher initial reactivities than the pure iron oxides (Fig. 2b) studied by Postma 1993 and Larsen and Postma 2001. They found that crystal structure governs reactivity of iron oxides rather than crystal surface area. Initial rates of dissolution are higher for less crystalline structures. Initial reactivities found

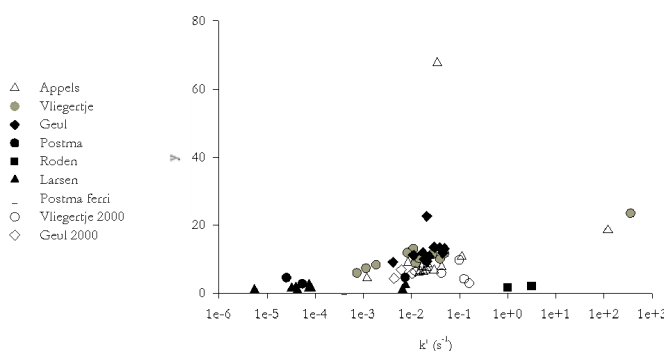**Fig. 7.** y vs. k' (s^{-1}) for Vliegertje, Geul, Appels and literature data on sediments and pure iron oxides: Postma - data from two sandy sediments, a mud sediment (Postma 1993), Postma ferri – data on ferrihydrite (Postma 1993), Larsen – data on ferrihydrite, lepidocrocite and goethite (Larsen and Postma 2001), Roden - data from a surface sediment (Roden and Wetzel 2002)

by Larsen and Postma 2001 varied between 5.4 x 10^{-6} to 7.3 x 10^{-3} s^{-1} for various iron oxides (Fig. 2b), below and at the lower end of our results for natural sediments (1.1 x 10^{-3} to 3.5 x 10^{2} s^{-1}, Fig. 2a). Consistently, Postma 1993 and Roden and Wetzel 2002 also observed a range of initial reactivities (5.3 x 10^{-5} to 3.1 10^{0} s^{-1}) including high values in natural sediments (see Fig.2b)

The range in the change of dissolution rate (γ) over time (indicating the distribution of iron oxide reactivity) is smaller than the range in initial reduction rate (Table 2). Homogeneous pure oxides have γ values approaching 1. Sediment values of Roden and Wetzel 2002 and Postma 1993 range between 1.7 for a freshwater wetland surface sediment to 4.6 in muddy surface sediment of Aarhus bay. This latter value is within the range found for our sediment samples (2.9 – 13.3, excluding outliners). The heterogeneous nature of our samples and the high initial reactivity indicates a mixture of aged and fresh iron oxides. Postma 1993 suggested that the kinetic characteristics of iron oxides would be site specific, which is not supported by our findings (Fig. 7). The range of initial reactivity and reactivity distribution within sediments is very large and sites do not show characteristic values.

Reactivity distributions might reflect the dynamics of sedimentary iron reduction. Van der Zee 2002 attributed the heterogeneity of Mn-oxide reactivity to physical perturbations in the sediment. The dynamic subtidal Vliegertje and Geul sediments have slightly more heterogeneous iron oxides than the Appels sediments. Especially the coarse grained Vliegertje sediment is affected by waves, consistent with the observed heterogeneity.

There appears to be no relation between the amorphous iron-oxide pool (Fe_{asc}) size and initial reactivity (Fig. 8a) or reactivity distribution (Fig 8b). Likewise there appears to be no relation with the total reactive iron oxide pool (i.e. dithionite extractable iron oxides). Thus reactivity based on the kinetic approach is not affected by nor related to pool size. However, a minimal amount of reactive iron oxide should be present to provide any results at all. Otherwise v and a, and thus the initial reactivity ($k = \dfrac{v}{a}$) and reactivity distribution ($\gamma = 1 + 1/v$), can not be derived, like in the Mediterranean samples. The results of the reactivity distribution (γ) are affected by the definition of $m0$ which is used to normalise the dissolution rate ($J/m0$, see eq. (2) and (3)). The method to define $m0$ should therefore be similar in order to compare results. Roden and Wetzel 2002 used a 0.5 M HCl extraction for the total amount of reactive iron oxides. This extraction liberates amorphous iron oxides and AVS, and results in a smaller $m0$ than the dithionite extraction, used here and by Postma 1993. The change in $m(t)/m0$ values over time also depends on the value and definition of $m0$. Using a smaller $m0$ will result in a

more homogeneous reactivity distribution, though initial reactivity values will remain rather similar. Applying HCl extractable iron for $m0$ in Appels sediments resulted in a y value approaching 1, consistent with y values of 2.0 and 1.7 as found by Roden and Wetzel 2002.

Initial reactivity and iron oxide reactivity distributions are not related to sediment surface area (Fig. 9). The lack of a sediment texture effect allows a cross system comparison of initial reactivity and reactivity distribution, which can not be made with the traditional iron oxide extractions as the latter are affected by the reactive surfaces of the sediment particles. For instance, the kinetic characteristics of Grevelingen and Appels sediments are in a similar range whereas absolute iron surface area. Larsen and Postma 2001 investigated the relation between iron oxide
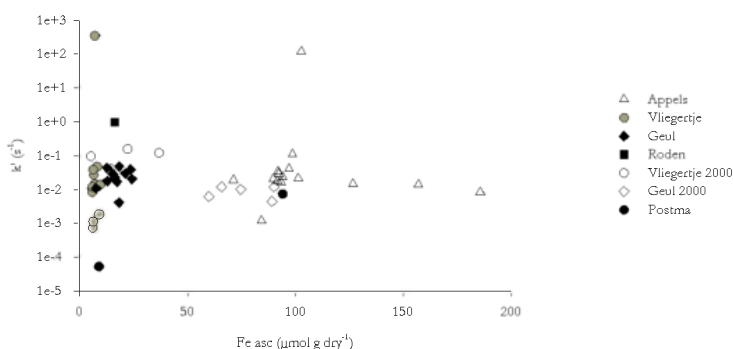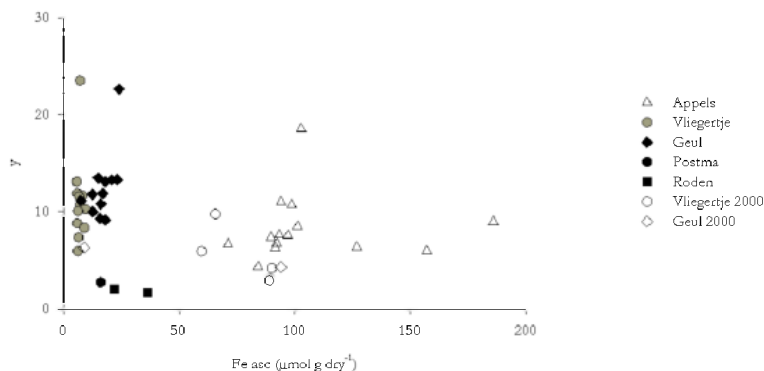
Fig. 8a

Fig 8b.

Fig. 8. a. k' (s^{-1}) vs. ascorbate extractable iron oxides (μmol g^{-1}), b. y vs. ascorbate extractable iron oxides (μmol g^{-1})

reactivity and specific surface area of pure iron oxides . They found no relation with specific surface area when investigating different types of iron oxides. It will be clear that the reactive continuum approach can be applied to quantify and compare iron oxide reactivity. However, the reactive continuum approach does not provide a simple well-defined view of the sedimentary reactive iron oxide pool because the two separate terms, initial reactivity and reactivity distribution, are necessary to indicate the pool characteristics. One term comprising both values will be more useful. However, the results of the reactive continuum approach should be combined with results from traditional extractions indicating absolute pool sizes in order to include the aspect of absolute pool size in sediment reactivity and iron oxide availability for (microbial) iron oxide reduction.

2.6. Conclusions

Iron oxide extractions have been shown to be useful in determining large differences in the composition of the sedimentary iron oxide pool between sediments. However small changes within the profile can not be discerned. Besides, results are affected by grain size and relative pool sizes and do not directly represent iron oxide reactivity. Results of a reactive continuum approach (Postma 1993) are independent of pool distribution and grain size and the approach allows a quantification of iron oxide reactivity in sediments. However, a minimal pool of
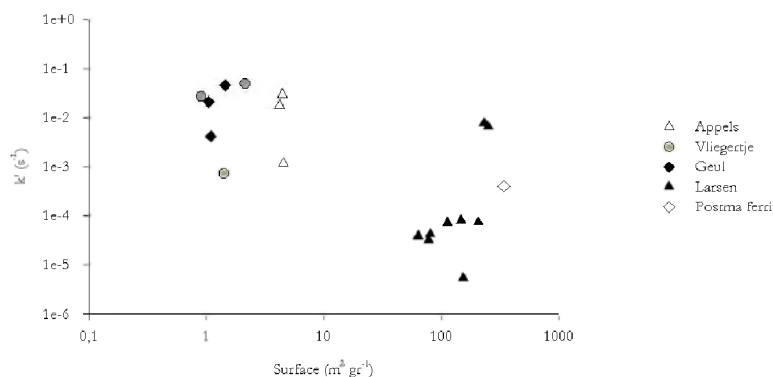
Fig. 9 k' (s^{-1}) vs. sediment surface area (m^2 g^{-1}). Data from Appels (open triangles), Vliegertje (closed circles), Geul (closed diamonds) and literature: Postma ferri (open diamonds) – data on ferrihydrite (Postma 1993), Larsen (closed triangles) – data on ferrihydrite, lepidocrocite and goethite (Larsen and Postma 2001).

reactive iron oxides should be present in the sediment to allow a reliable estimation of k', initial reactivity, and γ, distribution of initial reactivity. Therefore the method can not be applied to sediments which are poor in reactive iron oxides, e.g. Mediterranean Sea sediments. In sediments, with a sufficient pool size, the kinetic approach is a useful tool revealing relatively small differences in iron oxide composition and can therefore be used to discern sediment layers were freshly precipitated iron oxides are present. Even if profiles of sedimentary iron oxide composition are rather homogeneous, like found in a freshwater and an estuarine sediment, depth profiles of k' and γ appear to be very heterogeneous.

2.7. Acknowledgements

3. Factors limiting iron oxide reduction in a tidal freshwater and a Mediterranean deep sea sediment

Imola Ferro and Jack J. Middelburg

3.1. Abstract

The role of an oxidant in the degradation of organic matter is a function of the organic carbon and oxidant availability. Much knowledge is available for the dissolved oxidants (O_2, NO_3, SO_4 and CH_4). We describe results of a set of slurry experiments, with different additions of reactive iron oxides and labile organic carbon, to study the factors limiting iron-oxide reduction in tidal freshwater, estuarine and deep-sea sediments. In the freshwater sediments iron oxide reduction was limited by the availability of reactive iron oxides. Addition of organic carbon enhanced methanogenesis that was inhibited when iron oxides were added as well. The estuarine and deep-sea sediments were primarily limited by organic carbon. Maximum rates of iron oxide reduction (V_{max}) were between 2.3 and 5.6 x 10^{-5} mol m^{-3} sec^{-1} in freshwater slurries, irrespective of organic carbon loads. V_{max} in deep-sea slurries increased with higher organic carbon loads reaching about 3.8 x10^{-5} (mol m^{-3} sec^{-1}).

3.2. Introduction

Mineralization of sedimentary organic carbon involves a number of electron acceptors. After depletion of oxygen, micro-organisms utilise other electron acceptors including NO_3, Mn-oxides, Fe-oxides and sulphate and in their absence methanogenesis will dominate. In the past decades denitrification, sulphate reduction and methanogenesis have intensively been studied. More recently it has been shown that under specific conditions iron and manganese oxide reduction can be as important as one of the other anaerobic degradation pathways (Canfield et al. 1993b, Canfield et al. 1993a, Thamdrup and Canfield 1996).

Oxygen, nitrate, sulfate and methane are dissolved in the porewater and (molecular) diffusion is the dominant transport mode, whereas iron and manganese oxides are in a particulate form with the consequence that transport is impeded. Collection and analyses of porewater is relatively easy and the dissolved constituents have therefore been studied in detail (Jorgensen 1977, Revsbech et al. 1980, Sorensen 1987, Bakker and Helder 1993, Van der Nat and Middelburg 1998, Sansone et al. 1998). This resulted in a lot of knowledge about the factors

influencing rates of aerobic degradation, NO_3 and SO_4 reduction and methanogenesis in sediments. It has been found that i) the oxidants are used sequentially (Froelich et al. 1978) and ii) that the importance of a certain dissolved oxidant in total mineralization is a function of organic carbon and the oxidant (Canfield 1994, Wijsman et al. 2002).

The solid phase oxidants, Fe and Mn oxides, are more complicated to study, and they therefore have been studied much less than the dissolved oxidants. In this study we focus on the role of iron oxide as an oxidant in organic matter mineralization.

The particulate nature of iron oxides poses a number of complications. One, the details of iron oxide reduction have not been elucidated. Research is still going on to determine how important electron shuttling e.g. with humic substances (Lovley and Blunt-Harris 1999; King and Garey 1999, Nevin and Lovley 2000) and direct bacteria - oxide contact are for iron oxide reduction. Two, the nature, reactivity and availability of iron oxides varies considerably among sediments (Canfield 1989, Postma 1993), and there is no simple or straightforward method to measure the pool of available iron oxide. One therefore has to revert to operationally defined extractable pools (Lovley and Phillips 1987, Canfield 1989, Phillips et al. 1993, Kostka and Luther 1994). Three, measurement of iron oxide reduction rates is rather problematic. The size of the extractable iron-oxide pool is usually very large compared to the amount of iron reduced during incubations. Thus any changes in this pool are too small to detect. Another option is to measure the production of dissolved Fe (II) rather than the consumption of Fe (III) oxides. However a large amount of reduced iron may be adsorbed on clay particles, cell surfaces or iron oxides (Roden and Zachara 1996, Kostka et al. 1999a). Moreover precipitation reactions with carbonates and sulfides (Berner 1984, King and Farlow 2000) or re-oxidation and subsequent Fe-oxide precipitation (Postma 1985, Widdel et al. 1993, Murase and Kimura 1997, Postma and Appelo 2000) result in lower concentrations of Fe (II) as well. Finally, sedimentary iron reduction is not exclusively coupled to carbon oxidation, but may also result from chemical reduction, e.g. iron oxide reduction by dissolved sulfide (Pyzik and Sommer 1981, Murase and Kimura 1997).

Reduction rates of Fe-oxide have been reported for various sediments, e.g. marine sediments (Canfield et al. 1993b, Slomp et al. 1997, Thamdrup and Canfield 1996), freshwater sediments (Roden and Wetzel 1996), salt marshes (Kostka and Luther 1994, Luther et al. 1992), rice fields (Achtnich et al. 1995, Kruger et al. 2001) and landfills (Albrechtsen et al. 1995;Kennedy and Everett 2001). These studies revealed that there are a large number of factors governing the relative importance

of iron oxide reduction and its interaction with other pathways. The relation between the importance of iron reduction in mineralization and the flux of organic carbon to the sediment has been modelled by Wijsman et al. 2002. They have shown that iron oxide reduction contributes most to total mineralization at intermediate carbon loading and that different factors may control iron oxide reduction in different environments. It is difficult to elucidate the limiting factors for the iron oxide reduction in natural sediments, as interactions of controlling factors can not be controlled. Thereby, to identify the relation between iron oxide reduction and a controlling factor, one should use sediments differing in only one aspect. Because of co-variation of most biogeochemical processes this is impossible in natural sediments.

This research aims to investigate the optimal conditions for iron oxide reduction in sediments that differ in organic carbon and iron oxide concentrations. We have studied mineralization pathways in slurry incubations with addition of different amounts of Fe-oxides and organic carbon. Our results indicate that the controlling factors depend on the initial availability of iron oxide and organic carbon.

3.3.   Material and Methods

3.3.1.   Site description

Appels is a freshwater tidal sediment along the Scheldt river (Belgium) which is flooded twice a day (i.e. every tide), salinity is about 0.7. The sediment consists of silt (sediment specific surface area is 4.38 m^2 g^{-1}, medium grain size $\sim$ 63 μm). The sediment is rich in organic matter (2.13 wt. % org C (4.8 mg C m^{-2}), 0.19 wt. % N-total with a molar C/N-ratio of 12.9). This is due the particulate organic carbon supply from the river Scheldt, in situ benthic algal production and the high organic carbon input from the vegetated marsh adjacent to the flat. Samples were taken with Plexiglas cores (id. 10 cm). For the slurries 10-cm top-layer samples were pooled and thoroughly mixed.

Eastern Mediterranean sediment was collected by box-coring during the Smilable cruise with R.V. Logachev in 1999. Care has been taken not to collect sediment of distinctive layers like the manganese marker-bed, the sapropelic layer S1 or the anaerobic sediment beneath S1 (De Lange et al. 1989). Organic carbon degradation is very low (0.45 g. C m^{-2} yr^{-1}) (Moodley, personal communication) and organic carbon content is 0.27 wt. % (0.1 mg C m^{-2}). The eastern Mediterranean sediments are hemi-pelagic muds with a high (BET-N_2) specific surface area (31.2 m^2 g^{-1}) and salinity is 38. Samples were thoroughly mixed and then sealed in a N_2 atmosphere and stored at 4 °C until use.

The Grevelingen site is situated in Lake Grevelingen (The Netherlands), a constructed salt-water lake. It is a submerged site (51.45.78 N; 3.58.47 E, 3m) with a salinity of 28. The sediment contains 0.07-0.2 wt % organic carbon (~ 0.9 mg C m^{-2}). Median grain size was 334 µm, the (BET - N$_2$) specific average reactive surface area was 1.46 m^2 g^{-1}. The top-layer (0-0.25 cm) of the sediment was somewhat finer grained (median grain size 178.8 µm and sediment surface area 2.1 m^2 g^{-1}). Oxygen penetration measured with a microelectrode was ~0.9 cm, but depends on waterflow conditions in these coarse permeable sands. The site is prone to erosion and little sediment accumulation occurs. The sediment from this site was used in a pilot study and the number of treatments was limited.

3.3.2.   Solid-phase extractions

Sediment for solid phase extractions and determination of water content has been taken before each set of slurries. Porosity was calculated from water content assuming a sediment dry density of 2.5 g cm^{-3}. A number of extractions was used to characterise the pools of iron oxides. Ascorbate and HCl extractions were performed in an anaerobic chamber (Coy Laboratory Products) filled with > 97 % N$_2$ and < 3 % H$_2$.

The pool of amorphous Fe (III) oxides was extracted using ~ 0.3 g. wet sediment and 5 ml of an anaerobic solution of 10 g sodium citrate, 10 g sodium bicarbonate and 4 g ascorbic acid in 200 ml demineralised water (Kostka and Luther 1994). Extraction lasted about 24 hrs and the samples were continuously shaken in the dark. After filtration (0.45 µm cellulose acetate filter) iron concentrations were analysed in the filtrate. The amorphous iron extracted with this method is considered the most reactive part of the total sediment iron pool.

The pool of dithionite extractable iron represents amorphous iron (III) oxides, crystalline iron (III) oxides and the pool of iron bound to acid volatile sulfides (FeS) and was based on Canfield 1989. Leachant (5 ml, 0.35 M acetic acid/0.2M Na-citrate solution with 50 g l^{-1} dithionite) was added to ca. 0.3 g. wet sediment. After 2 hours shaking samples were filtered over a 0.45 µm filter and the filtrate was acidified with 0.1 ml 6 N HCl / ml filtrate.

The HCl extractable pool (amorphous iron oxides and AVS) sensu Kostka and Luther 1994 was measured through addition of 10 ml 0.5 M HCl solution to ca. 0.3 g. of wet sediment taken before or after use in a slurry. After 1 hour of incubation the sediment is filtered over 0.45 µm. Fe (II) and total Fe (FeHCltot) in the filtrate are measured with the ferrozine method (Stookey 1970) using HEPES buffer (12 g l^{-1} HEPES) for Fe (II) determination or reducing HEPES (12 g l^{-1} HEPES and 20 ml $H_3NO.HCl$ 10 g 100 ml^{-1}) buffer for total Fe determination (ferrozine

reagent 5 g l^{-1}, adsorbance measured at $\lambda = 562$). HCl extraction allows determination of adsorbed Fe (II) and the reduction state of the sediment. The difference between Fe (II) content before and after the experiment provides the amount of reduced iron that has precipitated or adsorbed to the sediment during the incubation. The ratio of Fe (II)adsorbed to Fe (II)dissolved has been used to correct iron oxide reduction rates based on the production of dissolved Fe (II).

The pool of total sediment iron (Fe_{total}) was measured on a freeze-dried split of the sediment. The method used HNO_3/HCl as destructant, a microwave for high temperature and a pressure digester (Nieuwenhuize et al. 1991).

3.3.3. Slurries

Sediment slurries were made and kept in an anaerobic chamber. About 30 g of wet sediment was weighed in 100 ml flasks and diluted with 50 ml (30 ml for Grevelingen slurries) N_2 flushed bottom water and closed with septa. Slurries were kept in the dark at room temperature and continuously shaken.

To identify the functional relationship between iron oxide reduction and carbon or Fe-oxide concentrations we have made a number of experimental additions to our slurries. Carbon was added as a glucose solution and iron oxides as freshly precipitated amorphous iron oxides prepared according to the method of Lovley and Phillips 1986. Fe concentrations in the oxides were determined by dissolution in a 20% sulphuric acid solution followed by Inductively Coupled Plasma – Optical Electron Spectrometry (ICP-OES) (Fe 1034 µmol g oxide[-1], Mn 34 µmol g oxide[-1]).The added amounts of glucose were based on the total amount of carbon mineralised in 3 days in the top 10 cm of Appels sediments (rate 40 mol m^{-2} yr^{-1}, value based data from Middelburg et al. 1996a). Resulting in final volume based concentrations of organic carbon of 1.64, 8.22 and 14.79 µmol cm^{-3} for the Appels slurries with 0, 4 and 8 times additional carbon. Final concentrations of carbon in Mediterranean slurries were 0.02, 0.18 and 6.59 µmol cm^{-3} for slurries which were named respectively 0, 0.1 and 4 additional carbon. For Grevelingen slurries carbon additions were based on the wt. % organic carbon value. Iron additions in Appels and Mediterranean slurries are based on the amount of Fe_{asc} in the Appels sediment in ($Fe_{asc} \sim 65$ µmol g^{-1}). The final iron-oxide load is calculated by the amount of added iron and the concentration of Fe_{asc} at the start of the experiment. In the Grevelingen slurries iron oxide addition is based on Fe_{asc} in the Grevelingen sediment (~ 6 µmol g^{-1}). All additions were duplicated except those of the pilot study (Grevelingen).

3.3.4. Sampling

Sampling started directly after the bottles had been closed and contents well mixed. Additional samplings were made at t = 10 hrs, and t = 1, 2, and 3 days. Samples, 4 ml (3 ml for Grevelingen sediments), were taken with a needle and a syringe, keeping the bottles closed and the headspace intact. After filtering (0.45 μm) samples were split as follows. A 2 ml portion was put in headspace vials, capped and acidified with 100 μl 20% H_2SO_4. CO_2 and CH_4 were analysed in the headspace. These data were used to calculate CO_2 and CH_4 concentrations in the slurries (Van der Nat and Middelburg 1998; Dauwe et al. 2001). After gas analyses vials were opened and water was used for metal analyses (ICP-OES). The un-acidified part of the sample was used for pH measurements and stored frozen afterwards. For Mediterranean samples a third sub-sample of 0.38 ml was taken and immediately fixed with 0.02 ml zinc-acetate (20 mM) for later sulphate analyses.

3.3.5. Analyses

Gases were analysed using a Carlo-Erba gaschromatograph, type MEGA 5340-00 equipped with a Haysep-Q column, 2m x 2 mm ID, mesh size 80-100 and a flame-ionisation detector for CH_4 and a hotwire detector for CO_2.

Concentrations of Fe, Mn, Ca, Na, Al, P in acidified samples were measured by Inductively Coupled Plasma –Optical Electron Spectrometry (Perkin-Elmer Optima 330 DV, using axial and radial view depending on concentration levels)

The sulphate concentration in porewater samples was analysed by ion-chromatography with a Dionex auto-suppressed anion system (Ion Pac AS11 column with ASRS suppressor).

3.3.6. Statistics & calculations

Reduction and production rates are calculated from initial slopes of the changes in concentration with time. Average reduction rates are shown; error bars represent the st.error of the 2 replicates. Statistical significance, based on regression statistics and analysis of variance have been performed with Statistica.

3.4.   Results

The sediment characteristics have been described in Material and Methods and are presented in Table 1. Appels is a freshwater silty sediment with a high carbon loading (4.8 mg C.m^{-2}), while the Mediterranean sediment is a hemi-pelagic mud with a low carbon loading (0.1 mg C m^{-2}). The Grevelingen sediment has an intermediate carbon loading (0.9 mg C m^{-2}) and is a coarse sand with a salinity of 28.

Table. 1. Characteristics of the sediments used in the slurries. Values of Fe$_{total}$ and Mntotal in the Mediterranean sediments are estimates based on other Mediterranean sediments

Site	Fe$_{asc}$ (µmol g^{-1})	Fe$_{dith}$ (µmol g^{-1})	Fe(III)HCl (µmol g^{-1})	Fe(tot)HCl (µmol g^{-1})	Fe$_{total}$ (µmol g^{-1})	Mn$_{dith}$ (µmol g^{-1})	Mn$_{total}$ (µmol g^{-1})	Carbon (mg C m^{-2})	surfacearea (m² g^{-1})	Salinity (‰)
Appels	64.6	74.2	39.4	54.1	381.6	5.8	12.9	4.8	4.38	< 1
Mediterranean	19.7	60.9	1.4	8.0	457	7.2	20.0	0.1	31.22	38
Grevelingen	7	13.3	na	na	48.2	na	1.2	0.9	1.46	28

3.4.1.   Mineralization rates

Rates of mineralization and production of anaerobic mineralization products of Appels slurries are presented in Fig 1a - 1c. Addition of iron oxides to sediments of Appels results in a rapid and large increase of the iron oxide reduction rate in the slurries with no carbon addition (Fig. 1a). At an iron concentration of about 70 µmol g^{-1} the reduction rates show no further increase and seem to reach a plateau. Reduction rates of manganese show a similar pattern as the iron oxide reduction rates, but with much lower values. In all Appels slurries the measured dissolved Mn production could very well result from the Mn liberated during the reduction of the Mn-bearing artificial iron oxides. However, due to side reactions like adsorption on to clay particles or precipitation measured Mn (II) probably does not correspond to real Mn (II) production (see discussion). Methane production was very low.
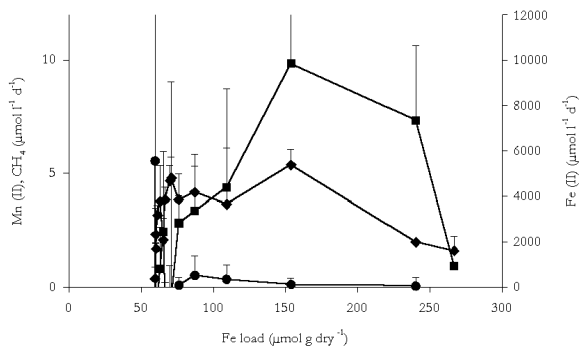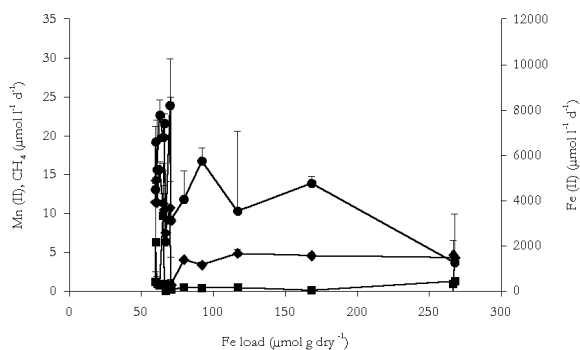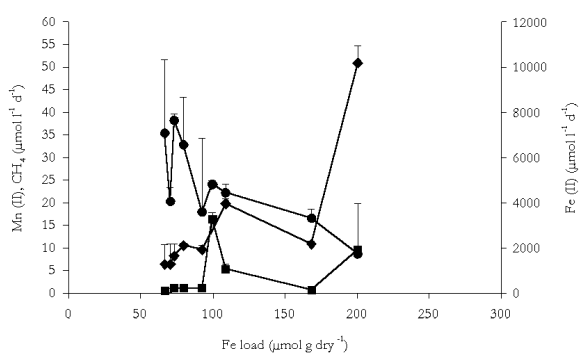
Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1: Production rates (μmol l^{-1} d^{-1}) in freshwater slurries in relation to the load of reactive iron. Left y-axis: reduced Mn (squares), CH_4 (circles), right y-axis: reduced Fe (diamonds).
a) no additional carbon, b) 4 times carbon added, c) 8 times carbon added.

With additional organic carbon the effect of iron addition is similar (Fig 1b), though details differ. For the 4C treatment some additions of iron oxide cause a large increase in iron reduction rates like in the 0C treatment. However the response curve is rather erratic, perhaps due to the fact that this data set consists of 2 sediment batches (one for 49.7 to 66.9 μmol Fe g^{-1} and one for 79.6 to 267.7 μmol Fe g^{-1}). This cause is not very likely, as control slurries (no Fe-oxide added) do not show such differences between the two batches. Moreover the site looks very homogeneous, cores are taken less than 1 m apart and we mixed the top 10 cm of several cores for each batch. Moreover none of the other analysed constituents showed this type of split response curve. Hardly any dissolved manganese is measurable. Methane production is evident and rates decrease with higher iron additions.

In the series with 8 times carbon addition (Fig. 1c) the small changes already seen between the 0 and 4 glucose treatments are more pronounced. Iron oxide reduction rates increase with higher iron addition. The plateau is at an iron oxide load of about 108 μmol g^{-1} with rates being slightly lower than for the 0 C addition. Manganese dissolution reappears in these slurries. Methane production is highest in the treatments with little iron addition and becomes suppressed at higher iron oxides loads. The inhibiting effect of iron addition on methanogenesis is most pronounced in the slurries with this carbon load.

The results of the 0 C treatment clearly indicate that in Mediterranean sediments organic carbon availability limits iron oxide reduction (Fig. 2). The slurries that were amended with a little carbon showed higher rates for Fe reduction. However
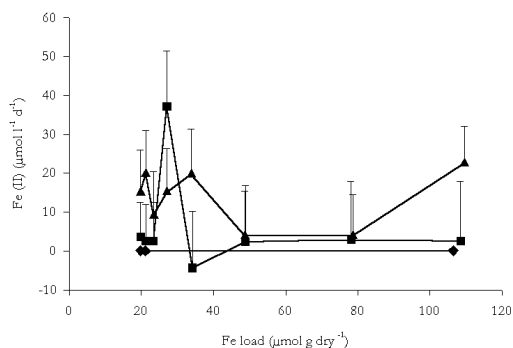

Fig. 2: Production rates of dissolved Fe (II) (μmol l^{-1} d^{-1}) in Mediterranean slurries in relation to the load of reactive iron at different additions of organic carbon. diamonds - no organic carbon added, squares - addition of 0.16 μmol cm^{-3}, triangles -addition of 6.6 μmol.cm^{-3} organic carbon.

there was a large difference between duplicates causing large error bars. This indicates that the process is only very minor. In the slurries with 4 times organic carbon the difference was significant (p <0.005) with 0 C addition (Fig 2c). Iron additions did not have any significant effect on Fe-reduction. Mn-oxide and sulfate reduction were too low to detect.

The Grevelingen slurry set is based on a pilot experiment and is not as elaborate as for the other two sites; however, it is illustrative and complements the other experiments (Fig.3). Iron-oxide reduction rates are low with no carbon additions irrespective of the addition of Fe-oxides. Fe-oxide and Mn-oxide reduction rates increase with carbon addition, with ~ 1200 μmol l^{-1} d^{-1} in the slurry with the largest iron-oxide and carbon addition. Methanogenesis is very low for the treatments with low organic carbon.
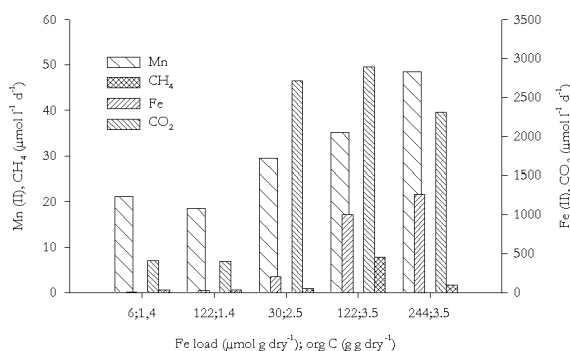
Fig. 3: Production rates (μmol l^{-1} d^{-1}) in estuarine slurries in relation to the load of reactive iron and organic carbon added. Left y-axis: reduced Mn ,CH$_4$, right y-axis: reduced Fe, CO$_2$. 6;0 stand for load of reactive iron is 6 μmol.g^{-1} and 0 μmol cm^{-3} organic carbon is added.

3.4.2. V$_{max}$ and Km

The metal oxide reduction rates versus Fe-loading date have been modelled using a Monod-type of equation to derive V$_{max}$ and Km of iron reducing communities (Van Bodegom and Scholten 2001, Liu et al. 2001).The V$_{max}$ of the iron reduction by the total community in the freshwater and Mediterranean sediments has been calculated for each set of organic carbon loads. V$_{max}$ represents the maximal iron reduction rates and Km values indicate the affinity of the microbes for the substrate (iron oxides). V$_{max}$ has been calculated for Fe$_{asc}$ (Table 2a) as well as Fe$_{dith}$ (Table 2b). As expected values did not differ significantly. For the Appels slurries with 4 times additional carbon the data from the first part (59 to 69 μmol Fe g^{-1}) has been used.

50

V_{max} values of freshwater slurries with 0 and 4 times additional carbon are similar. The values of 8 times organic carbon treatment are much higher, but this is due to outliners at highest iron addition (Fig 1c). If these extreme values are left out of the non-linear regression analysis V_{max} values are rather similar for all treatments (2.3-5.6 x 10^{-5} mol m^{-3} sec^{-1}) (Table 2a). Averaging iron-oxide reduction rates of high Fe addition treatments can also approximate maximum iron reduction rates (V_{max}). This is based on the implicit assumption that Km is smaller than added iron concentrations. These approximate V_{max} values based on the two highest additions are similar (1.8 – 3.5 x 10^{-5} mol m^{-2} sec^{-1}) to V_{max} values based on the Monod-equation

Table 2a. V_{max} and Km values of freshwater and Mediterranean slurries calculated with Fe_{asc} loads. V_{max} average rates refers to V_{max} calculations based on the 4 slurries with the highest iron additions.

Site	Analyte	C-load	N	V_{max} (10^{-5} mol m^{-3} s^{-1})	R^2	V_{max} average rates (10^{-5} mol m^{-3} s^{-1})
Appels	Fe	0	40	3.9 ± 1.2	0.11	1.85 ± 0.75
Appels	Fe	4	12	2.2 ± 2.1	0.18	1.75 ± 0.12
Appels	Fecorr	8	14	5.6 ± 3.7	0.46	3.5 ± 1.21
Medit.	Fe	0	6	2.65.10-1		
Medit.	Fe	0.1	16	1.23		3.18.10-3 ± 1.0
Medit.	Fe	4	16	3.82		1.53x10-2 ± 2.0

Table 2b. V_{max} and Km values of freshwater and Mediterranean slurries calculated with Fe_{dith} loads.

Site	Analyte	C-load	N	V_{max} (10^{-5} mol m^{-3} s^{-1})	R^2
Appels	Fe	0	40	4.1 ± 1.6	0.18
Appels	Fe	4	12	1.57 ± 1.4	0.18
Appels	Fe corr	8	14	4.5 ± 3.7	0.27
Medit.	Fe	0	6	8.4.10-2	
Medit.	Fe	0.1	16	5.3x10-1	
Medit.	Fe	4	16	1.3	

The Mediterranean slurries did show higher V_{max} values with increasing organic carbon loads: 0.26 x 10^{-5} (mol m^{-2} sec-) at 0 C, 1.2 x 10^{-5} (mol m^{-2} sec-) at 0.1 C and 3.8 x 10^{-5} (mol m^{-2} sec^{-1}) at 4C. In this sediment the iron oxide reduction rate is

51

clearly affected by organic carbon addition and by iron addition. V_{max} values for the Mediterranean started at lower values than the V_{max} of Appels, but gradually reached levels that were similar to those at Appels. This suggests that in Mediterranean sediments the iron oxide reduction capacity of the ambient bacterial community is primarily linked to organic carbon availability.

Km values for Appels slurries were estimated from figures and were all below 75 μmol Fe g^{-1}. Km value of Mediterranean slurries were all below 30 μmol Fe g^{-1}.

3.4.3. Relative importance in mineralization

Measured reduction and production rates can be used to calculate the relative importance of the different pathways in the mineralization of the organic matter. For these calculations traditional stoichiometry (Froelich et al. 1978) has been used where one mole of electron acceptor is reduced, 0.25 Fe (II), 0.45 Mn (II), 1 CH$_4$ and 2 SO$_4$ mol of CO$_2$ produced. In the absence of oxygen and nitrate the total sum of these estimates should be equal to the measured ΣCO2 production.

In the Appels slurries with no carbon addition iron oxide reduction is the most important pathway in the anaerobic mineralization (15-244 % of ΣCO$_2$ production, median 68 %). With addition of organic carbon the relative role of iron becomes smaller (11-143 %, median 46 % for 4 C treatment and 11-92%, median 18 % for 8C treatment). Besides, methanogenesis becomes more important. However, the percentage of methanogenesis in mineralization decreases on iron addition (Fig. 4) indicative of a competitive inhibition of methanogenesis by iron reducing bacteria.

In the Mediterranean slurries mineralization rates were rather low and iron-oxide reduction was not a major pathway of mineralization, accounting for 0.3 $\pm$ 0.5 %
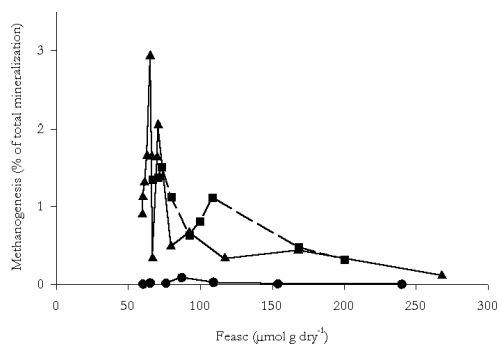

Fig. 4. Importance of methanogenesis in mineralization (%) in freshwater sediments in relation to the load of reactive iron (Fe$_{asc}$ μmol g^{-1}), for different amounts of organic carbon addition: o org C – circles, 4 org C - triangles, 8 org c – squares.

52

of the mineralization at the highest carbon additions. Similarly the other anaerobic degradation pathways at the highest carbon additions are also rather low: Mn 0.3 ± 0.5 %, SO_4 3.0 ± 5.9 and CH_4 0.3 ± 0.6 %.

In the Grevelingen slurries iron oxide reduction becomes relatively more important with higher carbon additions (from 0.6 to 13 %), partly at the expense of Mn-oxide reduction (highest value 2.5 %, lowest value 0.5 % of total mineralization). These two processes almost account for up to 13 % of the initial mineralization at the highest carbon loads. Importance of methanogenesis was neglectable (0.02-0.3 %).Thus indicating a relatively large role for the other processes. This may be either aerobic mineralization for the treatments without carbon addition because the aerobic sediment toplayer was included in the slurries, however this layer is very small and O_2 should be depleted very quickly. A more probable cause is enhanced sulphate reduction for the treatments with carbon addition, and side reactions resulting in lower dissolved Fe (II) analyses for treatments with and without carbon addition.

3.5. Discussion

3.5.1. Methodological

Amorphous iron oxides were used for reasons of consistency and comparability with previous studies. Munch and Ottow 1980have reported the preferential reduction of amorphous to crystalline iron oxides. Similarly Lovley and Phillips 1986 showed that the reduction rate of amorphous iron-oxide is much higher than the rate of hematite reduction. Energy gain and the efficiency of dissimilatory iron oxide reduction varies highly with the type of iron oxides that is reduced (Froelich et al. 1978, Lovley 1991, Thamdrup 2000). It will be clear that in order to compare experimental results it is necessary to use the same type of iron oxides.

Glucose was used as an additional source of organic carbon because a wide range of bacteria can use this carbohydrate. Lovley and Phillips 1986 showed the capability of iron reducing bacteria to use glucose as an electron donor, although the addition of acetate promoted amorphous iron reduction more. King and Garey 1999 also showed that glucose could be used as an electron donor in slurries with freshly precipitated iron oxides, glucose and plant roots (1143 μmol l^{-1} d^{-1} Fe-oxide reduction after lag-phase). More recently Coates et al. 1998, found an anaerobic metabolism for the complete oxidation of glucose with Fe (III) as the sole electron acceptor. It will be clear that not only iron reducing bacteria but also other bacteria may be stimulated by glucose addition. Lovley and Phillips 1989, showed that the complete oxidation of glucose in Fe (III) reducing sediments requires a consortium

of bacteria consisting of fermentative and Fe (II) reducing bacteria. As we used natural sediments we can assume both groups to be present. Using glucose ascertains a quick response of the microbial community to the enhanced C-load. In a pilot study with Grevelingen sediment we have also used cellulose as an organic source in one slurry. This resulted in a similar response though ΣCO_2 increased after a lag-phase because of the complex structure of cellulose.

In the Appels slurries the measured Mn dissolution was probably due to the co-release of Mn upon iron oxide reduction. However the exact amount of released Mn was difficult to estimate because Mn is probably adsorbed on the clay particles (Canfield et al. 1993b).

3.5.2. Iron oxide reduction rates

Iron-oxide reduction rates are rather high for Appels (1000 -10000 µmol l^{-1}, Fig 1) intermediate for Grevelingen (10-1200 µmol l^{-1} d^{-1}, Fig 3) and very low for most Mediterranean slurries (0.08 – 22 µmol l^{-1} d^{-1}, Fig 2). The reduction rates measured in Appels and Grevelingen slurries are comparable to reduction rates found by other researchers using cultured bacteria or sediment slurries. Glucose enriched estuarine sediments had initial iron oxide reduction rates of ca. 800 µmol l^{-1} d^{-1} (Lovley and Phillips 1986). They also enriched tidal river sediments with glucose and obtained Fe-oxide reduction rates (9090 µmol l^{-1} d^{-1}) comparable to our highest rates in the Appels sediments. Both enrichments of Lovley and Phillips 1986 were amended with amorphous iron oxides. Observed rates in Appels slurries are higher than those found by Kostka et al. 1999a for Fe (III) to Fe (II) reduction in smectite (1371 µmol l^{-1} d^{-1}). They measured the Fe (II) production in HCl extracts from cultured anaerobic bacteria in a minimal medium with addition of clay mineral as the sole electron acceptor. Dobbin et al. 1999 found intermediate values ($\sim$ 3200 µmol l^{-1} d^{-1}) in an anaerobic batch culture of the freshwater bacterium *Clostridium bejerinckii* using 50 mM Fe (II) citrate and 50 mM glucose. At the start of our freshwater slurry incubations iron concentrations were 19-72 mM and glucose concentrations were 0.27-2.5 mM (glucose molecule structure $C_6H_{12}O_6$). Van der Nat and Middelburg 1998 reported an iron-oxide reduction rate of ca. 370 µmol l^{-1} d^{-1} for unvegetated freshwater sediments. Their rate was estimated from measured *in situ* CO_2 production and methanogenesis, assuming that iron (III) reduction was the only other mineralization process. These results indicate that rates of iron-oxide reduction vary, due to iron and organic carbon availability.

Iron oxide reduction rates and V_{max} values are subject to over and under estimation. Values can be overestimated if Fe (II) is not only produced by iron-

oxide respiring bacteria but also by (chemical) reduction coupled to re-oxidation of reduced inorganic components. However, the latter would not be expected in freshwater slurries because no major pools of reduced components are available to reduce iron.

Iron oxide reduction rates and V_{max} can be underestimated if Fe (II) is precipitated (Berner 1970), sorbed on to particles or bacterial cells (Roden and Zachara 1996, Kostka et al. 1999a, Liu et al. 2001) or re-oxidized and then removed through precipitation (Postma 1985, Widdel et al. 1993, Murase and Kimura 1997, Postma and Appelo 2000).

Van Bodegom and Scholten 2001 found V_{max} values of iron oxide reduction in a rice paddy soil of 5.10^{-4} mol m^{-3} s^{-1}. They stated that this value was in the same range as values they derived from data in the literature (Lovley and Phillips 1986, Lovley and Phillips 1987, Lovley and Phillips 1988, Achtnich et al. 1995, and Roy et al. 1997). The V_{max} values for Appels ($2.4 - 5.6 \ 10^{-5}$ mol m^{-3} s^{-1}) and at highest carbon loading in the Mediterranean ($3.8.10^{-5}$ mol m^{-3} s^{-1}) are about 10 times lower than those estimated by van Bodegom and Scholten 2001.

V_{max} values in these freshwater slurries did not change significantly at higher organic carbon loads. This indicates that organic C is not limiting iron oxide reduction in these sediments. In contrast, V max values of Mediterranean samples increased after large organic carbon addition, V_{max} values approaching those of the freshwater sediments. This indicates that biogeochemical models should take into account at least two factors limiting iron oxide reduction rate in sediments. The incorporation of this carbon and Fe-oxide co-limitation of iron oxide reduction is usually done through a double Michaelis-Menten equation or a combined equation such as those used by van Bodegom and Scholten 2001 and Wijsman et al. 2002. Km values indicate the affinity of the microbial community for a substrate. Our Km values are not very well constrained but less than 75 µmol g^{-1}.The upper limit values are consistent with those van Bodegom and Scholten 2001 estimated from literature (61 mol.m^{-3} H$_2$O = 24 µmol g sediment^{-1} assuming a dry density of 2.5 g cm^{-3}) and those adopted by Meysman 2001 (20 µmol g^{-1}). Both their Km values were estimated whereas our values are experimentally determined, therefore some difference may exist.

The total carbon dioxide production estimated from stoichiometry and electron acceptor use is quite comparable to measured ΣCO_2 production (range 8-244% with a median of 55%) The values do not correspond exactly due neglect of denitrification, accumulation of errors, discrepancies between the calculated Fe (II) and the actual Fe (II) and the neglect of Mn (II) adsorption. Although Mn-oxide reduction can be important in some sediments (Canfield et al. 1993b, Thamdrup

and Canfield 1996, Van der Zee 2002) it is likely not important in these sediments because concentrations of Mn-oxides are very low (Appels 5.8 μmol Mn g^{-1}, Mediterranean 7.2 μmol Mn g^{-1}).

3.5.3. Factors limiting iron oxide reduction

Iron oxide reduction in Mediterranean sediments is primarily limited by the lack of degradable organic carbon. The Mediterranean sediments have very low organic carbon concentration, 0.1 mgC m^{-2}, and the organic material is already highly degraded (Dauwe et al. 1999). This is also reflected in a low mineralization rate (0.45 mol m^{-2} yr^{-1} total profile Moodley, L. personal communication; 0.56 mol m^{-2} yr^{-1} above sapropel S1, this thesis Chapter 5). Addition of organic carbon did not clearly indicate whether iron oxide availability is sufficient to result in significant iron oxide reduction provided higher carbon availability. Dithionite extractable iron-oxide concentration is not much lower in the Mediterranean sediment than in Appels (Table 1, Passier and De Lange 1998). However, the concentration of ascorbate extractable iron and reactivity of iron oxides in the Mediterranean sediments is very low (Chapter 2). An enhancement of iron oxide reduction rate without simultaneous addition of iron oxides and organic carbon therefore is not very likely.

V$_{max}$ values indicate that the potential of the iron oxide reducing community in Mediterranean sediment is similar to that of the iron-oxide reducing community in the freshwater sediment provided sufficient degradable organic carbon and reactive iron are available. However, observed rates are lower than those in Appels sediments. Since there is hardly any iron oxide reduction in the natural sediment (Chapter 5) there is likely no large active iron reducing population present at the start of the incubations. Incubation times were short with the consequence that there also was no time for significant build up of an iron reducing population and final rates were still much lower than those of Appels are. We do not have any counts on bacterial numbers to confirm this. van Bodegom and Scholten 2001 explained the lag-phase in their model by the presence of other more favourable electron acceptors which are used before iron reduction starts. As we used the aerobic part of the sediment this could be another reason though nitrate concentrations are low (~ 3 - 21 μmol l^{-1}, Slomp personal communication) and Mn-oxide reduction rates were measured to be low. Longer incubation times could have overcome both problems. However, results would not have been representative for the bacterial community active in the sediments.

Iron oxide reduction in Appels sediments is evidently limited by the availability of reducible iron, because Fe-oxide addition results in a clear increase (Fig. 1a to 1c).

Kostka et al. 1999a described the capability of an iron reducing bacterium, *Shewanella putrefaciens*, to couple Fe (III) -reduction in clay minerals to organic carbon oxidation. Those experiments were done with pure bacterial cultures and with clay minerals as the sole electron acceptors. In contrast, our experiments were conducted with natural sediments including the whole microbial community and other electron acceptors. Ernstsen et al. 1998 showed that the structural Fe (III) reduction in clay depends on the clay particle size (hence surface area) and the initial structural Fe (II) clay content. Addition of the amorphous iron oxides may increase iron oxide reduction rates because of an increase in surface area and thus the reaction sites. Roden and Zachara 1996 and Urrutia et al. 1998, determined the surface area to be the rate governing characteristic of iron oxides. An iron oxide surface control of iron oxide reduction is consistent with similar V_{max} values for all 3 carbon loads in Appels sediments and the highest carbon load in Mediterranean sediments.

Iron-oxide reduction in Grevelingen sediments is limited by organic carbon and probably by iron oxides as well. Iron reduction rates increase with addition of iron. In particular if carbon is added as well. The limited increase in iron oxide reduction if only iron oxides are added indicates the limitation of organic carbon. The lack of further increases in iron-oxide reduction rates upon iron addition at high carbon availability indicates that availability of iron oxides may also play a role. As natural sediments are very heterogeneous on temporal as well as small spatial scale the actual limiting factor can be variable. Based on other studies we expect SO_4 reduction to be the most important mineralization process in these marine sediment slurries (e.g. Jorgensen 1982, Canfield et al. 1993b, Moeslund et al. 1994). Although we did not measure sulphate reduction, the sediment in the org C amended slurries turned grey after some time. The high iron-oxide or low carbon amended slurries showed this change after a longer time, indicating a retarding effect on the shift to sulphate reduction as the dominant mineralization pathway. King 1990 also found that the addition of Fe-oxide can lower sulphate reduction with 47%.

3.5.4. Changes in mineralization pathways

In natural sediments changes in mineralization pathways will primarily occur due to the changes in the organic carbon loading. Higher supply of organic carbon to the sediment will cause a shift to a relatively higher importance of anaerobic mineralization (Heip et al. 1995). The conditions at the start of the experiment determine the impact of the induced changes and the final dominant pathway in the sediment.

Chances in mineralization pathways as a function of carbon loading are presented schematically in Fig. 5 (based on Monte-Carlo type numerical simulation of Wijsman et al. 2002). The total mineralization rate in a sediment is an indication of the input of degradable organic carbon. This figure visualises the shifts observed in the slurries due to addition of iron oxides and / or organic carbon. The precise carbon loading at which a particular mineralization pathway becomes important or the maximum relative contribution will clearly depend on numerous factors (Soetaert et al. 1996) but all sediments are subject to the same set of factors that play a role in the mineralization pathways.

Mediterranean sediments have low carbon loading and the anaerobic pathways are not important in these sediments (Pruysers et al. 1991, Passier and De Lange 1998, Dauwe et al. 1999, Thomson et al. 1999). Addition of organic carbon was not sufficient to shift the process to a higher importance of the anaerobic processes. Clearly conditions in these sediments were far away from those for optimal iron reduction.

The Grevelingen sediment had intermediate ΣCO_2 production. Addition of carbon results in enhanced iron oxide reduction and iron-oxide reduction rates approach those of freshwater Appels sediment. The sediment is on the brink of those conditions optimal for dominance of iron reduction. An additional input of labile carbon (e.g. spring bloom) may be sufficient to shift the sediment towards the domain where iron reduction prevails. If the carbon delivery is accompanied with delivery of reactive iron oxides the peak may become broader and iron reduction can account for a higher part of the total mineralization. The Grevelingen slurries

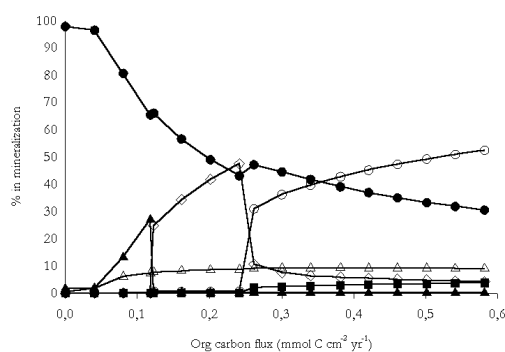**Fig. 5**. Changes in mineralization pathways (% in total mineralization) as a function of carbon loading (μmol C cm^{-2} yr^{-1}), based on numerical simulation of Wijsman et al 2002. Oxic mineralization - solid circles, nitrification - open triangles, Mn-oxide reduction - solid triangles, Fe-oxide reduction - open diamonds, Sulfate reduction - open circles, methanogenesis - solid squares.

are the only ones where analysed dissolved Mn (II) production really results from Mn-reduction and not from dissolution of the added iron oxides, because the Mn (II) concentration is higher than can be accounted for by iron-oxide dissolution only. In figure 5 can be seen that this supports the idea of carbon limitation in these slurries.

Mineralization rates in Appels sediments were highest of all and iron oxide reduction is already an important pathway in the natural sediment (74 % of total mineralization, Chapter 3). Roden and Wetzel 1996 reported an important role of iron oxide reduction for organic carbon mineralization in freshwater sediments. Addition of iron nevertheless results in a higher reduction and mineralization rate at the expense of methanogenesis. The sediment is capable of even higher iron oxide reduction without changes in organic carbon load. This can only happen if the sediment is in the optimal situation for iron-oxide reduction. The addition of organic carbon shifts the system past these conditions resulting in iron reduction becoming less important and increasing importance of methanogenesis. In this situation the addition of Fe-oxides shifts the system "backwards" to the more optimal conditions, resulting in an increasing importance of iron reduction and the observed inhibition of methanogenesis.

Methanogenesis in Appels sediment was stimulated when carbon was added, but this increase was limited if iron oxide was added as well. This may indicate that iron oxide reduction inhibits methanogenesis because of the competition for acetate or H_2 (Achtnich et al. 1995, Kruger et al. 2001). The Fe-reducing bacteria are clearly able to compete with the methanogenic bacteria as has already been reported for tidal river sediments (Lovley and Phillips 1986, Lovley and Phillips E.J.P. 1987). Moreover it has also been found in a number of other sediments for example in an anoxic paddy soil, (Achtnich et al. 1995), in rice fields (Frenzel et al. 1999, Kruger et al. 2001), and in tidal freshwater sediment along the Scheldt river (Van der Nat and Middelburg 1998).

3.6.   Conclusions

The factors limiting iron reduction depend on the characteristics of the sediments. The effects of iron and carbon addition also depend on the initial state. The position of the sediments in the "mineralization-sequence" determines the effect of additional inputs on the pathways. The Mediterranean sediments are aerobic and addition of organic carbon or iron moves the sediment a little towards conditions more favourable for iron reduction. In contrast, the freshwater sediment is at the optimal conditions for iron reduction already, the addition of organic carbon

moves the processes towards methanogenesis. Maximal V_{max} values can be reached as soon as sufficient degradable organic carbon is available. However realised iron reduction rates depend on the concentration of reducible iron oxides.

4. Effect of macrofauna, sediment ventilation and particle reworking on sedimentary iron and manganese pools in a mesocosm experiment

Imola Ferro, Paul van Nugteren, Jack J. Middelburg, Peter M. J. Herman, Carlo H.R. Heip

4.1. Abstract

Benthic fauna exerts a major impact on biogeochemical processes. Bioturbation can be partitioned into a pore-water irrigation and a particle-mixing component, which in most studies are lumped in an overall effect. Depending on sediment conditions, governing processes and studied constituents, the importance of each component may differ, as both have specific effects (e.g. oxygen input by irrigation, mixing of oxidized and reduced particulate metals by particle mixing). Here we present results from mesocosm experiments, using a marine sediment, in which we investigate the importance of both components for the sedimentary iron and manganese cycle. Solid-phase and dissolved iron and manganese were studied in sediment cores with four different treatments: control, ventilation, particle mixing and bioturbation by the polychaete *Nereis diversicolor*. Results for iron and manganese were similar for control cores and sediments bioturbated by worms with low metal concentrations in the porewater. In the latter sediments were oxygenated by the macrofaunal activity, resulting in an efficient retainment of both metals in the sediment. The effects of particle mixing and ventilation were different for both metals. The effect of ventilation on manganese cycling is larger than the effect of particle mixing, resulting in a better retainment of manganese in the sediment due to extensive oxidation of Mn (II). Despite the lack of a complete macrocycle or an external metal oxide source, indicate metal oxide reduction continued, likely due to a microcycle involving mobile oxidized metal phases. Particle mixing is more important for iron cycling than ventilation, resulting in a smaller flux to the overlying water than the ventilation treatment. The differences in the effect of ventilation and mixing on iron and manganese can be attributed to the differences in aquatic chemistry of the metals and are consistent with recent research on rate limiting steps in the sedimentary cycling of iron and manganese.

4.2.   Introduction

Organic carbon mineralization in sediments involves a number of electron acceptors including oxygen, nitrate, manganese- and iron oxides and sulphate. The presence of macrofauna and their structures is an important biogenic factor influencing organic matter degradation pathways (Kristensen 2000). Bioturbation can be split in two mechanistic components: (1) irrigation of the sediment and (2) sediment mixing, the latter resulting in mixing of both (aerobic and anaerobic) porewater and particles.

Irrigation of sediments by fauna activities or their structures involves the enhanced exchange of dissolved constituents between bottom water and pore-water. Irrigation itself can be divided in two components: (1) enhanced gas exchange (mainly O_2 and CO_2) between the sediment and the overlying water (aeration) and (2) enhanced exchange of dissolved components such as NH_4^+, Mn (II) and Fe (II). Ingalls et al. 2000 reported increased transport of dissolved solutes in the presence of bioturbating fauna. This resulted in a faster removal of degradation products and a higher input of oxygen and nitrate from the bottom water. Especially in systems with aerobic bottom waters and mostly anoxic sediment, ventilation can result in significant mineralization coupled to iron- and manganese-oxide reduction and thereby prevent a shift from sub-oxic mineralization to sulfide reduction (Hines et al. 1997). Moreover a higher oxygen input may result in a faster and more complete mineralization (Aller 1994a, Dauwe et al. 2001, Kristensen 2000).

Sediment mixing involves displacement of porewater and particles. Sediment mixing results in homogenisation of sediment properties, downward transport of oxidized elements and degradable organic carbon into the anoxic zone, and upward transport of reduced components into the oxidized zone. The downward transport of degradable organic carbon maintains mineralization in the deeper sediment layers. Herman et al. 1999 showed that sediment mixing results in a deeper average depth of organic matter mineralization.

Organisms exert through bioturbation multiple effects on degradation pathways. Aller and Aller 1998, Herman et al. 1999 and Kristensen 2000, provided an overview of recent knowledge on the role of bioturbating fauna in sediment diagenesis and mineralization. A number of studies have addressed the effect of bioturbation on (anaerobic) mineralization (Aller 1990, Aller 1994a, Banta et al. 1999, Burdige 2001, Kristensen and Holmer 2001). Aller 1994b showed that the role of Mn as intermediate oxidant in mineralization is largely determined by particle mixing which transports MnO_2 and degradable organic matter into reduced zones and by the efficiency of Mn (II) oxidation at the sediment-water interface.

Iron and manganese are both solid phase oxidants resembling each other in their role in diagenesis and iron is likely to be affected by bioturbation in a similar way as manganese.

The effect of bioturbation on iron-oxide reduction has not yet been investigated in detail. Aller and Aller 1998 investigated the dependence of mineralization on diffusive exchange. Net mineralization rates were calculated based on porewater - overlying water gradients using sediment plugs of different thickness. They found a strong dependence of anaerobic mineralization on diffusive transport distances and concluded that apparent small changes in burrow geometry are likely to have a significant effect on reaction balances. Recently Roden and Urrutia 1999 reported that removal of adsorbed Fe (II) promotes iron oxide reduction by retarding inhibiting effect of Fe (II) sorption on iron oxides or bacterial cell surfaces. The ventilation activity of benthic fauna may promote ferrous iron removal because of (1) higher porewater replenishment and (2) enhanced oxidation of ferrous iron and subsequent precipitation of ferric iron oxides. Therefore irrigation may also promote iron-oxide reduction. Glud et al. 2000 concluded that the high iron-oxide reduction rates they measured in a high-arctic sound needed an intense bioturbation. However, the dependence on particle mixing or porewater irrigation was not investigated separately.

The importance of iron and manganese oxide reduction in organic matter mineralization is often dependent on the rate and efficiency of their oxidation-reduction cycles (Wijsman et al. 2001b). To complete a cycle dissolved and particulate reduced components have to be transferred to a re-oxidation zone and particulate oxidized mineralization components have to be mixed into the reduction zone. The two components of bioturbation together provide a mechanism for this, but it is unknown, which of the two components is more important for the completion of the manganese and the iron cycle.

In this study we aim to elucidate the role of particle mixing and irrigation on iron and manganese oxide reduction. Therefore we studied the effect of a macrofauna species, *Nereis diversicolor*, and compared it with artificially enhanced transport of porewater-dissolved gases (ventilation) and particle mixing (without mixing of aerobic and anaerobic layers). We compared sediment-water fluxes, porewater profiles and metal oxide speciation of sediment cores among these 3 treatments. Differences were expected to be reflected in porewater profiles, fluxes across the sediment-water interface and metal oxide distributions. Based on the few literature studies to date we anticipated enhanced iron and manganese reduction at the end of the experiment in the sediment cores with *Nereis diversicolor* because this worm mixes and irrigates sediments. In the mixed and ventilated sediment lower or no

reduction were expected due to the lack of a complete cycling or another source of metal oxides to sustain metal oxide reduction after the sedimentary metal oxide pool has been used (i.e. after the number of days necessary for complete turnover of the pool).

4.3.   Material and Methods

4.3.1.   Set-up mesocosm experiment

For the experiment 30 cm Plexiglas cores (id =52 mm) were filled with ~ 15 cm sieved (1 mm mesh) sediment from Katsplaat, a tidal flat in the Oosterscheldt (a tidal inlet in the south-western part of the Netherlands). The sediment has a medium grain size of 93 µm, an organic carbon content of 0.36 wt.%, a carbonate content of 7.5 wt % and a sediment molar C/N ratio of 12.5. A 14 cm water column of Oosterscheldt seawater was put on top and cores were allowed to settle for 7 days at 20°C in a darkened aerated seawater tank. Following acclimation cores were transferred to a continuous flow system. The whole system was maintained in a climate controlled room. Sediment and specimens of *Nereis diversicolor* were collected simultaneously.

Nereis diversicolor is a common macrofauna species in the estuaries in the North Sea area. The worm lives on shallow soft bottoms in U-shaped burrows. *Nereis diversicolor* is considered to be a suspension feeder. The worm performs active porewater pumping. Riisgard 1991 showed that the total population-pumping rate could be several (upto 10) times the water column per day (Odense Fjord, Denmark). By its activities and high porewater pumping rate the animal can have an important effect on diagenesis.

Cores were divided into six groups with different treatments each consisting of three replicates. One group had no sediment and was used to measure non sediment related Fe (II) and Mn (II) production. The other five groups had a sediment column and were used for the following treatments: (1) t=0-group was sliced immediately at the beginning of the experiment, (2) control, (3) bioturbation (addition of 3 specimens of *Nereis diversicolor*, ~1500 ind m^{-2}), (4) ventilation (2 artificial U-shaped burrows constructed of silicone tubing to 8 cm depth) and (5) mixing (stirring device 50 mm id placed ca. 5 cm below the sediment surface). For an elaborate description we refer to the paper of VanNugteren et al. 2002.

Flux measurements were performed at 1, 5 10 and 16 days after start of the experiment by temporarily closing the circuit so that the continuous flow was stopped. In between incubations there was a continuous flow of aerated seawater. During incubations 4 ml samples of overlaying water were taken at 0, 30, 60, and

120 minutes. After collection samples were put in N_2 filled headspace vials and acidified with 20 %H_2SO_4 (20 µl ml sample[-1]).

At the end of the experiment cores were sliced in appropriate intervals. Porewater was collected by centrifugation (20 min, 355 G) using tubes like Saager et al. 1990. Porewater samples were capped in headspace vials, acidified and stored until analysis.

4.3.2. Solid-phase extractions

The centrifuged sediment was sub-sampled for solid phase extractions. A number of extractions were used to characterise the pools of particulate iron and manganese oxides. Ascorbate and HCl extractions were performed in an anaerobic chamber (Coy Laboratory Products) filled with > 95 % N_2 and < 5 % H_2. All extractions were done in duplicate. We were aware that these represent pseudo-replicates, but due to sample size restrictions it was not possible to do it otherwise.

The pool of amorphous Fe (III) oxides was extracted using ~0.3 g wet sediment and 5 ml of an anaerobic solution of 10 g sodium citrate, 10 g sodium bicarbonate and 4 g ascorbic acid in 200 ml demineralised water (Kostka and Luther 1994). Extraction lasted about 24 hrs and the samples were continuously shaken in the dark. After filtration (0.45 µm filter, cellulose / acetate) iron concentrations were analysed in the filtrate. The amorphous iron extracted with this method is considered the most reactive part of the total sediment iron pool.

The pool of dithionite extractable iron represents amorphous iron (III) oxides, crystalline iron (III) oxides and the pool of iron bound to acid volatile sulfides (FeS), i.e. the pool of chemically reactive iron oxides, and was based on Canfield 1988. Mn-oxides (crystalline oxides available for reduction) are extracted as well (Canfield 1988, Kostka et al. 1999b). Leachant (5 ml, 0.35 M acetic acid/0.2M Na-citrate solution 50 g l^{-1} dithionite) was added to ca. 0.3 g wet sediment. After two hours shaking, samples were filtered over a 0.45 µm filter and the filtrate was acidified with 0.1 ml 6 N HCl ml filtrate[1]. The excess Fe_{dith} and Mn_{dith} (Table 2a,2b) were calculated by substracting background concentrations of deep layer form the higher ones in the surface layers.

The HCl extractable pool (sensu Kostka and Luther 1994) was also measured. This involves the addition of 10 ml of a 0.5 M HCl solution to ca. 0.3 g of wet sediment. After 1 hour of incubation the sediment is filtered over 0.45 µm. Fe (II) and total FeHCl are measured in the filtrate with the ferrozine method (Stookey 1970) using HEPES or reducing HEPES buffer. HCl extraction allows determination of the reduction state of the sediment and adsorbed Fe (II). The difference between Fe (II) content before and after the experiment represents the amount of reduced iron

that is within the solid phase or has adsorbed to the sediment during the experiment.

The pool of total iron was measured on a freeze-dried split of the well-mixed sediment at the start of the experiment. The method used HCl/HNO_3 as destructant, a microwave for high temperature and a pressure digester (Nieuwenhuize et al. 1991).

4.3.3. Analyses & calculations

Except for the HCl-extraction solutes (Fe analysed using the ferrozine method (Stookey 1970), all Fe and Mn analyses were measured by Inductively Coupled Plasma – Optical Electron Spectrometry (ICP-OES, Perkin-Elmer Optima 330 DV, using either the axial or radial view method dependent on concentrations). Concentration changes of iron and manganese in the overlying water were used to calculate fluxes. In some of the cores the oxygen saturation in the overlying water decreased below 50% saturation during the incubations. As this affected iron concentrations in solution and induced more anoxic conditions in sediments, this resulted in extremely high fluxvalues. Therefore, these iron data (t = 120 minutes) have not been used in flux calculations.

4.3.4. Statistical analysis

Average values are shown for all results; error bars represent the standard deviation of the replicates. Statistical significance has been evaluated using analysis of variance (ANOVA with an LSD post-hoc comparison) and has been performed with Statistica.

4.4. Results

4.4.1. Fluxes to the overlying water during incubations

Concentration changes in the overlying water during incubations are used to calculate fluxes of Fe and Mn out or into the sediment. Fluxes (Fig 1) are the net result of all biogeochemical processes related to Fe and Mn taking place in the sediment and the water column. Differences among treatments and changes during the experiment have been investigated. Fe fluxes decreased significantly during the experiment (p = 0.005, F = 5.3, n=48) and did not depend on treatment (p = 0.09, F = 2.4, n=48). Whereas Mn fluxes depend on the treatment (p = 0.02, F = 3.8, n=48).

This is caused by the fluxes in *Nereis* cores being significantly lower than in the control cores (p=0.005). Additionally, Fe fluxes of the *Nereis* treatments were lower than those in the ventilated treatment (p < 0.05) and Mn fluxes of in *Nereis* treatments were lower than fluxes of the control and the mixed sediments (p < 0.05). The most remarkable feature is the large uptake in *Nereis* sediments of Fe (II) and Mn (II) at 5 days. This indicates the presence of extremely low concentrations of both solutes in the porewater of the top layers at that time due to well oxygenated sediments.
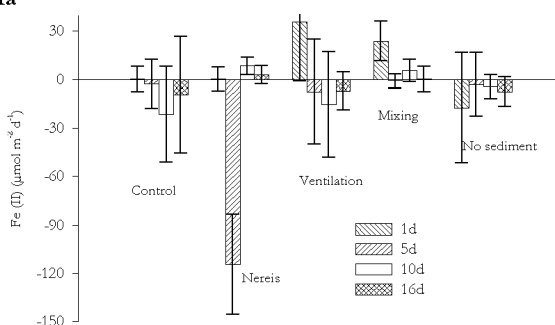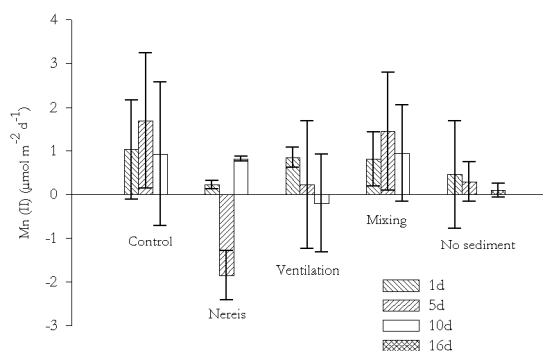
Fig. 1a

Fig. 1b

Fig.1 Fluxes of dissolved Fe (II) (a) and Mn (II) (b) from sediment cores to the overlying water ($\mu mol\ m^{-2}\ d^{-1}$) measured in incubations at 1, 5, 10 and 16 days.

4.4.2.   Porewater

Porewater concentration versus depth profiles of dissolved Fe (II) and Mn (II) are presented in Figure 2. Average values of the ventilation treatment are based on 2 replicates, as the third replicate showed indications of a sampling artefact (oxidation). These profiles reflect the final results of the processes going on in the cores during the experiment and profiles are the net result of all interactions within the sediment.

The overall effect of treatment, depth and their interaction was significant for the dissolved Fe (II) profiles (p=0.03, F = 2.4; p < 0.001, F = 11.7; p <0.001, F = 2.4; n = 88 respectively) (fig 2a). Profiles of control and *Nereis* were not significantly different from each other; both have low porewater iron concentrations and exhibit almost no changes with depth. However, these profiles were significantly (p< 0.05) lower than the t=0, ventilation and mixing profiles. These three treatments had classical profiles with subsurface maxima for dissolved Fe (II) concentrations. The ventilation and the mixing treatment differed significantly (p< 0.01) because the subsurface peak of the mixed profile was situated more to the surface and had higher values. Both treatments resembled the t=0 profile.

The manganese profiles only had an overall significant effect of treatment (p < 0.001, F = 21.5, n = 88) (Fig 2b). The *Nereis*, control and t=0 profiles of Mn (II) resembled each other and were significantly lower (p< 0.05) than those of the ventilation and mixing treatment.

4.4.3.   Solid phase extractions

The data on the solid phase iron extractions are rather variable. This variability depends on the iron fraction and the factors playing a key role in the distribution/formation of that fraction.

Fe_{dith} (dithionite extractable iron) had an overall effect of depth (p < 0.001, F = 6.7, n = 64) (Fig 3a) mainly due to the high values in the top layers of the control, *Nereis* and ventilation cores. No effect of treatment was discerned for Fe_{dith} profiles. Dithionite extractable Mn (Fig 3b) had a similar distribution and variance pattern as Fe_{dith} (p = 0.018, F = 2.9, n = 64).

Fe_{asc} (ascorbate extractable iron oxides) (Fig 4) had significant effects of depth (p < 0.001, F = 63.27, n = 64). This is the only profile with an effect of treatment (p < 0.001, F = 39.7, n = 64). This is caused by the ventilation profiles being on average slightly higher than the control (p< 0.05), *Nereis* and mixing profiles (p<0.01).

Fe (II) (HCl extractable Fe (II)) is an indicator of the reduction state of the sediment. No clear differences can be seen in this iron fraction. However values in the stirred ($p = 0.015$) and the ventilated cores ($p = 0.045$) are significantly lower than those in the control. Despite the fact that the mixing does not involve the aerobic top-layer, the mixing events affect the availability of oxidants, resulting in less reducing conditions and lower Fe (II) than in the control cores.

Total FeHCl (total HCl extractable sedimentary iron) changed significantly with

Fig. 2a
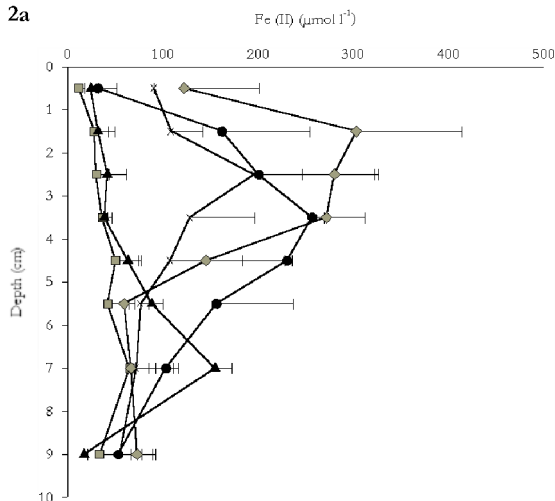
Fig. 2b
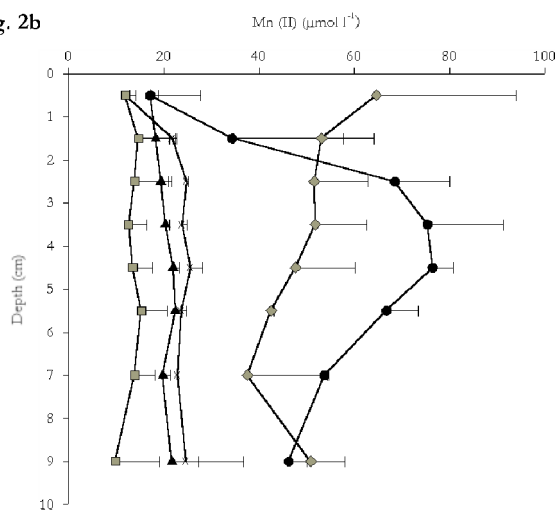
Fig.2 Porewater profiles of dissolved Fe (II) (a) and Mn (II) (b) (μmol l⁻¹) at the end of the experiment for control (squares) *Nereis* (triangles), ventilation (dots) and mixing (diamonds). Start profile at t=0 (asterix)

depth for *Nereis* and ventilation treatment (n= 16; p = 0.02, F = 5.0; p < 0.001, F = 13.2). The mixing treatments had significantly lower total FeHCl (p = 0.04) than the control cores (Fig. 6).

Fig. 3a
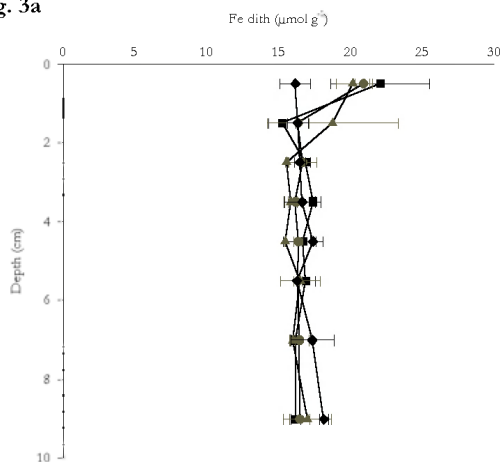
Fig. 3b
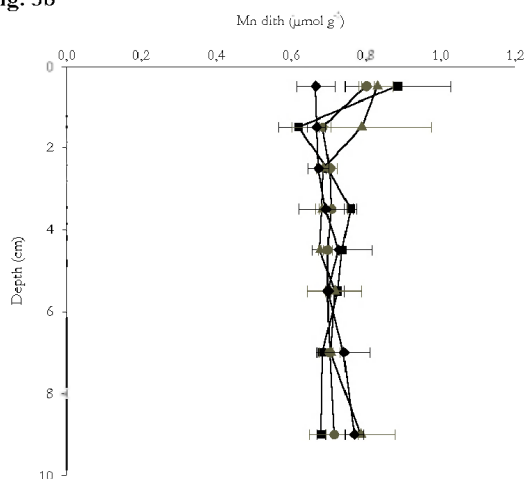
Fig. 3: Solid phase profiles (μmol g dry⁻¹) of dithionite extractable iron (a) and manganese (b) oxides for control (squares), *Nereis* (triangles), ventilation (dots) and stirring (diamonds).
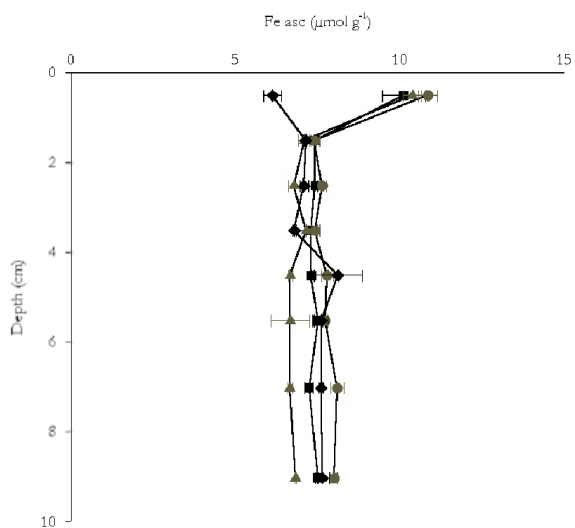
Fig. 4. Sediment profiles for ascorbate extractable Fe-oxides (μmol g dry^{-1}): control (squares), *Nereis* (triangles), ventilation (dots) and mixing (diamonds)
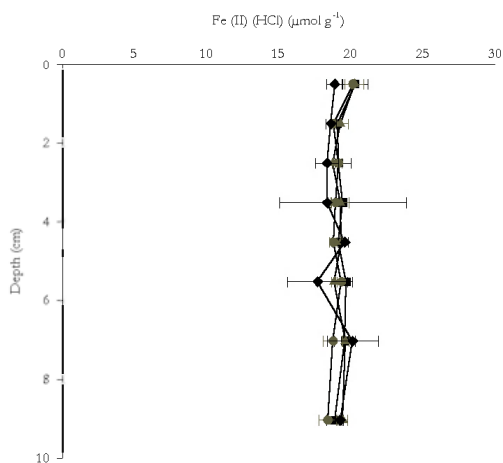
Fig. 5. Adsorbed Fe (II) profiles (μmol g dry^{-1}): control (squares) *Nereis* (triangles), ventilation (dots) and mixing (diamonds)

71
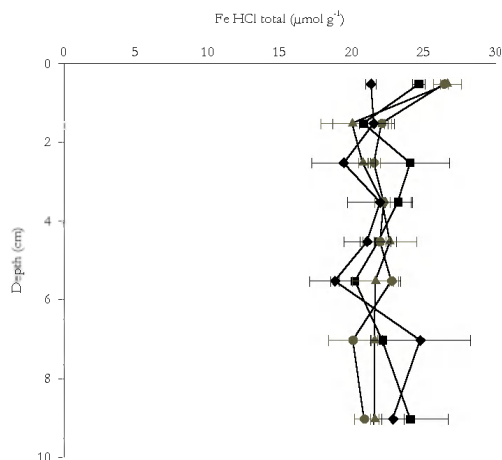
Fig 6. Total HCl extractable Fe (μmol g dry^{-1}) control (squares) *Nereis* (triangles), ventilation (dots) and mixing (diamonds)

4.5.   Discussion

The construction of burrows by benthic fauna and their ventilation activities affects the ratio of oxic to anoxic sediment. Kristensen 2000 calculated that, by combining data from Davey 1994, Kristensen 1984, Hylleberg and Henriksen 1980 and Fenchel 1996, the ratio of oxic sediment volume associated with Nereid burrows relative to the oxic surface layer is between 0.9 and 3.3. The U-shaped silicone tubing in our ventilation treatment also increased the entry of oxygen and the ratio of tube surface to cores surface was 1.47, thus the tubing allowed for a significant increase of the oxygenated area. However, silicone tubing only allows exchange of gases, and no dissolved constituents have been exchanged. Whereas in sediments active porewater pumping provides refreshment of the burrow water with oxic bottom water. This results not only in an enhanced gas-exchange but also enhances supply of electron acceptors such as nitrate and sulphate and removal of reduced porewater products. Rreduced metals are then transported into the oxic zone more readily. Davey and Watson 1995 have shown that the degree of transport enhancement is directly related to burrow wall surface area and thus to the increase in sediment-water contact zone. As Fe (II) oxidation and subsequent formation of iron oxide precipitates is essential to complete the sedimentary iron cycle the effect of irrigation in nature might be longer lasting than the effect of

ventilation observed in our cores because the treatment lacks a solute transport component.

Mixing was introduced by a stirring device placed ca. 5 cm below the sediment surface, i.e. in the anaerobic sediments (Van Nugteren et al. 2002). Although this treatment mainly involves particle mixing some porewater movement will occur as well. The thin oxic surface layer (about 2 mm) of the sediment was not mixed. However, in bioturbated sediments mixing includes the oxic layer. Therefore sediment mixing causes additional oxygen input and a higher incorporation of settled material (i.e. degradable organic matter and allochthonous metal oxides). The mixing of aerobic and anaerobic layers also enables adsorbed Fe (II) to be oxidized and recycled. This re-oxidation of Fe (II) may occur directly with oxygen or by re-oxidation with Mn-oxides, the latter will end when all Mn-oxides are reduced. Because the aerobic zone in our mixing treatment is not being mixed, the re-oxidation of Fe (II) most likely depends on reaction with Mn-oxides. When Mn-oxides are depleted iron recycling will come to an end and the barrier to retain reduced iron in the sediment will then disappear. The low fluxes in the mixing and *Nereis* treatment indicate that at least for the duration of the experiment large escape of dissolved Fe (II) to the overlying water is prevented.

Studies on the effect of bioturbation on sediment properties have used a large number of animals, for example the polychaete *Heteromastus filiformus*, the bivalves, *Macoma balthica* and *Tellina texana* (Aller and Yingst 1985), the amphipod *Corophium volutator* (Pelegri and Blackburn 1994), a heart urchin *Brissopsis lyrifera* Widdicombe and Austen 1999). Bioturbation studies have not been restricted to macrofauna, the effects of microfauna and meiofauna have been studied as well (Fenchel 1996, Aller and Aller 1992). *Nereis diversicolor* has been used in a number of studies (Davey 1994, Banta et al. 1999), e.g. to study the impact of benthic macrofauna on degradation of macroalgal detritus (Kristensen et al. 1992). Kristensen and Hansen 1999 reported that an important part of the irrigation effect of *Nereis* pumping activity in sandy sediments is based on the solute fluxes across the burrow wall. Banta et al. 1999 attributed the effect of macrofauna on the balance between aerobic and anaerobic microbial metabolism to the oxidising effect of irrigation. A clear bioturbation activity and the documented effects on sediment biogeochemistry in sediments justify the use of *Nereis diversicolor* to investigate the effect of bioturbation on sediment biogeochemistry.

4.5.1. Effect on total mineralization

In the companion paper the effects of the treatments on overall mineralization rates, carbon dynamics and their biogeochemical consequences have been

presented and discussed. We provide a short summary of these observations before discussing the effects of the treatments on iron and manganese.

Total inorganic carbon profiles of the treatments showed different patterns. At the start of the experiment ΣCO_2 profiles were classically shaped. Concentrations were low in the top-layer ($\sim$ 4 mM) and increased with depth ($\sim$ 10 mM). In the control cores, ΣCO_2 build up to 29 mM in the deepest layer during the experiment. The irrigation treatment resulted in a low ΣCO_2 concentration in the top 4 cm (3.5 - 7.5 mM) and a high concentration (up to 17.5 mM) deeper in the profile. Mixing treatment had a similar pattern as ventilation, but accumulation of ΣCO_2 in the deeper layers was slightly higher (22.2 mM). In the *Nereis* cores only low ΣCO_2 concentrations were observed in the top 6 cm (3.5 - 4.5 mM) but in the deepest layer ΣCO_2 increased to 9.5 mM. Mineralization rates, as calculated from the measured fluxes and changes in pore water inventories ΣCO_2, are given in table 1. Highest production rates were seen in the *Nereis* treatment, followed by ventilation, mixing and the control. Van Nugteren et al. 2002 concluded that improved transport of dissolved gases is more important with respect to mineralization enhancement than pure physical mixing of organic and mineral particles.

Table 1. Mineralization rates (μmol cm^{-2} d^{-1}), as calculated from the measured fluxes and changes in inventory porewater ΣCO_2, data from Van Nugteren et al. 2002.

Treatment	Mineralization rate (μmol cm^{-2} d^{-1})
Control	9.4
Nereis	21.2
Ventilation	14.9
Mixing	10.7

4.5.2.   Biogeochemical interactions

All results together provide an insight in the biogeochemical differences between the treatments.

Shape and concentration levels of porewater profiles of dissolved Fe (II) and dissolved Mn (II) contain information on the 'net' process and vertical distribution of iron- and manganese-oxide reduction and available sources of reducible metal-oxides. All iron and manganese related reactions are included in these profiles. Profiles with a clear subsurface maximum in Fe (II) or Mn (II) indicate a metal-oxide reduction zone (Thomas and Bendell-Young 1999). Reduced iron or manganese can diffuse out of the reduction zone. Downward diffusion of reduced

iron results in a reaction with sulphide and consequently acid volatile sulphide or pyrite (FeS_2) formation (Murase and Kimura 1997, Pyzik and Sommer 1981, Jorgensen 1982, King 1990). Upward diffusing reduced iron or manganese can be re-oxidized, chemically or microbial, with oxygen (Nealson 1978, Santschi et al. 1990, Canfield 1989) or, in the case of Fe (II) (Postma 1985, Postma and Appelo 2000), with Mn-oxides forming a (sub) surface peak of newly formed iron oxides (Aller 1990, Sundby and Silverberg 1985). Low porewater concentrations imply low reduction rates or fast Fe (II) / Mn (II) removal by one of the processes described above.

The fluxes out of the sediment to the overlaying water reflect net metal-oxide reduction and the ineffectiveness of the sediment processes to retain all reduced metals within the profile, either by re-oxidation or by reaction with other constituents such as products of sulphate reduction. Epping et al. 1998 studied iron and manganese oxidation in sediment covered by microalgae. During benthic oxygenic photosynthesis there is sufficient oxygen to oxidise reduced components. Thus there is precipitation of Fe and Mn oxides and a low flux of reduced metals to the overlying water. Thamdrup et al. 1994b indicated that the balance between oxidation of Mn (II) in the oxidized zone and escape to the overlying water depends both on oxygen penetration depth (i.e. area of oxygenated sediment) and the intensity of Mn-oxide reduction.

The porewater concentrations of dissolved Fe (II) in the control cores decreased from 196 μmol l^{-1} to 30 μmol l^{-1} in the subsurface maximum and from 54 μmol l^{-1} to 33 μmol l^{-1} in the deepest layer with lowest concentrations (Fig. 2a) indicating a lack of net metal oxide reduction. The cores supported no flux of Fe (II) to the overlying water and the flux of Mn (II) is rather large (Fig. 1a). The low porewater concentrations of iron and manganese, the low mineralization rates and the black colour of the sediment indicate that sulfate reduction is the dominant anaerobic mineralization process in these cores, and bacterial metal oxide reduction has decreased. Due to the inefficiency of processes causing retainment of manganese almost all manganese reduced in the control sediments could escape to the overlying water. Whereas Fe (II) reacts with products of sulfate reduction forming iron sulphide (AVS) and pyrite (FeS_2). These solid-phase sinks are not recycled so that no metal cycling occurred. As a consequence the sediment became depleted in reactive iron- and manganese oxides and bacterial metal oxide reduction diminished.

Literature data indicate that bioturbation stimulates benthic metabolism and that aerobic mineralization usually increases at the expense of anaerobic mineralization (Kristensen 2000; Banta et al. 1999, Herman et al. 1999). Consistently, *Nereis* cores

had highest mineralization rates (Table1). The low porewater concentrations of dissolved Fe (II) and Mn (II) (Fig. 2) and negligible fluxes of dissolved Fe (II) and Mn (II) (Fig. 1) all indicate a shift to more aerobic mineralization in the *Nereis* treatment. The decrease in dissolved Mn (II) in the porewater as a result of bioturbation was also observed by Watson and Frickers 1990 in their experiment of 14 days with the burrowing polychaete *Nereis (Hediste) diversicolor* (1400 individuals m^{-2}). Dissolved Mn (II) decreased with ~0.6 μmol Mn l^{-1} d^{-1} in the Mn-oxide reduction layer in their cores (0-5 cm depth), whereas we observed a decrease of ~ 0.25 μmol Mn l^{-1} d^{-1} (1-5 cm depth). In contrast to our results the decrease in their cores was accompanied by a flux of manganese to the water column. This difference can be attributed to bioturbation intensity and initial sediment conditions. In our *Nereis* treatment the iron-oxide reduction zone has moved into the sediment relative to the other treatments (Fig. 2a). The small dissolved Fe (II) maximum in the 6 through 8 cm layer of the *Nereis* sediment likely reflects the active zone of iron oxide redcution. However, the Mn-oxide reduction zone that would be expected above the Fe-oxide reduction zone was not found. This could be due to intensive particle mixing in these cores and the relatively large interval (1 or 2 cm) of the slices. The deepening of iron-oxide reduction zone is consistent with the model of Herman et al. 1999 that predicts a deeper average depth of mineralization and a less reduced sediment as a result of particle mixing accompanied with irrigation. Unfortunately our sediment cores were not long enough to reveal changes in deeper, more anaerobic layers.

The ventilation cores represent the effect of increased oxygen input and removal of dissolved carbon dioxide without concurrent mixing of sediment. The enhanced oxygen input results in an enlargement of the oxic and sub-oxic area (Aller 1983), which in turn supports a larger area with metal-oxide reduction and re-oxidation. This may account for the high reduced metal concentrations in the porewater of the ventilation cores when compared with the control and the *Nereis* treatment. The effects of an increased oxygenated surface are also reflected in the Fe$_{asc}$ profile (Fig. 4). These changes are consistent with documented effects of burrows on ammonium and total organic carbon cycling (Aller and Aller 1992, Kristensen and Hansen 1999, Van Nugteren et al. 2002). The mineralization rate in the ventilation treatment is higher than in the control (Table 1), indicating that the overall metabolism has been stimulated (Aller and Aller 1998, Kristensen 2000, Dauwe et al. 2001).

Porewater Fe (II) concentrations in the ventilation treatment are high (Fig. 2) likely due to the larger sub-oxic sediment volume. Dissolved metal concentrations are high and Fe (II) and Mn (II) escape to the overlying water mainly during the initial

period (Fig. 1). Although some reduced metal can be re-oxidized around the tubes, apparently this is not sufficient to retain metals in the sediment. Eventually, this results in a net decrease of available oxides in the sediments and consequently a decrease in metal-oxide reduction. In theory, despite of the additional oxygen, iron (or manganese) oxide reduction will end in due time, as no cycling occurs because of the lack of a particle mixing component. Moreover, in natural sediments porewater flushing in sediments results in the loss of degradation products like dissolved Mn (II) and Fe (II). This may result in less efficient iron and manganese cycling if significant amounts of iron and manganese are lost without replenishment from the overlying water or settling particles.

The high dissolved Fe (II) concentrations in the porewater of the mixing cores are indicative of iron oxide reduction and an active iron cycle. Apparently sediment mixing resulted in a shift towards the optimal conditions to maintain the iron cycle. Reduced iron (in dissolved and particulate form) is mixed upwards where it is re-oxidized with manganese oxides or oxygen. The formed iron-oxide minerals will subsequently be transported into the iron-oxide reduction zone. Iron is well retained in the mixing sediment since dissolved Fe (II) effluxes are very small (only little higher than those of the bioturbated sediments). A zone with a high concentration of reactive oxides can be expected in such a profile due to the re-oxidation of reduced metals and the subsequent precipitation in oxygenated surface layers (Burdige 1993, Slomp et al. 1997). Porewater profiles of dissolved Mn (II) and a high flux of dissolved Mn (II) indicate Mn-oxide reduction in the very near surface layer and support the idea of ferrous iron oxidation coupled to manganese oxide reduction. This re-oxidation of iron allows efficient retention of iron within the sediment. The efflux of dissolved Mn (II) is relatively large because the Mn-oxide reduction zone is close to the surface and the diffusion distance is too short for the slow Mn oxidation kinetics to completely re-oxidize Mn (II) (Sundby and Silverberg 1985).

The differential effects of ventilation and particle mixing on iron and manganese cycling are best illustrated by comparing the ventilation and mixing treatment. Particle mixing results in a redistribution of reduced particulate and adsorbed metals into the upper layers which otherwise would not have happened. This stimulated iron and manganese oxidation and reduction without a major change in the area of oxic to anoxic sediment with the consequence that more manganese could escape. The higher ammonium accumulation in the porewater of the mixed sediment (Van Nugteren et al. 2002) also indicate that these sediments are less oxic than the ventilation and *Nereis* treated sediments. In the ventilation treatments oxygen is transported into the sediment resulting in a longer time for re-oxidation

of the reduced metals before they reach the sediment surface. Van der Zee 2002, described the escape of reduced manganese to the water column when manganese reduction occurs near the sediment surface for Iberian margin sediments. In these sediments the rate limiting steps in the manganese cycle were slow oxidation kinetics and particle mixing whereas for the iron cycle only particle mixing was rate limiting. Our experimental observations support their field data in the importance of particle mixing for iron and manganese cycling and the importance of irrigation for Mn-oxidation.

4.5.3. Turnover times

To obtain insight in the metal cycling efficiency and dynamics it is instructive to calculate turnover times. These are calculated by dividing the pool of Fe_{dith} (here calculated by the excess of reactive iron-oxides compared to the background values) by the dissolved Fe (II) production, which is calculated from measured fluxes and porewater inventory changes. It should be mentioned that the metal cycling efficiency, i.e. the number of cycling times before burial, is more relevant than the turnover time because it gives the potential amount of organic matter that can be mineralised by one metal molecule (Canfield et al. 1993b, Wijsman et al. 2001b). Unfortunately we can not calculate this number because the natural processes of bioturbation and sedimentation are missing in most of the cores (except of bioturbation in the *Nereis* treatment). It should also be noted that turnover times as presented in Table 2a & 2b are maximum values because metal oxide reduction rates can be underestimated due to re-oxidation of reduced Fe and Mn and other side reactions. A long turnover time indicates a slow or incomplete cycling of the metals, whilst a short turnover time indicates either a high metal oxide reduction rate (i.e. a large flux or a large increase in porewater concentrations) or a small metal oxide pool. Due to low fluxes, turnover times of Fe and Mn calculated for the *Nereis* cores were longest, also in these cores flux direction is opposite (i.e. into the sediment) from the other fluxes (in Table 2a and b indicated by italic numbers).

The turnover times of Fe (< 10 days, Table 2a) are somewhat shorter than those reported for other sediments. Thamdrup and Canfield 1996 found Fe turnover times of 70 days in sediments from the slope of Chile. In the Skagerrak Canfield et al. 1993b calculated Fe and Mn turnover times of 70-250 days. Their turnover times are based on the dissimilatory iron-oxide reduction rates (i.e microbial iron oxide reduction coupled to carbon oxidation) only. Turnover times based on the total iron-oxide reduction (i.e. including the iron reduction coupled to sulfide oxidation) are faster, e.g. 46 to 61 days in the Slope of Chile. Our estimates

represent total iron-oxide reduction, because we have not differentiated the metal oxide reduction pathways. The fast turnover time in the control treatment resembles those of Van der Nat and Middelburg 1998. They reported a turnover time of 4 days for iron in a vegetated tidal freshwater marsh (without macrofauna). The pools of reactive iron oxides (dithionite extractable iron) in the different treatments ($\sim$ 430 µmol cm^{-2}) are similar to the values of Skagerrak, 350-400 µmol cm^{-2} (Canfield et al. 1993b) but higher than those of the Slope of Chile, 39-77 µmol cm^{-2} (Thamdrup and Canfield 1996). This is also the case for dissolved Fe (II) production rates (our cores, except the *Nereis* treatment: 0.9-1.1 µmol cm^{-2} d^{-1}); Skagerrak: 4.4-5.7 µmol cm^{-2} d^{-1}; Slope of Chile: 0.14-0.27 µmol cm^{-2} d^{-1}).

Table 2a. Fe-oxide reduction rates (µmol cm^{-2} d^{-1}), excess dithionite extractable Fe oxide (µmol cm^{-2}), pool size of ascorbate and dithionite extractable iron (µmol cm^{-2}), turnover times (days) and total importance iron plus manganese oxide reduction in organic carbon mineralization (%).

Treatment	Fe (II) production	Excess Fe$_{dith}$ (µmol cm^{-2})	Fe$_{asc}$ pool (µmol cm^{-2})	Fe$_{dith}$ pool (µmol cm^{-2})	Fe turnover time (days)	Fe & Mn in mineralization
Control	0.92.	5.61	195	433	6.10	0.6
Nereis	-2.61	22.78	183	429	*8.75*	0
Ventilation	2.93	11.26	206	430	3.83	8.1
Mixing	1.11	2.06	187	434	1.85	3.0

Table 2b. Mn-oxide reduction rates (µmol cm^{-2} d^{-1}), excess dithionite extractable Mn oxide (µmol cm^{-2}), pool size of ascorbate and dithionite extractable manganese (µmol cm^{-2}), turnover times (days) and total importance manganese oxide reduction in organic carbon mineralization (%).

Treatment	Mn (II) production (µmol cm^{-2} d^{-1})	Excess Mn$_{dith}$ (µmol cm^{-2})	Mn$_{dith}$ pool (µmol cm^{-2})	Mn$_{dith}$ pool (µmol cm^{-2})	Mn turnover time (days)	Mn in total mineralization
Control	0.15	0.33	2.0	18	2.23	0.1
Nereis	-0.02	0.91	1.3	19	*42.5*	0
Ventilation	0.04	0.26	1.5	18	6.35	0.2
Mixing	0.09	0.12	1.3	18	1.30	0.4

Mn turnover times (1.3 − 42.5 days, Table 2b) agree with those found in Long Island Sound, 0.37-344 days (Aller 1994b and the Skagerrak, 250 days Canfield et al. 1993b). In deep-sea sediments from Panama basin turnover times at high particle mixing were 365 days, low particle mixing resulted much longer turnover time, 1241 days (Aller 1990). The excess Mn_{dith} in our cores, 0.12-0.91 $\mu mol\ cm^{-2}$ (Table 2b) is lower than values of excess manganese in Long Island Sound and Panama basin, 5 -10 $\mu mol\ cm^{-2}$ and 173 $\mu mol\ cm^{-2}$ respectively. The pool of dithionite extractable manganese (~ 18 $\mu mol\ cm^{-2}$) is much smaller than in the Skagerrak, 900 $\mu mol\ cm^{-2}$, (Canfield et al. 1993b). Dissolved Mn (II) production rates (0.04 − 0.4 $\mu mol\ cm^{-2}\ d^{-1}$) were much higher than in Panama Basin, 0.002 $\mu mol\ cm^{-2}\ d^{-1}$,and lower than rates found in Long Island Sound, 13.3-29.0 $\mu mol\ cm^{-2}\ d^{-1}$ and Skagerrak (3.64 $\mu mol\ cm^{-2}\ d^{-1}$). The relatively small size of excess Mn_{dith} and high dissolved Mn (II) production underly the fast manganese turnover observed in our experiment.

4.5.4.   Mineralization pathways

The contribution of metal oxide reduction to carbon oxidation is variable among sediments. In most sediments the contribution of iron oxide reduction to the organic carbon mineralization is much higher than for manganese oxide reduction. The production of dissolved Mn (II) is often completely attributed to chemical reduction with Fe (II) (Postma 1985, Canfield et al. 1993b, Kostka et al. 1999b). Total iron oxide and manganese oxide reduction rates can be converted to carbon respiration rates via the stoichiometry of the dissimilatory reduction process (Froelich et al. 1978). Together with mineralization rates, based on total inorganic carbon during incubation, the relative importance of the metal-oxide reduction processes for the mineralization heve been calculated (Tables 2a & 2b). In control cores Fe and Mn together accounted for 0.6 % of mineralization. Calculations for ventilation and mixing treatments were 8% and 3 % respectively. In *Nereis* cores no net metal-oxide reduction was observed indicating that the overall effect of bioturbating fauna could not be described by either the ventilation or mixing alone and that their mutual effect was not additive. In treatments with detectable metal-oxide reduction the contribution of Mn-oxide reduction was very small, 0.1-0.4 %. The contribution of iron oxide reduction in the ventilation treatment approached but remain lower than those found for the Slope of Chile, 12-29 % (Thamdrup and Canfield 1996). Rates of metal oxide reduction in the Skagerrak (site S4 and S6) are much higher, 21-78 %, with a maximum contribution of Mn-oxide reduction of 5-14% (Canfield et al. 1993b). In Long Island Sound manganese-oxide reduction accounts for 3-4 % of mineralization (Aller 1980).

4.5.5. Porewater profiles

The porewater profiles contain clear indications for metal-oxide reduction. At the start of the experiment the profile indicate a lack of manganese oxide reduction and the presence of iron oxide reduction at 2-3 cm depth (t=0, fig. 2a and b). Because of the fast turnover rates, the presence of metal-oxide reduction at the end of the experiment reflects the presence of metal-oxide sources. In the control treatment metal-oxide reduction has diminished because of the lack of an oxide source and porewater concentrations of Fe (II) and Mn (II) are low throughout the profile . The porewater profiles of the *Nereis* treatments showed a small increase of Fe (II) at 6-8 cm depth. In these treatments the iron oxide availability is ensured by the particle mixing and irrigating activity of the worms. In contrast Mn concentrations were low in the whole profile, apparently the irrigation activity of *Nereis* is not sufficient for manganese to be re-oxidized efficiently and to complete the cycle (see paragraph on difference between iron and manganese).

Despite the lack of particle mixing the porewater profiles of iron and manganese in the ventilated sediments show clear indications of iron- and manganese oxide reduction. This implies the presence of a mechanism, either biotic or abiotic, to resource the reducible metal oxides in the reducing layer without particle mixing. Recently Sobolev and Roden 2001 reported on the suboxic deposition of ferric iron in opposing gradients of Fe (II) and O_2. They suggest that some type of Fe (II) oxidising bacteria produce mobile forms of Fe (III) as the initial product. These compounds which are formed at the oxic-anoxic interface are subject to diffusion, destabilise and hydrolyse and before they precipitate as Fe (III) oxides. The Fe (III) oxides can subsequently be used in iron oxide reduction, thereby providing a rapid and small scale coupling between oxidation and reduction. Furthermore, they suggested that the process was able to compete with abiotic iron oxide formation. Results of the ventilated sediment cores are consistent with the presence of such a microcycle. Due to the enlarged oxic-anoxic interface the process can cover a large surface and result in an significant role for iron oxide reduction despite the lack of a particle mixing component as required in the macro cycle with non-mobile oxidized metal oxides. The profiles of Mn could be explained by a similar process. However, the existence of a manganese micro-cycle has not yet been investigated.

4.5.6. Iron and manganese

Porewater profiles of iron and manganese differ among and within treatments, indicating the differential behaviour of these two metals. Known differences between iron and manganese include (1) manganese oxide reduction has a higher

energy gain than iron oxide reduction (Froelich et al. 1978) generally resulting in Mn-oxide reduction occurring before and thus above iron-oxide reduction, (2) Fe (II) can be re-oxidized in a micro cycle (Sobolev and Roden 2001), thereby decreasing the importance of bioturbation components for complete cycling (see above) (3) Fe (II) can be re-oxidized with Mn-oxides (Postma 1985, Burdige et al. 1992) (4) oxidation kinetics of Fe (II) are faster than that of Mn (II) (Stumm and Morgan 1981), (5) Fe (II) can react with hydrogen sulfide or disulphide with the result of sulfide mineral formation (Jorgensen 1977, Pyzik and Sommer 1981, Moeslund et al. 1994), while Mn (II) can react with a carbonate surface or precipitate in the form of Mn,Ca -carbonate (Middelburg et al. 1987). This differential behaviour of Mn and Fe has consequences for their recycling efficiency and their role in organic carbon mineralization.

While Mn in our experiments mainly escapes recycling by efflux to the overlying water, iron escapes recycling by a flux to the overlying water and reaction with sulphides to form solid phase iron sulphides. Therefore the sulfidic conditions in the control cores, had a larger effect on iron recycling than on manganese recycling.

Enhanced irrigation, in our experiment represented as enhanced ventilation, resulted in an additional oxygen input and an increased area of oxygenated surface. The effect on the biogeochemical processes depends on the specific conditions. Oxidation kinetics for iron is much faster than for manganese (Stumm and Morgan 1981). Thus addition of oxygen, even small amounts, affects iron on a very short time scale. Furthermore, a short period of enhanced oxygen penetration or a small oxygenated layer is sufficient to retain significantly more iron in a sediment. For Mn these conditions have to last longer and have to be more extensive (e.g. a larger aerobic layer). Irrigation may result in (1) a larger metal oxide pool (2) a smaller flux out of the sediment and (3) an increase in the number of times these metals are recycled. In our experiments the effect of ventilation is not straightforward. The Fe_{asc} concentrations indeed are significantly higher in the ventilation treatment than in the others, but for Mn-oxide such a difference is not observed, though the ventilation period was long and there was a steady supply of oxygen. Iron effluxes in the ventilated cores are not different from those in the other treatments. Although not significant, there seems to be a trend that Mn-fluxes are lower due to ventilation (Fig. 1b). A porewater peak of Mn (II) just below the sediment interface might reflect microbial Mn-reduction or chemical manganese reduction coupled to oxidation of reduced iron. This should be reflected in an increase of iron oxides at the depth of the dissolved Mn (II) peak and a decrease in Mn_{dith}. There is some indication for this (Fig 3a, 3b) but this is not very clear because of the coarse

82

resolution of our data and the large differences between relative changes in Mn-oxide concentrations and Fe-oxide pools. The enhanced oxygen supply increases the porewater concentrations of Mn (II) and Fe (II) about 3 and 2 times, respectively. Apparently, the effect of a larger sub-oxic area is relatively more important for Mn than for Fe. The limited increase of dissolved Fe (II) could however be due to a faster re-oxidation near the site of liberation. Altogether the effect of oxygen on manganese appears to be larger than that on iron.

In the mixing treatment re-oxidation of Fe (II) with Mn-oxides and the mixing of the particles are essential for maintaining the iron cycling. Manganese oxide reduction coupled to Fe (II) oxidation affects the relative importance of Mn-reduction in organic matter mineralization, because less Mn-oxides will be available for Mn-oxide based respiration. In the mixing treatment the Mn-oxide reduction layer is very close to the sediment surface with the results that most Mn (II) diffuses to the overlying water causing depletion of Mn in the sediment. This in turn results in a decreasing role, directly and indirectly, in the organic carbon oxidation. As long as Mn-oxides are present to re-oxidize reduced iron the mixing events stimulate the iron-oxide reduction more than the ventilation treatment. Furthermore, iron oxide reduction rate is increased 1.6 times relative to the ventilation treatment.

The study of Sundby and Silverberg 1985 , showed that the cycling rate of Mn in the Gulf of St. Lawrence was tightly coupled to Mn (II) production rates and depended mostly on particle mixing. In these sediments maxima of dissolved Mn (II) were at 4-10 cm depth. This is much deeper than in our cores and allows a better retainment of Mn in the sediment. Thus Mn-oxide reduction rates in the mixing cores could have been stimulated if the reduction layer would have been deeper in the sediment. Aller 1994b concluded that Mn can be important as intermediate electron acceptor during periods of high bioturbation with well oxygenated overlying waters and moderate organic carbon fluxes. Some diagenetic modelling studies have explored the importance of mineralization pathways as a function of organic matter load and provided an insight on the effect of the organic carbon load on the metal cycling (Soetaert et al. 1996, Wijsman et al. 2002). With increased organic matter loading the iron oxide reduction increases at the expense of manganese oxide reduction. In Aller 1990, Banta et al. 1999, Kristensen 1985 and our experiments it was shown that animal activity decreases the importance of anaerobic mineralization. Parallel increases of faunal biomass, bioturbation, and estuarine productivity allow for a longer lasting period with a high importance of the metal-oxide reduction pathways in organic matter mineralization. The resulting environmental conditions are more favourable for

benthic fauna than those resulting from sulfate reduction, with the consequence that there is positive feedback between animals and sediment biogeochemistry.

4.6.   Conclusions

The effect of bioturbation on iron and manganese cycling depends on particle mixing and sediment irrigation. Results show that both components are necessary to complete the cycle and maintain the sub-oxic mineralization pathways using metal-oxides. Particle mixing, in the experiment mimicked by a mixing device, is important for redistribution of (re) oxidized metal-oxides. Irrigation, here imitated by enhanced sediment ventilation (i.e. enhanced exchange of porewater dissolved gases), is essential for the input of dissolved oxidants. However, this aspect is more important for Mn than for Fe because Fe has a higher affinity for oxygen and can also re-oxidize with Mn-oxides, while, because of the slow oxidation kinetics, a longer oxygen-Mn (II) contact is needed for sufficient retainment in the sediment. Therefore the efficiency of Fe-cycling is determined primarily by particle mixing. Both particle mixing and irrigation determine Mn-cycling efficiency. Our results indicate that the combined effect of ventilation and particle mixing is not simply additive.

5.   Iron and manganese pools and reduction rates in tidal freshwater, estuarine and deep-sea sediments

Imola Ferro, Jack, J. Middelburg, Ralf R. Haese, Caroline P. Slomp

5.1.   Abstract

Recent studies have reported the importance of iron and manganese oxides as electron acceptors for the degradation of organic matter in a number of sediments. The contribution of metal oxide reduction in mineralization varies significantly and depends on the concentration of degradable organic carbon, the physical-chemical properties of metal-oxides and their transport to the depth of dissimiliatory reduction. Modelling studies and slurry incubations suggest that the importance of dissimilatory metal oxide reduction is highest at intermediate carbon fluxes to the sediment. However, no rate data have yet been presented to confirm these findings. Here, we present anoxic bag incubations to investigate the importance of metal-oxide reduction with respect to organic carbon degradation in three sediments covering a wide range of carbon inputs. Metal oxide reduction rates are compared with overall mineralization rates and other degradation processes. The relative importance of iron oxide reduction was highest in an organic carbon and iron oxide rich freshwater sediment and lowest in an organic carbon and iron oxide poor deep-sea sediment, with an estuarine sediment being intermediate. The manganese oxide reduction rates were low in all the sediments. Likewise, the importance of the other pathways changed up on conditions. With an important role for aerobic degradation and denitrification in organic poor sediment, a large role for sulphate reduction in the estuarine sediment and high denitrification rates in organic matter and nutrient rich freshwater sediment.

5.2.   Introduction

Following the discovery of iron oxide and manganese oxide reducing bacteria (Lovley and Phillips 1988, Ehrlich 1993) dissimilatory metal oxide reduction has intensively been studied (Thamdrup 2000). These studies not only cover microbial aspects but also early diagenetic processes (Canfield et al. 1993b). The relative importance of metal-oxide reduction in total organic matter mineralization has been found to depend on a number of factors: (1) The organic carbon load (Wijsman et al. 2001a, Wijsman et al. 2002, Canfield 1994). (2) The concentration

and reactivity of iron and manganese oxides (Chapter 2,Munch and Ottow 1980). (3) Interactions with other degradation pathways (Canfield et al. 1993b, Canfield and Thamdrup 1996, King 1990, Myers and Nealson 1988) and (4) Environmental and ecological conditions affecting the transport and availability of metal oxide and organic matter (e.g. sediment reworking, grain size and reactive surface area, Aller 1994b, Roden and Zachara 1996, Chapter 4)

In organic-rich freshwater sediments, iron oxide reduction and methanogenesis are generally the most important anaerobic mineralization pathways (Roden and Wetzel 1996, Van der Nat and Middelburg 1998). In contrast, in most organic-rich ocean margin sediments sulphate reduction accounts for the majority of mineralization (Jorgensen 1982, Canfield et al. 1993b, Thamdrup and Canfield 1996), but iron and manganese oxide reduction has been found to account for a relatively large part of the anaerobic organic carbon mineralization as well, e.g. in the Skagerrak (Canfield et al. 1993b). Solid phase intermediate electron acceptors may play an important role in organic carbon mineralization in highly bioturbated sediments (Aller 1994b). In contrast to organic rich sediments the pathways of organic carbon degradation in oligotrophic sediments are dominated by aerobic degradation and denitrification (Soetaert et al. 1996, Wijsman, Herman, Middelburg, and Soetaert 2002). Denitrification can also be an important pathway in nutrient rich environments, as found in the Scheldt estuary (Middelburg et al 1995).

In an experimental study (Chapter 3) we have elucidated relations between reactive iron-oxide and organic carbon availability and iron oxide reduction rates, using sediment slurries enriched with either iron oxide, organic carbon or both. The effects of iron oxide and organic carbon additions were different for different sediment types. Freshwater organic-rich sediment only showed increased iron-oxide reduction rates upon addition of iron oxides, an estuarine sediment primarily responded to organic carbon addition and a deep-sea surface sediment needed both iron and carbon additions to increase iron-oxide reduction rates. In this study we investigate whether differences in limiting factors as identified in slurry incubations agree with the size of iron and manganese oxide pools and iron and manganese oxide reduction rates in three sediments that differ in organic carbon loading. Additionally, we assessed the extent to which other reactions affect measured iron oxide reduction rates. Therefore, molybdate and ferrozine were used as inhibitors for sulfate reduction and the reaction of Fe (II) with other constituents, respectively.

5.3.   Material and Methods

5.3.1.   Sites

Sediment was collected at a tidal freshwater site, a sub-tidal estuarine site and two deep-sea stations. Geographical location and water depth are presented in Table 1. All sites were sampled between September 1998 and September 1999. Sediments were collected by box coring unless indicated otherwise.

The deep-sea sites are located in the Eastern Mediterranean Sea, Florence Rise (core SL10BC) and Eratosthenes Seamount (core SL22MC). Samples were taken during a cruise with R.V. Logachev in August-September 1999 with a boxcore (BC) or multi-core (MC). The sub-tidal estuarine site is situated in Lake Grevelingen (The Netherlands). This site was sampled twice: in September 1998 and in February 1999. The intertidal freshwater site is in the tidal reach of the Scheldt River, Belgium, and located near Appels. Cores were taken manually during low tide in October 1998.

Table 1. Characteristics and co-ordinates of sample sites. [a] Values in aerobic sediment (25) and sapropel (40) [b] Organic carbon content of the sapropel S1. [c] Value in top layer decreases to the value in the deeper layer

	Co-ordinates	Depth (m)	Salinity	BET-N2 specific surface area (m^{-2} g^{-1})	Median grain size (μm)	Organic carbon (mg g^{-1})
Florence Rise	34.47.17 N 31.31.38 E	2300	38	25-40[a]	Na	2.7 - 24.0[b]
Eratosthenes Seamount	33.45.04 N 32.50.41 E	970	38	na	Na	2.7 - 12.4 [b]
Grevelingen September	51.45.78 N 03.58.47 E	3	28		224 – 300[c]	2.0 – 0.8[c]
Grevelingen February	51.45.78 N 03.58.47 E	3	28	2.1-0.9 [c]	282	1.5 – 3.2[c]
Appels	51.02.43 N 04.05.37 E	0	0.7	4.8	63.5	22.9

5.3.2.   Sampling & slicing

Sub-samples were taken with Plexiglas cores (i.d. 10 centimetre for the bag incubations), except for those Mediterranean samples taken by multi-coring and manually cllected cores at Appels. Cores were cut into appropriate slices (0.25 – 4 centimetre). These were put into diffusion tight plastic bags (made of plastic coated

aluminium foil), flushed with nitrogen and sealed. Samples for solid phase iron extractions were kept at 4°C. At each site, parallel samples were taken and freeze-dried for determination of water content and total destruction. At Grevelingen and the Mediterranean stations a parallel core was used for microelectrode measurements (O_2 and HS- data). Additionally, for the Mediterranean sites the the porewater profiles result from another box core, with the sapropel starting at 31.5 centimetre.

The samples for the Mediterranean porewater profile were collected as follows. Subcores were sliced at in-situ temperature (13°C) in a nitrogen-filled glovebox immediately after collection. Pore water was collected by centrifugation of sediment in disposable polypropylene tubes (15 min., 4400g) and subsequent filtration of the overlying water (Acrodisc polysulfone filters, 0.2 μm). All pore water samples were split into several portions under nitrogen. Subsamples for Fe and Mn analyses were acidified to pH 1 with 12 M HCl (Slomp et al. 2002). Porewater collection of Appels and Grevelingen samples was similar to collection of incubated sediments (see section on incubation).

5.3.3. Incubations

Anoxic incubations were done to determine rates of iron and manganese oxide reduction. At each site 3 – 8 sediment cores were sliced, flushed with nitrogen and sealed in 5-8 series of profiles (for Mediterranean sediments slices were split over two incubation bags). All incubations were done at in situ temperature. The Grevelingen incubations consisted of three treatments: amendment with 20 mM molybdate (inhibition of sulphate reduction), amendment with 20 mM molybdate and 2 g l^{-1} ferrozine (inhibition of sulphate reduction and chelation of Fe (II)), and a control treatment. Mediterranean and tidal freshwater sediments were not amended.

Total incubation time was different for each series, ranging from 23 days for Grevelingen September 1998 series to 121 days for the series of Mediterranean deep-sea sediments.

All handling was done within an anoxic chamber (Coy Laboratory Products) filled with > 97 % N_2 and < 3 % H_2. Sediment of Appels and the Mediterranean Sea was transferred into tubes and centrifuged (15-30 min, at ~ 355 G). For the Grevelingen sediments, a modification of the system for sandy sediments (Saager et al. 1990) was used rather than normal tubes (15 min centrifugation, ~355 G). After centrifugation, samples were filtered over a 0.45 μm cellulose/acetate filter. One portion of the porewater was capped in headspace vials and acidified with 50

μl 20 % H_2SO_4 ml sample^{-1}. Σ CO_2 and CH_4 were measured in the headspace of acidified samples. Afterwards the samples were used for metal analyses. The other part of the porewater was stored frozen in Eppendorf vials and used for later SO_4 analyses.

Initial production rates of all measured constituents have been calculated from solute concentration changes in the bag incubations. Linear regression was done on the linear part of the concentration vs. time plot, starting at day 0.

5.3.4. Solid-phase extractions

Subsamples of sediment for solid phase extractions were taken before incubations. Samples for determination of water content were taken from parallel cores. A number of extractions were used to characterise the pools of iron and manganese oxides. Ascorbate extractions were performed in an anoxic chamber (Coy Laboratory Products, > 97 % N_2 and < 3 % H_2).

The pool of amorphous Fe (III) oxides (Fe_{asc}) was extracted using 5 ml of an anoxic solution of 10 g sodium citrate, 10 g sodium bicarbonate and 4 g ascorbic acid in 200 ml demineralised water (Kostka and Luther 1994) and ~ 0.3 g wet sediment. The extraction lasted about 24 hrs and the samples were continuously shaken in the dark. After filtration (0.45 μm cellulose / acetate filter) iron concentrations were analysed in the filtrate. The amorphous iron extracted with this method is considered the most reactive part of the total sediment iron pool.

The pool of dithionite extractable iron represents amorphous iron (III) oxides, crystalline iron (III) oxides and the pool of iron bound to acid volatile sulfides (FeS, Canfield 1989). Leachant (5 ml, 0.35 M acetic acid/0.2M Na-citrate solution 50 g l^{-1} dithionite) was added to ca. 0.3 g wet sediment. After 2 hours of shaking, samples were filtered over a 0.45 μm filter and the filtrate was acidified with 0.1 ml 6 N HCl ml filtrate^{-1}. Crystalline Mn-oxides extracted with this method were measured as well (Canfield 1989).

The pools of total sediment iron and manganese were measured on a freeze-dried split of the sediment. The method used HNO_3/HCl as destructant, a microwave for high temperature and a pressure digester (Nieuwenhuize et al. 1991).

5.3.5. Analyses

Gases in porewater were analysed using a Carlo-Erba gas-chromatograph, type MEGA 5340-00 equipped with a Haysep-Q column, 2m x 2 mm ID, mesh size 80-100 and a flame-ionisation detector for CH_4 and a hot-wire detector for CO_2.

Fe and Mn in acidified porewater samples were measured by Inductively Coupled Plasma –Optical Electron Spectrometry (ICP-OES, Perkin-Elmer Optima 330 DV,

using axial or radial views depending on concentration levels). In the Grevelingen September 1998 samples Fe and Mn were measured by Graphite-Furnace Atomic Adsorption Spectrometry with Zeeman background correction (Perkin-Elmer 3030 equipped with HGA 600 and AS 60). The first series (incubation time is zero) of (10x) diluted Mediterranean pore water samples were analysed for total Fe and Mn using a Perkin Elmer 4100 ZL Zeeman AAS. All samples were analysed in triplicate. Precision was generally better than 5% for Fe and 4% for Mn (Slomp et al. 2002). Mediterranean Sea samples were immediatey analyzed for NO_3^- on board with a Technicon TRAACS-800 autosampler (Grashoff et al. 1983). Precision was generally better than 1.5 %.

Dissolved iron and manganese concentrations in the total iron extracts were measured using a Flame Atomic Adsorption Spectrometre (Perkin-Elmer 2380, Grevelingen September) or ICP-OES (Appels, Grevelingen February, Mediterranean). The ferrozine method Stookey 1970 using a HEPES or reducing HEPES buffer was used for ascorbate and dithionite extractions in both Grevelingen September 1998 and Grevelingen February 1999 sediment. Results of ICP-OES and the ferrozine method were consistent.

The sulphate concentration in porewater samples was analysed by ion-chromatography with a Dionex auto-suppressed anion system (Ion Pac AS11 column with ASRS suppressor).

Pore water O_2 and HS^- were measured simultaneously with a gold amalgam voltametric micro-electrode (Brendel and Luther 1995) inserted in whole cores taken from the Mediterranean Sea and from Lake Grevelingen in February 1999. An elaborate description of this method can be found in Brendel and Luther 1995 and Luther et al. 1998.

5.3.6. General sediment properties

Total organic carbon was determined on freeze-dried and powdered sediment. Samples were combusted in a Carlo Erba Elemental Analyser NA-1500 after removal of carbonate within silver cups (Nieuwenhuize et al. 1994). Sediment specific surface area was measured on a surface area analyser (Quantachroom nova 3000 series) according to BET multipoint theory and using N_2 5.0 (produced by Hoek-Loos) as adsorbent. Sediment grain size spectrum was analysed on a Malvern Particle sizer 3600-EC with laser diffraction.

5.4. Results

5.4.1. General observations and sediment properties

An overview of the main site characteristics is provided in Table 1. The eastern Mediterranean sediments are hemi-pelagic muds. The Florence Rise cores contained 40 centimetre of sediment, and included the most recent sapropel (S1) starting at about 28.5 centimetre until the end of the profile. A sapropel is an anoxic organic-rich layer intercalated in organic-poor sediments (see Chapter 2 for a more elaborate description of a sapropel). The organic carbon content (Table1, Fig. 1a) was very low in the oxic sediment above the sapropel and increased in sapropel S1. Sediment profiles and observations during slicing indicated that a 2-3 centimetre thick layer of organic matter depleted sapropel S1 overlies the organic rich sapropel. This phenomena is well known and is caused by a post-depositional burn-down of organic matter by the oxygen (De Lange et al. 1989, Van Santvoort et al. 1996).Microelectrode profiles covered 25 centimetre of the profile and oxygen was measured until that depth. Visible signs of oxygen depletion (i.e. sediment colour changes from brown to dark-grey), were seen at 30 centimetre depth, i.e. down to the top of S1.

Characteristics of the Eratosthenes Seamount site are similar to those for Florence Rise. The largest difference is seen in the depth and thickness of the S1 layer. The core contained 28 centimetres of sediment and included the most recent sapropel (S1), which started at ca. 25.5 centimetre and had a thickness of about 2.5 centimetre. Organic carbon content (Table 1, Fig. 2a) was low in the oxic sediment and high in the sapropel. Depth profiles and observations during slicing indicated that a 3.5 centimetre thick layer of burnt down sapropel S1 preceded the sapropelic layer. Oxygen measurements reached to the whole profile. Visible signs of oxygen depletion were seen at 25.5 centimetre depth.

Grevelingen, the subtidal site in Lake Grevelingen had decreasing sediment organic carbon contents from the top-layer to the deepest layers in September. Concentrations February were more uniform (Table 1, Fig. 3a, Fig. 4a). Median grain size was different at both sampling events, and more variable in September than in February (Table 1). BET-N_2 specific surface area was 2.1 m^2 g^{-1} in the top-layer and decreased to 0.9 m^2 g^{-1} at 6-7 centimetre depth. The site is prone to erosion and little sediment accumulation occurs.

The sampling site at Appels is a freshwater intertidal sediment along the Scheldt River (Belgium) which is flooded twice a day (i.e. every tide). The sediment specific surface area is higher than in Grevelingen sediment, and the median grain size is smaller. The high organic carbon content (Fig.. 5a) is due to the high organic

carbon input by benthic algae, by the particulate organic carbon supply from the river Scheldt and from the vegetated marsh adjacent to the river bank.

5.4.2. Geochemistry

5.4.2.1. Florence Rise

The porewater oxygen concentrations gradually decrease until the end of the measured profile (Fig. 1.a).

Porewater Mn (II) is not detectable throughout the oxic part of the sediment (Fig. 1b), but increases with depth in the sapropel. Solid phase dithionite extractable Mn (Mn_{dith}) has slightly lower values than total Mn and shows the same basic profile with a double peak. The upper one indicates the marker bed (De Capitani and Cita 1996) at 24 centimetre, a Mn-rich layer that is an indicator of the top of the original sapropel (vanSantvoort et al. 1996,Thomson et al. 1999, Mercone et al. 2000). The lower Mn-peak is actively forming and is situated where dissolved oxygen and manganese disappear and is known to be diagenetically formed (Pruysers et al. 1993).

Porewater Fe (II) is also not detectable in the oxic sediment. The concentration increases at the transition from oxic to anoxic conditions i.e. at the top of the intact sapropel (Fig. 1c). The various iron oxide pools differ in size (Fig.. 1d). Ascorbate extractable Fe (Fe_{asc}) decreases about 50 % in the upper 5 centimetres and concentrations are very low throughout the whole core. Concentrations are significantly enriched around the upper boundary of the oxidized sapropel. Dithionite extractable Fe (Fe_{dith}, upper x-axis) concentration is approximately 10 times higher than Fe_{asc} in the oxic sediment and there is a clear peak in the oxidized sapropel. Moreover, Fe_{dith} abruptly decreases in the sapropel. Total iron (Fe_{total}, upper x-axis) concentrations in the oxic sediment are 2.5-4.5 times higher than Fe_{dith}. Fe_{total} in the sapropel is somewhat higher than in the overlying sediments.

Results of anoxic incubations with oxic and anoxic Florence Rise sediment are presented in Fig..1e. The hemi-pelagic sediments are poor in organic carbon and there is hardly any organic carbon input. This results in the low Σ CO_2 production observed for samples from the oxic part of the profile. The top-layer of the sediment contains some degradable organic carbon, there some mineralization was observed. The top of the organic carbon-rich sapropel had the highest rate of Σ CO_2 production. There was no significant manganese oxide reduction over the whole depth investigated. Besides a very small sub-surface peak, iron oxide reduction was detectable just above the sapropel and had highest rates in the upper

part of the sapropel and gradually decrease within the sapropel. This maximum in iron oxide reduction rates coincides with the peak in $\Sigma\,CO_2$ production.
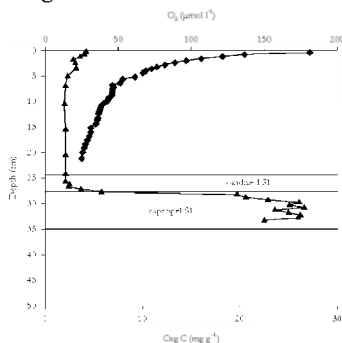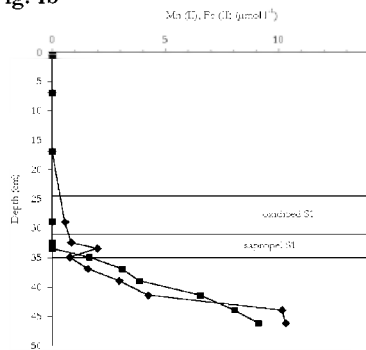
Fig. 1a

Fig. 1b

Fig. 1c
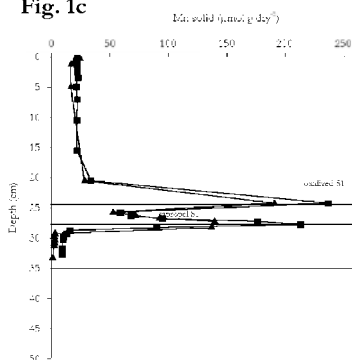
Fig. 1d
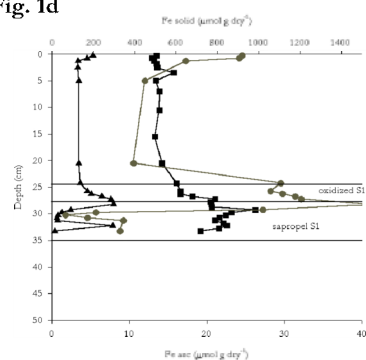
Fig. 1e
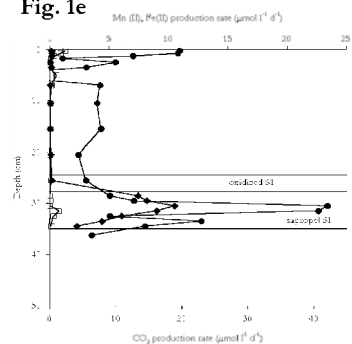
Fig. 1. Results of Florence Rise, N.B. porewater data result form a different box core. a. O2 (μmol l^{-1}) – diamonds, organic carbon (mg g sediment^{-1}) – triangles. b. Dissolved Fe (II) (μmol l^{-1}) – diamonds, dissolved Mn (II) (μmol l^{-1}) – squares. c. Mn dithionite (μmol g^{-1}) - triangles, Mn total (μmol g^{-1}) - squares d. Fe ascorbate (μmol g^{-1}) - circles, Fe dithionite (μmol g^{-1}) - triangles, Fe total (μmol g^{-1}) - squares e. Production rates (μmol l^{-1} d^{-1}) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares and Σ CO$_2$ - circles.

5.4.2.2. Eratosthenes Seamount

The sediments of Eratosthenes Seamount (Fig. 2) show similar patterns as the Florence Rise sediments, though the depth of the sapropel is shallower and the profiles are more confined. Oxygen decreases within the profile. Organic carbon content is low, except in the intact sapropel (Fig. 2a).

The Mn-oxide profile (Fig. 2c) is much broader at Eratosthenes Seamount than at Florence Rise, which might be due to a larger bioturbation effect. At 15 centimetre depth Mn-oxides rapidly increase to a maximum at 17 centimetre and then remain high till 24 centimetre depth. Dissolved Mn (II) concentrations (Fig. 2b) are below detection in the oxic sediment and start to increase after the transition from oxic to anoxic sediment at about 25 centimetre.

The different Fe pools (Fig. 2d) do not show a broad peak like Mn-oxide (Fig. 2c). Fe_{asc} values are uniform (~10 μmol g^{-1}) throughout the profile and only have a minor increase (to ca. 13 μmol g^{-1}) in the layer above the sapropel. Fe_{dith} concentrations increase three times from the oxic sediment (93 μmol g^{-1}) to the iron oxide rich layer above the sapropel (283 μmol g^{-1}).

Fe_{total} concentrations are much higher than Fe_{dith} concentrations and are maximal in sapropel S1. Porewater Fe (II) is below detection limit in the oxic part of the sediment (Fig. 2c) and increases at the transition from the oxic to anoxic sediment.

Incubation results are presented in Fig.2e. As for Florence Rise, Σ CO_2 production is very low in most of the intervals due to the low amounts of degradable organic carbon. Top-layer values are somewhat higher than the ones observed for Florence Rise. The organic-rich sapropel has the highest Σ CO_2 production, rates are higher than those of Florence Rise. Mn-oxide reduction rates were again not detectable. Two maxima in iron oxide reduction rate were seen in the subsurface layer and in the oxidized S1.

In both Mediterranean sediments porewater iron profiles indicate a consumption of iron at the same depth as the iron-oxide reduction. These contrasting results can be ascribed to the anoxic conditions of the bag incubations and the effect of some sediment mixing during sampling.
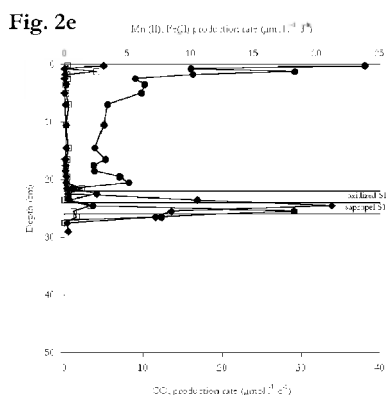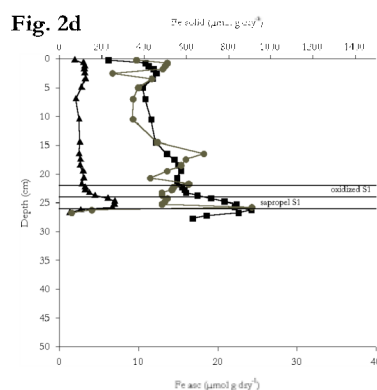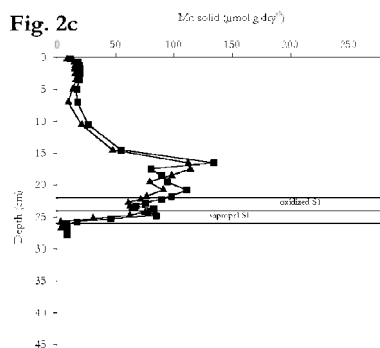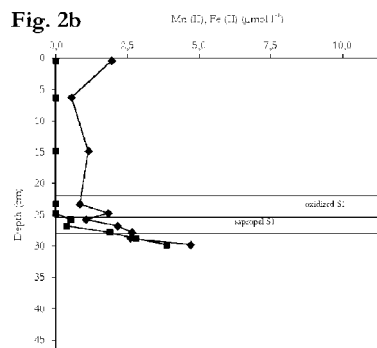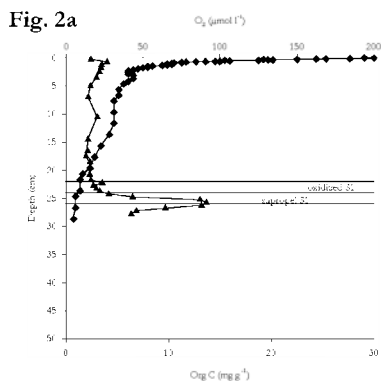
Fig. 2. Results of Eratosthenes Seamount, N.B. porewater data result form a different box core. a. O2 (µmol l⁻¹) – diamonds, organic carbon (mg g sediment⁻¹) – triangles. b. Dissolved Fe (II) (µmol l⁻¹) - diamonds, dissolved Mn (II) (µmol l⁻¹) – squares. c. Mn dithionite (µmol g⁻¹) - triangles, Mn total (µmol g⁻¹) - squares d. Fe ascorbate (µmol g⁻¹) - circles, Fe dithionite (µmol g⁻¹) - triangles, Fe total (µmol g⁻¹) - squares e. Production rates (µmol l⁻¹ d⁻¹) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares and Σ CO₂ - circles.

95

5.4.2.3. Grevelingen 1998

In September 1998, oxygen microprofiling revealed a surface layer (0 -1.35 centimetre) in which oxygen concentrations were high and exhibited a small gradient and a layer below (1.35-2.3 centimetre) in which oxygen was rapidly consumed (Fig. 3a).

Dissolved Mn (II) concentrations were below detection throughout the profile (Fig. 3b). Solid phase total Mn showed a sub-surface peak and uniform concentrations below.

Porewater iron concentrations ($\sim$10 μM) and the various iron oxide pools were rather uniform (Fig. 3c). Fe_{asc} ($\sim$ 4 μmol g^{-1}) was about 2.5 times smaller than the concentration of Fe_{dith} ($\sim$ 11 μmol g^{-1}). In turn, the Fe_{dith} concentration was about 2.5 times smaller than the total concentration of iron oxides (28 μmol g^{-1}).

Production rates in the unamended incubations are shown in Fig.3d. Rates of Σ CO_2 production were highest in the surface layers and decreased with depth. Mn-oxide reduction rates were also highest in the top 1 centimetre, but decreased with depth. Iron-oxide reduction rates had a, rather broad, subsurface peak from 0.75 to 2.25 centimetre depth and gradually decreased with depth.

5.4.2.4. Grevelingen 1999

Many of the results from the sampling event in February 1999 (Fig. 4) differed from those obtained in September 1998. In February oxygen penetration depth was only 9 mm (Fig. 4a).

Dissolved Mn (II) concentrations were highest ($\sim$ 53 μM) in the 0.5-0.75 centimetre interval and decreased downwards (Fig. 4b). Solid phase total Mn almost completely exists of dithionite extractable Mn-oxides. Concentrations were highest in the top 0.5 centimetre and had lower and rather uniform concentrations below.

Concentrations of dissolved Fe (II) were variable with maximal values at 1-3 centimetre depth (Fig. 4c). The various iron-oxide pools were rather uniform throughout the whole profile (Fig. 4c), consistent with September 1998 observations. However, ratios between the fractions differ from those in September. Fe_{asc} (7 μmol g^{-1}) was about 2 times smaller than Fe_{dith} (14 μmol g^{-1}), whereas Fe_{dith} values were 3.5 times smaller than the pool of total iron (49 μmol g^{-1}). In February Σ CO_2 production rates (Fig. 4d) were much lower than those in September were. This can probably be attributed to the lower temperatures and lower labile carbon contents. Rates of Mn and Fe oxide reduction (Fig. 4d) were lower as well. Mn-oxide reduction had the highest rate in the top-layer. Fe-oxide

reduction rates showed two subsurface maxima, one at about 0.5-0.75 centimetre and one at 3-5 centimetre.

Fig. 3a
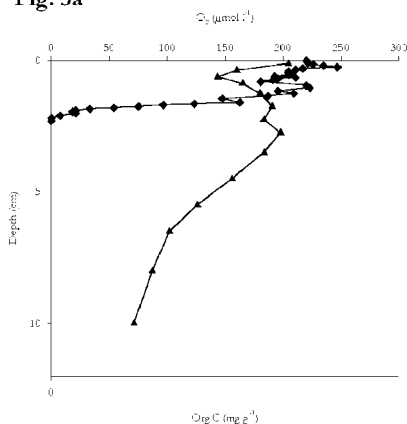
Fig. 3b
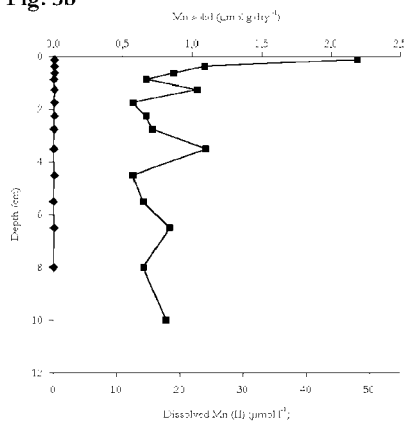
Fig. 3c
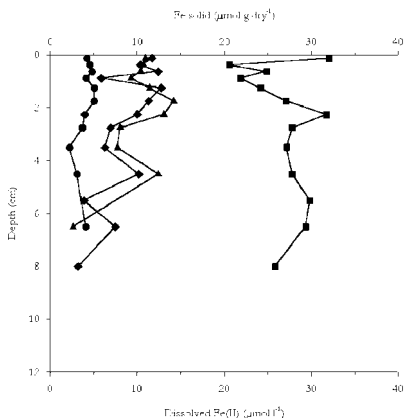
Fig. 3d
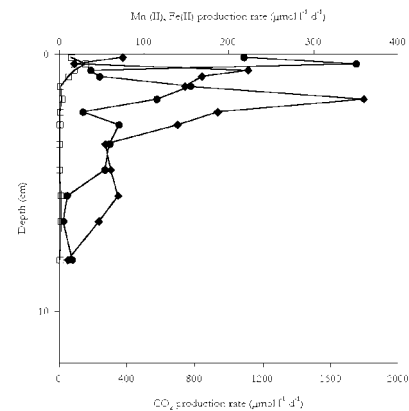
Fig. 3. Results of Grevelingen September 1998. a. O2 (μmol l^{-1}) – diamonds, organic carbon (mg g sediment[-1]) – triangles. b. Dissolved Mn (II) (μmol l^{-1}) - diamonds, Mn total (μmol g^{-1}) - squares c. Dissolved Fe (II) (μmol l^{-1}) - diamonds, Fe ascorbate (μmol g^{-1}) - circles, Fe dithionite (μmol g^{-1}) - triangles, Fe total (μmol g^{-1}) - squares d. Production rates (μmol l^{-1} d^{-1}) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares and Σ CO_2 - circles.

97

Fig. 4a
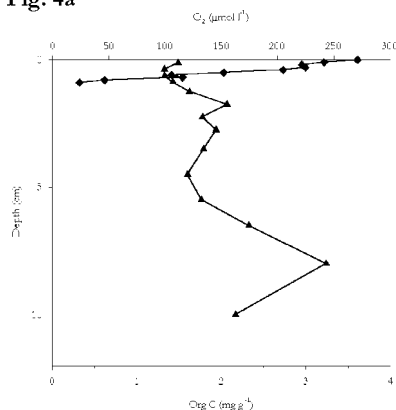
Fig. 4b
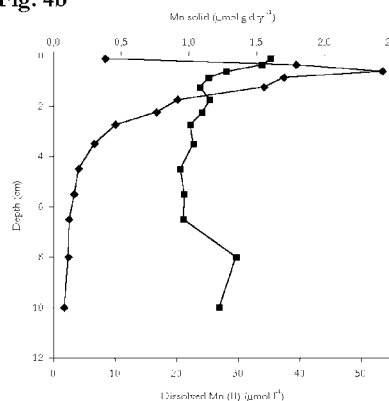
Fig. 4c
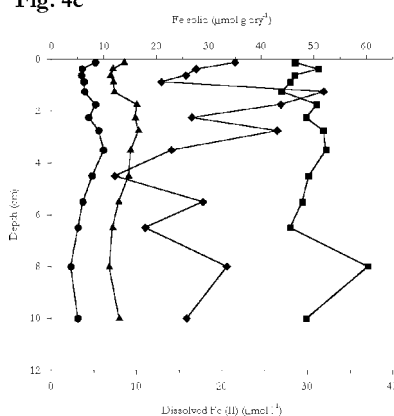
Fig. 4d
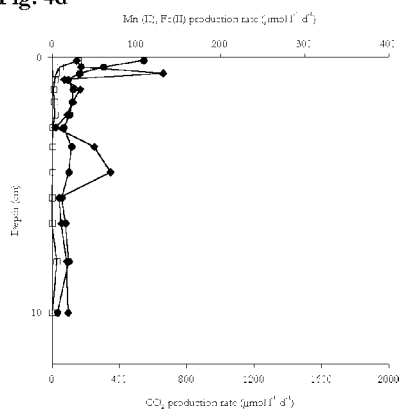
Fig. 4 Results of Grevelingen February 1999. a. O2 (μmol l^{-1}) – diamonds, organic carbon (mg g sediment^{-1}) – triangles, SO$_4$ (μmol l^{-1}) – open squares. b. Dissolved Mn (II) (μmol l^{-1}) - diamonds, Mn total (μmol g^{-1}) - squares c. Dissolved Fe (II) (μmol l^{-1}) - diamonds, Fe ascorbate (μmol g^{-1}) - circles, Fe dithionite (μmol g^{-1}) - triangles, Fe total (μmol g^{-1}) - squares d. Production rates (μmol l^{-1} d^{-1}) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares and Σ CO$_2$ - circles.

Fig. 5a

Fig. 5b

Fig. 5c

Fig. 5d

Fig. 5e
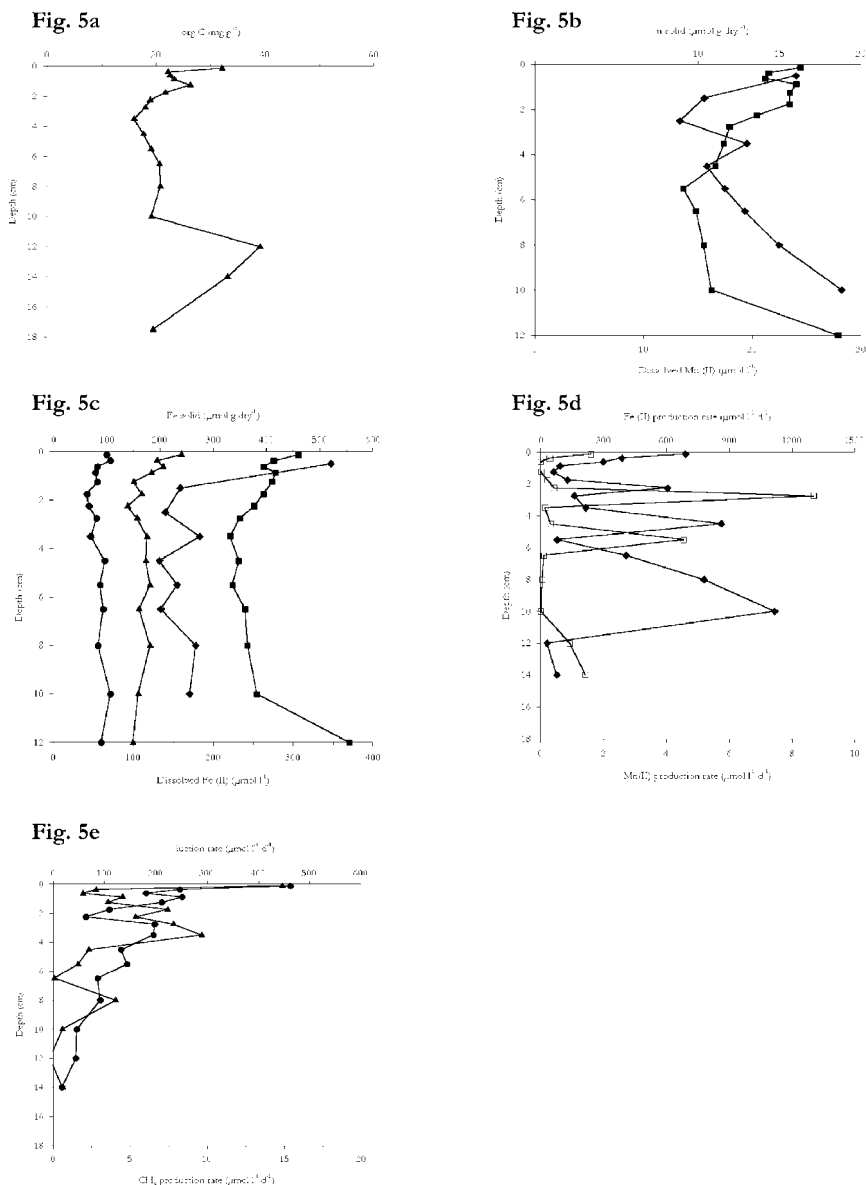
Fig 5. Results of Appels. a. O2 (μmol l^{-1}) – diamonds, organic carbon (mg r sediment^{-1}) – triangles. b. Dissolved Mn (II) (μmol l^{-1}) - diamonds, Mn total (μmol g^{-1}) - squares c. Dissolved Fe (II) (μmol l^{-1}) - diamonds, Fe ascorbate (μmol g^{-1}) - circles, Fe dithionite (μmol g^{-1}) - triangles, Fe total (μmol g^{-1}) - squares d. Production rates (μmol l^{-1} d^{-1}) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares CH$_4$ – triangles and Σ CO$_2$ – circles e. Production rates (μmol l^{-1} d^{-1}) of CH$_4$ – triangles and Σ CO$_2$ – circles

Fig. 6a

Mn production rate (μmol l^{-1} d^{-1})

Fig. 6b

Fe production rate (μmol l^{-1} d^{-1})

Fig. 6c

CO_2 production rate (μmol l^{-1} d^{-1})

Fig 6. production rates (μmol l^{-1} d^{-1}) in incubations Vliegertje 1998: control-diamonds, molybdate treatment – squares, molybdate & ferrozine treatment – triangles. a. Dissolved Mn (II) production. b. dissolved Fe (II) production. c. Σ CO_2 production.

Fig. 7a

Fig. 7b

Fig. 7c
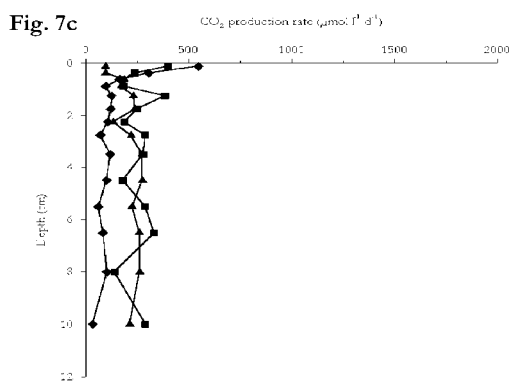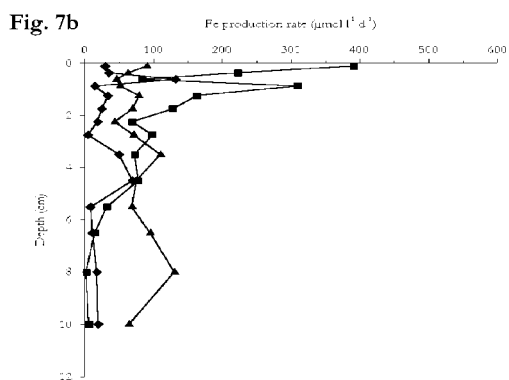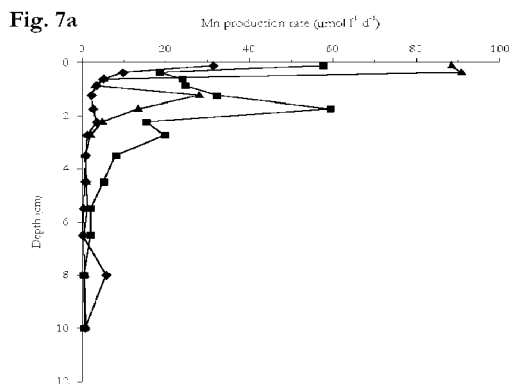
Fig. 7 production rates (μmol l^{-1} d^{-1}) in incubations Vliegertje 1999: control-diamonds, molybdate treatment – squares, molybdate & ferrozine treatment – triangles. Dissolved Mn (II) production. b. dissolved Fe (II) production. c. Σ CO_2 production.

5.4.2.5.   Appels

Dissolved and particulate Mn concentration profiles (Fig. 5 b) were rather similar, with a minor surface enrichment.

Dissolved Fe (II) concentrations (Fig. 5c) also had a surface maximum and were rather uniform thereafter. Various iron oxide pools showed rather uniform trends throughout the profile. Concentrations were very high for all three pools, values around 90, 170 and 400 μmol g^{-1} for Fe_{asc}, Fe_{dith} and Fe_{total} respectively (. 5c). On average, Fe_{dith} concentrations were 2 times higher than those of Fe_{asc} were. Total iron concentrations in turn were 2.5 times higher than the Fe_{dith}.

Production of Σ CO_2 decreased mostly from the surface (Fig. 5c). Mn-oxide reduction was detectable only in the surface layer and at 2.5 – 3 centimetre and 5-6 centimetre (Fig. 5d). Fe-oxide reduction rates were very variable and methanogenesis was very low throughout (Fig. 5c).

5.4.3.   Inhibitors

Figs 6 and 7 present the results of the Grevelingen incubations amended with molybdate and molybdate and ferrozine. The effect of the inhibitors differed between the two sampling events and depended on the main processes playing a role in the natural sediments.

In September 1998 (Fig. 6) addition of molybdate or molybdate and ferrozine resulted in lower Mn-oxide reduction rates (Fig. 6a), in particular in the subsurface layer. The effect of molybdate addition on iron-oxide reduction (Fig. 6b) was similar to that on Mn-oxide reduction. The subsurface Fe-oxide reduction peak of the control almost completely disappeared, but some Fe-oxide reduction still occurred in the top-lay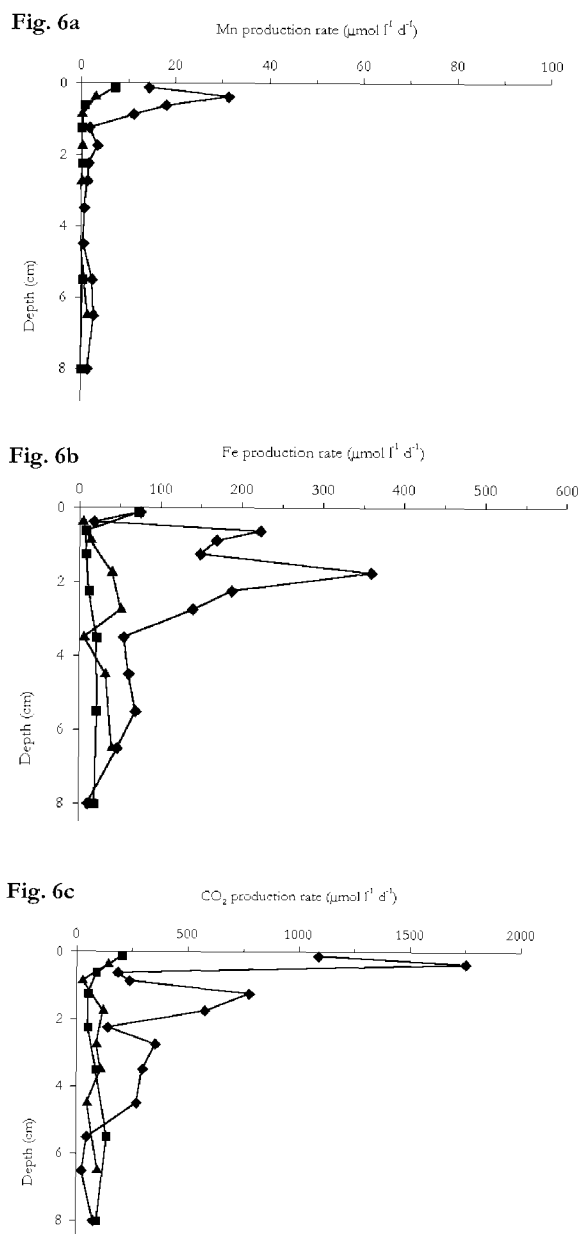er. Addition of both molybdate and ferrozine resulted in slightly higher Fe-oxide reduction rates relative to the molybdate treatment only. This might be an artefact because ferrozine can chelate adsorbed Fe (II) (Rasmussen and Nielsen 1996). Σ CO_2 production rates were also suppressed upon addition of molybdate or molybdate and ferrozine (Fig 6c).

Results of inhibitor amended incubations in February 1999 (Fig 7) were different from those obtained in September. Addition of molybdate increased rather than decreased Mn-oxide reduction (Fig. 7a). Moreover, Mn-oxide reduction rates showed not only a peak at the surface, but also a subsurface peak at about 2 centimetre depth. Addition of both molybdate and ferrozine lowered the subsurface peak of manganese reduction rates but increased the surface maximum.

102

The addition of molybdate or addition of molybdate and ferrozine (Fig. 7b) also increased iron-oxide reduction. However, addition of ferrozine besides molybdate lowered rates of iron-oxide reduction in the surface, but increased iron oxide rates below 5 centimetre. Total mineralization rates in the amended sediments were somewhat higher than those in the control (Fig. 7c). The surface peak that was present in the control and molybdate treatment is not observed after addition of molybdate and ferrozine.

5.5. Discussion

5.5.1. Sampling and methodological remarks

There is no simple and straightforward method for quantification of metal-oxide reduction rates. The bag incubation approach is now used by most researchers (e.g. Canfield et al. 1993b, Thamdrup and Canfield 1996, Hansen et al. 1998, Hansen et al. 2000, Kostka et al. 2002). However, there are some aspects to be kept in mind. Sediments are usually disturbed because of sampling, especially if inhibitors are added, as this requires thorough mixing. Disturbances can enhance mineralization processes because existing micro-niches are destroyed, resulting in a change in redox conditions that may enhance completeness and rate of mineralization (Aller 1994a). Incubation of the sediment profiles in slices prevents the exchange between zones varying in dominant pathways and electron acceptors. For example, transport of reduced components by molecular diffusion to a re-oxidation layer is not possible. To minimise the effect of these potential problems due to prolonged incubation, only initial concentration changes have been used for rate calculations.

All layers have been incubated anoxically despite that some of our sediments are oxic till a certain depth. These oxic layers may have proportionally high concentrations of solid-phase electron acceptors (i.e. Mn and Fe-oxides). In these layers, measured rates must be considered potential anaerobic mineralization rates, because these processes are normally out competed by aerobic mineralization (Canfield et al. 1993a).

A significant fraction of the reduced Mn and Fe may not appear in the dissolved pool because of sorption. Apels and Mediterranean sediments were extracted with a weak HCl extraction to determine the reduction state of the Fe in the sediments and the adsorbed Fe (II) (sensu Kostka 1994). In Appels the iron oxide reduction rates are corrected for the fraction Fe-adsorbed, based on the measured ratio of $Fe^{2+}/$ Fe (II). In the Mediterranean sediments almost no iron is adsorbed and no correction was applied (results not shown). Only few data on adsorbed Fe (II) are available for Grevelingen, in these sediments iron-oxide reduction rates are based

on the increase in dissolved Fe (II) assuming this fraction to be 10 % of the total Fe (II) production.

Differences in sampling time intervals and total incubation time may also have resulted in different initial reduction rates. The total incubation time of the Grevelingen September samples was 62 days, in February this was 23 days in the control treatments and 27 days in the amended sediments. The disturbances induced by sample handling are expected to be of less importance in incubation series that last long as these disturbances are masked by the other processes. However, a longer incubation time may move the sediment away from the natural characteristics. Finally, maximum activities may be lowered and smeared out due to the thickness of the incubated sediment slices and the incubation time.

5.5.2. Mineralization

The relative contribution of a specific electron acceptor in the organic matter degradation is determined by: 1) The organic carbon loading 2) the availability of alternative electron acceptors (Canfield 1994, Wijsman et al. 2001a), 3) interactions between degradation pathways and 4) transport processes.

On the basis of literature reviews (Canfield 1994, Heip et al. 1995, Thamdrup and Canfield 2000) and modelling efforts (Soetaert et al. 1996, Wijsman et al. 2002) it has been established that the relative importance of the organic carbon mineralization pathways is primarily a function of organic carbon loading. In their model for the continental shelf of the Black Sea Wijsman et al. 2002 clearly showed that the relative importance of the energetically less favourable pathways increases upon higher carbon loading (Fig. 8). At increasing organic carbon loading mineralization shifts from low to very high organic carbon loading there is a shift from aerobic mineralization, via denitrification and metal-oxide reduction pathways to sulphate reduction, and finally to methanogenesis. The absolute degradable organic carbon loading at which transition between dominant pathways occurs depends on the concentration of the electron acceptor and solute and particle transport rates. For instance, sulphate reduction accounts for most mineralization in organic rich marine sediments whereas the process is less important in organic rich freshwater sediments because sulphate concentrations are low. The sediments used in our study cover the total range from low to high organic carbon loading, thus similar changes in relative importance of pathways are expected. However, it should be remembered there are many environmental conditions that co-vary with organic matter loading. This complicates a direct comparison between field data and model results.

Depth integrated values of the iron and manganese reduction (Table 2a) and total mineralization were calculated for the upper 9 centimetre of the sediments and provide a simple insight in the relative importance of Mn and Fe-oxide reduction in the sediments. Besides, if relevant, rates and importance of aerobic mineralization and denitrification are presented.

The importance of each pathway in the organic carbon mineralization has been calculated using CO_2 equivalents for the mineralization products (Table 2 b). For these calculations traditional stoichiometry Froelich et al. 1978 has been used where 1 mol of produced CO_2 is accompanied by consumption of 1 mol O_2 or 1.25 mol NO_3^- or production of 0.25 mol of Fe (II), 0.45 mol of Mn (II), 1 mol of CH_4 and the reduction of 2 mol of SO_4. The total sum of the CO_2 equivalents should be equal to the measured $\Sigma\, CO_2$ production.

Calculated $\Sigma\, CO_2$ rates do not always correspond to measured $\Sigma\, CO_2$ production rates. This can be caused by: 1) lack of data on some pathways, 2) underestimation or overestimation of metal oxide production, carbon mineralization rates or oxygen consumption due to other reactions like adsorption, dissolution or re-oxidation (Berner 1964, Roden and Zachara 1996, Murase and Kimura 1997, Postma 1985, Postma and Appelo 2000, Widdel et al. 1993), 3) accumulation of errors of the individual terms and 4) differences in conditions during which rates are derived, e.g. Mediterranean O_2 consumption rates are derived under oxic conditions while CO_2 production rates are derived form anoxic bag incubations.

The organic poor Mediterranean sediments have the lowest depth integrated mineralization rates (48-140 nmol cm^{-2} d^{-1}). Oxygen is the most important electron acceptor for organic carbon mineralization in the organic poor sediment above the sapropel. However, rates of O_2 consumption based on modelling of porewater profiles (84 and 108 nmol cm^{-2} d^{-1} at Florence Rise and Eratosthenes Seamount, respectively) and CO_2 production (48 and 140 nmol cm^{-2} d^{-1} at Florence Rise and Eratosthenes Seamount, respectively) do not correspond exactly because the bag incubations were performed under anoxic conditions, so mineralization rates are not directly related to the oxic conditions. Correction for the O_2 due to re-oxidation of ammonium produced during the aerobic carbon degradation resulted in somwhat lower O_2 consumption rates (59.4 and 67.1 nmol cm^{-2} d^{-1} at Florence Rise and Eratosthenes Seamount, respectively). Denitrification has a relative important role, though much smaller than the role of aerobic degradation. Iron and manganese oxide reduction are of minor importance in these sediments (Florence Rise: Fe 0 %, Mn 3.4 % of mineralization; Eratosthenes Seamount: Fe 0.5 %, Mn 1.3 % of mineralization). These low contributions by metal oxides and the dominance of Mn over Fe-reduction are consistent with model predictions for

organic poor oxygenated sediments (Fig. 8) and slurry results of Chapter 3 indicating that organic carbon availability is the limiting factor for iron oxide reduction in these sediments. Sulphate reduction, and likewise methanogenesis, plays no role in the organic carbon mineralization of these oligotrophic sediments. Considering only the sapropel layer the importance of Fe-oxide reduction is higher (7.5% and 34 % for Florence Rise and Eratosthenes Seamount respectively). However, due to sampling disturbance the mineralization rates in the deeper sediment layers of our incubations are higher than real mineralization rates. For exemple an estimation of sediment mineralization rates based on the thickness of the burndown sapropel layer results in a rate of ca. 5.4 nmol cm^{-2} d^{-1}. Values for deep-sea sites based on organic carbon, O_2 and Db profiles were in the same order of magnitude (ca. 8 nmol cm^{-2} d^{-1}, Slomp and Meysman 2002).

Consistent with model predictions a shift in dominant processes is observed at an higher organic carbon concentration. In the Grevelingen sediments aerobic mineralization and denitrification processes become less important than in the Mediterranean sediments, as indicated by a higher relative importance of metal-oxide reduction (September 1998: Mn 0.6 %, Fe 7.6 %, February 1999: Mn 1.4%, Fe 6.2 %). Despite the availability of O_2 profiles we cannot calculate oxygen consumption rates because molecular diffusion can not be assesed and O_2 is used for reoxidation of reduced components. However, we expect that aerobic mineralization is only of minor importance because of the small oxygen penetration depths. Most oxygen consumption will probably be due to re-oxidation (Soetaert et al. 1996). Therefore, like in most shallow marine sediments, sulphate reduction likely accounts for most of the organic carbon degradation.

In the Grevelingen sediments the two sampling events have different dominant pathways. This results from differences in sediment characteristics due to heterogeneity and seasonal differences in temperetuare and carbon availbility.

Reduced S-compounds can be re-oxidized by reaction with iron or manganese oxides (Aller and Rude 1988, Thamdrup et al. 1993).The high mineralization rates in September may stimulate the importance of sulphate reduction and diminish the importance of the other anaerobic pathways. Molybdate addition results in a Σ CO_2-rate of only 33 % of the control rate, suggesting that 67 % of the mineralization is due to sulphate reduction. The large decrease in Σ CO_2 rate after molybdate addition suggests that no quick restoration of the other pathways occurs after inhibition of sulfate reduction. Probably because of a lack of sufficient other electron acceptors, as most of reactive iron and manganese oxides have already been reduced by sulfide. Furthermore, the addition of molybdate results in decreased iron and manganese-oxide reduction rates (the Fe-oxide and Mn-oxide

reduction rates are 23 % and 12 % of the control rate, respectively), suggesting that a large part of the iron and manganese reduction is due to reaction with sulfide or other reduced s compounds (Murase and Kimura 1997, Pyzik and Sommer 1981, Jorgensen 1982, King 1990) and that Fe^{2+} is not removed from the porewater by authigenic sulfide mineral formation in the unamanded sediment (Canfield et al. 1993b). The addition of both molybdate and ferrozine results in a similar decrease in mineralization rate when compared to control rates, whereas relatively to total mineralization both iron and manganese oxide reduction decrease less than on addition of molybdate only.

In February, inhibition of sulfate reduction resulted in a doubling of Σ CO_2 production. The manganese oxide and iron oxide reduction rates were 348 % and 227 %, respectively, of control rates, suggesting that sulphate reduction competes with the other pathways in these conditions. Canfield et al. 1993b also reported a stimulating effect of molybdate addition on Fe^{2+} liberation. According to these authors this could indicate either stimulation of Fe reducers upon inhibition by competition of sulphate reducers or lack of Fe^{2+} removal from the porewater by authigenic sulfide mineral formation in the deeper layers because sulfide is not produced any more and thus porewater Fe (II) can accumulate. In our study addition of ferrozine and molybdate increased the iron oxide reduction rates even more than molybdate addition alone. Roden and Urrutia 1999 also reported a stimulating effect of adsorbed Fe (II) removal and measured the iron-oxide reduction rate. They assessed that Fe (II) adsorbs on Fe (III) oxides and cell compounds, thus inhibiting iron oxide reduction. The direct chelation of Fe (II) inhibits this process.

Mn-oxide reduction rates decrease somewhat upon Fe (II) chelation and sulfate inhibition compared to sulfate inhibition only. Apparently the Mn (II) reduction is partly microbial and partly chemical. Canfield et al. 1993b also reported parallel occurrence of both processes in the Skagerrak.

Like in the Grevelingen sediment, the importance of aerobic mineralization in Appels is probably low, because oxygen consumption is likely due to re-oxidation reactions. In contrast to the Grevelingen denitrification rates are likely high in the Appels sediment, because NO_3 concentrations in de Scheldt are very high (a few hunderd µM). Middelburg and Nieuwenhuize 1998 modelled rates of 438 nmol cm^{-2}d^{-1}, which can account for a large part of the mineralization in Appels sediments. Iron oxide reduction is the pathway accounting for most of the other organic carbon degradation. However, the iron oxide reduction rate in Appels (2307 nmol cm^{-2} d^{-1}) was lower than the iron oxide reduction rate Roden and Wetzel 1996 found in an unvegetated freshwater sediment (8960 nmol cm^{-2} d^{-1}). Carbon

mineralization was much higher in their samples (5850 nmol cm^{-2} d^{-1}) than in Appels (776 nmol cm^{-2} d^{-1}) with the consequence that iron oxide reduction accounted for a smaller proportion in their study (38 % of total mineralization) compared to our sediments (74% of the total mineralization, Table 3). This is consistent with model results indicating a decrease in importance of iron oxide reduction at higher organic carbon fluxes. It should be noted that the values of Roden and Wetzel 1996 result from sediment slurries of a depth interval (0-3 centimetre for Fe, 0-10 centimetre for Σ CO$_2$) incubated at 22 °C which is higher than our ambient temperature (10 - 15 °C). Moreover, their sediment had a dry weight organic matter content of ~ 20 wt. %, almost an order of magnitude higher than that of Appels sediment (2.13 wt % organic carbon).

The organic poor sediments of the Mediterranean have low mineralization rates and iron oxide reduction is of minor importance,iron oxide reduction has a large share in the intermediate carbon receiving sediments of Lake Grevelingen and in the organic rich Appels sediment mineralization is almost completely governed by iron oxide reduction. The observations are consistent with literature reports and results of our slurry experiment (Chapter 3). Those indicated that in the Mediterranean sediments iron oxide reduction rates are limited by the organic carbon load, in Grevelingen both organic carbon load and iron oxide availability are limiting and in Appels sediments only iron oxide availability limits the reduction rate. The relative importance of iron oxide reduction rates in mineralization are consistent with these differences in limiting factors.

In most studies Mn-reduction is attributed to the chemical reduction by Fe (II) thus Mn-oxide reduction related to organic carbon mineralization is considered to be of no or limited importance. Exceptions are sites with a particular high deposition and concentration of Mn-oxides (Aller 1990, Canfield, Thamdrup, and Hansen 1993). In contrast, iron oxide reduction rates are more often considered in the studies of mineralization pathways. Rates of organic carbon oxidation and (contribution of) iron-oxide reduction in the studied sediments are highly variable (Table 3). Expressing literature and our data on the relative importance of iron oxide reduction as a function of mineralization rates, a proxy for organic carbon loading, (Fig. 9), shows that the overall trend seems to support model results (Fig. 8). Field data also indicate an initial increase of the contribution of iron oxide reduction to mineralization with increasing carbon inputs and a subsequent decrease (Fig.9). However, the trend in the field data is much more scattered than that of modelled data due to the effects of other factors affecting iron oxide reduction.
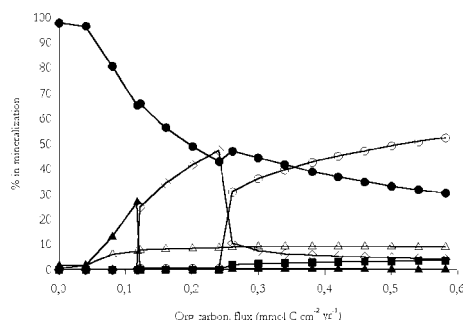
Fig 8. Changes in mineralization pathways (% in total mineralization) as a function of carbon loading (nmol C cm^{-2} yr^{-1}), based on numerical simulation of Wijsman et al. 2001a for the continental shelf sediments of the Black Sea. Oxic mineralization - solid circles, nitrification - open triangles, Mn-oxide reduction - solid triangles, Fe-oxide reduction - open diamonds, Sulfate reduction - open circles, methanogenesis - solid squares.
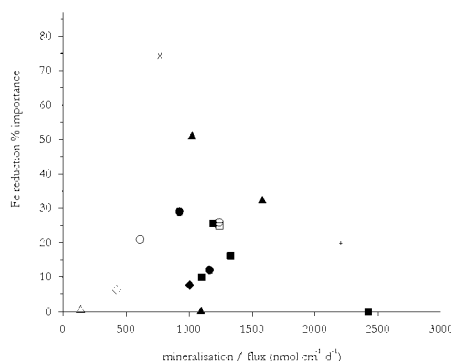
Fig. 9 importance of iron oxide reduction in organic carbon mineralization vs. total mineralization rate (nmol cm^{-2}d^{-1}) derived from experiments and literature: Appels x, Vliegertje September solid diamonds, Vliegertje February solid diamonds, Mediterranean – open triangles, Canfield et al. 1993a) – solid triangles, Thamdrup and Canfield 1996 – solid circles, Rysgaard et al. 1998 –open squares, Kostka et al. 1999b – solid squares, Glud et al. 2000 – open circles, Van der Nat and Middelburg 1998 – plusses.

5.6. Conclusion

This study elucidates differences in carbon mineralization pathways and rates in three different aquatic sediments. Moreover it shows the complexity of factors governing organic carbon mineralization rates, especially the processes using the intermediate electron acceptors. In the organic poor Mediterranean sediment mineralization in dominated by aerobic degradation and denitrification whereas metal oxide reduction can be neglected. Metal oxide reduction pathways are most important at intermediate to high organic carbon degradation rates. Intermediate organic carbon loaded estuarine sediments have higher rates of iron oxide reduction. Here, both iron oxide reduction and sulfate reduction are important. In organic rich freshwater sediments denitrification and iron oxide reduction account for most organic carbon mineralization, with only a minor role for the other pathways like manganese reduction and methanogenesis. These are among the first field data showing the effect of increasing organic carbon availability on pathways and degradation rates. Our findings confirm earlier model results and experimental data on factors limiting the importance of iron oxide reduction in mineralization

5.7. Acknowledgements

6. Discussion

6.1. Background

Bacteria capable of reducing and oxidising iron and manganese have been known for a long time (Jackson 1901 and Beijerinck 1913 in: Ehrlich and Salerno 1990) already reported bacterial oxidation of Mn (II). Although bacterial metal-oxide reduction was known, no research on the subordinate microbial ecology and biogeochemistry was performed yet.

In the late seventies marine geochemists (Froelich et al. 1978), studied the early oxidation of organic matter in pelagic sediment. Porewater profiles indicated that oxidants are consumed in order of decreasing energy production per mole of organic carbon oxidized (Table 1). These data suggested reduction of metal-oxide phases and also indicated that these processes have a higher energy production than sulfate reduction or methanogenesis. Therefore, metal-oxide reduction during early organic carbon oxidation occurs before sulfate reduction or methanogenesis. Despite these unequivocal porewater observations no dissimilatory iron- or manganese-oxide reducing bacteria had been isolated at that time.

Table 1. Free energy gain from mineralization reactions from Froelich et al. 1978. Energy gain is presented as kJ mol-1 glucose. Organic carbon is represented by CH_2O.

Process	Reaction	Energy gain (kJ.mole-1)
Oxic degradation	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-3190
Nitrification	$5\ CH_2O + 4NO_3^- \rightarrow CO_2 + 2N_2 + 4HCO_3^- + 3H_2O$	-2750
Mn-reduction	$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-3090
Fe-reduction	$CH_2O + 7\ CO_2 + 2\ Fe_2O_3 \rightarrow 4\ Fe^{2+} + 8\ HCO_3^-$	-1410
Sulphate reduction	$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-380
Methanogenesis	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-350

The oldest studies on iron-oxide reducing bacteria report cultures containing bacteria capable of fermentative metal-reduction (e.g. reviewed by Lovley 1987). In these cultures iron-oxide reduction is of limited importance for the overall process of organic matter oxidation. Organic carbon components are fermented resulting in an accumulation of fermentation products, but no complete organic carbon oxidation by iron-oxide reducing bacteria was reported. Lovley and Phillips 1986 provided the first proof of complete organic carbon oxidation by iron-oxide

reducing bacteria. They studied organic carbon mineralization in slurries of sediments from the River Potomac estuary.

Addition of amorphous iron oxides resulted in a complete oxidation of fermentation products to carbon dioxide with concomitant release of dissolved iron, whereas the addition of hematite resulted in modest iron-oxide reduction. The increase in iron-oxide reduction was accompanied by a decrease in methanogenesis. This pioneering study provided the first data on the potential of bacteria for dissimilatory iron-oxide reduction. It also revealed the potential importance of dissimilatory iron-oxide reduction, the importance of iron-oxide crystalinity and interactions with other mineralization processes. Thereby, the interest in bacterial metal-oxide reduction processes was raised.

Nowadays, a number of bacterial species is known to be capable of dissimilatory iron and manganese-oxide reduction. For an extensive review of the development of knowledge on iron and manganese reducing bacteria see Thamdrup 2000. Most of them have a large metabolic versatility and are capable of using one or more of the other electron acceptors for their respiration.

Lovley and Phillips 1986 identified a number of organic carbon sources for bacterial iron-oxide reduction including glucose, acetate, hydrogen, propionate, butyrate, ethanol, methanol and trimethylamine. Some of the more recent research especially focussed on contaminants like toluene (Langenhoff et al. 1997) or chlorinated carbon compounds and aromatic hydrocarbons (Lovley and Anderson 2000). Iron-oxide reducing bacteria are able to reduce a number of different iron oxides. Experiments show that amorphous oxides are preferentially used (Lovley and Phillips 1986) though ferric iron in clay minerals can be used as well(Kostka et al. 1999a).

Until recently iron and manganese-oxide reduction rates have been sparsely quantified in field studies on organic matter mineralization and biogeochemical processes. The first report on organic carbon oxidation pathways in natural sediment, including measurements on iron-oxide reduction, was presented by Canfield et al. 1993b. Their rates of iron-oxide reduction were based on anaerobic bag incubations. These contained sediments from various depth intervals in which the increase of Fe (II) concentration in a time period was measured. In order to discern rates of microbial reduction and chemical iron-oxide reduction molybdate was added. Molybdate inhibits the bacterial sulfate reduction (Oremland and Capone 1988) and thereby prevents chemical reduction of iron by sulfide. Canfield et al. 1993b also added ferrozine that chelates Fe^{2+} and prevents re-oxidation of the reduced iron (Stookey 1970). It can thus provide insight in the total iron-oxide reduction rate and the rate of Fe (II) oxidation. The study of Canfield and co-

workers showed large differences in iron and manganese-oxide reduction rates within and between sites and variable contributions of these processes to total mineralization. It was clear that the role of bacterial iron-oxide reduction in some marine sediment could potentially be very important.

Although metal-oxide reduction gained more interest in biogeochemical studies, direct measurements on iron-oxide reduction rates in sediments are still not regularly performed. Traditionally, the metal-oxide reduction was estimated from concentration profiles of the dissolved reduced metal and solid-phase concentration of reactive iron oxides. Solid-phase gradients are often rather small and combined with low particle transport rates, it resulted in the conclusion that these processes are not important in most sediments. This changed after recognition of active metal-oxide cycling which enables an important role for metal-oxide reduction although concentrations are. Despite this recognition, there are still only few studies that include direct measurement of sediment metal-oxide reduction. More often rates are investigated via bacterial tests, sediment slurries, and diagenetic models or by subtracting the importance of other respiration processes from the total mineralization thereby leaving an amount for metal-oxide reduction.

Nevertheless, the metal-oxide reduction pathways have been quantified for a number of different sediments. The role of metal-oxides in total mineralization of organic matter varies depending on a number of factors, like sediment type, interactions with product of other pathways, availability of other electron acceptors, organic carbon loading and availability and type of iron oxides.

For instance, the bag incubation study performed by Canfield et al. 1993b, showed iron-oxide reduction rates accounting for 0-78 % of (anaerobic) organic carbon reduction in marine Skagerrak sediments. Whereas metal-oxide reduction in Aarhus Bay was less important, iron-oxide reduction accounting for 3.5 % of anaerobic mineralization (Thamdrup et al. 1994a) and model results for North Sea sediments (Slomp et al. 1997) resulted in metal-oxide reduction rates accounting for < 4% of the mineralization. Thamdrup and Canfield 1996 described the pathways of organic carbon oxidation in continental margin sediments of Chile. Their anaerobic bag incubations revealed that at stations with high carbon oxidation rates, iron oxide reduction was unimportant for the total mineralization, whereas iron-oxide reduction accounted for 12-29 % of total mineralization at slope stations with lower organic carbon mineralization rates. Rysgaard et al. 1998 found similar results in an arctic coastal marine sediment of Young sound. On a yearly basis sulfate reduction in whole core incubations accounted for 33% of the total mineralization and iron oxide reduction accounted for 25 %. In microbial mats biologically

mediated iron-oxide reduction may accounts for > 50% of the reduction (Joye et al. 1996). In the salt marshes of Delaware Kostka et al. 2002 found that iron-oxide reduction was the dominant mineralization pathway in vegetated sediments. In unvegetated sediments no significant iron-oxide reduction could be detected.

Bacterial manganese-oxide reduction has been observed in a number of sediments as well. Compared to iron oxide reduction rates this process usually is of smaller significance in the organic carbon mineralization, because of the relatively small concentrations of manganese-oxides in most sediments. Increases in Mn (II) do not necessarily imply dissimilatory oxide reduction coupled to carbon oxidation because manganese-oxides can also be chemically reduced by Fe (II) and sulfide (Postma 1985, Aller and Rude 1988). In many studies Mn-oxide reduction is assumed to be completely chemical.

In the Arctic marine sediment studied by Rysgaard et al. 1998, manganese-oxide reduction below the 1 cm of surface sediment could be coupled to iron re-oxidation and was therefore not included in the organic matter mineralization budget

In Black Sea shelf sediments Thamdrup et al. 2000 found that microbial manganese reduction accounted for all non-sulfate based carbon oxidation. However, the relative importance under *in situ* conditions could not be established due to the competition with aerobic respiration and varied in response to varying bottom water oxygen concentrations. Data of two surface sediments from the Gulf of Maine indicated that iron-oxide and probably manganese-oxide reduction are significant processes in organic matter mineralization in these sediments as well (Hines et al. 1991). Likewise, the manganese efflux from microbial mats appears to be for a large part (> 50%) generated by microbial reduced Mn (II) (Joye et al. 1996). Whereas, in the sediments of Aarhus bay, manganese reduction appeared to be coupled to re-oxidation of iron and sulphur (Canfield et al. 1993b, Thamdrup et al. 1994a). Consequently dissimilatory manganese reduction plays no role in Aarhus sediments. In the hemi-pelagic sediments studied by Aller 1990, manganese reduction is capable of oxidising the entire estimated annual delivery of labile organic carbon. Likewise manganese reduction is an important electron acceptor in sediments of Long Island Sound during warm periods with intensive bioturbation, well-oxygenated bottom waters and a moderate organic carbon flux (Aller 1994b).

As indicated in the preceding paragraphs the importance of iron oxide and manganese-oxide reduction in organic carbon mineralization is variable among different environments. Microbial metal-oxide reduction may account for 0 - 100 % of the total carbon oxidation. A number of factors exist which interact with

114

microbial metal-oxide reduction, and thus govern the significance and rates of these processes in different sediments.

The availability of degradable organic carbon determines whether anaerobic mineralization occurs. At low organic carbon inputs the aerobic pathway can oxidize all organic matter. At increasing carbon concentrations most degradation is diverted into the anaerobic pathways. If anoxic degradation occurs, availability of microbial reducible metal-oxides should be such to support an active metal-oxide reducing community. This can be via high concentrations of reactive iron oxides or by a sufficiently fast metal-oxide cycle resupplying metal-oxides. Sediment disturbances like bioturbation and irrigation are important processes to enable an efficient cycling. Therefore, faunal activity is an important factor in relation to the role of metal-oxide reduction in organic carbon mineralization.

Interactions with other processes may be important as well. For example, sulfidic components can re-oxidise with iron- and manganese-oxides (Aller and Rude 1988, King 1990, Kostka and Luther 1995), thereby decreasing availability of the metal-oxides for the microbial reduction. Besides, products from sulphate reduction can react with Fe (II) and form iron sulfides and pyrite, consequently removing iron out of the short term biogeochemical cycle (Pyzik and Sommer 1981, Canfield et al. 1993b, Thamdrup et al. 1993).

Thus, in marine sediments the interactions between iron oxide and products of sulphate reduction are important factors determining iron-oxide reduction rates. In freshwater sediments, sulfate is (almost) absent, therefore sulfate reduction is of limited importance and the iron-oxide reduction may account for a large part of the anaerobic mineralization. Roden and Wetzel 1996 found that iron-oxide reduction accounts for 31 and 65 % of the total mineralization in an unvegetated and a vegetated freshwater sediment, respectively. Van der Nat and Middelburg 1998 report a considerable role for iron-oxide reduction in the freshwater sediment. The iron-oxide reduction seemed to decrease the importance of methanogenesis in total organic carbon mineralization. The study reported that this effect was probably caused by substrate competition between iron-oxide reducing bacteria and methanogens. In rice fields bacterial iron-oxide reduction has been shown to decrease the methane emission rates as well (Kruger et al. 2001). Achtnich et al. 1995 reported iron-oxide reduction rates accounting for ~ 91 % of total mineralization in anoxic fresh rice field paddy soil slurries. In landfills microbial iron-oxide reduction has also been observed in the horizontally occurring degradation sequence (Albrechtsen et al. 1995, Kennedy and Everett 2001).

6.2.   This thesis

The aim of this thesis was to address some of the questions concerning sedimentary metal-oxide reduction rates and the governing factors. In the preceding chapters four subjects have been tackled. First we addressed the problem of determining iron-oxide reactivity and quantifying the availability of iron oxides for dissimilatory iron-oxide reduction. We found that the reactive continuum method of Postma 1993 is a useful method to describe iron-oxide reactivity as long as the investigated sediments have a minimal amount of reactive iron oxides. The interaction of iron oxide and organic carbon availability was subsequently tackled. Depending on the type of sediment either iron oxide or organic carbon was the main factor limiting iron-oxide reduction rates. The third problem addressed was the effect of bioturbation and its two components (i.e. particle mixing and irrigation) on sedimentary iron and manganese cycling. Due to the differences in aquatic chemistry of Fe and Mn, the two components of bioturbation have a different effect on Fe and Mn. Iron is more affected by increased particle mixing whereas irrigation affects manganese most. Besides, results seemed to indicate the presence of a micro cycle thereby diminishing the importance of a particle mixing component to complete cycling. The last problem we tried to handle was the importance of iron and manganese reduction along an organic carbon gradient. We aimed to verify the findings of the first three questions in natural sediments and investigating the effect of the different interactions on metal-oxide reduction rates. The sediments we used were a fine grained freshwater tidal sediment with high organic carbon and iron-oxide concentrations, a coarse grained intertidal estuarine sediment with intermediate organic carbon and reactive iron concentration and a hemipelagic deep sea sediment containing hardly any organic carbon or reactive iron-oxides.

The reactive continuum approach resulted in similar reactivity characteristics (initial reactivity and heterogeneity) for the freshwater and the estuarine sediment, whereas the deep-sea sediments had extremely low reactivities, below the analytical window of the approach. Reactivity data were not correlated to the concentrations of amorphous iron oxides in the sediments, which were much higher in freshwater than in the estuarine and deep-sea sediments. The bag incubation results revealed much higher depth integrated iron-oxide reduction rates in freshwater sediments than in the estuarine sediments and hardly any reduction in the deep-sea sediments. If iron-oxide reactivity would be the sole rate determining factor governing iron-oxide reduction rates in estuarine and freshwater sediment then both rates should have been equal. The amount of reducible iron oxides determines the possible total

iron-oxide reduction. In sediments with a highly reactive but small iron-oxide pool, the increase in iron-oxide concentration over time will be limited (which when measured in anaerobic bag incubations imply low reduction rates).

The availability of degradable organic carbon ultimately determines degradation processes and the kinetic properties of the iron oxide pool are important for the role of iron oxide reduction in organic matter mineralization. This may imply that the iron oxide reduction rates increase with higher iron oxide initial reactivity, if the available organic matter and the quantity of iron oxides are not limiting. The experiments reveal that iron oxide reduction rates may perhaps depend on initial reactivity at in the freshwater sediment, but are independent of initial reactivity in the estuarine sediment (Fig. 1). At the freshwater site the iron oxide reduction rate is not related to the pools of ascorbate or dithionite extractable iron oxides (Fig. 1b), there by indicating the limited information on iron oxide availability provided by these extractions. On the basis of carbon and iron oxide additions to slurries of the same sediments (Chapter 3) we have shown that the concentration of easily reactive adsorption sites is limiting iron oxide reduction in the freshwater sediment and the availability of degradable carbon limits iron oxide reduction in the estuarine sediment. The combined results on initial reactivity, bag incubations and slurry incubations, indicate that although iron-oxide reactivity in freshwater sediments does matter, the importance of iron-oxide reduction depends on other factors,
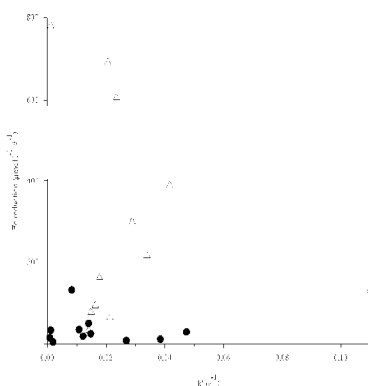
Fig. 1a   **Fig. 1b**
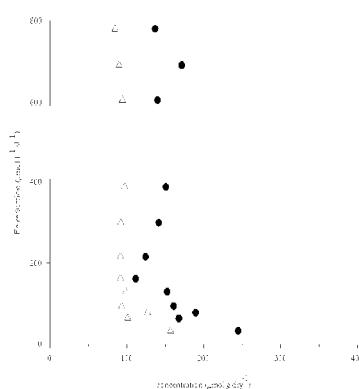
Fig. 1 a. Iron oxide reduction rate (μmol l^{-1} d^{-1}) vs. initial reactivity k' (s^{-1}) for a freshwater sediment (Appels, Belgium) - triangles and an estuarine sediment (Vliegertje, Lake Grevelingen, the Netherlands) – circles. b. Iron oxide reduction rate(μmol l^{-1} d^{-1}) vs. concentration of iron oxides (μmol gr^{-1}) in a freshwater sediment (Appels), Fe$_{asc}$ - triangles, Fe$_{dith}$ – circles.

such as concentrations of reactive iron oxides. Due to the large pool size of reactive iron oxides dissimilatory iron-oxide reduction accounted for ~ 74 % of the mineralization in freshwater sediments.

Apart form iron-oxide concentrations; an efficient and rapid iron cycle can also result in a large contribution of this pathway. Therefore in (estuarine) sediments bioturbation can stimulate and maintain metal-oxide reduction rates. The bioturbation experiment revealed that ventilation is most important for manganese reduction and particle mixing is the rate-limiting step for iron-oxide reduction. Thus the character of the bioturbation events, which is determined by composition and density of the benthic faunal community, may determine which metal is affected most. Efficient and fast bioturbation may leave a very minor role for sulfate reduction in the estuarine sediments. Besides the importance of sulphate reduction may be limited due to the existence of a micro-cycle mechanism. However, sediment observations in (i.e. the black colour of the sediment indicating sulphate reduction) did not support such an effective cycle.

In the freshwater sediments of Appels reactive iron oxides are in abundance and recycling is not necessary to maintain high reduction rates. Whereas in the deep-sea Mediterranean sediments all mineralization is aerobically, consequently no active cycling exists.

6.3.   Implications and scope for further research

The bioturbation data indicate that, due to intensive reworking, hardly any dissolved iron or manganese diffuses out of the sediment into the overlying bottom water. As long as the bottom waters are aerobic, bioturbation allows a more extensive aerobic sediment area and therefore a smaller metal efflux relative to the more anaerobic sediment. Moreover, bioturbation can increase the impact of metal-oxide reduction on organic matter degradation. In order to support sediment iron release, bioturbation should be such that the dissolved Fe^{2+} is not re-oxidized before it escapes the sediment, and the adsorption of the reduced iron on sediment articles should be limited.

The flux of organic carbon to the sediments is of important with respect to efficient recycling of iron, considering that once sulphate reduction is an important pathway, the sulphide liberates iron or manganese-oxides (Canfield et al. 1992, King 1990, Burdige 1993). The consumption of reactive iron- and manganese oxides decreases the sediment buffering capacity towards sulfide (King and Klug 1982, Heijs et al. 1999). Ultimately the sediments become sulfidic and toxic for fauna. Re-establishing conditions such that the sediment can be re-colonised by

infauna may require a long period. Some kind of ecosystem stability may be expected with respect to reactions in response to an increase of the organic carbon input. Higher carbon inputs can sustain a large faunal community with a larger bioturbation potential, thereby enhancing metal-oxide recycling and repressing sulphate reduction. Existence of such a mechanism still has to be investigated.

Besides, the direct effect of bioturbation on metal-oxide reduction, some indirect effects on sediment and water column processes can occur parallel with increased iron-oxide reduction. Research in freshwater sediments revealed a parallel release of phosphorous with iron-oxide reduction. In freshwater and some marine systems P is believed to be the limiting nutrient for primary productivity (Ryther and Dunstan 1971). Because a release of sedimentary phosphorous can stimulate primary productivity the organic carbon in put increases, consequently sustaining the anaerobic conditions.

The rate and importance of metal-oxide reduction in organic matter mineralization can be affected by a large number of factors that, in some cases, also affect each other by positive or negative feedback. To further our understanding of these complex interactions we have to refer to biogeochemical models. These models require data such as V_{max} (maximum rate of the process) and Km (affinity of the bacteria for a substance) to incorporate these complex interactions. The slurry experiments (Chapter 3) do provide information on maximum reduction rates (V_{max} values) in different sediments and what limits these. The experiments should be extended to other sediments and biogeochemical conditions. Km (the affinity of the bacteria for the substrates) should be estimated as well. Both Km and V_{max} depend on the reactivity of the iron oxides and the availability of iron oxides and organic carbon in the sediment. Consequently processes, such as re-oxidation of Fe (II) resulting in the formation of fresh highly reactive iron-oxides, are likely to affect Km and V_{max} and should be included in the modelling efforts as well. Some of the sediment features, like bioturbation or sediment patchiness, are rather complex to model, because of the different mechanisms playing a role at different scales. Recently, Meysman 2001, described a modelling theory to introduce the bioturbation process in diagenetic models.

With respect to understanding biogeochemistry of iron- and manganese oxides the next step should be the incorporation of reactivity, limiting factors and bioturbational aspects in diagenetic models. Meanwhile the set of directly measured iron and manganese-oxide reduction rates should be enlarged with a broader range of environments in which faunal and metal-oxide reactivity should be investigated parallel with organic degradation pathways.

6.3.1. Metal-oxides and the pelagic system

If the sediments of High-Nutrient-Low chlorophyll (HNLC) regions would have an efflux of iron to the overlying water as large as the efflux we measured in the non-bioturbated sediments this could have a positive effect on the pelagic productivity in these regions, as whole ecosystem additions have shown (Martin et al. 1994; Coale et al. 1996 and Boyd et al. 2000). Besides, the effects of global warming on the water circulation of the ocean's is such that the HNLC regions will receive water from more coastal area's or nutrient rich upwelling regions. This can enhance the iron availability and stimulate primary production, thereby CO_2 uptake from the atmosphere will be enhanced and the system forms its own feed back system. However, as argued by Chisholm et al. 2001, the total surface of the HNLC area's is not sufficient to have any global scale effect if CO_2 fixation would be enhanced. Sarmiento and Orr 1991 predicted that at most 15% of the anthropogenic production of CO_2 can potentially be sequestered. Moreover, increased primary productivity can result in anoxia in the deeper water layers due to the subsequent changes in biogeochemistry (Chisholm, 2001). This may results in enlarged N_2O or CH_4 emission which both are more effective greenhouse gases than CO_2. Besides, as stated by Johnson and Karl 2002, our knowledge of impact of higher iron availability on carbon cycling in the ocean is not enough to predict the effects

6.3.2. Iron-oxide cycling and methane emission

A number of studies deal with the microbial processes governing methane emission from freshwater sediments and soils (Frenzel et al. 1999, Kruger et al. 2001, Achtnich et al. 1995, :Van Bodegom 2000). Especially the contribution of rice fields to global methane emission received a lot of interest, because methane is one of the principal greenhouse gases. Rice paddies are a significant source of atmospheric methane and account for 9-30 % of the global methane emission (Cicerone and Oremland 1988; Houghton et al. 1996, Matthews et al. 1991 in:Van Bodegom 2000). Recent calculations indicate that from 1950 to 1990 the harvested area of rice increased from 104 to 146 million ha (International Rice Research Institute (IRRI) 1991 in: :Van Bodegom 2000). Because of this increasing area and the greenhouse gas potential of methane, prevention methods for methane emission have been investigated in detail.

Two approaches have been proposed to mitigate methane emissions: (1) reducing methanogenesis and (2) preventing emission of methane by e.g. methane oxidation. Methane production can be suppressed by stimulating other mineralization pathways like iron-oxide reduction. In submerged rice paddies this can be achieved

by fertilisation with easily reducible iron oxides, which will stimulate iron-oxide reduction. Recently the capability of methanogens to reduce Fe (III) oxide has been discovered (Bond and Lovley 2002). Thus the microbial community in the rice paddy is capable of iron-oxide reduction from the start without any change in conditions. In a rice microcosm addition of 15 and 30 g ferrihydrite per kg of soil resulted in a reduction of methane emission by 43 and 84 % (Jackel and Schnell 2000). Our slurry-experiments indicate that at high organic inputs a three times increase in iron-oxide load decreases methanogenesis ~ 5 times. The stimulation of methane oxidation in the soils, thereby reducing methane concentration might be another approach. In rice paddies and wetland sediments methanotrophs compete for oxygen with heterotrophic bacteria and oxygen consuming processes like iron oxidation (Van der Nat and Middelburg 1998, Van Bodegom et al. 2001).

Future research on the decrease of methane emission from freshwater sediments should investigate whether iron-oxide concentrations are actually limiting iron-oxide reduction rates. If so, experiments with iron-oxide fertilisation in field situation are necessary to test the decrease in methane emission. Besides, applicable methods to ensure an efficient iron-oxide cycle will have to be investigated.

6.3.3.   Metal-oxide cycling and trace metals

Trace metal cycling can be affected by the iron biogeochemistry in two ways. First the trace metals can be incorporated in iron-oxides or pyrite and released upon oxide dissolution or pyrite oxidation (Santschi et al. 2001, Zachara et al. 2001, Huerta-Diaz and Morse 1992, Morse 1994). Second trace metal solubility can be affected by redox changes induced by iron geochemistry.

Either way the changes in sediment biogeochemistry affect trace metal availability. Sediments that have been functioning as a sink for trace metals can have high concentrations. A change in the prevailing biogeochemical processes may lead to release of the metals into the pore- and overlying water and accumulation toxic concentration levels (e.g. see Muller 2002 for an example). Our study revealed that organic carbon may be a rate limiting for the metal-oxide reduction in some sediments. Trace metal loaded oxides in organic carbon poor sediments are likely not released. However, an occasional input of organic matter to organic poor aerobic sediments may stimulate anaerobic processes and results in a dramatic increase of trace metal concentrations.

If trace metals are not incorporated in iron oxides, their release can nevertheless be affected by changes in redox conditions and pH. Dissimilatory iron-oxide reduction and oxidation of reduced iron and manganese may change pH values and so affect trace metals solubility and availability in the sediment because of

desorption. Iron and manganese-oxides are sometimes regarded as means to dispose of trace metals and nuclear waste (Mott et al. 1993, inNealson and Little 1997, Means et al. 1978). However, a number of studies have shown the release of trace metals into the interstitial waters, the anoxic bottom water, or groundwater as a result of iron or manganese reduction (Lovley 1991, Nealson and Little 1997). Therefore the rate of reduction and factors affecting this rate are important in estimating the potential danger of using iron and manganese oxides as a means of disposal. Besides, knowing the exact process of scavenging and co-precipitation is of interest to gain more insight in the co-precipitation.

6.3.4.   Potential of metal-oxides in bio-remediation and waste water treatment

The two most relevant aspects related to bio-remediation of soils and wastewaters are: (1) removal of Fe^{2+} and Mn^{2+} and trace metals from the solution by oxidation and co-precipitation and (2) degradation of organic carbon contaminants by dissimilatory metal reduction pathways.

Fe^{2+} and Mn^{2+} can be removed from solution by precipitation due to either physico-chemical treatment or microbial oxidation. As described in the preceding paragraph, toxic trace metals can precipitate simultaneously with iron or manganese oxides The aspect of degradation of organic contaminants recently received a lot of interest because until recently known degradation pathways for organic contaminants were all aerobic, and therefore problematic to apply in poorly aerobic environments such as landfills. The degradation with dissimilatory metal oxide reduction offers perspectives for anaerobic bio-remediation methods, offering a solution to the problem of limited oxygen availability that is associated with aerobic remediation. Lovley and Anderson 2000 described the potential of iron reducing bacteria to degrade organic contaminants by degrading them anaerobically to carbon dioxide. Some iron-oxide reducing bacteria were found to be able to dechlorinate chlorinated carbon compounds or to oxidise aromatic hydrocarbons. Stimulation of the iron-oxide reducing community promoted the occurrence of this process in contaminated sediment. For continuous, steady anaerobic degradation of contaminants the supply of degradable iron oxides should be sufficient. We showed that this might be reached by the addition of reactive iron oxides. However this is not feasible in a simple way, because the oxides also have to be transported into the reduction layer, and a mode of on place re-oxidation would allow more perspective.

Means of remediation via "on place re-oxidation" are electrochemical regeneration, microbial re-oxidation including the micro-cycling performed by some bacteria (Straub et al. 1996, Sobolev and Roden 2001). Ohmura et al. 2002 reported the

increased growth of *Thiobacillus ferrooxidans* through exogenous electrolysis resulting in the electrochemical regeneration of iron. Re-oxidation also promotes iron-oxide reduction because it removes the Fe (II) from the adsorption sites (Roden and Urrutia 1999). The use of an anode is not feasible for large-scale in situ remediation, stimulation of bacterial processes offers better perspectives. Our results indicate that the iron-oxide reactivity is a factor determining the iron-oxide reduction rate if both organic carbon and microbial reducible iron-oxide availability are not limiting. The re-oxidation of reduced iron is therefore a factor that potentially will have a positive effect on degradation rates.

A number of iron reducing bacteria are able to reduce other metals, including uranium (Lovley et al. 1991, Lovley 1997, Lovley and Anderson 2000). This dissimilatory reduction could also provide a way of bio-remediation because some metals are solid in the reduced form (Kashefi et al. 2001, Lovley et al. 1991, Lovley and Anderson 2000). Recently the formation of intracellular iron-oxide minerals in a dissimilatory iron oxide reducing bacterium was reported (Glasauer et al. 2002). Stimulating this process could be another possibility of re-oxidising iron oxides.

Methods to stimulate the metal-oxide reducing bacteria will be very valuable for bio-remediation techniques. Re-use of metals from industrial waste materials may be possible by using metal reducing bacteria to recover metals from solids (Krebs et al. 1997). The main problems to be solved with respect to all methods of bio-remediation are how to promote the reduction and prevent the limiting conditions to arise. Depending on the type of remediation required the limiting factors can be either organic carbon concentration or metal-oxide availability. Especially the aspect of metal-oxide availability is complex, e.g. see the paragraph dealing with reduction of methane emission. Future research should focus on creation of favourable conditions without large material or laborious input. Some suggestions are the introduction of adapted fauna species thereby introducing bioturbation, resulting in metal cycling (Aller 1994a, Kristensen 2000) or stimulation of reduction-oxidation cycles like the ones existing around plant roots (King and Garey 1999) or bacterial micro-scale cycling (Sobolev and Roden 2001).

7.   References

1.   Achtnich, C., Bak, F., and Conrad, R., 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers and methanogens in anoxic paddy soil. Biology and Fertility of Soils 19: 65-72.

2.   Albrechtsen, H.J., Heron, G., and Christensen, T. H., 1995. Limiting factors for microbia Fe(III)-reduction n a landfill leachate polluted aquifer (Vejen, Denmark). Fems Microbiology Ecology 16: 233-247.

3.   Aller, R.C., 1980. Diagenetic processes near the sediment-water interface of Long Island Sound. II. Advances in Geophysics 22: 351-415.

4.   Aller, R.C., 1983. The importance of the diffusive permeability of animal burrow linings in determining marine sdeiment chemistry. Journal of Marine Research 41: 299-322.

5.   Aller, R.C., 1990. Bioturbation and manganese cycling in hemipelagic sediments. Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences 331: 51-68.

6.   Aller, R.C., 1994a. Bioturbation and remineralization of sedimentary organic-matter -effects of redox oscillation. Chemical Geology 114: 331-345.

7.   Aller, R.C., 1994b. The sedimentary Mn cycle in Long-Island Sound - its role as an intermediate oxidant and the influence of bioturbation, O2 and C(org) flux. Journal of Marine Research 52: 259-295.

8.   Aller, R.C. and Aller, J. Y., 1992. Meiofauna and solute transport in marine muds. Limnology and Oceanography 37: 1018-1033.

9.   Aller, R.C. and Aller, J. Y., 1998. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. Journal of Marine Research 56: 905-936.

10.   Aller, R.C. and Rude, P. D., 1988. Complete oxidation of solid [hase sulfides by manganese and bacteria in anoxic marine sediments. Geochimica et Cosmochimica Acta 52: 751-756.

11.   Aller, R.C. and Yingst, J. Y., 1985. Effects of the marine deposit-feeders Heteromastus filiformis (Plychaeta), Macoma baltoca (Bivalvia), and Tellina texana (Bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. Journal of Marine Research 43: 615-645.

12. Bakker, J.F. and Helder, W., 1993. Skagerrak (northeastern North-Sea) oxygen microprofiles an porewater chemistry in sediments. Marine Geology 111: 299-321.

13. Banta, G.T., Holmer, M., Jensen, M. H., and Kristensen, E., 1999. Effects of two polychaete worms, Nereis diversicolor and Arenicola marina, on aerobic and anaerobic decomposition in a sandy marine sediment. Aquatic Microbial Ecology 19: 189-204.

14. Behrenfeld, M.J. and Kolber, Z. S., 1999. Wide spread iron limitation of phytoplankton in the South Pacific Ocean. Science 283: 840843-

15. Beijerinck, M.W., 1913. Oxydation des Mangaancorbonate durch Bakterien und Schimmelpilze. Folia Microbiolo Holl Beitr Ges Mikrobiol Delft 2: 123-124.

16. Bender, L.M. and Conrad, R., 1992. Kintetics of methane oxidation in oxic soils exposed to ambient air and high CH_4 mixing. Fems Microbiology Ecology 101: 261-270.

17. Berner, R.A., 1964. Distribution and diagenesis of sulfur in some sediments from the Gulf of California. Marine Geology 1: 117-140.

18. Berner, R.A., 1970. Sedimentary pyrite formation. American Journal of Science 268: 1-23.

19. Berner, R.A., 1984. Sedimentary pyrite formaion: an update. Geochimica et Cosmochimica Acta 48: 605-615.

20. Bodelier, P.L.E. and Frenzel, P., 1999. Contribution of methanotrophic and nitrifying bacteria to CH4 and NH4+ oxidation in the rizospeher of rice plants determined by new mehtods of discrimination. Applied and Environmental Microbiology 65: 1826-1833.

21. Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., and Jorgensen, B. B., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623-626.

22. Bond, D.R., Holmes, D. E., Tender, L. M., and Lovley, D. R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295: 483-485.

23. Bond, D.R. and Lovley, D. R., 2002. Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. Environmental Microbiology 4: 115-124.

24. Boudreau, B.P. and Ruddick, B. P., 1991. On a reactive continuum representation of organic-matter diagenesis. American Journal of Science 291: 538-

25. Bowie, A.R., Withworth, D. J., Achterberg, E. P., Mantoura, R. F. C., and Worsfold, P. J., 2002. Biogeochemistry of Fe and other trace elements (al, Co,Ni) in the upper atlantic Ocean. Deep Sea Research Part I: Oceanographic Research Papers 49: 605-636.

26. Boyd, P.W., Watson, A. J., Law, C. S., Abraham, E. R., Trull, T., Murdoch, R., Bakker, D. C. E., Bowie, A. R., Beusseler, K. O., Chang, H., Charette, M., Croot, P., Downing, K., Frew, R., Gall, M., Hadfield, M., Hall, J., Harvey, M., Jameson, G., LaRoche, J., Liddicoat, M., Ling, R., Maldonado, M. T., McKay, R. M, Nodder, S., Pickmere, S., Pridmore, R., Rintoul, S., Safi, K., Sutton, P., Strezpek, R., Tanneberger, K., Turner, S., Waite, A., and Zeldis, J., 2000. a mesoscale phytoplankton bloom in the Polar southern Ocean stimulated by iron fertilization. Nature 407: 695-702.

27. Brendel, P.J. and Luther, G. W., 1995. Development of a Gold almalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O_2 and S^2 in porewaters of marine and freshwater sediments. Environmental Science & Technology 29: 751-761.

28. Burdige, D.J., 1993. The biogeochemistry of manganese and iron reduction in marine-sediments. Earth-Science Reviews 35: 249-284.

29. Burdige, D.J., 2001. Dissolved organic matter in Chesapeake Bay sediment pore waters. Organic Geochemistry 32: 487-505.

30. Burdige, D.J., Dhakar, S. P., and Nealson, K. H., 1992. Effects of manganese oxide mineralogy on microbial and chemical manganese reduction. Geomicrobiology Journal 10: 27-48.

31. Calvert, S.E., Nielsen, B., and Fontugne, M. R., 1992. Evidence from nitrogen isotope ratios for enhanced productivity during formation of Eastern Mediterranean sapropels. Nature 359: 223-225.

32. Canfield, D.E., 1988. Sulfate reduction and the diagenesis of iron in anoxic marine sediments.

33. Canfield, D.E., 1989. Reactive iron in marine sediments. Geochimica et Cosmochimica Acta 53: 619-632.

34. Canfield, D.E., 1994. Factors influencing organic-carbon preservation in marine-sediments. Chemical Geology 114: 315-329.

35. Canfield, D.E., Jorgensen, B. B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N. B., Thamdrup, B., Hansen, W., Nielsen, L. P., and Hall, P. O. J., 1993a. Pathways of organic carbon oxidation in 3 continental-margin sediments. Marine Geology 113: 27-40.

36. Canfield, D.E., Raiswell, R., and Bottrell, S., 1992. Reactivity of sedimentary iron minerals toward sulfide. American Journal of Science 292: 659-683.

37.   Canfield, D.E. and Thamdrup, B., 1996. Fate of elemental sulfur in an intertidal sediment. Fems Microbiology Ecology 19: 95-103.

38.   Canfield, D.E., Thamdrup, B., and Hansen, W., 1993b. The anaerobic degradation of organic-matter in danish coastal sediments - iron reduction, manganese redcution, and sulfate reduction. Geochimica et Cosmochimica Acta 57: 3867-3883.

39.   Chisholm, S.W., Falkowski, P. G., and Cullen, J. J., 2001. Dis-crediting ocean fertilization. Science 294: 309-310.

40.   Christoffersen, J. and Christoffersen, M. R., 1976. The kinetics of dissolution of calcium sulphate dihydrate in water. Journal of Crystal Growth 35: 79-88.

41.   Church, M.J., Hutchins, D. A., and Ducklow, H. W., 2000. Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean. Applied and Environmental Microbiology 66: 455-466.

42.   Cicerone, R.J. and Oremland, R. S., 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochemical Cycles 1: 299-327.

43.   Coale, K.H., Johnson, K. S., Fitzwater, S. E., Gordon, R. M., Tanner, S., Chavez, F. P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F. J., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W. P., Landry, M. R., Constantinou, J., Rollwagen, G., Trasvina, A., and Kudela, R., 1996. A massive phyoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383: 495-501.

44.   Coates, J.D., Councell, T., Ellis, D. J., and Lovley, D. R., 1998. Carbohydrate oxidation coupled to Fe(III) reduction, a novel form of anaerobic metabolism. Anaerobe 4: 277-282.

45.   Conrad, R. and Klose, M., 1999. Anaerobic conversion of carbon dioxide to methane, acetate and propionate wahsed rice roots. Fems Microbiology Ecology 30: 147-155.

46.   Dauwe, B., Middelburg, J. J., and Herman, P. M. J., 2001. Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area. Marine Ecology-Progress Series 215: 13-22.

47.   Dauwe, B., Middelburg, J. J., Herman, P. M. J., and Heip, C. H. R., 1999. Linking diagenetic alteration of amino acids and bulk organic matter reactivity. Limnology and Oceanography 44: 1809-1814.

48.   Davey, J.T., 1994. The architecture of the burrow of Nereis diversicolor and its quantification in relation to sediment-water exchange. Journal of Experimental Marine Biology and Ecology 179: 115-129.

49. Davey, J.T. and Watson, P. G., 1995. The activity of Nereis diversicolor (Polychaeta) and its impact on nitrient fluxes in estuarine waters. Ophelia 41: 57-70.

50. Davison, W., Fones, G. R., and Grime, G. W., 1997. Dissolved metals in surface sediment and a microbial mat at 100-mu m resolution. Nature 387: 885-888.

51. De Baar, H.J.W., Buma, A. G. J., Nolting, R. F., Cadee, G. C., Jacques, G., and Treguer, P. J., 1990. On iron limitation of the southern Ocean: experiemental observations in the Weddell and Scotia Seas. Marine Ecology-Progress Series 65: 105-122.

52. De Baar, H.J.W., de Jong, Jeroen T. M., Nolting, Rob F., Timmermans, Klaas R., van Leeuwe, Maria A., Bathmann, U., Rutgers van der Loeff, Michiel, and Sildam, Juri, 1999. Low dissolved Fe and the absence of diatom blooms in remote Pacific waters of the Southern Ocean. Marine Chemistry 66: 1-34.

53. De Baar, H.J.W., de Jong, Jeroen T. M., Nolting, Rob F., Timmermans, Klaas R., van Leeuwe, Maria A., Bathmann, U., Rutgers van der Loeff, Michiel, and Sildam, Juri, 1999a. Low dissolved Fe and the absence of diatom blooms in remote Pacific waters of the Southern Ocean. Marine Chemistry 66: 1-34.

54. De Baar, H.J.W., de Jong, Jeroen T. M., Nolting, Rob F., Timmermans, Klaas R., van Leeuwe, Maria A., Bathmann, U., Rutgers van der Loeff, Michiel, and Sildam, Juri, 1999b. Low dissolved Fe and the absence of diatom blooms in remote Pacific waters of the Southern Ocean. Marine Chemistry 66: 1-34.

55. De Capitani, L. and Cita, M. B., 1996. The "Marker-bed" of the Mediterranean Ridge diapiric belt: geochemical characteristics. Marine Geology 132: 215-225.

56. De Lange, G.J., Middelburg, J. J., and Pruysers, P. A., 1989. Middle and Late Quaternary depositional sequences and cycles in the eastern Mediterranean. Sedimentology 36: 151-156.

57. DeBaar, H.J.W., DeJong, J. T. M., Bakker, D. C. E., LOSCHER, B. M., Veth, C., Bathmann, U., and Smetacek, V., 1995. Importance of iron for plankton blooms and carbon-dioxide drawdown in the southern ocean. Nature 373: 412-415.

58. DeLange, G.J., Middelburg, J. J., and Pruysers, P. A., 1989. Middle and Late Quaternary depositional sequences and cycles in the eastern Mediterranean. Sedimentology 36: 151-156.

59. Deng, Y.W. and Stumm, W., 1994. Reactivity of aquatic iron(III) oxyhydroxides - implications for redox cycling of iron in natural waters. Applied Geochemistry 9: 23-36.

60. Dobbin, P.S., Carter, J. P., San Juan, C. G. S., von Hobe, M., Powell, A. K., and Richardson, D. J., 1999. Dissimilatory Fe(III) reduction by Clostridium beijerinckii isolated from freshwater sediment using Fe(III) maltol enrichment. Fems Microbiology Letters 176: 131-138.

61. Donaghay, P.L., 1991. The role of episodic atmospheric nutrients inputs in chemical and biological dynamics of oceanic ecosystems. Oceanography 4: 62-70.

62. Dong, D., Nelson, Y. M., Lion, Leonard W., Shuler, M. L., and Ghiorse, W. C., 2000. Adsorption of Pb and Cd onto metal oxides and organic material in natural surface coatings as determined by selective extractions: new evidence for the importance of Mn and Fe oxides. Water Research 34: 427-436.

63. Duce, R.A. and Tindale, N. W., 1991. Atmospheric transport of iron and its deposition in the ocean. Limnology and Oceanography 36: 1715-1726.

64. Ehrlich, H.L., 1993. Electron transfer from acetate to the surface of MnO2 particles by a marine bacterium. Journal of Industrial Microbiology 12: 121-128.

65. Ehrlich, H.L. and Salerno, J. C., 1990. Energy coupling in Mn2+ oxidation by a marine bacterium. Archives of Microbiology 154: 12-17.

66. Epping, E.H.G., Schoemann, V., and De Heij, H., 1998. Manganese and iron oxidation during benthic oxygenic photosynthesis. Estuarine Coastal and Shelf Science 47: 753-767.

67. Ernstsen, V., Gates, W. P., and Stucki, J. W., 1998. Microbial reduction of structural iron in clays - A renewable source of reduction capacity. Journal of Environmental Quality 27: 761-766.

68. Fein, J.B., Brady, P. V., Jain, J. C., Dorn, R. I., and Lee, J. U., 1999. Bacterial effects on the mobilization of cations from a weathered Pb-contaminated andesite. Chemical Geology 158: 189-202.

69. Fenchel, T., 1996. Worm burrows and oxic microniches in marine sediments .1. Spatial and temporal scales. Marine Biology 127: 289-295.

70. Frenzel, P., Bosse, U., and Janssen, P. H., 1999. Rice roots and methanogenesis in a paddy soil: ferric iron as an alternative electron acceptor in the rooted soil. Soil Biology & Biochemistry 31: 421-430.

71. Froelich, P.N., Luedtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., and Maynard, V. K., 1978. Early oxidation of

organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta 43: 1075-1090.

72.   Glasauer, S., Lanley, S., and Beveridge, T. J., 2002. Intracellular iron minerals in a dissimilatory iron-reducing bacterium. Science 295: 117-119.

73.   Glud, R., Hofmann, E. E., and Canfield, D. E., 1998. Benthic mineralization and exchange in Arctic sediments (Svalbard, Norway). Marine Ecology-Progress Series 173: 237-251.

74.   Glud, R.N., Risgaard-Petersen, N., Thamdrup, B., Fossing, H., and Rysgaard, S., 2000. Benthic carbon mineralization in a high-Arctic sound (Young Sound, NE Greenland). Marine Ecology-Progress Series 206: 59-71.

75.   Gran, H.H., 1931. On the conditions for the production of plankton in the sea. Rapp.P.-v.Cons.perm.Explor.Mer. 76: 37-46.

76.   Grashoff, K., Erhardt, M, and Kremling, C., 1983. Methods for seawater analysis. 0-419.

77.   Haese, R.R., Petermann, H., Dittert, L., and Schulz, H. D., 1998. The early diagenesis of iron in pelagic sediments: a multidisciplinary approach. Earth and Planetary Science Letters 157: 233-248.

78.   Haese, R.R., Schramm, J., Rutgers van der Loeff, M. M., and Schulz, H. D., 2000. A comparitive study of iron and manganese diagenesis in continental slope and deep sea basin sediments off Uruguay (SW Atlantic). International Journal on Earth Sciences 88: 619-629.

79.   Hall, J.A. and Safi, K., 2001. The impact of insitu Fe fertilisation on the microbial food web in the southern Ocean. Deep-Sea Research II 48: 2591-2613.

80.   Hansen, J.W., Thamdrup, B., and Jorgensen, B. B., 2000. Anoxic incubation of sediment in gas-tight plastic bags: a method for biogeochemical process studies. Marine Ecology-Progress Series 208: 273-282.

81.   Hansen, L.B., Finster, K., Fossing, H., and Iversen, N., 1998. Anaerobic methane oxidation in sulfate depleted sediments: effects of sulfate and molybdate additions. Aquatic Microbial Ecology 14: 195-204.

82.   Harper, M.P., Davison, W., and Tych, w., 1999. One dimenional view of three dimensional sediments. Environmental Science & Technology 33: 2611-2616.

83.   Heart, T.J., 1934. On the phytoplankton of the Southwest Atlantic and Bellinghausen Sea 1929-1931. 'Discovery' Report 8: 1-268.

84.   Heijs, S.K., Jonkers, H. M., van Gemerden, H., Schaub, B. E. M., and Stal, L. J., 1999. The buffering capacity towards free sulphide in sediments of a

coastal lagoon (Bassin d'Arcachon, France) - the relative importance of chemical and biological processes. Estuarine Coastal and Shelf Science 49: 21-35.

85. Heip, C.H.R., Goosen, N. K., Herman, P. M. J., Kromkamp, J., Middelburg, J. J., and Soetaert, K., 1995. Production and consumption of biological particles in temperate tidal estuaries. Oceanography and Marine Biology - An Annual Review, Vol 33 33: 1-149.

86. Henrichs, S.M. and Reeburgh, W. S., 1987. Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic porcesses in the oceanic carbon economy. Geomicrobiology Journal 5: 191-237.

87. Herman, P.M.J., Middelburg, J. J., Van de Koppel, J., and Heip, C. H. R., 1999. Ecology of estuarine macrobenthos. Advances in Ecological Research, Vol 29 29: 195-240.

88. Hines, M.E., Bazylinski, D. A., Tugel, J. B., and Lyons, W. B., 1991. Anaerobic microbial biogeochemistry in sediments from two basins in the Gulf of Maine: evidence for iron and manganese reduction: evidence for iron and manganese reduction. Estuarine Coastal and Shelf Science 32: 313-324.

89. Hines, M.E., Faganeli, J., and Planinc, R., 1997. Sedimentary anaerobic microbial biogeochemistry in the Gulf of Trieste, northern Adriatic Sea: Influences of bottom water oxygen depletion. Biogeochemistry 39: 65-86.

90. Houghton, J.T., Meira Filho, L. G., Callander, B. A., Harris, N., Kattenberg, A., and Marskell, K., 1996. Climate change 1995. The science of climate change. 1:

91. Huerta-Diaz, M.A. and Morse, J. W., 1992. Pyritization of trace metals in anoxic marine sediments. Geochimica et Cosmochimica Acta 56: 2681-2702.

92. Hylleberg, J. and Henriksen, K., 1980. The cenatrl role of biotrbation on sediment mineralization and element recycling. Ophelia Suppl.1: 1-16.

93. Ingalls, A.E., Aller, R. C., Lee, C., and Sun, M. Y., 2000. The influence of deposit-feeding on chlorophyll-a degradation in coastal marine sediments. Journal of Marine Research 58: 631-651.

94. International Rice Research Institute (IRRI), 1991. World rice statistics 1990.

95. Jackel, U. and Schnell, S., 2000. suppression of methane emission from rice paddies by ferric iron fertilization. Soil Biology & Biochemistry 32: 1811-1814.

96.   Jackson, D.D., 1901. The precipitation of iron, manganese and aluminium by bacterial action. J Soc Chem Ind 21: 681-684.

97.   Jakobsen, R. and Postma, D., 1999. Redox-zoning, rates of sulfate reduction and interaction with Fe-reduction and methanogenesis in a shallow sandy aquifer, Romo, Denmark. Geochimica et Cosmochimica Acta 63: 137-151.

98.   Johnson, K.S. and Karl, D. M., 2002. Is ocean fertilization credible or creditable ? Science 296: 467-469.

99.   Jorgensen, B.B., 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnology and Oceanography 22: 814-832.

100.   Jorgensen, B.B., 1982. Mineralization of organic matter in the sea bed - the role of sulphate reduction. Nature 296: 645-

101.   Joye, S.B., Mazzotta, M. L., and Hollibaugh, J. T., 1996. Community metabolism in microbial mats: The occurence of biologically mediated iron and manganese reduction. Estuarine Coastal and Shelf Science 43: 747-766.

102.   Kashefi, K., Tor, J. M., Nevin, K. P., and Lovley, D. R., 2001. Reductive precipitation of gold by dissimilatory Fe(III) reducing bacteia an archaea. Applied and Environmental Microbiology 67: 3275-3279.

103.   Kennedy, L.G. and Everett, J. W., 2001. Microbial degradation of simulated landfill leachate: solid iron/sulfur interactions. Advances in Environmental Research 5: 103-116.

104.   King, D.W. and Farlow, R., 2000. Role of carbonate speciation on the oxidation of Fe(II) by H2O2. Marine Chemistry 70: 201-209.

105.   King, G.M., 1990. Effects of added manganic and ferric oxides on sulfate reduction and sulfide oxidation in intertidal sediments. Fems Microbiology Ecology 73: 131-138.

106.   King, G.M. and Garey, M. A., 1999. Ferric tron reduction by bacteria associated with the roots of freshwater and marine macrophytes. Applied and Environmental Microbiology 65: 4393-4398.

107.   King, G.M. and Klug, M. J., 1982. Relation of soil water movement and sulfide concentration to Spartina alterniflora production in a Georgia salt Marsh. Science 218: 61-63.

108.   Knowles, R., Lean, D. R. S., and Chan, Y. K., 1981. Nitrous oxide concentrations in lakes: variations with depth and time. Limnology and Oceanography 26: 855-866.

109.   Kostka, J.E., Gribsholt, B., Petrie, E., Dalton, D., Skelton, H., and Kristensen, E., 2002. The rates and pathways of carbon oxidation in

bioturbated saltmarsh sediments. Limnology and Oceanography 47: 230-240.

110. Kostka, J.E., Haefele, E., Viehweger, R., and Stucki, J. W., 1999a. Respiration and dissolution of iron(III) containing clay minerals by bacteria. Environmental Science & Technology 33: 3127-3133.

111. Kostka, J.E. and Luther, G. W., 1994. Partitioning and speciation of solid-phase iron in salt-marsh sediments. Geochimica et Cosmochimica Acta 58: 1701-1710.

112. Kostka, J.E. and Luther, G. W., 1995. Seasonal cycling of Fe in salt-marsh sediments. Biogeochemistry 29: 159-181.

113. Kostka, J.E., Thamdrup, B., Glud, R. N., and Canfield, D. E., 1999b. Rates and pathways of carbon oxidation in permanently cold Arctic sediments. Marine Ecology-Progress Series 180: 7-21.

114. Krebs, W., Brombacher, C., Bosshard, P. P., Bachofen, R., and Brandl, H., 1997. Microbial recovery of metals from solids. Fems Microbiology Reviews 20: 605-617.

115. Kristensen, E., 1984. Effect of natural concentrations on nutrient exchange between polychaete burrow in estuarine sediment and the overlying water. Journal of Experimental Marine Biology and Ecology 75: 171-190.

116. Kristensen, E., 1985. Oxygen and inroganic nitrogen exchange in a Nereis virens (Polychaeta) bioturbated sediment-water system. Journal of Coastal Research 1: 109-116.

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

118. Kristensen, E., Andersen, F. O., and Blackburn, T. H., 1992. Effects of benthic macrofauna and temperature on degradation of macroalgal detritus - the fate of organic-carbon. Limnology and Oceanography 37: 1404-1419.

119. Kristensen, E. and Holmer, M., 2001. Decomposition of plant materials in marine sediment exposed to different electron acceptors (O-2, NO3-, and SO42-), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. Geochimica et Cosmochimica Acta 65: 419-433.

120. Kristensen, K. and Hansen, K., 1999. Transport of carbon dioxide and ammonium in bioturbated (Nereis diversicolor) coastal, marine sediments. Biogeochemistry 45: 147-168.

121. Kruger, M., Frenzel, P., and Conrad, R., 2001. Microbial processes influencing methane emission from rice fields. Global Change Biology 7: 49-63.

122. Langenhoff, A.A.M., BrouwersCeiler, D. L., Engelberting, J. H. L., Quist, J. J., Wolkenfelt, J. G. P. N., Zehnder, A. J. B., and Schraa, G., 1997. Microbial reduction of manganese coupled to toluene oxidation. Fems Microbiology Ecology 22: 119-127.

123. Larsen, O. and Postma, D., 2001. Kinetics of reductive bulk dissolution of lepidocrocite, ferrihydrite and goethite. Geochimica et Cosmochimica Acta 65: 1367-1379.

124. Lee, G., Bigham, J. M., and Faure, G., 2002. Removal of trace metals by coprecipitation with Fe, Al and Mn from natural water contasminated with acid mine drainage in the Ducktown Mining District, Tennesse. Applied Geochemistry 17: 569-581.

125. Liu, C.G., Zachara, J. M., Gorby, Y. A., Szecsody, J. E., and Brown, C. F., 2001. Microbial reduction of Fe(III) and sorption/precipitation of Fe(II) on Shewanella putrefaciens strain CN32. Environmental Science & Technology 35: 1385-1393.

126. Lovley, D.R., 1987. Organic Matter Mineralization with the Reduction of Ferric Iron: A Review. Geomicrobiology Journal 5: 375-399.

127. Lovley, D.R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiological Reviews 55: 259-287.

128. Lovley, D.R., 1997. Microbial Fe(III) reduction in subsurface environments. Fems Microbiology Reviews 20: 305-313.

129. Lovley, D.R. and Anderson, R. T., 2000. Influence of dissimilatory metal reduction on fate of organic and metal contaminants in the subsurface. Hydrogeology Journal 8: 77-88.

130. Lovley, D.R. and Blunt-Harris, E. L., 1999. Role of humic-bound iron as an electron transfer agent in dissimilatory Fe(III) reduction. Applied and Environmental Microbiology 65: 4252-4254.

131. Lovley, D.R. and Phillips E.J.P., 1987. competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Applied and Environmental Microbiology 53: 2636-2641.

132. Lovley, D.R. and Phillips, E. J. P., 1986. Organic Matter Mineralization with Reduction of Ferric Iron in anaerobic Sediments. Applied and Environmental Microbiology 51: 683-689.

133. Lovley, D.R. and Phillips, E. J. P., 1987. Rapid assay for microbially reducible Ferric Iron in aquatic sediments. Applied and Environmental Microbiology 53: 1563-1540.

134.   Lovley, D.R. and Phillips, E. J. P., 1988. Novel mode of micribial energy-metabolism - organic-carbon oxidation coupled to dissimilatory reduction of iron or manganese. Applied and Environmental Microbiology 54: 1472-1480.

135.   Lovley, D.R. and Phillips, E. J. P., 1989. Requirement for a microbial consortium to completely oxidize glucose in Fe(III)-reducing sediments. Applied and Environmental Microbiology 55: 3234-3236.

136.   Lovley, D.R., Phillips, E. J. P., Gorby, Y. A., and Landa, E. R., 1991. Microbial reduction of uranium. Nature 350: 413-415.

137.   Lovley, D.R., Woodward, J. C., and Chapelle, F. H., 1996. Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. Applied and Environmental Microbiology 62: 288-291.

138.   Luther, G.W., Brendel, P. J., Lewis, B. L., Sundby, B., Lefrancois, L., Silverberg, N., and Nuzzio, D. B., 1998. Simultaneous measurement of O-2, Mn, Fe, I-, and S(-II) in marine pore waters with a solid-state voltammetric microelectrode. Limnology and Oceanography 43: 325-333.

139.   Luther, G.W., Kostka, J. E., Church, T. M., Sulzberger, B., and Stumm, W., 1992. Seasonal iron cycling in the salt-marsh sedimentary environment - the importance of ligand complexes with Fe(II) and Fe(II) in the dissolution of Fe(III) minerals and pyrite respectively. Marine Chemistry 40: 81-103.

140.   Luther, G.W., Sundby, B., Lewis, B. L., Brendel, P. J., and Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. Geochimica et Cosmochimica Acta 61: 4043-4052.

141.   Magni, P. and Montani, S., 1998. Response of intertidal and subtidal communities of the macrobenthos to organic load and oxygen depletion in the Seto Inland Sea, Japan. Journal Recherche Oceanographique 23: 47-56.

142.   Martin, J.H., 1990. Glacia-interglacial CO2 change: the iron hypothesis. Paleoceanography 5: 1-13.

143.   Martin, J.H., Coale, K. H., Johnson, K. S., Fitzwater, S. E., Gordon, R. M., Tender, L. M., Hunter, C. N., Elrod, V. A., Nowicki, J. L., Coley, T. L., Barber, R. T., Lindley, S., Watson, A. J., Van scoy, K., Law, C. S., Liddicoat, M., Li, Yu, Stanton, T., Stockel, J., Collins, C., Anderson, A., Bidigare, R. M, Ondrusek, M., Latasa, M., Millero, F. J., Lee, K., Yao, W., Zhang, J. Z., Friedrich, G., Sakamoto, C., Chavez, F. P., Buck, K., Kolber, Z. S., Greene, R., Falkowski, P. G., Chisholm, S. W., Hoge, F., Swift, R., Yungel, J., Turner, S., Nightingale, P., Hatton, A., Liss, P., and Tindale, N. W., 1994. Testing the iron hyothesis in ecosystems of the equatorial Pacific Ocean. Nature 371: 123-129.

144. Matthews, E., Fung, I., and Learner, G., 1991. Methane emission from rice cultivation: Geographic and seasonal distribution of cultivated areas and emissions. Global Biogeochemical Cycles 5: 3-24.

145. Means, J.L., Crerar, D. a., and Borcsik, M. P., 1978. Radio nuclide adsorption by manganese oxides and implications for radioactive waste disposal. Nature 274: 44-47.

146. Mercone, D., Thomson, J., Croudace, I. W., Siani, G., Paterne, M., and Troelstra, S., 2000. Duration of S1, the most recent sapropel in the eastern Mediterranean Sea, as indicated by accelerator mass spectrometry radiocarbon and geochemical evidence. Paleoceanography 15: 336-347.

147. Meysman, F., 2001. Modelling the influence of ecological interactions on reactive transport processes in sediments.

148. Middelburg, J.J., 1989. A simple rate model for oganic matter decomposition in marine sediments. Geochimica et Cosmochimica Acta 53: 1577-1581.

149. Middelburg, J.J., 1991. Organic carbon, sulphur, and iron in recent semi-euxinic sediments of Kau Bay, Indonesia. Geochimica et Cosmochimica Acta 55: 815-828.

150. Middelburg, J.J., De Lange, G. J., and Van der Weijden, C. H., 1987. Manganese solubility control in marine porewaters. Geochimica et Cosmochimica Acta 51: 759-763.

151. Middelburg, J.J., Klaver, G., Nieuwenhuize, J., Wielemaker, A., deHaas, W., Vlug, T., and Van der Nat, J. F. W. A., 1996a. Organic matter mineralization in intertidal sediments along an estuarine gradient. Marine Ecology-Progress Series 132: 157-168.

152. Middelburg, J.J. and Nieuwenhuize, J., 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Scheldt Estuary. Marine Chemistry 60: 217-225.

153. Middelburg, J.J., Soetaert, K., Herman, P. M. J., and Heip, C., 1996b. Denitrififcation in marine sediments: a model study. Global Biogeochemical Cycles 10: 661-673.

154. Middelburg, J.J., Vlug, T., and Van der Nat, F. J. W. A., 1993. Organic matter mineralization in marine systems. Global and Planetary change 8: 47-56.

155. Moeslund, L., Thamdrup, B., and Jorgensen, B. B., 1994. Sulfur and iron cycling in a coastal sediment - radiotracer studies and seasonal dynamics. Biogeochemistry 27: 129-152.

156. Morse, J.W., 1994. Interactions of trace metals with autogenic sulfide minerals: Implications for their bioavailability. Marine Chemistry 46: 1-6.

157. Mott, H.V., Singh, S., and Kondapally, V. R., 1993. Res.Technol. 114:

158. Muller, A., 2002. Organic carbon burial rates, and carbon and sulfur relationships in coastal sediments of the southern Baltic Sea. Applied Geochemistry 17: 337-352.

159. Mulsow, S., Boudreau, B. P., and Smith, J. N., 1998. Bioturbation and porosity gradients. Limnology and Oceanography 43: 1-9.

160. Munch J.C. and Ottow, J. C. G., 1980. Preferential reduction of amorphous to crystaline iron oxides by bacterial activity. Soil Science 129: 15-21.

161. Munch, J.C. and Ottow, J. C. G., 1980. Preferential reduction of amorphous to crystaline iron oxides by bacterial activity. Soil Science 129: 15-21.

162. Murase, J. and Kimura, M., 1997. Anaerobic reoxidation of Mn2+, Fe2+, S-0 and S2- in submerged paddy soils. Biology and Fertility of Soils 25: 302-306.

163. Myers, C.R. and Nealson, K. H., 1988. Bacterial manganese reduction and growth with manganese oxide as sole electron-acceptor. Science 240: 1319-1321.

164. Namerhoff, T.J., Balistrieri, L. S., and Murray, J. W., 2002. Suboxic trace metal geochemistry in the eastern tropical North Pacific. Geochimica et Cosmochimica Acta 66: 1139-1158.

165. Nealson, K.H., 1978. the isolation and characterization of marine bacteria whoch catalyze manganese oxidation. 847-858.

166. Nealson, K.H. and Little, B., 1997. Breathin manganese and iron: Solid-state respiration. Advances in Applied Microbiology 45: 213-239.

167. Nevin, K.P. and Lovley, D. R., 2000. Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by Geobacter metallireducens. Applied and Environmental Microbiology 66: 2248-2251.

168. Nieuwenhuize, J., Maas, Y. E. M., and Middelburg, J. J., 1994. Rapid analysis of organic carbon and nitrogen in particulate materials. Marine Chemistry 45: 217-244.

169. Nieuwenhuize, J., Poley-Vos, C., Van den Akker, A. H., and Van Delft, W., 1991. Comparison of microwave and conventional extraction techniques for the determination of metals in soil, sediment and sludge samples by atomic spectroscopy. Analyst 116: 347-351.

170. Nixon, S.W., 1981. Remineralization and nutrient cycling in coastal marine ecosystems. 111-138.

171.   Ohmura, N., Matsumoto, N., Sasaki, K., and Saiki, H., 2002.
        Electrochemical regeneration of Fe(III) to support growth on anaerobic
        iron respiration. Applied and Environmental Microbiology 68: 405-407.

172.   Oremland, R.S. and Capone, D. G., 1988. Use of specific inhibitors in
        biogeochemitry and microbial ecology. Advances in Microbial Ecology 10:
        285-383.

173.   Pakulski, J.D., Coffin, R. B., Kelley, C. A., Holder, S. L., Downer, R., Aas,
        P., Lyons, M. M., and Jeffrey, W. H., 1996. Iron stimulation of Antarctic
        bacteria. Nature 383: 133-134.

174.   Passier, H.F. and De Lange, G. J., 1998. Sedimentary sulphur and iron
        chemistry in relation to the formation of Eastern Mediterranen sapropels.
        160:

175.   Pelegri, S.P. and Blackburn, T. H., 1994. Bioturbation effects of the
        amphipod Corrophium volutators on microbial nitrogen transfromation in
        marine-sediments. Marine Biology 121: 253-258.

176.   Phillips, E.J.P., Lovley, D. R., and Roden, E. E., 1993. Composition of non-
        microbially reducible Fe(III) in aquatic sediments. Applied and
        Environmental Microbiology 59: 2727-2729.

177.   Postma, D., 1985. Concentration of Mn and separation from Fe in
        sediments -I.Kinetics and stoichiometry of the reaction between birnessite
        and dissolved Fe(II) at 10oC. Geochimica et Cosmochimica Acta 49: 1023-
        2033.

178.   Postma, D., 1993. The reactivity of iron-oxides in sediments - a kinetic
        approach. Geochimica et Cosmochimica Acta 57: 5027-5034.

179.   Postma, D. and Appelo, C. A. J., 2000. Reduction of Mn-oxides by ferrous
        iron in a flow system: Column experiment and reactive transport modeling.
        Geochimica et Cosmochimica Acta 64: 1237-1247.

180.   Postma, D. and Jakobsen, R., 1996. Redox zonation: Equilibrium
        constraints on the Fe(III)/SO4- reduction interface. Geochimica et
        Cosmochimica Acta 60: 3169-3175.

181.   Pruysers, P.A., De Lange, G. J., and Middelburg, J. J., 1991. Geochemistry
        of eastern Mediterrranean sediment - primary sediment composition and
        diagenetic alterations. Marine Geology 100: 137-154.

182.   Pruysers, P.A., De Lange, G. J., Middelburg, J. J., and Hydes, D. J., 1993.
        The diagenetic formation of metal-rich layers in sapropel-containing
        sediments in the Eastern Mediterranean. Geochimica et Cosmochimica
        Acta 57: 527-536.

183. Pyzik, A.J. and Sommer, S. E., 1981. Sedimentary iron monosulfides: kinetics and mechanism of formation. Geochimica et Cosmochimica Acta 45: 687-698.

184. Rasmussen, H. and Nielsen, P. H., 1996. Iron reduction in activated sludge measured with different extraction techniques. Water Research 30: 551-558.

185. Revsbech, N.P., Sorensen, J., Blackburn, T. H., and Lomholt, J. P., 1980. Distribution of oxygen in marine sediments measured with microelectrodes. Limnology and Oceanography 25: 403-411.

186. Riisgard, H.U., 1991. Suspension feeding in the plychaete Nereis diversicolor. Marine Ecology-Progress Series 70: 29-37.

187. Roden, E.E. and Edmonds, J. W., 1997. Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron sulfide formation. Archives of Microbiology 3: 347-378.

188. Roden, E.E. and Urrutia, M. M., 1999. Ferrous iron removal promotes microbial reduction of crystalline iron(III) oxides. Environmental Science & Technology 33: 1847-1853.

189. Roden, E.E. and Wetzel, R. G., 1996. Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology and Oceanography 41: 1733-1748.

190. Roden, E.E. and Wetzel, R. G., 2002. Kinetics of microbial Fe(III) oxide reduction in freshwater wetland sediments. Limnology and Oceanography 47: 198-211.

191. Roden, E.E. and Zachara, J. M., 1996. Microbial reduction of crystalline iron(III) oxides: Influence of oxide surface area and potential for cell growth. Environmental Science & Technology 30: 1618-1628.

192. Rossignol-Strick, M., Nesteroff, W., Olive, P., and Vergnaud-Grazzini, C., 1982. After the deluge: Mediterranean stagnation and sapropel formation. Nature 295: 105-110.

193. Roy, R., Kluber, H. D., and Conrad, R., 1997. Early initiation of methane production in anoxic rice soil despite the presence of oxidants. Fems Microbiology Ecology 24: 311-320.

194. Rysgaard, S., Thamdrup, B., Risgaard-Petersen, N., Fossing, H., Berg, P., Christensen, P. B., and Dalsgaard, T., 1998. Seasonal carbon and nutrient mineralization in a high-Arctic coastal marine sediment, Young Sound, Northeast Greenland. Marine Ecology-Progress Series 175: 261-276.

195. Ryther, J.H. and Dunstan, W. M., 1971. Nitrogen, phosphorous and eutrophication in the coastal marine environment. Science 171: 1008-1013.

196. Saager, P.M., Sweerts, J. P., and Ellermeijer, H. J., 1990. A simple pore-water sampler for coarse, sandy sediments of low porosity. Limnology and Oceanography 35: 747-751.

197. Sansone, F.J., Rust, T. M., and Smith, S. V., 1998. Methane distribution and cycling in Tomales Bay, California. Estuaries 21: 66-77.

198. Santschi, P., Hohener, P., Benoit, G., and Bucholtztenbrink, M., 1990. Chemical processes at the sediment water interface. Marine Chemistry 30: 269-315.

199. Santschi, P., Wen, L. S., and Guo, L., 2001. Transport and diagenesis of trace metals and organic matter in Palos Verdes shelf sediments affected by waste water outfall. Marine Chemistry 73: 153-171.

200. Sarmiento, J.L. and Orr, J. C., 1991. xxx. Limnology and Oceanography 36: 1920-

201. Schippers, A. and Jorgensen, B. B., 2001. Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments. Geochimica et Cosmochimica Acta 65: 915-922.

202. Schwertmann, U. and Cornell, R. M., 1991. Iron oxides in the laboratory.

203. Skei, J.M., Loring, D. H., and Rantala, R. T. T., 1988. Partitioning and enrichment of trace metals in a sediment core from Framvaren, South Norway. Marine Chemistry 23: 269-281.

204. Slomp, C.P., Malschaert, J. F. P., Lohse, L., and Van Raaphorst, W., 1997. Iron and manganese cycling in different sedimentary environments on the North Sea continental margin. Continental Shelf Research 17: 1083-1117.

205. Slomp, C.P. and Meysman, F., 2002. Aquatic Chemistry (submitted)

206. Slomp, C.P., Thomson, J., and De Lange, G. J., 2002. Enhanced regeneration of phosphorous during formation of the most recent Mediterranean sapropel (S1). Geochimica et Cosmochimica Acta 66: 1171-1184.

207. Slomp, C.P., Van der Gaast, S. J., and Van Raaphorts, W., 1996. Phosphorus binding by poorly crystalline iron oxides in North Sea sediments. Marine Chemistry 52: 5-73.

208. Sobolev, D. and Roden, E. E., 2001. Suboxic deposition of ferric iron by bacteria in opposing gradients of Fe(II) and oxygen at circumneutral pH. Applied and Environmental Microbiology 67: 1328-1334.

209. Soetaert, K., Herman, P. M. J., and Middelburg, J. J., 1996. A model of early diagenetic processes from the shelf to abyssal depths. Geochimica et Cosmochimica Acta 60: 1019-1040.

210. Soetaert, K., Herman, P. M. J., Middelburg, J. J., and Heip, C., 1998. Assessing organic matter mineralization, degradability and mixing rate in an ocean margin sediment (Northeast Atlantic) by diagenetic modeling. Journal of Marine Research 56: 519-534.

211. Sorensen, J., 1987. Nitrate reduction in marine sediments: pathways and interactions with iron and sulfur cycling. Geomicrobiology Journal 5: 401-421.

212. Sorensen, J. and Jorgensen, B. B., 1987. Early diagenesis in sediments from Danish coastal waters: Microbial activity and Mn-Fe-S geochemistry. Geochimica et Cosmochimica Acta 51: 1583-1590.

213. Stookey, L.L., 1970. Ferrozine - a new spectrophotometric reagent for iron. Applied and Environmental Microbiology 42: 779-781.

214. Straub, K.L., Benz, M., Schink, B., and Widdel, F., 1996. Anaerobic, Nitrate-Dependent Microbial Oxidation of Ferrous Iron. Applied and Environmental Microbiology 62: 1458-1460.

215. Stumm, W. and Morgan, J. J., 1981. Aquatic Chemistry. an introduction emphasizing chemical equilibria in natural waters. 2:

216. Sundby, B. and Silverberg, N., 1985. Manganese fluxes in the benthic boundary layer. Limnology and Oceanography 30: 372-381.

217. Tessier, A., Rapin, F., and Carignan, R., 1985. Trace metals in oxic lake sediments: possible adsorption onto iron oxyhydroxides. Geochimica et Cosmochimica Acta 49: 183-194.

218. Thamdrup, B., 2000. Bacterial manganese and iron reduction in aquatic sediments. Advances in Microbial Ecology, Vol 16 16: 41-84.

219. Thamdrup, B. and Canfield, D. E., 1996. Pathways of carbon oxidation in continental margin sediments off central Chile. Limnology and Oceanography 41: 1629-1650.

220. Thamdrup, B. and Canfield, D. E., 2000. Benthic respiration in aquatoc sediments.

221. Thamdrup, B., Finster, K., Hansen, J. W., and Bak, F., 1993. Bacterial dispproportionation of elemental sulfur coupled to chemical-reduction of iron or manganese. Applied and Environmental Microbiology 59: 101-108.

222. Thamdrup, B., Fossing, H., and Jorgensen, B. B., 1994a. Manganese, iron and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. Geochimica et Cosmochimica Acta 58: 5129-

223. Thamdrup, B., Glud, R. N., and Hansen, J. W., 1994b. Manganese oxidation and in-situ manganese fluxes from a coastal sediment. Geochimica et Cosmochimica Acta 58: 2563-2570.

224. Thamdrup, B., Rosello-Mora, R., and Amann, R., 2000. Microbial manganese and sulfate reduction in Black Sea shelf sediments. Applied and Environmental Microbiology 66: 2888-2897.

225. Thomas, C.A. and Bendell-Young, L. I., 1999. The significance of diagenesis versus riverine input in contributing to the sediment geochemical matrix of iron and manganese in an intertidal region. Estuarine Coastal and Shelf Science 48: 635-647.

226. Thomson, J., Mercone, D., De Lange, G. J., and Van Santvoort, P. J. M., 1999. Review of recent advances in the interpretation of eastern Mediterranean sapropel S1 from geochemical evidence. Marine Geology 153: 77-89.

227. Urrutia, M.M., Roden, E. E., Fredrickson, J. K., and Zachara, J. M., 1998. Microbial and surface chemistry controls on reduction of synthetic Fe(III) oxide minerals by the dissimilatory iron- reducing bacterium Shewanella alga. Geomicrobiology Journal 15: 269-291.

228. Van Bodegom, P.M., 2000. Methane emissions from rice addies; experiments and modelling.

229. Van Bodegom, P.M. and Scholten, J. C. M., 2001. Microbial processes of CH4 production in a rice paddy soil: Model and experimental validation. Geochimica et Cosmochimica Acta 65: 2055-2066.

230. Van Bodegom, P.M. and Scholten, J. C. M., 2001. Microbial processes of CH4 production in a rice paddy soil: Model and experimental validation. Geochimica et Cosmochimica Acta 65: 2055-2066.

231. Van Bodegom, P.M., Wassmann, R., and Metra-Corton, T. M., 2001. A process-based model for methane esmission predcitions from flooded rice paddies. Global Biogeochemical Cycles 15: 247-263.

232. Van der Nat, F.J.W.A. and Middelburg, J. J., 1998. Effects of two common macrophytes on methane dynamics in freshwater sediments. Biogeochemistry 43: 79-104.

233. Van der Zee, C., 2002. Early diagenesis of manganese, iron and phosphorous in European continental margin sediments.

234. Van der Zee, C. and Van Raaphorst, W., 2002. Manganese oxide reactiviy in North Sea sediments. 57-76.

235. Van Nugteren, P., Herman, P. M. J., and Heip, C. H. R., 2002. Mesocosm.

236. Van Santvoort, P.J.M., De Lange, G. J., Thomson, J., Cussen, H., Wilson, T. R. S., Krom, M. D., and Strohle, K., 1996. Active post-depositional oxidation of the most recent sapropel (S1) in sediments of the eastern Mediterranean Sea. Geochimica et Cosmochimica Acta 60: 4007-4024.

237. Van Nugteren, P., Herman, P. M. J., Middelburg, J. J., and Heip, C., 2002. The effect of bioturbation on mineralisation of organic matter in marine sediments.

238. Van Santvoort, P.J.M., De Lange, G. J., Thomson, J., Cussen, H., Wilson, T. R. S., Krom, M. D., and Strohle, K., 1996. Active post-depositional oxidation of the most recent sapropel (S1) in sediments of the eastern Mediterranean Sea. Geochimica et Cosmochimica Acta 60: 4007-4024.

239. Warren, L.A. and Haack, E. A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. Earth-Science Reviews 54: 261-320.

240. Watson, P.G. and Frickers, T. E., 1990. A multilevel, insitu pore-water sampler for use in intertidal sediments and laboratory micrococms. Limnology and Oceanography 35: 1381-1389.

241. Wehausen, R. and Brumsack, H.-J., 1999. Cyclic variations in the chemical composition of eastern Mediterranean Pliocene sediments: a key for understanding sapropel formation. Marine Geology 153: 161-176.

242. Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Rassmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. Nature 362: 834-836.

243. Widdicombe, S. and Austen, M. C., 1999. Mesocosm investigation into the effects of bioturbation on the diversity and structure of a subtidal macrobenthic community. Marine Ecology-Progress Series 189: 181-193.

244. Wijsman, J.W.M., Herman, P. M. J., and Middelburg, J. J., 2001a. A model for early diagenetic processes in sediments of the continental shelf of the Black Sea. 43-66.

245. Wijsman, J.W.M., Herman, P. M. J., Middelburg, J. J., and Soetaert, K., 2002. A model for early diagenetic processes in sediments of the continental shelf of the Black Sea. Estuarine Coastal and Shelf Science 54: 403-421.

246. Wijsman, J.W.M., Middelburg, J. J., and Heip, C. H. R., 2001b. Reactive iron in Black Sea Sediments: implications for iron cycling. Marine Geology 172: 167-180.

247. Wijsman, J.W.M., Middelburg, J. J., Herman, P. M. J., Bottcher, M. E., and Heip, C. H. R., 2001c. Sulfur and iron speciation in surface sediments along the northwestern margin of the Black Sea. Marine Chemistry 74: 261-278.

248. Wilson, T.R.S., Thomson, J., Hydes, D. J., Colley, S., Culkin, F., and Sorensen, J., 1986. Oxidation fronts in pelagic sediments: diagenetic formation of metal-rich layers. Science 232: 972-975.

249. Wollast, R., 1991. the coastal organic carbon cycle: fluxes, sources and sinks. 365-381.

250. Zachara, J.M., Fredrickson, J. K., Smith, S. C., and Gassman, P., 2001. Solubilization of Fe (II) bound trace metals by a dissimilatory Fe (III) reducing bacterium. Geochimica et Cosmochimica Acta 65: 75-93.

8.   Dankwoord

Een dissertatie is een proeve van bekwaamheid waarmee de promovendus hoopt aan te tonen zelfstandig wetenschap te kunnen beoefenen. Echter, ondanks dit zeer individuele oogmerk is een proefschrift een product waaraan vele personen, direct of indirect, hebben meegewerkt.

Alle mensen die hebben bijgedragen aan de tot standkoning van dit boekje bedank ik hartelijk. Een aantal van hun noem ik hieronder, zij hebben een specifieke rol gehad die ik graag vermeld.

Als eerste mijn begeleider Jack Middelburg. Een prettiger en beter ingevoerd begeleider heb ik me niet kunnen wensen. Waar andere aio's nog wel eens klagen over gebrek aan tijd en input voor begeleiding of in een later stadium traagheid van correcties had Jack altijd tijd om even te praten en ligt zijn correctietempo ongeëvenaard hoog. Zijn input in het SMILE project was erg prettig. Jack bedankt.

Als tweede Carlo Heip. Als promotor en leider van onze werkgroep bood hij de faciliteiten voor het onderzoek, tijdens het laatste traject was hij de intermediair naar de universiteit en leidde me door de procedures.

Peter Herman en Paul van Nugteren hebben in grote mate bijgedragen aan de tot standkoming van hoofdstuk 4. Hun komt de eer toe van de experimentele opzet en uitvoer van het bioturbatie experiment.

Dit boekje experimenten met sedimenten van een drietal locaties: Grevelingenmeer; Appels aan de oever van de Schelde; en de Middellandse Zee. De bemanning van de Luktor en Ko Verschuren bedank ik voor de vaartochten naar vooral het Grevelingenmeer en de hulp bij het boxcoren. Mathieu Starink heeft me de weg gewezen naar de slikplaten van Appels. Tot slot de monsters van de Middellandse Zee. Leon Moodley introduceerde me in de wereld van de onderzoeks cruises en droeg als co-chief scientist bij aan het verkrijgen van de monsters. De SMILABLE tocht met R.V. Logachev is een bijzondere herinnering geworden, mede dank zijn de bemanning van de R.V. Logachev en de andere SMILABLE deelnemers.

Na het monsteren komt het labwerk. Pieter van Rijswijk hielp bij het puzzelen op de nieuwe anaërobe tent. Annette Wielemaker deed de HPLC analyses van mijn monsters en was een gezellige labgenoot.

Bij mijn labewerk zaten ook analyses van de vele extracties en poriewaters. Het analytisch lab heeft mij geholpen wegwijs te worden op de verschillende instrumenten. Speciale dank voor Joop Nieuwenhuize die als hoofd van het lab

145

9.   Samenvatting

9.1.   Inleiding en context

Dit proefschrift handelt over de cyclus van ijzer en mangaan in mariene sedimenten. Het draagt bij aan de fundamentele kennis over biogeochemische processen van ijzer- en mangaanoxide reductie in sedimenten. Deze kennis, en de inpasbaarheid ervan in modellen, is nodig om ideeën over eventuele toepassingen te kunnen uitwerken. Hierbij kan in eerste instantie gedacht worden aan microbiële bodemsaneringtechnieken, technieken voor vermindering van methaan emissie uit rijstvelden, en (nog zeer speculatief) verhoging van de CO_2 fixatie door algen en bacteriën in de oceanen.

IJzer is een belangrijk element voor levende organismen, het is bijvoorbeeld een belangrijk bestanddeel van veel enzymen, cytochroom en heamoglobine. In sommige delen van de oceanen is de ijzerconcentratie beperkt. In deze gebieden is ijzer een limiterende factor voor de groei van autotrofe organismen waardoor ook de voedselvoorraad voor heterotrofe organismen beperkt wordt, evenals de opname van CO_2 door de oceanen. Er is een beperkt aantal bronnen voor ijzer in de oceanen: 1) IJzer aangevoerd met de wind in terrestrisch materiaal, 2) IJzer van het land aangevoerd met rivier water, 3) IJzer dat vrijkomt door afbraak van organisch materiaal in de oceaan en 4) IJzer dat uit het sediment kan vrijkomen wanneer ijzeroxide gereduceerd wordt.

Autotrofe organismen leggen koolstofdioxide (CO_2) vast tijdens het assimilatieproces en vormen zo organisch materiaal. In de oceanen vindt dit proces voornamelijk plaats in de eufotische zone, de waterlaag waar het zonlicht doordringt. Het gevormde organisch materiaal dient als voedingsbron voor andere organismen, en zal daarom vaak in de vorm van faeces, samen met ander materiaal naar de bodem zinken. Tijdens de tocht naar beneden start de microbiële afbraak van het materiaal, hierbij worden de organische koolstof moleculen weer afgebroken tot CO_2. Als organisch koolstof het sediment oppervlak bereikt en, bijvoorbeeld door biologische activiteit, met het sediment vermengd wordt kan het verder worden afgebroken. Uiteindelijk komt de meeste koolstof weer vrij in de vorm van CO_2 en dit kan dan weer gebruikt worden door autotrofe organismen. Een klein gedeelte van het organisch materiaal komt buiten bereik van mineralisatie processen, bijvoorbeeld doordat de moleculen moeilijk en dus langzaam af te breken zijn voor bacteriën en het materiaal steeds dieper begraven wordt. Dit

147

organisch koolstof verdwijnt uit de relatief snelle biologische kringloop en gaat over naar de geologische kringloop, die een veel grotere tijdschaal omvat.

Afbraak van organisch materiaal kan via verschillende microbiële processen verlopen verschillende electron acceptors gebruikt worden. De energie winst die een bacterie haalt uit het proces is afhankelijk van de gekozen route. Routes die een hoge energie opbrengst hebben zullen daarom als eerste verlopen, minder gunstige routes worden pas gekozen als gunstigere routes niet meer mogelijkzijn, bijvoorbeeld wanneer één van de benodigde componenten niet meer beschikbaar is. Mineralisatie kan plaatsvinden in aan- of afwezigheid van zuurstof, de zogenaamde aërobe en anaërobe mineralisatie. De te onderscheiden processen zijn in volgorde van energieopbrengst: aërobe mineralisatie en de anaërobe processen, denitrificatie, reductie van mangaanoxide, ijzeroxide en sulfaat en als laatste treedt methanogenese op. Ook in mariene sedimenten verloopt de afbraak in volgorde van de energiewinst. In de toplaag is zuurstof aanwezig, dieper in het profiel is deze op en wordt overgeschakeld op de andere processen, waardoor er een diepte afhankelijke sequentie afbraakprocessen ontstaat. Dit moet in brede zin worden opgevat, want door de heterogeniteit van een sediment en het bestaan van verschillende microniches is van een strikte horizontale gelaagdheid in de praktijk geen spraken.

De ontdekking van de complete afbraak van organisch materiaal door anaërobe bacteriën met behulp van metaaloxide werd halverwege de jaren 80 gedaan. Op basis van lage concentraties van metaaloxiden in het sediment en de beperkte ophoping van gereduceerd metaal in het poriewater werd aangenomen dat metaaloxide reductie een beperkt aandeel in de mineralisatie had. Begin jaren negentig werd duidelijk dat onder bepaalde omstandigheden mangaan- en ijzeroxide reductie belangrijke afbraak processen kunnen zijn. Een belangrijk aspect hierin is de kringloop waarin beide metalen betrokken kunnen zijn (zie fig. 1 ter verduidelijking). Hierdoor kan het aandeel van metaaloxide(n) in het mineralisatieproces veel groter zijn dan op basis van concentraties verwacht wordt.

Totnogtoe zijn de factoren die de metaaloxide reductie beïnvloeden niet zo diepgaand opgehelderd als de factoren die de andere afbraakprocessen beïnvloeden. In dit proefschrift wordt een aantal nog onduidelijke aspecten behandeld. In de inleiding van dit proefschrift, **hoofdstuk 1**, wordt de context van deze aspecten beschreven.

De onderzochte sedimenten dekken een drietal geheel verschillende sedimenttypen. Het eerste, Appels (België), is een zoetwater sediment rijk aan organisch materiaal op een getijdenplaat aan de oever van de Schelde met een hoog reactief ijzergehalte. Het tweede type sediment is estuarien sediment (saliniteit 28),

148

afkomstig uit het Grevelingenmeer (Nederland), met een gemiddeld organische materiaal belasting en een gemiddelde reactief ijzerconcentratie. Het derde type sediment is diepzee sediment uit de Middellandse Zee (saliniteit 36), dit sediment is arm aan organisch koolstof en reactieve ijzeroxiden.

Er bestaan verschillende vormen van metaaloxiden, van amorfe en erg reactieve oxiden tot kristallijne en bijna inerte vormen. De bacteriële energie winst is het grootst voor hoog reactieve oxides. De compositie van de ijzeroxide stock is daarom een belangrijke factor voor de bacteriële ijzer reductie. Lange tijd was er geen eenduidige methode om de reactiviteit te bepalen en de relatie tot bruikbaarheid voor bacteriën goed weer te geven. In de meeste studies worden chemische extracties gebruikt om de stock grootte van de verschillende vormen ijzeroxide te bepalen, maar de traditionele extracties leggen enkel grote verschillen in ijzeroxidenpool bloot, meestal tussen sedimenten, terwijl kleine verschillen, vaak binnen een profiel, niet naar voren komen. Daarnaast worden de resultaten van de traditionele extractie beïnvloed door korrelgrootte en de relatieve poolgrootte en leveren geen data over de werkelijke reactiviteit. **Hoofdstuk twee** beschrijft de toepassing van een kinetische methode voor bepaling van initiële reactiviteit en de distributie van reactiviteit van ijzeroxiden in natuurlijke sedimenten. De methode benadert de reactiviteit als een continuüm en de resultaten zijn vrijwel onafhankelijk van korrelgrootte en relatieve poolgrootte. Gebruik van de methode leverde een kwantitatieve beschrijving van ijzeroxidenreactiviteit en maakte het mogelijk om kleine verschillen in reactiviteit te detecteren. Uit de resultaten bleek dat reactiviteit van ijzeroxiden in sedimenten soms gelijk is terwijl de absolute stock grootte erg verschilde. Andersom bleek dat de diepte profielen van reactiviteit vaak
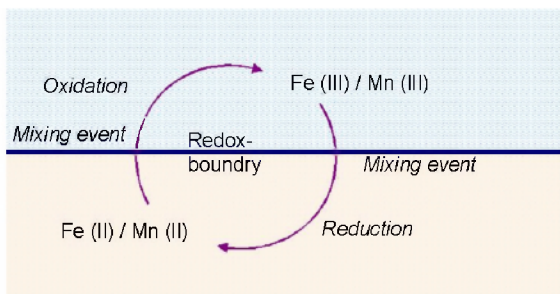

Fig 1. Metaal kringloop tussen de verschillende redoxzones. Een metaal cyclus kan plaatsvinden binnen een sediment of tussen sediment en water. Met de term 'menging' wordt gedoeld op mengmechanismen van zowel opgeloste als vaste stoffen, zoals respectievelijk diffusie / irrigatie en partikel menging.

149

veel contrastrijker waren dan de traditionele extracties aangaven. Een nadeel van de methode bleek dat een minimale pool van reactief ijzer aanwezig moest zijn om de methode te kunnen gebruiken. In de Middellandse zee sedimenten, die arm zijn aan reactief ijzer, bleek de methode daarom niet toegepast te kunnen worden.

Het aandeel van metaaloxide reductie in de afbraak van organisch materiaal is afhankelijk van de hoeveelheid en kwaliteit van afbreekbaar organisch materiaal, de metaaloxiden en van de interactie van beiden. Voor metaaloxide reductie is deze interactie niet vaak experimenteel onderzocht, onder andere doordat het proces beïnvloed wordt door de reactiviteit van oxiden. Terwijl voor stoffen als zuurstof en sulfaat de aanvoer van oxidanten naar de reductie sites via diffusie in het poriewater kan plaatsvinden, is een meer actief mechanisme nodig om de sedimentaire metaaloxiden aan te vullen en deze afbraakroute op gang te houden. Het feit dat de metaaloxiden zich grotendeels in de vaste fase bevinden is een extra complicerende factor. In **hoofdstuk 3** wordt een experiment beschreven waarin is onderzocht wat in de drie bemonsterde sedimenten de limiterende factoren zijn voor ijzeroxide reductie: beschikbaar reactief ijzer, de concentratie van afbreekbaar organisch materiaal of beiden. Door gebruik te maken van sediment slurries was er geen probleem met gelaagdheid van de monsters en transport van ijzeroxiden naar de reductie sites. De resultaten toonden dat in de Mediterrane sedimenten enkel afbreekbaar organisch koolstof limiterend is, in het zoetwater sediment is alleen de ijzerconcentratie limiterend terwijl in de estuariene sedimenten een interactie van beide bepalend is. Daarnaast leverden de data een waarde voor de maximale reductiesnelheid en de affiniteit van bacteriën voor reactief ijzer oxide, twee gegevens die nodig zijn voor modellering van de bacteriële processen en waar nog weinig direct gemeten waarden van bestaan.

Het bestaan van een (sedimentaire) ijzer en mangaan kringloop, met bijbehorende condities zoals de oxisch-anoxische overgangen, bepaalt voor een groot deel het mogelijke aandeel van de bacteriële metaaloxidereductie in de totale mineralisatie. Bioturbatie is een belangrijk proces om (sub)oxische condities in het sediment te houden. Daarmee speelt het proces ook een belangrijke rol in de anaërobe mineralisatie processen zoals ijzer- en mangaan reductie. Het effect van bioturbatie op metaaloxide reductie kan worden ontleed in twee aspecten: 1) Menging van partikels en 2) Irrigatie van het sediment. Beide aspecten voorzien in een stukje van de metaaloxide kringloop en zorgen ervoor dat de kringloop gesloten kan worden. De experimenten beschreven in **hoofdstuk 4** zijn opgezet om het afzonderlijke effect van partikel mening en irrigatie op sedimentaire mangaan- en ijzeroxiden te bepalen. De experimenten toonden aan dat beide componenten belangrijk zijn

voor de sedimentaire kringlopen. In deze experimenten was partikelmenging, hier nagebootst door een mengopstelling in het sediment, belangrijk voor het herdistribueren van gereoxideerde oxiden. Irrigatie, nagebootst door een verhoogde ventilatie (d.i. uitwisselingen van gassen), was belangrijk voor de aanvoer van opgeloste oxidanten in het sediment. De resultaten lieten zien dat het effect van beide aspecten voor ijzer en mangaan verschillend is. Verhoogde ventilatie had meer invloed op de mangaankringloop dan op de ijzerkringloop, omdat ijzer sneller oxideert en ook met mangaanoxiden kan re-oxideren terwijl, door een tragere oxidatie kinetiek, een langer contact van mangaan met zuurstof nodig is om mangaan te re-oxideren. De efficiëntie van de ijzerkringloop bleek meer afhankelijk te zijn van menging van partikels. Voor de mangaancyclus waren uiteindelijk zowel irrigatie als partikelmenging van belang. Naast de macrokringloop duidden de experimentele resultaten op het bestaan van een microcyclus waarin mobiele, geoxideerde metalen een rol spelen.

Metaaloxiden hebben veel interacties met andere mineralisatieprocessen, adsorberen aan partikels en zijn betrokken bij abiotische reductieprocessen. Hierdoor voldoen eenvoudige bepalingen niet om een schatting van het aandeel in de mineralisatie te maken. In incubaties moeten alle andere mineralisatie processen ook gevolgd worden, bijvoorkeur met parallelle sets waarin inhibitors gebruikt worden en is karakterisering van de metaaloxidestock in de gebruikte profielen nodig. Hierdoor is de bepaling van metaaloxide reductie (snelheden) in natuurlijke sedimenten een arbeidsintensieve bezigheid. Vanwege deze inspanning wordt er vaak voor gekozen om metaaloxide reductie indirect te bepalen of te modeleren aan de hand van de totale mineralisatie en de andere mineralisatieprocessen.

De hoofdstukken 2, 3 en 4 van dit proefschrift zijn elk gewijd aan een van de aspecten die een rol spelen bij (het onderzoek naar) metaaloxide reductie in mariene sedimenten en zijn gebaseerd op mesocosmos- en laboratoriumexperimenten. **Hoofdstuk 5** handelt over metaaloxide reductie in verschillende typen sedimenten gebaseerd op metingen onder meer natuurlijke omstandigheden. Hiervoor werd gebruik gemaakt van anoxische incubaties waarbij sedimentkernen in lagen werden gesneden en in diffusievrije zakken werden verzegeld. Uit de resultaten bleek dat ijzeroxiden reductie een belangrijk aandeel aan de afbraak van organisch materiaal kan leveren bij middelmatige tot hoge organische stof concentraties. In de organische arme Middellandse zee sedimenten speelde metaaloxide reductie over het algemeen geen rol omdat het grootste deel van de mineralisatie aëroob verliep. De estuariene sedimenten hebben een middelmatige organisch stof concentratie. Hier waren ijzeroxide en sulfaat reductie beide belangrijk, afhankelijk van de totale mineralisatie. Het gebruik van molybdaat

als inhibitor voor sulfaatreductie en ferrozine als chelator van gereduceerd ijzer (d.i. het bindt aan gereduceerd ijzer zodat dit niet kan reageren met bijvoorbeeld mangaanoxide) toonde aan dat de interactie tussen beide processen en de relatie met mangaanoxide reductie varieerde met de totale mineralisatie snelheid. In het organisch rijke zoetwater sediment aan Appels verliep de mineralisatie voornamelijk via bacteriële ijzeroxide reductie, mangaan reductie en methanogenese speelden slechts een kleine rol.

10.   Summary

10.1.   Introduction and context thesis

This thesis describes a research on the sedimentary cycling of iron and manganese in marine sediments. It extends the fundamental knowledge on the biogeochemical processes of sedimentary iron and manganese reduction. This knowledge, and the implementation in biogeochemical models, is necessary to explore utilisation of the processes for example in biological soil remediation, techniques to reduce methane emission from rice fields, and, still very speculative, as means to diminish the greenhouse effect by enhancing the oceans CO_2 fixation.

For the degradation of organic carbon, several microbial pathways exist. Different electron acceptors are used to oxidise the zero valent carbon in the organic matter to tetravalent organic carbon in carbon dioxide. In order of energy gain the processes are aerobic mineralization, nitrification, Mn-oxide -, Fe-oxide- and sulfate reduction and finally methanogenesis. The processes in which diffusive electron acceptors (O_2, NO_3, SO_4, and methanogenesis) are used are well studied. Research on the pathways using the solid phase electron acceptors iron and manganese oxide has been intensified more recently, after the iron and manganese reducing bacteria were shown to be able to complete degradation of organic carbon. Under specific conditions, these metal oxide reduction processes are relatively important for the sedimentary organic carbon mineralization.

The field and experimental studies are performed with sediments originating form different environments covering a gradient of salinity and organic carbon input. A freshwater site, Appels, was situated along the Scheldt River in the intertidal zone and was flooded twice a day. The sediment was very fine and had a high organic carbon content due to high organic carbon input from benthic algae and particles from the Scheldt River and the adjacent marsh. Two subtidal estuarine sites in Lake Grevelingen were selected. One, Vliegertje, is a sandy littoral site situated on a plain, the other, Geul, is situated in a gully. Due to focussing of fine material the sediment in the gully was finer than the Vliegertje sediment. However, at both sites sediments were coarser than at the freshwater site Appels. Organic carbon inputs were much lower as well. A deep-sea sediment was sampled in the Eastern Mediterranean. These sediments are hemipelagic muds with a low organic carbon content and small grain sizes.

10.2. Iron oxide reactivity

In contrast to the diffusive electron acceptors, metal oxides exist in different forms. Amorphous oxides are relatively reactive and provide a high energy gain for bacteria when used for organic carbon mineralization, whereas crystalline oxides are less reactive, therefore energy gain is less when used as an electron acceptor. As energy gain is an important aspect, determining occurrence of a pathway, the composition of the sedimentary iron oxide pool is important information to estimate the potential for bacterial iron oxide reduction. Chemical iron oxide extractions using different types of leachants are used to determine the pool of iron oxides. However, these traditional iron oxide extractions only reveal large differences in the composition of the sedimentary iron oxide pool (mostly between sediments) whereas small changes (often within a profile) are not discerned. Besides, extraction results are affected by grain size and relative pool sizes and do not directly represent iron oxide reactivity. A kinetic method was used to describe the composition of the iron oxide pools in the three types of sediment. The method is based on a reactive continuum approach and results are independent of grain size and relative pool size. Results of the method used in the three types of sediments showed to be independent of pool distribution and grain size and the approach allows a quantification of iron oxide reactivity in sediments. Although a minimal pool of reactive iron oxides should be present in the sediment, the method reveals relatively small differences in iron oxide composition.

10.3. Limiting factors

The two components in microbial iron oxide reduction are iron oxide availability and degradable organic matter load. These two factors and their interaction determine sedimentary iron oxide reduction rates and the relative contribution of the iron oxide reduction process to organic carbon mineralization. Which factor is limiting for this process depends on sediment characteristics. Experiments showed that addition of organic carbon to aerobic Mediterranean samples moved the sediment to more favourable conditions for iron oxide reduction. Whereas conditions in the freshwater sediment were already favourable and addition of organic carbon moved the sediment to methanogenic processes besides addition of reactive iron oxides increased iron oxide reduction rates. In the estuarine sediment, the iron oxide reduction rates are determined by the minute balance between iron oxide availability and organic carbon concentrations. Maximal V_{max} values can be reached as soon as sufficient degradable organic carbon is available. However,

realised iron reduction rates depend on the absolute concentration of reducible iron oxides.

10.4. Bioturbation

Bioturbation is an important process to preserve (sub) oxic sediment conditions and a relative important role for the sub-oxic organic carbon degradation processes such as metal oxide reduction. The effect of bioturbation on iron and manganese cycling depends on particle mixing and sediment irrigation. Experimental results show that both components are necessary to complete metal cycling and maintain the sub-oxic mineralization pathways using metal-oxides. Particle mixing, in the experiment mimicked by a mixing device, is important for (re) distribution of (re) oxidized metal-oxides. Irrigation, here imitated by enhanced sediment ventilation (i.e. enhanced exchange of porewater dissolved gases), is essential for the input of dissolved oxidants. However, the latter aspect is more important for manganese than for iron because iron has a higher affinity for oxygen and can also re-oxidise with manganese-oxides, while, because of the slow oxidation kinetics, a longer oxygen-Mn (II) contact is needed for sufficient retainment of Mn-oxide the sediment. Therefore, the efficiency of iron cycling is determined primarily by particle mixing. Whereas both particle mixing and irrigation determine manganese cycling efficiency. The experimental results indicate that the combined effect of ventilation and particle mixing on metal oxide cycling and reduction is not simply additive.

10.5. Field data

Determining organic carbon mineralization rates with the intermediate electron acceptors in sediments is complex because of interactions with other processes and constituents. Anaerobic bag incubations, to determine metal oxide reduction rates in different sediments, indicate that metal oxide reduction pathways may be important at intermediate to high organic carbon degradation rates. In organic poor Mediterranean sediments metal oxide reduction can be neglected as most of the mineralization occurred aerobically. However, slow metal oxide reduction will occur at greater depths. Intermediate organic carbon loaded estuarine sediments have higher rates of iron oxide reduction. In these sediments, both iron oxide reduction and sulfate reduction are important. In organic rich freshwater sediments, iron oxide reduction accounts for most organic carbon mineralization, with only a minor role for the other pathways like manganese reduction and

methanogenesis. These findings are consistent with model results and experimental data on factors limiting the importance of iron oxide reduction in mineralization.

11. Curriculum Vitae

Imola Ferro is geboren op 23 december 1972 in Bujumbura, Burundi. In 1991 behaalde ze haar VWO diploma aan Louise de Coligny Scholengemeenschap te Leiden. Daarna begon zij aan een studie Biologie aan de Landbouwuniversiteit in Wageningen. Tijdens deze studie, die zij in 1996 voltooide, deed zij een afstudeervak bodembiologie bij de vakgroep Terrestrische Ecologie en een afstudeervak microbiologische Ecologie bij de vakgroep Microbiologie. Daarnaast deed zij een stage bodembiologie in Burkina Faso. In 1997 kreeg zij een aanstelling als oio in het NWO project 'Sedimentary Iron and Manganese cycLEs' (SMILE) bij het Centrum voor Estuariene en Mariene Ecolgie van NIOO-KNAW, te Yerseke. Sinds begin 2002 werkt Imola bij de afdeling Milieu van NEN (het Nederlands normalisatie insitituut), als standaardisatie consultant.