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EFFECT OF MACROFAUNA, OXYGEN EXCHANGE AND PARTICLE REWORKING ON IRON AND MANGANESE SEDIMENT BIOGEOCHEMISTRY: A LABORATORY EXPERIMENT

I. FERRO, P. VAN NUGTEREN, J.J. MIDDELBURG,
*P.M.J. HERMAN, C.H.R. HEIP**
The Netherlands Institute of Ecology, P.O. Box 140, 4400 AC Yerseke, The Netherlands
** corresponding author: c.heip@nioo.knaw.nl*

BIOTURBATION
PARTICLE MIXING
IRRIGATION
IRON
MANGANESE
SEDIMENT
NEREIS DIVERSICOLOR

ABSTRACT. – The impact of bioturbation on iron and manganese cycling in marine sediments was studied in laboratory experiments in which faunal effects which combine in the field (particle mixing and oxygen exchange) were studied separately. Particle mixing and sediment aeration both enhance metal oxide reduction rates as reflected in pore-water and solid phase profiles, and in sediment-water exchange fluxes. We also studied the effect of *Nereis diversicolor* on iron and manganese biogeochemistry. The presence and activity of this worm resulted in more oxygenated conditions with reduced metal oxide reduction rates and lower rates of anaerobic degradation.

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RÉSUMÉ. – L'impact de la bioturbation sur le cycle du fer et du manganèse dans les sédiments marins a été étudié par une expérimentation en laboratoire au cours de laquelle les effets combinant dans la nature le remaniement particulaire et l'échange d'oxygène ont été étudiés séparément. Le remaniement des sédiments et l'aération accroissent la vitesse de réduction des oxydes métalliques comme l'indiquent les profiles d'eau interstitielle et de la phase solide, ainsi que les flux d'échanges sédiment-eau. L'effet de *Nereis diversicolor* sur la biogéochimie du fer et du manganèse a également été étudié. La présence et l'activité du Ver induisent des conditions d'oxygénation plus élevées avec des vitesses de réduction des oxydes métalliques réduites et une plus faible vitesse de dégradation anaérobie.

INTRODUCTION

A diverse heterotrophic community varying in size from protozoa to cm-sized animals inhabits marine sediments. Individual organisms as well as the concerted action of these organisms have a profound influence on sediment stability, erodability and texture, particle and solute transport processes, chemical reactions and the community structure and activity of micro-organisms (Herman *et al.* 1999). Bioturbation, the biological reworking of sediment, results from a wide range of animal behavior, including tube, burrow and mount construction, crawling, swimming and moving of interstitial fauna and sediment ingestion and defecation (Boudreau *et al.* 2001). These animal activities induce transport of sediment particles, pore-water packages and, if there is a gas phase, of gas parcels as well. Due to our limited understanding of sediment reworking by organisms, there is quite some confusion in the literature on bioturbation terminology and classification schemes. Recent developments in diagenetic modeling theory have provided insight into the relationships between different modes of bioturbation, non-local versus local particle mixing (Meysman *et al.* 2003) and biological exchange (due to feeding and defecation) versus mixing (due to animal movement) (Meysman 2001). The prime distinction is between fauna-induced mixing of particles (bioturbation *sensu stricto*) and mixing of solutes, either enhanced molecular diffusion due to interstitial protozoa and metazoans or irrigation of macrofauna tubes and burrows.

The presence of animals not only results in extensive reworking of sediments, but it has also major consequences for sediment biogeochemistry. Recent reviews (Aller & Aller 1998, Herman *et al.* 1999, Kristensen 2000) have summarized evidence convincingly showing that fauna affects organic matter mineralization pathways, rates and efficiencies. These faunal effects are related not only to direct assimilation of organic matter (~10-25%; Herman *et al.* 1999), but also to indirect effects related to facilitation of biogeochemical conditions for other mineralization agents, in particular bacteria, and to grazing of metazoans on bacteria and protozoa (Aller & Aller 1998). Most of these studies deal with oxygen, organic carbon, dissolved inorganic carbon and nitrogen; there are few that cover iron and manganese.

Organic matter mineralization involves a number of electron acceptors; the major ones being nitrate, nitrite, manganese oxides, iron oxides and sulfate. These alternative oxidants are utilized sequentially and sediments often exhibit a vertical redox zonation of oxidants depletion in the order oxygen nitrate > manganese oxide > ferric oxides > sulfate (Thamdrup & Canfield 2000). The relative contribution of a specific electron acceptor in organic matter mineralization depends on the organic matter delivery, the availability of electron acceptors, interactions between degradation pathways and particle and solute transport rates (Soetaert *et al.* 1996, Thamdrup & Canfield 2000, Wijsman *et al.* 2002). Manganese- and iron oxides are intermediate electron acceptors and differ from the other electron acceptors in their phase change upon redox changes. The oxidized forms of manganese (MnIII and MnIV) and iron (FeIII or ferric iron) are insoluble, with the consequence that they occur mainly as solid-phase oxides, while their reduced forms (MnII and FeII, or ferrous iron) are soluble. This difference in phase between the dissolved electron acceptors (oxygen, nitrate, sulfate) and solid-phase oxidants (manganese and iron oxides) suggests that their dependence on biologically induced transport is different. However, literature information on this subject is scarce. Aller (1990, 1994) 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.

Glud *et al.* (2000) concluded that the high iron-oxide reduction rates they measured in a high-arctic sound needed intense bioturbation, but the dependence on particle mixing or porewater irrigation was not investigated separately. Model studies show that 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 (Van Cappellen & Wang 1996, Wijsman *et al.* 2001). To complete a cycle reduced components have to be transferred to a re-oxidation zone and oxidized components have to be mixed into the reduction zone. Oxidized iron components are particulate, and therefore require particle mixing for this process to take place. Reduced iron components are both dissolved and particulate (as iron sulfides) and their transport to oxidized zones therefore depends on diffusion, porewater irrigation and particle mixing. Rates of iron oxide reduction are also influenced by the reactivity of the oxides, which can greatly vary within sediment (Postma 1993). Recently, Roden & Urrutia (1999) reported that adsorption of Fe(II) onto iron oxides retards the reduction of the iron oxides. Consequently, ventilation activity of benthic fauna may promote ferrous iron removal and iron oxide reactivity because of (1) higher porewater replenishment and (2) enhanced oxidation of ferrous iron and subsequent precipitation of ferric iron oxides.

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 (gas exchange) and particle mixing (without mixing of aerobic and anaerobic layers). We compared sediment-water fluxes, porewater profiles and metal oxide speciation of sediment cores between these 3 treatments. Differences were expected to be reflected in porewater profiles and fluxes across the sediment-water interface. 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.

MATERIAL AND METHODS

Set-up mesocosm experiment: For the experiment 16 Plexiglas cores (30 cm, internal diameter of 5.2 cm) were filled with ~ 15 cm sieved (1 mm mesh) sediment from Katsplaat, a tidal flat in the Oosterschelde (a tidal inlet in the south-western part of the Netherlands). The sediment had 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 0.2 µm filtered Oosterschelde 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 at 20 °C. Sediment and specimens of *Nereis diversicolor* were collected simultaneously.

N. diversicolor is a common macrofauna species in the estuaries in the North Sea area. By its activities and high pumping rate the animal can have an important effect on diagenesis. The worm lives on shallow soft bottoms in U-shaped, irregularly branching burrows. *Nereis diversicolor* can feed as a suspension feeder (Riisgard 1991), but is also reported to leave its burrows at low tide and feed actively on microphytobenthos, to feed as a deposit feeder on detritus in the sediment, and to act as a raptorial predator (Goerke 1966). The worm actively pumps water through its burrow, which may also lead to porewater pumping in the surrounding sediment. Riisgard (1991) showed that the total population-pumping rate could be several (up to 10) times the water column per day (Odense Fjord, Denmark).

After acclimation, four cores were processed to determine the initial conditions at the start of the experiment. The other cores were divided into four experimental treatments each consisting of three replicates: (1) *control* with defaunated sediments, (2) *Nereis diversicolor* (3 specimens corresponding to ~1500 ind m^{-2}), (3) *gas transport* (2 artificial U-shaped burrows) and (4) *mixing* (stirring device 50 mm internal diameter placed ca. 5 to 55 mm below the sediment surface). For an elaborate description we refer to the paper of Van Nugteren *et al.* in prep. Briefly, the artificial U-shaped burrows were constructed from polydialkylsiloxane silicone, reached 8 centimeters into the sediment and air was flowing through it. Mechanical mixing was achieved by manually rotating two times (i.e. by 720°) a perforated Plexiglas blade (50 mm internal diameter). Sediments at depth between 5 and 55 mm were mixed twice a day.

The experiment lasted 21 days and flux measurements were performed at 1, 5, 10 and 16 days after start of the experiment by temporarily short-cutting the circuit so that the continuous flow of aerated water was stopped. Water circulation was, however, maintained during incubations. In between incubations there was a continuous flow of aerated seawater resulting in water turnover time of approximately 6 minutes. 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 $sample^{-1}$). After the last incubation at day 16 a bromide solution was added to a final concentration of 7.1 mM. Bromide was added as a passive tracer. At the end of the experiment cores were sliced: 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-8 and 8-10 cm. Porewater of each slice 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 or stored frozen until analysis.

Solid-phase extractions: The centrifuged sediment of each depth interval was pooled and then sub-sampled for solid phase extractions. A number of extractions were used to characterize 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 on the pooled sediments. 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 demineralized water (Kostka & 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 analyzed 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 (Canfield 1988). Mn-oxides (crystalline oxides available for reduction) are extracted as well (Canfield 1988, Kostka *et al.* 1999). 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 HCl extractable pool sensu (Kostka & 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 (hydroxyethylpiperazine ethanesulfonate) 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).

Analyses: Except for the HCl-extraction solutes (Fe analyzed 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). Porewater concentrations of bromide were analyzed by means of ion chromatography (DIONEX) with electrochemical detection (ED40).

Flux calculations: 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 final stages of incubations. As this affected iron concentrations in solution and induced more anoxic conditions in sediments, this resulted in extremely high flux values. Therefore, these iron data (t=120 minutes) have not been used in flux calculations.

Statistical analysis: Average values are shown for all results; error bars represent the standard error 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.

RESULTS

Porewater profiles

At day 16 bromide was added as a passive tracer to identify modes of solute transport. Porewater bromide versus depth profiles were collected at day 21. The overall effect of treatment, depth and their interaction was significant ($p < 0.001$, $F = 222.8$; $p < 0.001$, $F = 524.4$; $p < 0.001$, $F = 38.3$;

n = 108 respectively). The bromide porewater profiles were significantly different between all treatments ($p < 0.001$) except the defaunated control and the gas transport treatment; they both bear the characteristics of a molecular diffusion dominated solute profile (Fig. 1a). Mixing resulted in enhanced solute transfer because bromide penetrated deeper into the sediments compared to control and gas transport treatment. Bromide penetration into *Nereis* treatment sediment was even higher indicating very efficient exchange of solutes between the overlying water and the porewater due to ventilation activity of the worms.

Porewater concentration versus depth profiles of dissolved Fe (II) and Mn (II) are presented in Figure 1b and c. Average values for the gas transport treatment are based on 2 replicates, as the third replicate showed indications of a sampling artifact (oxidation). These profiles reflect the final net results of all processes and their interactions going on in the cores during the experiment. The overall effect of treatment, depth and their interaction was

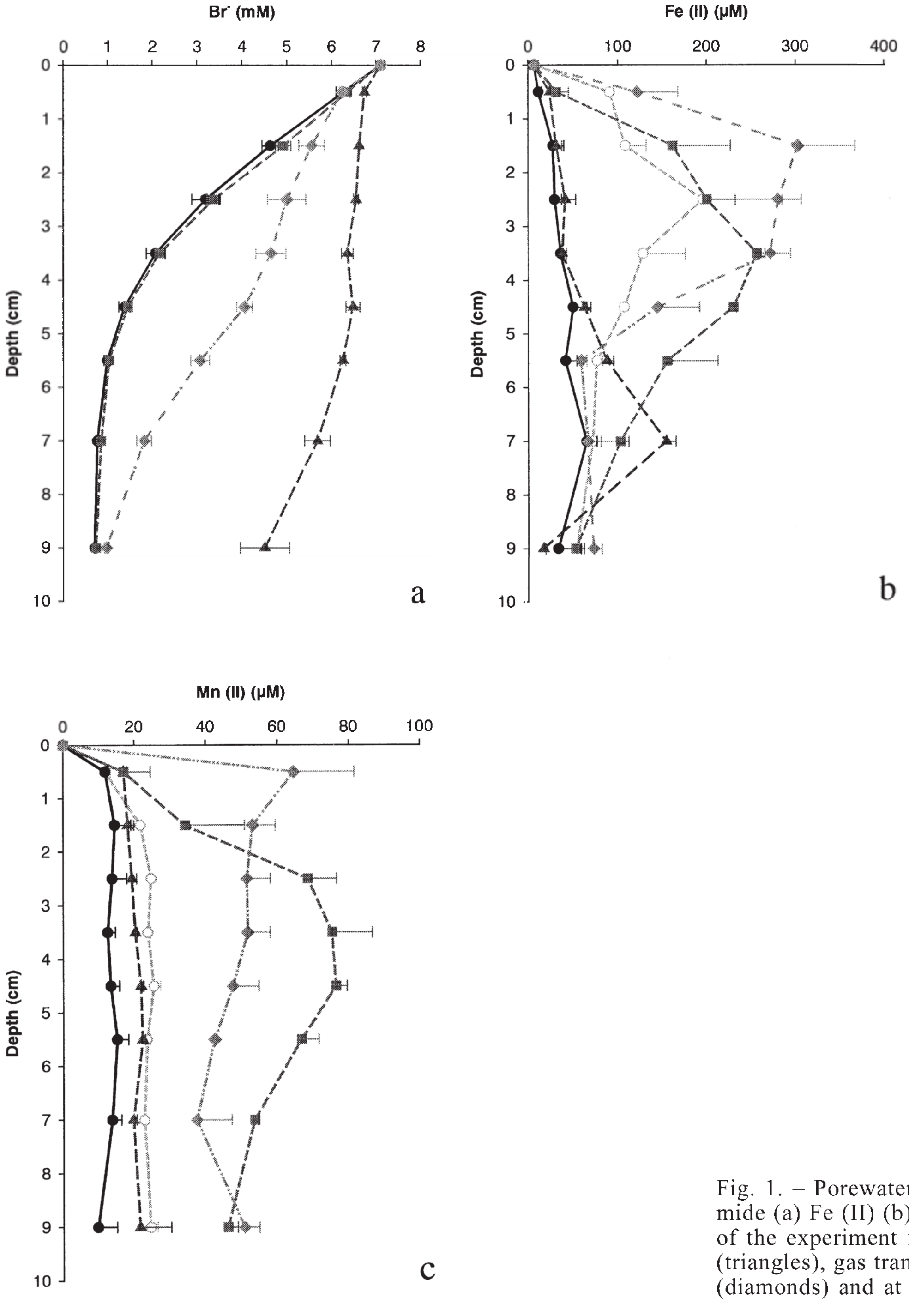

Fig. 1. – Porewater profiles of dissolved bromide (a) Fe (II) (b) and Mn (II) (c) at the end of the experiment for control (circles) *Nereis* (triangles), gas transport (squares) and mixing (diamonds) and at the start (open circles).

significant for the dissolved Fe (II) profiles ($p = 0.03$, $F = 2.4$; $p < 0.001$, $F = 11.7$; $p < 0.001$, $F = 2.4$; $n = 135$ respectively) (Fig 1b). 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 initial condition and gas transport and mixing profiles. These three had classical profiles with subsurface maxima for dissolved Fe (II) concentrations. The gas transport 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 initial condition. The manganese profiles only had an overall significant effect of treatment ($p < 0.001$, $F = 21.5$, $n = 144$) (Fig. 1c). The *Nereis* control and initial profiles of Mn(II) resembled each other and were significantly lower ($p < 0.05$) than those of the gas transport and mixing treatment.

Fluxes to the overlying water during incubations

Concentration changes in the overlying water during incubations were used to calculate fluxes of Fe and Mn out or into the sediment. Fluxes (Fig. 2) 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. Absolute Fe fluxes decreased significantly during the experiment ($p = 0.005$, $F = 5.3$, $n=48$) and did not depend on treatment ($p = 0.09$, $F = 2.4$, $n=48$). Absolute Mn fluxes depended on treatment ($p = 0.02$, $F = 3.8$, $n=48$). Fluxes in *Nereis* cores were significantly lower than those in the control cores ($p = 0.005$) and the mixing treatment ($p < 0.05$).

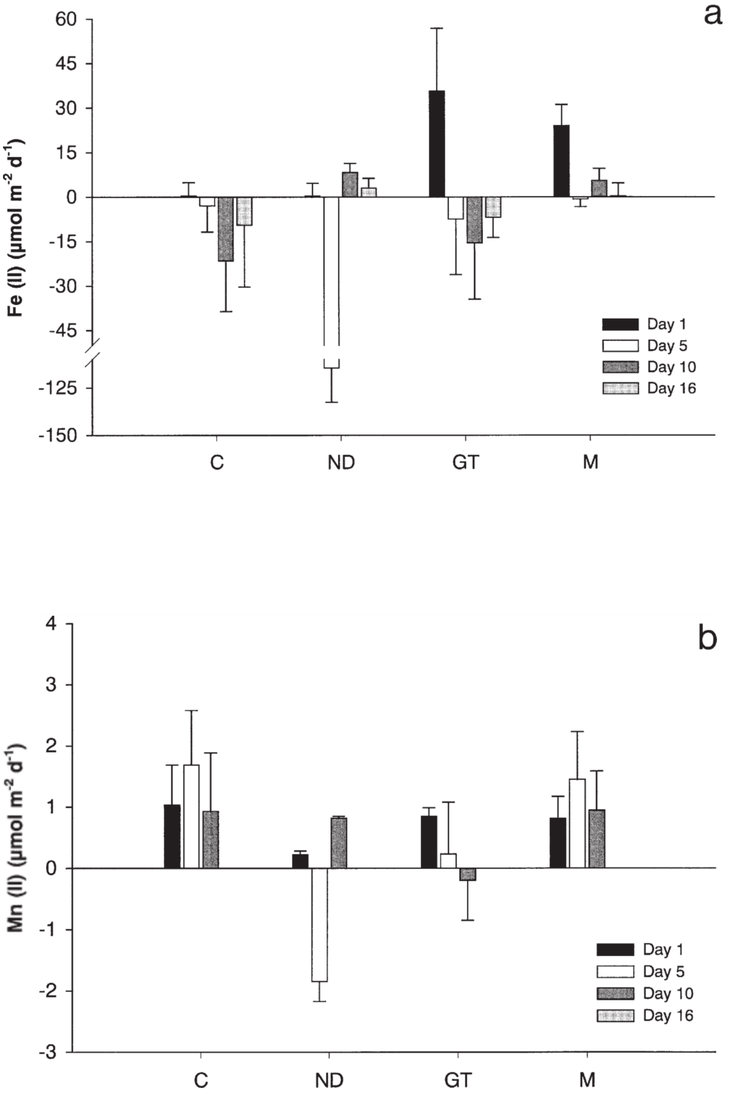

Fig. 2. – Fluxes of dissolved Fe (II) (a) and Mn (II) (b) from sediment cores to the overlying water (mmol m^{-2} d^{-1}) measured in incubations at 1, 5, 10 and 16 days (C = control, N = *Nereis*, GT= gas transport and M = mixing treatment).

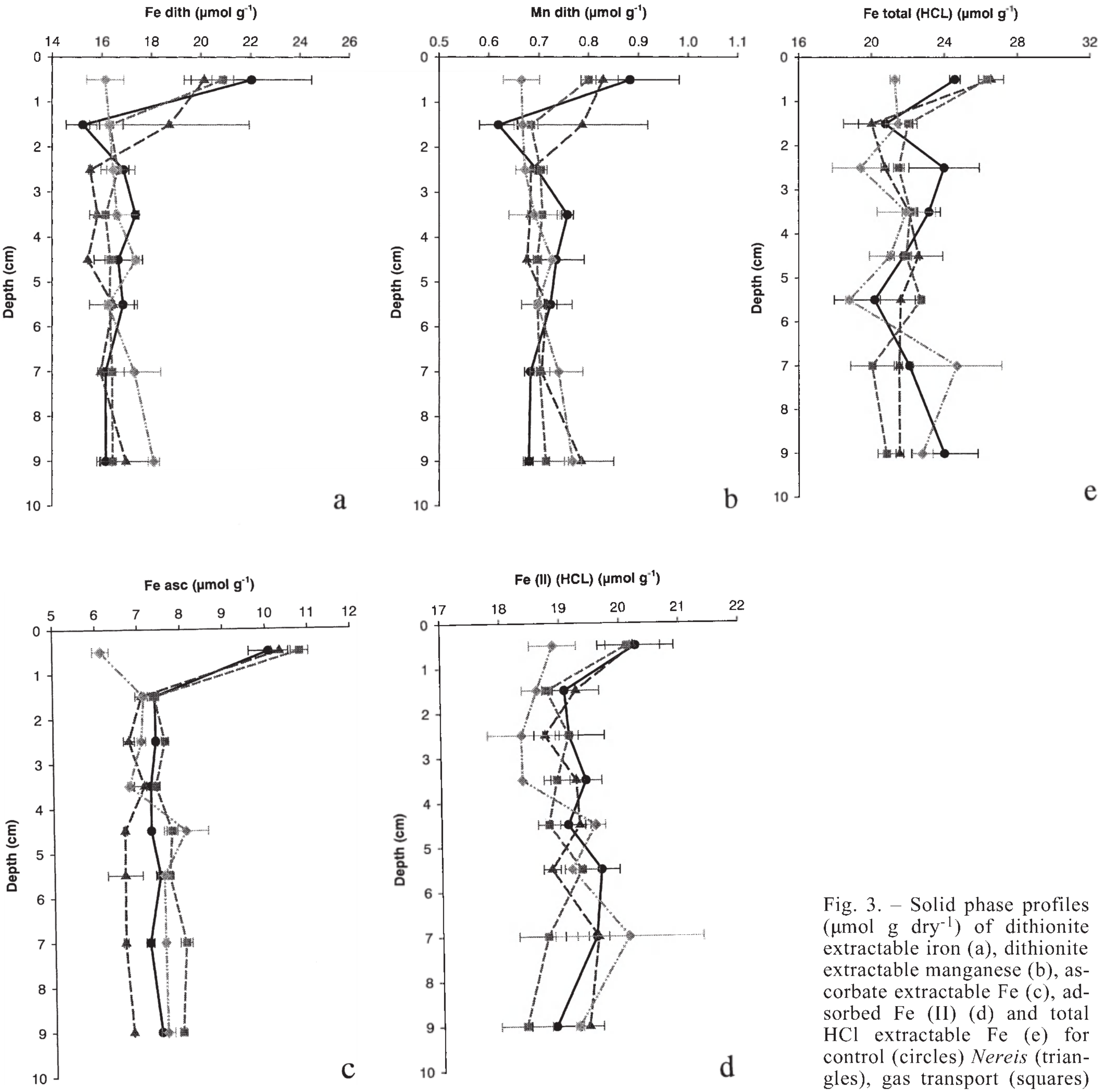

Fig. 3. – Solid phase profiles (µmol g dry-1) of dithionite extractable iron (a), dithionite extractable manganese (b), ascorbate extractable Fe (c), adsorbed Fe (II) (d) and total HCl extractable Fe (e) for control (circles) *Nereis* (triangles), gas transport (squares) and mixing (diamonds).

Solid phase extractions

The data on the solid phase iron extractions are rather variable, because sample heterogeneity dominates over changes due to diagenesis during the short incubation period. 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 gas transport 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) had significant effect 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 gas transport profiles that are 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 mixed ($p = 0.015$) and the gas transport 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 Fe_{HCl} (total HCl extractable sedimentary iron) changed significantly with depth for *Nereis* and gas transport treatment ($n = 16$: $p = 0.02$ $F = 5.0$; $p < 0.001$, $F = 13.2$). The mixing treatments had significantly lower total Fe_{HCl} ($p = 0.04$) than the control cores (Fig. 3e).

DISCUSSION

Before discussing in detail the consequences of bioturbation for sediment iron and manganese cycling, it is instructive to summarize the results on carbon cycling that are presented in the companion paper (Van Nugteren *et al.* in prep). Integrated rates of carbon mineralization were estimated from sediment-water exchange fluxes of total inorganic carbon and changes in total inorganic carbon inventories. While the control and mixing treatment had similar mineralization rates (~ 100 mmol m^{-2} d^{-1}), there was evident enhancement of total mineralization in the gas exchange (~ 1.5 times the control) and *Nereis* treatment (~ 2 times the control; Van Nugteren *et al.* in prep). The enhanced total inorganic carbon production was attributed to respiration of the worm, enhancement of gas transport (as evidenced by the gas transport treatment) and other bioturbation effects that stimulate organic matter mineralization such as removal of inhibitory substances and oscillating redox conditions (Aller & Aller 1998, Kristensen 2000). Van Nugteren *et al.* (in prep) also report that the volume of oxidized sediment in the mixing, gas transport and *Nereis* treatments were 1.2, 2.9 and 4.5 times higher, respectively, than that of the control. Increasing volumes of oxidized sediments will likely result in increasing contributions of aerobic respiration at the expense of anaerobic respiration and more extensive re-oxidation of reduced metabolites such as ammonium, iron(II) and manganese(II).

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) delineate condensed metal-oxide reduction zones. Reduced iron or manganese can diffuse out of the reduction zone. Downward diffusion of reduced iron results in a reaction with sulfide and consequently immobilization due to acid volatile sulfide (AVS) or pyrite (FeS_2) formation (Murase & Kimura 1997, Pyzik & Sommer 1981, Jørgensen 1982, King 1990). Downward diffusing reduced manganese is immobilized in the sediment matrix due to sorption to or precipitation as mixed calcium-manganese carbonates (Middelburg *et al.* 1987). Upward diffusing reduced iron or manganese can be re-oxidized, chemically or microbialy, with oxygen (Nealson 1978, Santschi *et al.* 1990, Canfield 1989) or, in the case of Fe (II) (Postma 1985), (Postma & Appelo 2000)), with Mn-oxides forming a (sub) surface peak of newly formed iron oxides (Aller 1990, Sundby & Silverberg 1985). Moreover, oxidation of Fe(II) is much faster than that of Mn (II). 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 overlying 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 sulfate reduction. Epping *et al.* (1998) studied iron and manganese oxidation in sediment covered by microalgae. During benthic oxygenic photosynthesis there is sufficient oxygen to oxidize 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.* (1994) 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.

Iron and manganese biogeochemistry in the absence of fauna

The porewater concentrations of dissolved Fe (II) in the control cores decreased from the initial 196 µmol l^{-1} to 30 µmol l^{-1} in the subsurface maximum and from the initial 54 µmol l^{-1} to 33 µmol l^{-1} in the deepest layer with lowest concentrations (Fig. 1b), indicating a lack of net metal oxide reduction. Similarly, porewater levels of Mn(II) in the control cores decreased as well. While the efflux of Fe (II) was very low, there was a significant flux of Mn(II) (Fig. 2).

The low porewater concentrations of iron and manganese and visual observation of a blackening of the sediment indicate that sulfate reduction was the dominant anaerobic mineralization process in these cores, and that bacterial metal-oxide reduction had decreased. This decrease in metal-oxide reduction rate is likely due to a lack of reactive iron– and manganese oxides regeneration. Reduced manganese in the control sediments could escape to the overlying water (Fig. 2), probably due to the inefficient retention in the oxidized surface, and the pool of reactive manganese oxides diminished. Reduced Fe (II) reacted with reduced sulfur components with the consequence that reactive iron became trapped as an iron sulfide phase. These solid-phase iron sinks are not recycled efficiently in the absence of particle mixing so that the sediment became depleted in reactive iron-oxides.

The sole effects of mixing and gas exchange

Mixing was introduced by a stirring device placed ca. 5 to 55 mm below the sediment surface, i.e. in the anaerobic sediments (Van Nugteren *et al.* in prep). Although this treatment mainly involves particle mixing some porewater movement occurred as well (i.e. intraphase mixing). This enhanced pore-water mixing is evident from the deeper penetration of the bromide tracer (Fig. 1a) Solid-phase iron and manganese phases were distributed homogeneously (Fig. 3). Moreover, the porosity increased in the mixed layer from 0.46 in the other treatments to 0.5 in the mixing treatment (Van Nugteren *et al.* in prep). Care was taken that the thin oxic surface layer (about 2 mm) of the sediment was not mixed. Pore-water concentrations of dissolved Fe(II) and Mn(II) increased relative to the initial and control profiles (Fig. 1b,c). These elevated pore-water concentrations occurred despite enhanced rate of pore-water mixing that would lower dissolve metal concentrations. This implies that sediment mixing stimulated iron and manganese oxide reduction rates, even though the oxic surface layer was not involved.

Maximum manganese pore-water concentrations were observed in the surface layer, while dissolved iron concentrations showed a maximum somewhat deeper in the sediment, consistent with the small Fe and significant Mn efflux (Fig. 2). This difference in effluxes can also be attributed to differences in oxidation rates and efficiencies between iron and manganese. Although our data clearly reveal the enhancement of metal oxide reduction rates due to mixing, it is not clear how much this is due to additional oxygen input (e.g. the volume of oxic sediment was enhanced by a factor 1.2 (Van Nugteren *et al.* in prep) and how much is due to enhanced transport of solid phase reactants.

The construction of burrows by benthic fauna and their gas exchange activities affects the ratio of oxic to anoxic sediment. The U-shaped silicone tubing in our gas transport treatment increased the oxic sediment surface and volume with a factor of about 3.7 and 2.9, respectively (Van Nugteren *et al.* in prep). This is similar to what has been reported for burrowing organisms (e.g., Kristensen 2000). However, silicone tubing only allows exchange of gases, and pore-water bromide profiles clearly show that there was no enhancement of solute exchange rates (Fig. 1a). Burrows populated by animals are actively ventilated and permeable for dissolved constituents. This results not only in enhanced gas-exchange but this also enhances supply of electron acceptors such as nitrate and sulfate and removal of reduced porewater products (Aller & Aller 1998).

Pore water concentrations of manganese and iron increased relative to the control and initial conditions (Fig. 1b,c). The shape of the profiles is rather classic: i.e. subsurface maxima with the one for dissolved manganese being somewhat shallower than that of dissolved iron. Surface sediment pore-water concentrations at the end of the experiment were rather low, consistent with the limited efflux of these metals (Fig. 2). Solute transport was not enhanced and consequently accumulation of manganese and iron reflects enhanced metal-oxide reduction. This enhancement of metal-oxide reduction could be explained by the enhancement of total carbon mineralization by a factor 1.5 relative to the control, if the availability of labile organic matter was limiting metal-oxide reduction. This is unlikely given the small contribution of aerobic processes and metal-oxide reduction to total carbon mineralization in these sediments (Ferro 2003). Alternatively, reduced iron could accumulate in the pore-water because the additional oxygen input (due to gas exchange) diminished anoxic iron sinks more than iron oxide sinks were stimulated.

Maintenance of sedimentary manganese and iron recycling requires not only transport of reduced manganese and ferrous iron to oxidation sites, but also transport of manganese (III, IV) and ferric iron to reduction sites (Wijsman *et al.* 2002). The oxidized forms of manganese and iron mainly occur as particulate phases and their transport consequently requires bulk sediment or particle mixing. There was no particle transport in the defaunated gas exchange treatment, yet there was significant metal oxide reduction. Two non-exclusive explanations can be invoked to explain this apparent discrepancy. One, metal-oxide reduction rates were very high at the start of the treatment and gradually decreased during the experiment because of limited resupply of particulate metal oxides to sites of reduction. Two, there is growing evidence (Taillefert *et al.* 2000, Sobolev & Roden 2001) that the initial products of iron oxidation are in a soluble phase that is subject to diffusion (like truly dissolved phases). Soluble ferric iron formed at sites of oxidation can then diffuse to anoxic sites where it can be consumed either directly or after ageing and precipitation (Taillefert *et al.* 2000). Formation and subsequent diffusion of soluble ferric iron provides a rapid and small scale coupling between oxidation and reduction without the requirement of particle transport (Sobolev & Roden 2001).

Nereis diversicolor and iron and manganese biogeochemistry

The polychaete *Nereis diversicolor* constructs semi-permanent branching galleries that are actively irrigated (Davey & Watson 1995, Kristensen 2000, Mortimer *et al.* 1999). It is an omnivore that can suspension-feed, predate and feed on surface as well as deeper sediment layers. This polychaete therefore affects sediments by particle mixing as well as by burrow and pore-water irrigation (Davey & Watson 1995, Petersen *et al.* 1998). The consequences for sediment biogeochemistry have been studied in quite some detail. The surface area of the burrow walls have been reported to increase total sediment surface area by about 1.3 to 5 times (Kristensen 2000), consistent with the 4.2 fold increase of surface area in our experiments (Van Nugteren *et al.* in prep). Similarly, the volume of oxidized sediments in our experiments increased about 4.5 times, somewhat more than the 1-3.3 times reported by Kristensen (2000). As discussed in detail in Van Nugteren *et al.* (in prep), there is an enhancement of organic matter degradation due to this additional oxygen input. This enhanced input of oxygen also causes an increase in iron hydroxides with the consequence that effluxes of phosphate and sometimes silicate are lowered (Mortimer *et al.* 1999). Watson and Frickers (1990) performed an experiment of 14 days with the *Nereis (Hediste) diversicolor*. They observed a decrease of pore-water concentration of manganese (~0.6 μmol Mn l^{-1} d^{-1}) in the upper 5 cm.

In our experiment porewater concentrations of dissolved Fe(II) and Mn(II) were low (Fig. 1) and there were negligible fluxes of dissolved Fe(II) and Mn(II) (Fig. 2). These observations are consistent with a shift to more aerobic mineralization in the *Nereis* treatment. In our *Nereis* treatment pore-water irrigation rates were high (Fig. 1a) and dissolved iron and manganese were rapidly transported from reduced to oxidized zones. As a consequence, pore-water concentrations were kept low, there were no significant effluxes and the net iron-oxide reduction zone has moved into the sediment relative to the other treatments (Fig. 1b).

Bioturbation comprises all effect of organisms on transport of particles, solutes and gases. In our experimental study we have tried to unravel the sole effect of particle mixing (mixing treatment) and sediment aeration (gas transport treatment). Our experiments clearly show that particle mixing and sediment aeration each result in enhanced rates of metal-oxide reduction. The main effects of *Nereis diversicolor* on sediment biogeochemistry are probably particle mixing, sediment aeration and irrigation. Pore-water irrigation by the worm results in more aerated conditions and the accumulation of metal oxides around the tubes. The worm also creates new burrows and the resulting particle mixing will, at least over somewhat longer periods, cause transport of metal oxides. The sole effect of aeration is to enhance metal-oxide reduction, but the underlying mechanism is not entirely clear. It appears that the combined effect of particle mixing, sediment aeration and irrigation is not simply additive with regards to iron and manganese recycling. However, it is very clear that the presence and activity of bioturbators such as *Nereis diversicolor* favor recycling of iron and manganese oxides at the expense of sulfate reduction and bioturbators thus have a positive effect on their environment by lowering the potential for sulfide accumulation.

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