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 dissimilatory 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, Wijerman, 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

<table>
<thead>
<tr>
<th></th>
<th>Co-ordinates</th>
<th>Depth (m)</th>
<th>Salinity</th>
<th>BET-N2 specific surface area (m² g⁻¹)</th>
<th>Median grain size (µm)</th>
<th>Organic carbon (mg g⁻¹)</th>
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</thead>
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<tr>
<td>Florence Rise</td>
<td>34.47.17 N 31.31.38 E</td>
<td>2300</td>
<td>38</td>
<td>25-40 b</td>
<td>Na</td>
<td>2.7 - 24.0 b</td>
</tr>
<tr>
<td>Eratosthenes Seamount</td>
<td>33.45.04 N 32.50.41 E</td>
<td>970</td>
<td>38</td>
<td>na</td>
<td>Na</td>
<td>2.7 - 12.4 b</td>
</tr>
<tr>
<td>Grevelingen September</td>
<td>51.45.78 N 03.58.47 E</td>
<td>3</td>
<td>28</td>
<td>224 - 300 c</td>
<td>2.0 - 0.8 c</td>
<td></td>
</tr>
<tr>
<td>Grevelingen February</td>
<td>51.45.78 N 03.58.47 E</td>
<td>3</td>
<td>28</td>
<td>2.1-0.9 c</td>
<td>282</td>
<td>1.5 - 3.2 c</td>
</tr>
<tr>
<td>Appels</td>
<td>51.02.43 N 04.05.37 E</td>
<td>0</td>
<td>0.7</td>
<td>4.8</td>
<td>63.5</td>
<td>22.9</td>
</tr>
</tbody>
</table>

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 collected 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
eextractions 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 (O₂ 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 % N₂ and < 3 % H₂. 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 % H₂SO₄ ml sample⁻¹, Σ CO₂ and CH₄ 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 SO₄ 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 % N₂ and < 3 % H₂).

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 HNO₃/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 CH₄ and a hot-wire detector for CO₂.

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 immediately analyzed for NO₃ 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 Spectrometer (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 O₂ 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 N₂ 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 (Table 1, 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-N2 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. 1a).

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$_{\text{dith}}$) 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 (van Santvoort 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$_{\text{asc}}$) 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$_{\text{dith}}$, upper x-axis) concentration is approximately 10 times higher than Fe$_{\text{asc}}$ in the oxic sediment and there is a clear peak in the oxidized sapropel. Moreover, Fe$_{\text{dith}}$ abruptly decreases in the sapropel. Total iron (Fe$_{\text{total}}$, upper x-axis) concentrations in the oxic sediment are 2.5-4.5 times higher than Fe$_{\text{dith}}$. Fe$_{\text{total}}$ 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 $\Sigma \text{CO}_2$ production.

Fig. 1. Results of Florence Rise, N.B. porewater data result form a different box core. a. O$_2$ ($\mu$mol l$^{-1}$) – diamonds, organic carbon (mg g sediment$^{-1}$) – triangles. b. Dissolved Fe (II) ($\mu$mol l$^{-1}$) – diamonds, dissolved Mn (II) ($\mu$mol l$^{-1}$) – squares. c. Mn dithionite ($\mu$mol g$^{-1}$) - triangles, Mn total ($\mu$mol g$^{-1}$) - squares d. Fe ascorbate ($\mu$mol g$^{-1}$) - circles, Fe dithionite ($\mu$mol g$^{-1}$) - triangles, Fe total ($\mu$mol g$^{-1}$) - squares e. Production rates ($\mu$mol l$^{-1}$ d$^{-1}$) of dissolved Fe (II) - diamonds, dissolved Mn (II) – open squares and $\Sigma$ 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.
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 (~10 µM) and the various iron oxide pools were rather uniform (Fig. 3c). Fe$_{asc}$ (~ 4 µmol g$^{-1}$) was about 2.5 times smaller than the concentration of Fe$_{dith}$ (~ 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 (~ 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. 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 CO₂ - circles.
Fig. 4 Results of Grevelingen February 1999. a. O2 (µmol l⁻¹) – diamonds, organic carbon (mg g sediment⁻¹) – triangles, SO₄ (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 CO₂ - circles.
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 CH₄ – triangles and Σ CO₂ – circles e. Production rates (µmol l⁻¹ d⁻¹) of CH₄ – triangles and Σ CO₂ – circles
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 CO₂ 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 dict and Fe total respectively (5c). On average, Fe dict concentrations were 2 times higher than those of Fe asc were. Total iron concentrations in turn were 2.5 times higher than the Fe dict.

Production of $\Sigma$ CO₂ 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-layer. 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). $\Sigma$ CO₂ 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.
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 Fe²⁺/ 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 CO₂ 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 CO₂ is accompanied by consumption of 1 mol O₂ or 1.25 mol NO₃⁻ or production of 0.25 mol of Fe (II), 0.45 mol of Mn (II), 1 mol of CH₄ and the reduction of 2 mol of SO₄. The total sum of the CO₂ equivalents should be equal to the measured Σ CO₂ production.

Calculated Σ CO₂ rates do not always correspond to measured Σ CO₂ 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 O₂ consumption rates are derived under oxic conditions while CO₂ 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 O₂ consumption based on modelling of porewater profiles (84 and 108 nmol cm⁻² d⁻¹ at Florence Rise and Eratosthenes Seamount, respectively) and CO₂ 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 O₂ due to re-oxidation of ammonium produced during the aerobic carbon degradation resulted in somewhat lower O₂ 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 example 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 assessed 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 temperatuare and carbon availibility.

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 \(\Sigma\) 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 \(\Sigma\) 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 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 unamended 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 $\sum$ 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 hundred 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 \(\Sigma CO_2\)) 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 is 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.

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