In vitro simulation of oxic/suboxic diagenesis in an estuarine fluid mud subjected to redox oscillations

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Abstract

Estuarine turbidity maxima (ETMs) are sites of intense mineralisation of land-derived particulate organic matter (OM), which occurs under oxic/suboxic oscillating conditions owing to repetitive sedimentation and resuspension cycles at tidal and neap-spring time scales. To investigate the biogeochemical processes involved in OM mineralisation in ETMs, an experimental set up was developed to simulate in vitro oxic/anoxic oscillations in turbid waters and to follow the short timescale changes in oxygen, carbon, nitrogen, and manganese concentration and speciation. We present here the results of a 27-day experiment (three oxic periods and two anoxic periods) with an estuarine fluid mud from the Gironde estuary. Time courses of chemical species throughout the experiment evidenced the occurrence of four distinct characteristic periods with very different properties. Steady oxic conditions were characterised by oxygen consumption rates between 10 and 40 μmol L⁻¹ h⁻¹, dissolved inorganic carbon (DIC) production of 9–12 μmol L⁻¹ h⁻¹, very low NH₄⁺ and Mn²⁺ concentrations, and constant NO₃⁻ production rates (0.4 – 0.7 μmol L⁻¹ h⁻¹) due to coupled ammonification and nitrification. The beginning of anoxic periods (24 h following oxic to anoxic switches) showed DIC production rates of 2.5–8.6 μmol L⁻¹ h⁻¹ and very fast NO₃⁻ consumption (5.6–6.3 μmol L⁻¹ h⁻¹) and NH₄⁺ production (1.4–1.5 μmol L⁻¹ h⁻¹). The latter rates were positively correlated to NO₃⁻ concentration and were apparently caused by the predominance of denitrification and dissimilatory nitrate reduction to ammonia. Steady anoxic periods were characterised by constant and low NO₂⁻ concentrations and DIC and NH₄⁺ productions of less than 1.3 and 0.1 μmol L⁻¹ h⁻¹, respectively. Mn²⁺ and CH₄ were produced at constant rates (respectively 0.3 and 0.015 μmol L⁻¹ h⁻¹) throughout the whole anoxic periods and in the presence of nitrate. Finally, reoxidation periods (24–36 h following anoxic to oxic switches) showed rapid NH₄⁺ and Mn²⁺ decreases to zero (1.6 and 0.8–2 μmol L⁻¹ h⁻¹, respectively) and very fast NO₃⁻ production (3 μmol L⁻¹ h⁻¹). This NO₃⁻ production, together with marked transient peaks of dissolved organic carbon a few hours after anoxic to oxic switches, suggested that particulate OM mineralisation was enhanced during these transient reoxidation periods. An analysis based on C and N mass balance suggested that redox oscillation on short time scales (day to week) enhanced OM mineralisation relative to both steady oxic and steady anoxic conditions, making ETMs efficient biogeochemical reactors for the mineralisation of refractory terrestrial OM at the land-sea interface.

1. Introduction

Mineralisation of terrestrially derived particulate organic matter (OM) is one of the major processes that drive ecosystem metabolism at the land-sea interface and makes near-shore coastal systems net heterotrophic environments and CO₂ sources for the atmosphere (Frankignoulle et al., 1998; Gattuso et al., 1998; Borges,
Land-derived particles often accumulate in particular coastal zones, like estuarine turbidity maxima (ETM) or deltaic mobile muds (DMMs) on the near-shore continental shelf, where they stay for months or years before being transferred back to coastal lagoons and wetland sediments or to the shelf break and deep seafloor (Jouanneau et al., 1999, 2008; Allison et al., 2000). These shallow estuarine and coastal environments are characterised by frequent resuspension of superficial sediments and the formation of transient benthic turbid structures called “fluid mud”, induced by changes in hydrodynamics. In ETMs, sedimentation and resuspension cycles are controlled by tidal currents and occur predominantly on semi-diurnal and neap-spring time scales (Abril et al., 1999). In DMMS, storms and waves are the major drivers of resuspension, which generally occur on time scales of several months (Aller, 1998; Allison et al., 2000). As a consequence, land-derived particles experience continuous redox oscillations, spending some time settled in anoxic sediments and some time suspended in the oxic water column. The relative proportion of POM in the solid phase that occurs in ETMs and DMMs suggests a link between redox oscillation and POM mineralisation efficiency (Aller, 1998; Abril et al., 2002; Blair et al., 2004; Aller and Blair, 2006; Middelburg and Herman, 2007).

The impact of redox oscillations induced by sediment resuspension on the mineralisation of terrestrial particulate OM is still only partially understood. Aller (1994, 1998) discussed that redox changes could enhance particulate OM mineralisation through two major groups of biogeochemical processes. First, mixing of terrestrial OM with much more labile marine or estuarine OM occurs frequently and promotes co-oxidation. Labile OM in muds includes living phytoplankton cells and detritus, bacterial biomass that is recycled after each redox change and transient fermentative compounds that become very labile inoxic conditions (Sun et al., 1993; Aller, 1994). Second, reduced inorganic compounds and nutrients are frequently recycled and become available again for diagenetic reactions and heterotrophic activities. The study of the vertical distribution of redox species in estuarine and deltaic fluid muds (Aller, 1998; Abril et al., 1999, 2000; Aller et al., 2004) has revealed that suboxic conditions dominate. This is probably because reduced nitrogen, manganese and iron are periodically reoxidised, forming nitrate and poorly crystallised oxides on the suspended particles, which are then available to oxidise OM during the next deposition phase (Aller, 1998; Aller et al., 2004). Consequently, sulphate reduction, the classical dominant heterotrophic process in marine sediments (Jorgensen, 1982), becomes minor in comparison with aerobic respiration, denitrification, and Mn and Fe reduction (Aller, 1998; Abril et al., 1999). In DMMS, where particles reside more than 90% of their time in anoxic conditions, and where reoxidation frequency is generally more than one month, iron oxides are the major electron acceptor (Aller, 1998; Aller et al., 2004; Aller and Blair, 2006). In contrast, in ETMs where particles spend 50% of their time in oxic conditions, and where the resuspension frequency is less than a week, vertical profiles in the fluid mud suggest that oxygen, nitrate and Mn oxides dominate as oxidants for OM degradation (Abril et al., 1999, 2000).

Although in situ studies give important information on the nature and time scales of processes occurring in fluid muds, in vitro approaches are necessary to quantify the rates of OM degradation and to investigate how redox oscillations could enhance OM mineralisation. Previous in vitro experiments designed to compare

The Gironde estuary is turbid almost all year, but experiences the most concentrated ETM in the summer, generally at low to intermediate salinities (Abril et al., 2002). A large majority of the particulate OM in the Gironde ETM is of terrestrial origin and has low reactivity (Etcheber et al., 2007). Fluid mud pools, in which the Total Suspended Solids (TSS) concentration ranges between 50 and 400 g L⁻¹, settle at neap tides and can reach 2 m in thickness (Abril et al., 1999). Available in situ data on the fluid mud of the Gironde estuary show that anoxia occurs when the TSS concentration is in the range of 50–150 g L⁻¹, depending on the geographical location (Abril et al., 1999). Concentration profiles of redox species suggest that organic matter mineralisation in the fluid mud is dominated by denitrification and reduction by oxides of manganese. In addition, nitrate is rapidly consumed in the upper layer of the fluid mud (150–250 g L⁻¹). Mn⁴⁺ appears in the more concentrated inner layer (250–450 g L⁻¹), and production of alkalinity, ammonium, and potentially labile dissolved organic carbon (DOC) were also observed in the fluid mud (Abril et al., 1999, 2000).

2. Materials and methods

2.1. Study site

We used a homemade incubator (Fig. 1), which enabled us to maintain a homogenised fluid mud under oxic or anoxic conditions for a determined period of time. The changes in concentrations of major chemical species were measured either continuously in the liquid or gas phases or at regular intervals by sampling, conditioning, and further analysis. The incubation system consisted of a 14.2 L glass vessel set on a magnetic stirrer and thermostatically controlled with a coiled silicone tube. Three orifices in the vial allowed hermetical connection of Teflon seams to a temperature, a pH, and an oxygen sensor. In addition, a tap connected to a 50 mL syringe allowed the sampling of aliquots without any contact with the atmosphere. The cap of the vessel was equipped with five apertures; two of them were connected by a closed circuit to a gas analyser, allowing sampling of the gas phase; two others were connected to two 5 L glass vessels set in parallel, one empty and one containing an oxygen trap (Aerocoat, Merck®). A peristaltic pump circulated the gas through the fluid mud sample at a flow of
0.17 L min\(^{-1}\) to equilibrate the liquid phase (total volume 10.6 L) with the gas phase (total volume 8.6 L). The last orifice was fitted with a rubber septum and permitted the injection of reagents with a syringe.

Oxic or anoxic conditions were obtained by occasionally purging the gas and water phases with either air or N\(_2\). There was no need to add CO\(_2\) to the purging gases to avoid too high of a pH value for the fluid mud. For anoxic periods, the N\(_2\) stream circulated through the vessel containing the oxygen trap to remove traces of O\(_2\). About 45 min was needed for complete conversion from oxic to anoxic conditions, or from anoxic to oxic conditions. When stable conditions were obtained, the system was closed and a peristaltic pump ensured gas circulation. Under oxic conditions, the release of CO\(_2\) from respiration strongly decreased the pH. To avoid too low of a pH value, purges with air were also performed at regular intervals during oxic periods. The selected criterion for these purges was to maintain the pH above 7.7, the typical lowest value observed in situ in the fluid mud of the Gironde (Abril et al., 1999).

Continuous measurements in the gas phase were performed with an infrared / photo-acoustic gas analyser (INNOVA 1312), which allowed recording of the partial pressures of carbon dioxide (pCO\(_2\)), methane (pCH\(_4\)) and nitrous oxide (pN\(_2\)O) every two minutes. In the present study, the gas analyser was calibrated against a Gas Chromatograph-Thermal Conductivity Detector and a Gas Chromatograph-Flame Ionization Detector for analysis on the 0–10,000 ppmv and 0–500 ppmv ranges for CO\(_2\) and CH\(_4\), respectively (\(R^2 > 0.98\), \(n = 20\)). No calibration was performed for N\(_2\)O, as this parameter was interpreted qualitatively only. In the liquid phase, pH and temperature were measured with a Ross combination pH electrode, calibrated every day with NBS buffers, and with a PT100 sensor, respectively. Oxygen was monitored with a polarographic YSI electrode calibrated in water-saturated air.

### 2.3. Experimental procedure

Fifteen litres of fluid mud were sampled with a Niskin bottle in April 2003 during a neap tide period in the Gironde ETM, 50 cm above the sediment surface. The particulate matter concentration of the sample was 140 g L\(^{-1}\), with an organic carbon content of 1.5 dry weight %, and the salinity was 1.3. Back in the laboratory, the sample was re-homogenised and 10.6 L was transferred into the incubator. Four litres were kept in a second hermetic glass vial, also equipped with a gas purging system. An 8-cm magnetic stirrer was introduced in the incubator, which was closed after a 3-h purge with air to reoxidise completely the fluid mud. Although the in situ temperature was 16.3°C, the incubator was thermostatically controlled at 20°C to limit thermal gradients and changes in gas adsorption/desorption to the vial wall. Throughout the experiment, the extra fluid mud in the second vial was purged at the same frequency with air or nitrogen. Two discrete 60 mL samples were taken at once from the incubator at variable time intervals with a 60 mL polypropylene syringe that had been previously filled with fluid mud from the second vial. Therefore, the liquid and gas volumes were constant in the incubator. The content of the syringe was homogenised 6 times with the fluid mud in the incubator before sampling. For DOC samples, fluid mud was centrifuged in pre-combusted glass vials. The supernatant was filtered through 0.7 μm glass fibre filters and the filtrates were poisoned with H\(_3\)PO\(_4\) (50 μL for 20 mL of pore water). For all other parameters, centrifugation was done in polypropylene vials and filtration through 0.45 μm cellulose acetate filters. The filtrates were then divided into four parts: one conditioned in a serum bottle without headspace and kept at ambient temperature for total alkalinity (TA); one frozen at −20°C for dissolved inorganic nitrogen (DIN) species; one kept at 4°C for sulphate (SO\(_4^{2-}\)) and one acidified with a 65% nitric acid drip for dissolved Fe\(^{2+}\) and Mn\(^{2+}\).
For anoxic samples, centrifugation, filtration and conditioning were performed under a nitrogen stream to limit oxidation.

The experiment described here was conducted for a total of 27 days. It started under oxic conditions for 6 days with 3 intermediate air purges. At day 7, anoxia was imposed until day 9. Then, oxic conditions were maintained for days 10, 11 and 12, with one intermediate purge. From days 13 to 22, anoxia was maintained for 10 days, followed by an injection of KNO₃ after 7 days to investigate redox reactions when Mn⁺² and nitrate are simultaneously present in anoxic fluid mud pore waters. Finally, from day 23 to 27, oxic conditions were maintained with three intermediate air purges.

2.4. Analytical techniques

Analytical techniques for nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₄⁺), TA, dissolved Mn and DOC have been described elsewhere (Abril et al., 1999, 2000). Briefly, DIN species were determined with classical spectrophotometric techniques, TA by Gran electro-titration with 0.1 N HCl, performed under a nitrogen stream for anoxic samples, Mn²⁺ by flame-atomic absorption spectroscopy and DOC with a Shimadzu TOC 5000 analyser. In addition, iron (II) was measured by the ferrozine method (Stookey, 1970) and sulphate was analysed according to a nephelometric method adapted from Rodier (1976), with a precision of about 5%.

2.5. Rate calculations

The rates of production or consumption of any chemical species during the experiment were defined as the loss of, or gain in, the incubation vial (liquid and gas) per volume of fluid mud incubated. For non-gaseous dissolved species (NO₃-, NH₄+, DIN and Mn²⁺) rates were simply calculated from the slope of the change in concentration versus time. Oxygen consumption rates were determined during steady oxic periods of linear oxygen decrease given by the solubility coefficient of carbonic acid from Mehrbach et al. (1973), and the CO₂ concentration versus time. Oxygen consumption rates were calculated the same way, except that measurements were done in the gas phase and that the solubility coefficient of methane given by Yamamoto et al. (1976) was used (Table 1). Dissolved inorganic carbon production rates were determined as the sum of the increase in CO₂ in the gas phase and DIC in the aqueous phase. The latter was calculated from the slope of the change in concentration versus time. Oxygen consumption rates were determined with classical spectrophotometric techniques, TA by Gran electro-titration with 0.1 N HCl, performed under a nitrogen stream for anoxic samples, Mn²⁺ by flame-atomic absorption spectroscopy and DOC with a Shimadzu TOC 5000 analyser. In addition, iron (II) was measured by the ferrozine method (Stookey, 1970) and sulphate was analysed according to a nephelometric method adapted from Rodier (1976), with a precision of about 5%.

Table 1
Overview of the parameters in the experimental setup.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid volume (L)</td>
<td>10.6</td>
</tr>
<tr>
<td>Gas volume (L)</td>
<td>8.6</td>
</tr>
<tr>
<td>Liquid and gas temperature (°C)</td>
<td>20</td>
</tr>
<tr>
<td>Liquid salinity</td>
<td>1.3</td>
</tr>
<tr>
<td>O₂ solubility coefficient (mmol kg⁻¹ atm⁻¹)</td>
<td>1.36</td>
</tr>
<tr>
<td>CO₂ solubility coefficient (mmol kg⁻¹ atm⁻¹)</td>
<td>38.9</td>
</tr>
<tr>
<td>CH₄ solubility coefficient (mmol kg⁻¹ atm⁻¹)</td>
<td>1.24</td>
</tr>
<tr>
<td>K₁ [H₂CO₃/HCO₃⁻] (mol⁻¹)</td>
<td>4.85 x 10⁻³</td>
</tr>
<tr>
<td>K₂ [HCO₃⁻/CO₃²⁻] (mol⁻¹)</td>
<td>8.58 x 10⁻¹¹</td>
</tr>
</tbody>
</table>

3. Results

3.1. Troubleshooting during the experiment

The experiment worked well throughout the 27-day period of observation with few exceptions. During days 13 and 14, at the beginning of the second anoxic period, however, the magnetic stirrer became blocked, which lead to a partial decantation of the fluid mud. As a result, redox conditions in the vial were different at the sampling height than at the electrode height. Because biogeochemical reactions occurred during this period, concentrations are still shown in the results (Figs. 3 and 4). The troubleshooting is indicated by a hashed zone, and the result was not considered for discussion. A second problem occurred during the first few days following the anoxic to oxic switch and was due to a limitation of the incubation technique used: oxidation processes were so intense during these periods that the oxygen electrode could not stabilise (Fig. 3A). In addition, during these reoxidation periods a large part of the CO₂ production obviously occurred during the purges with air and most of the CO₂ escaped from the system and could not be taken into account in mass balance calculations. Finally, during these reoxidation periods, but after the purge, pH and pCO₂ ranged linearly over periods of several hours. During transient periods following purges or when rapid changes in the pCO₂ trend occurred, DIC production rates derived from TA and pCO₂ would be affected by any delay in gas equilibration. For the same reason, it was not possible to provide a curve of DIC versus time throughout the whole experiment.

![Fig. 2](image.jpg)

**Fig. 2.** Equilibration delay between the liquid phase and the gas phase. Temporal evolution of pH in the liquid phase and pCO₂ (ppmv) in the gas phase following a 4.2 mmol injection of HCl.
and in addition to the oxic and anoxic criteria, we used the following definitions for four periods characteristic reproducible throughout the whole experiment. First, "steady oxic periods" referred to stable conditions occurring a few days after oxic conditions were installed, and during these periods concentrations of reduced compounds like NH$_4^+$ and Mn$^{2+}$ were null. Secondly, "beginning of anoxic periods" was used to designate the time right after the oxic to anoxic switches when NO$_3^-$/CO$_2$ and NH$_4^+$ showed extremely rapid changes (decreased and increased, respectively). Third, "steady anoxic conditions" referred to periods later in the anoxia when NO$_3^-$ remained low and constant. Lastly, the term "reoxidation periods" was used for the transient times just after the oxic to anoxic switches when NO$_3^-$ increased and NH$_4^+$ and Mn$^{2+}$ decreased very suddenly.

3.2. Time courses of chemical species throughout the experiment

During oxic periods, oxygen decreased linearly between purges (Fig. 3A). O$_2$ consumption rates that could be calculated from the measured data varied between 10.9 and 76.3 μmol L$^{-1}$ h$^{-1}$ and were always significantly higher after an anoxic period. During the last oxic period in particular, O$_2$ consumption rates progressively decreased between days 24 and 27. Throughout the whole experiment, pH and pCO$_2$ showed an anti-parallel correlation (Fig. 3B), except during and just after the troubleshooting period (days 13 and 14). This indicated that the liquid and gas phases were close to the equilibrium for CO$_2$. Values of pH varied between 7.65 and 8.24, except at the beginning of the last oxic period (day 22), when it reached 8.32 at the end of the purge. Values of measured pCO$_2$...
Fig. 4. Time courses of (A): NO$_3^-$ ($\mu$mol L$^{-1}$); (B) NH$_4^+$ ($\mu$mol L$^{-1}$); (C) NO$_2^-$ in the water phase ($\mu$mol L$^{-1}$) and pN$_2$O in the gas phase (ppmv); (D) DIN ($\mu$mol L$^{-1}$); and (E) Mn$^{2+}$ ($\mu$mol L$^{-1}$). Production and consumption rates are expressed in $\mu$mol L$^{-1}$ h$^{-1}$ ($R^2 > 0.95$). Shaded area same as in Fig. 3.
other processes affecting the pH and TA of the water. As TA remained constant or increased, DIC also increased during the oxic periods. DIC production rates that could be calculated from the evolution of pCO₂ in the gas phase and TA in the water phase were between 4 and 13 μmol L⁻¹ h⁻¹ during the first oxic period (Table 2), similar but lower than respiration deduced from oxygen during the same period (Fig. 3). DIC production rates calculated during reoxidation periods, and during the second and third oxic periods, were inconsistent with the evolution of oxygen (Table 2). This was probably due to the combined effect of equilibration delays and other processes affecting the pH and TA of the water.

During the anoxic periods, pCO₂ first decreased (days 7–9 and 15–16), then stabilised (day 17) and finally slowly increased again (days 18–19 and 21–22). The addition of nitrate under anoxic conditions (day 19 in Figs. 3 and 4) rapidly increased the pH and decreased the pCO₂. Throughout the experiment, TA varied between 2.60 and 4.10 mmol L⁻¹ (Fig. 3C) and showed a moderate increase during the first oxic period, followed by rapid increases during anoxic periods (days 7–9 and 13–19). Reoxidation periods (days 10 and 23–24), as well as the day following the nitrate injection (day 19) and the last day of the experiment under oxic conditions (day 27), were all characterised by decreases in TA. Under anoxic conditions, TA increases were concomitant to pCO₂ decreases (days 7–9 and 15–16), which resulted in a net positiveDIC production. During reoxidation periods (days 9 and 24) sudden TA decreases occurred during the purges with air and the resulting CO₂ escape from the system. The following pCO₂ increases were extremely fast, partly as the result of a delayed liquid-gas equilibration. As a consequence, DIC production rates during these reoxidation periods could not reasonably be used for the data interpretation. In contrast, after the nitrate injection and without any purge (day 19), both TA and pCO₂ decreased simultaneously even in the absence of any purge, showing a net DIC consumption in the system.

NO₃⁻ and NH₄⁺ species varied between 30 and 370 μmol L⁻¹ and 0 and 110 μmol L⁻¹, respectively, and showed a good anti-parallel correlation (Fig. 4A and B). Under steady oxic conditions, NH₄⁺ was less than 1 μmol L⁻¹ and NO₃⁻ increased regularly at rates of 0.4 and 0.7 μmol L⁻¹ h⁻¹ (during days 11–12, and during days 1–6 and 25–27, respectively). The first two days of the two anoxic periods (days 7 and 14), as well as the day following KNO₃ injection (day 19), NO₃⁻ rapidly decreased at rates of −6.3, −5.6 and −10.6 μmol L⁻¹ h⁻¹, respectively. In the same time period, NH₄⁺ increased at rates of +1.7, +1.4 and +2 μmol L⁻¹ h⁻¹, respectively. The resulting DIN decreases for the same days were −4.8, −2.6, and −9 μmol L⁻¹ h⁻¹ (Fig. 4D). Later in the anoxic conditions, NO₃⁻ stabilised at a constant value around 30 μmol L⁻¹ and NH₄⁺ increased at much lower rates (0.2 μmol L⁻¹ h⁻¹ for days 8–9 and 0.07 μmol L⁻¹ h⁻¹ for days 16–18). During the two reoxidation periods (day 10 and days 23–24), NO₃⁻ increased extremely fast at rates between 4.4 and 5 μmol L⁻¹ h⁻¹, and NH₄⁺ decreased at rates of −1.4 to −1.6 μmol L⁻¹ h⁻¹.

NO₂⁻ was near zero under oxic conditions, but showed some peaks just after the oxic to anoxic switch (Fig. 4C; 2.2 μmol L⁻¹ at day 7 and 15.1 μmol L⁻¹ at day 14). Under anoxic conditions, NO₂⁻ reached 59 μmol L⁻¹ at day 20 just after the KNO₃ spike and then decreased rapidly to zero. Additionally, N₂O in the gas phase was less than 0.1 ppmv during steady oxic conditions (days 1–6, 11–12 and 25–27). N₂O partial pressures were higher under anoxic conditions, as well as during reoxidation periods. In the anoxic conditions, pN₂O was constant at approximately 0.2 ppmv during days 7–9 and days 16–19. A similar value was observed during the first reoxidation period (day 10). Higher values revealed intense N₂O production after the second oxic to anoxic switch (up to 0.8 ppmv at day 15) and after the KNO₃ spike (up to 1.8 ppmv at day 20). Finally, N₂O was also produced during the second reoxidation period (days 23–24) when pN₂O increased rapidly.

Mn²⁺ was below 1 μmol L⁻¹ under steady oxic conditions and increased under anoxic conditions (Fig. 4E). Mn²⁺ production started as soon as anoxia was implemented, even in the presence of high concentrations of NO₃⁻. Production rates were linear around 0.3 μmol L⁻¹ h⁻¹ during the first 3 days of anoxia (days 7–9 and 14–16). During reoxidation periods, however, Mn²⁺ decreased rapidly to zero (rates between −0.76 and −2 μmol L⁻¹ h⁻¹). During the two days after the KNO₃ injection in the anoxic conditions (days 20–21), Mn²⁺ decreased at a rate of −0.18 μmol L⁻¹ h⁻¹. Furthermore, Fe²⁺ (data not shown) was less than 5 μmol L⁻¹ h⁻¹ (with two peaks at 14.4 and 9.5 μmol L⁻¹, but the changes in Fe²⁺ did not significantly follow the redox conditions) in both oxic and anoxic conditions throughout the whole experiment. Sulphate concentration (not shown) remained constant within the analytical error at 2.1 mmol L⁻¹. Further, the characteristic hydrogen sulphide odour was never perceived in any of the samples studied.

Table 2

Rates of changes of chemical species during the experiment. All rates were reported in μmol L⁻¹ h⁻¹, except for dpCO₂/dt, which was reported in ppmv h⁻¹ in the headspace. For O₂ and CH₄, rates included the changes in the gas and liquid phases. For DIC production, the rate included the change in DIC in the liquid phase and the change in CO₂ in the gas phase (see text).

<table>
<thead>
<tr>
<th>Days</th>
<th>O₂ cons.</th>
<th>DIC prod.</th>
<th>RQ</th>
<th>dpCO₂/dt</th>
<th>d[TA]/dt</th>
<th>CH₄ prod</th>
<th>d[NO₃⁻]/dt</th>
<th>d[NH₄⁺]/dt</th>
<th>d[Mn²⁺]/dt</th>
<th>d[DIN]/dt</th>
<th>O₂/DIN</th>
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<tbody>
<tr>
<td>1–2 ox</td>
<td>10.9</td>
<td>9</td>
<td>0.82</td>
<td>+26.7</td>
<td>+3.9</td>
<td>0</td>
<td>+0.7</td>
<td>0</td>
<td>0</td>
<td>+0.7</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>3–4 ox</td>
<td>13.3</td>
<td>7.1</td>
<td>0.53</td>
<td>+26.8</td>
<td>+3.9</td>
<td>0</td>
<td>+0.7</td>
<td>0</td>
<td>0</td>
<td>+0.7</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>5–6 ox</td>
<td>15.4</td>
<td>12</td>
<td>0.78</td>
<td>−24.4</td>
<td>+3.9</td>
<td>0</td>
<td>+0.7</td>
<td>0</td>
<td>0</td>
<td>+0.7</td>
<td>22</td>
<td>17</td>
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<tr>
<td>7 anox</td>
<td>8.6</td>
<td>−</td>
<td>−61.1</td>
<td>+9.2</td>
<td>0.012</td>
<td>−6.3</td>
<td>+1.7</td>
<td>+0.29</td>
<td>−4.8</td>
<td>−</td>
<td>−</td>
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<tr>
<td>8 anox</td>
<td>1.3</td>
<td>−</td>
<td>−12.1</td>
<td>+0.1</td>
<td>0.012</td>
<td>−0.02</td>
<td>+0.2</td>
<td>+0.29</td>
<td>+0.18</td>
<td>−</td>
<td>7</td>
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<tr>
<td>10 ox</td>
<td>4.6</td>
<td>h 1.11</td>
<td>b 115.5</td>
<td>−15.7</td>
<td>0</td>
<td>+4.4</td>
<td>−1.4</td>
<td>−2.0</td>
<td>+3.0</td>
<td>−</td>
<td>−</td>
<td></td>
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<tr>
<td>11–12 ox</td>
<td>b 33.8</td>
<td>b 4.6</td>
<td>0.12</td>
<td>+16.3</td>
<td>+3.5</td>
<td>0</td>
<td>+0.4</td>
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<td>−5.6</td>
<td>+1.4</td>
<td>+0.37</td>
<td>−2.6</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>16 anox</td>
<td>2.4</td>
<td>−</td>
<td>−54.2</td>
<td>+3.3</td>
<td>0.019</td>
<td>0</td>
<td>+0.07</td>
<td>+0.37</td>
<td>0.07</td>
<td>−</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>17–19 ox</td>
<td>0.6</td>
<td>−</td>
<td>−54.2</td>
<td>+3.3</td>
<td>0.019</td>
<td>0</td>
<td>+0.07</td>
<td>+0.18</td>
<td>0.07</td>
<td>−</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>20 spike</td>
<td>40.0</td>
<td>−</td>
<td>−10.6</td>
<td>−0.1</td>
<td>0</td>
<td>−0.25</td>
<td>+0.05</td>
<td>+0.02</td>
<td>−0.2</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>21 anox</td>
<td>23.1</td>
<td>b 82.1</td>
<td>0.01</td>
<td>+5.0</td>
<td>−1.6</td>
<td>−0.76</td>
<td>+3.3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>24 ox</td>
<td>11.1</td>
<td>b 0.14</td>
<td>+25.9</td>
<td>−12.4</td>
<td>0</td>
<td>+5.0</td>
<td>−1.6</td>
<td>0</td>
<td>+3.3</td>
<td>23</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>25 ox</td>
<td>18.2</td>
<td>0.62</td>
<td>+20.7</td>
<td>+0.1</td>
<td>0</td>
<td>+0.7</td>
<td>0</td>
<td>0</td>
<td>+0.7</td>
<td>41</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>26 ox</td>
<td>23</td>
<td>−</td>
<td>−27.3</td>
<td>−12.2</td>
<td>0</td>
<td>−0.7</td>
<td>0</td>
<td>0</td>
<td>+0.7</td>
<td>32</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

\* Rates totally (not shown) or potentially affected by drifts of the O₂ electrode.

\* Rates likely underestimated because of a delay in water-gas equilibration of CO₂ and because some CO₂ escaped the incubation vial during purges.

\* Rates affected by large TA changes likely due to carbonate dissolution/precipitation.
Finally, methane started to increase in the gas phase as soon as anoxia was implemented (Fig. 5A), even in the presence of NO₃⁻, and at a fairly constant rate of 0.012–0.019 μmol L⁻¹ h⁻¹. The NO₃⁻ addition immediately stopped the CH₄ production at day 19. When purging with air to create the oxic conditions, CH₄ was removed from the gas phase and was no longer detected in the oxic conditions. DOC was observed to vary between 313 and 592 μmol L⁻¹, the highest values appeared just after the anoxic to oxic switches (days 9 and 23), as well as after the troubleshooting at day 14.

4. Discussion

Temporal changes of chemical species, and the rates of these changes during the experiment, gave significant new information on the biogeochemical processes occurring during oxic/anoxic oscillations in fluid mud. Here, we first discussed the nature of processes occurring in anoxic and oxic conditions during the experiment. Next, we carefully analysed the data to perform a mass balance calculation to evaluate the rates of the various reactions involved and the impact of redox oscillation on OM mineralisation.

### 4.1. Processes in anoxic conditions

The fact that iron(II) and sulphate did not significantly change during the incubation revealed that, in contrast to DMM, where iron is the major oxidant (Aller, 1998), suboxic conditions in the estuarine fluid mud were dominated by the reactions in which nitrate, ammonium and manganese are involved (Abril et al., 1999, 2000). Methanogenesis also occurred, but it represented a minor part of the organic carbon mineralisation, with a CH₄ production rate of less than 20 mmol L⁻¹ h⁻¹ (Fig. 5, Table 2), compared to rates around 1–20 and 0.1–5 μmol L⁻¹ h⁻¹ for carbon and nitrogen, respectively (Table 2). There were two contrasting periods during the anoxic conditions: the first days after the oxic to anoxic switch.

### Table 3

Stoichiometry of possible reactions occurring in the fluid mud, with their respective standard free energies (ΔG°; kJ mol⁻¹). Values on the standard free energy of formation, ΔG°, were taken from Wagman et al. (1982) and Stumm and Morgan (1996).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG° (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Aerobic respiration: CH₄ + O₂ → CO₂ + H₂O</td>
<td>-478.9</td>
</tr>
<tr>
<td>b) Aerobic nitrification: NH₄⁺ + 2O₂ → NO₃⁻ + H₂O + 2H⁺</td>
<td>-299.1</td>
</tr>
<tr>
<td>c) Ammonification: R-NH₂ + H⁺ → R-OH + NH₄⁺</td>
<td></td>
</tr>
<tr>
<td>d) Denitrification: 4NO₃⁻ + 5CH₃O⁻ + 4H⁺ → 2N₂ + 5CO₂ + 7H₂O</td>
<td>-2423.7</td>
</tr>
<tr>
<td>e) DRNA: 2CH₃O + NO₃⁻ + 2H⁺ → NH₂ + 2CO₂ + H₂O</td>
<td>-688.7</td>
</tr>
<tr>
<td>f) Manganese oxides reduction: CH₃O + 4MnO₂ → 8H⁺ → CO₂ + 7H₂O + 4 Mn²⁺</td>
<td>-583.2</td>
</tr>
<tr>
<td>g) Manganese oxides reduction by ammonium: 8MnO₂ + NH₄⁺ + 14H⁺ → 8 Mn²⁺ + NO₃⁻ + 13H₂O</td>
<td>-586.5</td>
</tr>
<tr>
<td>h) Aerobic Mn²⁺ oxidation: 4 Mn²⁺ + 6H₂O + O₂ → 4MnOOH + 8H⁺</td>
<td>143.7</td>
</tr>
<tr>
<td>i) Mn²⁺ oxidation by NO₃⁻ to N₂: 10 Mn²⁺ + 2NO₃⁻ + 14H₂O → 10MnOOH + N₂ + 18H⁺</td>
<td>380.3</td>
</tr>
<tr>
<td>j) Anammox: NO₂⁺ + NH₄⁺ → N₂ + 2H₂O</td>
<td>-357.7</td>
</tr>
<tr>
<td>k) Methanogenesis: 2CH₃O → CO₂ + CH₄</td>
<td>-139.9</td>
</tr>
<tr>
<td>l) Calcium carbonate dissolution and precipitation: CaCO₃ + CO₂ + H₂O → Ca²⁺ + 2HCO₃⁻</td>
<td></td>
</tr>
</tbody>
</table>
showed a very fast NO\textsubscript{3} decrease and NH\textsubscript{4} increase, until the nitrate concentration stabilised at \(\sim 30 \text{ µmol L}^{-1}\). Later, with steady anoxic conditions achieved, NO\textsubscript{3} concentrations were constant and low and NH\textsubscript{4} continued to increase, but at a much lower rate. The fast production of NH\textsubscript{4} at the beginning of the anoxic period (days 7, 15 and 19, just after the KNO\textsubscript{3} spike) could have been the result of ammonification (reaction c in Table 3) and dissimilatory reduction of nitrate to ammonium (DRNA, reaction e). During these initial periods of anoxia, the production of NH\textsubscript{4} was between 1.4 and 1.7 \text{ µmol L}^{-1} h^{-1}, and the DIC production was between 2.4 and 8.6 \text{ µmol L}^{-1} h^{-1} (Table 2). The resulting DIC/NO\textsubscript{3} production ratio was 1.7–5.1, which appeared to be too low to result from ammonification alone because the POM in the Gironde had a C/N ratio around 10 (Middelburg and Herman, 2007). In addition, a heterotrophic organism needed to uptake DIN (Middelburg and Herman, 2007). DRNA were stopped when NO\textsubscript{3} use dissolved NO\textsubscript{3} interval and being a function of NO\textsubscript{3} ratio around 10 (Middelburg and Herman, 2007). In addition, ammoni mineralisation alone could not account for the fast NH\textsubscript{4} production at the beginning of the anoxic periods. The significance of DRNA as a NH\textsubscript{4} source was also attested to by the value of the NH\textsubscript{4} production rate, dNH\textsubscript{4}/dt, calculated for each measurement interval and being a function of NO\textsubscript{3} concentration during the beginning of the anoxic periods (Fig. 6B). This suggested that during these periods, NH\textsubscript{4} originates in part from the reduction of NO\textsubscript{3} by DNA. Several previous works have pointed out that DNA can consume between 7 and 98\% of nitrate in coastal sediments with a high OM content (Gilbert et al., 1998; Kelly-Gerreyn et al., 2001).

The increase in Mn\textsuperscript{2+} was linear and independent of NH\textsubscript{4} concentration during each anoxic period, which suggested dissimilatory Mn-reduction (reaction f) occurred dominantly, and Mn-reduction by ammonium was minor (reaction g), as previously interpreted from \textit{in situ} profiles (Abril et al., 1999).

During steady anoxic periods, nitrate concentration remained around 30 \text{ µmol L}^{-1}, which revealed that both denitrification and DRNA were stopped when NO\textsubscript{3} concentration reached this threshold concentration (Fig. 4A and B). This incomplete nitrate consumption was contradictory to measurements made in the Gironde fluid mud, where NO\textsubscript{3} concentration was zero when the SPM concentration reached 250 g L\textsuperscript{-1} (Abril et al., 2000). The SPM concentration of the fluid mud during our experiment was 140 g L\textsuperscript{-1}. Perhaps, in contrast to \textit{in situ} conditions in the fluid mud of the highly turbulent conditions of the incubation, bacteria fixed on particles and performing denitrification and DRNA, could not use dissolved NO\textsubscript{3} at a concentration below 30 \text{ µmol L}^{-1}. An alternative explanation was that nitrate consumption was balanced by nitrate production during these periods. Anoxic NO\textsubscript{3} production has been observed in several marine sediment cores (Anschutz et al., 2000, 2002; Deflandre et al., 2000, 2002; Mortimer et al., 2004) and \textit{in vitro} incubations of sediment (Hulth et al., 1999; Anschutz et al., 2005; Chailou et al., 2007). NO\textsubscript{3} could have been produced from anoxic oxidation of NH\textsubscript{4} using Mn (III, IV) oxides as the electron acceptor (reaction g). There was no evidence, however, of such processes occurring in the fluid mud, as the Mn\textsuperscript{2+} production rates remained very linear throughout the entire anoxic period (Fig. 4E). Anaerobic oxidation of NH\textsubscript{4} in the presence of NO\textsubscript{2} (anammox, reaction j) has also been recognised as an alternative pathway for benthic N\textsubscript{2} production in several anoxic aquatic environments (Dalsgaard and Thamdrup, 2002; Trimmer et al., 2003; Rysgaard et al., 2004). During our experiment, a NO\textsubscript{2} peak of 60 \text{ µmol L}^{-1} appeared just after the NO\textsubscript{3} spike during the anoxic period (Fig. 4, days 19). Then, the NO\textsubscript{2} concentration dropped back to zero while the NH\textsubscript{4} concentration kept increasing steadily. Accordingly, this suggested that the anammox process was minor.

The evolution of DIN species after the KNO\textsubscript{3} injection, and under prolonged anoxic conditions, followed a pattern similar to what we observed at the beginning of the anoxic period (Figs. 4 and 6). Both consumption of NO\textsubscript{3} and production of NH\textsubscript{4} showed the same dependency on NO\textsubscript{3} concentration as at the beginning of the anoxic periods (Figs. 6A and C). However, Mn\textsuperscript{2+}, DIC and TA had very different behaviours, as their concentrations decreased after the injection and increased at the beginning of each “normal” anoxic period (Figs. 3 and 4). After the KNO\textsubscript{3} injection, the concomitant high concentrations of NO\textsubscript{3} and Mn\textsuperscript{2+} possibly allowed the oxidation of Mn\textsuperscript{2+} with NO\textsubscript{3} to either N\textsubscript{2} (reaction i) or NH\textsubscript{4} (reaction h) (Luther et al., 1997; Hulth et al., 1999). These two reactions produced protons, and therefore, potentially decreased the TA, as was observed in the experiment. However, this alone could not explain the TA decrease because DNA and denitrification occurred simultaneously and involved many more protons (+2 \text{ µmol L}^{-1} h^{-1} NH\textsubscript{4} produced and \(-10 \text{ µmol L}^{-1} h^{-1}\) NO\textsubscript{3} consumed, compared to \(-0.18 \text{ µmol L}^{-1} h^{-1}\) Mn\textsuperscript{2+} consumed). These observations should have resulted in a net TA increase, according to the stoichiometry in Table 3, as was the case at the beginning of each anoxic period (Fig. 3C). An alternative and more plausible explanation was that the removal of Mn\textsuperscript{2+} was the result...
of precipitation associated with carbonates. Indeed, after the KNO₃ injection, concomitant TA and pCO₂ decreases occurred (Fig. 3). Such DIC consumption could only be attributed to carbonate precipitation (reaction k), which very likely occurred at that time as the pH reached 8.32. Dissolved manganese was most likely precipitated as a mixed calcium-manganese carbonate (Middelburg et al., 1987; Mucci, 1988).

The occurrence of methanogenesis in the presence of electron acceptors has been reported in several coastal sediments and was due to the presence of non-competitive organic substrates that are preferentially used by methanogens (Oremland and Polcin, 1982). This might be a reason why, before the KNO₃ injection, methanogenesis happened in the presence of NO₃ (Figs. 4 and 5). Organic substrate concentrations were probably sufficient in the system for both denitrification and methanogenesis to occur together. Methanogenesis, however, was suddenly stopped just after the injection of nitrate. There might be three reasons for this observation. First, denitrifying bacteria that could compete with methanogenic bacteria could have suddenly consumed the available substrates after injection, which would have stopped methanogenesis (Clarens et al., 1998). Second, N₂O concentrations were very high after the injection and this compound was known to be toxic for methanogens (Roy and Conrad, 1999). Finally, methane could have been suddenly oxidised with the added nitrate by anaerobic methanotrophic bacteria, as pointed out recently for anoxic fresh water environments (Raghoebarsing et al., 2006).

4.2. Processes in oxic conditions

Under oxic conditions, no loss of nitrogen as N₂ could occur and the DIN increase represented the net ammonification rate. During steady oxic conditions, NH₄ concentrations remained close to zero (Fig. 4), and nitrification was controlled by the supplied NH₄ from ammonification. In contrast, during reoxidation periods, the high initial concentration of NH₄ made the NO₃ gain (4.4–5 μmol L⁻¹ h⁻¹) higher than the NH₄ loss (−1.4 to −1.6 μmol L⁻¹ h⁻¹) (Fig. 4 and Table 2), with both rates being correlated to NH₄ concentrations (Fig. 6B and E). During reoxidation, one part of the nitrification occurred using the NH₄ initial pool and another part using NH₄ supplied simultaneously by ammonification. The fact that the DIN increase was much larger during the reoxidation periods (3–3.3 μmol L⁻¹ h⁻¹) than during the steady oxic periods (0.4–0.7 μmol L⁻¹ h⁻¹) (Fig. 4D and Table 2), gave evidence that OM mineralisation was much more intense during the reoxidation periods.

In addition to the nitrogen data, other evidence for an enhancement of OM mineralisation during reoxidation periods came from the carbon and oxygen data, although these were less quantitative. Indeed, carbon mineralisation was difficult to evaluate clearly because of some experimental limitations and the fact that chemical species were affected by a number of different processes. TA showed a decreasing trend during most reoxidation periods and also during the last days of the experiment, but an increasing trend during the two periods of steady oxic conditions (days 2–5 and 11–12 in Fig. 3C, Table 2). TA decreases during reoxidation could be attributed to the release of protons by nitrification and Mn²⁺ precipitation (reactions b and h). During steady oxic periods, nitrification coupled to ammonification could have slightly decreased the TA at a rate equal to the DIN increase (Abril and Frankignoulle, 2001), i.e., at −0.7 and −0.4 μmol L⁻¹ h⁻¹ at days 2–5 and 11–12, respectively (Table 2). Instead, TA increased at rates of 3.5 and 3.5 μmol L⁻¹ h⁻¹, respectively. Carbonate dissolution (reaction k) coupled to respiration was the most probable reason for this net TA production during steady oxic phases, a process already evoked for explaining in situ TA distribution in Gironde and Loire estuaries (Abril et al., 1999, 2003, 2004). When carbonate dissolution was coupled to aerobic respiration, one half of the TA produced originated from OM mineralisation and the other part from calcium carbonate. In our experimental setup, the CO₂ produced by aerobic respiration could have been involved in the dissolution reaction before the equilibration with the headspace was complete (Fig. 2). This TA production increased the buffer capacity of the carbonate system and might have slowed down CO₂ degassing to the headspace. During carbonate dissolution, the DIC production was not only related to OM mineralisation, but it might also have been underestimated due to gas equilibration.

The extremely high pCO₂ raises and the strong drift of the polarographic electrode immediately after anoxic to oxic switches both suggested an enhanced OM mineralisation during reoxidation. The oxygen consumption that could be calculated increased very significantly after anoxic periods (Table 2). For an unknown reason, this enhancement of the oxygen consumption following anoxic periods lasted longer than the enhancement of the DIN production (Figs. 3A and 4A, Table 2). DIC production rates, when they could be calculated, also showed this trend, with a maximum value of 23 μmol L⁻¹ h⁻¹ at days 22–23 (Table 3). In several cases, however, TA decreased after the oxic to anoxic switches (Fig. 3C) due to the reoxidation of various reduced compounds that produced protons. This observation was probably also partly due to the precipitation of calcium carbonate when pH rose during the purge with air. Besides these methodological limitations, carbon mineralisation rates in oxic conditions derived from DIC production or oxygen consumption were consistently higher after prolonged periods of anoxia (Table 2).

Finally, the DOC signal throughout the experiment (Fig. 5B) also supported an enhancement of OM mineralisation during reoxidation. If the first data point was excluded (which in fact also corresponded to a reoxidation, but from in situ conditions), as well as the data just after the troubleshooting, DOC concentrations were 509 ± 82 μmol L⁻¹ during the reoxidation periods (days 9 and 23) and 365 ± 34 μmol L⁻¹ the remainder of the experimental time. As DOC was generally an intermediate product during particulate OM mineralisation to CO₂ (e.g., Kristensen et al., 1995), the temporary DOC accumulation observed here suggested that the conversion of POC to DOC became faster than the conversion of DOC to CO₂. Additionally, the excess of 100–250 μmol L⁻¹ of DOC found during these periods was extremely labile, as it rapidly disappeared a few hours later. It was also probable that the background DOC of 365 μmol L⁻¹ that remained relatively constant throughout the whole experiment corresponded to a relatively refractory pool. Except during reoxidation periods, the supply of labile DOC from POC through exo-enzymatic activity or fermentative processes (Kristensen et al., 1995; Sun et al., 2002) probably strongly controlled the final rate of OM mineralisation. In contrast, the supply of labile DOC was enhanced during reoxidation, but it took some hours for the heterotrophic bacterial metabolism to respond and convert the available labile DOC to CO₂ and biomass.

4.3. Mass balance calculations

As a final objective to evaluate OM mineralisation rates, a simple model analysis of our experimental data was performed. The most robust rates were those derived from no gaseous species, i.e., NO₃, NH₄, DIN and Mn²⁺. Dominant reactions affecting OM and these species were aerobic respiration, nitrification, ammonification, denitrification, DRNA and Mn reduction. We could write for oxic conditions:

\[
\frac{d[NO₃]}{dt} = R_{NT} \quad \text{and} \quad \frac{d[DIN]}{dt} = R_{AM}
\]
where \( R_{\text{DENT}} \) and \( R_{\text{AM}} \) are the nitrification and ammonification rates, respectively.

For anoxic conditions:

\[
\frac{d [NO_3]}{dt} = -R_{\text{DNRA}} - R_{\text{DENT}}, \quad \frac{d [NH_4]}{dt} = R_{\text{AM}} - R_{\text{DNRA}}
\]

and

\[
\frac{1}{4} \frac{d [Mn^{2+}]}{dt} + \frac{5}{4} R_{\text{DENT}} + 2 R_{\text{DNRA}} = \frac{C}{N} R_{\text{AM}}
\]

where \( R_{\text{DENT}}, R_{\text{DNRA}} \) and \( R_{\text{AM}} \) are the denitrification, DNRA and ammonification rates, respectively, and \( C/N \) is an assumed C/N molar ratio for OM mineralisation.

In both the oxic and anoxic conditions, the rate of carbon mineralisation, \( R_{\text{C-MIN}} \), was related to the rate of ammonification according to the equation \( R_{\text{C-MIN}} = C/N R_{\text{AM}} \).

All rates could be calculated in this study if a C/N ratio was assumed. The C/N ratio of POM in the Gironde ETM was about 10, but the one for OM mineralisation could be higher, due to nitrogen uptake by heterotrophic bacteria (Middelburg and Nieuwenhuize, 2000). During the experiments, the ratio of \( O_2 \) consumption / DIN increased in the range between 15 and 41, excluding a rate potentially affected by electrode drift at days 11 and 12. DIC/DIN production ratios not affected by gas equilibration delays or large alkalinity changes were between 8 and 34. We have thus tested the dependency of calculated rates for C/N values between 10 and 40. This was particularly important at the beginning of anoxic periods, when denitrification, DNRA and ammonification occurred simultaneously. Fig. 7 describes the sensitivity of these three modelled rates to the C/N ratio during the periods of initial anoxia conditions. Modelled \( R_{\text{AM}} \) and \( R_{\text{DNRA}} \) were quite sensitive to the C/N ratio, decreasing respectively by 74% and increasing by 80%. Modelled \( R_{\text{DENT}} \) and \( R_{\text{C-MIN}} \), on the other hand, were much less sensitive, decreasing by 11% and increasing by 6%, respectively (Table 4). During the three other characteristic periods, \( R_{\text{AM}} \) was accurately obtained from the DIN increase and was independent of the C/N ratio. In contrast, modelled \( R_{\text{C-MIN}} \) was very sensitive (proportional) to the C/N ratio. Despite the uncertainty in the rates presented in Table 4, significantly different rates could be observed for different periods. For example, \( R_{\text{AM}} \) and \( R_{\text{C-MIN}} \) were significantly higher during reoxidation than during oxic steady state, than during steady anoxic conditions. However, beginning anoxic and steady oxic periods did not show significantly different modelled \( R_{\text{AM}} \) and \( R_{\text{C-MIN}} \).

4.4. Impact of redox oscillations on OM mineralisation rates

Several works based on in vitro experiments have investigated the impact of redox oscillations induced either by bioturbation or resuspension on sedimentary OM degradation. There is now clear evidence that for relatively refractory land-derived OM, aerobic respiration is almost always faster than anaerobic respiration (Aller, 1994, 1998; Kristensen et al., 1995; Andersen, 1996; Hulthe et al., 1998). This was also the case in our experiment as confirmed by both C and N mass balances (Table 3). Additionally, an accumulation of DOC occurred under anoxic conditions and this DOC was rapidly degraded under oxic conditions (Kristensen et al., 1995). There was also evidence that redox oscillations in coastal sediments could enhance OM degradation relative to steady oxic conditions and that OM degradation could go further under oscillating conditions. Aller (1994) incubated pieces of sediments under conditions of oscillating redox and found higher nitrogen storage in the form of NH4 extractable with KCl, than under steady oxic or steady anoxic conditions. The total number of bacteria was also higher at the end of the incubation under oscillating conditions, which could have been attributed to the fact that redox oscillations induced the death of the bacteria and consequently enhanced their production and the recycling of their biomass. Hulthe et al. (1998) found that buried sediments that had spent a long time under anoxic condition had much faster DIC production rates under oxic conditions than superficial, young oxic sediment. Aller (1998) discussed that the repetitive bacterial biomass recycling due to changes in redox conditions increased the amount of labile OM in the system and promoted the degradation of refractory organic compounds by a "co-oxidation" process. This mechanism, coupled to a permanent availability of poorly crystallised iron oxides available to oxidise OM, explained the efficiency of DMMs in mineralising refractory terrestrial OM at the land-sea interface (Aller, 1998). During our experiment, the short and transient DOC peaks observed during reoxidation periods (Fig. 5C) suggested that such processes also occurred in ETMs and that, owing to the redox oscillation, a large amount of highly labile DOC was always available for heterotrophs. Availability of NO3 as an oxidant was also maximised by the redox oscillations and the tight coupling between ammonification, nitrification, denitrification and DNRA (Fig. 3 and Table 4). The result of the NO3 spike confirmed this central role of nitrate-reducing organisms in OM degradation (Fig. 6).

The ability of redox changes to enhance OM mineralisation rates relative to steady oxic or anoxic conditions strongly depended on the frequency and duration of the oscillations. During our experiment, the duration of the reoxidation period seemed to depend on the former anoxic period, with the ratio between these two durations (reoxidation/preceding anoxia) being relatively constant: 0.55 for the first and shorter period (days 9–10), and 0.30 for the second and longer period (days 22–24). We have modelled the integrated \( R_{\text{AM}} \) resulting from different frequencies and durations of redox oscillations, using the rates in Table 4 and assuming reoxidation periods lasted 0.4 times the duration of the previous anoxic period. Table 5 summarises the results of modelled \( R_{\text{AM}} \), which were

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>( R_{\text{DENT}} ) (µmol L⁻¹ h⁻¹)</th>
<th>( R_{\text{DNRA}} ) (µmol L⁻¹ h⁻¹)</th>
<th>( R_{\text{AM}} ) (µmol L⁻¹ h⁻¹)</th>
<th>( R_{\text{C-MIN}} ) (µmol L⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>7.7</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>6.7</td>
</tr>
<tr>
<td>30</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>5.7</td>
</tr>
<tr>
<td>40</td>
<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Table 4 Results of calculated rates during the four characteristic periods. When a range was given, it resulted from the assumption of two C/N ratios of 10 and 40 for OM mineralisation.
compared to those under constant oxic and anoxic conditions. As already shown previously (Aller, 1994; Canfield, 1994; Kristensen et al., 1995), R\textsubscript{AM} rates were much slower under steady anoxic conditions (Case 2 in Table 4) than under oxic conditions (Case 1) or oscillating conditions (Cases 3–6). Our simple model also revealed that oscillating redox conditions induced a faster OM mineralisation than steady oxic conditions, except when the anoxic periods were too long (Case 5). The estuarine fluid mud in the Gironde estuary underwent redox oscillations at both tidal (1/2 day) and spring-neap (13 days) cycles (Abril et al., 1999). The fastest OM mineralisation occurred when the frequency of oxic/anoxic oscillations was short. For an equivalent duration of the oxic and anoxic periods, R\textsubscript{AM} was enhanced by 35–79% relative to steady oxic conditions when redox conditions changed every day (Case 3), whereas the enhancement was only 31–38% when the switches occurred only every 6 days (Case 6). This fact was due to the much lower rates after the first day of anoxia. If anoxic periods became much longer than the oxic period (Case 5), reoxidation conditions prevailed during the whole of the oxic period, but were not necessarily able to compensate for the slowing down that occurred under steady anoxic conditions. On the other hand, a short period of anoxia (less than one day) accelerated the N-mineralisation, regardless of the duration of the oxic period (Case 4). Indeed, because the R\textsubscript{AM} of the first day of anoxia was similar to that under oxic conditions, the effect of reoxidation became preponderant and increased the final total R\textsubscript{AM}.

5. Conclusion

Besides limitations due to gas exchange rates and complex inorganic carbon behaviour, the experimental setup presented here was an effective tool to study redox oscillations. We obtained significant new information about the major biochemical processes occurring in the fluid mud of the Gironde estuary. The incubation over 27 days, which included 3 oxic and 2 anoxic periods, confirmed the processes that explain in situ profiles in fluid mud: aerobic respiration, nitrification, ammonification, denitrification, dissimilatory reduction of nitrate to ammonium and manganese oxide reduction by OM. Iron and sulphate reduction, as well as methanogenesis, were minor. The most significant result was the observed enhancement of OM mineralisation during reoxidation periods, unequivocally evidenced by the DIN production rates. This period could not be studied in situ because it took place during the resuspension of the fluid mud, when particles and pore waters were diluted with the upper oxic water. Applying a mass balance model, we calculated that redox oscillations accelerated organic nitrogen mineralisation from 10% to 79% according to oscillation frequency.

Acknowledgments

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References


Table 5

<table>
<thead>
<tr>
<th>Time oxic (days)</th>
<th>Time anox (days)</th>
<th>Time oxic steady (days)</th>
<th>Time anox begin (days)</th>
<th>Time anox steady (days)</th>
<th>Time reox\textsuperscript{a} (days)</th>
<th>AM \textsuperscript{(μmol L\textsuperscript{-1} h\textsuperscript{-1})}</th>
<th>Acceleration relative to oxic (%)</th>
<th>Acceleration relative to anoxic (%)</th>
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\textsuperscript{a} The duration of the reoxidation period was assumed to be 0.4 times the duration of the previous anoxic period.


