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Anaerobic nitrification-denitrification mediated by Mn-oxides in meso-tidal sediments: Implications for N-2 and N2O production

Fernandes Sheryl Oliveira [1, 2], Javanaud Cedric [1], Aigle Axel [1], Michotey Valerie D. [1], Guasco Sophie [1], Deborde Jonathan [3], Deflandre Bruno [3], Anschutz Pierre [3], Bonin Patricia C. [1, *]

[1] Univ Toulon & Var, Aix Marseille Univ, CNRS, INSU,IRD,MIO,UM 110, F-13288 Marseille, France.
[2] Natl Inst Oceanog, CSIR, Div Biol Oceanog, Panaji 403004, Goa, India.
[3] Univ Bordeaux 1, CNRS, Lab Environm & Paleoenvironm Ocean, UMR 5805, F-33405 Talence, France.

* Corresponding author : Patricia C. Bonin, email address : patricia.bonin@mio.osupytheas.fr

Abstract :

Field measurements in the Arcachon Bay (southwest France) indicated anaerobic production of NOx via nitrification, which was coupled to the reduction of Mn-oxides. To prove the occurrence of this process, laboratory microcosm experiments were set up. A N-15 tracer-based approach was used to track if NOx produced through Mn-oxide-mediated anaerobic nitrification would be reduced to N-2 via denitrification or anammox. We also hypothesized the generation of the potent greenhouse gas nitrous oxide (N2O) during nitrification-denitrification in the presence of Mn-oxides. The microcosms were prepared using sediment sectioned at varying depths (0-2.5, 2.5-4.5, 4.5-8.5, 8.5-12 and 12-17 cm) during two sampling campaigns in October (fall) and January (winter). Labeling with (NO3-)-N-15 revealed low N-2 production originating from NO3- in the water column (P-w), which did not increase significantly on amendment with Mn-oxides during both sampling periods. However, for both seasons, a significant increase of N-2 produced via nitrification (P-n) was observed upon addition of Mn-oxides reaching 76-fold enhancement at <= 2.5 cm. To support these results, sediment slurries of October were subjected to amendment of (NH4+)-N-15, (NO3-)-N-14 with or without addition of Mn-oxides. A substantial production of P15 (N-2 production from (NH4+)-N-15) within 0-17 cm provided further evidence on nitrification-denitrification mediated by Mn-oxides probably with minimal intervention of anammox. In organically rich sediments, anaerobic nitrification-denitrification mediated by Mn-oxides could play an important role in lowering re-mineralized NH4+ levels in the benthic system. As hypothesized, significant production of N2O through the pathway was observed revealing newer mechanisms leading to the generation of the radiative gas.

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Highlights

► Anaerobic nitrification–denitrification occurred in Arcachon bay sediments. ► N_2 production through this pathway was up to ~ 76× higher in presence of Mn-oxides. ► Significant N_2O production was also observed through this process. ► The process could be important in lowering excess NH_4^+ in the aquatic system. ► Moreover, it helps to generate substrate for denitrifiers in NO_x-limited systems.

Keywords : Anaerobic, Anammox, Denitrification, Manganese oxides, Nitrification, Nitrous oxide, Sediments

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1. Introduction

In estuarine systems, nitrogen (N) budget calculations show that only 10 to 25% of anthropogenically-derived N is exported to the sea (Schaefer and Alber, 2007) suggesting its local elimination. Two anaerobic processes are known to be responsible for nitrogen removal: (i) denitrification, which is the reduction of NO_3^- or NO_2^- to N_2 and (ii) anammox (anaerobic ammonium oxidation) that couples oxidation of NH_4^+ to NO_2^- reduction with N_2 as the end product (Thamdrup and Dalsgaard 2002, Dalsgaard et al., 2003, Kuypers et al., 2003). Isotopic measurement (using ^{15}N enrichment as tracer) of di-nitrogen species can discriminate between these two processes (Minjeaud et al., 2008; Risgaard-Petersen et al., 2003). The production of N_2 via anammox has a 1/1 stoichiometry as follows:

$$^{14 \text{ or } 15}NH_4^+ + {}^{15 \text{ or } 14}NO_2^- \rightarrow {}^{29}N_2 + 2H_2O$$

whereas, the production of N_2 by denitrification follows a binomial distribution reflecting the frequency of ^{15}N and ^{14}N in the NO_2^- pool:

$$2^{(14 \text{ or } 15)}NO_3^- + 4\ H^+ \rightarrow {}^{(28,29,30)}N_2 + 2H_2O$$

In marine ecosystems (anoxic water column, particles, sediments), the removal of nitrogen is mainly dominated by denitrification (Michotey and Bonin 1997; Kuypers et al., 2005; Minjeaud et al., 2009, Ward et al., 2009; Fernandes et al., 2012). The percentage of N_2 production through anammox in sediments is variable (Dalsgaard et al., 2005). In coastal sediments, low anammox contribution has generally been recorded (Thamdrup and Dalsgaard 2002; Fernandes et al., 2012) while higher percentage of N_2 production has been observed offshore e.g. continental slope sediments (Thamdrup and Dalsgaard 2002). In coastal sediments, high denitrification rates of up to 0.22 μmol N_2 g^{-1} h^{-1} have been measured albeit pore water concentration of its substrates (NO_3^- and NO_2^-) being in micro molar range (Fernandes et al., 2012). Isotopic N_2 ratio measurements (Risgaard-Petersen et al., 2003) have solved this apparent contradiction. They showed that in sediments, denitrification from exogenous NO_3^- corresponds to less than 10% of the total rate (Hietanen and Kuparinen 2008; Minjeaud et al., 2009) while the main part of NO_3^- is formed *in situ* via nitrification. This suggests a close link between aerobic nitrification and denitrification in oxic-anoxic gradients. Among NH_4^+ oxidation processes, the role of oxidized metal compounds in alternative anaerobic processes has often been suggested to explain the presence of unexpected peaks of NO_3^- in deep layers of sediment. After excluding the hypothesis of an enhanced supply of NO_3^- due to bioturbation, a local production of the nutrient due to reduction of metal-oxides has been suggested (Anschutz et al., 2000). Among them, manganese oxides (Mn-oxides) are commonly described as powerful oxidants capable of modifying or creating new pathways of oxidation in the N cycle (Luther et al., 1997; Hulth et al., 1999; Anschutz et al., 2000; Thamdrup & Dalsgaard, 2000; Deflandre et al., 2002; Mortimer et al., 2002, 2004; Hulth et al., 2005; Bartlett et al., 2007, 2008). Luther et al. (1997) have shown a strong link between Mn and N cycles and described several reactions causing short-cuts in the N cycle. Hulth et al. (1999) have described a thermodynamically favorable reaction of nitrification in anaerobiosis using Mn-oxides as the oxidant of NH_4^+. Anaerobic nitrification could use manganese as the electron acceptor to form NO_3^- (Mortimer et al., 2002; 2004; Luther and Popp 2002; Clément et al., 2005; Bartlett et al., 2008), which is then denitrified by

biologically-mediated denitrification or chemo-denitrification (Sørensen 1987; Aller 1990; Schulz et al., 1994; Murray et al., 1995; Luther et al., 1997) to form N_2 (Javanaud et al., 2011).

$$8MnO_2 + 2NH_4^+ + 12H^+ \longrightarrow 8Mn^{2+} + 2NO_3^- + 10H_2O$$

$$5Mn^{2+} + 2NO_3^- + 4H_2O \longrightarrow 5MnO_2 + N_2 + 8H^+$$

However, these results are mainly based on concentration of solutes and there is still few concrete evidence for the occurrence of anaerobic nitrification mediated by Mn-oxides.

Mn-oxides are widely distributed in anoxic marine sediments and are very reactive leading to several secondary reactions of diagenesis (Deflandre et al., 2002). However, due to complex recycling of redox species, these processes are difficult to discern and therefore poorly understood. Javanaud et al. (2011) used a denitrifying strain from Arcachon Bay sediments to demonstrate N_2 production from NH_4^+ through anaerobic nitrification-denitrification in the presence of Mn-oxides. Under nitrate-limited conditions, the authors suggested N_2O accumulation through this pathway. In the present study, we used an isotopic approach (with $^{15}NO_3^-$ or $^{15}NH_4^+$) to investigate the following: (1) down-core variation in N_2 production through anaerobic nitrification-denitrification by introducing Mn-oxides in sediment slurries from the Arcachon Bay (2) to verify the extent of N_2 production from NH_4^+ in the presence of Mn-oxides and (3) to test the hypothesis that Mn reduction coupled to anaerobic nitrification-denitrification could be important for N_2O production in coastal sediments. Our results showed significant increase of N_2 production in slurry microcosms after addition of Mn-oxides which was mainly through anaerobic nitrification-denitrification rather than anammox. Elevated N_2O production through the former pathway was also observed strengthening our hypothesis and re-affirming earlier observations made by Javanaud et al. (2011).

2. Methods

2.1. Study area

Arcachon Bay is a shallow meso-tidal lagoon (Deborde et al., 2008) on the south-west (S.W.) coast of France (Atlantic Ocean; 44°40′ N, 1°10′ W). The total lagoon surface can be divided in two parts: the channels (57 km^2), and the intertidal area (117 km^2) (Plus et al., 2010) made up of large tidal flats (Deborde et al., 2008). These tidal flats are mainly composed of muddy sand (grain size = 15 – 40 µm). Only 40 km^2 remains submerged at low tide (De Wit et al., 2005). The sampling station was located on a sloping mud flat (Fig. 1) in the southeast part of the Arcachon Bay. This area remained exposed to the atmosphere for ~ 6 h d^{-1} and was composed of fine sediments of grain size ≈ 27 µm. This sampling site was chosen by all partners in the frame work of the PROTIDAL project. One of the aims of this project was to study the influence of successive emersion and immersion periods on benthic biogeochemical processes. The simultaneous determination of major redox biogenic and trace elements along with flux measurement at different interface (water-sediment, sediment-atmosphere, water-atmosphere, freshwater-seawater), macrofauna, seagrass and microbiological parameters in the transient conditions of the tidal environment were carried out.

2.2. Sampling and sample processing

Push cores of 8 cm diameter and 20 cm length were collected in October, 2007 and January, 2008 during low tide. Surface sediment temperature in October was around 10.0°C while in January it was 2.8°C. Eight sediment cores from an area of < 5 m^2 were collected, capped at both ends and transferred to the laboratory in an insulated box. For activity measurements, five replicate cores (core # 1 to 5) were sliced at 5 depth intervals i.e. 0-2.5, 2.5-4.5, 4.5-8.5, 8.5-12 and 12-17 cm within 3 h of retrieval. These depths were decided based on a preliminary profile of pore water Mn_2^+ (data not shown) in the sediment column. Immediately after slicing, each slice from same depth of the different cores were pooled and conserved in sealed bag flushed under N_2-atmosphere. The remaining three cores were processed for nutrient and metal analyses as follows:

Core # 6 – Analysis of pore water NO_3^- and NO_2^- (NO_x)

Core # 7 – Analysis of pore water Mn_2^+

Core # 8 – Analysis of sediment extractable NH_4^+ and Mn-oxides (particulate fraction).

Core # 6 and 7 were processed within an hour of transportation to the onshore laboratory. Core # 8 was preserved at -20ºC and processed for analysis of sediment extractable NH_4^+ and Mn-oxides within 48 h of collection.

2.3. Nutrient,dissolved and particulate Mn analyses

To gain a better resolution on the variation in nutrients with depth, sediment core was thawed and sectioned at 0.5, 1, or 2 cm intervals up 17 cm depth. Five millilitres of NO_x-free artificial seawater (Baumann and Baumann, 1981) was added to 5 mL of sediment. The contents were homogenised to form slurry and centrifuged for 10 min at 5000 rpm (× 1803 *g*). The supernatant volumes were measured upon filtering through a 0.2-µm filter. NO_3^- plus NO_2^-analyses corresponding to NO_x in the extracted pore water were analyzed with a continuous flow analyzer (AA3 autoanalyzer; SEAL Analytical; UK) according to Tréguer and Le Corre (1975). Ammonium concentrations were determined after KCl extraction (Laima 1993) using the fluorometric method (Kérouel and Aminot 1997). The dilution due to addition of synthetic seawater has been accounted for in the calculation of nutrients concentrations. Dissolved Mn was measured by flame atomic adsorption spectrometry (Model AA 300; Perkin Elmer; Waltham, MA) with ± 5% precision (Deborde et al., 2008). Sub-samples for analysis of Mn-oxides were dried in a hot air oven at 60 ± 2 °C for 48 h and disaggregated in an agate mortar before chemical treatment. An ascorbate reagent was used to extract the "amorphous readily microbially reducible phase" i.e. particulate Mn (Anschutz et al., 2005) from the sediment samples.

2.4. Synthesis of Mn-oxides

Since the crystallinity of MnO_2 provided by manufacturers does not correspond to MnO_2 freshly formed from oxidation of dissolved Mn (II) or particulate Mn (III) oxyhydroxides (Tebo et al., 2004), preparation of biologically active MnO_2 was performed in the laboratory using 0.1 M $KMnO_4$, 0.1 M NaOH and 0.1 M $MnCl_2$ (with ratio volume of 2/4/3, respectively) (Laha and Luthy

1990). Mn-oxides were collected by centrifugation and washed several times with distilled water. This method of synthesis produces Mn-oxides structurally similar to naturally occurring mineral birnessite ($(Na_{0.3}Ca_{0.1}K_{0.1})$ $(Mn^{4+}, Mn^{3+})_2$ $O_4 \cdot 1.5$ H_2O). Moreover, since the reactivity of Mn-oxide suspensions may change with long storage, they were used within 24 h after preparation to maintain bioavailability.

2.5. Estimation of N_2 production rates

Potential rates of anammox and denitrification were estimated by the method of Risgaard-Petersen et al. (2003), modified according to the work of Minjeaud et al. (2008). Briefly, 5 mL sediment samples from each section of the core were transferred to 22 mL headspace vials. Five mL of filter-sterilized seawater collected from the sampling site was added to the vials. The vials were capped with butyl rubber stoppers and crimped with aluminium seals. The headspace gas in the vials was replaced with helium gas by repeated vacuuming and purging. Vials containing oxygen free sediment suspension were pre-incubated for 3-5 h in order to eliminate any remaining traces of oxygen in the headspace. To re-affirm anaerobic conditions in the test vials, the oxygen level in gaseous phase was monitored by mass spectrometry until below the detectable limit of ~ 15 ppm. Positive pressure (0.15 MPa) was added to the headspace to prevent unintentional contamination with ambient air during the incubation and the gas sampling.

Two parallel incubations were also performed: (i) incubation with 100 µmol L^{-1} $^{15}NO_3^-$ (^{15}N atom%, 98%) and (ii) incubation with 100 µmol L^{-1} $^{15}NH_4^+$ plus 100 µmol L^{-1} $^{14}NO_3^-$. Each condition was duplicated with or without amendment of fresh Mn-oxides (final concentration adjusted to 100 µmol L^{-1}). This resulted in sets of twelve vials, which were prepared in five replicates for each station. Argon at a final headspace concentration of 1% was added to all test vials as an internal standard for mass spectrometry. Microbial activities in each set were terminated after 0, 2, 4, 6 or 8 h at *in situ* temperature by injecting 0.25 mL 7 M $ZnCl_2$.

The headspace gas samples were analysed by mass spectrometry (Quadrupole mass spectrometer *Anagaz* 100, MKS, Cheshire, UK) (Minjeaud et al., 2008) to quantify each N_2 isotope ($^{28}N_2$, $^{29}N_2$ and $^{30}N_2$). Potential N_2 production, anammox and denitrification rates were calculated from the production rates of $^{14}N^{15}N$ ($^{29}N_2$) and $^{15}N^{15}N$ ($^{30}N_2$) in the test vials according to the method of Risgaard-Petersen et al. (2003) and Minjeaud et al. (2008). In the first set of incubations, N_2 production based on NO_3^- originating from the overlying water (P_w) or produced in the sediment via nitrification (P_n) was quantified using the comprehensive method as outlined by Risgaard-Petersen et al. (2003). In the slurry systems, ^{14}N-NO_3^- would be produced only from ^{14}N-NH_4^+ via nitrification and P_n reflected the subsequent ^{14}N-NO_3^-reduction to N_2. Under strict anaerobic condition no P_n could occur except if ^{14}N-NO_3^- was produced by an alternative process. In the second set of incubations, we quantified N_2 production based on $^{15}NH_4^+$ (P15) to confirm the stimulation of anaerobic oxidation of NH_4^+ to N_2 in presence of Mn-oxides. Production of P15 (N_2 production from $^{15}NH_4^+$ labelling) permits to follow NH_4^+ oxidation to N_2 but does not discriminate anammox from P_n ($^{15}NH_4^+$ oxidation to NO_x and its subsequent reduction to N_2).

2.6. Nitrous oxide measurement

Nitrous oxide isotopes (molecular masses 45 and 46) produced in the incubation vials were quantified by mass spectrometry (Quadrupole mass spectrometer *Anagaz* 100, MKS, England). Total N_2O produced was obtained by correction from isotopic fractionation (Lloyd & Scott, 1983) and the concentration has been expressed as nmol L^{-1} h^{-1}.

2.7. Statistical analysis

Student's *t*-test was performed on the dataset to evaluate the differences between activities of the N cycle using XLSTAT software 2008 (*Addinsoft*, Paris, France).

3. Results

3.1. Sediment characteristics

The down-core variations in the concentrations of major compounds involved in the N and Mn cycle during October and January have been depicted in Fig. 2. In October, the NH_4^+ concentration-depth profile was characterized by different concentration gradients through the sediment column. The profile showed two main convex-shaped zones of NH_4^+ generation i.e. from 0-6 and 10-17 cm. In contrast, the NH_4^+ concentration-depth profile in January was characterized by a constant concentration gradient. The highest measured values of NH_4^+ were obtained at the deeper depth during October and January reaching up to 277.0 µmol L^{-1}. Profiles of oxidized forms of nitrogen (NO_x) which corresponds to the sum of NO_3^- plus NO_2^- showed lower values compared to NH_4^+ (Fig. 2). The maximum concentrations of NO_x were observed at the surface with values of up to 5.83 and 1.57 µmol L^{-1} during October and January respectively. Though NO_x concentration decreased to less than 1 µmol L^{-1} below the surface, a peak was observed at around 7 cm depth during both sampling times.

Concentration of ascorbate-extractable Mn-oxides (hereafter referred as Mn-oxides) revealed that whatever the sampling time, the highest concentrations were observed within the first few millimetres. Thereafter, it decreased with depth to remain constant throughout the sediment column. During October, concentration of Mn-oxides in the sediment decreased from 1.11 µmol g^{-1} at the surface to 0.63 µmol g^{-1} at 17 cm. In January, Mn-oxides varied between 0.47 µmol g^{-1} at the surface to 0.20 µmol g^{-1} at 5.5 cm. In contrast to the particulate form of Mn, the concentration of its soluble form (Mn^{2+}) was higher and its profiles presented varied patterns. In October, a major peak in pore water Mn^{2+} concentration was seen below the surface (< 1 cm) corresponding to the zone of decrease in Mn-oxides. Below this depth, Mn^{2+} concentration diminished except around 7 cm where concomitant peaks of Mn^{2+} and NO_x were noticed. However in January, concentrations of Mn^{2+} were lower and never exceeded 2 µmol L^{-1}. A peak in Mn^{2+} concentration was seen below the surface (1-3 cm depth). Below 3 cm, a sharp decline in Mn^{2+} concentration was observed.

3.2. Bacterial activities

Results for potential bacterial N_2-producing activities (P_{tot}) are tabulated in Table 1. Potential N_2 production rates varied by a factor of up to 3.2 (October) and 4.4 (January) along the sediment depth

(Table 1). This is largely due to high denitrification associated N_2 production (29.09 µmol L^{-1} h^{-1}) at the surface in January. Interestingly, the highest contribution of anammox to N_2 production (21%) was also observed at the same depth during this period. Thus, $^{15}NO_3^-$ labelling revealed denitrification to be the main process of N_2 production with minimal contribution from anammox.

Estimation of N_2 production based on NO_3^- from the overlying water (P_w) or produced through the oxidation of NH_4^+ in the sediment (P_n) was further discriminated in microcosms with or without amendment with Mn-oxides according to Risgaard's calculations (Risgaard-Petersen et al., 2003). In October, potential N_2 production through P_w was not significantly affected by addition of Mn-oxides (Fig. 3a). As expected under anaerobic conditions, very low natural P_n rates (without amendment of Mn-oxides) of ≤ 1.23 ± 0.80 µmol L^{-1} h^{-1} were observed except for the deeper layer where they were almost 3 × greater (Fig. 3b). In contrast, when Mn-oxides were added to the sediment slurries, a significant stimulation of P_n was observed between 0-4.5 cm with N_2 production i.e. 25.42 ± 3.29 µmol L^{-1} h^{-1} at 0-2.5 cm (*t*-test, n = 6, $p < 0.04$) and 9.04 ± 0.59 µmol L^{-1} h^{-1} at 2.5-4.5 cm (*t*-test, n = 6, $p < 0.00001$). During January, P_w presented a conventional pattern with maximum activity in the upper layers followed by a decrease with depth (Fig. 3c). The benthic system was highly heterogenous with elevated natural P_n rates under anaerobic conditions (Fig. 3d). On addition of Mn-oxides to the sediments, elevated N_2 production was observed at all depths. Highest N_2 production of 16.02 ± 9.94 µmol L^{-1} h^{-1} was recorded between 4.5-8.5 cm. It may be noted that larger standard deviation values for P_n rates observed in the presence of Mn-oxides are indicative of greater variability between test incubations during this sampling (Fig. 3d).

For samples collected during October, one set of test vials were spiked with $^{15}NH_4^+$ and $^{14}NO_3^-$ to examine N_2 production based on $^{15}NH_4^+$ (P15). The N_2 produced could originate from anammox and/or nitrification coupled to denitrification since the labelling procedure did not permit to discriminate these two pathways. A significant increase in P15 (*t*-test, n = 6, $p < 0.05$) was observed at all depths in the presence of Mn-oxides (Fig. 4).

The production of N_2O, a powerful greenhouse gas, was also investigated at both sampling times with or without amendment of Mn-oxides (Fig. 5). Without Mn-oxides, N_2O production was almost undetectable at both sampling times and at all depths. With Mn-oxides, significant higher production of N_2O was observed whatever the sampling time (*t*-test, n = 10, $p < 0.0001$ for all samples), except during January at 8.5-12 cm. During October, N_2O production in the presence of Mn-oxides was maximal at 8.5-12 cm occurring at a rate of 94.5 ± 30.8 nmol N_2O L^{-1} h^{-1}. In January, two maxima were observed i.e. 37.9 ± 5.2 nmol N_2O L^{-1} h^{-1} in the upper 2.5 cm layer in which a peak of Mn^{2+} has also been recorded in the sediment column and between 12-17 cm (66.60 ± 13.7 nmol N_2O L^{-1} h^{-1}) for which the concentration of nutrients at this depth are unfortunately not available.

4. Discussion

Manganese oxides can be generated by a variety of processes mediated by presence of oxygen (Van Cappellen et al., 1998) and alternative oxidants such as iodate or nitrate (Anschutz et al., 2000; Deflandre et al., 2002). Field observation undertaken in the present study revealed that concentration of Mn-oxides in Arcachon sediments varied from 0.20 to 1.11 µmol g^{-1} (Fig. 2). These values were 5 to 67 × lower than values reported from the Rhône estuary (Pastor et al., 2011) and continental shelf

sediment of Aquitaine merge (Hyacinthe et al., 2001; Anschutz et al., 2005). Maximum concentration of Mn-oxides was recorded in the first few millimetres of the sediment, followed by a decline with depth (Fig. 2). These Mn-oxides can be reduced to Mn_2^+ by indirect abiotic processes involving sulphur or intermediates of fermentation or by biotic processes such as bacterial respiration (Nealson and Saffarini 1994; Johnson et al., 1995). In the Arcachon sediments, Mn^{2+} concentration never exceeded 5.5 µmol L^{-1} (Fig. 2). This value is lower to that reported for organically-rich marine sediments where the concentration of Mn^{2+} can vary from 18-216 µmol L^{-1} (Mortimer et al., 2004). We also observed that between both sampling periods, relatively higher concentration of pore water Mn^{2+} was recorded in October (Fig. 2a) which could be attributed to reduction of Mn-oxides.

In Arcachon Bay, mineralization processes are intense because surficial intertidal flat sediments are rich in organic matter (Deborde et al., 2008). Products of microbial organic matter mineralisation, i.e. NH_4^+ diffuse in sediments. During both the sampling periods, pore water NH_4^+ concentrations showed different trends. In October, a strong vertical zonation was observed. Mineralization processes seemed to be more intense within 0-2 cm, whereas from 2-10 cm, production and consumption were more or less in equilibrium. In contrast, the NH_4^+ concentration depth profile in January was characterized by a constant concentration gradient indicating that above 9 cm, concentration of the nutrient could be controlled more by diffusive transport.

It is generally accepted that in the presence of oxygen, NH_4^+ can be oxidized to NO_2^- or NO_3^- through the nitrification pathway. These inorganic N compounds can diffuse from the oxic to anoxic layer and be subsequently reduced to N_2 via denitrification or anammox leading to a significant loss of N. NO_x concentration in the Arcachon sediments was < 1 µmol L^{-1} (Fig. 2) except at the surface where a continuous replenishment of these compounds from the ambient seawater could be expected. The observed NO_x profiles could therefore be due to contribution of diffusion of the compounds from the overlying seawater, their indigenous production by nitrification and their consumption by dissimilative processes like denitrification or anammox that lead to N_2 production. Our results have shown that denitrification was the main contributor to N_2 production in the Arcachon sediments whereas anammox was almost undetectable except at 0-2.5 cm in January (Table 1). NO_x concentration and the relative size of the anammox population or their activity are important factors controlling transformation of NH_4^+ through the anammox pathway (Trimmer et al., 2005). Therefore, favourable conditions for anammoxifiers mainly at < 2.5 cm could have lead to high anammox activity. For other layers and samples, N_2 production through denitrification superceeded that through anammox and ranged from 5 µmol L^{-1} h^{-1} to 29 µmol L^{-1} h^{-1} (Table 1). These values were of the same order of magnitude as those reported in coastal sediments from different geographic locations (Bonin et al., 1998; Thamdrup and Dalsgaard, 2002; Trimmer et al., 2003; Risgaard-Petersen et al., 2004; Dalsgaard et al., 2005; Trimmer et al., 2006; Minjeaud et al., 2009). In Mediterranean coastal sediments, Minjeaud et al. (2009) have also reported denitrification as the main process of N_2 production with occasional occurrence of anammox at low rates. The relatively low rate of anammox recorded in the present study could not be an artifact of NH_4^+ limitation in the slurries since concentration of the nutrient in the sediments was > 200 µmol L^{-1}. Thus, we can infer that NO_x reduction is routed through denitrification.

Earlier studies have reported coupling between nitrification and denitrification or anammox in an aerobic-anaerobic gradient. Under oxygen-depleted conditions, aerobic nitrification cannot sustain substrate for N_2 production. Bartlett et al. (2007) have reported the occurrence of nitrification coupled to the reduction of Mn-oxides in anoxic marine sediments based on concomitant peaks of NO_x and Mn^{2+}. We occasionally recorded such peaks in the anoxic layer of the Arcachon sediments as reported earlier by Deborde et al. (2008). Based on studies by Mortimer et al. (2004), we interpreted this finding as NO_x production by anaerobic NH_4^+ oxidation linked to reduction of Mn-oxides. Based on field observations, we assumed that NO_x produced due to anaerobic oxidation of NH_4^+ would be further reduced to N_2 via denitrification or anammox.

In this study, occurrence of anaerobic nitrification-denitrification mediated by Mn-oxides has been proved by development of an indirect approach using stable isotope tracer which permits to follow the origin of denitrified NO_3^- in the presence of Mn-oxides. ^{15}N labelling revealed that N_2 production based on NO_3^- originating from nitrification (P_n) in October occurred in the deeper layer (12-17 cm) (Fig. 3b) whereas in January, P_n values of around 5 μmol L^{-1} h^{-1} were observed through the sediment column (Fig. 3d). The detection of natural P_n in anoxic layers is therefore indicative of the occurrence of anaerobic oxidation of NH_4^+ leading to NO_x production via the nitrification pathway.

Since sediment from Arcachon contained low concentration of Mn-oxides, impact of Mn-oxides amendment was analyzed. We observed that addition of Mn-oxides stimulated P_n production mainly between 0 and 4.5 cm during October (Fig. 3b) while in January, P_n increase was observed at all depths (Fig. 3d). P_n stimulation at < 4.5 cm in October coincided with lowest values of natural P_n (without Mn-oxides addition) and the highest pore water Mn^{2+} concentration. This increase could be directly dependent on the lack of availability of substrate as outlined above along with a high abundance of reactive Mn in the sediments and NO_x reducing prokaryotes. The most prominent increase of P_n in the presence of Mn-oxides was observed for surface layers (< 4.5 cm) during October. We confirmed this hypothesis by counter-labelling with $^{15}NH_4^+$, $^{14}NO_3^-$ and amendment with Mn-oxides. The increase in P15 (N_2 production from $^{15}NH_4^+$) (Fig. 4) indicated that $^{15}NH_4^+$ was oxidized to NO_x and subsequently reduced to N_2. Thus, substantial stimulation of $^{15}NH_4^+$ oxidation to N_2 (based on increase in the rates of P_n and P15) provided indirect evidence on anaerobic nitrification-denitrification mediated by Mn-oxides. It could be argued that N_2 production could be due to a stimulation of denitrification or anammox. Quite clearly, that was not the case since potential N_2 production through NO_3^- from the water column (P_w) was not significantly affected by addition of Mn-oxides. Furthermore, the increase of N_2 production by Mn-oxides doesn't seem to be influenced by denitrification/anammox ratio. In consequence, we believe that Mn-oxides favour NO_x production and fuel N_2 producing processes.

In Arcachon Bay, Rysgaard et al. (1996) have reported aerobic nitrification rate of 3.3 mmol m^{-2} d^{-1} (Rysgaard et al., 1996) which is higher compared to those reported from other shallow marine sediments (Rysgaard et al., 1993, Gilbert et al., 1995). This high nitrification activity oxidized up to 22% of the NH_4^+ produced from organic matter mineralisation. It was suggested that these high levels were probably caused by high rates of NH_4^+ regeneration originating from decomposition of organic matter. Based on integration of anaerobic nitrification rates within 0-10 cm which were determined without amendment with Mn-oxides, we can estimate a rate of 0.7 mmol m^{-2} d^{-1}

corresponding to about 21% of aerobic nitrification rate previously reported (Rysgaard et al., 1996). The potential rates after amendment with Mn-oxides could reach up to 14.4 mmol m^{-2} d^{-1} indicating that the process could be ecologically important in controlling NH_4^+ accumulation in the benthic system.

For the first time, laboratory incubation experiments in the present study have shown a significant increase in N_2O production on addition of Mn-oxides during both samplings (Fig. 5). It should be noticed that maximum N_2O production in October corresponded to the zone where a secondary peak of NO_x and Mn^{2+} had been detected (Fig. 2a). Javanaud et al. (2011) have demonstrated bacterially-mediated N_2O production to occur in the absence of NO_3^- but with Mn-oxides and NH_4^+ amendment giving an indirect proof of NO_x production. In the presence of Mn-oxides, denitrifiers could switch over to performing nitrification under nitrate-limited conditions and produce NO_3^-, which is further reduced to N_2O (Javanaud et al., 2011). The radiative gas N_2O is known to be produced as an intermediate of multiple pathways in natural environment. However, the mechanisms and contribution of ammonia oxidation pathways (nitrifier nitrification, nitrifier denitrification, and coupled nitrification-denitrification) and heterotrophic denitrification to N_2O production is poorly understood as well as their dependence on environmental factors like concentrations of oxygen and inorganic N compounds. Zhu et al. (2013) have shown N_2O production via nitrification to increase as O_2 concentrations decreased from 21% to 0.5%. These authors suggest ammonia oxidation pathways and nitrifier denitrification as significant sources of N_2O.

5. Conclusion

Field observations from the Arcachon Bay provided indirect evidence on the occurrence of anaerobic nitrification coupled to the reduction of Mn-oxides. Further investigations using sediment slurry samples labelled with $^{15}NO_3^-$ and amended with Mn-oxides showed significant increase in rate of N_2 production mainly through anaerobic nitrification-denitrification. Elevated N_2 production in incubations amended with $^{15}NH_4^+$ and Mn-oxides further strengthened the origin of N_2 through this pathway. The contribution of anammox in N_2 production was found to be minimal. In oxygen-deficient zones, anaerobic NO_x production (through nitrification) mediated by Mn-oxides could be vital for the supply of substrate not only to denitrifiers but also to anammox bacteria that continuously need anaerobiosis and NO_2^- for maximal activity. As in the Arcachon Bay, where NH_4^+ concentration in the sediments are 100 × higher than those of NO_x, the importance of anaerobic NO_x production mediated by Mn-oxides in eutrophication control could be more important than previously thought as it prevents NH_4^+ accumulation. For the first time, our study revealed enhanced production of the greenhouse gas N_2O through anaerobic nitrification-denitrification in the presence of Mn-oxides. Thus, the process has the potential for stimulating N_2O productivity and its consequent emission from the benthic system during enhanced supply of Mn-oxides from the surficial layers via sediment re-working by bioturbators or physical mixing. Due to the complexity of the interactions between the different microbial communities involved in N and Mn cycles, further studies are necessary to identify major factors controlling the occurrence of anaerobic nitrification-denitrification mediated by Mn-oxides. This would provide a better understanding of the wider ecological significance of the process.

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References

Aller, R.C., 1990. Bioturbation and manganese cycling in hemipelagic sediments. Phil. Trans. R. Soc. 331, 51–68.

Anschutz, P., Dedieu, K., Desmazes, F., Chaillou, G., 2005. Speciation, oxidation state, and reactivity of particulate manganese in marine sediments. Chem. Geol. 218, 265–279.

Anschutz, P., Sundby, B., Lefrançois, L., Luther III, G.W., Mucci, A., 2000. Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments. Geochim. Cosmochim. Acta 64, 2751–2763.

Bartlett, R., Mortimer, R.J.G., Morris, K.M., 2007. The biogeochemistry of a manganese-rich Scottish sea loch: Implications for the study of anoxic nitrification. Cont. Shelf Res. 27, 1501–1509.

Bartlett, R., Mortimer, R.J.G., Morris, K.M., 2008. Anoxic nitrification: Evidence from Humber Estuary sediments (UK). Chem. Geol. 250, 29–39.

Baumann, P., Baumann, L., 1981. The marine gram negative eubacteria: genus *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In: Mortimer, P.S., Heinz, S., Truper, H.G., Balows, A., Schegel, H.G. (Eds.), The prokaryotes: a handbook on habitats, isolation and identification of bacteria. Springer-Verlag KG, Berlin, Germany pp. 1302–1330.

Bonin, P., Omnes, P., Chalamet, A., 1998. Simultaneous occurence of denitrification and nitrate ammonification in sediments of the french mediterranean coast. Hydrobiologia 389, 169–182.

Clément, J.C., Shrestha, J., Ehrenfeld, J.G., Jaffe, P.R., 2005. Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. Soil Biol. Biochem. 37, 2323–2328.

Dalsgaard, T., Canfield, D.E., Petersen, J., Thamdrup, B., Acuna-Gonzalez, J., 2003. N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature 422, 606–608.

Dalsgaard, T., Thamdrup, B., Canfield, D.E., 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Res. Microbiol. 156(4), 457–464.

De Wit, R., Leibreich, J., Vernier, F., Delmas, F., Beuffe, H., Maison, P., Chossat, J., Laplace Treyture, C., Laplana, R., Clave, V., Torre, M., Auby, I., Trut, G., Maurer, D., Capdeville, P., 2005. Relationship between land-use in the agro-forestry system of les Landes, nitrogen

loading to and risk of macro-algal blooming in the Bassin d'Arcachon coastal lagoon (SW France). Estuar. Coast. Shelf. Sci. 62, 453–465.

Deborde, J., Anschutz, P., Auby, I., Gle, C., Commarieu, M., Maurer, D., Lecroart, P., Abril, G., 2008. Role of tidal pumping on nutrient cycling in a temperate lagoon (Arcachon Bay, France). Mar. Chem. 109, 98–114.

Deflandre, B., Mucci, A., Gagné, J.P., Guignard, C., Sundby, B., 2002. Early diagenetic processes in coastal marine sediments disturbed by a catastrophic sedimentation event. Geochim. Cosmochim. Acta 66, 2547–2558.

Fernandes, S.O., Michotey, V.D., Guasco, S., Bonin, P.C., LokaBharathi, P.A., 2012. Denitrification prevails over anammox in tropical mangrove sediments (Goa, India). Mar. Environ. Res. 74, 9–19.

Gilbert, F., Bonin, P., Stora, G., 1995. Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral. Hydrobiologia 304, 49–58.

Hietanen, S., Kuparinen, J., 2008. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea. Hydrobiologia 596, 67–77.

Hulth, S., Aller, R.C., Gilbert, F., 1999. Coupled anoxic nitrification/manganese reduction in marine sediments. Geochim. Cosmochim. Acta 63, 49–66.

Hulth, S., Aller, R.C., Canfield, D.E., Dalsgaard, T., Engstr̈om, P., Gilberte, F., Sundb̈ack, K., Thamdrup, B., 2005. Nitrogen removal in marine environments: recent findings and future research challenges. Mar. Chem. 94, 125–145.

Hyacinthe, C., Anschutz, P., Carbonel, P., Jouanneau, J.M., Jorissen, F.J., 2001. Early diagenetic processes in the muddy sediments of the Bay of Biscay. Mar. Geol. 177, 111–128.

Javanaud, C., Michotey, V., Guasco, S., Garcia, N., Anschutz, P., Mathieu, C., Bonin, P., 2011. Anaerobic ammonium oxidation mediated by Mn-oxides: from sediment to strain level. Res. Microbiol. 162, 848–857.

Johnson, D., Chiswell, B., O'Halloran, K., 1995. Micro-organisms and manganese cycling in a seasonally stratified freshwater dam. Water Res. 29, 2739–2745.

Kérouel, R., Aminot, A., 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Mar. Chem. 57, 265–275.

Kuypers, M.M.M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B.M., Amann, R., Jørgensen, B.B., Jetten, M.S.M., 2005. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc. Natl. Acad. Sci. USA 102, 6478–6483.

Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jorgensen, B.B., Kuenen, J.G., Sinninghe Damsté, J.S., Strous, M., Jetten, M.S.M., 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422, 608–611.

Laha, S., Luthy, R.G., 1990. Oxidation of aniline and other primary aromatic amines by manganese dioxide. Environ. Sci. Technol. 29, 363–373.

Laima, M.J.C., 1993. Recovery of $^{15}NH_4$ in labeling experiments on coastal marine sediments. Mar. Chem. 44, 31–42.

Lloyd, D., Scott, R.I., 1983. Direct measurement of dissolved gases in microbiological systems using membrane inlet mass spectrometry. J. Microbiol. Meth. 1, 313–328.

Luther III, G.W., Popp, J.I., 2002. Kinetics of the abiotic reduction of polymeric manganese dioxide by nitrite: an anaerobic nitrification reaction. Aquat. Geochem. 8, 15–36.

Luther, G.W., Sundby, B., Lewis, B.L., Brendel, P.J., Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. Geochim. Cosmochim. Acta 61, 4043–4052.

Michotey, V., Bonin, P., 1997. Evidence for anaerobic process in the water column: denitrification and dissimilatory nitrate ammonification in the northwestern Mediterranean Sea. Mar. Ecol. Prog. Series 160, 47–56.

Minjeaud, L., Bonin, P.C., Michotey, V.D., 2008. Nitrogen fluxes from marine sediments: quantification of the associated co-occurring bacterial processes. Biogeochemistry 90, 141–157.

Minjeaud, L., Michotey, V.D., Garcia, N., Bonin, P.C., 2009. Seasonal variation in di-nitrogen fluxes and associated processes (denitrification, anammox and nitrogen fixation) in sediment subject to shellfish farming influences. Aquat. Sci. 71, 425–435.

Mortimer, R.J.G., Harris, S.J., Krom, M.D., Freitag, T., Prosser, J.I., Barnes, J., Anschutz, P., Hayes, P., Davies, I.M., 2004. Anoxic nitrification in marine sediments. Mar. Ecol. Progr. Ser. 276, 37–51.

Mortimer, R.J.G., Krom, M.D., Harris, S.J., Hayes, P., Davies, I.M., Davison, W., Zhang, H., 2002. Evidence for suboxic nitrification in recent marine sediments. Mar. Ecol. Progr. Ser. 236, 31–35.

Murray, J.W., Codispoti, L.A., Friederich, G.E., 1995. Oxidation-reduction environments: The suboxic zone in the Black Sea. In: Huang, C.P., O'Melia, C.R., Morgan, J.J. (Eds.), Aquatic Chemistry: Interfacial and Interspecies Processes. ACS Adv. Chem. Ser. 224, 157–176.

Nealson, K.H., Saffarini, D., 1994. Iron and manganese anaerobic respiration. Annu. Rev. Microbiol. 48, 311–343.

Pastor, L., Cathalot, C., Deflandre, B., Viollier, E., Soetaert, K., Meysman, F.J.R., Ulses, C., Metzger, E., Rabouille, C., 2011. Modeling biogeochemical processes in sediments from the Rhône River prodelta area (NW Mediterranean Sea). Biogeosciences 8, 1351–1366.

Plus, M., Dalloyau, S., Trut, G., Auby, I., de Montaudouin, X., Emery, E., Noël, C., Viala, C., 2010. Long-term evolution (1988–2008) of *Zostera spp.* meadows in Arcachon Bay (Bay of Biscay). Estuar. Coast. Shelf. Sci. 87, 357–366.

Risgaard-Petersen, N., Meyer, R.L., Schmid, M., Jetten, MSM, Enrich-Prast, A., Rysgaard, S., Revsbech, N.P., 2004. Anaerobic ammonium oxidation in an estuarine sediment. Aquat. Microb. Ecol. 36, 293–304.

Risgaard-Petersen, N., Nielsen, L.P., Rysgaard, S., Dalsgaard, T., Meyer, R.L., 2003. Application of the isotope pairing technique in sediments where anammox and denitrification coexist. Limnol. Oceanogr. Methods 1, 63–73.

Rysgaard, S., Risgaard-Petersen, N., Nielsen, L.P., Revsbech, N.P., 1993. Nitrification and denitrification in lake and estuarine sediment measured by the ^{15}N dilution technique and isotope pairing. Appl. Environ. Microbiol. 59(7), 2093–2098.

Rysgaard, S., Risgaard-Petersen, N., Sloth, N.P., 1996. Nitrification, denitrification and nitrate ammonification in sediments of two coastal lagoons in Southern France. Hydrobiologia 329, 133–141.

Schaefer, S.C., Alber, M., 2007. Temperature controls a latitudinal gradient in the proportion of watershed nitrogen exported to coastal ecosystems. Biogeochemistry 85, 333–346

Schultz, H.D., Dahmke, A., Schinzel, T., Wallmann, K., Zabel, M., 1994. Early diagenetic processes, fluxes and reactions rates in sediments of the South Atlantic. Geochim. Cosmochim. Acta 58, 2041–2060.

Sørensen, J., 1987. Nitrate reduction in marine sediment: pathways and interactions with iron and sulfur cycling. Geomicrobiol. J. 5(3/4), 401–421.

Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R., Webb, S.M., 2004. Biogenic manganese oxides: Properties and mechanisms of formation. Annu. Rev. Earth Planet. Sci. 32, 287–328.

Thamdrup, B., Dalsgaard, T., 2000. The fate of ammonium in anoxic manganese oxide-rich marine sediment. Geochim. Cosmochim. Acta 64, 4157–4164.

Thamdrup, B., Dalsgaard, T., 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol. 68, 1312–1318.

Tréguer, P., Le Corre, P., 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Lab. Océanogr. Chim. Univ. Bretagne Occid, Brest.

Trimmer, M., Nicholls, J.C., Deflandre, B., 2003. Anaerobic ammonium oxidation measured in sediments along the Thames Estuary, United Kingdom. Appl. Environ. Microbiol. 69, 6447–6454.

Trimmer, M., Nicholls, J.C., Morley, N., Davies, C.A., Aldridge, J., 2005. Biphasic behavior of anammox regulated by nitrite and nitrate in an estuarine sediment. Appl. Environ. Microbiol. 71, 1923–1930.

Trimmer, M., Risgaard-Petersen, N., Nicholls, J.C., Engström, P., 2006. Direct measurement of anaerobic ammonium oxidation (anammox) and denitrification in intact sediment cores. Mar. Ecol. Prog. Ser. 326, 37–47.

Van Cappellen, P., Viollier, E., Roychoudhury, A., 1998. Biogeochemical cycles of manganese and iron at the oxic-anoxic transition of a stratified marine basin (Orca Basin, Gulf of Mexico). Environ. Sci. Technol. 32, 2931–39.

Ward, B.B., Devol, A.H., Rich, J.J., Chang, B.X., Bulow, S.E., Naik, H., Pratihary, A., Jayakumar, A., 2009. Denitrification as the dominant nitrogen loss process in the Arabian Sea. Nature 461, 78–82.

Zhu, X., Burger, M., Doane, T.A., Horwath, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proc. Natl. Acad. Sci. USA 110(16), 6328–6333.

Legend to figures

Fig. 1. Location of sampling site (star) in the Arcachon bay. Grey areas indicate intertidal flats.

Fig. 2. Down-core profiles of principal compounds of N and Mn cycles (NH_4^+, NO_x, pore water Mn^{2+}, Mn-oxides) in the sediment of Arcachon Bay during October 2007 (a) and January 2008 (b). NO_x corresponds to NO_2^- and NO_3^- content. Due to analytical problems, no data could be generated in January at >10 cm.

Fig. 3. Down-core rates of potential N_2 production through anammox + denitrification using NO_3^- from seawater (P_w) (a) or NO_x produced in situ at the expense of ammonium (P_n) (b) during October. In January, N_2 production through P_w and P_n are shown in Fig. 3c and 3d respectively. Slurries have been subjected to $^{15}NO_3^-$ amendment only (white bars) or with $^{15}NO_3^-$ + Mn-oxides (hatched bars). Error bars represent SDs.

Fig. 4. Anaerobic P15 production rates in sediment slurry incubations during October. In the presence of $^{15}NH_4^+$ and $^{14}NO_3^-$ labelling, P15 corresponds to N_2 production arising from anammox + denitrification and originating from the added $^{15}NH_4^+$. Slurries have been subjected to $^{15}NH_4^+$ and $^{14}NO_3^-$ without (white bars) or with Mn-oxides (grey bars). Error bars represent SDs.

Fig. 5. Down-core variations in production of nitrous oxide during October (a) and January (b). Slurries have been subjected to $^{15}NO_3^-$ amendment only (white bars) or with $^{15}NO_3^-$ + Mn-oxides (dark bars). Error bars represent SDs.

Table 1

Potential N_2 production rates expressed in µmol L^{-1} h^{-1}. Values in bracket are the percentage of anammox production.

Depth (cm)	P_{tot} in October (µmol L^{-1} h^{-1})	P_{tot} in January (µmol L^{-1} h^{-1})
0-2.5	8.49 (3.2%)	29.09 (21%)
2.5-4.5	6.27 (0%)	10.62 (3.8%)
4.5-8.5	6.67 (0%)	12.79 (0%)
8.5-12	5.26 (0%)	6.57 (1.5%)
12-17	16.76 (0%)	19.84 (3.8%)

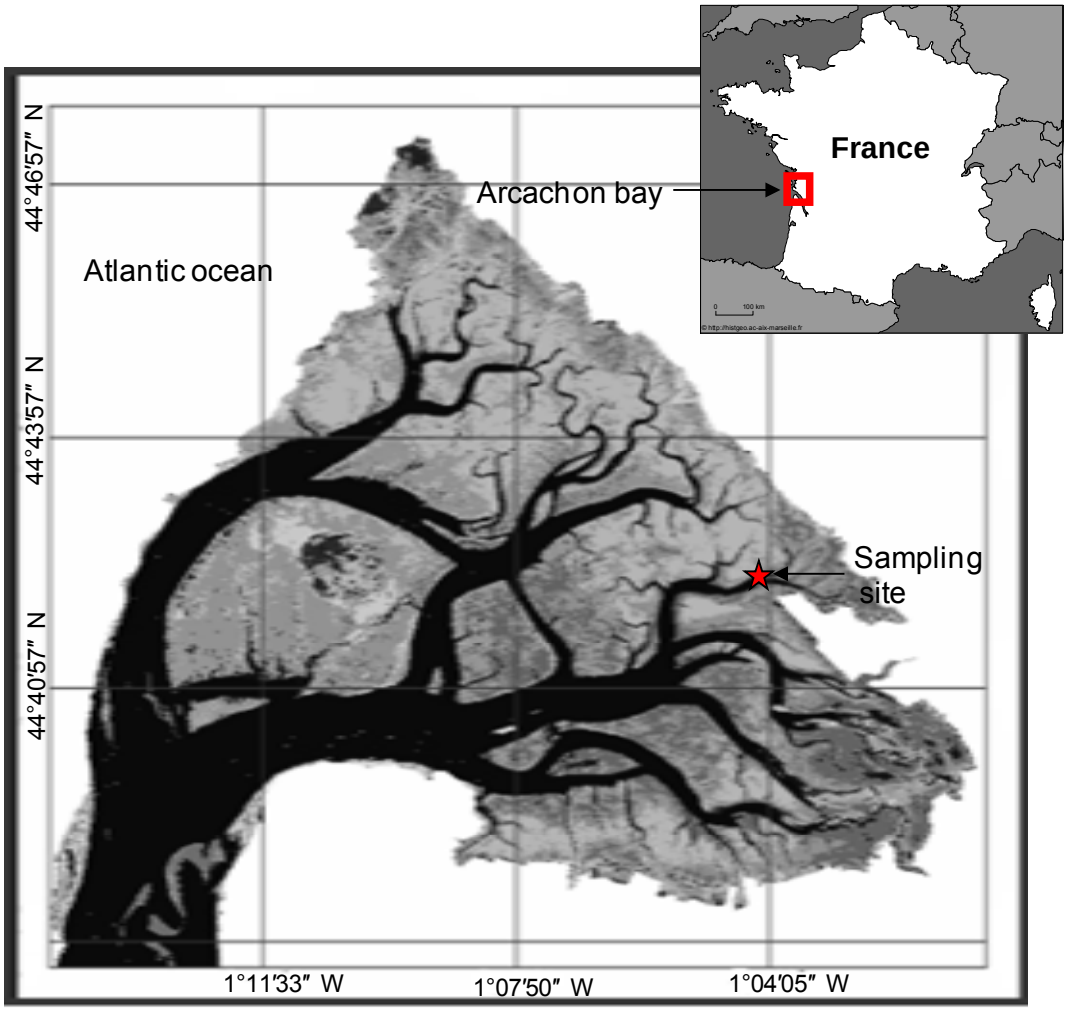

Fig. 1

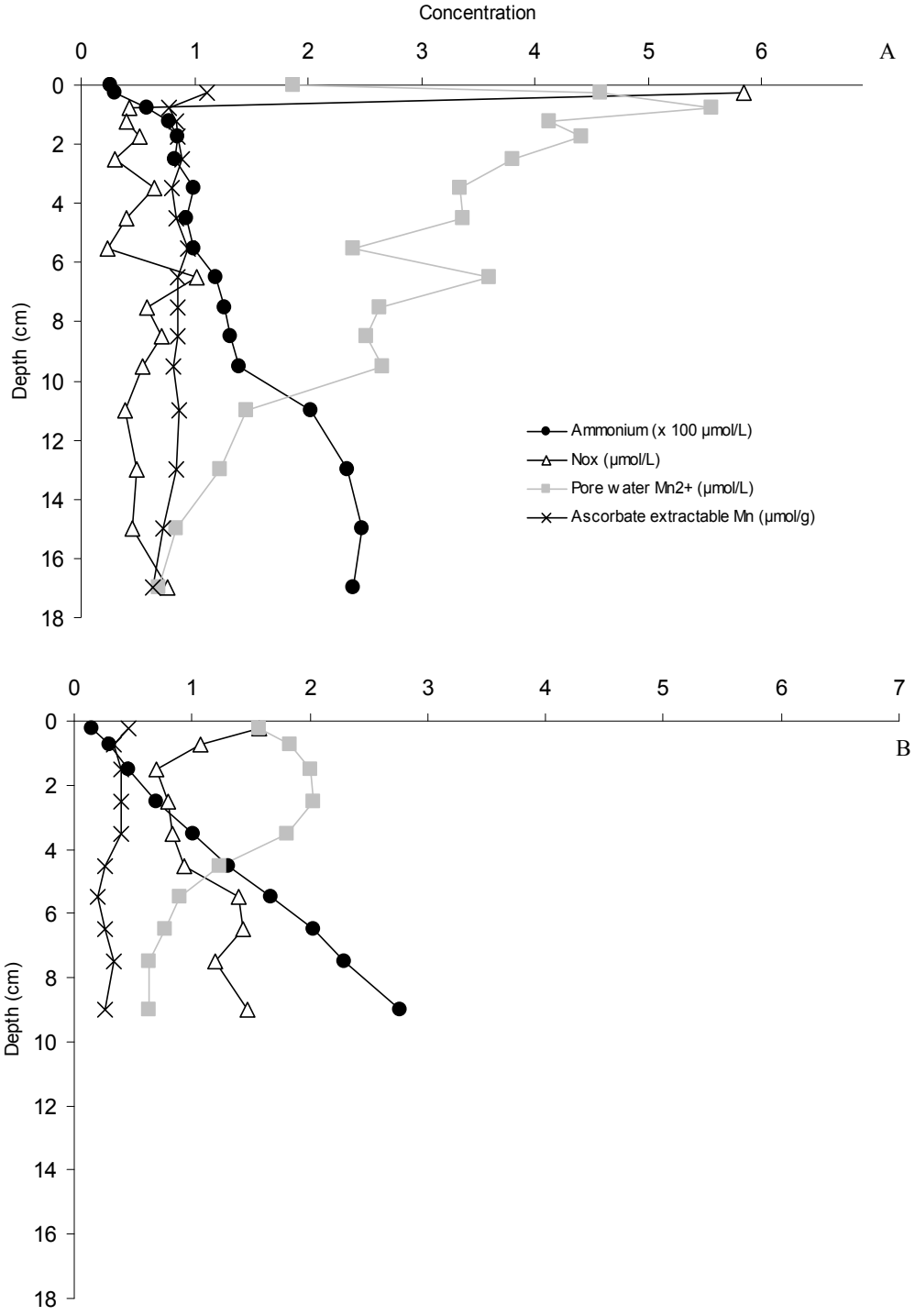
Concentration
A
Depth (cm)
Ammonium (x 100 µmol/L)
Nox (µmol/L)
Pore water Mn2+ (µmol/L)
Ascorbate extractable Mn (µmol/g)
B
Depth (cm)

Fig. 2

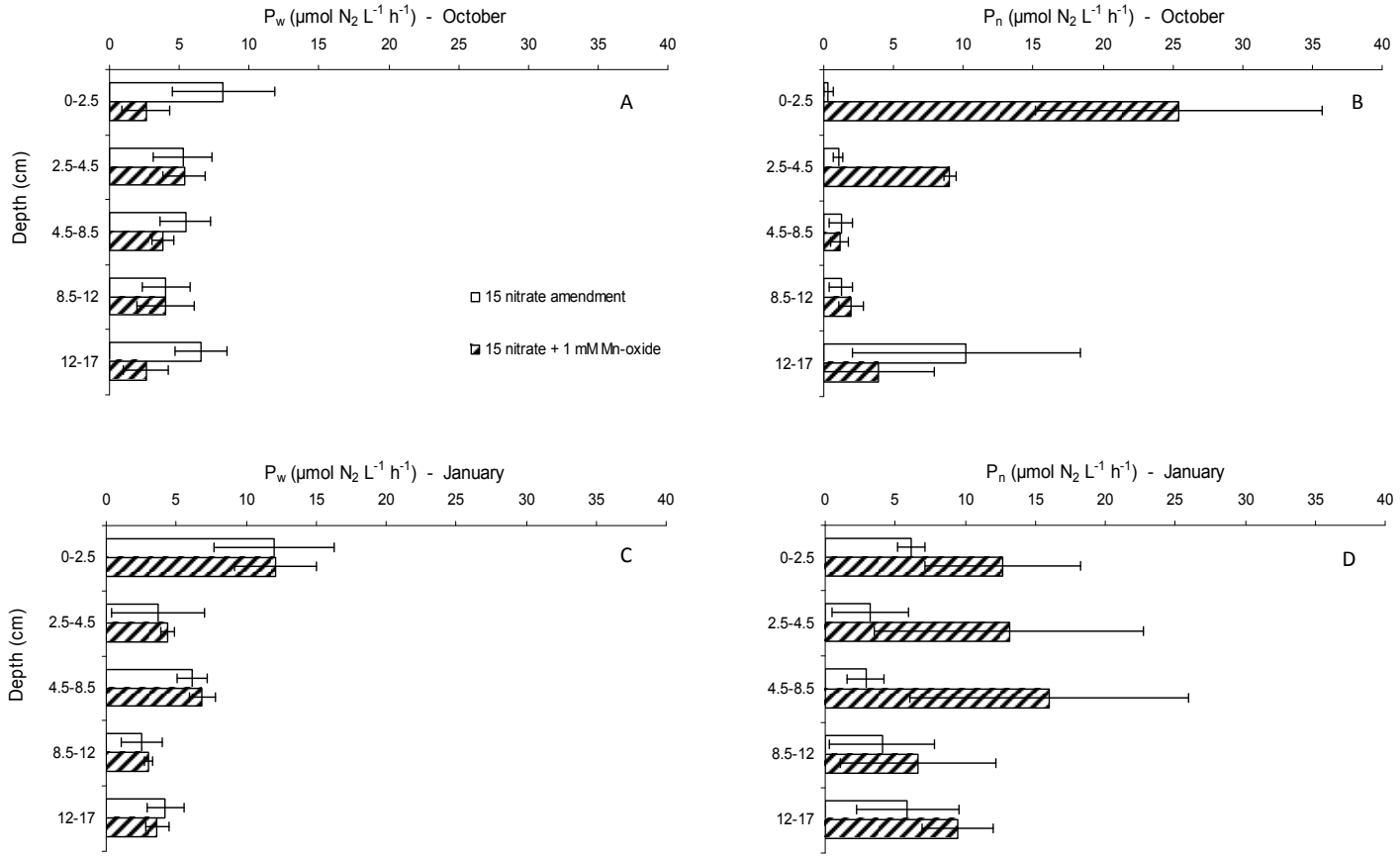
Pw (µmol N2 L-1 h-1) - October
Pn (µmol N2 L-1 h-1) - October
Pw (µmol N2 L-1 h-1) - January
Pn (µmol N2 L-1 h-1) - January
0 5 10 15 20 25 30 35 40
Depth (cm)
0-2.5
2.5-4.5
4.5-8.5
8.5-12
12-17
A
B
C
D
15 nitrate amendment
15 nitrate + 1 mM Mn-oxide

Fig. 3

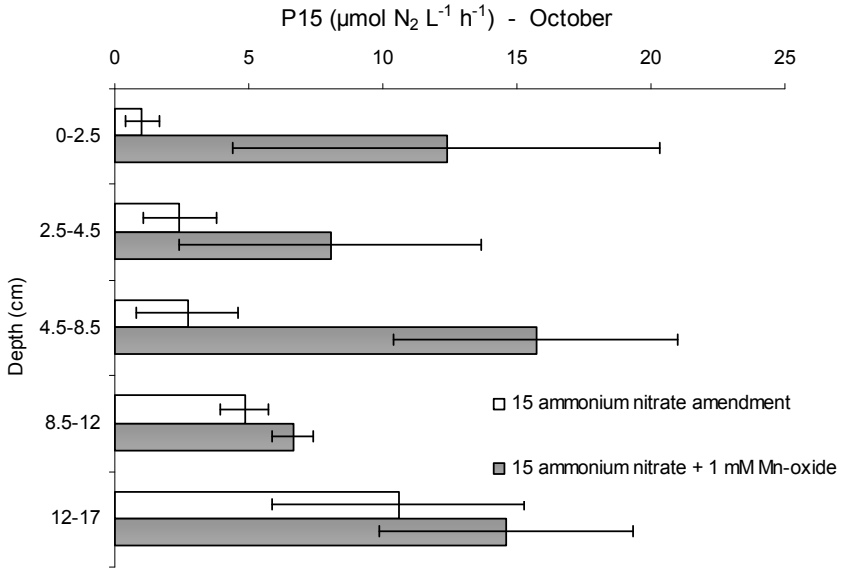
P15 (µmol N_2 L^{-1} h^{-1}) - October
0
5
10
15
20
25
0-2.5
2.5-4.5
4.5-8.5
8.5-12
12-17
Depth (cm)
15 ammonium nitrate amendment
15 ammonium nitrate + 1 mM Mn-oxide

Fig. 4

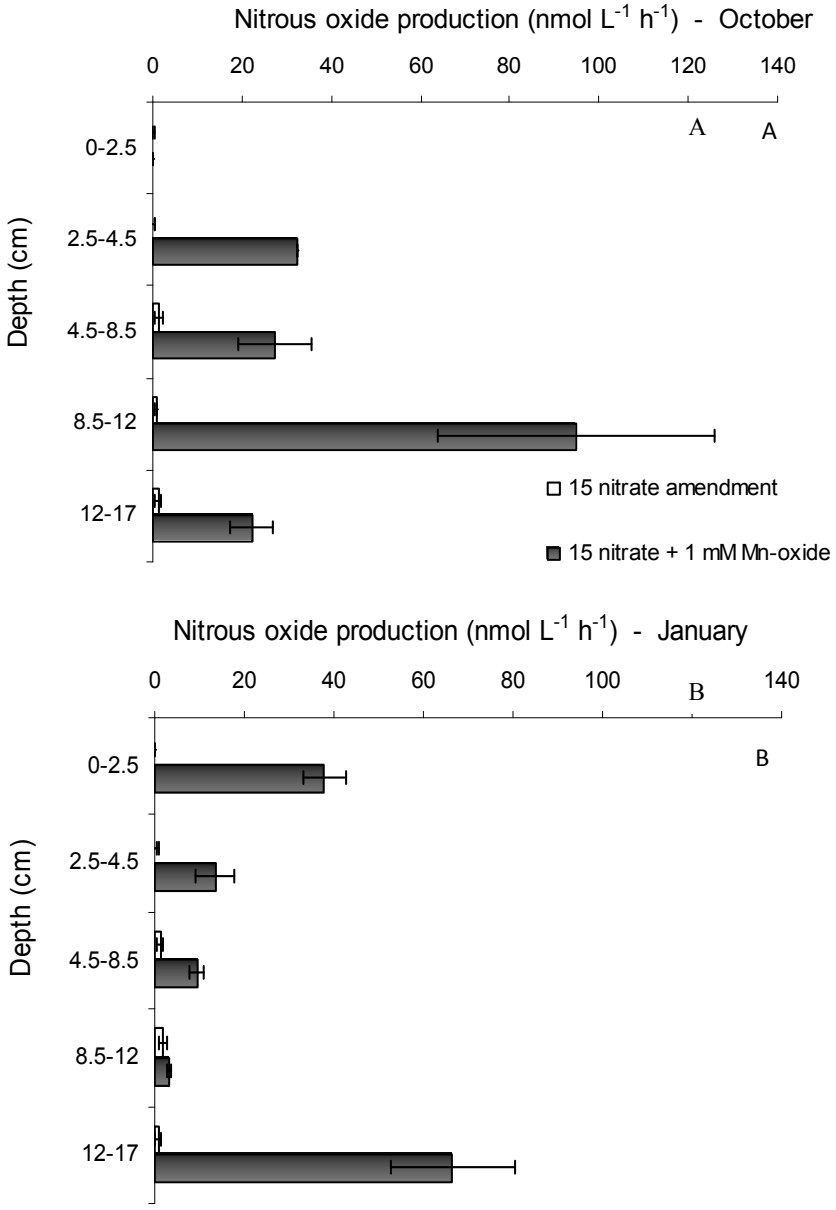
Nitrous oxide production (nmol L-1 h-1) - October
0
20
40
60
80
100
120
140
A
A
0-2.5
2.5-4.5
4.5-8.5
8.5-12
12-17
Depth (cm)
15 nitrate amendment
15 nitrate + 1 mM Mn-oxide
Nitrous oxide production (nmol L-1 h-1) - January
0
20
40
60
80
100
140
B
B
0-2.5
2.5-4.5
4.5-8.5
8.5-12
12-17
Depth (cm)

Fig. 5