

Influence of bioturbation on denitrification activity in Mediterranean coastal sediments: an *in situ* experimental approach

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ABSTRACT: An *in situ* experiment was conducted in the French Mediterranean littoral (Gulf of Fos) from July 1993 to January 1994 using controls without macrofauna or natural sediments. After 1, 4 and 6 mo, sediment reworking and denitrification activities (natural and potential rates) were studied. The bacterial processes were stimulated by the bioturbating activity of the autochthonous infauna. The natural and potential denitrification rates were 160 and 280% higher, respectively, than in the controls. The increase of denitrification, occurring at different depths in the sediment with respect to time, was directly dependent on the macrofaunal activity.

KEY WORDS: Bioturbation · Denitrification · *In situ* experiment · Mediterranean Sea

INTRODUCTION

Because of their activities in the sediment (feeding, burrowing, and bioirrigation), infaunal macro-organisms are known to play a crucial role in microbial activities and exchanges at the sediment-water interface (Aller 1980, Jørgensen & Revsbech 1985, Hüttel 1990, Binnerup et al. 1992). The effects of bioturbation on the nitrogen cycle and more particularly on denitrification have been extensively examined in *in vitro* studies (Chatarpaul et al. 1980, Kristensen & Blackburn 1987, Gilbert et al. 1995). Results from these studies have provided evidence for the quantitative role of bioturbation in various processes, e.g. enhanced nitrate supply (Kristensen et al. 1991) and stimulated coupling of nitrification-denitrification (Aller et al. 1983, Pelegrí et al. 1994), indicating that different, although mainly monospecific, macrofaunal populations may affect denitrification in coastal sediments. But the question remains whether a high denitrification potential which can be demonstrated *in vitro* is effective in the natural

environment. To date, only 1 *in situ* study carried out in Danish estuarine sediments, showing the *in situ* stimulation of denitrification by an amphipod population, has provided an answer to this question (Rysgaard et al. 1995).

The aim of our field experiment was to investigate the *in situ* effects of the activities of the whole of the autochthonous infauna on denitrification in Mediterranean coastal sediments.

MATERIALS AND METHODS

Experimental site. The site chosen was in the shallow water of Carteau cove situated on the western side of the Gulf of Fos (Fig. 1). Experiments were carried out at 5 m depth. The sediment is rich in organic matter (organic content ranging from 5.00 to 7.32%), and is classified as 'muddy sand sediment' (25 to 50% grains less than 63 µm), and is occupied by a macrofaunal assemblage characteristic of muddy sand in sheltered areas (Pérès 1982). During our experiment, polychaetes dominated the benthic macrofauna (70%) and crustaceans were the second most dominant group

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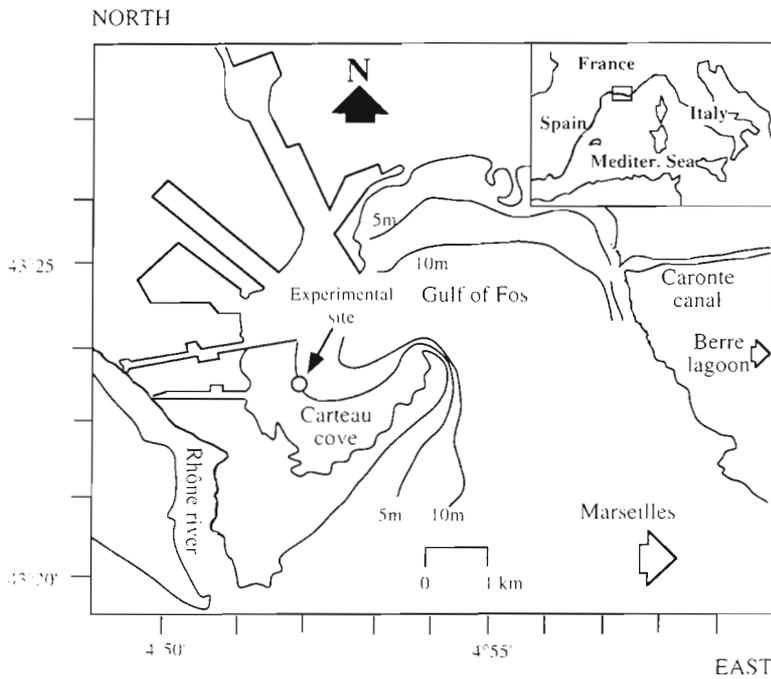


Fig. 1. Experimental site in the Gulf of Fos (Mediterranean Sea, France)

(25%). The other faunistic groups present were molluscs and echinoderms (5%). The total macrofaunal benthic density varied from 2580 to 3160 ind. m⁻². More than 80% of the organisms were located in the upper 4 cm of sediment (Gilbert 1994).

Experimental procedure. Twelve core tubes divided into 2 groups were inserted into the sediment (Fig. 2B): 6 C-core tubes (control sediment), containing macrofauna defaunated sediment, and 6 I-core tubes (inhabited sediment), containing natural sediment with autochthonous infauna. The core tubes were made from 27 cm long sections of 11 cm diameter PVC tubing (Fig. 2A). Caps were designed to slow down recolonization of the defaunated control sediments by macrofauna, without confining the area overlying the sediment surface. The caps were made from 6 cm long PVC tubing (diam.: 11 cm). The tops of the caps were closed with gauze (500 µm), and the sides contained 2 mesh-covered windows (11.2 cm long, 3.8 cm high, 1 mm mesh) to allow water circulation in the cores. To ensure standard experimental conditions, all the cores (defaunated and inhabited) were equipped with caps.

Analysis of sediment mixing. Mixing of the sediment was assessed by the luminophore tracer technique (Gerino 1990). Two size fractions of luminophores, 40 to 60 µm diameter and 150 to 200 µm diameter, were used. A mixture of the 2 luminophore fractions (1 g per fraction) was added to the surface of 6 cores (3 defaunated and 3 inhabited) in the form of a frozen 'sediment cake' (diam.: 10 cm, 1 cm thick; Gilbert et al. 1994). In order to

standardize the experimental conditions, 6 sediment cakes without luminophores were added to the remaining 6 cores.

Defaunation of control sediment. Control sediments were defaunated by an exclusion/transplant process: 6 sediment cores were taken by divers with experimental core tubes and transported to the laboratory in the following hour. In the laboratory, defaunation of sediments was achieved by sealing cores and stripping off O₂ present in the overlying water by a stream of N₂ ('N₂ method'; Kristensen et al. 1991). After 24 h at 24°C, dead and dying infauna that had climbed up to the sediment surface were collected and the sediment surface was gently homogenized to plug the burrow openings. The surface water was then aerated to restore oxic conditions after defaunation.

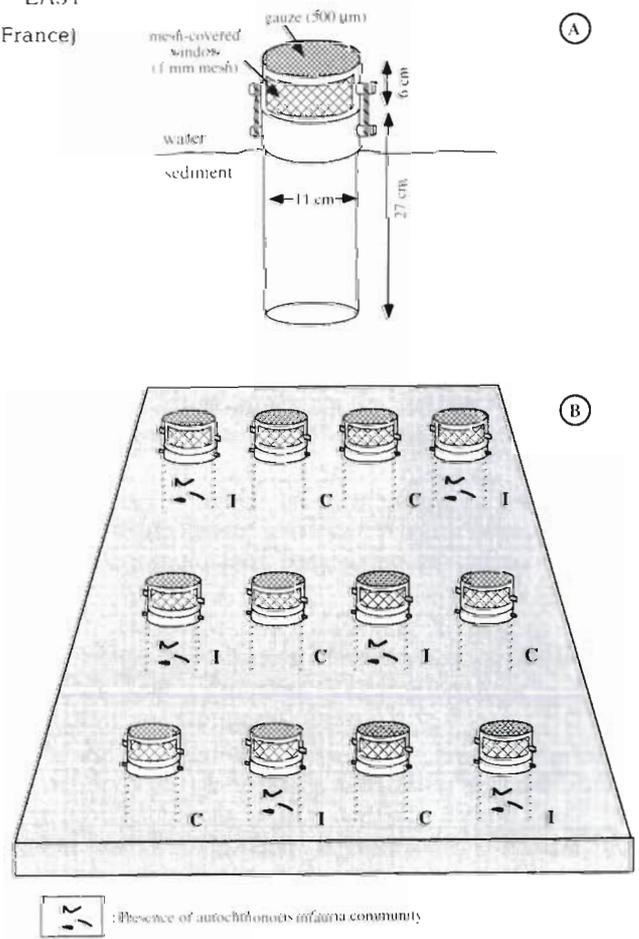


Fig. 2. (A) Experimental core design and (B) disposition of the different cores into the sediment (B). C: control defaunated cores; I: inhabited cores

The influence of this defaunation procedure on the microbial community is assumed to be insignificant (Kristensen et al. 1991).

Implantation of cores. After laboratory treatment, the defaunated sediment cores were transported back to the experimental site and transplanted. At the same time, 6 other core tubes (corresponding to the 'I-core tubes') were simply embedded in the sediment. Then the various additional cakes and the caps were added to the respective cores. The cores were checked weekly at the experimental site by divers. Dirty caps were brushed or replaced by new ones. During each sampling (after 1, 4, and 6 mo), 4 sediment cores were transported to the laboratory and the analyses were performed on 2 cm thick sediment segments from the top down to 10 cm.

Biological and chemical analyses. Natural and potential denitrification rates were assessed using the acetylene-blockage method (Raymond et al. 1992, Gilbert et al. 1997). Subsamples (4 ml) from each segment were transferred into 13 ml tubes containing 4 ml of natural sea water supplemented either with only chloramphenicol (1 g l^{-1}) to prevent bacterial growth during incubation (for the natural activity) or with chloramphenicol, glucose (1 g l^{-1}) and KNO_3 (1 mM) (for the potential activity, according to Tiedje et al. 1989). The tubes were sealed with rubber stoppers and anaerobic conditions obtained by flushing N_2 through the tube for 2 min. Acetylene, which inhibits the reduction of N_2O to N_2 (Balderston et al. 1976), was injected in the gas phase (final concentration 15 kPa) and the tubes were vortexed. Samples were incubated in the dark at *in situ* temperature for 0, 0.5, 1 and 3 h. After incubation each tube was treated with 0.1 ml of 1 M HgCl_2 solution, vigorously shaken by hand for 2 min, and then centrifuged at 2000 rpm ($350 \times g$) for 3 min. Denitrifying activity was considered as the linear initial rate of N_2O accumulation. After incubation, nitrous oxide was measured in the headspace and in sea water. A subsample (2.5 ml) of gas phase was injected into a 3 ml pre-evacuated tube (Venoject, Terumo, Leuven, Belgium) for later N_2O analysis. The extraction of N_2O from the liquid phase was carried out using the procedure of Chan & Knowles (1979) modified by the multiple equilibrium technique (Mac Aullife 1971). Nitrous oxide in the tubes was determined by gas chromatography (Girdel, series 30) using an electron capture detector. Chromatographic operating conditions were 8 ft length 'Porapak Q' column (mesh 50/80), oven temperature 80°C , injector temperature 180°C , detector temperature 250°C . Nitrogen was used as carrier gas at a flow rate of 20 ml min^{-1} (Bonin et al. 1987).

N compounds (nitrates and nitrites) were measured in the supernatant obtained after centrifugation at

$2000 \times g$ for 10 min. Nitrates were reduced on a Cu-Cd column adapted to Technicon II according to Tréguer & Le Corre (1975). Nitrite concentrations were determined colorimetrically by the method of Bendschneider & Robinson (1952).

Luminophore analyses were carried out as described by Gerino (1990). After sectioning, each slice of sediment was dried at 70°C for 1 wk, carefully mixed to homogenize sediment and luminophores, and sieved through a $500 \mu\text{m}$ mesh. For each segment, 3 subsamples of 0.25 g were taken. The luminophore counts were then conducted under an ultraviolet light source.

Data analysis. Differences between control and inhabited sediments and the space-time variations of denitrifying activity rates were studied using a 3-way analysis of variance (ANOVA). Bartlett's test was employed to test for homogeneity of variance. Heteroscedastic data were transformed and then evaluated using ANOVA.

RESULTS

Bioturbation

After 1 mo, no luminophore burial had occurred in control sediments (C-cores) (Fig. 3). The luminophore losses from the sediment due to the removal of particles into the water column were 53.7% (C-cores) and 36.8% (I-cores). The total recovery of luminophores in the deposit layer (0–2 cm) was 46.3% (926 mg). In inhabited sediments (I-cores), bioturbation displaced 17.7% (354 mg) of the luminophores between 2 and 10 cm depth, while 43.7% (874 mg) remained in the surface layer. In the bioturbated sedimentary column, the number of luminophores decreased with depth. After 4 mo, 14.7% (294 mg) of the luminophores were found in the C-cores. They were principally located in the 0–2 cm layer (Fig. 3). A very small quantity of luminophores (66 mg; 3.3%) was buried down to 6 cm ($<0.1\%$). In I-cores, where 13.0% (260 mg) were recorded in the surface layer, the number of luminophores distributed between 2 and 8 cm depth of sediment had increased compared to those found after 1 mo and reached 36.8% (736 mg). The maximum numbers of luminophores were found in the 2–4 cm layer. After 6 mo, 5.1% (102 mg) of luminophores were buried down to 4–6 cm in C-cores (Fig. 3), whereas 14.1% (282 mg) of luminophores were found down to 10 cm depth in I-cores. Since the previous samplings, increased losses of luminophores from sediments had occurred in all cores. Only 0.8% (16 mg) and 4.1% (82 mg) of luminophores were found in the initial deposit layer in C-cores and I-cores, respectively.

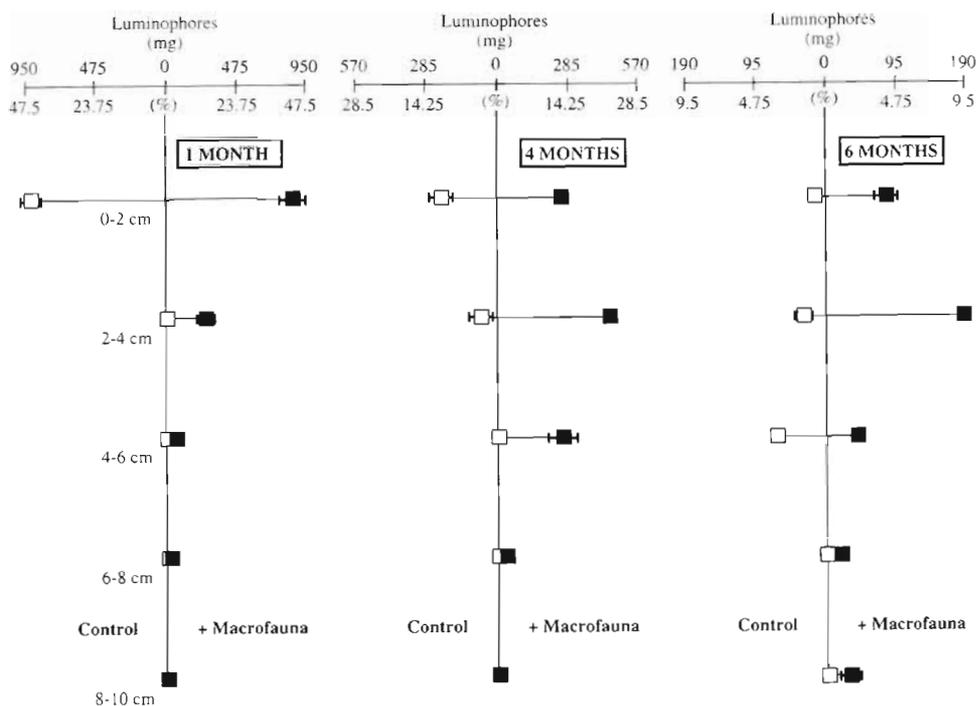


Fig. 3. Vertical distribution with time of luminophores in the sedimentary column for the different sediments, expressed in both milligrams and percentage of recovery from the initial deposit. Standard errors of the means are shown (n = 3). (□) Control defaunated sediment; (■) inhabited sediment

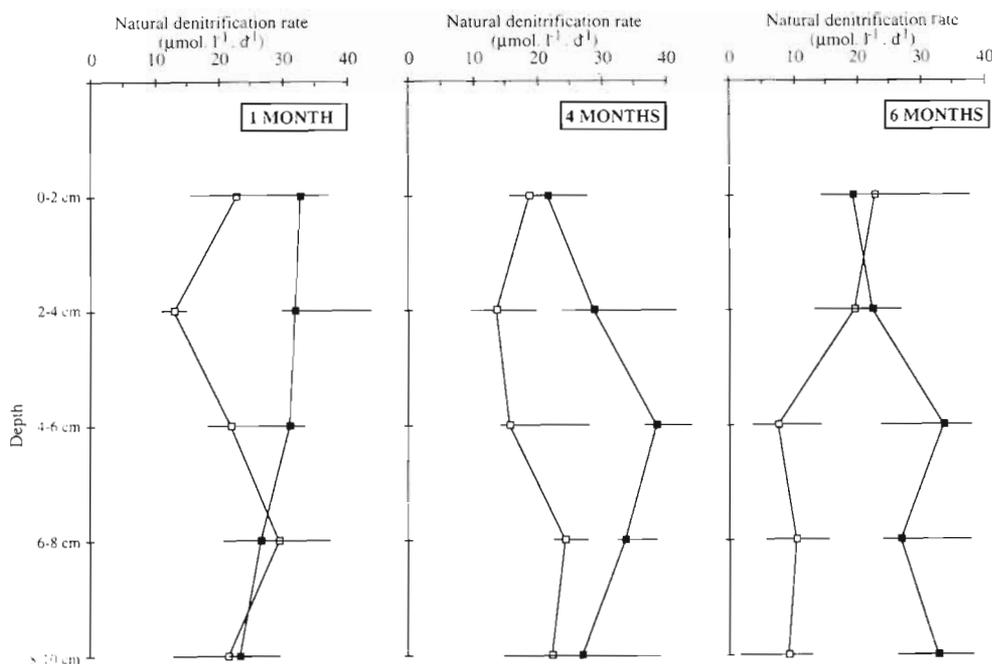


Fig. 4. Natural denitrification rate profiles for the different sediments with time. Mean values and observed ranges are shown. (□) Control defaunated sediment; (■) inhabited sediment

Denitrification

Natural denitrification. Natural denitrification rates measured in the sediments ranged from 10 to 40 μmol l⁻¹ d⁻¹ (Fig. 4) for NO₃⁻ and NO₂⁻ concentrations between 0.4 and 4.3 μM (Fig. 5) and 0.3 and 4.0 μM (Fig. 6), respectively. There was no difference in the nutrient profiles between control and inhabited sediments.

After 1 mo, denitrification was higher in presence of infauna down to 6 cm depth. Below this depth, control and inhabited sediments had the same denitrification rates. After 4 mo, the increase in the natural denitrification rate of the inhabited sediments was found throughout the sedimentary column. After 6 mo, in contrast, the increase in denitrification in the inhabited sediment occurred at greater depth (4–10 cm). The denitrification rates for the 2 types of sediment (control

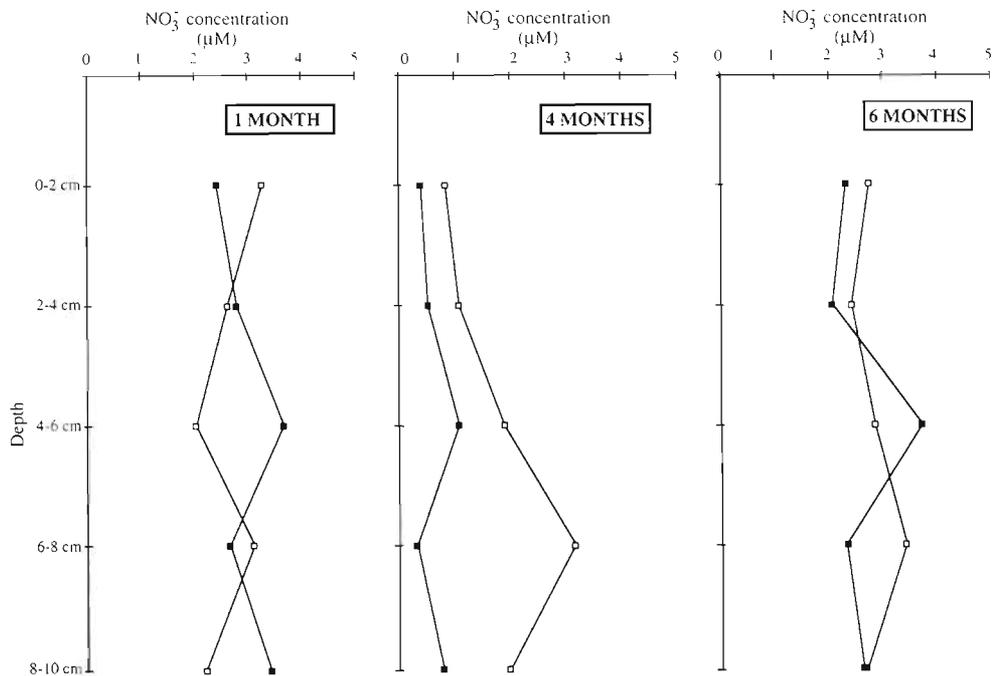


Fig. 5. Nitrate concentration profiles for the different sediments with time. (\square) Control defaunated sediment; (\blacksquare) inhabited sediment

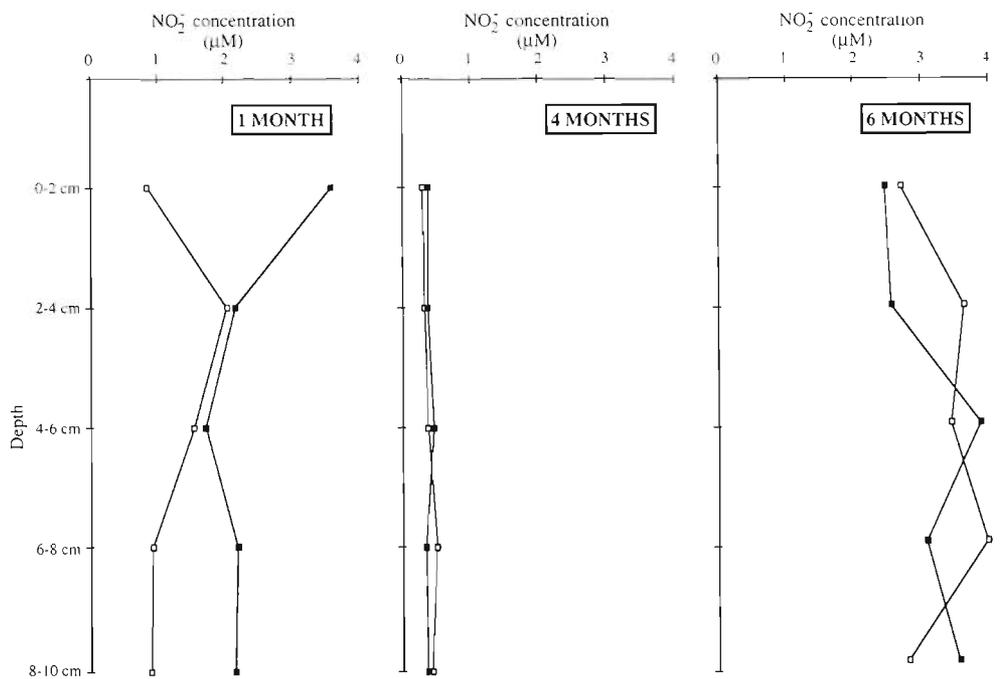


Fig. 6. Nitrite concentration profiles for the different sediments with time. (\square) Control defaunated sediment; (\blacksquare) inhabited sediment

and inhabited) were within the same range near the sediment surface.

Potential denitrification. After 1 mo, potential denitrification was higher in presence of infauna down to 4 cm depth (Fig. 7). Below this depth, control (C-cores) and inhabited (I-cores) sediments presented the same order of potential denitrifying activities, averaging $70 \mu\text{mol l}^{-1} \text{d}^{-1}$. After 4 mo, the increase of potential

denitrification in the inhabited sediments had spread throughout the sedimentary column. In the first 6 cm, potential denitrification was about 2-fold higher than 3 mo before. After 6 mo, a general decrease in the potential denitrification was found in inhabited sediments. However, denitrification rate was always higher compared to control sediment rate in the first 6 cm.

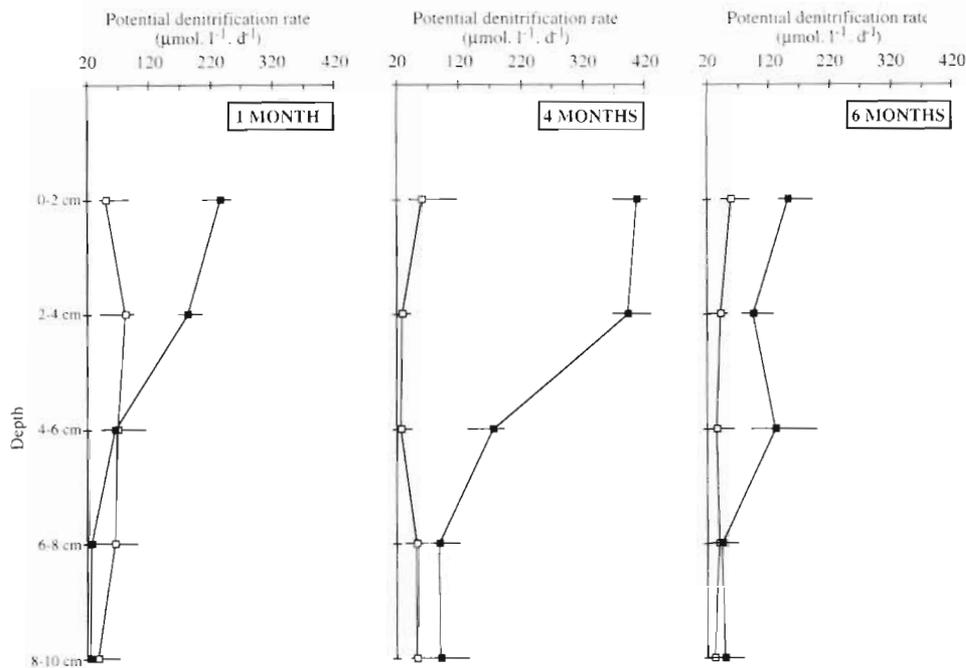


Fig. 7. Potential denitrification rate profiles for the different sediments with time. Mean values and observed ranges are shown. (□) Control defaunated sediment; (■) inhabited sediment

DISCUSSION AND CONCLUSIONS

Although the macrobenthic communities present in sediments may have been determined (major species: *Clymene oerstedii*, *Loripes lacteus*, *Microdeutopus anomalous* and *Venerupis aurea*; Gilbert 1994), in the course of this study, only the consequences of the macrobenthic activity in the sedimentary column are presented and discussed.

The caps were used to limit recolonization in defaunated sediments (C-cores). This physical barrier was chosen instead of chemical contamination (e.g. by tetraethyl lead as used by Gerino 1990) which may perturb the microbial activity rates in sediments. In order to detect and quantify the presence and activity of infauna in cores, we have followed the fate of luminophores deposited at the sediment surface. It appeared that, after 4 mo, no sediment mixing had occurred in control sediments (C-cores). On the other hand, after 6 mo, the burial of luminophores was found down to 6 cm depth, demonstrating a partial recolonization of controls.

Based on the luminophore results, the sedimentary column can be described as a '2 layer' system (Gerino et al. 1993): (1) a subsurface layer ('reworked layer') where the occurrence of reworking activity and macrofauna is highest (between the sediment surface and 4 cm depth for this experiment; Gilbert 1994); the sediment also being well bio-irrigated in this active layer. Below the active layer, the 'bioturbated layer' (2) extends further down to the maximum depth of the burrows.

In order to find evidence of the overall effects of bioturbation on denitrification, 3-way ANOVAs, taking into account the type of sediment (control or inhabited sediment), the biological layer and the time, were used to analyse the denitrification activities.

A 3-way ANOVA carried out on natural denitrifying activity data showed no significant seasonal or spatial variability in denitrification rate. On the other hand, a very significant ($p < 0.001$, $df = 1$, $F = 42.39$) increase of denitrification was found in inhabited sediment ($28.6 \pm 1.4 \mu\text{mol l}^{-1} \text{d}^{-1}$; mean \pm SE) compared to controls ($18.1 \pm 1.6 \mu\text{mol l}^{-1} \text{d}^{-1}$). While denitrification was enhanced 160% by bioturbation, the 'small-scale geometry' (each 2 cm sediment) of this stimulation in the sedimentary column was variable with time. At each sampling time, the stimulation of denitrification was located in the depth layers where the infauna was most active. In the first phase (1 mo), the macrofaunal activity occurred in the sediment down to 6 cm depth. Later (4 mo), the sediment reworking area was extended by infauna to the whole sedimentary column studied (down to 10 cm depth). After 6 mo, the infaunal activity in I-cores still occurred in the first 10 cm of the sediment. However, at this stage, the infauna had recolonized the upper layers of the initially defaunated sediment (C-core). A difference of macrofaunal activity and denitrification between the inhabited (I-core) and control (C-core) was at this stage only found in the deeper layers. As reported in other studies (Sørensen 1978, Law et al. 1991), the presence of burrowing activity of benthic macrofauna was reflected by high denitrification rates in the deeper layers of the sediment.

The 3-way ANOVA on potential denitrification showed significant interactions between the sediment treatment (animals or no animals) and time ($p < 0.001$), and between the sediment treatment and the sediment layer ($p < 0.001$). In control sediments (C-cores), potential denitrification was stable both with time (Fig. 8A) and as a function of the sediment layer (Fig. 8B). On the other hand, in inhabited sediments (I-cores), enhanced potential denitrification was demonstrated throughout the duration of the experiment, with increasing rates between 1 and 4 mo and a subsequent drop after 6 mo (Fig. 8A). Moreover, potential denitrification was higher in the 'reworked layer' than in the 'bioturbated layer' (Fig. 8B), the potential denitrification being from 177 to 529% higher in inhabited than in control sediments ($p < 0.001$). The 'small-scale geometry' pattern of macrofaunal activity and the associated stimulation of potential rate was less evident than that found with natural denitrification. Indeed, the potential denitrification rate reflects the state of the microbial denitrifying population, i.e. the enzyme content of the sediment, but not the actual rate (Tiedje et al. 1982).

In the natural sediments, different events depending on both the activity of macrofauna and its intensity operate in sediments and play a role in the denitrification. In the presence of infauna, sediment reworking and bioirrigation (into the burrows) increase the sediment porosity and the solute exchanges between the sediment and the overlying water. Because of the enhanced oxygen penetration, the oxidized surface layer of the sediment spreads more deeply, which favors nitrification. The nitrate exchanges are also increased by sediment reworking. The macrofauna therefore stimulates denitrification by providing more nitrate for bacteria from 2 sources: the overlying water and nitrification within the sediment. In addition, the presence of oxic (oxygen pocket in the reduced sediment) and anoxic micro-environments (fecal pellets deposited in the oxidized layer) strengthens the proximity and exchanges between nitrification and denitrification.

In our study, we can envisage the major mechanisms implicated in the stimulation of denitrification by the macrobenthos as a function of the dominant bioturbation processes in each 'biological layer'. In the 'reworked layer', the presence of a high sediment reworking activity may have preferentially induced the formation of micro-environments. On the other hand, in the 'bioturbation layer', the bioirrigation associated with the burrows may have resulted in the stimulation of denitrification by enhancing the exchanges of solutes between the water and the sediment. However, it appears too that there was no evidence of an increase of nutrient availability (NO_3^- and

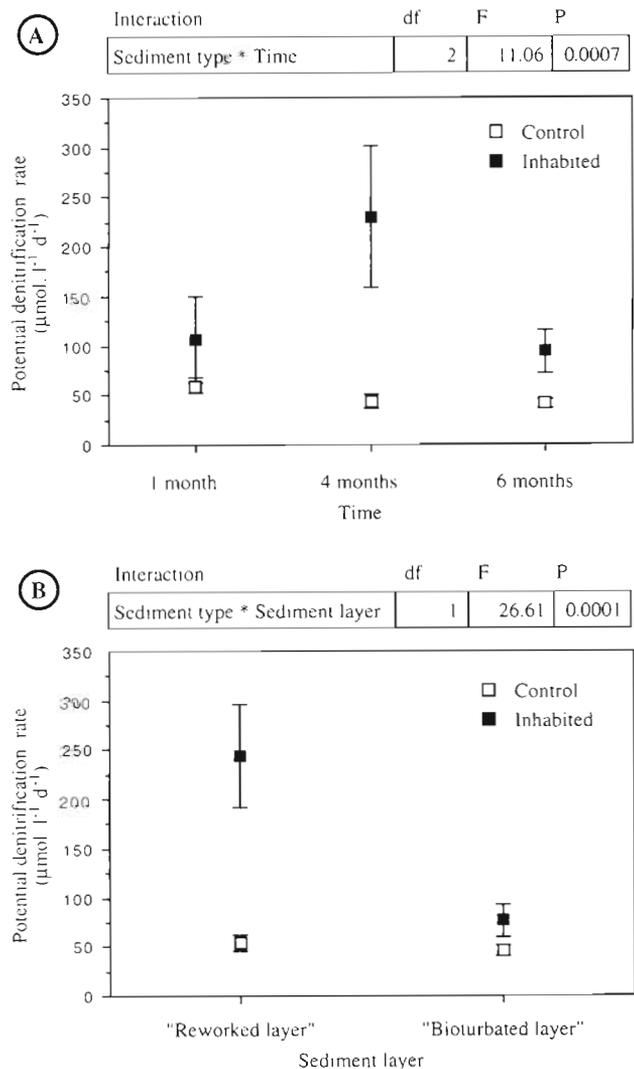


Fig. 8. Potential denitrification rate in the different sediments. Standard errors of the mean are shown. ANOVA comparison procedure indicates significant interactions ($p < 0.001$) between (A) the type of sediment (control or inhabited) and time, and (B) the type of sediment and the sediment layer (reworked or bioturbated layer) ($p < 0.001$)

NO_2^-) due to bioturbation. This could be explained by the rapid consumption of nutrients by denitrification (Sayama & Kurihara 1983, Hüttel 1990) but also by problems relative to the experimental procedure used (the thickness of sediment slice attenuating the nutrient profiles).

Our experiment was carried out in a natural sediment occupied by an assemblage characteristic of muddy sand in sheltered areas. It appears that the *in situ* increases of the denitrification rates induced by the whole macrobenthic community are within the same range as those found in previous monospecific laboratory and field experiments (Table 1). From their

Table 1. Denitrification stimulation rates induced by the benthic macrofauna in estuarine and coastal sediments. MSSA assemblage: macrofaunal assemblage in muddy sand in sheltered areas (Pérès 1982)

Experimental conditions	Bioturbating organism	Density (ind. m ⁻²)	Denitrification stimulation rate (%)	Source
Laboratory				
Columns	<i>Limnodrilus hoffmeisteri</i> and <i>Tubifex tubifex</i> (1/1)	12 000	180	Chatarpaul et al. (1980)
Aquaria	<i>Nereis virens</i>	2 000	171	Henriksen et al. (1980)
Aquaria	<i>Corophium volutator</i>	6 000	161	Henriksen et al. (1980)
Aquaria	<i>Neanthes japonica</i>	1 000	300	Sayama & Kurihara (1983)
Cores	<i>Nereis virens</i>	1 807	240	Kristensen & Blackburn (1987)
V-cores	<i>Nereis virens</i>	875	300	Kristensen et al. (1991)
Microcosms	<i>Corophium volutator</i>	19 800	300 to 500	Pelegri et al. (1994)
Cores	<i>Nereis diversicolor</i>	1 433	114	Gilbert et al. (1995)
Field				
Cores	Amphipods (<i>Corophium</i> spp.)	3 000 9 000	400 950	Rysgaard et al. (1995)
Cores	MSSA assemblage	3 160	160 to 529	Present study

in situ experiment, Rysgaard et al. (1995) concluded that the stimulatory effect of infauna during summer is of minor importance with regard to the annual denitrification budget due to low NO₃⁻ concentrations and nitrification potentials, although the benthic infauna density generally is highest in summer. Even so, our field study, based on the comparison between control defaunated and inhabited sediments, indicates that in presence of bioturbating macrofauna the denitrification rate is always higher than that recorded in uninhabited sediment.

We conclude that bioturbation by benthic macrofauna significantly stimulates *in situ* sediment denitrification and that this stimulation of denitrification, which is associated with the sediment layer where the infauna is most active, can occur at different depths in the sediment.

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