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**To link to this article:** DOI: 10.1023/A:1002931432250  
URL: <http://dx.doi.org/10.1023/A:1002931432250>

**To cite this version:** Gilbert, Franck and Stora, Georges and Bonin, Patricia and LeDréau, Yveline and Mille, Gilbert and Bertrand, Jean-Claude *Hydrocarbon influence on denitrification in bioturbated Mediterranean coastal sediments*. (1997) *Hydrobiologia*, vol. 345 (n° 1). pp. 67-77. ISSN 0018-8158

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# Hydrocarbon influence on denitrification in bioturbated Mediterranean coastal sediments

Franck Gilbert<sup>1,\*</sup>, Georges Stora<sup>1</sup>, Patricia Bonin<sup>1</sup>, Yveline LeDréau<sup>2</sup>, Gilbert Mille<sup>2</sup> & Jean-Claude Bertrand<sup>1</sup>

<sup>1</sup>Laboratoire d'Océanographie et de Biogéochimie, Centre d'Océanologie de Marseille (OSU), Université de la Méditerranée, UMR CNRS n° 6535, Campus de Luminy, Case 901, F-13288 Marseille Cedex 9, France

<sup>2</sup>Laboratoire de Chimie Analytique de l'Environnement, Université d'Aix-Marseille III, URA CNRS n° 1049, Faculté de Saint-Jérôme, avenue Escadrille Normandie-Niemen, Boite 312, F-13397 Marseille Cedex 20, France

\*Author for correspondence (e-mail: gilbert@com.univ-mrs.fr)

*Key words:* bioturbation, denitrification, hydrocarbons, macrofauna, organic matter, in situ experiment

## Abstract

An *in situ* experiment was carried out in bioturbated Mediterranean coastal marine sediments (Gulf of Fos) in order to study the influence of hydrocarbons on denitrification after 1, 4 and 6 months. In the absence of hydrocarbons in the control sediments, the presence of macrofauna stimulated denitrification by 160%. This stimulation is induced by sediment reworking that favours both direct NO<sub>3</sub><sup>-</sup> supply from the water column and the penetration of O<sub>2</sub>, which in turn stimulated nitrification, the other source of NO<sub>3</sub><sup>-</sup> in sediment. The presence of hydrocarbons in the experimental sediments either stimulated or inhibited the denitrification. The denitrification response to the presence of hydrocarbon is dependent on the quantity of matter buried by the macrofauna activity. In small quantities, the organic matter related to hydrocarbons 120% enhanced the denitrification compared to the controls. On the other hand, when buried hydrocarbon concentrations were higher (>100 mg saturated hydrocarbon fraction kg<sup>-1</sup> dry sediment), the denitrification was inhibited.

On the basis of the results obtained, a descriptive model of the patterns of denitrification in relation to the presence of macrofauna and the distribution of hydrocarbons in sediments is proposed.

## Introduction

In marine sediments, denitrification is regulated by the O<sub>2</sub> concentration (Betlach & Tiedje, 1981; Jørgensen et al., 1984), while the availability of NO<sub>3</sub><sup>-</sup> (King & Nedwell, 1987) or the concentration of available carbon (Slater & Capone, 1987; Brettar & Rheinheimer, 1992) control often the rate of this process.

Bioturbation plays an important role in the exchange at the water-sediment interface, while numerous works have demonstrated the influence of bioturbation on denitrification through enhanced NO<sub>3</sub><sup>-</sup> and O<sub>2</sub> supply and coupled nitrification-denitrification (Grundmanis & Murray, 1977; Chatarpaul et al., 1980; Aller et al., 1983; Sayama & Kurihara, 1983; Kristensen, 1984; Kristensen & Blackburn, 1987; Enoks-

son & Samuelsson, 1987; Kristensen et al., 1991; Pellegrini et al., 1994; Gilbert et al., 1995; Rysgaard et al., 1995). On the other hand, the effect of the organic matter redistribution at the sediment-water interface controlled by the benthic macrofauna on denitrification have received comparatively little attention (Li et al., 1990; Sloth et al., 1995).

Various works have focused on the reworking of sediment and adsorbed hydrocarbons induced by macrobenthos activity (Gordon et al., 1978; Gardner et al., 1979; Karickhoff & Morris, 1985; Bauer et al., 1988; Koerting-Walker & Buck, 1989; MacElroy, 1990; Gilbert et al., 1994; Gilbert et al., 1996). The effects of hydrocarbons on denitrification have also been studied (Haines et al., 1981; Griffiths et al., 1982; Bonin et al.,

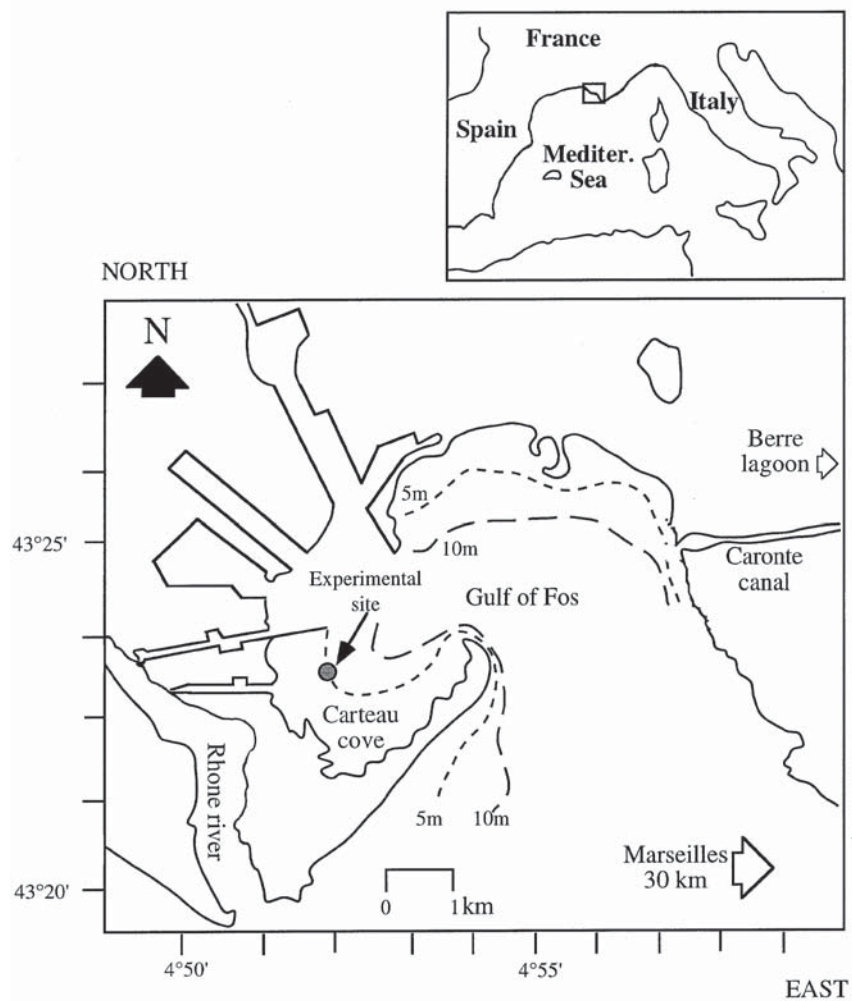


Figure 1. Experimental site in the Fos Gulf (Mediterranean Sea, France).

1990). However, the link between the two processes has not yet been established.

The aim of this work was to investigate experimentally the *in situ* impact of hydrocarbons on denitrification in bioturbated Mediterranean coastal sediments.

## Materials and methods

### *Experimental site*

The site was chosen on the shallow bottom of the Carteau cove situated on the western side of the Gulf of Fos (Figure 1). Experiments were carried out at 5 m depth. The sediment is rich in organic matter and is

classified as 'muddy sand sediment' (25 to 50% grains less than  $63 \mu\text{m}$ ), and is occupied by a characteristic muddy sand in sheltered areas assemblage (Pères, 1982). During our experiment, polychaetes dominated the benthic macrofauna (70%) and crustaceans were the second most dominant group (25%). The other faunistic groups present were the molluscs and echinoderms (5%). The total macrofaunal benthic density was from 2580 to 3160 ind.  $\text{m}^{-2}$ . More than 80% of the organisms were located in the first four cm of sediment (Gilbert, 1994).

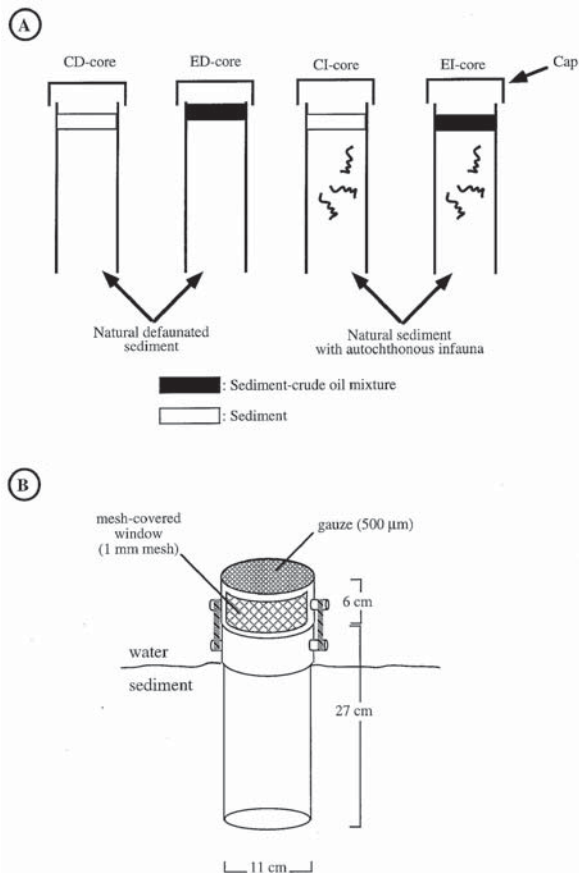


Figure 2. Presentation of the different types of experimental cores collected at each sampling time (A) and experimental core design (B). For the name of each core, see text.

### Experimental procedure

Twenty one cores divided into 4 groups (Figure 2A) were inserted into the sediment:

- 6 CD-cores (control defaunated sediment): natural, defaunated sediment, to assess sediment reworking resulting from non-biological processes (e.g. hydrodynamics);
- 6 CI-cores (control inhabited sediment): natural sediment with autochthonous fauna;
- 3 ED-cores (experimental defaunated sediment): natural, defaunated sediment with added crude oil (Arabian Light crude oil), to estimate possible hydrocarbon passive diffusion processes into the sedimentary column;
- 6 EI-cores (experimental inhabited sediment): natural sediment with autochthonous fauna and added crude oil.

### Experimental cores

The cores were made from 27 cm long sections of 11 cm diameter PVC tubing (Figure 2B). Caps were designed to slow down the recolonization of the defaunated control sediments by macrofauna, without confining the area overlying the sediment surface. The caps were made with 6 cm long PVC tubes (diam: 11 cm). The top of the caps were closed with gauze (500 µm mesh). The sides of the caps contain two mesh-covered windows (11.2 cm-long, 3.8 cm-high, 1 mm mesh). To ensure standard experimental conditions, all the cores (defaunated and inhabited) were equipped with caps.

### Sediment cake preparation

A mixture of Arabian Light crude oil and sediment was used to contaminate the cores. Nine hydrocarbon cakes (7048 mg saturated hydrocarbon fraction  $\text{kg}^{-1}$  dry sediment) were made by filling moulds (i.d.: 10 cm, 1 cm thick) (Gilbert et al., 1994). The non-contaminated cores received a similar sediment cake without added crude oil in order to standardize the experimental conditions. The mixing of sediment was assessed by means of the luminophore tracer technique (Gerino, 1990). A mixture of two size fractions of luminophores, 40–60 µm diameter and 150–200 µm (1 g per fraction), was added at the surface of the cakes. All the cakes were then frozen.

### Sediment defaunation

Sediments were defaunated by an exclusion/transplant process: nine sediment samples were taken by divers with experimental cores and transported to the laboratory. In the laboratory, sediments were defaunated by the  $\text{N}_2$  method, the impact of this defaunation procedure on the microbial community being assumed to be insignificant (Kristensen et al., 1991).

### Implantation of cores

After laboratory treatment, the defaunated sediment cores were transported to the experimental site and with 12 other cores 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. After each sampling (after 1, 4 and 6 months), sediment cores were transported

to the laboratory and sectioned to 2 cm thick segments from the top down to 10 cm before analysis.

### Analyses

#### Luminophores

When removed, each segment of sediment was dried at 70 °C for a week, carefully mixed to homogenize sediment and luminophores, and sieved through a 500 µm mesh. For each segment, 3 subsamples of 0.25 g were taken. The luminophore counts were then conducted under an ultra-violet light source (Gerino, 1990).

#### Hydrocarbons

The extraction of petroleum hydrocarbons from the sediment was achieved by alkaline digestion as described by Mille et al. (1988). The fractionation and capillary gas chromatography analyses of the saturated fraction of hydrocarbons (SF) were those used by Gilbert et al. (1994) using n-icosylene as internal standard (Carlo Erba 4160 FID chromatograph; glass capillary column SE 52 with helium as carrier gas; temperature programmed (i) from 70 to 285 °C at 5 °C min<sup>-1</sup>, (ii) 20 min-isotherm, (iii) from 285 °C to 295 °C at 10 °C min<sup>-1</sup>; (iv) 10 min-isotherm.

#### Denitrification

Denitrification rates were assessed using the acetylene-blockage method (Balderston et al., 1976). Each segment of sediment (4 ml) was placed into 13 ml tubes containing 4 ml of sterilized seawater. The tubes were sealed with rubber stoppers and rendered anoxic by flushing N<sub>2</sub> through the tubes. Acetylene (15% of the total volume of tube), which inhibits the reaction from N<sub>2</sub>O to N<sub>2</sub>, was flushed through the tubes. Two tubes were analysed after 0, 0.5, 1 and 3 hours of incubation at 20 °C. After incubation, each tube was vigorously shaken by hand for 2 minutes and harvested at 2000 × g for 3 min. Three millilitres of gas phase were injected into a pre-evacuated venoject tube for later N<sub>2</sub>O analysis. The extraction of N<sub>2</sub>O from the liquid phase was carried out using the procedure of Chan & Knowles (1979) modified by the technique of multiple equilibrium (Mac Aulife, 1971).

N-compounds were measured in the supernatant obtained after centrifugation at 2000 × g for 10 min. Nitrates were reduced on a Cu-Cd column adapted to Technicon II according to Treguer & Le Corre (1975). Nitrite concentrations were determined colorimetrically by the method of Bendschneider & Robinson (1972).

Table 1. In situ vertical concentration of added hydrocarbons (mg saturated fraction kg<sup>-1</sup> dry sediment) in the sedimentary column for the experimental sediments, with time. Values are mean ± standard error (n=3). For the name of each core, see text.

Depth	ED-core	EI-core
<b>1 month</b>		
0-2 cm	2482±51	2187±84
2-4 cm	0	274±77
4-6 cm	0	103±44
<b>4 months</b>		
0-2 cm	1387±40	1122±73
2-4 cm	0	258±53
4-6 cm	0	101±60
<b>6 months</b>		
0-2 cm	932±43	835±59
2-4 cm	72±15	467±68
4-6 cm	30±14	175±31

#### Data analysis

Differences between control and inhabited sediments and the space-time variations of denitrifying activities were studied using a three-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

Figure 3 presents, for the different experimental conditions, the vertical distribution of the luminophores in the sedimentary column with time. In inhabited sediments, although the sediment reworking may be different in presence (EI-core) or absence (CI-core) of petroleum hydrocarbons, the results showed the burying of luminophores down to 10 cm depth with a preferential accumulation with time in the 2-4 cm layer. On the other hand, in the different defaunated sediments (CD- and ED-core) the quantities of luminophores measured were very close. After 4 months, in defaunated cores (control and experimental), buried luminophores were found in the 2-4 cm layer, and after 6 months down to 6 cm depth.

The effects of sediment reworking by fauna on the distribution of added crude oil hydrocarbons sat-

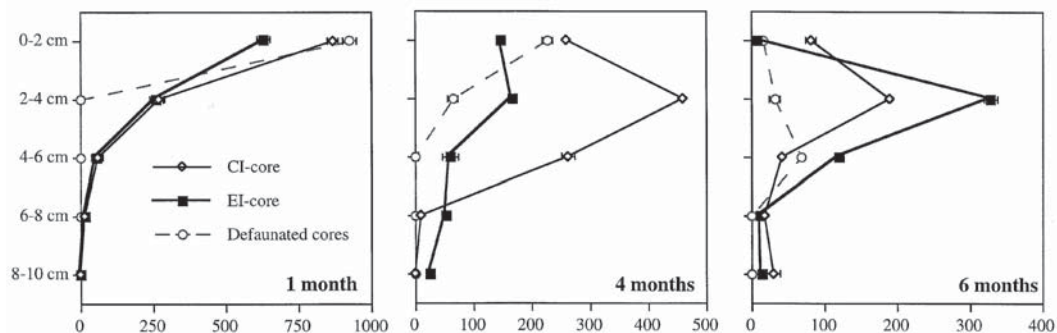


Figure 3. In situ vertical distribution of luminophores X-axis; (expressed in g per volume) in the sedimentary column for the different sediments, with time. Standard errors appear above the mean values ( $n=3$ ). CD- and ED-core values are regrouped in 'Defaunated cores'. For the name of each core, see text.

urated fraction (SF) in the sedimentary column are shown in Table 1, that presents the SF concentrations in the contaminated sediments, in presence (EI-core) or absence (ED-core) of macrofauna. In presence of reworking macrofaunal activity in contaminated sediment (EI-core), petroleum hydrocarbons were buried in the first 6 cm throughout the experiment duration; SF concentrations ranged from 101 to 468 mg kg<sup>-1</sup> dry sediment between 2 to 6 cm depth (Table 1). In experimental, defaunated sediment (ED-core), hydrocarbons remained at the sediment surface until the fourth month. After 6 months, only small amounts of hydrocarbon, 72 and 31 mg SF kg<sup>-1</sup> dry sediment for the 2–4 and 4–6 cm layer, respectively, were found in ED-core.

#### Denitrification

Figure 4 presents the denitrification rates measured in the five layers of the different sediments with time, which ranged from 2.8 to 11.1 nmol l<sup>-1</sup> h<sup>-1</sup> for NO<sub>3</sub><sup>-</sup> concentrations between 0.1 and 6.7 μM (Table 2).

After one month, in control sediments, denitrification was higher in presence of infauna down to 6 cm depth. Below this depth, defaunated (CD-core) and inhabited (CI-cores) sediments presented the same denitrification rates. In experimental sediments (ED- and EI-cores), denitrification rates were similar to or lower than those found in control defaunated sediment.

After 4 months, in control sediments, the increase of the denitrification in the inhabited sediments (CI-cores) was found in virtually the whole sedimentary column. In presence of crude oil, in both defaunated and inhabited sediments, denitrification rates presented the same pattern as previously observed.

Table 2. In situ vertical NO<sub>3</sub><sup>-</sup> concentrations (μM) profiles for the different sediments, with time. For the name of each core, see text.

Depth	CD-core	ED-core	CI-core	EI-core
<b>1 month</b>				
0–2 cm	3.2	3.2	2.4	6.7
2–4 cm	2.6	0.1	2.8	0.2
4–6 cm	0.9	0.5	4.3	0.3
6–8 cm	3.1	1.4	2.7	2.9
8–10 cm	2.2	2.0	3.4	2.3
<b>4 months</b>				
0–2 cm	0.8	3.8	0.4	2.1
2–4 cm	1.1	1.7	0.5	0.3
4–6 cm	1.9	1.1	1.1	0.4
6–8 cm	3.1	1.9	0.3	0.4
8–10 cm	2.0	2.4	0.8	0.4
<b>6 months</b>				
0–2 cm	2.7	2.4	2.3	0.8
2–4 cm	2.4	4.0	2.1	2.1
4–6 cm	2.8	3.0	3.7	2.1
6–8 cm	3.4	2.3	2.3	1.4
8–10 cm	2.6	2.2	2.6	1.0

After 6 months, in contrast to the first month's results, the enhancement of denitrification in the control inhabited sediment was located at depth (4–10 cm). Nearest the sediment surface, the denitrification rates of the two types of control sediment (defaunated and inhabited) were within the same range. While experimental, inhabited (EI-core) sediment always presented the lowest denitrification rates, the rates measured in experimental defaunated sediment were enhanced and reached the levels found in control inhabited sediments.

Table 3. In situ denitrification rates ( $\text{nmol l}^{-1} \text{h}^{-1}$ ). Minimal and maximum values obtained for each sediment. Values (linear initial rate of  $\text{N}_2\text{O}$  accumulation) were calculated from data ( $\text{N}_2\text{O}$  quantities) measured after 0, 0.5, 1 and 3 hours of incubation. The asterisk (\*) indicate that the number of data used to calculate the denitrification rate was lower than the total data number. For the name of each core, see text.

Depth	CD-core		ED-core		CI-core		EI-core		
	min	max	min	max	min	max	min	max	
<b>1 month</b>									
0–2 cm	4.7	6.4	2.6	8.4	6.6 *	12.1	3.2	8.7	
2–4 cm	3.5	6.6	3.2 *	6.1 *	5.1	9.2	4.0	6.5 *	
4–6 cm	5.8	9.0	3.4	5.9	8.1	9.8	0.5 *	8.0	
6–8 cm	4.9	10.5	2.0	9.9	5.8	10.0	2.2	7.7	
8–10 cm	3.6	6.8	3.4	7.9	5.7	8.0	0.9	7.5	
<b>4 months</b>									
	min	max	min	max	min	max	min	max	
0–2 cm	3.9	7.5	3.5	5.5	3.4	7.4	3.5	4.6	
2–4 cm	2.6 *	7.7	2.8 *	5.6	5.5	11.4 *	4.1	9.0 *	
4–6 cm	0.5	7.7	2.7	6.6	10.2	12.2	2.7	7.7 *	
6–8 cm	5.7	7.1	4.3	8.4	6.9	11.0 *	5.0	7.0	
8–10 cm	2.2	8.6	3.1	6.9	6.8	10.2	4.8	6.5	
<b>6 months</b>									
	min	max	min	max	min	max	min	max	
0–2 cm	3.7	10.2	3.5	7.2	4.2	5.7	0.2	4.7	
2–4 cm	1.6	5.9	3.6	7.3	3.2 *	7.5	2.1	4.0	
4–6 cm	0.0	4.5	6.4	11.3	6.8	12.9	0.5	1.7	
6–8 cm	2.8	2.9	8.3	13.6	6.7	10.4	0.2	4.4	
8–10 cm	2.2	3.6	5.3	7.7	7.3	10.6	0.4	4.6 *	

## Discussion

The purpose of our experiment was to determine the *in situ* effects of hydrocarbons on denitrification in bioturbated coastal sediments.

To achieve this, the sediment reworking activity of the macrofauna has been studied using the luminophore technique. On the basis of the luminophore results, the sedimentary column can be described as a 'two-biological layer' system, in view of the presence of a reworked layer where the occurrence of sediment reworking and macrofauna are highest (between the sediment surface and 4 cm depth for this experiment; Gilbert, 1994). In this layer, the sediment is well bio-irrigated (Gerino et al., 1993). Below this first layer, the bioturbated layer extends down to the maximum depth of the burrows (10 cm).

In order to find evidence on the overall effects of hydrocarbons on denitrification related to bioturbation, three-way ANOVA taking into account the type of sediment, the biological layer and time were used to analyse the denitrification activities.

The three-way ANOVA performed with denitrifying activity data showed no significant seasonal ( $P=0.2$ ) or spatial ( $P=0.09$ ) variability in denitrification rate. On the other hand, significant differences ( $P<0.001$ ) were found between the four types of sediments (Table 4).

Our results, by comparing inhabited and defaunated control sediments (without added crude oil), show a 160% stimulating effect of bioturbation on denitrification (Table 4). This rate of stimulation is in accordance with the results obtained *in vitro* by several authors who have indicated stimulation values ranging from 114 to 500% (Grundmanis & Murray, 1977; Chatarpaul et al., 1980; Henriksen et al., 1980; Aller et al., 1983; Sayama & Kurihara, 1983; Kristensen, 1984; Kristensen & Blackburn, 1987; Kristensen et al., 1991; Pelegri et al., 1994; Gilbert et al., 1995). Similarly, in the natural environment, Rysgaard et al. (1995) have measured a 400% stimulating effect of bioturbation on denitrification.

In the experimental inhabited sediments (EI-core), comparing to the control defaunated sediments, a 83% decrease of denitrification was demonstrated. This

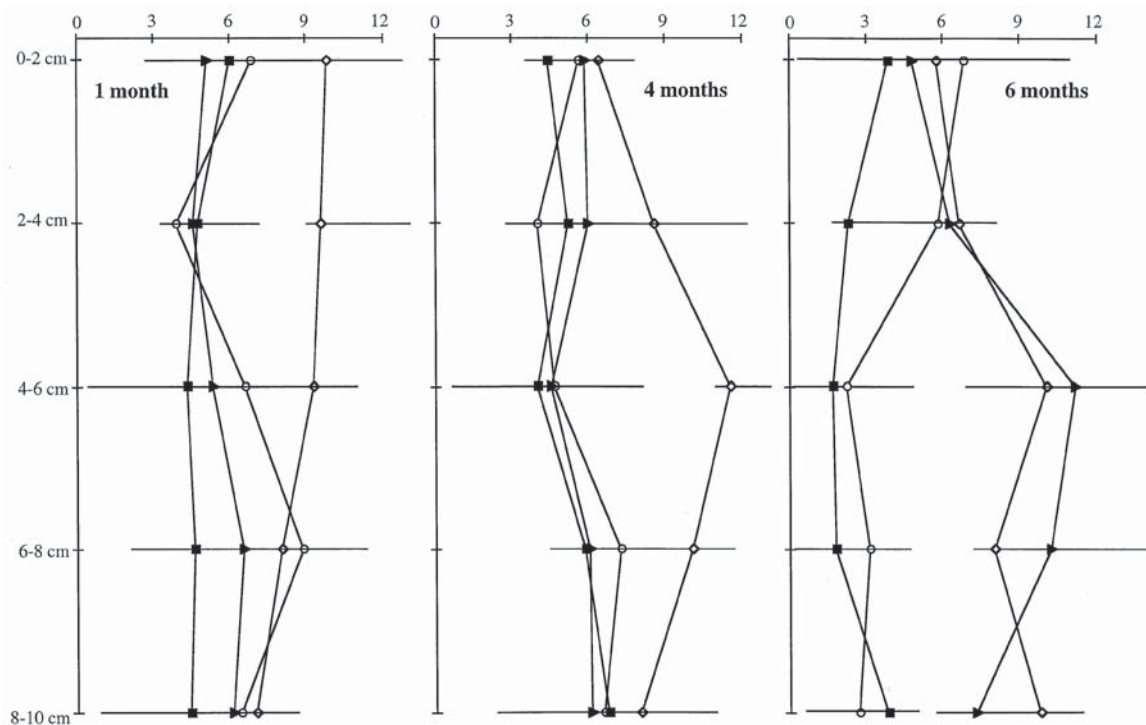


Figure 4. In situ denitrification rate X-axis; ( $\text{nmol l}^{-1} \text{h}^{-1}$ ) profiles for the different sediments, with time. The profiles present mean values associated with minimal and maximum values measured (cf. Table 2).  $\circ$ : CD-core;  $\square$ : CI-core;  $\blacktriangle$ : ED-core;  $\blacksquare$ : EI-core. For the name of each core, see text.

Table 4. In situ denitrification rates ( $\text{nmol l}^{-1} \text{h}^{-1}$ ). Values are mean  $\pm$  standard error for the sediments (A), time (B) and the biological layers (C). ANOVA analysis comparing the type of sediments, time and sediment biological layers for the in situ natural denitrification rates measured indicates a significant difference with 'Sediments' ( $P < 0.001$ ). No significant effect is found with 'Time' ( $p=0.2$ ) or 'Biological layer' ( $p=0.09$ ). For the name of each core, see text. Reworked layer: 0–4 cm; Bioturbated layer: 4–10 cm.

(A)			
CD-core	ED-core	CI-core	EI-core
$5.0 \pm 0.5$	$7.9 \pm 0.4$	$6.0 \pm 0.7$	$4.2 \pm 0.4$
(B)			
1 month	4 months	6 months	
$5.8 \pm 0.4$	$6.3 \pm 0.5$	$5.3 \pm 0.6$	
(C)			
Reworked layer	Bioturbated layer		
$5.4 \pm 0.3$	$6.1 \pm 0.5$		

result is in accordance with *in vitro* (Bonin et al., 1990) and *in situ* (Haines et al., 1981; Griffiths et al., 1982) previous work in which an inhibitory effect of crude oil on denitrification was found. On the other hand,

in experimental defaunated sediments (ED-core), in presence of hydrocarbons, the results showed a global 120% stimulation of denitrification compared to the control defaunated sediments (CD-core).

This variable overall sediment denitrification response to crude oil may be explained by the different rate of hydrocarbon burying in the sedimentary column due to the macrofauna, in ED- and EI-cores. The study of the small-scale geometry of the denitrification (Figure 4) and hydrocarbon distribution patterns (Table 1) in the sedimentary column shows that until the fourth month the denitrification was reduced in the whole sedimentary column by the presence of crude oil, either at the sediment surface (experimental, defaunated sediment) or both at the sediment surface and at depth ( $\text{SF} > 100 \text{ mg kg}^{-1}$  dry sediment; experimental, inhabited sediment), suggesting similar environmental conditions reducing denitrification, e.g. anoxic conditions and concomitant inhibition of nitrification and stimulation of sulfate reduction (Griffiths et al., 1982; Bauer et al., 1988; Bonin et al., 1990; Sloth et al., 1995). On the other hand, after 6 months, when more than 60% of the crude oil hydrocarbons initially deposited



had been removed to the water column (Gilbert et al., 1996), the denitrification rate patterns were different in the two experimental sediments. In the inhabited sediment (EI-core; 2118 ind. m<sup>-2</sup>), the concentrations of buried hydrocarbons were higher than previously and denitrification was always inhibited in the whole sedimentary column. At the same time, in defaunated sediment, the recolonization of defaunated sediment by macrofauna (ED-core; 1262 ind. m<sup>-2</sup>) induced the burying of smaller quantities of hydrocarbons at depth where denitrification was then stimulated.

As suggested in other works (Li et al., 1990; Sloth et al., 1995) in which the authors found that a moderate rate of organic matter mixing in the sedimentary column increased denitrification, which was decreased in presence of a high concentration of organic matter either mixed in the sediment or deposited at the sediment surface, our results demonstrate the important role of mixing processes (e.g. bioturbation) on the loading and distribution of organic matter and subsequent fate of nitrogen in the sediments.

However, this stimulation of denitrification and other microbial activities (CO<sub>2</sub> production, methanogenesis, N<sub>2</sub> fixation; Li et al., 1990) by low organic matter concentrations suggests that the microbes were carbon-limited and that the microflora could have easily degraded some of the added hydrocarbons. In our experimental sediments, the presence of biodegraded hydrocarbons both at the sediment surface and throughout the whole sedimentary column was noted from the fourth month (Gilbert et al., 1996). Considering the complexity of bioturbating activity, it appeared that this may result from:

- the aerobic biodegradation of hydrocarbons at the sediment surface followed by their burying in the reduced compartment of sediment by the infauna activity;
- the reworking of sediment by macrofauna, which allows aerobic biodegradation by O<sub>2</sub> in the sediment subsurface layers (Aller & Yingst, 1985; Kikuchi, 1986; Forster & Graf, 1992); moreover, bioturbation creates oxic micro-environments where aerobic microbial degradation occurs and induces a repetitive oscillation of redox conditions in the sediment, stimulating organic matter remineralization (Aller, 1995);
- the possible efficient anaerobic biodegradation of hydrocarbons (Bertrand et al., 1989; Rueter et al., 1994; Rabus & Widdel, 1995).

These results emphasize that in the natural sediments, the bioturbation process is very complex and

depends on both the different activities of macrofauna (e.g., sediment reworking, conveying, regeneration, bioirrigation) and their intensity, controlled by the abundance of the macro-organisms present. We can nevertheless envisage the different events that operate in sediments in the presence of macrofauna and propose a descriptive model of the patterns of change in denitrification in function of the presence of macrofauna and the distribution of hydrocarbons in sediments.

In non-bioturbated sediments, the oxidized layer where nitrification occurs is very thin (only a few millimeters). The distribution of the nitrate in the reduced lower layer (denitrification layer) is limited by the compactness of the sediment. Deeper down, sulfate reduction is the dominant bacterial metabolism (Figure 5A).

In the presence of infauna, sediment reworking increases the sediment porosity and the solute exchanges between the sediment and the overlying water (Figure 5B). Because of the enhanced O<sub>2</sub> penetration, the oxidized surface layer of the sediment spreads more deeply, which favors the expression of nitrification. The NO<sub>3</sub><sup>-</sup> are also increased by sediment reworking. The macrofauna therefore stimulates denitrification by providing more nitrate for bacteria from two 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. An strong loading of hydrocarbons at the surface of a non-bioturbated sediment rapidly induces the establishment of anoxic conditions at the surface through the consumption of the oxygen by microbes (for organic matter degradation), and the possible occurrence of a 'covering effect' limiting the penetration of the solutes. These anoxic conditions both inhibit nitrification and strongly stimulate sulfate reduction whose production of sulfide inhibits denitrification (Figure 5C).

The presence of bioturbating macrofauna in such sediments induces (Figure 5D):

- the penetration of the oxygen that reduces the anoxia of the sediment at the surface, stimulating nitrification and displacing the sulfate-reduction to the deeper layers;
- the increased penetration of nitrate in the sediment that favors denitrification;
- the creation of oxic and anoxic micro-niches;
- the removal of the hydrocarbons to the water column, allowing solute exchanges between sea water and sediment;

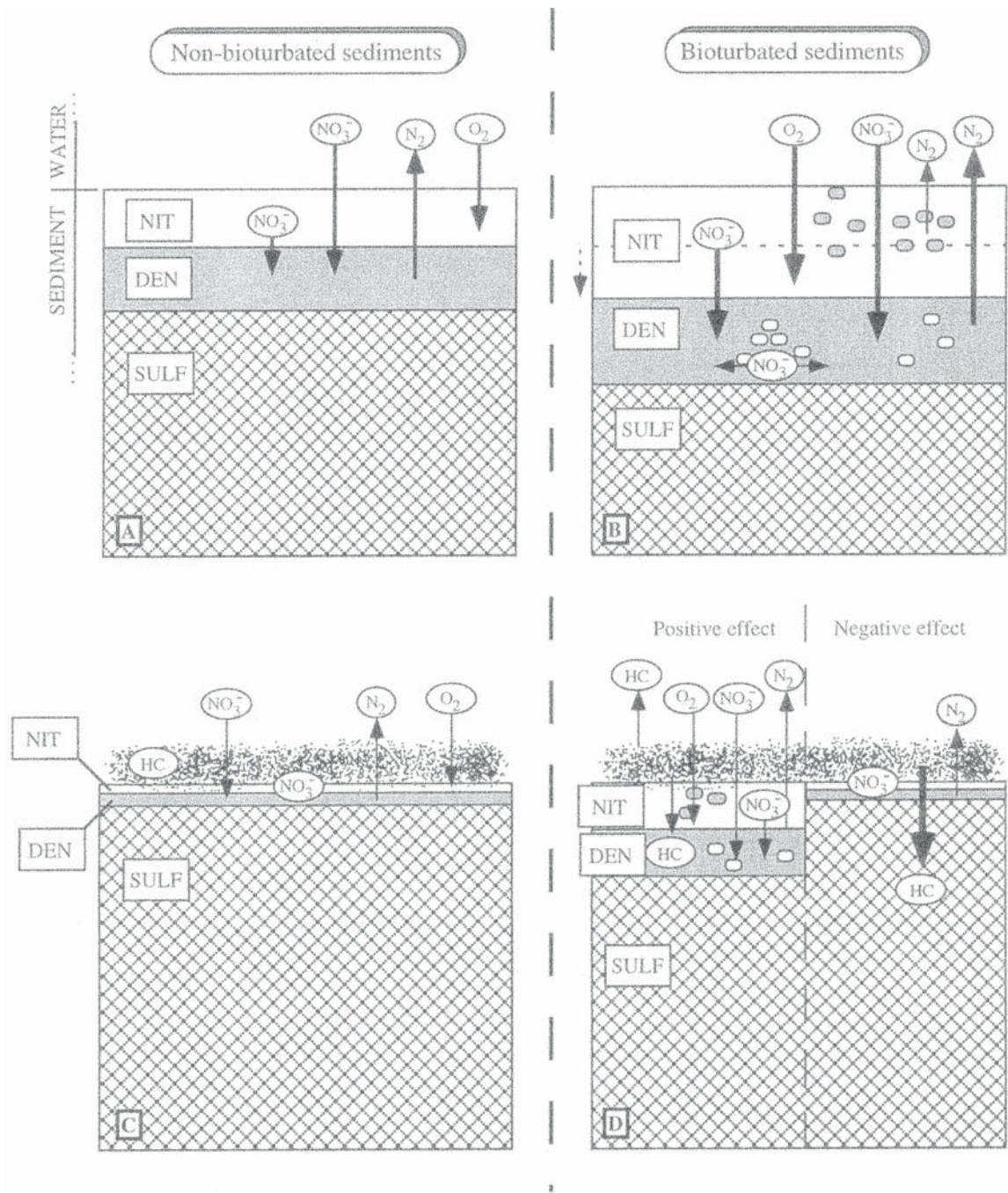


Figure 5. Denitrification as a function of the presence of macrofauna and the distribution of hydrocarbons in sediments. The thickness of layers and arrows are representative of the intensity of the processes. HC: hydrocarbons; NIT: nitrification, DEN: denitrification, SULF: sulfate reduction.

– hydrocarbon burying that either stimulates denitrification when it is weak or strengthens the inhibition of denitrification by intensifying anoxic con-

ditions and sulfate reduction when the quantities buried are large.

## Conclusions

In presence of a hydrocarbon loading at the sediment surface, the effect of bioturbation on denitrification is dependent on the quantity of matter buried by the macrofauna activity. In small quantities, the organic matter related to hydrocarbons enhances the denitrification. On the other hand, when buried hydrocarbon concentrations increase, the denitrification is inhibited. Works are now to be carried out in order to determine the precise hydrocarbon concentration at which the response of denitrification switches between stimulation and inhibition. Moreover, if the bioturbation efficiency to regulate the denitrification is well demonstrated, it is necessary to determine and quantify the processes involved on this regulation, e.g. are the stimulation and the inhibition of denitrification linked with the expression of nitrification, source of  $\text{NO}_3^-$  into the sediment?

## Acknowledgments

This work was carried out in the framework of the programme 'GDR HYCAR n°1123: Cycles biogéochimiques des hydrocarbures naturels et anthropiques en milieu marin' and supported by the Centre National de la Recherche Scientifique (CNRS), Universities and Elf Aquitaine.

## References

- Aller, R. C., 1995. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chem. Geol.* 114: 331–345.
- Aller, R. C. & J. Y. Yingst, 1985. Effects of the marine deposit-feeders *Heteromastus filiformis* (Polychaeta), *Macoma baltica* (Bivalvia), and *Tellina texana* (Bivalvia), on averaged sedimentary solute transport, reaction rates, and microbial distribution. *J. mar. Res.* 43: 615–645.
- Aller, R. C., J. Y. Yingst & W. J. Ullman, 1983. Comparative biogeochemistry of water in intertidal *Onuphis* (Polychaeta) and *Upogebia* (Crustacea) burrows: temporal patterns and causes. *J. mar. Res.* 41: 571–604.
- Balderston, W. L., B. Sherr & W. J. Payne, 1976. Blockage by acetylene of nitrous oxide production in *Pseudomonas perfectomarinus*. *Appl. envir. Microbiol.* 81: 504–508.
- Bauer, J. E., P. A. Montagna, R. B. Spies, M. C. Prieto & D. Hardin, 1988. Microbial biogeochemistry and heterotrophy in sediments of a marine hydrocarbon seep. *Limnol. Oceanogr.* 33: 1493–1513.
- Bendschneider, K. & R. J. Robinson, 1972. A new spectrometric method for determination of nitrite in sea water. *J. mar. Res.* 11: 87–96.
- Bertrand, J.-C., P. Caumette, G. Mille, M. Gilewicz & M. Denis, 1989. Anaerobic degradation of hydrocarbons. *Sci. Prog., Oxf.* 73: 333–350.
- Betlach, M. R. & J. M. Tiedje, 1981. Kinetic explanation for accumulation of nitrite, nitric oxide and nitrous oxide during bacterial denitrification. *Appl. envir. Microbiol.* 42: 1074–1084.
- Bonin, P., M. Gilewicz, E. Rambeloarisoa, G. Mille & J.-C. Bertrand, 1990. Effect of crude oil on denitrification and sulfate reduction in marine sediments. *Biogeochem.* 10: 161–174.
- Brettar, I. & G. Rheinheimer, 1992. Influence of carbon availability on denitrification in the central Baltic Sea. *Limnol. Oceanogr.* 37: 1146–1163.
- Chan, Y. K. & R. Knowles, 1979. Measurement of denitrification in two freshwater sediments by an in situ acetylene inhibition method. *Appl. envir. Microbiol.* 37: 1067–1072.
- Chatarpaul, L., J. B. Robinson & N. R. Kausmik, 1980. Effects of tubificid worms on denitrification and nitrification in Stream sediment. *Can. J. Fish. aquat. Sci.* 37: 656–663.
- Enoksson, V. & M.-O. Samuelsson, 1987. Nitrification and dissimilatory ammonium production and their effects on nitrogen flux over the sediment-water interface in bioturbated coastal sediments. *Mar. Ecol. Prog. Ser.* 36: 181–189.
- Forster, S. & G. Graf, 1992. Continuously measured changes in redox potential influenced by oxygen penetrating from burrows of *Callianassa subterranea*. In Hart, B. T. & P. J. Sly (eds), *Sediment/Water Interactions. Hydrobiologia* 235/236: 527–532.
- Gardner, W., R. F. Lee, K. Tenore & L. W. Smith, 1979. Degradation of selected polycyclic aromatic hydrocarbons in coastal sediments: importance of microbes and polychaete worms. *Wat. Air Soil Pollut.* 11: 339–347.
- Gerino, M., 1990. The effects of bioturbation on particle redistribution in Mediterranean coastal sediment. Preliminary results. *Hydrobiologia* 207: 251–258.
- Gerino, M., G. Stora & O. Weber, 1993. Potentialité de bioturbation dans le canyon du Cap Ferret: macrofaune et structures de bioturbation. *Actes III Coll. intern. Gasc.*, 265–269.
- Gilbert, F., 1994. Influence de la bioturbation sur l'activité dénitrifiante dans des sédiments marins côtiers. Effet d'un apport exogène de matière organique: les hydrocarbures. Thèse de Doctorat de l'Université d'Aix-Marseille II, ANRT-Grenoble: 94/AIX2/2053, 150 pp. (In French).
- Gilbert, F., G. Stora & J.-C. Bertrand, 1996. In situ bioturbation and hydrocarbon fate in an experimental contaminated Mediterranean coastal ecosystem. *Chemosphere* 33: 1449–1458.
- Gilbert, F., L. Rivet & J.-C. Bertrand, 1994. The fate of petroleum hydrocarbons in marine sediments: Influence of the burrowing polychaete *Nereis diversicolor*. *Chemosphere* 29: 1–12.
- Gilbert, F., P. Bonin & G. Stora, 1995. Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral. *Hydrobiologia* 304: 49–58.
- Gordon, D. C. Jr, J. Dale & P. D. Veizer, 1978. Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil. *J. Fish. Res. Bd Can.* 35: 591–603.
- Griffiths, R. P., B. A. Caldwell, W. A. Broich & R. Y. Morita, 1982. The long-term effects of crude oil on microbial processes in subarctic marine sediments. *Estuar. coast. Shelf. Sci.* 15: 183–198.
- Grundmanis, V. & J. W. Murray, 1977. Nitrification and denitrification in marine sediments from Puget Sound. *Limnol. Oceanogr.* 22: 804–813.
- Haines, J. R., R. M. Atlas, R. P. Griffiths & R. Y. Morita, 1981. Denitrification and nitrogen fixation in Alaskan continental shelf sediments. *Appl. envir. Microbiol.* 41: 412–421.

- Henriksen, K., J. I. Hansen & T. H. Blackburn, 1980. The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. *Ophelia Suppl.* 1: 249–256.
- Jørgensen, K. S., H. B. Jensen & J. Sørensen, 1984. Nitrous oxide from nitrification and denitrification in marine sediment at low oxygen concentrations. *Can. J. Microbiol.* 30: 1073–1078.
- Karickhoff, S. W. & K. R. Morris, 1985. Impact of turbidic oligochaetes on pollutant transport in bottom sediments. *Envir. Sci. Technol.* 19: 51–56.
- Kikuchi, E., 1986. Contribution of the polychaete, *Neanthes japonica* (Izuka) to the oxygen uptake and carbon dioxide production of an intertidal mud-flat of the Nanakita river estuary, Japan. *J. exp. mar. Biol. Ecol.* 97: 81–93.
- King, D. & D. B. Nedwel, 1987. The adaptation of nitrate-reducing bacterial communities in estuarine sediments in response to overlying nitrate load. *FEMS Microbiol. Ecol.* 45: 15–20.
- Koerting-Walker, C. & J. D. Buck, 1989. The effect of bacteria and bioturbation by *Clymenella torquata* on oil removal from sediment. *Wat. Air Soil Pollut.* 43: 413–424.
- Kristensen, E., 1984. Effect on natural concentrations on nutrient exchange between a polychaete burrow in estuarine sediment and the overlying water. *J. exp. mar. Biol. Ecol.* 75: 171–190.
- Kristensen, E., M. Hjorth Jensen & R. C. Aller, 1991. Direct measurement of dissolved inorganic nitrogen exchange and denitrification in individual polychaete (*Nereis virens*) burrows. *J. mar. Res.* 49: 355–377.
- Kristensen, E. & T. H. Blackburn, 1987. The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. *J. mar. Res.* 45: 231–257.
- Li, Y., J. T. Morris & D. C. Yoch, 1990. Chronic low level hydrocarbon amendments stimulate plant growth and microbial activity in salt-marsh microcosms. *J. appl. Ecol.* 27: 159–171.
- MacAuliffe, L., 1971. GC determination of solutes by multiple phase equilibration. *Chem. Technol.* 1: 46–51.
- MacElroy, A. E., 1990. Polycyclic aromatic hydrocarbon metabolism in the polychaete *Nereis virens*. *Aquat. Toxicol.* 18: 35–50.
- Mille, G., M. Mulyono, T. El Jammal & J.-C. Bertrand, 1988. Effects of oxygen on hydrocarbon degradation studies in vitro in surficial sediments. *Estuar. coast. Shelf Sci.* 27: 283–295.
- Pelegri, S. P., L. P. Nielsen & T. H. Blackburn, 1994. Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*. *Mar. Ecol. Prog. Ser.* 105: 285–290.
- Pérès, J. M., 1982. Major benthic assemblages. *Mar. Ecol.* 5: 373–522.
- Rabus, R. & F. Widdel, 1995. Anaerobic degradation of ethylbenzene and other aromatic hydrocarbons by new denitrifying bacteria. *Arch. Microbiol.* 163: 96–103.
- Rueter, P., R. Rabus, H. Wilkes, F. Aeckersberg, F. A. Rainey, H. Jannasch & F. Widdel, 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. *Nature* 372: 455–458.
- Rysgaard, S., P. B. Christensen & L. P. Nielsen, 1995. Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Mar. Ecol. Prog. Ser.* 126: 111–121.
- Sayama, M. & Y. Kurihara, 1983. Relationship between burrowing activity of the polychaetous annelid, *Neanthes Japonica* (Izuka) and nitrification-denitrification processes in the sediments. *J. exp. mar. Biol. Ecol.* 72: 233–241.
- Slater, J. M. & D. G. Capone, 1987. Denitrification in aquifer soils and nearshore marine sediments influenced by ground water nitrate. *Appl. envir. Microbiol.* 53: 1292–1297.
- Sloth, N. P., H. Blackburn, L. S. Hansen, N. Risgaard-Petersen & B. Aa. Lomstein, 1995. Nitrogen cycling in sediments with different organic loading. *Mar. Ecol. Prog. Ser.* 116: 163–170.
- Treguer, P. & P. Le Corre, 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Laboratoire d'Océanologie chimique, Université de Bretagne Occidentale, Brest, France, 110 pp.