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Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral

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Abstract

The *in vitro* effect of *Nereis diversicolor* on denitrification has been studied in PVC tubes filled with a coastal marine sediment defaunated by sieving. The first aim of the experiment was to determine the effect of sediment defaunation on denitrification (denitrifying population and Denitrifying Enzyme Assays). Sieving induced a loss of 70% of the initial DEA. The number of denitrifying bacteria was 10 times lower than in *in situ* sediment. In the top two centimetres, the DEA rose by 75% of its initial value, after 82 days. Polychaetes were only added after a return to near pre-disturbance levels to ensure that our data on the effects of their addition would not be disturbed by changes in the sediment.

Introduction of Polychaetes increased the denitrifying population and DEA in the first layer (0–2 cm) of the sediment after 15 days. After 45 days, the surface of the polychaete burrows in sediment was 1.3 to 1.5 times higher than after 15 days, resulting in an increase in solute exchange between seawater and the top layer of sediment. An inhibitory effect of oxygen on denitrification was detected in the uppermost layer only.

Introduction

Infaunal structures, such as worm burrows at the top of coastal sediments, are significant sites of exchange of solutes such as nitrate and oxygen across the water-sediment interface (Jørgensen & Revsbech, 1985; Kristensen, 1988).

The presence of burrows increases the area of oxic-anoxic interface and the transport of ions through the sediment and thus influences the microbial processes in the sediment column (Kristensen *et al.*, 1991; Binnerup *et al.*, 1992).

Under anaerobic conditions, NO_3^- and NO_2^- function as oxidants during mineralization of organic matter, but it is now accepted that denitrification can occur in the presence of O_2 (Robertson & Kuenen, 1984; Bonin *et al.*, 1989; Bonin & Raymond, 1990). Generally, denitrification takes place at aerobic-anaerobic interfaces so that nitrate can be produced by nitrification, an aerobic process, and is removed by denitrification in an adjacent O_2 limited zone. In sediment, near the surface, bioturbation can influence the alternance of aerobic-anaerobic conditions, hence, the denitrification processes.

Ecological studies are increasingly based on the use of microcosms to control factors that are uncontrolable in the natural environment. To study the influence of bioturbation on sediment conditions, defaunation procedures such as freezing (Kristensen & Blackburn, 1987), anoxic condition in the overlying water (Andersen & Kristensen, 1988) or sieving (Koerting-Walker & Buck, 1989), followed by the addition of selected macro-organisms, have often been used.

Defaunation by sieving and mixing destroys the original chemical and faunal profiles. Moreover, sieving influences the microbial biomass and metabolic activity in sediment (Findlay *et al.*, 1990). While the authors cited reported that microbial biomass returned to pre-disturbance levels 8 h after sieving, the influence

of sieving on microbial activities such as denitrification, has not been studied.

The objective of this study was first to determine the influence of sieving on microbial denitrification, and to assess the impact of nereid burrows and animal activity on denitrifying processes.

Materials and methods

Site and sampling

Sediment and worms were collected in the Saint-Antoine Canal during May and July 1991, respectively. This canal links the Rhone River and the Carteau Cove (Gulf of Fos) near Marseille, France (Fig. 1). Mean depth is 0.8 m. In May and July 1991, water temperature and salinity at the site were 13.2 °C and 35‰ S, and 20.5 °C and 30.7‰ S, respectively. Sediment and worms were sampled by digging at ~ 0.5 m water depth. In May, undisturbed sediment cores were also taken using a 30 cm PVC corer (i.d. 7.3 cm) to examine sediment characteristics before the start of the experiment.

Defaunated sediment and worms

In the laboratory, sediment collected in May was forced (by adding seawater) through a 1 mm sieve to remove macrofauna and thoroughly homogenized by gloved hands. In July, *Nereis diversicolor* was separated from sediment by gentle elution in sea water. The worms were acclimatized to experimental conditions, 17 °C and 30‰ S for 6 d in a recirculating seawater aquarium partially filled with natural sediment.

Seawater flow system

Experiments were carried out in flow-through cores (Fig. 2). Individual cores consisted of 7.3 cm-diameter PVC pipe (30 cm height) with closed bottom.

Six cores were filled to a depth of 20 cm with macrofauna-free sediment and placed in two aquaria thermostated at 17 °C under a LD 12:12 cycle.

Four multiple-head peristaltic pumps (Cole-Parmer, Chicago, IL) controled aerated and filtered (Millipak™ 200 GLCL 0.22 μm Disposable Filter Unit) seawater flow to the cores from two 50-litre thermostated tanks.

Individual feed lines (Tygon tubing) were suspended approximately 3 cm above the water surface of the cores.

Overlying the sediment of each core was approximatively 0.3 litre of seawater which was kept aerated by a small aquarium pump attached to an airstone. Seawater flow was ajusted to 0.6 ml min^{-1} resulting in three complete turnovers of water each day in each core. Water was evacuated through a 1 mm perforation bored 3 cm from the core top.

Introduction of macrofauna

After the first part of the experiment, in July, six selected *N. diversicolor* (0.64 to 0.81 g ind. wet weight) were added to 4 cores; two cores without nereids were used as controls. The density and biomass of *N. diversicolor* in the cores correspond to those observed at the sampling site (~ 1433 ind. m^{-2}; ~ 1 wt kg m^{-2}).

Animals that did not burrow within 12 hours were removed and replaced with fresh ones of the same weight.

Biological activity

Bacteria

Sediment cores were sectioned in 2 cm segments from the top to 10 cm.

For enumeration of denitrifying bacteria, 5 ml of sediment in 45 ml of sterile seawater were subjected to 60 min vigorous reciprocal agitation (96 rpm) at 4 °C. The suspension was used for enumeration by the most probable number method (MPN).

The cultures were prepared with medium containing lactate (1 g l^{-1}), acetate (1 g l^{-1}), succinate (1 g l^{-1}) and nitrate (3 g l^{-1}).

Anaerobic conditions were obtained by flushing nitrogen through the sealed flask for 20 min. After blockage with acetylene (20%) and incubation at 32 °C for 96 or 120 h, the production of nitrous oxide was measured.

For NO_3^- and NO_2^- concentrations measured *in situ*, in the range of 4 to 5 μM and 3.5 to 6 μM respectively, natural denitrifying activity was very low (0.9 μmol. N_2O l^{-1} d^{-1}). It is well known that the acetylene used to block N_2O reduction during denitrification also inhibits the nitrification that produces nitrate for denitrifying bacteria (Oremland & Capone, 1987). In view of this, we chose to study a potential activity. The denitrifying enzyme activity (DEA) was

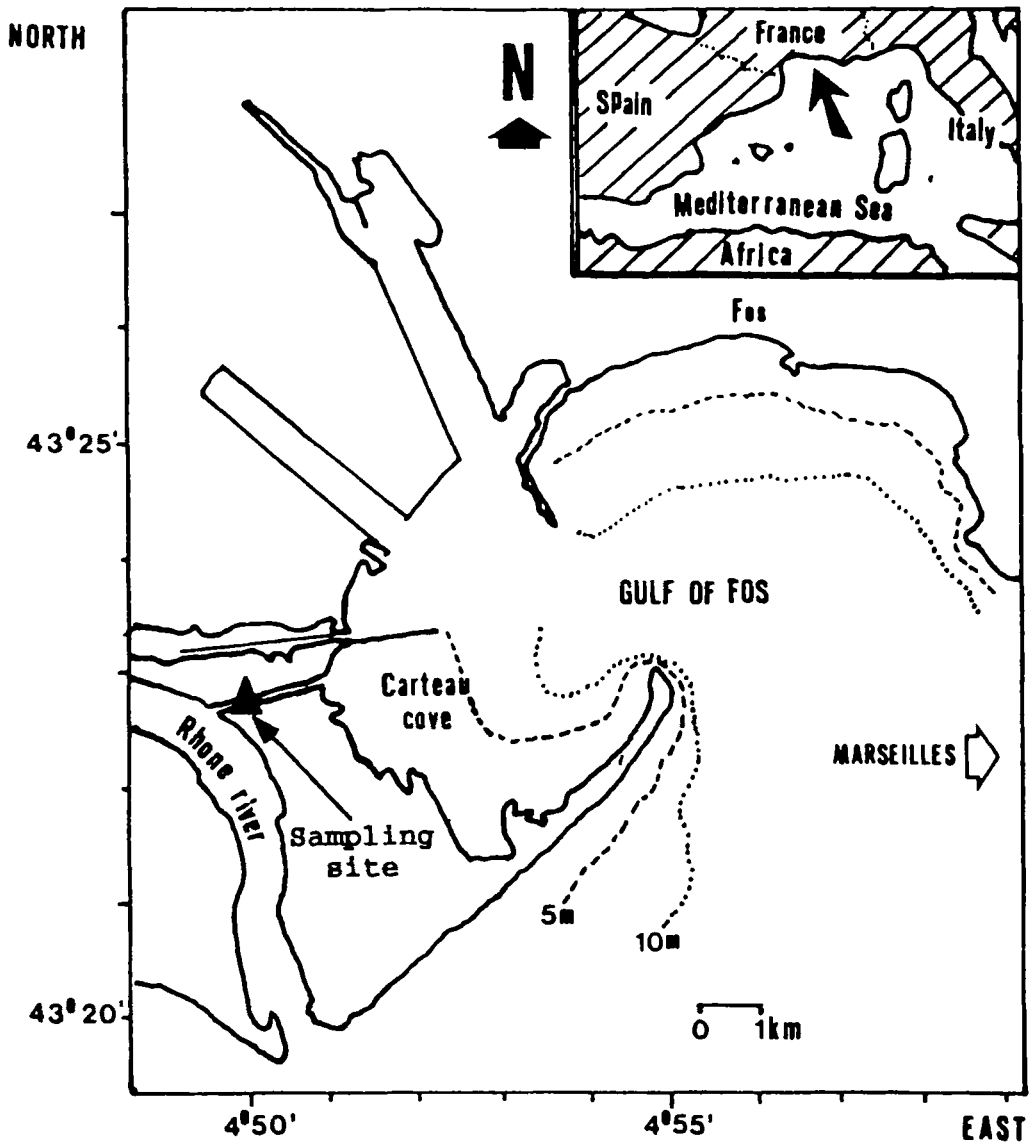

Fig. 1. Sampling site (▲) in the Northwestern Mediterranean basin.

measured by the procedure of Raymond *et al.* (1992). Four millilitres of each segment were distributed into 13 ml tubes containing 4 ml of sterilized seawater supplemented with glucose (1 mM), KNO_3 (1 mM), and chloramphenicol (1 g l^{-1}) according to Tiedje (1989). The tubes were sealed with rubber stoppers and rendered anaerobic by flushing N_2 through the tubes. Acetylene (15 kPa), which inhibits the reaction from N_2O to N_2, was distributed into the tubes. Two were sacrificed for analysis after 0, 0.5, 1 and 3 hours of incubation at 20 ° C. The linear initial rate of N_2O accumulation is considered as a measure of denitrification activity. 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_2O analysis. The extraction of N_2O from the liquid phase was carried out using the procedure of Chan & Knowles (1979) modified by the technique of multiple equilibrium (Mac Aulife, 1971).

Macrofauna

The reworking activity of *N. diversicolor* in microcosms was quantified by the luminophore tracer technique (Gerino, 1990). Luminophores are colored inert

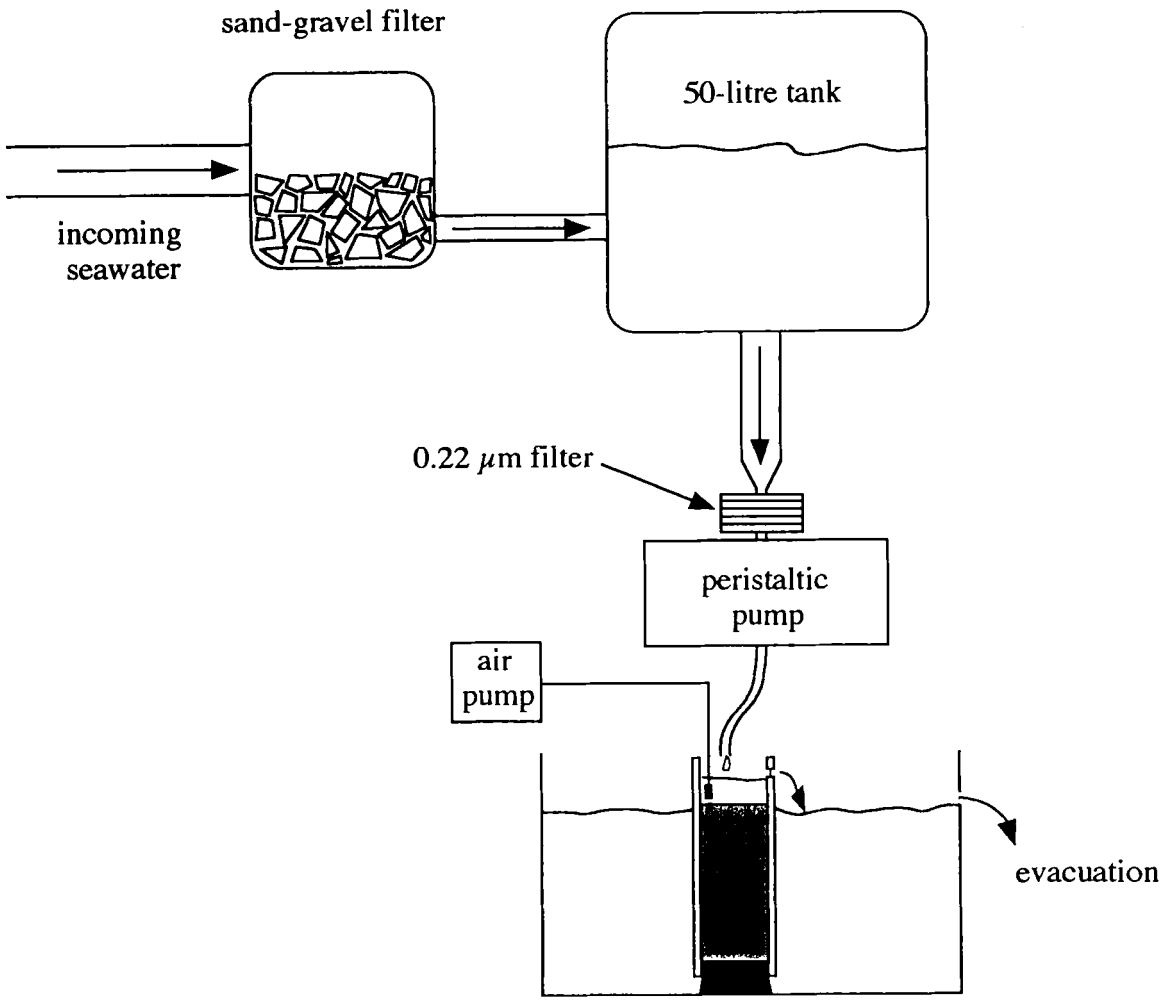

Fig. 2. Schematic diagram of the core and flow-through seawater system used in the present study. Refer to text for full description.

particles, fluorescent under U.V. light (Mahaut & Graf, 1987).

Two size fractions of luminophores, 40–60 μm diameter (red) and 150–200 μm diameter (yellow) were used. A mixture of the two fractions (0.5 g per fraction) was deposited at the sediment surface of four cores: two control cores without nereids and two cores with nereids.

Chemical analysis

Redox potential was recorded with a pH/mV (Metrohm 632) equipped with a combined Ag/AgCL reference and platinum electrode.

All nitrogen 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).

Nitrous oxide content was determined by a gas chromatograph (Girdel series 30) equipped with an electron capture detector. Chromatographic operating conditions: 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}.

Results

Influence of defaunation procedure on denitrifying enzyme activity

The sieving procedure to remove macrofauna offers the opportunity to homogenize the sediment and to compare directly results obtained from each microcosm sacrificed for analysis after incubation.

The denitrifying enzyme activity (Tiedje, 1988) was used to characterize the denitrifying activity. The potential N_2O production rate was measured by making all factors (electron donors and acceptors) non-limiting.

After acetylene blockage, the rate of nitrous oxide production is assumed to be proportional to DEA.

Before the start of the experiment, we determined the initial profiles of denitrifying activity and denitrifying population in undisturbed cores. The results are reported in Figs 3 and 4, respectively (dashed bars). In the top two centimetres, denitrifying activity was about 284 μmol. N_2O l^{-1} d^{-1}. Peak activity was found 4–6 cm below the sediment surface and reached 328.2 μmol. N_2O l^{-1} d^{-1}. The number of denitrifying bacteria was maximum (40×10^5 bact. cm^{-3}) in the 2–6 cm layer and was apparently associated with the main zone of denitrification activity.

After sieving, patterns of variation in the depth profiles of the number of denitrifying bacteria and their activities were monitored for 92 days (Figs 3 and 4) and compared with the initial data on denitrifying bacteria and DEA (dashed bars).

Analysis carried out after 15 days clearly showed that after sieving, DEA declined sharply, the level of N_2O production remaining approximately 30% below the initial activity whatever the depth. The same effect was observed on the bacterial population.

After 35 days, DEA increased. At depth, the amount of N_2O produced rose to 100% of the initial level, whereas at the surface it reached only 50% of that measured in undisturbed cores.

After 35 days, the increase of DEA continued, to rise at the top of the core, by 75% in about 82 days. After 82 days, N_2O production reached values close to those before sieving. However, the activity was never fully restored (Fig. 5). The increase of DEA between 82 and 92 days, in the first two centimeters, was weak. The curve obtained can be described by the equation:

$$D = 49.919 + 2.999t - 0.016t^2 (r^2 = 0.998),$$

where D is the DEA value in the first two centimeters of sieved sediment, and t is time.

With regard to the density of denitrifying bacteria, after 35 days of incubation, an increase of 25% in the population was observed in the whole core.

After 82 days, the number of denitrifying bacteria in the top two centimetres was up to five times higher than the corresponding value after 35 days.

Influence of Nereis diversicolor on denitrification

Figure 6 presents the number of denitrifying bacteria, the DEA and the nitrate concentration measured in cores with and without *Nereis diversicolor*, after 15 and 45 days.

After 15 days, the number of denitrifying bacteria increased from 25×10^4 to 140×10^4 bact. cm^{-3} in the first two centimetres of sediment, in the presence of *N. diversicolor*, without any effect in the lower layers.

In the same period, DEA in the first layer (0–2 cm) was enhanced to 14% compared to sediment without *N. diversicolor*. No significant effect on denitrification occurred as a result of the presence of the polychaetes in deeper layers. The nitrate concentration fell by 50% in the first layer, and nitrate concentrations from 2 to 10 cm depth were similar in presence or absence of polychaetes.

After 45 days, in the surface layer (0–2 cm) of the sediment with *N. diversicolor*, both denitrifying bacteria and DEA decreased, and NO_3^- concentration was higher than in the control (without *N. diversicolor*). At depth (>2 cm), bioturbation by polychaetes induced no significant effect on the denitrifying population but the denitrification was slightly enhanced.

Table 1 shows that the presence of polychaetes induced no variation in the negative redox potential profile of the sediment after 15 days. On the other hand, after 45 days, the redox potential progressed to +54 mV in the layer surface (0–2 cm), indicating oxic conditions.

Table 2 presents the distribution of added luminophores in the different sediments. In control sediment (without added polychaetes) no burrowing occurred. The absence of luminophores in the sedimentary column demonstrated that juvenile macrobenthos and meiofauna, which may have pass through the sieve and survive after sediment homogenization, did not have a major influence on sediment reworking. In cores with *N. diversicolor* the number of buried luminophores after 45 days was 1.3 to 1.5 times higher

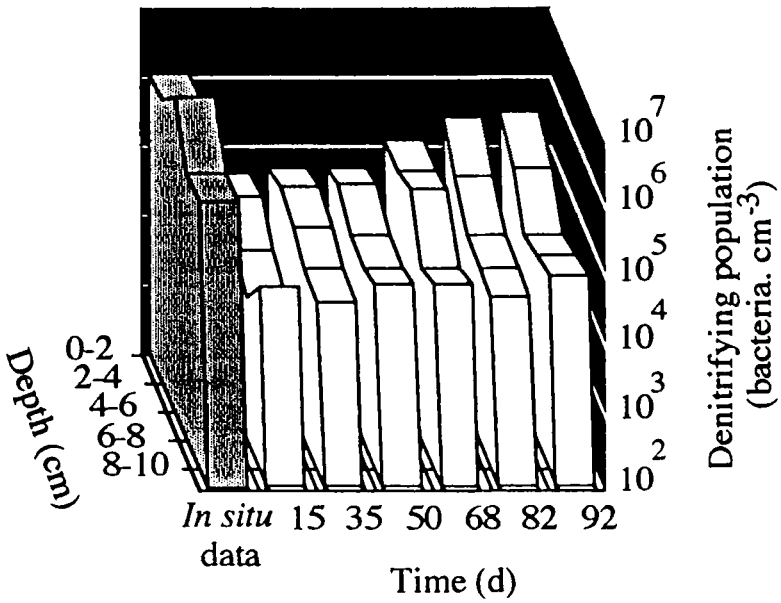

Fig. 3. Variation in the number of denitrifying bacteria in sediment after sieving (white bars) and *in situ* values before sieving (dashed bars).

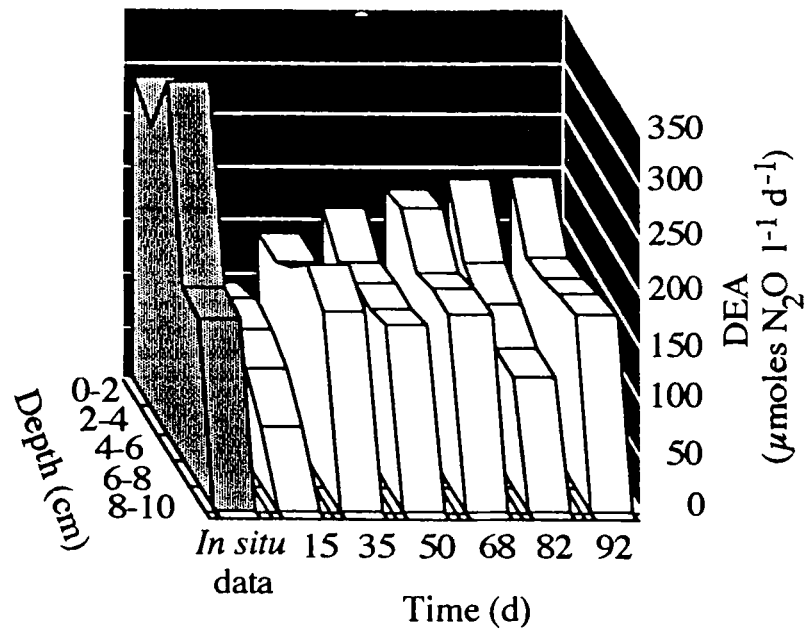

Fig. 4. Variation in the DEA in sediment after sieving (white bars) and *in situ* values before sieving (dashed bars).

than after 15 days over the whole depth of bioturbated sediment.

Discussion

The effect of defaunation on the sediment system has generally been neglected (Kristensen & Blackburn, 1987), or researchers have waited few days before starting their experiments (Bauer *et al.*, 1988). Our results show that sieving to remove macrofauna disturbs not only the original structure and chemical profiles (Kristensen & Blackburn, 1987) but also bacterial activity. Thus, after this kind of treatment, it seems essential to wait for the sediment parameters to return to initial levels before undertaking further studies. If

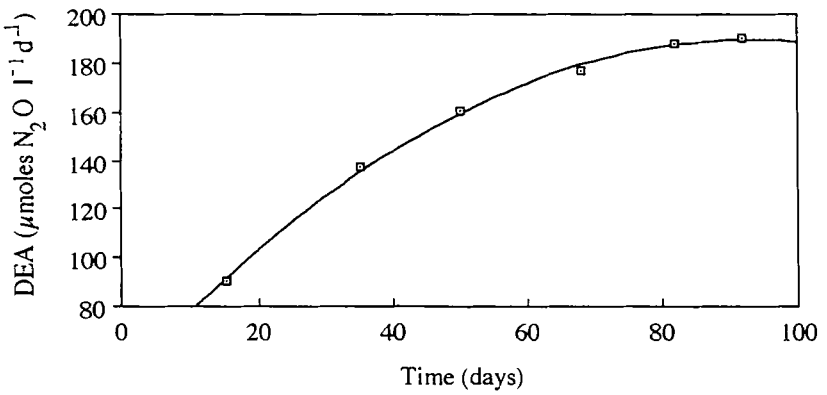

Fig. 5. Variation in the DEA in the first layer (0–2 cm) of the sediment after sieving. The curve obtained is described by $D = 49.919 + 2.999\ t - 0.016\ t^2$ ($r^2 = 0.998$), where D is the DEA value in the first two centimeters of sieved sediment, and t is time.

Table 1. Redox potential profile in control sediment without polychaetes and in sediment with the polychaetes population, 15 and 45 days after the introduction of 6 Nereis diversicolor. Values are expressed in millivolts.

Depth (cm)	15 days		45 days	
	Cores without polychaetes	Cores with polychaetes	Cores without polychaetes	Cores with polychaetes
0 – 2	−156	−145	−121	+54
2 – 4	−170	−165	−162	−151
4 – 6	−177	−178	−172	−152
6 – 8	−208	−192	−181	−167
8 – 10	−198	−195	−178	−174

Table 2. Repartition of the luminophores in control sediment without polychaetes and in sediment with the polychaetes population, 15 and 45 days after the introduction of 6 Nereis diversicolor. Values are expressed in milligrammes.

Depth (cm)	15 days		45 days	
	Cores without polychaetes	Cores with polychaetes	Cores without polychaetes	Cores with polychaetes
0 – 2	760.0	871.5	720.0	503.0
2 – 4	0.0	62.5	0.0	83.4
4 – 6	0.0	52.0	0.0	77.4
6 – 8	0.0	55.6	0.0	83.0
8 – 10	0.0	39.3	0.0	64.0

such changes are not taken into account, measurements must be considered unreliable to provide evidence of the effect of bioturbation on microbial activities. We show that 82 days were necessary to return to initial DEA values in sieved sediment (Fig. 5). Values after 92 days were of the same order as those measured 10 days before.

In systems where the initial values were reached, studies on the influence of *Nereis diversicolor* on denitrification were undertaken.

After 15 days, in agreement with the stimulatory effect of macrofaunal burrowing on microbial growth (Hines & Jones, 1985; Kikuchi, 1986; Reichardt, 1988), the number of denitrifying bacteria rose in presence of polychaetes. In the same period, the stimulation of denitrification in sediment with *N. diversicolor* was limited to the surface layer (Sayama & Kurihara, 1983) and contributed to the nitrate consumption of this layer (Kristensen, 1985; Chatarpaul *et al.*, 1980) because solute diffusion into the anoxic part of the sediment is increased by the presence of polychaetes (Henriksen *et al.*, 1983; Aller & Yingst, 1985; Koike, 1990). Nevertheless, this stimulation was slight, compared with other reports (Chatarpaul *et al.*, 1980; Kristensen *et al.*, 1991).

The decrease of DEA in the first sediment layer, after 45 days, could be explained by the inhibitory effect of oxygen on denitrification (Payne, 1976; Firestone *et al.*, 1980, Kapralek *et al.*, 1982) not observed after 15 days. Oxic conditions were in fact only obtained in the first 2-cm layer of the sediment and after 45 days.

The luminophore experiment was used to quantify the building of burrows by polychaetes. Gerino &

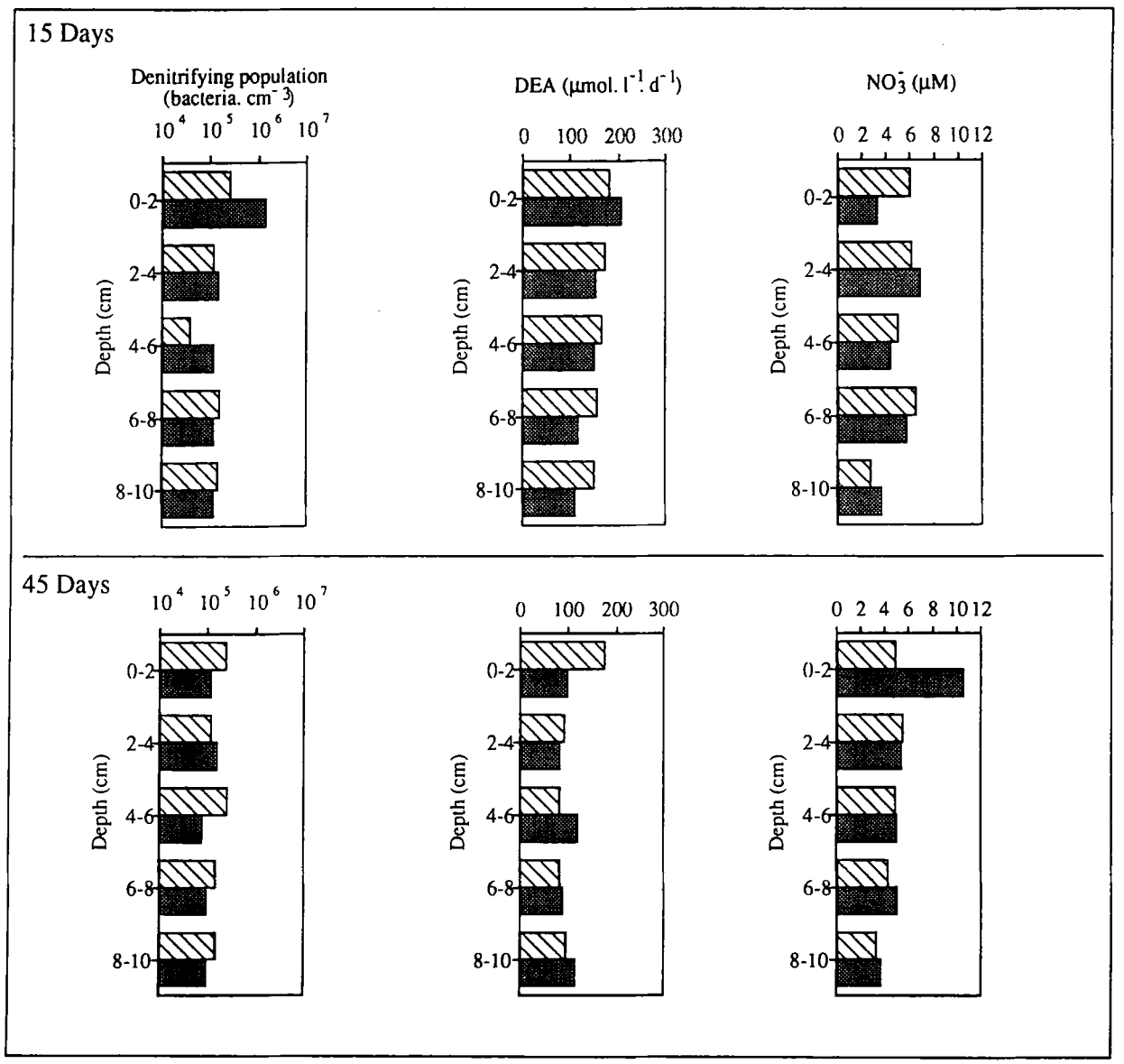

Fig. 6. Denitrifying bacteria, DEA and nitrate concentration in sediment 15 and 45 days after the introduction of the polychaetes. Control sediment without *Nereis diversicolor* (▨) and sediment with *N. diversicolor* population (■).

Stora (1991) have shown that there is a significant correlation between the surface of the burrow walls of *N. diversicolor* and the quantity of luminophores at each level, except in the first layer (0–2 cm) because luminophores are deposited in excess at the surface.

The increased surface of burrows resulted, after 15 to 45 days, in a better penetration of oxygen to the sediment. Yet, the oxygen level only approached that of the surface water during active ventilation periods, and was rapidly exhausted by consumption by the polychaetes and wall microbes in the burrows during the long resting periods (Kristensen, 1985).

The total oxygen concentration in the burrows can be considered as low, and without significant effect on denitrification in the bioturbated sediment in the conditions of our experiments ($\sim$ 1433 *N. diversicolor* m^{-2}). On the other hand, after 45 days, the presence of burrows in the first layer (0–2 cm), where the proximity of seawater allowed a certain oxygenation, induced an inhibitory effect of oxygen on denitrification by increasing the water-sediment interface.

In conclusion, these investigations demonstrate the complexity of the effects of *Nereis diversicolor* on denitrification. Two ranges of responses could be distinguished. After 15 days, denitrification rate exceeded that in defaunated sediment; in contrast, after 45 days, after the surface of the burrows had increased, the E_h value was positive and denitrification rate fell. This probably reflects the antagonistic effect of the transport of NO_3^- and O_2 from the overlying water to the sediment, favoured by irrigation of the burrows by the infauna. Animals can also have significant effects on the overall rates of coupled nitrification/denitrification within the sediment. Further work should elucidate the *in situ* effect of macrofauna on denitrification. Results obtained from our microcosms are not in fact directly comparable to those obtained *in situ*, but they should be regarded as evidence of the effect of bioturbation by *N. diversicolor* on denitrifying activity.

Acknowledgments

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