

Influence of shellfish farming activities on nitrification, nitrate reduction to ammonium and denitrification at the water-sediment interface of the Thau lagoon, France

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ABSTRACT: The seasonal patterns of nitrification, denitrification and dissimilatory ammonium production (DAP) rates were studied in the sediment of 2 stations in the Thau lagoon (south of France). The station ZA was located within the shellfish farming zone and the station B was the reference site. A marked effect of shellfish farming on bacterial activities was observed. Spatial differences were associated with discrepancies in the organic content and the reduction state of sediments, i.e. highest reductive processes (denitrification and DAP) were noted in shellfish farming area, whereas the oxidative process (nitrification) was predominant outside the farming zone. At both stations, the DAP activity increased in September (autumn) concomitant with an increase of the C/N ratio in the sediment due to the sedimentation of the summer phytoplanktonic production. Nitrification and denitrification rates exhibited maxima in November (winter) corresponding to dissolved inorganic nitrogen inputs from the surrounding land. In the shellfish farming site, 98% of nitrate was reduced to NH_4^+ and 2% to N_2O , showing that the most of the NO_3^- was reduced to ammonium and remained available for the ecosystem.

KEY WORDS: Shellfish farming · Nitrogen bacterial processes · Nitrification · Nitrate reduction to ammonium · Denitrification · Thau lagoon · Mediterranean Sea · Sediment-water exchanges

INTRODUCTION

The potential importance of nutrients in influencing the productivity of marine ecosystems is now well recognised, especially that of dissolved inorganic nitrogen, which appears to limit the primary production in coastal ecosystems (Ryther & Dunstan 1971). Benthic remineralisation is an important pathway in shallow ecosystems (Zeitzschel 1980, Nixon 1981), and the recycled N released from sediments can substantially contribute to the nitrogen requirement for phytoplankton growth (Nixon 1981).

Three major bacterial processes in sediments affect the nitrogen cycle, playing important roles in nitrogen budgets and fluxes of the ecosystem: (1) the ammonium oxidation by nitrification in sediments not only competes with heterotrophic processes for limited supplies of oxygen and nitrogen, but can also indirectly remove nitrogen through its coupling with the denitrification process (Koike & Hattori 1978, Jenkins & Kemp 1984). (2) During the remineralisation of organic nitrogen in sediments, 15 to 70% of N can be lost by denitrification (Seitzinger 1988). Furthermore, benthic denitrification can also decrease nitrate influxes from terrestrial origin (Jørgensen & Sørensen 1985, Jørgensen & Sørensen 1988). (3) Dissimilatory nitrate reduction to ammonium, referred to as dissimilatory

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ammonium production (DAP), competes for nitrate with denitrification in sediments (Sørensen 1978a, Enoksson & Samuelsson 1987). The relative contribution of denitrification and DAP to nitrate reduction is of considerable importance, as the former acts as a nitrogen sink for the ecosystem whilst the latter maintains the nitrogen levels.

The Thau lagoon (south of France) is the biggest shellfish breeding area in Europe with an estimated standing stock of 40 000 t of oysters (*Crassostrea gigas* and their epibiota) at a density of about 40 oysters m^{-2} of shellfish farming zone; the harvesting of oysters leads to the removal of large quantities of nitrogen from the ecosystem. Thus, the knowledge of the shellfish farming effect on bacterial activities associated with the N cycle is necessary for a better understanding of the N cycle. Kaspar et al. (1985) showed that the denitrification rate was 7 times higher in sediments from a mussel farm compared to from a reference site. Nevertheless, other experiments have shown that high loading of organic matter to sediments tends to decrease N removal by diminishing the coupling of nitrification and denitrification (Jenkins & Kemp 1984, Blackburn et al. 1988, Sloth et al. 1995, Jensen et al. 1996). This paper reports the first results on nitrification, DAP and denitrification measurements at the sediment-water interface of the shellfish farm and at a reference site in the Thau lagoon.

MATERIALS AND METHODS

Studied sites. The study was carried out in the Thau lagoon, a shallow lagoon of 75 km² located on the French Mediterranean coast (Fig. 1). This lagoon is connected to the sea through the narrow channels of Sète and receives freshwater mostly from the north shore. The Vène and Pallas are the major rivers representing 43% of the total watershed (280 km²). Two stations were chosen for their differences due to sediment alteration induced by biodeposition (Mesnage & Picot 1995, Mazouni et al. 1996). Stn ZA (8.5 m depth), located inside the eastern shellfish farming zone, has been previously described in Souchu et al. (1997). Stn B (8.5 m depth), which is the reference site, shows a particle size similar to that of Stn ZA, with the fraction below 63 μm constituting between 80 and 90%. The macrophytobenthos is almost totally absent at these relatively deep stations (Gerbal & Verlaque 1995), and a description of benthic macrofauna and meiofauna can be found in Guelorget et al. (1994). The stations were studied 4 times between June 1994 and April 1995, i.e. June, September and November 1994 and April 1995. Sediments from Stn ZA consisted of black mud giving off a hydrogen sulphide smell (H₂S). The

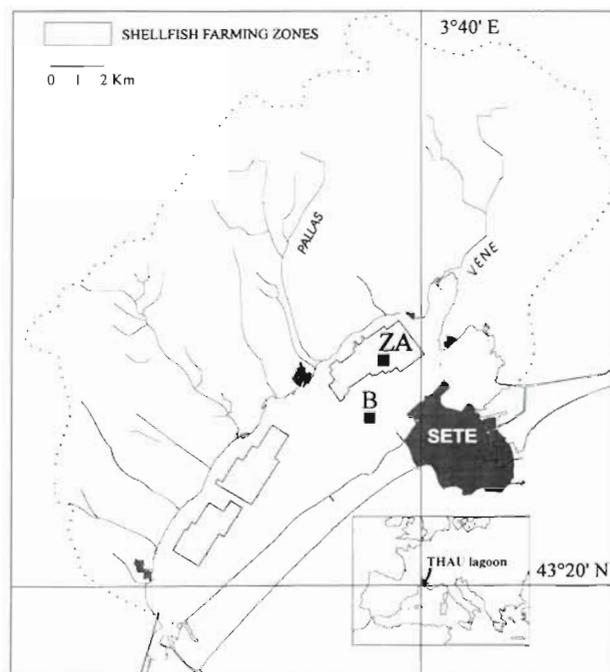


Fig. 1. Location of the study sites inside (Stn ZA) and outside (Stn B) the shell farming zone

interface was partly covered by filamentous cyanobacteria. Sediments from Stn B were made of a grey mud, lightly (1 or 2 ind. m^{-2}) colonised by the worm *Sabella pavonina* (Savigny). In September and November, the absence of macrofauna and the blackening of sediments at Stns ZA and B indicated that both stations suffered from bottom anoxia in August. In September, sediments from both stations were covered by a yellow-brown layer rich in suspended particles.

Water column. Bottom water (0.5 m above the bottom) was sampled with a Niskin bottle. Filtration and storage of samples were performed within 1 h after sampling. Samples for ammonium (NH₄⁺) determination were fixed immediately and measured by the colorimetric method of Koroleff (1976) using Bertelot's reaction. Samples were filtered (Whatman GF/F filter) and stored for later determination of NO₂⁻ and NO₃⁻ concentrations with a segmented flow analyser (Tréguer & Le Corre 1975). A detailed procedure of sampling, filtration and storage is described in Souchu et al. (1997). Records of daily rainfall at Sète were obtained from the National Meteorological Board of Montpellier. Salinity and temperature, recorded using a WTW LF 196 conductimeter (accuracy $\pm 0.5\%$), were provided by the observation network of IFREMER.

Sediments. Dry organic matter content and oxygen and redox profiles were measured in sediments collected in acrylic tubes by divers. At Stn ZA, sediments

were sampled 3 m outside the suspended lines of a shellfish farm.

For the percentages of dry organic matter (%OM), triplicate 8 cm wide cores were sliced into 1 cm sections, from which known volumes of sediment were freeze-dried and then heated at 450°C for 12 h.

For the pore water sediment extraction, triplicate 8 cm wide cores were cut into 1 cm segments inside a nitrogen-filled glove bag to avoid air oxidation of reduced nitrogen compounds dissolved in the water. Each slice of sediment was centrifuged at 10000 rpm ($8620 \times g$) for 15 min with a refrigerated centrifuge (Heraeus Megafuge 20R). Supernatant water was removed from the centrifuge tube, filtered through Whatman GF/F filters and frozen in precombusted Pyrex flasks for later determination of NH_4^+ , NO_2^- and NO_3^- concentrations as described for the water column. Interference of sulphides with Berthelot (NH_4^+) colorimetric reactions was avoided by a dilution ($\times 100$) with deionized water.

Redox potential and oxygen concentration were measured immediately after arrival in the laboratory with a pH/mV (Schott CG817T) meter equipped with combined platinum mini-electrodes and a Ag/AgCl reference electrode. Before each measurement, a delay of 2 min allowed the reading to become constant. The oxygen concentration in the sediment was measured with a mini-electrode with a tip diameter of 0.5 mm (Visscher et al. 1991) and an Ag/AgCl external reference electrode. The electrodes were connected to a picoammeter (Keithley 485), with a 750 mV polarisation charge positive to the reference electrode. Calibration under non-stirred conditions revealed a linear relationship between oxygen concentration and the observed signal. Typical 0 and 100% air saturation values were 1 and 58 nA. Positioning of the mini-electrode was accomplished with a micromanipulator (resolution 50 μm).

Nitrification activity. Nitrification rates were obtained by measuring the changes of NO_2^- concentration in sediment samples containing allylthiourea (ATU) or sodium chlorate (NaClO_3) to inhibit the oxidation of NH_4^+ and NO_2^- , respectively (Bianchi et al. 1992, 1994). The upper 2 cm of the sediment samples was divided into 3 sets of triplicate subsamples (30 g), and each was mixed thoroughly with filtered bottom water (Whatman GF/F) in a 1 l polycarbonate bottle. One triplicate set of bottles was unamended (control), one received 10 mg l^{-1} ATU (final conc.) and the third received 10 mM NaClO_3 . No substrate was added. The bottles were incubated in the dark with loosely fitted caps and without shaking at the *in situ* temperature in a Sanyo incubator. Concentrations of NO_2^- were measured every 4 h from zero time to 24 h. The nitrification activity rate was calculated from data obtained during

the linear NO_2^- or NO_3^- production phases in the first 12 h of incubation. The bottles were first vigorously shaken before 20 ml samples were extracted into screw cap polyethylene tubes for centrifugation at 5000 rpm ($2150 \times g$) for 15 min. No filtration was performed in order to avoid the leaching of particulate matter retained on the filter. The supernatant was fixed with HgCl_2 and stored at 4°C in precombusted Pyrex flasks for later analysis within 48 h. Analyses of NO_2^- were performed as described for the water column and averaged for triplicates. The NO_2^- accumulation and consumption were linear in time ($p < 0.05$), indicating that little or no growth of nitrifying bacteria occurred during the incubation. The slope of the NO_2^- curve in NaClO_3 and ATU bottles gave respectively NH_4^+ and NO_2^- oxidation rates in $\mu\text{mol N}$ per litre of wet sediment ($\text{l}^{-1} \text{WS}$) and per day. The accuracy was given by the standard error of the slope and if no significant correlation was found the rates were considered to be zero.

Denitrification and dissimilatory nitrate reduction to ammonium (DAP) activities. A combination of an acetylene blockage technique to assay for denitrification and a ^{15}N isotope tracer technique to measure dissimilatory nitrate reduction was performed. Subsamples (5 ml) from 2 cm segments of the 8 cm wide core were transferred into 13 ml tubes containing 5 ml of natural sea water supplemented either with only chloramphenicol to prevent growth during incubation (for the natural activities), or with chloramphenicol, glucose (1 g l^{-1}) and KNO_3 (1 mM) (for the potential activities; according to Tiedje et al. 1989). All subsamples were inoculated with $^{15}\text{NO}_3^-$. The amount of $^{15}\text{NO}_3^-$ (97.4 at.%; Isotec France) was added at a concentration lower than 10% of the guessed in-tube nitrate concentration. For calculation, the actual percentage was taken into account after measurement of the NO_3^- level during later analysis. No addition of NH_4^+ was necessary to prevent nitrate assimilation, because of the high ammonium content in the sediment (higher than 100 μM). 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 conc. 15 kPa) and the tubes were vortexed. Samples were incubated in the dark at *in situ* temperature for 0, 1, 3, 5 and 24 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 the sea water. A subsample (2.5 ml) of the gas phase was injected into a 3 ml pre-evacuated tube (Venoject,

Terumo, Leuven, Belgium) for later N_2O analysis. 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 Aullife 1971). Nitrous oxide concentrations in the tubes were determined by gas chromatography (Girdel, series 30) using an electron capture detector. Chromatographic operating conditions were: 8 ft (2.4 m) 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 with a flow rate of 20 ml min⁻¹ (Bonin et al. 1987).

According to Tiedje's (1988) procedure, we determined the rate of nitrate ammonifying activity by monitoring the progressive increase in isotopic enrichment of $^{15}NH_4^+$ with time as the substrate (NO_3^-) was used. In the sediment, it is usually assumed that NH_4^+ available to the bacteria is present in the pore water plus exchangeable- NH_4^+ pools (i.e. extractable NH_4^+). Pore water was removed by centrifugation of sediments and 2.5 ml of supernatant was collected in microdiffusion flasks. Then, 2 M KCl was added to the sediment pellet at a KCl:sediment proportion of 1:1 (v:v), the mixture was homogenised and then shaken for 1 h at 5°C (Laima 1993). The 2.5 ml of KCl extractable pools obtained after centrifugation at $3000 \times g$ for 5 min from this mixture was added to the microdiffusion flask. The NH_4^+ was removed by microdiffusion at 60°C as follows. The mixture was treated with MgO to convert NH_4^+ to NH_3 , which was trapped on quarter pre-combusted Whatman GF/C filter acidified with H_2SO_4 (50 µl, 0.5 N) (Brooks et al. 1989). The ammonium removal efficiency was greater than 98%. Since the amount of nitrogen present in the sample was below the sensitivity of the mass spectrometer, a known quantity (1 µmol) of unlabelled ammonium (carrier nitrogen) was added before microdiffusion. The amount of carrier nitrogen added was taken into account when calculating the ammonium production rates.

^{15}N calculations. N isotope analysis involved interfacing an automatic N/C analyser (ANCA) to a triple collector isotope-ratio mass spectrometer (ANCA-MS Tracer mass, European Scientific).

To calculate the rate of the flux from dissolved nitrate to dissolved ammonium, the following equations derived by analogy with that of Dugdale & Goering (1967) for a 2 compartment system were used:

$$DAP = \frac{\{\text{at.\% S excess in } NH_4^+\} (NH_4^+ \text{ concentration})}{\{\text{at.\% enrichment in } NO_3^-\} (\text{incubation duration})}$$

where at.% S excess in NH_4^+ is the at.% in excess of the sample without carrier, determined at the end of the incubation period from the measured at.%.

When carrier nitrogen was used, the measured at.% of the analysed sample includes the contribution of the

carrier as follows:

$$\text{Measured at.\%} = \frac{(M_s)(\text{at.\% S excess in } NH_4^+) + (M_c)(\text{at.\% C})}{(M_s + M_c)}$$

where M_s is the concentration of nitrogen in the sample, M_c is the carrier nitrogen, at.% S excess is the at.% in excess of the sample without carrier, and at.% C is the at.% of the carrier nitrogen (0.3662). The equation can be rearranged to calculate for at.% S excess in NH_4^+ as follows:

$$\text{At.\% S excess in } NH_4^+ = \frac{\{\text{Measured at.\%}\}(M_s + M_c) - M_c(\text{at.\% C})}{(M_s)}$$

At.% enrichment in NO_3^- is calculated according to:

$$\text{At.\% enrichment in } NO_3^- = \frac{\text{total } ^{15}N \text{ added}}{\text{total } ^{15}N \text{ added} + \text{total ambient N substrate}}$$

Data analysis. Differences between the areas inside (Stn ZA) and outside the shellfish farming zones (Stn B), and the space-time variations of nitrogen processes in sediments 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

Environmental data

The study period began in April during the establishment of warm and dry meteorological conditions; the temperature and salinity increased from April to August (Fig. 2, Table 1). The lack of wind to circulate the water in July and August was responsible for a bottom anoxia which occurred in the deeper eastern part of the lagoon (depth > 6 m) including the shellfish farming zone (Stn ZA). Later on, the period from September to early November corresponded to a rainy period with a drop of salinity down to 30.5 PSU. After November, the freshwater inputs were negligible, leading to the increase of salinity until April, with values close to levels measured in June (Fig. 2), but with a -8°C temperature difference (Table 1).

The concentrations of dissolved inorganic nitrogen (DIN) compounds in the water (NH_4^+ , NO_2^- , NO_3^- = DIN) showed a similar pattern at both stations for all compounds but were higher at Stn ZA, particularly in November (Table 1). The concentrations increased from June to November 10-fold or more and then dropped drastically in April.

Concerning the sediments, in June the %OM was significantly higher at Stn ZA than at Stn B (Fig. 3). In

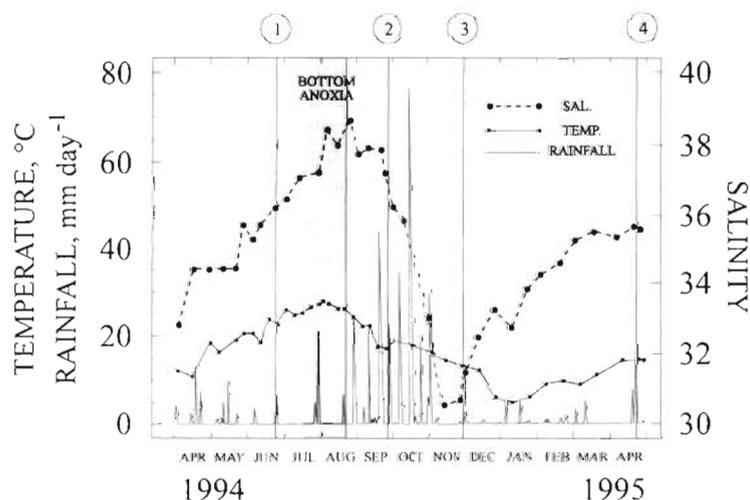


Fig. 2. Seasonal variation of rainfall monitored at Sète, salinity and temperature in the shell farming zone that contains Stn ZA, from April 1994 to April 1995. Sediment sampling times are indicated: 1, June 1994; 2, September 1994; 3, November 1994; 4, April 1995

September (after the bottom anoxia), %OM ranged from 15% (top of sediment) to 10% (at 40 mm depth) for both stations. The sediments collected in November were characterised by the minimum %OM (<10%), while values comparable to those in June were recovered in April with a similar difference between the 2 stations.

Sediments from Stn B displayed positive redox potential (Eh) values which ranged from 50 to 100 mV in June and were close to 200 mV during the next 3 study periods. Eh values were stable with depth whatever the season. On the other hand, at Stn ZA, the Eh was variable with a rapid change from positive to negative values within the top 20 mm for the 3 first study periods. Moreover, a decrease from -100 to -300 mV was observed between June and September (Fig. 3). In No-

vember, Eh values remained near -300 mV. In April, Eh values were positive and quite stable with depth and were close to levels measured at Stn B.

Oxygen profile measurements indicated that the depth of O₂ penetration ([O₂] > 6 μM) never exceeded 4 mm (Table 1) and was systematically deeper at Stn B than at Stn ZA. The maximum O₂ penetration was recorded in April at the reference Stn B, the minimum being found in winter at Stn ZA, where it was strictly limited to the interface itself.

Concentration of NH₄⁺ in pore water was stable with depth at Stn B. Pattern of changes with time of NH₄⁺ concentrations were similar for the 2 stations: (1) increase from June to September (from 50 to 100 μM and 220 to 550 μM for Stns B and ZA, respectively), (2) stability in November, and then (3) in April, a drop to levels close to those reported in June.

Bacterial activities

Nitrification

Rates of nitrification ranged from 0 to 77 μmol d⁻¹ l⁻¹ WS. The summer experiment (June) was characterised by insignificant nitrification rates both inside (Stn ZA) and outside (Stn B) the shellfish farming zone (Fig. 4A, B). At Stn ZA, both steps of nitrification in the sediment were only measurable in September, when the superficial layer rich in suspended particles was observed. In winter (November), the nitrification was enhanced at Stn B, where the first step of nitrification (NH₄⁺ oxidation; Fig. 4A) was 2.5 times higher than the second step (NO₂⁻

Table 1. Concentrations of DIN in the bottom water and sediments, temperature and salinity in the bottom water and depth of O₂ penetration in sediments ([O₂] > 6 μM) from June 1994 to April 1995 at Stns ZA and B

	June 1994		September 1994		November 1994		April 1995	
	ZA	B	ZA	B	ZA	B	ZA	B
Water								
Temperature (°C)	22.1	22.1	18.6	18.6	15.5	15.5	14.1	14.1
Salinity (PSU)	36.1	36.1	38.0	38.0	34.0	34.0	37.3	37.3
[NH ₄ ⁺] (μM)	0.66	0.34	5.3	3.4	7.7	1.62	0.84	0.69
[NO ₂ ⁻] (μM)	0.07	0.09	0.48	0.37	1.32	1.28	0.14	0.10
[NO ₃ ⁻] (μM)	0.12	<0.05	5.8	2.6	11.9	9.5	0.68	0.15
Sediment								
O ₂ penetration (mm)	1.7	2.7	1.2	3.2	0.1	2.0	1.5	4.0
[NO ₂ ⁻] (μM)	<0.05	0.1	0.09	0.11	1.3	1.3	0.43	1.1
[NO ₃ ⁻] (μM)	<0.05	<0.05	<0.05	<0.05	3.0	0.35	0.67	1.0

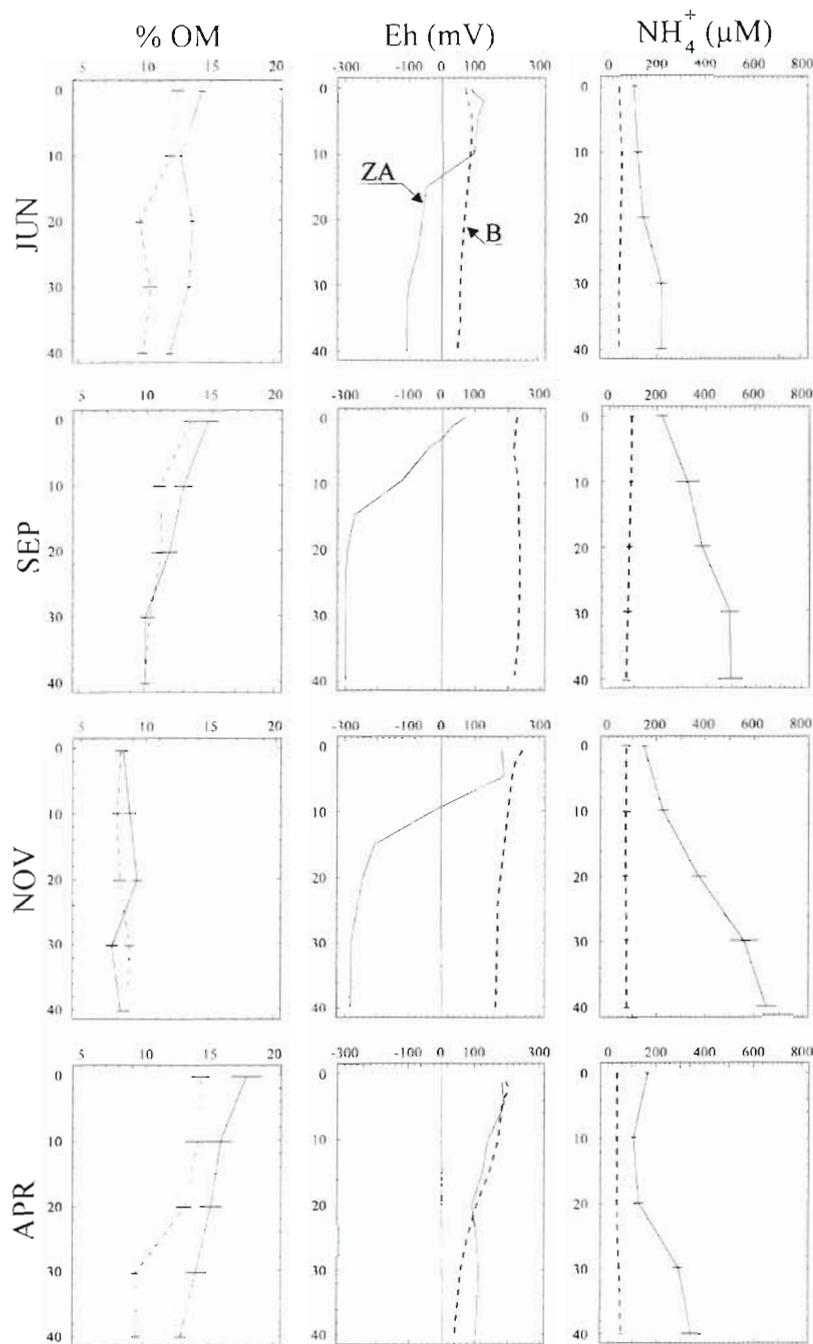


Fig. 3. Dry organic matter (%OM), distribution of redox potential (Eh), and concentration of ammonium in porewater (NH_4^+) in the upper 4 cm of sediment at Stn ZA (solid line) and Stn B (dashed line), from June 1994 to April 1995. y-axis: depth in sediment (mm). Values are mean \pm standard error ($n = 3$)

oxidation; Fig. 4B). These high rates of ammonium oxidation were also demonstrated by the significant net nitrifying activity ($23 \mu\text{mol}$ of N-NO_2 produced $\text{d}^{-1} \text{ l}^{-1}$ WS) in controls at Stn B in November (data not shown). A weaker but significant activity was also obtained outside the shellfish farming zone (Stn B) in April.

Dissimilatory processes: DAP and denitrification

In June, both the denitrification and DAP natural rates were very weak, averaging 0.26 and $1.57 \mu\text{mol d}^{-1} \text{ l}^{-1}$ WS, respectively, in Stn B (Fig. 4C, D). No significant differences were found between Stns ZA and B. In September, no denitrification activity was found at Stns ZA and B. On the other hand, highly enhanced DAP rates were measured, particularly at Stn ZA ($249 \pm 33 \mu\text{mol d}^{-1} \text{ l}^{-1}$ WS; mean \pm SE). In November, denitrifying rates were 2.1 ± 0.4 and $0.7 \pm 0.1 \mu\text{mol d}^{-1} \text{ l}^{-1}$ WS at Stns ZA and B, respectively, corresponding to the highest values obtained (Fig. 4C). At this sampling time, the DAP rates at the 2 stations dropped to very low values, corresponding to those found in June (Fig. 4D). Later on, between November and April, the denitrifying rates were back within the same range (around $0.6 \mu\text{mol d}^{-1} \text{ l}^{-1}$ WS) at both stations, whereas no significant changes in DAP rates were found in sediments.

For pore water NO_3^- and NO_2^- sediment concentrations never higher than $3.0 \mu\text{M}$, natural DAP and denitrifying activity may be very low. In view of this, we decided to complete the study by also taking into account their potential rates. Moreover, because of the oxygen penetration in the studied 0 to 2 cm layer (Table 1), which inhibits both the DAP and denitrifying activities, the activity rate measurements were extended down to 4 cm depth and separated into 2 layers (L1: 0 to 2 cm; L2: 2 to 4 cm). The natural denitrification and DAP rates measured in the 2 to 4 cm layer of the sediments of Stns ZA and B were of the same order of magnitude as those measured in the upper 0 to 2 cm layer (Table 2) and presented the same patterns of changes with time (data not shown).

The fluctuations with time of potential denitrification and DAP rates in the 2 layers of sediments from Stns ZA and B are presented in Fig. 5. In June, the potential denitrifying rates measured ($< 23 \mu\text{mol d}^{-1} \text{ l}^{-1}$ WS) were higher at Stn B than at Stn ZA in both layers of sediments (Fig. 5A, B). At the same time, the potential DAP

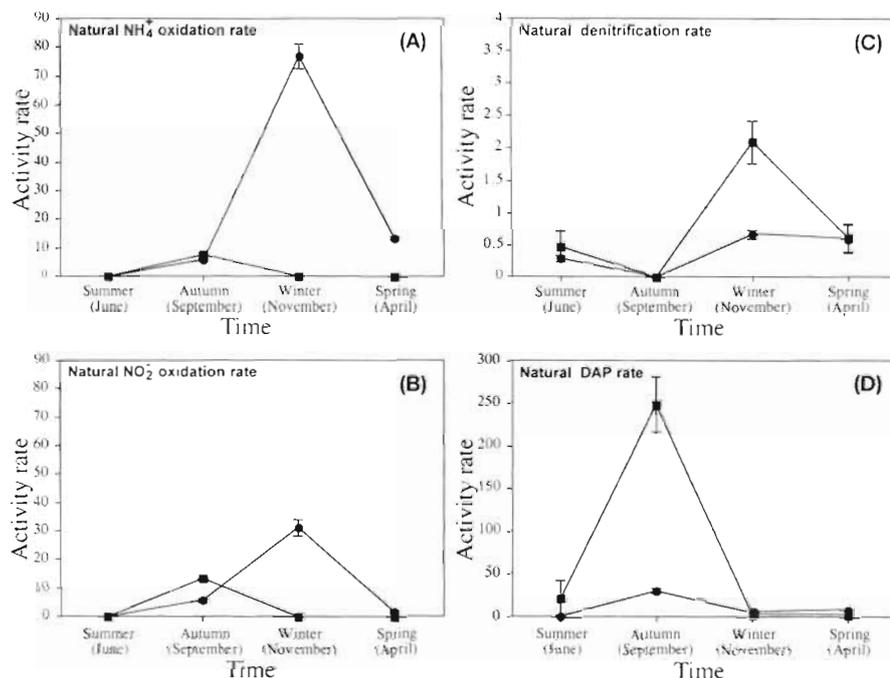


Fig. 4. Natural nitrification [both (A) NH_4^+ oxidation and (B) NO_2^- oxidation steps], (C) denitrification and (D) DAP rates in the 0 to 2 cm layer of the different sediments, from June 1994 to April 1995. Values are mean values \pm standard errors ($n = 3$) expressed in $\mu\text{mol d}^{-1} \text{l}^{-1} \text{WS}$ (wet sediment). (■) Stn ZA; (●) Stn B

rates at Stns ZA and B were about 27 and 40 $\mu\text{mol d}^{-1} \text{l}^{-1} \text{WS}$ in the 0 to 2 cm (Fig. 5C) and 2 to 4 cm layer (Fig. 5D), respectively. In September, the activity rates were either slightly (to 40 $\mu\text{mol d}^{-1} \text{l}^{-1} \text{WS}$; potential denitrification) or strongly enhanced (to 1300 $\mu\text{mol d}^{-1} \text{l}^{-1} \text{WS}$; potential DAP) at the 2 sites. In November, the potential activity rates came back within the same range as that measured in June. In April, both denitrification and DAP rates showed their highest values.

A 3-way ANOVA was applied to the data, in order to determine the space-time variations of bacterial activities (Table 2).

DISCUSSION

The wide fluctuations of environmental conditions, including the temperature, organic matter and DIN content, lead to considerable differences in nitrification, denitrification and DAP rates measured in the sediment of different ecosystems (Table 3). Because of the large variability in the values proposed in the literature, the nitrification, denitrification and DAP rates measured in the sediment of Thau lagoon sediment fall within the same range.

The results obtained have shown significant changes for the different bacterial processes with time and station localisation.

Seasonal variation

In June, the first sampling cruise was carried out during the typical summer situation. Dry periods correspond to low inputs of nutrients from the watershed, and primary production tends to be limited by nitrogen recycling process (Picot et al. 1990). Similar hydrological conditions were found in April, but the large difference in temperature (8°C) might have played a major role in the variation of benthic metabolism. However, in April, the nitrification, denitrification and DAP rates were on the same order as those of June. On the other hand, the concentration of O_2 at which NH_4^+ oxidation stops

Table 2. ANOVA analysis comparing the stations (Stns ZA and B), time (season) and sediment layers for the 3 bacterial N processes. When a significant effect is found with 'Time', the season with maximal activity is indicated. When a significant effect is found with 'Station' or 'Layer', the mean stimulation factor is indicated. NS: not significant; L1: 0 to 2 cm sediment core layer; L2: 2 to 4 cm sediment core layer; ND: not determined; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

	Station	Time	Layer
Natural rates			
NH_4^+ oxidation	ZA < B*** [$\times 12$]	Winter***	ND
NO_2^- oxidation	ZA < B*** [$\times 3$]	Winter***	ND
Denitrification	ZA > B*** [$\times 3.7$]	Winter***	NS ($p = 0.49$)
DAP	ZA > B* [$\times 3.3$]	Autumn***	NS ($p = 0.29$)
Potential rates			
Denitrification	ZA < B*** [$\times 1.4$]	Autumn, spring***	L1 > L2** [$\times 1.5$]
DAP	ZA > B** [$\times 1.5$]	Autumn, spring***	L1 > L2* [$\times 2$]

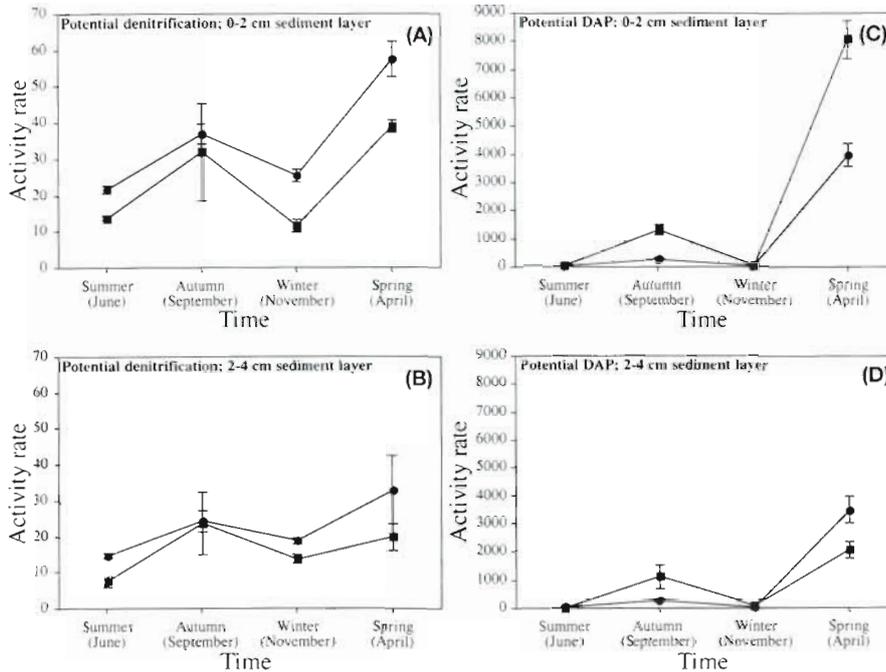


Fig. 5. Potential (A, B) denitrification and (C, D) DAP rates in the (A, C) 0 to 2 cm and (B, D) 2 to 4 cm layers of the different sediments, from June 1994 to April 1995. Values are mean values \pm standard errors (n = 3) expressed in $\mu\text{mol d}^{-1} \text{l}^{-1} \text{WS}$. (■) Stn ZA; (●) Stn B

Table 3. Nitrification, denitrification and DAP rates in various sediments ($\mu\text{mol l}^{-1} \text{d}^{-1}$). Both natural and experimentally enriched (*) activities are presented

Bacterial process	Source
Nitrification	
2.86	Koike & Hattori (1978)
0-150	Henriksen (1980)
15-70	Henriksen et al. (1981)
4.5-11.0	Szwerinski (1981)
760	Herbert (1986)
0.42	Enoksson & Samuelsson (1987)
0-77	Present study
Denitrification	
50-435*	Sørensen (1978a)
17.5	Sørensen (1978b)
60	Sørensen et al. (1979)
0-96	Andersen et al. (1984)
790*	Goeyens et al. (1987)
0-94.8	Raymond (1992)
23.2-35.4	Gilbert (1994)
0-19.8	Omnes (1996)
0-2.9	Present study
7.6-57.5*	Present study
DAP	
120-750*	Sørensen (1978a)
9-267	Blackburn (1979)
34	Jones et al. (1982)
2.8-110.4*	Enoksson & Samuelsson (1987)
790*	Goeyens et al. (1987)
1.3-83.2	Omnes (1996)
1.9-249	Present study
20.3-8054*	Present study

ranges from 1.1 to 6.2 mM (Henriksen & Kemp 1988) and as the oxygen penetration was only a few millimeters (Table 1), it is likely that nitrification in sediments was limited to the first centimeter. Due to conditions of low oxygen availability, H_2S inhibition and competition for NH_4^+ (Hansen et al. 1981, Joye & Hollibaugh 1995, Jensen et al. 1996), the nitrification rates decrease during summer

In autumn (September), the high salinity reported showed that the increase of DIN concentrations in the water could not be explained by freshwater inputs from the surrounding land. The mineralisation of the organic matter due to the primary producers of summer communities and NH_4^+ release from sediments after the anoxic period could have induced an increase in NH_4^+ concentrations in the water column, which would have probably enhanced pelagic nitrification (Berounsky & Nixon 1985, Collos et al. 1988), and thus increased NO_3^- concentrations. Higher NO_3^- concentrations at Stn ZA suggest that higher levels of NH_4^+ , coming from filter feeders and their epibiota, stimulated nitrification at the filter-feeder-water interface (Mazouni 1995), and then the NO_3^- produced was available for both dissimilatory processes (denitrification and DAP). However, at the same time, the highest %OM measured in the upper 20 mm of sediment confirmed the sedimentation of the phytoplankton bloom. In the presence of this organic matter supply, the elevation of the C/N ratio in sediments, already shown in autumn in the shellfish farming zone (Barranguet et al.

1994), may have favoured DAP compared to denitrification (Smith 1982, Tiedje et al. 1982, Rehr & Klemme 1989) that is predominant in low C/N conditions (Bowman & Focht 1974, Grabinska-Loniewska et al. 1985, Thomas & Lloyd 1995). As shown by the ANOVA results, in September (autumn), only the DAP was stimulated in sediments, denitrification being even lower than previously observed.

In November (winter), the 2 stations showed the same decrease in salinity, which reflected the influence of freshwater inputs from the watershed. Between April 1994 and April 1995, the total rainfall on the watershed was 456 mm, in comparison to a mean (\pm SE) of 623 ± 196 mm from 1961 to 1995. Therefore, the study period could not be considered as exceptionally wet, and a salinity of below 30 PSU with an NO_3^- concentration $>10 \mu\text{M}$ is reported to be common in this ecosystem (Tournier et al. 1982). The high NO_3^- concentrations in November probably induced diffusion into the sediment, which explains the significant levels of NO_3^- recorded in the pore water. The minimum %OM reported in November indicates efficient remineralisation. The combination of NO_3^- supply (by precipitation and streaming) and low levels of organic matter (inducing low C/N conditions) may thus have stimulated denitrification in the sediment, especially at Stn ZA. Moreover, if the nitrification rate was maximal, stronger stimulation of the NH_4^+ oxidation compared to the NO_2^- oxidation tended to induce accumulation of NO_2^- that can be used as substrate by denitrifying bacteria. In such conditions, the DAP rates were minimum. By comparing the patterns of change of the 2 dissimilatory nitrate reducing process rates, we have provided evidence, as already shown by Jørgensen (1989) and Zimmerman & Benner (1994), of some inverse seasonal variations between denitrification and DAP. Thus, it appears that the maximum nitrification rate (for both NH_4^+ oxidation and NO_2^- oxidation) took place in November, suggesting a high rate of production of NO_3^- substrate for the dissimilatory nitrate reducing processes, as measured in both water and sediment (Table 1). The maximum denitrification rate was also recorded at the same season. However, the highest DAP rate had already appeared in autumn when NO_3^- concentrations were lower.

Effect of shellfish farming activity

The ANOVA results (Table 2) also showed significant influence of the shellfish farming on bacterial activity. The dissimilatory processes (denitrification and DAP) were 3.5 times higher at Stn ZA than at Stn B. On the other hand, it appeared that Stn B presented an enhanced nitrification rate ($\times 12$ for NH_4^+

oxidation; $\times 3$ for NO_2^- oxidation) compared to that at Stn ZA. In fact, if the sediments were essentially similar, both being dominated by fine particles, sediments at Stn ZA had a slightly higher %OM, which reflects the greater deposition rates characteristic of shell farming areas (Dame et al. 1979). Moreover, the zone of rapid change from positive to negative Eh values situated in the upper 2 cm at Stn ZA indicated an organic enrichment in these sediments (Sampou & Oviatt 1991) compared with sediments from Stn B. The marked difference in concentration profiles of NH_4^+ between sediments from Stns ZA and B can be seen as resulting from different organic inputs (Sloth et al. 1995) combined with different nitrogen processes occurring in sediments (Enoksson & Samuelsson 1987). Because of oxygenated conditions at Stn B, aerobic nitrification could be observed. In contrast, the more reduced conditions at Stn ZA were more favourable for the anaerobic N processes (denitrification and DAP). In both stations, the end-product partitioning of dissimilatory nitrate reduction between nitrogen gas and ammonium was favourable to the DAP process (Fig. 4), suggesting that in the studied sediments, most of the nitrate was reduced to ammonium and recycled. Kaspar et al. (1985) estimated nitrate ammonification from the overall nitrate reduction minus denitrification. They also concluded that denitrification was the main dissimilatory nitrate reducing process, representing 76% of total nitrate reduction at the mussel farm and 93% at a reference site.

While the natural denitrification and DAP rates show the same pattern for the 2 sites, the ANOVA results obtained for the potential rates are different (Table 2). In contrast to the natural rate, the potential denitrification rate (corresponding to the enzyme content) in Stn ZA sediment was 1.4 times lower than at Stn B (Table 2), indicating the essential role of environmental conditions, such as carbon content, in the stimulation of the denitrification at Stn ZA, as already suggested by Kaspar et al. (1985). On the other hand, as for the natural rate, the potential DAP rate was higher at Stn B. For both denitrification and DAP, the 2 sites' sediments show similar natural rates in the 2 studied layers (0 to 2 and 2 to 4 cm), even if the respective potential rates were higher in the first (0 to 2 cm) than in the second layer (2 to 4 cm).

Conclusion

The changes in environmental conditions associated with the shellfish farming significantly influence the nitrogen cycle at the water-sediment interface of the Thau lagoon. The shellfish farming activity causes a lowering of the nitrification rate, whereas dissimilatory

nitrate reducing processes are stimulated. If the dissimilatory nitrate reducing processes are enhanced in such an ecosystem, denitrification remains a minor process versus DAP, suggesting that the loss of nitrogen for primary production is minimal. The DAP process maintains nitrogen available as ammonium, which plays a crucial role in the productivity of the coastal ecosystem.

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