Vol. 160: 47–56, 1997 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published December 15

Evidence for anaerobic bacterial processes in the water column: denitrification and dissimilatory nitrate ammonification in the northwestern Mediterranean Sea

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ABSTRACT: Bacterial anaerobic processes in particles in the water column of the northwestern Mediterranean Sea were investigated. Particles concentrated on a filter were incubated in oxygen-free sterile sea water. Results demonstrated that there is a weak but almost constant expression of bacterial nitrate-dissimilation processes [denitrification and dissimilatory nitrate ammonification (DAP)] associated with particles. Both activities were found from 30 m down to 615 m depth in coastal water off Marseilles, France, in autumn and from 100 to 700 m depth in spring and autumn in coastal water off Nice. In summer when oligotrophic conditions occurred, both activities were just detectable. In the other seasons, denitrification was the main process of nitrate dissimilation, with peak activity in autumn. This activity was associated with both large and small particles. In contrast, nitrate ammonification was associated only with large particles that were located in the upper layer in autumn and spread throughout the water column in spring.

KEY WORDS: Nitrate ammonification · Denitrification · Water column · Mediterranean Sea · Particles

INTRODUCTION

Particulate macroaggregates are often formed in the upper 50 m of the oceans (Alldredge & Gottschalk 1989). Typical aggregates contain rich communities of bacteria and protozoa, at densities 2 to 5 orders of magnitude higher than populations found in the surrounding water (Alldredge et al. 1986). This study was undertaken as part of the Mediterranean Targeted Project (MTP)-EMPS (European Microbiology of Particulate Systems); its purpose was to study the role of suspended particulate matter in microbial activities, and the main question addressed was 'the bacterial communities attached to the aggregates, their composition, their metabolic activities and their role in biogeochemical cycles'. With regard to the nitrogen cycle, dissimilatory reduction of nitrate by bacteria [comprising denitrification and dissimilatory nitrate ammonification (DAP)] is an important process. Reduction of nitrate to dinitrogen through denitrification leads to the production of gaseous products (N_2 or N_2O) that are rapidly lost for the ecosystem, whereas the alternative pathway (DAP) conserves nitrogen in a readily useable form (NH_4^+) and thus may cause nutrient enrichment. Therefore, depending on the relative intensity of both pathways, dissimilatory nitrate reduction can act as a source as well as a sink in the cycling of this element. Both corresponding pathways are generally described as strictly anaerobic processes, and their simultaneous occurrence has only been demonstrated in sediment (Jørgensen & Sørensen 1988, Gilbert et al. 1997) and in a generally oxygenated environment such as the particulate system from the very

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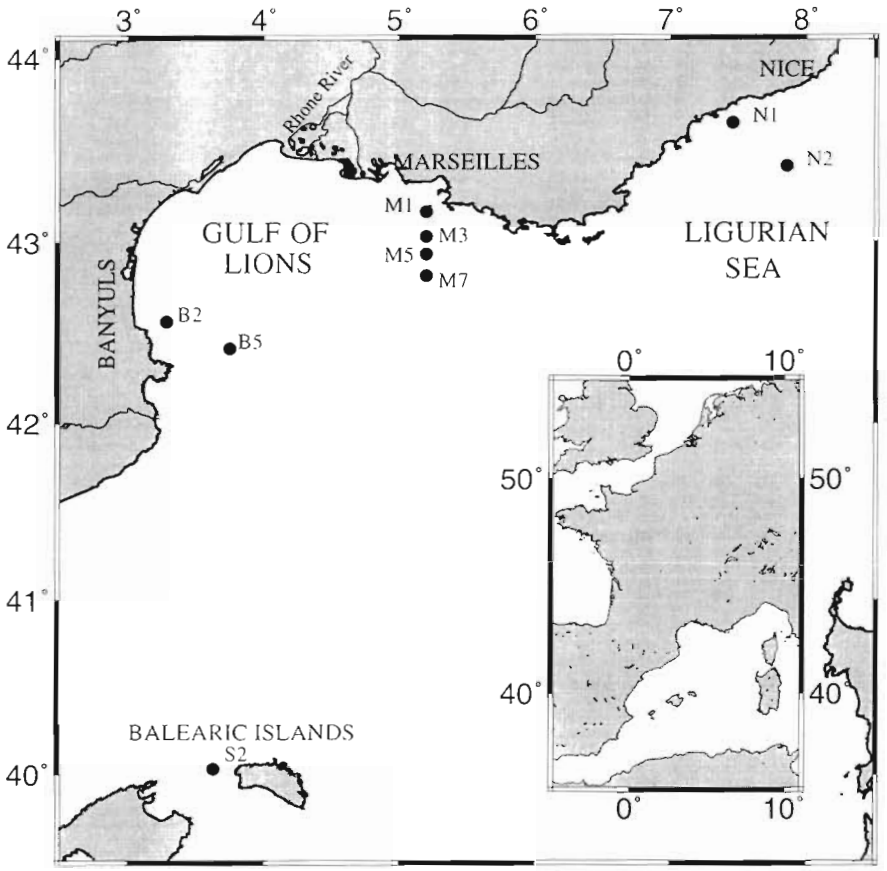

Fig. 1. Location of the sampling sites in the northwestern Mediterranean Sea

turbid plume water of the Rhone River (Omnes et al. 1996) but never in an oligotrophic ecosystem. The Rhone River plume water presents unusual features such as nitrate concentrations close to 100 mM and a very high number of particles in the water column, conditions which are far from being oligotrophic.

The aim of our work was therefore to investigate the occurrence and intensity of denitrification and DAP activities in the water column of the open sea and at coastal stations situated in the northwestern part of the Mediterranean (Gulf of Lions and Ligurian Sea) which are subjected to different land inputs at various periods of the year. These activities were investigated for particles of different sizes and, whenever possible, in phytoplankton peaks.

MATERIAL AND METHODS

Sampling strategy. Samples were taken in the Mediterranean Sea (Fig. 1). Table 1 gives the details of sampling. The cruises Pauline, Picnic, Mikel (EMPS project cruises; RV 'Thetys II' for the Pauline and Picnic cruises and RV 'Professeur Georges Petit' for the Mikel cruise) and Euromarge (EMPS and Euromarge joint cruise; RV 'Le Suroît') were organised as part of the MTP. During cruises the sampling depth was decided upon in real time according to the depth of the peak of large particles (>100 µm) determined by video profiler casts (Gorsky et al. 1992). For the Euromarge cruise, we sampled at the depth corresponding to the peak of fluorescence.

Temperature, fluorescence and salinity were measured using a CTD probe (Seabird SBE). Records of suspended particles larger than 100 µm were carried out using an underwater video profiler. Chlorophyll measurements were performed fluorimetrically.

For all cruises, particulate matter sedimenting through the water column was collected with an *in situ* pump (particle samplers: Challenger Oceanic) using 142 mm diameter filters. The pumps were run from 42 to 48 min. The filtration speed varied with the particle concentration and the resulting volume of filtered water varied from 80 to 400 l. For all cruises except Pauline, all particles greater than 0.8 µm were collected on a Millipore MF 0.8 µm filter For the Pauline cruise, particles were separated into 2 size ranges: >8 µm (Millipore AP 8 µm filter) and 0.8–8 µm (Millipore MF AP20). Except for the Pauline and Picnic (Stn N2, 28 miles) cruises, fecal pellets seemed to be

Table 1. Location, date and depth of samples collected during the different cruises

Cruise	Lat., long.	Date	Sampling depth (m)
Pauline (Marseilles transect)			
Stn M1	43°10'N, 5°12'E	12 Nov 94	25
			35
			65
Stn M3	43°02'N, 5°12'E	7 Nov 94	45
			55
			615
Stn M5	42°56'N, 5°12'E	15 Nov 94	30
			60
Stn M7	42°49'N, 5°12'E	14 Nov 94	45
			100
			620
Picnic (Nice transect)			
Stn N1, 5.5 miles	43°39'N, 7°27'E	7 Apr 95	140
			420
			700
Stn N2, 28 miles	43°25'N, 7°52'E	8 Apr 95	100
			250
			400
Euromarge			
Stn M1 (Marseilles)	43°10'N, 5°12'E	8 Jul 95	50
Stn B5 (Banyuls)	42°25'N, 3°45'E	1 Jul 95	40
Stn B2 (Banyuls)	42°34'N, 3°17'E	30 Jun 95	60
Stn S2 (Baleares)	42°02'N, 3°38'E	3 Jul 95	70
Mikel (Nice transect)			
Stn N1, 5.5 miles	43°39'N, 7°27'E	1 Dec 95	100
		2 Dec 95	300
		4 Dec 96	700

absent on the filters. Most of the particles collected with the *in situ* pump came from terrestrial sources; cuticular fragments were the only biogenic particles that could be found (Poulicek 1996).

Nutrient salts analysis. Subsamples of water collected with Niskin bottles and *in situ* pumps were frozen for later measurement of nitrate and nitrite concentrations using a Technicon autoanalyzer (Tréguer & Lecorre 1975).

Microbial activities. A combination of the acetylene blockage technique to assay for denitrification and a ^{15}N isotope tracer technique to measure DAP was used (Omnes et al. 1996, Gilbert et al. 1997). Measurement of the 2 bacterial activities was carried out independently with 100 ml subsamples kept in 130 ml serum flasks. Experiments were performed with water samples collected with a Niskin bottle or with particles collected on a filter using the *in situ* pump. For the latter samples, a fraction (1/10) of a filter (MF or AP20) was incubated with 100 ml of 0.22 mm filtered sea water. Flasks were sealed with butyl rubber stoppers and anaerobic conditions were obtained by flushing N_2 through the flask for 5 min. The incubations were performed in the dark and at a temperature corresponding to the *in situ* condition (±1°C). For each (denitrifying and DAP) activity, 2 measurements were determined: the natural and the potential (enzymatic) activity. The first (without any nitrate or carbon amendment) reflects the *in situ* metabolic rate, whereas the second reflects the amount of functional enzymes present in the sample at the time of sampling. For the latter activity, all factors affecting its expression (electron donor, i.e. glucose, and acceptor, i.e. nitrate) were adjusted to a non-limiting concentration (Tiedje et al. 1989). For measurement of natural activities, chloramphenicol was added to each sample (1 g l^{-1}) to avoid further protein synthesis; for measurement of potential activities, chloramphenicol, glucose (1 g l^{-1}) and KNO_3 (1 mM) were added (according to Tiedje et al. 1989). Activities were stopped by adding 100 µl of $HgCl_2$ (1 mM). Nitrate, nitrite, nitrous oxide and $^{15}NH_4^+/^{14}NH_4^+$ analyses were performed at time zero (starting time of incubation) and after 1, 3, 5, 10 and 24 h of incubation.

Denitrifying activity. The initial rate of nitrous oxide accumulation is considered to be the *in situ* denitrification activity. These assays were performed during a short-term incubation and N_2O was measured throughout the incubation period. The recommended period is 3 h. After incubation, 2.5 ml of the gas phase were sampled using a pre-evacuated Venoject tube. Extraction of nitrous oxide from the liquid phase was carried out using the procedure of Chan & Knowles (1979) modified by the technique of multiple equilibrium (McAullife 1971). Nitrous oxide was determined using a Girdel series 30 chromatograph equipped with an electron capture detector as previously described (Bonin et al. 1987).

Nitrate ammonifying activity. The procedure used has been extensively described by Omnes et al. (1996). The major criterion for identification of dissimilatory nitrate reduction to ammonium is the production of ammonium from nitrate in the presence of an excess of reduced nitrogen which is needed for growth (Tiedje 1988). Therefore, the formation of $^{15}NH_4^+$ was monitored in subsamples in which a small quantity of $^{15}NO_3^-$ (97.4% $^{15}NO_3/^{14}NO_3$; Isotec France) had been introduced. NH_4Cl corresponding to a final concentration of about 1 mM, was also added to the samples to block the nitrate assimilation pathway (Tiedje 1988). The progressive increase in isotopic enrichment of the ammonium and particulate fractions was monitored with time as the substrate (NO_3^-) was used. Ammonium was removed from the solution and trapped on a filter by microdiffusion. Filters containing NH_4^+ were then analyzed for ^{15}N content by mass spectrometry (Tracer mass, European Scientific) (Omnes et al. 1996, Gilbert et al. 1997).

RESULTS

Pauline cruise

The Marseilles transect was located on the Marseilles Canyon. Stn M1 was situated on the coastal shelf, and the other stations were in waters with depths of 1000 m or more.

Results of temperature and salinity profiles obtained during the Pauline Cruise are reported in Fig. 2. For all stations, a mixing of lower-salinity, warm upper water and colder, more saline deeper water was observed. The upper layer was 30 m deep for all stations except for Stn M1, where it was about 70 m deep. Stn M7 differed from the others in that the upper layer showed much lower salinity (37.5 vs 37.9‰). For Stns M1, M3, M5 and M7, the profiles of nitrate and nitrite concentration and the natural and potential activities are shown in Table 2 and Figs. 3 & 4.

Table 2. *In situ* denitrification and dissimilatory ammonium production (DAP) rates (nmol l^{-1} d^{-1}) in the Marseilles transect (Stns M1, M3, M5, M7) of the Pauline cruise. Minimum and maximum values shown for each sample. Values (initial linear rate of N_2O accumulation) were calculated from data (N_2O quantities) measured after 0, 1, 3, 5, 10 and 24 h of incubation. na: not available; nd: not detected

Stn	Depth (m)	Particle size (µm)	Denitrification rates Natural	Denitrification rates Potential	DAP rates Natural	DAP rates Potential
M1	25	>8	10.2–19.8	6.2–16.1	0.2–0.3	185.9–273.5
		0.8–8	11.9–12.3	7.1–15.8	<0.1	nd
	65	>8	na	na	<0.1	26.5–34.5
		0.8–8	6.7–11.9	6.9–10.8	<0.1	<0.1
M3	45	>8	15.8–39.1	18.9–28.9	0.9	78.2–79.1
		0.8–8	30.9–32.2	48.8–68.2	<0.1	<0.1
	615	>8	69.4–70.2	34.3–37.2	<0.1	13.3–17.4
		0.8–8	38.7–73.7	95.6–150.5	<0.1	7.1–9.9
M5	30	>8	79.2–166.7	74.2–153.1	<0.1	294.1–352.1
		0.8–8	50.1–67.8	43.8–79.2	<0.1	<0.1
	60	>8	53.9–107.8	55.1–83.7	<0.1	<0.1
		0.8–8	79.4–101.8	78.5–136.1	<0.1	<0.1
	150	>8	97.6–121.7	107.5–154.9	<0.1	<0.1
		0.8–8	38.5–94.9	43.12–106.6	<0.1	<0.1
M7	40	>8	17.9–30.3	20.8–35.7	<0.1	44.9–47.8
		0.8–8	45.5–73.7	54.4–62.6	<0.1	<0.1
	620	>8	41.9–83.7	45.5–79.2	<0.1	<0.1
		0.8–8	22.3–35.7	60.8–83.5	<0.1	<0.1

Concerning natural activity (Fig. 3), at all stations and depths, denitrification was the main process. This activity that was found at all sampled depths was within the same range of magnitude in small and in large particles. The maximum activities (mean value 90.6 and 122.9 nmol l^{-1} d^{-1} for small and large particles respectively) were found at Stn M5. In contrast, natural DAP was found only in the upper layer of Stns M1, M3 and M5 and was associated only with large particles. The maximum activity was found at Stn M3 (0.9 nmol l^{-1} d^{-1}).

By adding an electron donor (glucose) and acceptor (nitrate) to samples, the potential activities could be measured. For all stations, the potential denitrification activities were in the same range of magnitude as the natural ones, showing that the concentrations of nitrate and electron

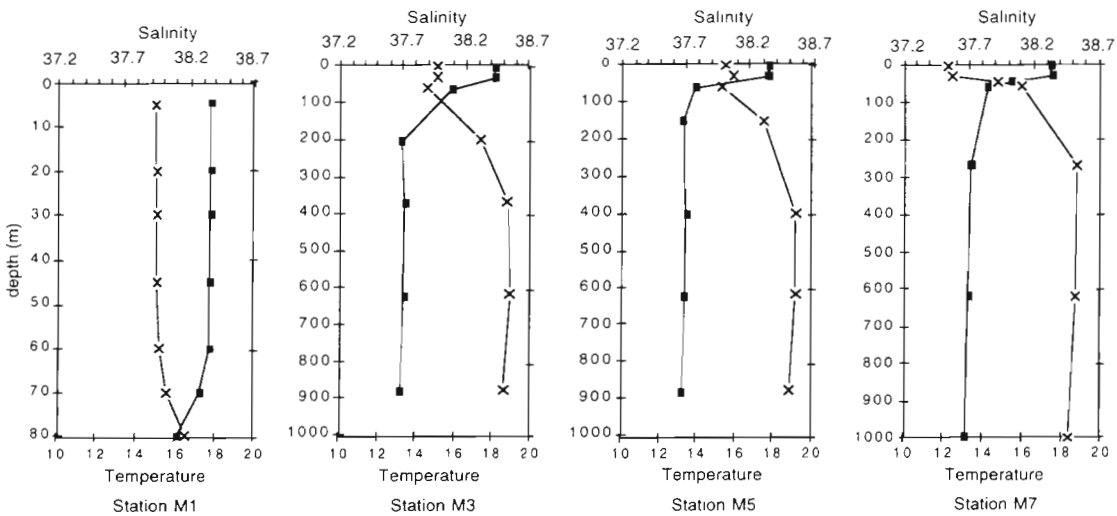

Fig. 2. Temperature (n) and salinity (×) profiles at Stns M1 to M7 of the Marseilles transect

donor were not the limiting factors for this process in the upper layer (Fig. 4). This suggests that if denitrifying bacteria were present they either occurred in small quantities or were not active. The activities were, however, very low.

In contrast, the addition of nitrate and electron donor resulted in an increase of the potential DAP activity, which then became the main process (Fig. 4). This activity was only found within large particles (>8 µm) and in the upper water layer, as was observed previously for the natural activity. In water samples collected with a Niskin bottle, we never observed DAP activity.

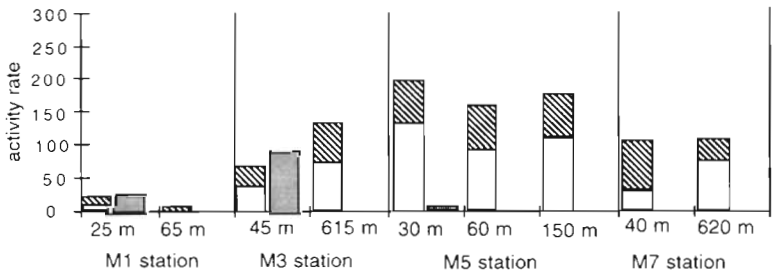

Fig. 3. Denitrifying and nitrate ammonifying (DAP) natural activities (mean values) in particles of different sizes at Stns M1, M3, M5 and M7 of the Marseilles transect. White bars: denitrifying activity in large particles (>8 µm); hatched bars: denitrifying activity in small particles (0.8–8 µm); grey bars: 100-fold DAP activity in large particles (>8 µm); DAP activity could not be detected in small particles. Activities expressed in nmol l^{-1} d^{-1}

For the other cruises, particles were not separated according to size.

Picnic and Mikel cruises (Table 3)

We investigated both denitrification and DAP activity on the Nice transect through the North Mediterranean Current (NMC) during the 2 periods of the year corresponding to its maximum flow. Two stations were sampled: N1, which is under coastal influence, and N2, which is an open sea station.

Comparison between results from the Pauline and Picnic cruises showed striking differences regarding the intensity and the distribution of denitrification and DAP activities. The natural denitrifying activities ranged from 1.8 to 44.8 nmol l^{-1} d^{-1} and were lower than those recorded from the Pauline cruise (Table 2) despite higher nitrate concentrations. The potential activities were 3- to 230-fold higher than the natural activities. For Stns N1 and N2, the natural activities decreased with depth whereas potential activities were maximum at the intermediate level (140 m) for Stn N1 and increased with depth for Stn N2.

For DAP activity, the natural rates ranged from 0 to 11.2 nmol l^{-1} d^{-1} and were higher than those observed for the Pauline cruise (0.9 nmol l^{-1} d^{-1}). Natural DAP rates increased with depth for both stations. The potential DAP activities were maximum at the intermediate level for Stn N1 and decreased with depth for Stn N2.

During the Mikel cruise, activities were measured only at Stn N1. Denitrification was of the same order of magnitude as that observed for the Pauline cruise which was carried out in the same season (autumn), and was higher than that recorded for the Picnic cruise

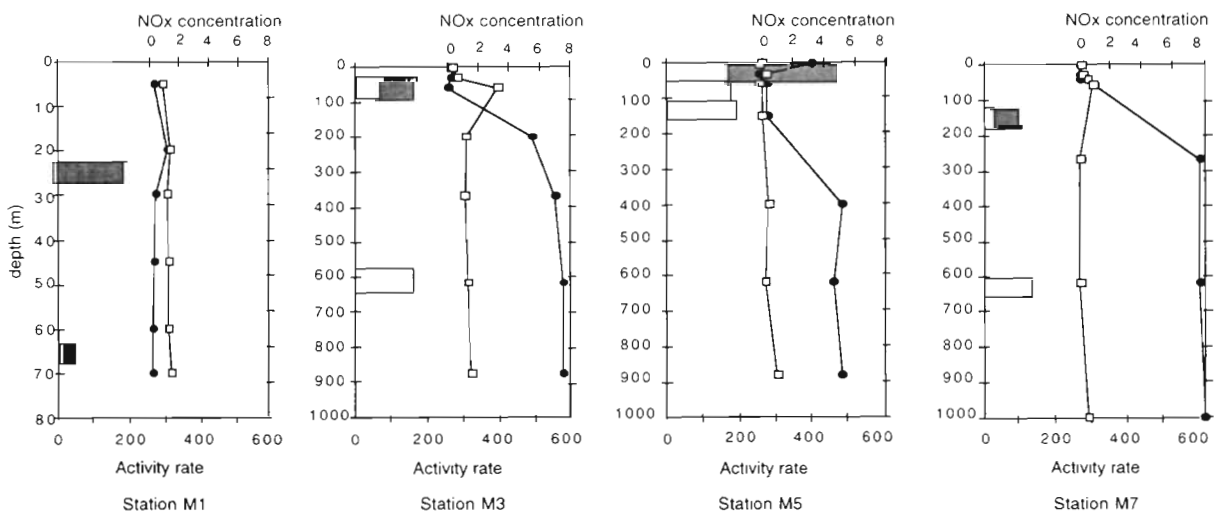

Fig. 4. Nitrate and nitrite profiles and potential denitrifying and DAP activities at Stns M1, M3, M5 and M7 of the Marseilles transect. (□) 10-fold nitrite concentration; (●) nitrate concentration; white bars: potential denitrifying activity in particles (>0.8 µm); grey bars: potential DAP activity in particles (>0.8 µm). Concentrations expressed in µmol l^{-1}; activities expressed in nmol l^{-1} d^{-1}

Table 3. Natural and potential denitrifying and nitrate ammonifying activities (DAP) in the Nice transect (Stns N1 and N2) of the Picnic (P) and the Mikel (M) cruises. Minimum and maximum values shown for each sample. Values (initial linear rate of N_2O accumulation) were calculated from data (N_2O quantities) measured after 0, 1, 3, 5, 10 and 24 h of incubation. na: not available

Stn	Depth (m)	NO_3^- (µmol l^{-1})	Denitrification rates		DAP rates	
			Natural	Potential	Natural	Potential
P-N1	140	4.74	10.9–15.7	285.3–369.4	<0.1	14.5–33.9
	420	9.99	2.4–3.3	338.8–461.5	<0.1	108.1–206.7
	700	8.05	3.9–4.3	267.4–270.8	2.48	10.4–15.5
P-N2	100	6.43	32.1–44.8	103.8–149.4	0.5–0.6	42.7–62.7
	250	6.25	1.8–2.7	134.6–190.2	3.9–7.5	na
	400	6.61	3.6–7.4	256.8–369.5	8.5–11.2	15.6–43.2
M-N1	100	1.28	19.4–33.5	185.9–223.6	0.8–0.9	110.6–149.2
	300	4.75	115.4–153.6	232.7–306.1	3.1–4.0	108.6–137.6
	700	6.44	84.6–113.1	273.8–343.3	2.6–4.3	na

Table 4. Natural and potential denitrifying and nitrate ammonifying (DAP) activities (nmol l^{-1} d^{-1}) in the Gulf of Lions (Euromarge cruise). Minimal and maximum values shown for each sample. Values (linear initial rate of N_2O accumulation) were calculated from data (N_2O quantities) measured after 0, 1, 3, 5, 10 and 24 h of incubation

Stn	Depth (m)	NO_3^- (µmol l^{-1})	Denitrification rates		DAP rates	
			Natural	Potential	Natural	Potential
B5	40	0.01	<2	<2	<0.1	<0.1
B2	60	2.09	<2	26.07–43.3	<0.1	<0.1
S2	70	0.63	<2	21.7–27.2	0.5–0.9	0.8–1.2
M1	50	1.23	<2	<2	<0.1	<0.1

(spring). Natural DAP measured for the Mikel cruise increased with depth, whereas the potential DAP showed little variation.

During the Euromarge cruise (Table 4), stations were sampled all over the northwestern part of the Mediterranean Sea in order to obtain an overview of the summer situation. Samples were taken at depths corresponding to the chlorophyll peaks. At these depths, nitrate concentration was very low except for Stn B2 where it reached 2.09 mM.

Natural denitrification rates were below the detection level for all the sampled stations. The potential rates were low (21.7 to 43.3 nmol l^{-1} d^{-1}) and could be detected only at Stns B2 and S2. Natural and potential DAP activities could be detected only at Stn S2 and were of similar intensity.

DISCUSSION

The Mediterranean Sea is characterized by an oligotrophic pattern increasing from west to east and approximately from north to south (McGill 1965). Durrieu de Mandron et al. (1990) have reported the existence of a gradient in total mass fluxes which appears to be related to the trophic level (Heussner et al. 1995): there is a west-east decrease of the total mass fluxes at the scale of the Mediterranean Basin which corresponds to the increase in the degree of oligotrophy. Heussner et al. (1995) have reported that the mass fluxes, averaged over a 6 mo summer period, were about 110 and 1080 mg m^{-2} d^{-1} for stations located on transects off Marseilles and Banyuls, respectively. In a water column that is generally oligotrophic, particulate matter containing sources of organic carbon provides a more favorable environment for microbial life (Cammen & Walker 1982, Koike et al. 1990). Furthermore, in a water column that is generally oxygenated, particles may provide microniches for a wide variety of metabolic processes, even anaerobic ones (Bianchi et al. 1992). In consequence, bacteria that are associated with particles, although minimal in number, exhibit metabolic activities that have a great impact on the mineral cycle of the water columns; this is especially so for activities that affect the nitrogen cycle (Tyrell & Law 1997).

Location of activities

The main hydrostatic motor in the studied area is the NMC. Conan (1996) has calculated that the annual flux of nitrate (0 to 200 m depth) of the NMC is around 3.1×10^5 t yr^{-1} compared to 3.5×10^5 t yr^{-1} for the Rhone River inputs. Nitrate and water fluxes varied with the same pattern. In July, nitrate fluxes were near zero. They increased from July to late autumn when they reached 4 g $N\text{-}NO_3^-$ l^{-1} s^{-1} and remained at this level until the spring. The NMC presents seasonal variations with 2 velocity maxima: at the beginning and at the end of the winter season (November/December and April/May) (Conan & Millot 1995). In summer, its speed is at a minimum. Its location also varies according to the period of the year. In late autumn (November), the NMC has an inshore position (30 km from land) that corresponds, for example, to a position between Stns M3 and M5 on the Marseilles transect (Conan & Millot 1995). The current has a width of 30 to 40 km and involves a water mass of 400 to 500 m depth. In summer the NMC runs offshore (about 50 to 60 km

from land), is wider and shallower (200 m) and the velocity is drastically reduced (down to 20 or 10 cm s^{-1}). All stations sampled in all cruises are potentially exposed to this geostrophic current.

For the Pauline cruise, the maximum natural denitrification activity was found at Stn M5, which is close to the core of the NMC at the period of the year when we sampled it (Conan 1996). Furthermore, it seems that denitrifying activity was associated with small and large particles, in contrast to DAP, which was associated with large particles only. Depending on the dynamics of large particles, DAP will be found at different depths. For the Pauline cruise, it was located only in the upper level, which is characterized by warmer, less saline water compared to the deeper water (S 37.5 to 38, temp. ca 17.5°C vs S 38.5‰, temp. ca 13°C). In contrast, for the Picnic and Mikel cruises, this activity was found throughout the water column. In these cases, the presence of large particles throughout the water column could explain the location where ammonifying activity was expressed. In this area and during the Picnic period, the NMC is very turbulent and unstable and causes the superimposition of different water layers (Millot 1990, Conan & Millot 1995). This phenomenon is also evidenced by an increase and decrease of the load of large particles (Gorsky et al. 1991). In fact a model of the behavior of particles on the same transect has shown that this sampling period (beginning at the transition period between bloom and oligotrophy) corresponds to an exportation time of large particles (Levy et al. 1997). This might also be the case for the Mikel cruise period, which corresponds, according to the same model, to a secondary bloom that is responsible for an export of phytoplankton cells through mixing processes.

In July, during the Euromarge cruise, the meteorological and hydrological conditions were typical of the relatively stable conditions found in this area during the summer: the NMC becomes wider and shallower, flows further offshore and becomes less susceptible to mesoscale variability such as meandering or internal waves. A similar pattern in the summer water structure occurs around the Balearic Islands (Font et al. 1988). The various stations differ in their water dynamics. The Banyuls transect has been shown to be under the influence of Rhone River input (Durrieu de Mandron et al. 1990). This could explain the higher nitrate concentration at Stn B2 (2.09 mM). During the sampling period the occurrence of both activities associated with the chlorophyll peak was investigated. Potential denitrification activity could be found only at coastal stations (B2 and S2) and DAP activity only at Stn S2. These results indicate that there was not a close relationship between the chlorophyll peaks and the occurrence of the 2 nitrate dissimilatory processes.

In the present study, the results of 4 cruises show that the location of denitrification activity in the Mediterranean Sea presents unusual features. This activity was found throughout the water column in almost every sampled station in the northwestern Mediterranean. In contrast, in the Baltic Sea (Brettar & Rheinheimer 1991) and the Arabian Sea (Naqvi et al. 1993) denitrification activity has been detected only in the minimum oxygen zone.

Intensity of activities

Our results indicate that denitrification and DAP activities are extremely weak in the open sea and can hardly be detected without first concentrating the samples.

Denitrification

Maximum natural denitrification rates were observed in autumn for the Pauline and Mikel cruises (234.5 and 153.6 nmol l^{-1} d^{-1} respectively). They were within the same range as that recorded in the Baltic Sea (110 to 140 nmol l^{-1} d^{-1}) (Brettar & Rheinheimer 1991) and the Arabian Sea (90 to 110 nmol l^{-1} d^{-1}) (Naqvi et al. 1993). This activity was, however, lower than that observed in sediment (ranging from 50 to 284 µmol l^{-1} d^{-1}) (Raymond et al. 1992, Omnes 1996, Gilbert et al. 1997) or in the Rhone River plume (1 to 4.3 µmol l^{-1} d^{-1}) (Omnes et al. 1996). In autumn, the natural and the potential denitrifying activities were of the same order of magnitude (for Stns M3, M5 and M7 the 2 activities were very similar) showing that the concentration of nitrate and electron donor was not the limiting factor for this process.

In spring, denitrifying activities were probably limited by the electron donors because potential activities and nitrate concentrations were within the same range as those observed in autumn.

Minimum denitrification rates were observed in July during the Euromarge cruise. During this period, nitrate concentrations were in the oligotrophic range for all sampled transects: Banyuls, Baleares and Marseilles. Denitrifying activity was below detectable values or just above. For Stns B5 and M1, the lack of activities was due to the lack of enzymes, since no potential activity was observed for these stations. In contrast, for Stns B2 and S2, potential denitrifying activities were observed but it may be noted that these were 15-fold lower than the activity obtained for Stn N1 (420 m) during the Picnic cruise. This result demonstrates that the small quantity of functional enzymes in the samples was probably due to the oligotrophic concentration of

nitrate. If denitrifying bacteria were present, they were not active, meaning that the associated enzymes were not present at the sampling time.

Nitrate ammonification

Natural DAP activity was very weak in the water column. The maximum natural activity was found for the Picnic cruise (11.2 nmol l^{-1} d^{-1}) and is 2×10^2 to 2×10^4 fold lower than that found in marine sediments (for review, see Gilbert et al. 1997). In contrast to denitrification, a seasonal pattern cannot be seen for DAP activity. For the Pauline samples, the addition of nitrate and an electron donor resulted in an increase of the potential DAP that became the main process. This result indicates that the weak natural DAP activity is limited by the amount of substrate. Except for Stn S2 during the Euromarge cruise, whenever potential DAP could be detected, it was always much higher than the natural rate. This result shows that, although nitrate-ammonifying bacteria might be present, their activity was usually limited by the lack of substrates.

Competition for nitrate use

The concentration of pigment was higher during the Picnic cruise than during the Pauline cruise (0.24 to 0.64 µg chlorophyll l^{-1} vs 0.07 to 0.20 µg chlorophyll l^{-1}). Below 60 m for Pauline and 100 m for Picnic, the pigments were undetectable (Iriberri 1995). During the Euromarge cruise the concentration of pigment was the highest (76 µg chlorophyll l^{-1} at Stn B5) (Bianchi et al. 1996). We can see that the higher concentration of chlorophyll corresponds to the lower dissimilatory nitrate reduction rates. This suggests a competition between nitrate-dissimilatory reducers and nitrate-assimilatory reducers in the photic zone.

Denitrification and nitrate ammonification are competitive bacterial processes. They occur in the same environmental conditions and compete for substrates (Tiedje et al. 1982, Omnes 1996, Gilbert et al. 1997). Studies on sediments have shown that DAP was maximum during summer whereas denitrification was maximum during winter (Jørgensen & Sørensen 1988, Omnes 1996, Gilbert et al. 1997). This phenomenon may be due to the variation of the bacterial community in relation with temperature. Ogilvie et al. (1997) suggest that at low temperature (<12°C) denitrifying bacteria predominate in estuarine sediments because of their ability to scavenge limited concentrations of nitrate, whereas nitrate ammonifiers are better competitors for nitrate at higher summer temperatures (>12°C). Our results show that in particulate matter in the northwest Mediterranean, the 2 processes did not present the same seasonal patterns as those described for sediments. Whatever the period of the year, natural denitrification was the main process. In a few cases, the natural DAP rate was equal to the denitrification rate. This phenomenon cannot be explained by temperature since, in the Mediterranean Sea, it is always higher than 12°C. *In vitro* studies have shown that depending on the nature of the (carbon) substrates, the one or the other process is favored (Bonin 1996). Competition for substrates is hard to elucidate in the water column ecosystem since natural nitrate ammonification rates are often below the detection limit. We have therefore focused our attention on Picnic cruise results, for which DAP activity could be measured at almost every sampled depth.

During this cruise, hydrological data showed that there was vertical mixing as described by Levy et al. (1997). The sampling period (April) corresponded to maximum fluxes (ammonium utilization by bacteria and phytoplankton, zooplankton activity, DOM assimilation, bacteria grazing) and a maximum fecal pellet production and high detritus export (Levy et al. 1997). Several studies have shown that degradation of fecal pellets is rapid (Andrews et al. 1984), suggesting that particle composition might vary with depth. Regarding the activities, it will be noted that the natural denitrification and DAP rates varied inversely to each other with changing depth. Whenever the denitrification rate decreased with depth (N1), ammonification rate increased and vice versa. This phenomenon does not result from a difference in density of functional bacteria, since the potential activities did not vary with the same pattern as the natural activities. Nor can the nitrate concentration account for this phenomenon, because it showed little variation. In consequence, microbial processes that take place in particles seem to be closely related not only to the availability of the electron acceptor but also to the quality and quantity of the organic carbon sources (electron donors). Depending on their chemical composition, the one or the other process could be favored. It has been suggested that bacteria such as *Pseudomonas* spp. that show an oxidative metabolism are responsible for denitrification activities, whereas bacteria such as *Vibrio* spp. that have a predominantly fermentative type of metabolism are responsible for DAP activity (Herbert & Nedwell 1990). During the sinking of the particles, the metabolism of microorganisms might alter the chemical constitution of particles, which in turn leads to changes in the bacterial processes taking place in the particles.

In conclusion, except in summer, a weak but almost constant expression of denitrification and nitrate ammonification in the water column was found during

this study. Associated bacteria are located in particles and their activities are often limited by the electron donor Our results in the Mediterranean Sea confirm those of Brettar & Rheinheimer (1992) that show the influence of carbon availability on denitrification in the Baltic Sea. The present work has also shed light on the influence of the electron donor on the competition between denitrification and nitrate ammonification in the water column. Rates of dissimilatory reduction of nitrate have been overlooked in the past in oceanic budgets. Both processes, together with the availability of electron donors and acceptors, should be taken into account in the calculation of nitrogen fluxes.

Acknowledgements. We thank the crew of the RVs 'Georges Petit', 'Le Suroît' and 'Thetys II' for their help during work at sea, as well as N. Garcia and D. Raphel for technical assistance; M. Bianchi was cruise leader of the Pauline and Picnic cruises; X. Durrieu de Madron was cruise leader of the Euromarge cruise. CTD measurements were carried out by M. Picheral and G. Gorsky for specific EMPS cruises and by X. Durrieu de Madron for the Euromarge cruise. M. Picheral and G. Gorsky recorded suspended particles. Chlorophyll measurements were performed by J. Iriberri (EMPS) and by Bianchi and co-workers (Euromarge). This work has been undertaken in the framework of the Mediterranean Targeted Project (MTP)-EMPS (European Microbiology of Particulate Systems) project (scientific coordinator: M. Bianchi). We acknowledge support from the European Commission Marine Science Technology (MAST) Program under contract MAS2-CT94-0090. We are grateful to M. Paul for his careful reading.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: July 25, 1997; Accepted: October 27, 1997
Proofs received from author(s): December 4, 1997