Regulation of nitrification in the land-ocean contact area of the Rhône River plume (NW Mediterranean)

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ABSTRACT: The study of natural conditions controlling nitrification processes was undertaken in the estuarine area of the Rhône River (NW Mediterranean Sea) over an annual cycle. Nitrification rates (N-oxidation rates, CO₂ fixation rates, specific counts of ammonium and nitrite oxidisers) as well as environmental parameters (temperature, salinity, suspended matter, inorganic nitrogen) were measured monthly over a 1 yr period. Depth profiles were obtained in low salinity plume water, at the edge of the plume (intermediate salinity) and in the sea, to study the evolution of nitrification processes from the mouth of the river to the sea of both the brackish surface layer and the bottom nepheloid layer. Nitrification rates in the surface plume generally declined with distance from the river and with increasing salinity, whereas nitrification rates in the benthic nepheloid layer exhibited little spatial variation. This was illustrated by high variability of activities in the plume (50 ± 37 and 33 ± 21 nM N oxidised h⁻¹ for ammonium and nitrite oxidation rates respectively) versus lower variability in the benthic nepheloid layer (30 ± 8 and 22 ± 6 nM N oxidised h⁻¹ for ammonium and nitrite oxidation rates respectively). Nitrification rates in both the plume and benthic nepheloid layer were higher in the summer (142 to 175 nM N oxidised h⁻¹ for ammonium oxidation and 64 to 96 nM N oxidised h⁻¹ for nitrite oxidation) than during the winter months (5 to 30 nM N oxidised h⁻¹ for both activities). Highest N-oxidation rates were related to low salinity in the surface layer, corresponding to the highest concentrations of ammonium from the river water. Ammonium oxidation (AO) is regulated by *in situ* ammonium concentration ($r^2 = 0.741$, $p = 0.0001$, $n = 132$) and nitrite oxidation (NO) by the ammonium oxidation rate ($r^2 = 0.850$, $p = 0.0001$, $n = 132$). AO rate = $14.5 + 10 [\text{NH}_4^+]$ and NO rate = $4.7 + 0.56 \text{ AO rate}$. Q_{10} values were calculated for ammonium oxidation (2.72) and nitrite oxidation (3.08) rates. The counts of nitrifiers reached 10^6 cell dm⁻³, representing less than 2% of the total bacterial counts. Nitrogen oxidation rates were correlated to nitrifying cell abundances ($r = 0.89$ to 0.99), and the activities per cell ranged from 1.2 to 1.9×10^{-15} mol N oxidised cell⁻¹ d⁻¹. The biomass production of the ammonium oxidisers was demonstrated by the decrease of N oxidised/C fixed ratio from 15.8 in winter to 10.8 in summer, in spite of the increase of AO rate in summer. A seasonal fluctuation of N oxidised/C fixed ratio for nitrite oxidisers was not demonstrated; however, the ratio increased from 21.8 ± 4.4 in winter to 29.3 ± 2.9 in summer. We estimated that 10% (winter) and 20% (summer) of ammonium was nitrified at the mouth of the river. During summer, nitrification is supported by the flux of ammonium from *in situ* mineralisation and contributes to nitrate excess in the plume. The contribution of nitrification to the N requirement of the primary producers was estimated to be 14% at the edge of the plume (intermediate salinity) and 66% at the sea station.

KEY WORDS: Nitrification rates · Mediterranean Sea · Plume · Seasonal cycle · Ammonium oxidisers · Nitrite oxidisers

INTRODUCTION

The understanding and further modelling of the impact of terrestrial inputs on the functioning of the

marine ecosystem, and more precisely on the functioning of the marine areas affected by the estuaries, depend on our capability to identify and classify the main factors regulating the bacterially mediated processes. Within the numerous processes involved in the functioning of ecosystems, nitrifiers play a central role in oxidising the more reduced form of nitrogen (NH₄⁺)

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into more oxidised forms, including NO_2^- and NO_3^- . Estuaries receive, via river discharge, dissolved and particulate organic and inorganic inputs that provide nitrogen and hold specific bacterial species. Spatial fluctuations of estuarine nitrification have been demonstrated through changes in the distribution and concentration of the different forms of inorganic nitrogen (Berounsky & Nixon 1990, Bronk & Glibert 1993, Pakulski et al. 1995). Nitrification rates have also been measured along the plume salinity gradient in several estuaries (Elkins et al. 1981, Helder & de Vries 1983, Owens 1986, Feliatra & Bianchi 1993).

The principal factors (substrate availability, temperature, oxygen, pH, light and substratum) regulating nitrifying activities have been described for pure cultures and for soil environments (Johnstone & Jones 1988, Guerrero & Jones 1997). The difficulty in extrapolating the response of pure cultures to the natural environment is however illustrated by the discrepancy between values of half-saturation constants of ammonium oxidisers in pure culture compared to those measured in the natural environment. Kaplan (1983) demonstrated that substrate affinity coefficients for marine nitrifying bacteria are much lower in *in situ* conditions than in culture conditions. For pure cultures, Prosser (1989) measured K_m (affinity constant) values ranging from 0.051 to 14 mM while Gentilhomme (1992) estimated K_m values of 0.017 μM in the Southwestern Mediterranean Sea. Relatively little attention has been paid to the relationships between apparent regulatory factors and *in situ* nitrifying rates. The predominant role of temperature on nitrification rates was demonstrated with *in situ* measurements during an annual cycle in Narragansett Bay, USA, by Berounsky & Nixon (1990). These authors estimated very high Q_{10} values for nitrification (6.8 to 17.6) in contrast to values of 2 to 3 measured for this activity by Carlucci & Strickland (1968) and Helder & de Vries (1983).

The Rhône River estuary is located in the northern part of the Western Mediterranean Sea. With an average flow of $1700 \text{ m}^3 \text{ s}^{-1}$, the Rhône discharges $5 \times 10^6 \text{ t yr}^{-1}$ of particulate matter, 76600 t yr^{-1} of inorganic nitrogen, 8400 t yr^{-1} of phosphorus and 92000 t yr^{-1} of silicate into the Northern Mediterranean Sea (Coste et al. 1985). The highest Rhône River outflows typically occur in spring and autumn and the lowest at the end of summer and in mid-winter. At the same time, nitrate displays maximum concentrations in winter and minimum in summer, while ammonium concentrations, coupled with dissolved organic nitrogen, are maximal in summer and autumn (Coste 1993). Due to the combination of light (controlled by the particulate load) and nutrient availability (controlled by nutrient concentrations), primary production at the plume boundary is 10 times the primary production within the

plume and 7 times greater than primary production in the oligotrophic marine area (Videau & Leveau 1990, Minas & Minas 1989). This localisation is similar to that noted in the Mississippi River plume (Lohrenz et al. 1990). At the same time, in the plume area, due to enrichments of ammonium and nitrate, bacterial processes such as nitrification, denitrification and nitrate reduction occur simultaneously (Bianchi et al. 1994).

The opportunity to look at the control of nitrifying activities in a natural environment was given, in the present work, by an annual cycle of measurements in the estuary of the Rhône River. The plume area provides highly variable conditions (salinity, inorganic nitrogen, organic carbon and suspended matter). The effect of these environmental parameters was estimated on nitrifying activities measured as N-oxidation rates, ^{14}C - CO_2 fixation rates and nitrifying bacteria counts. Furthermore, the importance of nitrification on the estuarine ecosystem could be assessed through studying the relationships between nitrifying activities and environmental factors.

MATERIAL AND METHODS

Sampling site and strategy. The 3 sampling stations were located in the vicinity of the Rhône River plume (Fig. 1), in the Northwestern basin of the Mediterranean Sea. Samples were taken monthly from November 1991 until October 1992. Following the hydrological structure of this area (see description in Feliatra & Bianchi 1993) we sampled 4 depths at each station: surface (plume), 1.5 m, 10 m and 2 m above the sediment (bottom nepheloid). The depth of the bottom increased from 50 m at the proximal station to an average of 200 m at the distal station. Water samples were collected using an 8 l Niskin bottle. All parameters were measured at each depth. Specific counts of nitrifying bacteria and the incorporation of $^{14}\text{CO}_2$ by nitrifiers were determined only once per season (January, May, July, October).

Physical and chemical parameters. Salinity and temperature were measured using a YSI 33 CTD probe (Yellow Springs Instrument Co, Yellow Springs, Ohio, USA). At each depth, subsamples were obtained for measurements of total suspended matter (Strickland & Parsons 1972), NH_4^+ (Solorzano 1969), NO_2^- (Bendschneider & Robinson 1952), and for NO_3^- determination by Technicon Analyzer (Tréguer & Le Corre 1975). Particulate carbon and nitrogen were measured with a CHN analyser (Perkin Elmer 204 C), using GF/F Whatman® filters combusted at 400°C for 4 h, and fumed with HCl prior to analysis.

Bacteriological parameters. Total bacterial counts were performed on samples (2 to 5 cm^3) filtered on

0.2 μm black Nuclepore filters following DAPI staining (Porter & Feig 1980), using an image analysis system (Van Wambeke 1988). Abundances of culturable ammonium oxidisers and nitrite oxidisers were estimated by the most probable number (MPN) technique (Tanaka et al. 1979). The mineral medium was amended with $(\text{NH}_4)_2\text{SO}_4$ (200 mg dm^{-3}) for ammonium oxidisers and NaNO_2 (100 mg dm^{-3}) for nitrite oxidisers. For each dilution, 1 to 10^{-7} , 2 cm^3 of sample was inoculated into 18 cm^3 of culture medium and incubated in the dark at 25°C for 22 and 49 d for ammonium and nitrite oxidisers respectively. The growth of ammonium oxidisers was indicated by the presence of nitrite in the medium as demonstrated by the Griess reagent Prolabo® and the growth of nitrite oxidisers by the disappearance of nitrite and presence of nitrate (reagent with diphenylamine). A statistical calculation of the MPN was performed (Rand et al. 1979).

Nitrification rates were determined as described by Feliatra & Bianchi (1993). Changes in nitrite concentration were measured in sub-samples containing allylthiourea (ATU) or NaClO_3 in order to inhibit the oxidation of ammonium and nitrite respectively. The sample was divided into $3 \times 1 \text{ dm}^3$ sub-samples. One (blank) was unamended, one received 10 mg ATU dm^{-3} (final concentration) and the third received 10 mM (final concentration) NaClO_3 . No substrate was added. The sub-samples were incubated in the dark in a culture chamber at *in situ* temperature (15°C). The nitrite concentration in the sub-samples was measured (Bendschneider & Robinson 1952) over 24 h at 4 h intervals, using 10 cm pathlength cells. The nitrification rates were derived from the linear relationship between the increase (ammonium oxidation: AO) or decrease (nitrite oxidation: NO) in nitrite concentration and time. In such short-term incubations, the effectiveness of inhibition with ATU treatment has been demonstrated for up to 2.5 d of incubation (Hall 1984).

The ^{14}C - CO_2 dark incorporation by nitrifiers was measured by the difference in $\text{H}^{14}\text{CO}_3^-$ incorporated between samples with and without the addition of the inhibitors ATU and NaClO_3 (Somville 1984). Each sample was divided into $6 \times 100 \text{ cm}^3$ sub-samples: 2 unamended blanks, 2 amended with 10 mg ATU dm^{-3} , and 2 amended with 10 mM NaClO_3 . $\text{NaH}^{14}\text{CO}_3$ (5 μCi [185 KBq] Amersham, specific activity 51.8 $\text{mCi mmol}^{-1} = 1.92 \text{ GBq mmol}^{-1}$) was added to every sub-sample. The bottles were incubated in a culture room at *in situ* temperature for 4 h. At the end of the incubation the samples were filtered through a 0.2 μm Nuclepore filter. The filters were rinsed with filtered sea water and 0.5 N HCl in order to remove any unincorporated ^{14}C and counted on a Beckman model LS 1800. The amount of C incorporated due to nitrification was

Fig. 1 Location of sampling stations. The shaded area shows the plume extension

calculated as the difference between the amount of ^{14}C activity in samples with and without ATU for the ammonium oxidisers and in samples with and without chlorate for the nitrite oxidisers. Previous tests have demonstrated the optimal incubation time for carbonate incorporation is 4 h as incubations of 12 or 24 h resulted in lower amounts of carbon incorporation due to losses of respiratory and excretory carbon and recycling processes (Feliatra 1994).

RESULTS

Environmental characteristics

Typical salinity depth profiles for each station are presented in Fig. 2. Throughout the year, the proximal station exhibited a low salinity upper layer of 1.5 m and a strong increasing gradient of salinity between 1.5 m and 10 m. This gradient was still present but attenuated at the distal station while at the sea station the influence of river water was no longer detected (Fig. 2). For this distribution of salinity, no annual cycle was observed.

Fig. 2. Typical depth profiles of salinity at the proximal, distal and sea stations

Water temperatures fluctuated from 10°C in winter to 18°C in summer. In winter, temperature globally increased from the plume water (11.2°C) to the sea water (12.0°C), while it was the reverse situation in summer (i.e. 17.7°C for plume water and 15.3°C for sea

Table 1. Seasonal average values ($\pm$ SD) of temperature (°C) at the 3 stations. Winter, spring and autumn: $n = 12$; summer: $n = 8$

Season	Proximal	Distal	Sea
Winter	11.2 $\pm$ 1	11.2 $\pm$ 0.9	12.0 $\pm$ 0.2
Spring	13.7 $\pm$ 0.3	13.5 $\pm$ 0.3	13.7 $\pm$ 0.2
Summer	17.7 $\pm$ 0.5	16.7 $\pm$ 1.6	15.3 $\pm$ 1.9
Autumn	16.9 $\pm$ 0.8	16.2 $\pm$ 1.8	15.3 $\pm$ 1.9

water). At the distal and the sea stations, a vertical gradient was observed in summer and autumn, demonstrated by an increased value of the SD of the average temperature (Table 1). The fluctuations of the suspended matter (SM) load demonstrated 2 trends: (1) at the proximal station, all depths showed a similar weight of suspended matter (34 to 39 mg dm⁻³) with non-seasonal variations, (2) at the 2 other stations, the bottom nepheloid layer demonstrated a SM load higher than those of the other depths, particularly the surface layer. The SM load of the surface layer exhibited a seasonal trend with an increase in spring and summer, a decrease in autumn, and low values in winter (Table 2). The load of SM in the bottom nepheloid layer did not decrease with increasing distance from the mouth of the river.

Particulate organic carbon (POC) fluctuations displayed different patterns (Table 2). In the surface layer the POC showed a drastic drop (70%) between the proximal and the sea stations, while the SM decreased by only 40%. The values of the POC/PON ratio in the surface layer and at 1.5 m depth of the sea station were significantly lower than those of the proximal and the distal stations (t -test, $df = 20$, $p = 0.05$), indicating the detrital composition of the particles in the plume water. In the bottom nepheloid layer, POC decreased by about 30% and the POC/PON ratio remained close to 11. The combination of the characteristics of SM, POC and C/N suggested an uncoupling of the bottom nepheloid layer from the water column.

The distinction between the surface water and the bottom nepheloid layer was confirmed by the concentrations of ammonium, nitrite and nitrate (Fig. 3). In the bottom nepheloid layer, concentrations of inorganic nitrogen forms were very similar and did not fluctuate during the year at the 3 stations, whereas concentrations of ammonium, nitrite and nitrate decreased from the proximal station to the sea in the superficial layer (Fig. 3). In the surface layer, nitrite and nitrate distribution exhibited the same pattern at the 3 stations despite a 3-fold increase between sea and distal stations and again a 3-fold increase between distal and proximal stations. Nitrite concentrations peaked in summer whereas nitrate peaked in winter and sum-

Table 2. Annual average values ($\pm$ SD) of suspended matter (SM), particulate organic carbon (POC) and C/N ratio of the particulate matter at the 3 stations ($n = 11$)

	SM (mg dm ⁻³)			POC (mg dm ⁻³)			C/N ratio		
	Proximal	Distal	Sea	Proximal	Distal	Sea	Proximal	Distal	Sea
Surface	37.4 $\pm$ 3.9	26.3 $\pm$ 6.8	22.6 $\pm$ 8.0	1.44 $\pm$ 1.03	0.74 $\pm$ 0.24	0.42 $\pm$ 0.19	12.6 $\pm$ 4.2	12.1 $\pm$ 5.2	9.7 $\pm$ 2.5
1.5 m	35.7 $\pm$ 3.3	32.5 $\pm$ 5.4	28.4 $\pm$ 2.9	0.80 $\pm$ 0.35	0.49 $\pm$ 0.23	0.31 $\pm$ 0.05	12.9 $\pm$ 2.6	9.4 $\pm$ 3.2	9.6 $\pm$ 2.5
10 m	34.1 $\pm$ 4.1	32.2 $\pm$ 4.4	31.0 $\pm$ 4.4	0.49 $\pm$ 0.29	0.43 $\pm$ 0.17	0.28 $\pm$ 0.10	10.0 $\pm$ 2.0	9.9 $\pm$ 1.9	10.4 $\pm$ 3.3
Bottom	38.9 $\pm$ 4.5	38.4 $\pm$ 5.0	37.0 $\pm$ 5.4	0.67 $\pm$ 0.48	0.35 $\pm$ 0.22	0.46 $\pm$ 0.25	10.9 $\pm$ 4.1	11.3 $\pm$ 2.9	10.6 $\pm$ 2.3

Fig. 3. Seasonal fluctuations of inorganic nitrogen species

mer. Ammonium distribution exhibited a different pattern at the 3 stations, with a late winter and a summer peak at the sea station, a single summer peak at the distal station, and high concentrations from late winter to late summer at the proximal station. There was a 3-fold increase between open sea and distal stations and an 1.5-fold increase between distal and proximal stations.

Bacterial counts

In the surface layer, total bacterial numbers decreased from the plume to the sea, while they were quite similar in the bottom nepheloid layer (Fig. 4). For all samples the number of bacteria fluctuated in a narrow range (5 to 6×10^6 bacteria cm^{-3}). A seasonal effect on total counts of bacteria could be demonstrated in the surface layer (surface and 1.5 m), with highest numbers in May and July (Fig. 4) and a trend to decrease from July to January. In winter, the bacterial abundance was similar at all stations. The number of culturable ammonium oxidisers fluctuated from 1.1 (winter, sea station, 10 m depth) to 35×10^3 bacteria cm^{-3} (summer, proximal station, surface layer). Abundance of culturable nitrite oxidisers was 3 to 5 times

lower than that of ammonium oxidisers (Fig. 4). As with total numbers of bacteria, numbers of nitrifiers decreased both from the proximal to the sea station and from July to January.

Distribution of nitrification activity

In the study area, nitrification displayed 3 major characteristics (Fig. 5). (1) The upper layers (surface and 1.5 m) demonstrated a behaviour different from the 10 m and bottom nepheloid layers. In the deepest layers, whatever the sampling station, the nitrifying rates were similar, while in the surface layers (0 and 1.5 m) there was a decreasing gradient of both rates from the proximal to the sea station. (2) At the sea station, the nitrifying rates were higher in the bottom nepheloid and 10 m depth layers than in the upper layers. (3) At all depths, the nitrifying rates exhibited seasonal trends with the lowest values in January-February (5 to 30 nM N oxidised h^{-1} for both activities) and the highest in July (between 142 and 175 nM N oxidised h^{-1} for AO and 64 to 96 nM N oxidised h^{-1} for NO at the distal and proximal stations, respectively).

As for the N oxidation rates, the CO_2 incorporated by the nitrifiers decreased from the proximal to the sea

Fig. 4. Spatio-temporal fluctuations of (A) ammonium oxidiser, (B) nitrite oxidiser (C) and total bacteria counts

Table 3. Seasonal average values ($\pm$ SD, $n = 4$) of N oxidised to C incorporated ratio at the 3 stations

Season	Ammonium oxidisers			Nitrite oxidisers		
	Proximal	Distal	Sea	Proximal	Distal	Sea
Winter	15.2 $\pm$ 1.5	14.3 $\pm$ 2.7	16.1 $\pm$ 2.3	23.5 $\pm$ 6.2	22.4 $\pm$ 2.7	19.7 $\pm$ 4.5
Spring	13.5 $\pm$ 0.1	14.1 $\pm$ 0.3	14.2 $\pm$ 2.3	29.3 $\pm$ 0.1	29.0 $\pm$ 0.3	26.1 $\pm$ 6.0
Summer	10.4 $\pm$ 0.1	11.0 $\pm$ 0.3	10.9 $\pm$ 0.1	32.4 $\pm$ 1.0	26.7 $\pm$ 7.6	30.6 $\pm$ 0.3
Autumn	13.0 $\pm$ 0.2	13.4 $\pm$ 0.1	13.5 $\pm$ 0.2	29.4 $\pm$ 0.3	28.8 $\pm$ 0.6	28.7 $\pm$ 0.2

station in the surface and 1.5 m layers, while the rates were similar in the 10 m depth and bottom nepheloid layers (Fig. 6). Seasonal fluctuations were demonstrated at the proximal and distal stations. At the sea station, the rates of CO_2 incorporation were low and only the summer rates (July) increased clearly (Fig. 6).

The molar ratio of nitrogen oxidised per carbon incorporated was measured 4 times during the year at

all stations. This ratio trended to decrease in summer. The average ratios for the surface layer were 15.8 ± 2.1 in winter, 13.9 ± 0.5 in spring, 10.8 ± 0.2 in summer and 13.5 ± 0.3 in autumn (Table 3). In contrast, the molar ratio of nitrogen oxidised per carbon fixed by the nitrite oxidisers globally increased in summer (21.8 ± 4.4 in winter, 28.1 ± 2.1 in spring, 29.9 ± 2.9 in summer and 28.9 ± 0.3 in autumn) and showed high variability.

Table 4. Relationships (Spearman coefficient) between nitrifying activities and environmental factors ($n = 132$). *** $p \leq 0.001$; ns: non significant. AOR: ammonium oxidation rate; NOR: nitrite oxidation rate; SM: suspended matter

	Month	Station	Depth	Salinity	SM
AOR	ns	0.14***	0.1***	0.34***	0.11***
NOR	ns	0.14***	ns	0.27***	ns

DISCUSSION

Environmental factors controlling the nitrification rates

Nitrification rates were independent of the geographical position of the sampling station, the depth of sampling and the season (Table 4). The salinity value

Fig. 5. Spatio-temporal fluctuations of ammonium oxidation and nitrite oxidation rates

rather than the location of the sampling point drove spatial distribution of nitrification rates. The highest rates of nitrification were related to the lowest salinity, indicating that nitrification in the plume was supported by riverine inputs of NH_4^+ . No linear or exponential decrease with depth of the nitrifying rates was observed.

The first step of nitrification, i.e. AO, was principally driven by the availability of substrates, irrespective of other environmental conditions (Fig. 7a). AO, in the present study, was regulated by the *in situ* ammonium concentration ($r^2 = 0.741$, $p = 0.001$, $n = 132$). The importance of substrate availability to AO and NO rates has been described previously in the Antarctic Ocean (Bianchi et al. 1997). NO rate was regulated by the AO rate ($r^2 = 0.850$, $p = 0.001$, $n = 132$) independently of *in situ* nitrite concentration (Fig. 7b). Such control of the NO rates by the AO rates in the natural environment has been demonstrated for the first time in the present work. Indeed, the distribution of nitrogen salts in this estuarine area is due to the combination of the Rhône River inputs, the role of biological processes such as nitrification and denitrification (Bianchi et al. 1994) and/or primary production uptake (Bianchi et al. 1993).

From the large data set ($n = 132$), a Model II regression was used to describe the mathematical relationship between NH_4^+/AO ($\text{AO} [\mu\text{M h}^{-1}] = 14.5 + 10 \text{ NH}_4^+ [\mu\text{M}]$) and AO/NO ($\text{nitrite oxidation} [\mu\text{M h}^{-1}] = 4.7 + 0.56 \text{ ammonium oxidation} [\mu\text{M h}^{-1}]$). Such relationships could be used for modelling the biological processes (including the regulation of primary production).

The nitrification rates showed a highly significant relationship with temperature ($r^2 = 0.247$, $p = 0.0001$, $n = 132$ for AO, and $r^2 = 0.361$, $p = 0.0001$, $n = 132$ for NO). Q_{10} values were determined for the temperature range 9 to 19.9°C, corresponding to 5°C either side of *in situ* temperature. The Q_{10} values have been calculated from the slope of the conventional Arrhenius plot (the logarithm of rate vs the absolute temperature) using the expression $Q_{10} = \exp(-10 \cdot m/T_{15}^2)$ (Raven & Geider 1988), where T_{15} is the *in situ* temperature (K) and m is the slope of the line at *in situ* temperature. Q_{10} values calculated from a large data set ($n = 132$) of *in situ* measurements were 2.72 for AO rates and 3.08 for NO rates. These values are close to the classical value of 2 for bacterial activities, but far from the apparent Q_{10} of 17.6 (*in situ* data) or 7.5 (experiments) calculated by Berounsky & Nixon (1990) in Narragansett Bay.

Fig. 6. Spatio-temporal fluctuations of carbon fixation rates of ammonium oxidising and nitrite oxidising bacteria

These authors used a fixed N oxidised to C incorporated ratio of 5.95 to extrapolate the NO rate from the C fixation rate, as they calculated the nitrification rates from the rates of ^{14}C - CO_2 incorporation. We have illustrated the spatial and seasonal variability of this ratio (Feliatra & Bianchi 1993 and the present work), which should be taken into account for Q_{10} calculations. If the values we measured were in the range of ammonium oxidising strains (reviewed in Ward 1986), the low summer values should imply that the biomass of these nitrifiers is mainly accumulated in summer. Using pure cultures of nitrifying bacteria (i.e. *Nitrosococcus oceanus* for AO and *Nitrococcus mobilis* for NO), Glover (1985) showed that the stoichiometric relationship between NH_4^+ or NO_2^- oxidation and the yield of organic C production did not remain constant and appeared to be dependent on the availability of the inorganic substrate for oxidation. This was true for ammonium oxidisers, for which C fixation rates were highest in July when ammonium concentrations peaked, inducing high AO rates. But, the relationship between substrate and organic C production was less clear for the nitrite oxidiser *N. mobilis* (Glover 1985). Considering the biomass production by nitrifiers in the study area, nitrite oxidisers displayed rates between 2 and 5 times lower than ammonium oxidisers, which

could be explained by the fact that NH_4^+ oxidation liberates more free energy than NO_2^- oxidation for an equivalent amount of C- CO_2 assimilation (Kelly 1978). However, in our measurements, AO as well as NO rates were positively correlated with temperature, while C- CO_2 fixation rates were positively correlated with temperature for ammonium oxidisers and negatively correlated with temperature for nitrite oxidisers, leading to a trend for the NO/C fixation ratio to increase during summer whatever the enhancement of NO rates (Fig. 8). In the Indian sector of the Southern Ocean, displaying low but measurable concentrations of ammonium (0.3 to 0.7 μM), the NO/C fixation ratio for ammonium and nitrite oxidisers showed significant negative correlation with temperature (Bianchi et al. 1997), as did the ratio for ammonium oxidisers in the Rhône River plume.

Due to the difficulty in selective counting of bacteria in natural environments, few data are available concerning the numbers of marine nitrifying bacteria (see review in Ward 1986). Despite the development of molecular probes, it is difficult to assess the actual number

Fig. 7. (a) Correlation between ammonium oxidation rate (AOR) and *in situ* concentration of NH_4^+ . (b) Correlation between nitrite oxidation rate (NOR) and AOR

of these bacteria in field samples (Ward 1995). Using the MPN technique, we were able to estimate that numbers of culturable nitrifiers in the Rhône River plume area were in the range of values reported in the literature (Ward 1995). The number of both nitrifiers increased in summer, concomitantly with the increase of the total number of bacteria. The average percentage of nitrifiers was less than 2% of the total counts. Counts of ammonium oxidisers were performed using nucleic probes at the same stations in this plume area, and showed the number of nitrifiers to be about 1 order of magnitude (Fosset 1998) higher than MPN counts. This discrepancy between 'total' and 'viable' counts could be in part explained by the contribution of non culturable bacteria to the total count. Nevertheless, the biomass production (CO_2 fixation rate) was well correlated with the living biomass increase (MPN numbers), i.e. $r^2 = 0.69$, $p = 0.0001$ and $r^2 = 0.64$, $p = 0.0001$, for ammonium and nitrite oxidisers, respectively. This indirectly validates the MPN method through an independent approach.

Nitrifying (AO and NO rates) activities were also well correlated with the number of corresponding culturable nitrifiers, independent of the spatio-temporal fluctuations. Thus, for ammonium oxidiser numbers and AO rates, r^2 values ranged between 0.89 in winter and 0.96 in summer and autumn. For nitrite oxidiser numbers and NO rates, r^2 values were 0.91 in spring and 0.99 in winter. Specific activities, calculated from the number of culturable nitrifiers obtained with the MPN method, fluctuated from 1.2 to 1.9×10^{-15} mol N-NH_4^+ oxidised d^{-1} for ammonium oxidisers. These rates were relatively low compared to the nitrite production rate per cell of about 5×10^{-13} mol $\text{cell}^{-1} \text{d}^{-1}$ reported by Ward et al. (1982) using immunofluorescent enumeration of the species *Nitrosomonas marinus* and *Nitrosococcus oceanus*. However, Ward et al. (1982) counted only 2 species of nitrifiers without knowing their relative importance within the overall ammonium oxidiser population in natural marine environments. For a pure culture and at its maximum growth rate *N. oceanus* demonstrated a per cell rate of 1.4×10^{-12} mol $\text{cell}^{-1} \text{d}^{-1}$ oxidised (Carlucci & Strickland 1968). It seems reasonable that natural rates would be lower than this value as the optimal conditions of growth may not exist in a marine environment. On the other hand, the activity per cell increased from the proximal to the sea station, showing an increase of specific activity in low substrate concentration conditions. Such modification of bacterial activity should be related to the lowering of substrate affinity (K_m) values in low nutrient environments, like that noted by Gentilhomme (1992) in the Southwestern Mediterranean Sea.

To summarise the control of nitrification in the estuarine environment, our data demonstrate that the rates

Fig. 8. Correlation between N oxidation/C fixation ratio and temperature for ammonium and nitrite oxidisers

of both AO and NO processes are mainly regulated by substrate concentration, the temperature being able to enhance or to reduce the resulting activity. The temperature increased the biomass production of both nitrifiers in summer, but it was less visible for nitrite oxidisers because C fixation rates showed a maximum increase (proximal and distal station in July) of 3 times while the NO rate increased by 5 times during the same period. The numbers of culturable nitrifiers were well correlated with their respective activities and the specific activity fluctuated in relation to the environmental concentration of substrate.

Implication of nitrification in the oligotrophic marine area of the Rhône River plume

In this estuary, we have seen that the behaviour of nitrification rates was clearly different in the water column and in the benthic nepheloid layer. So, the role of nitrification can be considered within each of these components of the estuarine area.

In the water column (surface and 1.5 m depth), for each month, we calculated, at the distal station, the difference between the concentrations of ammonium and nitrate measured *in situ* and those estimated from a conservative dilution as a function of the salinity (Fig. 9). The calculations took into account the concentration recorded at the mouth of the river on the same day, avoiding the possible seasonal influence of the river discharge. Positive values indicated a net source and negative values a net sink of ammonium and/or nitrate during the transit of the water from the river mouth to the edge of the plume. In winter, we observed a maximum of nitrate consumption despite the highest discharge rate (Coste et al. 1985). Previ-

Fig. 9. Seasonal fluctuations of $(\text{NO}_3^- - \text{NH}_4^+)$ departure from the salinity dilution line at the distal station. Positive values indicate a net source and negative values a net sink of NH_4^+ and NO_3^- .

ous studies at same station highlight a cyanobacterial bloom during the winter period (Soto et al. 1993), which could be held responsible for the nitrate consumption. The second period of consumption, in May, is due to the spring bloom composed of large phytoplanktonic cells and autotrophic flagellates that peaked at the distal station (Soto et al. 1993). From March, nitrification increases (Fig. 5), which gives rise to the nitrate production before the spring bloom. This nitrate production (about $1.5 \mu\text{M d}^{-1}$) continues over the spring period, compensating in part for the nitrate consumption in May (Fig. 9). While ammonium concentration is close to the value given by the theoretical dilution in winter and spring, enhanced ammonium production is noted in June and July. This production may originate from biological processes; either from mineralisation of the organic matter delivered from the river or from the primary production in the plume (Coste et al. 1985, Soto et al. 1993, and as noted by Pakulski et al. 1995 in the Mississippi River plume) or by dissimilative reduction of the nitrate (Bianchi et al. 1994). At the same time, an excess of nitrate (Fig. 9) corresponds to the maximum of nitrification rate (Fig. 5).

In the bottom nepheloid layer, the load of suspended matter was always higher than in the water column, despite inputs from the Rhône River in the surface layer at the proximal station. In the bottom nepheloid layer, nitrification did not vary spatially.

Rates were low at all stations and throughout the year, reflecting the constant composition of particulate material in the benthic nepheloid layer in this area. The benthic nepheloid layer is enriched with organo-mineral aggregations linked to primary productivity (Aloisi et al. 1982). In fact, the particulate matter of the bottom nepheloid layer is a mixing of 'fresh' river inputs, and 'old' river inputs. The latter remains on the wide continental shelf of the Gulf of Lion for a period of time, allowing a 'homogenisation' of the composition of this material after mineralisation (S. Heussner pers. comm.). This homogenisation of particulate material is confirmed by a POC/PON ratio around 11 and by similar concentrations of inorganic nitrogen at all stations and throughout the year. Thus, the production of ammonium from this particulate material is levelled whatever the space and time distribution. In contrast to processes associated with the water column, nitrification rates in the benthic nepheloid layer displayed an independent distribution: (1) rates decreased very slowly from the river mouth to the sea, and (2) the surface/bottom rate ratio was about 2 at the proximal to less than 1 at the sea station, exhibiting an increasing contribution of the nitrification of this layer to the functioning of the ecosystem.

Average nitrification rates and corresponding ammonium concentration have been used to estimate that 10% and 20% of the ammonium, respectively, for winter and summer was nitrified at the river mouth. The nitrification peak is located at the edge of the plume corresponding to the region of intense nitrification as measured by Pakulski et al. (1995) at intermediate salinity of the Mississippi River plume. The nitrate produced by nitrification was estimated to be 292, 237 and $156 \mu\text{M yr}^{-1}$ for proximal, distal and sea stations respectively. In the Rhône River estuary, influence of river inputs, combined with the hydrodynamical and meteorological conditions, stimulates primary production at the edge of the plume. Applying the Redfield ratio ($\text{C/N} = 106/16$) to the primary production measured by Bianchi et al. (1993) in the proximal ($73 \text{ mg C m}^{-3} \text{ d}^{-1}$), distal ($720 \text{ mg C m}^{-3} \text{ d}^{-1}$) and sea stations ($100 \text{ mg C m}^{-3} \text{ d}^{-1}$), nitrogen requirements for primary production were, respectively, 330, 3300 and $471 \mu\text{M N yr}^{-1}$. The estimated contribution of the nitrification to the N requirement of primary producers was 14% at the distal station and 66% at the sea station independent of *in situ* nitrate concentrations. On the other hand, the nitrification contribution to the N demand in the surface layer, in the NW Mediterranean, ranged from 16% to 61% for eutrophic to mesotrophic environments respectively (Bianchi et al. 1998a). Whether a coastal or an open ocean ecosystem, pelagic nitrification plays an important role in the nitrogen budgets and fluxes in the NW Mediterranean Sea.

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