Bacterial nitrification and denitrification rates in the Rhône River plume (northwestern Mediterranean Sea)

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ABSTRACT: Water samples were collected in May 1992 from turbid plume water along several transects of increasing salinity from the Rhône River mouth to the sea. Nitrogen salt concentrations (NH_4^+, NO_2^-, NO_3^-), nitrification, denitrification and nitrate reduction were determined. NH_4^+ values, measured in the plume water, were lower than those corresponding to a conservative dilution, demonstrating a loss of 2 µmol l^{-1} of NH_4^+. In situ concentrations of NO_3^- differed by 10 to 30 µmol l^{-1} from theoretical values (conservative dilution), showing a net consumption of this compound. NO_2^- concentrations stayed closed to the conservative dilution curve plots. Along the salinity gradient, ammonium and nitrite oxidation rates decreased from 2 to 0.2 and 1 to 0.1 µmol l^{-1} d^{-1} respectively. These 2 rates correlated well with in situ NH_4^+ concentrations. 15% of the allochtonous NH_4^+ was nitrified. Dissimilative nitrate and nitrite reduction rates displayed similar values, decreasing from 380 to 7 µmol l^{-1} d^{-1}. Denitrification ranged from 0 to 7 µmol l^{-1} d^{-1}, independently of the salinity value. 3.5% of the allochthonous NO_3^- was denitrified. In the plume, denitrification rates were 30 to 100 times lower than nitrite reduction, while at a salinity >20 psu, these 2 processes occurred at similar rates. A significant correlation was demonstrated between the nitrate reduction rate and the difference between theoretical and in situ NO_3^- concentrations. The deficit in nitrate in the plume could arise from the nitrate reduction process.

KEY WORDS: Nitrification · Denitrification · Nitrogen budget · Rhône River plume · Mediterranean Sea

INTRODUCTION

In contrast to most of the Mediterranean Sea, where evaporation processes dominate the freshwater inputs by rivers and rain, the northwestern Mediterranean basin is diluted by the Rhône River outflow. This river constitutes an important source of fresh water (average of 2000 m^3 s^{-1}). A layer of about 1 m vertical thickness and 1 to 6 km length, with a high load of dissolved and particulate matter, overlays the particulate- and nutrient-poor seawater. As the Mediterranean Sea is oligotrophic, the river runoff contribution to the nitrogen budgets and fluxes is of great importance. An increase in primary production has already been demonstrated near the edge of the plume (Coste et al. 1977, Minas et al. 1988, Videau & Leveau 1990), but the role of these nutrient enrichments in the global productivity of the northwestern Mediterranean Sea remains in question.

Nitrogen inputs from the Rhône River are mainly composed of nitrate (50 to 160 µM), the concentration of which displays annual fluctuations 1 to 3 times the average concentration (Coste 1974). Ammonium contributes about 10 to 15 µM, while nitrite inputs stay low (≤3 µM) and neither of them shows annual variations (Coste 1974). Chemical and physical processes such as flocculation, adsorption and desorption do not influence the concentrations of other mineral compounds, e.g. phosphorus (Coste 1974).

Ammonium and nitrate concentrations in estuaries are greatly influenced by anthropogenic inputs. In estuaries with high nutrient content (reviewed by Sharp 1983), conservative or nonconservative dilution can be demonstrated on the basis of nitrogen salt concentrations. Microbial processes, e.g. nitrification and denitrification, are able to influence the turnover of inorganic nitrogen inputs. In particular, ammonium could

be oxidized by nitrification processes, as was shown for estuaries of the Tamar River (Owens 1986), the Delaware River (Lipschultz et al. 1986) and Narragansett Bay (Berounsky & Nixon 1990). On the other hand, in the oxygenated water column, the particulate material provides microniches allowing anaerobic processes (Bianchi et al. 1992). In highly turbid estuarine waters which are nepheloid layers the abundance of particles, combined with high nitrate concentration, favours nitrate reduction and denitrification, which has to be taken into account for the nitrogen budget.

In the superficial water of the Rhône River plume, corresponding to a nepheloid layer (Aloïsi et al. 1979), nitrification, denitrification and nitrate reduction rates were measured along the gradient of increasing salinity. The influence of these bacterial processes on the behaviour of the nitrogen inputs in the marine area is discussed.

METHODS

In May 1992, water samples were collected from the surface water using polycarbonate bottles along several transects from the Rhône River to the sea. Salinity and temperature were measured with a YSI probe (Yellow Spring Instrument Co.). Subsamples were used for measurements of NH_4^+ (Solorzano 1969), NO_2^- (Bendschneider & Robinson 1952) and NO_3^- by Technicon Analyzer (method of Wood et al. 1967, modified according to Tréguer & Le Corre 1975). To counterbalance the salt effect, a first measure was carried out with sulfanilamide only, and the optical density was subtracted in each case from the value obtained with all the reagents together.

Total bacterial counts were performed by an image-analysis system on cells stained with DAPI (Porter & Feig 1980).

Nitrification rates were obtained by measurement of the increase or decrease of nitrite concentrations in subsamples containing allylthiourea (ATU) or $NaClO_3$ to inhibit the oxidation of ammonium and nitrite respectively The sample was divided into 3×1 l subsamples. One (control) was unaltered, one received 10 mg l^{-1} of ATU (final concentration) and the third received 10 mM of $NaClO_3$. No substrate was added, because of the high natural concentrations of ammonium and nitrite. The subsamples were incubated in the dark in a 200 l culture chamber (Facis S.A., France) at the *in situ* temperature. The nitrite concentration in the subsamples was measured every 4 h from time zero to 24 h, using 10 cm path-length cells. Nitrification rates were calculated from the exponential phase of increase or decrease of nitrite, as already described in Bianchi et al. (1992).

Denitrification rates were measured using 100 ml subsamples kept in 130 ml serum flasks. 100 μl of a NH_4Cl solution, giving a final concentration of about 1 mmol ammonium l^{-1} seawater, was added to repress the nitrate assimilation pathway (Tiedje 1988). Flasks were sealed with rubber stoppers and anaerobic conditions were obtained by flushing N_2 through the flask for 5 min. Acetylene (15 kPa), which inhibits the reaction from N_2O to N_2, was added to the flask (Balderston et al. 1976). NO_3^-, NO_2^- and N_2O analyses were performed after 0.5, 1, 2 and 3 h of incubation at *in situ* temperature in the dark.

The rate of nitrite reduction can be determined from the difference between the rate of nitrate consumption and the net rate of nitrite production (Bonin & Raymond 1990).

The initial linear rate of N_2O accumulation is considered as the *in situ* denitrification activity. After incubation, 3 ml of the gas phase was sampled using a pre-evacuated venoject tube. Extraction of N_2O from the liquid phase was carried out by the procedure of Chan & Knowles (1979) modified by the multiple equilibrium technique (McAullife 1971). Nitrous oxide was determined using a Girdel series 30 gas chromatograph equipped with an electron capture detector as previously described (Bonin et al. 1987).

RESULTS AND DISCUSSION

The distributions of ammonium, nitrite and nitrate concentrations versus salinity are reported in Fig. 1. The difference between theoretical and measured values demonstrates the net production (or loss) for the considered nitrogen compound. The measured *in situ* values of NH_4^+, NO_2^- and NO_3^- were in the same range as those reported by Coste (1974) for the Rhône River plume.

The maximum NH_4^+ concentration was 10 μmol l^{-1}, a low value compared to those from the Scheldt estuary in Belgium (Somville 1978), the Tamar estuary in England (Owens 1986) and Narragansett Bay, USA (Berounsky & Nixon 1990), where the NH_4^+ concentrations were 100 to 1000 μmol l^{-1}, 0 to 45 μmol l^{-1} and 10 to 20 μmol l^{-1} respectively. The NH_4^+ concentration decreased by a factor of 10 from the river mouth (10 μmol l^{-1}) to the sea (1 μmol l^{-1}) (Fig. 1a). Below a salinity of 30 psu (practical salinity units), most of the *in situ* values stayed below the value corresponding to a conservative dilution, demonstrating a loss of ammonium. This loss was around 2 μmol l^{-1}. Above 30 psu, a low production of ammonium (1 to 2 μmol l^{-1}) was noted (Fig. 1a).

The nitrite concentration fluctuated very little, between 3 and 0.5 μmol l^{-1} (Fig. 1b). Low concentrations

of nitrite are generally found in natural waters. The *in situ* values stayed close (± 0.5 µmol l⁻¹) to the theoretical ones.

Nitrate constituted the most important reservoir of inorganic nitrogen, ranging from 110 (mouth of the

river) to 2 (seawater) µmol l⁻¹. Near the river mouth, when the salinity was less than 20 psu, the *in situ* concentrations of NO_3^- were 10 to 30 µmol l⁻¹ lower than the conservative dilution, indicating a net consumption of this nitrogen salt (Fig. 1c).

From the river mouth to the sea, ammonium and nitrite oxidation rates decreased 10-fold, from 2 to 0.2 and 1 to 0.1 µmol l⁻¹ d⁻¹ respectively. Although the ammonium oxidation rates stayed 2 times higher than nitrite oxidation rates, these 2 rates were also well correlated (r = 0.871, n = 19, p < 0.01) (Table 1). In the Tamar estuary, Owens (1986) also measured higher oxidation rates for ammonium than for nitrite. This author suggested that in these estuarine areas the heavy loads of particulate material favour ammonium oxidizer activity because these bacteria exhibit a greater affinity for attachment to particles than do nitrite oxidizers. The ammonium oxidation rates stayed around 2 µmol l⁻¹ d⁻¹ in the plume, and correlated well with *in situ* ammonium concentrations (r = 0.888, n = 19, p < 0.01) (Table 1), which exhibited a 10-fold decrease in the salinity gradient. This significant correlation between the rate of ammonium oxidation and the environmental concentration of ammonium is also corroborated when comparing rates reported from different geographical sites. In the Scheldt estuary, rates of 0 to 24 µmol l⁻¹ d⁻¹ were reported for NH_4^+ concentrations of 100 to 1000 µmol l⁻¹ (Somville 1978); rates of 0.98 to 11.1 µmol l⁻¹ d⁻¹ for 45 µmol l⁻¹ were observed in Narragansett Bay (Berounsky & Nixon 1990) and rates of 1 to 2 µmol l⁻¹ d⁻¹ were reported in the Rhône River plume at NH_4^+ concentrations close to 10 µmol l⁻¹.

The amount of nitrified ammonium corresponded to the difference (2 µmol l⁻¹) between the NH_4^+ theoretical dilution curve and the *in situ* values. In the plume water, where the high load of particulate material drastically limits the light for photosynthesis (Videau & Leveau 1990), the lack of ammonium could be due to its removal by nitrification processes. Furthermore, the shadowing effect of particles removes the inhibition of nitrification by light (Ward 1985). The production of NO_3^- by nitrification was low (0.1 to
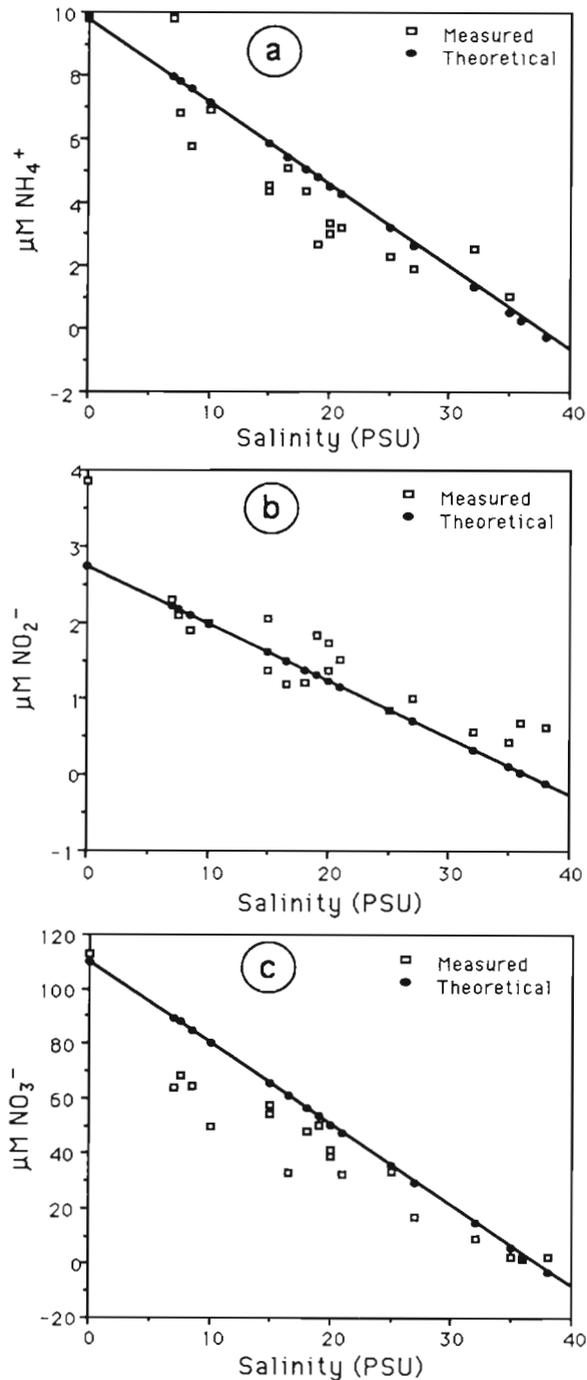

Fig. 1. Measured and theoretical concentrations of (a) ammonium, (b) nitrate and (c) nitrite, plotted against salinity. (□) Measured values; (●) theoretical values

Table 1. Spearman rank correlation coefficients for variables: NH_4^+ oxidation vs NH_4^+ concentration; NH_4^+ oxidation vs NO_2^- oxidation; nitrate reduction (NAR) vs nitrite reduction (NIR); nitrate reduction (NAR) vs difference between theoretical and *in situ* NO_3^- concentration [NO_3^-(T – *is*)]

Parameters	r	Slope	Intercept	p
NH_4^+ox : NH_4^+	0.888	4.96	−0.79	0.01
NH_4^+ox : NO_2^-ox	0.871	0.86	−0.08	0.01
NAR : NIR	0.780	1.54	−5.8	0.01
NAR : NO_3^-(T – *is*)	0.651	0.27	8.25	0.01

1 µmol l^{-1} d^{-1}). On account of the very high load of nitrate (20 to 60 µmol l^{-1}) in the plume water, the impact of nitrite oxidation was difficult to assess.

Bacterial density did not display great changes along the salinity gradient (Table 2).

Dissimilative nitrate and nitrite reduction rates displayed similar values, and demonstrated a strong decrease from plume water to seawater (>380 µmol l^{-1} d^{-1} to 7 µmol l^{-1} d^{-1}). Denitrification rates ranged from 0 to 7 µmol l^{-1} d^{-1}, regardless of the salinity values (Table 2). In the plume water, denitrifying activity was about 10-fold lower than that generally measured in marine sediments from the western Mediterranean coast (Raymond et al. 1992). Below a salinity of 20 psu, denitrification rates were 30- to 100-fold lower than nitrite reduction, while above this value, denitrification and nitrite reduction occurred at similar rates.

There was no correlation between the rates of reductive processes (nitrate and nitrite reduction, denitrification) and NO$_3^-$ concentrations. This suggests that nitrate did not limit either denitrification or nitrate and nitrite reduction. However, a significant correlation (r = 0.65, n = 10, p < 0.01) (Table 1) was observed between nitrate reduction rates and the difference between theoretical and measured NO$_3^-$ values. From this correlation, it can be suggested that the deficit in nitrate could arise from high rates of nitrate reduction. Nitrate and nitrite reduction rates were also well correlated (r = 0.78, n = 10, p < 0.01) (Table 1). There was no nitrite accumulation after nitrate reduction, as demonstrated by the similarity between theoretical and measured values of nitrite concentrations.

At the river mouth, data indicated significant nitrite consumption rates which could not be accounted for by denitrification, but which could be due to dissimilatory reduction of nitrite to ammonium. In fact, the rate of dissimilatory nitrite reduction to ammonium may be calculated as the difference between the overall nitrite reduction and denitrification (Jørgensen & Sørensen 1985) in samples amended with acetylene (to block nitrous oxide reduction) and ammonium which may act as an inhibitor of assimilatory reduction of nitrate via nitrite (Payne 1973). However, the activity of dissimilatory nitrite reduction to ammonium could be overestimated because of (1) the failure of the acetylene block (Tam & Knowles 1979) or (2) nitrate assimilatory uptake in spite of incubation conditions usually described as unfavourable to this process (increased ammonium salt concentrations and darkness). Indeed, Anderson & Kristensen (1988) have reported that phytoplankton (diatoms) may continue to assimilate nitrate up to 2 d after their last illumination period.

Near the river mouth, denitrification consumed only 1 % of the reduced nitrate, while 99 % was presumably recycled to ammonium through a dissimilatory reduction. This dissimilatory reduction process can overcome denitrification in some environments (Nishio et al. 1982). Near the edge of the plume, when salinity reached 30 psu, all the reduced nitrate was due to denitrification. The occurrence of denitrification was unexpected in a superficial water layer. But, recent reports have shown that this may be due to the occurrence of aerobic denitrification (Roberston & Kuenen 1984, Bonin et al. 1989, Bonin & Raymond 1990) and/or

Table 2. Rates of ammonium and nitrite oxidation, nitrate and nitrite reduction and denitrification and bacterial densities versus salinity. nd: not determined

Salinity (psu)	NH$_4^+$ oxidation	NO$_2^-$ oxidation	NO$_3^-$ reduction	NO$_2^-$ reduction	Denitrification	Bacteria (× 10^6 ml^{-1})
			(µmol N l^{-1} d^{-1})			
7.0	2.05	1.10	385	395	3.07	3.84
7.5	1.67	1.11	82	131	0	2.45
8.5	2.20	1.43	153	171	6.80	2.76
10.0	1.51	1.34	37	31.07	1.50	3.62
15.0	0.90	0.74	84	83	0	2.29
15.0	1.08	1.15	nd	nd	1.46	3.28
16.5	0.88	0.85	60	60	0	1.84
18.0	0.95	1.02	nd	nd	0	2.21
19.0	0.67	0.45	nd	nd	0	1.49
20.0	0.83	0.72	nd	104	2.40	2.08
20.0	0.92	0.69	3.4	0	0	3.01
21.0	1.22	0.64	nd	nd	0	1.67
25.0	0.57	0.35	0	0	0	2.17
27.0	0.50	0.26	14.80	2.18	2 10	1.40
32.0	0.86	0.60	6.60	6.60	6.60	2.16
35.0	0.23	0.14	7.05	7.05	5.90	1.32

the presence of anoxic microniches which may support denitrification in oxic environments (Jannasch 1960). Denitrification rates in seawater have been estimated in only one area, the oxygen-deficient water column of the Baltic Sea, and were reported to be from about 3.6 µmol N l^{-1} d^{-1} (Shaffer & Ronner 1984) to < 1 µmol N l^{-1} d^{-1} (Ronner & Sörenson 1985, Brettar & Rheinheimer 1992).

In the Rhône River plume, the suspended matter load is very high (>20 mg l^{-1}) allowing the presence of suboxic microzones in the oxygenated water column. Under such conditions, nitrification and denitrification processes could lead to the production of the greenhouse gas N_2O. At low oxygenation levels (ca 0.25 mg O_2 l^{-1}), the last step of denitrification can be inhibited, and the N_2O produced is accumulated (Bonin et al. 1989). Furthermore, at low oxygen concentrations and/or high NH_4^+ concentrations, nitrification may also enhance N_2O production. So, at reduced oxygen concentrations (ca 0.18 mg O_2 l^{-1}), about 10 % of the flux of ammonium through nitrification yields N_2O (Goreau et al. 1980). In addition to the N_2O produced by nitrification, Fuhrman & Capone (1991) have reported that 5 % of the denitrified NO_3^- could yield N_2O in suboxic water, where the nitrogen cycle is accelerated by the addition of fertilizer. According to these percentages and the rates of nitrification and denitrification we measured, and referring to the annual Rhône River inputs [about 55 000 metric tons (t) of nitrate and 5500 t of ammonium; Coste 1974], 82.5 and 96.2 t of N_2O yr^{-1} could be produced in the Rhône River plume area by nitrification and denitrification processes respectively. For these reasons, such bacterial processes affecting estuarine areas would have to be taken into account in future calculations of N_2O production.

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