Biogeochemistry 8: 135–151, 1989

Comparison of nitrification rates in three branches of the lower river Rhine

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Key words: nitrification, river Rhine, ammonium, nitrite, nitrate, hydrological conditions

Abstract. The nitrogen cycle in the lower river Rhine was analysed, using data on concentrations of ammonium, nitrite and nitrate, measured in the period from 1972 to 1986. The massive discharge of ammonium in densely populated areas in the Federal Republic of Germany led to microbial nitrification, detectable as decreases in ammonium and nitrite concentrations in the lower river Rhine over reaches 85–133 km long. The distribution of the nitrogen-rich Rhine waters over three different branches in the Netherlands permits some of the factors governing microbial nitrification in the river bed to be discriminated. In the fast-flowing main channel, intensively used by ships, nitrification is more important than in the smaller branches, despite the short residence time of the water in the main channel. Differences in the flow rate of water, in grain size distribution of sediments, and in intensity of shipping (aeration, turbulence) seemed to be responsible for the different rates of nitrification.

Introduction

The biology and chemistry of river water change dramatically while a river flows through urbanized areas. The river Rhine, flowing through a highly industrialized part of Europe, is no exception. In the last decade, reductions in the discharge of untreated domestic and industrial wastewaters have improved the oxygenation of the river water, but the concentrations of nitrogen compounds and phosphate are still very high and this leads to a disproportional chemical composition of the water and sediments. At present the lower river Rhine carries organic carbon and nitrogen compounds in a ratio C/N of less than 1, whereas in unpolluted rivers this ratio is over 10 (Meybeck 1982). The nitrogen status of the river Rhine as compared with other polluted and unpolluted rivers is illustrated in Fig. 1.

Various authors have studied the nitrogen cycle of polluted rivers and estuaries in NW Europe (Billen 1975; Curtis et al. 1975; Helder & De Vries 1983; Wolter et al. 1985; Müller & Kirchesch 1985). These studies indicate the importance of nitrification among the various nitrogen transformations

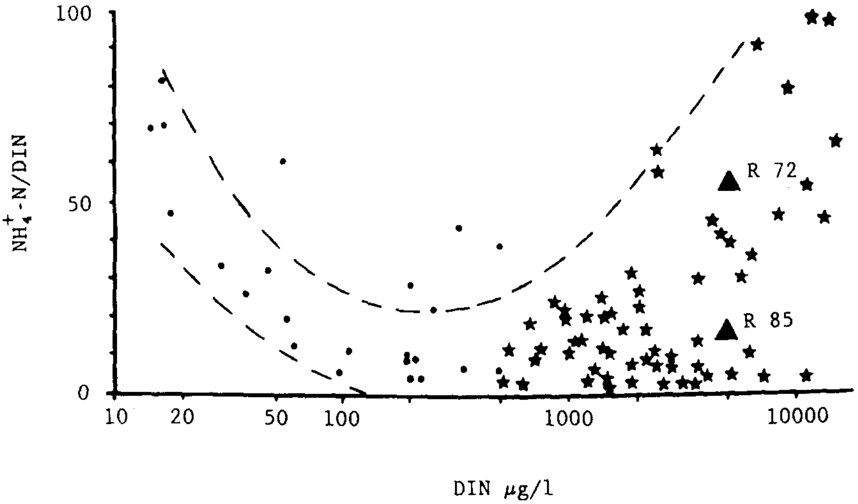

Fig. 1. Variation of the proportion of ammonium in dissolved inorganic nitrogen (DIN) in rivers with DIN (note the logarithmic scale for DIN). Common range found in rivers. Dots, uncontaminated rivers; black stars, contaminated rivers. R72, R85, values for the river Rhine in 1972 and 1985, respectively. Modified after Meybeck (1982).

in polluted rivers. Nitrification is the two-stage microbial oxidation of ammonium to nitrate via nitrite. In the sedimentation areas of rivers denitrification converts part of the massive nitrogen discharge of rivers (Billen et al. 1985; Wolter et al. 1985; Admiraal & Van der Vlugt 1988). Denitrification is the reduction of nitrate to molecular nitrogen carried out by bacteria oxidizing organic matter in anoxic layers of sediment.

In the present study we concentrate on reaches of the river Rhine downstream of a densely populated area where we expect nitrification to be intense. Here the river Rhine splits into three, thereby creating a natural experiment: the water is "incubated" in three different river beds. We tested the hypothesis that routine measurements of ammonium and nitrite in these channels can be used to calculate the in situ rate of nitrification. If this is the case it could be possible to detect some of the factors that regulate this microbial process. The impact of toxic materials on the nitrification process and the possibilities of using this microbial activity to monitor river water quality is reported elsewhere (Botermans & Admiraal 1989).

Materials and methods

Description of the river sections

Figure 2 shows the location of the reaches of the river Rhine investigated. Reach 1, the river Waal, is the main branch of the river Rhine in the

Netherlands and is an intensively used shipping route. Reach 2 is formed mainly by the rivers Nederrijn/Lek, and is equipped with two weirs that are closed at a low water discharge of the river to maintain a high and constant discharge of the river IJssel into lake IJssel. Finally, the river IJssel forms reach 3. All three reaches start at the Lobith sampling station. Reaches 1, 2 and 3 end at the Vuren, Hagestein, and Kampen sampling stations, respectively. The three branches of the river Rhine show hydrographic differences that are summarized in Table 1. Figure 3 shows the calculated residence times of water in the river reaches for the years 1980–1985.

The river Rhine leaves a densely populated area (the Ruhr district) in the Federal Republic of Germany ca. 70 km before passing the Lobith station. The river's load of ammonium ions peaks near the end of the Ruhr area (RIWA, 1986). Müller and Kirchesch (1985) noticed that in the German

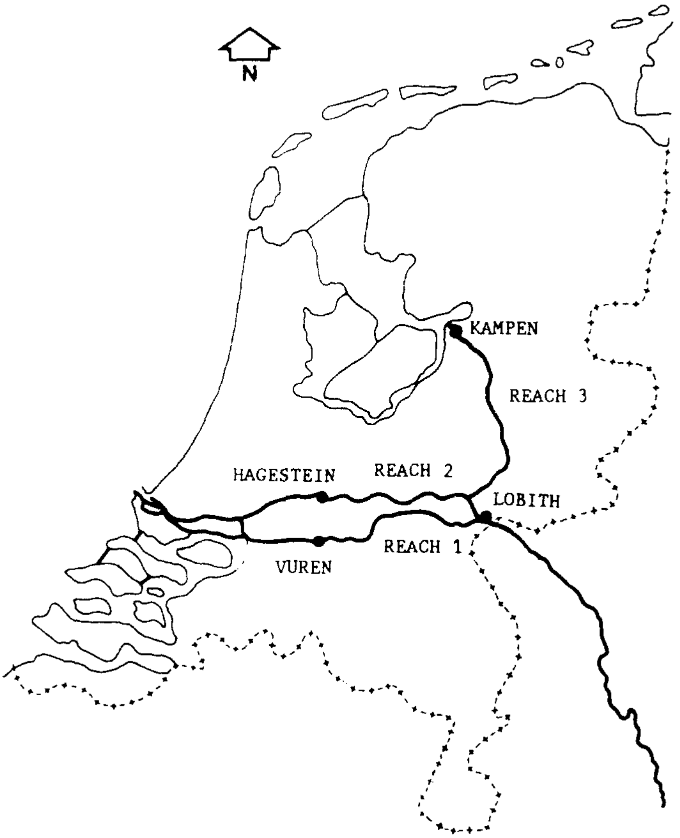

Fig. 2. Map of the Netherlands, showing the lower river Rhine with the four sampling stations. 1, 2 and 3: reaches of the river considered; note that these reaches overlap near the station Lobith.

Table 1. Morphological characteristics of three reaches of the river Rhine, numbered 1, 2 and 3 (Fig. 2) and the intensity of shipping in these three channels. Data from the State Department of Public Works.

		Reach 1	Reach 2	Reach 3
Length	km	85	85	133
Estimated water discharge (1980-1985)	m^3/s	1770	420	390
Average residence time (1980–1985)	h	22.4	36.1	44.0
Average flow rate (1980-1985)	km/h	3.9	2.5	3.2
Average depth	m	5.0	4.5	3.5
Median grain size (1974–1976):				
start	mm	5.2	5.2	5.2
middle	mm	1.8	2.7	1.3
end	mm	1.2	1.5	0.7
Intensity of shipping (1986)	10^9 ton/yr	266	15	25

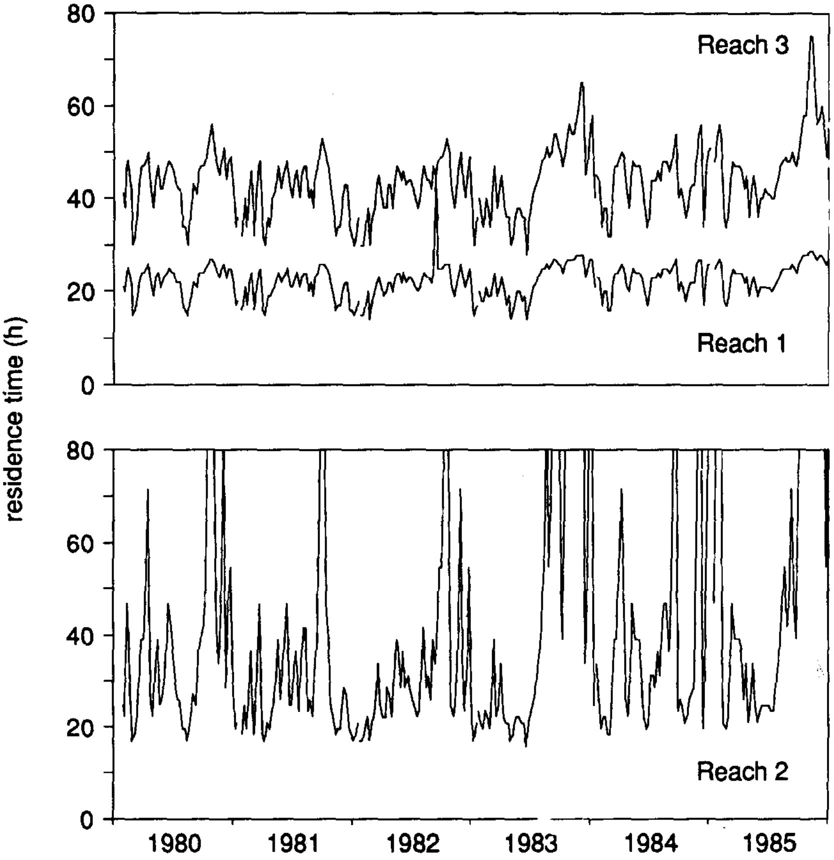

Fig. 3. Variation of the residence time of the water in reaches 1, 2 and 3 (see Fig. 2). Calculated after unpublished information of the State Department of Public Works.

part of the Rhine after 1976 the oxygen consumption associated with nitrification exceeded the oxygen consumption due to microbial oxidation of organic matter. Also Admiraal & Van Zanten (1988) indicated that nitrification is a quantitatively important process in the Lower Rhine. The branches of the Rhine in the Netherlands did not receive quantities of organically polluted waste water that altered the concentration of nitrogen compounds significantly. Indeed the ammonium load of the Rhine measured at station Lobith is currently ca. four times higher than the local input of ammonium in all Dutch surface waters together. Linders & Van de Wetering (1976) note that the river IJssel is the first river branch to be affected by municipal and industrial waste waters, because of its modest discharge. They compared the local discharges of a few compounds in the river IJssel with the load of these compounds already present in the river. The intermediately sized wastewater treatment plant of the city of Zutphen contributed 0.16% to the ammonium load of the river IJssel in 1974. The local wastewater plants and polluted tributaries together may contribute 10–20 times more ammonium than this wastewater treatment plant, so that the local addition to the ammonium concentration in the river IJssel is unlikely to exceed 5 percent.

Data analysis

Data on the concentrations of NH_4^+, NO_2^-, NO_3^-, and the percentage of oxygen saturation at the Lobith, Vuren, Hagestein and Kampen stations were derived from the quarterly reports of the Department of Public Works (Anonymous 1972–1986). Occasional checks were made on independent data collected at Lobith & Hagestein (RIWA, 1986). Changes in concentration over the river reaches were calculated from independent determinations at two stations. Usually, the time between the two samplings did not precisely match the residence time of a water parcel between the two stations. Pairs of observations with a large degree of mismatch in the times of sampling were discarded. Samplings at the downstream station on the same day or one day later were always accepted; samplings at the Kampen station made two days after Lobith station were also accepted, in view of the longer residence time of water in the river IJssel.

Changes in the concentrations of NH_4^+ and NO_2^- during transport of the water in reaches 1 to 3 were used to derive parameters for microbial nitrification, as explained later.

Lateral mixing in the river is known to be incomplete. The ammonium concentrations (introduced ca. 70 km upstream of Lobith station) of the right and the left banks are not identical. Therefore, the input to the left channel of the Rhine, the river Waal, may be overestimated by ca 15% (cf.

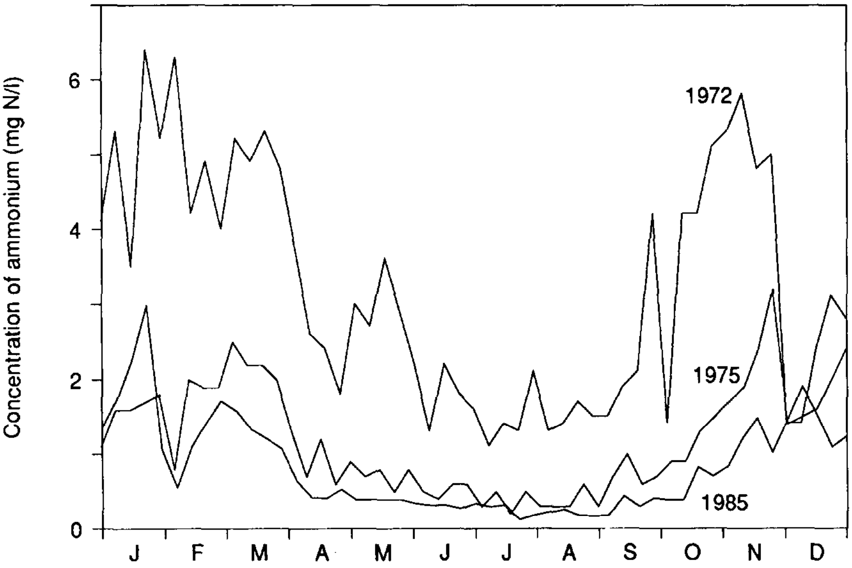

Fig. 4. Seasonal variation in the concentrations of ammonium measured at the Lobith station. Data for the years 1972, 1975 and 1985.

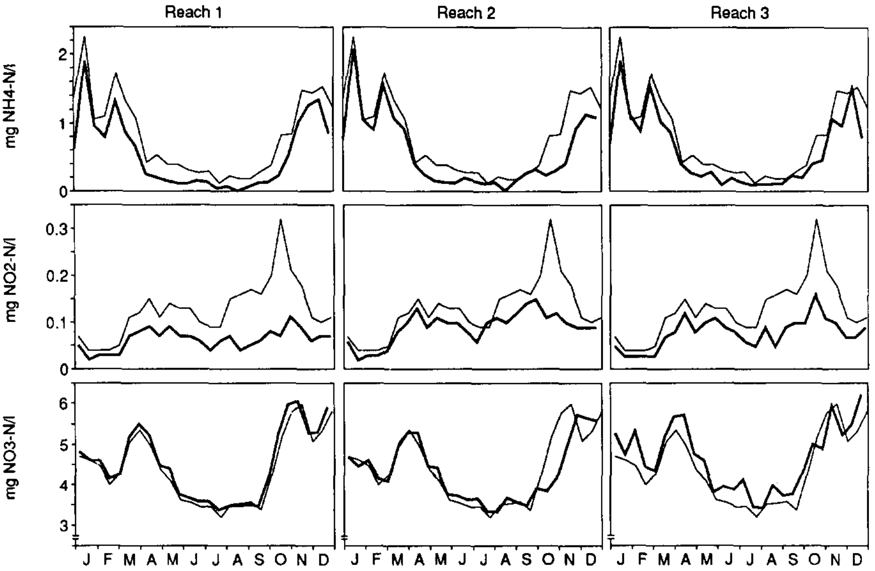

Fig. 5. Seasonal variation in the concentrations of ammonium ions (upper plots), nitrite (middle plots) and nitrate (lower plots) measured at the upper sampling station (Lobith, thin lines) and the lower sampling stations of river reaches 1, 2 and 3 (thick lines) in 1985.

Mazijk 1987). The observed concentration decreases of NH_4^+ in the Waal varied between 20% in winter to 90% in summer. Hence, the bias caused by sampling the right bank only may be significant in winter, but is relatively small in summer. However, the results suggest that even in winter the imperfect sampling of the river does not lead to abberations in the calculated rates of ammonium oxidation. Also representative samplings of the river's cross section carried out in the spring and summer of 1988 seem to indicate only a modest role of incomplete lateral mixing.

Results

Concentrations of nitrogen compounds

In the period 1972–1975 a rapid decrease in the ammonium concentrations was observed at the Lobith station, whereas only small reductions were

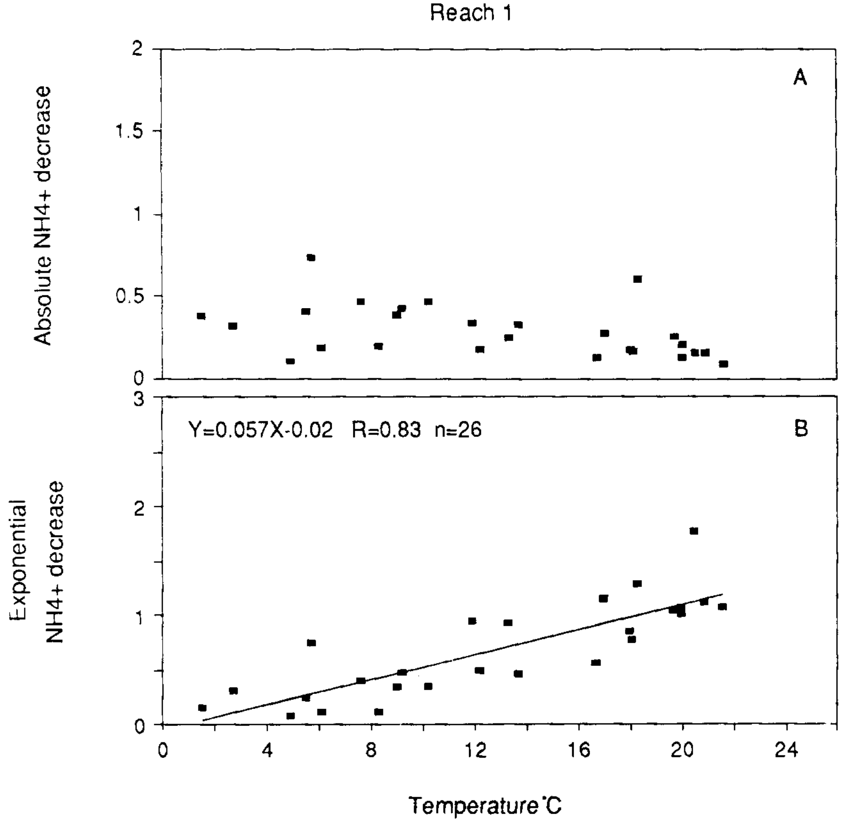

Fig. 6. Relation between ammonium decrease in river reach 1 with the water temperature in 1985. A: absolute decrease in ammonium concentration in mg N/l. B: logarithm of the ratio upstream concentration/downstream concentration (assuming exponential decrease).

observed from 1975 to 1985 (Fig. 4). This probably reflects the installation of purification plants reducing the organic load of the river Rhine.

Figure 5 shows for 1985 that the substrates for nitrifying bacteria, ammonium ions and nitrite, diminished in concentration during transport in reaches 1, 2 and 3. Fluctuations at the downstream stations generally followed those measured at the upstream station, Lobith, which indicates that the inadequacies in the sampling strategy (see "Materials and methods") did not greatly interfere with the determination of activities in the reaches. Irregularities were more frequent in the reaches 2 and 3 than in reach 1.

The simultaneous decrease in the concentrations of ammonia and nitrite

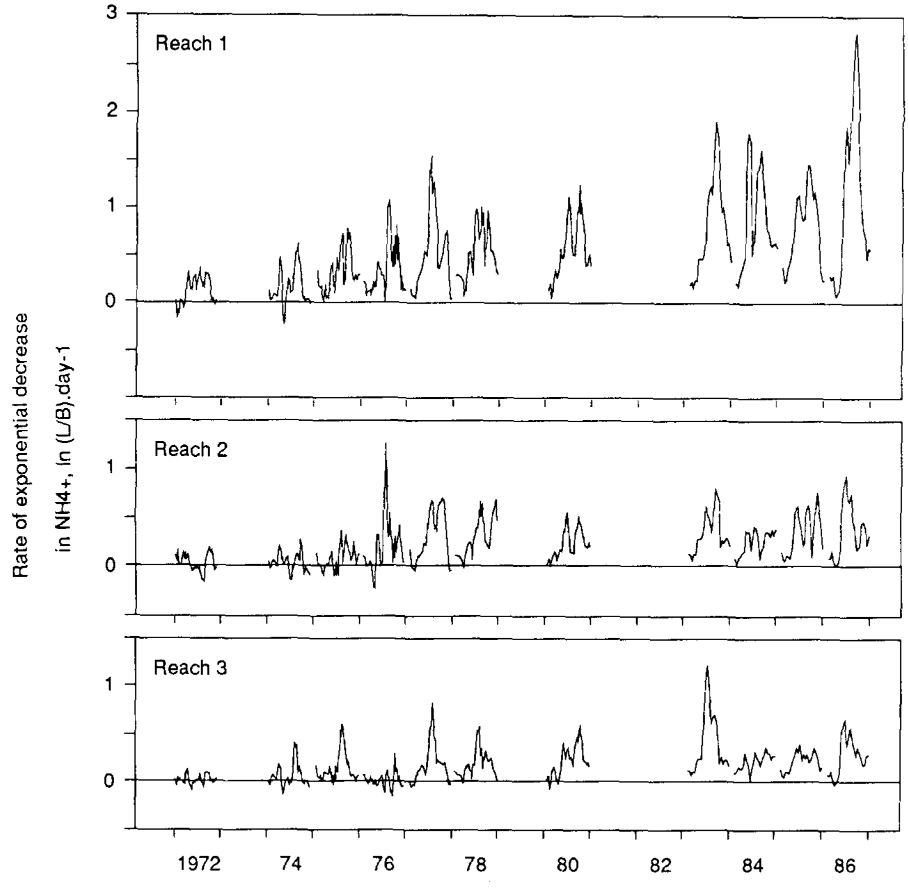

Fig. 7. Rate of exponential decrease in ammonium concentration in reaches 1, 2 and 3 for the period 1972–1986. L: ammonium concentration at station Lobith. B: ammonium concentration at downstream station. Rates given as moving average of order 3.

is attributed to nitrification in the river. Analogously, nitrification in the river upstream of Lobith station will have been responsible for the summer depression in ammonium concentration (Fig. 4). Assuming that nitrification was the only process affecting inorganic nitrogen species, one would expect that the quantities of ammonium-N and nitrite-N disappearing in the river reaches could be detected as increases in nitrate-N. This is only partly the case (Fig. 5). During 1985 the increase in nitrate-N in reach 1 was, on average, only 0.1 mg/l, whereas, on average, 0.3 mg/l of ammonium-N and 0.05 mg/l of nitrite-N disappeared. In reach 3 the increase of nitrate seems to match the disappearance of ammonium and nitrite reasonably well in the first three-quarters of 1985. However, in the last quarter, with low discharges and increased residence times in reaches 2 and 3 (Fig. 3), nitrate concentrations were significantly reduced during transport in reaches 2 and 3. The reasons for these phenomena are discussed later; for the moment it is assumed that the decreases in the concentrations of ammonium and nitrite can be attributed to nitrification, and that incomplete recovery of the nitrate produced did not interfere with the calculation of nitrification rate.

Reconstructions of nitrification rates

Two different expressions for the nitrification rate are examined here. Fig. 6A shows that the absolute decrease in the concentration of ammonium in reach 1 was only slightly dependent on temperature: it fluctuated around a level of ca. 0.3 $\mathrm{mg\,N.l^{-1}}$ with somewhat lower values at the highest temperatures. In contrast, the logarithmic parameter for nitrification rate (Fig. 6B) showed a linear relation with temperature (cf. Botermans & Admiraal 1989); this is consistent with the strong temperature dependence of microbial nitrification (Painter 1970). This process has been shown to follow first-order kinetics in river reaches with low concentrations of ammonium (Kennedy & Bell 1986) so that the rate of microbial oxidation of ammonium is appropriately expressed as an exponential function.

Figure 7 shows the appropriate rate parameter ($\mathrm{ln(L/B).day^{-1}}$) for the three river branches. Average residence times of the water in the reaches (Table 1) were used for the calculations accepting that some variation is introduced by the residence time (Fig. 3).

Since the absolute amounts of ammonium oxidized in the river seemed a relevant parameter too, we considered also this aspect. Figure 8 shows the amounts of ammonium dissappearing per m^2 of riverbottom per day; decreases per litre were recalculated using the average residence times and average water depths (Table 1) of the river branches. The rationale for

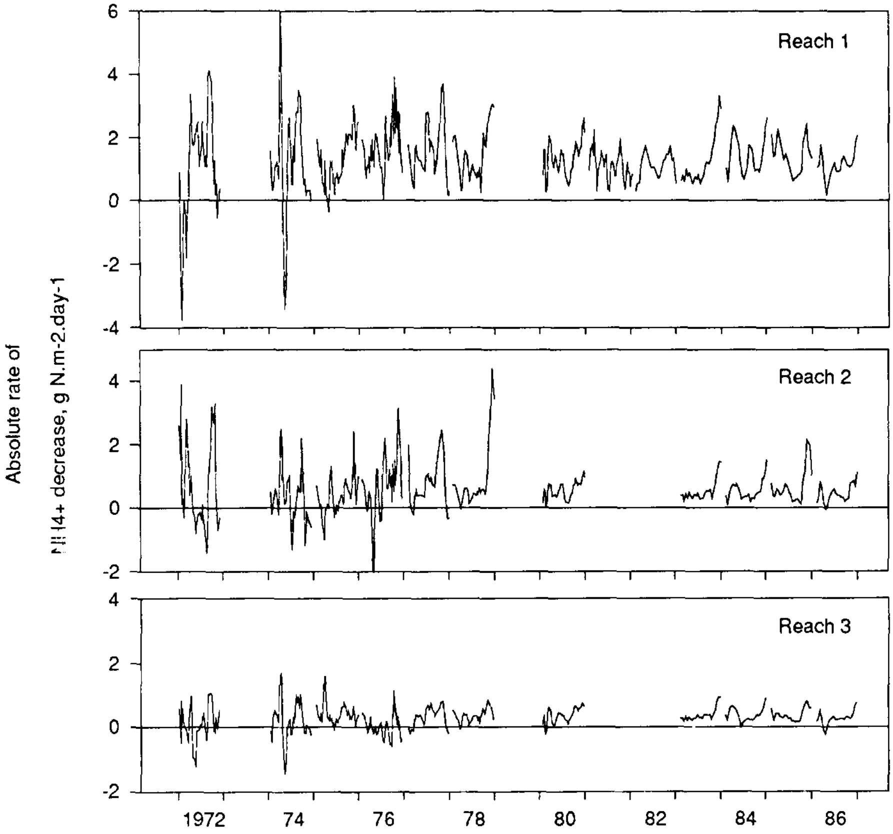

Fig. 8. Decrease in ammonium concentration in reaches 1, 2 and 3, calculated per m^2 of riverbottom per day. Rates given as moving average of order 3.

expressing the rate per surface area rather than per volume is given in the discussion section.

Figures 7 and 8 show the moving averages of three successive observations in order to reduce the prominence of short-term variations. It is evident that the fluctuations in the earlier years are larger than in recent years. However, this was to be expected: decreases in ammonium in 1972 and 1974 amounted to ca. 10% of the input, and any variation in sampling and analysis produces relatively large fluctuations.

Trends in the calculated nitrification rates

The nitrification rate in reach 1 was considerably higher than that in the reaches 2 and 3 independent of the nitrification parameter used (Figs. 7 and 8). Partly this difference is caused by the short residence time of the water

Table 2. Rate of exponential decrease of nitrite concentrations in the river reaches 1, 2 and 3. Yearly averages for the period 1978–1986.

		NO_2^-	
	Year	$\ln(L/B).day^{-1}$	SD
Reach 1	1978	0.35	0.21
	1980	0.48	0.18
	1983	0.58	0.30
	1984	0.54	0.22
	1985	0.70	0.34
	1986	0.65	0.46
Reach 2	1978	0.08	0.14
	1980	0.09	0.09
	1983	0.18	0.15
	1984	0.14	0.11
	1985	0.21	0.15
	1986	0.17	0.16
Reach 3	1978	0.20	0.10
	1980	0.14	0.08
	1983	0.19	0.12
	1984	0.13	0.11
	1985	0.24	0.11
	1986	0.21	0.14

L: concentration at Lobith station
B: concentration at downstream station
SD: standard deviation (n = 36 to 26)

in reach 1 that is included in the calculation. However, for many years the decreases of ammonium in the hydrographically short reach 1 are higher than in the other reaches (cf. Fig. 5). Also the decreases in concentration of nitrite expressed per litre (Fig. 5) or as $\ln(L/B).day^{-1}$ (Table 2) are more pronounced in reach 1 than in the reaches 2 and 3. In reach 1 the average rate of exponential reduction of the nitrite concentration was close to the value observed for ammonium (Table 2). However, in the other reaches, especially in reach 2, the decrease in the concentration of nitrite was lower than that of ammonium.

The Figures 7 and 8 emphasize different aspects of the nitrification process in the river, this is also evident when the long-term trends are compared. The absolute decrease in ammonium was more or less constant over the years. Figure 8 indicates for reach 1 a very high rate of ca. 1.6 g N oxidized per m^2 of riverbottom per day in early years as well as recently. In contrast, Fig. 7 shows that the rate of exponential decrease increased much during the period 1972–1986. The latter observation indicated a more efficient stripping of the lower concentrations of ammonium in recent years.

Discussion and conclusions

Use of chemical data sets

The present study reconstructs biological processes entirely from records of chemical and physical measurements in the river Rhine. The extensive documentation of the chemical qualities of the river contrasts with the paucity of biological information. Yet, biological processes interfere strongly with the geochemical cycles of nitrogen, phosphorus, silicon, sulphur and carbon. Davis (1986) argues that the current analysis of sets of chemical data on natural waters has poorly utilized the information inherent in the data. The geochemical characteristics of a drainage area (cf. Meybeck 1982; Kempe 1982) are causally related to natural biological cycles and the effects of man's activities and therefore the chemical data merit a detailed analysis. Such an analysis might indicate that future monitoring of natural waters should be based on a combination of chemical, physical and biological methods.

This study indicates that suitable parameters for the nitrification rate in the river Rhine could be obtained from routine determinations of ammonium and nitrite. This procedure is probably effective only in river reaches downstream of urbanized areas where the oxidation of massive discharges of ammonium takes place. Numerous studies are available for other water bodies showing, e.g. that nitrification in sediments is counterbalanced by denitrification (cf. Wolter et al. 1985; Cooke & White 1987), or that nitrification as well as assimilation by photoautotrophes may be responsible for the metabolism of ammonium in the water. Some of the findings of the present study indicate the role of nitrogen processes other than nitrification in the sections of the river Rhine considered. These processes must be checked for their potential effect on the calculated rates of nitrification.

1. Nitrate liberated upon the oxidation of nitrite and ammonium was not always qualitatively recovered. Nitrate concentrations even decreased, especially at low discharges, but the latter observation is consistent with earlier observations on denitrification rates as high as 25 g N.m^{-2}.yr^{-1} in sediments exposed to stagnant Rhine waters (Admiraal & Van der Vlugt 1988). Thus denitrification disqualifies nitrate production as a parameter of nitrification.

2. The concentration of total organic nitrogen in the river decreased from ca. 2.1 mg N/l in 1974 to ca. 0.8 mg N/l in 1985, whereas the concentration of ammonium decreased similarly from ca. 1.7 to 0.7 mg N/l (RIWA, 1986). The mineralization of organic nitrogen in the river water

may liberate NH_4^+ and this could affect the results for NH_4^+ oxidation obtained from calculations using concentration changes only. Hence, mineralization of organic nitrogen in the river may lead to increases of NH_4^+ in river reaches. However, these events were seen only incidentally. Furthermore, unlike the concentration of ammonium, the total content of organic nitrogen was not strongly reduced during transport of water in the river reaches. Thus it seems that the nitrification rates presented are only slightly underestimated by neglecting the in-river river mineralization.

3. Phytoplankton is able to deplete ammonium from surface waters. However, the density of the phytoplankton in the reaches of the river Rhine does not change significantly (Van Urk 1984). In view of the very high nitrogen content of the Rhine water we may assume that the phytoplankton is saturated with nitrogen. Furthermore our current observations indicate that even during algal blooms the concentration of particulate nitrogen changed less than 0.1 $mg.l^{-1}$, mostly there was no increase at all. Therefore it seems unlikely that ammonium uptake by phytoplankton affects the calculation of nitrification rates in the river reaches considered.

The comments 1–3 do not indicate that nitrogen processes other than nitrification are insignificant. Rather it seems that they do not change much the very high load of ammonium in the Rhine so that the role of the intense nitrification can be evaluated. Nitrification may be a dominant process in the reaches of the river Rhine we studied, though not to the extent that Cooper (1983) found in a stream receiving geothermal inputs of ammonium and as Schwert & White (1974) measured in coolant waters of a fertilizer manufacturing complex. The acid produced in nitrification is not detectable in Rhine water because of its high buffering capacity.

Ecological considerations

Cursory examination of Rhine water confirmed the role of suspended material and sediments as the substrate for nitrifying bacteria (cf. Curtis et al. 1975; Cooper 1983). In spring 1987 ca. 10% of the nitrification activity expected in the river Rhine could be measured in laboratory incubations of river water and suspended matter (Botermans, unpublished). Thus the river bed seems to be the active site for nitrification. The activity in the water was inhibited by 0.5 mg/l of L-histidine, as is the activity of nitrifying bacteria (Hockenbury & Grady 1977).

The hydrographic differences between the three branches of the river

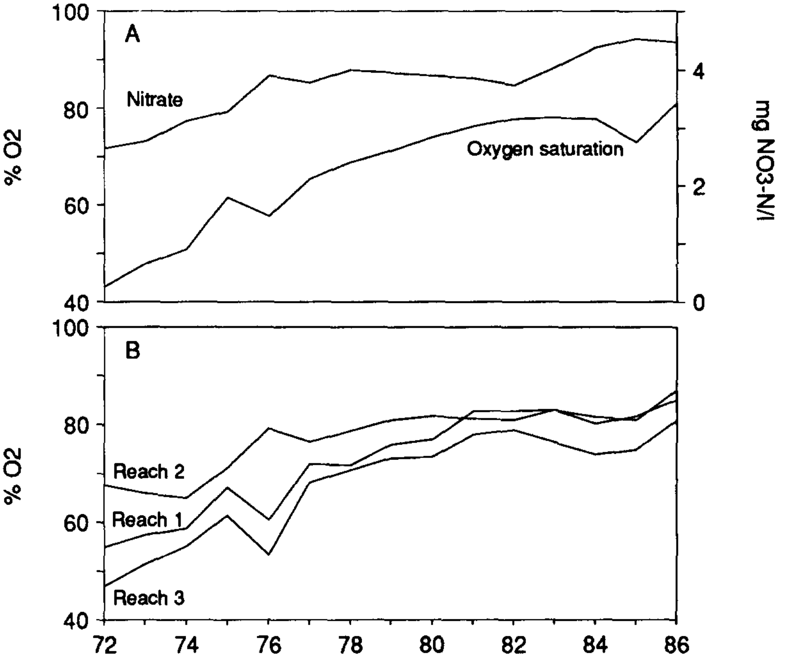

Fig. 9. Development of the yearly means of oxygen saturation and the concentration of nitrate at Lobith station (A), and the development of the yearly means of oxygen saturation at the lower sampling stations of reaches 1, 2 and 3 (B).

Rhine and the resulting differences in grain size and bottom-water exchange may now be regarded as steering factors for nitrification. The fast-flowing main branch of the river Rhine with coarse-grained sediments churned up by intensive shipping seems to function as an efficient sand-bed reactor. Analogously, Cooper (1983) found that gravel beds exhibited higher nitrification rates than the sand-bottoms of a stream. The very high nitrification rates of 1.6, 0.6 and 0.34 g NH_4^+-N.m^{-2}.day^{-1} calculated for reach 1, 2 and 3 require respectively 8.2, 3.1 and 1.7 g O_2.m^{-2}.day^{-1} for complete oxidation to nitrate. These oxygen consumption rates are very high as compared to seasonal maxima in overall oxygen consumption of 2.5 g O_2.m^{-2}.day^{-1} measured in estuarine sediments (Jørgenson & Sørenson 1985). Oxygen penetrates up to 5 mm deep into the coarse-grained sediments of reach 1 in September 1988 and O_2-consumption rates in the sediment of ca. 1 g m^{-2}.day^{-1} were estimated (Hofman & De Jong, personal communication). Thus the nitrification rates that we calculated seem to require all the oxygen consumption of the bottom and seem to depend on accelerated exchange of water and sediments.

The differences in the nitrification rates between the branches of the river

Rhine cannot be fully explained by differences in the presence of coarse sediment or gravel. These bottom types occur abundantly in the upper parts of all three reaches. The intensive shipping in the main channel may contribute to a more efficient sediment-water exchange, leading to higher nitrification rates in this river branch. In addition, the sediments in the winter bed of the river may contribute to the nitrification; since flooding is mostly restricted to the colder seasons, this contribution is not evident.

Several environmental conditions in the river changed in the period 1972–1986 and some of them will have affected the nitrification rates. Figure 9 surveys the changes in the oxygen saturation of the water and the concentration of nitrate. The yearly averages of oxygen saturation in the river Rhine increased from ca. 55% in 1972 to ca. 80% in 1985. The oxygen saturation in the slowly nitrifying reaches 2 and 3 is somewhat higher (reach 2) or somewhat lower (reach 3) than in reach 1, indicating that the oxygen concentration in the water per se does not regulate nitrification.

Oxygen concentrations over ca. 3 mg/l (or ca. 30% air saturation) are generally thought to saturate microbial nitrification (Painter 1970). However, in floccules of suspended material or in the river bed, higher levels of oxygen could enhance the conditions for nitrification. Thus, the rising oxygen levels in the river Rhine could have contributed to an increase of nitrifying activity during the period 1972–1986. This seems to be the case for the exponential decrease in ammonia concentration in the Rhine branches (Fig. 7), but not so for the absolute rates of nitrification (Fig. 8).

The increase in the concentration of nitrate tends to enhance the anaerobic degradation of organic matter in sediments, thereby improving the conditions for nitrification (Wolter et al. 1985). These effects parallel the reduced toxicity of waters and sediments. Toxicity used to inhibit the microbial nitrification in the river Rhine before 1975 (Müller & Kirchesch 1985; Botermans & Admiraal 1988). Now that extreme conditions such as oxygen depletion and poisoning of biological processes are less dominant, the physical conditions of the river bed seem to be the main steering factor for nitrification in the river. Both the continuously high input of ammonium to the river as well as the conditions of the river bed are influenced by human activity i.e. wastewater discharge and hydrographic changes of the river bed. Consequently, there are several ways that the unnatural "nitrogen state" of the river Rhine could be altered.

Acknowledgements

We are indebted to Ir. A. De Haas (Institute for Inland Water Management and Waste Water Treatment) for hydrographic information, and to Dr.

E.D. De Ruyter van Steveninck, Dr. P. Van Beelen, Drs D. De Zwart, and anonymous reviewers for critical comments. J. Burrough-Boenisch corrected the English text.

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