

Nitrogen behaviour and nitrous oxide emission in the tidal Seine River estuary (France) as influenced by human activities in the upstream watershed

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Abstract. The Seine River estuary (France) is the receptacle of a drainage basin characterised by high population density, heavy industrial activity and intensive agriculture. Whereas nitrate concentrations are high due to diffuse sources in the upstream drainage basin, ammonium mainly originates from the effluents of the Achères wastewater treatment plant (WWTP) downstream from Paris and its suburbs (6.5 million equivalent-inhabitants). Ammonium is mostly nitrified in the tidal freshwater estuary and nitrification causes a strong summer oxygen deficit. Average longitudinal summer profiles of oxygen and nitrogen concentrations for two periods, between 1993–1997 and 1998–2003 in dry hydrological conditions (excluding the wet years 2000 and 2001) clearly reflect the changes due to the improved treatment of wastewater from Paris and its suburbs. On the basis of daily water flux data and twice monthly nitrogen measurements at the boundaries of the upstream freshwater estuarine section (108 km), we calculated nitrification and denitrification fluxes, whose annual averages were 43 and 71×10^3 kg N d⁻¹ respectively from 1993 to 2003, with summer values (July–September) representing 73 and 57% of the annual fluxes, respectively. The degree of denitrification in the upper estuary appears to be closely related to the nitrification, itself more loosely related to the amount of reduced nitrogen (Kjeldahl) brought by the treated effluents from the Achères WWTP. We estimated the total N₂O emissions to about 40 kg N d⁻¹ (25–60 kg N d⁻¹) in the same sector.

Introduction

The ecological functioning of the Seine estuary is greatly affected by the input of a large amount of ammonium from treated effluents from Paris and its suburbs discharged 200 km upstream, which causes a strong summer oxygen deficit due to nitrification (Garnier et al. 2001). The treatment of the Paris effluents has been considerably improved in the last 10 years. Whereas the Achères wastewater treatment plant treated 8.5 million inhabitant-equivalents by the activated sludge process at the beginning of the 1990s, it now treats only 6.5 million inhabitant-equivalents, as the remainder is processed by

tertiary treatment in Valenton, Noisy and a newly constructed WWTP at Colombes. The implementation of a tertiary treatment, involving complete nitrification and partial denitrification at the Achères WWTP is programmed for 2007.

Both process of denitrification (as well as, to a lesser extent, dissimilatory nitrate reduction, Conrad 1996; Kelso et al. 1997) and nitrification, particularly nitrifier denitrification (i.e. autotrophic denitrification), at low ambient oxygen tension (Jorgensen et al. 1984; Poth and Focht 1985; De Wilde and de Bie 2000; Wrage et al. 2001; Dong et al. 2002; Punshon and Moore 2004), are known to cause emission of nitrous oxide (N_2O) as an intermediate product (Miller et al. 1993). The general concern for N_2O emissions arises from the fact that atmospheric N_2O significantly contributes to global warming. In addition, N_2O is stable in the troposphere and it can therefore reach the stratosphere where the NO_x by-products of its photochemical decomposition contribute to the destruction of the ozone layer (Crutzen and Ehhalt 1977; Bange 2000). Since 1970, N_2O has increased at a rate of $0.2\text{--}0.3\%$ yr^{-1} , reaching the present level of about 313 ppb (Rasmussen and Khalil 1986; Houghton et al. 1996; Garcia-Ruiz et al. 1998; Bange, 2000). The global rate of N_2O emission has increased dramatically in the last decade by about $2.8\text{--}4.3\text{ Tg N } yr^{-1}$ (Nevison et al. 1995), with most of the anthropogenic N_2O production being attributed to agricultural activities (Bouwman et al. 1993; Bouwman 1996). Nitrogen transformations in the soil are considered to be responsible for 65% of the increasing levels of N_2O in the atmosphere (Bouwman et al. 1993; Bouwman 1996; Bouwman et al. 2002; IPCC 2000), but estuaries may account for 60% of total marine N_2O emissions (Bange et al. 1996). Whereas N_2O emission from rivers and coastal zones has been generally assumed to be related to N loading at the global scale (Bouwman 1996; Bouwman et al. 2002; Seitzinger and Kroeze 1998), field and laboratory experiments have shown the complexity of the kinetic control exerted on this process by variables such as oxygen, nitrite and pH (Goreau et al. 1980; Anderson and Levine 1986; de Bie et al. 2002; Bonin *et al.* 2002; Cébron et al. 2006). Therefore, more studies are needed for a better understanding of the factors controlling the N_2O production, that appears to vary in time and to be dependent on specific conditions and cases (Mengis et al. 1996; De Bie et al. 2002).

Nitrification in the Seine river and estuary has been studied since the beginning of the 1990s (Chestérikoff et al. 1992; Brion et al. 2000; Garnier et al. 2001; Cébron et al. 2003). Recently, we have characterized and quantified the populations of nitrifying bacteria using molecular methods (Cébron et al. 2003, 2004), and showed that ammonium oxidizing bacteria were also responsible for the conversion of NH_4^+ into N_2O (Cébron et al. 2006). Although water column denitrification is probably of limited importance in the Seine fluvial and upper tidal estuarine sectors because oxic conditions are always maintained, benthic denitrification can have a role both in the nitrogen balance of the Seine river and estuary, and in the emission of N_2O , as shown for other estuaries (Robinson et al., 1998).

In this paper, we established the budget of nitrification and denitrification in the Seine estuary over a long time series (1993–2003) characterized by significant improvement of the parisian wastewater treatment, in order to (i) quantify nitrogen transformations and (ii) analyse the factors controlling *in situ* N₂O emission.

Material and methods

Site studied

Downstream from Paris, the Seine river is a large, regulated, 7th order river, draining a watershed of about 44,000 km², with intensive agriculture. Nitrate concentrations are as high as 4–5 mg N l⁻¹. 60 km downstream Paris, it receives the effluents from the wastewater treatment plant of Achères, treating, by the activated sludge process, 6.5 million equivalent-inhabitants of Paris and its suburbs (Figure 1). Immediately downstream, it receives a major tributary, the Oise river, with a catchment of 17,000 km². The fluvial sector of the Seine then continues without major tributaries nor point discharges down to the last navigation weir at Poses (km 202, distance from Paris *intra muros*), which represents the entrance of the estuarine sector and the upstream limit of tidal propagation (Figure 1). The freshwater part of the estuary extends to Caudebec (km 310), which represents the upstream limit of saline intrusion. This is a shallow estuary, well-mixed vertically.

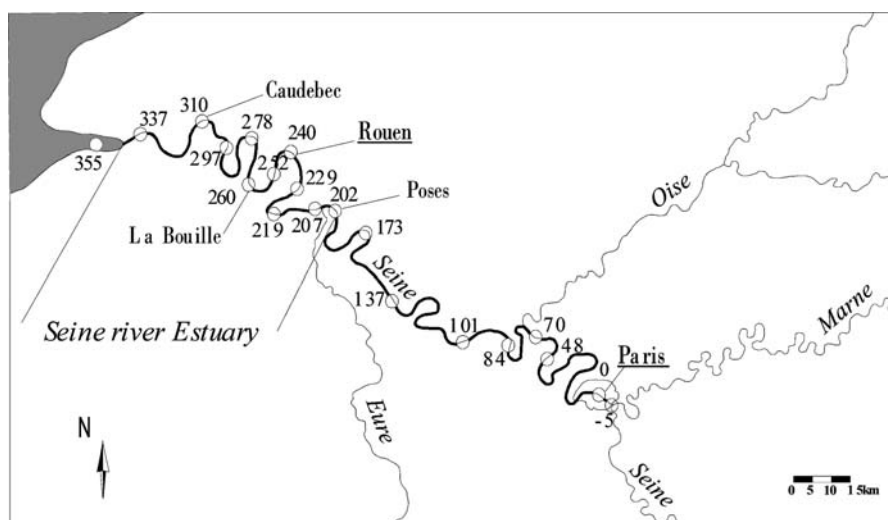


Figure 1. Map of the sampling locations in the freshwater estuary and the lower Seine River. The sampled stations are indicated by their km values.

Sampling strategy

Two different dataset have been gathered and discussed in this paper, (i) one at a twice monthly frequency at the inlet and outlet of the tidal estuary, (ii) another at 2–3 occasions during summer, for 17 stations covering the whole profile from Paris to Honfleur along the fluvial and estuarine sectors of the Seine. Except for N_2O , analysed only from 1997 on, all other variables were measured according to the same protocols over a 11-year-period from 1993 to 2003.

Seasonal sampling. Sampling was carried out at twice-monthly intervals, at the upstream and downstream limits of the tidal freshwater part of the estuary (Poses and Caudebec, respectively), (Figure 1). Besides classical water-quality variables (O_2 , PO_4^- , Si, etc.), the various forms of nitrogen were analysed (NO_3^- , NO_2^- , NH_4^+ , total organic nitrogen by Kjeldahl N, i.e. dissolved + particulate). The data used here are those from 1993 to 2003, during the period when a comprehensive scientific programme was launched by the Région Haute Normandie (Seine-Aval programme). Nitrous oxide (N_2O) was also determined seasonally at Poses and Caudebec in 1998 and 1999.

Longitudinal summer profiles were obtained at 17 stations from Paris to Honfleur at 2 or 3 occasions during summer (May, July and September). On day i , samples were collected at 7 stations from Saint Maurice to Poses, on day $i + 1$ at 8 stations from Poses to Caudebec, and on day $i + 2$ at 2 stations in the saline estuary. In the river and the upstream part of the estuary (km 0–250), samples were collected from bridges with a bucket, while, for the downstream part of the estuary, samples were collected from a ship, 1 m below the surface, with a peristaltic pump. Samples for dissolved N_2O analysis were immediately collected in a penicillin flask, poisoned with HgCl_2 (2%) and stoppered with a rubber septum excluding any headspace gas. For the other measurements and analysis, the water was kept in 10-l containers and brought to the laboratory within 2–4 h after sampling: activity measurements (see below) were initiated immediately, water was filtered and samples were deep frozen until chemical analysis.

Chemical measurements

Inorganic nitrogen. Ammonium was measured on filtered water (GF/F 0.7 μm of porosity) with the indophenol blue method according to Slavyck and McIsaac (1972). Nitrate was also measured on filtered water, after a cadmium reduction into nitrite, and nitrite was measured with the sulphanilamide method according to Jones (1984). Nitrite was measured prior to cadmium reduction.

Total organic nitrogen was determined according to the Kjeldahl method (Rodier 1984) by difference between Kjeldahl N and ammonium concentrations.

Nitrous oxide in water samples was determined with a gas chromatograph (Perichrom ST 200) equipped with an electron capture detector, after degassing an aliquot (20 ml) of the water sample.

Nitrifying activity was determined by the H^{14}CO_3 incorporation method in the presence of specific nitrification inhibitors, as described by Brion and Billen (1998) and Cébron et al. (2003). About 125 ml of a water sample is incubated in the dark for 20–24 h with the radiotracer ($0.5 \mu\text{Ci ml}^{-1}$) with and without inhibitors (N-serve or Nitrapyrin i.e. 2-chloro-6-trichloromethyl pyridine, 5 mg l^{-1} and chlorate, 10 mmol l^{-1}). Five replicate subsamples of 10–20 ml were filtered on $0.2\text{-}\mu\text{m}$ polycarbonate membrane filters. Radioactivity incorporation was counted by liquid scintillation. The carbon incorporation rate by the nitrifying bacteria was calculated by difference between non-inhibited and inhibited samples. As the incubations were carried out in optimum conditions (oxygen: 6 mg l^{-1} ; ammonium saturation: 2 mM ; pH: 7–8; temperature: 20°C), the values represent potential nitrifying activities. We used the yield factor of $0.11 \text{ molC molN}^{-1}$, as determined by Brion and Billen (1998), for calculating ammonium oxidation from carbon incorporation. Potential nitrifying activity values in the estuarine sector (downstream from km 202) were multiplied by a factor of $\times 2.5$ in order to take into account the sampling bias linked to the propensity of nitrifying bacteria to be attached on particles, as discussed in details by Brion et al. (2000).

Flux calculations

The measurements of the nitrogen forms at twice-monthly intervals and of daily water fluxes allowed the calculation of the N fluxes at the boundaries of the freshwater estuary (Poses-Caudebec) using the procedure described by Verhoff et al. (1980) and recommended by Walling and Webb (1985), and commonly used for the Seine (Meybeck et al. 1998; Némery et al. 2005).

$$\text{Flux} = \Sigma(C_i Q_i) \cdot Q_m / \Sigma Q_i,$$

where C_i , instantaneous concentrations; Q_i , corresponding instantaneous water flux; Q_m , mean water flux for the period considered (annual or summer).

These calculations assume that advection fluxes dominate the transfer. It is certainly so at the upstream limit of the sector, materialized by the weir of Poses. At the downstream limit, dispersion could play a role in the transfer. However, the choice of the station Caudebec, as a boundary is based on the fact that the longitudinal concentration gradients are minimal there (see Figure 3), owing to the fact that nitrification is completed several km upstream, and that sea water intrusion does not reach this point. Consequently, dispersion terms can be neglected.

The N_2O emission flux ($\text{kg N m}^{-2} \text{ d}^{-1}$) across the water–air interface was estimated using the classical gas transfer velocity approach (Frankignoulle et al., 1998; Raymond and Cole, 2001; Abril and Borges, 2004), in which the flux is calculated as the product of a gas transfer velocity (k , in m h^{-1}) by the excess of dissolved gas concentration over the equilibrium concentration with the atmosphere ($C - C_{\text{sat}}$). At given temperature and turbulence conditions,

the gas transfer velocity, k , is the same for any non reactive gaseous species, except for minor differences in their diffusivities. Previous analysis of the oxygen budget on the Seine estuary (Garnier et al. 2001) allowed to estimate the gas transfer velocity in the Seine estuary in the range from 0.04 to 0.06 m h⁻¹, in agreement with the estimates made by Thibodeaux et al. (1994) for the fluvial part of the Seine River (0.02–0.07 m h⁻¹). These values are in good agreement with the mean annual transfer velocity of 0.067 m h⁻¹ estimated by Law et al. (1992) for the Tamar estuary, and with the range of 0.03–0.07 m h⁻¹ proposed by Raymond and Cole (2001). The equilibrium concentration of N₂O was calculated on the basis of the relation with temperature and salinity established by Weiss and Price (1980), using the mean N₂O concentration in the atmosphere of 0.310 ppm by volume (Steffen et al. 2004).

Results

Changing conditions in the Seine river and estuary

The ten-year-period of study was characterized by changing conditions of water fluxes and effluent inputs, major factors controlling the spatial distribution and intensity of the biological processes in the lower Seine River (Chestérikoff et al. 1992; Servais and Garnier 1993; Garnier et al. 1995), (Figure 2a, b). The annual and summer average of discharges measured at Poses, shows that the years 1996 and 2003 were particularly dry, whereas the years 2000 and 2001 were wet (Figure 2a); these differences in the discharge affect the residence time of the water bodies and the spatial distribution of the processes.

Regarding the treated effluent fluxes of the Paris and suburbs wastewater treatment plants, a significant improvement during the period of study, from 1998 onwards can be observed (Figure 2b). This is evidenced both by the reduced nitrogen load (Kjeldahl) (reduction by 10% on average), which controls nitrification, and by the organic carbon load (BOD) (reduction by 40% on average), which controls denitrification (Figure 2b). The Achères wastewater treatment plant (one of the largest in Europe) represents more than 80% of the pollution by Paris of the lower Seine River. The recent changes are only part of a long-term plan of improvements in the Paris wastewater treatment (Billen et al. 2001).

Longitudinal summer profiles of oxygen and nitrogen in the estuary and the lower Seine River

To obtain a better understanding of the ecological functioning of the freshwater estuary, we have analysed the longitudinal pattern of oxygen and

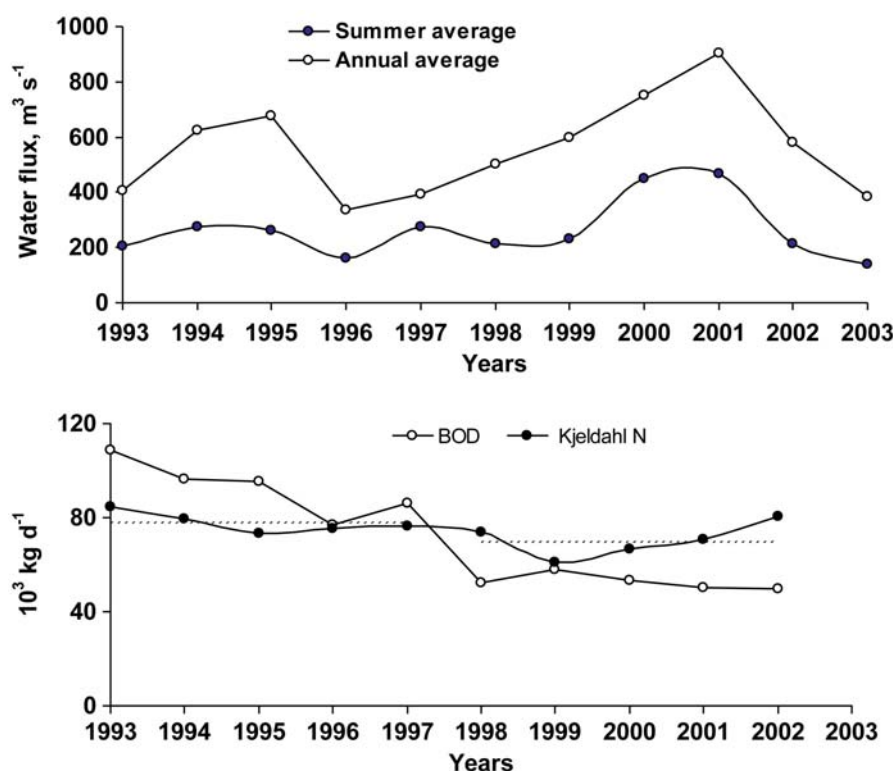


Figure 2. (a) Average annual and summer variations in the water fluxes from 1993 to 2003 ($\text{m}^3 \text{s}^{-1}$) at Poses, the entrance of the tidal freshwater estuary. (b) Mean annual fluxes of daily biological oxygen demand (BOD, 5 days, in $10^3 \text{ kg O}_2 \text{ d}^{-1}$) and Kjeldahl nitrogen (10^3 kg N d^{-1}) in the effluents of the WWTPs of the Parisian region (The dashed lines represent the mean values of the Kjeldahl nitrogen fluxes for the 2 periods (1993–1997 and 1998–2002) characterized by very different BOD values).

nitrogen concentrations along the continuum from the lower Seine upstream of the effluent outlet of the Paris WWTPs (Figure 3). The typical features of these profiles are (1) a sudden increase in ammonium, caused by the discharge of wastewater by the Achères treatment plant at km 70; (2) a limited oxygen drop immediately downstream of this input; and (3) the NH_4^+ disappearance, concomitant with an NO_3^- increase, evidence of nitrification, in the Seine freshwater estuary (from km 200 to 310); a severe oxygen deficit is also observed in this area.

The longitudinal profiles, observed in similar summer conditions during the two periods between 1993–1997 and 1998–2003 with different pollution loads, showed identical patterns; values were therefore averaged and shown with error bars (Figure 3). The water flow was low for dry summer conditions and averaged $220 \text{ m}^3 \text{s}^{-1}$, whereas it was more than $450 \text{ m}^3 \text{s}^{-1}$ during the wet

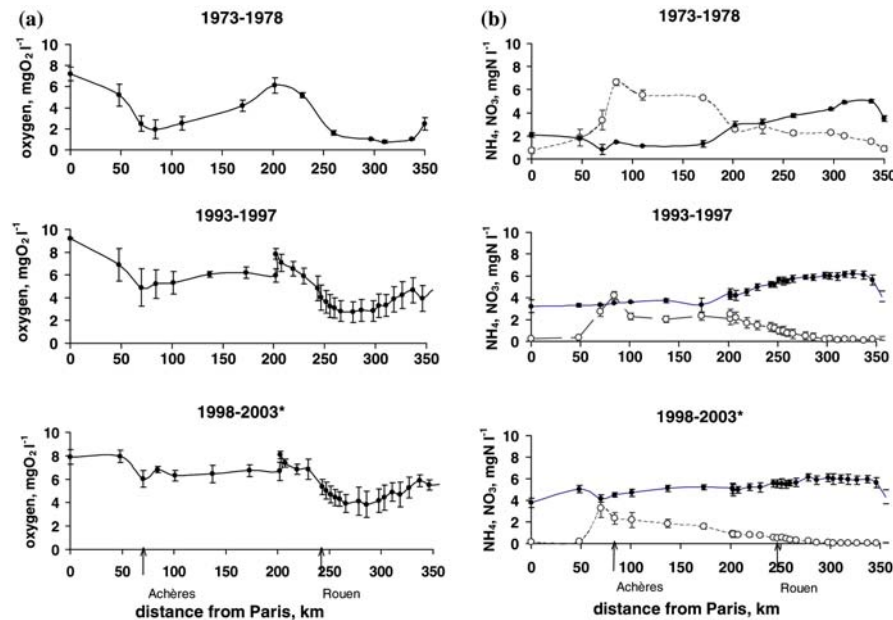


Figure 3. Summer longitudinal variations of (a) average oxygen concentrations ($\text{mg O}_2 \text{ l}^{-1}$) and (b) nitrate and ammonium (mg N l^{-1}) for the dry periods from 1993 to 1997 and from 1998 to 2003, excluding the wet years 2000 and 2001. For comparison, similar information are given for the period 1973–1978. Paris ('Pont Marie') is at km 0; the tidal estuary begins at km 202 from Paris (Poses weir).

years of 2000 and 2001, which we considered separately (profiles not shown here). For both considered periods, the ammonium brought by the Paris WWTPs (especially Achères) was completely nitrified 200 km downstream in the freshwater estuary and oxygen depletion was invariably observed in the estuarine sector (Figure 3). The fact that the oxygen depletion was less pronounced in the estuary during the recent period was obviously due to weaker nitrification because of the decreasing ammonium load. The decrease of organic matter inputs, led to better oxygenation immediately downstream of the effluent outlet during the recent period (Figure 3); previously, until the beginning of the 1990s, important heterotrophic bacterial degradation of organic matter led to frequent anoxia and fish mortality in the summer (Garnier et al. 1991, 1992; Servais and Garnier 1993).

Similar profiles are presented for potential nitrification rates, nitrite and N_2O concentrations (Figure 4). Potential nitrification rates do not reflect the effective *in situ* rate of the process, but are an expression of the nitrifying biomass. Whatever the period, values were maximum in the estuary, where ammonium completely disappeared, but increased already at the outlet of Achères effluents. At the mouth of the estuary in the turbidity maximum area, potential activity was again high; the longitudinal pattern of potential nitrifying activity

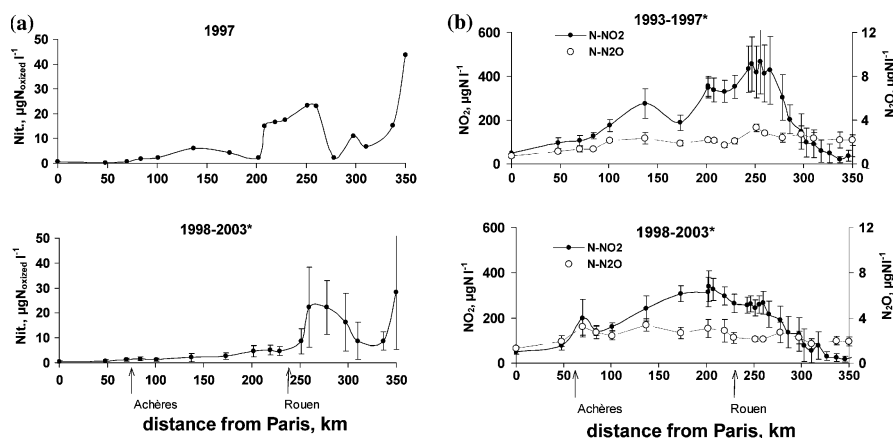


Figure 4. Summer longitudinal variations of (a) average potential nitrifying activity ($\mu\text{g N oxidized l}^{-1}$) and of (b) nitrite ($\text{mg N-NO}_2^- \text{ l}^{-1}$) and nitrous oxide ($\text{mg N-N}_2\text{O l}^{-1}$) for the dry periods from 1993 to 1997 (1997 only, for nitrous oxide) and from 1998 to 2003, excluding the wet years 2000 and 2001.

thus showed three sectors that were related to a shift in the bacterial community as supported by DGGE analysis (Cébron et al. 2004). The NO_2^- longitudinal pattern fits rather well with that of potential nitrifying activity but the N_2O concentrations were hardly higher in the estuary than in the lower Seine River downstream of the effluent outlet, revealing that N_2O might originate from other processes than nitrification.

Seasonal variations of oxygen and inorganic nitrogen in the estuary

Owing to the decrease in the pollution load, seasonal variations of NH_4^+ concentrations at the upstream and downstream limits of the estuary also showed a clear decrease since 1998, enhanced by greater dilution during the wet years of 2000 and 2001 (Figure 5). The NO_3^- concentrations, originating mainly from diffuse agricultural sources did not follow the same trend and even tended to increase due to the continuously rising nitrate contamination from agricultural sources in the Seine watershed (Billen and Garnier 1999; Billen et al. 2001). Particularly striking is the opposite variations between oxygen and nitrite concentrations (Figure 6). Taking into account the half-saturation constant for oxygen at the two stages of the nitrification process (0.2–0.5 $\text{mg O}_2 \text{ l}^{-1}$ for NH_4^+ oxidation, 0.8–2.4 $\text{mg O}_2 \text{ l}^{-1}$ for NO_2^- oxidation, Brion and Billen, 1998), oxygen depletion would limit the second stage of nitrification, allowing NO_2^- accumulation (and possibly an increase in N_2O emission). An inverse relationship between NO_2^- and O_2 was clearly shown by selecting the summer data, in the estuary as well as in the corresponding river data set upstream of the WWTPs and downstream of the Paris effluent outlet,

where NO_2^- increased as soon as the oxygen was depleted to 2 mg l^{-1} (Figure 7a). A similar relationship was found between N_2O and O_2 concentrations, i.e. an increase in N_2O concentrations at low oxygen (and high NO_2^-) concentrations (Figure 7b). Unfortunately, *in situ* observations did not give

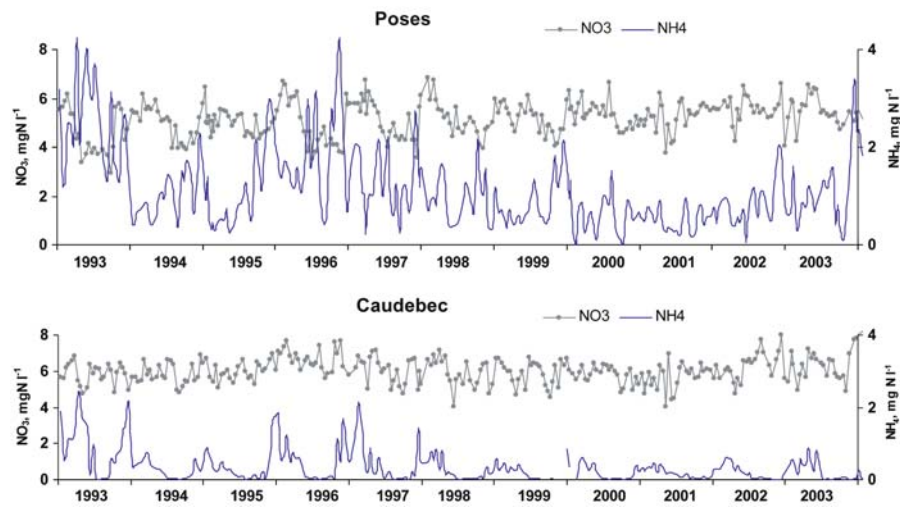


Figure 5. Seasonal variations of nitrate (mg N l^{-1}) and ammonium (mg N l^{-1}) at Poses and Caudebec from 1993 to 2003, the inlet and outlet of the freshwater estuary.

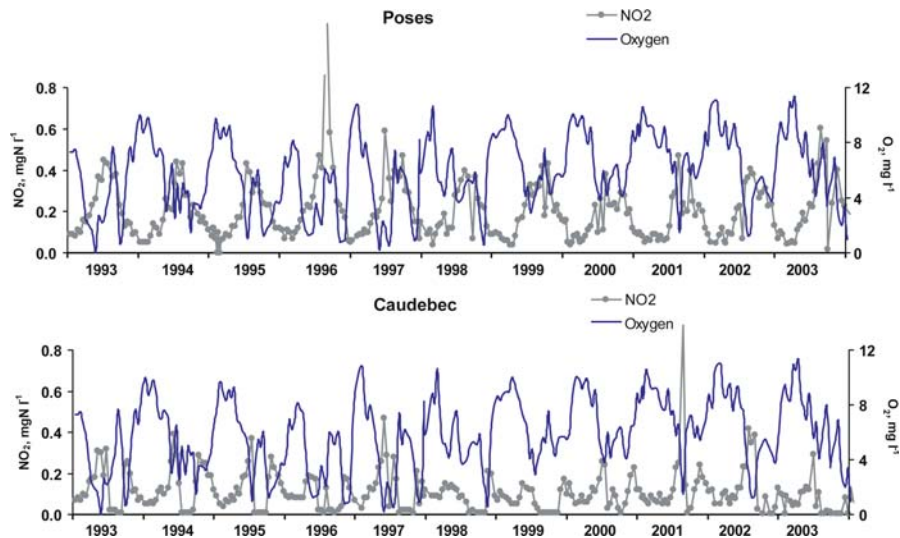


Figure 6. Seasonal variations of nitrite (mg N l^{-1}) and oxygen ($\text{mg O}_2 \text{ l}^{-1}$) at Poses and Caudebec from 1993 to 2003, the inlet and outlet of the freshwater estuary.

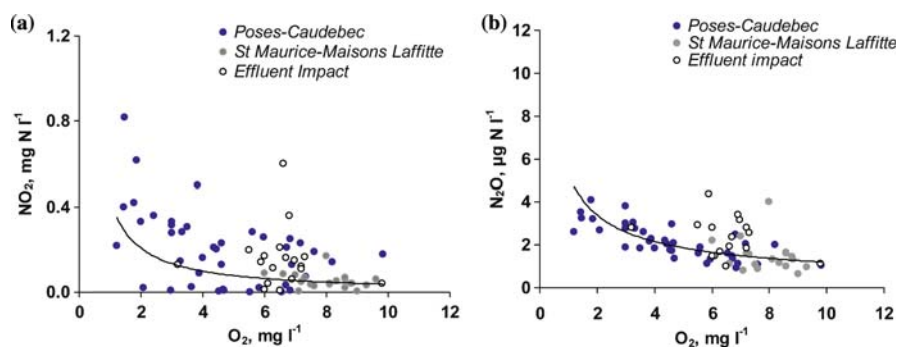


Figure 7. Relationships between (a) summer values of nitrite ($\text{mg N-NO}_2^- \text{ l}^{-1}$) and oxygen ($\text{mg O}_2 \text{ l}^{-1}$), (b) summer values of nitrous oxide ($\mu\text{g N-N}_2\text{O l}^{-1}$) and oxygen ($\text{mg O}_2 \text{ l}^{-1}$). Poses–Caudebec, the freshwater estuary; Saint Maurice – Maisons Laffitte the lower Seine upstream of the effluent outlet; Effluent Impact: station downstream of the effluent outlet.

any insight into NO_2^- and N_2O behaviour in the range of $0\text{--}2 \text{ mg O}_2 \text{ l}^{-1}$ and were therefore explored experimentally (Cébron et al. 2006).

Nitrification and denitrification in the Seine freshwater estuary

Nitrification and denitrification (in 10^3 kg N d^{-1}) in the tidal freshwater estuary were calculated by subtracting the output (the fluxes at the downstream boundary, Caudebec) from the inputs represented by the sum of the concerned N fluxes: (i) at the upstream boundary (Poses); (ii) at the outlet of the major tributary (the Eure River); and (iii) those by lateral point sources (Tables 1 and 2). All fluxes (10^3 kg N d^{-1}), were calculated as annual and summer (July–September) averages.

The estimate of nitrification flux (Table 1) is given by the output–input budget of reduced nitrogen forms (Kjeldahl N). Denitrification was determined by the difference between input–output budget of total nitrogen (sum of inorganic and organic N) (Table 2). As nitrate is the dominant form of total nitrogen in the whole estuary, the two budgets are largely independent from each other. Another way of estimating denitrification is to calculate the input–output budget of nitrate taking into account the estimates of nitrification as an additional input term. The two methods yield remarkably similar estimates of denitrification with an average variation coefficient of around 1% for both summer and yearly estimates (Table 2).

Depending on the years, the range of summer nitrification varied from $16 \times 10^3 \text{ kg N d}^{-1}$ to $55 \times 10^3 \text{ kg N d}^{-1}$ (Table 1), and represented on average 73% of the annual value (Table 1). More than half the N-NH_4 flux brought by the Achères treatment plant was nitrified in the estuary, and nitrification fluxes within the estuary appeared to be linearly oriented with the Kjeldahl N discharged with the Achères effluents (Figure 8a). This estimation compares well

Table 1. Budget of nitrification (10^3 kg N d^{-1}) in the freshwater Seine Estuary, from 1993 to 2003, averaged annual values (year) and summer values (Sum).

10^3 kg N d^{-1}	1993		1994		1995		1996		1997		1998		1999		2000		2001		2002		2003	
	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum
Poses	107	65	95	58	110	46	71	40	87	47	104	60	84	39	103	56	83	45	58	30	49	19
Eure R.	1.1	0.9	1.3	1.2	1.3	1.2	1.1	0.9	1.2	1.0	1.3	1.1	1.2	1.0	1.2	1.0	1.1	1.2	1.0	1.1	1.0	0.9
Lat. inputs	14.9	14.9	14.9	14.9	9.6	9.6	9.6	9.6	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
Total inputs	123	81	111	74	121	57	82	51	95	55	112	68	92	47	111	64	91	53	66	38	57	27
Output	68	26	61	25	57	23	38	23	47	32	85	51	51	15	59	25	52	26	40	16	35	11
(Caudebec)																						
Nitrification	-55	-55	-50	-49	-64	-34	-44	-28	-48	-23	-27	-17	-41	-32	-52	-39	-39	-27	-26	-22	-22	-16

Fluxes of Kjeldahl Nitrogen: inputs are represented by the sum of the fluxes at the estuarine upstream boundary (Poses), of the Eure, a main tributary within the sector and the lateral inputs (domestic effluents within the sector); outputs are represented by the fluxes at the estuarine downstream boundary (Caudebec).

Table 2. Budget of denitrification (10^3 kg N d^{-1}) in the freshwater Seine Estuary, from 1993 to 2003, averaged annual values (year) and summer values (Sum).

10^3 kg N d^{-1}	1993		1994		1995		1996		1997		1998		1999		2000		2001		2002		2003	
	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum	Year	Sum
Poses	285	136	384	169	424	161	234	110	277	162	344	153	364	139	486	259	488	273	332	135	233	76
Eure R.	18	12	27	21	26	21	17	12	19	15	23	17	24	15	29	20	29	20	23	15	19	13
Lat. inputs	36	36	36	36	22	22	22	22	22	20	20	20	20	20	20	20	20	20	20	20	20	20
Total inputs	340	184	448	226	472	204	273	144	316	198	387	190	408	175	535	299	537	314	375	171	272	109
Output	282	153	396	165	361	163	194	110	215	159	346	155	334	128	411	248	462	265	340	142	243	82
(Caudebec)																						
Denitrification	-58	-31	-52	-61	-111	-41	-79	-34	-101	-39	-41	-35	-74	-47	-124	-51	-75	-49	-35	-29	-29	-27
Denitrification-nit	-60	-32	-69	-66	-111	-41	-79	-33	-101	-38	-40	-36	-75	-48	-124	-51	-77	-50	-36	-30	-29	-29

Fluxes of total nitrogen (sum of inorganic and organic N): inputs are represented by the sum of the fluxes at the estuarine upstream boundary (Poses), of the Eure, a main tributary within the sector and the lateral inputs (domestic effluents within the sector); outputs are represented by the fluxes at the estuarine downstream boundary (Caudebec). In *italic* is shown, for comparison, the budget of denitrification calculated using nitrification (cf. Table 1) as an additional term to the $\text{NO}_3 + \text{NO}_2$ budget (denitrification-nit).

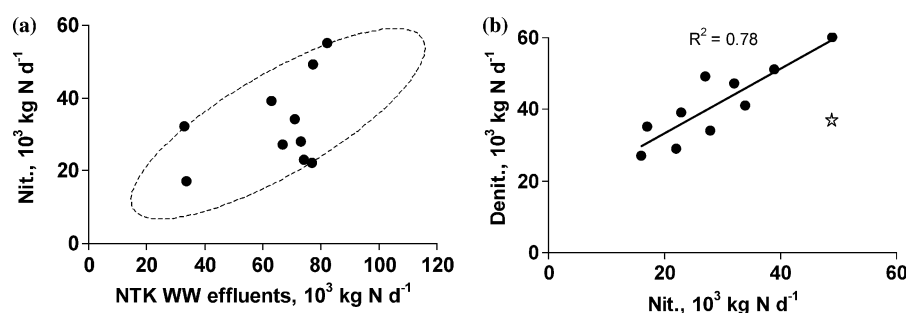


Figure 8. (a) Relationships between averaged summer values of nitrification (10^3 kg N d^{-1}) and nitrogen load from the Achères effluents (10^3 kg N d^{-1}). (b) Relationships between averaged summer values of denitrification (10^3 kg N d^{-1}) and nitrification fluxes (10^3 kg N d^{-1}).

with the mean integrated values of the direct potential nitrification measurements of the Figure 4 ($36 \times 10^3 \text{ kg N d}^{-1}$ during summer conditions). Summer denitrification ranged from 61 to $27 \times 10^3 \text{ kg N d}^{-1}$ from 1993 to 2003 and represented on average 57% of the yearly denitrification (Table 2). It is interesting to note that denitrification and nitrification are significantly positively related, a 1:1 ratio being found (Figure 8b).

Nitrous oxide fluxes in the Seine freshwater estuary

For the mean atmospheric N_2O concentration of 0.31 ppm, the equilibrium concentration in freshwater is between 0.48 and $0.24 \mu\text{gN-N}_2\text{O l}^{-1}$ in the temperature range from 0 to 20°C (Weiss and Price, 1980). Observed dissolved N_2O concentration are always above these values, indicating over-saturation, hence emission of N_2O from the water column to the atmosphere across the air–water interface. To calculate the magnitude of this emission flux (in terms of $\text{mg N-N}_2\text{O m}^{-2} \text{ h}^{-1}$), the difference between the measured N_2O concentration and the saturation value has to be multiplied by the gas transfer velocity, estimated between 0.04 and 0.06 m h^{-1} (see above, Materials and method section).

Summer N_2O emission to the atmosphere (kg N d^{-1}) for the estuarine sector (Poses-Caudebec, km 202–310, representing a surface of 16 km^2) was calculated from the concentrations measured in the water at the 8 stations sampled at 2–3 occasions during summer (see Figure 1 and Table 3). The variations were small for the studied period (1997–2003), averaging $2.7 \text{ mg N m}^{-2} \text{ d}^{-1}$ (1.5 – $3.5 \text{ mg N m}^{-2} \text{ d}^{-1}$ taking into account a wider range of 0.03 – 0.07 m h^{-1} for the gas transfer velocity as proposed by Raymond and Cole, 2001), i.e. 38 kg N d^{-1} for the whole estuarine sector area (24 – 57 kg N d^{-1}) (Table 3). These summer N_2O emission rates can be compared with our estimates of either nitrification or denitrification rates during the same periods (Tables 1 and 2). The N_2O emission to denitrification ratio is around 1% (0.7 – 1.1%).

Table 3. N_2O emission (kg N d^{-1}) within the freshwater Seine estuary (Poses-Caudebec: km 202–310) from 1997 to 2003, given as averaged summer values.

kg N d^{-1}	1997	1998	1999	2000	2001	2002	2003
Estuary	41	40	34		39	43	30
Downstream of the effluents	52	73	88		59	102	52
Upstream of the effluents	5	8	14		5	10	28
Total	99	121	136		103	156	111

See text for estimates. Emissions, within the downstream lower Seine sector, from downstream of the Paris effluent outlet to Poses (km 50–202), and the Seine upstream of the effluent outlet (km 0–50) are given for comparison.

The N_2O emission to nitrification ratio is around 1.5‰ (0.5–2.3‰). For comparison N_2O emission values expressed per m^{-2} are in average $2.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ ($1.8\text{--}4.1 \text{ mg N m}^{-2} \text{ d}^{-1}$) in the lower fluvial Seine sector (km 50–202) and $1.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ ($1.0\text{--}2.4 \text{ mg N m}^{-2} \text{ d}^{-1}$) in the river upstream of the Paris WWTPs (km 0–50), the range of the estimates being calculated following Raymond and Cole's gas transfer velocity values (2001). This pattern, i.e. maximum summer values increasing from upstream to downstream the studied sector, was confirmed by the annual variations in N_2O concentrations at various stations along the Seine river continuum (Figure 9).

Discussion

Improvement of the oxygenation conditions in the lower Seine River and the tidal estuary

A continuous improvement of wastewater treatment was observed over a much longer period than the one analysed in this paper (see Billen et al. 2001 for a 50-year reconstruction of the Seine ecological functioning). All the Parisian domestic wastewater was collected and transferred to the Achères WWTP from 1973, but during the period 1973–1978, incomplete BOD_5 treatment (and suspended matter, data not shown) led to a strong oxygen deficit immediately downstream the effluent outlet (km 80), whereas high NH_4^+ load discharged by the effluents were far from completely nitrified in the freshwater estuary, due to oxygen limitation (at around km 300). Improvement of wastewater treatment, involving first organic matter treatment, then reduction of the ammonium loading from the Achères WWTP, considerably reduced the oxygen deficit. For the two recent periods (1993–1997 and 1998–2003), the improvement averages $1 \text{ mg O}_2 \text{ l}^{-1}$ all along the longitudinal profile.

Given the already observed improvement in oxygenation, and the implementation of a planned tertiary treatment of all the effluents from Paris and its suburbs, we can expect a summer recovery of the oxygenation all along the upstream–downstream continuum which would attenuate the river discontinuity, linked to the discharge of the Paris effluents.

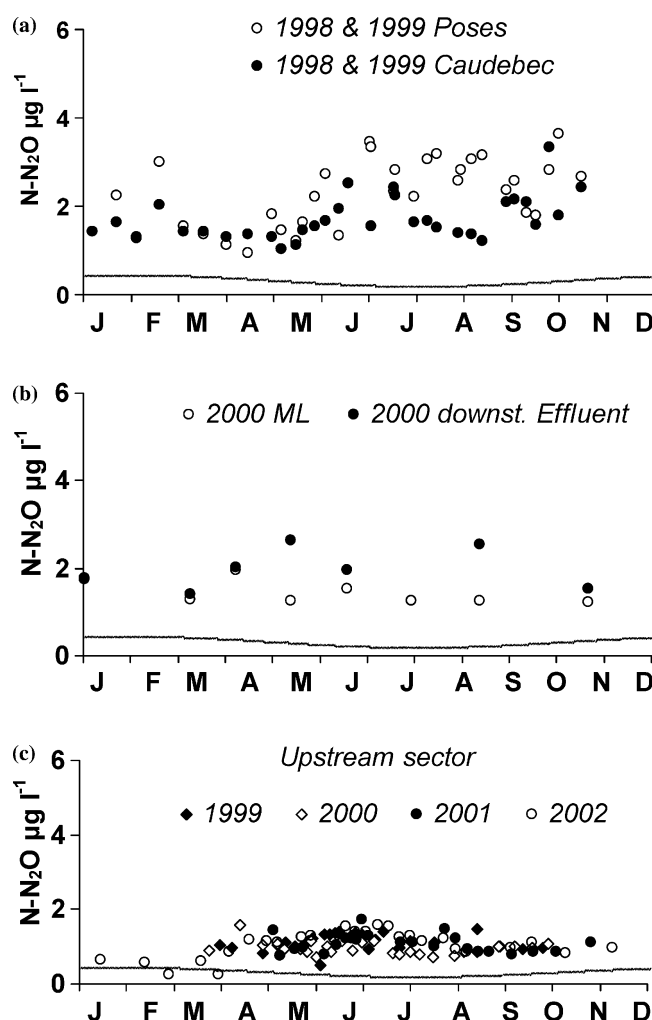


Figure 9. Annual variations of the nitrous oxide concentration ($\mu\text{g N-N}_2\text{O l}^{-1}$) (a) at Poses and Caudebec, the inlet and outlet of the freshwater estuary. Seasonal variations of values (b) upstream (ML: Maisons Laffitte) and downstream of the effluents, and (c) in the Marne sub-basin, are shown for comparison. The line of the variation in N_2O concentrations at saturation is drawn according to Weiss and Price (1980), taking into account the temperature.

Budget of nitrification, denitrification and nitrous oxide emission in the tidal estuary

Whereas the results of chemical analyses show a variation of less than 5% on nutrient values, estimates of the water flux vary within about 5–15%. Regarding the lateral input (treated domestic effluents) along the sector, a range of 10–15% also seems reasonable. Overall, the budget of nitrification and

denitrification are given with a maximum variation of 30%. For the nitrous oxide emission, the range of gas transfer velocity values leads to a variation of 50%. Year-to-year changes should be interpreted with this limitation in mind.

The budget calculations carried out in the freshwater tidal estuarine sector showed that nitrification and denitrification fluxes are of the same order of magnitude (Tables 1 and 2). Nitrification occurred mainly during the three summer months, whereas the period of denitrification was longer. Although previous work using natural isotopic composition of nitrate tended to show that no significant denitrification occurred in the water column in this sector (Sebilo 2003; Sebilo et al. in press), the budget calculations presented in this study reveal the occurrence of significant denitrification. This apparent contradiction can be easily explained if most of the denitrification revealed by our budget occurred in the benthic phase. Indeed, benthic denitrification, as it is controlled by the nonfractionating process of nitrate diffusion into the anoxic sediment layers, does not cause any significant change in the natural isotopic composition of nitrate (Sebilo et al. 2003). Some direct measurements of the benthic nitrate flux through the water sediment interface allowed Chesterikoff et al. (1992) to estimate a benthic denitrification of at least $3 \times 10^3 \text{ kg N d}^{-1}$ in the riverine sector downstream of the Achères WWTP outlet.

The positive relationship between the summer level of nitrification and denitrification throughout the 11 years, and the similarity of the absolute values of the corresponding nitrogen fluxes (Tables 1 and 2) should not lead to the conclusion that nitrate produced by nitrification was reduced by denitrification. The relationship should rather be interpreted as the double effect of the improvement of wastewater treatment which simultaneously led to better summer conditions of oxygenation and lower organic matter contamination, thus reducing denitrification, while, in parallel, the reduction in ammonium loading results in decreasing nitrification.

N_2O emission fluxes in the freshwater estuary (Table 3) amounted to less than 0.1% of both denitrification and nitrification fluxes, in agreement with the range of percentage obtained in laboratory experiments (Kester *et al.* 1997; Cébron et al. 2006), although our estimates refer to net field ratio, which may include the effect of N_2O consumption processes.

Mechanisms and control factors of N_2O emission

N_2O can be produced by nitrification (nitrifier denitrification) in a narrow range of low oxygenation, provided that NO_2^- is available (Ritchie and Nicholas 1972; Goreau et al. 1980; Jorgensen et al. 1984; Bock et al. 1995; Cébron et al. 2006). N_2O is also known as a by-product of denitrification or dissimilatory nitrate reduction (Conrad 1996; Kelso et al. 1997). Which mechanism (nitrification and/or denitrification) is responsible for the N_2O production observed in the lower Seine River and estuary, is a question that requires further investigations.

The present study however reveals a high N_2O production in the lower Seine river and estuary impacted by ammonium effluents. In the Scheldt estuary, where the ammonium contamination is still higher than in the Seine, De Bie et al. (2002) observed an inverse relationship between N_2O and oxygen, similar to the one we found in the Seine estuary (Figure 7). However, N_2O concentrations in the Seine estuary were much lower than those in the Scheldt (3 against $8 \mu\text{g N l}^{-1}$ respectively at maximum), just as the ammonium concentration that differed in a rather similar ratio (2 against 4 mg N l^{-1} respectively at maximum). This result supports the assumption by Seitzinger and Kroeze (1998), of a direct connection between the input of DIN (Dissolved Inorganic Nitrogen) to rivers and the output of N_2O gas. These results also support the findings by McMahon and Dennehy (1999) on the South Platte River (Colorado) that systems enriched in N by wastewater effluents are an important anthropogenic source of N_2O to the atmosphere. The lower Seine River and estuary, with a concentration above $4 \text{ mg N-NO}_3^- \text{ l}^{-1}$ ($300 \mu\text{M}$) and a N_2O emission amounting $0.6 \text{ g N m}^{-2} \text{ yr}^{-1}$ ($0.4\text{--}1.0 \text{ g N m}^{-2} \text{ yr}^{-1}$), have values in the higher range of those reported for 7 rivers and estuaries (Cole and Caraco 2001). These emissions to the atmosphere are of the same order of magnitude as the highest N_2O emissions reported for eutrophic lake water (Mengis et al. 1996).

Both nitrite and nitrous oxide concentrations in the water clearly depend on the oxygenation level, values increasing with decreasing oxygen concentration (see Figure 7). N_2O concentrations apparently increase less than those of NO_2^- , probably due to its escape to the atmosphere when the water is supersaturated. However, a scatter of data points demonstrated the complexity of the factors controlling NO_2^- and N_2O in the water. Because concentrations in the treated effluents were low ($0\text{--}1 \mu\text{g l}^{-1} \text{ N-N}_2\text{O}$), the N_2O present in the river was necessarily produced *in situ*. A fraction of the N_2O production might be due to denitrification occurring below the sediment–water interface, or even at microsites in suspended aggregates or fluid mud (Bonin and Raymond 1990; Bianchi et al. 1994; Middelburg et al. 1995; Abril et al. 2000; Bonin et al. 2002).

It is *a priori* surprising to find higher NO_2^- and N_2O concentrations at Poses, the upstream limit of the estuary, than at the downstream limit at Caudebec in similar oxygenation conditions. This may have several explanations. The first one would be ammonium limitation at Caudebec where more intermediate products are consumed than produced whereas at Poses conditions for nitrification were nonlimiting. This would argue in favour of nitrification as the predominant mechanism of N_2O emission in the tidal freshwater estuary. Another hypothesis would be a greater accumulation of biodegradable organic matter at Poses, which is the limit between the river and the upstream estuary, where the algal development is generally highest in the summer (Garnier et al. 2001); heterotrophic respiration of organic matter would deplete the oxygen in the bottom sediment layer; in this case, denitrification would be at the origin of the high NO_2^- and N_2O concentrations.

Although nitrification appears to be the dominant process at the origin of the N_2O emissions within the tidal freshwater estuary, denitrification at the anoxic water–sediment interface would be the major source of the N_2O emissions in the river immediately downstream of the effluent outlet. The resulting longitudinal pattern of N_2O concentrations in the lower Seine and the freshwater estuary is therefore a composite one, but the two processes may interfere with each other.

The significance of estuarine N_2O emission

Like many estuarine systems receiving high anthropogenic nutrient inputs, the conditions in the lower Seine River and estuary are ideal both for denitrification at the sediment–water interface and for nitrification in the water column, and consequently likely to contribute significantly to the N_2O emissions to the atmosphere (Bange et al. 1996; De Wilde and De Bie 2000).

According to our estimates, the N_2O emission in the upper Seine estuary amounted to about 40 kg N d^{-1} (Table 3). By adding the contributions of the fluvial riverine sectors, upstream and downstream of the Paris effluent discharge, a total emission of $120 \text{ kg N-N}_2\text{O d}^{-1}$ ($90\text{--}200 \text{ kg N-N}_2\text{O d}^{-1}$) was obtained for the investigated fluvial-estuarine continuum. If we extrapolate the observed emission rates, namely $1.2\text{--}2.2 \text{ mg N-N}_2\text{O m}^{-2} \text{ d}^{-1}$ in average, to the whole surface of the Seine drainage network ($310 \times 10^6 \text{ m}^2$, Guerrini et al. 1998) a daily emission rate of $370\text{--}680 \text{ kg N-N}_2\text{O}$ is obtained for the whole river system. Direct measurements of N_2O concentrations in small rivers and streams justify this extrapolation: N_2O concentrations remains as high in the head waters as the ones reported for the sector immediately upstream from the Parisian WWTPs (unpublished, see Figure 9). This would indicate that about 20–30% of total N_2O emissions by the whole hydrographic network occur in its fluvial-estuarine part. The currently available dataset does not, however, allow to estimate the possible role of the processes occurring in the most upstream sectors of the drainage network, including riparian wetlands.

Global climate scenarios consider that the major part of anthropogenic N_2O emissions is related to agricultural activities and represents, on average 1.25% (range 0.25–2.25%) of the total nitrogen content of applied organic and mineral fertilizers (IPCC, 2000). Of this, one third is assumed to occur as direct emission from agricultural soil, one third is linked to animal waste management, and one third is due to indirect N_2O emissions through ammonium and nitrate losses (IPCC, 2000). Our data on the Seine can be compared to these general numbers.

The nitrogen fertilizers used in the Seine watershed ($75,000 \text{ km}^2$) amount to $770 \times 10^6 \text{ kg N yr}^{-1}$, mostly (75%) of inorganic fertilizers, as animal farming in the Seine basin is restricted to the peripheral areas. Direct measurements of N_2O emission from representative cultivated soil, fertilized at a rate of $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, have revealed mean annual emission rates of

0.4–0.8 kg N-N₂O ha⁻¹ yr⁻¹ (data cited by Khalil, 2003; Khalil et al., 2004). This would represent the direct emission from agricultural soils themselves, and falls within the range of 0.2–0.4% of the used fertilizer. One can then propose the range of $1500\text{--}3000 \times 10^3$ kg N-N₂O yr⁻¹ (0.2–0.4% of 770×10^6 kg N yr⁻¹) for the total direct emission of N₂O from agricultural soils in the Seine watershed. The emission from animal waste management should be much lower because the agriculture in the Seine watershed specializes mostly in cereal and industrial crops. The above estimate of the ‘indirect’ emission from the river network represents an annual flux of 135–250 kg N-N₂O yr⁻¹, i.e. about 10% of the direct emission from agricultural soils.

Although speculative, these estimations of N₂O production caused by effluents or originating from agriculture are of major interest for managers responsible for the implementation of European Water Directive, the stakes for improving wastewater treatment competing those for changes in agricultural practices.

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