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Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system

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Abstract. Nitrogen budgets established for large river systems reveal that up to 60% of the nitrate exported from agricultural soils is eliminated, either when crossing riparian wetlands areas before even reaching surface waters, or within the rivers themselves through benthic denitrification. The study of nitrogen isotope ratios of riverine nitrates could offer an elegant means to assess the extent of denitrification and thus confirm these budgets, as it is known that denitrification results in a natural ^{15}N enrichment of residual nitrates. The results reported here, for the Seine river system (France), demonstrate the feasibility of this isotopic approach at the scale of large watersheds. On the basis of *in situ* observations carried out in a large storage reservoir in the upstream Seine catchment (Der Lake), where intensive benthic denitrification occurs, as well as on the basis of laboratory experiments of denitrification under controlled conditions, it is shown that the isotopic discrimination associated with benthic denitrification is minimal (ϵ of NO_3-N ranging from −1.5 to −3.6‰), probably because the rate-limiting step of the process consists of nitrate diffusion through the water-sediment interface. Riparian denitrification on the contrary, when it implies nitrate reduction during convective transfer through reducing environements, causes a much more significant isotopic enrichment of ^{15}N of residual nitrate (ϵ about −18‰). The authors report measurements of nitrogen isotopic composition of nitrate from rivers of various stream orders in the Seine river system under summer conditions. Anomalies in the data with respect to the values expected from the mixture of the various sources of nitrate are here attributed to riparian denitrification. However, the authors show that because of the patchy distribution of actively denitrifying riparian zones within the drainage network, the isotopic signature conferred to residual nitrate in river water intrinsically provides only a minimum estimate of the extent of denitrification.

Introduction

Increasing nitrate contamination of surface and groundwater is a potential problem in all regions of the world with intensive agriculture (Altman and Parizek 1995; Wassenaar 1995; Spalding and Exner 1993). Nitrogen budgets established for large river systems in North America and in Europe reveal that a significant part (sometimes over 60%) of nitrogen exported from watershed soils is eliminated by deni-

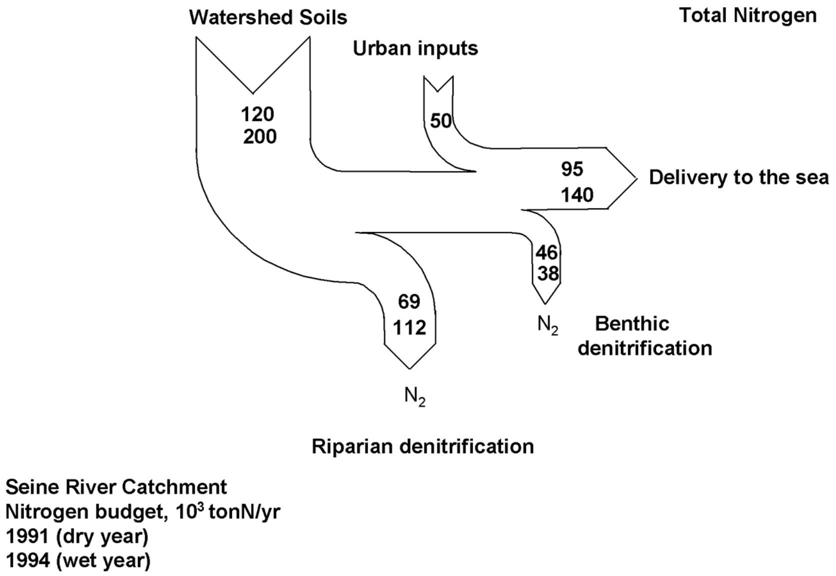


Figure 1. Budget of nitrogen transfers (in 10^3 tonN/yr) through the Seine River system, calculated by the RIVERSTRAHLER Model (Billen and Garnier 1999) for the hydrological conditions of year 1991 (upper figures) and 1994 (lower figures).

trification, either in riparian zones prior to reaching surface waters, or by anaerobic sedimentary deposits in the river bed itself (Lowrance et al. 1995; Simmons et al. 1992; Howarth et al. 1996; Billen and Garnier 1999; Behrendt and Opitz 1999; Seitzinger et al. 2002). Knowledge of the extent and the preferred location of denitrification is of considerable practical importance for management actions aimed at reducing nitrate contamination of surface water. From this point of view, it is essential to differentiate quantitatively between ‘retention’ of nitrogen within riparian wetlands and ‘retention’ due to benthic denitrification in the riverbed. In the case of the Seine basin, nitrogen balances, based on a general model of its circulation (Billen and Garnier 1999) suggest that elimination of nitrate by riparian areas represents more than 40% of the total nitrogen input to the watershed, while in-stream benthic denitrification eliminates 15 to 20% (Figure 1).

However, regional nitrogen balances are very sensitive to a number of assumptions and typically have a relatively large uncertainty. For this reason they should be tested through independent methods. Direct measurements of denitrification can be carried out at the station scale (Andersen 1997; Seitzinger et al. 1980; Christensen et al. 1989; Hill et al. 2000; Devito et al. 2000), but their extrapolation to the regional scale remains very delicate due to the large spatial variability. Since it is known that denitrification generally results in the enrichment of residual nitrates in heavy isotopes of nitrogen and oxygen (Mariotti et al. 1981, 1982; Böttcher et al. 1990; Aravena and Robertson 1998; Kellman and Hillaire-Marcel 1998; Fustec et al. 1991), the measurement of the isotopic composition of nitrate in surface water can potentially be a very powerful tool for assessing denitrification processes, integrating processes occurring at the scale of large river systems.

Isotopic ratios of riverine nitrate have previously been used to identify the sources of nitrate in surface water (Mariotti 1982; Mariotti et al. 1975, 1976; Mariotti and Létolle 1977; Battaglin et al. 1998; Katz et al. 1999). The variability of isotopic composition of the sources of nitrate (e.g., atmospheric deposition, synthetic fertilizer, animal wastes, urban waste water, etc...) often makes it difficult to distinguish mixing relationships from the effects of denitrification on the isotopic composition of nitrate. Kellman and Hillaire-Marcel (1998) reported significant increase in $\delta^{15}NO_3$-N attributable to in-stream denitrification in small agricultural streams. In a comparative study of 16 large watersheds of the northeastern USA, (Mayer et al. 2002) found that the isotopic composition of nitrate largely reflected the contribution of waste water and manure to the total nitrogen load. They did not, however, provide evidence for denitrification in the form of elevated $\delta^{15}NO_3$-N or $\delta^{18}O$-NO_3, even though in-stream denitrification was significant in all rivers.

The objective of this study was to determine under which conditions the isotopic composition of ^{15}N-NO_3^- can effectively be used as a tracer of denitrification in a large river system. We show that riparian denitrification in particular, but not benthic denitrification, can be characterized by a significant isotopic signal. Superimposed over the variations caused by the mixing of the different sources of nitrate, this signal can be detected in some, but not all, regions of the Seine watershed.

Study site, material and methods

The Seine river basin

The Seine river basin above Poses (last sluice lock before the entrance into the estuarine sector) covers an area of 65 000 km^2 (Figure 2). The watershed is characterized by concentric geological formations ranging from tertiary rocks in the central Parisian Basin to Jurassic limestones at the periphery, which consist of alternating layers of sandstones, limestones, chalk, clay, etc. These formations lie on a basement of ancient massifs outcropping at the extreme South-East and North-East.

The stream order structure of the river network, analysed according to Strahler (1957), can be summarized as follows: River Seine is 7th order from the confluence with River Yonne, and becomes 8th order after the confluence of River Oise, downstream from Paris. River Marne is 6th order. Typically, 4th order streams have a watershed area of 500–1000 km^2.

In the clayey area forming the ‘Champagne humide’ in the eastern upstream part of the basin, three major shallow storage reservoirs have been impounded in connection with the Seine (Lac de la Forêt d’Orient, 205 106 m^3), the Marne (Der Lake, 350 106 m^3), and the Aube rivers (Aube Lake, 170 106 m^3), respectively. These reservoirs are operated for the purpose of reducing winter floods and increas-

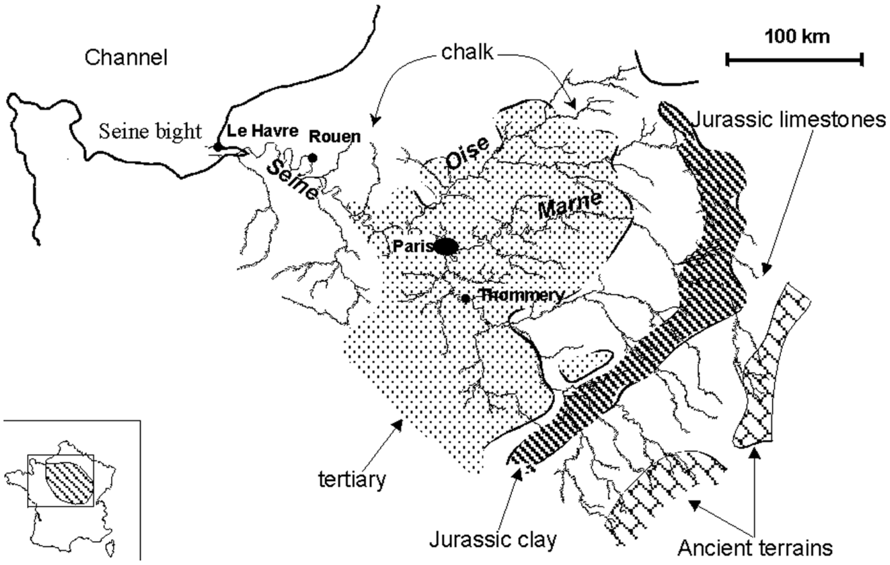


Figure 2. Geological structure of the Seine River watershed. The basin is constituted of successive convex layers: tertiary layers at the top in the center, then the secondary chalky layers, the jurassic formations and then the ancient terrains.

ing summer discharges. Der Lake, the largest of these reservoirs, is filled from November to April, to be emptied from July to October. During the period of water storage, from April to July, the reservoir can be considered a closed system. Atmospheric deposition of fixed nitrogen, the only external nitrogen input during this period, represents less than 2% of the initial nitrate pool. Intense benthic denitrification results in the nearly complete elimination of nitrate from the water column during that time (Garnier et al. 1998, 1999, 2000).

Sampling

River water samples were collected from a bridge with a bucket in the middle of the river. Water from Der Lake was collected from a boat using a pump to obtain integrated samples at depth. River bottom sediments were collected in River Seine at Thomery, upstream from Paris.

Methods

Analytical procedures

Nitrate concentrations were determined by colorimetry, according to the Griess-Ilosvay method (Greenberg et al. 1992), after reduction into nitrite on a copper cadmium column.

Isotopic composition of nitrate-nitrogen was measured by mass spectrometry after conversion of nitrate to molecular nitrogen. After concentration by evaporation, nitrate was reduced to ammonium salt according to the method of Bremner and Keeney (1965), using the reducing Dewarda alloy in alkaline medium. The ammonia formed was steam driven into 100 mL of M/40 sulphuric acid and the obtained ammonium sulphate was then oxidized to molecular nitrogen (N_2) by reaction with lithium hypobromite (LiOBr), in a vacuum preparation line. The gas was trapped on a silicagel molecular sieve placed in liquid nitrogen refrigerated sample collectors (Mariotti and Létolle 1977). The N_2 samples were subsequently introduced into the mass-spectrometer (VG Micromass SIRA 10) fitted with triple ion collectors and a dual inlet system equipped for rapid switching between reference and sample. The isotopic composition of NO_3^--N is expressed in relative units ($\delta^{15}N$, ‰), defined as:

$$\delta_{sample}(‰) = [(R_{sample} - R_{standard})/R_{standard}]*1000$$

where R is the isotopic ratio $^{15}N/^{14}N$. The international standard is atmospheric nitrogen (Mariotti (1983, 1984)), for which the ratio $^{15}N/^{14}N$ is 0.003676. Analytical precision is about 0.2‰.

Determination of the isotopic enrichment factor

In the case of a single step unidirectional reaction, the isotopic fractionation is defined as the ratio of the two rate constants:

$$\alpha = k^{15}N/k^{14}N.$$

The isotopic enrichment factor, ϵ, in ‰, is defined as:

$$\epsilon = 1000(\alpha - 1)$$

It is easily shown (Mariotti et al. 1981) from these definitions that, during a reaction Substrate (S) → Product (P), the production rates of the two isotopic forms (($d^{15}N$-P/dt) and ($d^{14}N$-P/dt) obey the relation:

$$(d^{15}NP/dt)/(d^{14}NP/dt) = (10^{-3}\epsilon + 1)(\ ^{15}NS\ /\ ^{14}NS\ ) \tag{1}$$

If the reaction takes place in a closed environment (i.e. without renewal of the substrate), the isotopic composition of the substrate (δ_S) at time t can be calculated from the following relation, known as the Rayleigh equation, which can be derived by integrating the Equation (1) above:

$$\delta_S(t) = \delta_{S0} + \epsilon \ln f = \delta_{S0} + \epsilon \ln Ct/C_0 \tag{2}$$

where δ_{S0} is the isotopic composition of the substrate at initial time, C_0 and Ct are the concentrations of the substrate at time zero and t, respectively. This relationship is used below to estimate the enrichment factor ϵ of denitrification by plotting measured values of $\delta_s(t)$ against ln Ct/C_0.

Results and discussion

Benthic denitrification in Der Lake

As a first test of the feasibility of using isotopic compositions of N-NO_3 as a tracer of denitrification, nitrate concentrations and δ^{15}N-NO_3 were followed from May to October 1998 in the water column of Der Lake. It is well established that nitrates are eliminated from the water column of this storage reservoir by benthic denitrification (Garnier et al. 1998, 1999, 2000). Despite the high decrease of nitrate concentration from more than 3 mg NO_3-N/L (3.33) in May 1998 to less than 1 mg NO_3-N/L (0.5) in September 1998 (Figure 3a), the corresponding isotopic enrichment remains relatively small, with the δ^{15}N of the residual nitrate remaining below 8‰ (Figure 3b). As the Lake does not receive any significant nitrate input during the observation period (atmospheric deposition and nitrate formed by nitrification are negligeable, Garnier et al. (2000)), the denitrification process can be modeled according to a Rayleigh process and Equation (2) is applicable. The corresponding isotopic enrichment factor (ϵ) is about −1.5‰ (Figure 3c). This ϵ value is lower than those reported in the literature for denitrifying systems, in laboratory and field experiments, which range from a minimum of −5‰ to as high as −33‰ (Böttcher et al. 1990; Mariotti 1982, 1983, 1994).

The low value of isotopic enrichment for denitrification in Der Lake can be explained by the following hypothesis. The rate-limiting step of the process of water column nitrate consumption in this system is probably not the benthic denitrification itself, but rather the diffusion of nitrates from the water column towards the lower layer of sediments where denitrification occurs. In these sediments, due to denitrification, the nitrate concentration will be lower than in water; in this manner a concentration gradient can be established, causing a molecular diffusion of nitrate towards sediments which effectively act as a nitrate sink. The molecular diffusion of the nitrate ion should present a low or null isotope effect due the solvation process of nitrate, which diminishes the mass difference between the two isotopic species: $^{15}NO_3^-(nH_2O)$ and $^{14}NO_3^-(nH_2O)$. In any case, this isotope effect is lower than the isotope enrichment factor associated with denitrification (rupture of a N-O bond)). Moreover, migration of nitrate through the water sediment interface is probably largely accelerated with respect to the molecular diffusion by bioturbation, resulting in still lower isotopic segregation. In this manner, as the direct sink function for nitrate in the water column of the lake is not denitrification but rather molecular or turbulent diffusion caused by the concentration gradient maintained by denitrification within the sediments, only very limited isotopic enrichment is observed in the residual nitrate pool of the water column.

In vitro *denitrification experiment*

In order to test this hypothesis, a laboratory experiment was set up to determine the possible difference in isotopic signature resulting from denitrification when it occurs: (i) in a system where diffusion is the limiting step (water column overlying a

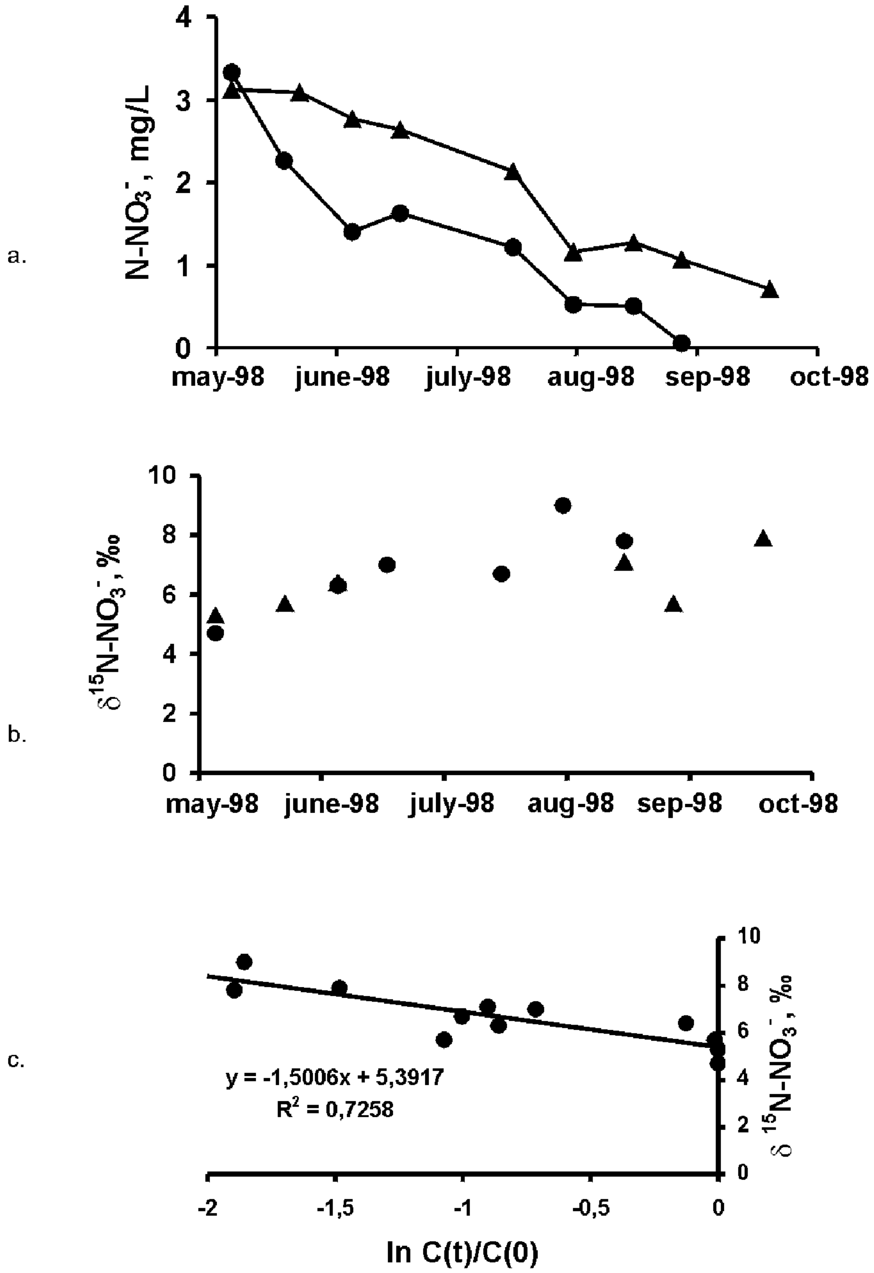
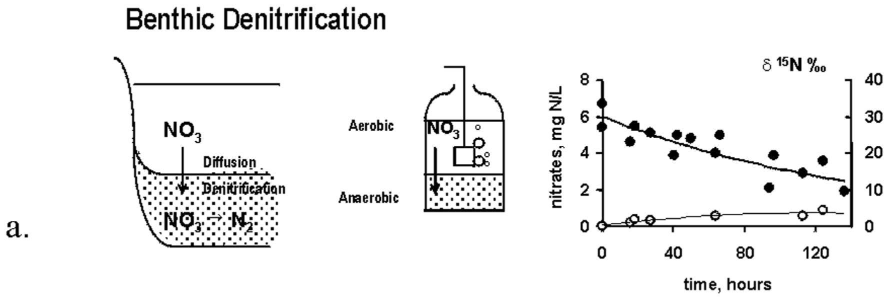


Figure 3. Nitrate concentration (a) and ^{15}N isotopic composition (b) during the period of water storage in the two sub-systems of the Marne Reservoir (● Der Lake; ▲ Champaubert Lake). Calculation of the isotopic enrichment factor (c).



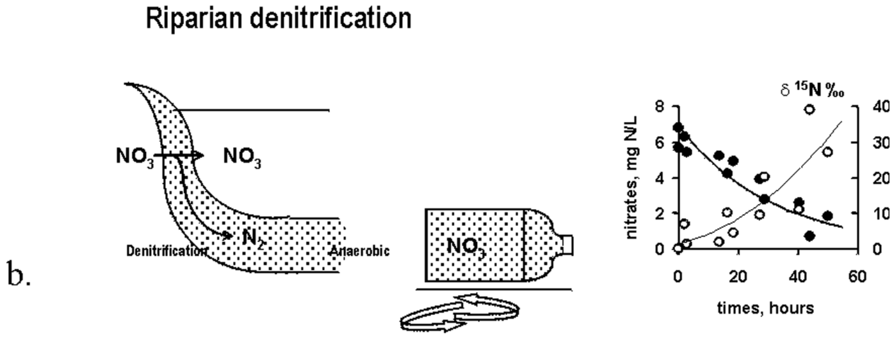


Figure 4. Variations of nitrate concentrations (●, mgN L^{-1}) and isotopic composition (○, $\delta^{15}N$) during two in vitro denitrification experiments respectively simulating (a) benthic denitrification after diffusive migration of nitrates from an aerobic water column, and (b) denitrification within an anaerobic environment without rate limitation by nitrate diffusion. (See text for explanation)

sediment layer: Der Lake analog), and (ii) in a system where diffusion is not limiting because denitrification occurs within the same phase where nitrates are present (analogue to a riparian zone crossed by a nitrate-rich water flux).

Organic rich muddy river bottom sediments were incubated in the dark at 20 °C (Figure 4).

1. in aerobic conditions in 2 L flasks, filled with river water; flasks were continuously aerated by an air pump adjusted to ensure aerobic conditions in the water phase without resuspending the river bottom sediments (Figure 4a). An inhibitor of nitrification (Nitrapyrine 5 mg/L, Brion and Billen (1998)) was added to prevent interference of mineralisation and nitrification of sediment organic matter.
2. in anaerobic conditions, in hermetically closed flasks, under permanent agitation in order to maintain the sediments in suspension (Figure 4b).

In the first experiment (Der Lake analogue; Figure 4a), nitrate reduction was slow and resulted in only slight isotopic enrichment of ^{15}N. In the second experiment (riparian zone analogue; Figure 4b), denitrification proceeded more rapidly and resulted in more substantial isotopic enrichment of ^{15}N. The corresponding mean isotopic enrichment factors calculated for the three replicate experiments were

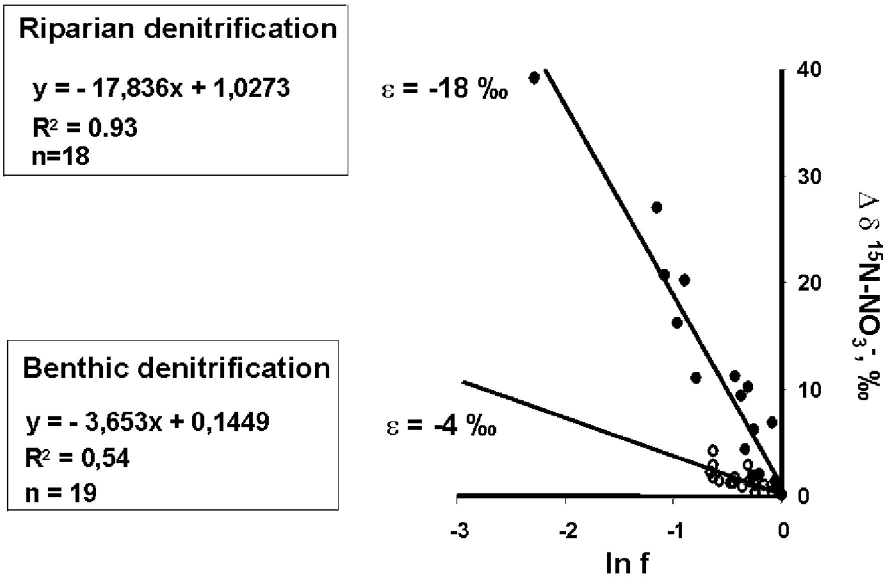


Figure 5. Isotopic enrichment factor of $^{15}NO_3^-$-N (ε ‰), for triplicates results of the experiments described in Figure 4.

−3.6‰ for the Der Lake analogue and −17.8‰ for the riparian zone analogue (Figure 5).

These results support the hypothesis that the diffusion of nitrate from the water column across the sediment-water interface is the limiting step, resulting in little or no isotopic discrimination. This implies that benthic denitrification occurring in river bottom sediments or within connected stagnant annexes, cannot be expected to lead to a significant isotopic enrichment of ^{15}N in river system nitrate. On the other hand, denitrification affecting the nitrate fluxes in riparian zones should result in a much more significant isotopic enrichment in ^{15}N (and ^{18}O) residual nitrate, because no rate limitation by diffusion of nitrate occurs in this case. Significant isotopic enrichment during riparian denitrification has indeed been experimentally shown by several authors including Hill et al. (2000) and Cey et al. (1999), Mengis et al. (1999). For the same reason, denitrification accompanying hyporheic flow also results in a strong isotopic signature (Hinkle et al. 2001).

Occurrence of an isotopic signal from riparian denitrification in nitrate composition in the Seine river system

About 100 samples were collected during summer low flow conditions in rivers and streams from the Seine drainage network and analysed for concentration and isotopic composition of nitrate. A possible signal from riparian denitrification should appear as a negative anomaly in nitrate concentration, and a positive anomaly in nitrate $\delta^{15}N$ with respect to the values expected from the mixing of the various nitrate sources in the system. As these sources are multiple and may be characterized by different concentration and isotopic composition, their close examination is necessary.

The case of low order streams

We compared concentrations and isotopic compositions of nitrate from small streams with those for nitrate in groundwater and in drainage water (Figure 6). We excluded from our data-base streams with population densities in their upstream watershed such that domestic wastewater contribution to the nitrate pool could be higher than 10%. For samples in agricultural areas, with nitrate concentrations above 5 mgN/l and specific flow rates around 5 L km^{-2} s^{-1}, this corresponds to a population density of 25 inhabitants/km^2 (on the basis of a specific loading of 10 gN inhab^{-1} day^{-1}, Garnier et al. (2000)). Neglecting domestic contributions, the nitrate pool in small streams can thus be considered a mixing of nitrates from groundwater and from more superficial runoff (surface runoff or drainage water).

In the chalky Champagne region, the contribution of superficial drainage water is very low, particularly in summer. The isotopic composition of groundwater nitrate is remarkably constant ranging between 3 and 5‰, although nitrate concentrations vary from 2 to 10 mg NO_3-N/L. Concentrations and isotopic compositions of nitrate in samples from small streams do not differ significantly from these values, and no isotopic anomaly can be detected. On the other hand, in the Brie area, nitrate $\delta^{15}N$ values in many stream samples are significantly higher than those of both groundwater and drainage water samples, particularly at low nitrate concentration. This difference of $\delta^{15}N$ value in the river samples and the values for the simple mixing can be considered as an indication of riparian denitrification.

The case of higher stream order rivers

The chemical and isotopic composition of water of large rivers mainly results from the mixing of water from smaller streams draining the various lithologic zones of their upstream watershed, with groundwater contributed by their direct watershed. Excluding rivers with population densities higher than 50 inhabitants/km^2 in their watershed, the concentration and isotopic composition from rivers of stream order higher than 4 can be compared with those of the contributing small streams in their watershed. Table 1 summarises the nitrate concentration and isotopic composition obtained for small streams in the various lithological zones of the Seine basin. Using these mean values and the relative areal contribution of the corresponding zones in their watershed, for each river we calculated from our database the concentration and isotopic composition of nitrate resulting from the simple mixing of water from the respective tributaries and compared these calculated values with the measured ones (Figure 7). Observed nitrate concentrations are lower and $\delta^{15}N$ values are consistenly higher than calculated values. This indicates, in all likelihood, the occurrence of significant riparian denitrification in the river sectors of stream orders higher than 4.

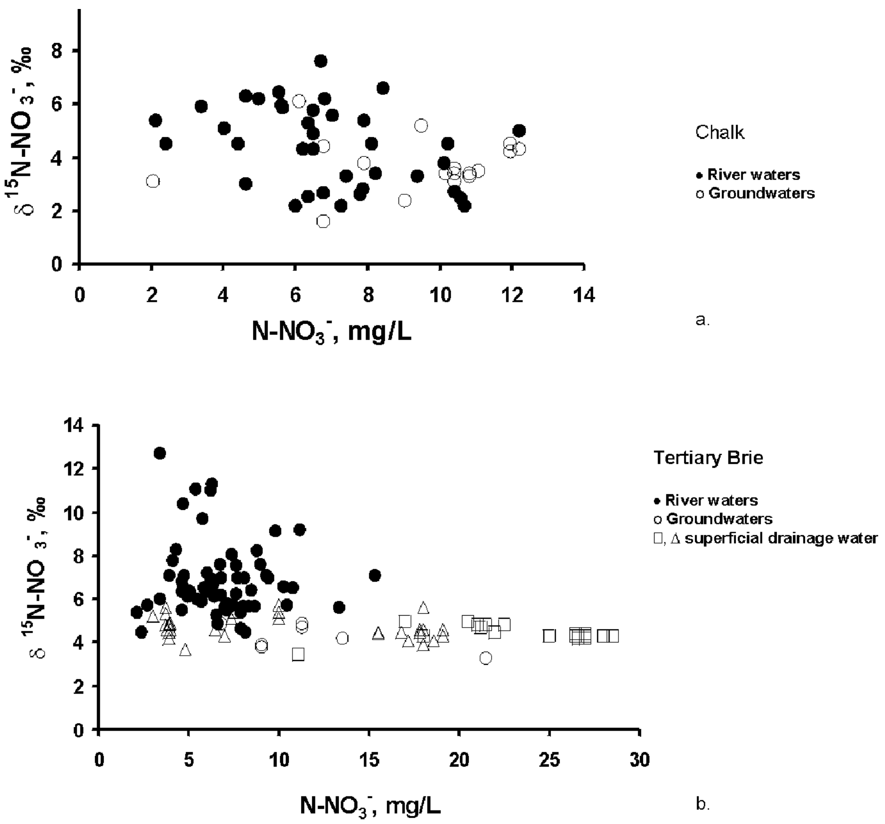


Figure 6. Isotopic composition and nitrate concentration for river water samples from the Seine drainage network. a. Comparison between river water and groundwater (Sebilo et al. 2002) from the Chalk area. b. Comparison between river water, groundwater and superficial drainage water (Dutriaux 1980; Mariotti 1982) in the Brie Region (Tertiary terrains). A distinct anomaly is apparent in the isotopic composition of nitrates of many river water samples.

Table 1. Concentrations and isotopic compositions for small streams in the various lithological zones of the Seine basin.

Order 1–4	NO_3 (mg/L)	$\delta^{15}N$‰	Number of sites
Ancient terrains	0,8	0,1	3
Jurassic	4,8	4,4	7
Chalk	7,9	4,1	22
Tertiary	7,1	6,8	56

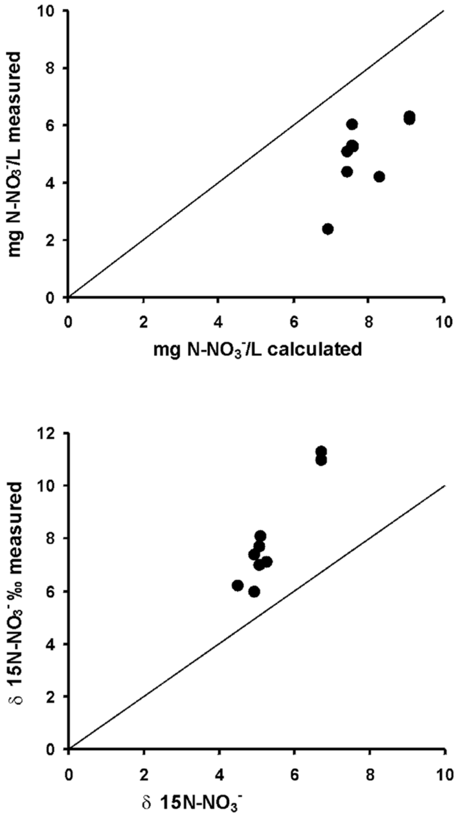


Figure 7. Comparison between nitrate concentration and isotopic composition from rivers of stream order higher than 4 and those of the contributing small streams in their watershed: the measured nitrate concentration and isotopic composition is plotted against the values calculated from the composition of the small streams draining the different lithologic zones of their upstream watershed.

The interpretation of the isotopic anomaly in terms of overall riparian denitrification at the scale of the river system

As stated in the introduction, our ultimate purpose of studying the isotopic composition of nitrates in river water was to provide a quantitative assessment of overall riparian denitrification at the scale of large river systems. When a significant difference is found between the isotopic composition of nitrate in river water and the composition calculated on the basis of mixing of the contributing sources, how can this anomaly be used to assess the intensity of riparian denitrification? If the denitrification capacity of riparian zones was uniformly distributed all along the network of upstream tributaries, always retaining the same fraction of the nitrate flux prior to entering river water, a simple relationship, derived from Equation (2), would hold between the extent of riparian 'retention' (R) and the isotopic anomaly

($\Delta\delta^{15}$N):

$$\Delta\delta^{15}N = \epsilon \ln(1 - R) \tag{3}$$

with (1–R) = Cr/Co, the ratio between nitrate concentration in river water (Cr) and in contributing water, either root-zone water or groundwater (Co).

This view of a uniform distribution and activity of riparian zones is obviously quite unrealistic. Riparian denitrification occurs in 'hot spots' of organic rich material patchily distributed within alluvial deposits (e.g., see Hill et al. (2000)). In the extreme case, if denitrification is complete in these hot spots, while no denitrification occurs elsewhere, no isotopic enrichment would be observed in riverine nitrate, whatever the magnitude of the resulting overall nitrate reduction might be. Thus we consider a theoretical situation where a fraction (g) of the flux of nitrates from the root-zone or groundwater crosses an active riparian zone, while the remaining part (1–g) is flowing directly into the river channel (Figure 8). The resulting nitrate concentration in the river water is then given by the relation:

$$C_r = C_0(1 - g) + C_0 g(1 - R') = C_0(1 - gR') \tag{4}$$

where R′ is the extent of denitrification affecting the part g of the flux joining the river water. By varying R′ in the active riparian zone, while maintaining the fraction g constant, the theoretical relationship between the resulting isotopic composition of nitrate in river water can be calculated from the following relationship:

$$\delta^{15}Nr = \frac{Co \times (1 - g)}{Cr} \times \delta^{15}No + \frac{Co \times (1 - R')}{Cr} \times (\delta^{15}No + \epsilon \ln(1 - R')) \tag{5}$$

The anomaly ($\Delta\delta^{15}$N = δ^{15} Nr – δ^{15} No) with respect to the isotopic composition of the source can thus be expressed by the following relationship:

$$\Delta\delta^{15}N = \frac{[g \times (1 - R') \times \epsilon \times \ln(1 - R')]}{1 - gR'} \tag{6}$$

On the other hand, the apparent overall nitrate retention (R) is given by

$$R = Cr/Co = (1 - g) + g \times (1 - R') = (1 - g \times R') \tag{7}$$

Obviously, the fraction g sets an upper limit to the overall nitrate retention.

Figure 8 shows how the isotopic anomaly (calculated with relation 6) varies with the overall retention (calculated with relation 7) for different hypothesis regarding the value of the fraction g. These curves provide evidence that the isotopic anomaly corresponding to the ideal uniform distribution of denitrifying zones (corresponding to g = 1, for which relation 6 is equivalent to relation 3), is always higher than for any other situation (with g < 1). A similar conclusion could be reached by considering a number of differently active riparian zones, each being crossed by a given

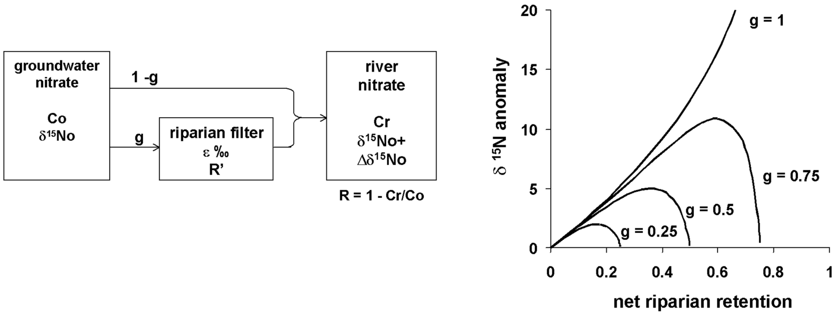


Figure 8. Theoretical relationship between the resulting isotope anomaly and the overall nitrate retention, in a case where only a fraction (g) of groundwater nitrate crosses active denitrifying areas.

fraction of nitrates. This implies that an observed anomaly in the isotopic composition of nitrates at a given station of a river provides a *minimum estimate* of the integrated riparian retention occurring upstream from this station in the drainage network. The absence of any anomaly, on the other hand, can never be interpreted as implying the absence of riparian retention.

Conclusion

Denitrification in river systems represents a significant sink to the global nitrogen cycle (Howarth et al. 1996; Billen and Garnier 1999). It occurs either in bottom sediments of rivers and their stagnant annexes after migration of water column nitrate through the benthic interface, or during hyporheic flow through gravel beds underlying the river course, or within riparian zones that the nitrate in groundwater or surface drainage water from the watershed has to cross before reaching the rivers. The respective role of these mechanisms in nitrate retention is difficult to assess at the regional scale, even by mass balance approaches.

The objective of this work was to assess the feasibility of using ^{15}N values of riverine nitrate as an indicator of denitrification at the scale of large river systems. We showed both through *in situ* observations on Der Lake and through laboratory experiments, that benthic denitrification, when it involves diffusive migration of nitrates through the water-sediment interface, does not affect the isotopic composition of riverine nitrate significantly because the rate limiting step is the non-discriminating process of nitrate diffusion (^{15}N isotopic enrichment factor about −2‰). On the other hand, riparian denitrification, as well as denitrification within hyporheic flow, are expected to strongly affect the isotopic composition of residual nitrates (^{15}N isotopic enrichment factor about −18‰), because they generally involve advective transport of nitrate to the site of reaction.

The nitrogen budget established by Billen et al. 1989 for the whole Seine river system implies that riparian denitrification plays an important role in nitrogen 'retention' in the upstream sectors of the Seine watershed, while in-stream, benthic denitrification is less effective. Our preliminary measurements of nitrogen isotopic composition of nitrates, in various rivers and streams from the Seine drainage network, support the view of a significant riparian (or hyporheic) denitrification. Although our data base of such measurements is not rich enough at the present stage to allow quantitative validation of the nitrogen budget on the basis of isotopic measurements, these results suggest that the study of natural isotopic composition of the nitrates in rivers constitutes a promising approach to assess the role of riparian denitrification at the scale of large river systems. We demonstrated, however, that because of the non-uniform distribution and activity of denitrifying riparian zones along tributaries, the isotopic signature conferred to river water nitrate can only be interpreted as a minimum estimate of the extent of nitrate reduction.

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