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Spatial and temporal patterns of denitrification in a riparian forest

G. PINAY, L. ROQUES and A. FABRE

Centre d'Ecologie des Ressources Renouvelables, CNRS 29 rue Jeanne Marvig, 31055 Toulouse, France

Summary

1. This study estimated the importance of *in situ* denitrification in the nitrate buffering capacities of a riparian forest.

2. Spatial and temporal patterns of *in situ* denitrification were investigated along a riparian catena.

3. Highest rates of *in situ* denitrification (up to 78 mg N m^{-2} day^{-1}) were measured in the riparian forest soils in late winter and early spring. Lowest rates (3 mg N m^{-2} day^{-1}) were measured in summer and autumn.

4. Whatever the season considered, 30 m of riparian buffer strip were enough to remove all the nitrates coming through the groundwater.

5. Rehabilitation of riparian zones with riparian vegetation together with the maintenance of waterlogged conditions induced by riverflow regulation appear to be a good point from which to start the restoration of buffering capacities of river ecosystems against nitrogen loads.

Key-words: catena, nitrogen, river floodplain, vegetated buffer strip.

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Introduction

Because of their position between aquatic and terrestrial ecosystems, riparian forests constitute a zone of transition of primary importance for the fluxes of matter and energy in longitudinal (Schlosser & Karr 1981; Cooper *et al.* 1987; Pinay *et al.* 1990), and lateral directions (Likens & Bormann 1974; Hynes 1975). The role of riparian forests in controlling the nutrients flowing from the upper catchment towards the river drainage system has been well documented since Kaushik *et al.* (1975) first stressed the retention capacity of riparian areas *vis-à-vis* nitrate fluxes from the drainage basin. Since then, several studies have demonstrated the high potential for removal of nitrogen in riparian areas (Yates & Sheridan 1983; Brinson, Bradshaw & Holmes 1983; Brinson, Bradshaw & Kane 1984; Lowrance, Todd & Asmussen 1984a, b; Peterjohn & Correll 1984; Jacobs & Gilliam 1985a, b; Lowrance *et al.* 1985; Davidson & Swank 1986; Haycock & Burt 1993). All these studies stressed the role of denitrification in nitrogen removal capacities, but very few have measured *in situ* denitrification in riparian areas (Pinay & Labroue 1986; Cooper 1990; Duff & Triska 1990). Although riparian vegetation can also be involved in nitrogen retention via uptake, it constitutes only a transitory retention system, since a large amout of nitrogen returns to the soil as litter, especially in matured forests (Vitousek & Reiners 1975). Thus, denitrification is the key biological process in reducing nitrogen loads by dissimilatory reduction of nitrate into gaseous nitrogen (Knowles 1982).

Denitrification as a microbiological process is now well understood and its limiting factors have been identified (see Knowles 1982; and Chalamet 1985 for a review). However, little is known of the factors controlling *in situ* denitrification or their effect in riparian forests. At the riparian forest scale three factors, namely anaerobiosis, the organic carbon energy source and nitrate supply, are controlled by soil topography, vegetation cover and local hydrology.

This study investigated spatial and temporal patterns of *in situ* denitrification along a riparian forest catena. The objective was to measure *in situ* denitrification under different soil waterlogging conditions and geographical positions with respect to the groundwater flow in order to identify the factors controlling denitrification.

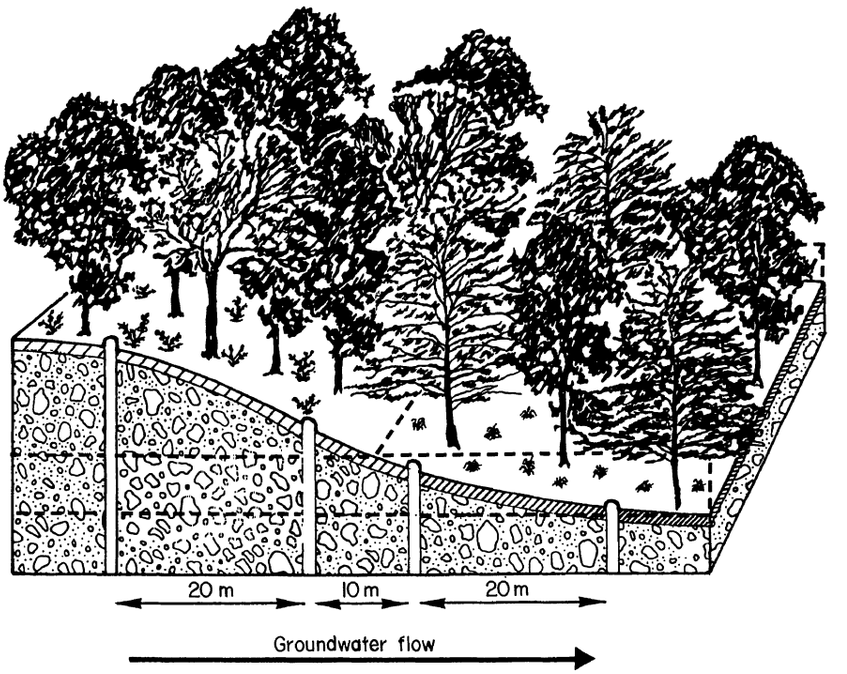
Study area

The study sites were selected along a riparian forest of the River Louge (fourth-order stream), a tributary of the River Garonne upstream of the city of Toulouse, south-west France. This forest lay on

an old terrace of the Garonne, above a 3-m thick aquifer of alluvial deposits. The groundwater table in the riparian forest lay between −1 and −0·5 m with respect to the soil surface in the Study Site 1 and from −0·5 to +0·5 m in the Study Site 4. The riparian forest was not flooded by the River Louge, but by the rising of the groundwater table (Pinay 1986). Average precipitation was 550 mm per year, with maximum in May and minimum in July. Air temperature averaged 14·5 °C monthly and extreme daily values ranged between 3° and 30 °C. Further details of the study sites are given by Pinay *et al.* (1989).

Four study sites were chosen along a 50-m-long transect parallel to the groundwater flow to represent the different features of the catena existing in the riparian forest (Fig. 1). The slope of the catena was about 3% (Pinay *et al.* 1989). Piezometric surfaces within the sites and the use of a tracer (NaCl) enabled dominant flow pathways to be identified. Duration of soil submersion varied from no submersion in Site 1 to up to 8 months p.a. of submersion by the groundwater table in Site 4. Clay and fine silt, which dominated in all the sites, increased the water holding capacities of these poorly drained clay loam soils (Pinay 1986). Vegetation distribution along the transect followed the topographic variations of the catena and in turn the duration of waterlogging conditions. On the upper part of the transect elm (*Ulmus minor* Miller), ash (*Fraxinus angustifolia* Vahl) and *Cardamine pratensis* (L.) dominated (Pautou *et al.* 1985). Lower down, alder (*Alnus glutinosa* (L.) Gaertn.), *Carex remota* (L.) and *Carex riparian* (Curtis) were dominant.

In the text the terms 'upslope' and 'downslope' refer to the catena as well as to the groundwater movement through the riparian forest.



	Site 1	Site 2	Site 3	Site 4	
Soil submersion					
Duration in months	0	4	6	8	
Grain size (%)					
Clay	34	40	44	46	
Fine silt	25	30	36	34	
Coarse silt	12	12	10	12	
Fine sand	14	10	7	5	
Coarse sand	15	8	3	3	
Vegetation cover					
Main tree species		Elm (*Ulmus minor*)		Alder (*Alnus glutinosa*)	
		Ash (*Fraxinus oxyphylla*)		Ash (*Fraxinus oxyphylla*)	
Understorey		*Cardamine pratensis*		*Carex remota*	*Carex riparia*

Fig. 1. Vegetation and soil characteristics of the four study sites along the catena. The two dashed lines represents the upper and lower limits of the water table. The hatched area above the aquifer symbolizes the soil layer which gets darker downslope due to organic matter accumulation. The vertical white columns represent the sampling wells.

Materials and methods

SOIL SAMPLING AND ANALYSIS

Three soil samples were taken monthly between January 1990 and February 1991 in each of the four study sites. The upper 10 cm of soil were taken after the litter was discarded. After collection, all samples were stored at 4 °C and processed within 48 hours. Soil temperature was measured weekly 10 cm below the soil surface in the four study sites with a Hanna temperature probe (HI 9063, Hanna Instruments, Paris France). Soil particle size was determined once in each site by the pipette sampling method (Day 1965), after pretreating the samples with hydrogen peroxide and dispersing with sodium hexametaphosphate solution. Soil subsamples were oven-dried for 24 hours at 105 °C in order to determine dry mass and percentage moisture by mass on a wet weight basis. Soil pH was determined on air-dried soils in 2·5:1 (litre:kg) water. Three subsamples of 10 g of fresh soil were treated with 20 ml of deionized water and filtered through a prewashed 0·45 μm filter after 30 minutes shaking and 20 minutes centrifugation (8000 *g*). Aliquots of the filtered solutions were used for the determination of 'extractable glucose equivalent' (EGE) considered as an index of carbon availability (Stanford, Vander Pol & Dzienia 1975; Reddy, Rao & Jessup 1982; Davidson, Galloway & Strand 1987), by the phenol method (Dubois *et al.* 1956). 10 g (fresh mass) of each soil sample were extracted with 150 ml of 2 mol l^{-1} KCl. The extract was filtered and analysed for NH_4-N and NO_3-N with a Technicon Autoanalyser (Technicon 1976). The NO_2-N form of inorganic nitrogen was not taken into account since it presented low and constant values whatever the site considered. Nitrogen mineralization potential (NMP) was performed on fresh subsamples by anaerobic incubation for 7 days at 40 °C (Waring & Bremner 1964). Total nitrogen (TN) was determined by digestion of air-dried subsamples following the Kjeldahl method (Bremner 1965).

In situ denitrification (DNT) was assayed by a static core acetylene inhibition method (Yoshinari & Knowles 1976). After the litter was discarded, three intact cores (length 10 cm, diameter 3 cm) sampled monthly in the four study sites were placed in glass jars five times larger than the soil cores, capped with rubber serum stoppers and then amended with acetone-free acetylene to bring core atmosphere concentration to 10 KPa (10% vol./vol.) acetylene and 90 KPa air. Denitrification rate was calculated as the rate of nitrous oxide (N_2O) accumulation in the soil core atmosphere between 4 and 8 h after amendment. Bulk density of each core was measured in order to express denitrification results on an area basis. Head space samples were removed from all cores and stored in evacuated collection tubes (Venoject, Terumo Scientific, 3030 Leuven, Belgium) before analyses were performed within few days. Gas samples were analysed by gas chromatography (Varian 3300 GC, Varian Associates, Sugar Land, Texas, USA) equipped with a ^{63}Ni electron capture detector (oven 80 °C, detector 350 °C, carrier gas N_2) and Porapak Q columns (Varian Associates, Sugar Land, Texas, USA). Estimates of annual N loss by denitrification were calculated by extrapolating measured rates during the period between sampling dates. Denitrification enzyme activity (DEA) was measured in three soil subsamples by transforming them into a slurry followed by anaerobic incubation in closed flasks for 8 h at average soil temperature (10 °C). Acetylene was added in the same proportion as for *in situ* denitrification. Nitrate was added to the soil suspension, 10 μg NO_3-N g^{-1} (soil fresh weight basis) following Smith & Tiedje's (1979) procedure.

WATER SAMPLING AND ANALYSIS

Wells made of PVC tubing were sunk into the aquifer up to the impermeable Molasse layer (4 m) in all the study sites. The base of each 8-cm diameter pipe remained open and its walls were converted to screens by perforating holes in the lower 50 cm. Monthly water samples were pumped through silicone rubber tubing using a peristaltic pump. Water samples (1 litre) were collected in prewashed glass bottles and filtered through 0·45-μm pore size Sartorius membranes. Ferrous iron analysis was performed in the field on sample aliquots immediately after sampling, using the colorimetric orthophenanthroline method (Golterman, Clymo & Ohnstad 1978). Samples were then stored at 4 °C and the other measurements were performed within 24 hours.

Nitrate (NO_3-N) was analysed colorimetrically using a Technicon Autoanalyser (Technicon 1976). Dissolved manganese (Mn) was measured by atomic absorption spectrometry (AAS Pye Unicam SP9 series).

STATISTICAL ANALYSIS

Statistical analyses were performed using the MGLH model from SYSTAT software (Wilkinson 1990). Multivariate regressions were calculated for each site, using the 11 monthly values of measured DNT after selection of the best explicative variables by stepwise regression (forward method, Draper & Smith 1966). For each multivariate regression we tested the residual normality using Lilliefors' test (Wilkinson 1990), the residual autocorrelation using Durbin-Watson's test (Wilkinson 1990), and the variance homogeneity by plotting the residuals against predicted values and independent variables (Dowdy & Wearden 1991). For all reported results

we considered only significant differences at $P < 0{\cdot}05$ (Elliott 1977).

Results

SOIL

Average moisture content in the four study sites increased significantly ($P < 0{\cdot}01$) from Site 1 to Site 4 (Fig. 2a). Soil water content decreased in the four sites between August and October, and then rose towards their maximum water-holding capacity. However, this seasonality was less apparent in Site 4 where the soils remained very moist all year. Soil temperature averaged 10 °C and fluctuated between 5 °C in February 1991 and 24 °C in July 1990 (data not shown). Extractable glucose equivalent (EGE) was not significantly different between the four study sites at $P < 0{\cdot}05$. Average soil pH values decreased along the catena from 7·4 in Site 1 to 6·05 in Site 4 (Site 1 being significantly different from Site 4 at $P < 0{\cdot}01$). Soil nitrate content was higher in Site 1 (6·38 $\mu g\, g^{-1}$ NO_3-N; SE = 1·34) than in the three other sites whose average values ranged between 2·62 and 3·38 $\mu g\, g^{-1}$ NO_3-N (Fig. 2), but in this respect the four study sites did not differ significantly ($P < 0{\cdot}05$). Although there was an increase in average values of soil ammonium content (NH_4-N) from

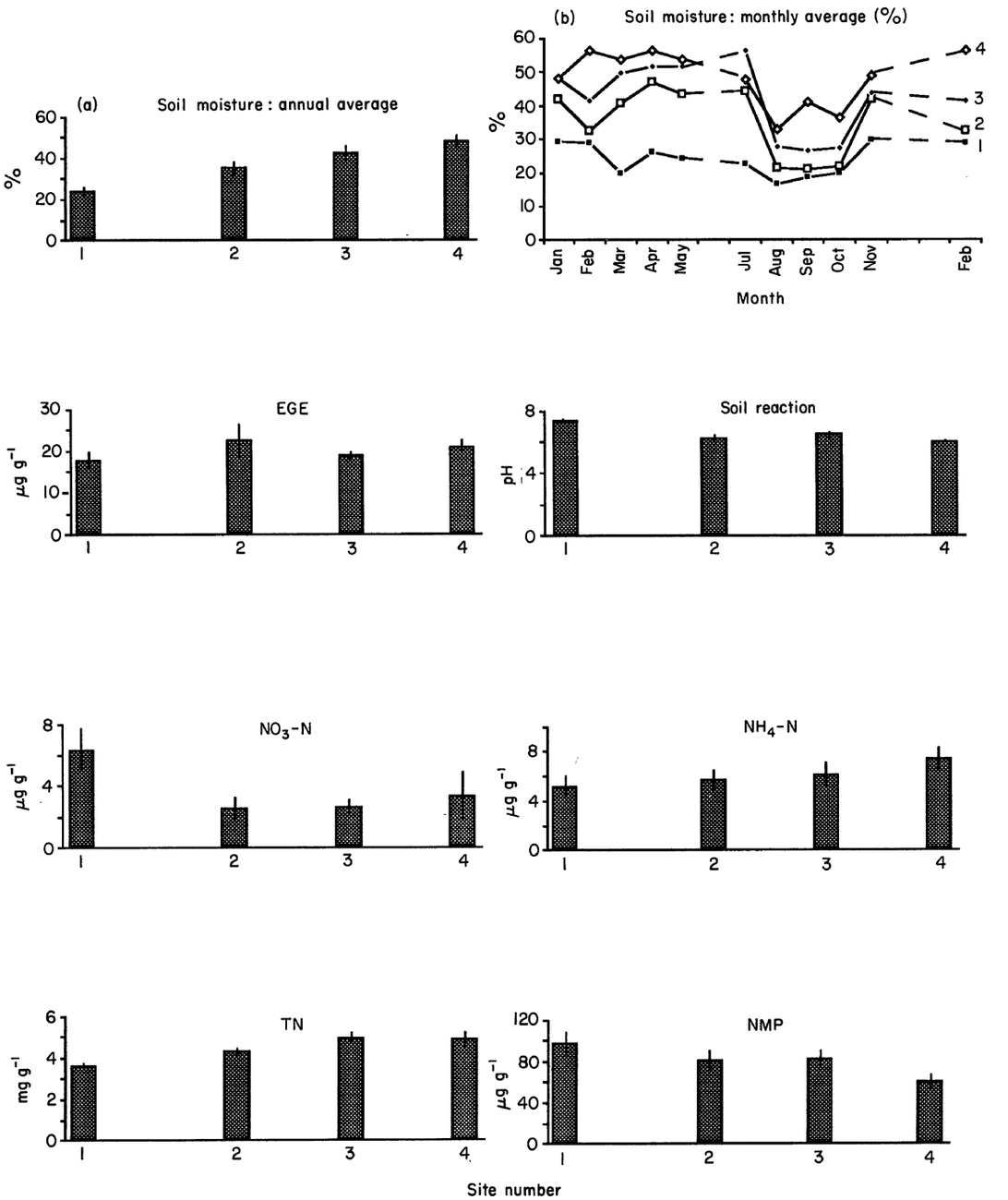


Fig. 2. (a) Annual average of soil characteristics in the four study sites. Vertical bars represent the standard error of the mean (SE). (b) Monthly variation of soil moisture: percentage moisture by mass on a wet weight basis (MOIST), at the four study sites. Continuous lines are broken whenever there was a break in the monthly sampling. EGE, extractable glucose equivalent; TN, total nitrogen; NMP, nitrogen mineralization potential.

Site 1 to Site 4, there was again no significant difference between the four study sites.

Average total nitrogen in soils (TN) increased significantly ($P < 0{\cdot}01$) from Site 1 to Sites 3 and 4, but the latter two did not differ from each other. Moreover, nitrogen mineralization potential (NMP) was significantly higher ($P < 0{\cdot}05$) at Site 1 than at Site 4.

DENITRIFICATION

Annual average *in situ* denitrification (DNT) was higher at the upslope site in the riparian forest (Site 1, 31·4 mg N m^{-2} day^{-1}) than at the downslope stations (Sites 3 and 4), but the latter two did not differ significantly (Table 1). Annual average DNT at Site 2 was within the range of the upper and lower sites.

On a monthly basis measured DNT was higher in winter and spring in the three upper study sites, while Site 4, the lowest, did not show such a marked seasonality (Fig. 3a). DNT was significantly correlated ($P < 0{\cdot}05$) with different soil variables in the four study sites (Table 2). Thus at Site 1, DNT was positively correlated with soil moisture (MOIST), soil nitrate content (NO_3-N) and EGE; and negatively correlated with soil temperature. At Site 2, DNT was positively correlated with MOIST, NO_3-N and Denitrification Enzyme Activity (DEA). At Site 3, DNT was only negatively correlated with soil pH. Finally at Site 4, DNT was positively correlated with NO_3-N and NH_4-N.

Annual denitrification was extrapolated by summing the monthly measurement of DNT (Fig. 3b). As had been observed for average *in situ* denitrification, total amount of nitrogen denitrified decreased from Site 1 (10·4 g N m^{-2} $year^{-1}$) to Site 4 (5·58 g N m^{-2} $year^{-1}$).

Monthly DNT was estimated by multiple regression analysis at the four study sites separately using the soil parameters measured monthly. The parameters which gave the best estimate soil DNT in each of the study sites are shown in the following equations:

Site 1 $\quad DNT = -51{\cdot}748 + 1{\cdot}198\,(MOIST) + +2{\cdot}098\,(NO_3\text{-}N) + 2{\cdot}3\,(EGE)$

$R^2 = 0{\cdot}766$ $P_{ANOVA} = 0{\cdot}013$

Table 1. Mean and range of *in situ* denitrification (DNT) and denitrification enzyme activity (DEA) in the four study sites

	Site 1	Site 2	Site 3	Site 4
DNT				
Number of measurements	33	33	33	33
Mean (mg m^{-2} day^{-1})	31·43	22·94	15·73	15·12
SE	7·88	5·41	4·21	3·50
Range (mg m^{-2} day^{-1})				
Minimal value	3·76	3·35	3·73	3·08
Maximal value	78·73	64·28	49·54	39·81
DEA				
Number of measurements	33	33	33	33
Mean (mg m^{-2} day^{-1})	278·94	266·44	271·57	278·18
SE	34·80	75·07	45·29	46·28
Range (mg m^{-2} day^{-1})				
Minimal value	118·09	95·46	87·05	72·76
Maximal value	491·01	979·59	515·62	507·49
DNT/DEA (%)	11·27	8·61	5·79	5·44

Table 2. Coefficient of correlation between *in situ* denitrification (DNT) and soil variables in the four different study sites. Values with * are significant at $P < 0{\cdot}05$

	Site 1	Site 2	Site 3	Site 4
MOIST (%)	0·721*	0·671*	0·505	0·340
pH	−0·322	0·547	−0·690*	−0·223
T (°C)	−0·646*	−0·487	−0·293	−0·206
NO_3-N (μg g^{-1})	0·607*	0·750*	0·236	0·706*
NH_4-N (μg g^{-1})	0·497	0·493	0·190	0·615*
TN (μg g^{-1})	0·366	−0·244	0·325	−0·303
NMP (μg g^{-1})	−0·034	−0·222	0·396	−0·283
EGE (mg g^{-1})	0·779*	−0·362	−0·076	−0·477
DEA (mg m^{-2} d^{-1})	0·113	0·759*	−0·008	0·083

Variable abbreviations: MOIST, moisture content; DEA, denitrification enzyme activity; others as in legend to Fig. 2.

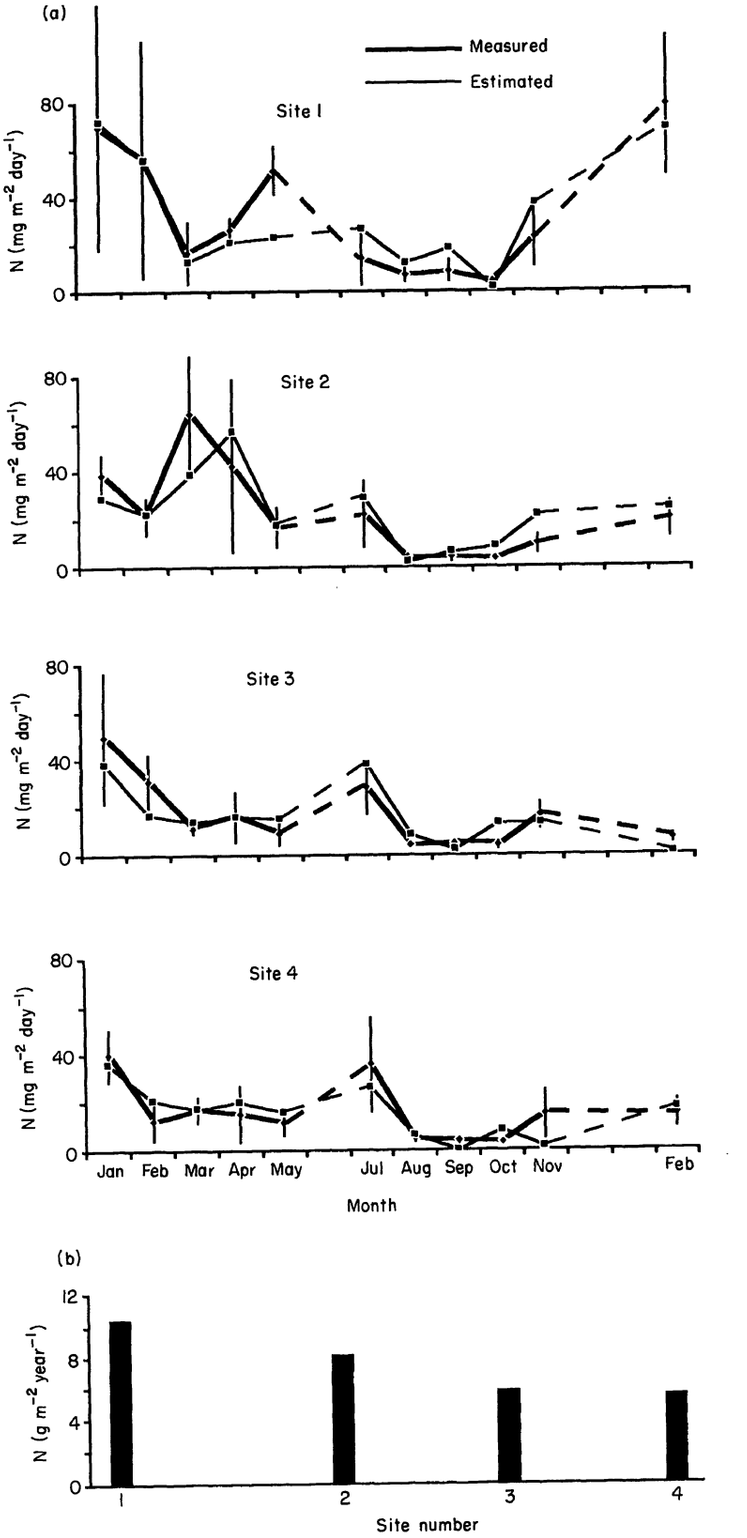


Fig. 3. (a) Monthly measured and estimated rates of *in situ* denitrification (DNT) in the four study sites. Vertical bars represent the standard error of the measured DNT means (SE). Continuous lines are broken whenever there was a break in the monthly sampling. (b) Annual N loss by *in situ* denitrification (DNT) extrapolated from monthly measured rates at the four study sites.

Site 2 $\quad DNT = -7{\cdot}684 + 1{\cdot}024\ (MOIST) + +2{\cdot}941\ (NO_3\text{-}N) - 0{\cdot}164\ (NMP)$

$R^2 = 0{\cdot}690$ P ANOVA $= 0{\cdot}034$

Site 3 $\quad DNT = 0{\cdot}015 + 0{\cdot}027\ (MOIST) + +0{\cdot}018\ (NO_3\text{-}N) + 0{\cdot}145\ (NMP)$

$R^2 = 0{\cdot}696$ P ANOVA $= 0{\cdot}032$

Site 4 $\quad DNT = 0{\cdot}125 + 0{\cdot}065\ (EGE) + +0{\cdot}185\ (TN) + 0{\cdot}011\ (NH_4\text{-}N) + +0{\cdot}037\ (NMP)$

$R^2 = 0{\cdot}784$ P ANOVA $= 0{\cdot}034$

Residual normality, residual autocorrelation and variance homogeneity were tested for each of

the multivariate regressions and resulted in the acceptance of the linear model. Moreover, all fitted regressions were significant at the $P < 0{\cdot}05$ level using ANOVA. Monthly estimates of DNT from the multiple regression analysis followed the seasonal trend noticed for the measured values with few significant differences (Fig. 3a).

Mean annual DEA ranged between 266·4 and 278·9 mg N m^{-2} day^{-1} at the four study sites and did not differ significantly between them (Table 1). Monthly measurements of DEA showed a seasonal trend with lower values in summer and autumn and higher ones in spring (Fig. 4). Nevertheless, nitrogen loss rates by DEA were always significantly higher ($P < 0{\cdot}01$) than measured values of DNT, whatever the site or the season considered. On average, rates of DNT represented only between 5·4 and 11·3% of the DEA (Table 1). The total amount that could be denitrified without nitrate and anaerobiosis limitation was calculated from the monthly DEA measurements (Fig. 4b) and ranged between 87 and 100 g N m^{-2} $year^{-1}$ in the riparian forest soils under study (Fig. 4b).

GROUNDWATER

The mean annual average of nitrate content (NO_3-N) in the aquifer decreased from Site 1 (1·06 mg l^{-1}, SE = 0·35) to Site 2; this ion was absent from the lower sites (Fig. 5a).

Dissolved manganese (Mn), absent in the aquifer under Site 1, increased (significant at $P < 0{\cdot}01$) from 0·69 mg l^{-1} (SE = 0·09) under Site 2, to 6·13 mg l^{-1} (SE = 0·87) under Site 4 (Fig. 5b). Dissolved iron (Fe) followed a similar pattern to manganese, but with a more marked increase in the wetter Sites 3 and 4 than in the drier ones (Fig. 5c). These two ions (manganese and iron) are present in a dissolved form when they are reduced, but precipitate under oxidized conditions. Their presence in a reduced, dissolved form gives an idea of the prevailing redox potential at a given site.

Discussion

RATES OF DENITRIFICATION AND TEMPORAL VARIATIONS

High rates of nitrogen loss by denitrification were found in the upper soil layers of the riparian forest (Table 1); although previous investigation in the deep sediments of this riparian forest (Pinay 1986; Pinay & Labroue 1986) have shown that denitrification occurred at very low rates down to a depth of at least 40 cm below the soil surface at all the study sites. The values measured in the upper soil layers, ranging from 3 to 78 mg N m^{-2} day^{-1}, are in accordance with those obtained by Groffman & Tiedje (1989) in comparable poorly drained clay

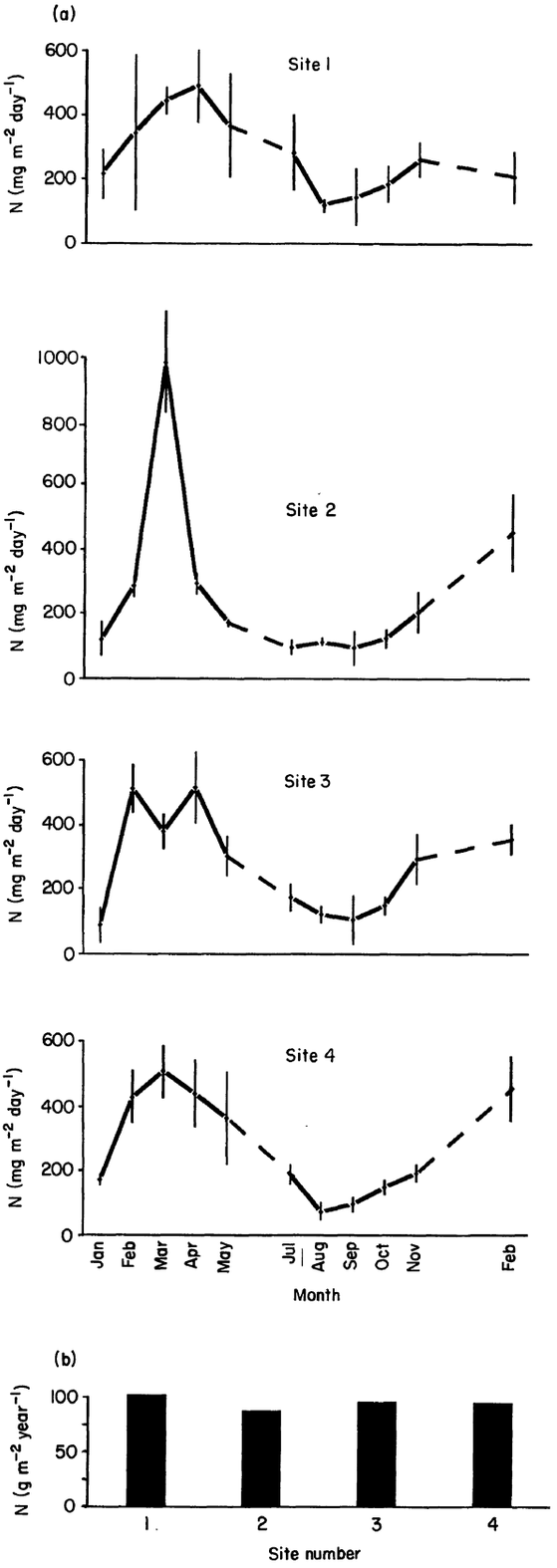


Fig. 4. (a) Monthly rates of denitrification enzyme activity (DEA) measured in the four study sites. Vertical bars represent the standard error of the mean (SE). Continuous lines are broken whenever there was a break in the monthly sampling. (b) Estimates of potential annual N loss by denitrification in the four study sites extrapolated from monthly DEA measurements.

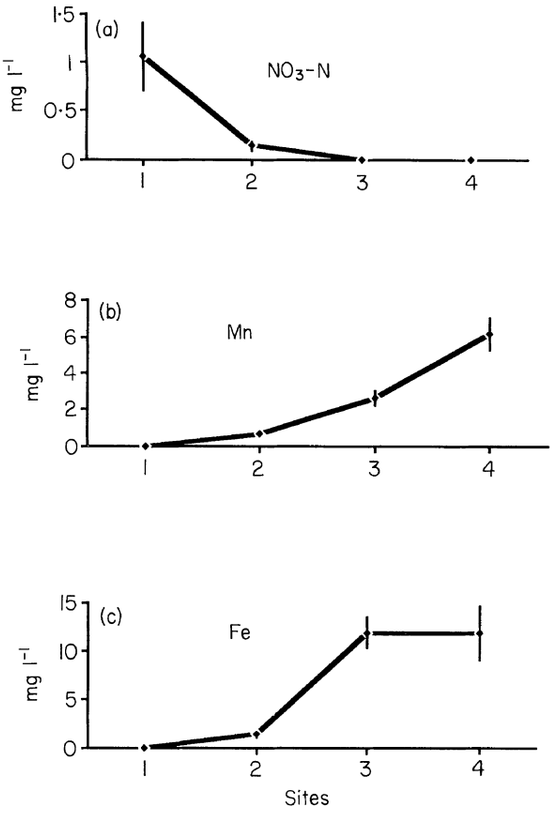


Fig. 5. Annual average of (a) nitrate, (b) reduced manganese and (c) ferrous iron in the groundwater flowing through the four study sites. Vertical bars represent the standard error of the mean (SE).

loam soils. These high rates have the potential for intercepting nitrogen that would otherwise be carried from upslope groundwater sources to the stream channel. The lowest denitrification rates were found in summer and autumn (Fig. 3a) corresponding to the period of low water tables (Fig. 2b) as well as to the period of vegetation growth. Thus, denitrification may be in competition with vegetation for uptake of nitrates during this time. In winter and early spring, assuming that plant uptake of nitrate is negligible during the dormant season, high water and soil temperatures compatible with microbiological activity (Bailey & Beauchamp 1973; George & Antoine 1982) favour a predominance of denitrification.

ESTIMATION OF *IN SITU* DENITRIFICATION ALONG THE CATENA

Rates of nitrogen loss by denitrification decreased along the riparian forest catena, Moreover, the estimates of *in situ* denitrification rates by multiple regression differed from site to site.

The annual loss of nitrogen by denitrification in the upslopes sites (Sites 1 and 2, Fig. 3b) was higher than nitrogen provision by litter fall measured as $7{\cdot}56\,g\,N\,m^{-2}\,year^{-1}$ in the same riparian forest by Chauvet (1989). This confirms the importance of allochthonous nitrate (i.e. groundwater load, but also nitrogen fixation, and wet and dry nitrogen deposition) in supplying nitrate for the denitrification process in upslope parts of the riparian forest.

The best estimate of denitrification at Site 1, which accounted for 76·6% of the total variance, is a linear combination of MOIST, EGE and NO_3-N. These three variables represent the three limiting factors of microbiological denitrification. A high soil moisture content leads to soil anaerobiosis by reducing the diffusion of free oxygen (Reddy, Rao & Patrick 1980). EGE constitutes an easily mineralizable source of energy for heterotrophic microbial activity like denitrification (Burford & Bremner 1975; Stanford, Vander Pol & Dzienia 1975; Reddy, Rao & Jessup 1982). Finally, soil nitrate content can also be a limiting factor for denitrification, especially at low nitrate levels (Bowman & Focht 1974; Reddy, Rao & Jessup 1982; Limmer & Steele 1982). The negative linear correlation (Table 2) between *in situ* denitrification and soil temperature (T °C) at Site 1 can be ascribed to three causes, namely:

1. soil temperature in this riparian forest is not a limiting factor (Bailey & Beauchamp 1973; Stanford, Dzienia & Vander Pol 1975);

2. high temperatures occur during the period of low water tables, when soil moisture is limiting the denitrification process;

3. low temperatures occur when soil has the highest moisture and possibly the most anoxic conditions due to low gas diffusion through saturated sediments.

The two intermediate sites (2 and 3) lay between Sites 1 and 4 with respect to groundwater flow. Therefore, they were transitional with respect to soil and groundwater nutrient content, as well as to denitrification rates (Figs 2 and 5; Table 1). In these two sites the estimation of denitrification was a linear combination of the same three factors: anaerobiosis through soil moisture content (MOIST), soil nitrate content (NO_3-N) and nitrogen mineralisation potential (NMP). Site 2 was the only site where DNT was significantly correlated with DEA (Table 2), although the average percentage of DNT rate to DEA was still very low (DNT/DEA = 8·6%, Table 1). One cannot interpret the linear correlation between DNT and pH at Site 3 (Table 2), since the soil pH was not a limiting factor for denitrification in the range (5·4 to 7·0) measured at Site 3 (Wang *et al.* 1978; Waring & Gilliam 1983).

Downslope from Site 2 (i.e. Sites 3 and 4) annual nitrogen loss by denitrification (Fig. 3b) was less than nitrogen input from litter fall (Chauvet 1989). Moreover, the constant absence of nitrate in the aquifer below these sites (Fig. 5a) suggested that nitrate supplied to the denitrification process came mainly from autochthonous nitrogen (i.e. litterfall mineralization) and probably from other potential allochthonous sources like nitrogen fixation or dry and wet deposition of nitrogen. However, the best

estimate of DNT at Site 4 was a linear combination of EGE, soil organic nitrogen (TN), NMP and NH_4-N; this accounted for 78·4% of the total variance. This suggested that *in situ* denitrification in this lower site was mainly a function of autochthonous nitrogen input (TN) from a litter rich in nitrogen (between 1·6 and 2·2% of the dry mass, Chauvet 1989) and with a high potential for mineralization (NMP) considered as an index of nitrogen availability in the forest ecosystems (Waring & Bremner 1964; Keeney 1980; Powers 1980). Although dissimilation of nitrogen by denitrification sustains microbial degradation of organic matter via the Krébs cycle under anaerobic conditions, organic matter accumulates in downslope soils of the catena (Pinay *et al.* 1989) since denitrification is less efficient than aerobic respiration (Wang *et al.* 1978; Stouthamer, Boogerd & Van Verseveld 1982). The role of NH_4-N as an estimator of denitrification as well as its linear correlation with DNT (Table 2) can be explained by the fact that the ammonium diffusion coefficient is six times less than that of nitrate (Reddy 1982); thus, ammonification rates and ammonium diffusion may control nitrification rate and denitrification in such soils (Reddy & Rao 1983).

IMPORTANCE OF *IN SITU* DENITRIFICATION FOR THE BUFFERING CAPACITY OF THE RIPARIAN FORESTS

Nitrate concentration decreased in the aquifer between Sites 1 and 2, and disappeared completely further down the aquifer (Sites 3 and 4; Fig. 5a). This constant absence of nitrate after 30 m of groundwater flow through the riparian forest was due to high rates of *in situ* denitrification measured during the dormant season; to vegetation uptake that one assumes to be high during the growing season; and to microbial immobilization.

Nitrogen loss to DNT represented only between 5·4 and 11·3% of what could be denitrified under unlimited nitrate supply and anaerobiosis (DEA, Table 1). These high potentials for denitrification underscored the possibility that this riparian forest may have had a nitrate removal capacity apparently higher than the nitrate load to which it was being subjected. Furthermore, the permanent presence of reduced manganese (Mn) and reduced iron (Fe) in the aquifer under the lower sites (Fig. 5b and 5c) indicated that microbiological demand for electron acceptors was higher than that provided by nitrate through denitrification. Thus, oxidized forms of manganese and iron were used as electron acceptors and, in turn, were reduced to dissolved forms. This suggests that nitrate and, in turn, the rate of organic nitrogen mineralization were the factors limiting denitrification in lower sites (Pinay & Décamps 1988).

It is suggested that a massive input of nitrates to the aquifer from cultivated fields upslope from the riparian wood would increase the denitrification rates in Sites 1 and 2. Any excess nitrate would then be denitrified in the downslope areas currently under reduced conditions (i.e. Sites 3 and 4).

Conclusions

High rates of *in situ* denitrification in the riparian forest soils underline the role of the denitrification process in the buffering capacity of riparian forests vis-à-vis nitrate loads. Riparian forests constitute buffering systems because they incorporate several physical and biological characteristics, which each contribute to the efficiency of the process. Denitrification rates vary through time according to soil moisture, which is dependent on soil texture, precipitation and groundwater table fluctuations. Soil texture and precipitation are similar throughout the riparian forest, but changes in the underlying aquifer influence *in situ* denitrification (DNT) in two ways. First, geographical position of the sites with respect to the groundwater flow (i.e. downslope) determine the relative importance of the allochthonous nitrate input (through groundwater flow) compared to the autochthonous input (from organic matter decomposition). Secondly, the topographic situation of the riparian forest soil close to the groundwater table will determine soil saturation and, in turn, its anaerobic status. Moreover, although vegetation cover constitutes a transitory retention system for the nitrate load, it has a key role in the buffering capacity of riparian forest in two ways:

1. seasonal variations of denitrification rate stress the role of vegetation in the buffering process through uptake of nitrate for growth in summer and autumn, corresponding to the period of low water tables when denitrification is limited by soil aeration;

2. perennial vegetation cover provides an easily mineralizable energy source, i.e. organic carbon, through litter fall and root decay or root exudates, to sustain soil microbial respiration processes and especially denitrification.

Thus, from a management point of view, we agree with the recommendations of several authors (Petersen, Petersen & Lacoursière 1992; Osborne & Kovacic 1993; Haycock, Pinay & Walker 1993), who think that the rehabilitation of riparian zones with vegetated buffer strip and the maintenance of their waterlogged condition through riverflow regulation appear to be good starting points for the restoration of buffering capacities of river ecosystems against nitrogen loads.

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