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Shallow groundwater nitrogen and denitrification in a newly afforested, subirrigated riparian buffer

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Summary

1. The EU 'Nitrates Directive' (Directive 91/676/EEC) and the WFD (Water Framework Directive 2000/60/EEC) introduced a series of measures designed to reduce and prevent water pollution caused or induced by nitrates from agricultural sources. Therefore, there is an urgent requirement to control the nitrate concentration in freshwater. The objective of this paper was to verify the potential capacity of a specifically designed afforested riparian zone in removing the excess of nitrogen from river water.

2. A buffer zone was set with irrigation ditches, to produce a subsurface water flow carrying water from the study river through the buffer strip to drainage ditches. This experimental system enables the co-occurrence of two main processes: vegetation/microbial nitrogen uptake and denitrification. Both *in situ* denitrification and denitrification potential were measured at different soil depths, and nitrogen removal of water passing through the buffer system was measured.

3. After the first year, high removal rates (63–64%) of total nitrogen in water were recorded. The lowest rate of denitrification took place in the upper soil layer, while maximum denitrification occurred in the medium layer (40–55 cm). Denitrification occurred mainly in the first few metres of the irrigation ditches leading away from the river. The denitrification rates clearly increased from the second to the third year, with highest rates in summer and autumn. Denitrification potential indicated that carbon availability was the most limiting factor.

4. *Synthesis and applications.* This study has demonstrated that nitrogen levels can be reduced in rivers by forcing water to circulate through afforested buffers. Nitrogen was removed both by plants and by microbial denitrification. Such activity can be supported by promoting anoxic conditions through appropriate water flow management. This could be achieved by creating semi-natural floodplains where water flows can be efficiently managed as in a drained wetland.

Key-words: denitrification, denitrification enzyme activity, eutrophication, nitrate remediation, potential denitrification, riparian zone, water quality

Introduction

The contamination of surface and groundwater by nitrates is a major factor affecting estuarine eutrophication (Howarth & Marino 2006; Hakanson, Bryhn & Hytteborn 2007) and drinking water supplies in many European countries (EEA 2005). The control of water pollution, especially nitrates, was an important concern for the Nitrates Directive (91/676/EEC). The WFD (Water Framework Directive 2000/60/ECC) has the specific aim of enhancing the status of all European water systems. The WFD (Art. 10) confirms and reinforces the need to reduce nonpoint pollution using the same strategy and the same actions proposed by the Nitrates Directive.

Agriculture is a significant source of combined nitrogen release to the environment, because fertilizer inputs to crops are generally higher than the amount of nitrogen required to maximize plant productivity (Driscoll *et al.* 2003). According to recent studies, agricultural practices are typically responsible for 50–80% of the total nitrogen load to groundwater and freshwater (EEA 2005; JRC 2006). Nitrate concentration in a number of intensive agricultural areas exceeds the maximum value of 50 mg NO_3 L^{-1} for drinking water. Although surface water quality trends have generally stabilized during the last few years, more effort is required to achieve the objectives of the Nitrates Directive. Regional estimates of the application

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rate of nitrogen from manure exceed 170 kg ha^{-1} year at the local level in several European countries. Approximately 16·5 million tons of nitrogen was applied to European soils in 2003, with 7·6 million tons per year derived from animal husbandry (mainly cows, pigs, poultry and sheep) and 8·9 million tons from mineral fertilizers.

Riparian zones, located at the interface between terrestrial human activities and aquatic ecosystems, play a key role as a buffer system (Lowrance, Todd & Asmussen 1983; Lowrance *et al*. 1984; Peterjohn & Correll 1984; Hunter & Faulker 2001; Spruill 2004; Carline & Walsh 2007; Pinay *et al*. 2007). A buffer zone can be defined as a transition area from one ecosystem to another, in this case from an agro-ecosystem to an aquatic ecosystem.

There are several biological mechanisms through which excess nitrogen is removed in riparian buffer zones: some act as temporary sinks, for instance uptake and assimilation by plants and microbes, and other permanently remove nitrogen from the soil in a gaseous form, i.e. denitrification processes (Hedin *et al*. 1998; Hefting & de Klein 1998). The two processes, vegetational/microbial uptake of available nitrogen and denitrification, can work together to provide a buffer zone protecting aquatic ecosystems from excessive nitrogen loads (Lowrance *et al*. 1984, 1997; Peterjohn & Correll 1984; Pinay, Roques & Fabre 1993; Haycock *et al*. 1997; Pinay *et al*. 2000, 2007; Sabater *et al*. 2003).

The effective removal of nitrate within riparian zones is dependent upon the presence of conditions conducive to high denitrification rates as well as to the growth of vegetation. Denitrification capacity of the soil can be evaluated and possibly enhanced in order to increase nitrogen (N) removal. Exploitation of *in situ* denitrification (DNT) to reduce nitrate load depends largely on local conditions such as the reduction capacity of the soils, the redox potential, temperature, nitrate concentration and organic carbon availability (Pinay, Burt & Gumiero 2006). Denitrification is most active in soils rich in organic matter and having high moisture content and low oxygen (Pinay, Ruffinoni & Fabre 1995), all characteristics commonly found in riparian zones.

Low-order streams are considered the most suitable for controlling nitrogen fluxes because of their great interaction potential with both riparian and agricultural areas (Décamps *et al*. 2004; Pinay, Burt & Gumiero 2006). However, in this study, we explored the possibility of reducing the in-stream nitrate concentration of high-order water courses flowing into the Venice Lagoon by creating semi-natural floodplains where water flow can be managed. In this case, water management was applied to pump river water into irrigation ditches so causing the water to flow through the riparian buffer.

North-east Italy includes one of the major drained reclamation regions of the country, and a considerable portion of the Venice Lagoon catchment area is located within this region. Over the past decades, nutrient loads delivered to the Venice Lagoon have attracted considerable concern, resulting in the establishment of a series of nitrogen and phosphorus reduction targets by the local government (Regional Authority) in 1995. For Dese and Zero Rivers, two of the main rivers managed by the local drainage authority (Consorzio di Bonifica Acque Risorgive), a reduction of 150×10^3 kg per year of total N and 40×10^3 kg per year of total P was established.

Several actions were undertaken to achieve these objectives, one of which was the conversion of a cultivated area of about 30 ha to a forested buffer strip, irrigated with freshwater from the Zero River. Inside this afforested area, a pilot experimental scale system was established in order to find the most suitable conditions for enhancing denitrification activity. The experimental forest buffer received almost continuous subsurface water flow with the aim of enhancing nitrate removal through denitrification.

The efficiency of the buffering capacity of this afforested area on nonpoint pollution sources of nitrogen was evaluated through detailed measurements of weather, hydrology, water quality, soil chemical parameters and denitrification rates. In this paper, we focus mainly on DNT and denitrification enzyme activity (DEA) to determine the processes responsible for reducing nitrogen in the whole system.

Materials and methods

The experimental site is located 15 km from Venice, Italy. The climate is subcontinental with temperatures ranging from a daytime average of 1 °C in January to 23 °C in July and August. The mean value of rainfall is 900 mm per year, peaking in autumn and spring and with lower values in winter and summer. Between 0 and 90 cm below the surface, the soil texture is extremely homogeneous, categorized as 'silty clay loam' (according to textural classification USDA-SCS 1984). An unstructured and calcic layer, with high content of loam and clay, occurs at around 90–150 cm depth, and owing to its very low permeability, it prevents interactions between alluvial groundwater and the near surface soil (ARPAV 2004).

The experimental area occupies a total area of about 0·70 ha (227 m long and 30 m wide) and was designed to rigorously monitor the hydrological fluxes and to carefully characterize the hydrology of the buffer system. It was planned according to the NICOLAS project (Burt *et al*. 2002) to examine a three-zone buffer system. Zone 3 is close to the irrigation ditch, zone 2 is at mid-distance between the two ditches and zone 1 is close to the drainage ditch. The two replicate sides of the drainage ditch were designated A and B, and all the measurements were carried out on both plots (see Fig. 1). Ridges and furrows facilitate subsurface water flow throughout the field from the inlet point, represented by two irrigation ditches where water is pumped through, to the parallel drainage ditches located at lower elevation (Fig. 1). The average slope between irrigation and drainage ditches is 4% (Fig. 2). Several tree and shrub species (white willow *Salix alba* L., almond willow *Salix triandra*, black alder *Alnus glutinosa* (L.) Gaertner, pedunculate oak *Quercus robur* L., field maple *Acer campestre* L., common hazel *Corylus avellana* L., common hawthorn *Crataegus monogyna* Jacq., manna ash *Fraxinus ornus* L. and black dogwood *Frangula alnus* L.) were planted in spring 1999 and were arranged in four parallel rows for each plot as indicated in Fig. 1.

HYDROLOGIC MONITORING

Two 5 × 3 m grids of piezometers (1·5 m depth and 38 mm diameter each) were installed in each plot in September 1999 giving a total of 30 piezometers. These were used to determine water-table depths and to collect water samples in monthly sampling.

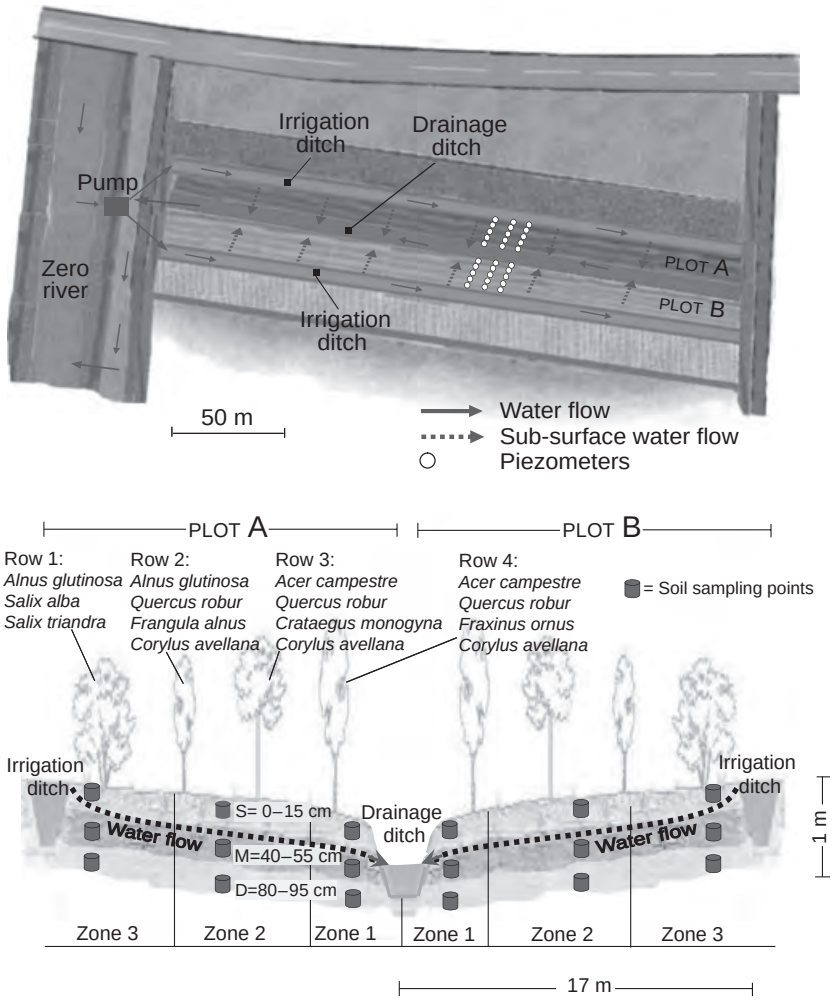


Fig. 1. Plan (above) and section (below) of the experimental site: each of the two plots is watered through an irrigation ditch carrying water from the Zero River. There is a difference in elevation between the irrigation ditches and the drainage ditch, resulting in a subsurface flow of water running through the wooded buffer strips. Soil sampling in each plot was located at nine points, three for each zone. Zone 3 is close to the irrigation ditch, zone 2 is at mid-distance between the two ditches and zone 1 is near the drainage ditch. For each sampling point, the soil is sampled at three layers at different depths (S: 0–15 cm, M: 40–55 cm, D: 80–95 cm below the soil surface).

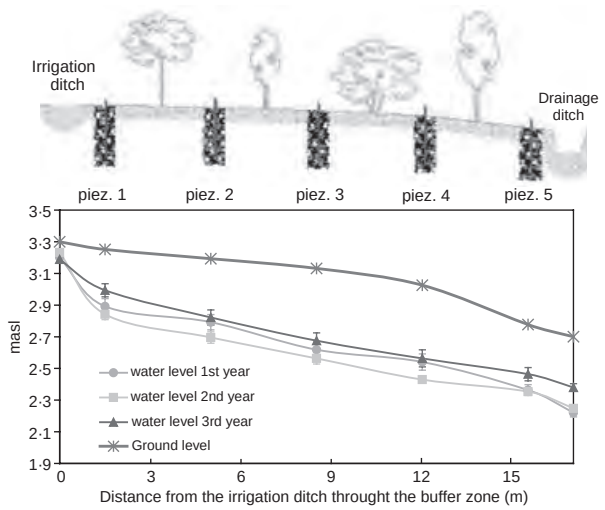


Fig. 2. Ground-level and mean annual water-table elevation measured in plot A (monthly measures in ditches and piezometers) during the three monitored years. Values for plot B were very similar. Bars represent standard error.

In each plot, two additional piezometers (one near the irrigation ditch and one near the drainage ditch) were equipped with a pressure transducer (Druck – PT-B1; GE Measurement & Control Solutions, Billerica, Massachusetts, USA) connected to a data logger (Smart-Reader 7 Plus; ACR Systems Inc., Surrey, BC, Canada) to record water-table elevations every 15 min. The data were collected from October 1999 to October 2002. Subsurface water depth was also measured monthly (using phreatimeters: Electric contact gauge KL 015; Ott Messtechnik GmbH & Co. KG, Kempten, Germany) in the 30 piezometers and during the entire period of study.

The volume of the introduced irrigation water was continuously measured by a flowmeter (Datawater WMPE; Maddalena S.p.A., Udine, Italy) inserted in the water supply line. Tracer experiments (Dierberg & DeBusk 2005) using the organic fluorescent tracer Rhodamine WT (RWT) were performed in 2007 to describe the hydraulic behaviour of the system. Two hundred grams of diluted (21·33%) RWT was injected into the irrigation ditch, and subsequently, 99 samples were collected every 60 min from the discharge ditch by an ISCO 6700 automatic sampler (Teledyne Isco Inc., Lincoln, NE, USA). All samples were filtered through a 0·45-μm fibreglass filter, and RWT concentration was determined by a fluorometer (SCUFA®; Turner Designs Inc., Sunnyvale, CA, USA).

The concentration of chloride, a biologically inert conservative tracer (Altman & Parizek 1995), was measured monthly to monitor dilution and dispersion (Sabater *et al.* 2003).

An automatic weather station near the experimental site recorded many climatic parameters (air temperature, rainfall, wind direction and velocity, air moisture, global radiation) and gave the opportunity to estimate the EPT potential by Penman–Monteith approach, using

a combination equation that combines the radiation and the aerodynamic terms (Allen *et al.* 1998).

The water balance of the afforested buffer area was estimated using the following formula

$$I + R - \mathrm{ET} = D,$$

where I = irrigation volume; R = rainfall; ET = evapotranspiration; D = drainage back to river.

WATER QUALITY

Water pumped from the Zero River into the irrigation ditches was sampled daily as a single discrete sample by using an automatic sampler (American Sigma – Portable sampler 900 standard, with 24 one-L bottles; Hach Company, Loveland, CO, USA). The irrigation and drainage ditches were sampled monthly by grab sampling of the general shallow flow. Piezometers were pumped using a hand pump (Kartell –MR 50 H *c*. 240 mL; Kartell S.p.A., Milan, Italy) first to remove two well volumes and then sampled after water had recharged the well. Field measurements were made of pH (pH meter handylab 1; Schott-Geräte GmbH, Mainz, Germany), temperature (°C) and electrical conductivity using a Schott-Geräte Conductivity meter handylab LF with integrated temperature sensor.

Water samples for analyses were filtered through a 0·45-μm PVDF filter in the laboratory and analysed within 24–36 h for N-NO_3, N-NO_2, N-NH_4, total nitrogen and chloride.

Dissolved anions (Cl, N-NO_3) were determined by ion liquid chromatography (Pfaff, Hautman & Munch 1997). Dissolved N-NO_2 was determined by the Griess-Illosvay method and spectrophotometric measurements (APHA AWWA WEF 2005a). Dissolved N-NH_4 was determined by the indophenol blue method and spectrophotometric measurements (APHA AWWA WEF 2005b). Dissolved total N was determined with the persulphate oxidation method (Valderrama 1981) followed by nitrate analysis. Nitrate was reduced to nitrite by cadmium reduction and determined as explained. Organic N was determined by calculation (Norg = Ntot−N-NH_3−N-NO_2−N-NO_3).

DENITRIFICATION AND CHEMICAL PARAMETERS OF SOIL

In each plot (A and B), soil samples were collected using a manual drill, from nine different places (three replications for each of the three zones) at three different depths [surface (S), 0–15 cm; medium (M) 40–55 cm and deep (D) 80–95 cm] (see Fig. 1). Samples were taken seasonally (every 3 months) for 3 years (October 1999–October 2002) with 54 soil samples per season and 12 total sample dates. Winter, spring, summer and autumn samples were taken in January, April, July and October, respectively. *In situ* DNT was assayed by the static core acetylene inhibition method (Yoshinari & Knowles 1976). One hundred grams of fresh soil was weighed into glass screw top jars (250 mL) capped with rubber serum stoppers and then amended with acetone-free acetylene to bring soil atmosphere concentration to 10 KPa (10% V/V) acetylene and 90 KPa air. Samples were incubated at field temperature, and denitrification rates were calculated as the rate of nitrous oxide (N-N_2O) accumulation in the head space between 1 and 4 h. Head space samples were removed from all cores and stored in 10-mL evacuated collection tubes (Venoject; Terumo Europe N.V., Leuven, Belgium). Gas samples were analysed via gas chromatography (Trace GC 2000; Thermo Fisher Scientific Inc. Waltham, Massachusett, USA), equipped with an electron capture detector (^{63}Ni) and a VARIAN CP7554 poraPLOT Q (VARIAN Inc.) column (27·5 m × 0·53 mm, film 20 μm).

Denitrification enzymatic activity was measured for each soil sample using Smith & Tiedje's procedure (1979) (see also Groffman *et al.* 1999). In the laboratory, four sets from each soil sample (30 g of fresh soil) were transferred into glass screw top jars (250 mL) capped with rubber serum stoppers, flushed with N_2 and incubated for 8 h with acetone-free acetylene to bring soil atmosphere concentration to 10 KPa (10% V/V) acetylene and 90 KPa N_2. One set was amended only with nitrate (10 μg N-NO_3 g^{-1} fresh soil); a second set was amended with organic carbon (4 mg C-glucose g^{-1} fresh soil); a third set was amended with both organic carbon and nitrate (10 μg N-NO_3 g^{-1} and 4 mg C-glucose g^{-1}), referred to as DEA. The last set was incubated with only acetylene under N_2 atmosphere. All the samples were under water-saturated conditions obtained by adding 1 mL of distilled water for each gram of soil. Denitrification rates (μg N-N_2O g^{-1} soil day^{-1}) were calculated as the rate of nitrous oxide (N-N_2O) accumulation in the head space between 4 and 8 h.

Each soil sample was further analysed for soil moisture, organic C and nitrate. Soil moisture was determined gravimetrically after drying subsamples at 104 °C for 24 h and by dividing the difference between wet and dry masses by the mass of the dry sample. The organic C content was determined by oxidizing the organic matter with acid dichromate reagent. The excess of chromate left after C oxidation was analysed by spectrophotometric measurements (Nelson & Sommers 1982). For determining the N-NO_3 contents, subsamples were extracted with 2 M KCl and quantified by the Griess-Illosvay method and spectrophotometric measurement (Keeney & Nelson 1982).

STATISTICAL ANALYSIS

All the results were statistically analysed by ANOVA (variance analysis).To analyse the effects of seasons, depth and zones (distance from irrigation ditch) on DNT rate, three-way factorial ANOVA with interaction terms was used. For this latter analysis only the second and third years were considered because of the lack of some data from the first year. For each of the three ANOVA factors, three soil replications in plot A and three in B were collected. Although the two plots of the experimental site (A and B) were designed as two exactly symmetrical sections that share the same drainage ditch and are very homogeneous in terms of soil and vegetation characteristics, the data should not be considered as completely independent. The analyses were conducted using STATSOFT ITALIA (StatSoft Italia S.r.l., Padova, Italy).

Results

HYDROLOGY

Irrigation volumes, rainfall, evapotranspiration and water balance for the 3 years are reported in Table 1. As a consequence

Table 1. Irrigation volumes, rainfall, evapotranspiration and water balance for the 3 years under study

Years	Irrigation volume (m-cu ha^{-1} per year)	Rainfall (m-cu ha^{-1} per year)	Evapotranspiration (m-cu ha^{-1} per year)	Drainage back to river (m-cu ha^{-1} per year)
1st	51917	7562	7274	52205
2nd	48060	8888	6963	49985
3rd	48600	11450	9611	50439

of the irrigation (an average of 17 500 m^3 $year^{-1}$ in each plot, about three times rainfall), a perched aquifer was created on the calcic layer located at around 90–150 cm depth. Thus, the water level in the experimental site was always between 25 and 60 cm below the soil surface (Fig. 2). While the 0–15 cm soil layer was subjected to the normal seasonal cycle, with water content (expressed as a percentage of the dry weight) of 13–24% to 21–31% in summer and winter, respectively, the 40 to 55cm and 80 to 95cm layers were often saturated.

The conservative tracer (chloride) concentrations measured in the water collected from different piezometers and ditches changed little through the buffer (from 10 to 15 mg Cl L^{-1}). Moreover, the mean value of chloride concentrations of regional groundwater is about 55 mg L^{-1} (Regional Environmental Agency, unpublished data). So, the lack of changes in the chloride concentration within the buffer and the large amount of water flowing into the shallow groundwater system makes the input of deeper groundwater to the shallow flow system unlikely. Therefore, it was assumed that dilution from existing groundwater was minor (see also Sabater *et al*. 2003).

The RWT injection, although carried out after the time period being reported, provides further evidence that the irrigation water was moving rapidly through the shallow perched aquifer and not seeping out into the alluvial aquifer. Indeed, by comparing mass balance in the irrigation and drainage ditches, a loss of only 9·6% of RWT was registered, with an average travel time through the shallow groundwater from the irrigation to the drainage ditch of 24·3 h. These results indicate that the deep seepage out of the shallow aquifer into the underlying alluvial aquifer is negligible.

NITROGEN RETENTION IN THE WATER

Figure 3 shows $N\text{-}NO_3$ concentration in the input water from the river Zero, through the 15 m of the buffer zone to the drainage ditch, for the three monitored years. It is evident that the systems did not remove nitrate during the first 6 months of monitoring. During the following months a considerable reduction in nitrate concentration was observed even at 3–4 m from the irrigation ditch. This performance was more evident during the warm season (April/May–November), while in the winter period (from December to March), the system was less effective.

The amount of the different chemical forms of combined nitrogen confirm that during the first year, the reduction in $N\text{-}NO_3$ remained below 40%, while in the second and third years, it reached and stabilized to more than 85% (Fig. 4). The same trend was evident for total nitrogen, with about 23% removed in the first year and more than 60% removed during the second and third years. Note that the leaching of organic nitrogen in the course of the first year (−152%) decreased considerably in the second and third years (−87% and −11%, respectively).

IN SITU DENITRIFICATION

The highest DNT rates took place in the 40–55 cm soil layer (Fig. 5). Denitrification rates in the different soil layers differed

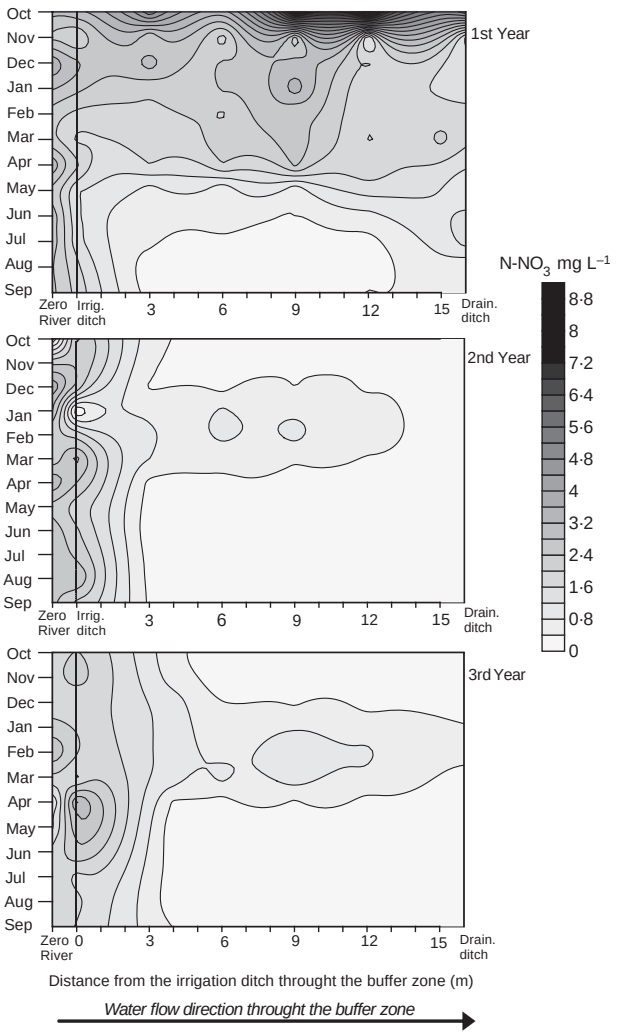
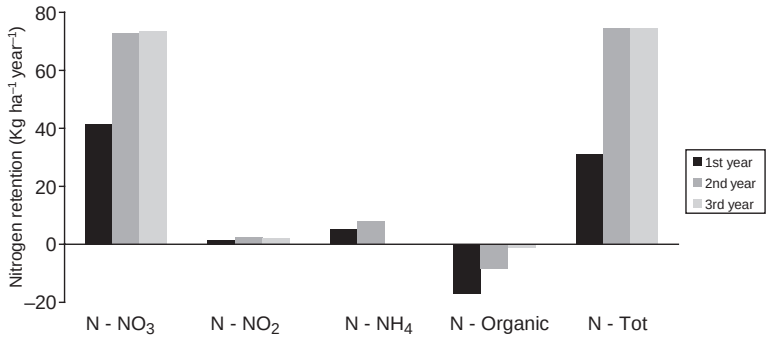


Fig. 3. $N\text{-}NO_3$ concentration in the input water from the river Zero, through the 15 m of the buffer zone to the drainage ditch, for the three monitored years. The grey scale on the right indicates the $N\text{-}NO_3$ concentration in mg L^{-1}. Data processing by the software 'Surfer® Version 8.01'. SSG-Surfer.com, a Division of Scientific Software Group, Sandy, Utah, USA.

significantly ($P < 0{\cdot}05$) during summer and to a lesser extent during autumn (Table 2, Fig. 5).

The *in situ* DNT activity was lowest during the second year but increased in the third year, with highest values recorded during the summer, demonstrating clear seasonal variations (Fig. 5 and Table 2). Overall, denitrification was higher in summer and autumn. This variability was significant for the 0 to 15cm and 40 to 55cm layers.

Denitrification activity in soil samples coming from the three different zones (see Fig. 1) was evaluated and compared. For the 40–55 cm soil layer, the highest rates of denitrification occur in zone 3 (located close to the irrigation ditch), causing a reduction in the amount of $N\text{-}NO_3$ moving through zone 1 (Fig. 6 and Table 2). The reduction in denitrification from zone 3 to zone 1 was especially pronounced during summer and autumn (Fig. 6), while in spring and winter, similar and very low denitrification activities were found through all the zones (data not shown).



	1st Year				2nd Year				3rd Year			
	Input	Output	Retention		Input	Output	Retention		Input	Output	Retention	
	kg ha^{-1} year^{-1}			%	kg ha^{-1} year^{-1}			%	kg ha^{-1} year^{-1}			%
N - NO_3	107·6	66·0	41·6	39%	85·0	12·3	72·7	86%	85·2	11·6	73·6	86%
N - NO_2	1·9	0·4	1·5	77%	2·6	0·3	2·3	88%	2·5	0·3	2·2	87%
N - NH_4	14·7	9·6	5·1	35%	18·7	10·8	7·9	42%	18·0	18·1	0·0	0%
N - Organic	11·2	28·2	–17·0	–152%	9·6	18·0	–8·4	–87%	12·0	13·3	–1·3	–11%
N - Tot	135·3	104·1	31·2	23%	115·9	41·4	74·5	64%	117·7	43·2	74·5	63%

Fig. 4. Combined nitrogen in terms of input, output and retention rates.

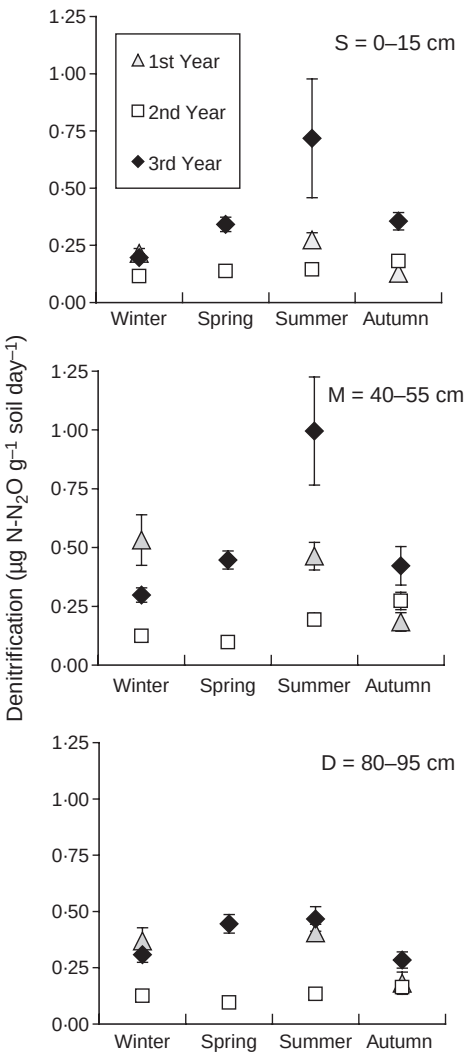


Fig. 5. Denitrification rates, as µg N-N_2O g^{-1} day^{-1}, for each year, season and layer at different depths. The values are the means of 18 measurements (nine in each plot) and bars represent standard errors.

Table 2. Three-way ANOVA exploring the differences in denitrification rate; the factors include seasons, soil depth (layers) and distance from irrigation ditch (zones)

Three-way ANOVA	d.f.	Mean square	*F*	*P*
Main effects (combined)				
Seasons**	3	1·225	8·846	0·000
Layers*	2	0·600	4·334	0·014
Zones	2	0·346	2·497	0·084
2-way interaction (combined)				
Seasons × layers	6	0·206	1·485	0·182
Seasons × zones*	6	0·322	2·324	0·032
Layer × zones	4	0·110	0·796	0·529
3-way interaction				
Seasons × layers × zones	12	0·067	0·486	0·923

Significant relationships and the level of significance are indicated by 0·05* and 0·001**.

DENITRIFICATION ENZYMATIC ACTIVITY AND THE EFFECTS OF CARBON AND NITROGEN

The 0–15 cm soil layer had the maximum potential for DEA (Fig. 7a). The addition of nitrate alone to the samples (Fig. 7b) resulted in increased denitrification in the 0–15 cm soil layer only. Adding only glucose to the soil resulted in a significant increase in denitrification rates in all the layers during the first year, but this effect was limited to the medium layer for the second and third years (Fig. 7c). There was no appreciable increase in denitrification activity in soil incubated without C and N addition but under anaerobiosis (Fig. 7d). In summary, (i) the limiting factor for the 0–15 cm soil layer, appears to be the availability of nitrate (Fig. 7b); (ii) the limiting factor for the 40- to 55-cm layer is the availability of organic carbon (Fig. 7c); (iii) microbial activity typically decreases toward the deep layer (80–95 cm) with no clear limiting factors (Fig. 7a–c).

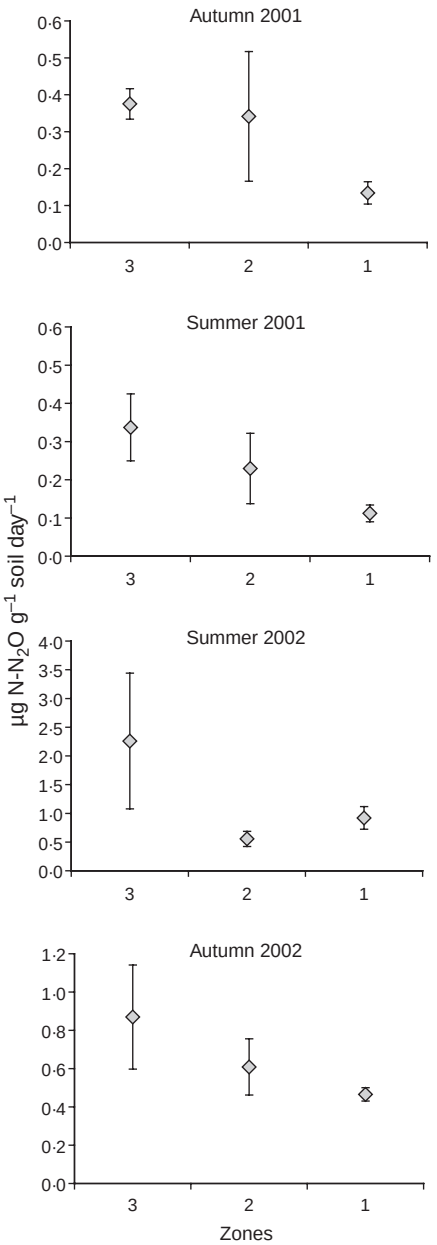


Fig. 6. Comparison of the denitrification rates, as μg $N\text{-}N_2O$ g^{-1} day^{-1}, of the three zones during autumn and summer of the second and third years. The vertical bars represent standard errors.

These effects become even more evident during the warmer seasons, although differences were smaller in winter, when temperature was an important limiting factor.

Discussion

The aim of this study was to explore the possibility of reducing the level of nitrogen in rivers by forcing water to circulate through afforested buffers. Nitrogen reduction can be achieved by creating semi-natural floodplains where water flows could be efficiently managed to support high nitrogen removal by microbial denitrification. An overall and detailed appraisal of the nitrogen retention by the system is given, even if the relative contribution of the different processes involved (i.e. plant/microbial uptake and denitrification) was not determined individually.

INFLUENCE OF SUBSURFACE FLOW REGULATION ON DENITRIFICATION ACTIVITY

Subsurface flow through both soil and deeper sediments of a riparian zone is known to be of crucial importance to denitrification and other nitrogen cycle processes (Mitsch, Dorge & Weimhoff 1977; LaBough 1986; Chescheir *et al.* 1988; Correll & Weller 1989; Dosskey & Bertsch 1994; Pinay *et al.* 2000). Because denitrification potential increases significantly towards the soil surface, water-table elevation can control the degree to which nitrate reduction by denitrification is optimized. Burt *et al.* (2002), reporting results from a pan-European experiment (NICOLAS), showed that denitrification process will be more effective within a riparian zone where topographic and soil conditions are conducive to a high water-table for as long as possible during the year. These conditions usually occur when permeable soil overlays impermeable bedrock and the land surface slope is low (5–10°) (Pinay & Burt 2001). Our results demonstrate that, even in the fine textured soil of the present experimental site, the anoxic conditions required for denitrification can be obtained by creating semi-natural floodplains where water flows can be suitably managed, i.e. by maintaining a slope of 4%. Under these hydrologic conditions, the higher denitrification rates were reached in the soil layer saturated by the perched aquifer. On the other hand, the lower values recorded in all soil layers in winter and spring indicate that the experimental design was unable to overcome other key limiting factors for denitrification such as low winter temperatures and plant competition in spring, even though a constant water flow was maintained.

THE CONVERSION OF THE SITE IN AN EFFICIENT BUFFER ZONE

Our results indicate that a buffer zone set-up for nitrate removal from river water starts to be effective during the second year (see Fig. 3). The area was rapidly converted from agricultural land to a tree-covered buffer even though there was no vegetation before the appropriate tree species were planted. At the start of the experiment, a considerable amount of residual combined nitrogen and organic carbon would have been present in soil (Table 3), derived from previous agricultural activities. During the first year, they would have been reduced by leaching, microbial activity and plant uptake. This may account for the limited denitrification activity detected in the study during the second year as compared with the first year. This trend may also be due to the higher nitrogen uptake by the plants (a mean of 104 g m^2 herbaceous vegetation biomass for the first year as compared with 298 g m^2 for the second), which started to grow quickly, thus reducing the amount of inorganic nitrogen available to the denitrifying bacteria and to the still limited organic carbon released by the young vegetation. Plant

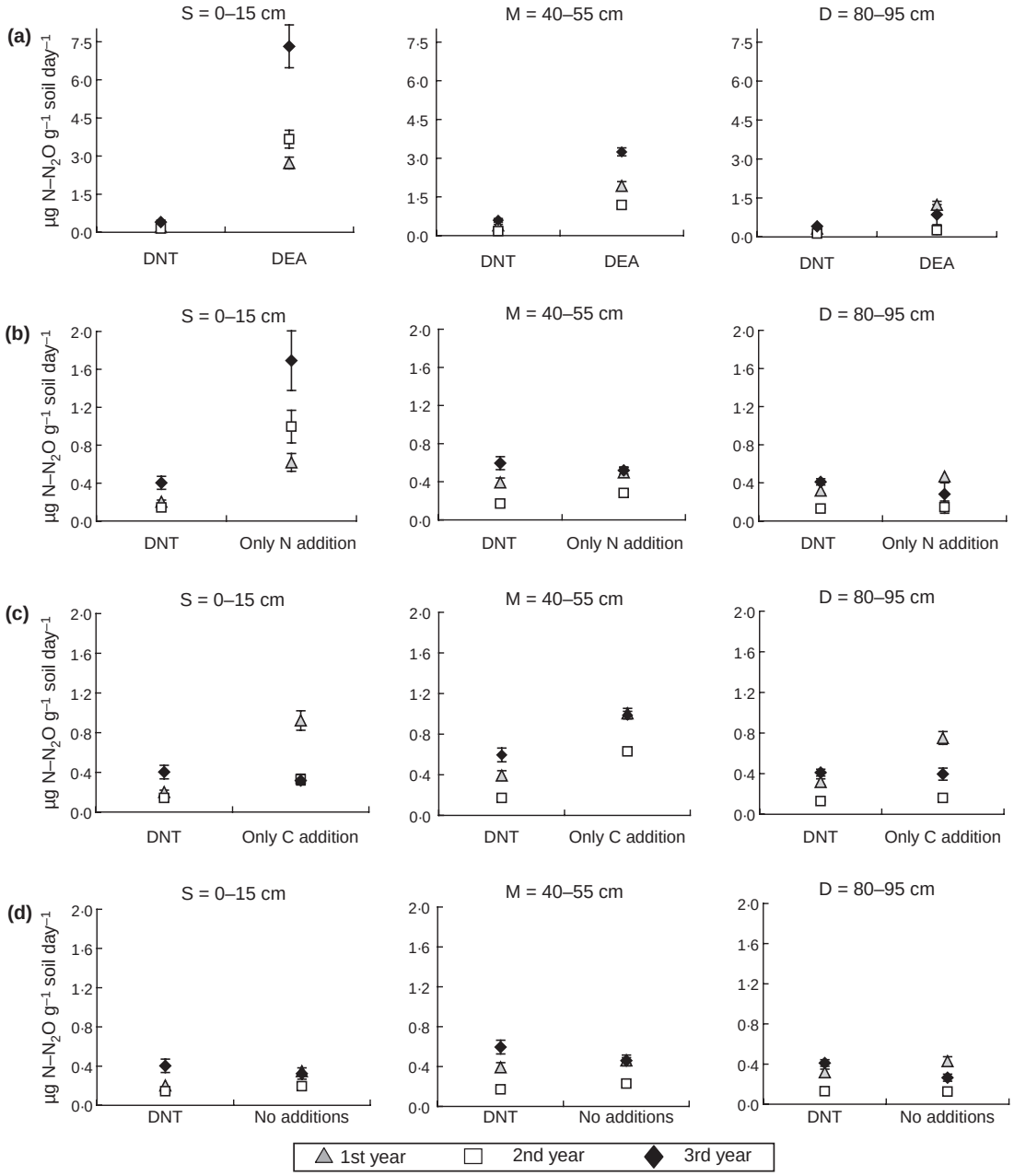


Fig. 7. Comparison of annual average in the three different layers between *in situ* denitrification rates (DNT) and potential denitrification obtained after incubation under anaerobic conditions with the addition of (a) both nitrate and organic carbon (denitrification enzyme activity), (b) only nitrate, (c) only organic carbon and (d) no additions. The vertical bars represent standard errors.

colonization and growth stabilized in the third year; hence, denitrification activity could take place effectively.

Our conclusions are further supported by the data reported in Fig. 4 where the reduction in $N\text{-}NO_3$ and total nitrogen during the first year was low, but reached and stabilized to significantly higher values in the second and third years, similar to values measured in other monitored European systems (see Pinay *et al.* 2007).

DENITRIFICATION POTENTIAL

As expected, although DNT was highest in the 40–55 cm soil layer, denitrification potential (measured as DEA) was highest in the top soil layer. Lower DEA in the deeper soil layer (80–95 cm) is generally attributed to the lower microbial populations living deeper in the soil. This is in agreement with a number of studies in natural forested riparian zones where DEA values are reported to generally decrease with soil depth (Hunt, Matheny & Stone 2004; Hunt, Matheny & Ro 2007). For example, Ambus & Lowrance (1991) reported that denitrification potential was mainly concentrated in the top 2 cm of soil in two riparian forest soils.

While nitrate represents the main limiting factor in the upper soil layer, organic carbon availability strongly affects denitrification activity at 40–55 cm depth. In deeper soil layers, even the addition of both nitrate and organic carbon is not always

Table 3. Soil nitrogen (mg N kg^{-1} soil) and organic carbon (%) content in the three layers of the buffer zone, for the three monitored years (± standard error)

Years	Layer	Inorganic N mg N kg^{-1} soil	Organic C %
1st	S (0–15 cm)	4·97 ± 0·56	1·03 ± 0·05
	M (40–55 cm)	5·71 ± 0·68	0·78 ± 0·05
	D (80–95 cm)	4·08 ± 0·45	0·71 ± 0·06
2nd	S (0–15 cm)	1·91 ± 0·10	0·87 ± 0·03
	M (40–55 cm)	2·71 ± 0·13	0·55 ± 0·02
	D (80–95 cm)	1·86 ± 0·14	0·31 ± 0·02
3rd	S (0–15 cm)	1·97 ± 0·12	0·82 ± 0·02
	M (40–55 cm)	2·40 ± 0·10	0·50 ± 0·02
	D (80–95 cm)	1·86 ± 0·10	0·31 ± 0·02

able to promote high denitrification potential. Indeed, the strong differences recorded between the seasons and the low DEA values in the deepest soil layer underline the importance of other key limiting factors for denitrification, such as the temperature and the distribution of the microbial population.

CRITICAL FACTORS AND OPTIMIZATION OF THE BUFFER CAPACITY

Our results confirm that a suitable irrigation system and an appropriate soil arrangement are crucial for optimizing the nitrogen removal potential of an afforested buffer site. Particular attention must be paid to maintenance of the shallow perched water-table as close as possible to surface soil layers.

Once the flow of nitrate enriched water was established through the shallow perched water-table, the key factor affecting the level of denitrification was carbon availability. In our experiment, the C supply in future will be ensured by litterfall and root production of several plant species expressly planted in the buffer zone. The selection of different fast-growing plant species may represent another critical factor in system design. McGill, Sutton-Grier & Wright (2010) indicated that plant communities clearly influence microbial activity and processes and that the diversity of plant communities can positively affect the stability of microbial processes, including DEA.

Finally, our results confirm that a maximum buffer strip width of 15 m can remove an excess of nitrate at concentrations typical of freshwater bodies (< 5 mg L^{-1} $N\text{-}NO_3$) and that narrower buffer strips (e.g. 5 m wide with only one row of trees) are likely to be adequate.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Ground level and mean annual water table depth from ground level measured in plot A (monthly measures in ditches and piezometers) during the three monitored years. Values for plot B were very similar. Bars represent standard error.

Fig. S2. Mean annual values of conservative tracer (chloride) concentrations measured monthly in the water collected from different piezometers and ditches.

Fig. S3. Denitrification rates for each season and layer at different depths. The values are the means of all the samples collected during 2001 and 2002. The vertical bars represent standard errors.

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