Nitrogen transformation and retention in riparian buffer zones

Stikstofomzettingen en -retentie in beekbegeleidende bufferzones

(met een samenvatting in het Nederlands)

Proefschrift

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NICOLAS

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Chapter 1

General introduction

with Jos Verhoeven

Introduction

In many parts of the western world, the drastic changes in agricultural practice in the 20th century have fundamentally changed the way in which landscapes function. Not only have many agricultural fields been drained and have stream beds been straightened and hardened, also the use of ever increasing amounts of inorganic fertilizer has augmented and altered patterns of nutrient flux between agricultural fields and the surrounding countryside. In the Netherlands, nutrients were historically transferred from large heathland areas and wet grasslands towards the crop fields, nowadays fertilizer applications are so high that considerable amounts of nutrients leach from the upland fields into the groundwater and further towards lower areas and aquatic systems, where they cause severe eutrophication. These problems need to be solved, and there is considerable interest in ways to strengthen natural capacities of landscape structures to retain or even remove nutrients. This thesis investigates the functioning of riparian buffer zones as habitats for nutrient removal. One of the study areas was North East Twente (the Netherlands), an area illustrative for the agricultural development, where the use of riparian buffer zones could make a difference in nutrient transport to streams.

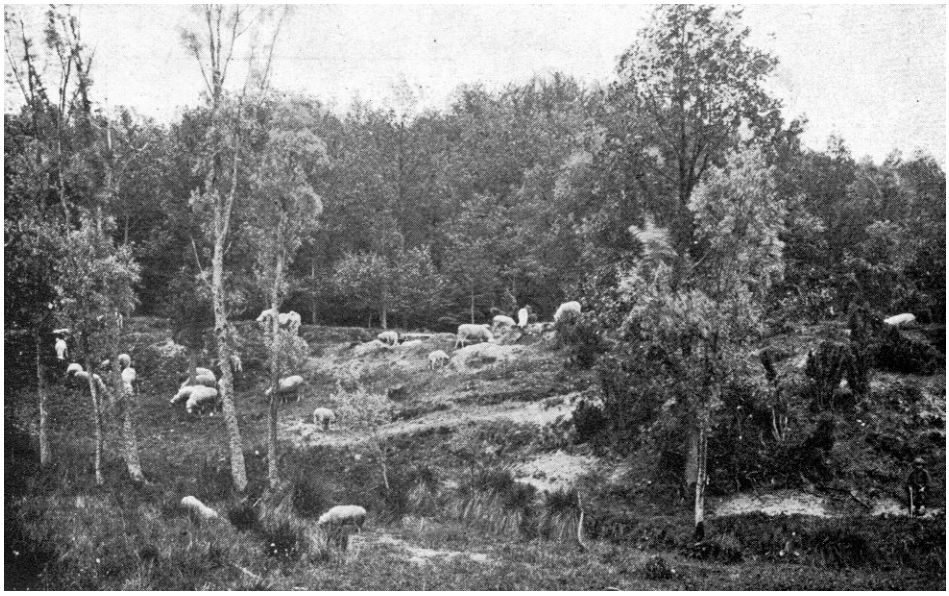

Figure 1 Sheep grazing in the Hazelbekke valley (Bernink, 1926).

Nutrient fluxes in Dutch agricultural landscapes; from converging to diverging flows

The Pleistocene sand area in the eastern part of the Netherlands, has been used by man since prehistoric times. The first agricultural activities started in the early Middle Ages, and from about 1200 rye was grown on communal crop fields ("essen") situated on the best drained parts of the landscape, mostly the higher flanks of glacial moraines and on sand dunes. Farmers kept herds of sheep, which grazed the surrounding heathlands and wet grasslands (Fig. 1, from Bernink, 1926). Overnight sheep were kept in stables ("potstal") on sods of heather, which were regularly cut from the heathland areas. To make the poor sandy soils more fertile, sods with sheep excrements were put on the communal crop fields. On these fields organic matter and nutrients accumulated over centuries, creating fertile soils ("eerdgronden") with layers rich in organic matter eventually reaching a thickness of about 1 metre. The heathlands on the other hand became nutrient poor, developing acid podzolic soils and eventually became overexploited, which led to bare sand "deserts".

With the introduction of chemical fertilizers in the beginning of the last century, patterns of nutrient flows in the landscape changed dramatically. The fertility of the crop fields became independent of the surrounding land and the use of fertilizers made it possible to use the nutrient-poor heathlands and moist grasslands for agricultural production. Reallotment schemes subsidized

by the Dutch government resulted in larger agricultural fields and better drainage. Consequently, most of the riparian buffer zones and hedgerows, landscape elements considered to be of little importance, disappeared. Nowadays, however, both are highly valued from the perspective of biodiversity and for their role as nutrient sink, maintaining water quality. Fertilizer levels increased, following the rapid intensification of agriculture, until the late 1980's to application rates of over 350 kg N ha^{-1}. The use of large amounts of inorganic fertilizer and import of high-energy fodder in agricultural practice resulted in considerable nutrient surpluses and consequently to considerable nutrient losses to the ground- and surface waters.

Diffuse nitrogen pollution

Today, agricultural activities are considered the main source of nutrient inputs, such as nitrogen (N) and phosphorus (P), to terrestrial and aquatic ecosystems. Leaching of nutrients leads to degraded water quality with negative potential impacts on human health and the environment. The contamination of surface water and groundwater with nitrogen is particularly severe in European agricultural environments (Meybeck and Helmer, 1989; Isermann, 1990). In the Netherlands, 70% of the nitrogen pollution originate from agriculture (Van Eerdt and Fong, 1998). The loss of nitrogen compounds from agricultural environments to the shallow groundwater and surface water has increased dramatically over the last decades (Oenema et al., 1998; Van Eerdt and Fong, 1998). Although several agricultural measures are being used to reduce the amount of excessive nitrogen in the soil profile after harvest, leaching of nitrate still takes place, polluting the shallow groundwater (Addiscot et al., 1991; Olsthoorn and Fong, 1998; Ondersteijn et al., 2002).
Possible health hazards of high nitrogen concentrations in drinking water are 'the blue baby syndrome' and stomach cancer (Harrisson, 1996). Although the probability of these hazards is very low due to thorough drinking water purification, the costs of water purification are high and will continue to increase in the future if nutrient pollution by agriculture continues. Diffuse nitrogen pollution of shallow groundwater remains of major concern since it causes eutrophication of fresh surface waters and marine waters affecting the functioning and biodiversity of aquatic ecosystems. Consequently, the reduction of diffuse nitrate pollution from intensive agriculture is one of the main issues in the EU Water Framework Directive. The Nitrate Directive (1991) prescribes an maximum concentration of 50 mg nitrate l^{-1} in groundwater, which is equivalent to 11.3 mg N l^{-1}. However in many agricultural areas in Europe this threshold is exceeded, especially in the

Netherlands, which has the most intensive agriculture in terms of external nutrient inputs (Van Bruchem et al., 1999; Ondersteijn et al., 2002). Because source-directed measures such as the Mineral Accounting System (MINAS), are not immediately reflected in changes in nutrient loads to the surface water, additional measures to directly reduce surface water enrichment should be implemented (Oenema et al., 1998; De Wit and Behrendt, 1999).

Riparian buffers are examples of habitats that can be used directly to decrease nutrient loading of surface waters. This thesis specifically deals with the nitrogen transformation processes in riparian buffer zones and their potential to improve water quality within different climatic zones and in areas with high nitrogen loading.

What are buffer zones?

Buffer zones are landscape elements, located at the interface between terrestrial and aquatic ecosystems, which can intercept nutrients from shallow subsurface runoff before it reaches the surface water. Buffer zones may differ in size and layout; hence several definitions and terms are used in the literature to indicate buffer zones, ranging from narrow fertilizer-free strips to large (natural) riverine wetlands. The wide range of possible landscape structures with potential for nutrient retention is indicated in Figure 2. In this study we focus on the buffer function of (semi) natural riparian zones (3a/d, Fig. 2) of 10-30 m width along lower-order streams (1st - 4th order). In this thesis we use the terms riparian buffer zone, riparian zone and buffer zone as synonyms. The term strip is used to distinguish specific parallel areas within the riparian buffer zone.

Processes of nitrogen transformation in riparian buffer zones

Riparian zones are important components of stream ecosystems as they are intimately linked to the functioning of the stream channel by influencing stream bank stability, water temperature, primary production and biodiversity (Hynes, 1983; Naiman and Décamps, 1997). Due to their position between upland and aquatic systems, riparian zones contribute to the control of energy, nutrients and organic matter fluxes both in longitudinal (Schlosser and Karr 1981; Pinay et al., 2000) and lateral directions (Peterjohn and Correll 1984; Haycock et al., 1997). In this perspective natural riparian zones can function

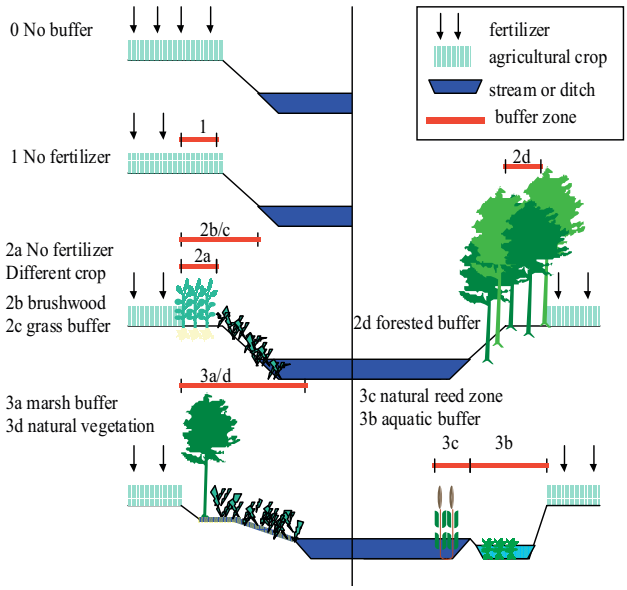

Figure 2 Bufferzones differ in size and layout (adapted from Orleans et al., 1994).

1 narrow fertilizer-free buffer strips
2 fertilizer-free buffer strips with adapted vegetation
  a different agricultural crop
  b with natural brushwood
  c with grass
  d forested
3 fertilizer-free buffer strip with an adapted layout
  a marsh buffer zone with a reduced slope and natural herbaceous vegetation
  b aquatic buffer zone with submerged aquatic vegetation
  c natural reed zone
  d forested marsh buffer zone with a reduced slope

as buffers to reduce the quantity of diffuse pollution that reaches streams (Lowrance et al., 1984; Peterjohn and Correll, 1984; Pinay and Décamps, 1988; Osborne and Kovacic, 1993; Vought et al., 1994; Kuusemets and Mander, 2001; Fig. 3). Nitrogen removal in riparian buffer zones is commonly attributed to denitrification (Groffmann et al., 1992a; Pinay et al., 1993; Verchot et al., 1997), immobilization and plant uptake (Kuusemets et al., 2001; Lyons et al., 2000; Fail et al., 1987). Despite extensive research, considerable uncertainty exists about the relative importance of principle removal mechanisms along climatic gradients and under varying hydrological regimes.

Denitrification

Denitrification is a microbial process involving the stepwise reduction of nitrate through nitrite, nitrogen oxide and nitrous oxide, ending with gaseous nitrogen (Reddy and Patrick, 1984; Tiedje, 1988). Denitrifiers are facultative

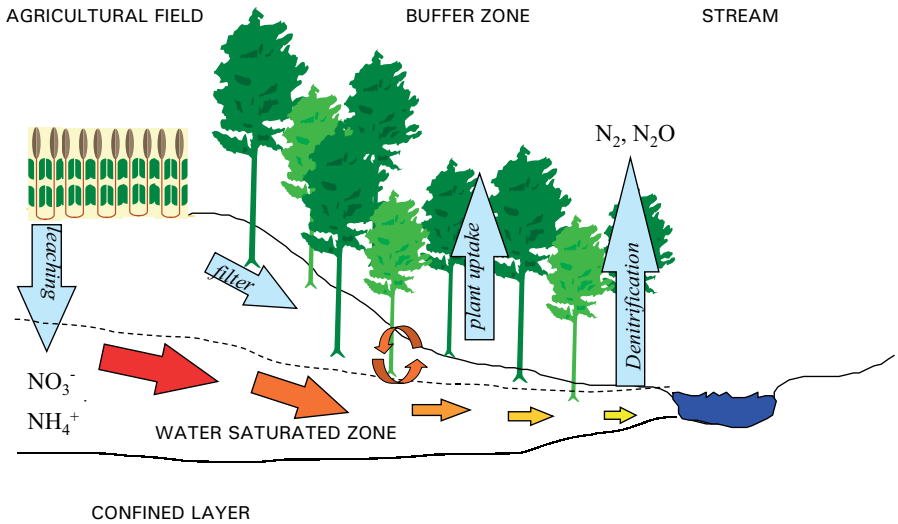

Figure 3 Schematic presentation of nitrogen transformation and retention in riparian buffer zones.

anaerobic organisms because they can use oxygen for their respiration when it is present, and change to nitrate when conditions become anoxic (Knowles, 1982; Tiedje, 1988). A number of environmental conditions are needed for denitrification to take place. Anoxic conditions and presence of nitrate as an electron acceptor are prerequisites for denitrification. In waterlogged soils anoxic conditions predominate because the chemical and microbial demand for oxygen greatly exceeds the supply and the solubility and diffusion of O_2 in water is poor. Contrary to oxygen, the diffusion rate of nitrate will increase under waterlogged conditions. Furthermore denitrification is an energy-demanding process and the required energy is usually derived from oxidation of organic matter (heterotrophic denitrification) as can be seen in the following equation (Reddy and Patrick, 1984):

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$$

Other factors of importance for denitrification are pH and temperature. The pH optimum for denitrification is in the range of 6 to 8, but considerable denitrification activity can be found at pH values up to 4.

Besides heterotrophic denitrification, several other denitrification pathways exist. For example in autotrophic denitrification, pyrite can be used as an electron donor leading to a reduction of nitrate and oxidation of sulphide by *Thiobacillus denitrificans* and oxidation of iron by *Gallionella ferruginea* (Blicher-Mathiesen and Hofmann, 1999).

Chemodenitrification is an abiotic process in which NO_2^- reacts with amines or amorphous iron compounds present in the soil. The nitrite for this reaction is produced as an intermediate product in both nitrification and denitrification, therefore it is difficult to determine the importance of chemodenitrification in the field. Chemodenitrification usually takes place in soils with low pH (3-4) and high amount of ferrous iron in solution (Van Cleemput et al., 1976; Van Cleemput and Baert, 1984). The main endproduct of chemodenitrification is NO (Van Cleemput and Baert, 1984), although N_2O may also be formed (Chalk and Smith, 1983). Nitrifier-denitrification by autotrophic ammonia oxidizers is yet another pathway of NO_2^- reduction which was recently studied by Wrage et al. (2001). Despite the fact that many types of denitrification exist, in riparian zones with high organic matter contents in the soil, heterotrophic denitrification is generally found to be the dominant nitrate reduction process (Blicher-Mathiesen and Hoffmann, 1999; Matheson et al., 2003).

Plant uptake and immobilization

Recent experimental studies have indicated that denitrification is probably more important than plant N uptake in the N removal of riparian zones (Verchot et al., 1997; Schade et al., 2001), mostly because plants only temporarily retain N which returns to the available N pool once mineralized. In contrast, denitrification permanently removes N from the soil to the atmosphere. Denitrification, however, cannot account for all inorganic N removal, suggesting that N storage in perennial plant tissue, and in soil as organic matter through peat formation are potentially important processes in riparian zones (Lowrance et al., 1984; Groffman et al., 1992a; Simmons et al., 1992; Haycock and Pinay, 1993; Zhu and Ehrenfeld, 2000). Moreover, the relative importance of vegetation in N mitigation may increase with biomass harvesting, e.g. mowing or logging (Mander et al., 1995). Results from studies of N retention efficacy in riparian zones dominated by different vegetation types (forest versus herbaceous) have not been consistent. In the past it has been assumed that a forest cover would be somewhat more effective in nitrate removal compared to a grass cover, due to a higher total biomass, (semi) permanent storage of nutrients in wood and a deeper root system. Deeper roots allow trees to take up nitrogen from a greater volume of groundwater, and produce organic matter deeper in the soil profile, which can be used by denitrifying bacteria (Cooper, 1990; Correll, 1991; Osborne and Kovacic, 1993; Haycock and Pinay, 1993) Opposite results, i.e. higher nitrate removal efficacies in grassland were found by Groffman et al. (1991), Schnabel et al. (1996) and Kuusemets et al. (2001). Other studies, however, have indicated that there is no significant difference in N removal between vegetation types (Vought et al., 1994; Lyons et al., 2000; Cosandey et al., 2001; Sabater et al., 2003; Syversen, 2002).

Apart from the role of vegetation in the long-term retention of nitrogen in riparian buffer zones, plant uptake in annual tissues results in a desynchronization of nitrogen availability caused by the time lag between plant N uptake and N release by decomposition and mineralization. Additionally, immobilization of N in litter during the first stages of the decomposition requires nutrients from external sources and may temporarily reduce the amount of inorganic nitrogen in the interstitial water (Berg and Staaf, 1981; Bowden, 1986; Downs et al., 1996).

Hydrology of riparian buffer zones

The hydrology of the riparian zone is known to be of crucial importance to denitrification and other nitrogen cycling processes. Redox conditions in wetland soils are strongly influenced by water table fluctuations. Spatial and temporal changes in the occurrence of oxic and anoxic conditions have drastic effects on the rates of ammonification, nitrification and denitrification (Reddy et al., 1980; Patrick, 1982; Reddy et al., 1989; Hill, 1996; Hedin et al., 1998; Clément et al., 2002b). Ammonification of organic nitrogen can be realized both under oxic and anoxic conditions but the nitrification process, which requires the presence of free oxygen, can only occur in aerated soils or sediments. Other processes involved in nitrogen cycling, such as dissimilative nitrate reduction or denitrification, are strictly anaerobic. Given their topographic location and sedimentary structure, most riparian zones are characterized by high water tables and anoxic soil conditions maintained by a variety of processes: inflow from adjacent hillslopes, upwelling groundwater, inflow from the river channel via bank seepage or overbank inundation, and rainfall. Nevertheless, especially during the growing season, the water table may lower significantly as inflows from nearby slopes are diminished and evapotranspiration rates increase. Since denitrification potential generally increases towards the soil surface, water table height strongly controls the degree of nitrate removal by denitrification (Burt et al., 1999). Furthermore hydrological pathways are important with respect to dilution, mixing and flow velocity influencing the nitrate loading and residence time within the riparian buffer. Flow patterns within riparian zones, with heterogeneous sediments, may be complex (Gold et al., 1998; Devito et al., 2000) and preferential flow paths conducting substantial quantities of water decrease the contact and residence time of water in the buffer. Moreover, bypass flow may occur, when nitrate-rich water short-circuits the biologically active saturated topsoil and reaches the stream without any improvement in water quality (Phillips et al., 1993; Wigington et al., 2003). Thus, detailed field data on hydraulic gradients,

flow patterns and mixing are essential for a correct evaluation of the N mitigation by riparian buffer zones (Nelson et al., 1995).

Climatic influences on nitrogen process rates

Soil moisture and temperature might both be affected by global climate change (Shaver et al., 2000; Georgakakos and Smith, 2001). Water table level and its dynamics may be altered both from upslope by land use/land cover change and from downstream areas by river discharge changes as a result of climate change (Nilsson and Berggren, 2000; Nijssen et al., 2001; Burt et al., 2002; Pinay et al., 2002). For instance in Europe, scenarios of change in the hydrological regime forecast an overall increase of the inter-annual variability of runoff, together with an increase of the average annual runoff in northern Europe and a decrease in the south (Arnell, 1999). Additionally, the timing and duration of high and low flow events might shift, especially in the eastern part of the continent.

Temperature is expected to rise as a result of an increase in the concentration of atmospheric carbon dioxide (IPCC, 1996). Higher temperatures would enhance mineralization of organic matter (Rustad et al., 2001) increasing the amount of nutrients in inorganic form (Freeman et al., 1994). Combined with increased runoff from upland fields in northern Europe, this may result in higher nutrient loading of riparian zones in agricultural environments. Ultimately, these changes will affect the rates of nitrogen cycling in riparian zones and their plant productivity.

Long-term sustainability and environmental consequences of highly N-loaded riparian buffer zones

N saturation

The nitrate concentration of subsurface runoff and the associated nitrate loading rate is another key factor influencing nitrate removal in riparian zones (Hanson et al., 1994a; Willems et al., 1997). In the Netherlands, the rate of fertiliser application is high (200-400 kg N ha^{-1} yr^{-1}) as are the losses to the groundwater (Oenema and Roest, 1997). Consequently, riparian buffer zones in agricultural watersheds in the Netherlands have been subject to prolonged nitrogen enrichment with high loading rates compared to other riparian zones in Europe. This increased nitrogen availability has resulted in changes in species

composition and possibly increased nitrogen cycling rates, reducing the N retention by the vegetation (Hanson et al., 1994b; Aerts et al., 1995; Verhoeven et al., 1996). Over time, the chronic N inputs may even cause saturation of N removal processes and riparian buffer zones may risk losing their beneficial function for water quality improvement (Hanson, 1994b).

Nitrous oxide emission

A predominantly anoxic environment rich in organic matter provides optimal conditions for denitrification and leads to a considerable denitrification potential. Consequently, denitrification has often been identified as the key N removal process in many riparian buffer zones. However as high nitrate availability usually inhibits or retards N_2O reduction, substantial quantities of N_2O may be emitted from riparian buffer zones in agricultural environments (Groffman et al., 1998; Heincke and Kaupenjohann, 1999). N_2O is a greenhouse gas with a high global warming potential (Bouwman, 1995); at present N_2O causes 6% of the radiative forcing of all greenhouse gases (IPCC, 2001). Furthermore, N_2O is also involved in the catalytic destruction of stratospheric ozone (Crutzen, 1970). The question therefore arises whether riparian zones, used as buffers to protect freshwater ecosystems, are a solution to an environmental problem or just partially substitute one environmental problem by another, i.e. by reducing water pollution but increasing N_2O in the atmosphere.

Nitrogen control by landscape structures, NICOLAS

The study described in this thesis was carried out as part of the European Union funded project NItrogen COntrol by LAndscape Structures in agricultural environments (NICOLAS ENV4-CT97-0395). This FP-4 project started in 1998 with a goal of evaluating the natural performance of riparian zones to sustainably buffer waterborne fluxes of diffuse agricultural nitrogen pollution of aquatic environments. The research described in this thesis was conducted within the framework of the objective "to measure the nitrogen retention and transformation processes of morphologically similar riparian structures within representative agricultural drainage basins along a climatic gradient" (Pinay and Burt, 2001).

More specifically the following research questions were addressed:

- What is the importance of groundwater flow path in the groundwater quality dynamics within a riparian buffer zone?;

- What is the importance of vegetation and litter in the effective retention of N in riparian buffer zones with various N loading and under different climatic conditions?;
- Is groundwater table a good predictor of quantitative aspects of the nitrogen cycle, and particularly the source-sink processes for nitrate?;
- Are chronically N-loaded riparian zones sustainable buffers or do they eventually lose their denitrification potential due to carbon limitation or N saturation?;
- Are riparian buffer zones useful in solving an environmental problem or rather cause a shift from groundwater pollution with nitrate towards air pollution with nitrous oxide?

Methodology and site description

Seven European countries participated in the NICOLAS research and scientists from different disciplines cooperated within the project. Study sites were selected in each country, and an identical experimental design was employed at each site, allowing climatic controls on riparian zone hydrology and nitrogen cycling processes to be explored.

A grid of piezometers at 10-metre spacing was installed at each site for measurements on groundwater level and quality. In addition, detailed measurements on nitrogen transformation rates were performed on soil and vegetation. For the process measurements a stratified random sampling strategy was chosen so as to divide each study site into three strips parallel to the stream. The strips were positioned across an elevation gradient from near the river edge towards the non-flooded upland bordering the agricultural field; in this thesis indicated with (S) stream, (I) intermediate and (F) field. Forested and herbaceous riparian buffers were selected in France (F), England (UK), Switzerland (CH), the Netherlands (NL) and Romania (R). In Spain (S) and Poland (PL) no herbaceous sites were available for study and only forested sites were selected.

In Table 1 the mean characteristics of the study sites are given. The range of sites provided a wide spectrum of climatic, hydromorphic and land-use conditions. For instance, the mean annual atmospheric temperature ranged from 6.8°C in Poland to 17°C in Spain and the mean effective precipitation ranged from 67 mm in Spain to 592 mm in Switzerland. Furthermore the lateral N loading rates by subsurface flow were highly variable ranging from 0.42 g N m^{-2} yr^{-1} in the forested site in Romania to 627 g N m^{-2} yr^{-1} in the

Table 1 **Main characteristics of the study areas (after Pinay and Burt, 2001).**

Country	France	United Kingdom	Netherlands
Geographic factors			
Catchment name	Vieux-Viel	Skerne	Twente
Discharge area (km^2)	10.00	8.00	0.15
Latitude	48°3N	54°4N	52°3N
Longitude	1°3W	1°2W	6°5W
Altitude (m)	20	100	64
Climatic variables			
Mean annual T °C	11.6	9	9.5
Maximum montly T °C	25	20	13
Minimum montly T °C	-2.6	1	5.6
Annual precipitation (mm)	880	800	761
Maximum monthly precipitation (mm)	164	68	136
Minimum monthly precipitation (mm)	12	42	16
Mean annual soil T °C	14.4	9.9	8.5
Land use			
% Agriculture	70	80	80
Fertilization rate (kg N ha^{-1})	200	20-50	270
Water quality			
Stream nitrate (mg N l^{-1})	4.6	4.0	5.0-10.0
Groundwater nitrate (input) (mg N l^{-1})	15	1	35
Maximum annual N loading (g N m^{-2} yr^{-1})	84	311	627
Geological substratum			
	Schist	Morenic sand	Glacial moraine
Soil Type			
	Silty clay loam, mixed, isomesic, Typic Haplaquoll	Stagnoluvic gley soil mesic, Typic Albaqualfs	Sandy loam, mixed, mesic, Entisol, Fluvent or mesic, Histosol, hemist
Vegetation Cover (main species)			
Meadow site	*Holcus lanatus* *Dactylis glomerata* *Juncus effusus*	*Lolium perenne* *Poa trivialis* *Trifolium repens*	*Glyceria maxima* *Urtica dioica*
Wooded site	*Salix alba* *Phalaris arundinacea* *Quercus sp*	*Acer sp.* *Fagus sylvatica* *Lolium perenne*	*Alnus glutinosa* *Urtica dioica* *Sambucus nigra*

forested site in the Netherlands. The dominant species in the herbaceous sites were grasses, and tree species in the studied riparian forests were all deciduous. A detailed description of the research sites in this project can be found in Pinay and Burt (2001), Burt et al. (2002) and Sabater et al. (2003). Additional measurements were performed in the chronically N loaded riparian buffer zones in the Netherlands, therefore these sites will be described in more detail.

Spain	Poland	Romania	Switzerland
Fuirosos	Jorka	Glavacioc	Montricher
16.80	65.00	26.00	8.00
41°4N	53°4N	45°5N	46°4N
2°3W	21°3W	23°4W	6°3W
80	150	200	650
17	6.8	10.3	7
29	23	22	19
3	-4.4	-2.7	1
885	580	600	1100
210	120	80	120
10	10	30	65
13.7	9.8	11.1	13.7
20	46	70	80
80	60-120	60	100
<1.00	2.2	?	6.2
11	0.9	0.4	7
7	1.1	0.52	27
Granite	Sandy clay	Loess	Glacial moraine
Sandy soil Sandy clay, mixed, isomesic, Typic Xerochrepts	Loamy sand , mixed Leached brown soils	Silty clay mixed, luvi-hemist	Loamy clay, mixed, hemic, Histosol Terric
No meadow site	No meadow site	*Lolium perenne* *Trifolium repens*	*Poa trivialis* *Ranunculus sp.* *Lolium multiflorum*
Platanus x Acerifolia *Alnus glutinosa* *Rubus ulmifolius*	*Alnus glutinosa* *Padus avium* *Quercus robur*	*Populus nigra* *Crataegus sp.* *Carex riparia*	*Alnus glutinosa* *Fraxinus excelsior* *Prunus padus*

The study in the Netherlands was conducted in two riparian buffer zones along permanent streams; a riparian forest on the Hazelbekke stream (Hefting and De Klein, 1998) and a grassland zone on the Ribbert stream (Hefting et al., 2003a). Both riparian zones were located along comparable first-order streams, in the northeastern part of the province of Overijssel in the Eastern part of the Netherlands (52°3'N, 6°5'W, Fig 4). In 1926 this area was described in detail by Bernink. A citation from this description in Dutch can be found below.

From: Bernink (1926)

"...Excelsior, al maar hooger Ootmarsum door, de molen voorbij, nog hooger, tot we weer bij de Nutter school waren, waar het klimmen eindigde. Dan gaat het vrijwel horizontaal voort in Noordelijke richting over heiwegen met een smal uitgeschuurd fietspaadje naar de Braakhuizen. Hier rollen wij over een oude hoogvlakte, kenbaar aan het vele grint, dat er in den bodem zit. Het is grint van Vecht en Eems, doorspekt met bonte granieten en gneisen, pikzwarte hoornblenden en roode kwartsieten uit noordelijke landen: Finland, Zweden. Hier ligt het bergpuin nog, eens van verre landen door het ijs meegebracht. Het ligt hier maagdelijk, niet door den mensch omgewoeld. De bodem is schraal, begroeid met Heide en Jeneverbes, met Berk en Buntgras. De herder hoedt er al eeuwen zijn witgewolde kudde en om de zooveel jaren steekt er de boer zijn plaggen voor de koestallen. Het smalle en uitgesleten paadje staat hier en daar onder water. Er zal een leemlaag onder zitten. 't Kan de keileem zijn, maar ik vermoed, dat de oude zeekleilaag hier heel hoog zit en dat die het wegzakken van het regenwater belet. Daar is alle reden voor dit te veronderstellen, want een tien minuten verder liggen de bronnen van de Hazelbeek, aan den rand der heide. Aan een geelbruinen, afgebrokkelde zandwal leggen wij onze fietsen neer. Wij staan aan het begin van een slingerend dal. Dat vrij stijl afloopt naar het Zuid-Westen. Het is een grasstrook, wel vijftien meter breed waarlangs elzen, eiken en berken in wilde wanorde gegroepeerd staan. Wij dalen af in het dal waar het vochtig is en de bodem trilt als een spiraalveeren matras. Een watertje van een handbreed slingert er zich doorheen. Onze vriend H. trotseert moedig het gevaar van natte voeten en een modderbad, want hij wil ons wijzen, waar het water overal uit den drassigen grond komt. Onder de hand noteer ik enkele planten: Kale Jonker, Parnassia, Montia, Waternavel, Moerasviooltje in vrucht; veel smalbladige Waterreppe, Watermunt en Moeraszoutgras." (...) "De steile kant bevat veel steenen en leemig zand. Op een paar plekjes liggen fosforieten. Ook die zijn dus hier gegraven een bewijs dat wij hier staan op tertiare formaties. Waar het moeras begint graaf ik een kuiltje en nog geen decimeter diep haal ik reeds de blauwe zeeklei naar boven. Daarom vindt men hier de bronnen, het regenwater, dat op de hoogvlakte valt -waar we over heen gefietst zijn- sijpelt door het zand (± zes meter dik?) tot op de kleilaag, waar het niet doorheen kan. Het volgt de helling van die laag tot het in een plooiing van de ondergrond te voorschijn kan treden...".

The vegetation of the forested buffer zone consisted of alder (*Alnus glutinosa*, L. Gaertn.) and elder (*Sambucus nigra* L.) with some oak (*Quercus* sp. L.) and willow (*Salix* sp. L.) and a herbaceous understorey dominated by nettle (*Urtica dioica* L.) and ferns (*Athyrium filix-femina* L. Roth.)(Fig. 5). The grassland buffer zone vegetation consisted of reed-grass (*Glyceria maxima* Hartm. Holmb), and nettle (*Urtica dioica*). On the hillslope of the buffer zone, bordering the agricultural field, were oaks (*Quercus* sp.) (Fig. 6).

At the upland edge both riparian zones bordered intensively managed arable land planted with maize, with high manure and fertilizer application rates, approximately 200-400 kg N ha^{-1} yr^{-1}, resulting in high concentrations of NO_3^- in the groundwater below the agricultural field (>30 mg NO_3^--N l^{-1}). Lateral nitrate influx rates to the riparian buffer zones were high, with approximately 630 g N $m^{-2}yr^{-1}$ into the forested buffer zone and 270 g N $m^{-2}yr^{-1}$ into the

Figure 4 Map of the research areas, scale 1:25.000. © Topografische Dienst, Emmen. A= Hazelbekke (forested riparian zone), B= Ribbert (Grassland riparian zone).

grassland buffer zone in 1999 (Sabater et al., 2003). High nitrate loading rates in the forested zone probably hampered the N_2 fixation by *Frankia* strains in *Alnus glutinosa* stands (Wolters et al., 1997).

A glacial moraine underlied the study area. The slopes of the glacial moraine consisted mostly of glacial till covered by a thin layer (0–4 m) of aeolien sand or fluvioglacial sediments. The heart of the moraine was dominated by the Dongen Formation (Van den Berg and Den Otter, 1993), dating from the Tertiary, which had a variable composition mostly of sediments with low conductivity, such as different clay types and loam. The sand layers in this formation were fine and contained clay and loam lenses. At some locations, bright green clay and fine sands were disclosed containing the mineral glauconite (Van den Berg and Den Otter, 1993). Both valleys were filled with

Figure 5 Hazelbekke in summer; photograph by Jos Verhoeven.

a 1-3 m thick layer of fluvioglacial deposits, aolien sands and Quaternary peat layers (Formation of Singraven) (Van den Berg and Den Otter, 1993).

The soil types in the study site have been strongly influenced by the historical land use system in this area as described above. On the higher ground, deep organic matter rich agricultural soils were created over centuries. The soils in the valleys were classified as entisols and histosols (USDA[1]). These soils had a peaty topsoil, 10-30 cm deep, upon a sandy substrate, and soils had clear hydromorphic features with high groundwater levels throughout the year. Groundwater levels in both buffer zones ranged from less than 20 cm to more than 50 cm depth. In the adjoining field, water table depth varied from 40 cm to more than 120 cm depth.

Due to the position of the glacial till layer, infiltrated rainwater quickly moves towards the streams in shallow groundwater. The first-order streams at the buffer zones had narrow streambeds (0.5-1.5 m) positioned in valleys of 20-50 m width, created by the erosive force of the melting water. This typical geomorphologic situation created optimal conditions for riparian buffer zone research due to the confined layer and the relatively wide riparian buffer zone compared with the stream size.

Figure 6 Ribbert in spring; photograph by Michiel van Dongen.

Outline of the thesis

This PhD thesis consist of 5 papers on the topics of hydrology, vegetation uptake and soil processes that influence nitrogen turnover in riparian buffer zones. Chapter two deals with the hydrology and nitrate removal in two Dutch buffer zones with special focus on the influence of flow paths and dilution on nitrate reduction. Two papers (Chapter 3-4) are about the risk of nitrous oxide emission from Dutch N-loaded riparian buffer zones and provide data for a more complete functional assessment of riparian buffer zones. Chapter 5 reports on the results of the EU-wide comparison, coupling nitrate process rates to water table height. Water table turned out to be a good predictor of nitrogen processes and three consistent water table thresholds were identified at very different riparian sites. Chapter 6 again builds on results from the NICOLAS project, now to evaluate the relative importance of vegetation and litter in the effective retention of nitrogen in the European buffer zones. Finally, chapter 7 attempts to synthesize the main results of the papers by integrating results from water, soil and vegetation measurements in a mass budget. In addition some recommendations for further research are given.

[1] (http:// www.usda.gov)

Chapter 2

Water quality dynamics and hydrology in riparian zones in the Netherlands

with Boudewijn Beltman, Derek Karssenberg, Karin Rebel, Mirjam van Riessen and Maarten Spijker

Abstract

Riparian zones are known to function as buffers, reducing non-point source pollution from agricultural land to streams. Riparian ecosystems in agricultural catchments in the Netherlands are subject to increasingly high nitrate inputs. Nitrate loading rates have become so high that nitrate retention may level off. In this research we combined hydrological, chemical and soil profile data with groundwater modeling to evaluate whether chronically N-loaded riparian buffer zones were still mitigating diffuse nitrate fluxes. We were also interested in spatial differences in nitrate removal in riparian zones with complex lithology. A forest and a grassland zone along first order streams were selected for this research. Hydraulic parameters and water quality were monitored in both riparian zones on a monthly basis over two years in 50 piezometers. Average nitrate loadings were high in the forested buffer zone with 87 g NO_3^--N m^{-2} yr^{-1} and significantly lower in the grassland buffer zone with 15 g NO_3^--N m^{-2} yr^{-1}. Chloride was used as a conservative tracer to separate between dilution and nitrate removal. Groundwater from a second aquifer played an important role in diluting the shallow nitrate-loaded agricultural runoff causing a significant decrease in nitrate concentration and a significant increase in chloride concentration along its flow path towards the stream. Tracing the groundwater flow paths and dilution along these pathways revealed that clear spatial differences occurred in biological N removal within riparian zones. Both riparian zones were capable of reducing nitrate in subsurface runoff by biological N removal, the grassland riparian zone as a whole removed about 63% of the incoming nitrate load whereas in the more heavily loaded forested zone clear symptoms of saturation were visible and only 38% of the incoming nitrate load was removed.

Introduction

Research of riparian ecosystems in agricultural catchments with high nitrate concentrations in subsurface runoff has often shown a substantial decrease in nitrate concentrations within the riparian zone (Lowrance et al., 1984; Peterjohn and Correll, 1984; Pinay and Décamps, 1988; Simmons et al., 1992; Hill et al., 2000; Dhondt et al., 2002). The importance of natural processes such as vegetation uptake, denitrification and microbial immobilization in the removal of nitrate from shallow groundwater in riparian zones has been demonstrated in a number of studies, e.g. Groffman et al. (1996a); Haycock and Pinay (1993); Martin et al. (1999). These results have led to the conclusion that riparian zones are crucial to the control of non-point source pollution of surface waters in agricultural environments.

However, flows of groundwater from a semi-confined aquifer with low nitrate concentrations and surface water in the near stream (hyporheic) zone may significantly contribute to the decrease in nitrate concentrations in the shallow groundwater through dilution or mixing (Vought et al., 1994; Altman and Parizek, 1995; Pinay et al., 1998). The relative contribution of groundwater from a semi-confined aquifer to the water and solute budgets in riparian zones is largely dependent on the geological setting, and can be substantial in glacial terrain due to the scaled and folded deposits in the moraine (Roulet, 1990).

Another factor that needs to be considered is that flow patterns within riparian zones with heterogeneous sediments may be complex and create spatial differences in both residence time and material encountered by the groundwater traveling within the riparian zone (Gold et al., 1998; Devito et al., 2000). Preferential flow paths may occur as a result of local differences in permeability of the soil, and these may conduct substantial quantities of water and decrease the overall residence time of water in the buffer zone. Hefting and De Klein (1998) observed large spatial variation in nitrate concentrations in stream surface water, probably caused by channeled subsurface runoff from an agricultural field through a forested riparian zone. Another example of the importance of the hydrological flow paths in nitrate removal is bypass flow, when nitrate-rich groundwater passes underneath the riparian buffer zone. The nitrate-rich groundwater then short-circuits the biologically active saturated topsoil and reaches the stream without any improvement in water quality (Phillips et al., 1993; Wigington et al., 2003). Thus, knowledge of groundwater flow paths and physical water mixing is essential for a correct evaluation of the N mitigation by riparian buffers (Nelson et al., 1995).

Besides these hydrological processes, the intensity of nitrate loading is another key factor influencing the removal efficiency of riparian zones (Hanson et al., 1994a; Willems et al., 1997). In the Netherlands, the rate of fertiliser application is high (200-400 kg N ha^{-1} yr^{-1}) as are the losses to the groundwater (Oenema and Roest, 1997). Consequently, riparian ecosystems in Dutch agricultural watersheds have been subject to prolonged nitrogen enrichment with high loading rates compared to other riparian ecosystems in Europe (Sabater et al., 2003; Chapter 5). This increased nitrogen availability has resulted in increased N cycling rates and possibly reduced N retention (Hanson et al., 1994b). Over time, the chronic N inputs may even have caused the riparian ecosystems to become saturated with N and consequently lose their beneficial function on water quality improvement. Sabater et al. (2003) found a negative relation between nitrate loading and riparian zone removal efficiency over a wide range of riparian sites in Europe, suggesting an N saturation effect for the forested zone in the Netherlands. In this study we examined buffer zones in the Netherlands in more detail, focusing primarily on nitrate loading rates and hydrological processes affecting water quality in riparian buffer zones with prolonged nitrate loading.

Our aims were to determine the effect of groundwater pathways, both in horizontal and vertical direction, on measured changes in nitrate concentration within the riparian zone with special attention to the possible occurrence of N saturation effects along these pathways. We also investigated whether dilution effects with water sources other than subsurface runoff contributed significantly to the decline in groundwater nitrate concentrations.

In this research the following step-wise approach was pursued:

1. determination of the groundwater flow paths at a regional and local scale using equi-potential contours of measured hydraulic heads and a groundwater flow model (MODFLOW; McDonalds and Harbaugh, 1984);
2. investigation of changes in groundwater nitrate concentrations along flow paths by monitoring water quality on a monthly basis;
3. determination of the mixing of water from different sources along flow paths within the riparian zone on the basis of chemical signatures, especially chloride concentrations;
4. calculation of the contribution of dilution removal processes to the groundwater nitrate decrease along flow paths;
5. analyses of the effects of hydraulic gradient (water flux) and N-loading on the N removal capacity along flow paths.

Material and Methods

Site descriptions

The study was conducted in two riparian buffer zones; a riparian forest on the Hazelbekke stream (Hefting and De Klein, 1998) and a grassland zone on the Ribbert stream (Hefting et al., 2003a; Chapter 3). Both riparian zones were located along comparable first-order streams, in the northeastern part of the Twente province in the Eastern part of the Netherlands (52°3' N, 6°5' W). The vegetation of the forested buffer zone consisted of alder (*Alnus glutinosa* L. Gaertn.) and elder (*Sambucus nigra* L.) with some oak (*Quercus* sp. L.) and willow (*Salix* sp. L.) and a herbaceous understorey dominated by nettle (*Urtica dioica* L.) and ferns (*Athyrium filix-femina* L. Roth.). The grassland buffer zone vegetation consisted of reed-grass (*Glyceria maxima* Hartm. Holmb), and nettle (*Urtica dioica*). On the hillslope of the buffer zone, bordering the agricultural field, were oaks (*Quercus* sp.). Both riparian zones border intensively managed arable land planted with maize, with high manure and fertilizer application rates, approximately 200-400 kg N ha^{-1} yr^{-1}, resulting in high concentrations of NO_3^- in the groundwater below the agricultural field (>30 mg NO_3^--N l^{-1}). Lateral nitrate influx rates to the buffer zones were high, with approximately 630 g N m^{-2} yr^{-1} into the forested buffer and 270 g N m^{-2} yr^{-1} into the grassland buffer in 1999 (Sabater et al., 2003).

A glacial moraine underlies the study area. The slopes of the glacial moraine consist mostly of glacial till covered by a thin layer (0-4 m) of aeolien sand or fluvioglacial sediments. The heart of the moraine is dominated by the Dongen Formation (Van den Berg and Den Otter, 1993), dating from the Tertiary, which has a variable composition mostly of sediments with low conductivity, such as different clay types and loam. The sand layers in this formation are fine and contain clay and loam lenses. At some locations, bright green clay and fine sands are recovered containing the mineral glauconite (Van den Berg and Den Otter, 1993). The first-order streams at the buffer zones have narrow streambeds (0.5-1.5 m) positioned in valleys of 20-50 m width, created by the erosive force of the melting water. Both valleys are filled with a 1-3 m thick layer of fluvioglacial deposits, aolien sands and Quaternary peat layers (Formation of Singraven) (Van den Berg and Den Otter, 1993). Soils in the buffer zones are classified as entisols and histosols (USDA[1]) and are characterized by high groundwater levels throughout the year. In the adjacent agricultural field and at the upslope edge of the riparian zone the topsoil consists of organic matter rich loamy fine sands and water table depth varies from 0.4 m to more than 1.2 m below the soil surface.

Lithology

To obtain insight into the groundwater flow paths in the complex glacial terrain, we carried out a detailed study of the lithology. Over 110 drillings with depth ranging from 1.2-5.0 m were performed using hand auger equipment (Eijkelkamp, the Netherlands). On a regional scale 70 auger holes were divided over 8 transects, and 20 additional drillings were performed both in the forested and grassland riparian zone. Distinct layers found in the drillings were mapped and grouped according to the texture class. Additional information was used from a study using georadar in the same area (Van der Aa et al., 1999) and a hydrological field study (Hendriks et al., 1996). Information on hydraulic conductivities of the different texture classes was obtained from the unsaturated soil hydraulic database (UNSODA[2]). To verify the saturated hydraulic conductivity (K) chosen from the database, pumping tests were performed in the field. Values were in the same order of magnitude although high spatial variability was observed in the field measurements.

Groundwater monitoring

A grid of dipwell piezometers (5 transects with at least 3 wells, at approximately 10 m intervals) was installed in both study sites over an elevational gradient (slope 10%) from the agricultural fields towards the stream (Fig. 1). Dipwell piezometers were installed into the phreatic groundwater on top of the Tertiary clay layer and tubes were perforated along the whole length. The length of the dipwell piezometers varied from 1-3 m according to the depth of the phreatic aquifer. Additional standpipe piezometers with short filters were installed below (and into) the semi-confined clay layers, to monitor water quality in the second aquifer (depth >5 m), this will hereafter be called the "deep groundwater". This deep groundwater was also monitored to determine the possible existence of bypass flow underneath the riparian soils. Groundwater levels were recorded fortnightly from spring 1998 to mid-November 2000 and seasonally to July 2001. Groundwater flux along flow paths was estimated using Darcy's formula:

$$Q = K.(dh/dl)\ A$$

Where Q is the flow volume per day through a cross-sectional area perpendicular to the direction of flow ($m^3\ d^{-1}$), K is the saturated hydraulic conductivity ($m\ d^{-1}$), dh is the difference in hydraulic head between upslope and down slope piezometers in each flow path (m), dl is the length of distance between the piezometers in the flow path (m) and A is the cross-sectional area perpendicular to the direction of the flow (m^2).

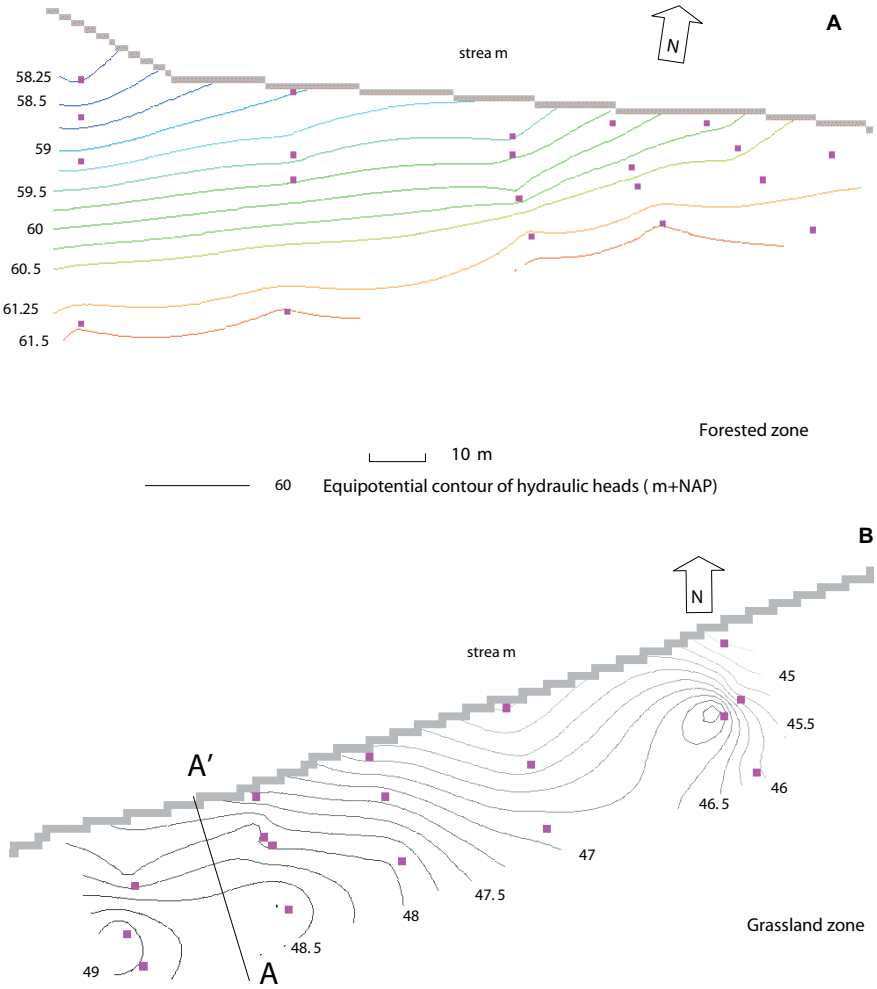

Figure 1 Average annual phreatic water table elevation contours and piezometer locations within the forested (A) and grassland (B) riparian zones. The transect A-A′ in Fig. 1(B) is the location of the cross section shown in Fig. 2.

The lithological surveys and water table elevations (Fig. 1, 2) revealed a more complex hydrological system in the grassland riparian zone compared to the forested system. On the basis of these results we decided to study the groundwater flow pattern of the grassland zone in more detail. In the forested zone groundwater flow paths were determined directly from equi-potential contours of hydraulic heads in the piezometers (Fig. 1).

Groundwater modelling for the grassland riparian zone

A spatial database was constructed for groundwater flow modelling with MODFLOW (McDonalds and Harbaugh, 1984). Digitized land use maps were based on topographical maps (scale 1:10.000) and field observations. Daily

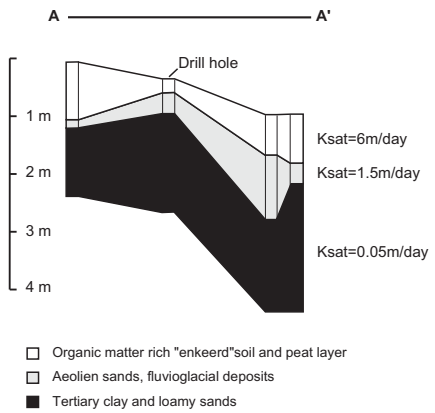

Figure 2 Cross-section of the grassland riparian zone showing the three main formations encountered in the detailed lithological study.

precipitation data were used from the grassland zone in 1999 and from a nearby weather station at Tubbergen (at a distance of 5 km). Evapotranspiration data were used from the regional (Twente) weather station at a distance of approximately 20 km (Dutch Royal Meteorological Institute). These values were converted to an evapotranspiration value for each land use type, using crop coefficients. The digital elevation model for this study area was based on elevation data measured on a 5×5 metre grid using laser altimetry. These data were obtained from the Ministry of Transport, Public works and Water management.

We first created a regional groundwater model in MODFLOW (McDonalds and Harbaugh, 1984) to calculate the boundary conditions (fixed head) for the local model. The size of the regional model was 2 by 1 km with grid cells of 10×10 m, and 5 layers. To study the groundwater flow paths in the riparian zone, a local model was built, which was only 60 by 110 m , using a grid size of 1×1 m and 5 layers. The thickness of the layers and hydrological conductivity values were derived from the field investigation described above. Both forward and backward particle tracking was performed with PMPath (1994-1996) which uses the algoritm described by Pollock (1988), from each piezometer filter location in the grassland riparian zone to interpret groundwater flow paths. In the particle tracking algorithm, dispersion and diffusion are not taken into account. Therefore we assumed that, besides the piezometers that were directly linked by simulated flowlines, also neighboring piezometers were influenced by the passing groundwater flow, only in cases when the filter depth coincided with the depth of the flow paths.

Water quality measurement

Groundwater was sampled monthly from June 1998 to February 2000, and seasonally from May 2000 to July 2001, using a peristaltic pump. Piezometers

were emptied prior to water sampling to remove the standing water. Water samples for cation and DOC analyses were collected in glass flasks and acidified in the field (with 0.1 ml 37% HNO_3 in 10 ml) to prevent precipitation of iron. Water samples for pH, conductivity and anion analyses were transported in PVC flasks. Additional surface water samples were taken mid-stream at a fixed position. Water samples were transported to the laboratory at approximately 4°C. Measurements of pH (WTW pH 540 GLP) and conductivity (WTW LF 539) were made upon arrival in the laboratory. Samples for cation and anion analyses were filtered in the laboratory, using glass fiber filters (Schleicher and Schuell, GF 52) and analyzed within 24 h of sampling for NO_3^-, NH_4^+, SO_4^{2-}, Cl^-, Ca^{2+}, and Mg^{2+} using a continuous flow autoanalyser (SKALAR SA-40, Breda, the Netherlands). Chloride was measured to detect possible dilution with the chloride: nitrate ratio, considering chloride as a conservative tracer (Altman and Parizak, 1995; Cey et al., 1999; Clément et al., 2002b). Additional measurements on Na^+, K^+, and HCO_3^- were performed at an irregular basis to further characterize the chemical signatures of the water for conclusions on the origin of the water. Measurements on groundwater DOC and organic N contents were performed to study possible organic N losses (Prior and Johnes, 1999;Van Breemen, 2002).

Statistical analysis

Water quality data were tested for normality and homogeneity of variance. If data did not meet the requirements they were log transformed before statistical analysis. Variables were tested using Student T-tests, Regression analysis, ANOVA, Repeated Measures Analysis of Variance and Tukey post hoc tests, using SPSS 8.0 for Windows (SPSS 1997, Chicago, Illinois, USA).

Results

Groundwater table

Fluctuations in groundwater levels were rather similar between the two study sites (Fig. 3). Close to the stream and in the middle of the riparian zones the water table remained close to the soil surface (-15 cm to 0 cm) and fluctuated little during the year. Water tables at the field border were always well below the soil surface (>40 cm) and showed a clear seasonal variation. However, only a weak relation could be found with precipitation data, indicating a more regional groundwater flow system (Altman and Parizak, 1995; Caissie et al., 1996). Differences in hydraulic heads between the field border and the middle of the riparian zone were significantly different during summer and winter periods, resulting in significantly higher water fluxes, calculated using

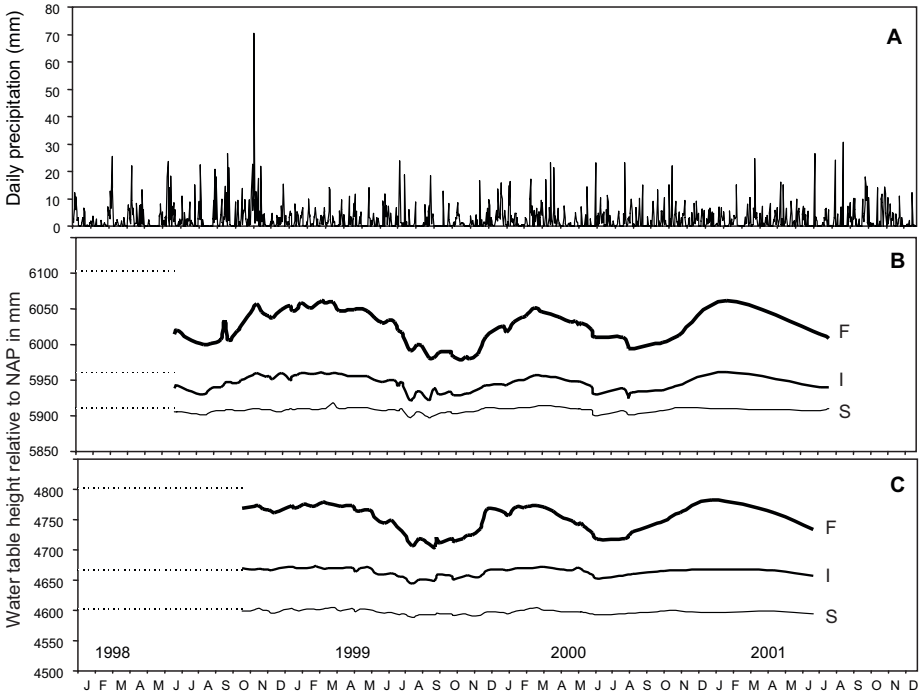

Figure 3 **Precipitation in mm (A) and water table elevation (referred to NAP=Amsterdam Ordnance datum in cm) from 1998 to July 2001 in strips parallel to the stream in the forested (B) and grassland (C) riparian zone. Water table data are averages of 2 weekly recordings in 5-12 dipwell piezometers per strip i.e. field (F), intermediate (I) and near stream (S). Dotted lines indicate the height of the soil surface.**

equation 1, through the riparian buffer zones in winter periods (Fig. 2, paired-sample t-test, p=0.002).

Lithology

The glacial history of the area has resulted in a complex lithology with many discontinuous folded and fractured layers. The major layers are shown in Figure 2. Tertiary clay layers and loamy sand layers containing glauconite were encountered at depths varying from 1 m close to the stream to 4 m below the agricultural fields. Saturated hydraulic conductivities of these layers varied between 0.05 m d^{-1} and 10^{-6} m d^{-1}. On top of the semi-confined tertiary material, mixed fluvioglacial deposits and aolien sands were found with saturated hydraulic conductivities of about 0.5-1.5 m d^{-1}. The soil layer consisted of 1 m thick organic matter rich agricultural soils ("enkeerdgronden") on the higher grounds and Quaternary peat layers in the riparian zones with depth varying between 0.2-1 m with saturated conductivities of 0.35-0.5 m d^{-1} and 5.0-6.0 m d^{-1}, respectively (Hendriks et

al., 1996). The typical black agricultural soils were formed by the historical land use system in this area; heathland sods and peat sods with sheep manure were added as fertilization, creating thick, organic matter rich topsoils (Ebbers and Van het Loo, 1992).

Groundwater flow paths

Results from the local model of the grassland riparian zone showed that water infiltrating in the agricultural fields directly adjacent to the stream valley was transported, as subsurface flow, through the grassland riparian zone towards

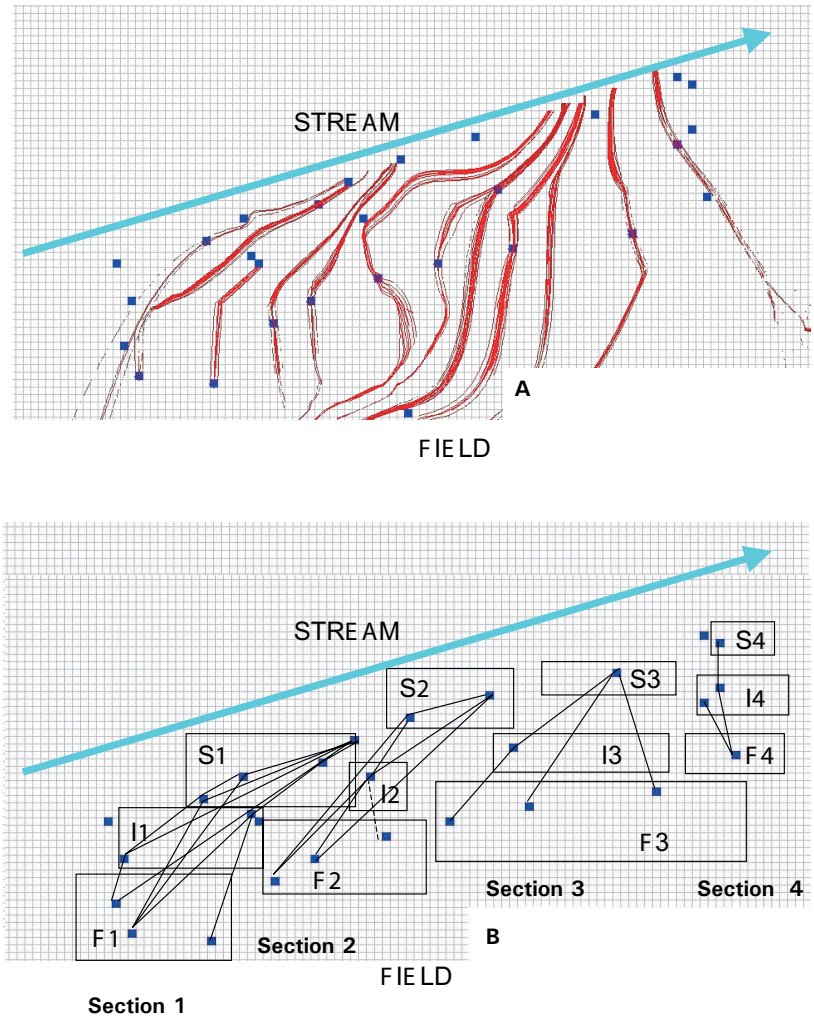

Figure 4 The overall result of a virtual tracing experiment using particle tracking in PMPath (A) showing 4 sections of connected piezometers. Each section has groups of piezometers in three strips parallel to the stream, i.e. field border (F), intermediate (I) and near stream (S).

the stream. However, groundwater flow patterns appeared to be complex (Fig. 4 A). Based upon the piezometer grid, we distinguished four flow paths sections (Fig. 4 B). The four flow path sections were determined by particle tracking connecting piezometer locations in three dimensions. To cope with the complex flow pattern, we grouped piezometer observations along the observed flow paths in three major groups (Fig. 4 B), i.e. field (F), intermediate (I) and near stream (S).

Determination of piezometric surfaces within the forested riparian zone (Fig. 1) revealed that the dominant flow path was perpendicular to the stream. Within the forested research area, five flow path sections were identified. The forested riparian zone piezometers were also clustered into three groups, field (F), intermediate (I) and near stream (S).

Nitrogen inflow

Mean groundwater nitrate concentrations over the study period were significantly higher in the field border of the forested buffer zone (average 35 mg NO_3^--N l^{-1}, median 36 mg NO_3^--N l^{-1}), compared to the field border in the grassland buffer zone (average 11 mg NO_3^--N l^{-1} median 8 mg NO_3^--N l^{-1}) (repeated measures F 10.613, p=0.012) (Fig. 5). NH_4^+ concentrations in

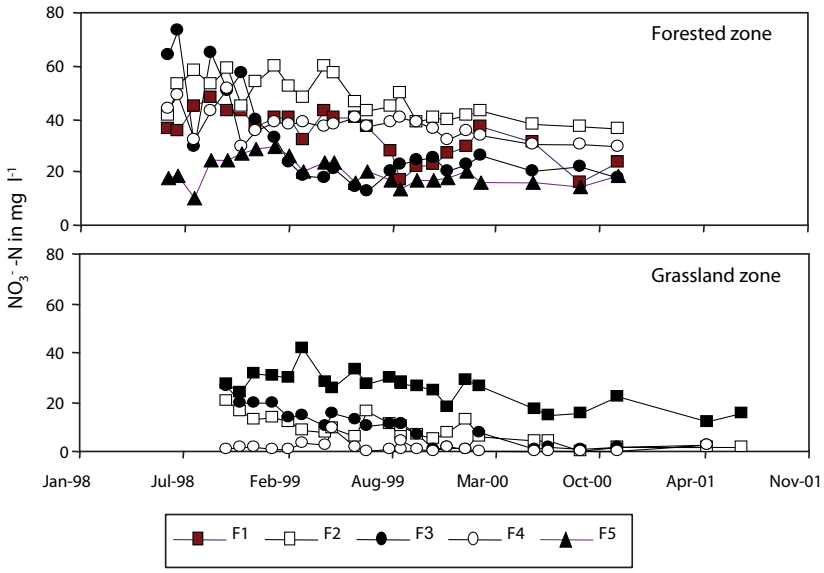

Figure 5 **Nitrate-N inflow concentrations at the field border (F) over the study period in the forested and grassland buffer zone. Five groups (n=2) of piezometers at the field border were analyzed in the forested zone (F1-F5) and four main groups (n=2-3) of piezometers were analyzed at the field border of the grassland zone (F1-F4 see Fig. 4).**

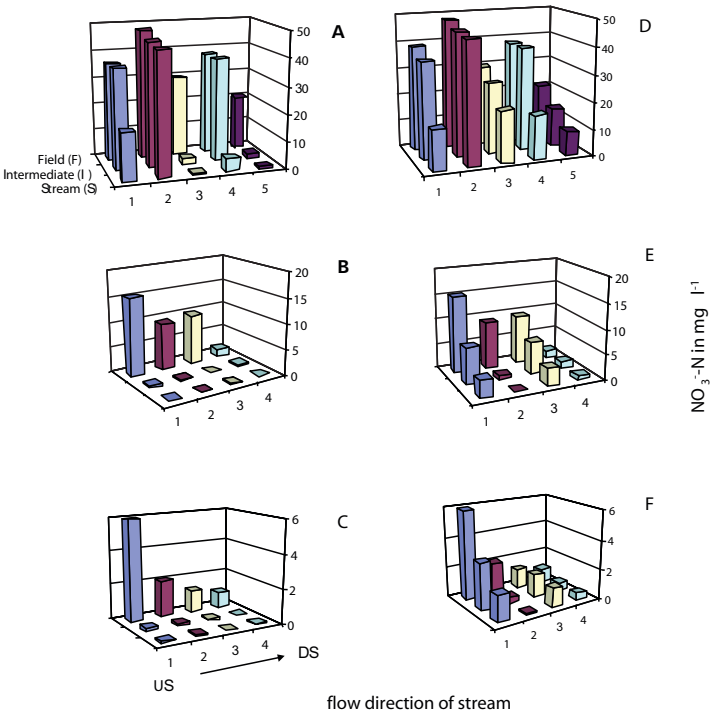

Figure 6 **Average nitrate-N concentrations observed along flow paths June 1998-February 2000 in the forested riparian zone (A) and in the grassland riparian zone (B). Average nitrate concentrations observed along flow paths in the grassland riparian zone from July 2000-July 2001 with significant lower nitrate inflow concentrations (C). US, upstream and DS down stream location of the flow paths (parallel to the stream). Average nitrate-N concentrations corrected for dilution with deep groundwater using chloride concentration in the forested riparian zone (D) and in the grassland riparian zone (E). Average corrected nitrate concentrations observed along flow paths in the grassland riparian zone from July 2000-July 2001 with significant lower nitrate inflow concentrations (F).**

the shallow groundwater were low in both buffer zones with mean concentrations below 0.4 mg N l^{-1}. No significant seasonal variation was measured in the mean nitrate concentration at the field boundary, and no direct response of nitrate concentrations to agricultural practice in the adjacent farmlands (e.g. manure application, ploughing, harvesting) was observed. However, inflow nitrate concentrations at the field border were significantly higher in 1998 compared to 1999-2000, in both buffer zones, due to extremely wet weather conditions in 1998. Furthermore, nitrate concentration of the input to the grassland buffer zone decreased significantly over the period January 1999 to July 2001 (ANOVA, F=73.510, p=0.04). This was probably caused by the construction of a 12 m unfertilised zone in the agricultural field on the upland edge of the riparian zone in spring 1999. Moreover, nitrate concentrations in the field border of the Ribbert grassland area were significantly higher at the "upstream" field border piezometers compared to

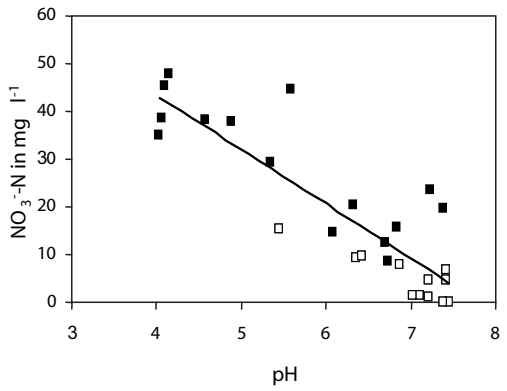

Figure 7 Relationship between the nitrate concentration and the pH. Open symbols indicate the grassland riparian zone, filled symbols indicate the forested riparian zone.

"downstream" field border piezometers (ANOVA, F=60.697, p<0.0001, Fig. 6 A,B,C). In the forested riparian area no systematic difference in inflow concentrations could be observed in the flow direction of the stream. Nitrate concentrations at the field border were, however, significantly different between the measured transects (ANOVA, F 26,353, p<0.0001, Fig. 6 A,B,C).

Nitrogen concentrations along flow paths

A decrease in nitrate concentration was observed along flow paths in both riparian areas (Fig. 6 A,B). Such a decrease also occurred in periods with lower nitrate inflow concentrations (Fig. 6 C). Nitrate concentrations were significantly lower in the near-stream strips compared to the field border strips (Table 1). Furthermore, high spatial variation in the decrease in nitrate concentrations was found in the forested buffer zone. In contrast to the low nitrate concentrations observed at the stream border of the grassland buffer zone, the mean nitrate concentration in the stream border of the forested buffer zone showed a strong spatial variation with a range of concentrations between 0.5 and 45 mg N l^{-1}. The higher nitrate concentrations were strongly correlated to lower pH values (Fig. 7, R^2=0.754, p<0.001).

To separate the contribution of dilution and biological removal processes responsible for the decrease in nitrate, chloride was used as a conservative tracer. We observed a significant increase of chloride concentration with decreasing nitrate from the field border to the stream (Table 1). This strong increase of 20-150% was not likely to be caused by evapotranspiration in the riparian zone, because increases were too high and no seasonal differences were observed in chloride concentrations. Moreover, observed differences in other macro ionic compounds, expressed in STIFF diagrams (Beltman and Rouwenhorst, 1991; Freeze and Cherry, 1979,) and pH in the shallow groundwater flowing through the riparian zones indicated that there was a

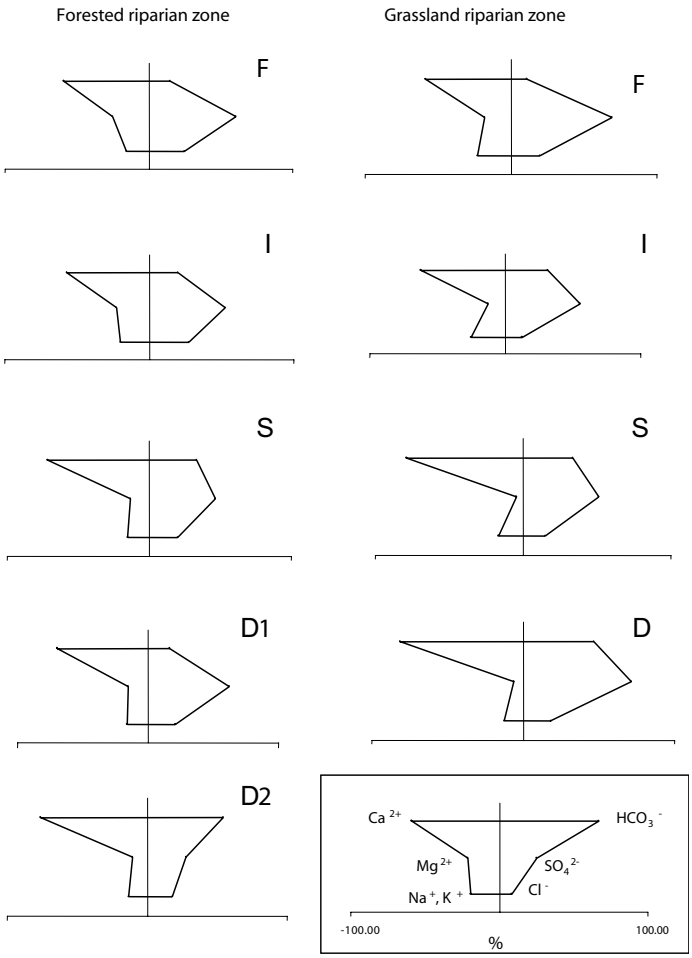

Figure 8 **Average macro ionic composition of water samples along the flow paths expressed in STIFF diagrams. Concentrations are expressed as % of the sum of cations (left) or anions (right) (meq l^{-1}). Abbreviations indicate the location within the riparian zone, i.e. field (F), intermediate (I) and near stream (S). D1 represents the water quality of the deep groundwater at the upstream location in the forested riparian zone, D2 represents the water quality in the middle and downstream location. D represents the deep groundwater in the grassland zone.**

change in groundwater composition down the flow paths, most probably caused by dilution with deep groundwater in both study sites (Fig. 8; Table 1). Chloride concentrations measured in this groundwater were used to correct for dilution. This correction revealed that the nitrate dynamics in both riparian buffers was significantly affected by dilution with deep groundwater. In the grassland zone an average dilution of 65% and in the forested zone an average dilution of 47% was calculated on the basis of the chloride concentrations. Large spatial differences in dilution occurred in both sites, although no

Table 1 Average macro ionic composition of the groundwater in strips parallel to the stream. Standard errors are given in brackets. D1= upstream location of deep groundwater. D2= downstream location of deep groundwater. n= number of observations. #l= number of piezometer locations.

Forested riparian zone	Field border	Intermediate	Near stream	Surface water	Deep groundwater D1	Deep groundwater D2
NO_3^--N	34.78 (1.99)	21.48 (3.02)	11.47 (2.43)	6.42 (1.72)	15.51 (4.63)	0.83 (0.35)
Cl^-	21.6 (1.5)	31.3 (1.9)	31.5 (1.2)	19.1 (2.3)	51.3 (10.5)	38.3 (2.1)
SO_4^{2-}	81.1 (6.2)	158.7 (13.0)	151.8 (11.1)	64.9 (6.5)	202.3 (44.6)	201.3 (23.3)
Ca^{2+}	56.4 (3.0)	94.6 (8.0)	98.6 (5.5)	93.4 (29.2)	148.4 (31.5)	121.0 (17.8)
PH	5.00 (0.12)	5.65 (0.2)	6.66 (0.12)	7.24 (0.22)	5.97 (0.08)	6.98 (0.17)
EC	534 (21)	691 (25)	635 (20)	406 (27)	992 (166)	748 (83)
n	198	141	144	21	47	44
# l	9	5	5	1	2	2
Grassland riparian zone	**Field border**	**Intermediate**	**Near stream**	**Surface water**	**Deep groundwater**	
NO_3^--N	15.29 (3.6)	0.12 (0.03)	0.11 (0.05)	3.05 (1.91)	0.31 (0.21)	
Cl^-	21.1 (1.3)	30.1 (2.3)	33.6 (2.2)	31.8 (3.2)	41.4 (5.6)	
SO_4^{2-}	88.0 (7.3)	86.6 (4.7)	85.9 (3.5)	50.3 (7.0)	103.4 (7.0)	
Ca^{2+}	49.5 (3.5)	69.8 (4.8)	82.2 (7.9)	66.7 (19.3)	72.5 (8.3)	
PH	6.28 (0.16)	7.28 (0.07)	7.39 (0.07)	7.31 (0.10)	7.32 (0.07)	
EC	368 (12)	426 (18)	426 (10)	440 (15)	462 (13)	
n	160	150	132	21	23	
# l	12	7	5	1	3	

significant differences were observed in dilution rates between summer and winter (paired sample t-test p=0.67 and p=0.96 for the forested and grassland riparian zone). In the forested zone two types of groundwater were encountered below the semi-confined clay layer (D1, D2 Fig. 8). Deeper groundwater (>5 m) in the upstream part (D1 Fig. 8) of the riparian buffer still contained about 15 mg l^{-1} of nitrate-N and the STIFF diagram was more or less comparable to groundwater characteristics of recently infiltrated water at the field border, whereas deep groundwater rich in calcium bicarbonate in the middle and downstream part of the riparian zone (D2) was significantly different from groundwater at the field border. The major difference between the groundwater characteristics is the considerable amount of bicarbonate (170 mg l^{-1}) present in the middle and downstream deep groundwater whereas the deep groundwater at the upstream part only contains 77 mg l^{-1}.

Nitrate concentrations corrected for dilution with deep groundwater along the flow paths in both research sites indicate that there is a considerable "real" nitrate removal along most of the flow paths (Fig. 6 D,E,F). In the forested zone 6-77% of the inflowing nitrate was removed compared to a range of 28-

99% of the inflowing nitrate in the grassland riparian zone. The decrease of nitrate was not reciprocated by an increase in ammonium concentration, although a slight increase in ammonium concentration in the shallow groundwater was observed in the forested riparian zone from 0.3 to 0.6 mg l^{-1}. Moreover, dissolved organic N concentrations in the groundwater were low at both research sites, often below the detection limit of 0.5 mg l^{-1}.

Mean nitrate loading rates and removal rates were calculated using the corrected concentrations, the hydraulic conductivity of 0.5 m d^{-1} (assuming a homogeneous, isotropic soil) and the hydrological gradient between strips (F and I and I and S, respectively) in each flow path using equation 1. Absolute nitrate removal rates (expressed as the difference between the input and output nitrate loading in relation to the lengths of the flow paths in the riparian zone) were mainly positive, ranging from 1-20 g NO_3^--N per metre flow path per year. Values close to zero were found along flow paths connecting the intermediate (I) and near stream (S) strips of the grassland zone with extremely low N inputs and for some highly loaded flow paths in the forested zone. Average absolute nitrogen removal rates were higher in the forested zone compared to the grassland riparian zone (independent sample t-test, $p=0.015$). Nitrogen removal efficiencies expressed as % m^{-1} were not significantly different between the forested and grassland riparian zones with average values of 1.9 and 2.7% m^{-1} respectively.

Clear spatial differences were observed in the absolute nitrate removal along the different flow paths in the riparian zones. This was mainly the case in the forested riparian zone, where nitrate removal was almost absent along one of the flow paths (Fig. 9, flow path 2). Furthermore a spatial pattern of nitrate removal was visible with significant higher absolute nitrate removal at the upper edge of the grassland riparian zone (ANOVA, $F=5.161$, $n=16$, $p=0.042$), whereas in the forested riparian zone nitrate removal was concentrated between the intermediate (I) and the near-stream (S) strip. The differences in the forested area were however not significant (ANOVA, $F=3.893$, $n=10$, $p=0.084$).

In the grassland riparian zone the absolute nitrate removal was significantly positively related to the water flux ($R^2=0.686$, $p<0.000$), in contrast to the forested riparian zone, where no significant relationship was found ($R^2=-0.048$, $p=0.558$). The relative nitrate removal showed an opposite pattern, with a significant negative relation between nitrate removal and the water flux in the forested riparian zone ($R^2=0.781$, $p=0.001$) and no significant relation in the grassland zone ($R^2=-0.017$, $p=0.427$). Although significant differences in flow

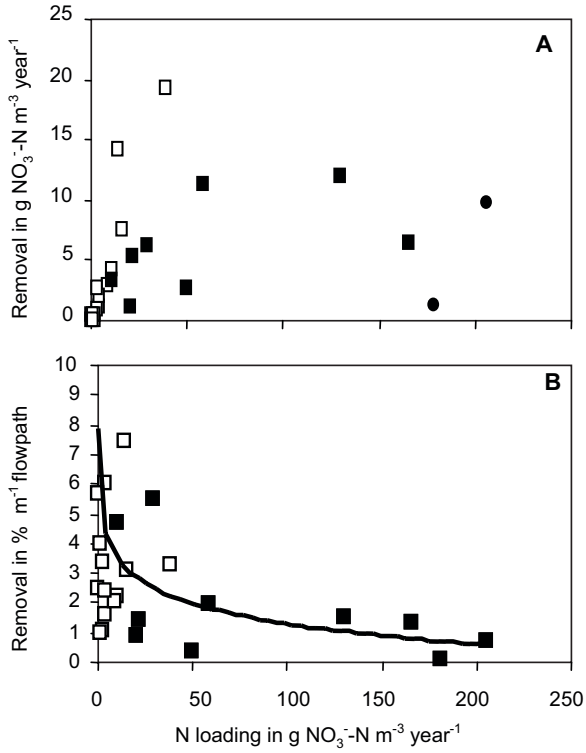

Figure 9 **Relationship between absolute nitrate removal (A) and nitrate removal efficiency (B) versus annual N loading. Open symbols indicate the grassland riparian zone, filled symbols indicate the forested riparian zone. Filled circles indicate the flow path 2 in the forested site with extremely low nitrate removal on concentration basis (see Figure 6).**

rate were observed in summer and winter, no significant differences were found in nitrate removal between these periods (ANOVA, F=0.607, p=0.442).

Nitrate removal rates were significantly correlated with nitrate loading (Fig. 9 A). In the grassland riparian zone, subject to significantly lower nitrate loadings compared to the forested zone, a positive relation was found between the absolute nitrate removal and N load (R^2=0.761, p<0.0001) whereas higher nitrate loading in the forested riparian zone did not result in higher absolute nitrate removal.

The relative nitrate removal expressed as % m^{-1} flow path (Fig. 9 B) showed a negative relation with nitrate loading in the forested zone (R^2=0.634, p=0.011). In the grassland zone no clear relation was observed between relative removal and nitrate loading.

Discussion

Dilution and nitrate removal

Seasonal variations in groundwater table levels in the intermediate (I) and near stream (S) strips were marginal in both riparian zones studied (Fig. 3). Constant discharge of deeper groundwater precluded rapid water table responses to weather conditions in these strips. This is a common situation in glacially mantled areas (Roulet, 1990; Hill, 1993; Hill and Waddington, 1993; Cirmo and McDonnell, 1997). The constant discharge of deeper groundwater also played an important role in diluting the shallow nitrate loaded runoff causing a significant decrease in nitrate concentration and a significant increase in chloride concentration along flow paths towards the stream. The higher chloride concentration found in the deep groundwater probably originated from the tertiary clay in the subsoil, which was formed under marine conditions (Van den Berg and Den Otter, 1993).

Spatial differences occurred in the groundwater quality of the semi-confined aquifer in the forested riparian zone. The STIFF diagrams (Fig. 8) indicate that the groundwater at the upstream part of the forested riparian zone is likely to be recently infiltrated groundwater bypassing a part of the riparian buffer at greater depth. In our lithological survey we encountered some gravel lenses at this upstream part of the riparian zone that are likely to contribute to this flow pattern.

Inflow nitrate concentrations were significantly higher in the forested buffer zone compared to grassland buffer zone. A larger infiltration area and a higher percentage of intensively managed arable fields in the catchment are assumed causes for this higher loading. Increased atmospheric deposition of ammonium due to turbulence at the interface between the agricultural field and the riparian forest might also contribute to the higher N loading (Draaijers et al., 1988).

Corrected nitrate concentrations along the flow paths at both research sites indicate that, besides the dilution effect, a considerable nitrate reduction took place. Average nitrogen removal efficiencies of 2-3% m^{-1} were found in both the grassland and the forested riparian zone (see Fig. 9 B, but note the high spatial variation).

The overall nitrate removal effect of the riparian zones as a whole, corrected for dilution and calculated on the basis of input and output nitrate load, resulted in a nitrate removal of 63% for the grassland riparian zone and 38% for the forested riparian buffer zone. The nitrate removal percentage of the

grassland riparian zone is within the range found of 60-100% for wet riparian buffer zones (Cooper, 1990 (64-94%); Haycock and Burt, 1993 (82%); Haycock and Pinay, 1993 (60-90%); Mander et al., 1995 (100%); Clément et al., 2002b (76-99%)). The lower removal percentage at the forested riparian zone can be attributed to nitrogen saturation due to the relative high nitrate loading. Scenario calculations with the agricultural nitrogen model (ANIMO, Berghuijs-Van Dijk et al 1985) calibrated with input data from a nearby forested riparian zone resulted in a comparable low removal percentage of 28% at a total buffer width of 10 m (Kruijne, 1996).

Processes responsible for nitrate removal

The lack of seasonal difference in nitrate removal between the winter and summer period, indicates the importance of nitrate removal processes other than plant uptake. Generally, heterotrophic denitrification is the dominant nitrate removal process during winter if the shallow subsurface groundwater flows through near-stream substrates rich in organic matter (Haycock and Pinay, 1993). Especially when groundwater levels are close to the soil surface, redox conditions are optimal for heterotrophic denitrification. Nitrate can, however, also be reduced through the oxidation of pyrite (FeS_2), if carbon availability is limited. Böhlke and Denver (1995) found a significant nitrate reduction in groundwater flowing through glauconite-rich sediments. The higher sulfate concentration observed in the deep groundwater (Table 1) may be partly attributed to this autotrophic denitrification by *Thiobacillus denitrificans* (Blicher-Mathiesen and Hoffmann, 1999; Lamers et al., 1998). Other possible causes for the higher sulfate concentrations are pyrite oxidation due to groundwater drawdown for drinking water preparation and high concentrations of sulfate in manure and in tertiary (marine) clay layers. Nitrate removal under reduced conditions is also possible by dissimilatory nitrate reduction to ammonium. This process is not very likely to occur at our study sites because the decrease in nitrate was certainly not reciprocated by an increase in ammonium.

Our results, in particular the negative relation between pH and nitrate, indicate that heterotropic denitrification is probably the major mechanism of nitrate removal from the shallow groundwater. Results from detailed process studies on N transformation, denitrification and vegetation N uptake confirm this conclusion (Chapter 5; Chapter 6).

Effects of flow rate on nitrate removal

In the grassland riparian zone the absolute nitrate removal significantly increases with increasing water flux. Increased flow rates increased the nitrate loading and consequently the absolute amount of nitrate that could be

removed. In the forested zone no significant increase in absolute nitrate removal was observed with increasing water flux. In general an increased water flux did increase the nitrate loading in the forested zone. However, nitrate was not limiting the denitrification and consequently no effect on absolute removal rates was observed. The relative nitrate removal in this zone, however, was significantly negative related with the water flux indicating reduced removal efficiencies due to shorter contact time between groundwater and the organic matter rich topsoil with its high denitrification potential. This negative relation between nitrate removal and flow rate was observed earlier in lab experiments by Willems et al. (1997). In the grassland zone no significant relation was found between the relative nitrate removal and water flux, due to the large variation in the relative nitrate removal found at low nitrate concentrations.

Spatial differences in nitrate removal

The higher absolute nitrate removal rates in the upper part of the grassland riparian zone were caused by a higher nitrate loading due to a combination of higher nitrate concentrations and higher flow rates. This spatial pattern with nitrate removal concentrated in the upper parts of the riparian zone is well known (Peterjohn and Correll, 1984; Pinay and Décamps, 1988; Cooper, 1990; Haycock and Pinay, 1993). However, this pattern is not caused by differences in denitrifying potential but rather by a lack of nitrate available for denitrification in the groundwater further down in the riparian zone.

Although nitrate loadings in the forested zone were significantly higher compared to these in the grassland riparian zone, nitrate removal was not significantly higher. Moreover, some highly loaded flow paths at the forested zone had absolute nitrate removal rates close to zero. Furthermore, the spatial pattern of the absolute nitrate removal rates at the forested zone showed a different pattern with higher nitrate removal rates close to the stream in most flow paths. These observations in absolute nitrate removal in the forested riparian zone can largely be explained by a pH effect. The groundwater in the flow paths with low absolute removal rates had a pH around 4, whereas groundwater in other flow path with clear removal had pH values ranging from 5 to 6. Furthermore, dilution with deeper groundwater might significantly have increased the pH to 7.3. This has resulted in the spatial pattern with higher nitrate removal rates close to the stream.

The higher absolute nitrate removal values found in the grassland zone compared to the forested zone may also be related to a pH effect, as pH values in the shallow groundwater in the grassland riparian zone were significantly higher than in the forested zone (independent sample t-test $F=11.977$, $n=27$, $p=0.002$) with values ranging from 5.5 to 7.5. The lower absolute nitrate

removal rates in groundwater with low pH values can be explained by a decrease in denitrification activity. Heterotrophic denitrification, has been found to be low to non-existent at pH values around 4.0 (Bremner and Shaw, 1958) and pH values below 3.5 are found to totally inhibit the denitrification activity (Aulakh et al., 1992). However, significant denitrification activity at these low pH values, was found by Willems et al. (1997). In this low pH range interpretation of results can be complicated by the occurrence of chemo-denitrification (Van Cleemput and Baert, 1984).

Relation between pH and nitrate concentration

The significant negative relation found between pH and nitrate concentration can be explained by the acidifying effect of nitrification (Van Breemen et al., 1982; Correll, 1997) possibly combined with a de-acidifying effect of heterotrophic denitrification (Appelo and Postma, 1993; Correll, 1997). The dominant form of fertilization on the agricultural fields is organic manure. The ammonia from this manure is transformed into nitrate by nitrification producing two protons for every nitrate molecule formed. Furthermore ammonium can originate from atmospheric ammonium deposition that is known to be higher at forested interfaces due to turbulence effects (Draaijers et al., 1988). When heterotrophic denitrification occurs one proton is consumed for every reduced nitrate molecule, resulting in a decrease in nitrate combined with an increase in pH.

Effect of vegetation type on nitrate removal

The type of vegetation may affect the nitrate removal capacity of riparian areas. Forests have been reported to be more effective than herbaceous vegetation (Cooper, 1990; Osborne and Kovacic, 1993; Haycock and Pinay, 1993). Opposite results, i.e. higher nitrate removal efficiencies in grassland were found by Groffman et al. (1991), Schnabel et al. (1996) and Kuusemets et al. (2001). Recent Europe-wide studies, including our research, have indicated that there is no significant difference in N removal efficiency between vegetation types (Cosandey et al., 2001; Sabater et al., 2003). Thus, observed differences in nitrate removal between the two riparian zones studied are probably not caused by difference in vegetation type.

Results from this study show a significantly higher nitrate removal in the forested riparian zone compared to the grassland riparian zone, probably caused by the higher N loading in the forested zone, because nitrogen removal efficiencies expressed as % m^{-1} were not significantly different between the two zones. No general conclusions on the effect of the type of vegetation could be drawn from this research with only two research sites.

Nitrogen saturation effect

Riparian ecosystems in agricultural watersheds in the Netherlands have become subject to increasingly high nitrate inputs, which has led to increased nitrogen cycling rates and probably to a reduced nitrate retention (Aerts et al., 1995; Verhoeven et al., 1996; Bobbink et al., 1998). In this study, a significant negative relation was found between nitrate removal efficiency (Fig. 9 A) and nitrate loading for the forested zone (R^2=0.409 p=0.010). This is consistent with Nichols (1983) who observed a rapid decline of N removal efficiency with increasing N loading rates over a broad range of natural ecosystems receiving applications of secondary wastewater effluent. The low nitrate removal efficiency at high nitrate loading rates in the forested zone suggests a nitrate saturation effect; chronical high nitrate loading finally exceeds the buffering capacity (Aber, 1992; Sabater et al., 2003) of the riparian buffer zone. Although nitrate removal still occurs, the efficiency is significantly decreased at higher nitrate loadings. Besides a significantly reduced nitrate removal in the forested zone, other symptoms of nitrogen saturation such as high soil nitrogen mineralization rates (Pinay and Burt, 2001; Chapter 5) and significantly higher nitrous oxide emissions and nitrate concentration in pore water (Chapter 3) were observed in the forested riparian zone.

However, in this saturation trajectory even a decrease in absolute removal capacity was observed. This decrease suggests an inhibitory effect of nitrate on the removal process (i.e. denitrification) at extremely high loadings. Such inhibiting effect on denitrification is, however, not known for nitrate. It has well been established in literature that absolute nitrogen retention in lakes, rivers and wetlands increases with nitrogen loading (Saunders and Kalff, 2001), and no upper limit of the riparian buffering capacity had previously been reported. Hanson et al., (1994b) observed clear symptoms of nitrogen saturation in a forested riparian zone subjected to long-term enrichment but high rates of nitrate removal were still possible under enriched conditions. It seems that denitrification can continue at the same rate as the nitrate loading unless the carbon availability becomes limited (Saunders and Kalff, 2001). Yet, studies on denitrification potential in the highly N-loaded wetland soils have generally indicated that the vast supply of organic carbon in these soils does not limit the denitrification process, whereas amendment studies in the laboratory have shown that denitrification was still nitrate-limited (Nichols, 1983; Hanson et al., 1994b) even in the saturated forested zone (Chapter 7). Data from our field study, however, suggest an inhibitory or limiting effect above an input flux of approximately 50 g NO_3^--N m^{-2} per year. The fact that groundwater high in nitrate generally had a low pH (see above) might explain the observed decrease in absolute nitrate removal.

Conclusions

Groundwater from the semi-confined aquifer played an important role in diluting the shallow nitrate-loaded agricultural runoff causing a decrease in nitrate concentration within the riparian zone. If this physical process is not taken into account in these study sites, it leads to a significant over-estimation of the nitrate removal capacity varying between 0-60% depending on the flow path. A detailed understanding of the flow system in riparian zones is therefore necessary to make a general assessment of nitrate removal. However, besides this dilution effect, biological removal processes also significantly reduced the nitrate concentration in the shallow groundwater in both riparian zones studied. While nitrate-loading rates were high, on average a considerable percentage (38-63%) of the nitrate could be removed, although clear symptoms of "saturation" were visible in the forested riparian zone. Concentrations at this zone were not reduced sufficiently to prevent eutrophication of the surface waters. The low pH of the nitrate-rich groundwater in the forested zone probably limited the denitrification activity, thus explaining the saturation effect in this case.

Riparian buffer zones receiving high nitrate loads in shallow groundwater with low pH values may not fully protect the stream ecosystem. Liming of the agricultural fields and riparian zones might increase the nitrate removal efficiency of these riparian zones, but source-directed measures to reduce leaching of nitrate in agricultural are still to be preferred to protect aquatic ecosystems from eutrophication.

Acknowledgements

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[1] **(http://www.usda.gov)**
[2] **(http://www.ussl.ars.usda.gov/models/unsoda.htm)**

Chapter 3

Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones

(J. ENVIRON. QUAL., VOL. 32, 1194-1203 2003)
with Roland Bobbink and Hannie de Caluwe

Abstract

Riparian buffer zones are known to reduce diffuse N pollution of streams by removing and modifying N from agricultural runoff. Denitrification, often identified as the key N removal process, is also considered as a major source of the greenhouse gas nitrous oxide (N_2O). The risks of high N_2O emissions during nitrate mitigation and the environmental controls of emissions have been examined in relatively few riparian zones and the interactions between controls and emissions are still poorly understood. Our objectives were to assess the rates of N_2O emission from riparian buffer zones that receive large loads of nitrate, and to evaluate various factors that are purported to control N emissions. Denitrification, nitrification, and N_2O emissions were measured seasonally in grassland and forested buffer zones along first-order streams in the Netherlands. Lateral nitrate loading rates were high, up to 470 g N m^{-2} yr^{-1}. Nitrogen process rates were determined using flux chamber measurements and incubation experiments. Nitrous oxide emissions were found to be significantly higher in the forested (20 kg N ha^{-1} yr^{-1}) compared with the grassland buffer zone (2-4 kg N ha^{-1} yr^{-1}), whereas denitrification rates were not significantly different. Higher rates of N_2O emissions in the forested buffer zone were associated with higher nitrate concentrations in the groundwaterwater. We conclude that N transformation by nitrate-loaded buffer zones results in a significant increase of greenhouse gas emission. Considerable N_2O fluxes measured in this study indicate that Intergovernmental Panel on Climate Change methodologies for quantifying indirect N_2O emissions have to distinguish between agricultural uplands and riparian buffer zones in landscapes receiving large N inputs.

Introduction

The loss of nitrogen compounds from agricultural environments to the shallow groundwater and surface water has increased dramatically over the last decades (Van Eck, 1994; Olsthoorn and Fong, 1998). Although several agricultural measures are being taken to reduce the amount of excessive N in the soil profile after harvest, high leaching of nitrate still takes place, polluting the shallow groundwater (Addiscot et al., 1991; Olsthoorn and Fong, 1998). This diffuse pollution of shallow groundwater is a major cause of the eutrophication of freshwater environments in agricultural landscapes. Riparian buffer zones positioned between the terrestrial and aquatic environments are known to remove large amounts of N from shallow groundwater (Peterjohn and Correll, 1984; Pinay and Décamps, 1988; Jordan et al., 1993; Hill, 1996; Groffman et al., 1996a). The major mechanisms of N retention in riparian zones are plant uptake, denitrification, and microbial immobilization. Denitrification has been studied extensively in these ecosystems (Lowrance, 1992; Pinay et al., 1993, 2000; Schipper et al., 1993; Hanson et al., 1994b; Jordan et al., 1998; Watts and Seitzinger, 2001). The predominant anaerobic environment rich in organic matter provides optimal conditions for denitrification and leads to a considerable denitrification potential. Consequently, denitrification has often been identified as the key N removal process in many riparian buffer zones. However, we have to consider the possibility that riparian zones may contribute to the enhanced greenhouse effect (Crutzen, 1981; Lloyd, 1995).

Nitrous oxide (N_2O) can be an important intermediate of denitrification. Nitrous oxide is known as an effective greenhouse gas with a warming potential of approximately 300 compared with CO_2 and is also involved in the catalytic destruction of stratospheric ozone. The question then arises if riparian zones, used as buffers to protect freshwater ecosystems, are a solution to an environmental problem or if they partially substitute one environmental problem by another, that is, reducing water pollution but increasing greenhouse gas emissions. Although many studies have quantified denitrification in riparian buffer zones in agricultural landscapes, N_2O emissions from buffer zones receiving large nitrate loads have received surprisingly little attention (Groffman et al., 1998, 2000). Accordingly, the current recommended methodologies of the Intergovernmental Panel on Climate Change (1997, p. 4.107) to calculate N_2O emissions from groundwater and agricultural drainage water do not account for possible high N_2O production in riparian buffer zones (Mosier et al., 1998).

The flux of N_2O from soils can be due to nitrification or denitrification (Stevens et al., 1997). Generally, denitrification and nitrifier denitrification

(Wrage et al., 2001) are believed to be of major importance in the emission of N_2O (Firestone et al., 1980; Knowles, 1982; Robertson and Tiedje, 1987). Nitrous oxide is an obligate intermediate in denitrification and more than 5% of the gaseous end-product is N_2O, whereas N_2O production from nitrifying bacteria is less than 1% of the oxidized NH_4^+ (Lipschultz et al., 1981). However, soil incubation studies have shown that nitrification can be the dominant process producing N_2O in aerobic soils (Stevens et al., 1997). Due to the predominantly wet conditions and the high nitrate and low ammonium inputs via the shallow groundwater, nitrification would contribute little to N_2O emissions from riparian buffer zones. Nevertheless, high mineralization rates in aerobic parts of riparian buffer zones may enhance nitrification and it is thus also important to quantify the relative contribution of nitrification to the total N_2O flux.

There is considerable uncertainty about the ecosystem properties and environmental conditions affecting N_2O production. The N_2O production depends on a number of factors, such as nitrate concentration, anoxia, pH, temperature, organic matter availability, and microbial populations (Blackmer and Bremner, 1978; Firestone et al., 1980; Firestone and Davidson, 1989; Weier et al., 1993; Van Cleemput, 1998). The effect of the individual parameters on N_2O production by denitrifiers is quite well understood. In general, N_2O production is relatively higher under conditions that are suboptimal for denitrification. However, under field conditions there is a simultaneous and interactive influence of different factors, resulting in a great variability. It is therefore difficult to predict which circumstances are enhancing or limiting N_2O emissions. Insight into the ways in which soil variables control the absolute amounts of N_2O emitted is, however, crucial to determine risks of greenhouse gas emissions in zones with a high potential for N transformations.

In this study we quantified N_2O emissions from a forested and a grassland riparian buffer in an agricultural landscape with high nitrate loading. Specifically, this study aimed to (i) assess the significance of N_2O emissions from natural riparian buffer zones in agricultural landscapes; (ii) assess the relative role of denitrification and nitrification as sources of N_2O emission from riparian buffer zones receiving large loads of N from adjacent agricultural fields; (iii) reveal the environmental factors controlling N_2O emission from these ecosystems; and (iv) evaluate the emission factors measured in the perspective of the Intergovernmental Panel on Climate Change emission factors for indirect agricultural sources. Results from this study may contribute to the knowledge base for a future risk assessment of the emissions of N_2O from riparian buffer zones.

Material and Methods

Research Sites

Two riparian buffer zones along first-order streams were selected on the sandy Pleistocene deposits in the eastern part of the Netherlands (52°3' N, 6°5' W). One of the buffer zones was covered with a natural alder (*Alnus glutinosa* (L.) Gaertn.) ("forested riparian zone") (Hefting and De Klein, 1998). The grassland buffer zone was dominated by reed meadow grass (*Glyceria maxima* (Hartm.) Holmb.). Both riparian buffer zones border intensive agricultural fields planted with maize (*Zea mays* L.). Nitrate loading rates were high, with a lateral input flux of approximately 630 g N m^{-2} yr^{-1} into the forested buffer zone and a lateral input flux of 270 g N m^{-2} yr^{-1} into the grassland buffer zone in 1999 (Sabater et al., 2003). High nitrate loading rates in the forested zone hamper the N_2 fixation by *Frankia* strains in alder stands (Wolters et al., 1997).

Soils in the two riparian buffer zones were classified as Entisols at the upland riparian boundary and Histosols close to the stream. In both riparian buffer zones a tertiary clay layer containing the mineral glauconite was found at 1 to 3 m below the soil surface. The study area is underlain by a glacial moraine that has gentle slopes facing west and east. These slopes consist mostly of impermeable glacial till covered by a thin layer of aeolien sand. Due to the position of the glacial till layer, infiltrated rainwater quickly drains toward the permanent streams. These first-order permanent streams have narrow streambeds (0.5-1.5 m) positioned in relatively wide valleys (20-50 m) created by the erosive force of the melting water. This typical geomorphologic situation created optimal conditions for riparian buffer zone research due to the confined layer and the relatively wide riparian buffer zone compared with the stream size.

Groundwater monitoring

A grid of dipwell piezometers (five transects with at least three piezometers, at 10-m intervals) were installed in both study sites over an elevational gradient from the agricultural fields toward the stream. Piezometers were installed into the phreatic groundwater with depth ranging from 0.5 to 3.5 m (depending on the depth of the impermeable clay layer). Tubes were perforated along the bottom 50 cm. Groundwater levels were recorded fortnightly from mid-January to mid-November 2000, and water samples were taken seasonally for water quality analyses. Piezometers were emptied prior to water sampling to remove the standing water. Water samples were filtered in the laboratory using glass fiber filters (GF 52; Schleicher & Schuell, Dassel, Germany) before colorimetric analysis of NO_3^- and NH_4^+ using a continuous-flow autoanalyzer (SA-40; Skalar Analytical BV, Breda, the Netherlands). Water samples were kept

at 4°C during transport and storage, and were analyzed for NO_3^- and NH_4^+ within 24 h of sampling.

Experimental setup

Measurements on N process rates and controlling variables were performed in February, May, August, and November 2000. The riparian buffer zones were divided into three strips, which were each parallel to the stream. A strip that formed the boundary between the stream and the riparian buffer zone will hereafter be called the "stream border". The strip that was established approximately midway within the riparian buffer zone will be called the "intermediate strip". The third strip, indicated hereafter as "field border," was located just inside the buffer zone and downslope of the agricultural field. In each strip, eight permanent frames were installed upon which flux chambers could be attached for N_2O flux measurements. In line with the technical layout of the field analyzer, the frames of each strip were placed in two groups of two frames and one group of four frames. For statistical analysis these groups were interpreted as pseudo-replications and average values of emissions were used, resulting in three replicates per strip.

Because of the destructive sampling method, intact cores for the denitrification assay were collected within 1 m^2 of the position of the frames. For the denitrification assay, three cores were collected near each of the three groups of frames in each strip. The three cores from each sampling station were pooled, resulting in three replicates per strip, in analogy with the gas flux measurements.

Nitrous oxide emission measurements

Fluxes of N_2O were measured between 09:00 and 16:00 h using vented, closed flux chambers with an inner diameter of 15.2 cm and a height of 24.2 cm. The flux chambers were attached to preinstalled, permanent frames to minimize disturbance of the soil structure. These frames did not to hamper the groundwater flow, because of their perforation and the dominant vertical groundwater flow in the topsoil due to upwelling. Emissions were measured continuously with 2-min intervals, with two gas samples per flux chamber, over a period of approximately 1 h using a multisampler and a photoacoustic (spectroscopic) infrared gas analyzer (Brüel & Kjær, Nærum, Denmark). Emission rates were calculated from the increase in N_2O concentration over time using linear regression analysis. Besides N_2O emissions, measurements on CH_4 and CO_2 emissions were performed. Carbon dioxide measurements were used to correct for a possible interference between N_2O and CO_2. Soil temperature was measured immediately after the flux chamber was removed, at a depth of 10 cm, using an Eijkelkamp soil thermometer. Pore water was

collected next to each flux chamber using Rhizon samplers (Rhizon SMS, 10 cm; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Pore water was analyzed for NH_4^+ and NO_3^- as described above.

Denitrification, nitrification and soil properties

"Actual" denitrification was measured using an intact core incubation method with acetylene inhibition (Yoshinari and Knowles, 1976; Ryden et al., 1987). Three 10-cm-deep cores with a diameter of 3.5 cm were collected near each ring, wrapped in perforated aluminum foil, and placed in 1-l preservation jars. The jars were closed with a glass lid containing a rubber gasket and fitted with two rubber septa. In the laboratory, jars were flushed with N_2 for 5 min, leaving the lid open to remove accumulated N_2O and to lower the oxygen content considerably to make conditions more comparable with the soil atmosphere. At the start of the incubation, jars were amended with acetone-free acetylene to bring the soil atmosphere concentration to 10 kPa (10% v/v) acetylene and 90 kPa air. Samples were incubated at average field temperature, and denitrification rates were calculated as the rate of N_2O accumulation in the head space between 1 and 4 h. Gas samples were analyzed directly via a gas chromatograph (Model 3300; Varian, Palo Alto, CA) equipped with an electron capture detector (ECD ^{63}Ni) and Porapak Q columns (2-m-long packed columns; Alltech Associates, Deerfield, IL). Nitrous oxide dissolved in water was taken into account by using the Bunsen coefficient (Wilhelm et al., 1977).

Following completion of the incubation experiments, soil cores from each jar were thoroughly mixed and large stones, roots, and twigs were removed. Soil NH_4^+ and NO_3^- contents were determined after extraction (1 h) of 20 g of fresh soil with 100 ml of 0.4 M KCl. After extraction, the pH of the soil suspension was measured using a pH meter (WTW Measurement Systems, Ft. Myers, FL). The suspension was filtered over a glass fiber filter and the extract was analyzed colorimetrically. Soil moisture content was determined gravimetrically after drying approximately 20 g of fresh soil at 105°C for at least 48 h. Organic matter content was determined by loss on ignition of dry (105°C) ground soil at 550°C for 2.5 h.

Net nitrification rates (0-20 cm) were estimated by measured changes in the NO_3^--N content of soil extracts during 30-d incubations of largely undisturbed topsoil inside in situ buried polyethylene bags (Binkley and Hart, 1989; Eno, 1960).

Statistical analysis

Both denitrification rates and N_2O fluxes were approximately lognormally distributed. Therefore, these data were log transformed before statistical

analysis. Due to large differences in environmental conditions between strips within each riparian buffer zone, the variances of both soil properties and soil processes (i.e. denitrification, nitrification, N_2O emission) were not homogeneously distributed. Unequal variances were still observed after log transformation of the data. To test differences between strips within the buffer zones, nonparametric Kruskal-Wallis or Mann-Whitney U tests were used. In case a significant difference was found with the Kruskal-Wallis test, we used the nonparametric Tukey type multiple comparison Nemenyi test to distinguish differences between strips (Zar, 1998). Variables with a normal distribution and homogeneous variances were tested with analysis of variance (ANOVA) and Tukey post hoc tests. We used a principle component analysis to examine relations between N_2O emission and controlling soil factors. Statistical analysis were performed using SPSS 8.0 for Windows (SPSS, 1997).

Results

Groundwater monitoring

Annual fluctuations in groundwater levels were rather similar at the two study sites (Fig. 1). The water table remained close to the soil surface (-15 to 0 cm) and hardly fluctuated throughout the year within the stream border and intermediate strips. Seasonal variations in groundwater levels were only observed at the field boundary and agricultural upland sites.

Mean groundwater nitrate concentrations over the study period were higher in the field border of the forested buffer zone (23-30 mg N l^{-1}) compared with the field border in the grassland buffer zone (4-9 mg N l^{-1}) (Fig. 2). In the grassland buffer zone the nitrate concentrations in shallow groundwater decreased rapidly; after passing the first 10 m of the buffer zone, the mean nitrate concentration in the groundwater was reduced by 95% to an average of 0.3 mg N l^{-1}. Within the stream border the mean nitrate concentration was reduced to 0.08 mg N l^{-1}. The decrease in nitrate concentration between the field border and the intermediate zone was significant (Kruskal-Wallis, $p=0.008$). In the forested buffer zone there was a more gradual decrease in mean nitrate concentration with a significant decrease between the field border and the stream border (ANOVA, $F=4.727$, $p=0.013$). The large standard errors in nitrate concentrations in the forested buffer zone were caused by a high spatial variation in nitrate concentrations. In contrast to the low nitrate concentrations observed at the stream border of the grassland buffer zone, the mean nitrate concentration in the stream border of the forested buffer zone is approximately 10 mg N l^{-1}.

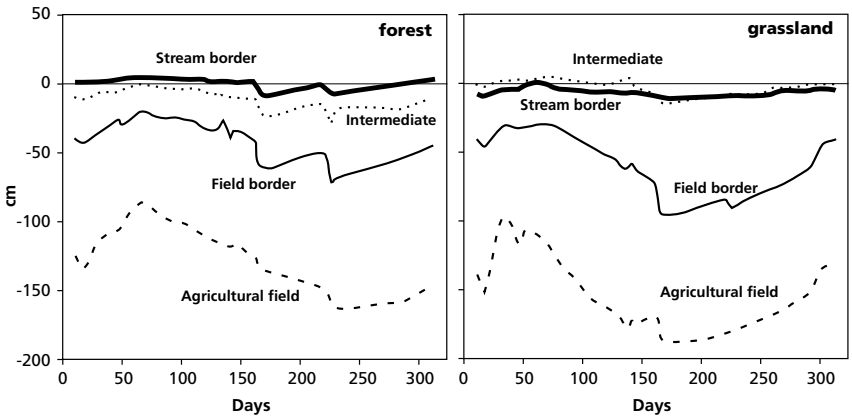

Figure 1 Water table levels relative to the soil surface in strips parallel to the stream in the forested and grassland riparian buffer zones. Water table level was recorded from 11 Jan. 2000 to 13 Nov. 2000. Values are means; n=5.

No significant seasonal variation was measured in the mean nitrate concentration (Fig. 2), and no response of nitrate concentrations to agricultural practice in the adjacent farmlands (e.g. manure application, plowing, harvesting) was observed. This constant nitrate loading was consistent with earlier monthly measurements on groundwater quality in 1998 and 1999 (unpublished data). The NH_4^+ concentrations in the shallow groundwater were low in both riparian buffer zones, with mean concentrations of 0.3 mg N l^{-1}. Apart from concentrations of N in the shallow groundwater, we calculated the mean loading rates using the concentrations, the hydraulic conductivity (approximately 0.5 m d^{-1}), and the hydrological gradient for each riparian buffer zone. During the course of this experiment nitrate loading rates were high, with a lateral input flux of 467 g N m^{-2} yr^{-1} into the forested buffer zone and a lateral input flux of 192 g N m^{-2} yr^{-1} into the grassland buffer zone. Ammonium inflow rates were negligible.

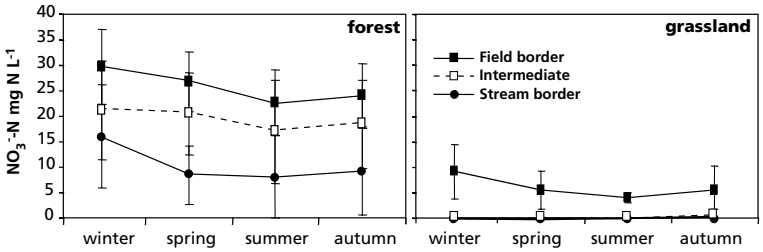

Figure 2 Nitrate concentrations in the shallow groundwater in three strips parallel to the stream in the forested and grassland riparian buffer zones. Values are means with standard errors; n=5.

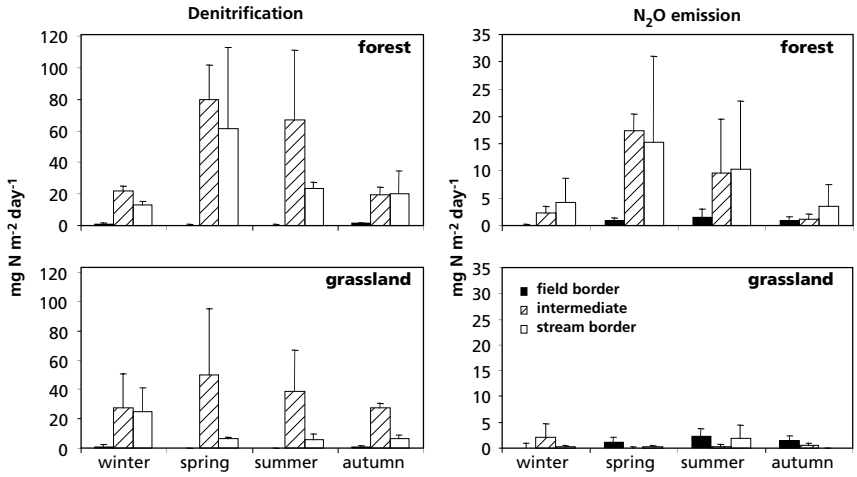

Figure 3 Denitrification in strips parallel to the stream in the forested and grassland riparian buffer zones, and N_2O emission in zones parallel to the stream in the forested and grassland riparian buffer zones. Values are means with standard errors; n=3. Note differences in scale between the denitrification and N_2O emission graphs.

Denitrification, nitrification and nitrous oxide emissions

High denitrification activities were measured in both buffer zones, with average rates as high as 80 mg N m^{-2} d^{-1} (Fig. 3). Denitrification rates did not differ significantly between the forested and the grassland buffer zones, and there was no significant effect of season. However, rates seemed to follow a seasonal trend, with higher rates in spring and summer. This trend was not significant for the whole riparian zone due to the high variation in the denitrification measurements (Fig. 3; Table 1), but a significant seasonal effect was observed within the intermediate strip of the forested zone (Kruskal-Wallis, p=0.036). Significant differences were observed for denitrification activity between the field border (with low rates) and the intermediate strip and the stream border (with much higher activities) (Fig. 3; Table 2). Maximum denitrification rates were measured in the intermediate strip, particularly in local upwelling areas where groundwater with high nitrate concentrations

Table 1 Results of two-way analysis of variance (ANOVA) for each process with zone and season as main effects.

	Buffer zone			Season			Buffer zone × season		
	df	F	p	df	F	p	df	F	p
Ln (denitrification)	1	2.477	0.120	3	0.198	0.897	3	0.615	0.608
Ln (nitrification)	1	0.235	0.629	3	1.088	0.361	3	0.481	0.697
Ln (N_2O emission)	1	11.923	0.001*	3	2.041	0.117	3	1.465	0.233

* Significant main effect.

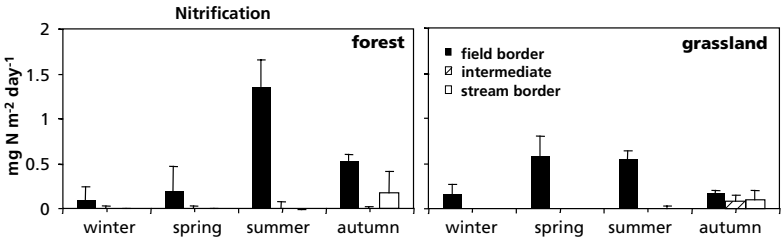

Figure 4 Net nitrification in strips parallel to the stream in the forested and grassland riparian buffer zones. Values are means with standard errors; n=3.

discharged to the surface. The spatial pattern of net nitrification rates was opposite the pattern of denitrification. In both riparian buffer zones nitrification differed significantly between the strips and nitrification was almost exclusively restricted to the field border, while rates were negligible in the intermediate and stream border strips (Fig. 4; Table 2). Net nitrification rates did not differ significantly between the forested and grassland buffer zones (Fig. 4; Table 1) and rates were very low compared with the rates of denitrification, except in the field border where nitrification and denitrification were in the same order of magnitude. No significant seasonal effects were found in the nitrification activity (Fig. 4; Table 1).

In contrast with denitrification and nitrification rates, N_2O emissions were significantly different between the grassland and the forested buffer zone. Nitrous oxide emissions from the grassland buffer zone were seven times lower compared with the emissions from the forested site (Fig. 3; Table 1). Emissions were not significantly affected by the measurement time during the day. A seasonal trend, with higher emissions in spring and summer, comparable with the trend observed in denitrification rates, was found for the measured emissions in the forested buffer zone. The patterns were, however, not significant. In the grassland buffer zone no obvious differences could be observed in N_2O emission rates between the seasons (Fig. 3). A spatial pattern

Table 2 Results of a Kruskal-Wallis nonparametric test with differences between strips within the two riparian zones.

	Forest riparian zone			Grassland riparian zone		
	n	χ^2	p	n	χ^2	p
Denitrification	36	24.794	< 0.0001*	36	24.222	< 0.0001*
Nitrification	36	14.468	0.001*	36	18.743	< 0.0001*
N_2O emission	36	4.749	0.093	36	3.754	0.153

* Significant effect.

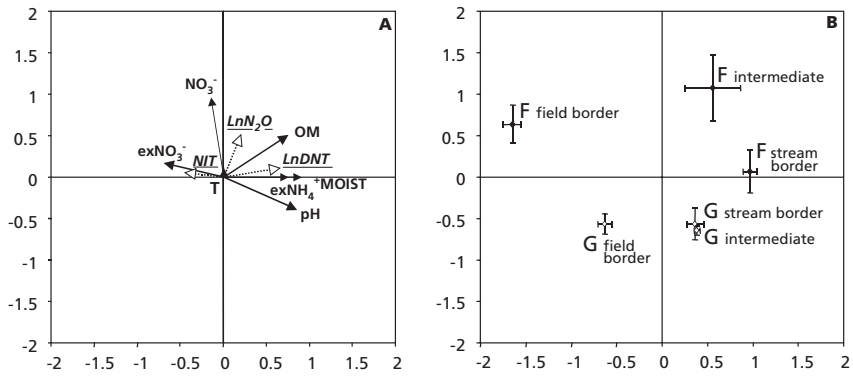

Figure 5 **(A) Correlation bi-plot from the standardized principal component analysis (PCA) on soil variables. Components are given in Table 3. Correlations of the soil variables with the main axes are given by arrows. The term NO_3^- is the nitrate concentration in soil water, OM is the organic matter content, MOIST is the moisture content, and $exNO_3^-$ and $exNH_4^+$ are the amounts of extractable nitrate and ammonium in the soil. Correlations with soil processes are given by dotted arrows and underlined abbreviations. The term LnDNT stands for denitrification, LnN_2O is N_2O emission, and NIT is the nitrification. (B) Correlation bi-plot from the standardized PCA with cluster centroids for the strips parallel to the stream in each of the two riparian buffers. The term F indicates the forest buffer zone, and G indicates the grassland buffer zone. Subscripts indicate the strip.**

similar to the denitrification activity, with lower rates in the field border strip compared with intermediate and stream border strips, was observed for N_2O emission rates. However, differences were not significant, probably due to the large standard errors and the contribution of nitrification to N_2O emission in the field border strip (Fig. 3; Table 2). In the grassland buffer zone, the relatively low N_2O emission showed no spatial pattern, neither from Fig. 3 nor statistically.

Environmental controls

A principal component analysis (PCA) on soil variables for both riparian buffer zones resulted in three components with eigenvalues larger than 1, which explained 76% of the total variance (Table 3). A correlation bi-plot of the results from a standardized PCA on the soil variables for both riparian zones is given in Fig. 5 A. Additional correlations of the soil N transformation processes with the main components are included. Cluster centroids (average score on each component, with standard errors) for the sites are presented in Fig. 5 B. As can be seen from the bi-plot, some soil variables were strongly interrelated (arrows pointing in the same direction). The first component explained about 43% of the observed variance. Several soil variables were significantly correlated with this first component, namely soil moisture, organic matter, and pH, but also extractable NH_4^+ (Table 3). The second component explained about 18% of the observed variance and the only "soil" variable that

Table 3 Results of a principal component analysis (PCA) of the soil variables and nitrate in pore water.

Variable	Abbreviation	Component 1	Component 2	Component 3
Moisture	Moist	0.892*	0.012	-0.143
pH extract	pH	0.815*	-0.394	0.036
Extractable NH_4^+	$ExNH_4^+$	0.748*	-0.026	0.119
Organic matter	OM	0.734*	0.515	-0.129
NO_3 in pore water	NO_3^-	-0.156	0.896*	-0.053
Extractable NO_3^-	$ExNO_3^-$	-0.660*	0.171	-0.035
Temperature	T	0.015	0.055	0.981*
Variance explained, % of total		43.3	17.7	14.5

*** Significant correlation ($p<0.001$).**

correlated strongly with this component was the nitrate concentration in the pore water (Table 3). The third component explained 15% of the variation and only correlated significantly with soil temperature (Table 3). This third component can be seen as influenced by the seasonal variation. It is clear from the cluster centroids that the grassland and forested buffer zones were generally separated by the second component (Fig. 5 B), which was strongly associated with the nitrate concentration of the pore water (Fig. 5 A). Nitrate concentrations in the pore water were significantly lower in the grassland buffer zone (mean=2.8 mg l^{-1}) compared with the forested buffer zone (mean=15 mg l^{-1}) (one-way ANOVA, $F=19.534$, $p<0.001$). The three strips within the riparian buffer zones were clearly separated by the first component, and only partly by the second (forested stream border strip). The stream border and intermediate strips differed considerably in the forested buffer zone while in the grassland buffer zone the same strips were not significantly different.
A forward regression between PCA components and N transformation processes showed that denitrification was significantly related to the first component, and dominantly influenced by the soil variables influencing this component (i.e. moisture and/or oxygen status, pH, and extractable N compounds of the soil). Nitrous oxide emission was significantly correlated with the second component (i.e. the nitrate concentration of the pore water) (Table 4). The net nitrification activity was related to the first and third component (i.e. associated with moisture status, pH, extractable N compounds, and the soil temperature). The dominant effect of soil moisture content on both denitrification and nitrification activity is also illustrated in Fig. 6. A lower soil moisture content (less than 0.5, as occurred at the field border) limits denitrification rates but stimulates the net nitrification (Fig. 5 A, 6).

We also performed PCA analyses for each buffer zone separately (data not shown). The results of the within-zone PCA were largely comparable with those for both riparian buffer zones. Nitrous oxide emissions were, however,

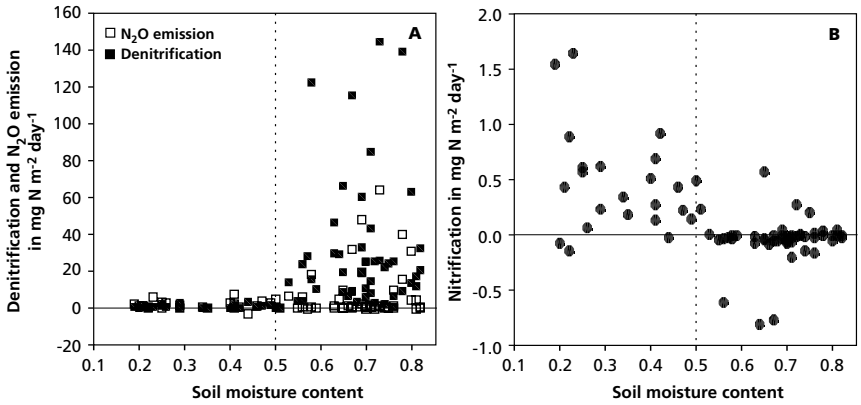

Figure 6 (A) Denitrification and N_2O emission versus soil moisture content in the two riparian buffer zones. (B) Net nitrification versus soil moisture content in the two riparian buffer zones.

not as strongly regulated by nitrate concentrations in the forested buffer zone compared with the grassland buffer zone, which had a lower nitrate loading rate. Results of forward regression illustrate that the nitrate concentrations in the pore water explained less than 10% of the variability in N_2O emission in the forested buffer zone, whereas nitrate concentration explained more than 23% of N_2O emission in the grassland buffer zone. In contrast to the overall PCA, soil moisture content did play a significant role controlling N_2O emissions in the forested zone. Fluxes from the forested buffer zone largely determined the significant bivariate relation found between N_2O emissions and soil moisture (Fig. 6).

Discussion

Because of the high nitrate loading found in shallow groundwater in Dutch agricultural landscapes, we had suspected that riparian buffer zones protecting freshwater ecosystems could potentially contribute to atmospheric

Table 4 Results from a forward regression of process rates versus the principle components of soil variables (see Table 3).

Dependent variable	Component	Slope	R^2	F	p
Ln (denitrification)	1	0.617	0.372	43.008	< 0.0001*
Ln (N_2O)	2	0.489	0.229	22.058	< 0.0001*
Nitrification					
Model 1	1	-0.410	0.156	14.119	< 0.0001*
Model 2	3	0.290	0.230	11.607	< 0.0001*

* Significant effect.

contamination by having high rates of N_2O emissions. Studies on N_2O emissions from seminatural ecosystems and temperate fen ecosystems and the few existing studies on riparian zones show emissions within a range of 0.1 to 5.3 kg N_2O-N ha^{-1} yr^{-1} (Weller et al., 1994; Augustin et al., 1996; Groffman et al., 1998; Skiba et al., 1998; Sozanska et al., 2002). The N_2O fluxes found in this study for the grassland buffer zone were within this range with 2 to 4 kg N ha^{-1} yr^{-1}. However, N_2O fluxes found in the forested buffer zone were much higher with a yearly average emission of 20 kg N ha^{-1} yr^{-1}. Particularly high fluxes were found in the intermediate and stream border strips in the forested buffer zone, a yearly average of 30 kg N ha^{-1} yr^{-1} and local spots exceeding rates of 100 mg N m^{-2} d^{-1}. Fluxes up to 26.9 kg N ha^{-1} yr^{-1} were found by Merbach et al. (2001) in a drained alder swamp. Higher annual fluxes have only been measured in specific cases with direct fertilization, for instance on grazed fertilized peaty grasslands (36-42 kg N ha^{-1} yr^{-1}; Velthof et al., 1996) or in subtropic fertilized mires with a maximum flux of 157 kg N ha^{-1} yr^{-1} (Duxbury et al., 1982). The relatively high emissions found in the forested zone clearly confirm the risk of "natural" riparian zones in nitrate-loaded agricultural landscapes as a significant source of greenhouse gas emission. This risk is, however, a relatively recent phenomenon, since historical records from 1944 indicate that this riparian zone used to be a low-productivity natural ecosystem with species-rich plant communities (Westhoff and Jansen, 1990).

In the literature, N_2O emissions are reported to be controlled by the availability of mineral N, soil temperature, and soil water content (Skiba et al., 1998; Van Cleemput, 1998; Heincke and Kaupenjohann, 1999). These controlling variables are universal, but operate in different combinations and in different orders of importance in both space and time (Skiba and Smith, 2000). In our study, the difference in N_2O fluxes between the two study sites could mainly be explained by nitrate availability. Although the input of nitrate via shallow groundwater in the grassland zone was certainly not low, nitrate concentrations in the pore water of the topsoil were significantly lower in the grassland than in the forested buffer zone. Lower nitrate concentrations in the grassland buffer zone were probably caused by dilution of the nitrate-enriched shallow groundwater with deeper seepage water and possibly by dilution with recharge water from the channel in dry summer periods (Chapter 2; Sabater et al., 2003). Lower nitrate concentrations in the pore water limited N_2O emission rates in the grassland buffer zone, as indicated by the PCA analysis. In the forested buffer zone soil moisture content also played a role in controlling N_2O emissions. In many studies (Heincke and Kaupenjohann, 1999), a positive relation was found between moisture content and N_2O emissions. High water contents with increasingly anoxic conditions stimulate denitrification activity and thus facilitate N_2O production (Davidson and Firestone, 1988; Schnabel

and Stout, 1994). On the other hand, high soil moisture content will also increase the residence time of N_2O in the soil, by restricting diffusion, and may consequently enhance the reduction of N_2O to nitrogen gas (Blicher-Mathiesen and Hoffmann, 1999; Jacinthe et al., 2000). Due to the microbial preference for the reduction of nitrate above N_2O, the further reduction of N_2O would only be prominent in soil solutions that are relatively low in nitrate (Davidson and Swank, 1986; Arah et al., 1991). This can partly explain the lower N_2O fluxes found in the grassland riparian zone.

Although N_2O fluxes from the forested riparian buffer zone were high, data from Rusch and Rennenberg (1998) indicate that our flux data underestimated N_2O emissions. They showed that alder trees, which were dominant in our forested riparian zone, could mediate N_2O emissions from the soil to the atmosphere by an efflux from the stem. This efflux was not taken into account with our flux chamber setup, while a possible plant-mediated transport of N_2O oxide via aerenchyma of reed meadow grass in the grassland buffer zone was included (Mosier et al., 1990; Yan et al., 2000).

Denitrification was most certainly the major source of N_2O emission from the wet strips in both riparian areas. The significantly lower denitrification activity in the field border was closely related to the lower soil moisture contents in this zone. The relatively higher N_2O fluxes compared with the denitrification found in the field border (Table 2) are consistent with results from Webster and Hopkins (1996) and may have been caused by a combination of two processes. First, the denitrification end-product is known to shift toward N_2O when the soil oxygen status is less favorable for denitrification (Reddy et al., 1989; Jacinthe et al., 2000). Second, an additional N_2O flux can occur from the nitrification activity in the aerobic field border strip (Stevens et al., 1997). Net nitrification rates measured in this zone were in the same order of magnitude as the low denitrification rates; however, net nitrification is the difference between nitrification, immobilization, and denitrification and can only be used as a "qualitative" measure. Gross nitrification rates can exceed net rates by an order of magnitude (Verchot et al., 2001). Burt et al. (1999) also found that nitrification is the major source of N_2O emission in aerobic soils with a relatively low absolute N_2O emission. The distinct pattern of spatially decoupled denitrification and nitrification activity observed in this study (Fig. 3, 4) was probably influenced by groundwater level. As shown in Fig. 1, the average groundwater level in the intermediate and stream border strips was continuously close to the soil surface, resulting in anaerobic soil conditions, conductive to denitrification, whereas the groundwater level in the field border strip was lower, which resulted in aerobic soil conditions that stimulate nitrification. The relation between N process rates and soil moisture

content also illustrates this pattern (Fig. 6). Even though nitrification might occur in aerobic spots in the wet soils, we presume that the nitrification activity is very limited and nitrification is an insignificant source of N_2O under these water-saturated conditions. This presumption is confirmed by the negative relation between nitrification and extractable NH_4^+ (Fig. 5 A), which is due to the accumulation of NH_4^+ (originating from mineralization) under these wet soil conditions. These results are consistent with an Europe-wide study demonstrating the key role of the groundwater table depth in soil N cycling processes in riparian zones (Chapter 5; Pinay and Burt, 2001).

The seasonal trend in denitrification with higher rates in spring and summer in the wet strips (Fig. 3) did not correspond to the trends observed in studies by Burt et al. (1999) and Haycock and Pinay (1993), who found higher denitrification rates in autumn and winter under comparable climatic conditions. The observed difference in seasonal dynamics can probably be attributed to the higher availability of nitrate in the Dutch sites, decreasing the competition between denitrifiers and vegetation in summer, and the stable high groundwater level in the Dutch sites providing permanent optimal conditions for denitrification in the topsoil (specifically in the intermediate strips). Consequently, denitrification rates will then be influenced by temperature following the Arrhenius equation (Maag and Vinther, 1996).

Apart from the hazardous N_2O emissions from buffer zones that receive high N inputs, and in spite of beneficial nitrate removal from the shallow groundwater, concentrations will in some cases not be reduced strongly enough to prevent eutrophication of the surface waters. This phenomenon was observed in our forested riparian buffer zone with a rather high average nitrate concentration of 10 mg N l^{-1} close to the stream. Thus, a realistic evaluation of the total environmental effect of riparian zones is needed. Results from this study indicate that riparian buffer zones that receive large nitrate loads may not fully protect the stream ecosystem.

Current methodologies of the Intergovernmental Panel on Climate Change (1997, p. 4.107) to calculate national N_2O emission from indirect (agricultural) sources do not account for N_2O production in riparian buffer zones. We expected that the emission factor from groundwater (EF5-g) underestimates the indirect N_2O emission from riparian buffer zones in Dutch agricultural landscapes with high nitrate concentrations in the shallow groundwater and suboptimal soil temperatures. The EF5-g calculated from this study, on the basis of N_2O flux measurements and the yearly incoming NO_3^- flux in the groundwater, ranges from 0.028 to 0.058 in the forested riparian zone and from 0.016 to 0.031 in the grassland riparian zone. The ranges found in this

study are significantly higher than the proposed 0.015 (EF5-g). Due to the high solubility of N_2O in water, additional research is needed to distinguish between transported and locally produced N_2O. However, on the basis of our denitrification measurements, we conclude that there is a significant amount of N_2O production in riparian buffer zones and, depending on the surface area of riparian buffer zones within agricultural landscapes, these areas can significantly increase the indirect N_2O emissions. Groffman et al. (2000) already suggested that emission factors (EF5-g) based only on N_2O losses from supersaturated concentrations in groundwater and agricultural drainage water are unrealistically low. On the other hand, Nevison (2000) has reevaluated the emission factor and proposed to reduce the EF5-g from 0.015 to 0.001 kg N_2O-N per kg N input because the Intergovernmental Panel on Climate Change agricultural source estimate now significantly overestimates the observed atmospheric increase. In accordance with Groffman et al. (2000) and Nevison (2000), we suggest that the Intergovernmental Panel on Climate Change inventory might be improved by separately considering emission factors for groundwater flowing through riparian areas versus groundwater under upland agricultural fields. As indicated by Groffman et al. (1998, 2000) and Well et al. (2001), there is an urgent need for more data on N_2O emissions from riparian wetland buffer zones to adjust the existing EF5-g emission factor. The results from this study in nitrogen-stressed riparian zones clearly contribute to a more realistic basis for future N_2O emission inventories.

Conclusions

When nitrate loading in riparian buffer zones is high, N_2O is an important end-product of denitrification. In these cases N transformation by buffer zones results in an unfavorable shift from water pollution to an increase in greenhouse gas emission.

Until now, only the beneficial function of riparian zones on water quality improvement has received a lot of attention. To perform a full assessment of riparian ecosystem functioning, however, we have to evaluate the precise consequences of both forms of environmental pollution to determine the environmental risks.

Acknowledgements

The results reported here were collected as part of a European project on nitrate buffer zones, the NICOLAS (NItrogen COntrol by LAndscape Structures in agricultural environments) project, which was funded by the European Commission DG XII (H. Barth, Scientific Advisor), Grant ENV4-CT97-0395. The manuscript was critically reviewed by Jos Verhoeven and Dennis Whigham.

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Chapter 4

Spatial variation in denitrification and N_2O emission in relation to nitrate removal efficiency in an N-stressed riparian buffer zone

with Roland Bobbink and Merlijn Janssens

Abstract

Spatial variability in hydrological flow paths and nitrate removal processes complicates the overall assessment of riparian buffer zone functioning in terms of water quality improvement as well as enhancement of the greenhouse effect by N_2O emissions. This research aimed at quantifying spatial patterns of N_2O emissions associated with nitrate removal in riparian buffer zones. Specifically, we wanted to assess the degree of spatial variability in denitrification and N_2O emissions in habitats where nitrate removal rates were high or low. Denitrification and emissions of N_2O were measured in winter and summer along two groundwater flow paths in a forested riparian zone using flux chambers and incubation experiments. In winter, N_2O emissions were significantly higher (12.4 mg N m^{-2} d^{-1}) along the flow path with high nitrate removal compared with the flow path with low nitrate removal (2.58 mg N m^{-2} d^{-1}). In summer a reverse pattern was observed, with higher N_2O emissions (13.6 mg N m^{-2} d^{-1}) from the flow path with low nitrate removal efficiencies. Distinct spatial patterns of denitrification and N_2O emission were observed along the high nitrate removal transect compared to no clear pattern along the low nitrate removal transect, where denitrification activity was very low. Spatial variability of both denitrification and N_2O emission, expressed as coefficient of variation, was significantly higher (140-233%) in the low nitrate removal transect compared to the high nitrate removal transect (72-109%). Results from this study indicate that spots with a high nitrate efficiency also significantly contribute to an increased N_2O emission from riparian zones.

Introduction

Riparian buffer zones, located at the interface of the terrestrial and aquatic environment, are valued for their capacity to remove nitrate from subsurface agricultural runoff. Denitrification in riparian buffers is often found to be the major process responsible for the nitrate removal (Groffman et al., 1992a; Pinay et al., 1993;Verchot et al., 1997). However, denitrification is known to generate various gaseous end products, i.e. besides N_2, also NO and N_2O can be emitted. N_2O is an intermediate product of the denitrification process and may be emitted in substantial quantities from riparian buffer zones (Groffman et al., 1998). N_2O emission is of serious concern because it is a trace gas that contributes to the enhanced greenhouse effect (Wang et al., 1976). Estimates of the global N_2O budget indicate that soils are globally a major source of N_2O emission with 3.3-9.9 Tg N yr^{-1} for natural soils and 0.6-14.8 Tg yr^{-1} for agricultural soils on a total of 14.9-17.7 Tg N yr^{-1} (IPCC, 2001). Because N_2O is a greenhouse gas with a high global warming potential, it is very important to know whether the enhanced denitrification in nitrate loaded riparian buffer zones leads to harmless emissions of N_2 or produces substantial N_2O. In discussions of the environmental benefits of riparian zones, it is paramount that the risk of N_2O emission is taken into account and weighed against the ecosystem function of water quality improvement.

N_2O is also a by-product of nitrification (Williams et al., 1992) and of nitrifier denitrification (Wrage et al., 2001) and the relative contribution of nitrification, nitrifier denitrification and denitrification to the N_2O emission is still unclear. Although it is impossible to completely unravel the contribution of these different processes in a field experiment, it is likely that denitrification is the major process causing N_2O emissions in long-term nitrate-loaded and permanently wet riparian zones (Groffman et al., 1998, 2000; Hefting et al., 2003a, Chapter 3). Research over a whole range of riparian zones in Europe has revealed an accumulation of ammonium under wet soil conditions with groundwater levels less than 10 cm below the soil surface, indicating that nitrification was marginal under these circumstances (Hefting et al., 2003b, Chapter 5).

In general, denitrification and N_2O emission are regulated by the availability of oxygen, nitrate and organic matter (Martikainen and De Boer, 1993; Martikainen et al., 1993). Theoretically, the factors controlling the relative proportions of the different gaseous end products of denitrification are also well known. All conditions whereby the denitrification process is marginal are favorable for the formation of N_2O rather than N_2 (e.g. temperatures below 4

degrees, pH values below 5.0, low moisture contents and low availability of metabolizable carbon (Knowles, 1982; Wrage, 2003). High nitrate availability usually inhibits or retards N_2O reduction, because nitrate is preferred as an electron acceptor, also resulting in relatively high N_2O emission (Blackmer and Bremner, 1978; Schlegel, 1992; Van Cleemput, 1998). We hypothesized that within the nitrate-loaded riparian buffer zones the relative contribution of the N_2O emission to the total denitrification flux would be significantly higher along flow paths with low nitrate removal efficiency, due to the higher nitrate concentrations and sub-optimal conditions for denitrification, compared with flow paths with a high nitrate removal efficiency.

However, field measurements of denitrification and N_2O emissions have been notoriously complicated because of technical difficulties in measuring denitrification rates and, even more difficult to solve, the large spatial variation in process rates measured (Weller et al., 1994). The large spatial variability in production of N_2 and N_2O in soils is influenced by various environmental factors, which are each subject to spatial variability. Furthermore, spatial variability of gas emissions is aggravated by irregular transport of gaseous products to the soil surface. There is a need for better understanding of the sources of variability and the factors that control denitrification and N_2O emission at the site scale. It would be ideal if we could assess the balance between environmental benefits (water quality improvement) and environmental hazards (N_2O emissions) of buffer zones through detailed spatial information of the factors controlling these processes in a specific riparian ecosystem.

In riparian zones regulating factors are typically spatially distributed, with gradients from the hill slope down towards the stream. In large portions of the riparian zones, soils are permanently wet and rich in organic matter, thus providing optimal conditions for denitrification. As a consequence, the distribution of denitrification and N_2O emission in riparian zones with clear nitrate removal is expected to show a clear spatial pattern (regular or structured variation) in contrast to the almost random variation found for denitrification and N_2O emissions in drained, fertilized soils with suboptimal conditions for these processes (Velthof et al., 1996a,b; Koops et al., 1997; Ball et al., 1997; Van den Pol-Van Dasselaar et al., 1998).

We hypothesized that within riparian zones, the spatial variability of denitrification and N_2O emission would be lower and less random along flow paths which exhibit high nitrate removal compared to flow paths with sub-optimal conditions for denitrification and consequently with low nitrate removal.

In this study, we evaluated the spatial variability in denitrification and nitrous oxide emission in a riparian buffer heavily loaded with diffuse subsurface agricultural runoff, and related the variability in these processes to the spatial variability of controlling factors, i.e. nitrate concentration, water-filled pore space (WFPS), soil pH, organic matter and temperature. Our objective was to find clues for explaining spatial variability in nitrate removal, denitrification and N_2O emission, and to use this insight to help assess the balance between environmental benefits and risks in these habitats. Our approach was that we selected two transects in a riparian woodland receiving high nitrate inputs through agricultural subsurface runoff, along flow paths which clearly differed in intensity of nitrate removal.

Material and Methods

Site description

The study area was located in a riparian buffer zone covered with natural Alder (*Alnus glutinosa* L. Gaertn.) carr in the eastern part of the Netherlands (Hefting and De Klein, 1998). At the upland riparian boundary the buffer bordered an intensive agricultural field planted with maize. Soils in the riparian buffer zones developed in sandy, Pleistocene deposits and would be classified as entisols at the upland-riparian boundary and as histosols close to the stream (USDA[1]). A glacial moraine with gentle slopes, underlied the study area. Due to the position of the glacial till layer, infiltrated rainwater quickly drained towards the permanent first order stream. Nitrate loading rates via the shallow inflowing groundwater were high, up to 200 mg N m^{-2} d^{-1} (730 kg N ha^{-1} yr^{-1}).

Setup of the experimental plots

Two locations were selected in the forested riparian zone on the basis of their difference in nitrate removal efficiencies (Table 1, Chapter 2). In the first location we measured nitrate removal from the shallow groundwater along the flow path, with rates up to 80 mg N m^{-2} d^{-1}. In the second location nitrate concentrations in the shallow groundwater did not decrease significantly along the flow path. In each location a transect was established from the agricultural field to the stream. The transects were 15×4 m and each was divided in 60 (1×1 m) grid cells. The transect along the flow path without nitrate removal is indicated hereafter as LR (Low Removal) transect and the other transect with clear nitrate removal as the HR (High Removal) transect. Measurements on soil processes and environmental parameters were performed twice for each flow path, once in winter and once in summer, to obtain insight in spatial patterns in different seasons. With this sampling design we did not aim to

quantify annual N_2O fluxes from the riparian zone, only differences in spatial patterns at times of low and high microbial activity. The winter sampling was performed on 27, 28 February for the LR transect and on 12, 13 March 2001 for the HR transect. Measurements for the summer sampling were performed on 17, 18, 31 July and 01 August 2001 for the LR and HR transect respectively. Flux measurements were performed in the center of each grid cell and soil samples were collected within the measurement frame (after removal of the flux chambers), for laboratory measurements of denitrification and other soil parameters. All parameters measured in the same grid cell were considered to be spatially linked. N_2O emissions and soil variables were measured on the first measurement day, denitrification was measured the day after. Due to the destructive sampling of soil cores, transect was relocated 25 cm downstream (parallel to the stream) before we conducted the summer sampling.

N_2O emission and denitrification measurements

Fluxes of N_2O were measured between 9:00 and 16:00 h each sampling date using vented, closed flux chambers with an inner diameter of 15.2 cm and a height of 24.2 cm. No significant variation in time was observed within the sampling period. The flux chambers were attached to pre-installed, perforated frames to minimize disturbance of the soil structure. Twelve flux chambers were measured simultaneously using a multisampler attached to a photo-acoustic infrared gas analyzer (Bruel and Kjaer, Danmark). The measurement regime was stratified random, with four strata and three flux chambers in each stratum. Within each flux chamber, three gas samples were taken over a period of 1 h. Emission rates were calculated from the increase in N_2O concentration over time using linear regression analysis. Besides N_2O emissions, measurements on CO_2 emissions were performed. CO_2 measurements were used to correct for a possible interference between N_2O and CO_2.

Denitrification was measured using an intact core incubation method with acetylene inhibition (Yoshinari and Knowles, 1976; Ryden et al., 1987). Three 10 cm deep cores with a diameter of 3.5 cm were taken inside each measurement frame. Earlier studies, both on actual and potential denitrification activity, showed a sharp decrease of activity with depth (Pinay and Burt, 2001; Consandey et al., 2001; Chapter 7). Cores were wrapped in perforated aluminum foil and placed in 1-liter preservation jars. The jars were closed with a glass lid containing a rubber gasket and fitted with two rubber septa. In the laboratory, jars were flushed with N_2 for 5 minutes, leaving the lid open to remove accumulated N_2O and to lower the oxygen content to a level comparable to the soil atmosphere. At the start of the incubation, jars were amended with acetone-free acetylene to bring the soil atmosphere

concentration to 10 KPa (10% v/v) acetylene and 90 KPa air. Samples were incubated at average field temperature, and gas samples were taken from the head space after 1 and 5 h. Gas samples were stored in Venoject tubes (Terumo, Leuven Belgium) for a maximum period of 5 days. Gas samples were analyzed via gas chromatography (GC Hewlett Packard 5890) equipped with an electron capture detector (ECD ^{63}Ni) and Hayesep Q columns. N_2O dissolved in water was taken into account by using the Bunsen coefficient (Wilhelm et al., 1977).

Soil and pore water measurements

We measured nutrient concentrations in interstitial water, soil pH, soil temperature, organic matter content, soil moisture content, bulk density and extractable nitrogen compounds in each grid cell. In addition to measuring these variables to determine their relationship to N_2O emissions under field conditions, the data was also used to gain insight into the small scale variability of soil parameters. Soil temperature was measured, immediately after the flux measurement, at a depth of 10 cm using an Eijkelkamp soil thermometer. Interstitial water was collected next to each flux chamber using Rhizon samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment Giesbeek, the Netherlands). Pore water was analyzed for pH (WTW) and NH_4^+ and NO_3^- content using a continuous-flow auto-analyzer (SKALAR, Breda, the Netherlands). Following completion of the denitrification experiments, soil cores from each jar were thoroughly mixed, and large stones, roots and twigs were removed. Soil NH_4^+ and NO_3^- contents were determined after extraction (1h) of 20 g of fresh soil with 100 ml of 0.4 M KCl. After extraction, the pH of the soil suspension was measured using a (WTW) pH meter. The suspension was filtered over a glass fiber filter and the extract was colorimetrically analyzed using a continuous-flow auto-analyzer (SKALAR). Soil moisture content was determined gravimetrically after drying approximately 20 grams of fresh soil at 105°C for at least 48 hours. Water-filled pore space (WFPS) was calculated from the moisture content and data on bulk density analog to De Klein and Logtestijn (1996). Organic matter content was determined by loss on ignition of dry ground soil at 550°C for 2.5 hours.

Calculation of the N_2O:N_2 ratio of the denitrification end product

The N_2O to N_2 gas ratio of denitrification end-products was calculated using estimates of N_2 emissions calculated as the difference in N_2O produced between acetylene amended cores and flux measurements from the field ($N_2 = N_2O_{incubation} - N_2O_{field}$) (Ryden et al., 1979; Weier et al., 1993). Due to the possible contribution of nitrification to the N_2O emission in the drier hills slope area of the riparian zone, the calculation of N_2O emission to the total N removal by denitrification was restricted to the permanently wet

Table 1 Nitrate concentration, loading and removal in the experimental transects.

Transect (code)	Low nitrate removal (LR)		High nitrate removal (HR)	
Season	Winter	Summer	Winter	Summer
NO_3^- inflow mg N l^{-1}	62.28	57.41	36.31	31.95
NO_3^- loading mg N m^{-2} d^{-1}	194.1	209.6	155.5	93.5
NO_3^- removal mg N m^{-2} d^{-1}	0.95	41.1	37.1	80.3
NO_3^- removal %	0.49	18.7	31	85.9
Flow rate (m^3 d^{-1})	0.023	0.03	0.026	0.024

floodplain were N_2O emission from nitrification activity is assumed to be insignificant (Chapter 3; Chapter 5). Only positive N_2O fluxes were included in the calculation.

Statistical analysis

All data were tested for normality and homogeneity of variance. If data did not meet the requirements they were transformed before statistical analysis. Denitrification and N_2O fluxes were approximately lognormally distributed. Variables were tested using Pearson correlation and ANOVA. To minimize the problems with the highly correlated variables (multicollinearity, Draper and Smith, 1981) a principle component analysis with varimax rotation was used on soil variables to cluster highly correlated variables to obtain independent components. The PCA components were related to the process rates and the N_2O:N_2 ratio using a forward multiple regression. All statistical analysis were performed using SPSS 8.0 for Windows (SPSS 1997, Chicago, Illinois, USA).

Results

Denitrification

The denitrification activity was significantly different between the two transects (Table 2, 3), with higher rates in the HR transect. No significant difference in denitrification activity were observed between seasons, but a trend with higher activities during the summer measurements was found in the HR transect (Table 2; ANOVA, $F=3.692$, $p=0.057$, $n=59$). Spatial patterns in denitrification also differed significantly for the two transects. Denitrification in the LR transect was mainly concentrated in the near stream zone, whereas denitrification in the HR transect was measured over the entire width of the riparian buffer (Fig. 1). However, in both transects the denitrification rate in the upper part of the riparian zone bordering the agricultural field was negligible to non-existent. The portion of the transects with insignificant denitrification activity was greater in the transect with a

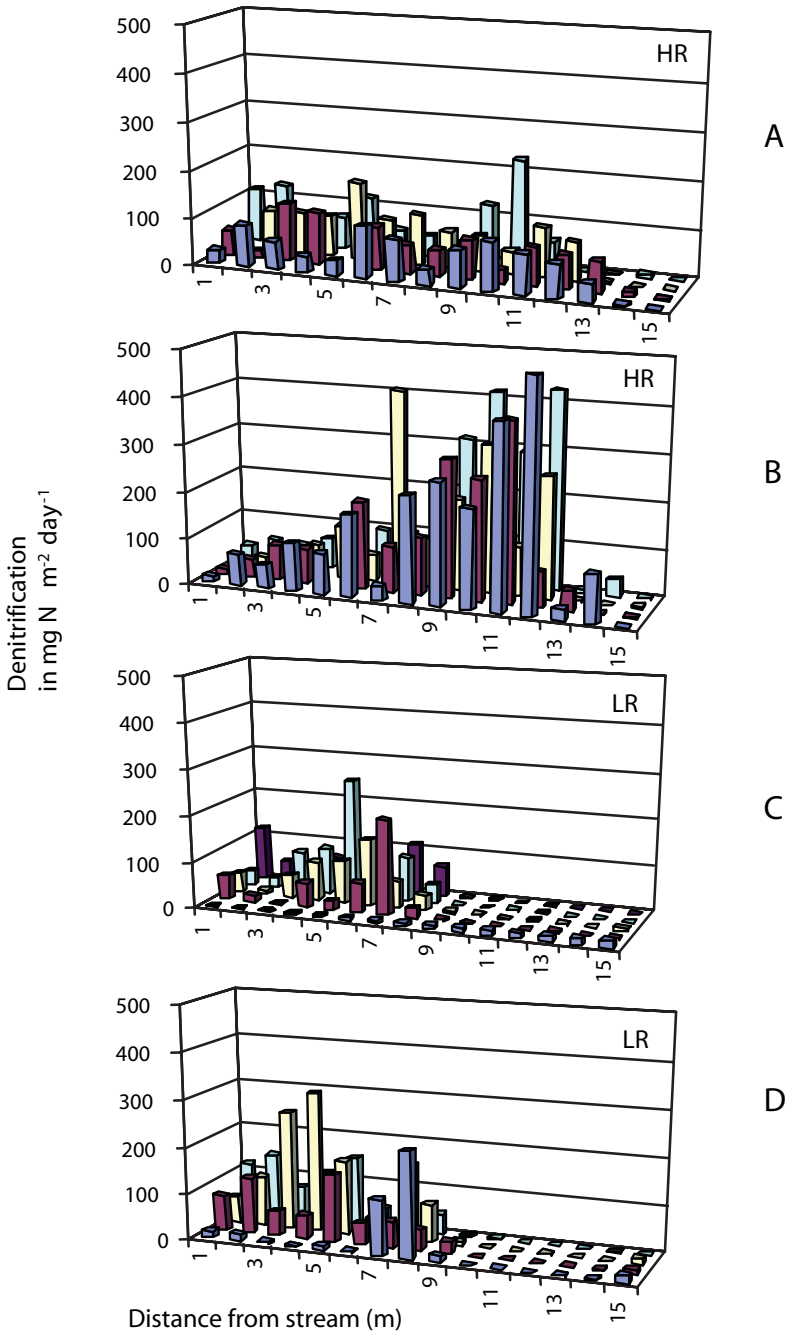

Figure 1 Denitrification rates (mg N m^{-2} d^{-1}) from the stream to the agricultural field in the HR (high nitrate removal transect) and LR (low nitrate removal transect) in winter (A, C) and summer (B, D).

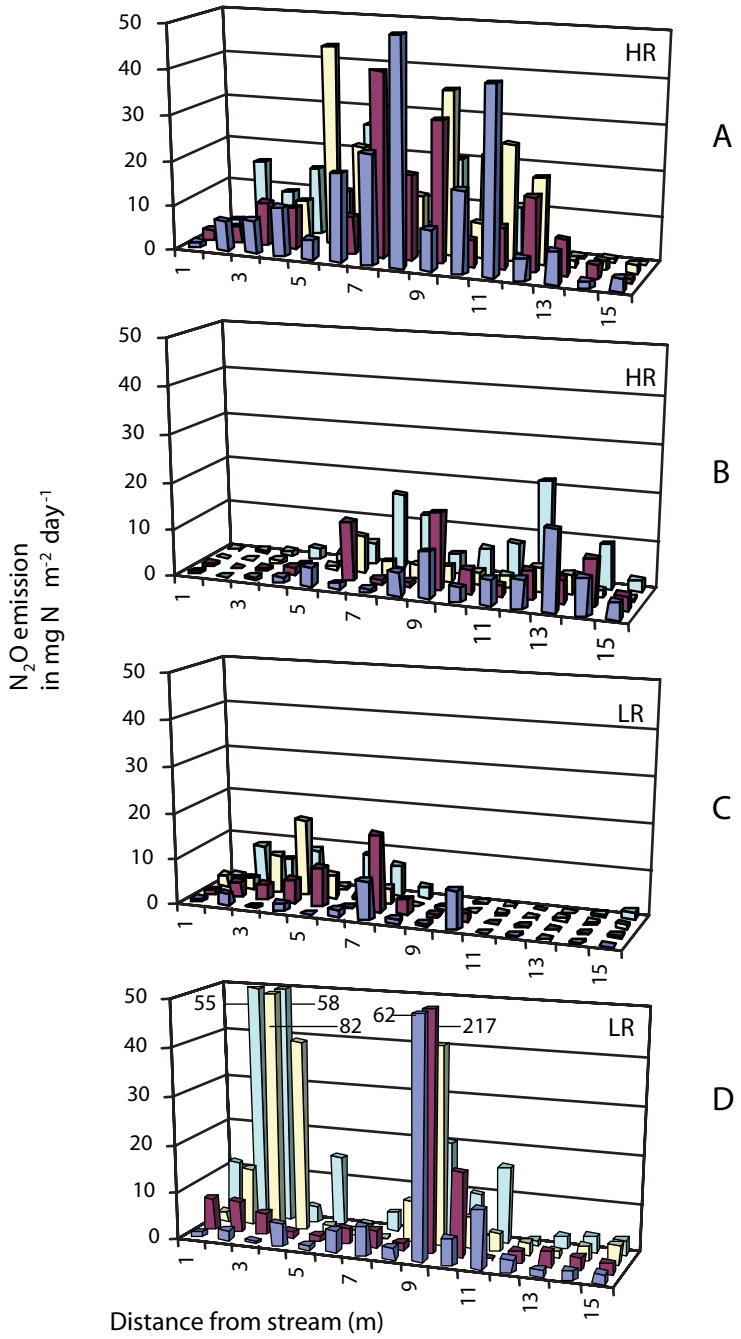

Figure 2 N_2O emission rates (mg N m^{-2} d^{-1}) from the stream to the agricultural field in the HR (high nitrate removal transect) and LR (low nitrate removal transect) in winter (A, C) and summer (B, D). Note that some bars are cut short in Fig. 2 D for scaling reasons, values indicate their real value.

Table 2 Average fluxes in mg N m^{-2} d^{-1}. Standard errors are given between brackets. N_2O‡ indicates the fluxes from the permanent wet sites only. DNT is denitrification, N_2O is the nitrous oxide emission and ratio is the N_2O:N_2 ratio from the permanent wet sites. CV is the coefficient of variation.

Transect (code)	Low nitrate removal (LR)		High nitrate removal (HR)	
Season	Winter	Summer	Winter	Summer
Date DNT	28-feb-01	13-march-01	18-July-01	01-August-01
Date N_2O	27-feb-01	12-march-01	17-July-01	31-July-01
Average DNT mg N m^{-2} d^{-1}	36.4 (6.8)	49.9 (9.1)	64.3 (6.0)	133.0 (17.3)
Average N_2O mg N m^{-2} d^{-1}	2.58 (0.51)	13.6 (4.1)	12.4 (1.6)	4.44 (0.63)
Average ratio (-)	0.93 (0.78)	0.22 (0.11)	0.29 (0.07)	0.032 (0.01)
Average N_2O‡ mg N m^{-2} d^{-1}	3.81 (0.69)	18.57 (6.0)	14.78 (2.3)	3.44 (0.8)
CV N_2O (%)	153	233	102	10
CV DNT (%)	144	140	72	100
CV ratio (%)	521	268	139	131

low nitrate removal (approximately 7 m) compared to the HR transect (1–3 m). The spatial variability, expressed as coefficients of variation, was clearly higher in the LR transect with values above 100% (Table 2). A clear pattern in denitrification activity was observed in the HR transect in summer (Fig. 2). A bell shaped curve was observed in the denitrification activity with a peak in the "middle" zone and a decrease in activity towards the agricultural field and the stream. No such spatial dependency could be observed in the LR transect or in the HR transect in winter.

N_2O emissions

Significantly different N_2O emission rates were measured in the two transects (Table 2, 3). In winter N_2O emissions were higher in the HR transect with an average rate of 12.5 mg m^{-2} d^{-1}corresponding to the higher denitrification activity found in this transect. The average N_2O emissions in the LR transect was 2.6 mg m^{-2} d^{-1} in winter. In summer higher, and highly variable, N_2O emission rates were measured in the LR transect (average 13.6 mg m^{-2} d^{-1}) compared to the HR transect (4.44 mg m^{-2} d^{-1}) (Fig. 2, Table 3). No significant diurnal variability was observed within the sampling periods.

A bell-shaped spatial pattern of N_2O emission was found in winter in the HR transect (Fig. 2). The highest N_2O fluxes were measured in the center of the riparian zone, decreasing towards the stream and towards the agricultural field. In comparison to this spatial pattern, N_2O emissions in the LR transect were highly variable. The spatial variability of N_2O emissions was generally higher in the low nitrate removal transect with coefficients of variation above 100% both in winter and summer measurements (Table 2).

Fluxes of N_2O emission were significantly correlated with denitrification

Table 3 Results of one-way (¶ winter, ¥ summer) and two-way ANOVA for denitrification and N_2O.emission with transect and season (winter, summer) as main effects. Significant main effect is printed in bold, n=238 for two way ANOVA and 119 for one way ANOVA. N_2O‡ indicates the fluxes from the permanent wet sites (excluding the possible N_2O emission from nitrification).

	transect			season			transect * season		
	df	F	p	df	F	p	df	F	p
Ln (Denitrification)	1	40.944	0.000	1	2.711	0.101	1	0.658	0.418
Ln (N_2O emission)	1	7.928	0.005	1	0.271	0.603	1	42.888	0.000
Ln (N_2O‡ emission)	1	0.096	0.758	1	2.230	0.138	1	39.652	0.000
Ln (ratio)	1	0.668	0.415	1	4.672	0.032	1	1.333	0.250
Ln (N_2O emission) ¶	1	44.735	0.000						
Ln (N_2O emission) ¥	1	6.839	0.010						
Ln (N_2O‡ emission) ¶	1	26.996	0.000						
Ln (N_2O‡ emission) ¥	1	15.106	0.000						

activity in winter in both transects (Pearson correlation coefficients 0.635, 0.682, $p<0.0001$). In summer N_2O emission from the HR transect was still significantly correlated with denitrification (Pearson correlation coefficient, 0.295, $p=0.023$) whereas no significant correlation was found in the low removal transect.

N_2O:N_2 ratio

The calculation of the N_2O:N_2 ratio was restricted to the permanently wet parts of the transects to exclude N_2O produced by nitrification based on the assumption that the bulk of N_2O emission measured was produced by denitrifier activity. The N_2O:N_2 ratio depended primarily on the N_2O emission, however, no significant differences between the transects were observed in winter due to the large variability. In summer, significantly higher ratio's were found in the LR transect (Table 2; ANOVA, $F=6.308$, $p=0.015$, $n=64$). In both transects a seasonal effect was observed with significantly higher ratio's in winter compared to the summer (Table 3).

Environmental controls on process rates and the N_2O:N_2 ratio

The observed spatial pattern in denitrification (i.e. very low activity levels) in the part of the riparian buffer zone bordering the agricultural field coincides with the spatial patterns of pH of the pore water and WFPS (Fig. 3; Table 4). In both transects the area with low denitrification activity coincided with soil pH values below 4.0 and WPFS values below 70%. In both transects, pH and WFPS were significantly correlated (Pearson correlation coefficients, 0.713 and 0.611, $p<0.001$). Nitrate availability had been expected to be of more importance in determining the denitrification activity in the HR transect. However, no clear resemblance was observed between the pattern of nitrate

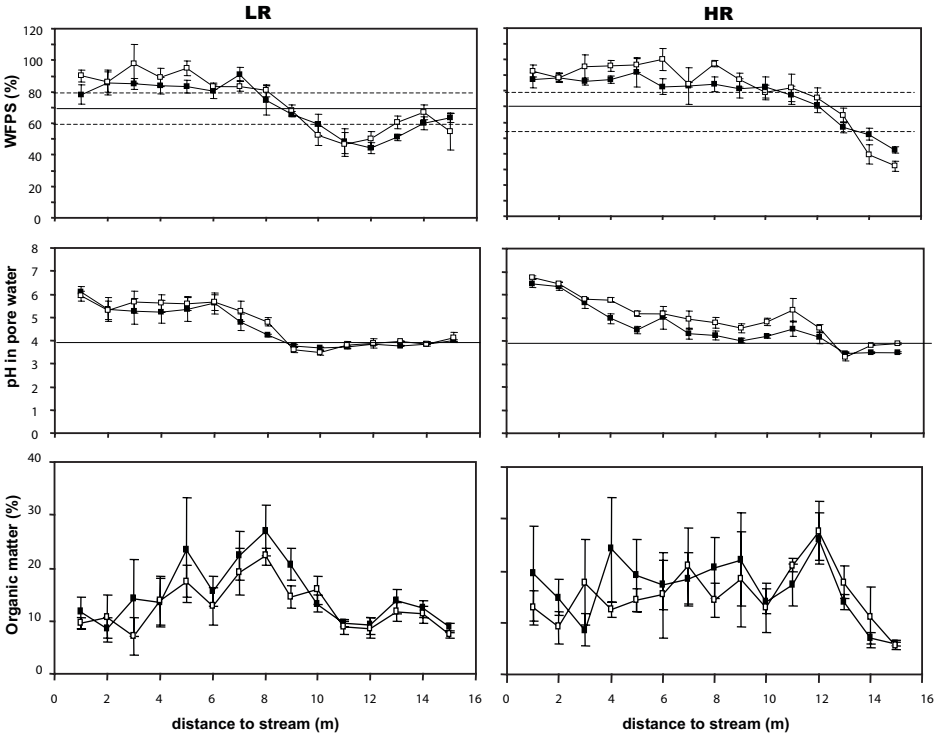

Figure 3 Water filled pore space (WFPS), pH and organic matter content (%) (mean ± SE, n=4) found in the transects from the stream to the agricultural field in winter (closed symbols) and in summer (open symbols). LR indicates the low nitrate removal transect and HR indicates the high nitrate removal transect Lines indicate possible threshold values of 70% WFPS and a pH value of 4.0.

concentration in pore water and spatial patterns of denitrification rates (Fig. 4). Nitrate availability was also significantly correlated to other controlling soil and environmental factors. To deal with highly correlated variables, a principal component analysis was used on soil variables (PCA, Table 5; Fig. 5). The first component explained about 33% of the total variance and was significantly correlated with WFPS, organic matter, pH of the pore water and negatively correlated with the nitrate concentration (Table 5). The second component

Table 4 Main environmental characteristics of the experimental transects. Abbreviations: T, temperature; WFPS, water filled pore space in %; NIT, nitrate concentration in mg Nl-1 in the pore water.

Transect (code)	Low nitrate removal (LR)		High nitrate removal (HR)	
Season	winter	summer	winter	summer
Average soil T	3.21 (0.13)	13.76 (0.08)	7.67 (0.05)	16.45 (0.09)
Average pH	4.57 (0.12)	4.7 (0.13)	4.59 (0.13)	5.00 (0.13)
Average WFPS	70.3 (2.2)	73.7 (2.5)	76.7 (2.2)	80.4 (2.8)
Average NIT	26.3 (1.3)	21.8 (2.8)	13.0 (0.8)	7.4 (1.3)

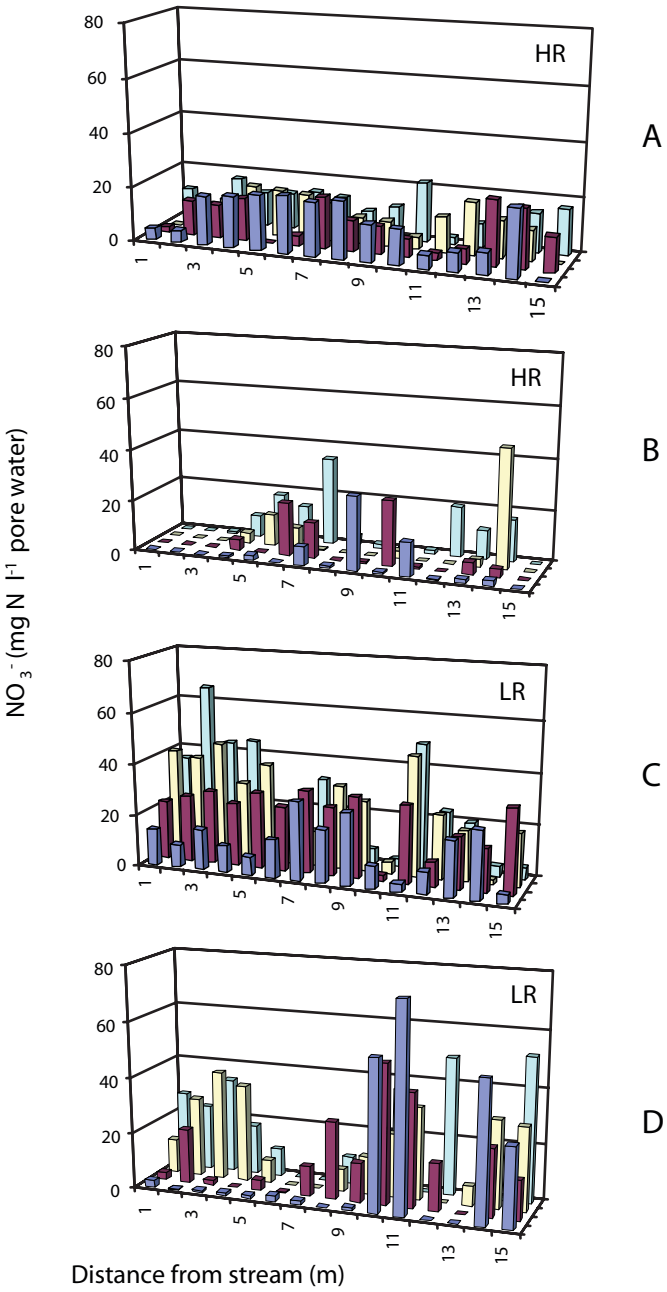

Figure 4 Nitrate concentration in pore water (in mg N l^{-1}, mean ± SE, n=4) from the stream to the agricultural field in the high nitrate removal transect (HR) and the low nitrate removal transect (LR) in winter (A, C) and summer (B, D).

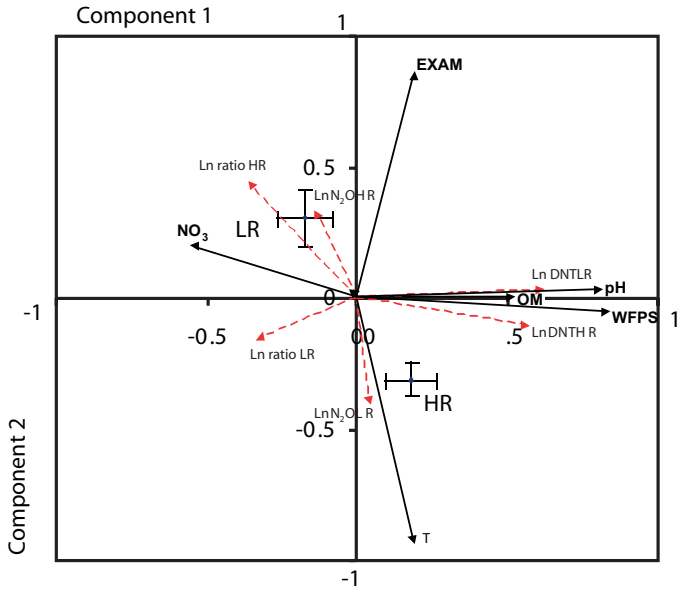

Figure 5 Correlation bi-plot from the standardized PCA on soil variables. Components are given in Table 4. Correlations of the soil variables with the main axes are given by arrows. NO_3 is the nitrate concentration in soil water, OM is the organic matter content, WFPS is the water filled pore space and EXAM is the amount of extractable ammonium in the soil. Correlations with flux measurements are given by dotted arrows. LnDNT stands for denitrification, LnN_2O is the Nitrous oxide emission and ratio is the N_2O:N_2 ratio from the permanent wet sites. Cluster centroids for the two transects are given with error bars, LR indicates the low nitrate removal transect and HR indicates the high nitrate removal transect.

explained about 23% of the observed variance and correlated strongly with soil temperature and extractable ammonium (Table 5; Fig. 5). The third component explained 16% of the variance and correlated significantly with nitrate concentration in the interstitial water and nitrate concentration in soil extracts (Table 5; Fig. 5).

Table 5 Results of a Principal Components Analysis of the soil variables and nitrate in pore water. Significant correlations are indicated with ** when significance is at the 0.01 level and * when significance is at the 0.05 level.

Variables	Abbreviation	Component 1	Component 2	Component 3
Water filled pore space	WFPS	0.842**	-0.037	-0.112
pH of pore water	pH	0.819**	0.023	-0.156*
Organic matter	OM	0.533**	0.009	0.086
Temperature	T	0.192**	-0.923**	0.137*
Extractable NH_4^+	EXAM	0.182**	0.839**	0.314**
Extractable NO_3^-	EXNIT	-0.019	0.022	0.968**
NO_3^- in pore water	NO_3^-	-0.540**	0.199**	0.531**
Variance explained (% of total)		32.89	22.66	15.94

Table 6 **Results from a multiple linear regression of process rates versus the principle components of soil variables using a forward procedure (see Table 4).**

Transect	Model	R^2	n
LR	Ln Denitrification=0.614 (C1) + 2.751	0.371	120
	Ln N_2O=-0.410 (C2) + 0.235 (C3) + 1.427	0.221	120
	Ln ratio=-0.296 (C1) + 0.162	0.073	66
HR	Ln Denitrification=0.582 (C1) + 3.749	0.333	118
	Ln N_2O=0.329 (C2) + 1.888	0.100	118
	Ln ratio=-0.255 (C1) + 0.375 (C2) + 0.211	0.244	69

Additional correlations between N_2O emission, denitrification and N_2O:N_2 ratio with the main components are included (dotted arrows) as well as the cluster centroids for the LR and HR transect scores. Denitrification was significantly related to the first component (Table 6; Fig. 5), and was dominantly influenced by the soil variables influencing the moisture / oxygen status and pH of the soil, whereas N_2O emission is significantly correlated with the second and third component i.e. soil temperature, extractable ammonium and extractable or pore water nitrate (Table 6; Fig. 5). The ratio of N_2O:N_2 in the LR transect is significantly, although weakly, related to the first component. In the HR transect N_2O:N_2 ratio was more clearly influenced by component 1 and 2. Bivariate correlations between the ratio and separate soil variables in the HR transect indicate that pH, nitrate in pore water and temperature are important variables controlling the ratio (Pearson correlation coefficients -0.412, 0.329 and 0.506, respectively, $p<0.001$). However, as was already clear from the PCA result, significant correlations exist between nitrate in pore water and both variables pH and temperature.

Discussion

Differences in nitrate removal from the shallow groundwater between the two transects (Table 1) can largely be attributed to differences in denitrification activity (Table 2). Denitrification rates in the high nitrate removal (HR) transect were approximately twice as high as those in the low nitrate removal (LR) transect. In both transects quite distinct areas with no denitrification were observed at the upland portions of the transects close to the agricultural field. In the LR transect extremely low process rates were found in an area that extended 7 meters into the riparian buffer. In the HR transect the lower activity at the hill slope was visible only in the first two meters (Fig. 1). We assume that the lower WFPS was a crucial factor in limiting denitrification

activity in the upper part of the buffer. At WFPS values below 70%, denitrification rates were found to be insignificant (Table 4; Fig. 3). Soil moisture content or WFPS is often identified as the most important factor controlling denitrification activity (Grundmann et al., 1988; Klemedtsson et al., 1991; De Klein and Van Logtestijn, 1994). De Klein and Van Logtestijn, (1996) found a comparable critical threshold of 71% WFPS for denitrification in drained peat soils in agricultural use. Moreover, other studies conducted at the study site (Chapter 2) demonstrated that the low pH of the incoming groundwater entering the riparian buffer retarded denitrification. This pH effect was also observed in this study (Table 4, Fig. 3) and may have contributed to the low denitrification activity. The major factors limiting denitrification in our hill slope zone (WFPS, pH and organic matter) are so strongly mutually correlated that it is hard to determine the relative importance of separate factors in the low denitrification rates. Results of the PCA show this in a different way because these three factors all correlated strongly with the first principal component. Hence, this method is therefore not helping to distinguish between effects of these factors.

The same distinct pattern with low emission rates near the agricultural field was observed for N_2O emissions (Fig. 2). From the largely analogous spatial patterns in denitrification and N_2O emission (Fig. 1, 2) we conclude that N_2O emission is strongly associated with denitrification. This confirmed our hypothesis that denitrification is the major process causing N_2O emissions in long-term nitrate-loaded and permanently wet riparian zones. The low N_2O emissions from the drier hill slope area at WFPS values of 60% (optimal condition for nitrification; Bollmann and Conrad, 1998) indicated that N_2O emission originating from nitrification is not very important in these soils.

Theoretical models assume a maximum N_2O emission from denitrification at WFPS values of 70-80% (Davidson, 1991; Potter et al., 1996). The WFPS values in this range, combined with a pH 4, limiting N_2O reductase completely (Knowles, 1982), can explain the peak of N_2O emission pattern in the middle of the HR transect in winter. A further reduction of N_2O to N_2 with increasing pH and WFPS explains the lower emission rates at the stream border. It was expected that nitrate availability in the pore water also significantly contributed to the bell-shaped pattern in N_2O emission found in the HR transect. However, no clear decrease in nitrate availability was observed in the HR transect in winter (Fig. 4).

As expected, N_2O emissions rates were significantly different between transects. Unfortunately, measurements on the LR and HR transects could not

be performed on the same day. This complicates the comparison between transects, especially in winter due to the differences in temperature between the sampling dates (Table 4). The relatively low N_2O emissions in the LR transect in winter can partly be explained by a temperature effect on N_2O solubility. Lower temperatures result in a higher solubility of N_2O in soil water and a decrease in emission. Therefore N_2O emissions can be significantly lower than the microbial N_2O production at these low soil temperatures (Heincke and Kaupenjohann, 1999). However, when emissions were corrected for soluble N_2O, using the temperature dependent Bunsen coefficient and the water content of the top 10 cm, emissions were still significantly higher in the winter series of the HR transect (data not shown).

N_2O emission rates in the LR transect were positively influenced by temperature, whereas those in the HR transect were negatively influenced by temperature (Fig. 5; Table 5, 6). The temperature response can be explained by differences in availability of electron acceptors between transects. In the HR transect lower nitrate inflow concentrations and higher denitrification rates in summer resulted in limited nitrate availability stimulating the reduction of N_2O, whereas higher nitrate availability in the LR transect resulted in a higher N_2O production with increasing denitrification activity.
Results from the PCA showed that the variance in N_2O emission rates could not be explained by WFPS, pH, or organic matter (Table 5; Fig. 5). This is remarkable because WFPS and pH are often used as the main predictors of N_2O emission (Velthof et al., 1996b; Sozanska et al., 2002). N_2O emissions were, however, partly explained by temperature and nitrate in soil pore water. The increase of N_2O emission with higher nitrate availability is in agreement with results from another study at the same site (Chapter 3). The strong effect of temperature on N_2O emission rates was however not consistent between the two studied transects. In the LR transect significantly higher N_2O emission was observed at higher temperatures whereas in the HR transect significantly lower N_2O emissions were measured at higher temperatures.

We hypothesized that there would be distinct spatial patterns in rates of denitrification and N_2O emission along the HR transect but that the distribution of rates of both processes would be more random along the LR transect as a result of a limited denitrification activity. In summer a clear unimodal spatial pattern in denitrification was observed along the HR transect whereas denitrification activity within the active part of the LR transect was more randomly distributed (Fig. 1). The spatial pattern of denitrification within the HR transect can be explained by a peak in activity at the spots were the shallow groundwater (containing nitrate) comes into contact with the

biologically active topsoil. The decrease towards the stream coincides with a decrease in nitrate availability. In winter no such pattern was observed in the HR transect, possibly because nitrate availability was not limiting due to overall lower denitrification activities and absence of plant uptake. A similar spatial difference was found for N_2O emission; a distinct spatial pattern in the HR transect, and highly random emissions in the LR transect. Exceptionally high emission rates occurred in a few grid cells along the LR transect in summer, while in most grid cells the activity was comparable to the emissions in winter. As was hypothesized, denitrification is generally limited in the LR transect by several factors each exhibiting spatial variation. This pattern of different limiting factors might also create a random spatial pattern with local hot spots of favorable conditions for denitrification (Parkin, 1987). Due to the high nitrate availability in the LR transect, high N_2O fluxes can occur in these hot spots. No explanation for these hot spots could however be found on the basis of the possible controlling factors that we measured. Local patches of labile organic matter might be the cause of higher process rates, although, amendment studies in the laboratory have shown that denitrification was not C-limited (Chapter 7). Given the significant influence of hot spot activity to the average N_2O emissions, a mechanistic understanding of hot spots occurrence might improve predictive relationships (McClain et al., 2003).

The mean and median values for the N_2O:N_2 ratio, 0.38 and 0.06 respectively were both within the ranges reported in the literature (0.002-3.000) (Blackmer and Bremner; 1978; Maag and Vinther, 1996; Velthof et al., 1996 b; Rudaz et al., 1999; Groffman et al., 2000, Well et al., 2001). We hypothesized that the ratio would be significantly higher in the portion of the LR transect that had high nitrate levels in the groundwater because N_2O is expected to be further reduced to N_2 as long as NO_3^- availability is not extremely high and environmental factors are not impeding denitrification (Blackmer and Bremner, 1978). This hypothesis appeared to be valid in the summer only, when conditions in the HR transect are optimal for denitrification and nitrate becomes limiting due to high denitrification rates and competition with plant uptake. The higher ratio's found in the HR transect in winter compared to the summer values indicate that soil conditions in winter were sub-optimal for denitrification. The effect of temperature on the ratio is also clear from the PCA for the HR transect (Fig. 5; Tables 5, 6). In winter, no significant differences in ratio could be observed between transects. However, higher solubility of N_2O at the lower soil temperature in the LR transect and high variation in the winter series found in the LR transect (coefficient of variation of 521%, Table 2) may have obscured differences between the two transects.
High variation in the N_2O:N_2 ratio during denitrification also complicates a

full understanding of the environmental factors that control the ratio in riparian zones (Groffman et al., 2002). Firestone et al., (1980) indicated that the further reduction of N_2O was strongly impeded by low pH and high soil O_2 levels, because of denaturation of the N_2O reductase enzyme. We found a significant relationship between the N_2O:N_2 ratio and the first two PCA components in the HR transect (Table 5) thus precluding any clear distincton between the effects of the main controlling factors. Bivariate correlations indicated that nitrate in pore water, pH and temperature were the most important factors influencing the N_2O:N_2 ratio but relations were not very strong. This is consistent with results from a Rhode Island study described in Groffman et al. (2000) where the ratio was found to be correlated to pH. It is also consistent with the theory that high nitrate availability retards N_2O reduction. In our study only weak relationships were found between the N_2O:N_2 ratio and environmental conditions, despite our expectations that relatively constant conditions (e.g. high nitrate in soil water and permanently wet soils) would minimize the spatial variability and increase our ability to find predictive relationships. This result is in accordance with later studies by Groffman et al. (2002) who stated that none of the studied variables were significant predictors of the ratio under field conditions. Several explanations can be given for the wide range in ratios and the poor predicitive relations found. First the use of acetylene in the denitrification measurements is known to inhibit nitrification and possibly even NO reduction (Bollmann, 1997). Second, spatial discrepancies in vertical sample location may introduce differences. Both denitrification and soil variables were measured in the upper 10 cm of the soil, while N_2O emissions were an integrated measurement over the whole soil profile. Since earlier experiments in our sites showed a sharp decrease in denitrification activity with depth, we do not expect this to be of major influence (Pinay and Burt, 2001).

Groffman et al. (2000) suggested that the N_2O:N_2 ratio in denitrification is a critical controller of N_2O emissions from riparian zones. However, our study indicates that, despite a relatively low ratio within the denitrification end-product, the absolute amounts of N_2O emission in the HR transect were still considerable, whereas comparable or higher N_2O:N_2 ratio's found in the LR transect under circumstances with low gaseous losses did result in significantly lower absolute N_2O fluxes in the permanently wet zone (Tables 2, 3). Moreover, the poor relations between the N_2O:N_2 ratio and environmental conditions reduces the value of the ratio as a proxy to evaluate the environmental consequences of riparian buffer zone management. Therefore we conclude that the N_2O:N_2 ratio in denitrification is not an important indicator of N_2O emissions from riparian buffers. This result is consistent with

later results from Groffman et al., (2002) in which he found no coherent patterns between the ratio and environmental variables in the field. Focusing on the absolute N_2O emission rates and the nitrate removal from the shallow groundwater seemed to be preferable for a full assessment of riparian zone functioning (Van Cleemput, 1998).

Conclusions

The N_2O emission measured in the riparian buffer zones was clearly associated with denitrification rates and in general N_2O emissions seemed to be higher when any factor was reducing the denitrification rate, i.e. temperature, pH and WPFS. On the basis of earlier studies (Chapter 2; Hefting et al., 2003a, Chapter 3), ineffective groundwater flow paths in buffer zones (with high nitrate loading rates and low nitrate removal rates) were expected to be detrimental for the environment since they fail to protect the stream ecosystem and show a relatively high contribution to the emission of the greenhouse gas N_2O. This study indicates that denitrification rates were indeed quite different between the studied flow paths with more than 2 times higher rates in the flow path with high nitrate removal. On the contrary, total N_2O emissions were quite similar for both flow paths, indicating that high nitrate removal transects can also significantly contribute to an increased N_2O emission from riparian zones. Riparian zone management aiming at an increased denitrification activity in buffers is worthy from the perspective of water quality improvement, however a certain risk of N_2O emission remains inevitable. Simultaneous minimization of N_2O emissions is only possible if riparian zone management is combined with source-directed measures to drastically reduce the nitrate concentration in agricultural runoff.

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[1] **(http:// www.usda.gov)**

Chapter 5

Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient

(BIOGEOCHEMISTRY, 67(1), in press (2004))

with Jean-Christophe Clément, David Dowrick, Anne-Claude Cosandey, Suzanna Bernal, Carmen Cimpian, Andrew Tatur, Tim Burt and Gilles Pinay

Abstract

Riparian zones have long been considered as nitrate sinks in landscapes. Yet, riparian zones are also known to be very productive ecosystems with a high rate of nitrogen cycling. A critical factor regulating processes in the N cycle in these zones is groundwater table fluctuation, which controls aerobic/anaerobic conditions in the soil. Nitrification and denitrification, key processes regulating plant productivity and nitrogen buffering capacities are strictly aerobic and anaerobic processes respectively. In this study we compared the effects of these factors on the nitrogen cycling in riparian zones under different climatic conditions and N loading at the European scale. No significant differences in nitrification and denitrification rates were found either between climatic regions or between vegetation types. On the other hand, water table elevation turned out to be the prime determinant of the N dynamics and its end product. Three consistent water table thresholds were identified. In sites where the water table level is within -10 cm of the soil surface, ammonification is the main process and ammonium accumulates in the topsoils. Average water tables between -10 and -30 cm favour denitrification and therefore reduce the nitrogen availability in soils. In drier sites, i.e. water table level below -30 cm, nitrate accumulates as a result of high net nitrification. At these latter sites, denitrification only occurs in fine textured soils probably triggered by rainfall events. Such a threshold could be used to provide a proxy to translate the consequences of stream flow regime change to nitrogen cycling in riparian zones and consequently, to potential changes in nitrogen mitigation.

Introduction

Riparian zones are important components of stream ecosystems since they are intimately linked to the functioning of the stream itself (Hynes, 1983; Naiman and Décamps, 1997). Due to their position between upland and aquatic systems, riparian zones contribute to the control of energy, nutrients and organic matter fluxes both in longitudinal (Schlosser and Karr, 1981; Pinay et al., 2000) and lateral directions (Peterjohn and Correll, 1984; Haycock et al., 1997). Riparian zones are often nutrient-rich systems with a high productivity and rapid nutrient cycling (Mitsch and Ewel, 1979; Brinson et al., 1981; Brinson et al., 1984; Mitsch and Rust, 1984). The extent of riparian zones and their high productivity is also largely controlled by the timing and duration of flooding and low flow events (Salo et al., 1986; Gregory et al., 1991; Nilsson and Svedmark, 2002). Odum et al., (1979) hypothesized in their subsidy-stress model that plant productivity in wetlands will be highest when periodic flooding of short duration occurs because of subsidies of nutrients and water; long-lasting floods will cause physiological stress to the plants, while complete lack of flooding will limit production due to the lack of nutrient inputs. However, in recent studies this adaptation of the theory of intermediary perturbation has been questioned. For instance, in a field study Megonigal et al. (1997) did not find any significant difference in above-ground production between moderately wet and dry sites. They hypothesized that periodically dry and flooded conditions require additional morphological and physiological trade-offs such that trees cannot tolerate both drought and flooding. Moreover, when considering multiple indices, i.e. below-ground biomass, litter fall and current annual increment of woody biomass, Clawson et al. (2001) found that the wettest sites had the greatest net primary productivity due to the woody biomass increment. This is consistent with previous studies where they found that biomass allocation was strongly influenced by flooding gradient with significantly higher above-ground production compared to below-ground under flooded conditions (Day and Megonigal, 1993). However, apart from the study of Burke et al. (1999), which related the lowest net primary production observed to the low nutrient availability in the wet transition zone, most of these studies have mainly focused on the importance of oxygen stress for plants as the primarily driver of plant productivity in such fluctuating environments. Biogeochemical processes, especially those related to nitrogen and phosphorus, are sensitive to redox conditions of the soil, and differences in nutrient availability as a result of these moisture-driven redox conditions may also be a key factor for plant production. In riparian zones subject to considerable N loading from the adjacent upland fields, the redox conditions of the soil determine the nutrient removal capacity of the riparian zones by

controlling plant uptake and the dominant biogeochemical processes (Cirmo and McDonnell, 1997).

Redox conditions in wetland soils are strongly influenced by water table fluctuations. Spatial and temporal changes in the occurrence of oxic and anoxic conditions have drastic effects on the rates of ammonification, nitrification and denitrification (Reddy et al., 1980; Patrick, 1982; Reddy et al., 1989; Hill, 1996; Hedin et al., 1998; Clément et al., 2002a). Ammonification of organic nitrogen can be realized both under aerobic and anaerobic conditions but the nitrification process, which requires the presence of free oxygen, can only occur in aerated soils or sediments. As a consequence, under permanently anaerobic conditions the organic nitrogen mineralization process results in the accumulation of ammonium. Other processes involved in nitrogen cycling, such as nitrogen dissimilation or denitrification, are strictly anaerobic. Therefore, the end products of nitrogen cycling available for plants in wetlands are controlled by soil moisture. Soil temperature also has a significant influence on the rate of nitrogen cycling processes with relationships more or less according to the Arrhenius equation (Maag and Vinther, 1996).

Soil moisture and temperature might both be affected by global climate change (Shaver et al., 2000; Georgakakos and Smith, 2001). Indeed, water table level and its dynamics may be altered both from upslope by land use/land cover change and from below by river discharge changes as a result of climate change (Nilsson and Berggren, 2000; Nijssen et al., 2001; Burt et al., 2002; Pinay et al., 2002). At the same time, temperature is expected to rise as a result of an increase in the concentration of atmospheric carbon dioxide (IPCC, 1996). For instance in Europe, scenarios of change in the hydrological regime forecast an overall increase of the inter-annual variability of runoff, together with an increase of the average annual runoff in northern Europe and a decrease in the south (Arnell, 1999). Additionally, the timing and duration of high and low flow events might shift, especially in the eastern part of the continent. Moreover, higher temperatures would enhance mineralization of organic matter (Rustad et al., 2001) increasing the amount of nutrients in inorganic form (Freeman et al., 1994). Combined with increased runoff from upland fields in northern Europe, this may result in higher nutrient loading of riparian zones in agricultural environments. Ultimately, these changes will affect the rates of nitrogen cycling in riparian wetlands and their plant productivity.
In this context, our objective was to determine in a pan-European study called NICOLAS (NItrogen COntrol by LAndscape Structures in agricultural environments), if there was a threshold of water table level above which the redox conditions shift from aerobic to anaerobic conditions in riparian zones

and whether this threshold was consistent in a wide range of climatic conditions and for different vegetation types. Indeed, the determination of such a threshold could be used to provide a proxy to translate the consequences of stream flow regime change to nitrogen cycling in riparian zones and, consequently, to potential changes in nitrogen mitigation. The hypothesis to be tested was whether the water table level in riparian zones is a good predictor of the relative importance of net ammonification, *in situ* denitrification and net nitrification, irrespective of climatic conditions or vegetation cover.

The study was conducted in 13 riparian sites with a vegetation cover of either forest or meadow along a climatic gradient in West and Central Europe. The main processes involved in the nitrogen cycle, i.e. ammonification, nitrification and *in situ* denitrification were measured seasonally and related to the average water table level.

Site Descriptions

The study sites were located in 7 European countries fairly evenly distributed along a climatic gradient (Table 1), with widely different conditions represented by Mediterranean (i.e., Spain), continental (i.e., Poland and Romania), and Atlantic (i.e., France and United Kingdom) climates. The study sites were chosen in order to obtain a wide spectrum of conditions to test hypotheses regarding the importance of the groundwater table versus the soil temperature on soil N cycling processes. Indeed, climatic parameters varied among sites and exhibited major differences in temperature and precipitation (Table 1). For instance, mean annual air temperature ranged from 6.8°C in Poland to 17°C in Spain. Mean annual soil temperature ranged from 8.5 in the Netherlands to 14.4°C in France. Mean annual precipitation ranged from 580 mm in Poland to 1100 mm in Switzerland, and seasonal rainfall patterns varied widely between countries (Table 1). The riparian zones were selected along lower-order streams (1-4).

In each region, a wooded riparian site and a wet meadow riparian site were selected except in Spain and Poland, where only forested riparian zones were available at the sites. The vegetation of each site has been documented and was characterized by typical wetland trees and herbaceous species in the wooded and grassed riparian sites, respectively (Pinay and Burt, 2001; Table 1). Lateral N loading rates by subsurface flow (input fluxes) were highly variable ranging from 0.52 g N m^{-2} yr^{-1} in the forested site in Romania to

over 600 g N m^{-2} yr^{-1} in the forested site in the Netherlands and England (Table 1; Sabater et al, 2003).

Methods

Water table elevation

At each site we followed the same experimental design to monitor groundwater table movements and nutrient fluxes (Burt et al., 2002; Sabater et al., 2003). Basically, 3 transects of 4 piezometers were installed across an elevation gradient from near the river edge towards the non-flooded upland bordering the agricultural field. Water table elevation was measured at least once a month for at least one year. At several sites water table level was continuously recorded with a data logger (Campbell CR10, Logan UT, USA). By convention, water table level is expressed in centimetres below the soil surface. Positive values refer to situations where the water table is above the soil surface while negative values refer to situations where it is below the soil surface.

Soil processes

At each of the 13 study sites, 3 replicate soil samples were taken 4 times a year from 3 different locations corresponding to a transect from the near-stream strip to the upland-riparian wetland interface. These transects corresponded to a gradient of soil moisture conditions. Sample locations were named after their position along the transect i.e. stream strip, intermediate strip and field strip. Soil analysis focused on the upper 20 centimetres which corresponds to the most active zone in a biological sense (Pinay et al., 2002; Clément et al., 2002a). *In situ* denitrification rates were measured using an intact core incubation method with acetylene inhibition (Yoshinari and Knowles, 1976; Ryden, 1987). Intact soil cores were inserted in gas-tight jars. At the start of the incubation, jars were 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_2O) accumulation in the head space between 1 and 4 h. Gas samples were analysed directly via gas chromatography (GC Varian 3300) equipped with an electron capture detector (ECD ^{63}Ni) and Porapak Q columns (2 m long packed columns).

Net nitrogen mineralization was calculated from measured changes in the mineral-N content of largely undisturbed soil isolated inside polyethylene bags

Table 1 Main characteristics of the study areas (after Pinay and Burt, 2001).

Country	France	United Kingdom	Netherlands
Geographic factors			
Catchment name	Vieux-Viel	Skerne	Twente
Discharge area (km^2)	10.00	8.00	0.15
Latitude	48°3N	54°4N	52°3N
Longitude	1°3W	1°2W	6°5W
Altitude (m)	20	100	64
Climatic variables			
Mean annual T °C	11.6	9	9.5
Maximum montly T °C	25	20	13
Minimum montly T °C	-2.6	1	5.6
Annual precipitation (mm)	880	800	761
Maximum monthly precipitation (mm)	164	68	136
Minimum monthly precipitation (mm)	12	42	16
Mean annual soil T °C	14.4	9.9	8.5
Land use			
% Agriculture	70	80	80
Fertilization rate (kg N ha^{-1})	200	20-50	270
Water quality			
Stream nitrate (mg N l^{-1})	4.6	4.0	5.0-10.0
Groundwater nitrate (input) (mg N l^{-1})	15	1	35
Maximum annual N loading (g N m^{-2} yr^{-1})	84	311	627
Geological substratum			
	Schist	Morenic sand	Glacial moraine
Soil Type			
	Silty clay loam, mixed, isomesic, Typic Haplaquoll	Stagnoluvic gley soil mesic, Typic Albaqualfs	Sandy loam, mixed, mesic, Entisol, Fluvent or mesic, Histosol, hemist
Vegetation Cover (main species)			
Meadow site	*Holcus lanatus* *Dactylis glomerata* *Juncus effusus*	*Lolium perenne* *Poa trivialis* *Trifolium repens*	*Glyceria maxima* *Urtica dioica*
Wooded site	*Salix alba* *Phalaris arundinacea* *Quercus sp*	*Acer sp.* *Fagus sylvatica* *Lolium perenne*	*Alnus glutinosa* *Urtica dioica* *Sambucus nigra*

allowing air to pass through but preventing leaching (Eno, 1960; Pastor et al., 1987; Binkley and Hart, 1989). After one month of incubation in the field, nitrogen content in the incubated bags was compared to the soil nitrogen content at the beginning of the incubation. Net nitrification and net ammonification were estimated from measured changes in NO_3^--N and NH_4^+-N content respectively.

Spain	Poland	Romania	Switzerland
Fuirosos	Jorka	Glavacioc	Montricher
16.80	65.00	26.00	8.00
41°4N	53°4N	45°5N	46°4N
2°3W	21°3W	23°4W	6°3W
80	150	200	650
17	6.8	10.3	7
29	23	22	19
3	-4.4	-2.7	1
885	580	600	1100
210	120	80	120
10	10	30	65
13.7	9.8	11.1	13.7
20	46	70	80
80	60-120	60	100
<1.00	2.2	?	6.2
11	0.9	0.4	7
7	1.1	0.52	27
Granite	Sandy clay	Loess	Glacial moraine
Sandy soil Sandy clay, mixed, isomesic, Typic Xerochrepts	Loamy sand , mixed Leached brown soils	Silty clay mixed, luvi-hemist	Loamy clay, mixed, hemic, Histosol Terric
No meadow site	No meadow site	*Lolium perenne* *Trifolium repens*	*Poa trivialis* *Ranunculus sp.* *Lolium multiflorum*
Platanus x Acerifolia *Alnus glutinosa* *Rubus ulmifolius*	*Alnus glutinosa* *Padus avium* *Quercus robur*	*Populus nigra* *Crataegus sp.* *Carex riparia*	*Alnus glutinosa* *Fraxinus excelsior* *Prunus padus*

Soil analysis

Before and after incubation, 20 g of fresh soil were extracted with 100 ml of either 0.2 M K_2SO_4 or 0.4 M KCl, for 1 hour. The extracts were filtered and analysed for NH_4^+-N and in NO_3^--N and dissolved N organic using an auto analyser (Technicon, 1977). Nitrate was analysed by the Griess-Ilosvay colorimetric method (Keeney and Nelson, 1982) after reduction by percolation on a copperized cadmium column. NH_4^+ was measured following the

colorimetric Indophenol Blue Method (Keeney and Nelson, 1982). Dissolved N organic was measured on the extract by oxidation to in NO_3^- with potassium persulphate at 120°C, and analysed by the above-mentioned procedure for nitrate. Soil moisture content was determined gravimetrically after drying approximately 20 grams of fresh soil at 105°C for at least 48 hours. The Pipette Sampling Method was used to determine soil grain-sizes (Day, 1965). Soil samples were pre-treated with hydrogen peroxide and hydrochloric acid and dispersed in a sodium hexametaphosphate solution.

Data analysis

Statistical procedures were performed using SPSS 8.0 for Windows (SPSS, Chicago, Illinois, USA, 1997). Variables were analysed using ANOVA and Tukey's post hoc tests. Data were tested for homogeneity of variance; denitrification rates were log-transformed prior to statistical analysis to meet these requirements.

Water table levels were averaged over 4 weeks preceding the process measurements to relate to the measured soil N cycling processes. Thresholds were identified with trial and error using the adjusted regression coefficient and r^2 of the linear regressions between ammonification, denitrification and nitrification versus the sum of soil N cycling process rates as decision criteria. The data set for groundwater levels was separated into three groups of process rates with maximum differences between the slopes and r^2 and values closest to one; values closest to the 1:1 relationship between the process rates and the sum of all main N cycling rates indicated that the process was the dominant N cycling processes under these conditions.

Table 2 **Number of days in which the groundwater table is within the specified groundwater table class, specified for each strip along the piezometer transects. GWT classes are in cm. Italic site information is from a forested site in Poland with an abundant herbaceous undergrowth.**

Country	France			United Kingdom			Netherlands		
GWT class I > -10 cm, -10cm >II>-30 cm, III>-30 cm	I	II	III	I	II	III	I	II	III
Meadow site									
Stream strip	273	16	76	47	60	258	269	96	0
Intermediate strip	215	12	138	107	94	164	258	107	0
Field strip	0	138	227	0	47	318	0	67	298
Forested site									
Stream strip	133	108	124	0	0	365	337	28	0
Intermediate strip	0	208	157	0	28	337	222	124	19
Field strip	0	162	203	0	75	290	0	168	197

Results

There was a significant seasonal pattern in water table elevation at each of the 13 sites. However, the amplitude of the water table fluctuations varied widely within and between sites depending on the local topographic and geomorphic context (Table 2). Therefore, there were no significant relationships between geographical location of the site, i.e. latitude and longitude, and the average water table level. In most cases, the water table remained closer to the soil surface in the near-stream and intermediate strips than in the near-field strips. Overall, the forested sites in England and Spain had lower water tables than the other sites. At each site, water table variations followed a seasonal pattern but at the European scale it was not related to average monthly temperature or precipitation (Burt et al., 2002).

Nitrogen cycling process rates did not show any significant differences between the forested and the wet meadow sites (Table 3). However, significant seasonal patterns in process rates were found at the different study sites (Table 3). On the other hand, no patterns were detected that related to climatic differences, expressed as latitude (Fig. 1 A-C). Similarly, no significant trends could be found between climatic parameters such as average annual soil temperature or precipitation and the annual rates of N cycling processes (Fig. 1 D-I). A significantly higher average net nitrification rate was measured at the Spanish site (0.9 mg N kg^{-1} dry soil d^{-1}), whilst the highest average denitrification rates (0.6-0.9 N mg kg^{-1} dry soil d^{-1}) were measured in the Netherlands, France and Switzerland (Fig. 2). No significant positive relation was found between N cycling process rates and annual N loading rates or extractable inorganic nitrogen (Fig. 3). For further details on this aspect see the data analysis by Cosandey et al. (2001).

Spain			Poland			Romania			Switzerland		
I	II	III	I	II	III	I	II	III	I	II	III
			365	*0*	*0*	0	221	144			
			243	*122*	*0*	125	85	155	34	210	121
			0	*96*	*269*	79	113	173	0	230	135
0	0	365	128	159	78	174	146	45	48	237	79
0	0	365	96	151	118	80	213	72			
0	0	365	0	0	365	0	57	308			

Table 3 Results from a three way ANOVA with differences between the study sites,vegetation cover and season.

Process	Ammonification			Denitrification			Nitrification		
	df	F	p	df	F	p	df	F	P
Study site	5	15.238	0.000*	5	43.160	0.000*	5	11.443	0.000*
Vegetation type	1	2.588	0.109	1	2.038	0.155	1	0.440	0.508
Season	3	4.797	0.003*	3	3.392	0.019*	3	4.195	0.000*
Site*Vegetation	3	1.908	0.129	3	2.580	0.054	3	2.553	0.056
Site * Season	15	4.054	0.000*	15	3.196	0.000*	15	3.183	0.000*
Vegatation * Season	3	0.722	0.540	3	0.916	0.434	3	0.862	0.461
Site*Vegetation*Season	9	1.653	0.100	9	3.684	0.000*	9	1.457	0.164

*** indicate significant effects.**

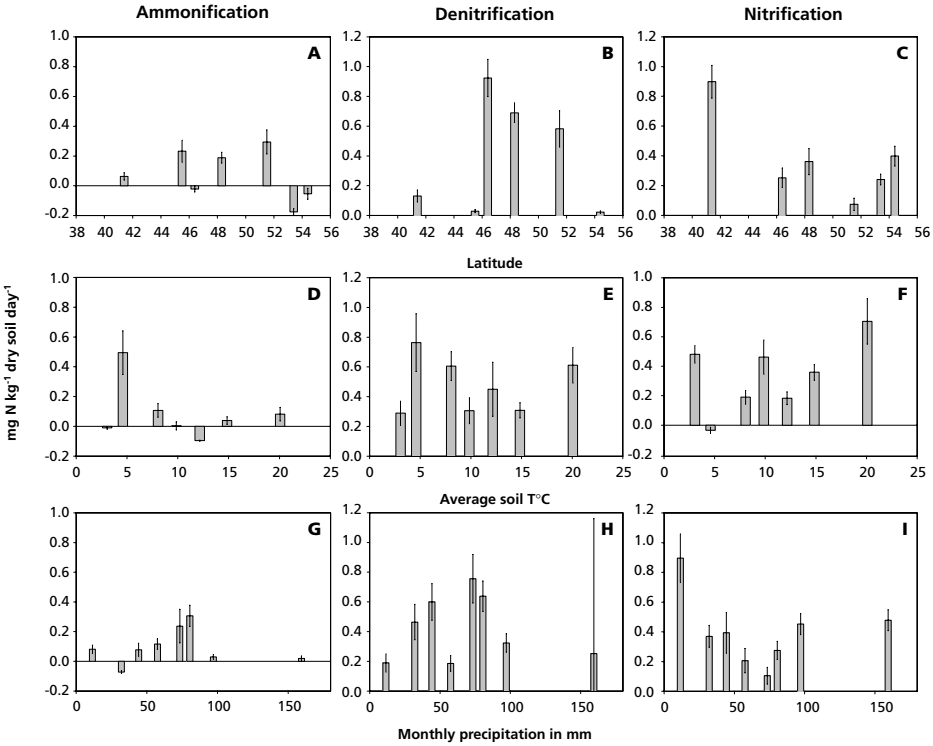

Figure 1 Climatic influence (latitude, average soil temperature and average monthly precipitation) on soil N cycling processes in the topsoil (0-20 cm). Values for soil temperature and monthly precipitation are values measured in the month prior to the process rate measurements. Means and standard errors of process rates are given (n>10).

At most sites net ammonification rates were significantly lower than nitrification and denitrification rates (Wilcoxon rank test $p<0.0001$). Highest average net ammonification rates measured at the Dutch sites were in the same

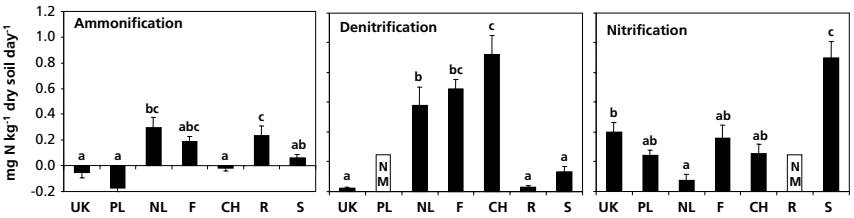

Figure 2 Nitrification, denitrification and ammonification in riparian top soils (0-20 cm) (NM, process not measured). Means and standard errors of process rates are given (n>30) Study sites in England (UK), Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S). Letters (a, b, ab, c) indicate significant differences (Tukey's a posteriori test).

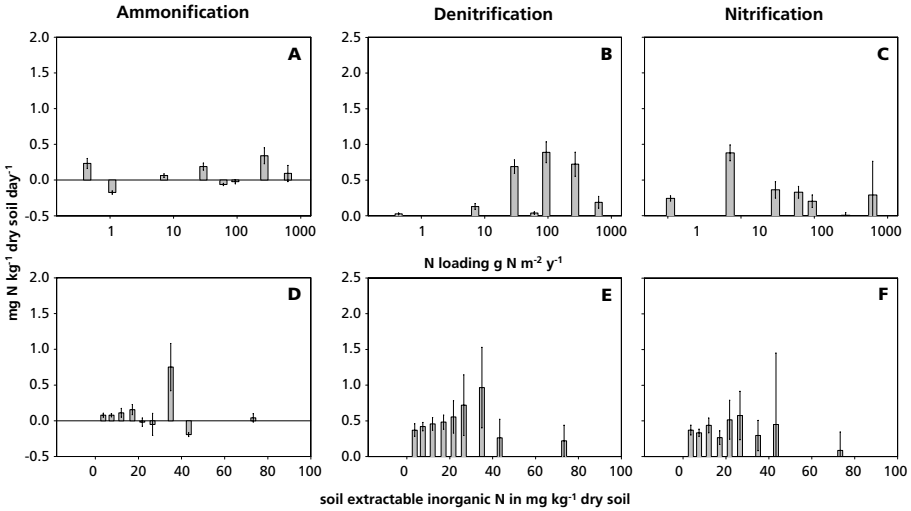

Figure 3 Influence of N loading and N availability on the soil N cycling processes in the topsoil (0-20 cm). Means and standard errors of process rates are given (n>10).

order of magnitude as nitrification and denitrification (0.3 mg N kg^{-1} dry soil d^{-1}). Global analysis of the data set, i.e. collating results from all sites, resulted in a significant relationship (r^2=0.908, p<0.001) between net ammonification and total mineralization rate in the riparian top-soils when groundwater levels were above -10 cm (Fig. 4 A). Ammonium was the main end product of the N mineralization under these waterlogged conditions. Below this -10 cm groundwater level threshold, no relationship was found between net ammonification and total mineralized N in topsoil. However, when groundwater table was below -10 cm we measured a significant relationship (r^2=0.917, p<0.001) between net nitrification and total N mineralization (Fig. 4 B), with nitrate as the predominant end product of N mineralization.

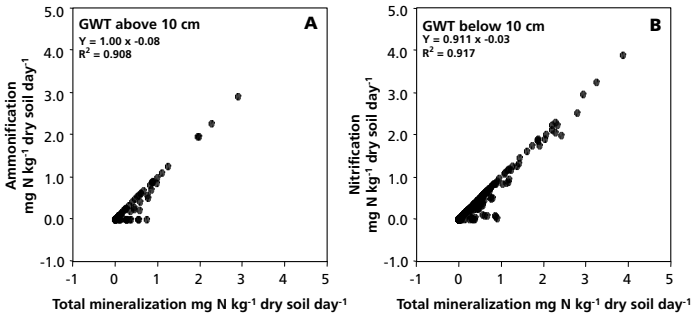

Figure 4 **Relationship between ammonification and nitrification versus the total N mineralization in top-soils (0-20 cm) separated by groundwater level at a threshold value of 10 cm below the soil surface (63<n<249).**

Relationships between the sum of the rates of the main N cycling microbial processes (SMP) in the top 20 cm of the riparian soils were correlated with each of the processes, i.e. net ammonification, net nitrification and denitrification, in order to evaluate their respective contribution under different groundwater conditions (Fig. 5). When water table level was above -10 cm, a significant positive relationship occurred between ammonification and SMP (Fig. 5 A) and between denitrification and SMP (Fig. 5 B). Net nitrification was negligible at all SMP values (Fig. 5 C). When the water table level was located between -10 and -30 cm, net ammonification rates were no longer significant (Fig. 5 D) but denitrification exhibited a highly significant positive relationship with SMP (Fig. 5 E) with a regression slope close to 1. Net nitrification rates were measurable but low at all values of SMP (Fig. 5 F). Where water table levels were below -30 cm ammonification was again very low (Fig. 5 G) but high rates of nitrification were measured (Fig. 5 I), representing the highest proportion of the microbial processes of N cycling measured (r^2=0.77, $p<0.001$). There was still some denitrification activity, even when the groundwater table level was below -30 cm (Fig. 5 H). On closer inspection, these higher denitrification rates were measured in soil with high silt + clay content (Fig. 6 A). This relationship between soil grain size and denitrification did not exist when the groundwater table level was above -30 cm (Fig. 6 B).

Discussion

Results from this pan-European study confirmed the key role of the groundwater table level in soil N cycling processes in riparian zones. This direct control over the rates of soil N cycling processes overrides other key

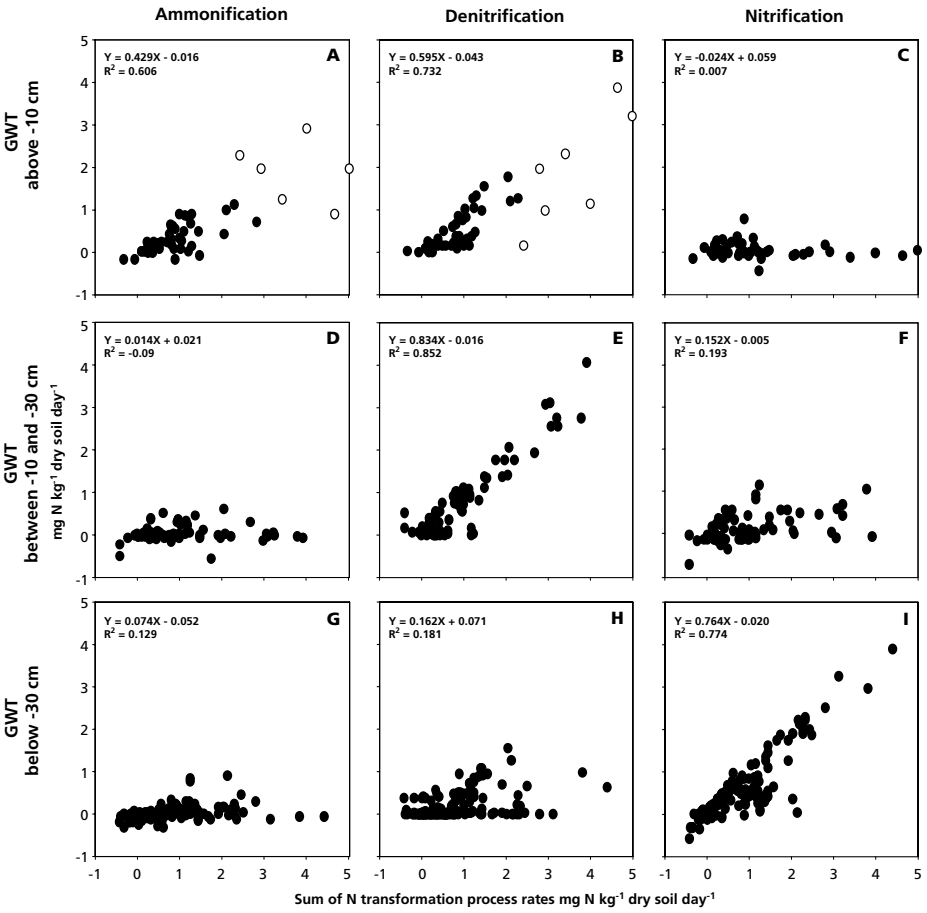

Figure 5 **Relationship between ammonification, denitrification and nitrification versus the sum of N transformation processes in top 20 cm, separated for three groundwater classes based on thresholds calculated from r^2 values (50<n<169). Open symbols indicate specific sampling spots in the Dutch riparian zones with a high allochtonous nitrate input to the stream.**

factors often mentioned in the literature such as soil texture (Groffman and Tiedje 1989; Pinay et al., 1995), soil type (De Klein and Van Logtestijn, 1994), geomorphic context (Pinay et al., 2000; Johnston et al., 2001), climatic conditions (Groffmann et al., 1987; Tiedje, 1988), N input (Hanson et al., 1994; Verchot et al., 1997) or vegetation cover (Daniels and Gilliam., 1996; Groffman et al., 1996b). It is already well known that waterlogging limits oxygen diffusion by filling the soil pore space and, in turn, that it triggers anoxic conditions (Ponnamperuma, 1972). Therefore, soil flooding or drainage type are often used as a proxy to determine the redox conditions, and denitrification potential, or to identify riparian sinks for nitrate in watersheds (Rosenblatt et al., 2001; Gold et al., 2001). In this European study, we found different water

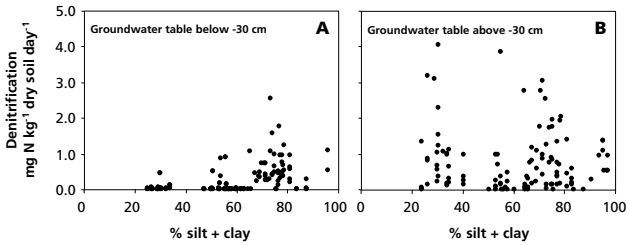

Figure 6 **Denitrification rates in riparian top-soils (0-20 cm) as a function of the silt and clay content, separated for dry (A) and wet (B) sites using the groundwater table threshold value of -30 cm (62<n<249).**

table level thresholds, i.e. -10 and -30 cm, which characterized the predominance of different microbial N cycling processes in the soils. Denitrification activity occurred at all groundwater table levels; even in soils with groundwater levels below -30 cm (Fig. 5). However, the rates varied widely, and the results provided evidence that the limiting factors of denitrification were directly related to the water table level.

When the water table was within -10 cm of the soil surface, the major end product of N mineralization was ammonium (Fig. 5 A). Net nitrification was insignificant (Fig. 5 C) because of the shortage of free oxygen in the soil. Even though it might occur in aerobic spots, the nitrate end product will have been denitrified. Therefore, under these conditions nitrification can be considered as the rate-limiting step for the denitrification process (Davidson and Swank, 1986; Van Oorschot, 2000). The very high denitrification rates measured at the Dutch sites occurred because of an extremely high allochtonous nitrate input from the adjacent upland fields so that there was a high nitrate availability even in the saturated near-stream strip (Fig. 5 B). The high ammonification rates found at the Dutch sites under these reduced conditions (Fig. 5 A) may have partly been caused by microbial dissimilatory reduction of NO_3^- to NH_4^+ (Howard-Williams and Downes, 1993).

When water table levels were between -10 and -30 cm from the soil surface we measured the highest rates of denitrification. Under these conditions, aerobic and anaerobic hot spots co-exist in the soil profile allowing both nitrification and denitrification to occur (McClain et al., 2003). The nitrification activity was demonstrated indirectly by the lack of net ammonification (Fig. 5 D), which revealed that most ammonium being released was further nitrified. However, net nitrification was still limited (Fig. 5 F) since its nitrate end product was denitrified as soon as it was formed (Fig. 5 E). Therefore water table fluctuations within the upper soil horizons, i.e. ca -10 to -30 cm, allow the co-existence of both nitrification and denitrification

microbial processes in close proximity, which results in a large removal of nitrogen from the riparian soils via denitrification (average values range from 0.62-1.04 mg N kg^{-1} dry soil d^{-1}).

In drier sites or periods, i.e. when water table levels were below -30 cm, the end product of N mineralization was nitrate (Fig. 5 I). At such sites denitrification can only occur in fine-textured soils and is probably triggered by short-term events such as rainfall or flash floods that generate partial anaerobiosis in these fine-textured soils. This significant relation between soil texture and denitrification activity in floodplain soils has been observed elsewhere (e.g. Groffman and Tiedje, 1989). For instance Pinay et al. (2000) found a threshold value of 65% silt and clay above which significant denitrification rates were found. In our study no such clear threshold value could be observed, although the highest denitrification rates under these dry conditions occurred in sites with a silt and clay content above 70% (Fig. 6 B).

According to Burt et al. (2002), water table movement is regulated by upslope hydrology in steep (headwater) riparian zones and by the adjacent stream level in flat floodplains. Under natural conditions the hydrological regime of riparian wetlands often entails large seasonal fluctuations in water table elevation (Naiman et al., 2002; Nilsson and Svedmark, 2002). Our results show that water level variations can enhance nutrient losses by denitrification in wet riparian zones leading to a decrease of N availability. In riparian zones with low N loading rates this will lead to a decrease of plant production compared to permanently wetter or drier sites. This result is consistent with previous studies by Clawson et al. (2001) who found the highest primary productivity in the wettest zones and Burke et al. (1999) who related the lowest net primary production in the intermittently flooded zone to nutrient deficiency.

In riparian zones subjected to considerable N enrichment, increased water level variations will enhance the nitrogen removal efficiency. Indeed, several studies have demonstrated that alternating aerobic and anaerobic conditions affect soil microbial activity (Mamilov and Dilly, 2002), enhancing organic matter mineralization and nitrogen loss through denitrification (Reddy, 1975; Groffman and Tiedje, 1988). In a recent study Clément et al. (2002a) found that the potential denitrifying community of the upper soil horizons of riparian zones did not vary significantly between the near-stream strip and the non-flooded upland bordering the agricultural field, despite the large seasonal groundwater table fluctuations. This large and ubiquitous potential denitrification activity even in drier sites reveals that any change in the hydrological regime might affect the denitrification activity in riparian soils.

Scenarios of climate change on the hydrological regime forecast an increase of the inter-annual variability of runoff (Arnell, 1999). Therefore, water table level and its dynamics can be altered both from the upslope by land use/land cover change influencing the runoff response and from the changes in river discharge.

Although it is difficult to forecast all the consequences of climate change on N cycling in riparian ecosystems, the prevalent role of water table dynamics in N cycling provides some basis for predictions of possible changes. Indeed, an increase in runoff variability will result in larger fluctuations in water table level and consequently larger fluctuations in soil redox conditions, which in turn will stimulate N removal by denitrification. Moreover, enhanced temperatures may increase rates of N mineralization (Rustad et al., 2001), nitrification and denitrification (Maag et al., 1997). In northern Europe, Arnell (1999) expected an increase in average annual runoff, which may result in an increased nutrient loading of riparian zones. Thus, in terms of water quality enhancement riparian buffer zones in the north are expected to become even more effective under the new climatic conditions. In southern Europe, however, drier soil conditions as a result of climatic change, are expected to compensate the effects of temperature increase on mineralization and N removal by denitrification (Leiros et al., 1999; IPCC, 2001). Furthermore, the total area of wetlands is expected to decrease in the south, which could reach the point that their nutrient amendment function would become insignificant from the catchment perspective.

Conclusions

In this study, three consistent water table thresholds were identified at very different riparian sites in terms of climate and N loading. When water table levels are within -10 cm of the soil surface, ammonification prevails and ammonium accumulates in the topsoil. Average groundwater tables between -10 and -30 cm favour denitrification and therefore reduce the nitrogen availability in soils. At sites with water table levels below -30 cm, nitrate is the main end product as a result of high net nitrification. At these latter sites, denitrification is triggered by rainfall events in fine-textured soils. These threshold values provide a proxy to evaluate the consequences of water level variations under human or natural changes on nitrogen processes and N availability in riparian wetlands.

Acknowledgements

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Chapter 6

The role of vegetation and litter in the nitrogen dynamics of riparian buffer zones in Europe

(ECOLOGICAL ENGINEERING. SPECIAL ISSUE, 2004 Proceedings of the International Workshop on Efficiency of Purification Processes in Riparian Buffer Zones November 5-9 2001, Kushiro city, JAPAN, accepted)

with Jean-Christophe Clément, Piotr Bieñkowski, David Dowrick, Claire Guenat, Andrea Butturini, Sorana Topa, Gilles Pinay and Jos Verhoeven

Abstract

Plant uptake and denitrification are considered to be the most important processes responsible for N retention and mitigation in riparian buffer zones. In many riparian buffer zones, however, nutrients taken up by plants remain in the system only temporarily and may be gradually released by mineralization later. Still, plants increase the residence time of nutrients considerably by reducing their mobility. We investigated the importance of plant N uptake and N immobilization in litter for N retention in riparian buffer zones. Nitrogen uptake in vegetation and N dynamics in litter were measured over a two-year period in a range of forested and herbaceous riparian buffer zones along a climatic gradient in Europe, receiving different loadings of N-enriched groundwater. Plant production, nitrogen uptake and N retention were significantly higher in the forested buffer zones compared to the herbaceous buffer zones. However, in herbaceous buffer zones periodic harvesting of herbaceous biomass contributed considerably to the N retention. No relationship between external N loading and plant productivity or N uptake was observed; this indicated that plant growth was not N-limited. In the winter period, decaying leaf litter had a small but significant role in N retention in a majority of the riparian ecosystems studied. Moreover, no responses to the climatic gradient were found. Generally, we can state that annual N retention in the vegetation and litter compartment is substantial, making up 13-99% of the total N removed from through-flowing water.

Introduction

Natural riparian zones are known to influence many aspects of stream ecosystems, including stream bank stability, water temperature, primary production and water and nutrient inputs from terrestrial runoff. See Naiman and Decamps, (1997) for a review. In agricultural landscapes both surface runoff and subsurface runoff are major sources of sediments, nutrients and pesticides for streams. Nitrogen (as nitrate) is considered the most important and widespread water pollutant in agricultural runoff (Meybeck, 1989; Isermann, 1990; Olsthoorn and Fong, 1998) and N removal can be highly efficient in riparian zones (Cooper, 1990; Gilliam, 1994; Hill, 1996; Mander et al., 1995). Indeed riparian zones function as buffers to reduce the quantity of diffuse pollution that reaches streams (Lowrance et al., 1984; Peterjohn and Correll, 1984; Pinay and Décamps, 1988; Osborne and Kovacic, 1993;Vought et al., 1994).

The N removal of nitrogen in riparian ecosystems is commonly attributed to both denitrification and plant uptake. There is, however, little agreement on the relative importance of these two processes in N removal. Most studies on nitrogen dynamics in riparian ecosystems have focused on denitrification and nitrogen mass balances in shallow groundwater. Although there are numerous studies on plant nutrient uptake and plant biomass accumulation in riparian ecosystems, few authors have attempted to quantify the net annual retention of nutrients in plant biomass and litter (Johnston, 1991). Recent experimental studies on N removal in riparian zones indicated that denitrification is probably more important than plant N uptake in this respect (Verchot et al., 1997; Schade et al., 2001). This may be in accordance with expectation, because plants only temporarily retain N which returns to the available pool once mineralized, whereas denitrification permanently removes N from the soil to the atmosphere. Denitrification, however, cannot account for all inorganic N removal, suggesting that N storage in perennial plant tissue, and soil as organic matter through peat formation are potentially important processes in riparian buffer zones (Lowrance et al., 1984; Groffman et al., 1992a; Simmons et al., 1992; Haycock and Pinay, 1993; Zhu and Ehrenfeld, 2000). Moreover, the relative importance of vegetation in N mitigation may increase with biomass harvesting, e.g. mowing or logging.

Apart from the role of vegetation in the long-term nitrogen retention in riparian buffer zones, plant uptake into annual tissues results in a desynchronization of nitrogen availability caused by the time lag between plant N uptake and N release by decomposition and mineralization. Additionally, immobilization of N in litter during the first stages of the decomposition

requires nutrients from external sources and may temporarily reduce the amount of inorganic nitrogen in the interstitial water. Therefore, net-immobilization by decomposing litter contributes to short-term N retention in riparian zones (Berg and Staaf, 1981; Bowden, 1986). As litter decomposition predominantly begins with plant senescence in autumn, the immobilization and retention of N in the litter fraction is most important during the dormant (winter) period. This coincides with the period when the risk of nutrient losses from agricultural fields is high due to excessive rainfall and the absence of a crop (Burt and Arkell, 1987). Moreover, low temperatures in this period limit N removal by denitrification activity (Maag et al., 1997). Hence, in winter, immobilization may be more important than denitrification in the N retention in riparian buffer zones.

Riparian ecosystems in agricultural watersheds are subject to increasingly high nitrate inputs, which will lead to changes in species composition and nitrogen dynamics. Increased nitrogen availability is known to result in increased nitrogen cycling rates (Aerts et al., 1995; Verhoeven et al., 1996). Enhanced nitrogen cycling may reduce the importance of plant uptake and litter immobilization in the nitrogen retention capacity of riparian buffer zones. Consequently, the role of vegetation and litter in the net annual N retention is expected to be relatively small in sites with higher N loading (Zhu and Ehrenfeld, 2000). Besides external N loading rates, differences in climatic conditions are known to influence decomposition rates, which will also affect nitrogen retention in litter and soil organic matter (Swift et al., 1979).
Results from studies of N retention efficacy of different vegetation types (forest versus herbaceous) are not consistent. In the past it has been assumed that a forest cover would be somewhat more effective in nitrate removal compared to a grass cover, due to a higher total biomass, (semi) permanent storage of nutrients in wood and a deeper root system. Deeper roots allow trees to take up nitrogen from a greater volume of groundwater, resulting in higher organic matter production deeper in the soil profile, which can be used by denitrifying bacteria (Cooper, 1990; Osborne and Kovacic, 1993; Haycock and Pinay, 1993). Opposite results, i.e. higher nitrate removal efficacies in grassland were found by Groffman et al. (1991), Schnabel et al. (1996) and Kuusemets et al. (2001). Other studies, however, have indicated that there is no significant difference in N removal between vegetation types (Vought et al., 1994; Lyons et al., 2000, Cosandey et al., 2001; Sabater et al., 2003; Syversen, 2002).

In this chapter, we evaluate the importance of vegetation and litter in the effective retention of N in riparian buffer zones with various N loading and under different climatic conditions.

The specific objectives of this study were to:

- quantify the direct plant N uptake in herbaceous and forested riparian ecosystems and the annual N removal by biomass harvesting;
- to elucidate the role of litter in the retention of N in riparian ecosystems;
- to determine the relative importance of plant N uptake in the N mitigation of riparian buffer zones.

This study evaluates the importance of plant uptake relative to the denitrification process, as if processes are strictly independent, we have to keep in mind however, that plants provide organic matter needed in the denitrification process. This indirect role of the vegetation is not quantified in this study but certainly contributes to the significance of vegetation in the N mitigation of riparian buffer zones.

Material and Methods

Site description

This study was conducted within the framework of the research project NICOLAS funded by the EU, in which six European countries participated. Forested and herbaceous riparian buffer zones were selected in France (F), Switzerland (CH), the Netherlands (NL) and Romania (R). In Spain (S) and Poland (PL) no herbaceous sites were available for study and only forested sites were selected.

In the Dutch herbaceous riparian site, data were collected both under undisturbed conditions and under a mowing regime (indicated with NL and NL mown respectively). Mowing took place once a year in August. The Swiss herbaceous riparian buffer zone was mown twice a year, in July and September; unfortunately no data exists on the undisturbed vegetation. This article describes the survey on the role of the vegetation in N mitigation in 10 riparian buffer zones. In Table 1 the mean characteristics of the study sites are given. The range of sites provided a wide spectrum of climatic, hydromorphic and anthropogenic conditions for which to evaluate the relative importance of plant N uptake. For instance the mean annual atmospheric temperature ranged from 6.8°C in Poland to 17°C in Spain and the mean effective precipitation ranged from 67 mm in Spain to 592 mm in Switzerland. Furthermore the lateral N loading rates by subsurface flow were highly variable ranging from 0.42 g N m^{-2} yr^{-1} in the forested site in Romania to 627 g N m^{-2} yr^{-1} in the forested site in the Netherlands. The dominant species in the herbaceous

sites were grasses, and tree species in the studied riparian forests were all deciduous. A detailed description of the research sites in this project can be found in Pinay and Burt (2001) and Burt et al. (2002).
A stratified random sampling strategy was chosen so as to divide each study site into three strips parallel to the stream. The strips were positioned across an elevation gradient from near the river edge towards the non-flooded upland bordering the agricultural field. strips were named after their position, i.e. stream, intermediate and field.

Biomass productivity and N uptake in the vegetation

Above-ground biomass of herbaceous vegetation and herbaceous undergrowth in the forested sites was measured by randomly harvesting five plots (50×50 cm) within each strip. Biomass was sampled at least three times per year in 1998 and 1999. Biomass samples were separated into living biomass and standing dead fractions, hereafter called litter. Note that surface litter was not included in this study. Furthermore no distinction was made between different species. Biomass production was calculated from increases and decreases in living and dead fractions between sampling dates according to McClaugherty et al. (1982). Nitrogen uptake and loss were calculated in a similar way from increases and decreases in the amount of nitrogen in living and dead plant material (Van Oorschot et al., 1998). To obtain the net plant N uptake, the total uptake was corrected for the re-use and re-translocation using the difference in N content between summer and autumn leaves.

The biomass production of wooded species was determined following the methods of Whittaker and Woodwell (1968). Litter was collected monthly in each strip within the forested sites using 0.20 m^2 litter traps, (n=5) over a two-year period. Further sample treatment was identical to herbaceous biomass samples as described above. Litter production was used as a measure for leaf biomass production. N uptake and re-translocation were calculated from the difference in N content between leaves harvested from the trees in summer (approximately at time of maximum standing biomass) and dead leaves collected in the litter traps. The biomass production of wood was measured with a combination of sylvimetric calculation methods and coring. In each strip trees were counted and the diameter at breast height (DBH) and tree height were determined. Groups of trees were selected for coring on the basis of DBH (Whittaker and Woodwell, 1968). Differences in DBH over a two-year period and the size of annual rings in cores were used to calculate the annual stem production. Afterwards, increment cores were used for nitrogen analysis.

Table 1 Main characteristics of the study areas (after Pinay and Burt, 2001).

Country	Poland	Netherlands	France
Geographic factors			
Catchment name	Jorka	Regge & Dinkel	Vieux-Viel
Discharge area (km^2)	65.00	0.15	10.00
Latitude	53°4N	52°3N	48°3N
Longitude	21°3W	6°5W	1°3W
Altitude (m)	150	64	20
Climatic variables			
Mean annual T°C	6.8	9.5	11.6
Maximum montly T°C	23	13	25
Minimum montly T°C	-4.4	5.6	-2.6
Annual precipitation (mm)	580	761	880
Effective precipitation (mm)	309	398	239
Total buffer width (m)			
	No herbaceous buffer Forested buffer: 15	Herbaceous buffer: 20 Forested buffer: 18	Herbaceous buffer: 20 Forested buffer: 20
Vegetation Cover (main species)			
Herbaceous site	No herbaceous site	*Glyceria maxima* *Urtica dioica*	*Holcus lanatus* *Dactylis glomerata* *Juncus effusus*
Forested site	*Alnus glutinosa* *Eupatorium cannabinum* *Urtica dioica*	*Alnus glutinosa* *Urtica dioica* *Sambucus nigra*	*Salix alba* *Phalaris arundinacea* *Quercus sp.*
External N loading			
via lateral inflow (g N m^{-2} yr^{-1})*			
Herbaceous site	No herbaceous site	627	87
Forested site	1.1	271	27

*** Sabater et al., 2003**

Below-ground biomass was sampled using a root corer with a diameter of 16 cm. Soil cores varied in depth depending on the rooting depth of the herbaceous vegetation (max 40 cm). Roots were washed by hand over a 0.5 mm sieve and separated into living and dead roots visually. The main features used to distinguish living and dead roots were the root color, the elasticity of the roots, and the presence of cortex and lateral roots.

All biomass samples were dried (70°C, 48 h), weighed, ground and stored before nitrogen analysis. Ash content was determined from subsamples to correct for mineral content. Nitrogen concentrations of the collected vegetation samples were determined using either acid digestion (Bremner and Mulvaney, 1982) or C and N analysis by dry combustion (CHN elemental analyzer, Interscience CE Instruments).

Country	Switzerland	Romania	Spain
Geographic factors			
Catchment name	Montricher	Glavacioc	Fuirosos
Discharge area (km^2)	8.00	26.00	16.80
Latitude	46°4N	45°5N	41°4N
Longitude	6°3W	23°4W	2°3W
Altitude (m)	650	200	80
Climatic variables			
Mean annual T°C	7	10.3	17
Maximum montly T°C	19	22	29
Minimum montly T°C	1	-2.7	3
Annual precipitation (mm)	1100	600	885
Effective precipitation (mm)	592	240	67
Total buffer width (m)			
	Herbaceous buffer: 15 Forested buffer: 5	Herbaceous buffer: 12 Forested buffer: 12	No herbaceous buffer Forested buffer: 8
Vegetation Cover (main species)			
Herbaceous site	*Poa trivialis* *Ranunculus sp.* *Lolium multiflorum*	*Lolium perenne* *Trifolium repens*	No herbaceous site
Forested site	*Alnus glutinosa* *Fraxinus exclesior* *Prunus padus*	*Populus nigra* *Crataegus sp.* *Carex riparia*	*Platanus x Hispanica* *Alnus glutinosa* *Rubus ulmifolius*
External N loading			
via lateral inflow (g N m^{-2} yr^{-1})*			
Herbaceous site	93	0.52	No herbaceous site
Forested site	93	0.42	34

Litter decomposition

Litter decomposition rates and patterns of nitrogen dynamics in decomposing litter were measured using the litterbag method in the field. Experiments lasted for at least one year. In the forested sites, senescent leaf material was collected in litter traps in autumn 1998. In the herbaceous sites, standing dead leaf material was collected by hand in the same period. Litter was air-dried to a constant weight and exactly one gram (1.000 g) of litter was placed into 10×10 cm polyethylene bags with a mesh size of 0.3 mm. Litterbags were placed on the soil surface in each of the three strips in every study site in autumn 1998. For each litter type, groups of 6 bags were placed at 5 random plots within each strip. Each sampling date, 15 litterbags (one from each plot) were harvested. After collection, the litter was carefully rinsed with water and fresh roots and soil fauna were removed. Litter samples were dried, weighed and prepared for nutrient analysis analogous to biomass samples. From the litterbag

study annual decay rate constants were calculated assuming a negative exponential model (Olsen, 1963). Leaching, immobilization and mineralization of nitrogen during litter decay were calculated from the differences in average absolute N content over time.

Additionally, reference leaf litter (*Phragmites australis*) collected in autumn 1998 in the Oostvaardersplassen (the Netherlands) was incubated in one riparian site in each country. Reference litter was used to compare the decomposition rates and nutrient dynamics of standard litter without any interference of differences in litter quality between the sites.

Soil analysis

Soil NO_3^--N and NH_4^+-N content were measured by extraction of 20 g of fresh soil with 100 ml extractant (either 0.2 M K_2SO_4 or 0.4 M KCl) for 1 hour. After filtering the suspension, the extract was analyzed for NO_3^- and NH_4^+ on a continuous flow auto analyzer (Skalar-40) using a colorimetric method (Keeney, 1982). Soil moisture content was determined gravimetrically after drying approximately 20 grams of fresh soil at 105°C for at least 48 hours. The Pipette Sampling Method (Day, 1965) was used to determine the soil grain-sizes. Organic matter content in the soil was analyzed with a CHN analyzer or through loss on ignition. To determine the relative importance of N retention in the vegetation and litter compartments versus N removal by denitrification, we used seasonally collected denitrification data from the NICOLAS sites for comparison. Detailed protocols for soil analysis used in this study can be found in Pinay and Burt (2001). Denitrification rates and N contents of the soil, measured per gram of dry soil were converted to a square meter basis using soil bulk densities estimates based on measurements of organic matter content and grain size distribution.

Statistical analysis

For analysis of N uptake and decomposition rates, statistical analyses were based on all 15 replications in each site (5 for each strip). Due to persistent unequal variances after transformation, non-parametric tests (Wilcoxon-Mann-Whitney and Spearman correlations) were used to analyze the data. In other comparisons and calculations, processes rates were not coupled at this detailed scale (due to different numbers of replicates and/or different spots), in these cases average values for each strip were used as replications (n=3). Due to the use of average values, parametric tests (two-way ANOVA's followed by Tukey's post hoc tests, T-test comparisons and linear regressions) could be used to analyze these condensed datasets. Statistical procedures were performed using SPSS 8.0 for Windows (SPSS, Chicago, Illinois, USA).

Results

Biomass production

The total biomass production in the herbaceous sites was significantly lower than the total biomass production in the forested sites (ANOVA, F=33.587, p<0.0001). Moreover significant differences could be observed between the aboveground biomass productions within a vegetation type (see Fig. 1). The Romanian riparian site showed a significantly lower production in the herbaceous riparian buffer zone and a significantly higher production in the forested riparian buffer zone (Fig.1 A,C). As measurements of the below ground biomass did not show any seasonal patterns and standard deviations were large (data not shown), plant production and N uptake were based only on the seasonal dynamics of the above-ground biomass.

Despite the large range of climatic conditions and N loading between the study sites, we found no evidence of increasing biomass production with in-

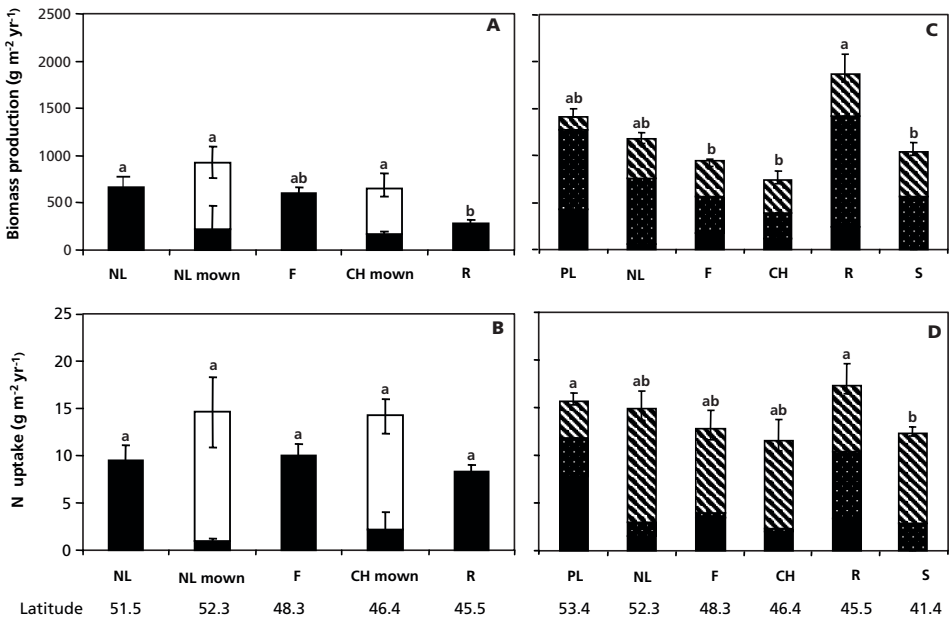

Figure 1 Above-ground biomass production and cumulative plant nitrogen uptake in different vegetation pools in herbaceous (A, B) and forested (C, D) sites in Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S). Black sections of the bars indicate the herbaceous vegetation, white sections the biomass (A, C) and N removal by mowing (B, D), stippled sections indicate biomass (A, C) and the amount of N in wood (B, D), striped sections indicate the biomass (A, C) and the amount of N in tree leaves (B, D). Letters indicate significant differences within a vegetation type (p<0.05). Numbers at the bottom indicate the latitude of the different study sites.

creasing N loading (Fig. 2) (Linear Regression, adjusted R^2=-0.072, p=0.834) or systematic differences between the regions (Fig. 1 A,C; Table 1). Furthermore no relation was found between the aboveground biomass production and N availability expressed as soil extractable nitrate or extractable ammonium.

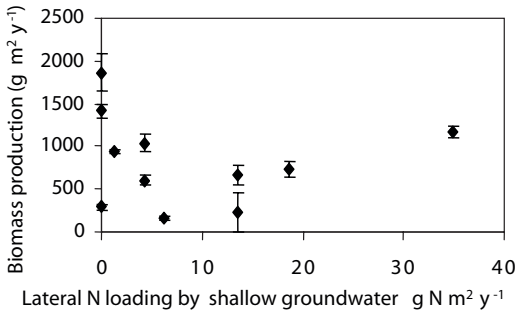

Figure 2 **Above ground annual biomass production versus the N loading by enriched groundwater (annual N loading expressed in g N per m^2 buffer zone area, lateral N loading corrected for the buffer width).**

Table 2a **N uptake and N release (g N m^{-2} yr^{-1}) in the vegetation and litter of herbaceous riparian sites (means with standard errors in brackets, n=3, average values per zone as replications).**

Country	Netherlands	Netherlands mown	France	Switzerland mown	Romania
Vegetation (living)					
N-uptake	9.51 (0.86)	14.61 (2.57)	10.01 (0.87)	14.21(0.53)	8.30 (0.68)
N retranslocation	1.83 (0.10)	0.33 (0.06)	2.51 (0.18)	0.86 (0.50)	3.19 (0.61)
N export -mowing		13.61 (2.42)		12.06 (0.98)	
Litter					
N leaching	-	-	0.12 (0.1)	-	2.51 (0.15)
N immobilization	1.86 (0.52)	0.16 (0.08)	0.88 (0.86)	-	0.19 (0.51)
N mineralization	4.71 (1.58)	0.33 (0.07)	1.94 (0.74)	1.13 (0.04)	1.61 (0.45)
Retention					
	4.83 (2.67)	0.51 (0.34)	6.32 (2.38)	0.16 (0.04)	1.19 (0.50)

Table 2b **N uptake and N release (g N m^{-2} yr^{-1}) in the vegetation and litter of forested riparian sites (means with standard errors in brackets, n=3, average values per zone as replications).**

Country	Poland	Netherlands	France	Switzerland	Romania	Spain
Vegetation (living)						
N-uptake	15.68 (0.87)	14.87 (1.12)	12.83 (1.86)	11.51 (2.24)	17.35 (2.29)	12.28 (0.60)
N retranslocation	2.97 (1.40)	3.37 (0.41)	5.43 (0.88)	2.66 (1.26)	1.34 (0.80)	2.57 (0.58)
Litter						
N leaching	1.30 (0.16)	0.29 (0.35)	0.09 (0.03)	-	2.12 (1.90)	0.54 (0.36)
N immobilization	6.03 (1.30)	0.32 (0.22)	0.84 (0.15)	0.39 (0.16)	1.64 (0.43)	2.95 (1.10)
N mineralization	1.83 (0.63)	4.97 (0.78)	1.21 (0.41)	5.16 (0.79)	4.42 (1.00)	3.22 (1.11)
Total retention						
	15.62 (1.40)	6.56 (1.31)	6.95 (0.78)	4.08 (1.80)	11.11(1.36)	8.89 (0.59)

Table 3 Decomposition rates k (yr^{-1}) (means with standard errors in brackets, n=3, average values per zone as replications).

	Native leaf litter	Reference litter
Herbaceous sites		
NL	4.48 (2.71)	2.30 (1.26)
F	1.01 (0.09)	1.34 (0.33)
CH	1.53 (0.10)	0.42 (0.08)
R	0.67 (0.18)	0.56 (0.05)
Forested sites		
PL	1.11 (0.22)	0.26 (0.04)
NL	1.66 (0.36)	0.28 (0.76)
F	0.50 (0.19)	
CH	0.82 (0.10)	
R	1.44 (0.40)	
S	0.43 (0.07)	0.42 (0.09)

Plant N uptake

Biomass production and N uptake generally followed the same pattern. The N uptake was significantly higher in the forested sites than in the herbaceous sites (ANOVA, F=7.471, p=0.014) although differences were less pronounced compared to the differences in biomass, due to lower N concentrations in the wood fraction (Fig. 1 B,D; Table 2 a,b). The N storage in wood ranged from 0.4 g N m^{-2} yr^{-1} in Switzerland to 7 g N m^{-2} yr^{-1} in Romania. This long-term retention accounted for 3-44% of the yearly plant N uptake.

Mowing of herbaceous sites removed 85% and 93% of the plant N uptake in Switzerland and the Netherlands, respectively. N uptake was not correlated with N loading, though N concentrations in plant tissue correlated significantly with N loading (Spearman correlation coefficient 0.448, p=0.25). The retranslocation of N in autumn was calculated from the difference in N content of summer and autumn leaves. N retranslocation or re-use ranged from 0.33 g N m^{-2} yr^{-1} in the mown herbaceous site to 5.43 g N m^{-2} yr^{-1} in the French forested site. N retranslocation was not affected by differences in N loading or soil extractable N. Furthermore differences in climatic zone could not explain the observed variance in N uptake between the study sites.

Litter decomposition

A wide range of decomposition rates (k) were measured (Table 3), with the highest average value of 4.48 yr^{-1} in the Dutch herbaceous buffer zone, and the lowest average values of 0.43 yr^{-1} in the Spanish forested buffer zone. In general, leaf litter decomposition rates were found to be significantly higher in the herbaceous sites than in the forested sites (Mann-Whitney U test, p=0.023, Table 3). The decomposition rate (k) was significantly correlated with

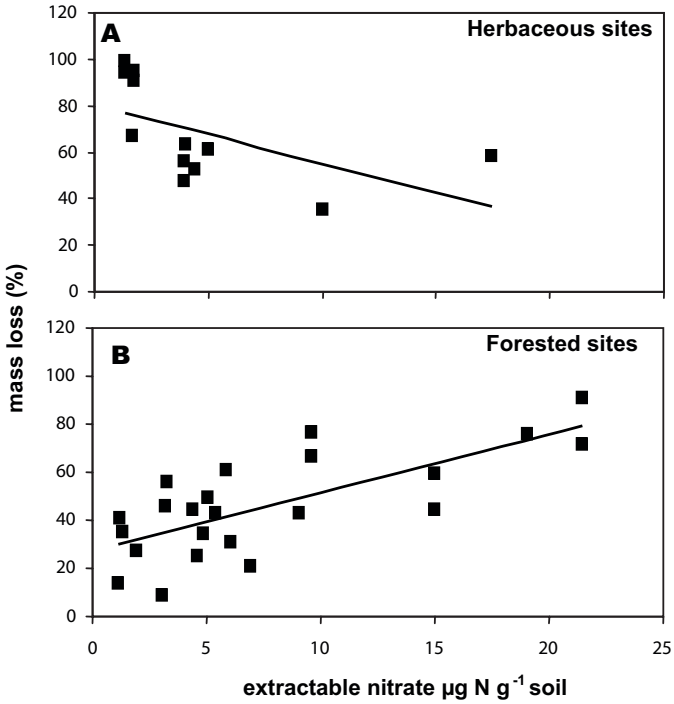

Figure 3 Annual mass loss of decomposing litter versus the average yearly extractable soil nitrate content for herbaceous (A) and forested (B) buffers.

Table 4 Spearman's bi-variate correlations between the decomposition rate k and several relevant variables.

		K	
Climatic variables			
	precipitation	-0.131	ns
	effective precipitation	-0.442	ns
	Average temperature	0.399	ns
	Evapo-transpiration	0.325	ns
Litter quality variables			
	N initial	0.057	ns
	C/N initial	-0.096	ns
	P initial	-0.449	ns
Soil variables			
	Sand %	0.430	*
	Soil pH	-0.166	ns

*** correlation significant at the 0.05 level (2 tailed)**
ns correlation not significant

the extractable soil nitrate for both forested and herbaceous sites. There was a difference between the forested and the herbaceous sites, in that the forested sites correlated positively (Spearman correlation coefficient 0.643, p=0.001) and the herbaceous sites negatively (Spearman correlation coefficient -0.659,

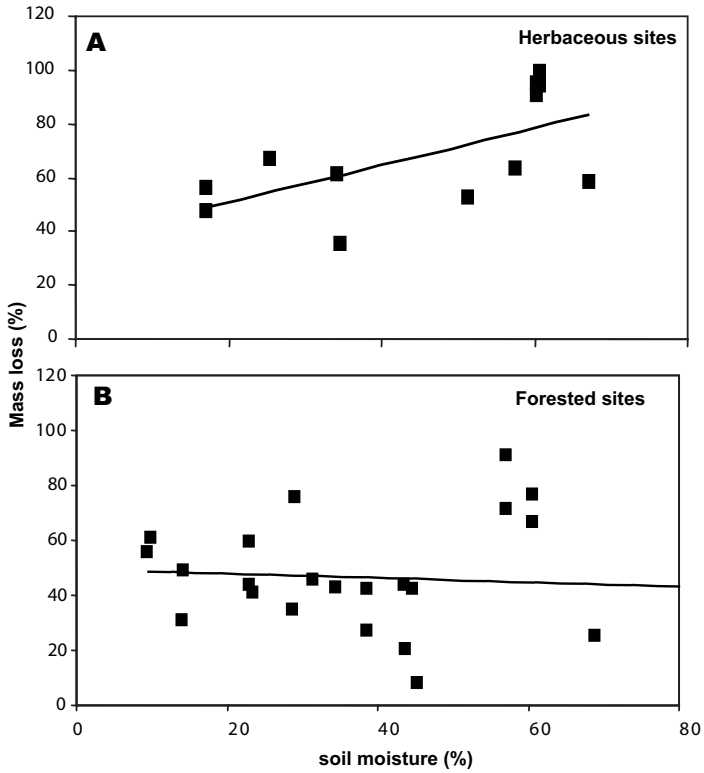

Figure 4 **Annual mass loss versus the soil moisture content in the herbaceous (A) and forested (B) buffers.**

p=0.014) with soil nitrate. Percent mass loss of decomposing litter in the first year and soil nitrate showed even more significant correlations (Spearman correlation coefficient 0.678, p<0.0001, -0.752 p=0.005, Fig. 3 A,B respectively). Another factor correlating significantly with the decomposition in the herbaceous site was soil moisture (Spearman correlation coefficient 0.671, p=0.017, Fig. 4 A,B). Other variables related to climatic conditions (soil temperature, precipitation or effective precipitation), litter quality (initial N content, C:N ratio or initial P content) or soil pH could not explain the observed variance in decomposition rates (Table 4). The % sand did however significantly correlate with the decomposition rates.

Decomposition rates of reference litter *Phragmites australis* leaves from NL placed in situ in all sites) varied between 2.3 and 0.26 yr^{-1} (Table 3). The difference between the lowest and highest rates is approximately tenfold, analogous to the difference in decomposition rates of native plant litter between sites. Comparison of the decomposition rate of reference litter with the decomposition rate of native litter in the same site showed a significant correlation (Spearman correlation coefficient 0.496, p<0.001). This implies

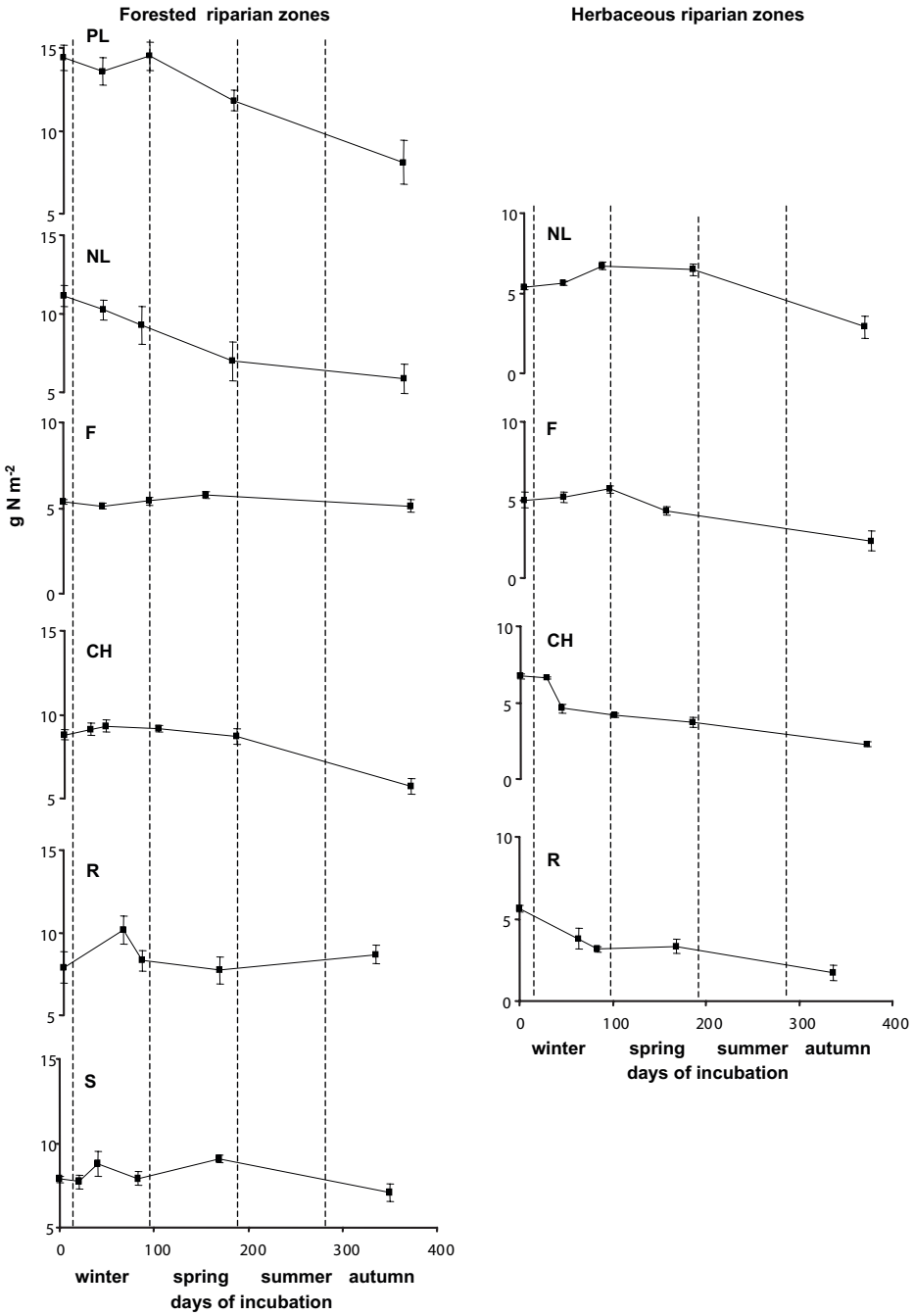

Figure 5 Absolute nitrogen dynamics in decomposing litter in the first year after senescence for forested riparian buffers (left) and herbaceous riparian buffers (right). Study sites in Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S).

Table 5a Spearman's bi-variate correlations for native litter N dynamics and influencing factors.

	Nload	ex NO_3^-	CN litter	N initial	Leaching	Immobili-zation	Minerali-zation
N in environment							
Nload	•						
ex NO_3^-	0.585**	•					
N in litter (initial)							
CN litter	-0.092 ns	-0.571**	•				
Ninitieel	0.097 ns	0.578**	-0.997**	•			
N dynamics in litter							
Leaching	-0.482 *	-0.131ns	-0.359ns	0.353ns	•		
Immobilization	-0.272 ns	-0.286ns	0.187ns	-0.186ns	0.098ns	•	
Mineralization	0.527**	0.520**	-0.545**	0.531**	-0.086ns	-0.069ns	•

*** correlation significant at the 0.05 level (2 tailed)**
**** correlation significant at he 0.01 level (2 tailed)**
ns correlation not significant

Table 5b Spearman's bi-variate correlations for reference litter N dynamics and influencing factors.

	Nload	ex NO_3^-	Leaching	Immobili-zation	Minerali-zation
N in environment					
Nload	•				
ex NO_3^-	0.310ns	•			
N dynamics in litter					
Leaching	0.199ns	0.371ns	•		
Immobilization	0.755*	-0.024ns	0.048ns	•	
Mineralization	0.810*	-0.122ns	-0.110ns	-0.732*	•

*** correlation significant at the 0.05 level (2 tailed)**
**** correlation significant at he 0.01 level (2 tailed)**
ns correlation not significant

that the environmental conditions in the riparian buffer zones had a distinct influence upon the decomposition rates. However, none of the environmental parameters studied in this research significantly correlated with the measured decomposition rate for reference litter. The decomposition rates found in the forested site in the Netherlands were lower than those in the herbaceous site. The effect of the vegetation type on the decomposition rate of reference litter could, however, not be tested, since reference litter was incubated at only one site in each country.

N dynamics during litter decomposition

Results on nitrogen dynamics in the decomposing native litter presented three distinct phases: leaching (N release), immobilization (N uptake) and mineralization (N release) as described by Berg and Staaf (1981). In this study, the immobilization of N is defined as the incorporation of N from external

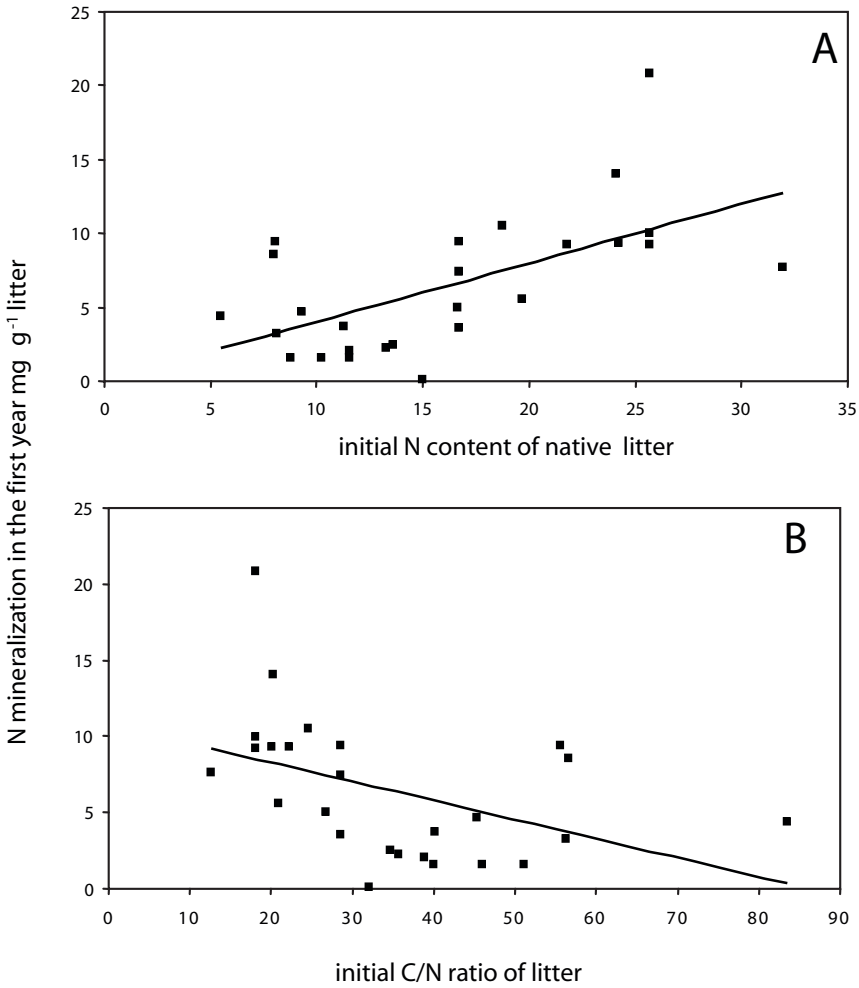

Figure 6 **Nitrogen mineralization rates from decomposing litter versus initial N content of the litter (A) and versus the initial C/N ratio of the litter (B).**

sources into an organic form by microorganisms during decomposition. This should not be confused with the common process of increasing nitrogen concentration in decomposing litter, which only results in the preservation of N that had already been taken up in plant material during the growing season.

Average changes in the absolute N content in the litter layer are given in Fig. 5. The contribution of each of the phases to the annual N retention is given in Table 2. The leaching phase defined as the rapid release of initially labile nitrogen was generally observed in the first 3-6 weeks after litter incubation, although it seemed to last longer in the herbaceous site in Romania.
In three sites (e.g. the Dutch forested site, and the herbaceous sites in Switzerland and Romania) the immobilization phase was almost absent and

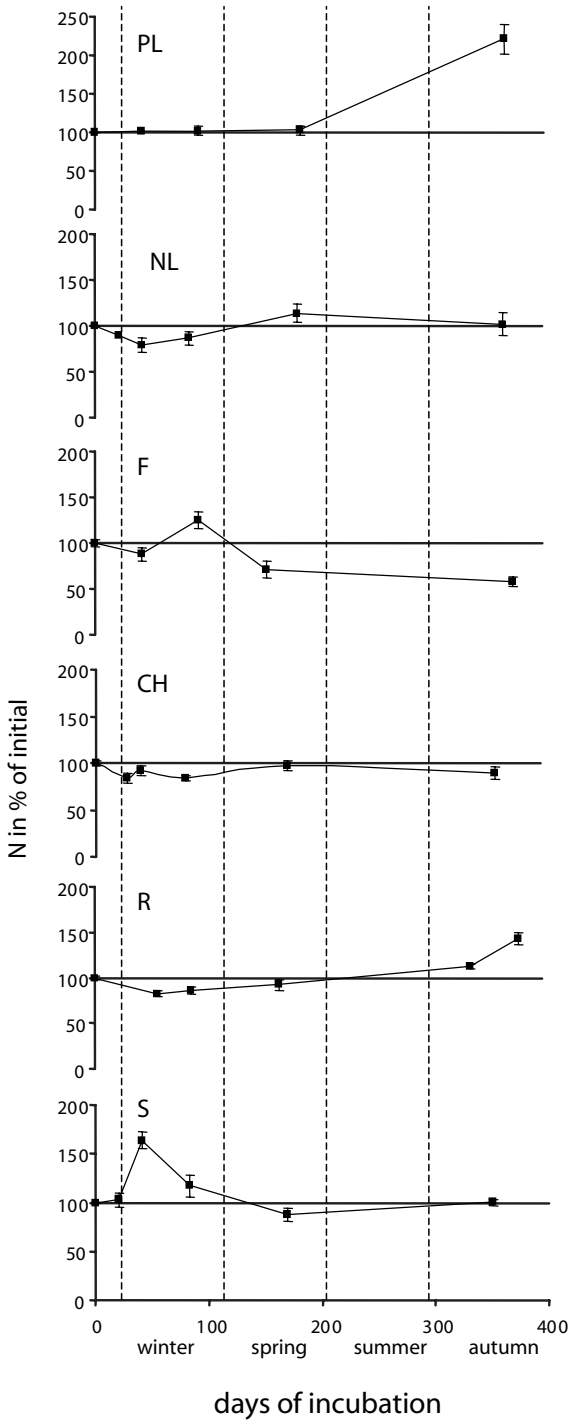

Figure 7 Average relative amount of nitrogen in decomposing reference litter (Dutch *Phragmites australis* leaves) in the first year of field incubation in several riparian zones in Europe. Study sites in Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S).

the distinction between leaching and mineralization is hard to make. In the other six sites the dominant immobilization phase generally occurred in the first 3 months of the incubation, coinciding with the winter period. Maximum net immobilization of N ranged from 115 to 172% of the absolute initial amount of nitrogen. In some buffer zones, however, the immobilization phase occurred later in the year or lasted over a longer period (Fig. 5). In the forested sites in Spain and Romania a second immobilization phase could be distinguished, this is caused by a difference in the timing of the immobilization phases depending on the position of the litterbags. Mineralization rates generally increased 6 months after incubation, coinciding with the spring period.

The occurrence of leaching and immobilization could not be explained by the N availability expressed as extractable nitrate or N loading. Moreover, neither leaching nor immobilization were related to the initial N content of the litter (either expressed as the absolute amount in mg g^{-1} or the C/N ratio, Table 5 A). On the other hand mineralization rates were significantly correlated with both; the external N supply and the initial N content of the litter (Fig. 6; Table 5 A). This implies that mineralization could be either directly stimulated through higher N availability to microbes or indirectly through higher N content of the litter, or both. The N dynamics in the reference litter, however, was clearly influenced directly by the N loading of the study sites (Table 5 B; Fig. 7), although no correlation was found with the soil extractable nitrate concentration.

Although significant differences in litter decomposition rate occurred in the herbaceous versus the forested sites, vegetation type did not significantly influence the N immobilization and mineralization rates (ANOVA, $F=0.310$, $p=0.582$; $F=0.715$, $p=0.411$).

Discussion

Primary production and N uptake

Despite the large differences in external N loading between the sites, there were no signs of an increased biomass production with higher external N loading. This was in line with the absence of a relation between the aboveground biomass production and N availability expressed as soil extractable nitrate or extractable ammonium. Observed differences in biomass production must be due to other environmental factors, including the degree of disturbance. Plant species composition (e.g. presence of *Urtica dioica,*

Glyceria maxima and *Rubus spp.*) and primary production indicated that all riparian zones studied were eutrophic (Cronk, 2001) Similarly; there was no sign of plant N uptake increase with increasing N loading. N uptake was generally related to the biomass production and varied from 8 to 17 g N m^{-2} yr^{-1}. The N:P ratios in above ground herbaceous vegetation were below 10, indicating N limitation of plant growth (Koerselman and Meuleman, 1996). Yet, average absolute N concentrations in herbaceous plant biomass was high ranging from 13-35 mg g^{-1}. This is equal and above the mean values of 13.3 mg Ng^{-1} in wetland plants given by Güsewell and Koerselman (2002). Hence, N probably did not limit plant production in these study sites (Aerts et al., 1995), but rather light or climatic factors (Spink et al., 1998). This is in contrast to several studies on the role of vegetation in N cycling in riparian ecosystems from the US where N pollution levels are often lower compared to Europe (Verchot et al., 2001). Nitrogen re-translocation did not relate to the N availability, which could be seen as another indication that the study sites were not N-limited. However, the nutritional control on nutrient re-translocation is not always apparent (Aerts, 1996).

Unfortunately the role of roots in direct plant N uptake could not be elucidated in this study since variations in biomass between sampling dates were small compared to variations between replicate samples. However, the N uptake and N release from roots is known to be a more continuous (year around) process in contrast to the seasonality of the above-ground fraction (Lowrance, 1992; Ehrenfeld et al., 1997). Consequently, uptake and release of N from roots will overlap in time, probably reducing the net effect of roots in the total N retention of riparian buffer zones.

Harvesting of biomass

The harvesting of herbaceous biomass and the storage of nutrients in woody tissue results in a (semi) permanent removal of nitrogen from the system, whereas the uptake of nitrogen in non-harvested herbaceous vegetation and in deciduous tree leaves is only short-term removal process, as it will be remineralized within a few months to a few years. Mowing of herbaceous sites removed up to 93% of the plant N uptake. The long-term N storage in wood accounted for 3-44% of the yearly net plant N uptake. N storage in wood was generally within the range of 1.2-4.2 g N m^{-2} yr^{-1}, as reported by Fail et al. (1987) and Lowrance et al. (1997) for woody sites in the southeastern US. Nevertheless, extremely low values were found in the Swiss and French sites (0.4-0.5 g N m^{-2} yr^{-1}). In the French forested riparian zone, this low value can be explained by the low tree density (less than 30 trees in the 2500 m^2 study area). The total N uptake in the forested sites, including the N uptake by tree leaves and by the herbaceous undergrowth, was higher in the European

forested sites compared to the rates reported for the US sites by Fail et al. (1987).

Information on tree age is incomplete for the studied sites, so that the effect of tree age on N retention could not be tested. However, in other reported studies, lower N retention rates were found in sites with older tree stands (Boggs and Weaver, 1994; Vitousek and Reiners, 1975; Syversen, 2002). Therefore periodical clear-cutting can increase and sustain the retaining capacity of the riparian vegetation. However, disturbances by logging often results in a short-term increased mineralization rate of soil organic matter and a limited N uptake in the first years because young trees have only a limited root system (Vitousek and Melillo, 1979, from Aber and Mellilo, 1980). Proper streamside forest management with selective harvesting of mature trees and avoidance of disturbance of the soil may be needed to maintain the water quality function of forested riparian zones (Lowrance et al., 1984; Fail et al., 1987).

Litter decomposition

In accordance with Aerts (1997), no general predictor of litter decomposability could be found in our dataset either in terms of chemical litter quality or climate. Factors other than climate, such as land use, history of the sites and site dynamics (e.g. flooding) may have obscured the influence of climate on decomposition rates. As an example, the highest decomposition rates were found in the Dutch herbaceous buffer zone (4.48 yr^{-1}), which is probably related to the much higher N loading and denitrification activity compared to sites in other countries. On the other hand the lowest decomposition rate, found in the Spanish forested buffer zone (0.43 yr^{-1}), was most likely influenced by high evapotranspiration rates, resulting in low soil moisture contents. A significant positive correlation was found with the soil sand content. This is probably related to the soil moisture and oxygen status of the soil.

Furthermore significant correlations were found between decomposition rate (k or mass loss) and extractable soil nitrate for both forested and herbaceous sites. In the forested sites a positive correlation was found which was as expected. Higher soil nitrate availability for the microbes is known to stimulate the decomposition rate and consequently the mass loss (Swift et al., 1979). In the herbaceous sites, however, a significant negative correlation was found between the decomposition rate and extractable nitrate. Decomposition in the herbaceous site was also under the control of soil moisture whereas no significant differences were found between decomposition at different soil moisture levels in the forested site (Fig. 4). The significant negative correlation

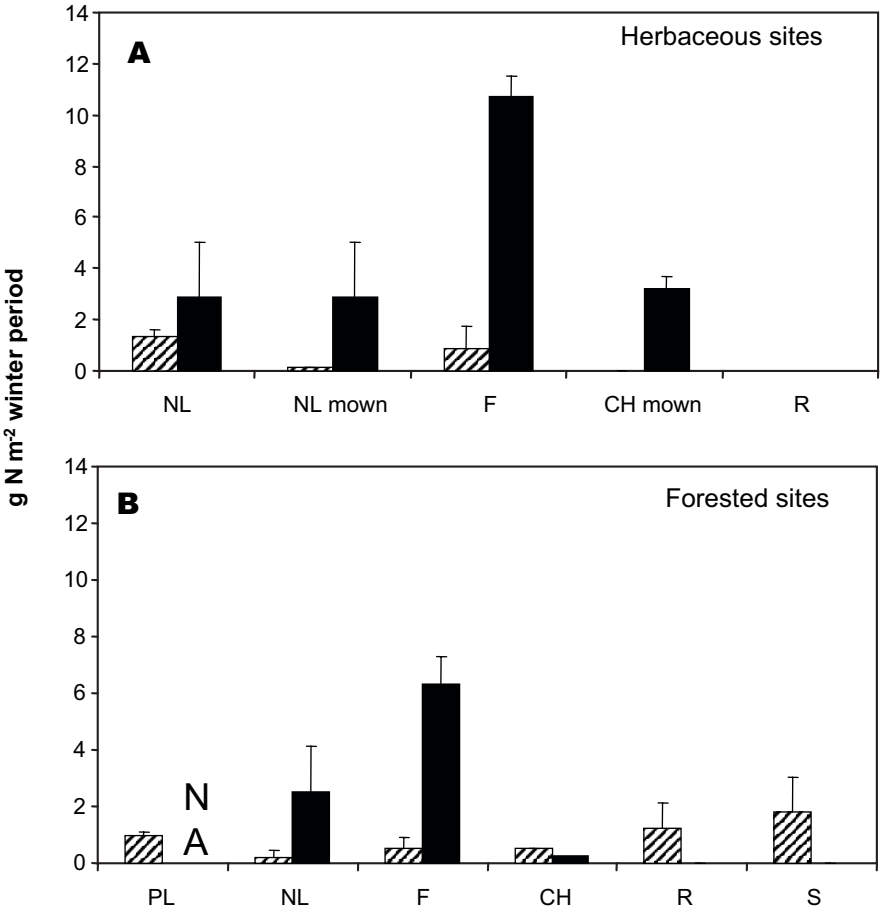

Figure 8 **Net immobilization over the winter period in decomposing litter in the first year of field incubation (striped bars) compared with N removal by denitrification (dark bars) over the same period, in herbaceous (A) and forested sites (B). NA= not analyzed. Study sites in Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S).**

of decomposition with extractable nitrate may therefore be explained by a combination of high denitrification and decomposition rates in wet sites.

Leaf litter decomposition rates were significantly higher in the herbaceous sites than in the forested sites. This difference might have been caused by shading in the forested sites, which was found to decrease the decomposition rates (Köchy and Wilson, 1997). Besides this microclimate effect, differences in litter quality may significantly have influenced the decomposition rates (e.g. the presence of ligneous leaf veins in tree leaves and other resistant substrates as phenol). Initial litter N content in the more refractory forest-derived litter was higher than in herbaceous litter; therefore this aspect of litter quality cannot explain the differences in decomposition rate. Differences in

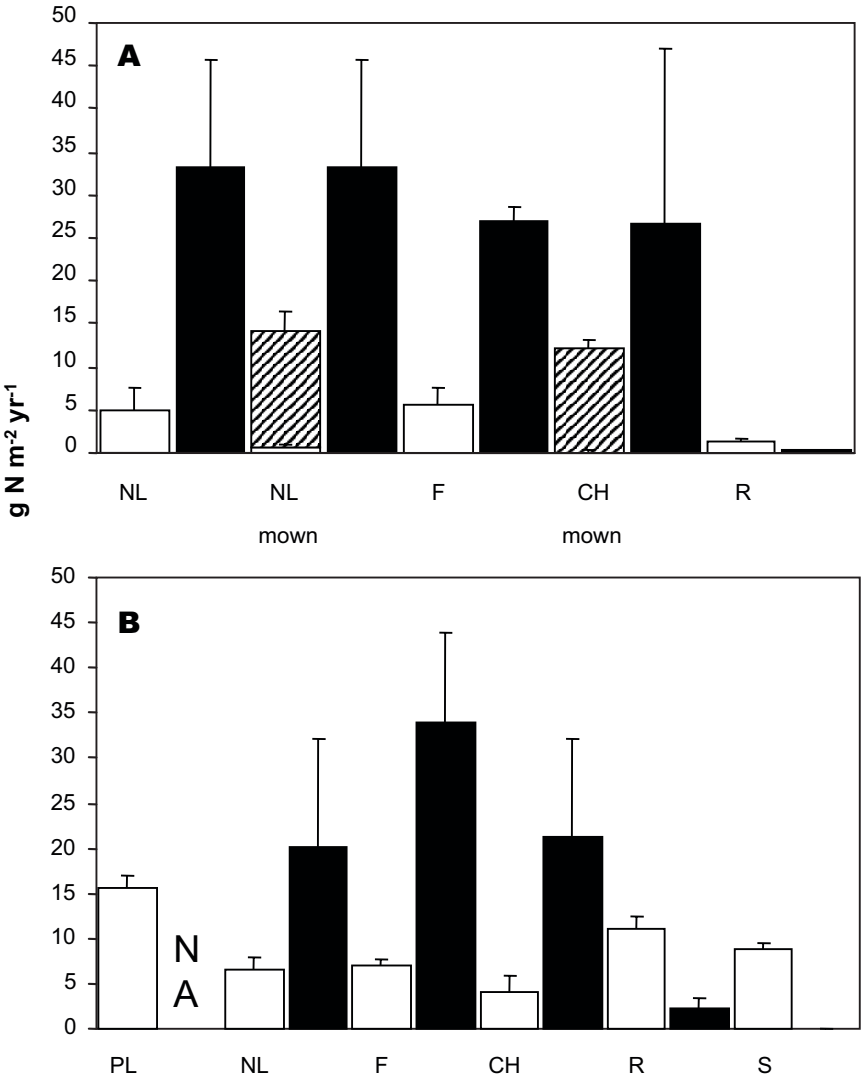

Figure 9 **Annual net retention of nitrogen in vegetation and litter (white bars), compared with annual removal of N by denitrification (black bars) in herbaceous (A) and forested (B) zones. Striped sections indicate the amount of N removal by biomass harvesting. NA= not analyzed. Study sites in Poland (PL), the Netherlands (NL), France (F), Switzerland (CH), Romania (R) and Spain (S).**

denitrification rates found between forested buffer zones and grassland buffer zones are often attributed to a higher quality of organic matter in the forested sites (Haycock and Pinay, 1993; Hefting and De Klein, 1998). The higher decomposition rates found in herbaceous sites and the negligible effect of vegetation type on denitrification rates (Pinay and Burt, 2001) observed over the range of NICOLAS study sites, suggest a higher organic matter quality in the herbaceous sites rather than the forested sites.

Table 6 Annual N retention in the vegetation in % of the annual sum of N removal processes (denitrification and plant uptake).

Herbaceous sites	
NL	12.6
NL-mown	29.7
F	17.5
CH-mown	31.0
R	73.0
Forested sites	
PL	Unknown
NL	24.5
F	17.0
CH	16.1
R	82.7
S	99.6

N accumulation and release from litter

As hypothesized, the phase of maximum net N immobilization often coincided with the winter period when plant uptake was a negligible process in N retention and denitrification activities were low due to low soil temperatures. In this period, net immobilization contributed significantly to the N retention in most of the riparian ecosystems studied, particularly in sites with low denitrification capacities such as Spain and Romania (Fig. 8). However, immobilization rates in winter were lower than expected, ranging from 0.19 to 6.03 g N m^{-2} yr^{-1}. Immobilization rates were also significantly lower relatively to the denitrification activity in the corresponding period for the herbaceous sites (Fig. 8 A). More specifically biomass harvesting decreased the amount of litter production and consequently the contribution of immobilization to the N retention (Fig. 8 A; Table 2). In the forested sites, the role of net immobilization in the winter N retention was larger when compared to most of the herbaceous sites (Fig. 8). A phase of high mineralization rates followed the immobilization phase in the spring period, when both denitrification and plant N uptake increased, thus compensating for the higher release from litter and reducing extensive N losses to through-flowing water. Our data on N dynamics in decomposing litter in relation to the N availability support the hypothesis of a positive litter feedback (Van Breemen, 1995; Aerts, 1997; Miki and Kondoh, 2002). Species adapt to high nitrogen availability by production of litter with a higher N content and consequently a faster N release.

The role of plants and litter in the overall N mitigation in riparian buffer zones

The overall picture of N retention in the vegetation (Fig. 9; Table 2) clearly shows that the above-ground vegetation and litter can be considered as net

sinks for N on an annual basis in all riparian areas studied with a retention capacity ranging from 1 up to 16 g N m^{-2}. Moreover, it is clear from this between-sites comparison that denitrification is the dominant process of N removal in most riparian sites, except for the Spanish and Romanian sites where denitrification rates were significantly lower than plant uptake. Generally we can state that annual N retention in the vegetation and litter compartments is substantial, making up at least 13% and up to 99% of the total N mitigation. Periodic harvesting of herbaceous biomass considerably increased the N retention in herbaceous sites (Table 6). Apart from the combined effect of direct N uptake and N incorporation in litter, vegetation has also a significant indirect role on N removal, by stimulating denitrification activity through the supply of organic matter.

When both litter decomposition and mineralization are low in these buffer zones, N will be sequestered in soil organic matter. Global change might negatively affect the N sequestration in organic residues in soils because higher temperatures and drier soil conditions may enhance mineralization of more complex organic matter structures (i.e. hemicellulose and lignin), consequently changing the functioning of riparian buffer zones from a sink of exogenous N to a source of endogenous N (Pinay et al., 2002).

Results of this study, however, did not show any clear effect of climate on N process rates. As stated above other factors such as historic site conditions, differences in catchment hydrology, or differences in land use might have obscured the influence of climate. This effect of entangled site conditions is a drawback that is ultimately inherent to comparative field studies over a wide range of countries. It appeared hard to find suitable study sites that are both morphologically similar and representative for their climatic zone, country and agricultural land use. On the other hand using a wide range of study sites can be an advantage since it provides a realistic evaluation of riparian zone functioning in Europe and enables us to elucidate some general trends.

Biomass production differed significantly between the vegetation types, with a higher production in the forested sites. Consequently, plant N uptake was also significantly higher for the forested sites compared to the herbaceous sites. Differences in N uptake were less pronounced in comparison to differences in biomass production due to the relatively low N concentration of woody tissue. Combined with the significantly lower decomposition rates and high N accumulation rates in litter (especially in Poland and Spain) the total N retention in the vegetation-litter compartment was significantly higher in forested sites compared to herbaceous sites. This higher N uptake and higher

N retention in forested buffer zones is in agreement with the literature reviewed by Osborne and Kovacic (1993). Cooper (1990) compared the efficiency of grass buffer zones and forested buffer zones in a small headwater catchment and found that the forested buffer zone was more efficient to reduce nitrate concentration in shallow groundwater. Our data on the retention of N in the vegetation-litter system seem to support this, but data on denitrification and groundwater nitrate removal in our sites did not show any significant differences between the vegetation types (Sabater et al., 2003; Clément et al., 2002).

Conclusions

Plant uptake is often considered less important in N mitigation of riparian buffer zones than denitrification due to the temporary character of the retention i.e. most of the nutrients taken up by the vegetation are released once the vegetation dies and decomposes. This pan-European study demonstrated that annual N retention in vegetation and litter accounts for 13-99% of the total N mitigation. Higher N uptake and higher N retention was found in the forested buffer zones, however, periodic harvesting of herbaceous biomass contributes considerably to the N retention in herbaceous sites. Generally, the contribution of N immobilization in decomposing litter to the annual N retention was small, though it contributed significantly to the temporary retention of N in winter.

Acknowledgement

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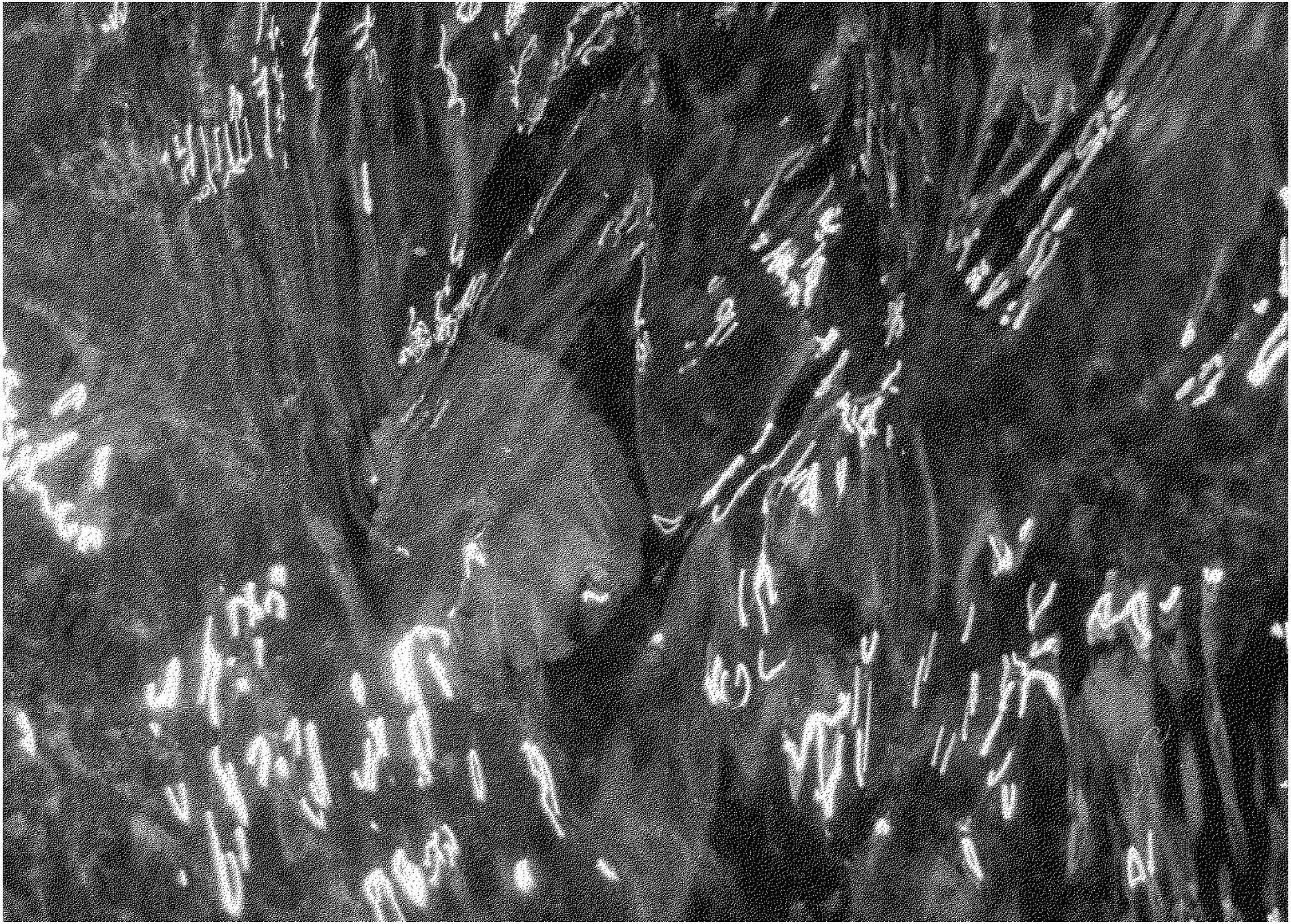

Chapter 7

Nitrogen transformation and retention in riparian buffer zones: a synthesis

Introduction

The efficiency of nitrate removal from groundwater passing through riparian zones can vary with climate and landscape setting (Devito et al., 2000). This study was conducted to determine the influence of nitrate loading rate, vegetation and hydrologic regime on the mechanisms of nitrate removal in riparian zones along a climatic gradient. We studied nitrate removal in nitrate-loaded riparian buffer zones that were down-gradient from intensively fertilised agricultural fields. The research was conducted in the Netherlands and other locations across Europe within the framework of a joint European research project called NICOLAS (NItrogen COntrol by LAndscape Structures in agricultural environments).

What is the relative significance of various nitrate removal mechanisms within herbaceous and forested riparian zones?

Although riparian zones have been shown to reduce nitrate in contaminated groundwater since the early 1970s (Correll, 1997), many unanswered questions remain regarding the complexity of hydrologic and biogeochemical interactions in these ecotones. Despite extensive research, considerable uncertainty exists about the relative importance of the principle removal mechanisms along climatic gradients and under varying hydrological regimes. Generally, nitrate reduction has been attributed to plant uptake (Lowrance et al., 1984; Groffman et al., 1992a; Lyons et al., 2000; Zhu and Ehrenfeld., 2000) and denitrification (Hanson et al., 1994; Jordan et al., 1998; Martin et al., 1999). However, several authors (i.e. Hill, 1990; Pinay et al., 1993; Vought et al., 1994; Böhlke and Denver, 1995) indicate that dilution and mixing of groundwater can significantly contribute to the decrease of nitrate concentrations in groundwater as it passes through riparian zones. Therefore, one objective of this study was to determine the effect of groundwater flow paths and dilution of groundwater on nitrate removal.

Groundwater dilution

Dilution of shallow nitrate-loaded agricultural runoff with groundwater from a deeper aquifer caused a significant decrease in nitrate concentrations in the riparian buffer zones in the Netherlands. If this physical process is not taken into account, there would be a significant over-estimation of the nitrate removal capacity, varying between 0-60% depending on the flow path. A detailed understanding of the flow system in riparian zones is therefore necessary to assess nitrate removal. In the European wide study, dilution was low to moderate in eight sites, and relatively high in four sites including the forested site in the Netherlands. However, besides this dilution effect, biological removal processes also significantly reduced the nitrate concentration in the shallow groundwater in all of the riparian zones studied (Chapter 2; Sabater et al., 2003).

Plant uptake

In the NICOLAS programme we studied the role of vegetation and litter in the nitrogen retention in herbaceous and forested riparian areas along a climatic gradient. The annual N retention in vegetation and litter accounted for 13-99% of the total N removal (Chapter 6). Higher N uptake and higher N retention was found in forested buffers but periodic harvesting of herbaceous biomass contributed considerably to the N retention in sites

dominated by herbaceous vegetation. We found no differences in the effectiveness of specific vegetation types (forest or herbaceous) on nitrate removal from shallow groundwater. This result is in agreement with our water quality studies in which we found no significant differences between removal efficiency with vegetation type (Sabater et al., 2003). Neither did we find significant differences in denitrification rates between the forest or herbaceous vegetation (Pinay and Burt, 2001; Consandey et al., 2001).

Role of N immobilization in litter

Generally, the contribution of exogenous N immobilization in decomposing litter to the annual N retention was small, though it contributed significantly to the temporary retention of N in winter. On an annual basis, N retention by immobilisation in litter was compensated for by litter N mineralization, with the exception of the Polish sites where immobilisation rates of about 40 kg N ha^{-1} yr^{-1} were found. The immobilization values measured in Poland are extremely high compared to potential N immobilisation (1-1.5 kg N ha^{-1} yr^{-1} in decomposing beech litter calculated on the basis of a ^{15}N isotope experiment) measured by Downs et al. (1996). Apart from the combined effect of direct N uptake and N incorporation in litter, vegetation has also a significant indirect role in N removal by stimulating denitrification activity through the supply of organic matter by litter and root exudates.

Denitrification

Studies on nitrogen mitigation in wet riparian buffer zones have largely focused on nitrate reduction by denitrification. Denitrification has been studied in many different riparian zones and the significance of the process is that it provides the only permanent sink for excess nitrate (Pinay et al., 1993; Groffman et al., 1996; Hill, 1996). *In situ* denitrification rates varied between 0.11 and 91 mg N m^{-2} d^{-1} in the studied European riparian zones, a range comparable to those measured in other European studies and in the US (0.15-214 mg N m^{-2} d^{-1} measured in soil cores with the acetylene inhibition method, as compiled by Hoffmann, 1998). Much higher in situ denitrification rates (i.e. 1120 and 8112 mg N m^{-2} d^{-1}) were found in riparian zone soils from New Zealand (Cooper, 1990; Schipper et al., 1993) using a different in situ incubation method with disturbed soil samples instead of undisturbed soil cores. The denitrification rate is however strongly influenced by measurement method. The method of Cooper (1990) and Schipper et al (1993) has more resemblance with the (unamended) denitrification enzyme activity rate (DEA-A, Box 1), which is often more than 10-fold higher compared to denitrification rates under "natural" conditions due to strict anaerobic conditions and enhanced diffusion of nitrate and carbon substrate.

Box 1
Potential denitrification in riparian buffer zones in the Netherlands

Denitrification enzyme activity (DEA) was measured seasonally in 1999 and 2000 at three distinct depths in the soil profile (0-20, 40-60, 80-100cm), using Smith and Tiedje's (1979) procedure and according to the NICOLAS protocols (Pinay and Burt, 2000). Subsamples were amended with demi water (DEA-A), nitrate solution (10 µg NO_3-N g^{-1}, soil fresh wt basis DEA-N), C solution (2 mg C-glucose g^{-1} and 2 mg C-acetate g^{-1}, soil fresh wt basis DEA-C) or C + N solution (10 µg NO_3-N g^{-1}, and 2 mg C-glucose g^{-1}, and 2 mg C-acetate g^{-1}, soil fresh weight basis, DEA-CN).

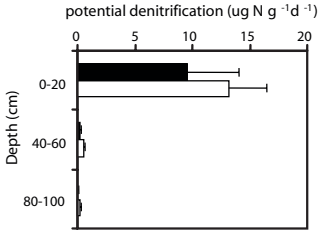

Figure 1 **Vertical distribution of the average potential denitrification activity in the Dutch riparian zones. Black bars indicate the forested zone, white bars indicate the herbaceous zone.**

The vertical distribution of DEA activity within the soil profile showed a significant decrease of activity with depth. At 40-60 cm only 3-10% of the top soil activity was observed. At a depth of 80-100 cm potential activities decreased even more with average values of 0.8-5% of the topsoil activity (Fig. 1).
Carbon addition did not significantly increase the DEA compared to non-amended soil samples in both riparian sites (Fig. 2). This indicated that denitrification was not C limited, a comparable pattern was found at greater depth (data not shown). In contrast, N amended soil samples showed a clear increase in DEA compared to the non-amended samples. This was most obvious in the herbaceous zone (Fig. 2 D,E,F) but could also be observed in the higher N-loaded forested site (Fig. 2 A,B,C).

We expected to find significantly higher *in situ* denitrification rates in riparian zones in the Netherlands that received very high rates of nitrate loading. However, average values (50–70 mg N m^{-2} d^{-1}) were comparable to denitrification rates found in several other studies where nitrate loading rates were significantly lower. For example Pinay et al. (1993) measured denitrification rates of 78 mg N m^{-2} d^{-1} with input concentrations of 1.06 mg N l^{-1} and Lowrance et al. (1995) measured rates of 56 mg N m^{-2} d^{-1} with an N loading of 5.1 g N m^{-2} yr^{-1}.

Comparison of N retention and removal processes

The relative contribution of denitrification, plant uptake and other processes as immobilization and dilution to the NO_3^- removal from shallow groundwater has been a major question in riparian buffer zone research. Hansen et al. (1994)

Box 1 continuation

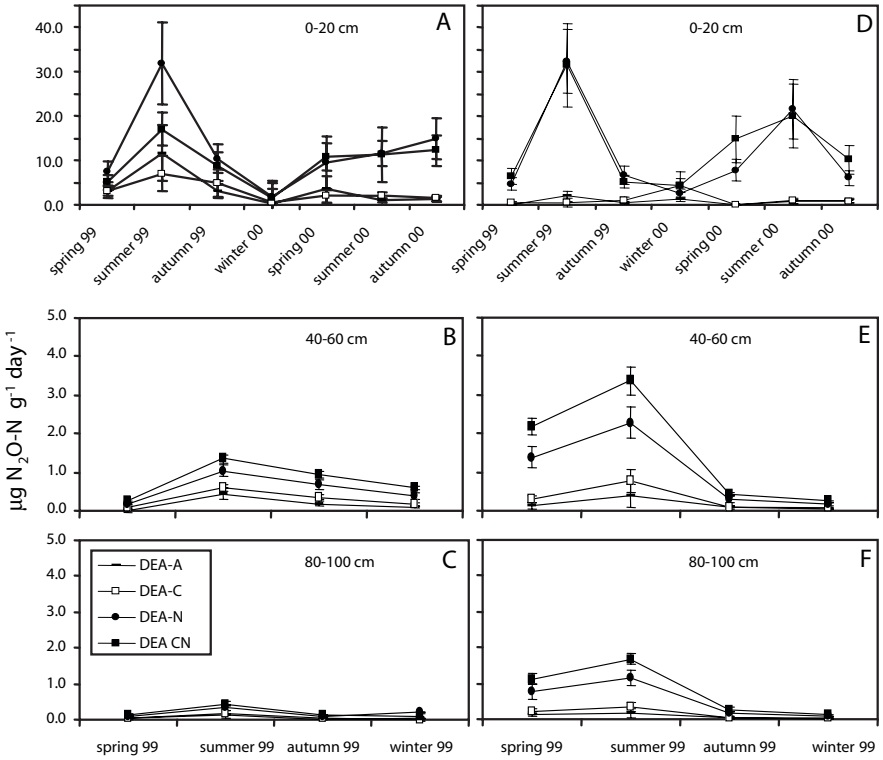

Figure 2 **Effect of different amendments on the DEA activity in time. The activity in the top 20 cm was measured for two years in 1999 and 2000. Deeper soil layers were only measured in 1999.**

calculated that denitrification removed approximately 59% of the NO_3^- that entered an enriched riparian site. In studies of Peterjohn and Correll (1984) 33% of NO_3^- removal from the groundwater was accounted for by plant uptake, and they hypothesised that denitrification is a significant process in explaining the remaining 67% nitrate N removal from the groundwater. More recent literature on riparian zone nitrate retention from Blicher-Mathiesen and Hoffmann (1999), Clément et al. (2002) and Dhondt et al. (2002) all underlined the important role of denitrification in nitrate removal from the groundwater. However, Devito et al. (2000) indicated that effective nitrate removal from groundwater by denitrification is restricted to sites with shallow lateral subsurface flow. Less effective nitrate retention was found when riparian zones are connected to large upland aquifers with a more vertical direction of flow (Hill, 1990; Devito and Dillion, 1993).

Table 1 N removal from the shallow groundwater compared with removal process rates.

g N m^{-2} yr^{-1}	Forested zone	Herbaceous zone
Measured removal from groundwater	32.79	11.76
Measured removal from process rates	33.77	39.48

Lowrance et al. (1984) measured higher removal NO_3^- rates than NO_3^- inputs by shallow groundwater. In the Dutch sites we also measured higher NO_3^- removal rates by denitrification and plant uptake compared to the NO_3^- removal measured through hydrological studies of the subsurface water (corrected for dilution) (Table 1). However, when other nitrogen "input" sources are included in the budget as nitrogen deposition and soil N mineralization, N input balances the N output in the grassland site (Table 2). For the forested site we end up with a residual term indicating that there was nitrogen that was not accounted for, although input and output are still in the same order of magnitude (Table 2). The residual term is not surprising, as a full evaluation of processes that contribute to NO_3^- removal requires detailed mass balances for water and nitrogen, whereas our research was not designed as a mass balance study.

Table 2 Annual N input and N output (g N m^{-2} yr^{-1}) in the studied riparian zones in the Netherlands.

N input (g N m^{-2} yr^{-1})	Forested zone	Herbaceous zone
Groundwater	86.41	14.58
N deposition (from literature)	4.0	4.0
Soil N mineralization	20.4 (0.03)	24.7 (0.03)
Litter N leaching	0.29 (0.35)	0
Litter N mineralisation[1]	4.97 (0.78)	0.33 (0.07)
Plant N retranslocation	3.37 (0.41)	0.33 (0.06)
N fixing (alnus, from literature)	(-)	(-)
Total in	**114.47**	**43.61**
N output (g N m^{-2} yr^{-1})	**Forested zone**	**Herbaceous zone**
Groundwater	53.62	2.82
Denitrification	18.90 (3.16)	24.87 (5.09)
Plant uptake[2]	14.87 (1.12)	14.61 (2.57)
Litter N immobilization	0.32 (0.22)	0.16 (0.08)
Total out	**87.71**	**42.46**
Residual	**(+) 26.76** (23% of total input)	**(+) 1.15** (2.6% of total input)

[1] litter N mineralisation is not included because this is already included in the soil mineralisation
[2] due to difficulties in determining the belowground production (see Chapter 5) production and decomposition rates are restricted to the above ground biomass

Complete mass balance studies in natural systems are very rare because they necessitate an enormous research effort to measure all the fluxes and pools accurately (Koopmans, 1996; Meuleman et al., 2003). In many mass balance studies, the residual term has been used to estimate non-measured input or output processes. Another factor that may contribute to uncertainty is that we have not been able to measure the below-ground plant uptake and turnover because of the high spatial variability and limited temporal (seasonal) differences of root biomass. We therefore assumed N uptake in newly formed roots and N release from dead roots to be continuous (year around), thereby implicitly assuming that N uptake in new roots was not very significant in the total N retention of riparian buffers (Lowrance, 1992; Ehrenfeld et al., 1997). Furthermore, we assumed that water-saturated soil conditions and high nutrient availability contributed to relatively low investments in below ground biomass as previous studies indicated that biomass allocation was strongly influenced by flooding gradients with significantly higher above-ground production compared to below-ground under flooded conditions (Day and Megonigal, 1993). However, given the slower decomposition rate of fine *Alnus glutinosa* roots compared to roots of *Glyceria maxima* (Table 3) we may speculate that tree roots contribute more to the N retention, explaining part of the residual found in the forested site. Abiotic immobilization of nitrate by iron (Iron Wheel hypothesis), recently described by Davidson et al. (2003) as an important process in forest soils might also explain part of the residual found.

There are other factors that make it difficult to develop an accurate and complete N budget in riparian zones. First, are problems associated with the extrapolation of measured process rates to rates per unit area. Riparian zones cannot be considered as homogeneous closed systems, which implies that spatial and temporal extrapolation of process rates inevitably induces errors. Moreover the translation of hydrologic flux data ($m^3 h^{-1}$) to area based process rates (m^2) may also induce errors. Uncertainties as described above and

Table 3 Decomposition rates k (yr^{-1}) (means with standard errors in brackets, n=5 for leaves, n=8 for roots).

	Stream zone	Intermediate zone	Field zone
Herbaceous site			
Leaf decomposition	3.84 (0.91)	8.60 (3.77)	1.01 (0.09)
Root decomposition	1.62 (0.12)	1.61 (0.07)	1.51 (0.08)
Forested site			
Leaf decomposition	2.21 (1.19)	1.53 (0.23)	1.19 (0.15)
Root decomposition	0.27 (0.03)	0.26 (0.02)	0.37 (0.02)

inaccuracies in methods generally accumulate in the residual term. In this study we considered the hydrological inputs and outputs as the most uncertain terms due to the heterogeneous sediments and consequently the occurrence of preferential flow paths with deviating hydraulic conductivity and flow velocity. Therefore, we chose to focus on the relative contribution of the dominant processes plant uptake and denitrification to N removal. This comparison is described in Chapter 6. Denitrification was the dominant process contributing to N removal at all of the sites studied along the climatic gradients in Europe, except for riparian sites with low soil moisture contents where denitrification rates were significantly lower than plant uptake (Chapter 6; Cosandey et al., 2001). Denitrification might even play a more important role than indicated in Chapter 6 of this thesis because acetylene used for denitrification measurements is known to inhibit nitrification and contribute to NO reduction, leading to a significant underestimation of the process (Bollmann, 1997). The methods used to make measurements also introduce a source of uncertainty. Measurements of denitrification were performed on soil cores collected in the field and thus taken out of the soil matrix. This physical disturbance could also have induced a significant underestimation of the process rates because cores were cut off from the continuous nitrate supply by groundwater.

We anticipated that riparian zones that are relatively dry would be less efficient in removing NO_3^- from the groundwater. Our data from a variety of European riparian zones, however, did not support this hypothesis (Sabater et al., 2003). We conclude that while denitrification is the dominant process accountable for N removal in riparian buffer zones, the direct role of vegetation in the annual N retention is considerable and cannot be ignored in the water quality functioning of riparian buffers.

What is the importance of groundwater flow path in the spatial variability of nitrate removal?

Tracing the groundwater flow paths and nitrate removal along groundwater pathways revealed high spatial differences within the forested riparian buffer zone in the Netherlands. The occurrence of ineffective flow paths could partly be explained by differences in water flux and routing causing reduced removal efficiencies due to shorter contact time between groundwater and the organic matter rich topsoil with its high denitrification potential (Chapters 2, 4). Groundwater table levels and hydrological flowpaths are known to be important factors controlling nitrate removal processes (Hill, 1996; Cirmo and

McDonell, 1997). Hydrological pathways in riparian zones are generally heterogeneous due to complex sedimentology (Devito et al., 2000; Clément et al., 2002). However, the observed spatial variability in nitrate removal between groundwater pathways measured in the forested riparian zone is not commonly found. An additional explanation for the lower nitrate removal could be found in a pH effect limiting the denitrification activity in high nitrate loaded flowpaths (Chapters 2, 4).

Is it possible to use water table as a common descriptor of soil N transformation processes in riparian zones under a wide range of climatic conditions?

The influence of individual environmental factors on the denitrification process is quite well known from individual sites within a climatic zone. However, the importance of these controlling factors in riparian zones across a wide range of climatic conditions had never been evaluated. One of the main regulating factors of denitrification is the soil moisture content (Tiedje, 1988). Soil moisture strongly influences the O_2 availability by restricting the diffusion, besides it increases the availability of nitrate and dissolved organic matter. Soil moisture content in turn was expected to be highly dependent on the local climate (i.e. precipitation and evaporation). Overall however, no significant effect of climate has been observed in measurements of N removal efficiency or denitrification rates in a range of European sites (Cosandey et al., 2001; Sabater et al., 2003, Chapter 6). The lack of any demonstrable relationship between climate and N removal does not exclude the possibility that denitrification rates are influenced by climatic conditions. At the scale of our analysis, however, and given the large variation between study sites, factors other than climate (e.g., land use, site history and flooding regime) may have obscured the influence of climate on measured process rates.

Cosandey et al. (2001) found that the intensity of denitrification in European riparian study sites was strongly related to total soil organic matter content and soil moisture regime. These factors appeared to be more important than climate, type of vegetation or season in predicting denitrification rates. Kaiser et al. (1996) performed a comparable pan-European study on N_2O emission and N-loss through denitrification from agricultural soils. Similarly, this study showed no clear relation between denitrification rates and climatic variables, and denitrification was clearly related to organic carbon content. This relation can be explained by the fact that organic carbon is required as a substrate for denitrification. Furthermore the content of organic matter in soils is often

closely related to the water table level, e.g. under permanently wet conditions, decomposition rates are low and organic matter accumulation can take place. Because water table level is easy to measure and is often indicated on soil maps, we wanted to know whether N process rates could be predicted on the basis of certain water table thresholds. In Chapter 5, we used the NICOLAS data to test whether water table level was a good predictor of nitrogen cycling, particularly regarding sources and sinks of nitrate.

Indeed, water table level turned out to be a good predictor of nitrogen processes and N availability in riparian ecosystems. Three consistent water table thresholds were identified at very different riparian sites in terms of climate and N loading and vegetation. When water table levels are within -10 cm of the soil surface, ammonification prevails and ammonium accumulates in the topsoil. Average groundwater tables between -10 and -30 cm favor denitrification and therefore reduce the nitrogen availability in soils. At sites with water table levels below -30 cm, nitrate is the main end product as a result of high net nitrification. At these latter sites, denitrification might occur in fine-textured soils, where it is triggered by rainfall events. These water table level thresholds can be used as a proxy to translate the consequences of stream flow regime change to nitrogen cycling in riparian zones and, consequently, to potential changes in nitrogen mitigation.

Are chronically N-loaded riparian zones sustainable buffers or do they eventually lose their denitrification potential due to carbon limitation or N saturation?

Few investigators have examined the nitrate removal of riparian zones along headwater streams that receive high groundwater nitrate inputs (Hanson et al., 1994 a,b; Hoffmann, 1998; Devito et al., 2000). In the heavily nitrate-loaded forested riparian zone in the Netherlands, nitrate removal efficiencies were lower than those measured in other European sites with lower N loadings (Sabater et al., 2003). In this respect, questions arise about the sustainability of riparian buffer zones for nutrient retention. Some studies suggest that they might become less efficient with time, due to a reduced effectiveness for sediment-bound N removal (Dillaha et al., 1989) or due to N saturation and carbon limitation (Brinson et al., 1984; Hanson et al., 1994 a,b; Haycock et al., 1997; Hoffmann et al., 1998). The question whether the water quality function could potentially be jeopardized by prolonged excessive nutrient and hydrologic inputs needs to be resolved before decisions for utilizing riparian buffer zones for polluted subsurface runoff treatment are made.

Carbon-limitation

Denitrification is an energy demanding process and the energy required is usually derived from a carbon source (heterotrophic denitrification) as illustrated in the following equation (Reddy and Patrick, 1984):

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O$$

Often a positive correlation between denitrification rate and the amount of easily degradable organic carbon (Burford and Bremner, 1975; Bijay-Singh et al., 1988) has been found. Haycock and Pinay (1993) therefore recommended that future studies on nitrogen retention in riparian zones should focus on the carbon turnover. An increased nitrate loading enhances litter decomposition because plants produce litter with low lignin contents and high nutrient contents (Van Oorschot., 1996). Decomposition of organic matter takes place in sequential steps. First oxygen is used as the electron acceptor, followed by nitrate when oxygen is depleted. Consequently, anaerobic decomposition (denitrification) rates are also enhanced with an increased nitrate loading as the availability of the electron acceptor increases. In chronically N-loaded riparian zones the C demand of aerobic and anaerobic decomposition might exceed the yearly production of easily degradable C. As litter production is mainly taking place in autumn, a temporal C limitation might occur during summer leading to periodically lower nitrate removal by denitrification.

On the other hand increasing N load enhances primary productivity and litter quality, which may compensate for the increasing demand of easily degradable carbon by decomposition and denitrification (Van Oorschot, 1996; Malhi et al., 2003; Van Groeningen et al., 2003; Palm et al., 2002). A critical element in this discussion is whether or not the annual input of carbon by plant growth in riparian zones is adequate to meet the carbon demand of denitrification. If denitrification in buffer zones were to become C-limited, harvesting of biomass as a management practice to remove nutrients from the system might have an adverse affect on nutrient removal by denitrification.

Table 4 gives a rough indication of the order of magnitude of C consumption by denitrification activity in comparison with the annual above-ground C production. The C consumption of above-ground litter breakdown is also given based on litterbag studies. For the extrapolation of measured decomposition rates in mg C per gram litter per year to C consumption per unit area, we had to make use of the annual litter production data, complicating the comparison of the C production and C consumption terms because they are not independent. We can, however provisionally, conclude from these figures that the annual C production exceeds C decomposition rate. Even in wetland soils containing high organic matter contents a large part of carbon

Table 4 Annual carbon production and consumption rates in g C m^{-2} yr^{-1}.

Site	C production Plant production[1]	Balance	C consumption by denitrification
Forest	528.9 (62.0)$_{\text{incl wood}}$ 214.6 (14.0)$_{\text{leaves \& herbs}}$	57.9	20.3 (3.4)
Herbaceous	358.3 (6.6) 23.60 (6.3)$_{\text{after mowing}}$	12.4	26.7 (5.5) 17.6 (15.0)

[1] due to difficulties in determining the belowground production (see Chapter 5) production and decomposition rates are restricted to the above ground biomass

can be refractory, and will not be available for microbial respiration (Bridgham and Richardson, 1992). Still, this comparison indicates that the C consumption by denitrifiers is very small compared to the C production. Apart from these indications of C dynamics we suspect that below-ground C production and rhizo-deposition might play a very important role in providing substrate for denitrifiers. Unfortunately, no below-ground production were measured in our study due to high spatial variability and limited temporal (seasonal) differences of root biomass. Below-ground decomposition experiments were performed (Table 3) and indicated a slower decomposition of root material compared to above ground litter, due to predominantly anaerobic conditions in the soil. Under these circumstances competition for C sources is probably not as strong as at the soil surface where both aerobic and anaerobic decomposition could take place (Van der Lee et al., 1999). Furthermore, knowledge about the amount, the composition, and the turnover of root exudates is still very limited. Hutsch et al. (2002) demonstrated with different plant species that up to 20% of photosynthetically fixed C was released into the soil during the vegetation period. Lynch and Whipps (1990) indicated that depending on plant species, age and environmental conditions, rhizodeposition can even account for up to 40% (or more) of the dry matter produced by plants. A large percentage (64-86%) of this C source is easily degradable and can quickly be respired by micro-organisms as denitrifiers (Hutsch et al., 2002).

Additionally, measurements on denitrification enzyme activity (Box 1) did not show any signs of C-limitation in the forested riparian zone. We conclude that there is no clear indication that buffer zones with prolonged N loading might become C-limited due to the high C respiration activity of denitrifiers.

N saturation

In Chapter 2 we stated that clear symptoms of saturation were visible in the forested site because i) flow paths with high N loading showed a low N removal efficiency, ii) observed in situ denitrification rates at the highly N-

loaded flow paths were not significantly higher than at flow paths with lower nitrate input rates (Chapter 4), iii) rates of N mineralization were extremely high in the Netherlands sites compared to other riparian areas (Chapter 5; Aber et al., 1989), iv) a decrease in absolute removal capacity was observed along the highly N-loaded flow paths, suggesting an inhibitory effect of nitrate on denitrification. Results of denitrification enzyme activity (DEA) measurements in the laboratory (Box 1), however, still showed an increase of denitrifier activity in the nitrate amended soil samples (although effects were not always significant). The significant negative relation found between pH and nitrate concentration in the groundwater may be explained by an inhibitory effect of low pH on denitrification. Detailed studies on denitrification along selected groundwater pathways with high nitrate loading and low nitrate removal efficiencies (Chapter 4) underlined the pH effect on denitrification although low WFPS also contributed to the low denitrification activity in the low nitrate removal flow path.

Clear symptoms of N saturation were observed in the forested riparian zone in the Netherlands, but indirect effects of pH and water content on denitrification rates are probably decisive in the lower nitrate removal. Data on DEA activity in the laboratory indicated that the upper limit of denitrification activity was not yet exceeded. This large and unlimited denitrification potential was also found in a recent study by Clément et al. (2002).

Are riparian buffer zones solving an environmental problem or do they cause a shift from groundwater pollution with nitrate towards air pollution with nitrous oxide?

Results from the comparison between the forested and grassland site in the Netherlands (Chapter 3) revealed that high nitrate loading of riparian buffer zones leads to high N_2O emission rates when the NO_3^- availability is high. In these cases N transformation by buffer zones may result in an unfavorable shift from water pollution to an increase in greenhouse gas emission.

What is the influence of nitrate removal efficiency on the nitrous oxide emission (and denitrification) from nitrate-loaded riparian buffer zones?

As expected, significant N_2O emissions occurred along the flow path with low nitrate removal efficiency and high nitrate loading rates. Our results also indicated that flow paths with a high nitrate removal capacity significantly

contribute to an increased N_2O emission from riparian zones under sub-optimal temperatures. Analyzing the spatial patterns found in this detailed study we concluded that N_2O emission was higher when any factor was reducing the denitrification rate i.e. temperature, water filled pore space or pH.

Is the N_2O:N_2 ratio of denitrification end products a valuable proxy for the evaluation of riparian zone functioning? Which (combinations of) environmental factors control this ratio in riparian systems?

On the basis of results described in Chapter 3, we might expect that nitrate availability is the strongest controller of the N_2O:N_2 ratio during denitrification. However, N_2O:N_2 ratios calculated with data collected in 2000 turned out to be only affected by pH (Fig. 3). This is in agreement with studies by Christensen (1990) indicating that N_2O is the main end product of denitrification at pH values below 4. Flessa et al. (1998) and Groffmann et al. (2000) found similar effects of pH on N_2O emission and the denitrification end-product ratio. Results from the detailed field experiment performed in 2001 within the nitrate loaded forested riparian zone (Chapter 4) did not show any coherent pattern between the N_2O:N_2 ratio and environmental factors as nitrate availability or pH due to multiple factor interactions. From this study we concluded that high variability in N_2O:N_2 ratio and poor relations with environmental conditions reduce the value of the ratio as a proxy to evaluate the environmental consequences of riparian buffer zone management (Chapter 4; Groffman et al., 2002). Measurements of the absolute N_2O emission rates and the nitrate removal from the shallow groundwater are required for a full assessment of riparian zone functioning (Van Cleemput, 1998).

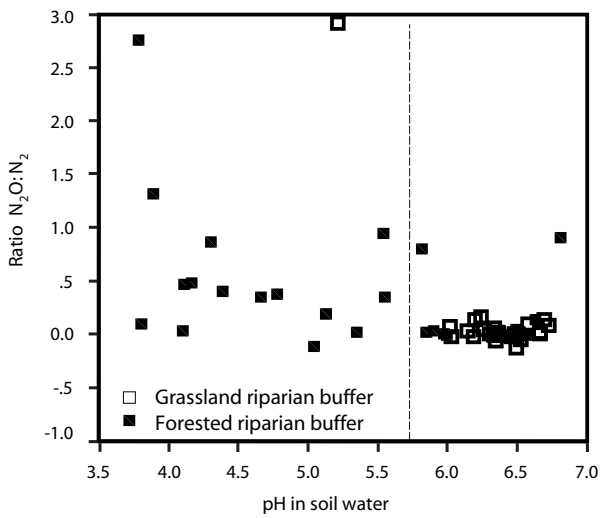

Figure 3 **N_2O:N_2 ratio in denitrification versus pH in the soil water from two riparian buffer zones.**

Does the Intergovernmental Panel on Climate Change (IPCC) emission factor for indirect N_2O emission from agriculture by groundwater and drainage water (EF5-g, IPCC, 2001) account for N_2O emissions from N-loaded riparian buffer zones?

Increasing attention has been paid to the so-called indirect anthropogenic sources of N_2O as they are considered the most uncertain in national N_2O inventories (Well, 2002; Rypal, 2002; Groffman, 2002). It is assumed that a considerable fraction of the indirect N_2O emission caused by agriculture is emitted from aquifers and riparian zones during transport of leached nitrogen from agricultural fields to groundwater and surface waters. Riparian zones are considered as hotspots of N_2O emissions with disproportionately high rates relative to the surrounding landscape because nitrate loaded groundwater flow path converge with anaerobic C rich substrate (McClain et al., 2003). Biogenic N_2O formation in forested riparian zones may even be enhanced by higher atmospheric deposition of nitrogen oxides (NO_x) or ammonia (NH_3) due to turbulence at the interface between the agricultural field and the riparian forest (Draaijers et al., 1988; Kroeze and Mosier, 2002).

In Chapter 3 we commented on the proposed Intergovernmental Panel on Climate Change (IPCC) emission factor (EF5-g) of 0.015 for quantification of indirect N_2O emissions from groundwater. The EF5-g value calculated in this study, on the basis of the N_2O flux measurements and the yearly incoming NO_3^- flux in the groundwater, were significantly higher than the proposed 0.015, with ranges of 0.028-0.058 and 0.016-0.031 in the forested and grassland riparian zone, respectively. In Chapter 4, N_2O emissions measured were spatially linked to N loading from two specific groundwater flow paths. The EF5-g values calculated on the basis of this detailed study ranged from 0.01-0.08. Although these measurement series were highly limited in terms of temporal scope, and cannot directly be extrapolated to annual emission rates, these ranges again indicate that the proposed 0.015 (EF5-g) is probably too low for nitrate-loaded, wet riparian buffer zones. We therefore concluded that considerably higher emission factors are needed to describe the emission from nitrate loaded groundwater passing through riparian buffer zones and we propose the use of separate EF5-g factors for groundwater flowing through riparian areas, versus groundwater under upland agricultural fields.

The beneficial functions that improve water quality in riparian zones have received a lot of attention. This study indicated that high nitrate loading of riparian buffer zones may, result in an unfavourable shift from water pollution to an increased N_2O emission. However, available data on N_2O formation and emission during transport of agricultural N from the soil to the surface water

is limited. There is a strong need for more data on N_2O emissions from riparian zones and groundwater, induced by agricultural activities in watersheds. To perform a full assessment of riparian ecosystem functioning, we have to evaluate the precise consequences of both forms of environmental pollution to determine the environmental risks.

What are the opportunities for water quality improvement and N_2O mitigation while managing N flows across the landscape?

From the perspective of water quality and N_2O emission, source-directed measures aiming at a reduction of nitrate leaching from agricultural soil are obviously the best management practice to protect the aquatic ecosystem from eutrophication. Although several agricultural measures are already being taken to reduce the amount of excessive nitrogen in the soil profile after harvest, considerable leaching of nitrate still takes place (Addiscot et al., 1991; Oenema et a1, 1998; Olsthoorn and Fong, 1998). Therefore additionally end-of-pipe-measures as riparian zones reducing diffuse nitrate pollution of surface waters need to be implemented and optimized. Management options to mitigate N_2O emissions while stimulating denitrification in riparian zones are limited. Results from Chapter 2 and 4 of this thesis indicate that liming of the riparian zones might possibly reduce the N_2O emission and increase the denitrification activity and consequently increase the nitrate removal efficiency in buffer zones throughout the Netherlands. The positive effect of lime on denitrification rates was already observed in an experiment by Groffman et al (1991) comparing two grass and two forest vegetated filter strips (VFS) in Rhode Island. Their results showed significantly higher denitrification rates in the grassland strips due to the use of fertiliser and lime for grass production. The possible mitigation of N_2O emission by liming is however restricted to the wet riparian zones. In the drier hill slope area liming may even significantly increase N_2O emissions due to enhanced ammonification and nitrification activity as was observed in a spruce forest by Papen and Butterbach-Bahl (1993).

Although this study (Chapter 6) confirms the importance of denitrification in riparian zone nitrate removal, the role of N uptake by the vegetation is not insignificant. As stated before, mowing and harvesting can importantly contribute to N export from the system. Comparison of C production by the vegetation and consumption by denitrifiers indicates that the risk of C-limitation due to C consumption and C export by mowing is probably small. Removal of plant material is therefore still recommended as a management

practice, it will decrease the systems susceptibility of N loss following disturbance by clear-cutting (Yeakley et al., 2003) or extreme weather events (Prior et al., 1998).

Reduction of fertilizer use in the upland agricultural fields is an essential measure to protect the aquatic ecosystem from eutrophication. Riparian buffer zones can add to the water quality protection but are certainly no replacement of source-directed measures. A combination of methods is definitely needed in watersheds with intensively managed agricultural fields, because high N loading of riparian zone leads to unwanted effect of high N_2O emissions.

However, apart from the water quality function, riparian ecosystems have other essential functions including: i) stream bank stability and erosion protection, ii) regulation of water temperature and growth of aquatic macrophytes by canopy shading, iii) increasing connectivity in landscapes and iv) increasing biodiversity. Therefore we support the general belief that riparian buffer zones are highly valuable landscape elements, far in excess of their relative surface area, and need to be protected, restored or re-established.

Recommendations for further research

Use of ^{15}N isotopes

In the NICOLAS project we used a combination of complementary methods to evaluate the relative importance of denitrification and plant uptake in the groundwater nitrate removal within the riparian zones. Although these approaches were necessary for a first evaluation, it appeared difficult to draw general quantitative conclusions from our results. Clément et al. (2003c) and Dhondt et al. (2003) extended their riparian zone study with a study on natural stable nitrogen isotope abundance along the groundwater flow paths to evaluate the respective role of denitrification and plant uptake on the groundwater nitrate removal. Results from these studies seem very promising to obtain insight in the relative importance of several N removal processes in riparian zones. However, care must be taken not to use this method in sites with N fixating plant species. Moreover, additional evidence that plants do not fractionate nitrogen during uptake is needed as a firmer base for this method.

Another possibility is the use of artificially ^{15}N-enriched nitrate in sites with already high nitrate loading. An elegant spiking method to quantify denitrification rates in groundwater at specific locations under field conditions

is described by Addy et al. (2002). They injected groundwater with ^{15}N and conservative tracers into a mini-piezometer and extracted it from the same mini-piezometer after an incubation period. When this method is combined with detailed field data on hydraulic gradients and flow patterns this method can contribute to the mechanistic understanding of denitrification in heterogenous riparian zones.

Predicting the effect of changing water regimes on nitrous oxide emission at the landscape scale

Currently, flood protection in the Netherlands is changing from exclusion strategies (reinforcement of dikes and dams) to allowing a more natural situation by the creation of water retention zones. The development of areas for water retention, thereby widening the actual riverbed and excavating flood plains, is generally being coupled to the development of natural riparian ecosystems with adventitious positive effects on water quality. High nitrogen concentrations in river water and groundwater in flood plains originating from (former) agricultural activities combined with the altered hydrological regimes may however considerably enhance nitrous oxide emissions.

The vast majority of the N_2O flux measurement, denitrification measurements and modeling activity that has taken place in riparian zones focused on the field scale, with hourly and daily measurements of fluxes. It appeared to be very difficult to use these data at a broader spatial and temporal scale and establish strong predictive relationships between fluxes and landscape-scale parameters such as presence and area of riparian wetlands within a catchment or distribution of soil types (Burt et al., 1988; Consandey et al., 2001). The poor relationships found in this study can partly be explained by the extreme temporal and spatial variability in fluxes and non-linearity between environmental variables and N_2O fluxes at different scales. It may in this respect be questioned to what extent data collected at the field scale allow for solid conclusions on estimation emissions and mitigation measures on the catchment scale. Sustainable water management in drainage basins therefore requires suitable scaling techniques. The use of deterministic models linked with GIS may improve the prediction of denitrification and N_2O emissions on a catchment scale as was done by Plant (1998) to study the effect of landuse on N_2O emissions in Costa Rica. However, more insight is needed in mechanistic relationship between key model parameters and processes at different scales as Plant (1998) suggested that linear scaling involved in the extrapolation of emissions from microsites to regional scale may cause serious aggregation errors. Furthermore, we need to assess the role of spatial and temporal hot spots at different spatio-temporal scales (McClain et al., 2003).

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Summary

Introduction

Diffuse pollution of nutrients and pesticides from agricultural areas is increasingly recognised as a major problem in water management. Ecotechnological measures such as constructed wetlands and riparian buffer zones clearly have an important role in the reduction of diffuse pollution by removing and modifying pollutants from agricultural runoff. However, the processes that account for the pollution retention capacity are diverse and the performance of buffer zones along climatic gradients and under varying hydrological regimes is largely unknown.

The study described in this thesis was conducted to determine the influence of N-loading rate, vegetation and hydrologic regime on the mechanisms of nitrogen removal in riparian zones along a climatic gradient. We focused our research on nitrogen removal in riparian buffer zones that were down-gradient from intensively fertilised agricultural fields. The research was conducted in the Netherlands and other locations across Europe within the framework of a joint European research project called NICOLAS (NItrogen COntrol by LAndscape Structures in agricultural environments ENV4-CT97-0395). This FP-4 project started in 1998 with a goal of evaluating the natural performance of riparian zones to sustainably buffer waterborne fluxes of diffuse agricultural nitrogen pollution of aquatic environments. Partners in this project were researchers from Rennes (F), Durham (GB), Barcelona (S), Lausanne (CH), Boekarest (R) and Warschau (PL). The main aim of the research described in this thesis was to further improve the understanding of nitrogen transformation processes occurring in natural buffer zones and to evaluate the risk of greenhouse gas emissions during pollutant mitigation. In this summary an

overview is given of the most important results of this research described in the following major themes;

- dilution or removal,
- plant uptake and denitrification,
- groundwater level and nitrogen transformation,
- nitrous oxide emission, and
- sustainability of buffer zones.

Dilution or removal

In the past, the majority of the studies on buffer zones relied on concentration based input-output analyses to evaluate the water quality functioning. Since dilution of shallow nitrate-loaded agricultural runoff with groundwater from a deeper aquifer may cause a significant decrease in nitrate concentrations, this study focused on the importance of groundwater flow paths in the groundwater quality dynamics. A forest and a grassland zone along first-order streams in the Netherlands were selected for this research (Chapter 2). Hydraulic parameters and water quality were monitored in both riparian zones on a monthly basis over two years in 50 piezometers. Average nitrate loadings were high in the forested buffer zone with 87 g NO_3^--N m^{-2} yr^{-1} and significantly lower in the grassland buffer zone with 15 g NO_3^--N m^{-2} yr^{-1}. Groundwater from a second aquifer played an important role in diluting the shallow nitrate-loaded agricultural runoff causing a significant decrease in nitrate concentration and a significant increase in chloride concentration along its flow path towards the stream. Tracing the groundwater flow paths and dilution along these pathways revealed that clear spatial differences occurred in N removal within riparian zones. The observed dilution could cause an over-estimation of the nitrate removal capacity of up to 60% if this physical process is not taken into account. Besides the dilution both riparian zones were capable of reducing nitrate in subsurface runoff by biological N removal, the grassland riparian zone as a whole removed about 63% of the incoming nitrate load whereas in the more heavily loaded forested zone clear symptoms of saturation were visible and only 38% of the incoming nitrate load was removed.

Plant uptake and denitrification

As stated before, the efficiency of nitrate removal from groundwater passing through riparian zones can vary with climate, landscape setting and nitrate

loading, therefore results often seem somewhat site-specific. The range of sites within the NICOLAS project provided a wide spectrum of climatic, hydromorphic and anthropogenic conditions ideal to evaluate the relative significance of various nitrate removal mechanisms within herbaceous and forested riparian zones (Chapter 6). Plant uptake and denitrification are considered to be the most important processes responsible for N retention and mitigation in riparian buffer zones. However, nutrients taken up by plants remain in the system only temporarily and may be gradually released by mineralization later. Still, plants increase the residence time of nutrients considerably by reducing their mobility. Denitrification is a microbial process involving the stepwise reduction of nitrate through nitrite, nitrogen oxide and nitrous oxide, ending with gaseous nitrogen. The significance of the denitrification process is that it provides a permanent sink for excess nitrate. In our NICOLAS study the annual N retention in vegetation and litter accounted for 13-99% of the total N removal (Chapter 6). Higher N uptake and higher N retention was found in forested buffers but periodic harvesting of herbaceous biomass contributed considerably to the N retention in sites dominated by herbaceous vegetation. We found no differences in the effectiveness of specific vegetation types (forest or herbaceous) on nitrate removal from shallow groundwater. Denitrification rates measured in soil cores with the acetylene inhibition method varied between 0.11 and 91 mg N m^{-2} d^{-1} in the studied European riparian zones. The between-sites comparison showed that denitrification was the dominant process of N removal in most riparian sites, except for the Spanish and Romanian sites where denitrification rates were significantly lower than plant uptake.

Groundwater level and nitrogen transformation

Overall, no significant effect of climate has been observed in measurements of N removal efficiency or denitrification rates in a range of European sites. Cosandey et al. (2001) found that the intensity of denitrification in our NICOLAS riparian study sites was strongly related to total soil organic matter content and soil moisture regime. These factors appeared to be more important than climate, type of vegetation or season in predicting denitrification rates. Because water table level is easy to measure and is often indicated on soil maps, we wanted to know whether N process rates could be predicted on the basis of certain water table thresholds. In Chapter 5, we used the NICOLAS data to test whether water table level was a good predictor of nitrogen cycling, particularly regarding sources and sinks of nitrate. Indeed, water table level turned out to be a good predictor of nitrogen processes and N availability in

riparian ecosystems. Three consistent water table thresholds were identified at very different riparian sites in terms of climate and N-loading and vegetation. When water table levels are within -10 cm of the soil surface, ammonification prevails and ammonium accumulates in the topsoil. Average groundwater tables between -10 and -30 cm favor denitrification and therefore reduce the nitrogen availability in soils. At sites with water table levels below -30 cm, nitrate is the main end product as a result of high net nitrification. At these latter sites, denitrification might occur in fine-textured soils, where it is triggered by rainfall events. These water table level thresholds can be used as a proxy to translate the consequences of stream flow regime change to nitrogen cycling in riparian zones and, consequently, to potential changes in nitrogen mitigation.

Nitrous oxide emission

Denitrification was identified as the dominant process of N removal in most riparian zones studied, denitrification is however also considered as a major source of the greenhouse gas nitrous oxide (N_2O). We therefore questioned if riparian buffer zones were useful in solving an environmental problem or rather cause a shift from groundwater pollution with nitrate towards air pollution with nitrous oxide. We assessed the rates of N_2O emission from riparian buffer zones that receive large loads of nitrate, and evaluated various factors that are purported to control N emissions (Chapter 3). Denitrification, nitrification, and N_2O emissions were measured seasonally in grassland and forested buffer zones in the Netherlands. Nitrogen process rates were determined using flux chamber measurements and incubation experiments. Nitrous oxide emissions were found to be significantly higher in the forested (20 kg N ha^{-1} yr^{-1}) compared with the grassland buffer zone (2-4 kg N ha^{-1} yr^{-1}), whereas denitrification rates were not significantly different. Higher rates of N_2O emissions in the forested buffer zone were associated with higher nitrate concentrations in the groundwater. We conclude that N transformation by nitrate-loaded buffer zones results in a significant increase of greenhouse gas emission.

Tracing the groundwater flow paths and nitrate removal along such groundwater pathways in the Dutch riparian zones revealed high spatial differences within the forested riparian buffer zone. The spatial variability in hydrological flow paths and nitrate removal processes complicates the overall assessment of riparian buffer zone functioning in terms of water quality improvement as well as enhancement of the greenhouse effect by N_2O

emissions. The objective of the research described in chapter 4 was therefore to find clues for explaining spatial variability in nitrate removal, denitrification and N_2O emission, and to use this insight to help assess the balance between environmental benefits and risks in these habitats. Denitrification and emissions of N_2O were measured in winter and summer along two groundwater flow paths in a forested riparian zone using flux chambers and incubation experiments. In winter, N_2O emissions were significantly higher (12.4 mg N m^{-2} d^{-1}) along the flow path with high nitrate removal compared with the flow path with low nitrate removal (2.58 mg N m^{-2} d^{-1}). In summer a reverse pattern was observed, with higher N_2O emissions (13.6 mg N m^{-2} d^{-1}) from the flow path with low nitrate removal efficiencies in comparison with the flow path with high nitrate removal (4.44 mg N m^{-2} d^{-1}). Distinct spatial patterns of denitrification and N_2O emission were observed along the high nitrate removal transect, whereas no clear pattern was found along the low nitrate removal transect, where denitrification activity was very low. On the basis of the studies described in Chapters 2 and 3, ineffective groundwater flow paths in buffer zones (with high nitrate loading rates and low nitrate removal rates) were expected to be detrimental for the environment, because they fail to protect the stream ecosystem and show a relatively high contribution to the emission of the greenhouse gas N_2O. Results described in chapter 4 indicate that denitrification rates were, indeed, quite different between the studied flow paths with more than 2 times higher rates in the flow path with high nitrate removal. On the contrary, total N_2O emissions were quite similar for both flow path, indicating that high nitrate removal transects can also significantly contribute to an increased N_2O emission from riparian zones. Riparian zone management aiming at an increased denitrification activity in buffer zones is worthy from the perspective of water quality improvement, however a certain risk of N_2O emission remains inevitable. Simultaneous minimization of N_2O emissions is only possible if riparian zone management is combined with source-directed measures to drastically reduce the nitrate concentration in agricultural runoff.

Sustainability of buffer zones

Few investigators have examined the nitrate removal of riparian zones along headwater streams that receive high groundwater nitrate inputs. In the heavily nitrate-loaded forested riparian zone in the Netherlands, nitrate removal efficiencies were lower than those measured in other European sites with lower N loadings (Sabater et al., 2003). In this respect, questions arise about the sustainability of riparian buffer zones for nutrient retention.

We questioned in our synthesis (Chapter 7) if chronically N-loaded riparian zones were at risk in eventually losing their denitrification potential due to carbon limitation or N saturation. Denitrification is an energy demanding process and the energy required is usually derived from a carbon source (heterotrophic denitrification). In chronically N-loaded riparian zones the C demand of aerobic and anaerobic decomposition might exceed the yearly production of easily degradable C. As litter production is mainly taking place in autumn, a temporal C limitation might occur during summer leading to periodically lower nitrate removal by denitrification. To check this statement the order of magnitude of C consumption by denitrification activity was compared with the annual above-ground C production. This comparison indicated that the C consumption by denitrifiers is very small compared to the C production. Although it is a rather rough comparison we can provisionally conclude from these figures that the annual C production exceeds C decomposition rate and C limitation is not very likely in this system. Additionally, measurements on denitrification enzyme activity (potential denitrification) did not show any signs of C limitation in the forested riparian zone. This leads to the conclusion that there is no clear indication that buffer zones with prolonged N-loading might become C limited due to the high C respiration activity of denitrifiers.

In Chapter 2 we stated that clear symptoms of N saturation were visible in the forested site because i) flow paths with high N loading showed a low N removal efficiency, ii) in situ denitrification rates observed at the highly N-loaded flow paths were not significantly higher than at flow paths with lower nitrate input rates (Chapter 4), iii) rates of N mineralization were extremely high in the Dutch sites compared to other riparian areas (Chapter 5; Aber et al., 1989), and iv) a decrease in absolute removal capacity was observed along the highly N-loaded flow paths, suggesting an inhibitory effect of nitrate on denitrification. Results of denitrification enzyme activity (potential denitrification) measurements in the laboratory (Chapter 7), however, still showed an increase of denitrifier activity in the nitrate-amended soil samples. The significant negative relation found between pH and nitrate concentration in the groundwater may be explained by an inhibitory effect of low pH on denitrification. Detailed studies on denitrification along selected groundwater pathways with high nitrate loading and low nitrate removal efficiencies (Chapter 4) underlined the pH effect on denitrification although low water-filled soil porosity also contributed to the low denitrification activity in the low nitrate removal flow path. Liming of the agricultural fields and riparian zones might therefore increase the nitrate removal efficiency of these riparian zones.

Conclusion

Riparian buffer zones are important additional measures to protect water quality from diffuse pollution in agricultural environments but reduction of fertilizer use in the upland agricultural fields is still essential to protect the aquatic ecosystem from eutrophication. A combination of methods is needed in watersheds with intensively managed agricultural fields, because high N loading of riparian zones may lead to the undesired effect of high N_2O emissions and nitrate concentrations in high loaded riparian zones may not be reduced sufficiently to prevent eutrophication of the surface waters.

Apart from the water quality function, riparian ecosystems have other essential functions including: i) stream bank stability and erosion protection, ii) regulation of water temperature and growth of aquatic macrophytes by canopy shading, iii) increasing connectivity in landscapes and iv) increasing biodiversity. Therefore we support the general belief that riparian buffer zones are highly valuable landscape elements, far in excess of their relative surface area, and need to be protected, restored or re-established. Furthermore we recommend performing additional research on greenhouse gas emission from natural and constructed wetlands that are used for water purification. Until now, only the beneficial function of wetlands on water quality improvement has received a lot of attention. To perform a full assessment, however, we have to evaluate the precise consequences of both forms of environmental pollution to determine the environmental risks.

Nederlandse samenvatting

Inleiding

Diffuse verontreiniging van oppervlaktewater met nutriënten en pesticiden afkomstig uit landbouwgebieden is een groot knelpunt in het waterbeheer. Nutriënten als stikstof en fosfaat stimuleren de algengroei en verstoren het oorspronkelijk veel voedselarmere en soortenrijkere oppervlaktewater. Ondanks het strenge mestbeleid van de afgelopen jaren is de belasting van het oppervlaktewater met nutriënten afkomstig van de landbouw nog steeds aanzienlijk. Ecotechnologische maatregelen zoals zuiveringsmoerassen, helofytenfilters en beekbegeleidende bufferstroken kunnen in belangrijke mate bijdragen aan de reductie van diffuse verontreiniging door het verwijderen en omzetten van verontreinigingen uit afspoelend water en ondiep grondwater. Een bufferstrook is een strook grond langs een beek, sloot of andere watergang die zo ingericht is dat meststoffen afkomstig van aanliggende landbouwgrond hier verwijderd kunnen worden.Voorgaande buitenlandse onderzoeken lieten een duidelijke afname zien van de stikstof- en fosforconcentratie in het ondiepe grondwater nadat het water een bufferstrook is gepasseerd. Hoewel er de afgelopen jaren relatief veel onderzoek is verricht naar de nutriënten-verwijdering in bufferstroken bleef het moeilijk om iets te zeggen over de effectiviteit van dergelijke stroken. De processen die verantwoordelijk zijn voor het waterzuiverend vermogen zijn complex en het is grotendeels onbekend hoe bufferstroken functioneren onder verschillende omstandigheden qua nutriëntenbelasting, hydrologie en klimaat.

De studie naar bufferstroken die in dit proefschrift wordt beschreven beoogde inzicht te krijgen in de invloed van stikstofbelasting, vegetatietype en hydrologie op de stikstofverwijdering. Het onderzoek beperkte zich tot de

stikstofverwijdering in bufferstroken die grenzen aan intensief bemeste akkers. Het onderzoek is uitgevoerd in Nederland en in zes andere Europese landen in het kader van het NICOLAS project, gefinancierd door het 4e Kaderprogramma van de EU. Partners in het project waren onderzoekers van de universiteiten van Rennes (F), Durham (GB), Barcelona (S), Lausanne (CH), Boekarest (R) en Warschau (PL). De onderzoeksgebieden in de deelnemende landen lagen langs een klimaatgradiënt en waren tevens zeer uiteenlopend voor wat betreft de bemestingsniveaus van aangrenzende landbouwgebieden. De belangrijkste doelstelling van het onderzoek was om de processen die verantwoordelijk zijn voor het waterzuiverend effect beter te begrijpen, en om na te gaan of het waterzuiverend effect niet leidt tot het vergroten van het broeikaseffect. In deze samenvatting zijn de belangrijkste onderzoeksresultaten op een rij gezet. Daarbij zijn de volgende thema's onderscheiden;

- verdunning of verwijdering,
- plantopname en denitrificatie,
- grondwaterstand en stikstofprocessen,
- lachgasemissie, en
- duurzaamheid van bufferstroken.

Verdunning of verwijdering

Veel eerder uitgevoerde studies naar het nitraatverwijderend vermogen van bufferstroken baseerden hun conclusies op verschillen in nitraatconcentraties tussen het instromende en uitstromende water. Verdunning van het ondiepe verontreinigde grondwater met dieper schoon grondwater kan echter in belangrijke mate bijdragen aan de lagere concentraties die vaak in het uitstromende water gevonden zijn zonder dat er echte waterzuivering heeft plaatsgevonden. Om deze reden is in hoofdstuk 2 de grondwaterstroming in detail bestudeerd. Metingen van waterstanden en waterkwaliteit werden maandelijks uitgevoerd in meer dan 50 peilbuizen in een met bos en een met gras begroeide bufferstrook langs eerste orde beekjes in Noordoost Twente. De gemiddelde nitraatbelasting was hoog in de bosbufferstrook met 87 g NO_3^--N m^{-2} j^{-1} en significant lager in de grasbufferstrook met 15 g NO_3^--N m^{-2} j^{-1}. Verdunning van het ondiepe verontreinigde grondwater met schoon grondwater droeg in belangrijke mate bij aan de lagere nitraatconcentraties in het grondwater na passage van de bufferstrook. Daarnaast was er een duidelijke toename te zien in de chlorideconcentratie. De gevonden verdunning had in ons geval kunnen leiden tot een overschatting van de nitraatverwijdering van

maximaal 60% wanneer dit proces niet was meegenomen in de analyse. Naast verdunning bleken de bufferstroken ook in staat om daadwerkelijk nitraat te verwijderen. De gehele grasbuffer verwijderde zo'n 63% van de inkomende nitraatbelasting en de bosbuffer verwijderde 38% van de inkomende nitraatbelasting.

Plantopname en denitrificatie

Zoals al eerder beschreven hangt de efficiëntie van de nitraatverwijdering af van hydrologische en klimatologische condities en van de belasting. De vergelijking van de data afkomstig van de verschillende deelnemende landen in hoofdstuk 6 laat zien dat er een groot verschil is in nitraatconcentraties in het ondiepe grondwater van vrijwel 0 in Oost-Europa tot 50 mg nitraat-N per liter in Nederland. Deze brede range was ideaal om het belang van verschillende nitraatverwijderende processen te evalueren. Over het algemeen zijn denitrificatie en plantopname de belangrijkste processen die deze afname veroorzaken. Nutriënten die worden opgenomen door planten verblijven echter slechts tijdelijk in de plantenbiomassa en komen terug in het systeem wanneer planten afsterven en het strooisel wordt afgebroken. Denitrificatie is de microbiologische omzetting van nitraat naar stikstofgas. Denitrificatie is belangrijk als waterzuiverend proces omdat nitraat na omzetting door denitrificerende microorganismen het systeem verlaat in de vorm van stikstofgas, hierdoor wordt de stikstof dus echt uit het systeem verwijderd. Bos- en grasbufferstroken in al deze landen bleken effectief in het verwijderen van nitraat uit het ondiepe grondwater en denitrificatie bleek daarbij veruit het belangrijkste verwijderingsprocess. Plantopname en vastlegging in plantenstrooisel zorgde voor een tijdelijke stikstofretentie van 13-99% van de totale stikstofretentie. Over het algemeen werd een hogere N opname en retentie gemeten in de bosbufferstroken. Regelmatig maaien van grasbufferstroken en het afvoeren van het maaisel zorgt echter voor een substantiele extra N verwijdering in het grasland zodat het verschil in retentie van anorganisch N tussen de vegetatietypen verwaarloosbaar wordt. Denitrificatiesnelheden gemeten in bodemkolommen met de acetyleen-inhibitiemethode varieerden tussen de 0.11 and 91 mg N m^{-2} dag^{-1} in de Europese onderzoeksgebieden. Denitrificatie was het dominante N-verwijderingsproces in de meeste bufferstroken, behalve in de Spaanse en Roemeense bufferzones, waar de N-verwijdering door denitrificatie significant lager was dan de N-verwijdering door plantopname.

Grondwaterstand en stikstofprocessen

Geen significant effect van klimaat kon worden vastgesteld op de denitrificatiesnelheden en de stikstofverwijdering in de reeks van ver uiteengelegen onderzoeksgebieden in Europa. Consandey et al. (2001) vonden een duidelijke relatie tussen de denitrificatiesnelheden en milieuvariabelen zoals het organisch stofgehalte en het vochtgehalte van de bodem. Deze factoren bleken belangrijker te zijn dan het klimaat, het type vegetatie of het seizoen in het verklaren van de denitrificatiesnelheid. Omdat grondwaterstanden gemakkelijk te meten zijn en op bodemkaarten worden weergegeven is nagegaan of de processnelheden van stikstoftransformatie konden worden voorspeld op basis van de grondwaterstand.
In hoofdstuk 5 is de gehele NICOLAS dataset gebruikt om te testen of grondwaterstand een goede voospellende variabele is voor de stikstoftransformatie. Drie consistente drempelwaarden in grondwaterniveau werden geïdentificeerd over de brede reeks van onderzoeksgebieden. Wanneer grondwaterstanden binnen de 10 cm van het bodemoppervlak zijn dan is ammonificatie het belangrijkste proces in de stikstofkringloop. Hierdoor hoopt ammonium zich op in de bovengrond. Als de gemiddelde grondwaterstand tussen de 10 en 30 centimeter van het bodemoppervlak staat zijn de omstandigheden optimaal voor denitrificatie; onder deze omstandigheden kan er een stikstoftekort op gaan treden. Bij een grondwaterstand onder de 30 centimeter treedt er nitraataccumulatie op. Denitrificatie kan onder deze omstandigheden periodiek optreden in bodems met een fijne textuur (hoog klei- en siltgehalte). De gevonden drempelwaarden kunnen worden gebruikt als een indicator om te voorspellen wat er gebeurt met de stikstofverwijdering bij veranderingen in grondwaterstanden (bijvoorbeeld door waterberging).

Lachgasemissie

Denitrificatie bleek het dominante proces van nitraatverwijdering in de meeste NICOLAS onderzoeksgebieden. Denitrificatie is echter ook een belangrijke bron van lachgas (N_2O). Lachgas is een broeikasgas en het vernietigt de ozonlaag. De vraag rees daarom of het totale milieurendement van bufferstroken positief is wanneer men naast het waterzuiverende effect ook het effect van verhoogde lachgasemissies zou beschouwen. In hoofdstuk 3 worden de metingen beschreven van de denitrificatie, nitrificatie en lachgasemissies in hoogbelaste Nederlandse bufferstroken. Lachgasemissies zijn gemeten met behulp van fluxkamers die in het veld opgesteld worden en

aangesloten worden op een akoestische infra-rood gas-analyser. De denitrificatiesnelheid tussen de gras- en bosbufferstroken was vergelijkbaar maar het uitstromende water in het bos bevatte nog steeds veel nitraat. Daarnaast bleek in de bosbufferstrook een groot deel van de stikstof het systeem te verlaten in de vorm van lachgas (20 kg N ha^{-1} j^{-1}in vergelijking tot 2-4 kg N ha^{-1} j^{-1}in het grasland). De hoeveelheid lachgas die werd geproduceerd bleek voornamelijk af te hangen van de nitraatconcentratie. Bij de lagere nitraatbelasting zoals gevonden in het grasland nam de hoeveelheid lachgasproductie meer dan evenredig af omdat er relatief meer nitraat in het onschadelijke stikstofgas werd omgezet. Met andere woorden, als de nitraatconcentraties maar niet al te hoog zijn prevaleert het milieuvoordeel van bufferzones.

In de bosbufferstrook werden duidelijk verschillende stroombanen gevonden met verschillende stikstofverwijdering (hoofdstuk 2). De ruimtelijke variabiliteit van stroombanen met verschillende nitraatverwijderingsefficiëntie compliceert de evaluatie van het milieurendement van bufferstroken. In hoofdstuk 4 is nagegaan wat de lachgasproductie was bij stroombanen met een verschillende nitraatverwijderingsefficiëntie. Daarnaast werden de ruimtelijke patronen van denitrificatie en lachgasemissie bij de verschillende stroombanen bestudeerd. Denitrificatie en lachgasemissie werden gemeten in de winter en in de zomer op 60 plekken per stroombaan. In de winter was de N_2O emissie significant hoger bij de stroombaan met hoge nitraatverwijdering (12.4 mg N m^{-2} d^{-1}) in vergelijking tot de emissie van de stroombaan met lage nitraatverwijdering (2.58 mg N m^{-2} d^{-1}). In de zomer was er een omgekeerd patroon zichtbaar, met hogere N_2O emissies (13.6 mg N m^{-2} d^{-1}) bij de stroombaan met lage nitraatverwijdering in vergelijking tot de stroombaan met hoge nitraatverwijdering (4.44 mg N m^{-2} d^{-1}). Onze hypothese was dat stroombanen met een lage nitraatverwijdering slechter waren voor het milieu omdat daar naast de hogere nitraatconcentratie in het uitstromende water ook nog meer lachgas zou worden geproduceerd. Het in hoofdstuk 4 besproken onderzoek liet echter zien dat de lachgasproductie zeer vergelijkbaar was bij de verschillende stroombanen. Hieruit kunnen we concluderen dat bufferstroken een belangrijke effect-gerichte maatregel vormen om de belasting van het oppervlaktewater met nutriënten te verminderen, waarbij het milieurendement echter nooit bevredigend kan worden zonder de bestaande brongerichte maatregelen zoals de gebruiksnormen en het MINAS systeem. Deze brongerichte maatregelen zijn noodzakelijk om de nitraatbelasting zover te verminderen dat lachgasemissies verwaarloosbaar worden, zodat bufferstroken voornamelijk nog positief werken door hun gunstige effect op de waterkwaliteit.

Duurzaamheid van bufferstroken

Tot nu toe zijn slechts weinig onderzoeken aan bufferstroken uitgevoerd aan hoog-belaste systemen. De resultaten van de hoofdstukken 2 en 4 en die van andere onderzoekers in het NICOLAS-project (Sabater et al. 2003) leken te wijzen op een stikstofverzadiging van de bufferstroken. De vraag rijst nu dus hoe duurzaam bufferstroken eigenlijk zijn bij een langdurige nitraatbelasting.
Denitrificatie is een proces waar organische koolstof bij nodig is. In chronisch belaste systemen kan er een situatie ontstaan waarbij de koolstofvraag groter is dan de jaarlijkse productie van gemakkelijk afbreekbare organische stof door de vegetatie. Hierdoor zal een tijdelijke koolstoflimitatie (in de zomer) het denitrificatieproces kunnen remmen.
Om deze uitspraak te testen is een een ruwe vergelijking gemaakt van de koolstofproductie en -consumptie in hoofdstuk 7. Hieruit blijkt dat de jaarlijkse koolstofconsumptie door denitrificerende microörganiscmen vele malen kleiner is dan de koolstofproductie. Hieruit kan voorzichtig geconcludeerd worden dat er geen gevaar is dat hoogbelaste bufferstroken op termijn minder goed zullen gaan functioneren door een koolstoflimitatie van het denitrificatieproces. Naast koolstoflimitatie kan er ook mogelijk een stikstofverzadiging optreden. Aanwijzingen hiervoor werden gevonden in hoofdstuk 2, terwijl een dergelijke verzadiging voor het proces van denitrificatie niet eerder is gevonden. Mogelijk werkt de lage zuurgraad die geassocieerd is met de hoge nitraatconcentratie remmend op het denitrificatie-proces. Bekalken van de akkers en de bufferstroken zal in deze gevallen de nitraatverwijderingsefficiëntie kunnen verhogen.

Conclusie

Beekbegeleidende bufferzones kunnen worden ingezet als belangrijke aanvullende maatregel om het oppervlaktewater te beschermen tegen diffuse verontreiniging met nutriënten. Bufferstroken kunnen echter nooit gezien worden als vervanging van de bestaande brongerichte maatregelen zoals de gebruiksnormen en het MINAS-systeem. Deze maatregelen zijn noodzakelijk om de nitraatbelasting zover te verminderen dat lachgasemissies verwaar-loosbaar worden, zodat bufferstroken voornamelijk nog positief werken door hun gunstige effect op de waterkwaliteit. Daarnaast hebben bufferstroken bijkomende voordelen voor het aquatische ecosysteem, ze zorgen voor de stabiliteit van oevers, reguleren de watertemperatuur en algengroei door beschaduwing. Bufferstroken kunnen ook worden gezien als belangrijke

verbindingszones tussen natuurgebieden. Al met al zijn bufferzones met een gemiddelde nutriëntenbelasting zeer waardevolle landschapselementen die beschermd of gerestaureerd dienen te worden.
De resultaten in dit proefschrift geven aanleiding om met grote voorrang nader onderzoek te doen naar broeikasgasemissie uit moerassen die worden ingezet voor waterzuivering. Dit is nodig om een goede afweging te maken van het totale milieurendement van degelijke voorzieningen.

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Curriculum vitae

Mariet Hefting werd geboren op 8 december 1967 in Ede. Na het doorlopen van de IVO-MAVO in Oosterbeek en HAVO en VWO aan het Thomas A Kempis college in Arnhem verbleef ze gedurende een half jaar in India, Hoshangabad, om te werken op een biologische boerderij. Geïnspireerd door de landbouwpraktijk begon ze in 1989 haar studie Bodem Water Atmosfeer aan de Landbouwuniversiteit in Wageningen (LUW). In haar afstudeervakken bestudeerde ze de uitspoeling van zuren uit kattekleigronden in de Mekong Delta, Vietnam, de accumulatie van zware metalen door regenwormen en het effect van temperatuur op de stikstof stromen in een subboreaal bodemecosysteem. In 1995 behaalde ze haar ingenieurs diploma (*cum laude*) in de richting procesbodemkunde. In datzelfde jaar begon ze aan een onderzoek bij de vakgroep Waterkwaliteitsbeheer en Aquatische Ecologie, LUW waarbij ze onderzoek verrichtte aan de ruimtelijke variabiliteit van denitrificatie in grasland bodems en de betekenis van deze variabiliteit voor de stikstof balans.
In 1997 werkte ze gedurende een half jaar bij het Staring Centrum Wageningen (Alterra) waar ze een literatuur studie en systeemanalyse uitvoerde naar de nutriëntenopname door helofyten tbv het waterkwaliteitsmodel NUSWA. In 1998 begon ze als Assistent In Opleiding bij de projectgroep Landschapsecologie, departement Geobiologie van de Universiteit Utrecht. Ze werkte in het kader van het Europese project NICOLAS (NItrogen COntrol by LAndscape Structures in agricultural environments) aan de stikstof transformatie en retentie in beekbegeleidende bufferstroken. Een onderzoek dat resulteerde in het voorliggende proefschrift. Met ingang van 22 maart 2003 kreeg ze een tijdelijke aanstelling als junior docent voor het ontwikkelen en (deels) geven van de nieuwe Mastercursus "Ecology of Plant Communities and Landscapes". Vanaf eind november zal ze zich gaan bezighouden met het voorbereiden en uitvoeren van een workshop ter afronding van het NWO stimulerings programma Biodiversiteit.

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Hoed vol gaten

Ik wil niets
meer bewijzen,

vooral
mezelf niet,

ik wil reizen
door de tijd,

een hoed op
vol gaten,

licht
in het hoofd.

Hans Andreus, Holte van licht (1975)