Chapter 3

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

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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 (N₂O). The risks of high N₂O 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 N₂O 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 N₂O 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 N₂O 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. Considerable N₂O fluxes measured in this study indicate that Intergovernmental Panel on Climate Change methodologies for quantifying indirect N₂O 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$_2$O) 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$_2$O 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$_2$O emissions from groundwater and agricultural drainage water do not account for possible high N$_2$O production in riparian buffer zones (Mosier et al., 1998).

The flux of N$_2$O 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$_2$O (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$_2$O, whereas N$_2$O 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$_2$O 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$_2$O 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$_2$O flux.

There is considerable uncertainty about the ecosystem properties and environmental conditions affecting N$_2$O production. The N$_2$O 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$_2$O production by denitrifiers is quite well understood. In general, N$_2$O 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$_2$O emissions. Insight into the ways in which soil variables control the absolute amounts of N$_2$O 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$_2$O 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$_2$O emissions from natural riparian buffer zones in agricultural landscapes; (ii) assess the relative role of denitrification and nitrification as sources of N$_2$O emission from riparian buffer zones receiving large loads of N from adjacent agricultural fields; (iii) reveal the environmental factors controlling N$_2$O 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$_2$O 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 N₂ 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 aeolian 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 NO₃⁻ and NH₄⁺ 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\textsubscript{3} and NH\textsubscript{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\textsubscript{2}O 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\textsuperscript{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\textsubscript{2}O 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\textsubscript{2}O concentration over time using linear regression analysis. Besides N\textsubscript{2}O emissions, measurements on CH\textsubscript{4} and CO\textsubscript{2} emissions were performed. Carbon dioxide measurements were used to correct for a possible interference between N\textsubscript{2}O and CO\textsubscript{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$_2$O 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$_2$O 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$_2$O 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\(^{-1}\).
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 NH₄⁺ 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 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.

**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.
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.

<table>
<thead>
<tr>
<th></th>
<th>Buffer zone</th>
<th>Season</th>
<th>Buffer zone x season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Ln (denitrification)</td>
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<td>2.477</td>
<td>0.120</td>
</tr>
<tr>
<td>Ln (nitrification)</td>
<td>1</td>
<td>0.235</td>
<td>0.629</td>
</tr>
<tr>
<td>Ln (N(_2)O emission)</td>
<td>1</td>
<td>11.923</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* Significant main effect.
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$_2$O 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$_2$O emission rates between the seasons (Fig. 3). A spatial pattern

![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.](image)

| Table 2 Results of a Kruskal-Wallis nonparametric test with differences between strips within the two riparian zones. |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| **Forest riparian zone** | **Grassland riparian zone** |
| Denitrification | n | $\chi^2$ | p | n | $\chi^2$ | p |
| 36 | 24.794 | < 0.0001* | 36 | 24.222 | < 0.0001* |
| Nitrification | 36 | 14.468 | 0.001* | 36 | 18.743 | < 0.0001* |
| N$_2$O emission | 36 | 4.749 | 0.093 | 36 | 3.754 | 0.153 |

* Significant effect.
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
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,
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\textsubscript{2}O emission in the forested buffer zone, whereas nitrate concentration explained more than 23% of N\textsubscript{2}O emission in the grassland buffer zone. In contrast to the overall PCA, soil moisture content did play a significant role controlling N\textsubscript{2}O emissions in the forested zone. Fluxes from the forested buffer zone largely determined the significant bivariate relation found between N\textsubscript{2}O 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

![Figure 6](image-url) (A) Denitrification and N\textsubscript{2}O emission versus soil moisture content in the two riparian buffer zones. (B) Net nitrification versus soil moisture content in the two riparian buffer zones.

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

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Component</th>
<th>Slope</th>
<th>R\textsuperscript{2}</th>
<th>F</th>
<th>p</th>
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</tr>
<tr>
<td>Ln (N\textsubscript{2}O)</td>
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<td>0.229</td>
<td>22.058</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Nitrification</td>
<td>Model 1</td>
<td>-0.410</td>
<td>0.156</td>
<td>14.119</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
<td>0.290</td>
<td>0.230</td>
<td>11.607</td>
<td>&lt; 0.0001*</td>
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</table>

* Significant effect.
contamination by having high rates of N$_2$O emissions. Studies on N$_2$O 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$_2$O-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$_2$O 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$_2$O 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$_2$O 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$_2$O 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$_2$O 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$_2$O emissions. In many studies (Heincke and Kaupenjohann, 1999), a positive relation was found between moisture content and N$_2$O emissions. High water contents with increasingly anoxic conditions stimulate denitrification activity and thus facilitate N$_2$O 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\textsubscript{2}O in the soil, by restricting diffusion, and may consequently enhance the reduction of N\textsubscript{2}O to nitrogen gas (Blicher-Mathiesen and Hoffmann, 1999; Jacinthe et al., 2000). Due to the microbial preference for the reduction of nitrate above N\textsubscript{2}O, the further reduction of N\textsubscript{2}O 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\textsubscript{2}O fluxes found in the grassland riparian zone.

Although N\textsubscript{2}O fluxes from the forested riparian buffer zone were high, data from Rusch and Rennenberg (1998) indicate that our flux data underestimated N\textsubscript{2}O emissions. They showed that alder trees, which were dominant in our forested riparian zone, could mediate N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O when the soil oxygen status is less favorable for denitrification (Reddy et al., 1989; Jacinthe et al., 2000). Second, an additional N\textsubscript{2}O 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\textsubscript{2}O emission in aerobic soils with a relatively low absolute N\textsubscript{2}O 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 $10 \text{ mg N l}^{-1}$ 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 N₂O in water, additional research is needed to distinguish between transported and locally produced N₂O. However, on the basis of our denitrification measurements, we conclude that there is a significant amount of N₂O 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 N₂O emissions. Groffman et al. (2000) already suggested that emission factors (EF5-g) based only on N₂O 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 N₂O-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 N₂O 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 N₂O emission inventories.

Conclusions

When nitrate loading in riparian buffer zones is high, N₂O 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.

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