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Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon

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Abstract. The influence of hydrology and patterns of supply of electron donors and acceptors on subsurface denitrification was studied in a forest riparian zone along the Boyne River in southern Ontario that received high nitrogen inputs from a sand aquifer. Two hypotheses were tested: (1) subsurface denitrification is restricted to localized zones of high activity; (2) denitrification zones occur at sites where groundwater flow paths transport NO_3^- to supplies of available organic carbon. A plume of nitrate-rich groundwater with concentrations of 10–30 mg N L^{-1} flowed laterally at depths of 1.5–5 m in sands beneath peat for a horizontal distance of 100–140 m across the riparian zone to within 30–50 m of the river. *In situ* acetylene injections to piezometers revealed that significant denitrification was restricted to a narrow zone of steep NO_3^- and N_2O decline at the plume margins. The location of these denitrification sites in areas with steep gradients of groundwater DOC increase supported hypothesis 2. Many of these denitrification "hotspots" occurred near interfaces between sands and either peats or buried river channel deposits. Field experiments involving *in situ* additions of either glucose or NO_3^- to piezometers indicated that denitrification was C-limited in a large subsurface area of the riparian zone, and became N-limited beyond the narrow zone of NO_3^- consumption. These data suggest that denitrification may not effectively remove NO_3^- from groundwater transported at depth through permeable riparian sediments unless interaction occurs with localized supplies of organic matter.

Introduction

The role of riparian zones in regulating the transport of nitrate in groundwater flow from uplands to streams has received considerable attention (Hill 1996). Although most researchers have reported that riparian zones in agricultural landscapes effectively remove NO_3^- from groundwater (Peterjohn & Correll 1984; Lowrance et al. 1984; Pinay & Decamps 1988; Osborne & Kovacic

1993), there is considerable uncertainty about the processes involved in NO_3^- removal. Most researchers suggest that the primary mechanisms of NO_3^- removal in riparian zones are plant uptake and denitrification, the microbial conversion of NO_3^- to gaseous forms of nitrogen. Studies using acetylene amended incubations of soil slurries and intact soil cores indicate high levels of denitrification activity in the organic-rich surface soils of riparian zones (Cooper 1990; Pinay et al. 1993; Schipper et al. 1993). These data suggest that denitrification is a major mechanism of NO_3^- loss at sites where nitrate-rich water interacts with surface soils. However, nitrate is often effectively removed from riparian aquifers which extend to depths of several meters. In these riparian areas, there is often no interaction between groundwater and surface soils when the water table is low, while during periods of maximum water table elevation the surface soil is only in contact with a small part of the aquifer.

The nature and extent of denitrification in groundwater and subsurface riparian sediments is highly uncertain. Laboratory measurements of denitrification potentials in subsurface soils at and below the water table have found low or undetectable activity which could not account for the amounts of nitrate removal observed in groundwater (Ambus & Lowrance 1991; Groffman et al. 1992; Lowrance 1992; Simmons et al. 1992; Groffman et al. 1996). Other studies have used injection experiments or increases in the ^{15}N abundance of groundwater NO_3^- to identify subsurface denitrification (Duff & Triska 1990; Bohlke & Denver 1995; McMahon & Bohlke 1996; Verchot et al. 1997; Cey et al. 1999). In view of these conflicting results we need to develop a better understanding of subsurface denitrification in riparian zones.

Riparian zones are complex environments that are spatially heterogenous in a horizontal and vertical dimension with respect to hydrology, sediment characteristics and biogeochemical processes. Currently, little information is available on the variability of denitrifying activity at depth in riparian zones. Laboratory studies of denitrification potentials in subsurface sediments have used small soil samples which may not be representative of aquifer conditions (Gold et al. 1998). Small patches of organic matter representing less than 2% of the soil mass were critical “hotspots” of denitrification at a depth of 0.6 m within the zone of seasonal water table fluctuation of a poorly drained riparian soil (Jacinthe et al. 1998).

Laboratory-based studies of sediments and *in situ* injection experiments have been generally restricted to the upper 1.5 m of riparian sediments. Moreover, patterns of NO_3^-, dissolved oxygen (DO) and dissolved organic carbon (DOC) concentrations which control denitrification have often not been measured in detail at these sampling sites. Microbial populations generate energy for growth and maintenance by using organic matter as the dominant electron donor and a range of electron acceptors such as O_2 and NO_3^- in a predictable sequence as a function of redox state. Recently, Hedin et al. (1998) have suggested that the ability to understand and predict the biogeochemical function of stream riparian zones can be enhanced by studying how microbial communities interact with variations in the supplies of electron donors and acceptors. These researchers found that denitrification was restricted to a narrow zone at 20–40 cm depth adjacent to a stream bank where a horizontal flow of water with high DOC from the organic surface soil horizon interacted with vertical upwelling of nitrate-rich subsurface water.

In a previous study we focused on nitrate chemistry in relation to the lithology and hydrology of a large forest riparian zone which received high N inputs from a sand aquifer in southern Ontario (Devito et al. 2000). A plume of nitrate-rich groundwater flowed laterally in sands beneath surface riparian soils. Declines in NO_3^- concentrations occurred along this subsurface flow path at depths of 2–5 m and analysis of ^{15}N in a few groundwater samples suggested that denitrification could account for the NO_3^- removal. In this paper, we examine how variations in hydrology and patterns of supply of electron donors and acceptors influence subsurface denitrification in this riparian forest. Two hypotheses were tested: (1) subsurface denitrification is restricted mainly to localized zones of high denitrifying activity within the riparian aquifer; (2) denitrification zones occur at sites where groundwater flow paths transport NO_3^- to supplies of available organic carbon. Hydrologic data were related to spatial variability in the supply of electron donors (organic carbon) and electron acceptors (DO NO_3^- and N_2O) within the aquifer. In situ injections of acetylene into piezometer nests were used to identify sites of denitrification activity. We also used *in situ* experimental additions of glucose and NO_3^- to investigate the role of C and N limitation on subsurface denitrification.

Study area

The study site was located along the Boyne River, a 5th order river in the Nottawasaga drainage basin, approximately 70 km north of Toronto, Ontario (Figure 1). In this area, the Boyne River flows in a forested valley incised to depths of 9–12 m below the surface of an extensive sand plain which is under intensive agricultural production with potatoes as the main crop. The sand plain forms an unconfined aquifer that is underlain regionally by a thick sequence of clays and silts (Sibul & Choo-Ying 1971). Long term use of commercial fertilizers has resulted in high concentrations of NO_3^- and chloride in the shallow aquifer which discharges groundwater to the Boyne River (Hill 1982; Gillham 1991).

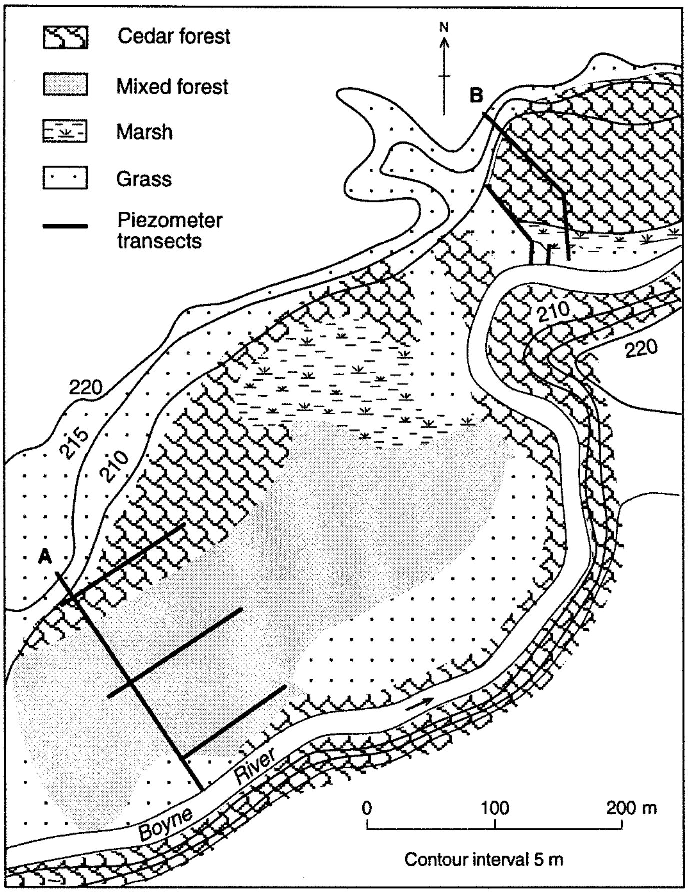

Figure 1. Boyne River floodplain and location of the sampling transects at the upstream and downstream sites.

This research was conducted on a 100–200 m wide forested floodplain on the north side of the Boyne River (Figure 1). This site is separated from cropland by a 12 m high valley side slope. Vegetation on the floodplain consists of conifer forest dominated by northern white cedar (*Thuja occidentalis*), and mixed deciduous and conifer forest interspersed with patches of marsh and grasses.

Materials and methods

Groundwater hydrology and chemistry

Piezometer installation and hydrometric methods are described in detail by Devito et al. (2000). Briefly, a network of piezometer nests was installed in two areas of the riparian zone approximately 400 m apart (Figure 1). The main transects were parallel to the direction of groundwater flow within the riparian zone which was approximately perpendicular to the river in the upstream area. In the downstream area, groundwater flowed from the valley side in an oblique downvalley direction which became more perpendicular near the river (Devito et al. 2000). Most piezometer nests were installed in May–June 1996 and consisted of PVC pipes (1.25 cm ID) with a 20 cm slot zone at depths ranging from 0.5 to 5.5 m. Detailed groundwater hydrology and chemistry information was collected on 3–9 sampling dates in June–December 1996, 3–6 dates in April–November 1997 and 1 date in April and August 1998. Floodplain stratigraphy and sediment properties were determined from cores collected during piezometer and well installations.

Prior to chemical sampling, piezometers were purged using a hand-operated pump. Fresh groundwater samples were withdrawn using polypropylene syringes. Samples were transported in coolers to the laboratory and stored at 4 °C prior to analysis for NO_3^--N and chloride. Water samples collected for DOC analysis were filtered through GF/C filters and stored in glass vials at 4 °C. DOC samples were preserved by adding phosphoric acid to a pH of less than 2. For dissolved oxygen determination, water samples were collected with tygon tubing connected to a three way stopcock fitted to the Luer end of a syringe to prevent contact with ambient air during pumping. Dissolved oxygen was measured in the field with a DO meter and oxygen probe inserted into the syringe. Samples were also analyzed on some dates in the field using a modified micro-Winkler method (Wetzel & Likens 1991). The two methods measured similar DO concentrations in groundwater samples. Groundwater samples for dissolved N_2O analysis were pumped from piezometers with syringes fitted with a three way stopcock to prevent N_2O outgassing. Dissolved N_2O was extracted from water using the syringe shaking method of Rudd et al. (1974). The gas phase was then transferred to 5 cc evacuated containers and analyzed for N_2O within 24 hours.

Acetylene injection experiments

Subsurface *in situ* denitrification activity was examined by the injection of acetylene saturated groundwater into 27 piezometers to inhibit the conversion of N_2O to N_2. The resulting accumulation of N_2O provides evidence of the occurrence of denitrification (Yoshinari & Knowles 1976). After one piezometer volume of groundwater was pumped from each piezometer, a fresh water sample was collected by syringe for N_2O analysis prior to acetylene injection. Then either 2 or 4 L of water, depending on sediment hydraulic conductivity, was pumped from each piezometer and calcium carbide was added to produce an acetylene saturated solution. This acetylene amended groundwater was then reinjected slowly into the piezometer over several hours. Samples of fresh groundwater were withdrawn from the same piezometer at intervals over 7 days using syringes and analyzed for N_2O.

Experimental carbon and nitrate additions

In August and September 1997, we conducted experiments to evaluate the role of carbon and NO_3^- in limiting subsurface denitrification activity in the riparian zone. Carbon additions involved adding glucose to 4 litres of groundwater pumped from each piezometer to produce a total of 300 and 100 mg L^{-1} of C in the solutions for piezometers on transect A and B respectively. Nitrate additions involved adding KNO_3^- to 4 litres of groundwater to produce a total of 100 and 50 mg L^{-1} of N in piezometers on transects A and B respectively. We included Br^- in the C and NO_3^- solutions to determine the extent to which physical processes such as dispersion and dilution contributed to reduction in groundwater DOC and NO_3^- concentrations.

Laboratory techniques

Percentage organic matter of subsurface sediment samples was determined by a loss on ignition method (Nelson & Sommer 1982). Groundwater samples were analyzed for NO_3^--N + NO_2^--N and chloride (Cl^-) by automated wet chemistry on a Technicon AutoAnalyzer system using standard techniques (Technicon 1977; Environment Canada 1979). Nitrite was insignificant and is included as NO_3^-. Samples were analyzed for Br^- using an electrometric method with an Orion Research 720A Ionanalyzer (Orion Research 1992). DOC analysis was performed in the Department of Geography laboratory at McGill University using a Dorhman Carbon Analyzer. Nitrous oxide was determined by using a Hewlett Packard gas chromatograph equipped with an electron capture detector. Gases were separated with a Poropak Q column using a carrier gas of ultra-high purity (UHP) oxygen-free nitrogen. All N_2O concentrations were corrected for the amount of N_2O dissolved in water.

Results

Site stratigraphy

The stratigraphy along the two transects extending from the upland perimeter to the river is shown in Figure 2. The regional clay that forms an aquitard below the sand plain aquifer was detected at several locations on transect B that range from 2 m beneath the river bed to 4 m beneath the ground surface at inland sites. On transect A the clay was found at the river bank, elsewhere on this main transect it was $>$5–5.5 m deep. Clay was also found at 4.5–5.5 m at two locations along the transect running parallel to the river at the base of the valley side slope (Figure 1). Peat deposits along transect A increased from 0.5 m at site 3 to a maximum depth of 2.5 m at site 6 approximately 100 m from the river bank and then graded into organic-rich organic loams which became increasingly sandy and lower in organic matter near the bank. Peat deposits were similar along transect B and reached maximum depths of 2.8 m at site 93. In contrast to transect A this maximum depth on transect B occured only 40 m from the river bank and due to the shallow depth of the sands the peat extended to less than 1 m above the regional clay deposit. The riparian peat layer on both transects was underlain by fine beige and grey sands which contained layers of coarse sand and fine gravel. A more complex subsurface stratigraphy was evident in a zone extending up to 50 m inland from the river bank on both transects (Figure 2). Thin clay lens were underlain by a 1–2 m thick sequence of interbedded curved layers of yellow-brown sands and olive-brown muds. The beds were commonly 0.5–2.0 cm thick and included considerable amounts of fine woody debris and larger tree branches near the base. This sediment sequence formed when channel bar deposits were preserved during lateral migration of the river channel. Sediment organic content of bulk samples which included several sand and mud layers ranged from 14 to 100 g kg^{-1}. Underlying this deposit, is a grey-black coarse-grained layer of shell fragments mixed with small lens of mud and occasional pieces of wood which indicates the base the of the former channel. Organic matter content of this channel lag gravel ranged from 5 to 22 g kg^{-1}.

Groundwater flow

Observations in 1996–1998 indicated that general groundwater flow patterns are similar throughout the year in the riparian zone (Devito et al. 2000). Groundwater flow patterns along the two transects are shown for late summer 1997 in Figure 2. The flow nets indicate predominantly horizontal flow from the upland boundary of the riparian zone towards the river. Hydraulic head

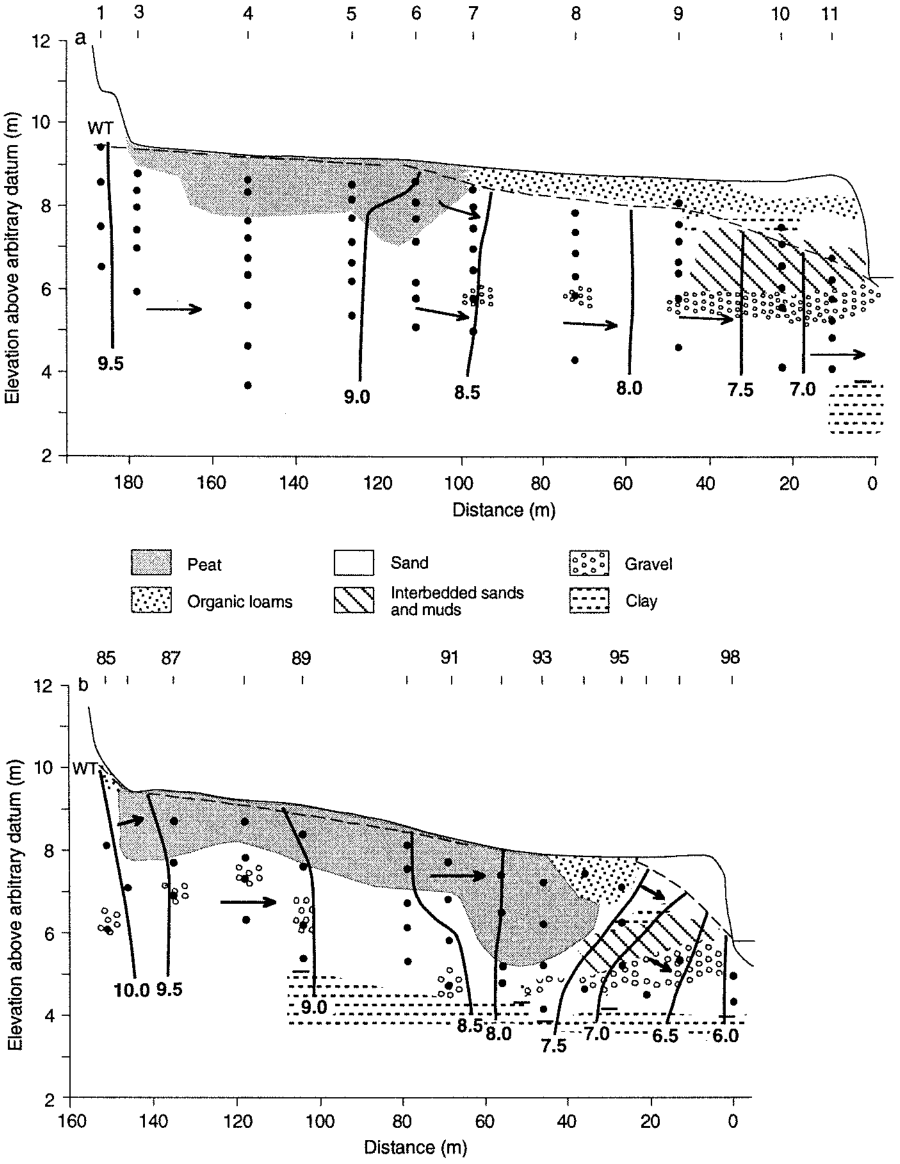

Figure 2. Vertical cross-section along (a) transect A at the upstream site and (b) transect B at the downstream site showing stratigraphy, piezometer slot zones (dots) and hydraulic head contours (m). Dashed line represents location of the water table in late summer 1997. Numbers above each cross-section represent piezometer nest locations. Vertical exaggeration = 10 ×.

contours indicate upward groundwater flow at 1–2 m depth near the upland perimeter on transect B, whereas near the river the contours shift direction indicating downward flow to deeper sediments at the river bank. Between sites 6 and 7 on transect A, groundwater near the surface recharged from the peats into areas of fine sands. The water table remained near or just below the peat surface on both transects year round. Seasonal variations in water table were evident near the river with maximum water table elevations in spring varying from 0.5–1.0 m above late summer levels (Figure 2).

Spatial patterns of subsurface water chemistry

Spatial and temporal patterns in riparian aquifer NO_3^- and chloride concentrations are reported in Devito et al. (2000). Nitrate-N concentrations for late summer 1997 are representative of trends which were recorded throughout 1996–1998 (Figure 3). Groundwater NO_3^--N concentrations entering the riparian zone from the sand plain aquifer ranged from 20–60 mg L^{-1}. This plume of nitrate-rich groundwater extended at depths of 1.5–5.0 m beneath the riparian peat deposits. High NO_3^--N concentrations of >10 mg L^{-1} persisted in the sands for a horizontal distance of 100 m (transect B) and 140 m (transect A) from the hillslope-riparian boundary and approached within 30–50 m of the river bank. Nitrate-N levels decreased abruptly at the plume margins. For example, at sites 8 and 9 on transect A, NO_3^--N declined from 12–16 mg L^{-1} to <1 mg L^{-1} over a vertical distance of 1 m (Figure 3(a)). Groundwater NO_3^--N concentrations in peat and buried channel deposits were usually <1 mg L^{-1}, but localized areas of higher concentration were present in peats at several sites near the upland perimeter where groundwater discharges to the peat surface.

High groundwater chloride concentrations of 40–75 mg L^{-1} were found throughout a major portion of the riparian zone and variations with depth and distance from the perimeter were considerably less than for NO_3^- (Figure 4). In most locations where NO_3^- concentrations declined abruptly, chloride values either remained constant or showed smaller, gradual declines. Chloride concentrations in the peats and buried channel sediments were usually 40–50 mg L^{-1} at sites where NO_3^--N concentrations were 0.1–2.0 mg L^{-1} (Figure 4).

We observed significant spatial variations in DO, DOC and N_2O within the riparian zone. Patterns shown for late summer 1997 in Figures 5 to 7 are representative of trends recorded during April–December 1996–1998. Trends in dissolved oxygen (DO) concentrations were similar to the NO_3^- distribution in the riparian aquifer (Figure 5). Groundwater with 5–8 mg L^{-1} DO extended laterally from the hillslope-riparian margin in sands beneath the peat for 50 and 80 m on transects B and A, respectively. With increased

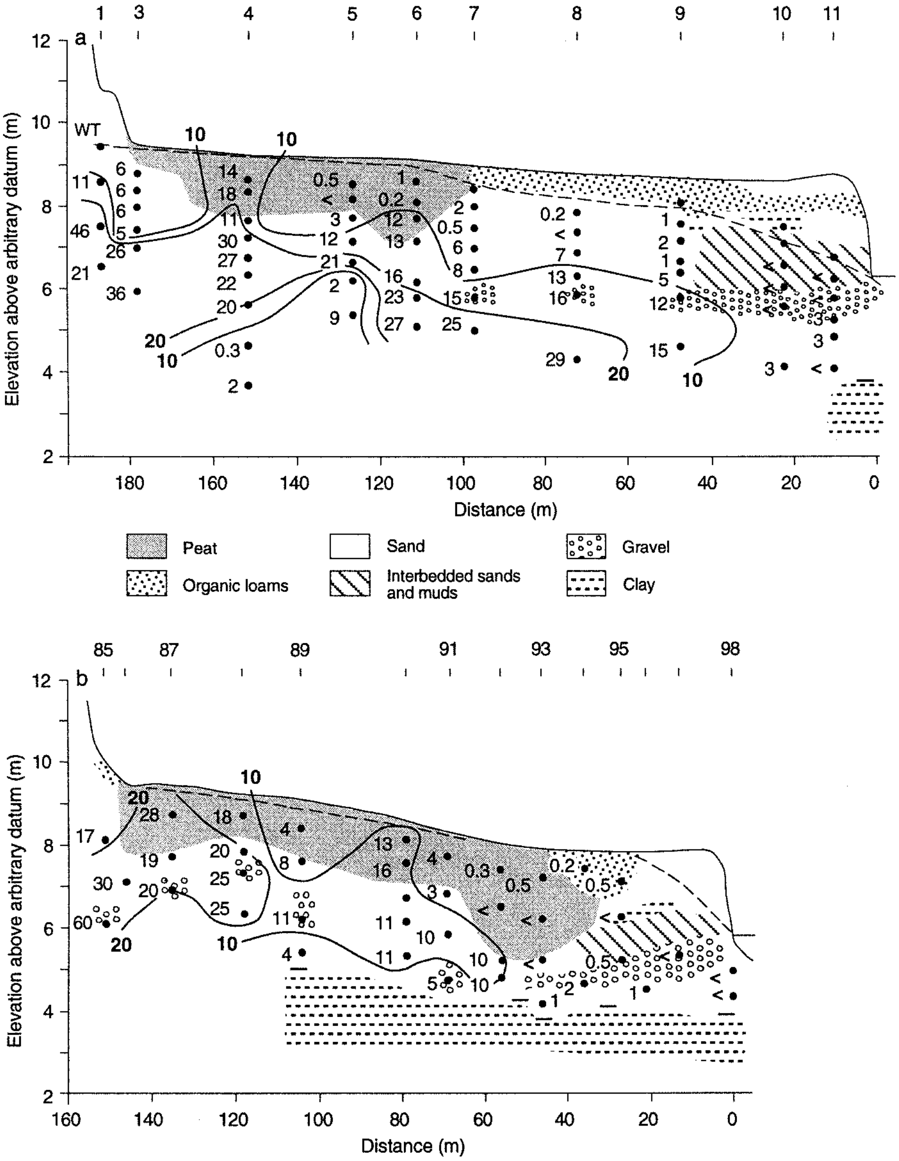

Figure 3. Groundwater NO_3-N concentrations (mg L^{-1}) in late summer 1997 along (a) transect A and (b) transect B. < indicates NO_3-N concentrations <0.1 mg L^{-1}. Lines are contours of equal nitrate concentration.

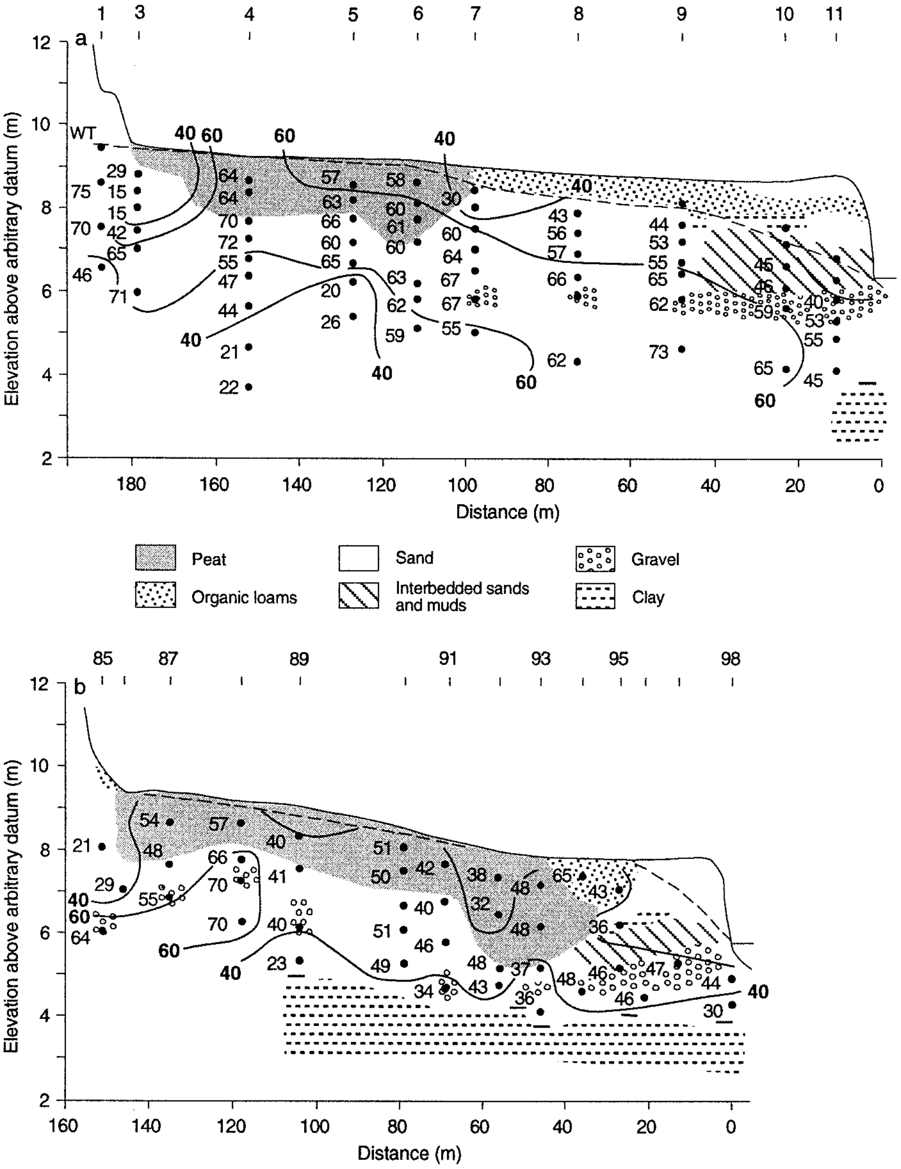

Figure 4. Groundwater chloride concentrations (mg L^{-1}) in late summer 1997 along (a) transect A and (b) transect B. Lines are contours of equal chloride concentration.

distance from the riparian perimeter DO gradually declined to 1.3–2.5 mg L^{-1} in the buried channel sediments and underlying sands adjacent to the river. Dissolved oxygen concentrations in the peats ranged from 2.1–4.3 mg L^{-1} and were often less than the concentrations in the sands beneath.

The spatial distribution of DOC concentrations showed a well defined pattern in the riparian zone (Figure 6). DOC levels in sands at depths of 1.5–5.0 m were usually <4.0 mg L^{-1} whereas, concentrations were frequently >6.0 mg L^{-1} in peats and interbedded sands and muds. A well defined zone of high DOC (13–21 mg L^{-1}) concentrations occurred in the upper 1–2 m of the aquifer between site 7 and the river on transect A (Figure 6(a)).

Variability of N_2O-N concentrations in riparian groundwater was high ranging from 0 to 1190 μg L^{-1} (Figure 7). These N_2O values were as much as 3 orders of magnitude higher than those expected in water at equilibrium with the atmosphere. Groundwater N_2O-N concentrations along transect A were generally lower at the base of the valley slope and increased at depth and along the flow path in the sand aquifer (Figure 7(a)). A zone where N_2O-N ranged from 100–250 μg L^{-1} was located in the deeper sands between sites 5–9. Nitrous oxide levels were <30 μg L^{-1} within the upper 1–2 m of the sands between site 7 and the river. Groundwater N_2O-N concentrations were also low in the surface peat, but higher levels were observed near the peat-sand interface. On transect B, N_2O concentrations were low in the river-side sediments and at depths of >2 m in sands near the upland perimeter (Figure 7(b)). In contrast N_2O-N concentrations at some locations in the peat and near the peat-sand contact exceeded 500 μg L^{-1}. These "hotspots" were persistent, for example at site 87 (0.5 m) N_2O-N values were 890–1190 μg L^{-1} in 1996 to 1998.

Correlations between different electron acceptors and donors were analyzed based on groundwater samples collected on both transects between May 1996 and August 1998. This analysis revealed several strong relationships. A strong inverse relationship occurred between both NO_3^- and N_2O and DOC (Figure 8). Nitrate-N concentrations >5 mg L^{-1} were only found in water that contained <8.0 mg L^{-1} DOC, whereas N_2O levels only exceeded 100 μg N L^{-1} where DOC was <8 mg L^{-1}. We found a positive linear correlation between DO and NO_3^--N ($r = 0.73$; $P < 0.05$). Higher NO_3^--N concentrations coincided with increased DO and more oxidizing conditions, whereas groundwater with <2 mg L^{-1} DO usually had NO_3^--N concentrations below 1 mg L^{-1} (Figure 8(a)). Groundwater NO_3^--N levels were not significantly correlated with N_2O (data not shown). Although N_2O concentrations did not exceed 30 μg N L^{-1} in water with NO_3^--N concentrations below 1 mg L^{-1}, there was no clear relationship at higher concentrations (Figures 3 and 7).

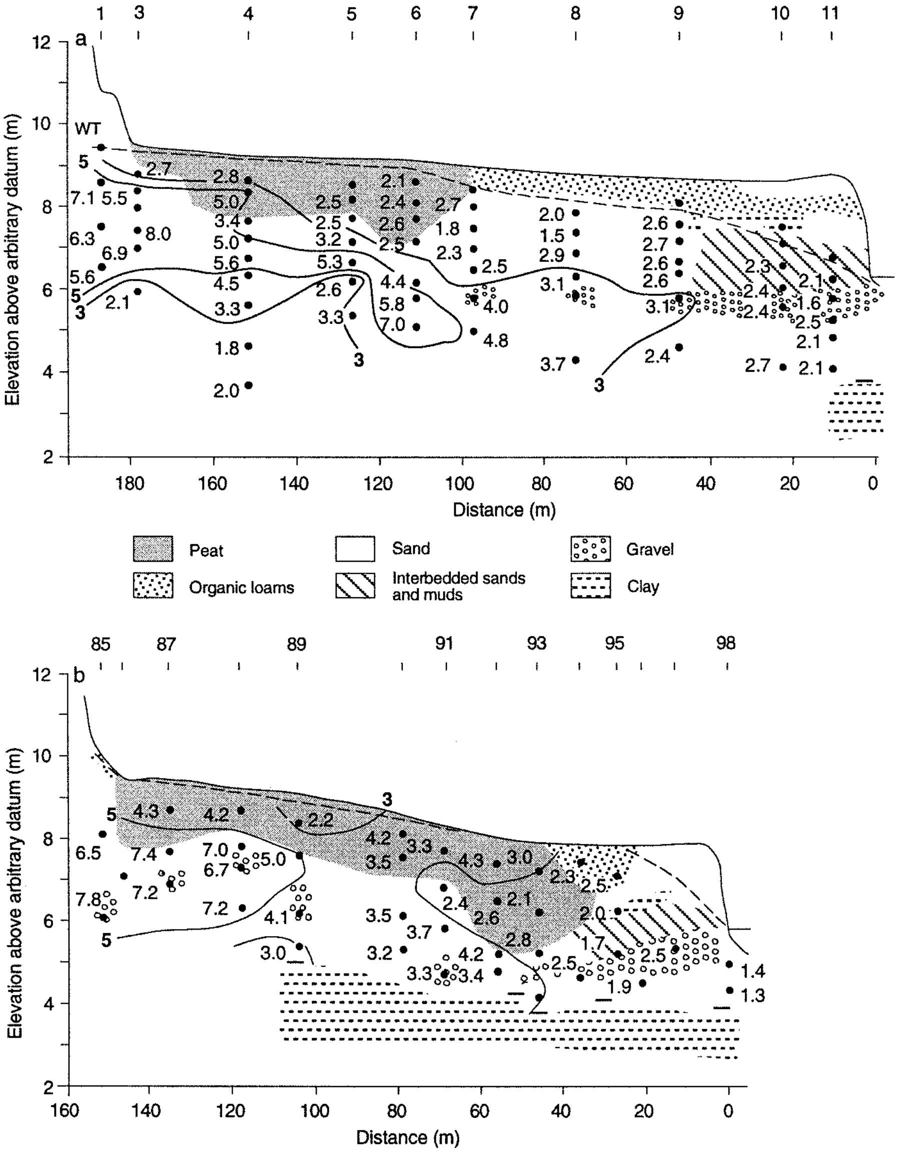

Figure 5. Groundwater dissolved oxygen concentrations (mg L^{-1}) in late summer 1997 along (a) transect A and (b) transect B. Lines are contours of equal dissolved oxygen concentration.

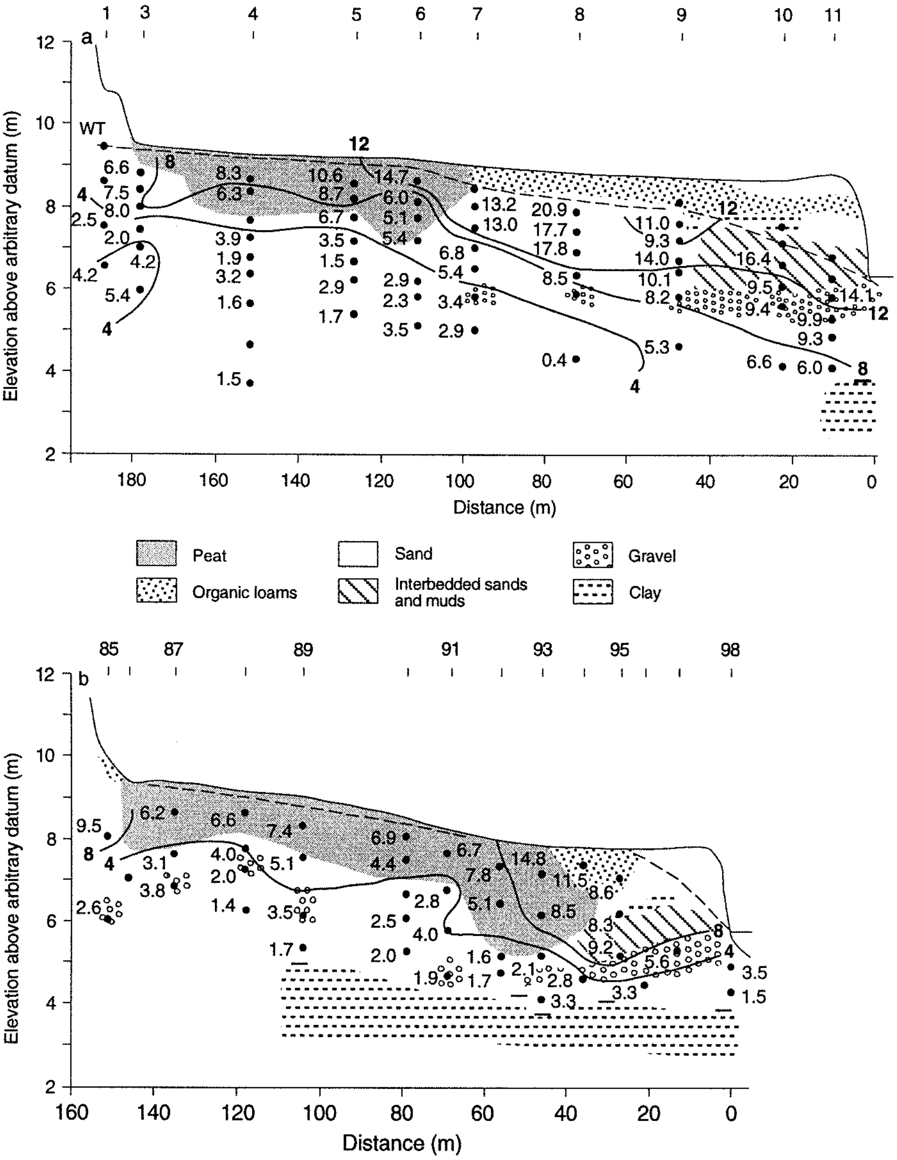

Figure 6. Groundwater DOC concentrations (mg L^{-1}) in late summer 1997 along (a) transect A and (b) transect B. Lines are contours of equal DOC concentration.

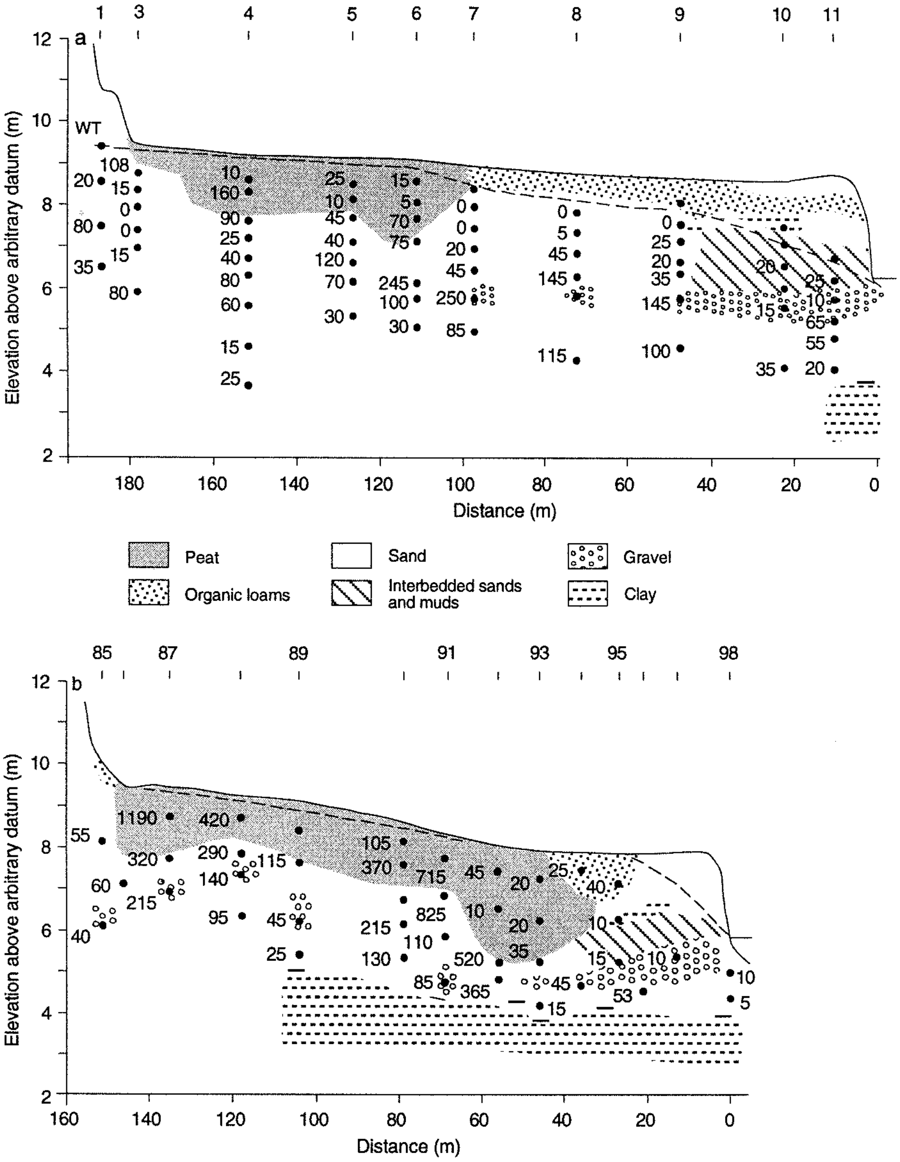

Figure 7. Groundwater N_2O-N concentrations (μg L^{-1}) in late summer 1997 along (a) transect A and (b) transect B. Lines are contours of equal nitrous oxide concentration.

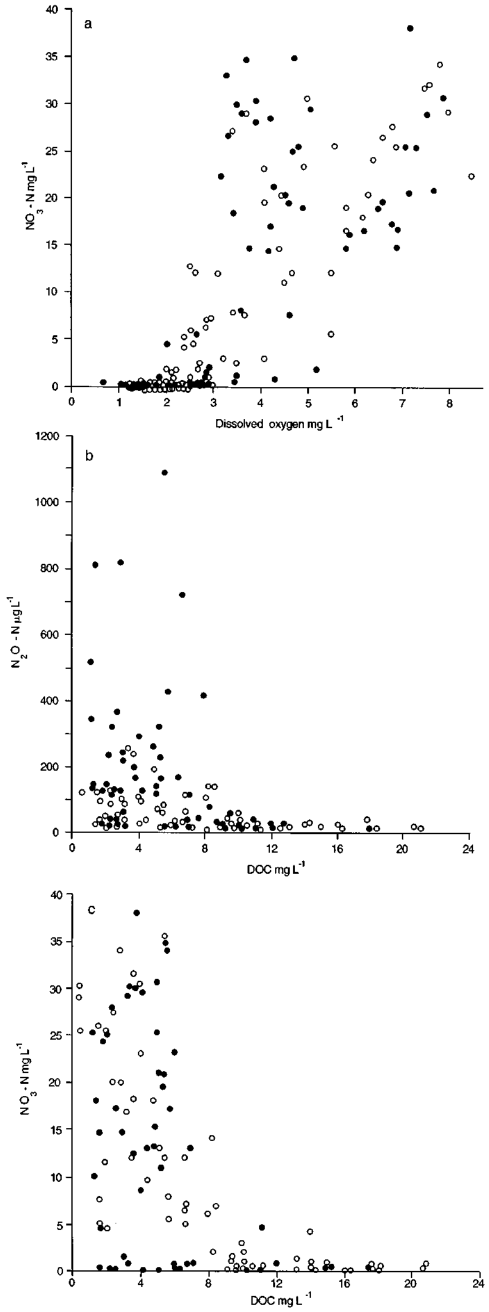

Figure 8. Correlations between (a) NO_3^- and dissolved oxygen, (b) N_2O and DOC and (c) NO_3^- and DOC in groundwater under background conditions. Transect A (○), transect B (●).

Experimental acetylene injections

Piezometers selected for injection were located within the plume of NO_3^--rich groundwater and in areas where NO_3^- concentrations declined to low concentrations. Acetylene additions caused considerable increases in N_2O in 15 piezometers. Various representative patterns of increase were evident. At site 9 (1.5 m) N_2O-N concentrations increased from pre-injection levels of 0 to 540 μg L^{-1} after 1 day, whereas at site 11 (3.5 m) concentrations rose gradually from pre-injection values of 40 μg L^{-1} to 120 μg L^{-1} after 3 days (Figure 9). Most piezometers which had a response to acetylene addition showed peak concentrations after 1–3 days followed by a slow decline towards background levels by 7 days. However, the response was not always immediate. Groundwater N_2O-N concentrations at site 5 (1.5 m) increased slowly only after 1 day and reached a level of 470 μg L^{-1} after 7 days (Figure 9).

Most piezometers which showed a large N_2O response to acetylene injections were in areas of the riparian zone where steep declines in NO_3^- were associated with increased DOC concentrations and DO levels of $<$3 mg L^{-1}. Many of these sites were adjacent to the boundaries between either peat or buried channel deposits and sands (Figure 10). In contrast, most piezometers which showed no N_2O response to acetylene additions were located in the plume of oxic, nitrate-rich groundwater which extended through the sands. Values of δ^{15}N-NO_3^- measured in a previous field study (Devito et al. 2000) are also shown in Figure 10. Values of δ^{15}N were strongly enriched (+17 to +36.4‰) at or adjacent to piezometers that showed a large N_2O response to acetylene, whereas δ^{15}N-NO_3^- was not elevated in the nitrate-rich plume.

Experimental carbon and nitrate injections

Short-term injections of acetylene plus glucose into 13 piezometers located in the deep sands beneath the peat where background groundwater DOC concentrations were $<$4.0 mg L^{-1} produced a range of responses (Figure 11). Peak N_2O-N concentrations of 145–500 μg L^{-1} were 2–5$\times$ greater than pre-injection levels in 5 piezometers and 12$\times$ larger in 1 piezometer. A larger N_2O increase after the acetylene plus glucose addition in comparison to an injection of only acetylene several weeks earlier in piezometers 5 (3.0 m) and 96 (3.3 m) is representative of the response in the six piezometers (Figure 12). Groundwater N_2O concentrations after glucose additions usually increased to maximum levels after 1–2 days before declining rapidly to background values after 5–7 days. In most piezometers, glucose addition raised DOC from pre-injection concentrations of 1.7–2.7 mg L^{-1} to 6–17 mg L^{-1} on transect B

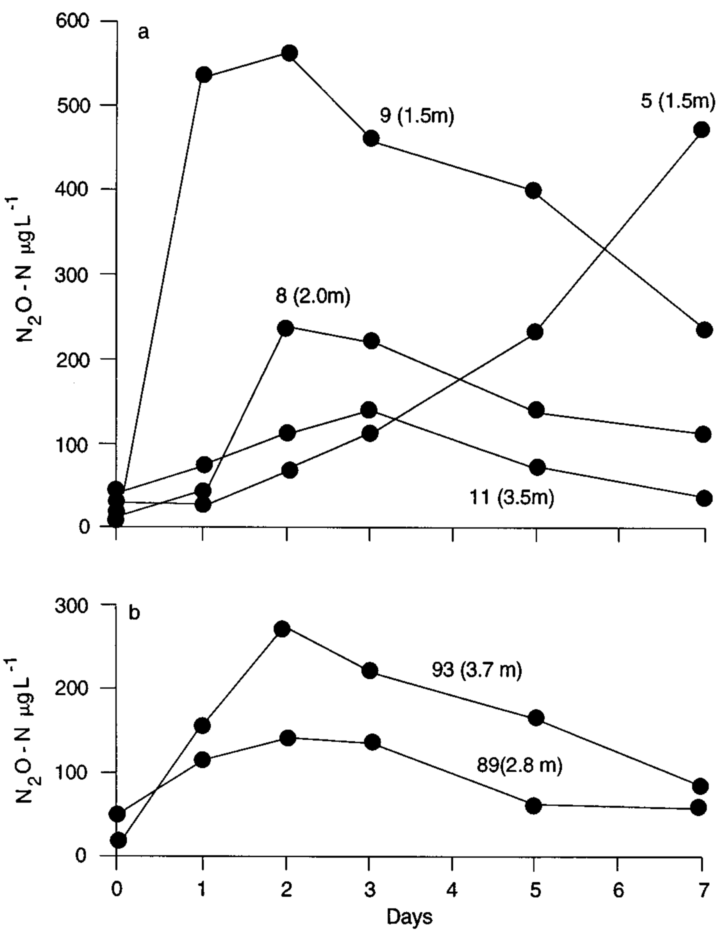

Figure 9. Nitrous oxide concentrations in response to experimental injections of acetylene into piezometers located along (a) transect A and (b) transect B. Numbers indicate piezometers nests and depth (m).

and 15–55 mg L^{-1} on transect A which received a higher dose. These DOC peaks occurred after 1 to 2 days before a rapid return to background levels after 5 to 7 days. The DOC/Br^- ratio during the experiments were similar to the application ratio indicating that the rapid declines in DOC were caused mainly by dilution. Groundwater DO concentrations prior to DOC additions were <4.0 mg L^{-1} in 5 of 6 sites exhibiting an increase in N_2O concentrations, whereas DO concentrations were usually >4.0 mg L^{-1} in sites which did not respond. DO concentrations remained relatively constant at all sites during the experiments.

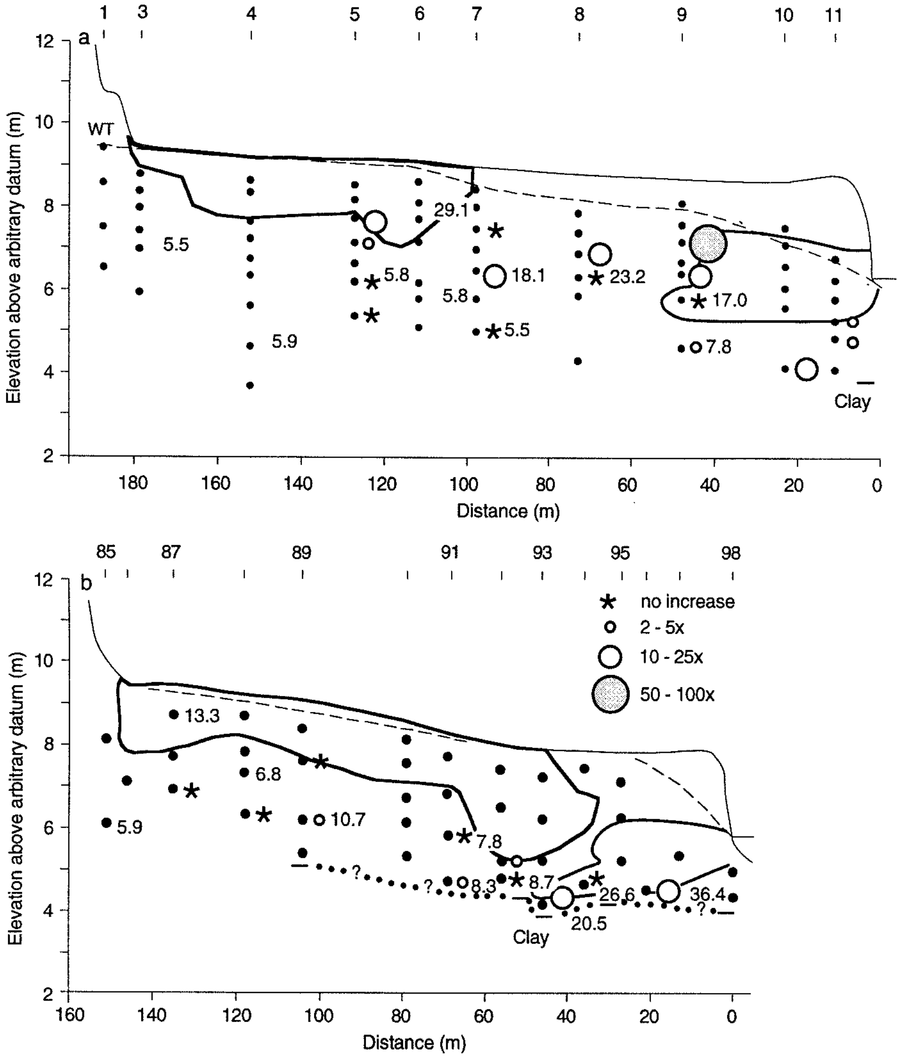

Figure 10. Maximum increase in nitrous oxide concentrations during a seven day period after injection of acetylene into piezometers located along (a) transect A and (b) transect B. Numbers indicate δ^{15}N natural isotope abundance of NO_3^- in piezometers reported by Devito et al. (2000). Solid lines indicate the location of the peats and buried channel sediments.

Additions of NO_3^- plus acetylene caused very strong increases of N_2O-N from 5–20 to 200–1500 μg L^{-1} in 7 of 8 piezometers (Figure 11). Groundwater N_2O-N concentrations which increased from pre-injection values of <20 μg L^{-1} to 520 μg L^{-1} after 1 day at site 11 (3.0 m) and to 1200 μg L^{-1} after 3 days at site 95 (2.6 m) are representative of the response of these seven

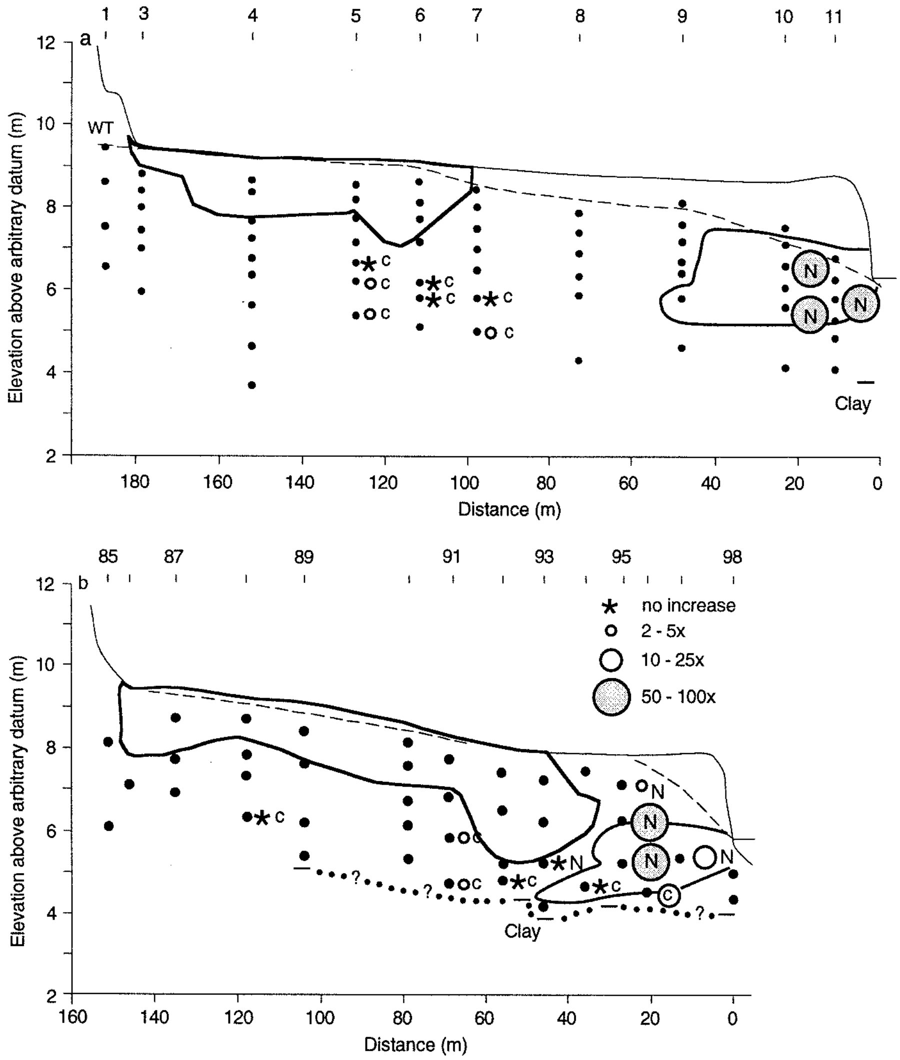

Figure 11. Maximum increase in nitrous oxide concentration during a seven day period in response to injection of either acetylene and glucose or acetylene and NO_3^- into piezometers along (a) transect A and (b) transect B. Solid lines indicate the location of the peats and buried channel sediments. C = glucose injection; N = nitrate injection.

piezometers (Figure 13). High N_2O concentrations were maintained until the end of the experiments. The NO_3^--N/Br^- ratio indicated that declines in NO_3^- after initial peaks were mainly a result of dilution, but a component of the decline was a result of removal of NO_3^- rather than dilution.

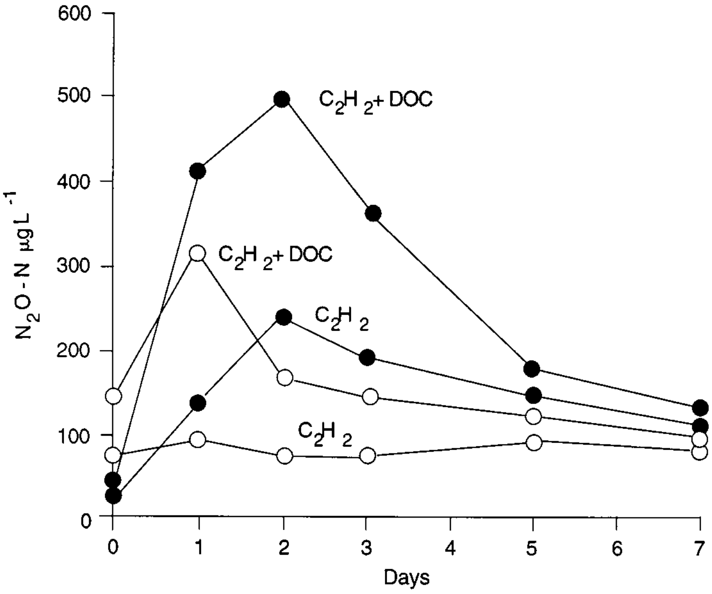

Figure 12. Nitrous oxide concentrations in response to an initial experimental injection of acetylene and a second injection of acetylene and glucose several weeks later into two piezometers. ● = piezometer nest 95 (3.3 m); ○ = piezometer nest 5 (3.0 m).

Discussion

Our data reveal strong spatial patterns of NO_3^-, DO and DOC in riparian groundwater with distance from the hillslope-riparian perimeter and with depth in sediments. These patterns provide support for the view that biogeochemical variations in riparian zones are determined by interactions between the metabolism of microbial communities and supplies of electron donors and acceptors (Hedin et al. 1998). A significant positive correlation between O_2 and NO_3^- and the strong inverse relationship between oxidized N (NO_3^- and N_2O) and DOC suggests that oxidized forms of N are depleted in more reduced environments which have high supplies of DOC. This provides further evidence that microbial transformations that involve the sequential reduction of electron acceptors influence riparian groundwater chemistry.

The spatial sequence of electron donors and acceptors across the Boyne River riparian zone suggests the occurrence of an initial zone dominated by aerobic respiration as nitrate-rich oxic groundwater enters from the adjacent upland aquifer. With greater distance along the subsurface horizontal flow path, a second zone is dominated by denitrification. This spatial pattern is consistent with the simple conceptual model of Correll and Weller (1989) which proposed that lateral subsurface flow beneath a riparian forest to the

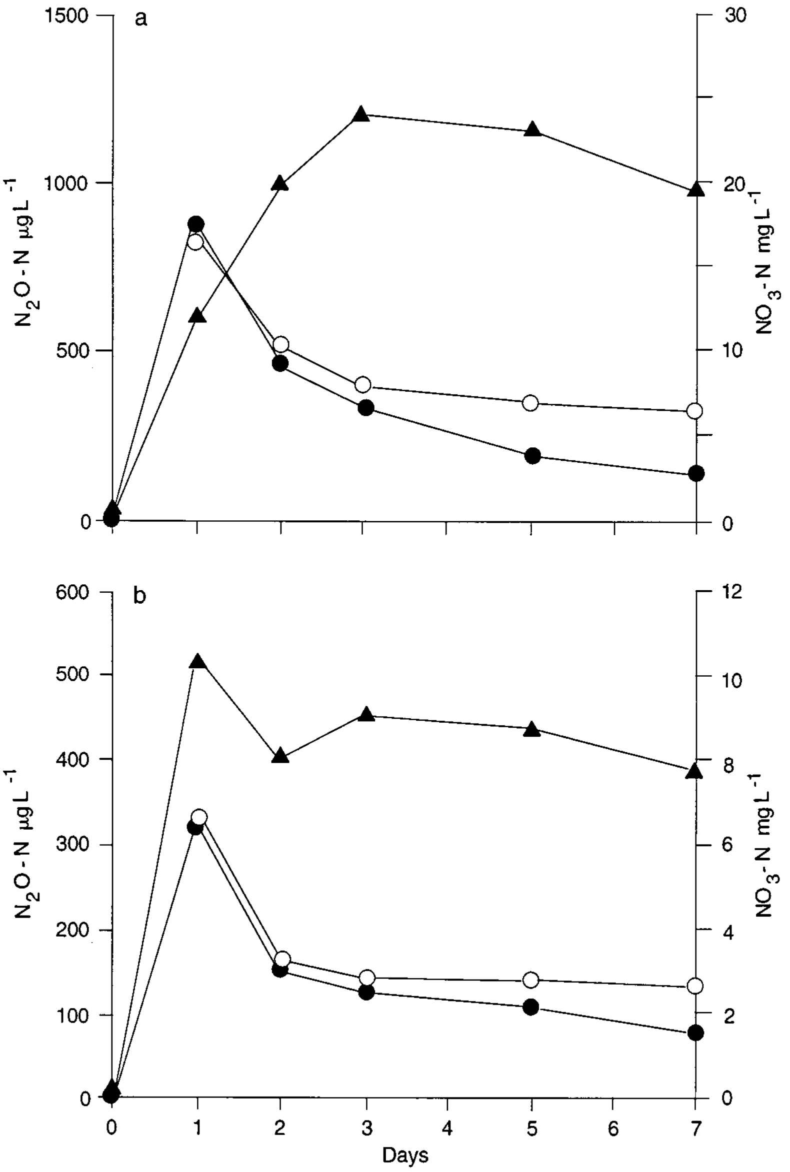

Figure 13. Concentrations of N_2O and NO_3^- in response to injection of acetylene, bromide and NO_3^- into groundwater at (a) piezometer nest 95 (2.6 m) and (b) piezometer nest 11 (3.0 m). ▲ = N_2O concentration; ● = observed NO_3^- concentration; ○ = predicted NO_3^- concentration from bromide.

stream is dominated in sequence by respiration, denitrification and sulfate reduction as a function of redox state of the environment.

Our study of the Boyne River riparian zone supports the hypothesis that significant denitrification is confined to narrow regions of the subsurface environment. Steep gradients of NO_3^- decline in relation to chloride which suggest NO_3^- depletion rather than dilution, occurred over short distances within the aquifer. Groundwater N_2O concentrations also declined sharply at these locations indicating consumption of N_2O. The use of *in situ* acetylene injection to locate sites of denitrifying activity revealed that significant denitrification was restricted to these zones of steep decline in NO_3^- and N_2O concentration. The hydraulic conductivity of sediments at sites where piezometers showed a large N_2O increase after acetylene injection was similar to sediments where piezometers had no N_2O response. Consequently, the patchy distribution of denitrification was not simply a function of the residence time of the injected acetylene in the sediments. The pattern of elevated $\delta^{15}N\text{-}NO_3^-$ reported by Devito et al. (2000) in the vicinity of piezometers that showed a large N_2O response to acetylene confirmed the occurrence of considerable microbial denitrification at these sites. Isotopic fractionation during denitrification causes any NO_3^- that remains to be progressively enriched in ^{15}N (Blackmer & Bremner 1977; Mariotti 1986).

We cannot delimit precisely the areal extent of denitrification in sediments around each piezometer tip. The volume of sediment affected by the injection of acetylene amended groundwater and subsequent withdrawal of this water by pumping during the experiments varies in relation to the hydraulic conductivity of the sediments. Nevertheless, adjacent piezometers separated by vertical distances of only 0.5 to 1.0 m frequently showed different responses to acetylene additions that ranged from zero N_2O increase to increases of 10–25 and 50–100×. These contrasts suggest that our injections detect denitrification in the vicinity of the piezometer tip.

The acetylene inhibition technique may underestimate *in situ* denitrification in the Boyne River riparian sediments. Previous studies have shown acetylene was only 50–70% effective at blocking N_2O reduction to N_2 (Nielsen et al. 1990; Seitzinger et al. 1993). The blockage of nitrification by acetylene is frequently reported as a major limitation of the acetylene inhibition method for detecting denitrification (Bremner & Blackmer 1979; Knowles 1990). However, this limitation is not important in our study because the major source of nitrate is the discharge of nitrate-rich groundwater from the adjacent upland sand aquifer rather than *in situ* nitrification at depth in the riparian sediments.

The limited areal extent of strong denitrifying activity in the Boyne River riparian saturated zone which is 4–6 m thick and up to 200 m wide underscores the difficulty of detecting subsurface denitrification by laboratory studies of small sediment samples. Narrow zones of denitrification associated with redox fronts have also been reported in extensive shallow aquifers in non-riparian landscapes (Smith et al. 1991; Spalding & Parrott 1994) and in riparian areas at the soil-stream interface (Hedin et al. 1998) and upland-riparian interface (Blicher-Mathiesen & Hoffman 1999). Several other studies have found rapid NO_3^- loss in the initial 5 to 20 m of the riparian zone (Peterjohn & Correll 1984; Jacobs & Gilliam 1985; Haycock & Burt 1993). Although these studies did not investigate patterns of electron donors and acceptors, the rapid depletion of NO_3^- suggests the presence of a narrow redox front at the upland perimeter of these riparian zones.

The spatial pattern of groundwater N_2O concentration suggests that the N_2O is produced by denitrification rather than nitrification. Extensive areas of the Boyne river riparian aquifer inland from the zone of high denitrifying activity have N_2O of >50 μg L^{-1}. High groundwater N_2O-N concentrations of 100–250 μg L^{-1} on transect A and 300–500 μg L^{-1} on transect B occurred near sites of high denitrifying activity. At site 87 (0.5 m) where N_2O-N concentrations were >800 μg L^{-1} throughout 1996–1998, a $\delta^{15}N\text{-}NO_3^-$ value of 13.0 in comparison to values of 5.5–5.8 in groundwater entering the riparian zone indicated denitrification activity (Figure 10). Although factors influencing the proportion of N_2O produced during denitrification are not well understood, ratios of N_2O to N_2 are thought to increase in groundwater with a high concentration of NO_3^- relative to organic carbon (Firestone & Davidson 1989). Riparian zone groundwater N_2O concentrations in the range of 0–500 μg L^{-1} have been reported previously for sites receiving elevated NO_3^- inputs from agricultural land (Weller et al. 1994; Gilliam et al. 1997; Hedin et al. 1998; Blicher-Mathiesen & Hoffman 1999).

The spatial distribution of the narrow zones of high denitrification in riparian sediments strongly supports the hypothesis that the location of denitrification "hotspots" is linked to the supply of oxidizable organic carbon. Most sites at which *in situ* acetylene blockage demonstrated considerable denitrification were located in areas with steep gradients of groundwater DOC increase. Analysis of subsurface stratigraphy (Figure 2), also indicates that many of the denitrification "hot spots" (Figure 10), occurred near interfaces between sands and either peats or buried river channel deposits. Large quantities of solid organic carbon are present in the peats and the buried channel sediments which contain layers of organic muds and large quantities of coarse woody debris. The source of the high concentrations of groundwater DOC in sands at depths of 1–2 m between sites 7 and 9 is less certain (Figure 6). No evidence of former channel sediments were found in this area although localized patches of organic matter may be present in the aquifer sands.

Alternatively, shallow groundwater discharges from the peats into the sands in this area (Figure 2), and it is possible that the high DOC concentrations may result from transport of DOC from the adjacent peat deposits.

Rapid removal of NO_3^- from subsurface water in a buried floodplain channel has been reported (Haycock & Burt 1993) and high rates of denitrification have been measured in a former meander bend (Fustec et al. 1991). However, our study is the first to analyze in detail the patterns of subsurface NO_3^- transport and denitrification activity associated with buried channel sediments or deep riparian peat deposits. Large areas of the peat and buried channel deposits have very low NO_3^- concentrations indicating that denitrification can deplete NO_3^- in a narrow zone at the boundaries of these subsurface organic-rich materials. Rapid removal of NO_3^- by denitrification in a 1.5 m wide subsurface zone in which soil was mixed with sawdust as a carbon source previously demonstrated that narrow carbon-rich zones can effectively deplete NO_3^- in groundwater (Schipper & Vojvodic-Vukovic 1998). Localized areas of high groundwater NO_3^- within the Boyne River riparian peat occur at sites where cavities create pipes in the peat (Devito et al. 2000). These pipes permit flow of groundwater to the surface which bypasses the peat matrix where denitrification takes place.

Low groundwater DOC concentrations within the extensive zone of high NO_3^- concentration beneath the peat suggest that denitrification of NO_3^- and N_2O to N_2 gas was limited by the supply of organic carbon in the deep riparian sands. Other studies have also suggested that subsurface denitrification can be limited by carbon supply (Bradley et al. 1992; Starr & Gillham 1993; Groffman et al. 1996). Carbon supply influences denitrifiers directly by providing energy and indirectly through the consumption of O_2 by heterotrophic microbes which depletes O_2 in the sediments (Groffman 1994). Our injections of glucose to piezometers in the low DOC sands confirmed a carbon limitation at several sites. Experimental glucose + acetylene injections at these locations increased N_2O concentrations significantly above levels measured several weeks earlier when only acetylene was injected. However, the addition of succinate or acetate which are less stimulatory than glucose as C substrates for denitrifers (Beauchamp et al. 1989), may have produced a smaller N_2O response. Elsewhere, glucose additions to piezometers located in sands did not result in a definite N_2O increase. This absence of a denitrification response may reflect limitations of the short-term injection method used rather than the absence of carbon limitation. Our injections produced only a temporary 1 to 2 day DOC increase at these sites and initial DO concentrations of 4.0 to 7.0 mg L^{-1}. remained stable during the experiments. Continuous carbon additions for a longer period may be necessary to stimulate heterotrophic activity and reduce O_2 to levels were denitrification can occur.

Groundwater NO_3^--N concentrations were often <0.1 mg L^{-1} in a zone that extended 30–40 m inland from the river bank (Figure 3). Low DO concentrations and the presence of DOC-rich buried channel deposits in this near-river zone create favourable conditions for denitrification and it is therefore likely that denitrifying activity is limited by the supply of NO_3^-. Experimental *in situ* additions of NO_3^- and acetylene which induced large sustained increases in N_2O concentrations in groundwater confirm the importance of NO_3^- limitation on subsurface denitrification near the river (Figure 11). Other studies that have noted rapid NO_3^- removal near the upslope edge of the riparian zone have also suggested that riparian areas closer to the stream have a large unused potential for denitrification (Peterjohn & Correll 1984; Jacobs & Gilliam 1985; Cooper 1990; Haycock & Burt 1993).

Our data indicate that significant subsurface denitrification is restricted to narrow zones within an extensive riparian aquifer. The ability of stream riparian zones to effectively remove NO_3^- depends on whether these “hotspots” of denitrifying activity occur in hydrologically active areas where most subsurface NO_3^- transport occurs (Hedin et al. 1998). The major subsurface flowpath for NO_3^- transport in the Boyne River riparian zone occurs in the deep permeable sediments where hydraulic conductivities generally ranged from 10–100 cm day^{-1} in the sands and from 200–750 cm day^{-1} in the fine gravel layers, in comparison to 0.1–10 cm day^{-1} in the peats (Devito at al. 2000). Although denitrification activity occurs in the peat and in marls at the interfaces between the peat and sands, these sites have little effect on riparian zone NO_3^- removal because the transport of NO_3^- contaminated groundwater is minor in these low permeability materials. Narrow zones of high denitrification in sands between sites 7 and 9 and at the perimeter of the interbedded sands and muds on transect A are more important in regulating NO_3^- transport because of the greater groundwater flow rates which transport more NO_3^- to these locations.

The most critical denitrification sites for limiting the flux of NO_3^- from the riparian zone to the river are located in the vicinity of the channel lag gravels which extend inland from the river bank in a relatively continuous layer for up to 40–50 m. These coarse sands and fine gravel are a major conduit of groundwater transport to the river. The channel gravels contain small patches of organic matter and receive flows of DOC from overlying interbedded sands and muds in some locations. Acetylene injection revealed considerable denitrification at some sites, but not at others in the gravel. At one site denitrification was increased by additions of carbon. Denitrification may be restricted by the low retention time of groundwater in these highly conductive sediments and by the variability of solid carbon content. This patchy distribution of denitrification in the gravel layer is reflected in patterns of NO_3^- concentration. Groundwater NO_3^--N levels in the channel lag gravels near the river on transect A were often 3–4 mg L^{-1}, whereas only trace concentrations were recorded in the gravel layer on transect B (Figure 3).

Our results suggest a conceptual model of environmental controls on denitrification in the Boyne River riparian zone (Figure 14). Denitrification is carbon limited in a large subsurface area of the riparian zone that extends from the upslope margin laterally for up to 120–140 m towards the river. In this zone horizontally flowing groundwater has high NO_3^- and N_2O concentrations and aerobic respiration only depletes DO slowly because of low levels of available carbon in the sands. Significant denitrification is confined to a narrow redox zone where groundwater transports a NO_3^- supply to sites of organic carbon which occur in peats overlying the sands, or at depth in buried channel sediments near the river. In areas of the riparian zone beyond this redox front low DO concentrations and elevated levels of DOC in groundwater favour denitrification, but this activity is limited by an absence of NO_3^-. This model can probably be applied to other riparian zones where large groundwater NO_3^- fluxes are transported through permeable riparian sediments that extend to considerable depths. Deeper NO_3^--rich groundwater can bypass vegetation and surface riparian soils in these riparian zones. Consequently, if NO_3^- is not transported to localized subsurface sites of carbon supply, denitrification may not effectively remove NO_3^-.

The location of narrow zones of NO_3^- consumption by denitrification and the portion of the riparian zone where denitrification is N or C limited in this model do not apply to riparian zones which are underlain at a shallow depth by impermeable materials. In this hydrogeologic setting, groundwater flowing laterally at shallow depths interacts with organic-rich soils so that a zone of C limitation is often absent and denitrification occurs mainly at the upland perimeter of the riparian zone. The portion of the riparian zone where denitrification is limited by NO_3^- may be large in these riparian zones.

Conclusions

This study illustrates the role of interactions between hydrology and patterns of supply of electron donors and acceptors in influencing subsurface denitrification in riparian zones. Denitrification in the Boyne River riparian zone occurred at depths of several meters in a very narrow zone within a 150–200 m wide floodplain. In this zone, flow paths transported oxidized nitrate-rich groundwater to a redox interface associated with high supplies of DOC.

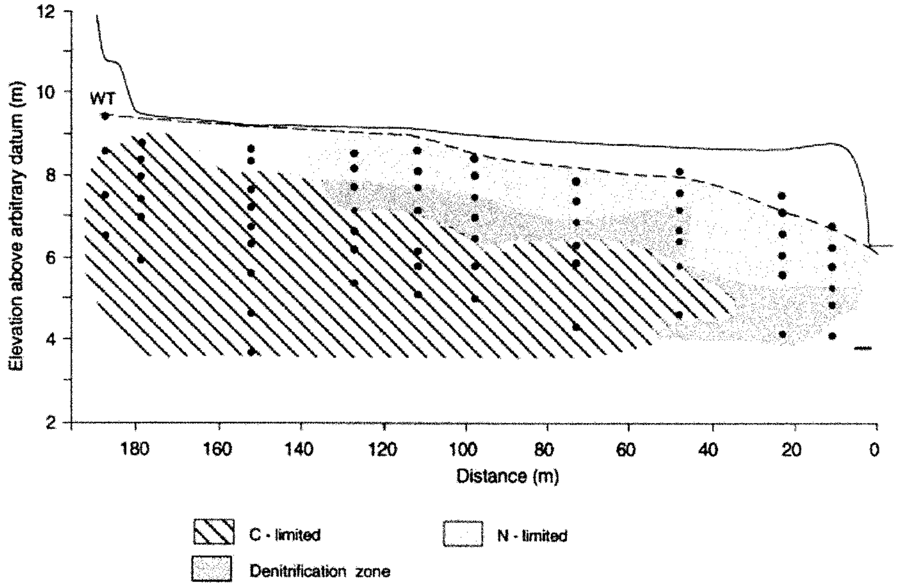

Figure 14. Environmental controls of denitrification in the Boyne River floodplain based on transect A at the upstream site.

Current uncertainty about subsurface denitrification as a mechanism of nitrate removal in riparian zones may reflect the difficulty of detecting localized patches of denitrifying activity at depth in these environments.

These data further emphasize the importance of lithology and hydrology in understanding NO_3^- dynamics within riparian zones. The depth of the aquifer in the Boyne River flood plain resulted in a large plume of groundwater with high NO_3^- concentrations bypassing surface riparian soil horizons and extending laterally across the riparian zone at depths of several meters in permeable sands and gravels. Within this extensive zone of high hydrologic throughput, low groundwater DOC concentrations and responses to *in situ* additions of glucose indicated a carbon limitation on denitrification. We suggest that in riparian zones with deep permeable sediments, the ability to remove NO_3^- by denitrification is critically dependent on localized supplies of oxidizable carbon. These carbon supplies may occur in buried river channel deposits, or where DOC is transported by groundwater recharge from overlying organic soils to deeper sediments. The influence of depth of permeable riparian sediments overlying aquitards and the location of subsurface deposits formed by fluvial processes may be more important than the width of vegetated riparian strips in understanding the ability of riparian zones to remove nitrates.

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