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Nitrous oxide generation, denitrification, and nitrate removal in a seepage wetland intercepting surface and subsurface flows from a grazed dairy catchment

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Abstract. Little is known about seepage wetlands, located within agricultural landscapes, with respect to removing nitrate (NO_3^-) from agricultural catchments, mainly through gaseous emissions of nitrous oxide (N_2O) and dinitrogen (N_2) via denitrification. These variables were quantified using a push–pull technique where we introduced a subsurface water plume spiked with ^{15}N-enriched NO_3^- and 2 conservative tracers [bromide (Br^-) and sulfur hexafluoride (SF_6)] into each of 4 piezometers and extracted the plume from the same piezometers throughout a 48-h period. To minimise advective and dispersive flux, we placed each of these push–pull piezometers within a confined lysimeter (0.5 m diameter) installed around undisturbed wetland soil and vegetation. Although minimal dilution of the subsurface water plumes occurred, NO_3^--N concentration dropped sharply in the first 4 h following dosing, such that NO_3^--limiting conditions (<2 mg/L of NO_3-N) for denitrification prevailed over the final 44 h of the experiment. Mean subsurface water NO_3^- removal rates during non-limiting conditions were 15.7 mg/L.day. Denitrification (based on the generation of isotopically enriched N_2O plus N_2) accounted for only 7% (1.1 mg/L.day) of the observed groundwater NO_3^- removal, suggesting that other transformation processes, such as plant uptake, were responsible for most of the NO_3^- removal. Although considerable increases in ^{15}N-enriched N_2O levels were initially observed following NO_3^- dosing, no net emissions were generated over the 48-h study. Our results suggest that this wetland may be a source of N_2O emissions when NO_3^- concentrations are elevated (non-limited), but can readily remove N_2O (function as a N_2O sink) when NO_3^- levels are low. These results argue for the use of engineered bypass flow designs to regulate NO_3^- loading to wetland denitrification buffers during high flow events and thus enhance retention time and the potential for NO_3^--limiting conditions and N_2O removal. Although this type of management may reduce the full potential for wetland NO_3^- removal, it provides a balance between water quality goals and greenhouse gas emissions.

Additional keywords: bromide, denitrification, ^{15}N, NO_3^- removal, N_2O, N_2, wetland, SF_6.

Introduction

Nitrogen (N) losses from applied chemical fertilisers, dairy effluent irrigation, and grazing animal excreta potentially cause eutrophication in receiving waters (Phipps and Crumpton 1994; Naiman *et al*. 1995; Carpenter *et al*. 1998). The export of N via surface and subsurface runoff to water bodies can either be minimised by best farm management practices: applying N fertiliser with N inhibitors (Zaman *et al*. 2008*a*), and using riparian zones along river and stream banks (Cooper 1990; Ambus and Lowrance 1991; Haycock and Burt 1993; Carpenter *et al*. 1998; Burt *et al*. 1999), seepage wetlands (Blackwell *et al*. 1999), or permanently wet swales located at gullies of agricultural hillslopes (Burns and Nguyen 2002; Rutherford and Nguyen 2004).

Nitrate moving through riparian and seepage areas is subject to denitrification, plant uptake, dissimilatory reduction of NO_3^-

Abbreviations: BD, bulk density; DEA, denitrification enzyme activity; DNRA, dissimilatory reduction of nitrate to ammonium; I.D., internal diameter; LOI, loss on ignition; PVC, polyvinyl chloride.

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to ammonium (NH_4^+) (DNRA), and microbial immobilisation (Bowman *et al*. 1989*a*, 1989*b*; Hefting *et al*. 2003; Matheson *et al*. 2003). Denitrification is considered to be the major NO_3^- removal process, in which NO_3^- is enzymatically reduced to N_2O and N_2 (Hoffmann *et al*. 2000). Nitrous oxide is a long-lasting greenhouse and potential ozone (O_3) depleting gas, whose generation rates from highly organic enriched wetlands/seepage zones are likely to be substantial, particularly if they receive high NO_3^- pulses in seepage and runoff from adjacent, intensively grazed dairy farming systems (Fig. 1).

Nitrous oxide has been found to be an important end product of denitrification in riparian soils receiving high NO_3^- loadings (Hefting *et al*. 2003). Nitrous oxide in wetlands is produced not only by denitrification but also by nitrification and DNRA (Stevens and Laughlin 1998; Hefting *et al*. 2003; Smith *et al*. 2003). Nitrous oxide produced by various processes might form 1 pool before being reduced to N_2 by nitrous oxide reductase, the enzyme involved in reduction of N_2O to N_2 (Stevens and Laughlin 1998), which suggests that wetlands can act as a source or a sink of N_2O (Blicher-Mathiesen and Hoffmann 1999; Well *et al*. 2001). A wide range of $N_2O : N_2$ ratios has therefore been reported for wetlands, depending on factors (e.g. soil pH, carbon, anaerobicity, NO_3^- concentrations) that govern the nitrification–denitrification–DNRA processes (Groffman *et al*. 2000, 2002; Smith *et al*. 2003). Thus, the use of wetlands in decreasing diffuse N pollution of streams and rivers may shift the potential environmental problem from water pollution to greenhouse gas emission (Well *et al*. 2001; Hefting *et al*. 2003).

Several techniques have been used to investigate NO_3^- removal in wetlands. Many studies have focused on quantifying NO_3^- removal by measuring the rates of processes such as denitrification in laboratory incubation and microcosm studies (Seitzinger 1994; Groffman and Hanson 1997; Zaman *et al*. 2008*b*). These studies suffer from the effects of soil disturbance on oxygen (O_2) status and hence N transformation processes, and from the difficulty of obtaining sediments from depths below the water table for microcosms (Jacinthe *et al*. 1998; Addy *et al*. 2002).

In-situ studies conducted by Burns and Nguyen (2002) have compared NO_3^- movement in wetland groundwater to that of a more conservatively transported ion such as bromide (Br^-). Nitrate removal was then estimated from the changes in $NO_3^- : Br^-$ ratios and NO_3^- and Br^- mass with time, assuming that Br^- is not taken up by plants or processed by soil microorganisms (Schnabel *et al*. 1996; Whitmer *et al*. 2000). Addy *et al*. (2002) successfully demonstrated the use of the push–pull method to determine *in-situ* subsurface water denitrification and NO_3^- removal rates in riparian zones. In that study, collected wetland subsurface water was spiked with Br^-, sulfur hexafluoride (SF_6), and ^{15}N-enriched NO_3^- (potassium nitrate). The amended subsurface water was pushed (i.e. injected) into a mini-piezometer (diameter 18 mm with screen length 20 mm) and then pulled (i.e. extracted) from the same piezometer after an incubation period ranging from 6 to 48 h. Sulfur hexafluoride, an inert and slightly water-soluble gas with an extremely low atmospheric background concentration of 3 parts per trillion by volume, was found to behave as conservatively as Br^-, indicating negligible degassing, thus allowing the authors to confidently use the sum of ^{15}N dissolved gas ($^{15}N_2O$ and $^{15}N_2$) generation and changes in NO_3^- concentration (NO_3^- consumption) as estimates of the denitrification rate and NO_3^- removal.

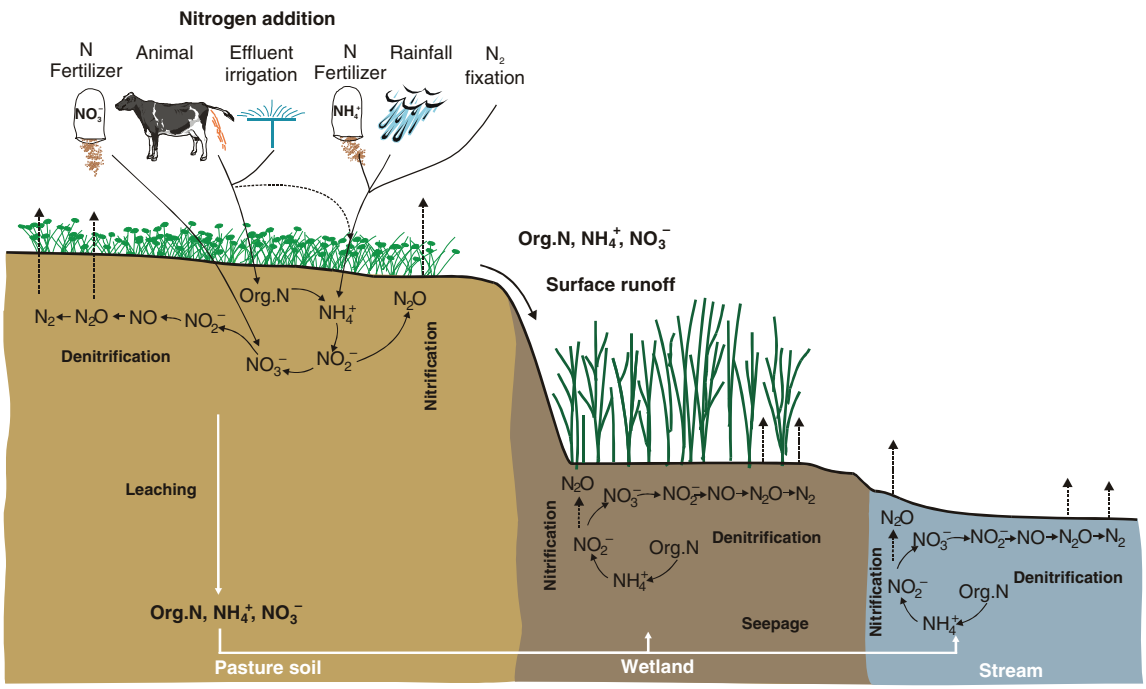

Fig. 1. Nitrogen inputs and losses across the agricultural landscape.

The objectives of this study were to (*i*) quantify *in-situ* NO_3^- removal, N_2O and N_2 emissions, and denitrification rates in a wetland swale in a dairy landscape using the push–pull technique; (*ii*) compare *in situ* denitrification rates as estimated by either the ^{15}N tracer technique or the decrease in added NO_3^- concentrations with time; and (*iii*) provide information on $N_2O : N_2$ ratios in emitted gas from wetlands under non-limiting and limiting NO_3^- conditions.

Materials and methods

Description of study site

The studied seepage wetland (6817 m^2) was located at a footslope of 3 pasture paddocks of a grazed dairy farm (3 cows/ha) in a dairy catchment at Kiwitahi, about 32 km from Hamilton (37°44′S, 175°35′E), New Zealand. The climate is humid–temperate with mean annual temperature of 15°C and annual rainfall of 1150 mm.

The wetland comprises a broad, low gradient (<1° slope) area that receives water from a spring (approximately 1 m from the wetland inlet), natural seepage, and discharges from shallow channels that intercept surface runoff and overland flow from the adjacent pasture paddocks. Grazing animals have been excluded from the wetland since 1999. An artificial swale that is now filled with sediment, organic floc, and wetland plants runs through the wetland and carries most of the flow. The entire wetland complex developed due to a flow constriction at the end of the permanently wet swale which creates partially flooded conditions within the area surrounding the swale (with a slope of <1° across the wetland).

There are 2 major soil types in the catchment, the Topehaehae silt loam and the Kiwitahi silt loam. The Topehaehae silt loam, which is derived from volcanic ash alluvium, is a gley recent soil (Aeric Haplaquent; USDA Soil Taxonomy) with silt loam topsoil and blocky clay loam at 0.3–0.75 m depth. It is a poorly drained soil with very slow subsoil permeability (<0.5 cm/h; Wilson 1980). The Kiwitahi silt loam is a yellow-brown loam (Typic Andept) with a brown silt loam soil texture and moderately permeable subsoil. Its parent material is volcanic ash over late Pleistocene terrace deposits. The soil is well drained and friable with well-developed fine crumb structure. Our study focused on the wetland swale. The soil within the wetland swale is saturated to the surface and is composed of very loose organic material approximately 0.2 m deep. The top 0.1 m depth comprises mainly a thick root mat of wetland plants plus unconsolidated organic mucks. It is followed by a 0.1 m layer of unconsolidated organic flocs and decayed plant materials. Beyond the 0.2 m depth, sediment is more condensed with a mixture of organic matter, silt, and clay, probably originating from the eroded soil materials that have been washed in from the adjacent pasture paddocks plus organic matter decay from wetland vegetation. At a depth of 0.5 m, there is a transition to bluish grey silty clay that increases in its firmness and density with depth. A dense silty clay layer of low permeability particularly at 0.7–0.9 m depth acts as an aquiclude, restricting water movement to a deeper subsurface water. Beyond 0.9 m depth, sediments consisted of a sand and clay mixture. Using tracer tests in a similar seepage wetland, Rutherford and Nguyen (2004) found rapid dilution and pore water velocities of approximately 0.5 m/day within the upper layers of soil. Wetland vegetation consists mainly of soft brome (*Bromus hordaceus* L.) with some floating glaucous sweet grasses (*Glyceria declinata* Breb.) and soft rush (*Juncus effuses* L.) and wiwi (*Juncus edgariae* L.) in areas around the wetland channel and the remaining wetland area. The herbage in pasture paddocks was a mixture of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.).

Lysimeter installations

Push–pull studies are poorly suited to wetlands with high advection and dispersion that can carry the introduced plume away from the dosing piezometer. Because seepage wetlands have high advective and dispersive flux, we installed 4 large lysimeters made up of polyvinyl chloride (PVC) (0.5 m inner diameter and 1.2 m length with a sharpened bevel at the bottom end) to create a confined control volume for push–pull experiments in January 2003. This lysimeter set-up allowed us to investigate denitrification and to follow changes in N_2O and NO_3^- with time. To facilitate the installation of each lysimeter into the wetland, a serrated knife and 0.6-m machete were used to cut through the wetland plant roots and sediments around the lysimeter perimeter and to remove above ground wetland herbage within each lysimeter to 0.05 m height above the wetland surface. The lysimeter was then gently pushed through the wetland media to the underlying silt-clay layer and then slowly pounded into the aquiclude layer. Each lysimeter extended 0.25 m above the wetland surface and at least 0.25 m into the consolidated aquiclude layer of silty clay. Thus, in each lysimeter the depth of wetland sediment above the aquiclude material was approximately 0.7 m.

In the centre of each of the 4 lysimeters, a PVC piezometer (PVC pipe 0.03 m internal diameter and 1.25 m long screened with 0.25-mm slots at 6-mm intervals over the 0.15-m length between 0.20 and 0.35 m depth below the surface of the wetland subsurface water) was installed for the purpose of dosing tracers and water sampling. An impermeable barrier (PVC sleeve) was created within the piezometer at 0.35 m from the wetland ground surface. The estimated well volume of the piezometer over the top 0.35 m depth of the wetland was 247 mL (thereafter a well volume was designated as 250 mL). The piezometer was installed into a PVC sleeve, 0.08 m diameter and 0.40 m deep, hollowed out from the wetland sediment with an auger. The piezometer was inserted into this PVC sleeve and pounded into the clay aquiclude for stability. Each piezometer extended 0.35 m above the wetland ground surface and over 0.2 m deeep into the aquiclude. After installation, the lower 0.2 m of space between the 0.08-m PVC sleeve and the piezometer was backfilled with quartz drilling sand (36% pore space), and the upper top 0.2 m was filled with bentonite before removal of the PVC sleeve. Added bentonite acted as a seal to minimise water flow along the side of each piezometer. Two additional piezometers, which were not confined within lysimeters, were also installed at the site to obtain ambient subsurface water to supply the amended dosing volumes used in the push–pull test. The screened depth, construction, and installation of these piezometers were identical to the piezometers in the lysimeters.

Pre-testing the modified push–pull technique in the wetland lysimeters

A preliminary study was conducted about 6 weeks before the initiation of the ^{15}N-tracer push–pull study. The objective of this study was to provide background information on the physical behaviour of an introduced tracer plume and the approximate rate of NO_3^- removal within the studied lysimeters. We dosed 4 lysimeters through their piezometers with 10 L of wetland subsurface water at a rate of 0.2 L/min., using a peristaltic pump. This subsurface water was amended with 200 mg/L of chloride (Cl^-) and 8 mg/L of NO_3^--N). Surface and subsurface water samples were then obtained 1, 2, 3, 4, 24, 48, 72, and 96 h after dosing and analysed for NH_4^+, NO_3^-, and Cl^- concentrations. During and immediately following this study we extracted a total of 15 L from each lysimeter to remove most of the introduced plume.

Preparation of the dosing NO_3^- tracer solution for push–pull technique

To prepare a dosing solution for 4 lysimeters (10 L per lysimeter), about 42 L of clear wetland water was extracted from the 2 additional piezometers outside the lysimeters. To achieve clear wetland water, the dead volume (muddy water) was pumped until clear water was visible. The collected water was brought back to the laboratory in insulated boxes with ice cubes and stored <4°C until used for preparation of the dosing solution. The dosing solution contained 30 mg/L of Br^- (as LiBr) and 12 mg/L of ^{15}N-labelled NO_3^- as KNO_3 (99 atom% ^{15}N). The level of Br^- was higher than the background level (<1 mg/L) typically found in wetlands in the studied area while the NO_3-N level was comparable to that found in the studied wetland inflow (Nguyen *et al.* 2002). The dosing solution for each lysimeter was then transferred to a polypropylene carboy with a 3-port lid for sparging with 1 ppmv SF_6 through a sparge stone for 5 min to lower the dissolved oxygen (DO) to 3 mg/L. After SF_6 sparging, the headspace was sparged with helium (He) gas to lower the DO to <2 mg/L, to minimise the potential for artificially aerating the soil solution during dosing. The lid on the carboy was then securely closed to avoid any gas leakage during the transportation of the dosing solution to the field.

Push–pull of the dosing solution into lysimeters and water sampling for analyses

The push–pull techniques of Addy *et al.* (2002) was modified to measure NO_3^- removal and N_2O and N_2 generation during late summer (March 2003), the time of the year when no major inflows to and outflows from the studied wetland occurred. Surface water from each lysimeter was collected for ion (NH_4^+, NO_3^-, and Br^-) and gas (N_2O and SF_6) analyses prior to injecting dosing solution into each piezometer. The dosing solution was again sparged with SF_6 to bring the DO <1 mg/L in the field. Two subsamples of dosing solution were then taken to determine the concentration of SF_6. Dosing solution was injected between 1100 and 1200 hours. into the piezometer of each lysimeter at the rate of 250 mL/min using a peristaltic pump. This injection was found to increase the surface water level in each lysimeter to about 0.03–0.05 m. Replicated samples of surface water and subsurface water (piezometer) were removed from each lysimeter after 1, 2, 3, 4, 24, and 48 h of dosing for analysis of dissolved gases ($^{15}N_2O$, $^{15}N_2$, N_2O, SF_6) and ions (NH_4^+, NO_3^-, and Br^-). Before subsurface water was taken from each piezometer for gas and ion analyses, 2 well volumes of water (500 mL) at the first sampling (i.e. 1 h after dosing) and 1 well volume at subsequent samplings (i.e. 2, 3, 4, 24, and 48 h after dosing) were pulled from each lysimeter using a peristaltic pump and discarded. Another 200 mL was then taken from each piezometer and stored in two 100-mL plastic bottles for ion analyses. For gas analyses, duplicate 20-mL subsurface water samples were collected from each piezometer using a 60-mL syringe through a closed system to avoid exposure to air, and injected into 2 separate 120-mL pre-evacuated gas bottles. All samples were brought back to the laboratory in insulated boxes with ice cubes and stored at 4°C before analyses.

Wetland plant N status and sediment characteristics

Four samples of mixed ryegrass–white clover pasture herbage (standing pasture herbage of 0.1–0.15 m height) were randomly taken from adjacent paddocks by cutting to 20 mm height. Similarly, grab samples of vegetation in the wetland swale (mainly sweet grass) were also collected. Pasture herbage and wetland vegetation were dried at 60°C for 7 days, ground, sieved <2 mm, and analysed for total N. Four sediment samples per replicate (85 mm diameter) were taken from 0–0.1, 0.1–0.2, 0.2–0.4, and 0.4–0.7 m depths outside each lysimeter for bulk density (BD), porosity, and saturated hydraulic conductivity (Ks). Estimates of Ks for each horizon were obtained from undisturbed cores using a constant head laboratory method described in Rutherford and Nguyen (2004). Soils were then extruded and oven-dried at 105°C for 48 h to determine BD and porosity. The unconsolidated nature of the top 0–0.1 m sediment depth made it difficult to use the soil core technique for measuring Ks, and hence the pump test technique was used (Klute 1986). Four additional sediment samples (0.05 m diam.) were taken from the same depths and analysed for denitrification enzyme activity (DEA) (Tiedje *et al.* 1989), pH, organic matter, moisture, and NH_4^+ and NO_3^- contents.

Analytical methods and laboratory procedures

Water samples collected from the preliminary test were analysed for Cl^- and Br^- by ion chromatography (American Public Health Association 1998), and for total Kjeldahl-N, NO_3^--N, and NH_4^+-N with a flow injection analyser (FIA). The DO and temperature of surface and subsurface waters were recorded at every sampling event using a WP-82Y Model DO/temperature meter (TPS). Water samples for ion analyses during the main experiment were filtered through GFC filters (1 μm pore size) to remove suspended materials and subsequently analysed for Br^- by inductively coupled plasma emission spectroscopy-mass spectrometry (American Public Health Association 1998), and for NH_4^+, NO_2^-, and NO_3^- by FIA.

Immediately after arrival at the laboratory, He gas was injected into the 120-mL gas bottle samples collected for dissolved gas analyses, to bring them to atmospheric pressure. After storage overnight at 4°C, the sample bottles

were shaken for 30 s to equilibrate SF_6, N_2O, and N_2 between the aqueous (subsurface water) and gaseous phases (headspace). Two gas samples of 12 mL each were then collected from each bottle headspace and stored in pre-evacuated 12-mL glass vials fitted with a screw cap and a rubber septum (Exetainers; Labco, High Wycombe, UK), one for $^{15}N_2O$ and $^{15}N_2$ analyses and another for N_2O and SF_6 analyses. The ^{15}N analyses of N_2O and N_2 were performed by automated continuous flow isotope ratio mass spectrometry (CF-IRMS) and N_2O and SF_6 concentrations were determined using a gas chromatograph (GC) (Shimadzu GC-17A, Japan) equipped with a ^{63}Ni-electron capture detector operating at column, injector, and detector temperature of 55, 75, and 330°C, respectively. Correction for dissolved N_2O and N_2 in the water phase at 5°C was achieved by using the Bunsen solubility coefficients of 1.06 and 0.021 for N_2O and N_2, respectively (Weiss and Price 1980).

Sediment water content was determined gravimetrically by oven drying at 105°C for 48 h. Sediment NH_4^+ and NO_3^- contents were determined by shaking freshly collected sediment (10 g on oven-dried basis) with 20 mL of 2 M KCl solution for 1 h, followed by centrifugation at 4000*G*, filtration through Whatman no. 42 filters, and analysis by FIA. Sediment organic matter content was determined as the proportion of the weight of oven-dried soil based on loss on ignition (LOI) at 550°C for 4 h. Sediment pH was determined using a combination glass electrode after equilibrating freshly collected samples (10 g on oven-dried basis) with 10 mL of deionised water for 30 min. All sediment analyses were corrected for sediment water content.

Wetland sediment was bulked on a soil depth basis and 3 replicate samples from each depth were analysed for DEA using a Bunsen coefficient of 0.632 for 20°C (temperature of the laboratory) to account for N_2O in aqueous solution (Tiedje *et al*. 1989). Sediment (5 g fresh weight) was amended with 5 mL solution containing NO_3^- (KNO_3 0.1 g/L) and glucose (0.2 g/L). The headspace was flushed with N_2 gas and 10 mL acetylene (C_2H_2) to inhibit the reduction of N_2O to N_2. After 15 and 60 min of incubation at laboratory ambient temperature (20°C), duplicate headspace samples were transferred to 12-mL exetainers. The samples from exetainers were then analysed for N_2O by the Shimadzu GC as described above.

Plant samples (0.1 g per sample) were digested with 5 mL of Kjeldahl mixture for 3 h at 350°C and N content was determined by automated analysis with a Technicon Auto Analyzer II (Technicon Instruments Corp., Tarytown, NY). Total S and total P content in plant samples were determined using the technique of Quin and Woods (1976) in which plant samples were digested with nitric and perchloric acid mixture for 1 h at 200°C.

Estimating denitrification rate and NO_3^- removal

Measured concentrations and ^{15}N atom% in $^{15}N^{15}N^{16}O$ ($^{46}N_2O$) and $^{14}N^{15}N^{16}O$ ($^{45}N_2O$) in dissolved gases extracted from surface and subsurface water samples were used to determine denitrification rates after correcting for the ^{15}N background (natural abundance) of 0.3663 atom%. The dissolved N_2 concentration in water samples (obtained from CF-IRMS) was converted to the amount (μg/L) as described in Tiedje (1982) taking into account the volume of an exetainer, the volume of water samples collected from each lysimeter for N_2 analyses, and the volume of a headspace in a 0.12-L bottle (i.e. 0.012, 0.020, and 0.10 L, respectively). The amount of N_2 produced was then converted to a rate of N_2 generation by multiplying it by the ratio of applied ^{15}N (99% atom) present as ^{15}N atom % in dissolved N_2. The rate was expressed as μg/L.day after taking into account the time interval between each sampling event.

Similarly, the N_2O concentration in water samples as measured by GC was converted to the amount of N_2O produced (μg/L), which was then converted to the rate of N_2O generation (i.e. the amount of N_2O that was derived from the denitrification of labelled NO_3^-) by multiplying it by the ratio of applied ^{15}N (99% atom) present as ^{15}N atom% in dissolved N_2O. The data on ^{15}N atom% in $^{46}N_2O$ was used in this calculation since it was significantly correlated ($r^2 = 0.86$; $P < 0.001$) with the ^{15}N atom% in $^{45}N_2O$ of the same samples.

Nitrate removal attributed to denitrification and other biological processes was calculated as the difference between measured and estimated 'conserved' NO_3^- concentrations in subsurface water at a sampling time (t_s). The 'conserved' NO_3^- estimate at t_s was based on concentration reductions associated with the introduced Br^- tracer and represented an estimate for NO_3^- concentration reductions resulting from physical processes such as dilution and not denitrification or other biological processes. Based on this assumption, the estimated NO_3^- concentration was calculated by multiplying NO_3^- concentration at the time of dosing (t_0) by the ratio of Br^- concentration at t_s and t_0 (Burns and Nguyen 2002).

Statistical analyses

Standard deviation and standard error were calculated for different parameters using data collected from the 4 lysimeters. The first-order kinetic equation was used to fit the curve in the surface and subsurface data of NO_3^- concentration and ratio of NO_3^- over Br^- concentrations.

Results and discussion

Wetland sediment physical and chemical characteristics and plant nutrient status

The studied wetland sediment had low BD and high organic matter (LOI) content (Tables 1 and 2). Its high porosity and high hydraulic conductivity in the top 0.1 m depth (Table 1) made it difficult to use the push–pull technique of Addy *et al*. (2002) without the use of a confined lysimeter to investigate NO_3^- removal and gaseous emissions of N_2O and N_2 due to rapid dispersion of the introduced tracer plume. Using a confined

Table 1. Sediment physical properties
Values are means ± standard deviations

Depth (m)	Bulk density (g/cm^3)	Porosity (%)	Saturated hydraulic conductivity (cm/day)
0–0.1	0.15 ± 0.02	77.5 ± 3.5	94.5 ± 35
0–0.2	0.20 ± 0.02	65.5 ± 3.6	50.6 ± 36
0.2–0.4	0.31 ± 0.04	50.4 ± 4.5	22.5 ± 1.3
0.4–0.7	0.95 ± 0.05	32.1 ± 3.6	2.5 ± 0.4

Table 2. Sediment chemical properties
Values are means ± standard deviations

Depth (m)	Loss on ignition (%)	pH	KCl-extractable (mg/kg soil): NH_4^+-N	NO_3^--N
0–0.1	48.5 ± 2.5	5.4 ± 0.1	50.3 ± 3.5	0.12 ± 0.013
0–0.2	26.6 ± 4.5	5.2 ± 0.2	31.2 ± 4.5	0.05 ± 0.013
0.2–0.4	12.2 ± 2.2	5.0 ± 0.3	13.5 ± 2.5	0.02 ± 0.010
0.4–0.7	4.5 ± 1.4	4.7 ± 0.2	5.7 ± 2.1	0.01 ± 0.006

lysimeter minimised advection–dispersion of the introduced Br^- tracer plume in the subsurface water. The major form of N in surface and subsurface depths of wetland sediments was NH_4^+-N (Table 2) because anaerobicity inhibits nitrification (Zaman *et al*. 2007).

Both surface water and subsurface water had low DO levels (<1 mg O_2/L; Table 3), suggesting that anaerobic conditions favourable for denitrification (Smith *et al*. 2003; Zaman *et al*. 2008*b*) were prevalent in this wetland. Various workers (Tiedje 1988; Patrick and Jugsujinda 1992; Achtnich *et al*. 1995; Blicher-Mathiesen and Hoffmann 1999) have reported that NO_3^- reduction occurs mainly when subsurface water DO falls below 0.5–1.6 mg O_2/L.

The low level of NO_3^- in wetland waters (Table 3) suggests that any NO_3^- that enters the wetland via seepage springs and runoff is readily removed by denitrification (Zaman *et al*. 2008*b*) and other biological processes (Seitzinger 1994; Hill 1996; Fennessy and Cronk 1997; Matheson *et al*. 2002, 2003). Ammonium and organic N were the predominant N fractions in both surface and subsurface waters (Table 3), likely due to inputs from farmland runoff and/or the incomplete breakdown of organic matter originating from wetland organic sediments and/or plant vegetation under anaerobic conditions (Nguyen 2000).

Denitrification enzyme activity in the upper 0.4 m of wetland sediment ranged from 53 to 205 mg N_2O-N/kg soil.day (Table 4). Highest DEA was measured in the top 0.1 m and decreased sharply with depth, probably due to a reduction in the level of organic matter and denitrifier populations with depth. Since NO_3^- was unlikely to reach the lower depths because of active denitrification in the top sediment layer, DEA at the lower depths was also likely limited by low NO_3^- concentrations (Xue *et al*. 1999; Hoffmann *et al*. 2000; Well *et al*. 2001).

At the time of the experiment, nutrient status of the sweet grass collected from the wetland channel was lower than that in pasture herbage of the adjacent paddocks (Table 5). These results were similar to those obtained in the following spring (6 months later in September) when wetland plants were at their most active growing stage. The lower N (and also P and S) status in wetland vegetation was likely to be due to the difference in plant species of wetland and pasture soils. Perennial ryegrass (*Lolium perenne* L.) is a highly nitrophyllous species, selected for high production potential, whereas wetland plants do not require as much N as pastures; therefore, their roots systems are less adventitious in accessing N. The high N concentrations (Table 5) of pasture herbage with a mixture of ryegrass and white clover (*Trifolium repens* L.) suggest a high level of soil fertility; the paddock results are consistent with those reported in New Zealand conditions (Machado *et al*. 2005; Blennerhassett *et al*. 2006; Zaman *et al*. 2008*a*).

Table 3. Surface and subsurface water characteristics before the commencement of the study
Values are means ± standard deviations of 4 measurements

Chemical properties	Surface water	Subsurface water
Dissolved oxygen (mg/L)	0.38 ± 0.02	0.41 ± 0.02
Total Kjeldahl N (mg/L)	3.6 ± 0.012	3.2 ± 0.015
NH_4^+-N (mg/L)	0.67 ± 0.016	1.55 ± 0.018
NO_3^--N (mg/L)	0.010 ± 0.004	0.012 ± 0.002
NO_2^--N (mg/L)	0.008 ± 0.001	0.007 ± 0.001
Cl^- (mg/L)	41.1 ± 0.03	18.5 ± 0.14
Br^- (mg/L)	0.09 ± 0.012	0.10 ± 0.065
pH	5.1 ± 0.03	4.9 ± 0.03
Water temperature (°C)	19.4 ± 0.02	17.5 ± 0.03

Table 4. Denitrification enzyme activities at different depths in the studied wetland sediment
Values are means ± standard deviations (n = 3)

Depth (m)	mg N_2O-N/kg soil.h	mg N_2O-N/kg soil.day
0–0.1	8.5 ± 1.01	205 ± 24.0
0–0.2	5.6 ± 0.20	134 ± 4.8
0.2–0.4	2.2 ± 0.17	53 ± 4.2
0.4–0.7	0.54 ± 0.111	13.0 ± 2.68

Table 5. Nutrient status (g/100 g dry matter) of wetland sweet grass and pasture herbage in the adjacent paddocks
Values are means ± standard deviations

Nutrient status	Wetland plant		Mixed pasture herbage	
	February	September	February	September
N	2.6 ± 0.1	2.7 ± 0.02	4.1 ± 0.05	4.3 ± 0.2
P	0.27 ± 0.03	0.24 ± 0.02	0.47 ± 0.02	0.45 ± 0.04
S	0.19 ± 0.01	0.19 ± 0.02	0.36 ± 0.04	0.37 ± 0.06

Preliminary testing of the push–pull technique within a confined wetland lysimeters environment

The Cl^- concentration of the introduced plume changed <25% over a 72-h period (data not shown), suggesting that the introduced subsurface water plume in the *in-situ* wetland lysimeters was subject to minimal dilution. The low hydraulic conductivity of the wetland sediment at depths beyond the top 0.4 m (Table 1) may limit any water transfer out of, or into, this sediment zone. This was further confirmed by the slow drop in the level of the ponded water that occurred after injection of the dosing solution. No water was observed emerging around the piezometers or the bentonite seals, arguing against any significant short-circuiting of flow between surface and subsurface water in the piezometer–lysimeter system. Thus, the modification of the push–pull technique of Addy *et al*. (2002) with the use of 0.03-m internal diameter piezometers, instead of mini-piezometers in conjunction with *in-situ* lysimeters, was appropriate in our wetland environment.

Nitrate in both surface and subsurface water returned to background levels (<0.015 mg/L) within 24 h after dosing

(data not shown). As a result of this rapid removal rate, we chose to use a higher initial concentration (12 *v.* 8 mg N/L) during the denitrification study with ^{15}N-enriched NO_3^-.

Nitrate dynamics in wetland waters after dosing with ^{15}N-enriched NO_3^-

Following dosing with ^{15}N-enriched NO_3^-, Br^-, and SF_6, NO_3^- concentrations (Fig. 2*a*, *b*; Table 6) and NO_3^-/Br^- ratios (Fig. 3*a*, *b*) in both surface and subsurface waters significantly ($P < 0.001$) decreased with time, indicating an active NO_3^- removal from surface water and particularly from subsurface water. Bromide is a conservative tracer and its fate over a short period of time (48 h) is probably governed by

Table 6. Measured and theoretical (based on the changes in bromide concentrations with time) concentrations of NO_3^--N (g/cm^3) in wetland waters sampled over a 48-h study period

Values are means ± standard deviations

Time (h)	Measured		Expected	
	Surface water	Subsurface water	Surface water	Subsurface water
1	1.75 ± 1.84	9.97 ± 0.30	1.91 ± 1.95	9.98 ± 0.42
2	1.37 ± 1.15	7.64 ± 1.33	1.70 ± 1.35	8.92 ± 0.94
3	1.20 ± 0.97	5.80 ± 1.64	1.54 ± 1.15	7.78 ± 1.11
4	0.96 ± 0.75	2.70 ± 2.29	2.67 ± 1.46	4.64 ± 3.13
24	0.19 ± 0.31	0.38 ± 0.47	2.77 ± 2.22	4.13 ± 3.06
48	0.01 ± 0.01	0.01 ± 0.01	2.15 ± 0.98	3.72 ± 1.80

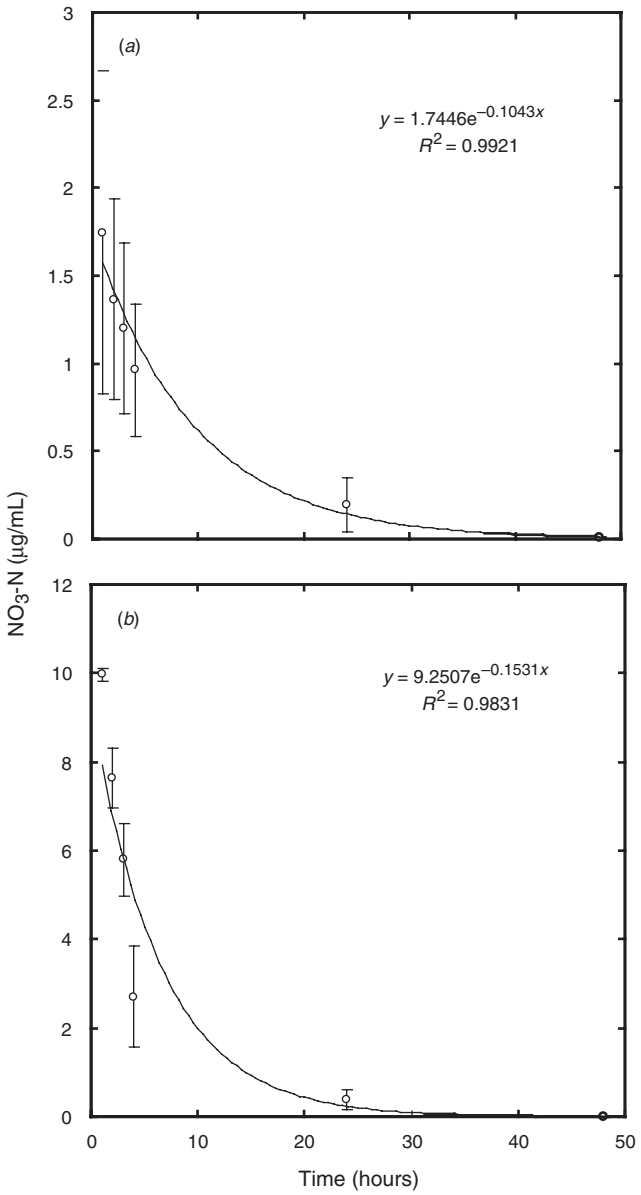

Fig. 2. Nitrate concentration in (*a*) surface and (*b*) groundwater samples collected over a 48-h period after the dosing of nitrate-bromide and sulfur hexafluoride. Error bars represent standard error of the mean. The fitted line represents a least square regression of the first-order kinetics equation.

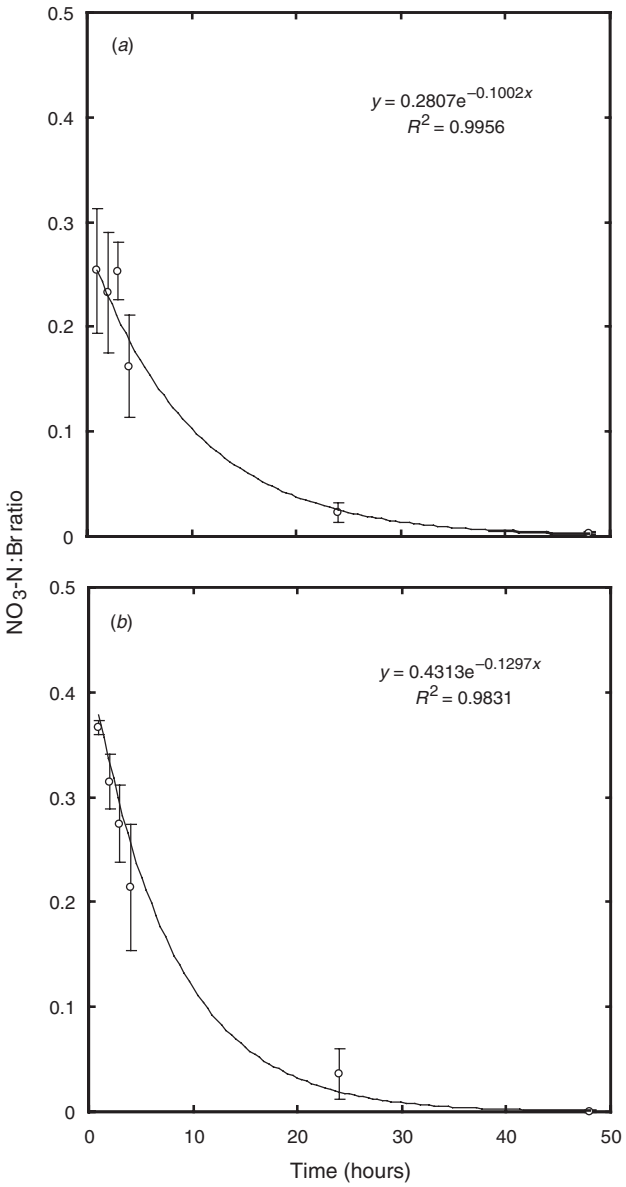

Fig. 3. Ratios of nitrate over bromide concentrations in (*a*) surface and (*b*) subsurface water samples collected over a 48-h period after the dosing of nitrate-bromide and sulfur hexafluoride. Error bars represent standard error of the mean. The fitted line represents a least square regression of the first-order kinetics equation.

hydrologic conditions and not by transformations from microbial processes or plant uptake (Xue *et al*. 1999; Addy *et al*. 2002; Burns and Nguyen 2002). If decreases in NO_3^- concentrations were strictly due to physical processes, changes in NO_3^- concentration with time would mirror the reduction in Br^- concentration with time, yielding a constant NO_3^-/Br^- ratio over the sampling period (Addy *et al*. 2002).

Changes in the ratio of Br^- (C_i/C_0) concentration between t_i and t_0 can be used to estimate the expected concentrations at time t_i of 'conserved' NO_3^- (the concentration resulting from physical processes such as dilution and dispersion). The conserved concentration estimates based on changes in the Br^- (C_i/C_0) ratios were higher than measured NO_3^--N concentrations within each lysimeter during virtually all (46 out of 48) of the sampling periods (Table 6). Measured NO_3^--N concentrations remained >2 mg/L, the level at which NO_3^--N availability begins to limit denitrification (Schipper and Vojodic-Vukovic 1998), for the first 3–4 h following dosing in all subsurface water samples and in the surface water of 2 of the 4 lysimeters. The surface water was a mixture of the dosing solution and displaced water and exhibited considerable dilution as it moved from the 0.2–0.35 m dosing depth to the surface, with relative Br^- concentrations ranging from 0.1 to 0.3 (C_i/C_0) 1 h after dosing. All samples from all locations obtained after 24 and 48 h had low NO_3^--N concentrations.

The average NO_3^- removal rates during non-limiting conditions (i.e. NO_3^--N > 2.0 mg/L) were 3.96 and 15.7 mg/L.day for surface and subsurface water, respectively (Table 7). These rates were substantially higher than the average rates of ^{15}N-enriched denitrification gas production (N_2O-N plus N_2) over the same period (0.25 and 1.1 mg/L.day in the surface and subsurface water, respectively; Table 7). Denitrification only accounted for 6–7% of NO_3^- removal during non-limiting conditions, suggesting that other transformation processes were responsible for most of the NO_3^- removal. Because many studies expressed the rates on a real basis, we transformed the rates by assuming that the observed groundwater rates reflect conditions within the upper 0.4 m of the wetland soil. Below that depth, organic matter and Ks decline precipitously. Given the measured porosity of 0.65 (Table 1), the upper 0.4 m of a 1-m^2 area contains 260 L of water, so areal subsurface water denitrification and NO_3^- removal rates were 289 and 4094 mg N/m^2.day.

The observed groundwater denitrification rates are comparable to those (quoted below in mg N/m^2.day) reported for other wetland sediments (76.8–115.2, Christensen and Sorensen 1986; 33.6–336, Lindau *et al*. 1990, Lowrance *et al*. 1995; 48–283, Xue *et al*. 1999; 20–80, Hefting *et al*. 2003; 50–741, Pinay and Decamps 1988, Cooper 1990, Haycock and Burt 1993). The elevated rates we observed reflect environmental conditions conducive to denitrification, i.e. low DO (<1 mg O_2/L; Table 3) plus a highly enriched organic matter sediment (Table 2) and optimum sediment–water contact time within the confined lysimeter environment (Hill 1996; Fennessy and Cronk 1997; Hoffmann *et al*. 2000; Smith *et al*. 2003). The observed high denitrification rates could be attributed to the elevated DEA values (Table 4) in the wetland soils (Groffman *et al*. 1999).

The amount of N_2O and N_2 generation (and hence denitrification) did not account for most of the NO_3^- removal; however, it is unlikely that we have underestimated the amount of N_2O and N_2 generation. The SF_6 concentration in wetland waters (data not shown) did not significantly change with time after its dosing; the transport of N_2O and N_2 to atmosphere via stem and aerenchyma of wetland vegetation (Well *et al*. 2001; Hefting *et al*. 2003), or through the interface between the sediment and the edge of piezometers or channels in the sediment or around the dead roots (Blicher-Mathiesen *et al*. 1998), is assumed to be insignificant in our study.

Given that denitrification was a minor pathway for NO_3^- transformation, the rapid NO_3^- removal observed in the first several hours following dosing may be attributed to several processes that we did not measure; specifically DNRA (Silver *et al*. 2001; Matheson *et al*. 2002, 2003), abiotic immobilisation (Davidson *et al*. 2003), and microbial immobilisation and plant uptake (Hill 1996; Fennessy and Cronk 1997). Because the wetland soils likely included eroded materials from the adjacent Allophanic volcanic soils of the paddocks, there is potential for some portion of the dosed NO_3^- to be subject to anionic sorption (Magesan *et al*. 1998). However, considerable NO_3^- leaching has been observed in the paddock soils (Wilcock *et al*. 1999), suggesting that any sorption is likely to account for a limited proportion of the NO_3^- removal. Uptake of NO_3^- by wetland plants in our study appears to be plausible even over a short period of 48 h, particularly because the vegetation appeared to be N-deficient at the time of the experiment. Bowman *et al*. (1989*a*,

Table 7. Nitrate-N removal rates (μg/L.day) and corresponding flux (μg/L.day) of ^{15}N-enriched denitrification gases (N_2O-N and N_2) during non-limiting phase of push–pull experiments

Nitrate-N removal rates calculated from changes in Br^- : NO_3^--N ratios. Non-limiting phase defined as NO_3^--N concentrations >2.0 mg/L. Values are means ± standard deviations. n.d., No values obtained for NO_3^--limiting conditions

Time (h)	NO_3^--N removal rate		Flux of denitrification gases		Prop. of NO_3^--N removal due to denitrification	
	Surface water	Subsurface water	Surface water	Subsurface water	Surface water	Subsurface water
1	5923 ± 3285	4752 ± 5489	118 ± 37	704 ± 447	0.02	0.15
2	5580 ± 877	26105 ± 26952	441 ± 359	1416 ± 490	0.08	0.05
3	378	16662 ± 8250	176	1777 ± 1148	0.46	0.11
4	n.d.	15470 ± 561	n.d.	544 ± 101	n.d.	0.04
Mean	3960 ± 3107	15747 ± 8739	245 ± 172	1110 ± 584		

1989*b*) found extremely high, short-term N uptake rates by moderately N-deficient turfgrass. In particular, they noted that N uptake rates by N-deficient ryegrass during the first 6 h of exposure to NO_3^--enriched solution was >4-fold greater than the rates that occurred after 96 h of exposure.

The pasture growth rate in the studied Waikato region during February–March is at least 45–50 kg dry matter (DM)/ha.day and the standing biomass in our wetland site was estimated to be 3–4 times that of the adjacent pastoral land (data not shown). Assuming a daily wetland plant above-ground growth rate of 135–200 kg DM/ha.day (assuming that production rate is proportional to standing biomass), the amount of N that can potentially be taken up by wetland vegetation with N concentration of 2.6–2.7% (Table 5) is 3.5–5.4 kg N/ha.day (351–540 mg N/m^2.day). In our study approximately 680 mg NO_3^--N/m^2 was depleted over a 4-h period, suggesting that short-term plant uptake by N-deficient plants could account for a substantial portion of the NO_3^- removal.

Although considerable increases in ^{15}N-enriched N_2O-N levels were initially observed following NO_3^- dosing, no net emissions were generated over the 48-h study (Fig. 4*a*, *b*). The wetland served as a source of N_2O during the non-NO_3^--limiting phase of the experiment, but functioned as a sink for

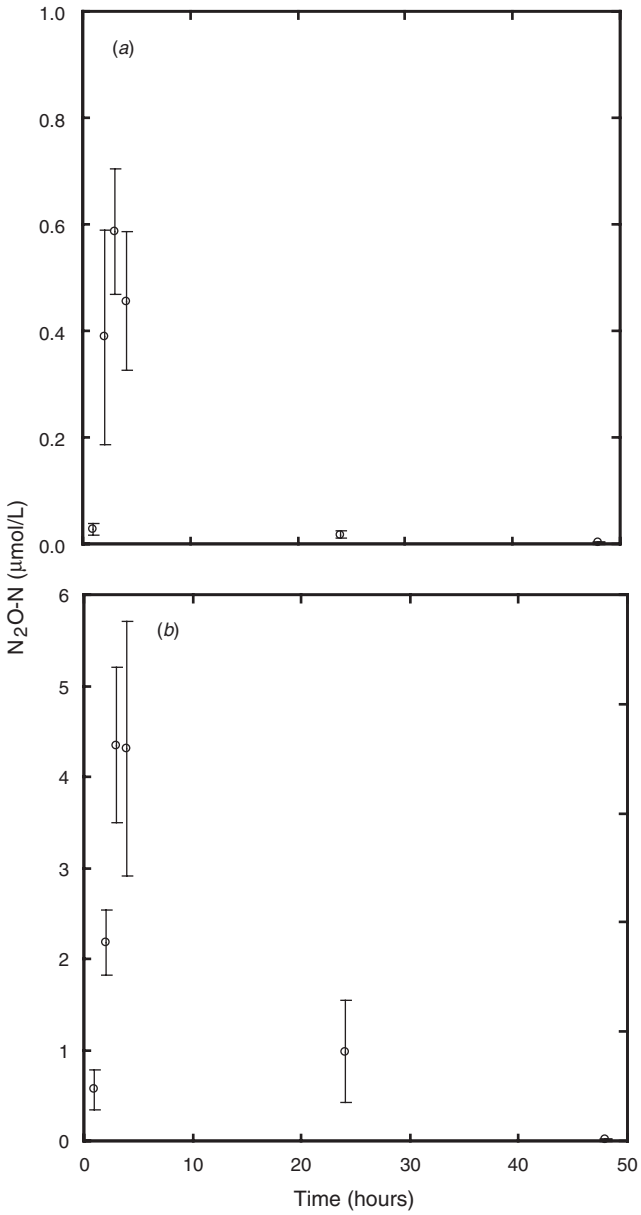

Fig. 4. Dissolved isotopically enriched nitrous oxide concentration in (*a*) surface and (*b*) subsurface water samples collected over a 48-h period after the dosing of nitrate-bromide and sulfur hexafluoride. Error bars represent standard error of the mean.

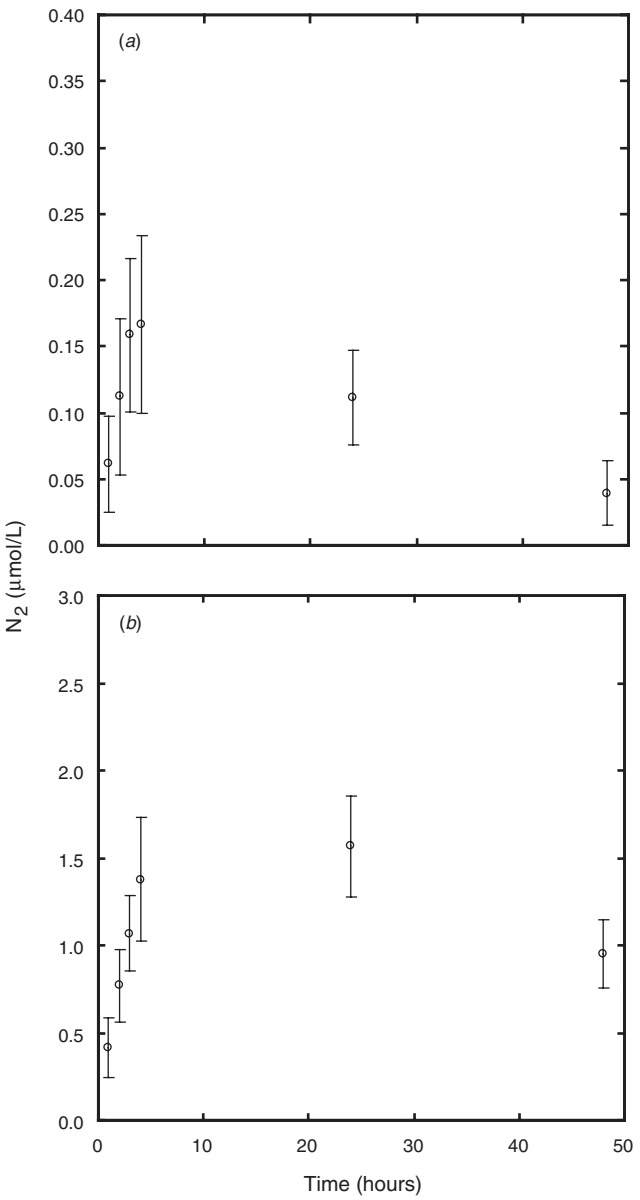

Fig. 5. Dissolved isotopically enriched dinitrogen concentration in (*a*) surface and (*b*) subsurface water samples collected over a 48-h period after the dosing of nitrate-bromide and sulfur hexafluoride. Error bars represent standard error of the mean.

Table 8. Nitrous oxide (N_2O-N) fluxes, their ^{15}N atom%, and ratios of N_2O-N : N_2 in wetland waters sampled over a 48-h study period
Flux is computed as the difference in concentration of dissolved gases between each sampling period per time interval during this period. Values are means ± standard deviations. n.a., N_2O concentrations declined while negligible N_2 generated during sampling interval

Time (h)	N_2O-N fluxes (μg/L.day)		^{15}N atom %		N_2O-N : N_2 ratios	
	Surface water	Subsurface water	Surface water	Subsurface water	Surface water	Subsurface water
1	19.9 ± 14.2	404.2 ± 313.2	38.7 ± 18.2	53.6 ± 25.1	0.33 ± 0.056	4.6 ± 7.0
2	259.4 ± 275.9	1162.0 ± 424.5	69.6 ± 17.5	87.3 ± 2.8	188.4 ± 370.7	4.1 ± 3.0
3	143.1 ± 273.8	1561.9 ± 988.6	77.1 ± 15.1	90.3 ± 1.2	99 : 1[B]	5.0 ± 4.9
4	–94.0 ± 317.7[A]	–24.2 ± 1297.7[A]	59.1 ± 19.6	70.6 ± 20.6	n.a.	n.a.
24	–15.8 ± 8.9[A]	–119.8 ± 118[A]	39.4 ± 7.3	69.7 ± 17.7	n.a.	n.a.
48	–0.5 ± 0.4[A]	–29.2 ± 33.8[A]	21.2 ± 5.1	45.1 ± 16.6	n.a.	n.a.

[A]All these values generated under NO_3^--N limiting conditions with <1 mg N/L.
[B]N_2 generation was negligible during sampling interval.

N_2O during the NO_3^--limited phase of the study. Concentrations of N_2O rapidly increased with time, reaching a peak 4 h after dosing and declining to background by 24 h after dosing (Fig. 4*a*, *b*). This was observed for both surface and subsurface waters and was related to the decline in NO_3^- concentration with time ($r = 0.41$–0.46; $P < 0.05$). Nitrous oxide generation was more predominant than isotopically enriched N_2 generation (Fig. 4*a*, *b* *v.* Fig. 5*a*, *b*) but this predominance declined with time (after 3–4 h of tracer dosing; Table 8), suggesting that the generation of N_2O and N_2 is dependent on the level of NO_3^- in sediments. With a decline in NO_3^- concentration with time (Fig. 2*a*, *b*), the N_2O : N_2 ratio decreased, and more N_2 instead of N_2O was emitted (Fig. 5*a*, *b*), probably because of the reduction of N_2O to N_2. Our results are in agreement with several studies (Swerts *et al*. 1996; Cho *et al*. 1997; Dendooven *et al*. 1997; Blicher-Mathiesen and Hoffmann 1999; Well *et al*. 2001; Zaman *et al*. 2008*b*) which found that during the initial stages of denitrification and in the presence of significant NO_3^- inputs, NO_3^- may be denitrified to N_2O and not fully reduced to N_2. However, when NO_3^- becomes limited, N_2O that is dissolved in wetland water is reduced to N_2 by microbes due to the demand for electron acceptors when NO_3^- in wetland waters but also by the depth from which N_2O was produced. If N_2O is produced at sites just below the top sediment layer, it may readily diffuse into sediment–air water interface as N_2O, instead of being reduced to N_2. In contrast, N_2O produced in subsoils may be entrapped in subsurface waters and subsequently be reduced to N_2 by sediment denitrifiers (Hefting *et al*. 2003; Smith *et al*. 2003). In addition to NO_3^- concentration and depth, sediment pH, C, and O_2 content and temperature may also affect the generation rates of N_2O and N_2 (Del Grosso *et al*. 2000; Dobbie and Smith 2001; Smith *et al*. 2003; Zaman *et al*. 2004, 2007, 2008*b*). The influence of this variety of sediment and environmental factors as discussed above could explain why a range of N_2O : N_2 ratios (0–20) has been reported in the literature (Rolston *et al*. 1978; Weier *et al*. 1993; Maag and Vinther 1996; Cho *et al*. 1997; Well *et al*. 2001; Rochester 2003) and in our study (0.33–188; Table 8) and in other associated laboratory studies using pastoral and wetland sediments collected from areas within the same catchment (0.9–1.4) (Zaman *et al*. 2007).

Since the potential contribution of a wetland to greenhouse emissions is dependent on the amount and fraction of N emitted as N_2O, and these parameters are likely to vary depending on sediment and environmental factors as outlined above, the net annual production (net balance between source and sink) of N_2O from the studied wetland with temporal variation in NO_3^- inputs is not known. This aspect needs to be evaluated in future studies. Various studies have shown that the extent and duration of contact time between wetland inflows and wetland sediment (Hill 1996; Cirmo and McDonnell 1997; Devito *et al*. 2000; Hill *et al*. 2000) can affect the extent of denitrification and hence the wetland capacity to remove NO_3^-. During high flow events, low removal is expected because high NO_3^--containing water bypasses microbially active wetland sediments by flowing across the top of wetlands (Gold *et al*. 2001; Burns and Nguyen 2002; Rutherford and Nguyen 2004). The use of engineered bypass flow designs to regulate NO_3^- loading may enhance sediment–water contact time, thus promoting NO_3^- removal and the potential for NO_3^--limiting conditions. Our results suggest that the studied wetland may be a source of N_2O emissions when NO_3^- concentrations are elevated (non-limited), but can readily remove N_2O (function as a N_2O sink) when NO_3^- levels are low. Although the proposed bypass flow design may short-circuit some of wetland NO_3^- inflows and hence reduce the full potential for wetland NO_3^- removal, it provides a balance between water quality goals and greenhouse gas emissions. Future research is therefore required to investigate this balance issue and assess temporal variations in NO_3^- removal rates, denitrification, and N_2O generation in responses to changes in NO_3^- inputs and water-sediment contact time.

Conclusions

The studied seepage wetland can remove substantial amount of NO_3^- under conditions where sediment water contact time is optimum (confined lysimeter) for denitrification. The amount of NO_3^- removal was much higher than the amount of denitrification gases (N_2O and N_2) produced, suggesting that additional processes (e.g. plant uptake) were responsible for NO_3^- removal. The push–pull method, in combination with confined lysimeters to minimise advection and dispersion of

water, is a promising tool for quantifying NO_3^- removal and N_2O and N_2 generation rates under a variety of conditions.

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