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RCM

Using $\delta^{15}N$ and $\delta^{18}O$ to evaluate the sources and pathways of NO_3^- in rainfall event discharge from drained agricultural grassland lysimeters at high temporal resolutions†

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The origin of NO_3^- yielded in drainage from agricultural grasslands is of environmental significance and has three potential sources; (i) soil organic mater (SOM), (ii) recent agricultural amendments, and (iii) atmospheric inputs. The variation in $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- was measured from the 'inter-flow' and 'drain-flow' of two 1 ha drained lysimeter plots, one of which had received an application of 21 m^3 of NH_4^+-N-rich agricultural slurry, during two rainfall events. Drainage started to occur 1 month after the application of slurry. The concentrations of NO_3^--N from the two lysimeters were comparable; an initial flush of NO_3^--N occurred at the onset of drainage from both lysimeters before levels quickly dropped to <1 mg NO_3^--N L^{-1}. The isotopic signature of the $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- during the first two rainfall events showed a great deal of variation over short time-periods from both lysimeters. Isotopic variation of $\delta^{15}N$-NO_3^- during rainfall events ranged between −1.6 to +5.2‰ and +0.4 to +11.1‰ from the inter-flow and drain-flow, respectively. Variation in the $\delta^{18}O$-NO_3^- ranged from +2.0 to +7.8‰ and from +3.3 to +8.4‰. No significant relationships between the $\delta^{15}N$-NO_3^- or $\delta^{18}O$-NO_3^- and flow rate were observed in most cases although $\delta^{18}O$-NO_3^- values indicated a positive relationship and $\delta^{15}N$-NO_3^- values a negative relationship with flow during event 2. Data from a bulked rainfall sample when compared with the theoretical $\delta^{18}O$-NO_3^- for soil microbial NO_3^- indicated that the contribution of rainfall NO_3^- accounted for 8% of the NO_3^- in the lysimeter drainage at most. The calculated contribution of rainfall NO_3^- was not enough to account for the depletion in $\delta^{15}N$-NO_3^- values observed during the duration of the rainfall event 2. The relationship between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- from the drain-flow indicated that denitrification was causing enrichment in the isotopes from this pathway. The presence of slurry seemed to cause a relative depletion in $\delta^{18}O$-NO_3^- in the inter-flow and $\delta^{15}N$-NO_3^- in the drain-flow compared with the zero-slurry lysimeter. This may have been caused by increased microbial nitrification stimulated by the presence of increased NH_4^+-N. Copyright © 2008 John Wiley & Sons, Ltd.

Nitrate (NO_3^-) is the predominant form of inorganic N lost from soils to aquatic systems and losses are particularly associated with agricultural systems. Analysis of the long-term trends of NO_3^- in UK rivers indicates that the amount of N leached per unit area of agricultural land continues to increase in the order of 0.1 to 0.2 mg N L^{-1} $year^{-1}$.[1,2] As a result of this over half of the land area of England is now designated as falling within areas designated by the European Union as 'Nitrate Vulnerable Zones'. These are areas of surface water or ground water that have, or are at risk of having, concentrations of NO_3^- in excess of 50 mg L^{-1}. Within these zones farmers must reduce their NO_3^- inputs and control their application of N to the land, including the application of farm organic wastes. However, the presence of NO_3^- in these wastes is minimal, and it is the conversion of abundant ammonium-N (NH_4^+-N), particularly in slurries, into NO_3^--N through microbial nitrification within the soil profile that contributes to large losses of NO_3^- in discharge from land. If additional NO_3^--N losses from slurry disposal are to be minimised, the timing and effectiveness of

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dissolved N sources and the mechanisms involved in the inter-conversions between N sources and NO_3^- export must be better understood, particularly during hydrological events occurring after slurry application.

Stable isotope abundances of N have been used extensively to provide information on the origins and transformations of N in soils, surface waters and ground waters.[3–9] It has also been demonstrated by a number of studies that a wide variety of chemical and biological processes (i.e. denitrification) can alter the enrichments of the $\delta^{15}N\text{-}NO_3^-$.[10,11] In this context one long-term study of $\delta^{15}N\text{-}NO_3^-$ in leachate from agricultural soils concluded that $\delta^{15}N$ may provide more information on the predominance of microbial processes in soils than on N origins.[6] Taken alone, however, $\delta^{15}N$ values fail to distinguish among the various N sources due to overlapping ranges and though isotopic fractionations which occur during N transformations. Further studies have suggested that the oxygen (O) isotope content of NO_3^- may provide additional information on microbial processes, such as denitrification,[12] as well as better distinguishing the sources of NO_3^- in waters, particularly discrimination between atmospheric, microbial and synthetic fertiliser NO_3^-.[8,13–19] Hence, one can expect that a dual isotope approach with respect to the identification of NO_3^- sources may provide more reliable information with respect to the NO_3^- sources and biogeochemical interactions during NO_3^- production. These studies are typically long term, examining samples collected at temporal scales measured in weeks or months.

Work conducted by Kellman[9] found that the $\delta^{15}N\text{-}NO_3^-$ in the tile drainage of fields receiving different sources of N with different $\delta^{15}N$ signatures differed between fields in accordance with the $\delta^{15}N$ sources applied to those fields; however, all fields were enriched in relation to their respective sources. These drains flow only following rainfall events and samples were collected over several days following such events. Such rainfall events drive the movement of NO_3^- through the soil systems and the soil moisture regime also affects the $\delta^{15}N\text{-}NO_3^-$ produced in soils.[10] Potentially, variations in the isotopic composition of NO_3^- in the short term, such as during a rainfall event leading to drainage, may reveal more information on the sources and pathways of agriculturally derived NO_3^-.

Nitrate losses for a given agricultural system are determined by rainfall and by soil texture. Losses from sandy, unstructured, soils are typically driven by the movement of water vertically through the soil profile, with a generally uniform wetting front carrying NO_3^- downwards towards the groundwater. This process is typically described as 'piston flow' with rainfall pushing water and NO_3^- further down into the soil.[20] However, the mechanisms for NO_3^- movement in more clay-rich, structured, soils are less certain.[21] In clay-rich soil the smaller pore sizes can restrict vertical movements of water (and NO_3^-) through the profile, making them more retentive of NO_3^-. However, during rainfall events, water generally moves rapidly laterally, either across the surface or through the surface layers via cracks, channels and, ultimately if installed, drains. Importantly, this movement known typically as 'macro-pore flow', can result in rapid water movement through soils that would at first be considered impermeable.[22] Thus, water and NO_3^- movement through clayey soils can occur both as rapid movements through cracks, macro-pores and as more slow vertical movements through the bulk of the soil. The concentration of NO_3^- in drainage water depends on the amount of contact between the drainage water and the sources of NO_3^-. This can result in more complex NO_3^- leaching profiles from clayey soils, compared with the relatively smooth NO_3^- leaching pattern from sandy soils. More recently a model has been developed to try to better understand the movement of water and NO_3^- through such clay rich soils by viewing the system as two 'domains': a mobile domain representing rapid vertical flow pathways such as macro-pores and fissures, and an immobile domain representing micro-pore storage within the soil matrix. This latter immobile domain can be further subdivided into a zone of interaction between immobile and mobile water and a non-interactive zone.[23]

In order to evaluate the contribution of NH_4^+-N-rich cattle slurry application on NO_3^- in drainage water from agricultural grassland during rainfall events, a dual isotope approach using the $\delta^{15}N$ and $\delta^{18}O$ of the NO_3^- produced was applied on the field-scale lysimeters (1 ha) of the Rowden experimental research platform (RERP). Agriculturally derived slurry that has been collected and stored is rich in NH_4^+ as all urea excreted by farm livestock rapidly becomes hydrolysed to NH_4^+ by the abundant enzyme urease.[24] Within these stores NH_4^+ is in equilibrium with NH_3 gas which, over the typical storage time from uncovered stores, is lost to the atmosphere. The exchange and loss of NH_3 from the slurry to the atmosphere are subject to a fractionation whereby $^{14}NH_3$ is lost preferentially thus leaving the residual slurry enriched in $^{15}NH_3$ and $^{15}NH_4^+$ with $\delta^{15}N$ values of typically >10‰.[9,25–27]

During April to May 2006, NO_3^--N concentrations and $\delta^{15}N\text{-}NO_3^-$ and $^{18}O\text{-}NO_3^-$ of water draining via two different pathways from two agricultural-managed field-scale lysimeters were measured during the occurrence of a rainfall event, where one lysimeter received an application of cattle slurry while the other one remained untreated. The paper focuses on the following three objectives: (i) to determine whether the isotopic signatures of NO_3^- leaving the two lysimeters could be attributed to different N sources due to slurry application and (ii) to determine if the $\delta^{15}N$ and ^{18}O values of the produced NO_3^- are also affected by the drainage pathway, and (iii) to observe the range of isotope fluctuations occurring over short time scales.

EXPERIMENTAL

Site

The study was sited on the field-scale lysimeters (1 ha) of the Rowden Experimental Research Platform (RERP) in Devon, southwest England (National Grid Reference: SX 650995). The experimental site is maintained as permanent grassland and is grazed by beef heifers at stocking densities of approximately 4 livestock units per hectare in order to manage sward height during the months of June to September. The soil is a clayey non-calcareous pelostagnogley of the Hallsworth series (Dystic Gleysol, FAO), overlying

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clay shales of the Crackington Formation (Culm measures) and the site slopes between 5 and 10% westwards. For details of soil properties, see Scholefield *et al*.[28] and Armstrong and Garwood.[29] The annual rainfall at this site averages 1055 mm, where the majority falls between October and March. As a consequence of the virtually impermeable clay layer below 30 cm the soil remains waterlogged for much of the winter period. Half of the lysimeters at the RERP are drained by 55 cm deep mole drains crossing 85 cm deep permanent pipe drains and are termed 'drained' (Fig. 1), while the remaining lysimeters, dependent on natural drainage via surface and lateral through-flow pathways, are termed 'undrained'. All lysimeters have V-notch weirs for measuring surface runoff plus lateral through-flow to a depth of 30 cm that runs into perimeter drains (termed inter-flow) (Fig. 1). The drained lysimeters have additional and separate weirs for measuring water that flows through the drainage system (termed drain-flow) (Fig. 1). Both inter-flow and drain-flow were measured using solar powered Starlevel flow sensors (Star Instruments, Royston, UK) with data recorded by Campbell radio loggers (Campbell Scientific Ltd., Shepshed, UK)and subsequently transmitted via radio modem to a central computer.

We focused on two drained lysimeters; one was to receive an application of cattle slurry the other would remain untreated. These lysimeters are referred to as 'slurry amended' and 'zero slurry'. The lysimeters have historically received an application of phosphorus and potassium as a standard management strategy and this occurred on 24 April 2006. Neither lysimeter received any inorganic N fertilisers during 2006 nor over the study period; however, the zero-slurry lysimeter had received mineral N as part of its standard management in the previous year with the last application occurring in August 2005. On 18 April 2006 the slurry-amended lysimeter received an application of 21 m^3 of cattle slurry using a conventional vacuum tanker fitted with a splash plate. The maximum recommended application rate for UK grassland systems is 50 m^3;[30] however, due to environmental restrictions at the RERP at the time of application only 21 m^3 could actually be applied. The slurry was sourced from a local dairy farm and was extracted from an open-topped, above-ground, slurry tower. Slurry had been collected in this store since the start of the winter period with constant additions throughout the winter. This slurry was then transported to a smaller 25 m^3 above-ground store where it was kept for 2 months before application to the lysimeter. Analysis of the slurry showed that this application was equivalent to applying 51 kg of total N where the inorganic fraction of NH_4^+ and NO_3^- accounted for 23 kg N and <2 g N, respectively. The slurry was derived from cattle fed on a grass silage system from a local dairy farm, and had been collected and stored in an open air tank over the previous winter period. This was applied leaving a 10 m margin around the edge of the lysimeter in order to prevent contamination of the surface drains in accordance with the 'Code of Good Agricultural Practice'.[30]

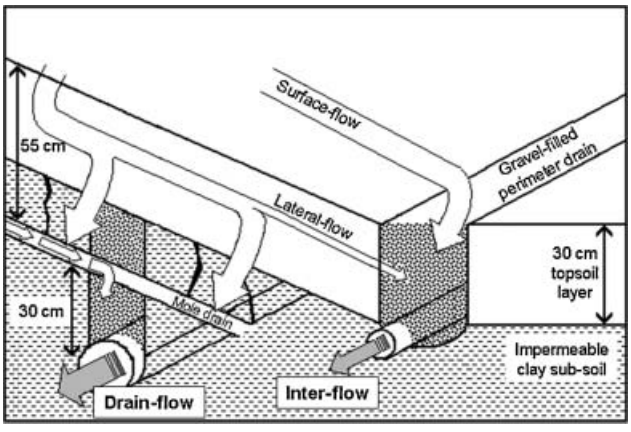

Figure 1. Hydrological pathways through the drained lysimeters at the Rowden Experimental Research Platform.

Sampling

Soil cores were collected to ascertain the $\delta^{15}N$ of the total soil N from which NO_3^- would be produced through microbial mineralisation and subsequent nitrification. Five points within four of the lysimeters at the RERP were used which included the two lysimeters used in this study. At each point 10 cores were taken to a depth of 7.5 cm (2.5 cm diameter) and these cores were then bulked.

Samples of drainage water were taken as follows. All V-notch weir systems were fitted with an internal stainless steel mixing plate below the fresh drainage input to the weir. The fresh drainage flowing through the mixing plate was then sampled on hourly or sub-hourly time-steps either through an automated sampler or through the collection of manual grab samples. Auto-samplers were set to sample using the same atomic clock time as the flow loggers. At very small flows the auto-samplers were unable to collect sufficient sample for analysis.

Slurry was applied on 18 April 2006 when soil conditions were dry enough to take mechanical equipment without damaging the soil surface. Under these conditions drainage from the lysimeters was minimal or nil (Fig. 2). Sub-samples of the slurry were taken for isotopic analysis; however, these bottles were broken by the courier while in transit to the mass spectrometry laboratories. Sampling of drainage water was undertaken upon the first rainfall event to initiate drainage from the lysimeters. This occurred on 19 May 2006, some 31 days after slurry application (Fig. 2). A bulk rainwater sample was also collected over the period 11 April to 5 June to establish the isotopic composition of any NO_3^- being delivered to the site in precipitation.

Analysis

Bulked soil cores were sieved through a 2 mm sieve to remove large stones and organic fragments before being dried overnight at 30°C to a constant weight and ground into a fine powder. Afterwards $^{15}N/^{14}N$ ratios of total soil N was analysed by oxidative combustion using an ANCA /SL 20/20 continuous flow isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, UK).

Drainage samples were collected and delivered to the laboratory within 24 h of being sampled. They were then refrigerated at 5°C until analysis for total oxidised nitrogen (NO_2^--N + NO_3^--N) which is typically assumed to be NO_3^--N.

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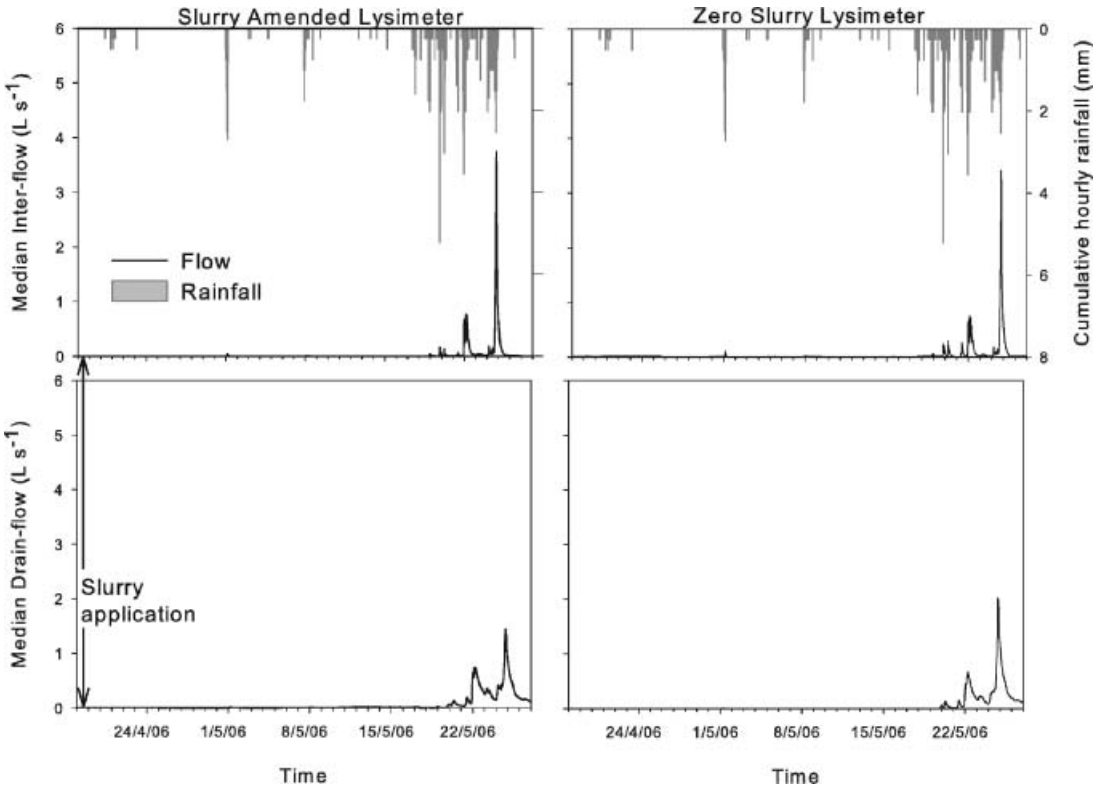

Figure 2. Rainfall and rate of flow from the lysimeter pathways from time of slurry application until the end of sampling.

Analysis for NO_3^- occurred within 4 weeks of sample collection. Nitrate was analysed photometrically after reduction to NO_2^- and reaction with sulphanilamide and naphthylethylenedamine dihydrochloride to an azo dye using a continuous flow analyser (Skalar, Breda, The Netherlands). Drainage water samples were stored frozen (−15°C) until ready for isotopic analysis, prior to which they were filtered (<0.45 μm).

Concentrations of NO_3^--N and NH_4^+-N were determined at an external laboratory (NRM, Bracknell, UK). Slurry was passed through a 1 mm screen before NO_3^--N and NH_4^+-N were extracted using deionised water. Slurry NO_3^--N and NH_4^+-N were also analysed photometrically on a rapid flow analyser (Alpkem, Silver Springs, MD, USA); NO_3^--N after reduction to NO_2^- and reaction with sulphanilamide and naphthylethylenedamine dihydrochloride to an azo dye, and NH_4^+-N by reaction with alkaline hypochlorite and phenol to produce indophenol blue.

Sufficient sample volume of both the drainage and rainwater to yield about 35 microequivalents of NO_3^- was passed through cation- and anion-exchange resins, and processed to form silver nitrate in the manner described elsewhere.[31–33] Where samples contained less than 35 microequivalents of NO_3^- consecutive samples were bulked. The silver nitrate was analysed for $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios by oxidative combustion and high-temperature pyrolysis, respectively, in a ThermoFinnigan (Bremen, Germany) system: 'Flash EA' and 'TC/EA' linked to a Delta+XL mass spectrometer. Sample purity was monitored by concurrent determination of C/N and N/O ratios.

The isotope ratios are reported as δ values where:

$$\delta^{15}N \text{ and } \delta^{18}O(\text{in per mile}) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

for $R = {}^{15}N/{}^{14}N$ and $^{18}O/^{16}O$, respectively, and the standards are atmospheric N_2 (AIR) and Standard Mean Ocean Water (SMOW), respectively. Corrections to these standards were undertaken by comparison of samples with within-run IAEA (Vienna, Austria) standards: IAEA-N-1 ammonium sulphate, with assumed $\delta^{15}N = +0.4‰$ versus AIR; and IAEA-NO_3 potassium nitrate, with assumed $\delta^{18}O = +25.6‰$ versus SMOW.[34] Replication for duplicate splits of water samples put through the entire resin extraction, silver nitrate preparation and mass spectrometry was typically better than ±0.4‰ for $\delta^{15}N$ and ±0.6‰ for $\delta^{18}O$.

Water $^{18}O/^{16}O$ ratios were determined on CO_2 equilibrated with the water samples in a Micromass Isoprep 18 (Middlewich, UK) coupled to a Micromass SIRA mass spectrometer. The ratios are reported as $\delta^{18}O$ values versus VSMOW, based on comparison with laboratory standards calibrated against IAEA standards VSMOW and SLAP.

Correlation and regression analyses between $\delta^{15}N$, $\delta^{18}O$ and flow were performed using Genstat.[35]

RESULTS AND DISCUSSION

Flow rates

The rates of water discharge though the inter-flow and drain-flow pathways from both lysimeters can be seen in Fig. 2. Three rainfall events were sampled, occurring on 19,

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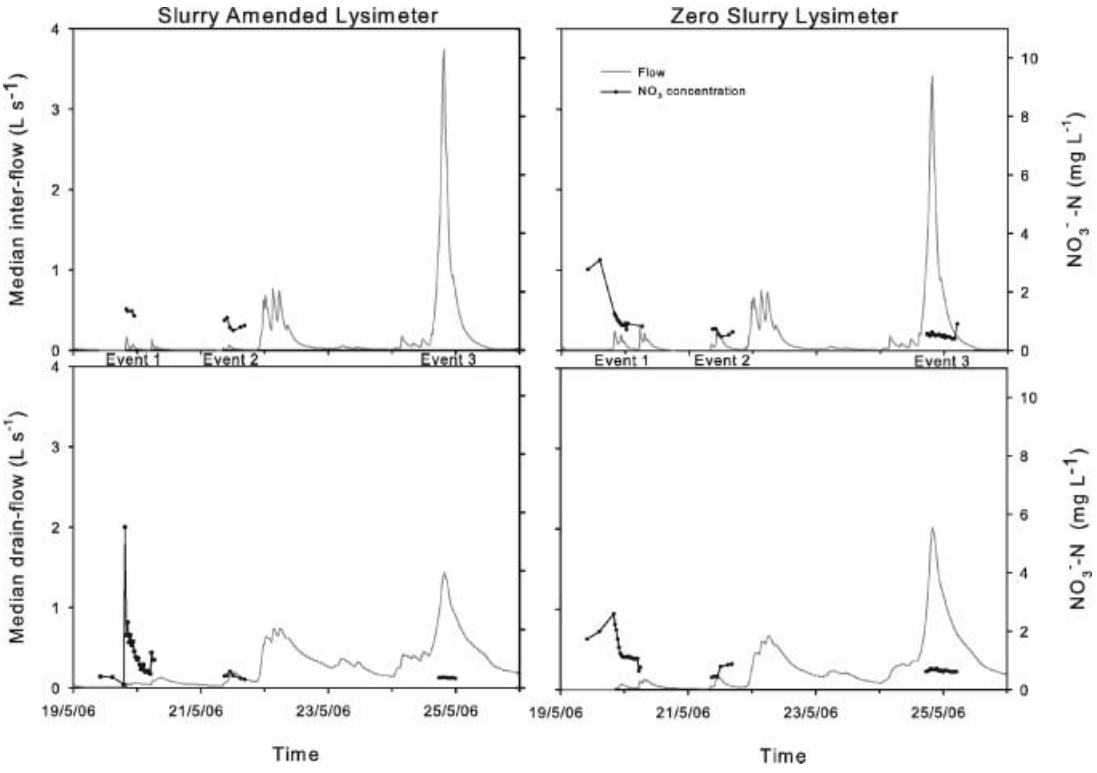

Figure 3. Nitrate concentrations in drainage from the lysimeters during events 1, 2 and 3.

21 and 24 May, referred to as events 1, 2 and 3, respectively (Fig. 3). The discharges from the pathways show good agreement with other, and any discrepancies in the flow rates from each pathway between the lysimeters could be accounted for in terms of small differences in lysimeter topography (especially slope). The rapid response of the drainage and the peaky nature of the resultant hydrographs are typical of this site and of other clay soil sites, both with and without mole drainage.[29,36,37] When mole drains have been installed, one could expect the drainage pathway to carry the greater proportion of storm water.[29,37] However, should the drainage system not be operating efficiently through collapse of the moles, the peaky drain-flow hydrograph would be expected to be replaced by a flatter response while the inter-flow pathway would carry a greater percentage of the total drainage.[29] As Fig. 2 shows, while drain-flow from the lysimeters remained peaky, the hydrographs are more smoothed than the inter-flow hydrographs. Furthermore, the volume of water carried by the drain-flow pathway was approximately equal to that of the inter-flow. This indicated that the mole drainage within the lysimeters was working, albeit not with complete efficiency.

Nitrate concentrations

From the zero-slurry lysimeter the first small storm (event 1) produced initial maximum concentrations of NO_3^- through both the inter-flow and the drain-flow pathways (maximum values of 3.1 and 2.6 mg NO_3^--N L^{-1}, respectively) before concentrations rapidly declined during the event to values of about 1 mg NO_3^--N L^{-1} (Fig. 3). Subsequent storm events continued to produce small concentrations of NO_3^- from both pathways, with values that range between 0.4 and 0.9 mg NO_3^--N L^{-1}. Similar trends were observed in the NO_3^- concentrations derived from the slurry-amended lysimeter. Again, an initial NO_3^- maximum was observed through the drain-flow pathway (5.5 mg NO_3^--N L^{-1}) before values declined to about 1 mg NO_3^--N L^{-1}. Subsequent events produced low concentrations, in the range of 0.3–0.6 mg NO_3^--N L^{-1}. Sample numbers collected from the inter-flow pathway of the slurry-amended lysimeter were limited due to technical difficulties, and as a result no samples were taken that corresponded to the maximum concentrations of the other pathways at the start of event 1, but concentrations from events 1 and 2 range between 0.7 and 1.4 mg NO_3^--N L^{-1}.

The initial 'high' NO_3^--N concentrations observed probably represent NO_3^- that had been produced during the preceding warm dry spell in which no significant drainage had occurred. The mineralisation and nitrification of soil organic matter (SOM) had led to an increase in soil water NO_3^- before the onset of drainage. The onset of rainfall led to soil water movement and caused a rapid increase in NO_3^- concentrations, potentially from readily accessed pockets of soil water. The subsequent rapid decline in NO_3^- concentrations was probably due to the exhaustion of this readily mobilised NO_3^--rich water. The initial NO_3^- flush observed from the two zero-slurry lysimeter pathways were of a similar size, while a larger flush was observed from the drain-flow pathway of the slurry-amended lysimeter which could have been due to the increased availability of NH_4^+-N supplied in the slurry. However, the difference between the two lysimeters is not so large as to be easily accounted for by the differences between plot treatments.

The concentrations of NO_3^- yielded from both lysimeters were very small and are not considered to be environmentally significant. They can be compared with the concen-

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trations of 0.1–0.2 mg NO_3^--N L^{-1} typically recorded in rainfall at the site and the range 0.5–2.5 mg NO_3^--N L^{-1} measured in the nearby River Taw throughout the year.[38] Concentrations of NO_3^- from other lysimeters at the RERP site, which have received regular and high levels of mineral N, often greatly exceed the EC limit of 11.3 mg NO_3^--N L^{-1} for drinking water each autumn. However, even the concentrations of NO_3^- observed from these lysimeters decline over the winter period although often remaining above the EC limit.[28]

Isotopic characterisation

Rainwater and soil

Soil sampling across the RERP site indicated there was no significant variation between the δ^{15}N values of the total N of the lysimeters, with the average isotopic composition of the soil profile down to 7.5 cm being +5.4‰ ± 2.4. Isotopic analysis of the bulk rainfall samples collected over the experimental period gave a δ^{15}N-NO_3^- value of +0.9‰ which falls within the typical range expected for other parts of Europe.[33] The δ^{18}O-NO_3^- value of +68.8‰ was also within the range (+47 to +86‰) of values already reported from the few studies that have measured δ^{18}O-NO_3^- from non-polar regions.[39,40]

Drainage water

Due to experimental constraints isotopic analysis of drainage NO_3^- was limited to events 1 and 2. Samples were collected during event 1 using automated samplers; however, due to the low flow rates generated, extremely low sample volumes were obtained restricting the number of samples that could be analysed isotopically. Event 2 was of an equivalent magnitude; however, samples were obtained manually enabling sufficient volume to be collected at each sampling to allow more detailed isotopic temporal variation to be observed. The range of values measured from each lysimeter is presented in Table 1, along with the mean and two standard deviations of the data. This data appears to show that the δ^{15}N-NO_3^- values in the drain-flow of the zero-slurry lysimeter are enriched when compared with the slurry-amended lysimeter. This enrichment is not observed in the inter-flow pathway of the lysimeters. In contrast δ^{18}O-NO_3^- values show no such enrichment in the drain-flow from either lysimeter, while in the inter-flow, δ^{18}O-NO_3^- values appear enriched from the zero-slurry lysimeter compared with the slurry-amended lysimeter (Fig. 5). The isotopic values for δ^{15}N-NO_3^- and δ^{18}O-NO_3^- exhibit a great deal of variation from all plots and all pathways over relatively short temporal periods (Fig. 4). No significant relationships could be found between δ^{18}O-NO_3^- and flow rate from any of the pathways from either event 1 or 2, or from the data *en masse*, although an apparent positive trend in δ^{18}O-NO_3^- values and flow rate appears to exist within the inter-flow samples from both lysimeters. A negative correlation between δ^{15}N-NO_3^- and flow rate also appears to occur during event 2 in all pathways except for the drain-flow from the zero-slurry lysimeter (Fig. 4). This negative trend is only significant in the drain-flow pathway from the slurry-amended lysimeter ($r_4 = 0.84$, $n = 6$, $p < 0.05$).

Pathway variation between treatments

Inter-flow NO_3^-

The isotopic signatures of the inter-flow pathways are both similar; δ^{18}O-NO_3^- first increases then decreases through the duration of event 2, while δ^{15}N-NO_3^- exhibits the opposite pattern (Fig. 4). This pattern would suggest the increasing importance of the contribution of rainfall-derived NO_3^- during event 2 from this pathway. This would seem logical as the inter-flow pathway would be most susceptible to effects caused by rainwater. Due to the highly enriched nature of atmospheric δ^{18}O-NO_3^-, it is possible to assess the rainfall contribution of NO_3^- to the NO_3^- in the drainage leaving the lysimeters by calculating the theoretical δ^{18}O-NO_3^- values expected through microbial nitrification of soil organic matter. This can be calculated because one O atom of microbial NO_3^- has been shown to originate from atmospheric O_2 and two atoms from soil water.[41,42] The δ^{18}O of the drainage water from the lysimeters ranged between –8.0 and −5.4‰ (−6.0 ± 1.2‰) and, with the δ^{18}O of atmospheric O_2 taken to be +23.5‰,[43] the δ^{18}O-NO_3^- formed through microbial nitrification should be in the range of +2.5 to +4.2‰ (Fig. 5). This theoretical δ^{18}O-NO_3^- range for microbial nitrification relies on several assumptions:[5] (1) that the proportion of oxygen on microbial NO_3^- is 2:1 from soil water and from O_2; (2) no fractionations occur during the incorporation of oxygen from soil water and O_2; (3) the δ^{18}O of the drainage water leaving the lysimeters is equivalent to the soil water from which microbial NO_3^- derives its oxygen; and (4) the δ^{18}O of the soil O_2 is identical to that of atmospheric O_2. However, several studies[5] have reported δ^{18}O-NO_3^- values higher than expected and it has been suggested that the theoretical microbial δ^{18}O-NO_3^- should be considered as a minimum.[44] Several reasons have been suggested for actual δ^{18}O-NO_3^- values being slightly higher than expected; that evaporation of soil water could lead to enrichment of δ^{18}O-H_2O,[13,45,46] that under certain

Table 1. Values of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- recorded during events 1 and 2 from the slurry-amended lysimeter and the zero-slurry lysimeter. The mean and two standard deviations are presented in parentheses

	Slurry-amended lysimeter		Zero-slurry lysimeter	
	Inter-flow	Drain-flow	Inter-flow	Drain-flow
δ^{15}N-NO_3^-	−1.6 to +5.2‰ (+0.3‰ ± 4.3)	+0.4 to +4.1‰ (+2.1‰ ± 2.3)	+0.1 to +3.8‰ (+1.2‰ ± 2.6)	+7.4 to +11.1‰ (+8.9‰ ± 2.3)
δ^{18}O-NO_3^-	+2.0 to +4.5‰ (+3.4‰ ± 1.7)	+4.4 to +7.4‰ (+5.8‰ ± 2.1)	+6.0 to +7.8‰ (+6.6‰ ± 1.3)	+3.3 to +8.4‰ (+6.5‰ ± 4.1)

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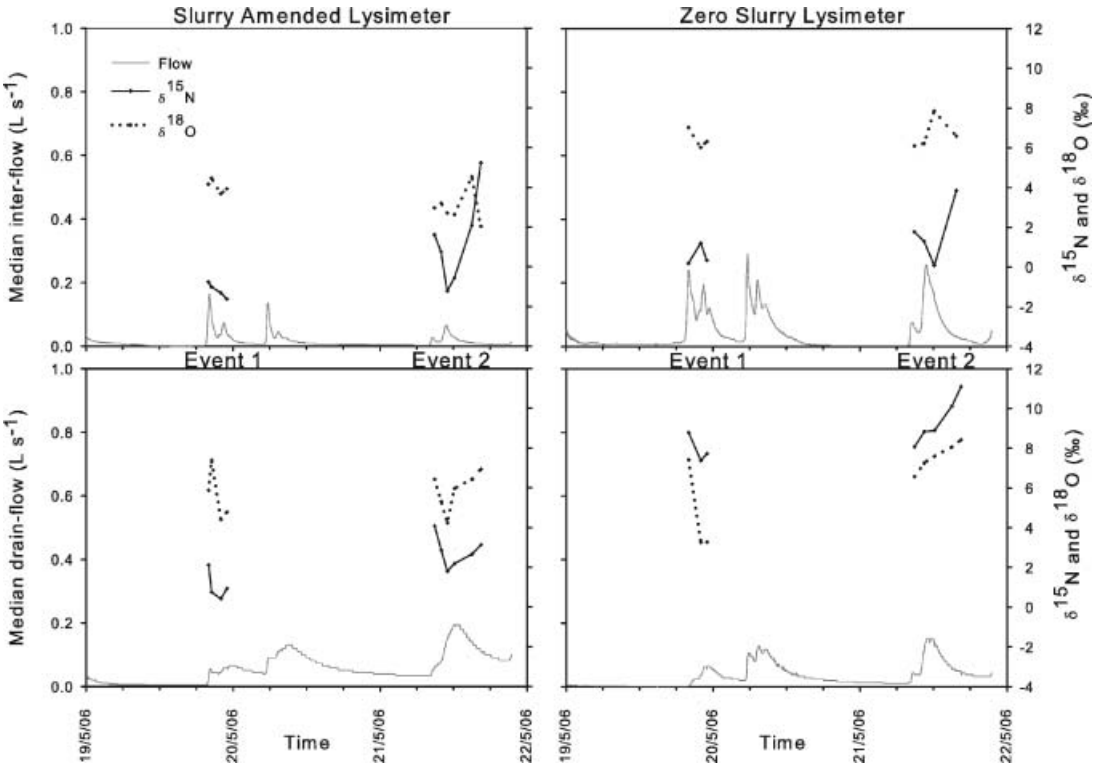

Figure 4. The δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values during events 1 and 2.

conditions less than two-thirds of the oxygen is derived from soil water,[44] or that microbial respiration may lead to enrichment in the δ^{18}O of soil O_2 over time.[5] However, if it is assumed that the δ^{18}O-NO_3^- values in drainage that exceed this calculated range might have a component of atmospheric NO_3^-, then taking the highest δ^{18}O-NO_3^- value measured in the inter-flow from the zero-slurry lysimeter of +7.8‰ and using the minimum calculated value of +2.5‰ for δ^{18}O-NO_3^- for microbial-derived NO_3^-, we can calculate that, at most, atmospheric NO_3^- only contributes a maximum of 8% of the NO_3^- in drainage, the majority of which is microbial in origin. This is in agreement with other authors, who typically report rainfall as only significantly contributing to NO_3^- during major flood events or during periods of snow melt.[16] The relative depletion of the δ^{18}O-NO_3^- seen in the inter-flow pathway of the slurry-amended lysimeter compared with the zero-slurry lysimeter could be explained by increased nitrification occurring in the surface of the plot due to the application of cattle slurry. This would lead to a 'dilution' of the relative contribution of rainfall δ^{18}O-NO_3^- seen in this plot compared with the zero-slurry lysimeter.

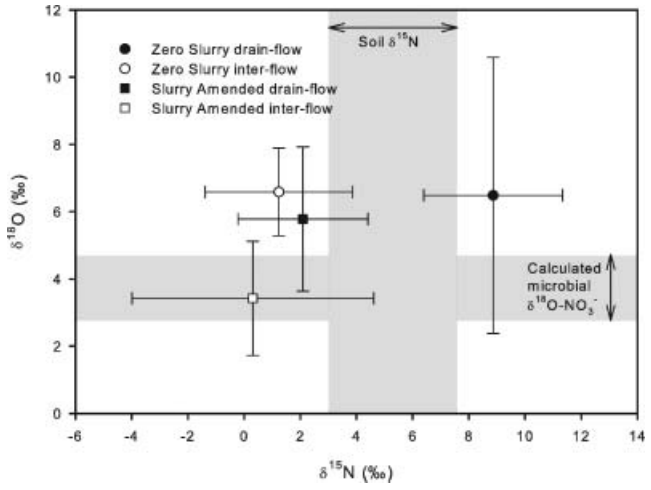

Figure 5. Mean (± 2 Std. Dev.) of the δ^{15}N-NO_3^- and δ^{18}O-NO_3^- time series values obtained from the two lysimeters shown against soil δ^{15}N range and the calculated δ^{18}O of microbial NO_3^-.

The use of δ^{18}O-NO_3^- to calculate the maximum contribution of rainfall NO_3^- at 8%, however, does not explain the marked depletion in δ^{15}N-NO_3^- seen during event 2. Assuming that the δ^{15}N-NO_3^- values of soil microbial NO_3^- were derived from SOM (+5.4‰), a 8% contribution of rainfall δ^{15}N-NO_3^- (+0.9‰) would yield depleted δ^{15}N-NO_3^- values of +5‰; however, values of δ^{15}N-NO_3^- in drainage drop to ≈+0.1‰. One possible cause for this maybe a significant underestimation of the contribution of rainfall NO_3^- due to depletion of the δ^{18}O-NO_3^- through the biochemical oxygen exchange between NO_3^- and H_2O.[47] Yet for rainfall to cause the observed depletion in δ^{15}N-NO_3^- values, it would need to account for the majority of the NO_3^- evolved in drainage at the peak of flow rate and, if this were the case, we would expect to see highly enriched δ^{18}O-NO_3^- values (≈+60‰). This level of enrichment is not seen in the δ^{18}O-NO_3^- data and it seems unlikely that oxygen exchange would have depleted it to the extent seen. Even assuming an underestimation in the contribution of rainfall, a larger than expected depletion in δ^{15}N-NO_3^- occurs. Due to the similar δ^{15}N-NO_3^- profiles for both inter-flow pathways which occur over the duration of the rainfall event this could be due to a fractionation occurring between the rapidly moving rainfall water which is having limited contact with the soil water

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across the boundaries of which NO_3^- is diffusing; however, this short-term depletion cannot be fully accounted for within the remit of this study.

Drain-flow NO_3^-

The δ^{15}N-NO_3^- values obtained from the drain-flow pathway from the zero-slurry lysimeter were greatly enriched compared with all the other pathways (Fig. 5). The mean value of the δ^{15}N-NO_3^- in the drain-flow here was +8.9‰ ± 2.3 which was far higher than in the other pathways whose means were ≤+2.1‰. The enrichment in δ^{15}N-NO_3^- could be explained by the different trend in δ^{15}N-NO_3^- relative to flow in this pathway during event 2. The δ^{15}N-NO_3^- values through other pathways indicated an apparent negative relationship to flow, albeit non-significant in most cases; however, δ^{15}N-NO_3^- values from the drain-flow of the zero-slurry lysimeter showed no such pattern and increased steadily regardless of flow. Furthermore, no other pathways during events 1 and 2 showed any significant relationships between δ^{15}N-NO_3^- and δ^{18}O-NO_3^- except for the drain-flow from the zero-slurry lysimeter during event 2. During this event both δ^{15}N-NO_3^- and δ^{18}O-NO_3^- exhibited a significant ($r_3 = 0.95$, $n = 5$, $p < 0.05$) positive correlation (Fig. 6). Such a relationship is characteristic of the isotopic enrichment of NO_3^- due to denitrification where the slope of the relationship is typically about 0.5;[25] the slope observed for the drain-flow of the zero-slurry lysimeter during event 2 was 0.57. This process would also account for the high δ^{18}O-NO_3^- values observed from this pathway which would appear unrelated to rainfall NO_3^-. This scenario is given further credence by the similar evolution profiles of δ^{18}O-NO_3^- and δ^{15}N-NO_3^- during events 1 and 2. When this trend is compared with that for the slurry-amended llysimeter it is also apparent that δ^{15}N-NO_3^- and δ^{18}O-NO_3^- follow a similar pattern and, although non-significant, a positive correlation between the two is also observable (Fig. 6). This would suggest that in both drain-flow pathways rainfall NO_3^- is not contributing and that this pathway is more affected by soil water and with denitrification occurring at depth.[48] The difference in the enrichment of the two drain-flow pathways could be explained again by the presence of slurry N. Typically, the δ^{15}N-NO_3^- derived from the SOM is the same as or slightly lower than the δ^{15}N value of the SOM, where the mineralisation of SOM is the rate-limiting step. This leads to all NH_4^+ generated being nitrified to NO_3^- with only minor fractionation against the δ^{15}N-NH_4^+. When large amounts of NH_4^+ become available, such as in cattle slurry, the mineralisation of SOM is no longer the rate-limiting step in NO_3^- production. With a large pool of NH_4^+ available to nitrifying bacteria the $^{15}NH_4^+$ is readily fractionated against, despite its increased abundance in naturally enriched cattle slurries, and the δ^{15}N-NO_3^- produced under such conditions is depleted relative to SOM-derived δ^{15}N-NO_3^-.[3] This may have caused the pool of δ^{15}N-NO_3^- within the slurry-amended plot to have become depleted relative to the zero-slurry lysimeter.

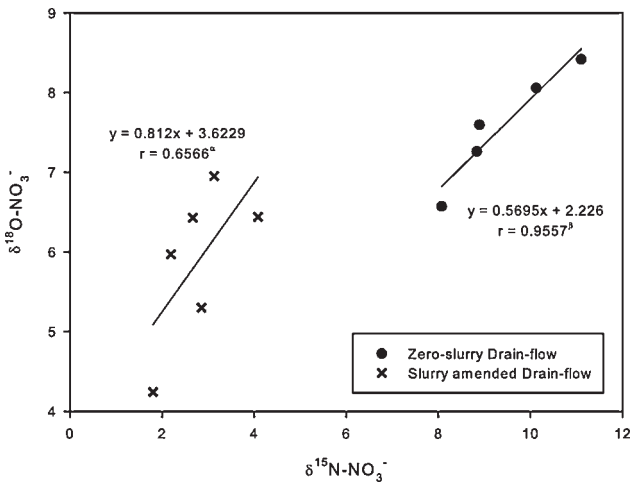

Figure 6. Denitrification relationships between δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the drain-flow from the lysimeters during event 2. $^{\alpha}$non-significant, $^{\beta}p < 0.01$.

Aside from the apparent denitrification enrichment of the δ^{15}N-NO_3^-, the mean δ^{15}N-NO_3^- values observed from the other lysimeter pathways are lower than the mean δ^{15}N of the soil with depletion ranging from 3.3 to 5.1‰ (Fig. 5). Low δ^{15}N-NO_3^- values can indicate an inorganic N fertiliser origin; however, the slurry-amended lysimeter has never received inorganic fertiliser N and, although the zero-slurry lysimeter had received such amendments up to the previous year, values from both are comparable, indicating that in the zero-slurry lysimeter there was no residual fertiliser N signal. This is to be expected as the RERP site receives a high annual rainfall and N in soil systems is readily cycled and lost to atmosphere and water. Instead, the depletion of δ^{15}N-NO_3^- relative to SOM is probably in response to ^{15}N fractionation during mineralisation.[6]

CONCLUSIONS

From the data presented in this study it is impossible to determine completely the relative importance of the differing sources of NO_3^--N evolving from these grassland systems. However, it is clear that the lysimeter that received an application of animal slurry rich in NH_4^+-N had a distinctly different NO_3^- response isotopically from that which received none. Furthermore, the data also indicate that the pathway by which water leaves these systems also affects the isotopic nature of the NO_3^- evolved. What is not clear, however, is whether these pathways are affected through differing N sources of the NO_3^-, or whether differing processes that occur in that pathway are altering the final isotopic signal.

This study would indicate that the majority of NO_3^- derived from the lysimeter plots is microbial derived from SOM. The NO_3^- derived through the inter-flow pathways would appear to show a small (<8%) contribution from rainfall-derived NO_3^-; however, depletions in the δ^{15}N-NO_3^- which occurred during the storm could not be accounted for by an increased contribution from rainfall NO_3^-. It is suggested that fractionation may be occurring during the diffusion of NO_3^- from the soil water to the rainfall water. The contribution of rainfall NO_3^- could not be observed in the NO_3^- derived from the drain-flow pathways. Nitrate from this pathway would appear to be subject to a greater degree of microbial fractionation through denitrification, which

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produced a distinctly different isotopic signal from that from the inter-flow pathway.

The application of NH_4^+-N rich animal slurry does appear to be contributing to the NO_3^--N being derived from both inter-flow and drain-flow pathways from the slurry-amended lysimeter, although the specific nature of the contribution was undetermined. However, depleted δ^{18}O-NO_3^- values from the inter-flow and depleted δ^{15}N-NO_3^- values from the drain-flow pathways in the slurry-amended lysimeter could indicate that extra NH_4^+-N in the system was stimulating nitrification.

From this study we can also conclude that the isotopic make-up of NO_3^- evolved from grasslands can exhibit a high degree of variation over temporal scales of hours. This would indicate that those involved with studies which take low numbers of samples to represent temporal scales of weeks and months need to be cautious in the interpretation of that data. Such studies may not be capturing the important detail of the processes that affect the sources and production of NO_3^-.

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REFERENCES

1. Johnes PJ, Burt TP. In *Nitrate: Process, Patterns and Management*, Burt TP, Heathwaite AL, Trudgill ST (eds). John Wiley: Chichester, 1993.
2. MacDonald AM, Edwards AC, Pugh KB, Balls PW. *Water Res.* 1995; **29**: 837.
3. Feigin A, Shearer G, Kohl DH, Commoner B. *Soil Sci. Soc. Am. Proc.* 1974; **38**: 465.
4. Heaton THE. *Chem. Geol. (Isotope Geoscience Section)* 1986; **59**: 87.
5. Kendall C. In *Isotope Tracers in Catchment Hydrology*, Kendall C, McDonnell JJ (eds). Elsevier: Amsterdam, 1998; 521.
6. Ostrom NE, Knoke KE, Hedin LO, Robertson GP, Smucker AJM. *Chem. Geol.* 1998; **146**: 219.
7. McTiernan KB, Jarvis SC, Allen D, Bol R, Scholefield D. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1803.
8. Oelmann Y, Wilcke W, Bol R. *Rapid Commun. Mass Spectrom.* 2005; **19**: 3177.
9. Kellman LM. *Nutrient Cycling in Agroecosystems* 2005; **71**: 131.
10. Choi W-J, Ro H-M, Lee S-M. *Soil Biol. Biochem.* 2003; **35**: 1289.
11. Kellman LM, Hillaire-Marcel C. *Agriculture, Ecosystems and Environment* 2003; **95**: 87.
12. Böttcher J, Strebel O, Voerkelius S, Schmidt H-L. *J. Hydrol.* 1990; **114**: 413.
13. Amberger A, Schmidt H-L. *Geochim. Cosmochim. Acta* 1987; **51**: 2699.
14. Wassenaar LI. *Appl. Geochem.* 1995; **10**: 391.
15. Spoelstra J, Schiff SL, Elgood RJ, Semkin RG, Jeffries DS. *Ecosystems* 2001; **4**: 536.
16. Burns DA, Kendall C. *Water Resources Res.* 2002; **38**: 9.
17. Mayer B, Boyer EW, Goodale C, Jaworski NA, Van Breemen N, Howarth RW, Seitzinger S, Billen G, Lajtha K, Nadelhoffer KJ, Van Dam D, Hetling LJ, Nosal M, Paustian K. *Biogeochemistry* 2002; **57/58**: 171.
18. Deutsch B, Liskow I, Kahle P, Voss M. *Aquat. Sci.* 2005; **67**: 156.
19. Oelmann Y, Kreutziger Y, Bol R, Wilcke W. *Soil Biol. Biochem.* 2007; **39**: 3024.
20. Burns IG. *J. Soil Sci.* 1974; **25**: 165.
21. Armstrong AC, Leeds-Harrison PB, Harris GL, Catt JA. *Soil Use and Management* 1999; **15**: 240.
22. Bevan K, Germann P. *Water Resources Res.* 1982; **18**: 1311.
23. Gooday R, Anthony S, Fawvett L. *Environmental Modelling and Software* 2008; in press.
24. Sommer SG, Zhang GQ, Bannink A, Chadwick D, Misselbrook T, Harrison R, Hutchings NJ, Menzi H, Monteny GJ, Ni JQ, Oenema O, Webb J. *Adv. Agronomy* 2006; **89**: 261.
25. Kendall C, Aravena R. In *Environmental Tracers in Subsurface Hydrology*, Cook P, Herczeg AL (eds). Kluwer Academic Publishers: Dordrecht, 2000; 261.
26. Karr JD, William JS, Jennings GD. *Agriculture, Ecosystems and Environment* 2003; **95**: 103.
27. Curt MD, Aguado P, Sánchez G, Bigeriego M, Fernández J. *Water Air and Soil Pollution* 2004; **151**: 135.
28. Scholefield D, Tyson KC, Garwood EA, Armstrong AC, Hawkins J, Stone AC. *J. Soil Sci.* 1993; **44**: 601.
29. Armstrong AC, Garwood EA. *Hydrological Processes* 1991; **5**: 157.
30. Defra. *Code of Good Agricultural Practice for the Protection of Water*. Defra Publications: London, 2002.
31. Chang CCY, Langston J, Riggs M, Campbell DH, Silva SR, Kendall C. *Can. J. Fish. Aquat. Sci.* 1999; **56**: 1856.
32. Silva SR, Kendall C, Wilkison DH, Ziegler AC, Chang CCY, Avanzino RJ. *J. Hydrol.* 2000; **228**: 22.
33. Heaton THE, Wynn P, Tye AM. *Atmos. Environ.* 2004; **38**: 5611.
34. *IAEA Analytical Quality Control Services, Reference Materials Catalogue 2004–2005*. International Atomic Energy Agency: Vienna, 2004.
35. Baird DB, Harding SA, Lane PW, Murray DA, Payne RW, Soutar DM. *Genstat for Windows* (6th edn), Introduction, VSN International: Oxford, 2002.
36. Trafford BD, Rycroft DW. *J. Soil Sci.* 1973; **24**: 380.
37. Harris GL, Goss MJ, Dowdell RJ, Howse KR, Morgan P. *J. Agric. Sci., Cambridge* 1984; **102**: 561.
38. Tyson KC, Hawkins JMB, Stone AC. *Final Report on the AFRC-ADAS Drainage Experiment. 1982–1993*, Institute of Grassland and Environment Research: Okehampton, Devon, 1993.
39. Hastings MG, Sigman DM, Lipschultz F. *J. Geophys. Res. – Atmospheres* 2003; **108(D24)**: 4790.
40. Pardo LH, Kendall C, Pett-Ridge J, Chang CCY. *Hydrological Processes* 2004; **18**: 2699.
41. Kumar S, Nicholas DJD, Williams EH. *FEBS Lett.* 1983; **152**: 71.
42. Andersson KK, Hooper AB. *FEBS Lett.* 1983; **164**: 236.
43. Kroopnick P, Craig H. *Science* 1972; **175**: 54.
44. Mayer B, Bollwerk SM, Mansfeldt T, Hütter B, Veizer J. *Geochim. Cosmochim. Acta* 2001; **65**: 2743.
45. Böhlke JK, Eriksen GE, Revesz K. *Chem. Geol.* 1997; **136**: 135.
46. Cappa CD, Hendricks MB, DePaolo DJ, Cohen RC. *J. Geophys. Res. – Atmospheres* 2003; **108**: Art. No. 4525.
47. Kool DM, Wrage N, Oenema O, Dolfing J, Van Groenigen JW. *Rapid Commun. Mass Spectrom.* 2007; **21**: 3569.
48. Handley LL, Johnston AM, Hallett PD, Scrimgeour CM, Wheatley RE. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1274.

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