

Assessing multiple novel tracers to improve the understanding of the contribution of agricultural farm waste to diffuse water pollution

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Received 5th August 2009, Accepted 30th November 2009

First published as an Advance Article on the web 2nd March 2010

DOI: 10.1039/b915929k

A study was undertaken on drained and undrained 1 ha grassland lysimeters to assess the effectiveness of multiple novel tracing techniques in understanding how agricultural slurry waste moves from land to water. Artificial fluorescent particles designed to mimic the size and density of organic slurry particles were found to move off the grassland *via* inter-flow (surface + lateral through-flow) and drain-flow. Where both pathways were present the drains carried the greater number of particles. The results of the natural fluorescence and $\delta^{13}\text{C}$ of water samples were inconclusive. Natural fluorescence was higher from slurry-amended lysimeters than from zero-slurry lysimeters, however, a fluorescence decay experiment suggested that no slurry signal should be present given the time between slurry application and the onset of drainage. The $\delta^{13}\text{C}$ values of $>0.7\ \mu\text{m}$ and $<0.7\ \mu\text{m}$ material in drainage were varied and unrelated to discharge. The mean value of $>0.7\ \mu\text{m}$ $\delta^{13}\text{C}$ in water from the drain-flow pathways was higher from the lysimeter which had received naturally enriched maize slurry compared to the lysimeter which received grass slurry indicating a contribution of slurry-derived material. Values of $<0.7\ \mu\text{m}$ $\delta^{13}\text{C}$ from the same pathway, however, produced counter intuitive trends and may indicate that different fractions of the slurry have different $\delta^{13}\text{C}$ values.

Introduction

With the recent introduction of legislation and policy aimed at improving aquatic ecology and water quality in river basins (e.g. EU Water Framework Directive), attention is increasingly focusing on efforts to decrease inputs of pollutants into surface waters (*i.e.* rivers and lakes) and groundwater. Agricultural areas typically represent a large proportion of the land surface in many

countries, and in Europe and North America this land often receives large quantities of agricultural amendments such as inorganic fertilisers or managed animal manure (e.g. farmyard manure (FYM) and slurry) which are applied to improve plant production. However, with the intensification of farming, and subsequent increases in stocking densities, managed manures are often applied to land in excess. These organic amendments can have a significant impact if released into river systems as they contain high levels of nutrients, particularly nitrogen (N) and phosphorus (P) and have a high biological oxygen demand which can lead to a decrease in river oxygen levels.¹ Increases in plant nutrients in aquatic systems can lead to the eutrophication of an ecosystem with a host of associated detrimental effects.² The application of these amendments to land has long been implicated by studies as a source of aquatic diffuse N and P pollution.^{3–7} However, few studies actually provide evidence of a direct link between the organic sources of pollution applied to land and the occurrence of pollutants in water bodies.⁸ The potential to apply tracing techniques—particularly the simultaneous use of multiple tracers—to provide substantive evidence for the nature of this link at the field-scale has previously been highlighted.⁹

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Environmental impact

Based on largely anecdotal evidence, agricultural amendments have been linked to the degradation of surface water quality through diffuse pollution. This study assesses a suite of novel tracing approaches which could be used to provide a more definitive link between different contaminant sources and the occurrence of pollution in drainage water. More specifically from an artificially drained intensively managed grassland on a heavy soil type which has received an application a liquid farm slurry waste. The tracing approaches used were artificial fluorescent particles designed to mimic organic slurry particles, the natural fluorescence properties of agricultural wastes, and the difference in the natural abundance C isotopes of both grass and maize derived slurries.

To provide proof of concept, a tracer study was undertaken on 1 ha intensively managed, drained and undrained, grassland lysimeters in the southwest UK. Three tracing techniques were chosen: (i) natural abundance carbon (C) isotopes, (ii) labelled synthetic particles, and (iii) natural fluorescence.

It is believed that the use of stable C isotopes at natural abundance should be able to provide evidence for the movement of slurry-derived C from land into drainage waters. A previous unreported study undertaken at the small plot scale had shown that the amount of slurry-derived organic C lost in drainage varied from 10 to 75%, with the maximum loss occurring shortly after slurry application during a rainfall event. Furthermore, slurry-derived C continued to be detectable in drainage waters for over 2 months, long after pollutants had returned to pre-slurry levels (Bol, personal communication). The principles behind the stable C isotope tracing technique have been described in detail elsewhere.^{10–12} In short, all grasslands in the UK are C₃ systems ($\delta^{13}\text{C} \approx -26\%$). Cattle overwintered on silage produced from this herbage produce a slurry of similar enrichment while cattle overwintered on a C₄ plant-based silage produce an isotopically higher slurry ($\delta^{13}\text{C} \approx -13\%$).¹³ Thus, any increase in the $\delta^{13}\text{C}$ signal of C present in the drainage water leaving a C₃ lysimeter which receives C₄ slurry, when compared to a C₃ lysimeter which receives C₃ slurry, can be attributed to the presence of the C₄ slurry. A lysimeter receiving C₃ slurry is used as a control rather than a zero-slurry lysimeter to account for any differences in the soil biogeochemical processes due to the presence of slurry and it is assumed that the processes that affect both C₃ and C₄ slurries are the same. By examining the $\delta^{13}\text{C}$ of both particulates and filtrates in the drainage waters, this technique may be extended to provide evidence for differing loss processes.

Synthetically labelled particles have been previously used in maritime and terrestrial aquatic environments.^{14–16} These particles typically comprise a polymer combined with a fluorescent dye manufactured with a density matched to that of the particles which are being mimicked. To an extent, similar size distributions may also be achieved by initial sieving, with further control over particle size being applied at the sample analysis phase. Thus particles of roughly equivalent size and density to particles typically present in slurry can be created and subsequently added to and mixed with slurry before its application to the grassland lysimeters. The presence, or the absence, of these particles in water draining the lysimeters is a direct indication that particulate material applied with the slurry is able to move, and has moved from the system.

Recent advances in fluorescence spectroscopy have enabled rapid automated collection of fluorescence intensity data across a wide range of excitation and emission wavelengths. The observed centres of fluorescence in river water samples correspond to those produced by known fluorophores, predominantly the amino acids, tryptophan and tyrosine, and high molecular weight organic molecules, such as humic and fulvic acids. Hudson *et al.*¹⁷ provide an excellent review of both the phenomenon of natural fluorescence and its measurement. Farm wastes have also been analysed for their fluorescent properties and have been found to have very high intensities of tyrosine-like and tryptophan-like fluorescence and high ratios of these to fulvic/humic-like fluorescence compared to stream waters.¹⁸ This suggests that pollution of waters by these agricultural wastes could leave

a distinct fluorescence signature reflecting both the source of the dissolved organic matter and its concentration.

This combination of tracers may therefore be able to provide not only evidence of a direct connection between pollutant losses and organic amendments applied to agricultural grassland but also to discriminate between dissolved and particulate slurry fractions. The aim of this paper is to present the results from the field experiment in terms of both the evidence provided by the tracers for direct slurry losses into drainage waters and to provide an assessment of the tracing techniques used.

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 four 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; HOST class 24¹⁹), overlying 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.*²⁰ and Armstrong and Garwood.²¹ 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 have been agriculturally drained while the other half has no drainage installed. Lysimeters in which no drainage has been installed are dependent upon natural drainage *via* surface-flow and lateral through-flow pathways, and are termed ‘undrained’ (Fig. 1a). Lysimeters which have drainage installed are termed ‘drained’ and have mole drains at a depth of 55 cm which are intercepted by gravel filled trenches with 85 cm deep permanent pipe drains (Fig. 1b). All the lysimeters have perimeter gravel filled ditches to a depth of 30 cm which collect any surface runoff plus any lateral through-flow (combined referred to as inter-flow). The flow is channelled through 45° V-notch weirs. The drained lysimeters have additional and separate V-notch weirs for measuring water that flows through the drainage system (termed drain-flow) (Fig. 1b). The stage height (*h*) of both inter-flow and drain-flow was measured using solar powered Starlevel flow sensors on a 5 min time step with data recorded by Campbell radio loggers and subsequently transmitted *via* radio modem to a central computer. To convert *h* to discharge (*Q*) stage–discharge relationships were produced for each weir, including estimates of the errors involved in the calibration.²² These were used to produce hydrographs with associated uncertainty bands (*Q*_{max} and *Q*_{min}) through a technique developed by Krueger *et al.*²³ Rainfall at the site was measured using a tipping-bucket rain gauge (Rainwise, Bar Harbor, ME), which recorded the total number of tips per minute (each tip equivalent to 0.254 mm rainfall).

Experimental design

The experiment began on the 18th April 2006. Prior to this, slurry was sourced from two local farms where cattle were overwintered

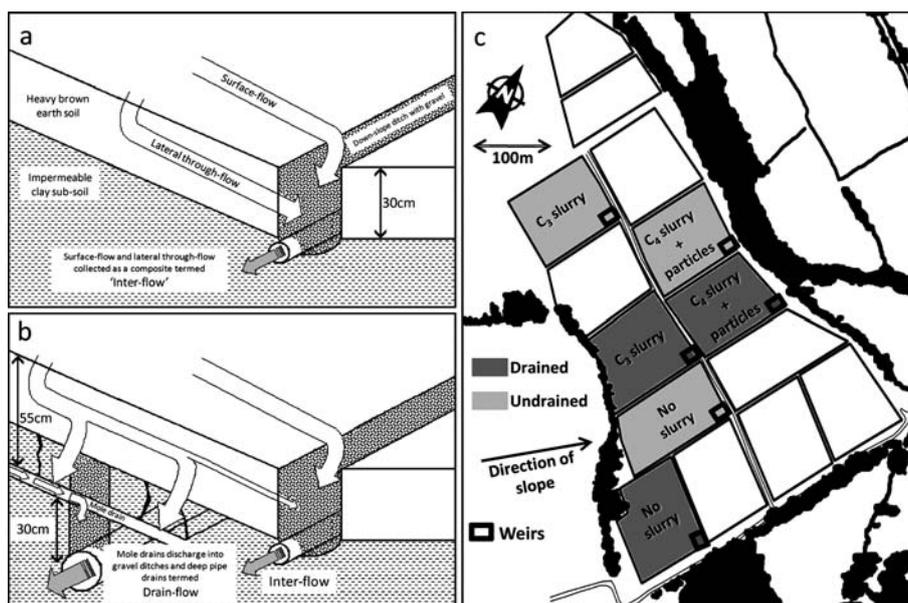


Fig. 1 The hydrological pathways of the undrained (a) and drained (b) lysimeters at the Rowden experimental research platform (adapted from Granger *et al.* 2008³⁹). The location of the lysimeters used for each tracer application is presented in (c).

on a diet of either ryegrass (*Lolium perenne* L., a C₃ plant with a $\delta^{13}\text{C}$ value of approx. -26‰) or maize (*Zea mays* L., a C₄ plant with a $\delta^{13}\text{C}$ value of approx. -13‰) based silage. After collection, and prior to application, the slurries were stored in two slurry mini-stores so that they could be well mixed. Each store was used exclusively for the storage and mixing of either the C₃ or the C₄ slurry to avoid cross-contamination.

Prior to mixing and application to the lysimeters, the C₄ slurry received a dose of fluorescent tracer particles. These were manufactured by Environmental Tracing Systems Ltd, Helensburgh, UK with a particle size distribution (PSD) based on the PSD of an initial test slurry (Fig. 2a). These data showed a bimodal distribution with one peak between 27 and 37 μm . The artificial fluorescent particles were manufactured to match this size range and have a density of 1.15 g cm^{-3} so as to be comparable with the organic particles within the slurry. The PSD data for the raw

tracer particles (Fig. 2b) show a relatively large proportion of very fine particles below 2 μm . Given that these were not present in the initial test slurry, all particles below 2 μm were discounted during the analysis of any samples. Excluding the $<2\text{ }\mu\text{m}$ particles, the D_{50} of the tracer was $\sim 12\text{ }\mu\text{m}$ with the main peak between 16 and 19 μm . Analysis of the two stock solutions showed that 4.4×10^{13} particles were applied per release. Mixing was accomplished through the pump and return of slurry out of, and back into the slurry towers using a conventional vacuum tanker. Tracer was then added to the slurry before further mixing was undertaken.

The experimental design comprised six hydrologically isolated lysimeters at the RERP consisting of three drained and undrained pairs. Two pairs received an application of either C₃ or C₄ slurry while the third pair received nothing (Fig. 1c). The maximum recommended application rate for UK grassland

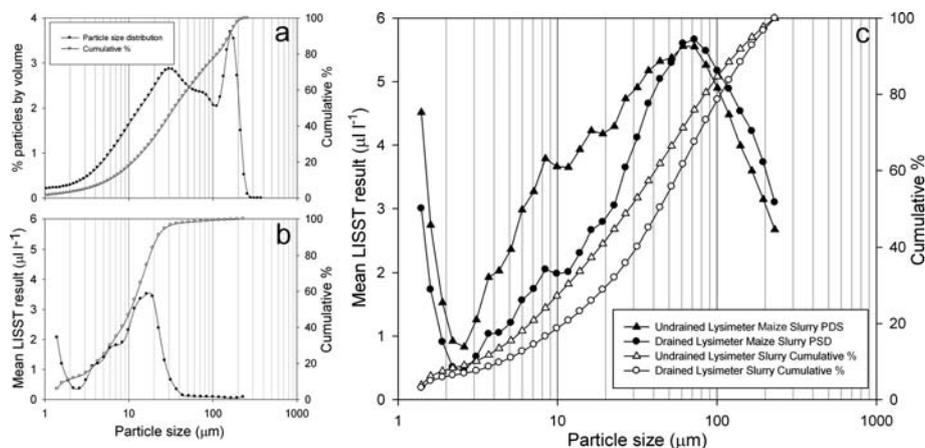


Fig. 2 Particle size distributions of (a) the initial test dairy slurry, (b) the raw artificial fluorescent tracer particles and (c) the maize slurries applied to drained and undrained lysimeters after the addition of the tracer particles.

systems is $50 \text{ m}^3 \text{ ha}^{-1}$, however, due to environmental constraints at the RERP at the time of application only $21 \text{ m}^3 \text{ ha}^{-1}$ were applied. The C_3 and C_4 slurries were applied to the drained lysimeters on the 18th April, while application to the undrained lysimeters occurred one week later on the 25th April. Slurry was applied using a conventional vacuum tanker fitted with a splash plate in three applications starting with the third of the lysimeter furthest away from the access point. Slurry was remixed before each tanker load was removed from the slurry mini-stores. A 10 m margin was left around the edge of each lysimeter in order to prevent contamination of the surface drains in accordance with the 'Code of Good Agricultural Practice'.²⁴

Sampling

Soil cores were collected from across the RERP to ascertain the $\delta^{13}\text{C}$ of the total soil C prior to slurry application. Five points within four of the lysimeters at the RERP were used which included two of the lysimeters used in this study. At each point 10 cores (2.5 cm diameter) were taken to a depth of 7.5 cm and these cores were then bulked.

Sub-samples of slurry were collected from each batch of slurry that was applied to each of the four lysimeters and stored in a refrigerator at $4 \text{ }^\circ\text{C}$ until analysis.

Samples of drainage water were taken as follows: all V-notch weir systems were fitted with an internal stainless steel mixing plate below the drainage input to the weir. The drainage flowing through the mixing plate was then sampled on hourly or sub-hourly time steps either using an automated sampler or through the collection of manual grab samples. Sampling of drainage water commenced after the first rainfall event to initiate drainage from the lysimeters. This occurred on the 19th May 2006, some 31 days after slurry application. A further two rainfall events were sampled, occurring on the 21st and 24th of May, and these three events are referred to as events 1, 2 and 3 respectively. Events 1 and 3 were sampled using automated samplers while event 2 was sampled manually by taking hourly grab samples. This was undertaken as during event 1 it was found that at low flows the auto-samplers were unable to collect sufficient sample for analysis. Event 3 was sufficiently large for resumption of automated sampling. Auto-samplers were set to sample using the same atomic clock time as the flow loggers. Samples were stored in a refrigerated environment in 1000 ml polyethylene bottles.

In addition, temporal changes in the natural fluorescence signal of the slurry were monitored by applying slurry to

a compartmented tray of a sterile medium (vermiculite) at the same rate (*i.e.* depth) as to the lysimeters. The use of a sterile medium was chosen so as not to introduce additional sources of fluorescence. There was no attempt to simulate soil processes. Four replicate sub-samples and two controls were collected each week and extracted using de-ionised water for analysis.

Analysis

Chemistry. Slurry samples were analysed for total solids by drying a pre-weighed sample in an oven at $100 \text{ }^\circ\text{C}$ and subsequently re-weighing until a constant weight is obtained. Organic matter was determined through weight loss on ignition at $500 \text{ }^\circ\text{C}$ of the dried slurry.

Within 24 h of arrival at the laboratory, drainage waters had sub-samples for total phosphorus (TP) analysis transferred to polypropylene autoclavable bottles. Concentration of TP was then determined through an acid persulfate digestion using a method described by Eisenreich *et al.*²⁵ Absorbance was measured colourimetrically on a spectrophotometer (Cecil Instruments, Cambridge, UK) calibrated by standards prepared fresh on the day of analysis. Concentrations of suspended solids (SS) and volatile solids (VS) were determined through vacuum filtration and subsequent drying at $105 \text{ }^\circ\text{C}$ for at least 1 h, of a known sample volume through a pre-weighed GF/F filter paper (Whatman), with particle size retention of $0.7 \text{ }\mu\text{m}$. Filter papers were then heated to $500 \text{ }^\circ\text{C}$ for at least 30 min to determine the total residual ash. The difference between SS and total ash is considered to represent VS.²⁶

Artificial fluorescent particles. Particle size distributions for both the raw tracer particles and tracer particles mixed with slurry were measured using a LISST 100 laser diffraction particle size analyser (Sequoia Scientific, USA). In both cases, ten analyses were undertaken and a mean result generated. Water samples collected from the lysimeters were agitated and sonified to disaggregate and suspend any solids and tracer particles. A measured volume, between 5 and 25 ml depending on turbidity, of the sample was then filtered through a $0.45 \text{ }\mu\text{m}$ cellulose nitrate filter paper (Whatman). The filter paper was carefully transferred using forceps from the filter housing to a labelled microscope slide. Each slide was then analysed manually using a specially modified scanning fluorescent microscope (Leica, Germany). The total counts for the measured volume were then converted to tracer counts per ml (cts ml^{-1}).

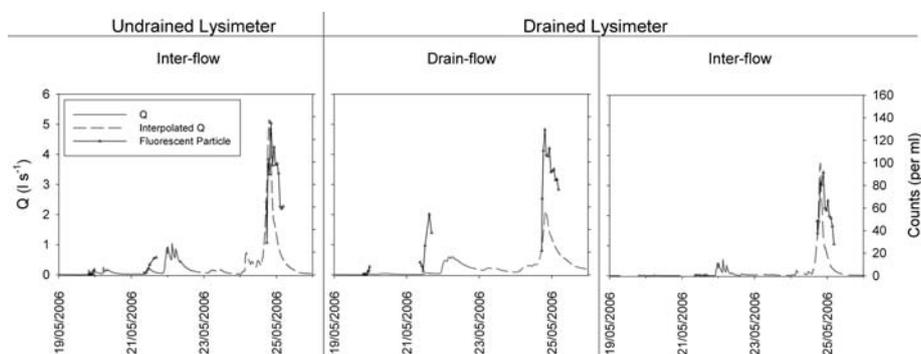


Fig. 3 The response of the fluorescent tracer particles to Q during the three monitored rainfall events.

Replicate analyses were carried out on 15% of the samples analysed with the results indicating less than 9% error on all samples.

Prior to the release of the tracer particles some raw slurry samples and background water samples were collected from the RERP to determine whether there was any background fluorescence. While some weakly fluorescent particles were present in these samples, likely to be either inorganic minerals and/or biological material, they did not interfere with the very much brighter fluorescent yellow particles applied due to the different excitation and emission wavelengths.

Natural fluorescence. Sub-samples of drainage water were taken from the ISCO sample bottles in acid-washed brown glass bottles and kept in cool, dark conditions. Samples were analysed at room temperature and before analysis, samples were filtered using pre-ashed GF/C glass fibre filters (Whatman) with particle size retention of 1.2 μm .

The fluorescence measurements were carried out on a Varian Cary Eclipse fluorescence spectrophotometer fitted with a Xenon flash lamp using slit widths of 5 nm, an integration time of 12.5 ms and voltage of 725 V. Excitation wavelengths from 240 to 400 nm (in steps of 5 nm) and emission wavelengths from 280 to 500 nm (in steps of 2 nm) were used to generate excitation/emission matrices (EEMs). The absorbance of each sample was measured in a 1 cm cuvette on an Agilent 8453 spectrophotometer at 1 nm intervals from 200 to 800 nm. Samples with an absorbance value of $>0.02 \text{ cm}^{-1}$ at 650 nm were omitted from the analysis as this was indicative of inefficient filtering. Samples which had an absorbance of 0–0.02 cm^{-1} at 650 nm had their absorbance corrected using the long-wavelength scatter-correction method of Blough *et al.*²⁷ Raw fluorescence data were then corrected for lamp output and for instrument sensitivity following manufacturer's instructions (Haines, personal communication). Inner-filtering corrections, calculated from the corrected absorbance data using the method of Lakowicz,²⁸ were then applied. The fluorescence data were finally converted to Raman units using the method of Lawaetz and Stedmon.²⁹

Following investigations using known mixtures of test solutions prepared with ultra-pure water and DL-tyrosine, L-tryptophan, IHSS Suwannee River Fulvic Acid Standard II and Humic Acid Standard II, indices representing tryptophan-like and fulvic/humic-like fluorescence were calculated as the mean fluorescence intensity over the following ranges:

tryptophan index (TI): excitation 275–285 nm/emission 346–354 nm

fulvic/humic index (FI): excitation 325–375 nm/emission 450–500 nm

This provides a ratio of TI : FI which is typically >2 for slurry and <1 in drainage waters in the absence of slurry (Naden *et al.*, in press³⁰). Thus, slurry losses should be identifiable in the enrichment of the TI : FI ratio. Where results are presented as means, two standard errors are presented in parenthesis.

Isotopic. Bulk 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. Slurry sub-samples were dried to a constant weight in an oven at 100 °C before being milled to a fine powder. Drainage samples were collected and delivered to the laboratory within

24 h of being sampled. They were then refrigerated at 5 °C until ready for preparation. Samples were subject to vacuum filtration through pre-combusted (500 °C), washed and dried 0.7 μm glass fibre filters (GF/F, Whatman). The resultant filtrate was then frozen and freeze-dried prior to analysis. Filter papers upon which $>0.7 \mu\text{m}$ particulate material was collected were dried in an oven at 105 °C and stored individually in airtight bags and placed in a desiccator until ready for analysis.

Total C (TC) and ^{13}C content were determined by dry combustion on a Carlo Erba NA 1500 elemental analyser connected to an automated continuous flow ANCA 20/20SL system (Europa, Crewe, UK). Prepared soil and slurry samples were weighed into 8 × 5 mm tin capsules and combusted using 25 ml of O_2 . Samples of particulate material on glass fibre filter papers were cut to fit inside 10 × 10 mm tin capsules and combusted in the elemental analyser *via* a large bore auto-sampler (AS128) using a 50 ml oxygen loop. Despite the 10 × 10 mm capsule being able to hold significantly more sample than an 8 × 5 mm capsule, the actual amounts of sample C were still found to be low. To test the precision of the mass spectrometer (MS) with small amounts of C, a series of 63 flours (42.2% C; -26.4‰ $\delta^{13}\text{C}$) from 5 mg down to 0.25 mg (*i.e.* 5, 4, 3, 2, 1, 0.5, and 0.25) were used where each weight was weighed to within $\pm 5\%$ of the target. Sample data were statistically adjusted based on the exponential curves generated by results up to 5 mg flour. Freeze-dried $<0.7 \mu\text{m}$ filtrates were also weighed into 10 × 10 mm tin capsules and combusted using 50 ml of O_2 , however, in this case sufficient sample C was supplied to not warrant statistical adjustment of the data.

Ground wheat flour referenced against CaCO_3 (NBS-19, Gaithersburg, MD, USA) was used as a calibration standard. The natural abundance values were expressed as δ values, which represent the ratios of $^{13}\text{C}/^{12}\text{C}$ relative to the international VPDB standard.

The $\delta^{13}\text{C}$ values (per mil) are defined as:

$$\delta^{13}\text{C} = \left[\frac{\text{atom}\%^{13}\text{C}_{\text{sample}} - \text{atom}\%^{13}\text{C}_{\text{VPDB}}}{\text{atom}\%^{13}\text{C}_{\text{VPDB}}} \right] \times 1000$$

Internal quality controls were included with samples during analysis comprising no less than 10% of the total number of samples and drift correction was made against internal standards during the run. Replication of samples was typically better than 0.8‰ for GF/F particulate samples and 1‰ for freeze-dried filtrates with mean values of 0.1 (± 0.2) and 0.1 (± 0.5) respectively (two standard errors are presented in parenthesis).

Results and discussion

Hydrological response

Three storms were monitored during the period 19th–26th May 2006 which generated hydrographs from the nine hydrological pathways contained within the six paired lysimeters. Due to a logger failure, discharge data from the zero-slurry and maize slurry lysimeters were not recorded from 22nd May until the end of the study. The Q record from the grass slurry lysimeters was unaffected. To estimate the missing Q record for this time period, Q has been taken from other lysimeters at the RERP site which

have been shown to behave in a hydrologically similar fashion. These lysimeters were selected by visual comparison of the hydrographs and assessment of the monthly flow statistics recorded from all lysimeters compared to those with missing data over the period March–May 2006.

The amounts of rainfall leading to the Q events sampled were 13, 7 and 14 mm occurring on the 19th, 21st and 24th May respectively. The previous month, during which slurry had been applied to the lysimeters, had been unusually dry having received just 18 mm rainfall compared to the mean April rainfall of 68 mm. For the month of May, up to the 19th, 30 mm of rainfall had fallen which was still lower than average for the time of year. By the time drainage caused by event 1 on the 19th May occurred the soil moisture deficit (SMD) for the site, as calculated by the Penman equation,³¹ was approximately 25 mm. This deficit explains why a rainfall event of 13 mm over 6 h did not produce significant Q for event 1. By event 2 on the 21st May the SMD had been reduced to ~4 mm, however, the relatively small amount of rainfall received meant that Q was low from the lysimeters. However, by event 3 on the 24th May the soil had no SMD and the 14 mm of rainfall that fell at the site caused Q to be substantially greater than event 1.

The hydrological response of the lysimeters is typical of this site and of other clay soil sites both with and without mole drainage.^{21,32,33} Discharge responds rapidly to the onset of rainfall forming characteristically peaky hydrographs, especially from the undrained lysimeters. Where mole drains have been installed, one could expect this drainage pathway to carry the greater proportion of storm water.^{21,33} However, should the drain system not operate efficiently through collapse of the moles the peaky drain-flow hydrograph would be attenuated while the inter-flow pathway would carry an increased percentage of the total drainage.²¹ During this study, while drain-flow from the lysimeters remained peaky, the hydrographs are more smoothed in comparison to the inter-flow hydrographs. Furthermore, the volume of water carried by the drain-flow pathway was approximately equal to that of the inter-flow.²²

Chemical response

A summary of the chemical data for each lysimeter and hydrological pathway for the three monitored drainage events is presented in Table 1. These data show that there was considerable variation in TP concentrations between events, slurry treatments and drainage status of the lysimeters with no consistent treatment effect evident. The mean concentrations of TP from the zero-slurry undrained lysimeters were 146, 540 and 247 $\mu\text{g l}^{-1}$ for events 1, 2 and 3 respectively. Mean concentrations from slurry-amended undrained lysimeters were higher during events 1 and 3, but lower during event 2. Mean concentrations of TP from the drained lysimeters were always higher in the drain-flow than the inter-flow. Concentrations in both pathways were also found to be higher from the zero-slurry lysimeter compared to both the slurry-amended lysimeters during events 1 and 2 although during event 3 concentrations were higher in the drain-flow of both the slurry-amended lysimeters.

Suspended sediment concentrations also showed cross-site variation with no distinct event or slurry treatment effects. Mean event concentrations from the zero-slurry undrained lysimeters

were 78, 185 and 51 mg l^{-1} while from the grass slurry-amended lysimeter they were 44, 38 and 90 mg l^{-1} and from the maize slurry-amended lysimeter 116, 78 and 45 mg l^{-1} . Mean concentrations from the drained lysimeters showed a similar lack of event or slurry effect although concentrations were typically higher in the inter-flow than in the drain-flow for any given lysimeter. Concentrations of VS also showed no distinct event or slurry treatment effect although as with SS mean concentrations tended to be slightly higher in the inter-flow than the drain-flow. The composition of the SS is typically mineral with maximum mean VS from the zero-slurry lysimeter of 24% from the undrained and 26/24% from the drain-flow/inter-flow pathways of the drained lysimeters. The proportion of VS was generally higher in lysimeters which received slurry compared to those which did not for any given event, with the drain-flow of the drained lysimeters having a larger percentage of VS to SS compared to the inter-flow.

The highest concentrations of SS and TP typically coincide with or occur just before peak Q and then decrease with decreasing Q as previously described by Bilotta *et al.*²² The concentrations reported here are comparable with those reported previously during a more comprehensive study of SS and TP from zero-slurry lysimeters at this site.²² The number of significant correlations between SS and TP increases through events 1 to 3 but shows no link to drainage status of the lysimeter, the hydrological pathway, or the application of slurry (Table 1). During event 1 there were virtually no significant relationships while all pathways in event 3 show highly significant correlations. This perhaps indicates that the size of the drainage event may be more important in SS and TP mobilisation rather than the drainage status of the grassland and that the soil, not the slurry, was the dominant source of SS and TP.

Artificial fluorescent particles

The PSD data for the slurries mixed with tracer are presented in Fig. 2c and given the much smaller volume of tracer added to a large volume of slurry the PSD data essentially reflect the slurry used for each study. The data indicate that the maize slurry used to mix with the fluorescent tracer was not the same as the slurry used as an initial guide. The maize slurry used for the undrained and drained plots shows mainly a unimodal distribution with a peak around 60–80 μm and not a bimodal distribution as the initial guide slurry had shown. The PSD data for the slurries applied to drained and undrained lysimeters show differences although given they were different batches and not collected at the same time this is perhaps not surprising. The fluorescent tracer particles used tended to represent the initial 40–50% of the slurry based on PSD data with more than 50% of the slurry consisting of particles larger than the tracer used. The PSD data of the slurry tracer mixture also show that both have an increase in the number of particles $<2 \mu\text{m}$, a feature not present in the initial test slurry. This is interpreted as purely a function of the addition of the tracer which has a high number of $<2 \mu\text{m}$ particles, which were excluded from analysis.

The number of tracer particles lost from each pathway increased through events 1 to 3 (Table 1); the mean number of particles lost during each event from the inter-flow pathway of the undrained lysimeter was 1, 8 and 84 cts ml^{-1} , while

Table 1 Summary of data obtained from RERP lysimeters during the three monitored events. Drainage status: UD = undrained, D = drained. Pathway: IF = inter-flow, DF = drain-flow. Correlations: ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Event ID	Treatment	Event 1												Event 2												Event 3											
		Zero Slurry				Grass Slurry				Maize Slurry				Zero Slurry				Grass Slurry				Maize Slurry				Zero Slurry				Grass Slurry				Maize Slurry			
		UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D	UD	DF	IF	D
TP ($\mu\text{g l}^{-1}$)	Min	83	168	85	20	182	321	109	288	171	540	313	303	267	114	178	253	113	61	202	154	220	210	180	139	221	107	202	154	220	210	180	139	221	107		
	Mean	146	346	161	60	360	321	109	288	171	540	313	303	267	114	178	253	113	61	247	206	261	359	204	281	142	247	206	261	359	204	281	142				
	Max	269	516	359	8	680	615	140	439	283	707	353	355	305	348	207	348	316	236	398	255	326	818	237	307	601	307	255	326	818	237	307	601	185			
	r^2	6	8	8	4	4	8	7	8	6	6	6	4	6	6	6	6	6	5	20	20	20	7	8	13	13	20	20	20	7	8	13	13				
SS (mg l^{-1})	Min	37	36	40	20	6	28	49	3	104	43	58	23	17	44	60	20	30	28	30	48	15	11	21	35	17	28	30	48	15	11	21	35	17			
	Mean	78	60	81	44	28	33	116	22	185	50	73	38	21	56	78	33	43	51	44	66	90	22	45	45	23	51	44	66	90	22	45	45	23			
	Max	303	108	292	75	95	45	154	41	361	72	104	54	26	71	97	49	68	121	86	121	271	38	38	128	66	121	86	121	271	38	38	128	66	33		
	r^2	8	8	4	4	4	10	9	9	6	6	6	4	6	6	6	6	4	4	20	20	20	7	8	13	13	20	20	20	7	8	13	13				
Correlation SS v. TP	r^2	-0.08	0.23	-0.32	0.96	0.04	0.82	-0.07	0.64	0.96	0.87	0.22	-0.04	0.22	0.89	0.94	0.94	1.00	1.00	0.97	0.86	0.82	0.96	0.88	0.99	0.75	0.97	0.86	0.82	0.96	0.88	0.75					
	p=	ns	ns	ns	*	ns	ns	ns	ns	***	*	ns	ns	ns	*	***	***	***	***	***	***	***	***	**	***	**	***	***	***	***	***	**					
		6	8	10	10	1	9	9	3	3	20	12	11	8	8	9	13	7	9	5	6	10	7	4	5	14	10	17	20	27	12	13					
VS (mg l^{-1})	Min	6	14	16	30	31	29	17	41	14	19	16	21	29	15	14	23	23	23	16	18	14	20	21	16	29	33	16	18	14	20	21	16				
	Mean	12	14	16	30	31	29	17	41	16	26	21	28	40	19	18	30	30	30	19	24	22	34	31	25	38	42	19	24	22	34	31	25	38			
	Max	34	19	48	31	25	14	26	13	61	14	20	15	9	15	15	15	15	16	19	15	20	54	9	20	27	12	20	27	12	20	27	12	13			
	r^2	8	8	4	9	4	4	10	9	6	6	6	4	6	6	6	6	4	4	20	20	20	7	8	13	13	20	20	20	7	8	13	13				
% VS	Min	11	16	16	30	4	26	14	21	14	19	16	21	29	15	14	23	23	23	16	18	14	20	21	16	29	33	16	18	14	20	21	16				
	Mean	18	24	22	39	31	29	17	41	16	26	21	28	40	19	18	30	30	30	19	24	22	34	31	25	38	42	19	24	22	34	31	25	38			
	Max	25	32	26	49	43	31	21	100	19	29	25	42	51	21	21	33	32	32	40	31	29	47	40	33	47	67	33	47	40	33	47	67	67			
	r^2	8	8	8	4	9	4	10	9	6	6	6	4	6	6	6	6	4	4	20	20	20	7	8	13	13	20	20	20	7	8	13	13				
Correlation SS v. VS	r^2	0.99	0.59	1.00	0.95	0.86	0.96	0.86	0.84	0.97	0.62	0.65	0.68	-0.46	0.82	0.45	0.93	0.97	0.90	0.89	0.84	0.99	0.76	0.97	0.78	0.64	0.90	0.89	0.84	0.99	0.76	0.97					
	p=	***	ns	***	*	**	*	**	**	***	ns	ns	ns	ns	*	ns	**	*	***	***	***	***	*	*	***	**	*	***	***	***	***	*					
		6	8	10	10	1	9	9	3	3	20	12	11	8	8	9	13	7	9	5	6	10	7	4	5	14	10	17	20	27	12	13	13				
$\delta^{13}\text{C}$ ($>0.7\mu\text{m}$)	Min	-28.2	-27.8	-27.8	-28.2	-27.3	-27.8	-28.9	-27.1	-27.3	-27.8	-27.2	-27.8	-27.8	-27.2	-27.8	-26.8	-28.1	-28.1	-28.8	-26.8	-27.4	-27.4	-28.1	-28.3	-28.7	-29.1	-28.8	-29.4	-28.4	-27.5	-27.6	-28.3				
	Mean	-27.3	-27.0	-27.1	-27.0	-27.0	-27.1	-27.6	-27.6	-27.1	-27.1	-26.6	-26.3	-26.9	-26.7	-26.6	-27.2	-26.5	-27.4	-27.4	-26.2	-26.9	-26.9	-26.9	-27.6	-27.6	-27.2	-28.2	-28.2	-27.7	-27.8	-27.5					
	Max	-27.0	-26.0	-27.1	-27.0	-27.0	-27.1	-27.6	-27.6	-27.1	-26.9	-26.3	-26.3	-26.9	-26.7	-26.6	-27.2	-26.2	-27.4	-27.4	-26.2	-26.9	-26.9	-26.9	-27.6	-27.6	-27.2	-28.2	-28.2	-27.7	-27.8	-27.5					
	r^2	4	9	4	9	4	4	8	6	4	4	4	4	6	6	6	6	4	4	6	6	6	4	4	6	6	7	7	8	8	13						
$\delta^{13}\text{C}$ ($<0.7\mu\text{m}$)	Min	-28.9	-26.1	-28.9	-30.5	-28.9	-26.9	-30.5	-29.6	-28.7	-26.9	-28.4	-29.0	-28.0	-28.0	-28.4	-29.0	-30.0	-30.0	-29.5	-26.9	-28.7	-29.5	-26.9	-28.7	-29.3	-28.8	-29.5	-26.9	-28.7	-29.3	-28.8					
	Mean	-27.6	-21.9	-26.9	-27.7	-27.1	-25.6	-27.7	-27.1	-25.6	-25.4	-28.0	-28.4	-27.5	-29.1	-28.4	-27.5	-29.1	-29.1	-29.0	-25.6	-28.7	-29.0	-25.6	-28.7	-28.8	-27.9	-27.3	-28.8	-28.8	-27.9						
	Max	-24.9	-4.8	-24.4	-20.1	-20.9	-17.3	-22.4	-20.9	-17.3	-22.4	-27.6	-27.6	-26.8	-28.2	-27.6	-26.8	-28.2	-28.2	-28.4	-21.7	-28.7	-28.4	-21.7	-28.7	-28.1	-24.5	-24.5	-28.1	-24.5	-25.0						
	r^2	4	9	3	8	6	8	6	6	4	4	4	4	6	6	6	4	4	6	6	6	6	4	4	6	6	7	7	8	8	13						
Tracer Particles (cts ml^{-1})	Min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
	Mean	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
	Max	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4				
	r^2	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8				
TI:FI	Min	0.36	0.40	0.45	0.70	0.48	0.39	0.40	0.57	0.37	0.41	0.49	0.80	0.53	0.47	0.41	0.61	0.59	0.68	0.38	0.43	0.54	0.94	0.56	0.68	0.50	0.37	0.41	0.49	0.80	0.53	0.47	0.41				
	Mean	0.37	0.41	0.49	0.80	0.53	0.47	0.41	0.61	0.38	0.43	0.54	0.94	0.56	0.68	0.50	0.37	0.41	0.49	0.37	0.41	0.49	0.80	0.53	0.47	0.41	0.49	0.80	0.53	0.47	0.41	0.61					
	Max	0.38	0.43	0.54	0.94	0.56	0.68	0.50	0.37	0.41	0.49	0.80	0.53	0.47	0.41	0.61	0.59	0.68	0.38	0.43	0.54	0.94	0.56	0.68	0.50	0.37	0.41	0.49	0.80	0.53	0.47	0.41	0.61				
	r^2	5	6	6	4	6	6	6	5	6	6	6	4	6	6	6	6	5	5	5	6	6	6	4	6	5	5	6	6	6	6	5					

a comparable response is also present in the drain-flow of the drained lysimeter (2, 23 and 90 cts ml⁻¹). While there are no data for event 1 from the inter-flow pathway of the drained lysimeter, there is a marked increase in tracer particle numbers in event 3 compared with event 2, however, the numbers of particles lost in events 2 and 3 are notably lower from this pathway than from the other two pathways (0 and 58 cts ml⁻¹), particularly during event 2. During event 1 no relationship between tracer particles and Q was observed in the undrained lysimeter, however, a significant positive relationship ($r_7 = 0.83$, $p < 0.01$) occurs in the drain-flow from the drained lysimeter. Similarly during event 2 no relationship was present between tracer particles and Q in the inter-flow from either lysimeter, although tracer particle numbers had increased compared to event 1 from the undrained lysimeter and were in turn greater than those from the drained lysimeter inter-flow pathway. No significant relationship was found between particle tracer number and Q from the drain-flow pathway during event 2; however, the number of particles lost was greatest from this pathway during this event. As event 3 Q from these two lysimeters is interpolated Q and not true Q from these lysimeters, no statistical analysis of Q vs. tracer particles has been undertaken, however, a clear trend is apparent. From all three pathways particle counts per ml appear to exhibit a positive relationship with Q with the maximum counts per ml occurring at, or shortly after, the maximum Q (Fig. 3). The increase in numbers is rapid on the rising limb of the hydrograph, while numbers decline more slowly on the falling limb, mirroring the nature of the Q itself.

Natural fluorescence

It is important to analyse samples for natural fluorescence as quickly as possible after sampling as it is known that fluorescence may change over time and this may be related to many factors (e.g. microbial activity, oxygen availability, temperature in sampler) which make the magnitude of change for any given sample very difficult to predict. Therefore the data presented here are restricted to storm 2 (Table 1) for which the period between sampling to analysis was <3 days.

Spatial sampling in a nearby first order catchment has shown that drainage waters not directly contaminated by farm waste have TI : FI ratios of 0.3 to 0.4; at the RERP subsequent sampling of non-slurry affected lysimeters gave mean TI : FI ratio of 0.45 (± 0.01). For the samples discussed here, the mean value of TI : FI in inter-flow from the zero-slurry lysimeter was 0.37 (± 0.00) while mean values from the two lysimeters which received slurry were higher, one marginally so at 0.41 (± 0.01) while the other was much higher at 0.80 (± 0.06). Similarly mean values recorded in the inter-flow of the drained lysimeters were the same (0.47 (± 0.02)) or higher (0.59 (± 0.03)) from slurry-amended lysimeters compared to the zero-slurry lysimeter (0.49 (± 0.1)). Mean values of TI : FI from the drain-flow appear to be more consistently affected by the presence of slurry; the value from the zero-slurry lysimeter was 0.41 (± 0.00), while values from the two slurry-amended lysimeters were 0.53 (± 0.01) and 0.61 (± 0.02). This suggests some evidence for the loss of slurry-derived material in a dissolved phase during event 2. However, results from the tray experiment indicate that within 4–5 weeks much of the natural fluorescence signal from the slurry

(TI and FI) has been lost and the results from the TI : FI are therefore inconclusive.

Isotopic response

No significant difference in the $\delta^{13}\text{C}$ of the soil was found between lysimeters at the RERP and therefore a grand mean of -28.7‰ (± 0.2) is taken to represent the $\delta^{13}\text{C}$ of the soil at the site. Slurry was applied to the lysimeters at two distinct times, with the drained lysimeters receiving an application of grass and maize slurry one week before the undrained lysimeters. The $\delta^{13}\text{C}$ of the grass slurries were -28.2‰ (± 0.3) and -27.3‰ (± 0.1) applied to drained and undrained lysimeters respectively, while the maize slurries had $\delta^{13}\text{C}$ values of -18.9‰ (± 0.2) and -18.0‰ (± 0.2) having a higher $\delta^{13}\text{C}$ value of $\sim 9\text{‰}$ which is less than reported elsewhere.¹²

Summary values of $\delta^{13}\text{C}$ measured in material >0.7 and <0.7 μm leaving the lysimeters are presented in Table 1. There were no overall significant relationships between the $\delta^{13}\text{C}$ of the <0.7 and >0.7 μm fractions. Variation in $\delta^{13}\text{C}$ values is greatest in the <0.7 μm fraction compared to the >0.7 μm fraction and both fractions show no clear trends occurring within individual storm events. There were also no clear trends in the $\delta^{13}\text{C}$ of >0.7 and <0.7 μm fractions across the three events in the inter-flow of both the undrained and drained lysimeters. However, due to auto-sampler failure no data from event 1 on the maize slurry-amended and event 3 on the grass slurry-amended drained lysimeters were collected from this pathway on the drained lysimeters which make it impossible to delineate trends. Values of $\delta^{13}\text{C}$ from the drain-flow pathway of the drained lysimeters do, however, show a consistent trend across all three events in both the grass and maize slurry-amended lysimeters. Here values of $\delta^{13}\text{C}$ in both >0.7 and <0.7 μm material become lower with each subsequent event (Fig. 4). The trend is clearest in the >0.7 μm material and less so in the <0.7 μm fraction, which is strongly influenced by a few samples which have a far higher $\delta^{13}\text{C}$ than the bulk of the data especially during event 1. This trend of lower values across subsequent storms does not appear to be affected by the slurry treatment.

To establish what slurry-derived organic material is leaving the lysimeters a comparison between the $\delta^{13}\text{C}$ of lysimeters that received grass slurry and naturally enriched maize slurry was made. To do this the mean value of $\delta^{13}\text{C}$ (± 2 standard errors) from the maize slurry-amended lysimeters during each event was taken and compared to the mean obtained from the grass slurry-amended lysimeters from the corresponding event and pathway (Fig. 4). The >0.7 and <0.7 μm materials obtained from the undrained lysimeter were similar in that although there were apparent differences between grass and maize slurry treatments, there was no specific direction to the trends. A more pronounced trend was observed in the $\delta^{13}\text{C}$ of material derived from the drain-flow of the drained lysimeter. The $\delta^{13}\text{C}$ values of the >0.7 μm fraction from the maize slurry lysimeter were always higher relative to that from the grass slurry lysimeter. This increase was only markedly different during events 2 and 3 with increases of 0.6‰ (± 0.20 standard error of the difference (SED)) and 0.9‰ (± 0.16 SED) respectively. The reverse was true for the $\delta^{13}\text{C}$ values of the <0.7 μm material which were higher from the grass slurry lysimeter relative to the maize slurry lysimeter:

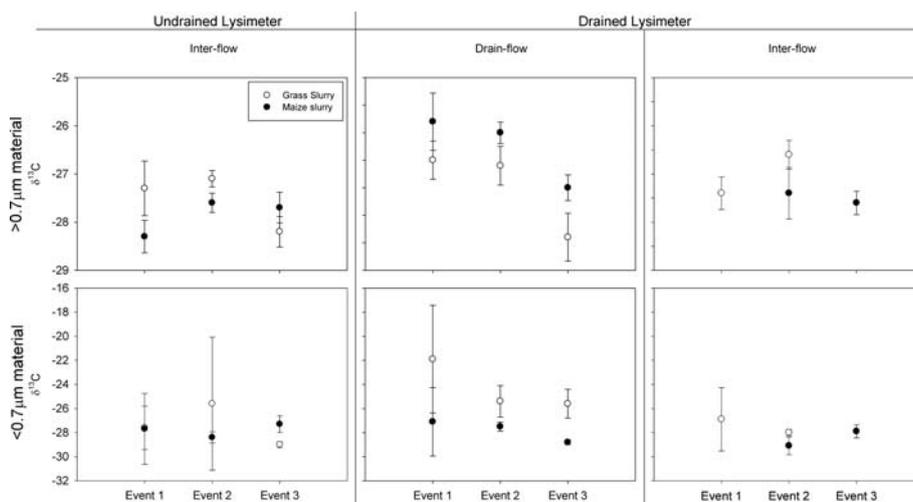


Fig. 4 Mean $\delta^{13}\text{C}$ (± 2 standard errors) of the <0.7 and >0.7 μm material in waters from the undrained and drained lysimeters which received an application of either grass or maize based slurry.

2.1‰ (± 0.68 SED) and 3.2‰ (± 0.37 SED) for events 2 and 3 respectively. Assuming that the increase in $\delta^{13}\text{C}$ of the >0.7 μm particles was caused as a result of maize slurry material becoming entrained in the drain-flow; it is estimated that between 6% (± 2) and 10% (± 2) of this material originated from the slurry during events 2 and 3 respectively.

What is less clear is why an unexpected and counter intuitive difference in $\delta^{13}\text{C}$ values was present within the <0.7 μm fraction derived during events 2 and 3. We have no simple explanation for this behaviour. The above observation may point to the fact that: (i) the maize slurry was not homogeneously naturally enriched with the <0.7 μm fraction being less labelled than the particulate >0.7 μm fraction, (ii) the finer material of the surface applied <0.7 μm fraction is predominantly held in the soil matrix at the soil surface, hence what is found in the drains is soil derived material which might have a different $\delta^{13}\text{C}$ value between the two plots, (iii) the <0.7 μm material contained forms of C that were significantly different in their isotopic composition from that of C_3 and C_4 plant matter in the slurry; this material may have been sourced from either, or both, of the slurries themselves or from the soil/soil–water system or (iv) the differential fractionation in the soil of the <0.7 and >0.7 μm material differs between maize and grass slurry.³⁴ All of these tentative suggestions partly counter the assumption on which the natural abundance labelling technique is based³⁵ and require further investigation.

Analysis of the tracing approaches

The effectiveness of any tracer is wholly dependent on the degree to which it represents the material that is being traced. All the tracers being applied to this study are novel to some extent; natural fluorescence signals at the field lysimeter scale have not been widely studied and reported previously, artificial fluorescent particles have been used previously but not in the terrestrial environment, while natural abundance C isotopes have been used previously to assess the contribution of dung derived C to soil systems but not to aquatic ones nor at this scale. To evaluate fully

the effectiveness of the tracers being used we need to reconsider the strengths and limitations of the individual approaches.

The artificial fluorescent particles appear to have been a success in terms of improving our understanding of the processes and pathways that organic material sourced from agricultural amendments follow. However, while these particles may mimic the size and density of a large fraction of slurry particles there are other aspects which remain uncertain such as the shapes of slurry particles and the nature of their surface charge (the particles being manufactured with a positive charge). This obviously has implications for the way such particles may flocculate and move,³⁶ however, unpublished data indicate that the particles do adsorb natural charge and therefore behave similarly when looking at hydraulic and flocculation processes (J. K. Marsh, personal communication). Furthermore, the distribution and number of tracer particles mixed with the slurry may not represent the distribution and number of particles within the slurry, and counts of particles in drainage water were made irrespective of particle size. Therefore it is uncertain whether the sizes of particles yielded in drainage are representative of the initial tracer as a whole or whether one particular size range dominates. Additionally, to better understand the consequences of slurry particle movement more information is needed on what pollutants are associated with these particles.

While natural abundance C isotopes have been used as tracers with a great deal of success elsewhere,³⁷ results from this application of the technique were inconclusive. This may have resulted from the low initial slurry application reducing the strength of the maize slurry $\delta^{13}\text{C}$ signal, or may be a consequence of the long dry period between slurry application and drainage causing slurry-derived material to be (partly) decomposed or sorbed onto soil surfaces. Furthermore, the nature of the label within the C_4 slurry was uncertain, appearing to be only partly labelled with a δ value of -18‰ compared to other C_4 slurries with δ values of *ca.* -12‰ .¹² Unlike direct excreta, slurry is a complex composite of potentially many materials and while the ‘particulates’ in the slurry might be considered to represent pieces of undigested or partially digested C_4 plant material and therefore fully labelled,

the colloidal and soluble components may represent material not sourced from the C₄ substrate and therefore not labelled to the same extent. These different size fractions are also subject to different processes and rates of decomposition,³⁸ with smaller organic particles being metabolised differently to larger ones. Other issues were methodological such as the separation of the >0.7 and <0.7 μm material. Collecting and analysing >0.7 μm material collected on GF/F papers severely restrict the mass of actual particulate material available to the MS and although increasing the volume of sample filtered would have increased the amount of particulate C on the filters, they would become rapidly clogged leading to large sample preparation times and the retention of material <0.7 μm. Furthermore, the freeze-dried <0.7 μm material represented a composite of all colloids and solutes <0.7 μm, thus may have contained a component of inorganic C, which if present may have increased the δ¹³C values.

Natural fluorescence has potential to act as an indicator for slurry-derived material, however, the nature of the signal is time dependent and probably only persists for 3–4 weeks after slurry application. Thus, only direct losses may be traced using natural fluorescence rather than any longer term impacts. There is also a requirement for the samples to be analysed quickly after sampling which may also limit the potential applications of the technique.

Conclusions

The artificial fluorescent particles show that organic slurry particles have the potential to move readily from land to water and numbers increase and decrease with *Q*. Particles were lost *via* the inter-flow pathway of both the undrained and drained lysimeters. Where both inter-flow and drain-flow pathways were present the drain-flow carried the greater number.

Natural fluorescence TI : FI ratios were inconclusive. Although ratios were higher in slurry-amended lysimeters when compared to the zero-slurry lysimeter, given the time interval between the application of slurry and the monitored runoff it is likely that most of the natural fluorescence signal from the slurry *per se* would have been lost.

The data obtained from the stable C isotope tracing approach were also inconclusive. The δ¹³C of both particulate and dissolved phases were highly variable over time and only the particulate material in the drain-flow appeared to carry a slurry signature.

While many questions have been raised concerning the interpretation of the evidence from individual tracers, the simultaneous use of multiple tracers has been useful in helping to corroborate the available evidence. One of the main limitations in the experiment was the low application rate of slurry and further work needs to be carried out using a higher rate of application. Another limitation was the length of time between the applied slurry and the first rainfall event which meant that the use of some of the tracers was limited. There is a need to follow this up by more controlled experiments looking specifically at the mechanisms behind the tracers and the environmental processes through which they are changed.

Acknowledgements

The work was supported through a Department of Environment, Fisheries and Rural Affairs (Defra) grant (PE0120). The first

author acknowledges the support of Cranfield University and North Wyke Research through his PhD studentship award as well as advice from Dr Phil Owens of the University of Northern British Columbia. North Wyke Research is grant aided by the Biotechnology and Biological Sciences Research Council (BBSRC). We acknowledge the work of Tobias Krueger, Dr Jim Freer, Dr John Quinton (University of Lancaster), and Dr Kit Macleod (N. Wyke) in establishing the flow measurements and uncertainties for the RERP.

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