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Assessment of natural fluorescence as a tracer of diffuse agricultural pollution from slurry spreading on intensely-farmed grasslands

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ABSTRACT

The value of natural fluorescence in tracing diffuse pollution, in liquid phase, following slurry application to land was assessed by field experiment using twelve one hectare lysimeters on a heavy clay soil in Devon, UK, during autumn 2007. A strong linear relationship was found between natural fluorescence intensity and slurry concentration. The ratio of indices of tryptophan-like and fulvic/humic-like fluorescence (TI:FI) varied between 2 and 5 for a range of slurries sampled from Devon farms and allowed slurry to be distinguished from uncontaminated drainage waters (TI:FI<1). Incidental losses of slurry, indicated by significantly enhanced TI:FI ratios, high TI and high ammonium levels, occurred via the drain flow pathway of the drained lysimeters during the first small event following slurry-spreading. The maximum estimated loss from a single lysimeter was 2-8 kg or 0.004-0.016% of the applied slurry. In the second larger storm event, some five weeks later, significantly enhanced TI:FI ratios in the drain flows were not associated with high TI but with high nitrate levels and, compared to the earlier storm, an increase in the humification index. This implies the loss of slurry decomposition products during this event but further work is needed to validate this. There was no significant enhancement of TI:FI in the surface/throughflow pathways of the drained or undrained lysimeters in either of the events. The observed change over a period of weeks in the strength and nature of the fluorescence signal from spread slurry restricts quantification of slurry losses to those immediately after slurry spreading. Nonetheless, this study demonstrates the utility of fluorescence as an indicator of slurry in drainage waters and the importance of field drains in diffuse agricultural pollution.

Keywords

fluorescence; animal slurry; diffuse agricultural pollution; field drainage

1. INTRODUCTION

Intensively-farmed grasslands pose a number of concerns for river water quality which have been highlighted by Hooda *et al*. (2000). These concerns arise from intensification of livestock production with associated increases in stocking density, demand for fodder and the need to manage and dispose of animal wastes such as farmyard manure, slurry, dirty water and silage effluent. The quantity of farmyard manure and slurry produced annually from beef and dairy cattle in the UK amounts to about 73 million tonnes (Smith *et al*., 2001), with roughly 45% being applied to grassland (Smith *et al*., 1998). While this can be a valuable source of crop nutrients and organic matter necessary to maintain soil fertility and structure, it also needs careful management in terms of its storage and application to land in order to prevent diffuse pollution either directly from farmyards and hard standings, or more indirectly following spreading.

On average cattle slurry contains roughly 6% dry matter, 3 kg N m^{-3}, 0.5 kg P m^{-3} and 3 kg K m^{-3} and has a biochemical oxygen demand (BOD) of 10,000-20,000 mgL^{-1} i.e. about 50 times greater than the BOD of untreated domestic sewage (Hooda *et al*., 2000; Defra, 2009). While there is guidance as to best practice for both the management and spreading of slurry (Defra, 2001a; 2001b; 2009), the timing and rates of application are often determined by storage and weather considerations. Leakage of farmyard stores, drainage from hard standings, and runoff following slurry application to land can all lead directly to losses of organic matter, nutrients and pathogenic micro-organisms, with potential consequences for both stream ecology and human health. Losses from land have been variously attributed to storm events immediately following slurry spreading, surface application of slurry in winter, and the role of under-drainage in heavy clay soils (Withers *et al*., 2003). These diffuse losses

have mainly been characterised in terms of nutrients (e.g. Misselbrook *et al*., 1995; Heathwaite *et al*, 1998; Vadas *et al*., 2007), although attention has recently turned to pathogenic micro-organisms (e.g. Oliver *et al*., 2005; Kay *et al*., 2008). However, the link between slurry amendment and nutrient losses is, at least in part, circumstantial and tracing techniques are required to substantiate this link and provide an improved understanding of the key pathways and their controls (Granger *et al*., 2007).

One potential way forward is through the use of natural fluorescence. Recent advances in fluorescence spectroscopy have enabled rapid automated collection of fluorescence intensity data across wide ranges of excitation and emission wavelengths. This technological development has led to the widespread use of fluorescence to characterise dissolved organic matter (DOM) in water. An excellent review is provided by Hudson *et al*. (2007). In river water samples, both protein-like (tyrosine- and tryptophan-like) and fulvic-like and humic-like fluorescence have been detected. The relative strength of these signals has been used to indicate the presence of different sources of DOM and the extent of contamination by sewage or other effluent (e.g. Baker, 2001; Baker and Inverarity, 2004; Galapate *et al*., 1998; Reynolds and Ahmad, 1997). Baker (2002) has also shown that animal wastes not only have very high protein-like fluorescence but also very high ratios of protein-like to fulvic/humic-like fluorescence compared to stream waters. The signal is similar to that from human sewage (Henderson *et al*., 2009), so other tracers (e.g. boron; Jarvie *et al*., 2006) would need to be used in conjunction with fluorescence to separate out agricultural from sewage or septic tank sources. However, in the context of purely agricultural headwaters, there is considerable potential for exploring natural fluorescence as a tracer for agricultural pollution in the liquid phase from both hard standings and following slurry application to land and, thus, reinforce the existence of a direct link between agricultural amendments and nutrient export.

The purpose of this paper is to (i) demonstrate the use of natural fluorescence as an indicator of diffuse slurry loss, in the liquid phase, following application to land and (ii) assess the prospects for its use in quantifying such losses.

2. MATERIAL AND METHODS

This assessment of the use of natural fluorescence to trace slurry losses, following application to land, was carried out by field experiment on the Rowden Experimental Research Platform (RERP) at North Wyke Research, Devon, UK during autumn 2007. The data from this experiment were supplemented by analysis of nine different slurries sampled from dairy farms across Devon in June 2007 and of drainage waters from the nearby catchment of Den Brook during January 2007.

2.1 Description of the field experiment

The Rowden Experimental Research Platform (Figure 1) was established in 1982 on old unimproved pasture on poorly drained sloping land (5-10%). Full details are provided in Armstrong and Garwood (1991), Scholefield *et al*. (1993) and Bilotta *et al.* (2008). The site consists of fourteen plot-scale lysimeters which are approximately one hectare in size. The soil type is a clayey non-calcareous pelostagnogley of the Hallsworth Series (Findlay *et al*., 1984) which overlies the clay shales of the Crackington Formation and is typical of much of the permanent grassland in the south-west of England (Haygarth *et al*., 1998). At a depth of 30 cm, there is an impermeable clay horizon that prevents water from percolating downwards. The lysimeters are hydrologically isolated from their neighbours by gravel-filled ditches to 30 cm and at their upslope boundaries by deep interceptor drains. Half of the lysimeters are

drained, with mole drains at 2 m spacing to a depth of 55 cm. The mole drains cross permanent pipe drains (>100 mm diameter) at 40 m spacing and a depth of 85 cm, with permeable backfill to within 30 cm of the surface. The lysimeters are managed as intensive grassland with annual applications of NPK fertiliser in line with the Code of Good Agricultural Practice (Defra, 2009). They are grazed by beef cattle between June and October, with an average stocking density of four livestock units per hectare, to give a sward height of between 8 and 10 cm. Each lysimeter has had a slightly different management history, in terms of liming, fertiliser application and re-seeding, since its inception.

Insert Figure 1 here

Surface flow and throughflow from both the undrained and drained lysimeters is collected in gravel-filled ditches installed at 30 cm depth at the lower lysimeter boundary. This flow then passes over a standard 45˚ V-notch weir (BSI, 1981). The drained lysimeters have a second V-notch weir which is used to monitor the flow from the subsurface mole drains and pipe drainage network. Stage is recorded at 1 minute intervals by Starlevel sensors (Unidata, O'Connor, WA, Australia) connected to Campbell dataloggers (Model CR215, Campbell Scientific, Loughborough, UK). Rating curves were developed for the weirs by field experiment during July 2006. Full details are given in Krueger *et al*. (in press). Rainfall was measured hourly by tipping bucket rain gauge connected to an automatic weather station.

Twelve of the lysimeters were used in the experiment. Six lysimeters (three drained and three undrained) were selected for slurry application as shown in Figure 1. Slurry was sourced from a nearby dairy farm where it had been stored in an open-air lagoon. It was applied at a rate of 46 $m^3 ha^{-1}$ between 11 and 13 October 2007 using a trailing-shoe spreader which applied the

slurry directly to the soil surface. A 5 m buffer zone was left around the perimeter of the lysimeters to avoid any transfer of material directly into the collection ditches. Grab sampling of the drainage water from the twelve lysimeters was undertaken every two hours during storm events. Two storm events were sampled. The first storm event was a relatively small event with 11.6 mm rainfall on 16-17 October 2007, which occurred immediately after spreading. This was only the third storm of the autumn with over 10 mm rainfall and, as indicated by the small and varied flow response, the lysimeters were in different states of wetness prior to this event. The second storm event sampled was a more substantial double storm totalling 42.0 mm (16.4 mm plus 25.6 mm) rainfall some five weeks later on 18-22 November 2007. The intervening period had been relatively dry with only 23.4 mm rainfall spread over several small events, all less than 5.6 mm.

In setting up the experiment, we were aware of potential changes over time in the fluorescence signal from the applied slurry. These may be a result of microbial activity, photodegradation, oxidation, mineralization and preferential adsorption (Baker, 2002; Ohno and Bro, 2006; Hunt and Ohno, 2007; Ohno *et al*., 2007). In order to track these temporal changes, the slurry was also applied to a compartmented tray of sterile medium (vermiculite) at the same rate as to the lysimeters. The use of a sterile medium was chosen in order not to introduce additional sources of fluorescence. There was no attempt to simulate soil processes. The trays were located in the field adjacent to the lysimeters. Sub-samples were taken from the trays on a weekly basis using four replicates and two no-slurry controls.

2.2 Laboratory methods

Samples of drainage waters and slurry were collected in acid-washed brown glass bottles and kept cool. Slurry samples were gently mixed by inversion of the bottles before taking 0.5 g

slurry which was then made up to 50 ml with Milli-Q water. The sub-samples from the tray experiment were individually bagged in the field and, once received in the laboratory, 200ml Milli-Q water were added. Samples were then shaken for 1 minute and left to stand for 9 minutes. This was repeated before swirling for a few seconds prior to extracting a sample for measurement.

All samples were analysed at room temperature within 24-48 hours of collection from the field. Before analysis, samples were filtered using pre-ashed Whatman GFC (1.2µm) glass fibre filters. 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. The short integration time was necessary in order to measure the large numbers of storm samples within the required 24-48 hours. Excitation wavelengths were varied from 200 to 400 nm in steps of 5 nm and emission wavelengths from 280 to 500 nm in steps of 2 nm. Absorbance was measured in a 1 cm cuvette on a Varian Cary 50 UV-Vis spectrophotometer at 1 nm intervals from 800 to 200 nm. All absorbance spectra were referenced to a blank of Milli-Q water. Samples with absorbance at 254 nm greater than 1.5 AU cm^{-1}, were diluted using Milli-Q water. This threshold was adopted for pragmatic reasons. It is considerably higher than the value of 0.3 AU cm^{-1} quoted by Ohno (2002) but within the limits found by Holland *et al*. (1977).

The absorbance measurements were long-wavelength scatter-corrected using the method of Blough *et al*. (1993). Instrument corrections (Holbrook *et al*., 2006) were applied to the fluorescence data to account for lamp output and for instrument sensitivity, following manufacturer's recommendations. Inner-filtering corrections were calculated from the corrected absorbance data (Lakowicz, 1983; Ohno, 2002) and applied to the fluorescence

data. These corrected data were finally converted to Raman units (RU) using the method of Lawaetz and Stedmon (in press). This step normalizes the fluorescence intensity by the area under the Raman peak between emission wavelengths of 380 and 410 nm for an excitation wavelength of 348 nm and, therefore, incorporates both a daily Raman correction and a conversion to standard units. Over the entire period of measurement (January 2007 to January 2008), the area under the Raman peak was measured on 55 days; these measurements have a mean value of 380.4 and a standard deviation of 5.7 arbitrary fluorescence units.

To represent the signal of tryptophan-like and fulvic/humic-like fluorescence, indices (TI and FI) were derived based on results from known standards and their mixtures. Thus, instead of simply using the fluorescence at, or in the region of, the peak for each of the anticipated fluorophores, optimal regions of the excitation-emission matrix (EEM) were identified, taking into account the overlap between the separate fluorescence centres. The wavelengths defining each region are given in Table 1 and the index is simply the average of the corrected fluorescence intensities in the given region. A further approach using PARAFAC analysis (Stedmon *et al*., 2003; Stedmon and Bro, in press) is also being conducted but is not presented here.

Insert Table 1 here

3. RESULTS

In order to assess the potential for using fluorescence as a general indicator of slurry losses, we need to be able to demonstrate five points: (i) that there is a relationship, preferably linear, between the fluorescence indices and slurry concentration; (ii) that there is a difference in the fluorescence signal between slurry and drainage waters with no slurry contamination; (iii) that

fluorescence characteristics are similar in different slurries; (iv) that uncertainty in the data, from sampling through to post-processing, is much smaller than the differences which we hope to detect and (v) that temporal changes in the fluorescence signal are either minimal or predictable.

Figure 2 shows data from a dilution series prepared from an example slurry. It demonstrates that both the TI and the FI extracted from the corrected fluorescence data are linearly related to slurry concentration. The results thus validate the fluorescence correction methods used and confirm the linearity of the selected indices for natural slurry.

Insert Figure 2 here

Corrected EEMs for a slurry and for drainage waters (with no slurry input) are given in Figure 3. Data with an excitation wavelength below 240 nm are unreliable and are not shown. Figure 3a clearly shows the large protein-like fluorescence signal found in slurry consisting of large tyrosine-like and tryptophan-like peaks at an excitation wavelength (Ex) of 280 nm and emission wavelengths (Em) of 304 and 348 nm respectively. Drainage waters, by contrast, show a large fulvic/humic-like signal at higher excitation and emission wavelengths (Figure 3b) with peaks typically quoted in the literature at Ex 220-250 nm / Em 400-480 nm and Ex 300-380 nm / Em 400-480 nm (e.g. Baker and Spencer, 2004). Figure 3 thus confirms the distinction between the fluorescence signals from slurry and drainage waters found by Baker (2002). In these two samples, the ratios of TI:FI are 2.4 for the slurry and 0.3 for the uncontaminated drainage water. This eight-fold difference suggests that there is at least potential for using the enrichment of the TI:FI ratio in drainage waters as an indicator of slurry losses, even down to relatively small amounts of slurry.

Insert Figure 3 here

Figure 4 shows the fluorescence and absorbance characteristics of the slurry used in the tracing experiment in the context of nine different slurries sampled from farms around Devon in June 2007. To give some indication of the variability in the measurements, box and whisker plots of the TI, FI, TI:FI ratio and absorbance at 254 nm are shown. For the slurry applied during the RERP experiment (A), they were derived from triplicate measurements carried out on individual samples of the slurry applied to each of the six lysimeters. In the case of the farms (B-J), three samples of slurry were taken from each farm and triplicate measurements were carried out on some of the individual samples. For these samples, it was not possible to thoroughly mix the slurry prior to sampling and the slurries varied in both age and method of storage on the farm. There were also differences in the farm systems, with potentially different feed stuffs and bedding material as well as livestock numbers and types. Figure 4 shows that there are considerable differences in the values of fluorescence and absorbance between the different slurries. The slurries which have very low TI, FI and absorbance were very watery and the low values are thought to represent considerable dilution during storage on the farm. However, all slurry samples had a TI:FI ratio greater than 2 which is much greater than that found in uncontaminated drainage waters which tend to have ratios much less than 1, or example pore waters collected from the three no-slurry drained lysimeters in January 2008 (TI:FI about 0.3). The slurry used in the RERP experiment is at the lower end of the TI:FI range with a lower than average TI. The observed variation between the slurries implies that the prospects for general quantification of diffuse losses of slurry, in terms of the proportion of slurry, are low as it will be important to know the fluorescence characteristics of each applied slurry. However, the fact that the TI:FI ratio

across all slurries is consistently high compared to drainage waters suggests that significant slurry losses should be identifiable in the enrichment of the TI:FI ratio, confirming the potential to use fluorescence, in cases with no sewage effluent, as an indicator of the existence, if not the magnitude, of losses.

Insert Figure 4 here

Uncertainties in fluorescence measurements of drainage waters from sampling through to analysis were estimated by taking ten consecutive samples from four sampling sites with different levels of slurry contamination in the nearby catchment of Den Brook. With little or no contamination (TI<1), measurements of TI and FI were respectively within ±0.06 and ±0.14 RU, with the TI:FI ratio, as calculated from each EEM, being within ±0.01. At high levels of contamination (TI>3), measurements of TI and FI were within ±0.32 and ±0.17 RU, with the TI:FI ratio being within ±0.08. In the context of the values shown in Figure 4 for the variation between slurries, this measurement uncertainty is negligible.

3.1 Slurry tracing experiment

The results of the slurry tracing experiment are shown in Figure 5 in terms of a summary of the TI:FI ratio for all the samples from all drainage paths for all the lysimeters. The data are separated out for the baseflow prior to slurry spreading, the two storm events sampled after slurry spreading, and baseflow on two occasions following the storm sampling. Within each sampling period, the data are arranged in treatment pairs, i.e. slurry (shaded) and no slurry, for each flow pathway. The flow pathways, labelled on Figure 5, are surface/throughflow from the undrained lysimeters (U), surface/ throughflow from the drained lysimeters (S), and drain flows from the drained lysimeters (D). There are very few baseflow samples as the lysimeters

only flow during storm events or very wet periods. Each of these samples is shown as an individual point. The results from the storm-event samples are shown as box and whisker plots with the box defining the inter-quartile range and the whiskers extending to the furthest data point within a distance of 1.5 times the interquartile range away from the box. Any outliers beyond this are shown as individual points. The horizontal line in each of the boxes shows the position of the median value. The number of samples which fall in each of the categories is given below the plot.

Insert Figure 5 here

Most of the samples have TI:FI ratios less than 1.0. The exceptions to this are mainly samples taken during the storm immediately after slurry spreading from the drain flow pathway of the drained lysimeters receiving slurry. The TI:FI ratios for these samples have a median value of 1.58 and tend towards the values found in the raw slurry (Figure 4). They are, therefore, thought to indicate the occurrence of incidental slurry losses through the drain flow pathway. Values of the TI:FI ratio for the drain flow pathway of the lysimeters in receipt of slurry remain relatively high in the second storm with a median value of 0.77. Prior to slurry spreading, two samples, one each from the drain flow pathways of lysimeters 3 and 9, showed somewhat elevated TI:FI ratios. All lysimeters had been grazed by cows during the summer prior to slurry spreading. However, this was not expected to have had a big effect on the TI:FI ratios or a preferential effect on lysimeters 3 and 9. Drained lysimeters 4 and 9 had received slurry in May 2006 but, after 18 months, no residual signal is expected (see below). Thus, the reason for these elevated values prior to slurry spreading is unknown. Samples of baseflow taken after the two sets of storm samples tend to suggest that TI:FI ratios in the drain flow pathways of lysimeters receiving slurry remain relatively elevated, although to gradually

lesser degrees. However, the number of baseflow samples taken before and after slurry application is too small to draw any substantive conclusions.

In the case of the samples from the surface/throughflow pathways of both the undrained (U) and drained lysimeters (S) shown in Figure 5, there is a slight tendency towards higher values of TI:FI in those in receipt of slurry compared to those without. However, this is a very minor effect compared to the results from the drain flow pathway of the drained lysimeters (D).

In order to test the hypothesis that there is a significant difference in the TI:FI response for the lysimeters where slurry was applied, an analysis of variance (ANOVA) test was performed under the assumption of a completely randomised design, two treatment levels (slurry/no slurry) and three replicates. The data for each pathway in each storm were considered separately. To satisfy the assumptions of the ANOVA test, the reciprocal of the TI:FI ratio was used and outliers removed until a Shapiro-Wilk test for normality was satisfied. For the samples taken during the October storm, Table 2 shows that no significant difference was detected (p-value>0.05) between the two treatments for the surface/throughflow pathway of either the undrained or drained lysimeters. However, the null hypothesis is rejected (p-value<0.05) for the drain flow pathway indicating that there is a statistically significant enrichment of the TI:FI ratio in the drain flows from those drained lysimeters in receipt of slurry. As shown in Table 3, a similar result is obtained for the samples taken in the November storm with the null hypothesis being rejected (p-value<0.01) in the case of the drain flow pathway.

Insert Tables 2 and 3 here

These results clearly show that there is a marked difference in the fluorescence signal, expressed in terms of TI:FI, of water from the drain flow pathway for those lysimeters to which slurry was applied compared to those without slurry. In the first storm, this difference is associated with large values of TI which is consistent with a slurry source. Elevated TI:FI ratios in the drain flow pathway of slurry-amended lysimeters during the first storm is also associated with elevated concentrations of ammonium which has an average value of 4.6 mg N L^{-1} compared to 0.65 mg N L^{-1} for drain flows without slurry. This again is indicative of a slurry source. In the second storm, the higher TI:FI ratios in the slurry-amended drain flow pathways are the result of only a slightly higher TI coupled with a much lower FI. Ammonium levels are only slightly elevated but there is a substantial difference in the concentrations of nitrate with an average value of 9.6 mg N L^{-1}, compared to 5.5 mg N L^{-1} for the drain flows with no slurry-amendment. These findings imply that there is potential to use fluorescence as a direct indicator of diffuse slurry losses immediately following slurry application but that over subsequent weeks, the nature and strength of the signal changes.

3.2 Tray experiment

The tray experiment was designed to provide information on the change in the fluorescence signal from the applied slurry over time and the results are presented in Figure 6. Also shown are the values measured in the applied slurry. Comparison of these values and those of the tray sub-samples immediately after application suggests that about 80% of the fluorescence signal is lost once the slurry is applied to the vermiculite. This implies significant adsorption of the chemicals onto the surface of the vermiculite which our extraction method did not release. The adsorption onto the soil or a vegetated soil surface is unknown. Further work is needed to explore other extraction methods and leaching experiments with vegetated soil cores in order to fully quantify this initial effect. However, when focussing on the TI:FI ratio,

the first set of tray samples shows only a small reduction in TI:FI compared to the applied slurry (Figure 6) and, therefore, this initial adsorption does not invalidate the results based on the TI:FI ratio.

Insert Figure 6 here

Looking at the change over the succeeding weeks, Figure 6 shows that there is a preferential loss of tryptophan-like fluorescence over the first two or three weeks which quickly reduces the TI:FI ratio to about 0.7, which is more commensurate with that found in the drainage waters (Figure 5). This loss is assumed to be due to microbial activity which breaks down the molecules associated with the protein-like fluorescence. Other decay experiments (not shown here) using different application rates and including examples with a covering of vermiculite over the top of the slurry to simulate subsurface (i.e. in the absence of light) degradation, showed similar findings with a strong tryptophan-like fluorescence signal one week following application which was reduced by a factor of about four some four weeks later. The similarity of findings from the surface and subsurface experiments implies that there is little effect of photodegradation on the fluorescence signal. Experiments simulating conditions which may be found in drains or pipes were not performed.

The decay over time shown in Figure 6 substantiates in part the findings from the field experiment. The initial storm event was only 3-5 days after the slurry application, i.e. about the same time as the first tray samples, so we can be confident that the fluorescence data shown for this storm reflect the slurry signal and are consistent with direct incidental losses through the field drains. The second storm event, however, occurred some five weeks after the slurry application. The results from the tray experiment shown in Figure 6 suggest that by

this time most of the tryptophan-like signal has disappeared while the fulvic/humic-like signal remains somewhat elevated, thus giving a minimal TI:FI ratio of around 0.7. While this does not explain the elevated TI:FI ratio found in samples of drain flow from the slurry-amended lysimeters during the second storm, it does reinforce the need for careful interpretation of these results.

4. DISCUSSION

The data presented here have demonstrated the potential for using natural fluorescence as an indicator of diffuse slurry losses, in liquid phase, following application to land. Measurements of natural fluorescence are highly reliable, provided that samples are stored in the cool and in the dark and measured within 24-48 hours of collection. There is a strong linear relationship between fluorescence intensity, as expressed in the TI and FI indices, and slurry concentration; the TI:FI ratio for slurry is substantially higher than that found in drainage waters which are uncontaminated by slurry; and the combined sampling and measurement uncertainty is low.

Prospects for quantifying diffuse slurry losses, in terms of the proportion of the applied slurry, however, are limited except for incidental losses in specific experiments or from specific sources. There are two issues here. The first of these is the variability in the fluorescence signal, including the TI:FI ratio, between different slurries. This variability is presumably dependent on the age of the slurry and the method of storage on the farm, as well as differences in the farm system (feed stuffs, bedding material, livestock etc). Despite this variability, the results shown highlight the distinction between the fluorescence signal from slurry and that from drainage water uncontaminated by slurry. This is consistent with the

findings of Ohno and Bro (2006) who also showed that DOM from manures was quantitatively very variable but had a distinct fluorescence characteristic compared to DOM from soils, on the one hand, and biota (crops, wetland plants and tree leaves) on the other.

The second issue, which relates to the use of natural fluorescence in quantifying slurry losses, is the change in the fluorescence signal over time following its application to land. The TI:FI enrichment measured in the drain flows in the second storm may be an indicator of slurry loss, involving temporary storage in the drains and subsequent re-mobilisation. However, it is not associated with high levels of TI and the tray experiment, investigating the change in the fluorescence signal from slurry over time, suggests that high levels of tryptophan-like fluorescence may be largely lost by the time of the second storm, giving a reduced TI:FI ratio of 0.7. This is very close to the median value for the sampled slurry-amended drain flows of 0.77. An alternative explanation for the signal from the drain flows is that the addition of slurry enhances microbial activity in these pathways and that it is the products from this secondary effect which are being measured. Support for this hypothesis comes from the fact that the elevated TI:FI ratios in the first storm are associated with elevated concentrations of ammonium whereas, in the second storm, they are associated with high nitrate concentrations.

The fluorescence characteristics of fresh and decomposed DOM have been investigated by Hunt and Ohno (2007). Their work is based on extracted DOM and they report a reduction in tryptophan-like fluorescence and in the ratio of tryptophan-like to humic-like fluorescence for dairy manure after only 10 days of decomposition. They also report an increase in the humification index (i.e the ratio of the fluorescence at emission wavelengths 435-480 nm to that at 300-345nm for an excitation wavelength of 255 nm). Median values of the humification index from our tray experiment increase from 3.3 (the raw slurry has a value of

1.4 implying some preferential adsorption to vermiculite) for the first set of tray samples to 4.7 at the time of the second storm. For the sampled drain flows, the humification index for the slurry-amended lysimeters has a median value of 1.1 in the first storm and 6.3 in the second storm, compared with values of 11.4 and 10.3 respectively from the drains with no slurry amendment. This implies that the fluorescence data from the second storm may well be indicative of the loss of decomposed slurry. Hunt and Ohno (2007) explain the increase in the humification index by the synthesis or release of higher molecular weight, more aromatic, moieties into solution. Extracellular polymeric substances produced by microbial excretion and cell lysis are known to contain large molecular weight aromatic substances and the production of these during decomposition may be partly responsible for the change in the fluorescence signal. Further work is needed to clarify the fluorescence signals associated with the processes of slurry transport, decomposition and carbon sequestration within soils as well as the transport and decomposition processes within drains.

In terms of incidental losses, the experimental evidence presented here has shown the importance of field drains as a pathway for slurry losses in heavy clay soils. This concurs with the findings of Hodgkinson *et al*. (2002) and Withers *et al*. (2003). Incidental losses during the first storm were particularly apparent from the drain flow pathway of lysimeter 9. Here the enrichment of the TI:FI ratio is associated with TI values around 9.6 RU. These high levels of TI were measured in four successive samples over a period of 5.4 hours. With the exception of one value (from the drain flow from lysimeter 5), all other measurements of TI are less than 1.64 RU. Assuming that this represents the maximum background level of TI and taking into account uncertainty in the flow data, from the TI calibration curve for the RERP slurry, we estimate that some 2-8 kg slurry were lost in liquid phase via the drain flow pathway from lysimeter 9 during the first storm. Furthermore, assuming a slurry density of approximately

1000 kg m^{-3}, we can calculate that this represents 0.004-0.016% of what was applied to the 1 hectare lysimeter. This is clearly only a very small fraction of the applied slurry and demonstrates the sensitivity of the technique. In the context of other published incidental losses (e.g. Withers *et al*., 2003), it should be noted that the October storm fell on relatively dry ground which meant that this was a very minor flow event with the total volume of the drain flow from lysimeter 9 being only 1.7% (0.5-2.8%) rainfall. The high soluble reactive phosphorus and BOD content of slurry (Hooda *et al*., 2000; Defra, 2009), however, suggests that even this small amount may have a significant impact in terms of stream ecology, especially in headwater streams.

One of the key advantages of natural fluorescence is that measurements are relatively quick and inexpensive, compared with that of some nutrient fractions, BOD and faecal indicator organisms (FIOs). At the catchment scale, Baker and Inverarity (2004) and Hudson *et al*. (2008) have shown a strong relationship between tryptophan-like fluorescence and BOD. In this larger scale context, the sources of BOD include sewage and industrial effluents which individually also show high correlations between tryptophan-like fluorescence and BOD (Reynolds and Ahmad, 1997; Baker and Curry, 2004). Little or no work has been done to relate the fluorescence from agricultural sources to BOD or to FIOs and further work is needed in this area. Indeed, in terms of river monitoring, it is the impact which matters rather than the proportion of slurry (raw or decomposed) which is lost. Hence, it may be that fluorescence has greater potential to quantify impact than loss. This, coupled with the potential to development techniques for *in situ* instrumentation (cf. Baker *et al*., 2004; Spencer *et al*., 2007), is particularly important in the context of the very short-lived intense spikes of diffuse pollution which we have measured from agricultural sources both from areas

of hard standing (Granger *et al*., in press) and, as described here, through drains in heavy clay soils following slurry-application to land.

5. CONCLUSIONS

The results presented have demonstrated the use of natural fluorescence as an indicator of diffuse slurry losses, in liquid phase, following slurry application to land. Specifically, they show that

- there is a strong linear relationship between fluorescence intensity (as expressed by TI and FI) and slurry concentration;
- the TI:FI ratio is a good indicator of slurry contamination with values from slurry consistently greater than 2 and values from uncontaminated field drainage less than 1;
- there is considerable variability in the TI:FI ratio ($2<TI:FI<5$) between different slurries which limits quantification to cases where the signal from the applied slurry is known;
- the combined sampling and measurement uncertainty for fluorescence is low;
- incidental slurry losses, indicated by significantly enhanced TI:FI ratios, high TI and high ammonium levels, occurred via the drain flow pathways of the slurry-amended lysimeters in the small storm immediately following slurry spreading – the maximum estimated loss from a single lysimeter was 2-8 kg or 0.004-0.016% of the applied slurry;
- there are large changes in the strength and nature of the fluorescence signal from slurry over time periods of days to weeks, with a preferential reduction in TI;
- significantly enhanced TI:FI ratios in the drain flows during the second larger storm event, some five weeks after slurry spreading, were not associated with high TI but with high nitrate levels and an increase in the humification index which is consistent with the loss of decomposed slurry.

Further work is needed to fully characterise the fluorescence signals associated with slurry, particularly in the context of its transport and decomposition within soils and field drains. Data are also required to define the relationships between fluorescence, BOD and FIOs for agricultural wastes. The key advantages of fluorescence as an indicator of slurry loss compared to these other determinands are its ease of measurement and the potential for *in situ* monitoring which could capture very short-lived spikes of diffuse pollution such as the incidental losses through field drains highlighted here.

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13

Table 1 Definition of indices

	Excitation wavelengths (measured at 5 nm intervals)	Emission wavelengths (measured at 2 nm intervals)
Tryptophan index (TI)	275-285 nm	346-354 nm
Fulvic/humic index (FI)	325-375 nm	450-500 nm

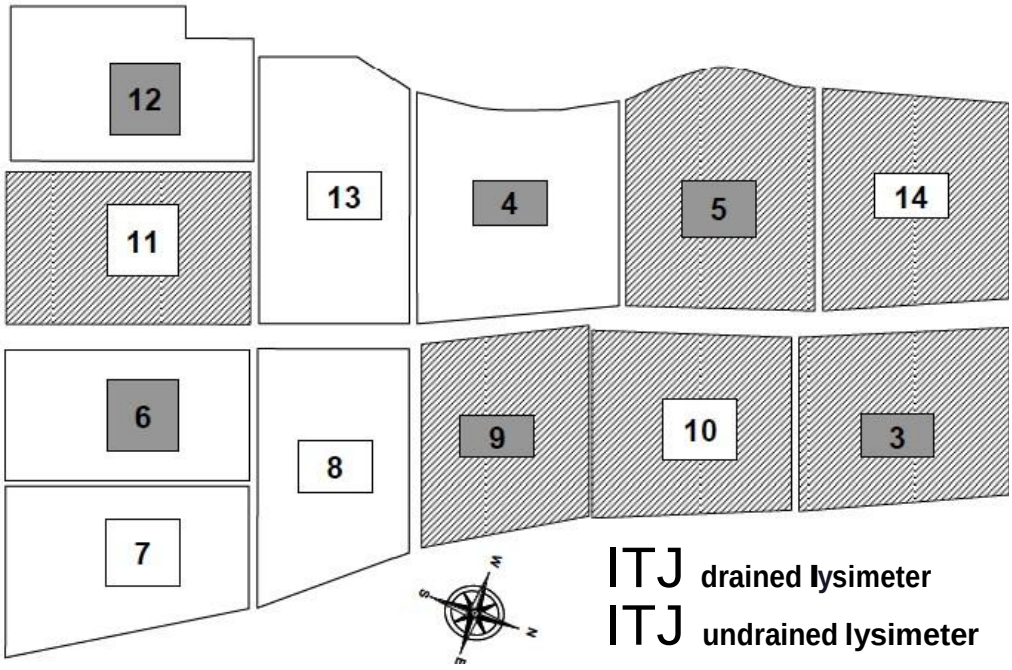
Table 2 Analysis of variance table for the October storm

Undrained lysimeters: surface/interflow pathway					
Source	Sums of squares	df	Mean square	F	p
Treatment	0.029	1	0.029	0.014	0.912
Error	8.331	4	2.083		
Total	8.360	5	1.672		
Drained lysimeters: surface/interflow pathway					
Source	Sums of squares	df	Mean square	F	p
Treatment	0.210	1	0.210	4.72	0.118
Error	0.134	3	0.045		
Total	0.344	4	0.069		
Drained lysimeters: drain pathway (2 outliers removed)					
Source	Sums of squares	df	Mean square	F	p
Treatment	12.361	1	12.361	26.61	**0.014**
Error	1.394	3	0.465		
Total	13.755	4	3.439		

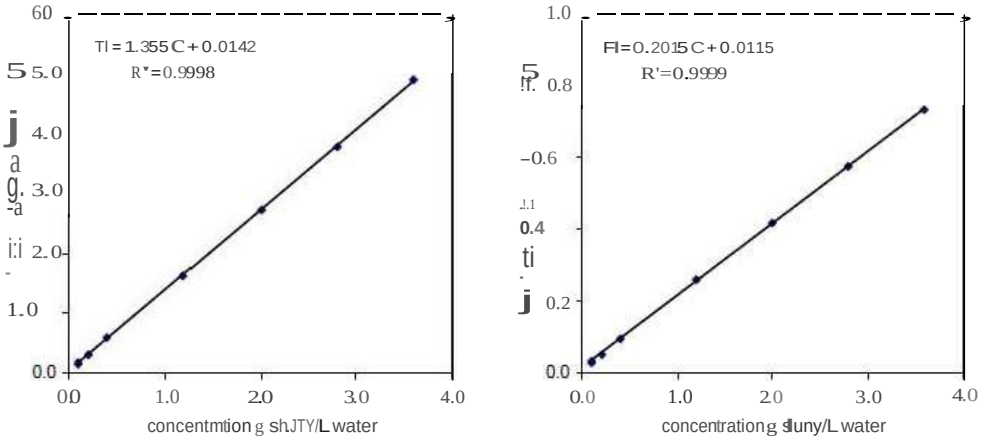
Table 3 Analysis of variance table for the November storm

Undrained lysimeters: surface/interflow pathway (2 outliers removed)					
Source	Sums of squares	df	Mean square	F	p
Treatment	0.684	1	0.684	0.446	0.541
Error	6.140	4	1.535		
Total	6.824	5	1.365		
Drained lysimeters: surface/interflow pathway					
Source	Sums of squares	df	Mean square	F	p
Treatment	1.005	1	1.005	1.647	0.269
Error	2.441	4	0.610		
Total	3.446	5	0.689		
Drained lysimeters: drain pathway (3 outliers removed)					
Source	Sums of squares	df	Mean square	F	p
Treatment	25.431	1	25.431	31.272	**0.005**
Error	3.253	4	0.813		
Total	28.684	5	5.737		

Figure 1 Rowden Experimental Research Platform (RERP), Devon, UK (adapted from Armstrong and Garwood, 1991); reference numbers of lysimeters are shown-suffix D denotes drained lysimeters and hatched areas indicate lysimeters where slurry was applied.



1 **Figure 2 Tryptophan index** (TI) **and fulviclhumic index** (FI) vs. **sluny concentration** (C).



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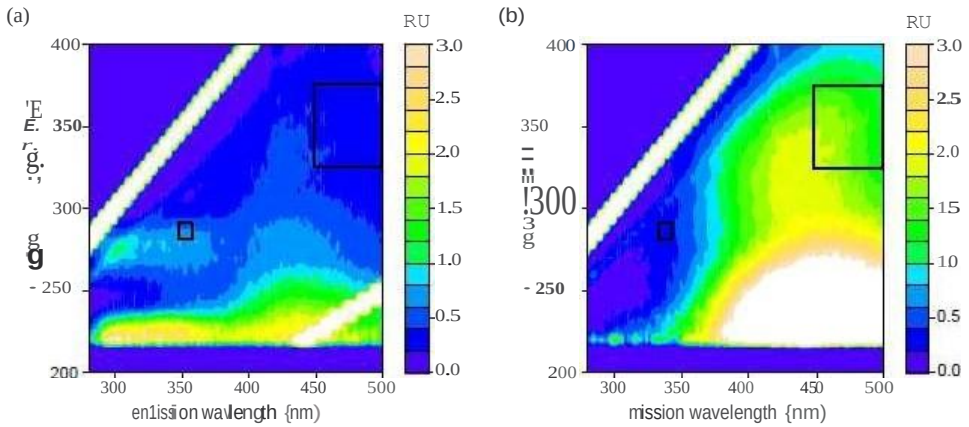
1 Figure 3 Example conected excitation-emission matrices in Raman units: (a) fi·esh slurry

2 1 g L·' water; (b) drain water from lysimeter no. 4 (no slurry). Boxes show areas used to

3 calculate the tryptophan (TI) and fulviclhumic (FI) indices as defined in Table 1.

4



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6

Figure 4 Fluorescence characteristics (tryptophan index, fulvic/humic index and their ratio) and absorbance at 254 nm of the slurry used in the Rowden tracing experiment (A) in the context of other sampled fann slurries (B-J). All values relate to 1 g slurry per Litre of added Milli-Q water.

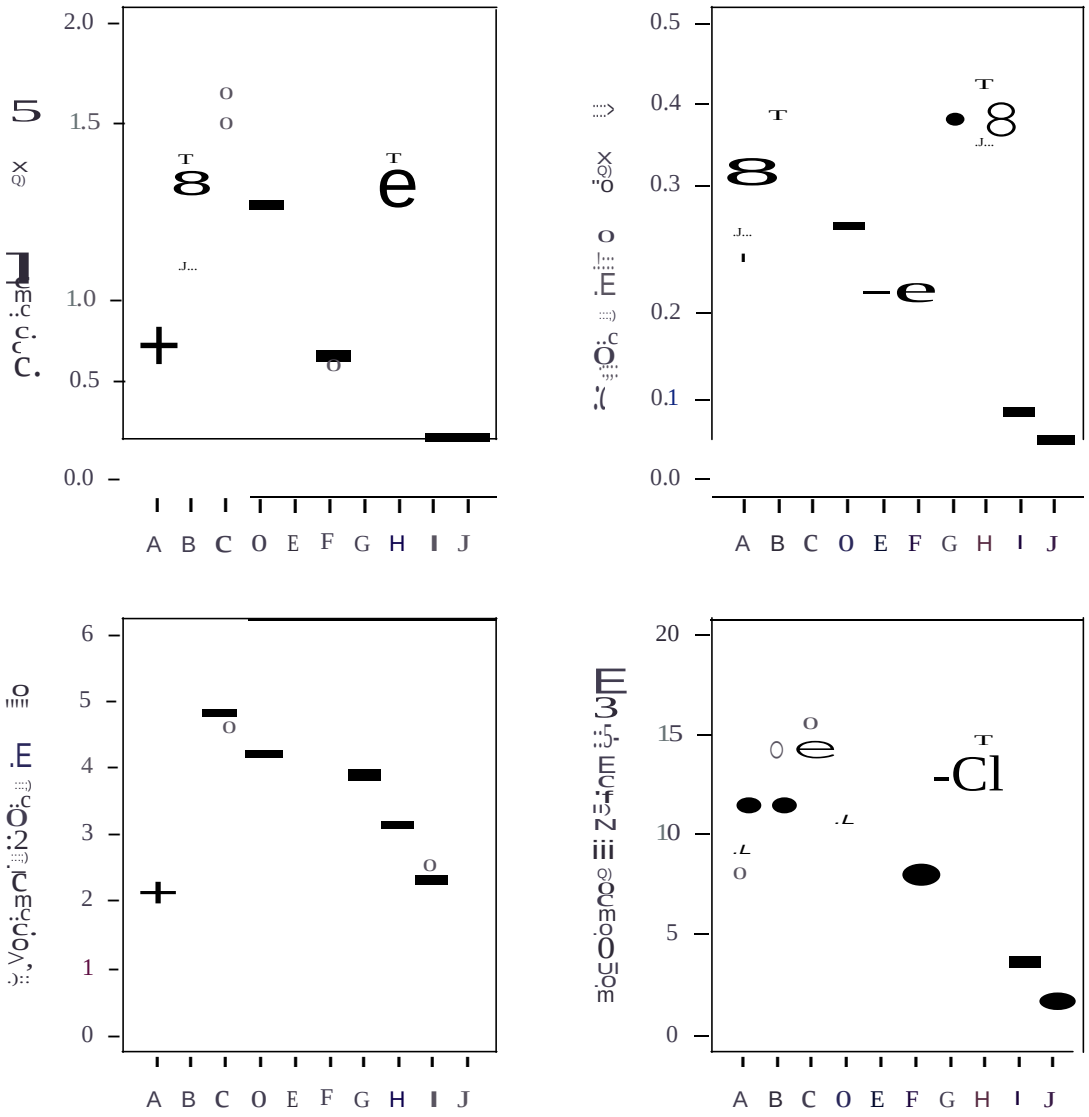

Figure 5 Summary plot of results from RERP experiment autumn 2007. Grey shading indicates applied slurry. Data are arranged in pairs (slurry/no slurry) by drainage path: surface/throughflow pathway of the undrained lysimeters (U), surface/throughflow pathway of the drained lysimeters (S), drain flow pathway of the drained lysimeters (D). For small sample numbers, individual data points are shown (squares); for storm samples, data are summarised by box and whisker plots showing median (thick horizontal line), interquartile range (box), data spread up to 1.5 times the interquartile range beyond the box (dashed line), and outliers (empty circles). The number of samples measured for fluorescence is shown below the graph; the arrow shows when slurry was applied.

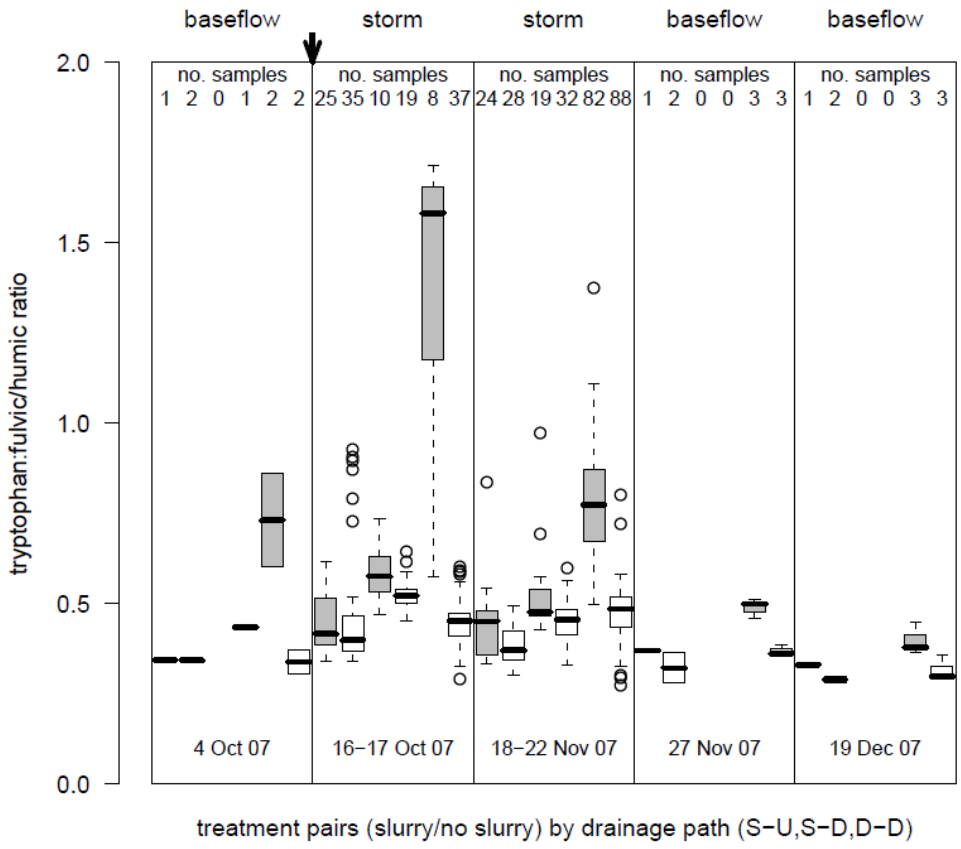

Figure 6 Example results from slurry tray experiment: black filled diamonds show results from tray compartments where slurry was applied; empty diamonds are controls; grey triangles denote applied slurry; all measurements are expressed in terms of 13 g slurry in 100 mL water. Note the different scales for the tray experiment (left-hand axis) and applied slurry (right-hand axis).