Understanding groundwater, surface water and hyporheic zone biogeochemical processes in a Chalk catchment using fluorescence properties of dissolved and colloidal organic matter

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Abstract

Understanding groundwater–surface water (GW–SW) interaction in Chalk catchments is complicated by the degree of geological heterogeneity. At this study site, in southern England (United Kingdom), alluvial deposits in the riparian zone can be considered as a patchwork of varying grades and types with an equally varied lateral connectivity. Some display good connection with the river system and others good connection with the groundwater system and by definition poorer connectivity with the surface water. By coupling tangential flow fractionation (TFF) with fluorescence analysis we were able to characterise the organic matter in the river and hyporheic zone. There is a significant proportion of particulate and colloidal fluorescent organic matter (FOM) within the river system, and at depth within the gravels beneath the river channel. At depth in the hyporheic zone the surface water inputs are dampened by mixing with deeper groundwater FOM. The shallow (0-0.5 m below river bed) hyporheic zone is highly dynamic as a result of changing surface water inputs from upstream processes. Labile C in the form of protein-like FOM appears to be attenuated preferentially compared to fulvic-like fluorescence in the hyporheic zone compared to the adjacent gravel and sand deposits. These preliminary findings have important implications for understanding nutrient and trace element mobility and attenuation within the groundwater, surface water and hyporheic zone of permeable

Chalk catchments. Fluorescence analysis of dissolved organic matter has been shown to be a useful environmental tracer that can be used in conjunction with other methods to understand GW-SW processes within a permeable Chalk catchment.

Keywords

Dissolved organic matter (DOM); Colloids; Fluorescence; Hyporheic Zone; Groundwater–surface water processes; Tangential Flow Fractionation (TFF); Chalk

1. Introduction

The importance of groundwater (GW), surface water (SW) and hyporheic zone processes has been appreciated for some time [*Winter et al.*, 1998]. These processes may vary depending on the hydrology, landuse, ecological sensitivity and management of a particular system. Understanding these processes is therefore important: to determine the sustainable limits of abstraction in a water sensitive system [*Cook et al.*, 2003]; to understand the hydrological function of the riparian zone [*Fernald.*, 2001; *Krause and Bronstert*, 2007; *Krause et al.*, 2007]; and/or to assess the function of the riparian zone in regulating biogeochemical processes [*Findlay et al.*, 1993; *Hill*, 1996; *Jones et al.*, 1995; *Jarvie et al.*, 2006; *Rassam et al.*, 2006]. The hyporheic zone is a hydrobiologically defined region below the river bed where surface and subsurface water mix. In the hyporheic zone nutrients (e.g. C, N and P) and weathering products may be exchanged due to upwelling or downwelling. Dynamic gradients exist at different scales in this zone, e.g. chemical and microbiologically mediated nutrient and weathering transformations on particle surfaces [*Boulton et al.*, 1998; *Gooseff et al.*, 2002; *McKnight et al.*, 2002; *Greenwald et al.*, 2008].

Dissolved organic matter (DOM) includes compounds such as carbohydrates and proteins, which are structurally well-defined, and less well chemically defined compounds with higher molecular weight such as humic substances which include fulvic and humic acids [*Thurman*, 1985]. Recent studies suggest that humic substances are a complex mixture of both microbial and plant biopolymers, with their various breakdown products, and as such are highly heterogeneous in nature [*Kelleher and Simpson*, 2006]. In the natural environment DOM is a complex mixture of many compounds and as such is difficult to characterise [*Leenheer and Croue*, 2003]. DOM has a range of important functions in the aquatic environment as both a source of energy for micro-organisms and in its role in the transport of metals and organic contaminants [*McKnight et al.*, 1992; *Benedetti et al.*, 1996]. The structure, molecular weight, composition and abundance of DOM in the environment controls many processes including mobility and chemical reactivity as well as water treatment [Her et al., 2003].

Fluorescence spectroscopy can be used to quantify and understand the composition and temporal–spatial variability in DOM [*Jaffe et al.*, 2008]. It has been used in many different ecosystems to understand the source and composition of DOM including marine, wastewater, surface water, groundwater and terrestrial studies [*Coble*, 1996; *Reynolds and Ahmad*, 1997; *Baker,* 2001; *McKnight et al.*, 2001]. Organic matter (OM) fluorescence has been used to trace flow in groundwater systems including recharge processes in karstic aquifers [*Baker and Genty*, 1999; *Baker and Lamont-Black*, 2001; *Eniko et al.*, 2004]. It has been used to determine the source of stormflow in granitic systems [*Katsuyama and Ohte*, 2002], and to investigate flow processes and sources of dissolved organic matter in sandstone systems [*Lapworth et al.*, 2008a]. The rapid collection of high-resolution fluorescence data at multiple excitation-emission wavelengths has led to the development of excitation-emission matrices (EEMs) to represent the fluorescence intensity of a sample in optical space. This method is rapid, requires small sample volumes (<5 mL) and is non-destructive. Together these factors make it an appropriate method for characterisation of fluorescent OM (FOM) in natural waters.

Historically many studies of GW–SW interaction in the UK have focussed on upland catchments [e.g. *Soulsby et al.*, 2002; *Lapworth et al.*, 2008b]. However, there has been a growing focus on the hydrological function of permeable aquifers [*Wheater and Peach.*, 2004] due in part to a lack of fundamental understanding of GW–SW processes in these systems and the increasing management pressures on lowland aquifers. The contribution to river flow of groundwater sources in Chalk catchments is significant, in some cases as high as 95% [*Sear et al.*, 1999] sustaining river flow during periods of reduced rainfall. A greater understanding of GW–SW processes is also required in light of the European Water Framework Directive [*CEC*, 2000], which demands that all water bodies achieve targets for good chemical and ecological status. This necessitates a holistic approach to the management and understanding of catchment hydrology. Recent studies have highlighted the complexity of GW-SW processes both in terms of spatial scales and temporal variability [*Grapes et al.*, 2005; *Gooddy et al.*, 2006; *Griffiths et al.*, 2006; *Pretty et al.*, 2006; *Krause et al.*, 2007]. These have shown that the traditional classification of a particular river section as either gaining or losing is over-simplistic.

The aim of this paper is to demonstrate the value of organic matter fluorescence as a tracer understanding GW–SW processes in a lowland Chalk catchment. The paper focuses on characterising the mixing processes within the wider riparian zone, the sand and gravel deposits adjacent to the river, and within the hyporheic zone below the river channel. The study also investigates the lateral extent of GW–SW processes in the alluvial groundwater system in terms of spatial and temporal variability. The combined use of tangential flow fractionation and organic matter fluorescence is used to characterise and investigate the fluorescence properties of the suspended colloidal organic matter within the hyporheic zone. This has important implications for contaminant transport, transformation and possible attenuation at the GW-SW interface.

2. Site Description

2.1 Study site, Regional Geology and Hydrogeology

The Westbrook Farm study site is located in rural Berkshire (NGR 442900 172200), southern England (UK) and is one of the observation sites from the LOwland CAtchment Research (LOCAR) network [*Wheater and Peach*, 2004]. The site has a network of piezometers (some of which are multi-level) completed in the underlying Chalk and shallow deposits located on either side and below the River Lambourn (Figure 1). The site is located ~13 km downstream of the source of the river Lambourn at Lynch Wood, and has a catchment area of 234 km^2.

The catchment comprises Chalk (Upper Cretaceous) underlain by Upper Greensand (Lower Cretaceous) below which are mud rocks of Jurassic age. The Chalk is overlain by Palaeogene deposits and Quaternary superficial deposits. The Chalk is in hydraulic continuity with the Greensand which is sealed at the base by the Jurassic mud. A detailed geological description of the Pang-Lambourn catchment is given in *Aldiss and Royse* [2002]. The upper reaches of the River Lambourn show ‘bourne’ or ephemeral behaviour, while the lower reaches show perennial behaviour. This is supported by groundwater inputs and a slow hydrological response to rainfall that is typical of lowland permeable catchments.

2.2 Conceptual hydrological model at the study site

Gooddy et al., [2006] investigated groundwater mixing processes in the same location as this study using both CFCs (CFC-11 and CFC-12) and SF_6 in a limited array of piezometers. Three different regimes were identified: Regime 1, in the interfluve where piston flow dominates in the deep unsaturated zone; Regime 2, within a thinner unsaturated zone where there is mixing between up-gradient older groundwater and recent recharge; Regime 3, a zone of GW–SW interaction below and adjacent to the river channel. This conceptual model provides a framework within which the fluorescence data can be understood.

3. Methods

3.1 Groundwater sampling

Groundwater samples were collected using a submersible pump following prolonged purging of at least three piezometer volumes. On-site parameters (dissolved oxygen, pH, redox potential, temperature and specific electrical conductance) were measured and allowed to stabilise prior to sampling. Samples for fluorescence analysis were filtered through 0.45 µm silver filters (Millipore™) into sterile acid washed glass containers and stored refrigerated in the dark at 4° C before analysis.

Sampling sites (piezometers and river samples), response zones for the piezometers, lithology, and number of samples are detailed in Table 1. Sampling for chemical analysis was carried out at the study site in two phases, the first being between November 2005 and November 2006, and the second between February 2008 and May 2008. Two sampling rounds (July 17th and 5th August 2008) in the hyporheic zone were carried out for the tangential flow fractionation work.

3.2 Tangential flow fractionation

Suspended colloidal material can be separated from the dissolved aqueous fraction in two principal ways: filtration and centrifugation. Some studies have used a combination of the two to investigate colloidal particles [*McDowell and Sharpley*, 2001]. Conventional filtration methods such as 0.45µm membrane filters have been used for environmental studies to separate the dissolved and solid fractions. However colloidal particles span a wide range of sizes and are therefore difficult to study using this method. Ultrafiltration (using high pressure) has been used to fractionate samples, but this suffers from concentration polarisation effects and membrane clogging due to colloid aggregation on the surface of the membrane [*Heathwaite et al.*, 2005]. This is a particular problem for anoxic samples with high concentrations of Fe^{2+}, which is not the case at this site.

Tangential-Flow Fractionation (TFF) offers some improvement [G*uéguen et al.*, 2002; *Morrison and Benoit*, 2004], as the tangential arrangement minimises the clogging at the membrane surface. Although better than the classical method of filtration it does not avoid coagulation altogether. Coagulation prior to filtration nonetheless can be minimised if the method is employed in the field. Previous work by *Gooddy et al.* [2007] has used this method successfully in a Chalk groundwater system.

A mass balance for the fulvic acid like fluorescence (FA-like) and tryptophan like fluorescence (TRP-like) was carried out on a Chalk groundwater to assess the potential for adsorption and/or contamination during the TFF [*Mopper et al.*, 1996, *Ross and Sherrell.*, 1999]. Table 2 shows the results (% recovery) for both the FA-like fluorescence and TRP-like fluorescence, see *Ross and Sherrell* [1999] for details on mass balance calculations. In addition blank samples (Ultrapure water - ASTM type I reagent grade water, including a UV cracker) were also used to assess contamination within the system during TFF and these showed no signs of contamination or leaching of fluorescent organic matter from the membranes. It can be seen that reasonable recoveries for the FA-like fluorescence were obtained (101-111 %) but relatively poor recoveries were obtained for the TRP-like fluorescence (84-117 %), implying issues with both adsorption and possible cross-contamination, consequently only the FA-like results from the TFF are presented in this paper.

A Pellicon 2 Millipore™ system was used for TFF with a range of large surface area composite regenerated cellulose filters (nominal cut of, 0.45 µm, 1000 kDa, 10 KDa). A thorough protocol for cleaning membranes was followed [*Guéguen et al.*, 2002].

Fractionation was carried out in the field to avoid any microbial changes to the sample or aggregation of colloidal material. An unfiltered sample and permeate were collected and stored in acid washed glass vials.

3.3 Chemical analysis

DOC analysis was carried out using a Thermalox™ C analyser after acidification and sparging. Bicarbonate analysis was carried out by titration in the field. All fluorescence analysis was carried out within two weeks of sampling, and usually within 48 hours. Repeat analysis of groundwater and river samples after 3 hours and after 1 month showed relative changes of <5 % for protein and FA-like intensities, thus giving confidence in short term sample stability and measurement precision. A Varian™ Cary Eclipse fluorescence spectrometer was used for the fluorescence analysis. Excitation (Ex) wavelengths were set between 200 and 400 nm with a 5 nm bandwidth. Emission (Em) wavelengths were set between 250 and 500 nm with a 2 nm bandwidth. The detector voltage was set to 900 V, and all analysis was done in quartz vials with a path length of 1 cm. All the samples had low DOC concentrations (<3 mg/L) and low absorbance (A_{254} <0.03), and the fluorescence analysis was in the linear range for all components of the Ex/Em matrix, precluding the need for absorbance correction [*Ohno*, 2002]. All fluorescence results are reported in Raman units (R.U), normalised to the area under the water Raman peak of Ultrapure water blanks at Ex350 [*Stedmon et al.*, 2003], and have been blank subtracted. Two fluorescence peaks within the EEM were used in this study to compare intensities between sites: FA-like maximum (Ex330 nm, Em410 nm–460 nm) and the TRP-like maximum (Ex280 nm, Em346 nm–354 nm). These were selected based on the use of tryptophan standards and by visual inspection of the sample EEMs. Data were processed using R software [*R Development Core Team*, 2008; *Lapworth and Kinniburgh*, in press]. Ultrapure water (ASTM type I reagent grade water, including a UV cracker) was used to make up reagents and clean the quartz cell between samples.

4. Results

4.1 Long term DOC concentrations in the River Lambourn

Dissolved organic carbon (DOC) results for the river Lambourn, a site in the shallow gravels (D2) and a deeper piezometer (D1) in the Chalk sampled between November 2003 and March 2008 are shown in Figure 2 (see Table 1 for site details). It can be seen that the DOC concentrations measured in this study are typical of those found at the site over the last four years and are representative of longer term concentrations. Low recharge during the winter months in 2004 and 2005, or high periods of recharge and flooding in late 2007 do not seem to have significantly affected the DOC concentrations in the Lambourn or the local groundwater system. This highlights the dominance of the groundwater component in the River Lambourn and its role in moderating and controlling stream chemistry and composition.

4.2 Organic matter fluorescence and DOC

DOC, FA-like and TRP-like fluorescence (one standard deviation given in brackets where appropriate), TRP:FA, specific electrical conductance (SEC) and bicarbonate ($HCO3^-$) results are shown in Table 3. The results are presented in groups: shallow groundwaters in the alluvial deposits (0–4.7 mbgl), deeper groundwaters (>6.3 mbgl), hyphoreic zone (from beneath the river bed, 1.27–4.25 mbgl) and surface waters. Results are expressed as single mean values; mean values for each group are also shown where appropriate. The fluorescence and DOC results follow the same overall trends where deep groundwater <shallow groundwaters <hyporheic zone <river. Bicarbonate and conductivity results show little variation either within or between groups. There is some evidence that the deep groundwaters show less variability than the shallow groundwaters and river water.

4.3 Temporal variations in fluorescence and DOC

The temporal variations in FA-like and TRP-like fluorescence and DOC over a twelve month period between November 2005 and December 2006 are shown in Figure 3. Results from five sites are shown: A2 and D2 in the shallow sand deposits and gravels adjacent to the river, A1 and D1 within the underlying Chalk aquifer, and the river Lambourn. All three parameters show similar trends for the river and shallow groundwaters. Higher FA-like fluorescence was observed during the winter/autumn months (September-February) in the river and to a less extent in the shallow groundwaters compared to spring and summer months. This could be due to higher surface run-off inputs during winter/autumn, however, the limited amount of available fluorescence data, and the discrete nature of the sampling does make seasonal interpretation difficult and longer data series are needed to verify these observations. The deeper groundwaters show little temporal variation, except for DOC results at site A1 which are anomalously high during the later months of sampling. On half the occasions the gravel site (D2) showed significantly higher FA and TRP-like intensities than sand site A2. During the other sampling rounds there was no significant difference between the two sites. Higher intensities were observed for both sites during the later sampling rounds.

4.4 Spatial variations in fluorescence

Changes in FA and TRP-like fluorescence with depth in the sands, gravels and Chalk piezometers are shown in Figure 4. Intensities of both parameters decrease with depth, although the changes in TRP-like intensities are smaller than those of FA-like. Results for A2 (FA-like) fall to the left of the overall trend and show intensities characteristic of those in the deeper Chalk groundwaters. Results for E2 (TRP-like) fall to the right of the trend and show results that are more characteristic of the river water.

Changes in mean FA-like intensities within the alluvial deposits and in the deeper Chalk along an W–E transect from the road (see Figure 1) are shown in Figure 5. The Chalk groundwaters show a decreasing eastward trend, while the gravels show no consistent trend. At site W and A2 in the sand and gravel deposits the FA-like intensities appear suppressed and more characteristic of Chalk groundwaters, while at Y, E2 and Q the intensities are higher and more characteristic of the River Lambourn.

4.5 Fluorescence variations and size fractionation in the hyporheic zone

FA-like intensity for the whole sample (unfiltered) and permeate fractions in the hyporheic zone sites (R, S and T) and the River Lambourn (see Figure 1 for site details) are shown in Figure 6. Data from two sampling rounds are included in Figure 6 (A). Figure 6(B) shows the changes with depth for the different fractions from samples taken on the August 5th 2008. Overall there is a decrease in FA-like intensity with depth in the hyporheic zone (sites R–T, 0.5–2.5 m below river bed), and a decrease in intensity with progressively smaller nominal TFF cut off sizes. Each sampling round (July 17th and August 5th, 2008) shows distinct size-fraction characteristics which were common in both the hyporheic zone (site R) and the river sample for the higher sized cut off fractions. There was less difference between the FA-like intensities in the <1000 KDa and <10 KDa fractions for samples S and T, which were deeper in the hyporheic zone.

Comparison of the ‘whole’ (unfiltered) sample and the <0.45 µm fraction shows that for most samples, except one of the river samples (August 5th), there was only a small component of FA-like intensity associated with the >0.45 µm fraction (Figure 6(A)). Similar FA-like fluorescence profiles across the different fractions were observed in both the river and the shallow hyporheic zone on the two separate sampling occasions implying that there is connectivity between the river and the hyporheic zone to a depth of at least

1.5 m below the river bed. The second round of sampling (August 5th) showed higher FA-like intensities that were associated with particulate material (defined as >0.45 µm) and a large proportion associated with colloids >1000 KDa. While both rounds showed FA-like intensities indicative of colloidal material, the results show the dynamic nature of the river system and the hyporheic zone, and the temporal variation in the nature of organic matter that is transported within it.

5. Discussion

5.1 *Tracing groundwater-surface water processes*

DOC concentration alone is not a very useful parameter within this Chalk groundwater dominated catchment to understand GW–SW processes (Table 3). However, fluorescence analysis has been able to identify different sources and mixing within the riparian zone. DOC and HCO_3^- show no significant difference across the sites and groups (as is the case for most major and trace elements except Cl^- and SO_4^{2-}) while there is a significant difference between the groups for FA-like fluorescence ($p < 0.05$). DOC is not able to differentiate between the different sources of organic carbon, and the concentrations are attenuated because the flow in the river system is predominantly from groundwater sources. *Abesser et al.*, [2008] reported Cl^- and SO_4^{2-} concentrations that are significantly higher in the alluvial groundwaters than surface water in certain locations (e.g. W, Y, X, N4), in close proximity to a cattle barn, (see Figure 1).

FA-like fluorescence and two dissolved gasses that are groundwater residence time indicators, CFC-12 and SF_6 (see *Gooddy et al.*, [2006]), show a reasonable positive correlation, r^2 of 0.57 and 0.59 respectively. The older the groundwater the lower the FA-like fluorescence, this was also reported by Lapworth et al., [2008] within a Sandstone system in the UK. The fluorescence results are broadly consistent with the conceptual model presented by *Gooddy et al.*, [2006] where the shallow alluvial deposits and weathered Upper Chalk in the riparian zone show flow regimes consistent with GW–SW interactions.

5.2 Temporal changes in groundwater-surface water processes

There is a high degree of temporal variability in fluorescence intensities in both the surface waters and shallow groundwaters (Figure 3). There is no clear seasonality in either the TRP-like fluorescence or the DOC in river samples or shallow groundwaters. The deeper groundwater samples show much less variability. There is also evidence that some alluvial deposits have good connectivity with the groundwater system and poorer connectivity with the surface water system (e.g. A2) showing reduced temporal variability. The variability in fluorescence intensity gives an indication of the mixing and GW–SW processes. This result is perhaps not surprising as the damped signal from a groundwater component would have the effect of moderating any temporal variability from the surface water component. While higher FA-like fluorescence was observed during the winter/autumn months in the river, and to a less extent in the shallow groundwaters, compared to spring and summer it is clear that longer time-series are needed to properly assess the temporal variability of FOM. The poor temporal resolution of discrete sampling methods used in this study limits the process understanding. In situ continuous monitoring of FOM [*Chen,*1999] would greatly aid in understanding the temporal variability, particularly in the surface waters and hyporheic zone which appear to be highly dynamic.

5.3 Spatial changes in groundwater-surface water processes

The degree of GW–SW interaction within the riparian zone at this site seems to be controlled by lithology, topography, and the regional groundwater flow. Sand deposits adjacent to the river (A2) appear to be poorly connected to the river system compared to gravel deposits (e.g. D2), although the variability within the gravels suggests that lateral connectivity with the river system is spatially complex and dynamic in nature (Figure 3 and 5). This is not surprising as the alluvial deposits can be considered as a patchwork of varying grades and types with an equally varied lateral connectivity. Some regions displaying good connection with the river system and others better connection with the groundwater system. Some gravel deposits (e.g. E2) show very good connectivity with the river system (Figure 5), possibly controlled by the regional groundwater flow conditions and topographical features. These findings corroborate other studies that have established that the Lambourn does not simply gain uniformally along its reach [*Grapes et al*., 2005; *Griffiths et al*., 2006] but rather shows a complex mixture of gain and loss which varies over relatively small distances.

A cross plot between FA-like and TRP-like intensities for each group is shown in Figure 7. There is a weak linear relationship between the two components ($R^2 = 0.4$, $p < 0.05$). The relationship can be understood in terms of mixing (exponential) between groundwaters of differing residence times in the underlying Chalk system, and mixing between the groundwater system (with a range of residence times) and the river water (also with a range of residence times). The shallow groundwaters in the hyporheic zone and sand and gravel deposits adjacent to the river channel fall broadly between the river water and the deeper groundwaters, although there seems to be some attenuation of protein like fluorescence in the hyporheic zone compared to other alluvial deposits adjacent to the river system.

The river water is a complex mixture of waters of various residence times, as is the groundwater system. These waters mix within the shallow groundwater system and hyporheic zone to differing extents depending on seasonal changes in heads within the groundwater and its relation to the river stage, as well as the localised controls on hydraulic conductivity within the alluvial system.

While there is separation between the different groups based on mean TRP-like and FA-like fluorescence, there is some overlap between the sand and gravel sites and the Chalk sites (Figure 7). For example; sites A2 and W fall closer to the Chalk groundwater end member and this may indicate that they are poorly connected (laterally) to the river system compared to the other localised shallow deposits. The Chalk samples with the most river-like signal are from sites N15 and X which can be understood by two possible causes. Site N15 is open-cased from the rest water level to 15 mbgl and therefore represents a mixture of water from a range of different depths and lithology, not purely groundwater from the underlying Chalk at 15 mbgl. Site X is the shallowest Chalk site (6.3–9.7 mbgl) and is therefore also in closest proximity to the weathered zone of the Upper Chalk (ca. 7 mbgl). This site may have good connectivity with the gravels at site Y which show the highest FA-like intensities, possibly from a local source as was suggested by *Abesser et al.*, [2008] based on the Cl^- and SO_4^{2-} and NO_3^- anomalies.

There are changes in landuse along the transect (Figure 1), some areas are wooded and others are covered by grazing. The wooded area (largely covered by poplars) is mature and several trees have been felled in recent years due to their instability. It is possible that the elevated Cl^-, SO_4^{2-} and NO_3^- concentrations found within close proximity to the wooded site are a result of felling activities [*Neal et al.*, 2003] rather than contamination from fertilisers as suggested by *Abesser et al.*, [2008]. The higher FA-like fluorescence results at site Y and E2 (Figure 5) could be the result of breakdown and release of organic matter from litter horizons and felling debris, and insufficient biological uptake [*Hughes and Reynolds*, 1991; *Thiffault et al.*, 2008]. Although this is a possibility a more detailed assessment of the effects of landuse on the export of organic matter at this site is needed to verify this hypothesis. Examination of the location of the FA-like (maximum) across the transect W–Q showed that there was no significant change in terms of excitation or emission wavelength, maxima were clustered around Ex330, Em422, suggesting little variation in the nature and source of FA-like organic matter along the transect [*Her et al.*, 2003]. There are no trends in TRP:FA ratios along the transect either to indicate a different source of DOM.

5.4 Tangential flow fractionation

The results from the TFF indicate that there is a significant proportion of particulate and colloidal fluorescent organic matter (FOM) within the river system, and at depth within the hyporheic zone in the gravels beneath the river channel (Figures 6 (A) and (B)). Using a range of different filters on two occasions with contrasting proportions of colloidal FOM enabled us to investigate the FOM and show that there is good connectivity between the gravels (>0.5 m below river bed) in the hyporheic zone and the river channel. The proportion of particulate (>0.45 μm) and colloidal (<0.45–1000 KDa) FOM decreased with depth in the hyporheic zone. In contrast the dissolved component (<1000–<10 KDa) shows little change with depth (Figure 6 (B)).

The proportional change in FA-like fluorescence with depth in the whole sample and <0.45 μm fraction remained approximately constant (Figure 6 (A) and (B)). One possible reason for this is that this change is due to mixing from two end members, upwelling groundwater and downwelling river water. Piezometric head data in the hyporheic zone shows an increase with depth and this also suggests that mixing is possible. Another reason for this observation may be that colloidal and particulate FOM is being attenuated in the hyporheic zone by physicochemical processes, adsorption onto clays and organic matter, and biogeochemical processes, being used by microbes as a source of energy. Based on field Eh/pH and dissolved oxygen measurements and water chemistry, the hyporheic zone does not appear to have distinct oxic/anoxic interfaces, pH gradients or the associated oxide precipitates. Therefore, attenuation of FA-like organic matter by adsorption onto surface oxides may not be a major factor in controlling the mobility of organic matter in the hyporheic zone as it is in other sites [*McKnight et al.*, 2002].

Maximum values for all fractions of samples were centred at Ex330-Em422, indicating that the fractionation did not separate various components of the FA-like signature that were otherwise overlapping in the bulk sample, and the same fluorophores responsible for the FA-like intensity were associated with particles and colloids across a wide range of molecular sizes. The TRP:FA ratios in the hyporheic zone show a decrease in the shallow gravels (0.19) compared to the river system (0.34), and an increase thereafter with depth to values consistent with Chalk groundwaters. This suggests that the labile protein-like FOM is being preferentially removed within the shallow hyporheic zone (0-0.5 m below river bed). Attenuation of labile C is likely to be occurring within the natural biofilm of the stream bed, as well as on clay surfaces, acting as a sink, and possibly a source of labile C (and dissolved organic nitrogen) under changing temporal conditions [*Marmstrong and Barlocher*, 2006]. Clearly more detailed work is needed to explore these preliminary findings further, and properly understand the spatial and temporal variability of the labile C dynamics in the hyporheic zone.

6. Conclusions and Implications

Fluorescence analysis has been able to pick out unique signatures from different water sources and mixing within the alluvial deposits and hyporheic zone. While DOC concentration (and major ion chemistry, see *Abesser et al.*, [2008]) are of limited use as environmental tracers to understand GW-SW processes within this Chalk groundwater dominated catchment. Alluvial deposits in the riparian zone can be considered as a patchwork of varying grain sizes and types with an equally varied lateral connectivity.

Some display good connection with the river system and others better connection with the groundwater system.

The riparian zone can be considered as a zone of mixing with relatively high spatial and temporal variability. The variability in fluorescence intensity gives an indication of the mixing and GW–SW processes in the alluvial deposits and hyporheic zone. The groundwater component has the effect of moderating temporal variability from the surface water component.

Coupling the TFF with fluorescence analysis was useful for investigating the FOM in the river and hyporheic zone. This suggested that there is good connectivity between the surface water to depth within the underlying gravel deposits. There is a significant proportion of particulate and colloidal fluorescent organic matter (PFOM, CFOM) within the river system, and at depth within the shallow gravels beneath the river channel. Changes in TRP: FA from within the river and the shallow hyporheic zone suggest that labile protein-like FOM is being preferentially attenuated. Deeper in the hyporheic zone the surface water inputs are diluted by greater mixing with older groundwater FOM. The composition of organic matter in the shallow (0-1.5m) hyporheic zone is temporally dynamic in nature as a result of changing upstream processes such as waste water inputs, surface runoff and landuse practices which deliver variable concentrations and types of dissolved, colloidal and particulate FOM. While these preliminary findings have important implications for understanding biogeochemical processes and nutrient attenuation within the hyporheic zone, more detailed work is needed to verify these findings, and properly understand the spatial and temporal variability of the labile C dynamics in the hyporheic zone.

Coupling fluorescence EEM analysis to TFF, and other techniques such as size exclusion chromatography, provides powerful ways to characterise organic matter, and further the understanding of processes in the natural environment. Future work should focus on understanding the nature and dynamics of organic matter and trace element mobility within the shallow hyporheic zone and soil zone, as well as the impacts of landuse change and waste water inputs into the river and groundwater system. This would be aided greatly by the use of in-situ FOM monitoring techniques [*Chen*, 1999] which would enable much better understanding of these dynamic processes.

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Figure captions

Figure 1. Location of the Westbrook Farm study site and piezometer array. Hyporheic zone piezometers are labelled R-T.

Figure 2. Long term dissolved organic carbon (DOC) time series in the river Lambourn and selected groundwaters, site D1 is in the Chalk aquifer and site D2 in the shallow gravels adjacent to the Lambourn.

Figure 3. Fluorescence and DOC time series for selected sites in the Chalk (D1 and A1), the gravel deposits (D2) and the sand deposits (A2) at Westbrook Farm. Error bars represent the maximum estimated relative standard deviation (+/- 10%) from a combination of the sampling and analytical error, which was estimated by repeated sampling and analysis (n=2) at all the sites on two separate occasions.

Figure 4. Changes in average FA-like and TRP-like fluorescence intensity with depth. Sites A2 and E2 are indicated on the plot, mbgl = meters below ground level.

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Table 1. Summary of site details, response zones, lithology, number of samples, and sampling phase.

Table 2. Tangential flow fractionation mass balance recoveries for FA-like and TRP-like fluorescence in a Chalk groundwater sample.

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Tables

Table 1. Summary of site details, response zones, lithology and number of samples.

Site ID	Response zone (m)	Lithology	Samples
Shallow groundwaters			
A2	0-1.8	Sand	9
Q	1.4-2.5	Sandy gravel	2
Y	1.5-3.6	Gravel	1
P	1.6-3.3	Gravel/sand	2
D2	0.7-3.8	Gravel	8
N4	RWL-4	Gravel	3
E2	0-4.7	Gravel	1
V	2.3-5.3	Gravel	1
W	1.5-5.6	Gravel	1
Deeper groundwaters			
N7	RWL-7	Putty Chalk	3
X	6.3-9.7	Chalk	1
N15	RWL-15	Chalk	3
A1	13.2-24	Chalk	9
D1	10.7-25	Chalk	9
E1	11.5-25.2	Chalk	1
Hyphoreic Zone			
R	1.27-1.47	Gravel	3
S	3.01-3.21	Gravel	3
T	4.05-4.25	Gravel	3
Surface waters			
River Lambourn			12

RWL = Rest Water Level, depth in meters below ground level

Table 2. Tangential flow fractionation mass balance recoveries for FA-like and TRP-like fluorescence in a Chalk groundwater sample.

TFF Filter*	**FA-like**	**TRP-like**
	Recovery%	
0.45µm	101	84
1000 KDa	101	117
10 KDa	111	84

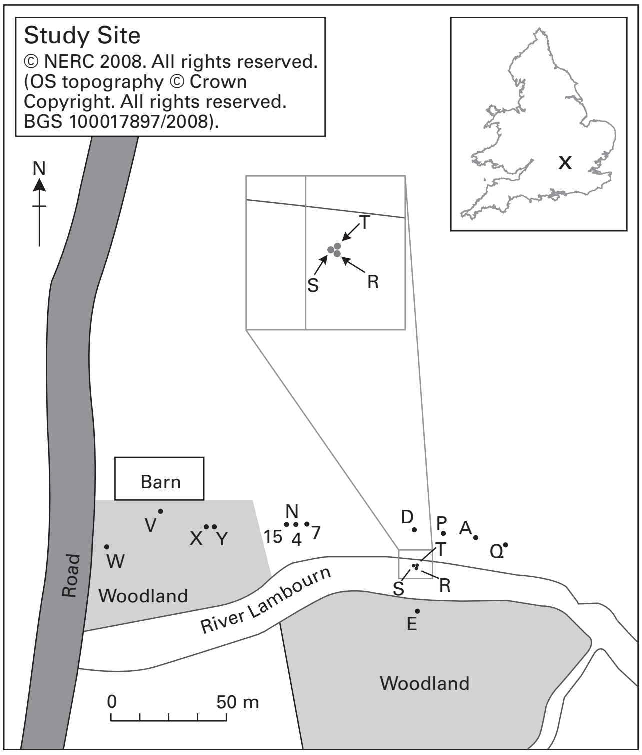
* Nominal cut-off

Table 3. Summary of dissolved organic carbon (DOC), fluorescence intensity and chemistry results collected at the Westbrook Farm site (November 2005–May 2008).

Site ID	**DOC** $mg\ l^{-1}$	**FA-like** R.U	**TRP-like** R.U	**TRP:FA**	**T** °C	**SEC** $\mu S\ cm^{-1}$	HCO_3 $mg\ l^{-1}$
Shallow groundwaters							
A2	1.0	0.24	0.08	0.34	10.8	590.5	301.5
Q	0.9	0.30	0.09	0.29	9.8	625.0	300.2
Y	0.7	0.34	0.08	0.24	10.3	649.0	298.7
P	0.8	0.27	0.07	0.25	9.6	618.2	295.2
D2	0.8	0.29	0.09	0.32	10.6	594.6	296.9
N4	0.7	0.28	0.09	0.31	10.2	645.4	299.6
E2	0.7	0.32	0.20	0.61	10.7	615.5	302.1
V	0.6	0.28	0.08	0.28	10.6	642.0	287.1
W	0.9	0.25	0.06	0.22	9.6	655.0	307.2
Mean (SD)	0.8 (+/- 0.13)	0.29 (+/- 0.03)	0.09 (+/- 0.04)	0.32	10.2	626.1	298.7
Deep groundwaters							
N7	0.7	0.27	0.11	0.40	10.5	608.0	291.3
X	0.6	0.26	0.06	0.24	10.8	630.0	298.0
N15	0.7	0.23	0.08	0.34	10.3	599.8	300.9
A1	1.0	0.20	0.07	0.36	10.6	582.2	297.4
D1	0.9	0.22	0.06	0.26	10.6	577.5	291.0
E1	0.5	0.18	0.07	0.39	10.6	448.2	300.2
Mean (SD)	0.8 (+/- 0.19)	0.22 (+/- 0.03)	0.07 (+/- 0.02)	0.32	10.6	567.5	297.5
Hyphoreic zone							
R	0.8	0.38	0.07	0.19	10.0	626.2	295.7
S	0.6	0.30	0.09	0.29	10.2	561.3	294.6
T	0.7	0.28	0.09	0.32	9.9	617.8	301.6
Mean (SD)	0.7 (+/- 0.10)	0.32 (+/- 0.05)	0.08 (+/- 0.01)	0.27	10.0	601.8	297.3
Surface water							
R. Lambourn	1.2 (+/- 0.3)	0.45 (+/- 0.08)	0.15 (+/- 0.09)	0.34	9.6	538.8	270.1

FA-like = fulvic acid like intensity, TRP-like = Tryptophan like intensity, R.U = Raman Units, SEC = Specific Electrical Conductance, SD = one standard deviation

Figures



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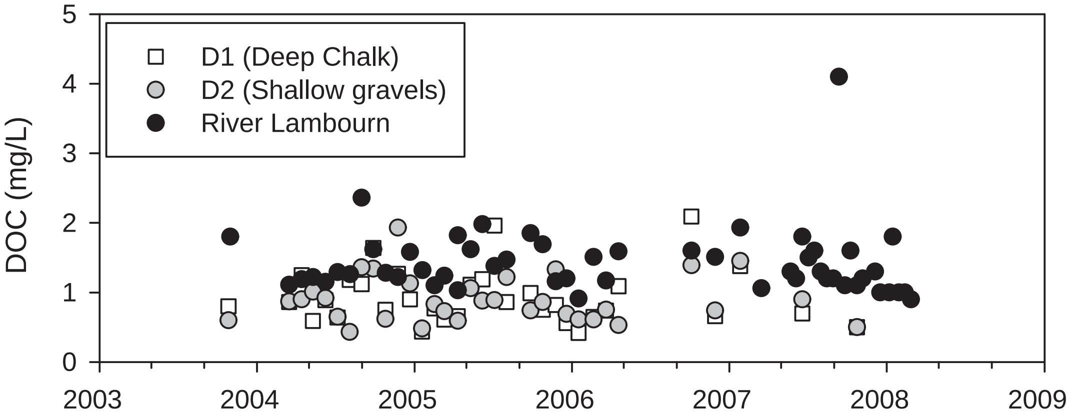

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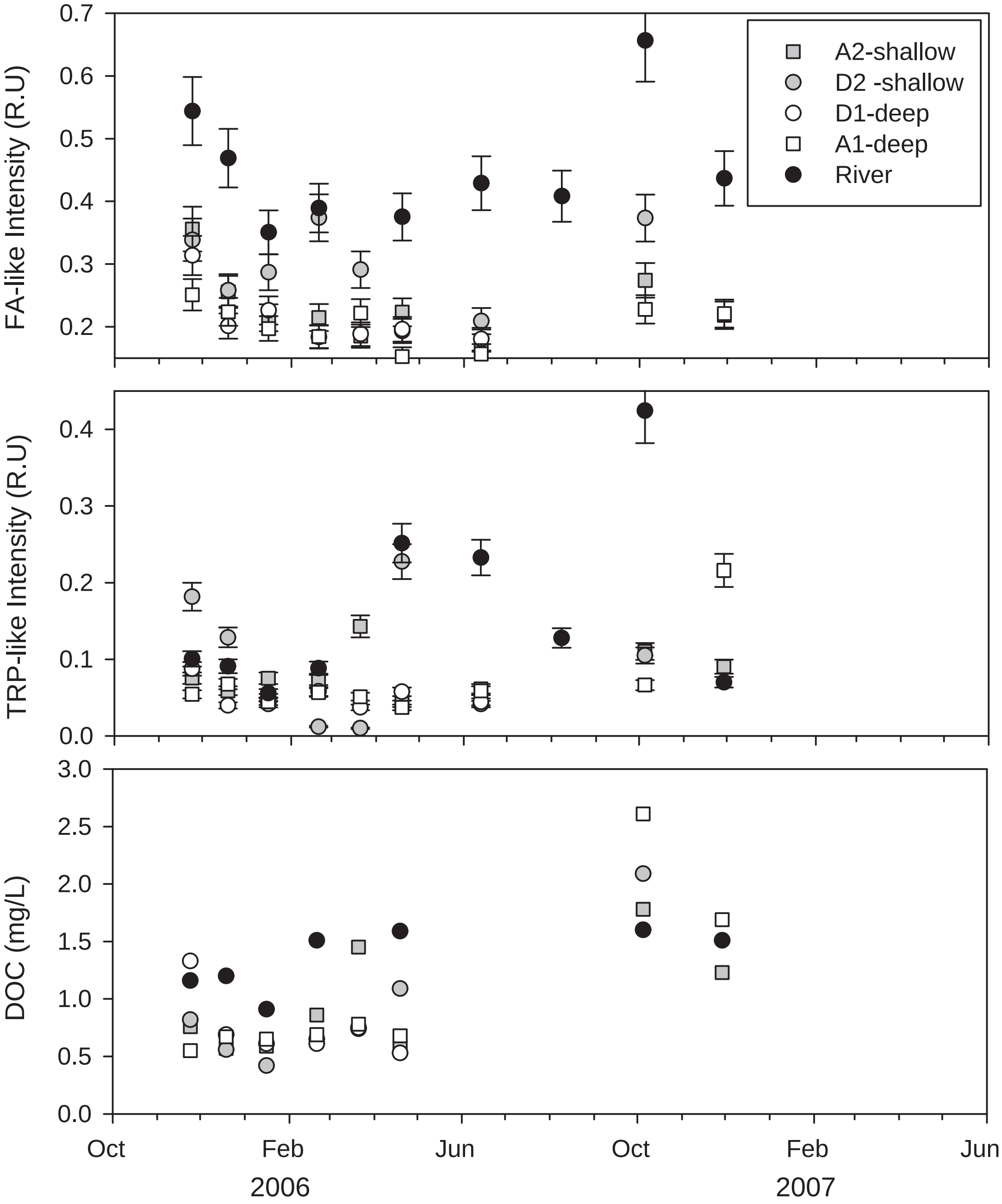

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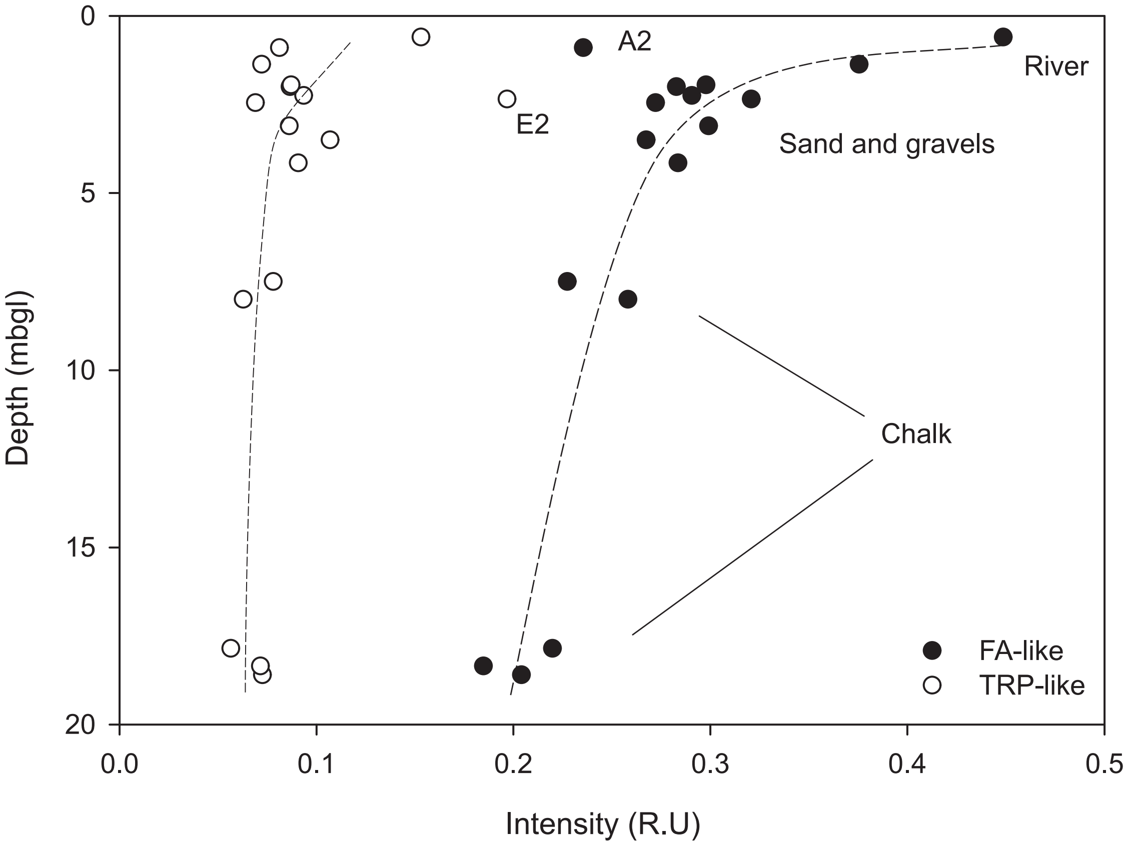

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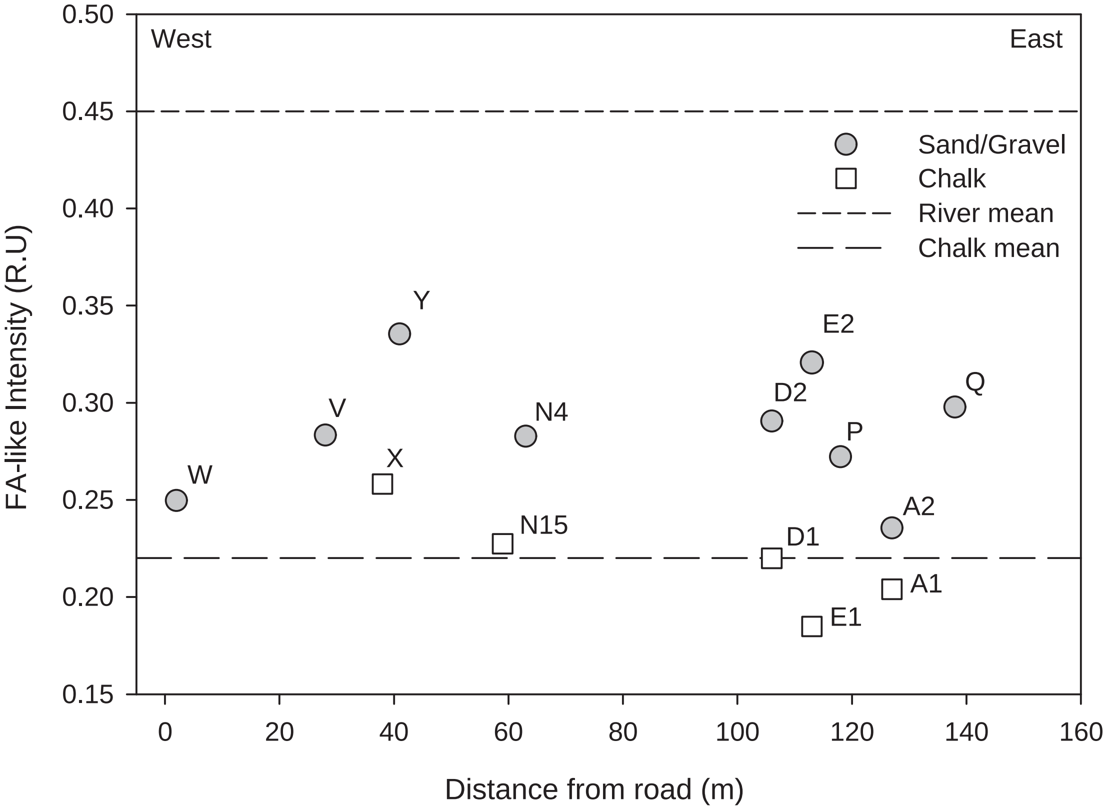

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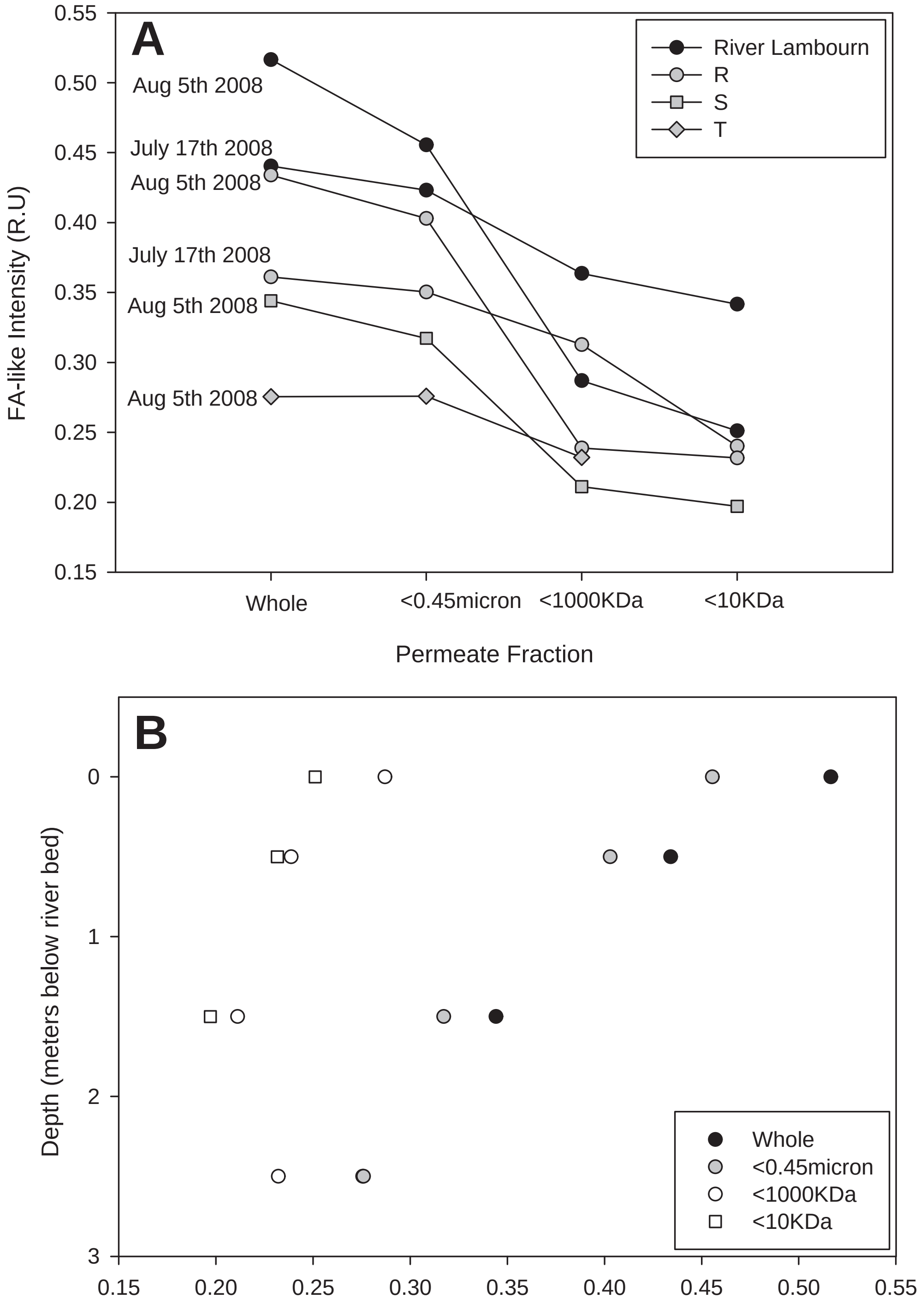

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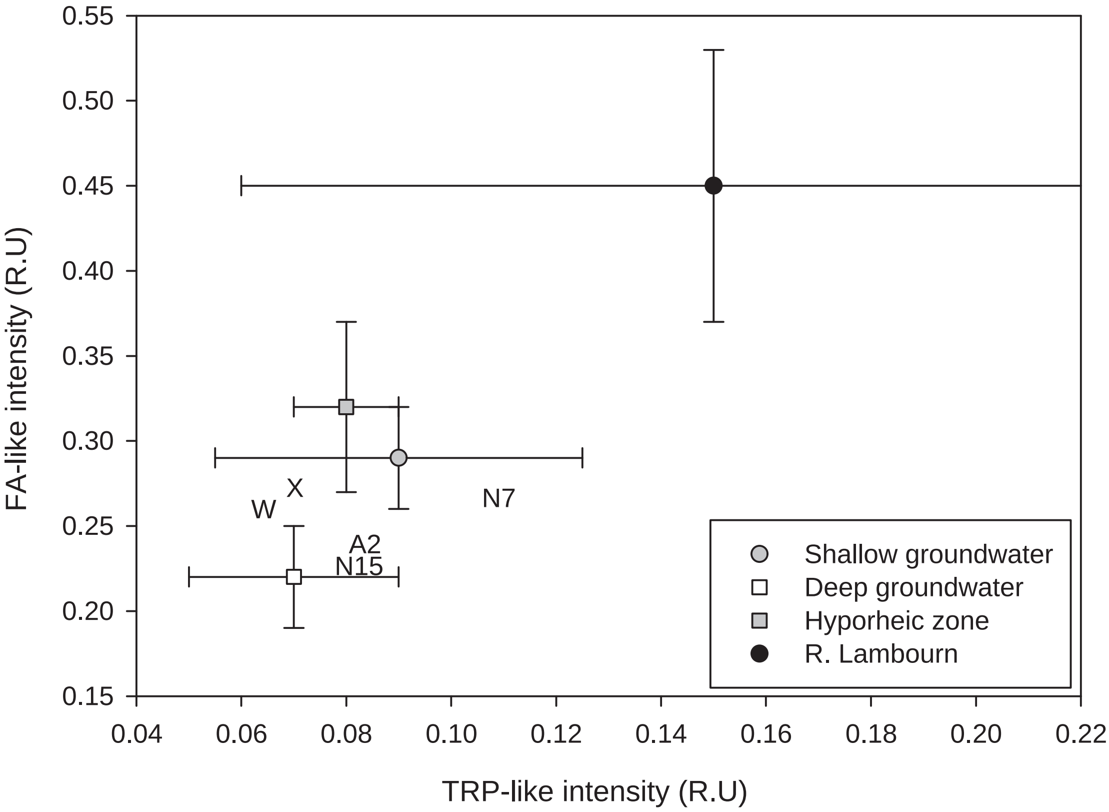

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