Characterising changes in fluorescence properties of dissolved organic matter and links to N cycling in agricultural floodplains

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ABSTRACT

Sand and gravel aquifer systems are common features below and adjacent to river networks and are important in providing a hydrological link between terrestrial and aquatic ecosystems. They are often used for intensive agriculture and therefore provide a conduit for the transport of nutrients to aquatic systems. Understanding the biogeochemical cycles of C and N in such systems is essential in efforts to improve water quality, with a major link being the transfer and properties of DOM which drives microbial processing of nutrients. In this work undertaken in the Trent Valley, U.K., the differences in dissolved organic matter (DOM) properties in the soil, aquifer ground water and river water are examined using excitation–emission matrix fluorescence spectroscopy (EEM), in addition to chemical analysis. In the aquifer system studied the water table is ~1.5 m below the land surface, suggesting that DOM and nutrient transfer would occur between the soil and groundwater. Nitrate levels in the ground water were ~50 mg L^{-1} NO$_3$-N. Soil DOM properties were measured in KCl extracts used to extract inorganic and organic N so that further information could be obtained regarding possible sources and properties of the DON and the biodegradability of the DOC pool. Within the soils tested, fulvic-like properties and the humification index (HI) were significantly ($P < 0.05$) higher in sandy soils suggesting that microbial accessibility to organic matter was greater. The applicability of assessing DOM characteristics in the different matrices were assessed and quantitative or qualitative comparisons made. Properties of DOM in both the soils and aquifer waters all showed a typical terrestrial source with principal component analysis (PCA) showing strong correlations between DOC, fulvic-like properties, the HI and tryptophan-like properties. A major finding was that the HI was generally higher in the groundwater, suggesting continued processing of DOM within the aquifer whilst qualitative comparisons showed the groundwater possessed a higher fluorescence index (FI). Whilst DON and the tyrosine-like proteins were correlated in the soil, the tryptophan-like proteins were strongly correlated to fulvic-like substances in both the soil and groundwater. This suggested that phenolic compounds were also being identified in this part of the EEM matrix, emphasising the need for additional compound analysis to fully understand the nature of DOM in these systems.

1. Introduction

Dissolved organic matter (DOM) represents the most mobile phase of organic matter in the environment, consisting of a range of organic compounds (e.g. carbohydrates, proteins, fulvic and humic acids) each with different reactivity. The flux of DOM from soils and plants is the dominant link between terrestrial and aquatic systems where it plays a major role in a wide range of ecological processes and geochemical reactions in waters, soils and sediments (Jaffe et al., 2008). Whilst the concentration of dissolved organic carbon (DOC) provides information regarding variation in seasonal and annual fluxes of carbon, the characterisation of DOM provides information regarding the source, composition, reactivity and potential utilisation in biogeochemical processes. Whilst DOM provides an energy source, it also participates in light absorption, the transport and buffering of metals, organic contaminants, radionuclides and aluminium, initiating photoreactions, producing hydrophobicity and determining particle surface and colloidal chemistry, ionic balance and pH (Gondar et al., 2007; Thacker et al., 2005, 2008). Identifying its source, transformations and preservation mechanisms are therefore important when assessing terrestrial C cycling,

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particularly in aquatic systems where it generally represents the largest pool of detrital carbon (Jaffe et al., 2008).

Spatial and temporal changes in DOM properties as it passes through hydrological components in a landscape can provide insights into biogeochemical processes and the potential transfer of energy sources of C. One technique that has been used extensively to compare DOM properties within aquatic ecosystems is that of UV visible absorbance and fluorescence spectroscopy (Hood et al., 2006; Fellman et al., 2008). The method provides a rapid assessment of DOM properties such as aromaticity, humification and protein-like molecule composition and has been applied to analysis of marine, surface, ground and terrestrial waters (Hood et al., 2005; Baker and Lamont-Black, 2001). It is also used to determine the DOM source, primarily distinguishing between DOM of microbial or plant origin and has been used to identify farm yard wastes in aquifers and streams (Lapworth et al., 2008; Old et al., 2012). Previous work using fluorescence spectroscopy has examined changes in DOM within and across ecosystems where biogeochemical reactions, the hydrological regime, land cover and land management practices can determine DOM biogeochemistry (e.g. Jaffe et al., 2008; Rhymes et al., 2015). Comparing DOM composition across 12 biomes in the US, Jaffe et al. (2008) found the optical properties of DOM to be highly variable and influenced by physical (hydrology), chemical (photo-reactivity/redox status) and biological (primary productivity) factors. They found no general correlation between DOM quantity and quality, but the variation in fluorescence signature was mainly a function of DOM source and biogeochemical processes controlling redox state. A study by Wilson and Xenopoulos (2009) found that DOM characteristics in 34 watersheds were related to agricultural land use, nitrogen loading and wetland loss. They found that the degree of structural complexity of DOM decreased as the ratio of continuous cropland to wetland

increased. Concomitantly, the amount of bacterially produced DOM increased with agricultural land use. Temporal changes have been found in DOM properties in streams reflecting seasonal events such as snow melt and storm flushing (Fellman et al., 2009; Hood et al., 2005). Hosen et al. (2014) recently investigated changes in DOM fluorescence properties as a function of urbanisation within 8 watersheds in the US and found a link between urbanisation and changes in DOM quality in surface waters. Singh et al. (2014) used fluorescence spectroscopy to trace C inputs from poultry farms and changes in DOM quality in surface waters.

In this paper we focus on the use of UV visible absorbance and fluorescence spectroscopy to determine the differences in DOM properties that occur between different components of a soil–shallow aquifer–river system in the River Trent valley, Nottinghamshire, UK. These types of shallow aquifers are common in the UK, and act as an important interface between terrestrial and aquatic ecosystems as they are hydraulically linked to river systems (Stuart and Lapworth, 2011; Gooddy et al., 2014). They can act as both a source of DOM to the river, e.g. during base flow conditions, or a sink during high flow conditions (Pretty et al., 2006; Lapworth et al., 2009, 2011; Allen et al., 2010). With the addition of DOM from the soils above to drive microbial processes there is the potential for significant nutrient transformation in the aquifer (e.g. denitrification). Therefore, because the N and C cycles are intimately connected (Grant, 1995) it is important to understand their linked inputs, geochemical processes and outputs within these aquifer systems, so that improved land management decisions can be made to achieve compliance with legislative drivers (e.g. Nitrates Directive 91/676/EEC, 1991; Water Framework Directive 2000/60/EC). The aims of this work were to (i) examine the differences in DOM characteristics in the soil, aquifer groundwater and river water components of these systems, (ii) to assess the relationships between C and N within each component and
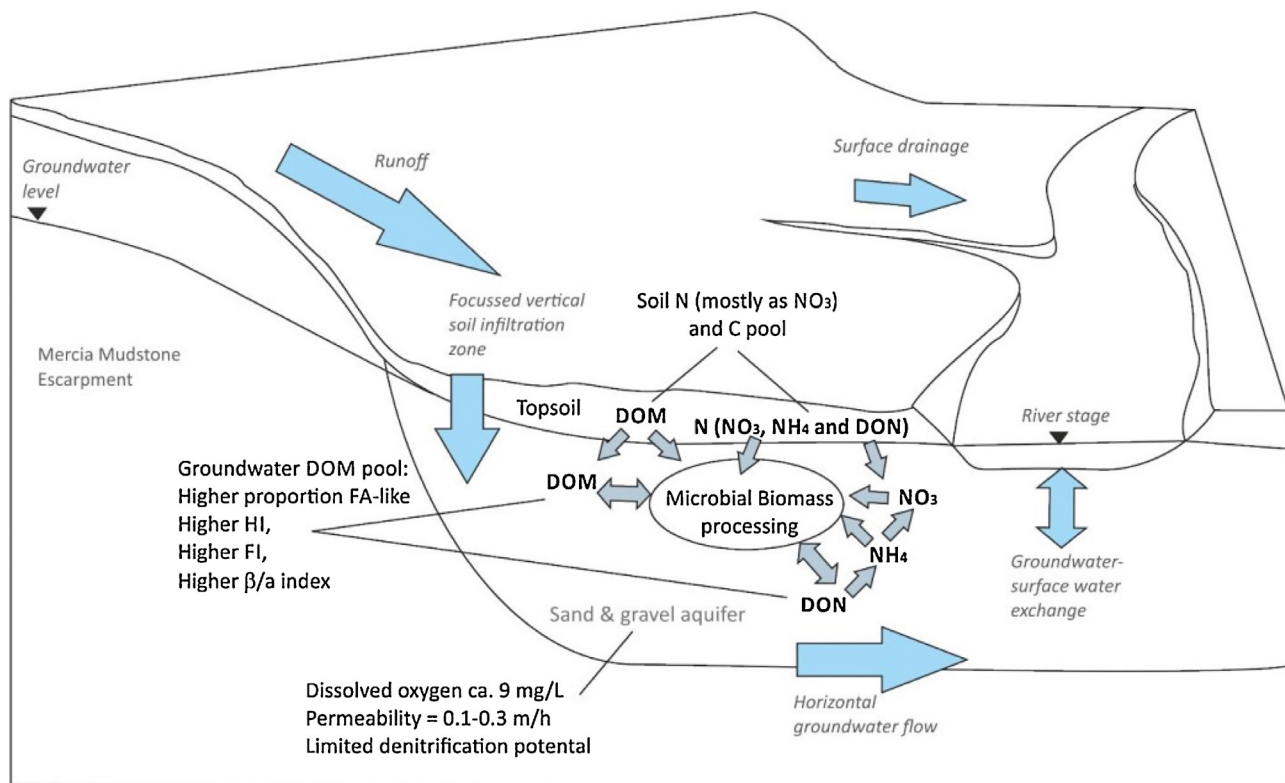

Fig. 1. Conceptual model of N and C dynamics within a shallow sand and gravel aquifer of the River Trent at Shelford.

(iii) to examine the differences in DOM and N characteristics between components of the soil–aquifer–river system.

2. Materials and methods

2.1. Background

Sand and gravel aquifers in the Trent valley are derived from glacial outwash and are largely used for intensive arable and cattle farming. The high permeability of these aquifers allows rapid transport of water and nutrients (e.g. C, N and P) as has been demonstrated in other similar settings (Arauzo and Valladolid, 2013; Lorite-Herrera and Jinénez-Espinosa, 2008). Within the aquifers and hyporheic zones beneath and adjacent to the river channel, zones of active nutrient processing may exist that help protect aquatic ecosystems from inputs of these nutrients. Nutrient processing is predominately microbially driven, thus the transfer of DOM through the hydrological components of these ecosystems will provide a significant energy source for processes such as heterotrophic dentrification (Rivett et al., 2008; Welch et al., 2011).

In the aquifer studied, the water table is shallow (∼1.5 m below ground level) and the soil is likely to contribute greater amounts of DOM to the aquifer than is commonly found in deeper aquifer systems (e.g. Lapworth et al., 2008). The dominant source of water, nutrients and DOM into the alluvial aquifer is via infiltration of diffuse recharge from rainfall as well as surface runoff and focussed infiltration in zones where the break in slope or natural topographic lows occur. Thus, we hypothesise that microbial activity will change the properties of DOM within the aquifer and combined with knowledge of the hydrochemistry, will identify the role the aquifer plays in the transport, storage and processing (e.g. denitrification) of N species (inorganic N, organic N). A conceptual model of the potential processes linking N and C cycling in the aquifer is given in Fig. 1 where DOM has an important role in providing energy. However, these processes depend not only on the quality of DOM entering the aquifer but also on other physico-chemical conditions such as nutrient availability (N, P) and redox status. Upon leaving the aquifer through subsurface transit towards natural discharge points such as the river system or springs, the resulting DOM quality will contribute towards both a potential transfer of C energy, as well as the many functions described earlier.
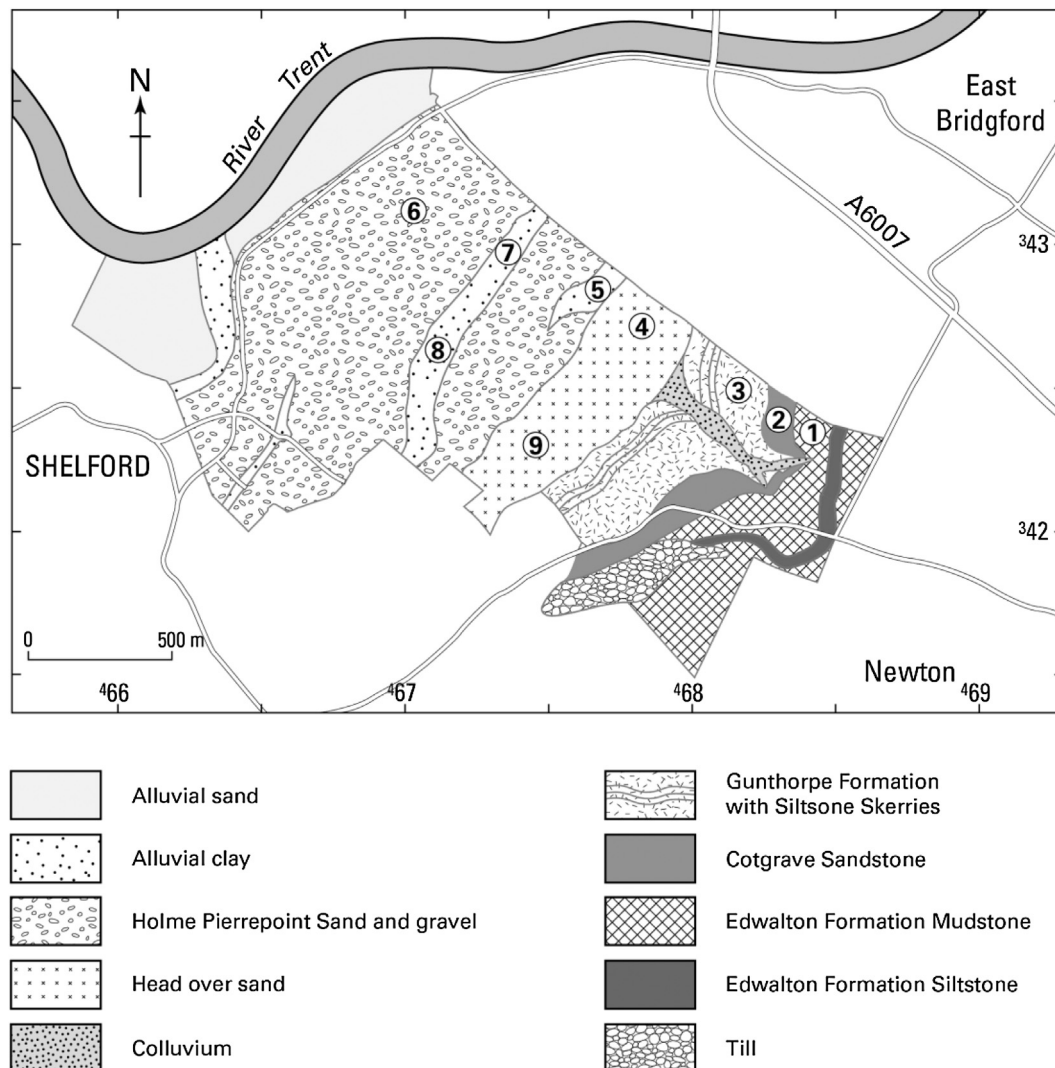

Fig. 2. Bedrock and superficial geology domains that make up the soil parent material domains. Contains Ordnance Survey data ©Crown Copyright and database rights [2015]. The numbers on geological domains represent areas where soil samples were taken for analyses (see Table 1).

2.2. Study area

The study area is centred round the village of Shelford, lying ~4 km east of the city limits of Nottingham in the valley of the River Trent, the third largest river in England. The land is predominantly used for arable agriculture, with the crop rotation including wheat, barley and oilseed rape. Sugar beet is also grown on the sandy soils above the aquifer. The site consists of a Triassic Mercia Mudstone escarpment (60 m AOD) which is overlain in the river valley by sand and gravel deposits (20 m AOD) formed as Quaternary river terraces ~24000 years BP (Howard et al., 2009). Closer to the river is the modern Holocene floodplain consisting of alluvial sand and gravel deposits overlain by alluvial clays. The surface geology is shown in Fig. 2, each representing the parent material of the different soil types. Full descriptions of the soils can be found in Tye (2010). The Triassic rocks include mudstones and siltstones whilst the superficial deposits include alluvial clays, colluvium, head (solifluction deposits) over sand, Holme Pierrepoint sand and gravel (HPP) as well as modern day alluvial sands. Descriptions of the soils can be found in Tye (2010). Flooding occurs on the Holme Pierrepoint sand and gravels which make up Terrace 1 on a frequency of approximately 1 in 30 years (EA Flood Risk Map). More frequent flooding occurs on the alluvial sand (Fig. 1) which constitutes the modern flood plain.

Within this floodplain setting the depth of the water table below the surface of the soil typically ranges between 0.5 and 2 m throughout the year (Tye, 2010). The depth of the sand and gravel aquifer overlying the Mercia mudstone ranges from 4 to 6 m below ground level (mbgl) (Tye et al., 2011). Inputs of nutrients are via areal recharge of precipitation, focussed infiltration via overland flow from the slopes of the escarpment and surface water–groundwater interactions in the hyporheic zone. Groundwater and DOM and nutrients will leave the aquifer through direct horizontal flow into the river or via a series of small drainage ditches that ultimately connect to two major drainage channels exiting to the River Trent. The three major DOM reservoirs within the landscape are the soil, the aquifer and the river.

2.3. Sampling

2.3.1. Soil samples

In October 2008, 4 samples were taken at depths of 0–15 cm from 9 of the different soil parent material domains shown in Fig. 2 using a Dutch auger. The soil parent material domains sampled were those developed on the Edwalton mudstone, Cotgrave sandstone, Gunthorpe mudstone, head over sand (×2), alluvial clay (×3) and the Holmepierrepoint sand and gravel (HPP). Inorganic nitrogen (NO_3-N and NH_4-N) extractions were undertaken on fresh samples using 2 M KCl. it was important to extract fresh soils because Jones and Willet (2006) found that air drying soils caused a 3–10 fold increase in the extraction of DOC and dissolved organic nitrogen (DON). As further suggested by Jones and Willet (2006) DON analyses were undertaken on these extractions so that a complete balance of N species could be obtained (DON, NO_3-N, NH_4-N). Concentrations of extractable DOC were determined on these extracts along with DOM characteristics using fluorescence excitation emission matrix spectroscopy (EEM), the intention being to obtain further information regarding possible sources and properties of the DON and the biodegradability of the DOC pool.

Whereas DOM properties in soil pore waters have been measured (e.g. Santin et al., 2009; Fellman et al., 2008), it is less common for DOM properties to be measured in the standard 2 M KCl extracts. As the soils are in such close proximity to the aquifer water it was decided to analyse DOM in the KCl extracts because (i) Jones and Willet (2006) describe this fraction of DOC and DOM
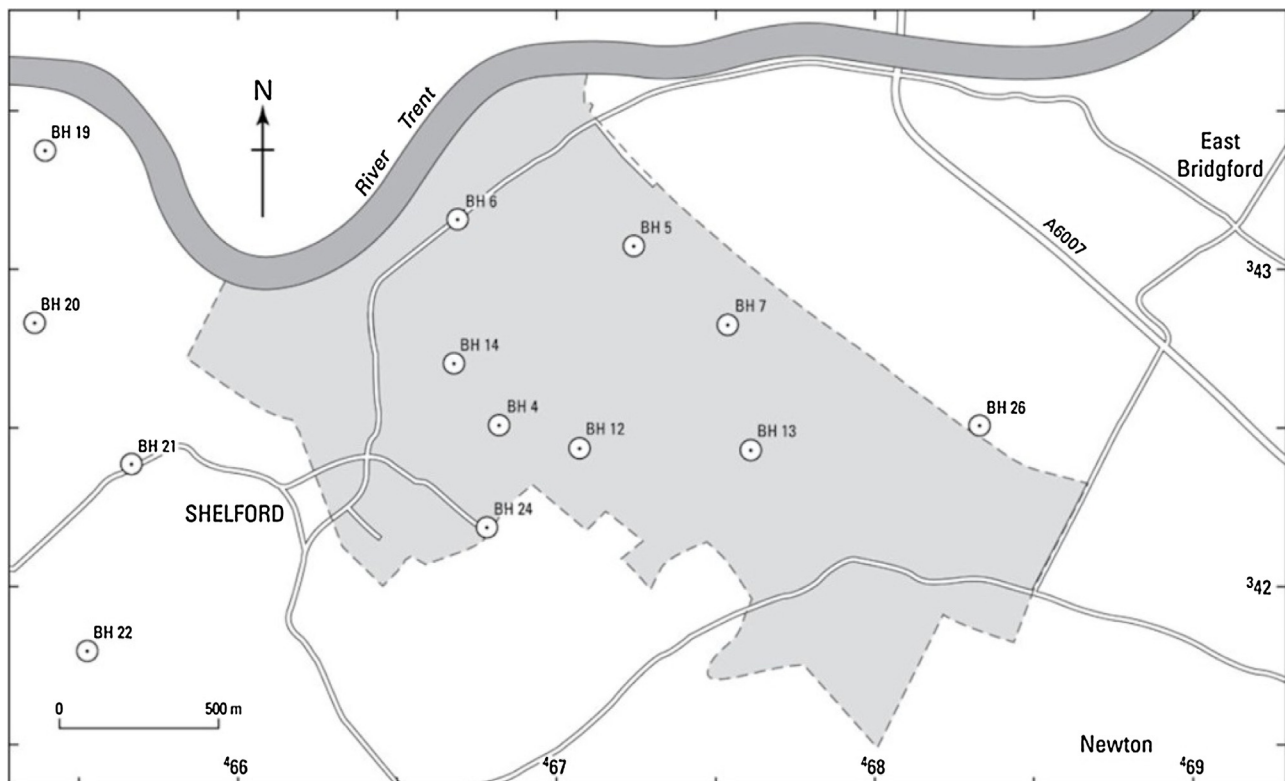

Fig. 3. Positions of piezometers across the sand and gravel aquifer at Shelford. Contains Ordnance Survey data ©Crown Copyright and database rights [2015].

as the readily soluble and exchangeable components of soil SOM, which is most likely to interact with soil pore water and be subject to transport (via run-off or leaching) to the shallow aquifer and (ii) it will provide DOM information alongside the complete N inventory which may provide further information regarding the interacting processes of the C and N cycles. As EEM has rarely been used on KCl extracts, we initially tested the technique to assess whether a robust fluorescence signal of DOM properties could be obtained. Potential interferences were examined including the molarity of the extraction solution or signal quenching and or enhancement through the extraction of cations such as aluminium (Al) or calcium (Ca) from the soil matrix. The remainder of each soil sample was air-dried and sieved to <2 mm for subsequent analysis.

2.3.2. Groundwater

Groundwater samples were collected from a series of 13 piezometers installed across the study area (Fig. 3). The piezometers were slotted and installed to the depth of the sand and gravel or other deposit they were inserted into; the deepest being 7 m (BH5) and shallowest 2 m (BH22). Piezometers were sampled three times (July 2008, October 2008 and February 2009) to gain an understanding of seasonal changes in groundwater hydrochemistry and in particular N and C species. Piezometers were purged fully 3 times with a groundwater pump before collection of the sample for analysis. Field measurements of dissolved oxygen (DO), pH, temperature and conductivity were taken at the time of sampling using HANNA field instruments. Samples were filtered using 0.45 μm nylon membrane filters and the sample split for various analyses. Samples for alkalinity and pH measurements were filled to overflowing before capping the bottle. Samples for cation analysis were acidified to 1% v/v HNO_3. Samples for anions were stored un-acidified. All bottles were Nalgene LDPE. Samples for DOC, DON and DOM analysis were stored in brown, Teflon capped glass bottles.

2.4. Analytical techniques

2.4.1. Soils

Total organic C and total N in soil samples were determined using a Costech ECS4010 Elemental Analyser (EA) calibrated against an Acetanilide standard. Replicate analysis of well-mixed samples indicated a precision of ±0.1%. Organic C samples were analysed after leaching of the sample with 10% HCl to remove inorganic C. Organic N was calculated as the difference between total N – inorganic N. Results are reported on a dry weight basis after correction for moisture. Inorganic N in 2 M KCl extracts was determined was analysed by Scalar at the Eurofin Laboratories in Wolverhampton. Samples for particle size analysis were oven-dried prior to the removal of organic matter using H_2O_2. Particle size distribution (<2 mm) was determined using a Micromeritics sedigraph III particle size analyser.

2.4.2. Groundwater

Water pH and alkalinity were analysed by titration using a Radiometer TIM 865 Titra-Lab analyser. Major cations were analysed using a Varian Vista ProAX ICP-AES and major anions (Cl^-, SO_4^{2-}, NO_3^-, NO_2^-) were analysed using a Dionex DX600 ion chromatograph. Ammonium was analysed by Scalar at the Eurofin Laboratories in Wolverhampton. Analysis of DOC was carried out using a Shimadzu TOC analyser. Total N in groundwater was determined using a Thermalux TOC/TN analyser. Organic N concentrations were estimated by subtracting inorganic N from the total dissolved N determinations.

2.4.3. EEM fluorescence spectroscopy in soils and waters

EEM works by producing high resolution matrices of excitation-emission wavelengths that represent the 3D fluorescence landscape of a particular sample. Speciation of DOM in soil extracts and groundwater was undertaken using a Varian Cary Eclipse Florescence spectrometer. For soil extracts, this method had not been previously been tested. Therefore we undertook comparisons between MQ-water, 0.5 M KCl and 2 M KCl with additions of (a) Suwannee River fulvic acid (FA)—1 mg/L, (b) Tryptophan—100 μg/L and (c) tyrosine—100 μg/L standards. Results in Fig. 4 show that measurement of these standards in 2 M KCl extract produced similar results to those measured in MQ-water, suggesting that the EEM measurements could effectively be carried out on the inorganic N extractions. Concentrations of aluminium (Al), calcium (Ca) and magnesium (Mg) in the soil extracts were also measured because high concentrations of these elements may alter the FA
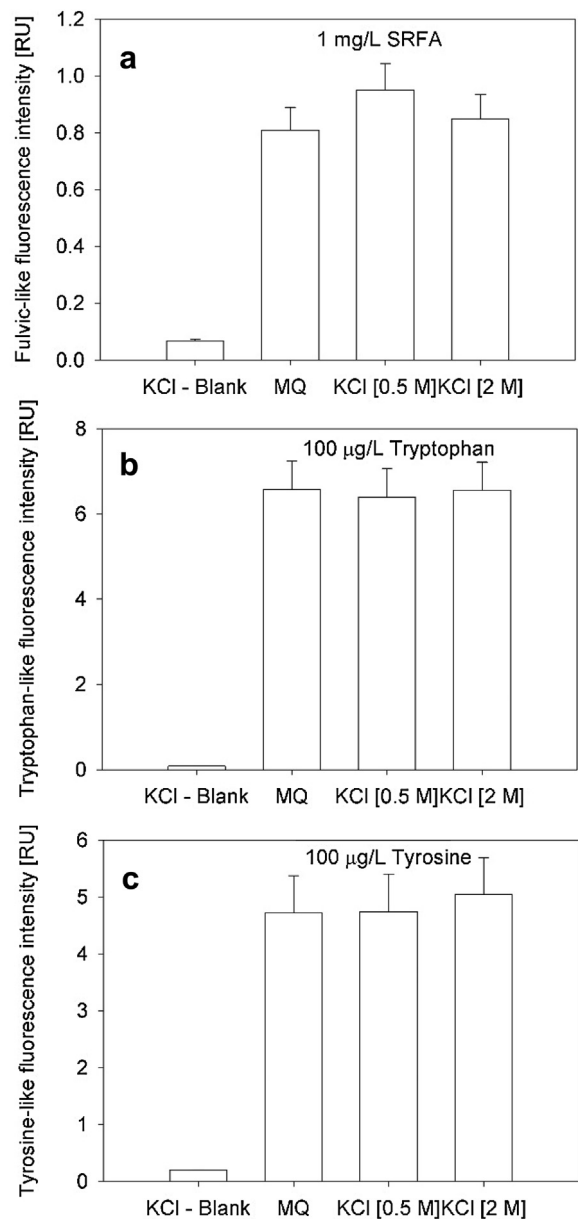

Fig. 4. Results of testing 2 M KCl for quenching effects prior to analysing soil extracts for DOM quality. Components tested are (a) fulvic-like intensity, (b) tryptophan-like intensity and (c) tyrosine-like intensity. The blank results in the plots are for analysis in 2 M KCl, error bars are 3sigma.

Table 1
Mean values ($n = 4$) and significant differences between DOM properties of soils sampled on different parent material domains. Statistical analysis was performed by one way analysis of variance followed by LSD. Differences in superscript letters signify significant differences between samples as calculated using least significant differences ($P < 0.05$). *See Fig. 2 for sample position.

Soil parent material	*Sample	Clay (%)	C (%)	N (%)	C:N	DOC (mg L^{-1})	TDN (mg L^{-1})	NO$_3$-N (mg L^{-1})	NH$_4$-N (mg L^{-1})	DON (mg L^{-1})	Tryptophan-like (RU)	Tyrosine-like (RU)	Fulvic-like (RU)	FI	β/α	HI
Edwalton mudstone	1	6.6[a]	1.23[a]	0.16[a]	7.6[a]	9.3[ab]	3.0[a]	0.4[a]	0.78[a]	1.86[c]	0.64[a]	0.35[a]	1.59[b]	1.34[a]	0.47[ab]	8.49[ab]
Cotgrave sandstone	2	8.6[ab]	1.85[bc]	0.18[abc]	10.1[bc]	11.3[b]	4.0[a]	1.44[a]	1.03[abc]	1.74[b]	1.20[b]	0.35[a]	2.79[c]	1.36[ab]	0.52[ab]	9.74[ab]
Gunthorpe mudstone	3	14.1[c]	2.01[cd]	0.20[a]	9.9[c]	9.1[ab]	3.4[a]	1.66[a]	0.80[a]	0.86[a]	0.44[a]	0.30[a]	1.05[ab]	1.40[ab]	0.54[abcd]	9.38[ab]
Head over sand	4	9.5[b]	1.67[b]	0.17[ab]	9.6[bc]	10.2[ab]	9.3[c]	7.55[c]	1.15[bc]	1.68[bc]	0.62[a]	0.31[a]	1.60[bc]	1.51[c]	0.59[cd]	9.95[b]
Alluvial clay	5	14.4[c]	2.11[b]	0.21[b]	9.9[bc]	10.7[b]	4.05[a]	2.38[a]	0.89[ab]	0.69[a]	0.70[a]	0.31[a]	1.71[c]	1.43[ab]	0.52[abc]	9.27[ab]
Holmepierrepoint S&G	6	8.7[b]	2.38[d]	0.22[c]	10.7[d]	14.2[c]	4[a]	0.84[a]	1.07[abc]	1.96[c]	1.04[b]	0.33[a]	2.73[c]	1.32[a]	0.46[a]	12.41[c]
Alluvial clay	7	14.3[c]	3.18[e]	0.28[d]	11.4[d]	7.3[a]	2.75[a]	0.21[a]	0.83[ab]	1.73[bc]	0.54[a]	0.31[a]	1.21[abc]	1.38[a]	0.55[bcd]	7.57[ab]
Alluvial clay	8	17.5[d]	3.04[e]	0.29[d]	10.6[cd]	6.9[a]	2.8[a]	0.21[a]	0.85[ab]	1.68[bc]	0.46[a]	0.37[a]	0.99[a]	1.50[d]	0.61[d]	7.29[a]
Head over sand	9	9.14[b]	1.54[ab]	0.17[a]	8.9[b]	8.5[ab]	6.8[b]	5.00[b]	1.26[c]	0.84[a]	0.58[a]	0.28[a]	1.33[abc]	1.53[c]	0.56[bcd]	7.37[a]
LSD ($P < 0.05$)		2.00	0.43	0.04	1.16	3.33	2.31	2.26	0.33	0.98	0.26	0.10	0.55	0.10	0.08	2.40

RU = Raman units.

intensities; Al can enhance FA intensities while Ca can cause minor blue shifts for FA and tryptophan (Elkins and Nelson, 2001; Determann et al., 1994). Aluminium concentrations were found to be below ICP-AES detection limits and the potential effects were considered insignificant. Calcium concentrations varied between 452 and 1170 mg L^{-1} and Mg varied between 21 and 168 mg L^{-1} and would not significantly alter humic-like fluorescence intensities at these concentrations (Elkins and Nelson, 2001).

The EEM method used has previously been reported by Lapworth et al. (2008). Briefly, the excitation wavelengths (Ex) were examined between 200–400 nm with a 5 nm bandwidth. Emission wavelengths were set between 250 and 500 nm with 2 nm bandwidth. The scan rate was 9600 nm/min and the detector voltage was 900 V. Analysis was undertaken using quartz cells with a 1 cm path length. The Raman peak of water at 348 nm was used to check for instrument stability prior to analysis. Ultrapure water (ASTM type 1 reagent grade water including a UV cracker) was used for the blank sample. Analysis of the Raman lines involved correcting for the blank and masking Raleigh–Tyndell lines for data processing purposes by multiplying the sample matrix by a matrix of '0's and '1's. Only florescence data above Ex250 nm were used due to instability in the region below Ex250 nm. Fluorescence analysis was carried out on diluted samples (absorbance was <0.3 at 254 nm) to ensure there were no inner filter effects. All the post processing of florescence data was carried out using the statistical package R (R Development Core Team, 2008; Lapworth and Kinniburgh, 2009).

Three fluorescence organic matter components within the EEM were used in this study to compare intensities between sites, and sampling rounds: the fulvic-like (FA) maximum (Ex330−340 nm, Em410−460 nm), the tryptophan-like (TLF) maximum (Ex270−290 nm, Em320−360 nm) and the tyrosine-like (TY) maximum (Ex280−270 nm, Em294−302 nm). DOM characteristics were investigated through the use of three indices which have been shown to relate to DOM structure: (i) the fluorescence index (FI) which is commonly used to differentiate between terrestrial and microbial DOM sources (McKnight et al., 2001), (ii) the humification index (HI), an indication of humicity, and the condensing of fluorescing molecules (Zsolnay, 2001), (iii) the ratio of two fluorescing components (β/α), where β represents recent labile OM (often microbially produced or autochthonus) and α represents recalcitrant OM (allocthonous) (Wilson and Xenopoulos, 2009; Parlanti et al., 2000). As β/α values increase it suggests a greater input of autochthonous carbon production.

2.5. Statistical analysis

Principal component analysis (PCA) was undertaken on the soils and groundwater datasets using the statistical package 'R'. The datasets included the carbon and nitrogen species as well as the properties and indices obtained from the laser fluorescence analysis. Prior to the PCA, all data was normalised by dividing by the maximum value to derive values between 0 and 1. Individual soil and groundwater datasets were then analysed. Other statistical analysis (linear correlations and one way ANOVA) were undertaken in Excel.

3. Results

3.1. C and N species in soils

Table 1 shows the mean ($n = 4$) results for each of the measured N and C components in soils from the 9 soils sampled (see Fig. 2). Significant differences ($P < 0.05$) were found between some of the different soil types for % clay, % C, % N and C:N ratios as determined by one way analysis of variance, with least significant difference
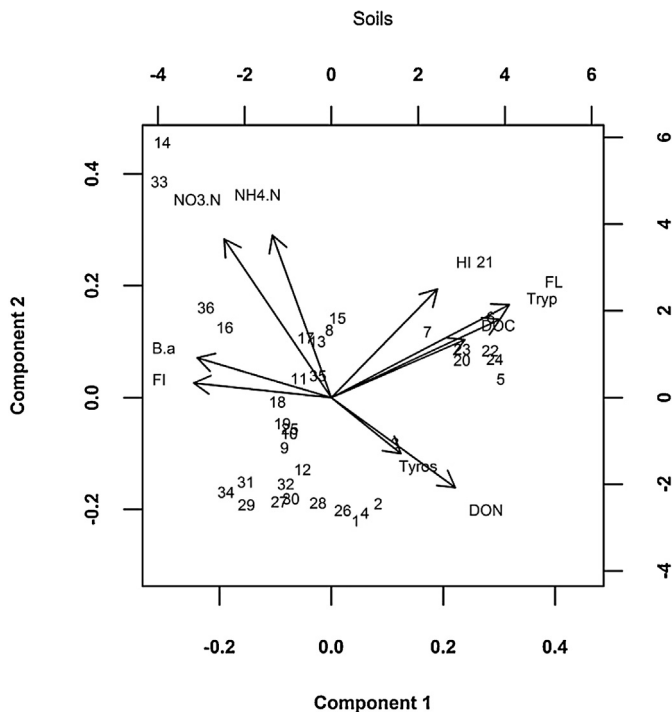
Fig. 5. PCA showing relationships between measured carbon and nitrogen parameters and DOM properties measured in different soils sampled in October 2008.

tests being used to determine significant differences ($P < 0.05$). Results from the initial survey of different top-soils in this study indicate that there were linear relationships between the organic C, N and clay concentrations (0–15 cm). These were % C = 0.611 + 0.131 × % clay, $P < 0.001$, $r^2 = 0.51$ and % N = 0.0974 + 0.009 × % clay, $P < 0.001$, $r^2 = 0.55$. Soil organic C and organic N also had a strong positive linear correlation (% N = 0.0603 + 0.071 × % C, $P < 0.001$, $r^2 = 0.94$). Below the depth of 30 cm, the soil organic carbon concentration was <0.1% C (Tye, 2010) for the alluvial sand and gravels overlying the aquifer. After extraction with KCl the soils formed from the head over sand parent material had significantly ($P < 0.05$) greater concentrations of NO_3-N and NH_4-N (Table 1) than the other soils. The likely explanation is that these soils occur at the break of the slope, and are subject to runoff that includes NO_3^- from the escarpment slope. Thus, the break of slope represents a significant zone for focussed recharge and input for DOM and NO_3 into the aquifer. Elevated NH_4 concentrations are

likely due to the greater NO_3 availability leading to greater ammonification. Concentrations of DOC in the KCl extracts varied between 6.9 to 14.2 mg l^{-1} whilst DON values ranged from 0.69 to 1.86 mg L^{-1}. Unlike the solid phase of the soil, there were no correlations between the DOC and DON concentrations in the extracts. The results obtained demonstrate that the DOC and DON pools between soils are highly variable, with some significant differences between soil types found (Table 1). There were no statistically significant correlations between soil physical properties e.g. % clay content and the amount of soluble or exchangeable DOC and DON extracted.

3.2. DOM characteristics in soils using EEM

For most of the measured DOM characteristics there were only small differences in reported Raman Units (RU) for soil from the different sites sampled (Table 1). However, significant differences ($P < 0.05$) in fulvic-like and tryptophan-like values (RU) were found for those soils derived from sandy parent materials (Cotgrave sandstone and HPP) compared to the other soils. In addition, soil samples from the HPP parent material also had a significantly ($P < 0.05$) greater HI index. The FI values are close to 1.4 indicating a predominantly terrestrial source (McKnight et al., 2001). There were no relationships between % clay and the properties measured using the EEM.

Within the soluble and exchangeable pool of DOM extracted using KCl extracts there were several strong groupings found using PCA. A scree plot suggests 3 components could be identified accounting for 70.6% of the variance. Fig. 5 shows the bi-plot between components 1 and components 2, of which there are 4 groups of variables that appear to be well correlated. These are (Group 1) the inorganic N species (NO_3-N and NH_4-N), (Group 2) the ratios β/α and FI, (Group 3) tyrosine-like proteins and DON and (Group 4) HI, FL, tryptophan-like and DOC. There was a linear correlation between fulvic-like substances and tryptophan-like substances (tryptophan-like = 0.0379 + 0.3926 × fulvic-like, $P < 0.001$, $r^2 = 0.92$). The close grouping of the β/α ratio and FI suggests that where an increase in the FI occurs, it is accompanied by an increase in the labile carbon (α) in a system that appears to be dominated by recalcitrant terrestrial carbon (β). Thus this pairing may represent a more labile DOM than is found in Group 4. The inorganic N species which make up Group 1 are likely to be correlated because of ammonification and nitrification processes. The relationship between tyrosine-like and DON is probably because the tyrosine-like proteins are likely to form a component of DON. In Fig. 5, two sets of soils (5–8 and 21–24) in particular cluster closely around Group 4. These are the soils based on sandy

Table 2
Results of laser fluorescence analysis on groundwater and River Trent samples taken in October 2008.

	DOC (mg L^{-1})	NO_3-N (mg L^{-1})	NH_4-N (mg L^{-1})	NO_2-N (mg L^{-1})	DON (mg L^{-1})	Fulvic-like (RU)	Tryptophan-like (RU)	Tyrosine-like (RU)	FI	β/α	HI
BH4	11.5	11.18	<0.3	0.16	1.55	3.76	1.09	0.10	1.63	0.66	18.65
BH5	4.9	10.86	<0.3	<0.004	0.56	2.38	0.63	0.05	1.73	0.69	18.69
BH6	6.0	23.30	<0.3	<0.004	0.13	0.92	0.30	0.04	1.59	0.65	14.94
BH7	14.3	3.87	<0.3	0.03	0.25	2.76	0.76	0.10	1.58	0.54	20.35
BH12	14.7	0.81	<0.3	<0.004	0.21	2.62	0.64	0.29	1.50	0.56	16.73
BH13	8.5	15.11	<0.3	<0.004	0.51	0.58	0.25	0.18	1.68	0.72	6.72
BH14	7.2	41.86	<0.3	<0.004		1.29	0.47	0.27	1.66	0.70	9.47
BH19	4.3	7.42	<0.3	0.01	0.13	1.14	0.40	0.19	1.49	0.66	11.70
BH20	2.1	13.67	<0.3	0.01	0.04	0.44	0.15	0.20	1.41	0.61	6.56
BH21	2.6	22.85	<0.3	<0.004	0.03	0.65	0.21	0.19	1.65	0.69	8.60
BH22	9.1	10.84	<0.3	0.02	0.47	1.18	0.35	0.06	1.66	0.64	16.83
BH24	11.2	3.60	<0.3	<0.004	0.48	2.36	0.63	0.17	1.59	0.64	19.01
BH26	13.8	17.10	<0.3	0.01	1.41	4.74	0.97	0.18	1.47	0.55	24.65
R. Trent	11.5	4.16	<0.3	0.05	0.77	2.70	0.84	0.19	1.35	0.55	14.04

Table 3
Results of laser fluorescence analysis on groundwater and River Tent samples taken in February 2009.

	DOC (mg L^{-1})	NO$_3$-N (mg L^{-1})	NH$_4$-N (mg L^{-1})	NO$_2$-N (mg L^{-1})	DON (mg L^{-1})	Fulvic-like (RU)	Tryptophan-like (RU)	Tyrosine-like (RU)	FI	β/α	HI
BH4	11.0	34.62	<0.3	<0.004	1.24	4.06	1.00	0.05	1.67	0.65	22.8
BH5	5.02	12.67	<0.3	<0.004	0.81	2.16	0.53	0.05	1.73	0.67	20.57
BH6	3.41	18.10	<0.3	<0.004	0.65	1.24	0.33	0.04	1.59	0.70	21.45
BH7	3.04	2.04	<0.3	0.01	0.07	1.32	0.31	0.05	1.31	0.57	22.67
BH12	3.61	9.28	<0.3	0.02	0.13	1.18	0.37	0.08	1.58	0.61	19.49
BH13	6.61	14.93	<0.3	<0.004	0.16	0.61	0.18	0.05	1.72	0.63	20.41
BH14	5.39	37.33	<0.3	<0.004		2.01	0.64	0.40	1.65	0.65	12.23
BH19	2.91	5.43	<0.3	<0.004		0.99	0.31	0.05	1.69	0.72	17.01
BH20	1.15	13.12	<0.3	<0.004	0.66	0.35	0.13	0.06	1.42	0.69	13.14
BH21	1.59	27.15	<0.3	<0.004		0.57	0.17	0.06	1.60	0.74	18.4
BH22	6.79	2.26	<0.3	0.01	0.12	1.09	0.36	0.07	1.49	0.71	17.61
BH24	4.82	4.30	<0.3	0.01		1.82	0.49	0.06	1.50	0.66	23.02
BH26	8.63	20.36	<0.3	<0.004	0.55	3.31	0.76	0.06	1.34	0.53	29.57
R. Trent	5.82	5.88	<0.3	0.02	0.43	1.88	0.64	0.13	1.46	0.55	14.26

parent materials (Cotgrave sandstone and HPP) which had previously been identified (Table 1) as having significantly ($P < 0.05$) higher relative units of HI and fulvic-like substances.

Further information was obtained by plotting PCA component 1 against component 3 and this generally reinforced the results seen in Fig. 5. Group 1 showed that NH$_4$-N, FI and the β/α ratio were reasonably well correlated, suggesting a possible microbial influence on N ammonification. The HI shows a strong negative correlation to FI and the β/α ratio, again suggesting that the source of the more labile DOM is derived microbially, and not from recently lysed plant cells. The last major group is that of DON, DOC, tryptophan-like and fulvic-like and represents a similar grouping as previously found, but with DON. Overall the PCA analysis suggests that that the DOM in soils is largely terrestrial plant derived and fulvic-like in origin, with the identified more labile components probably being associated with microbial activity.

3.3. C and N species in groundwater and river water

The N and C species from the July 2008 sampling, along with the hydrochemistry for the aquifer water and river is summarised in Supplementary information 1. No EEM measurements were undertaken for these samples. It reports typical values of DO, pH, major cations and anions found. Tables 2 and 3 summarise the concentrations and ranges of C and N species and DOM characteristics obtained using laser fluorescence for the aquifer water for samples taken in October 2008 and February 2009. Nitrate is the most abundant form of N at all three sampling dates, indicative of its use in the agricultural systems and with a concentration range of 0.81 to 41.86 mg L^{-1}. Ammonium was always below detection

limits (0.03 mg L^{-1}), and NO$_2$-N where found, was typically <0.004 mg L^{-1}. Water samples generally had low concentrations of Fe and Mn, as would be expected in the presence of DO. Organic N was present and measured at October 2008 and February 2009 and was typically < 2 mg L^{-1}. Dissolved organic carbon (DOC) was typically < 10 mg L^{-1} in the aquifer water and of a similar concentration range as found in the soil extracts. There were no significant differences in the concentrations of DOC, NO$_3$-N and NO$_2$-N between the sampling dates. Typical values of HPO$_4^-$, essential for driving microbial activity were generally below detection limits (<0.1 mg L^{-1}).

3.4. Dissolved organic matter properties in groundwater using EEM fluorescence

Tables 2 and 3 show fluorescence results obtained from the groundwater samples taken in October 2008 and February 2009. The major difference between the sampling dates is that the relative units of the HI and fulvic-like characteristics have generally increased between the two samplings dates. PCA analysis was undertaken for both sets of groundwater data. For October 2008, the scree plot suggests 2 principal components accounting for 65% of the variance. The PCA plot where component 1 is plotted against component 2 is shown in Supplementary information 2. Firstly Group 1 comprises the HI, tryptophan-like proteins, DOC, DON and fulvic-like substances, suggesting that the DOM is predominantly terrestrially derived. Within this group there were several highly correlated parameters. These included (i) HI and tryptophan-like (tryptophan-like $= -0.0626 + 0.0414 \times$ HI, $P < 0.001$, $r^2 = 0.60$), fulvic-like and tryptophan (tryptophan–like =

Table 4
Summary of DOM properties in soil extracts, groundwater and the River Trent.

| Sample		Fulvic like (RU)	Tryptophan like (RU)	Tyrosine-like (RU)	FI	β/α	HI
Soil	Mean	1.66	0.69	0.33	1.42	0.54	9.06
($n = 36$)	SD	0.67	0.26	0.03	0.08	0.05	1.63
Groundwater October 2008	Mean	1.91	0.5	0.2	1.6	0.60	14.80
($n = 14$)	SD	1.29	0.29	0.08	0.09	0.06	5.72
Groundwater February 2009	Mean	1.59	0.43	0.08	1.56	0.66	19.87
($n = 14$)	SD	1.05	0.25	0.09	0.14	0.07	4.58
Trent October 2008 ($n = 1$)		2.7	0.84	0.19	1.35	0.55	14.00
Trent February 2009 ($n = 1$)		1.87	0.63	0.12	1.46	0.54	14.25
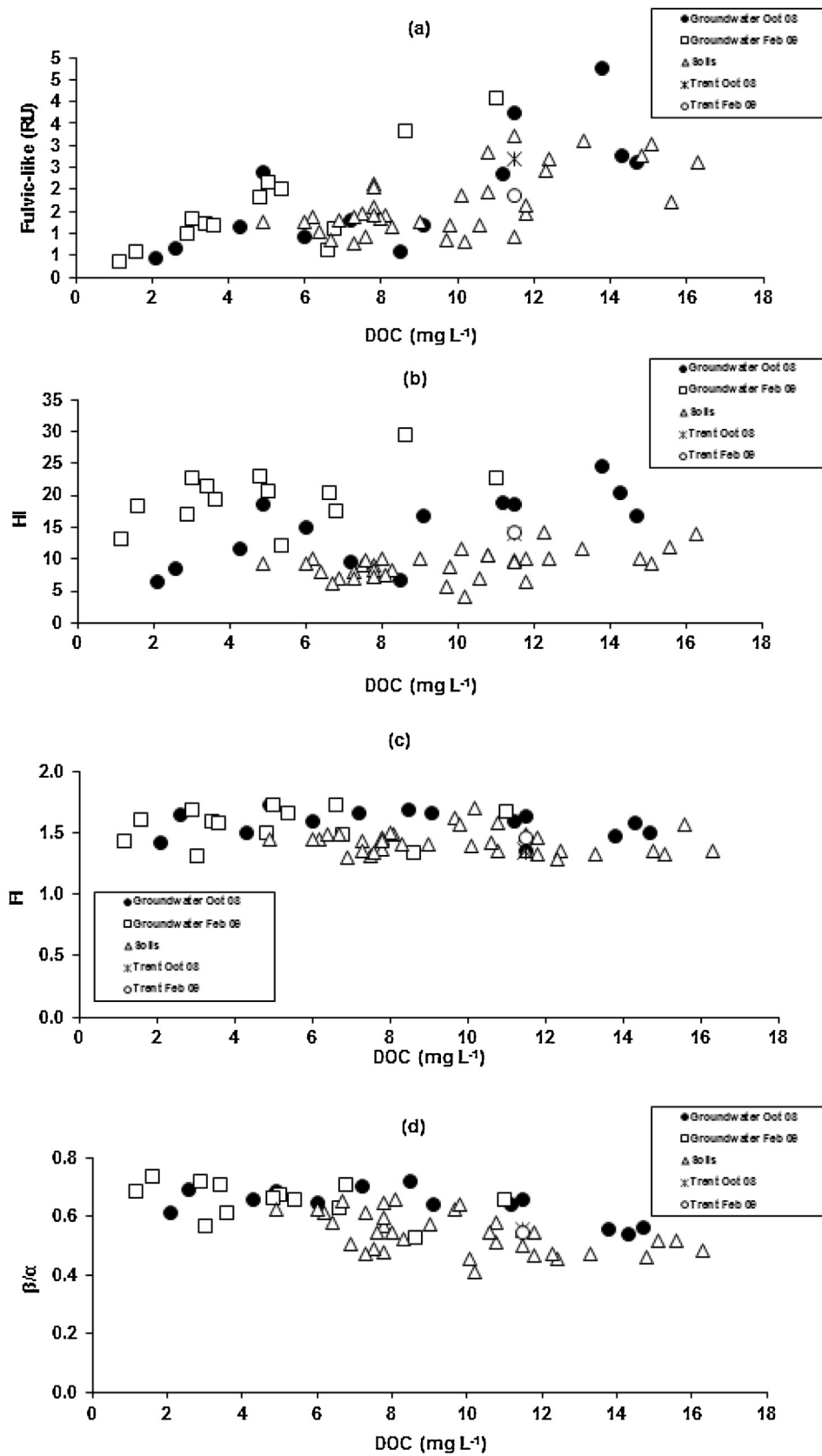
Fig. 6. Comparison of (a) DOC vs. HI, (b) DOC vs. fulvic-like, (c) DOC vs. FI and (d) DOC vs β/α for soils, aquifer waters and river waters to assess the effects concentratin may have on DOM property.

$0.1222 + 0.21 \times$ fulvic like, $P < 0.001$, $r^2 = 0.91$) and fulvic-like and HI (HI $= 7.622 + 3.460 \times$ fulvic like, $P < 0.001$, $r^2 = 0.73$). A second group (Group 2) consisted of a poorer correlation of NO_3, the β/α ratio and the fluoresence index.

The PCA scree plot for the groundwater collected in February 2009 (Supplementary information 3) showed two components accounting for 62% of the variance. The first group of variables (Group 1) consisted of a group that included fulvic-like substances, DON and DOC. Linear correlations between DOC and fulvic-like (FL $= 0.031 + 0.31 \times$ DOC, $P < 0.001$, $r^2 = 0.67$), DOC and tryptophan (tryptophan-like $= 0.067 + 0.073 \times$ DOC, $P < 0.001$, $r^2 = 0.67$), fulvic-like and tryptophan (tryptophan-like $= 0.066 + 0.22 \times$ fulvic-like, $P < 0.001$, $r^2 = 0.96$) were found. The second major group is the tyrosine-like proteins and the FI. In a similar manner to the soils these suggest that they represent a more labile fraction of DOM such as microbial or recent lysed plant cell sources. Lastly, HI and β/α are negatively correlated on this plot which may be expected as the β/α represents a more recent DOM component. In a similar manner to the soil analyses, there was little indication that DOM properties consistently reflected the inorganic or organic N status of the groundwater. With the exception of a linear correlation between DON and tryptophan-like proteins (tryptophan-like $= 0.445 + 0.584 \times$ DON, $P < 0.01$, $r^2 = 0.73$) in October 2008 there were no further correlations. No relationships between DON and tyrosine in the groundwater were evident but the measured tyrosine-like protein intensity values measured were much lower than in the soils.

3.5. DOM properties of river water

Carbon, nitrogen and DOM properties of the River Trent water are reported in Tables 2 and 3. Whereas DOC and DON concentrations are similar to the ground water, the DOM properties and indices β/α and FI are generally lower or towards the lower end of the range of values for the groundwater.

3.6. Comparison of DOM properties of soils, groundwater and river water with DOC concentration

Table 4 compares the mean and Standard deviation (SD) of the DOM in the soil and groundwater samples along with the River Trent samples. The SD of the mean values in the groundwater are generally higher than those of the soil, especially for the HI, reflecting the range of possible different sources of DOM and speeds of transport through the different sediment architectures and textures that make up the shallow aquifer. Fig. 6 shows the complete datasets for soil and groundwater of DOM EEM properties plotted against DOC concentrations for the fulvic-like properties, the HI and FI and the ratio β/α. In (Fig. 6a) the HI which is associated with the condensing of fluorescing molecules as a result of a lower H/C ratio was found to be largely independent of DOC concentration for both the soils and groundwater. It is evident that the HI had a larger range in groundwater (5–30) than in soils (4–14). Fig. 6(b) shows that the fulvic-like component of the DOM in the groundwater has a greater range of values than the various soil types, but again both are largely independent of DOC concentration. Fig. 6(c) and (d) shows that for both groundwater and soils the FI and β/α properties are again independent of DOC concentration.

4. Discussion

The aims of this work were to (i) examine the DOM characteristics in a range of soils associated with a shallow river aquifer and assess the relationships between C and N within a recognised extraction procedure (2 M KCl) for inorganic and organic N in soils,

and (ii) to examine the differences in DOM characteristics between components of a soil–aquifer–river system. An assessment was then made regarding how DOM may interact with the storage and processing of nitrogen in the aquifer, as outlined in Fig. 1.

The general pattern of Total C and N in the catena soils conforms to previously published work where % C was related to texture and suggests that OC is protected to a greater degree by clay sized material through aggregation of particles (Guggenberger and Kaiser, 2003). Total N and total C were related with C:N ratios within the expected range for agricultural soils. There was no relation between solid phase C and N and that extracted in the KCl extracts. Within the extracts there were no relationships between DON and DOC concentrations. Jones and Willet (2006) also found highly variable DOC and DON contents between soils without any one factor being the obvious cause. The intention behind measuring the DOM properties within the KCl extracts was to obtain further information regarding possible sources and properties of the DON and the biodegradability of the DOC pool. This is most likely through the relationships between the protein-like fluorescence properties and DON concentrations, whilst the calculated indices of HI, FI and β/α will provide an indication of the biodegradability of the DOC and its source. However, unlike some aquatic ecosystems (e.g. Lutz et al., 2011), there appears to be no tendency for DON concentrations to be influenced by the concentration of inorganic N in soil. Principal component analysis demonstrated that all components of the aquifer system (soils, groundwater and river water) were dominated by terrestrial DOM type properties. In the soil extracts, it was found that soil DOM properties were linked to the soil texture; those with higher sand contents and a more open structure having a significantly greater HI. This may arise from there being less protection for organic matter from biogeochemical processing. It was also found that some DOM properties (FI, β/α and HI) in the soil extracts appeared to be independent of DOC concentration. This was also found by Jaffe et al. (2008).

The relationships between N species and the properties of the DOM in the KCl soil extracts were assessed. The soil DON was generally <50% of the total dissolved N. Key findings found were that (i) that there was no correlation between inorganic N and the DOM properties identified through EEM analysis and (ii) a strong correlation existed between DON and tyrosine-like proteins. The strong relationship between the tyrosine-like protein and DON is because it will be a constituent of the DON pool. However, the tryptophan-like proteins did not show a similar correlation to the DON pool, instead being correlated to the fulvic-like pool. It may be expected that the tryptophan-like component would also show a relationship towards the DON pool, as found for the tyrosine-like component. A reason for this result maybe that the area of the excitation-emission matrix where the tryptophan-like component is measured is also shared with other molecules. Work by Maie et al. (2007) suggests that phenolic moieties, leached from senescent plant materials may also fluoresce in this area of the excitation–emission matrix. These compounds are important precursors of humic substances. The presence of these compounds may therefore explain the relationship between the trypothan-like and fulvic-Like substances within the PCA. A similar situation may also have occurred in the groundwater where DON concentrations were generally lower than in the soils. In these samples, the trypotophan-like substances appears in a group with DOC, DON and fulvic-like substances, whilst samples also had a high HI. In addition, in March 08 there was a strong relationship between DOC and DON suggesting that the DON may be contained with more recalcitrant DOC molecules. This would suggest that the trypto-phan-like measurement may again be including these phenolic groups. It would appear that caution needs to be exercised in interpreting the tryptophan-like substances and more direct

comparisons with known biomolecules should be undertaken to identify the molecular composition of specific fluorescent components.

Prior to comparing measured DOM properties between the soil-aquifer-river components of the ecosystem (Table 4), assessments of whether analysing EEM output from solutions with different matrices (e.g. ground water and KCl extracts) is valid. Previous work by Jones and Willet (2006) demonstrated that KCl extracted greater concentrations of DOC and DON than water extractions, but they found that the ratio between DON and amino acids remained similar. Our laboratory tests suggested that fulvic-like, tryptophan-like and tyrosine-like properties produced very similar results between H_2O and KCl. In addition, Gabor et al. (2015) tested soils extracted in KCl and H_2O and their results suggested tryptophan and tyrosine produced only slightly higher intensities in water extractions as compared to KCl. Thus from assessing the results of our laboratory tests and those of Gabor et al. (2015), it would appear that the results between KCl and H_2O for these DOM properties are reasonably comparable. With respect to other DOM properties measured, Gabor et al. (2015) compared EEM characteristics in water, KCl, K_2SO_4 and $CaCl_2$. They found that between the salt extractions the fluorescence properties were generally comparable. However, higher intensities in HI and FI were found in KCl than in waters. Therefore the implications for our study where we are comparing KCl extracts with groundwater are that direct comparisons can not be made for the FI and HI indices but qualitative comparisons can. Thus, despite the potential matrix effects, the values for HI were found to be higher in groundwater than in the KCl extracts, suggesting that an increase in groundwater HI has occurred. This is likely to have arisen as a result of (i) continued microbial processing as the DOM travelled through the soil profile and (ii) microbial processing within the aquifer. Several authors have suggested that fractionation of DOM occurs as it passes through a soil profile, with a more labile DOM component found deeper in the profile as a result of aromatic and humified DOM components being held on the soil matrix, through a combination of chemical and physical interactions (Inamdar et al., 2012). In addition, Corvasce et al. (2006) found that aromaticity and the HI decreased dramatically down a soil profile whilst there was an increase in the FI. Considering the rapid transport of water through the sand and gravel soil profile into the aquifer the increase in HI is likely a result of further microbial decomposition in the aquifer. Similarly, there were higher FI values found in the groundwater, compared to the soil extracts. With knowledge that FI may be higher in the KCl extracts we can suggest qualitatively that there was a greater microbial influence in the groundwater (FI values moving closer to 1.9, suggesting a more microbial influence). Whereas differences in the DOM properties between soils and groundwater were apparent, possible seasonal factors were also identified between the October and February groundwater samples. In particular there was an increase in groundwater HI found between October and February. The lower HI found in October may result from (i) a greater input of root exudates when the crop had been growing or (ii) an input of less humic plant like material entering the aquifer after autumn cultivations. After winter (February 2009), when microbial action had continued through the winter, an increase in the HI was found in the groundwater. This suggests that bacteria within the aquifer are processing the carbon of the DOM for respiration.

Despite sand and gravel deposits making up considerable areas of the Trent valley and being a source of surface water flow via baseflow (base flow index of 0.63 (NRFA 2015)), the DOM properties of the river water possessed values different to those of the aquifer groundwater and soil. The river will reflect both baseflow and soil runoff DOM contributions as well as other anthropogenic contributions (e.g. sewage treatment works (STW)).

Differences between the aquifer and river water DOM properties included higher tryptophan-like and tyrosine-like components and lower FI, β/α and HI components. In addition there was one very high fulvic-like component found in the river water in October 2008. This combined with the higher tryptophan and tyrosine-like values are typical of releases of processed waters from STW's (Baker, 2001), of which one exists a short distance upstream (<2 km).

Part of this work was also to examine the possible role DOM may play in nutrient processing within the aquifer. As expected, the intensive agricultural practices on the soils overlying the aquifer, combined with run off from the valley slopes, resulted in the transport of fertiliser N (predominantly as NO_3-N) into the shallow sand and gravel aquifers. The shallow groundwater had high concentrations of NO_3-N, being close to or exceeding the UK drinking water standard of $50\,mg\,L^{-1}$. Concentrations of NH_4-N were below detection limits ($<0.3\,mg\,L^{-1}$) whilst small concentrations of organic N were found (range: 0–$1.55\,mg\,L^{-1}$; median $0.47\,mg\,L^{-1}$). The predominance of nitrate-N as the transported species of N is likely because ammonium-N is readily sorbed to clay particles whilst organic-N is largely composed of rapidly processed molecules such as proteins. Soils above the aquifer have an unsaturated hydraulic conductivity (using a tension Infiltrometer) (K) of $\sim0.34\,m\,h^{-1}$ and a hydraulic conductivity (using a pressure infiltrometer) of $\sim0.1\,m\,h^{-1}$ (A. Tye, Unpublished results). This is likely to lead to rapid transport of NO_3^- through the soil profile and into the aquifer without substantial subsoil denitrification taking place; unlike in soils with a greater silt or clay content (Baker and Vervier, 2004; Pinay et al., 2000). In addition, beneath the top 30 cm of soil the organic C concentration was <0.1% C (Tye, 2010). This is likely to restrict the size of the microbial populations that the carbon substrate can support.

Low denitrification rates in sand and gravel aquifers have been reported by Robertson and Schiff (2008). However, in this work despite measurements of DO suggesting it is generally a well oxygenated aquifer, the extent of denitrification that occurs in it cannot be ascertained without measurement. Typical conditions for denitrification to take place, are a DO concentration of $<3\,mg\,L^{-1}$, and a supply of labile organic matter and nitrate (Vidon and Hill, 2004), all of which are found in some of the pumped aquifer waters. However, the low P concentrations found in the groundwater may limit bacterial growth which could also limit denitrification. In addition, when considering the extent of denitrification that may occur in the aquifer water it needs to be appreciated that water was sampled via piezometers. This type of sampling may preferentially sample water that flows along paths that rapidly respond to the pumping and which may have greater oxygenation. Thus, whilst well oxygenated water with associated low concentrations of Fe and Mn in the ground water may suggest that anaerobic conditions are generally not found through much of the aquifer, widespread oxygenated conditions cannot be assumed. The presence of NO_2^- in a small number of samples suggests that small pockets of sediment or biogeochemical hotspots may exist within the aquifer where denitrification may occur. These could be related to changes in sediment architecture, (e.g. infilled clay channels—the alluvial clay sections in Fig. 2) or in the riparian zone where an increased supply of more labile carbon in the thinner soil profile may lead to greater redox sensitivity (Robertson and Schiff, 2008). The existence of biogeochemical hotspots within wider areas of riparian zones will influence denitrification as suggested by Vidon and Hill (2004) and McClain et al. (2003).

In the aquifer biogeochemical hotspots where denitrification may occur, the quality and composition of DOM will be important as it will be the carbon substrate for heterotrophic denitrifying bacteria (McClain et al., 2003). Siemens et al. (2003) found that DOC leached from soil was generally a poor source of carbon for

bacteria to use in denitrification as it had low biodegradability, possibly because of its age. They suggested that the transport of root exudates would provide a more reliable carbon source for denitrification. Baker and Vervier (2004) suggested that the rate of denitrification in an alluvial aquifer was best predicted by the concentration of low molecular weight organic acids including acetate, formate, lactate, butyrate and propionate.

If not denitrified losses of the accumulated NO_3-N is likely through (i) effluent flow to the river through the hyporohozic zone or (ii) losses to the River Trent through the ditches and streams that form part of the drainage system. Using the ground water Model ZOOM (Mansour and Hughes, 2004) with a particle tracking facility ZOOPT (Jackson, 2004), for the area in which our site is situated, estimates were made for the amount of time it may take for NO_3^- to leave the aquifer (Wang et al., 2010). The results are based on advection movement only, and with an estimate of porosity for the aquifer. Results showed that transport from the aquifer to the aquifer was slow, and it may take up to 10 years for a particle entering the aquifer via run-off at the furthermost edge of the aquifer (head over sand domain) to reach the river. The results of this study suggest that as DOM is passed from the soil to the aquifer there is an increase in the humification of the DOM, potentially decreasing the transfer of energy between different components of the ecosystem, through the decrease in its bioavailability (Marschner and Kalbitz, 2003). Thus the carbon released to the river from the aquifer is less likely to provide an energy source to the river ecosystems or participate in CO_2 degassing or photo-chemical reactions because of the increase in its humification. This may have implications for in-stream processing of N under baseflow conditions. It is also likely that some nitrate within the aquifer is assimilated into the microbial biomass. This however is likely to be mineralised back into NO_3 via the pool of organic N found in the aquifer water.

5. Conclusions

Shallow sand and gravel alluvial aquifer systems are common globally and are potential sources of nutrients to river ecosystems. The N and C cycles are linked in these systems through the transfer of DOM from the soil to the aquifer, providing an energy and nutrient source for the microbial processing of the nutrients. DOM properties were examined in the soils associated with the aquifer in KCl extracts and in the groundwater and River Trent. All three components of the system showed that DOM was typically of a terrestrial nature, but small changes occurred between ecosystem components. In soils the greatest change was in soils with a sandier texture where the HI and fulvic-like properties were higher suggesting that microbial greater decomposition occurs where accessibility to SOM is highest. Compared to soils, the groundwater had DOM with a higher HI index suggesting further processing of leached DOM from the soils. The results demonstrated that DOM analysis in the same extracts as used to extract inorganic and organic N has potential to provide information regarding the biodegradability of the DOC pool and the source of DON. However, analysis across different ecosystems would provide information as to its greater value, as compared to the soils in our work which are dominated by nitrogen fertilisation. A greater knowledge of the organic molecules that make up the EEM matrix is also required, although the use of PCA analysis appears to be an effective way to start understanding the data and picking out discrepancies as was found with the trypotophan-like components in soils and ground waters.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.01.033.

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