Unravelling organic matter and nutrient biogeochemistry in groundwater-fed rivers under baseflow conditions: uncertainty in *in situ* high-frequency analysis

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Abstract

In agricultural catchments, diffuse nutrient fluxes (mainly nitrogen N and phosphorus P), are observed to pollute receiving waters and cause eutrophication. Organic matter (OM) is important in mediating biogeochemical processes in freshwaters. Time series of the variation in nutrient and OM loads give insights into flux processes and their impact on biogeochemistry but are costly to maintain and challenging to analyse for elements that are highly reactive in the environment. We evaluated the capacity of the automated monitoring to capture typically low baseflow concentrations of the reactive forms of nutrients and OM: total reactive phosphorus (TRP), nitrate nitrogen (NO_3-N) and tryptophan-like fluorescence (TLF). We compared the performance of *in situ* monitoring (wet chemistry analyser, UV-Vis and fluorescence sensors) and automated grab sampling without instantaneous analysis using autosamplers. We found that automatic grab sampling shows storage transformations for TRP and TLF and do not reproduce the diurnal concentration pattern captured by the *in situ* analysers. The *in situ* TRP and fluorescence analysers respond to temperature variation and the relationship is concentration-dependent. Accurate detection of low P concentrations is particularly challenging due to large errors associated with both the *in situ* and autosampler measurements. Aquatic systems can be very sensitive to even low concentrations of P typical of baseflow conditions. Understanding transformations and measurement variability in reactive forms of nutrients and OM associated with *in situ* analysis is of

great importance for understanding in-stream biogeochemical functioning and establishing robust monitoring protocols.

Keywords

Macronutrients, Nitrogen, Phosphorus, Organic matter, Baseflow, Monitoring, High-resolution sampling, Autosamplers, Optical sensors, Groundwater-fed streams

1. Introduction

A growing number of studies report applications of *in situ* wet chemistry analysers and optical sensors for providing improved understanding of nutrient dynamics at the scales of hydrological responses. Fewer studies, however, evaluate the uncertainties associated with the *in situ* nutrient measurements and their focus mainly on the effects of sampling frequency on load estimation (Carey et al., 2014; Cassidy and Jordan, 2011; Rozemeijer et al., 2010) and on unravelling nutrient dynamics in response to storm flows (Bieroza and Heathwaite, 2015; Mellander et al., 2015). There is little work reporting uncertainty in high temporal resolution nutrient measurements during baseflow conditions when nutrient export is limited and in-stream processes become dominant.

Despite typically limited transfers of particulates and solutes during baseflow conditions, nutrient concentrations can exhibit a large temporal variation (Halliday et al., 2012; Pellerin et al., 2009; Scholefield et al., 2005; Wade et al., 2012). In rural streams subject to diffuse pollution and without major point sources, baseflow nutrient concentrations are generally low (Jarvie et al., 2010; Rothwell et al., 2010) and show diel cycles driven by the daily photoperiod (Nimick et al., 2011). In-stream processing and hyporheic exchange of reactive nutrients and organic matter (OM) controls baseflow nutrient concentrations in groundwater-fed streams (Lansdown et al., 2015). During baseflow conditions, the biogeochemical cycles of nutrients and OM become closely interlinked sustaining or limiting primary production, metabolic processes and controlling many biogeochemical processes in the water column and bed sediments. Many of these processes are

controlled by diel changes in temperature, light conditions and redox potential e.g. denitrification, sorption and desorption from the benthic sediments, metabolic uptake and release of nutrients e.g. assimilation of NO_3-N and release of labile OM by the autotrophs (Nimick et al., 2011; Trimmer et al., 2012). These in-stream transformations can potentially lead to a large variation in nutrients and OM on fine temporal scales and thus necessitates monitoring at high temporal resolution.

To date, high-resolution baseflow nutrient and OM dynamics are typically captured using autosamplers for water sample collection with subsequent laboratory analysis and automated wet chemistry instruments and optical sensors that undertake the measurements *in situ*. Autosamplers are routinely used for automated collection of time- or flow-based stream samples but show limitations of small sampling capacity, preferential sampling effects and storage transformations in unfiltered samples due to the time delay between sample collection and retrieval (Bende-Michl and Hairsine, 2010; Harmel et al., 2006; Kotlash and Chessman, 1998; McMillan et al., 2012). *In situ* analysers and sensors can address these issues by undertaking immediate chemical analysis in the field and thus removing the storage step (Bende-Michl and Hairsine, 2010). However, the wet chemistry analysers are prone to underestimation of concentrations compared with conventional sampling due to lower extraction of particulates, incomplete colour development in phosphorus (P) determinations (Bieroza et al., 2014; Jordan et al., 2005; Wade et al., 2012), temperature effects e.g. on reagent stability (Bende-Michl and Hairsine, 2010) and a requirement for a reliable power supply (Wade et al., 2012). Optical (absorbance and fluorescence) sensors are prone to interferences from light absorbing dissolved and particulate material (turbidity), temperature and self-quenching effects (Downing et al., 2012; Khamis et al., 2015). These uncertainties in measurements of baseflow nutrient concentrations with autosamplers and *in situ* instruments can potentially impair our understanding of baseflow nutrient dynamics e.g. limiting factors for primary production.

Here, we examine the variation in baseflow nutrient and OM concentrations determined by automatic *in situ* (wet chemistry analyser, UV-Vis and fluorescence sensors) and automated grab

sampling without instantaneous analysis using autosamplers. We evaluate and compare the performance of the automatic sampling techniques and their ability to capture typically low baseflow concentrations of highly reactive forms of nutrients and OM: (1) total reactive phosphorus (TRP), (2) nitrate nitrogen (NO_3-N) and (3) tryptophan-like fluorescence (TLF) which is an autochthonous fraction of OM linked to microbial production (Coble et al., 2014). We hypothesise that during summer baseflow conditions when nutrient and OM concentrations are typically low but variable due to diel cycling and primary production is at its highest, nutrient measurements with *in situ* analysers and autosamplers can be highly uncertain. In particular, we examine the effects of low nutrient concentration and temperature on *in situ* automatic determinations and autosampler storage effects for hourly samples retrieved daily for laboratory analyses. We compare concurrent measurements of TRP, NO_3-N and TLF on unfiltered samples obtained with *in situ* wet chemistry analyser (TRP), absorbance (NO_3-N) and fluorescence (TLF) sensors and autosampler with subsequent laboratory analyses and evaluate storage changes in both unfiltered and filtered autosamples for a range of nutrient and OM parameters (Table 1).

2. Methods

2.1. Study area

The lowland reach of the River Leith (Supplementary Figure A; catchment 54 km^2, annual rainfall 957 mm, 1999-2014, average annual evapotranspiration 480 mm, 1990-2010 (Allen et al., 2010)) in NW England is subject to diffuse nutrient pollution from agricultural land use (Bieroza and Heathwaite, 2015; Bieroza et al., 2014) and is a zone of strong surface-groundwater interactions (Binley et al., 2013; Lansdown et al., 2015). Since 2009, the river reach in Cliburn has been monitored using automated high-frequency *in situ* bank side analysers for nutrients and water quality parameters. Flow discharge is measured at 15 min intervals by an automated Environment Agency gauging station (NY 5896 2444) located approximately 200 m downstream of the monitoring unit (Supplementary Figure A) (Bieroza and Heathwaite, 2015). Previous research of

storm flow biogeochemical data revealed a seasonal transition in nutrient delivery from episodic delivery and supply limitation in summer to chemostatic delivery and transport limitation in winter and showed an important role of subsurface P and N delivery pathways (Bieroza and Heathwaite, 2015; Bieroza et al., 2014).

2.2. *In situ* and autosampler data collection

The bank side monitoring unit in the River Leith comprises a Nitratax Plus probe (UV-Vis sensor, Hach Lange, DE) measuring NO_x-N (NO_3-N and NO_2-N), a MicroMac C analyser (wet-chemistry analyser, Systea, IT) measuring TRP and WaterWatch unit (Systea, IT) measuring a range of water quality parameters (dissolved oxygen, pH, turbidity, specific conductivity, redox potential, water temperature) on an hourly to 15 min basis (Supplementary Table A). *In situ* TRP analysis (0.005-2.0 mgl^{-1}) is based on the phosphomolybdenum blue method (Murphy and Riley, 1962) and typically underestimates TRP concentrations by 3-8% compared to grab samples (Bieroza et al., 2014). *In situ* NO_3-N (in the River Leith mean NO_3-N 2.37 and NO_2-N 0.02 mgl^{-1} based on N=67 grab samples, 2009-2012) analysis (0.1-100 mgl^{-1}) is based on measurements of the raw UV-Vis absorbance spectrum of water and typically overestimates NO_3-N concentrations by 2-8% compared to conventional grab sampling (Bieroza et al., 2014). In addition, a UviLux Tryptophan sensor (fluorescence sensor, Chelsea Technologies Group Ltd, UK) was deployed at 1 s measurement interval for one week during baseflow conditions in July 2014. It measures fluorescence at 280 nm excitation and 360 nm emission wavelengths in a range of 0.0002 – 0.80 mgl^{-1} ± 0.02 mgl^{-1} determined from dose response curve of L-Tryptophan in deionised water. The MicroMac C, Nitratax and WaterWatch analysers are contained in a bank side unit and the UviLux sensor was installed directly in the stream with a sun protection cap. The bank side unit's inlet pipe is positioned at a mid-channel location, attached to the bottom of a floating buoy at a fixed depth of 15 cm below the water surface and equipped with a coarse filter to prevent clogging. The instruments were regularly serviced and maintained on a weekly basis including cleaning the

instruments, sampling line and tubes in MicroMac C and calibration to minimise drift in the *in situ* instruments.

To test the performance of automatic analysers during baseflow conditions, concurrent hourly water samples were collected with the ISCO® autosampler (Teledyne Isco, US) during two weekly sampling campaigns: *Baseflow 1* in 2013 and *Baseflow 2* in 2014 (Table 2). For TRP and NO_3-N additional concurrent samples were available for baseflow conditions in 2009-2014. The hourly autosamples were retrieved daily and analysed in the laboratory using standard analytical methods for nutrients and OM concentrations and using the same reagents for P determination as in the wet chemistry *in situ* analyser (Bieroza et al., 2014). Additional field and laboratory experiments were conducted to assess temperature effects on the performance of the *in situ* analysers and storage effects in autosamples collected hourly and retrieved daily for laboratory analyses.

Temperature effects on in situ nutrient and OM measurements

As the phosphomolybdenum blue method has been shown to be temperature dependent (Sjösten and Blomqvist, 1997), the *in situ* TRP determination is potentially susceptible to problems with maintaining a stable temperature within the monitoring unit. The TRP Systea Micromac C analyser is equipped with a small heater in the colorimeter set at 35 °C maintaining a consistent reaction temperature between the samples. However, the low power of the heater and short travel times of the reagents and the sample through the system (1 minute for sample mixing with reagents and 1 minute for colour development) potentially mean that ambient temperature changes can affect the performance of the analyser. Thus the performance of the TRP analyser was tested for a combination of four standard concentrations (0.01, 0.02, 0.05 and 0.10 mgl^{-1}) and five temperatures (5, 10, 15, 20 and 25 °C) using a water bath to maintain constant temperature. For each concentration repeated measurements were carried out keeping the temperature of sample, reagents and distilled water constant (Supplementary Table B). In the same experiment the temperature effects on the *in situ* determinations of NO_3-N were also evaluated for three concentrations of 0.5,

2.5 and 4.5 mgl^{-1} (Supplementary Table B). The *in situ* TLF measurements were also shown to be temperature dependent, thus a temperature correction factor was calculated following procedures described in the literature (Khamis et al., 2015; Watras et al., 2011) using L-tryptophan calibration stock 1000 mgl^{-1} solution and a 5-point dilution series (0.005, 0.010, 0.025, 0.050 and 0.100 mgl^{-1}).

Storage effects in water samples collected with autosamplers (autosamples)

Four 24 hour experiments were conducted to assess variation in baseflow nutrient and OM concentrations due to short-term storage in water samples collected with a 24 bottle ISCO® autosampler. The simulated sampling regime involved automatic collection of 24 x 1 l water samples on an hourly basis, filtration of the 50 ml aliquots (using 0.45 µm polyethersulfone membrane filter, Sarsted, DE, pre-washed with deionised water) once all samples had been collected and transportation of samples to the laboratory on a daily basis. Once in the laboratory, the samples were kept refrigerated and analysed on the day of collection for a range of nutrient and OM determinands (Table 1). The time delay between sample collection and laboratory analysis varied between 27-30 hours for the oldest sample (collected first) and 3-6 hours for the newest sample (collected last) with 23 hours and 1 hour respectively before the filtration step.

To assess the magnitude of storage changes, coefficients of variation (*CV*) were determined for three repeated measurements of each determinand (analytical precision) and compared with the variation between three repeated samples (sampling precision; Table 1) for all experiments.

Storage changes experiments 1-2

On each occasion, 25 l of unfiltered water was collected from the River Leith and transported to the laboratory within 3 hours keeping the sample cool and at dark. Upon delivery, water was separated into 4 x 1 l ISCO® autosampler bottles and kept in the dark at constant temperatures of 10 and 20 °C for 24 hours. For each temperature, four 50 ml unfiltered and filtered aliquots were collected:

two after 1 hour (1st bottle, the newest sample) and two after 24 hours (24th bottle, the oldest sample). All samples were then analysed for a suite of determinands listed in Table 1.

Storage changes in filtered samples experiment

Similarly to the experiments above, a large volume of river water was collected, transported to the laboratory and separated into two sets of 24 x 1 l l ISCO® autosampler bottles kept at 10 and 20 °C for 24 hours. Every hour two 50 ml filtered aliquots, one for each temperature, were removed and kept refrigerated until all 24 samples had been collected. All samples were analysed for determinands in Table 1.

Field experiment

Finally, a field experiment was carried out for 24 hours to assess the storage changes in hourly autosamples under ambient hydro-meteorological conditions with ambient temperatures between 14.6 and 21.2 °C. An autosampler was set to collect river water samples on an hourly basis, with samples being filtered once all samples had been collected. Simultaneously, a 50 ml river grab sample was manually collected and filtered once an hour for the duration of the experiment. In addition, hourly *in situ* measurements were also conducted for TRP, NO_3-N and TLF using the analyser and sensors described above.

2.3. Spectroscopic laboratory and data analyses

A detailed description of the acquisition and analysis of fluorescence and absorbance data is given in Supplementary Text A. Laboratory fluorescence measurements were carried out on Varian Cary Eclipse spectrophotometer with temperature controller and absorbance measurements were obtained from Varian Cary UV-Vis spectrophotometer. A number of fluorescence and absorbance parameters that characterise the quantity and quality of OM (Coble et al., 2014; Hudson et al., 2007) were calculated (Table 1 and Supplementary Text A), including a 6-component parallel factor analysis (PARAFAC) model (Supplementary Figure B) obtained with the *drEEM* toolbox (Murphy

et al., 2013). Freshwater OM fluorescence exhibits increased intensity in a number of regions called peaks (Table 1) that can be attributed to both terrestrial OM (peak A and C) and microbially-derived OM (peak T and B) with transitional peak M linked to microbial processing of terrestrial OM (Coble et al., 2014).

2.4 Data analysis

All data analyses were performed in Matlab (MathWorks, US) using standard statistical methods. All time series were checked for a presence of outliers. Linear least-squares regression with 5% confidence intervals was used to quantify the relationship between *in situ* and autosampler determinations of TRP, NO_3-N and TLF. Residuals were tested for normality and heteroscedasticity. Linear trends were obtained by least-squares first degree polynomial fitting.

3. Results

3.1. Uncertainty in *in situ* nutrient and organic matter concentrations

Mean baseflow concentrations in the River Leith (Table 2) are consistent between hourly *in situ* automated sampling (2009-2014) and monthly routine Environment Agency (EA) monitoring (1990-2014) for the sampling point located 100 m downstream of the *in situ* unit (Bieroza et al., 2014). There is a greater variation in concentrations determined with the *CV* for the EA monitoring compared with the *in situ* measurements and for TRP (79.3% (2009-2014) for *in situ* and 193.8% (1990-2014) and 144.1% (2009-2014) for the EA monitoring) compared with NO_3-N (12.2% for *in situ* and 20.7% and 17.7% for the EA monitoring). The TRP and NO_3-N concentrations observed during two weekly baseflow sampling campaigns (*Baseflow 1* in July 2013 and *Baseflow 2* in July 2014) are consistent with the results from the long-term monitoring. There are, however, marked differences in nutrient concentrations between the two sampling periods with lower TRP and higher NO_3-N in 2013 (0.010 and 2.67 mgl^{-1}) compared to 2014 (0.029 and2.28 mgl^{-1}) but with similar *CV* values for both determinands: ~20% for TRP and ~6% for NO_3-N (Table 2). These differences in

nutrient concentrations are likely driven by the differences in antecedent flow conditions (Supplementary Table C). The baseflow TLF concentrations measured as peak T_2 fluorescence intensity based on hourly autosamples showed similar patterns with lower concentrations in 2013 (0.06 in the Raman Units R.U.) compared with 2014 (0.28 R.U.). The latter value corresponded to a mean, temperature-corrected TLF concentration determined *in situ* of 0.022 mgl^{-1} (standard deviation ρ=0.003 mgl^{-1}, number of samples *N*=10500 and *CV*=13.9%) but a distinctively higher *CV* was observed for hourly autosamples (*CV*=41.1%, *N*=192).

A large variation in baseflow TRP and TLF concentrations compared with NO_3-N is apparent when comparing correlations between concurrent *in situ* and autosampler determinations (Figures 1). There is a large variation in TRP *in situ* and autosampler concentrations indicating a presence of relative under- and over-estimations (Figure 1a) with the slopes of the correlation distinctively below a 1:1 line. There is a particularly poor agreement between *in situ* and automatic *Baseflow 1* measurements (R^2=0.05, *N*=162) indicating potential storage effects in autosamples that mask a diurnal pattern observed in the *in situ* TRP concentrations (not shown here). The *in situ* TRP concentrations for the *Baseflow 1* campaign are close to the lower detection limit of the MicroMac C analyser which has been experimentally established as 0.0029 mgl^{-1} for *CV*=32% and *N*=5 (Supplementary Table B). For the NO_3-N concentrations (Figure 1b) the relative errors between *in situ* and automatic measurements do not exceed on average 10% and the automatic sampling replicates diurnal cycling observed in the *in situ* concentrations (not shown here). The slopes of the relationship are consistent and close to a 1:1 line (0.95 for *Baseflow 1* and 0.96 for *Baseflow 2*) and the majority of the variance observed in the *in situ* samples is explained by the automatic measurements (R^2=0.84, *N*=159 and R^2=0.95, *N*=167). The *Baseflow 1* dataset shows a relative 20% underestimation and *Baseflow 2* a relative 10% overestimation of the automatic samples (Figure 1a). The relative errors between *in situ* and autosampler NO_3-N measurements appear to be a

function of storage time and flow discharge: the longer the storage and higher the flow discharge, the greater the errors, but only the flow discharge relationship was significant at α=0.05 (Figure 2).

A relationship between automatic peak T_2 measurements and temperature-corrected *in situ* TLF concentrations (Figure 1c) showed a large amount of scatter reflected in the moderate strength of the correlation (R^2=0.45, N=168). Similarly to TRP, autosamples do not replicate the diurnal pattern observed in the *in situ* TLF measurements (not shown here).

3.2. Temperature effects on *in situ* determination of nutrients and organic matter

Only NO_3-N *in situ* measurements were not temperature-dependent (Supplementary Table B). The temperature effect on the *in situ* TRP determinations was significant for concentrations below 0.1 mgl^{-1} (Figure 3). There was a negative linear relationship between TRP concentration and temperature in the range of 5-15 °C and the slope of the relationship was concentration dependent. The highest decrease in the TRP concentrations was observed for the lowest concentrations of 0.01 mgl^{-1} (30%); for 0.02 mgl^{-1} it was 20% and for 0.05 mgl^{-1} 10% (Figure 3). In the range of 15-25 °C there was a <5% increase in TRP concentrations with temperature but this effect was not statistically significant. For the lowest tested concentration of 0.01 mgl^{-1}, the accuracy was calculated as -8.8% at 5 °C, -8.0% at 10 °C, -5.9% at 15 °C, -6.6% at 20 °C and -6.5% at 25 °C. The *in situ* TRP concentrations were underestimated compared to laboratory-based determinations and the effect was greater for lower concentrations. For the lowest TRP concentration of 0.01 mgl^{-1} at 5 °C the underestimation was 10% whereas for temperatures >15 °C it was as much as 60%.

The temperature quenching effect on TLF was evaluated based on the lab calibration of the *in situ* sensor; for TLF concentrations of 0.025 mgl^{-1} and temperatures 5-35 ºC a negative correlation with a slope value of -0.86 and intercept 0.042 mgl^{-1} was found (R^2=-0.83, N=30). The correlation was used to determine a temperature correction factor ρ of -0.0203 following a temperature compensation equation for *in situ* sensors described in detail elsewhere (Khamis et al., 2015; Watras et al., 2011). The value of ρ obtained here was in agreement with the estimations of Khamis *et al.*

(2015) for the same type of fluorometer – for TLF concentrations of 0.025 mgl^{-1} the authors found $\rho 1$=-0.0254 (slope -1.6, intercept 0.063 mgl^{-1}) and $\rho 2$=-0.0215 (slope -1.1, intercept 0.053 mgl^{-1}) and R^2>0.90.

3.3. Uncertainty in autosamples due to storage effects

The mean TRP, NO_3-N and TLF as peak T_2 fluorescence measured during the storage experiments (Table 1) were in the range of baseflow concentrations observed in the River Leith (Table 2). The analytical precision was typically lower than the variation between repeated samples: for NO_3-N 0.4% and 1.6% respectively, TRP 2.5% and 2.9%, peak T_2 3.0% and 3.4% and for the corresponding PARAFAC C4 component 1.3% and 4.5% (Table 1). Fluorescence variables showed a similar range of *CV* values for terrestrially-derived OM (peaks A and C and corresponding components C1 and C3, analytical precision 2.4% and sampling precision 2.7%) and higher values for microbially-derived OM (peaks T_1, T_2, B_1 and B_2, 5.8% and 8.3%; Table 1). The highest variation was observed for the fluorescence index (*FIX* 7.9% and 9.9%) and the freshness index ($\beta:\alpha$ 4.5% and 5.3%; Table 1).

These values can be compared with the variation in concentrations due to short-term (24 hour) storage of river water at two temperatures of 10 and 20 °C, reflecting the changes occurring in autosamples retrieved daily (Table 3 for unfiltered waters and Supplementary Table D for filtered waters). We found that the differences in concentrations after 24 hour storage compared to original concentrations for both unfiltered and filtered samples were statistically significant at α=0.05 for TRP/SRP, protein-like fluorescence (peaks T_1, T_2, B_1, B_2 and components C4, C5 and C6) and the freshness index $\beta:\alpha$. No significant differences were observed for NO_3-N, DOC, terrestrially-derived fluorescence (peaks A and C and components C1, C2 and C3) and related fluorescence and absorbance parameters (*FIX*, *HIX*, *a254*, S_R, *SUVA*) although a large between-sample variation was observed for DOC (*CV* up to 24.9%) and FIX (*CV* up to 6.1%). The latter variation can be explained by poor analytical and sampling precision of both determinands: DOC (*CV*=6.0 and

7.8%) and *FIX* (*CV*=7.9 and 9.9%; Table 1). The magnitude of storage changes was temperature dependent with a higher loss/gain in concentrations at higher temperature e.g. TRP loss of -13.5% at 10 °C and -23.4% at 20 °C (Table 3). For a given determinand, we also observed differences in the magnitude of storage changes between two experiments potentially reflecting differences in water sample composition.

Fluorescence peaks and PARAFAC components showed an initial relative increase in concentrations followed by a decrease over time, similarly to TOC, and a greater magnitude of change for *Storage experiment 1* and 20 °C (Table 3, Figure 4 and Supplementary Figure C). Terrestrially-derived OM showed lower variation compared with microbially-derived OM in both experiments (Figure 4 and Supplementary Figure C). Relative increases in peak A fluorescence did not exceed 8.6% (4.8% for the equivalent PARAFAC component C1) and 11.2% for peak C (4.5% for C3). Both TLF regions showed a marked increase in concentrations of 64.4% for peak T_1 (125.6% for C5) and 118.1% for peak T_2 (313.0% for C4). Tyrosine-like fluorescence (peaks B_1 and B_2 and component C6) showed both losses up to -41.1% and increases up to 117.7% (Table 3).

The pattern of storage changes were similar in filtered samples, with distinctively higher relative increases in microbially-derived OM fluorescence compared with unfiltered samples (Supplementary Table D and Supplementary Figures D-E). The effect of sample filtration was significant for differences between TRP/SRP (-36.2%), TOC/DOC (43.6%) and microbially-derived peaks T_1-T_2 (-31.0%) and peaks B_1-B_2 (Figure 5). In contrast, filtration did not introduce significant differences for TON/NO_3-N and terrestrially-derived OM (peaks A and C).

The total fluorescence measured as a sum of individual PARAFAC component scores (Table 3) and a relative contribution of PARAFAC components (Table 4) changed significantly, showing a relative increase in TLF e.g. C4 from 9.3% to 27.2% at 20 °C and corresponding decrease in all other fluorescence components.

There was a clear effect of storage changes on dissolved fractions of nutrients and OM, particularly at higher temperature (*Storage changes in filtered samples experiment*; Figure 6 and Supplementary Figure F). Determinands showed a variable sensitivity to storage conditions, with several exhibiting linear negative trends over time (Supplementary Table E) indicating a decrease in concentrations e.g. SRP loss of 50% and NO_3-N loss of 3.8% at 20 °C. Spectroscopic parameters showed negative trends which were significant at 20 °C for *a254* (7.4%), peak C and C3 (7.0%) and C2 (6.1%) (Supplementary Table E). The TLF showed the largest variation in concentrations 83.9% at 20 °C and 44.3% at 10 °C (Figure 6) and the relative contribution in the total fluorescence (Supplementary Table F).

Finally, for TRP and TLF the diurnal pattern in concentrations captured by the *in situ* measurements (*Field experiment*) was masked by the storage changes in the autosamples retrieved daily (Figure 7). The TRP/SRP diurnal pattern was reproduced with hourly autosamples both unfiltered and immediately filtered but even this procedure was not successful in reproducing the TLF diurnal signal. Although *in situ* measurements are carried out on unfiltered samples, there is a good agreement with the filtered samples as SRP and NO_3-N constitute majority of the total fractions in the River Leith (Bieroza et al., 2014).

4. Discussion

4.1 Uncertainty in *in situ* baseflow determinations of nutrients and organic matter

Automated *in situ* sampling technologies enable collection of biogeochemical data at timescales that are sensitive to changes in flow (Bieroza et al., 2014; Halliday et al., 2015; Jordan et al., 2005; Wade et al., 2012) and have improved our understanding of biogeochemical patterns and processes (Bende-Michl et al., 2013; Bieroza and Heathwaite, 2015; Halliday et al., 2012; Outram et al., 2014), and nutrient load estimation (Cassidy and Jordan, 2011; Rozemeijer et al., 2010). The wet chemistry *in situ* analysers are particularly useful in providing insights into P fractions dynamics in

response to varying flow conditions (Mellander et al., 2015) and management practices (Perks et al., 2015).

A better understanding of the sources of analytical and sampling uncertainties would help support wider uptake of *in situ* wet chemistry and optical analysers in freshwater systems. Key here are: (1) underestimation of suspended sediments and sediment-bound solutes due to lower extraction of particulates (Bieroza et al., 2014; Jordan et al., 2005) particularly during storm events, (2) sensitivity to changes in physicochemical water properties, mainly ambient temperature and pH (Coble et al., 2014; Wade et al., 2012), (3) loss of signal due to light attenuation by both coloured dissolved material (the inner-filter effect) and suspended particles (Downing et al., 2012; Khamis et al., 2015; Saraceno et al., 2009) potentially leading to underestimation of *in situ* spectroscopic determinations of OM fluorescence and NO_3-N concentrations. For baseflow, low concentrations near the detection limit of *in situ* analysers and the temperature-dependence on the *in situ* determinations are also important and are discussed below.

Low concentrations

In groundwater-fed streams, the relative importance of aquifer and subsurface pathways on water column nutrient and OM concentrations increases during baseflow conditions. We observed low baseflow nutrient concentrations compared with similar studies of agricultural streams (Jarvie et al., 2010; Rothwell et al., 2010). Earlier work has shown the importance of groundwater sources for NO_3-N with concentrations typically higher (4.5 mgl^{-1}) than the stream (Bieroza et al., 2014; Lansdown et al., 2015), and shown that as flow decreases, TRP concentrations typically decrease and NO_3-N concentrations typically increase (Bieroza and Heathwaite, 2015). The different flux pathways for N and P help to account for the observed differences in the two baseflow sampling campaigns: lower TRP and higher NO_3-N concentrations at lower flow discharge for *Baseflow 1* compared with *Baseflow 2*. The presence of the concentration effect controlling the TRP concentrations means that at baseflow conditions the TRP concentrations can reach the lower limit

of detection of the *in situ* analyser. As discussed by Bende-Michl and Hairsine (2010), the potential error in *in situ* analysis increases during flows with low concentrations and the accuracy of the instruments decreases at the detection limits. In our study, the TRP analyser's accuracy was both concentration and temperature dependent and much lower than the accuracy of ±3% claimed by manufacturer MicroMac C (Bende-Michl and Hairsine, 2010). However, the latter value was achieved in a laboratory-controlled conditions and a wider calibration range 0.001-1.0 mgl^{-1}. Low accuracy at the lower detection limit leads to a high degree of variation in low TRP concentrations and can also result from a two-point calibration in wet chemistry analysers compared to a typical five-point calibration used in the benchtop laboratory instruments. This highlights that P *in situ* measurement in freshwater systems is still an analytical challenge as there is a need to accurately capture a wide range of P concentrations, from very low concentrations important for the ecological functioning of the aquatic ecosystems to very large concentrations in order to provide an accurate estimation of loads and compliance with the environmental programmes.

In contrast to TRP, the NO_3-N *in situ* determinations were in good agreement with the autosampler determinations suggesting both low errors in *in situ* NO_3-N analysis and low storage-related errors in autosamples. The environmental range of observed NO_3-N concentrations in freshwater aquatic ecosystems subject to diffuse pollution (Bieroza et al., 2014; Halliday et al., 2012; Wade et al., 2012) is typically much narrower compared with P, thus allowing a better accuracy of the *in situ* analysers. *In situ* NO_3-N measurements are also based on a relatively simple spectroscopic determination compared with a more complex wet-chemistry determination of P. A consistency in the 1:1 slope of the relationship between *in situ* and grab NO_3-N concentrations was observed also in other studies (Bieroza et al., 2014; Carey et al., 2014; Wade et al., 2012). Carey et al. (2014) linked the varying intercept of the relationship with the seasonal differences in the chemical matrix and flow conditions and our study focused on baseflow conditions showed a ±20% variation potentially related to storage time and flow discharge.

Observed differences between *in situ* and laboratory-based determinations of nutrients and OM can also potentially result from a number of analytical differences between *in situ* and laboratory measurements that can be eliminated by a regular maintenance of the *in situ* instruments, use of the same analytical methods and reagents. However, as the above results show, a perfect match between *in situ* and laboratory-based measurements is difficult to achieve for low concentration samples for which measurements are more uncertain.

Temperature effects

The second source of uncertainty in *in situ* baseflow nutrient and OM determinations can result from temperature effects on the formation rate of the phosphomolybdenum complex (Sjösten and Blomqvist, 1997), reagent stability (Bende-Michl and Hairsine, 2010) for wet chemistry analysers, and fluorescence intensity quenching for optical sensors (Coble et al., 2014; Khamis et al., 2015). Here, both TRP and TLF showed the temperature effects due to the temperature dependence of the phosphomolybdenum blue method (Sjösten and Blomqvist, 1997) and a negative linear relationship between temperature and fluorescence intensity (Watras et al., 2011). The formation rate of the blue phosphomolybdenum complex has been shown to decrease with decreasing orthophosphate concentration and decreasing reaction temperature (Sjösten and Blomqvist, 1997). The authors found that at 15 °C only samples with orthophosphate concentrations above 50 mgl^{-1} reached full colour development in 5 min. For lower concentrations and temperatures, the reaction took longer. As shown by Jarvie et al. (2002) large errors in low P concentration samples can be a combination of a slower rate of the phosphomolybdenum blue complex formation and larger sensitivity to matrix interferences effects due to low intensity of colour formation. Our finding that the temperature effect was present for ambient temperatures <15 °C is consistent with the study of Wade et al. (2012) for the Systea Micromac C analyser. The temperature-related underestimation of TRP concentrations was the greatest at 15 °C: 40% for 0.01 mgl^{-1} and 25% for 0.02 mgl^{-1} and remained relatively constant for temperatures >15 °C, typically observed during summer baseflow

conditions in the River Leith. Thus, the *in situ* TRP concentrations in our study are potentially underestimated by ~40% for *Baseflow 1* and ~20% for *Baseflow 2* and their correction could improve, to an extent, the slope of the relationship with the automatic samples in Figure 1a.

All automatic *in situ* analysers used in our study show similar ambient operating conditions: MicroMac C TRP analyser 4-40 °C, Nitratax Plus NO_3-N sensor 2-40 °C and UviLux tryptophan sensor -2-40 °C. Bende-Michl and Hairsine (2010) showed that rapid temperature changes accelerate the decay of some reagents even within the range recommended by the manufacturer. These effects can potentially be eliminated by installation of temperature-controlled housing of the instruments but as shown by Wade et al. (2012) it does not completely remove the issue particularly during the winter months.

Organic matter fluorescence intensity is inversely related to temperature at the rate of 7 0.8-1.5% per 1 °C for CDOM (Downing et al., 2012; Watras et al., 2011) and 0.0011-0.0016 mgl^{-1} per 1 °C for TLF (Khamis et al., 2015). Thus *in situ* fluorescence measurements need to be temperature-corrected in order to provide correct interpretation of biogeochemical patterns (Watras et al., 2011). The temperature correction of *in situ* fluorescence sensors is typically based on laboratory trials that do not account for interferences resulting from matrix effects introduced by field deployments (Khamis et al., 2015). We found that temperature-corrected TLF was temperature dependent (R^2=-0. 36, N=818) and exhibited linear correlations with the temperature-corrected specific conductivity (R^2=0. 62), redox potential (R^2=-0.63), turbidity (R^2=0. 30) and pH (R^2=0. 25, N=818, data not shown here). The above relationships can be indicative of diurnal cycling driven by temperature dynamics or sensitivity of the sensor technology to even subtle changes in water matrix as those observed during baseflow conditions.

Interference of turbidity on *in situ* TLF measurements has been shown to be negligible at low concentrations (Khamis et al., 2015), however, turbidity in our study explained 30% of the variance in the *in situ* temperature-corrected TLF measurements.

The interference from pH has been shown to have little impact on *in situ* determinations of CDOM (Spencer et al., 2007) but it was acknowledged that it can affect TLF associated with colloidal material through pH-related conformational transformations (Baker et al., 2007) that lead to shifts in excitation and emission wavelengths. As shown in our study, there is a potentially large pool (20-40%) of baseflow TLF associated with particulate and colloidal fractions in freshwater samples. As the spectra of *in situ* sensors are fixed, they might not resolve the quantity of fluorescent OM correctly.

4.2 Uncertainty in autosampler baseflow determinations of nutrients and organic matter

The daily variation in *in situ* concentrations in our study was generally low: TRP *Baseflow 1* 19.1% ±3.6% and *Baseflow 2* 10.2% ±3.2%, NO_3-N *Baseflow 1* 1.2% ±0.4% and *Baseflow 2* 2.0% ±0.6% and TLF during *Baseflow 2* for 1 s temperature-corrected time series 5.8% ±2.3% and for 5 min moving averages 3.1% ±1.7%. These subtle solute signals detected by the *in situ* analysers, with SRP diurnal amplitude detectable as low as 0.003 mgl^{-1} (Cohen et al., 2013), can therefore be easily masked by errors associated with the automated sampling. Our study corroborates this observation; we found that the variation due to short-term storage changes in autosamples is significantly larger than diurnal variation in *in situ* concentrations. Many studies utilising autosamplers implicitly assume accurate representation of the *in situ* sample composition and negligible storage changes up to 24 hours from collection (Ghazaleh et al., 2014) and therefore the literature documenting short-term storage changes in autosamples is scarce. Multiple physical, chemical and biological processes in autosamples including sorption, hydrolysis, precipitation, complexation, microbial uptake and release can modify nutrient and OM concentration and their speciation during the time delay between sample collection and analysis (Harmel et al., 2006; Jarvie et al., 2002; Kotlash and Chessman, 1998). For low nutrient concentration sites (e.g. rural catchments without major sewage effluents) and seasons (e.g. summer baseflow conditions), absolute losses can be smaller but percentage losses are generally higher compared to more polluted catchments and storm events

(Kotlash and Chessman, 1998). The biogeochemical effects constitute the majority of uncertainty in autosamples e.g. for total P (TP) 64-92% compared to 0-17% errors due to preferential sampling and lower extraction of particulates and sediment-bound P (McMillan et al., 2012). The highest losses of dissolved nutrients occur from low-concentration samples, with up to 50% of NO_3-N, and 67% of SRP potentially lost after 6 days of storage without refrigeration (Kotlash and Chessman, 1998). In our study, the NO_3-N concentrations (μ=2.6 mgl^{-1}) were the least sensitive to short-term storage in autosamples with absolute changes similar to the analytical precision of laboratory-based instruments (*CV*=0.4%) and not exceeding the sampling precision (*CV*=1.6%). The TRP/SRP concentrations (μ=0.0036 mgl^{-1}) showed significant linear losses over the short-term that can result from adsorption associated with microbial uptake and adsorption onto particulates or autosampler bottle walls due to high P charge density (Harmel et al., 2006; Jarvie et al., 2002). As differences between TP and SRP and thus the concentration of particulates were low (16.7%), samples were shaken before removing aliquots and storage losses were temperature dependent, the microbial uptake is a plausible cause of SRP depletion during storage.

The fluorescence signature of autosamples exhibited significant shifts in relative OM composition towards a higher percentage of labile, tryptophan-like material. TLF is an indicator of algal and microbial activity and biogeochemical oxygen demand (Hudson et al., 2007) and in general is more reactive than recalcitrant, terrestrially-derived OM fractions (peaks A, C, M) (Coble et al., 2014). Thus TLF storage changes potentially suggest enhanced microbially mediated nutrient and OM transformations and large sensitivity of TLF to storage conditions. As shown by Ghazaleh et al. (2014) the temperature and not the storage duration was an important factor in controlling the faecal bacteria abundance in autosamples and variation in microbial concentrations. We observed a similar pattern in our study, with rapid initial temperature-dependent increase in TLF (within 1-6 hours) followed by a gradual decrease in concentrations accompanied by incidental increases not related to storage duration.

Filtration through a 0.45 μm filter reduced the TLF intensity by 20-40% screening out particulate OM and larger micro-organisms (bacteria, phytoplankton). Baker et al. (2007) showed that particulate and colloidal microbial material > 1.2 μm accounts for a large portion of the TLF (peak T_1). We tested the hypothesis that incidental increases in tryptophan-like OM could be related to mechanical effects of sample filtration and potential damage to microbial cell aggregates (Ghazaleh et al., 2014; Harmel et al., 2006). However, as similar patterns were observed in both unfiltered and filtered samples and we did not observe corresponding spikes in SRP suggesting cell lysis, we concluded that the filtering artefacts were negligible and incidental increases in TLF are evidence of underlying biogeochemical process.

Thus, we suggest that microbial activity in autosamples can play an important role in controlling TRP/SRP and TLF concentrations during short-term storage. There is potentially a scope for correction of short-term storage changes in autosamples for TRP/SRP concentrations as they follow a linear trend, however due to large variation at higher temperatures the correction might not be feasible. For determinands exhibiting large variation and both increases and decreases in concentrations (tryptophan- and tyrosine-like fluorescence), short-term storage correction might not be possible and there is a need for *in situ* analysis. As P and micro-organisms can attach to the autosampler bottle surface, shaking the sample prior to analysis will reduce the potential losses (Ghazaleh et al., 2014; Harmel et al., 2006; Jarvie et al., 2002).

5. Conclusions and implications - Towards robust automated high-frequency baseflow monitoring

Water samples with low P and OM concentrations appear most vulnerable to *in situ* analytical errors and storage changes in autosamples, likely due to increased microbial processing and chemical precipitation, since percentage errors are greatest when initial concentrations are low (Jarvie et al., 2002). Use of preservatives e.g. acidification, although effective in minimising the storage effects for N, are not appropriate for P analysis as they enhance desorption of P from

particulates. Refrigeration of automatic samples during the time before collection and retrieval is possible but requires power supply that may not be available in remote locations.

Automated high-frequency sampling brings new understanding to the complex biogeochemical processes in freshwaters and their coupling with hydrological controls. Baseflow and storm events form boundary conditions for functioning of stream ecosystems and provide a testing ground for a range of rapidly emerging *in situ* sensor technologies enabling fast determination of nutrients and OM. During baseflow conditions, the main sources of uncertainty in nutrient and OM *in situ* determinations are the persistence of low (near detection limit) concentrations and confounding effects of diurnal variation in temperature, pH and redox potential. Capturing baseflow nutrient and OM dynamics is important for the understanding of diffuse pollution, the role of biogeochemical controls on nutrient and OM processing and the importance of potential legacy stores in the catchment. High-resolution *in situ* nutrient and OM monitoring is critical in bridging the gap between existing monitoring networks operating at coarse spatial and temporal resolutions and scientific needs requiring data at fine resolution. For example, existing routine water quality monitoring networks are generally not fit for the purpose of providing evidence of diffuse pollution (Sharpley et al., 2015). In England and Wales, the current water quality monitoring network managed by the Environment Agency (EA) evolved from the General Quality Assessment initiated in 1990 which was aimed at targeting point-sources e.g. effluents from sewage treatment works. As a result of this legacy, the routine monitoring network does not provide evidence of diffuse pollution on appropriate high temporal and spatial scales (Bieroza et al., 2014). Sensor technology could potentially address these issues subject to better understanding of measurement uncertainty, catchment-specific correction factors for interference effects of temperature, turbidity and pH along with their seasonal variations. For fluorescence sensors in particular, although the exact chemical nature of fluorophores is not yet well understood (Coble et al., 2014), the research on ultrahigh-resolution mass spectrometry (Kellerman et al., 2015) can provide chemical meaning for

correlations observed between fluorescent OM and environmental determinands (Hudson et al., 2007). Finally, as the sensor measurements are typically based on unfiltered samples, to utilise the benefits of the technology (online measurements and *in situ* deployment), further studies characterising both particulate and dissolved fractions of nutrients and OM fluorescence and matrix effects on *in situ* measurements are needed.

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