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Focus Article

Understanding dissolved organic matter dynamics in urban catchments: insights from *in situ* fluorescence sensor technology

K. Khamis,* C. Bradley and D.M. Hannah

Dissolved organic matter (DOM) is critically important for catchment biogeochemical cycling, yet the DOM dynamics of many river systems remain poorly characterized. Recently, DOM mobilization and transport in forested and agricultural catchments have received increased attention; however, for urban catchments, our understanding of spatio-temporal variability in DOM concentration and composition is very limited. This is a particular concern as urbanization can increase and alter labile DOM fluxes leading to a shift from downstream transport of stream carbon to increased microbial production and respiration of stream carbon in headwaters. Furthermore, the anthropogenic modification of the water cycle and the flashy hydrology of urban rivers have constrained attempts to characterize intra- and inter-seasonal variability in DOM across the spectrum from low to storm flows. In this *focus article*, we synthesize the contemporary literature on urban DOM sources, flow paths, and spatio-temporal variability and present a conceptual model to unravel system dynamics and inform future monitoring efforts. The potential of field deployable fluorescence sensor technology to overcome monitoring challenges in urban rivers is highlighted. We use a case study of a relatively well-studied UK urban river to illustrate the potential of *in situ* fluorescence to reveal DOM dynamics in a system with marked inter-event variability in DOM sources and pathways. Finally, we outline future directions for this research, particular the need to standardize field and laboratory protocols and advance new sensor development.

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INTRODUCTION

Globally urbanization is increasing at a rapid rate with urban areas forecast to triple between 2000 and 2030.[1] This change in landcover is having a profound effect on freshwater ecosystems, modifying water flow pathways and strongly altering the quantity and quality of nutrients and dissolved organic matter (DOM) in rivers. DOM is a complex, heterogeneous, combination of compounds that vary in their solubility and reactivity: the quality and quantity of allochthonous DOM is derived from plant and soil organic matter (OM), and reflects landscape, vegetation, hydrology, and climate, while autochthonous DOM is produced by microbial processing of OM.[2] The transport, processing, and transformation changes according to the river network properties and

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the reactivity of the DOM.[3] DOM quality and quantity are determined by a combination of their origin and the biogeochemical processes associated with their production and transport.[4] To date, DOM studies have focused on semi-'natural' or agricultural catchments[5,6] neglecting the effects of urbanization, although, urban rivers and streams have been associated with a unique OM composition,[7] with a higher proportion of microbial OM and trace pollutants such as pharmaceuticals, endocrine disrupters, and polycyclic aromatic hydrocarbons (PAHs). DOM in urban water-courses is also highly dynamic reflecting the interaction and nonlinear mixing of DOM derived from varying sources, transport along diverse pathways over different timescales, and variation in the extent and nature of subsequent transformation.[8] Unraveling this complexity is essential in the context of satisfying regulatory requirements for water quality, particularly given the role of DOM in the associated transport of heavy metals, persistent organic pollutants, and as a precursor of disinfection byproducts[9,10] Furthermore, given the global increase in urbanization, greater understanding of the key drivers of urban biogeochemistry is needed urgently to: (1) enable urban catchments to be managed and developed more sustainably and (2) assess the potential implications for carbon cycling, particularly in rivers with significant urban landcover.

The DOM of urban rivers is composed of a greater fraction of low molecular weight and proteinaceous material relative to rural systems reflecting the importance of microbially derived OM from human and animal waste, and algal-derived OM from eutrophication.[11,12] However, in urban catchments DOM is highly variable, and characterized by non-stationarity at different scales given the interaction between: (1) the anthropogenic sewerage and drainage systems, which are designed for the rapid conveyance of rainfall through urban catchments and (2) waters that have passed through the 'natural' or undrained areas of the catchment. Additional DOM inputs include effluent from sewage treatment works, and untreated sewage which may be intermittently discharged via stormwater sewers and combined sewer overflows during rain events, or regularly discharged if the sewage treatment infrastructure is insufficient, for example, in some rapidly developing 'mega-cities'.[13,14] The conventional model suggests that DOM from urban sources is highly bioavailable and subject to rapid processing characterized by the rapid loss of labile, low-molecular weight material, such as proteins, carbohydrates, and organic acids, with the production of more refractory DOM of higher molecular weight. However, the high molecular weight DOM may be more reactive than previously thought (according to the size-reactivity continuum model) due to the extent of microbial processing.[12] Labile DOM is readily processed by microbial communities in-stream, potentially leading to oxygen depletion with implications for other ecosystem functions, such as nitrogen cycling, decomposition rates, and secondary production.[15] New approaches to understand and resolve problems of poor urban water quality are needed urgently, particularly related to measurement resolution and repeatability[16] given projections of a continued and sustained global increase in urban populations.[17,18]

Fluorescence spectroscopy has the potential to make a significant contribution to research on urban water quality as specific DOM components can be characterized and quantified rapidly.[19] The physical basis for this approach lies in the absorption and subsequent emission of energy (i.e., fluorescence) by organic molecules when excited at specific wavelengths. Fluorescent DOM (FDOM) is a subset of the absorbent component of DOM, colored dissolved organic matter, and can be used to trace important compositional changes.[20] Determination of a full excitation–emission matrix (EEM) in optical space, can identify broad groups of fluorophores including humic and fulvic acids, proteins, and amino acids (e.g., tryptophan and tyrosine). Further information can be derived from FDOM indices,[21] such as the Fluorescence Index (FI), which is indicative of DOM source (higher values indicating increased autochthonous as opposed to allochthonous DOM), and the Humification Index (HIX), which is indicative of more humic, complex, DOM. These qualitative indices are more useful when evaluated in combination[22]; however, they provide a basis for developing *in situ* applications of fluorescence spectroscopy.

Recently developed submersible fluorescence sensors offer huge opportunities to significantly improve our understanding of urban biogeochemistry by quantifying seasonal and event dynamics, in the context of continually varying DOM sources, pathways, and processing in urban catchments. In this *focus article*, we explore these interrelationships in more detail by:

1. synthesizing recent research on urban biogeochemistry and deriving a conceptual model describing urban DOM sources, pathways, and processing;
2. providing a detailed overview of field deployable fluorescence sensor technology and outlining methods to overcome challenges to water quality monitoring in urban rivers and streams;

3. presenting a case study on the use of *in situ* fluorescence monitoring for a relatively well-studied urban river system, highlighting how this technology can be used to develop our fundamental knowledge of urban DOM dynamics.
4. highlighting current uncertainties and detailing promising future directions for work with respect to standardizing monitoring programs and capitalizing on recent advances in sensor technologies.

URBAN RIVER BIOGEOCHEMICAL PROCESSES

Urban catchments are characterized by a high diversity of DOM sources ('natural' but additionally anthropogenic sources) and a complex drainage system in which engineered structures (storm drains, sewers, ditches, and retention tanks) complement 'natural' flow pathways. When considered in terms of transport, or hydraulic routing, engineered pathways are (very) fast and natural pathways are generally slower. The fast, engineered pathways are primarily active during periods of rainfall via the storm drainage and combined sewer overflow (CSO) networks. This structural complexity (both above and below ground) combined with the flashy flow regime complicates the quantification of urban hydrological and biogeochemical process interactions. Detailed monitoring of urban drainage systems is also problematic and limited by the constraints of conventional sampling methods, particularly in the catchment headwaters given their spatial extent and variability.[23] Hence, in reviewing urban hydrological and biogeochemical processes for temperate regions, we focus on two specific components of the hydrograph: *low flow* (Figure 1(a)) and stormflow (Figure 1(b)) for headwater systems, recognizing that there will inevitably be differences in the process dynamics that characterize urbanized low-gradient and floodplain areas downstream.

During low flows, river flow predominantly comprises groundwater and waters that have followed deep sub-surface flow pathways through the soil matrix associated with areas of natural and semi-natural vegetation including parks, woodland, and gardens.[24] This baseflow may be augmented by agricultural areas bordering urban regions, effluent from waste water treatment works (WwTW) and industry with additional, but less predictable, inputs from cross-connected sewers and leaky drainage pipes.[25,26] These water sources all have a distinct DOM composition, often associated with specific flow paths to the

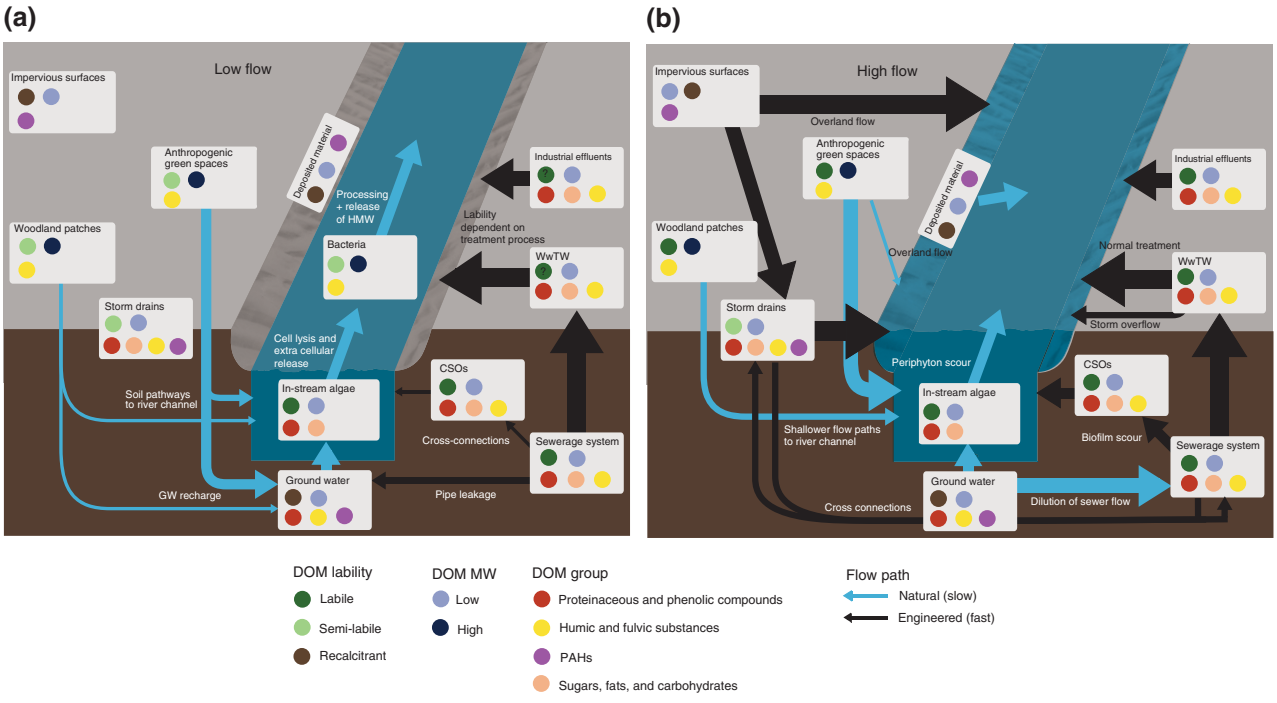

FIGURE 1 | Conceptual representation of dissolved organic matter (DOM) and water fluxes to headwater urban river system during: (a) low flow and (b) high flow. CSO, combined sewerage overflow; GW, ground water; HMW, high molecular weight; MW, molecular weight; PAH, polycyclic aromatic hydrocarbon; WwTW, waste water treatment works; ?, DOM lability is dependent on waste water treatment process. Size of arrows are proportional to the magnitude of the flux.

river network, which vary in residence time and potential for photodegradation and microbial processing of DOM.[27] Conversely, during storm flow events, runoff from impervious surfaces and flow through the storm drainage system are increasingly important[24] and can contribute >50% of total river flow. Under these conditions, even rainfall on areas of woodland, or other urban green space, is likely to be routed rapidly to the river channel through shallower water flow pathways.[28,29]

During baseflow conditions, in-stream sources and processing of DOM are important: reflecting a 'transformation-focus' in the river where the DOM mainly comprises material from algal biomass.[8] Extracellular release and cell lysis provide a highly labile source of DOM consisting of predominately sugars and amino acids,[30] while transformation of DOM by bacteria can lead to production of humic material.[31] Effluent from water treatment plants and industrial activities generally have high DOM concentrations, particularly low molecular weight material (e.g., phenols, indoles, and proteinaceous material), however, their lability will largely depend on the treatment processes prior to discharge.[32] Groundwater inputs are particularly important during baseflow, the DOM of which can be enriched with protein-like material through leakage from the sewerage system[33,34] or labile humic material from recharge waters associated with urban green space.[35] Inputs from deeper soil flow paths with water sourced from woodland/green spaces are proportionately less important but will be high in aromatic compounds and hence will be more recalcitrant than other DOM sources.[36]

During storm events, DOM sources and pathways are altered, with previously disconnected DOM stores may be re-connected to the main channel (Figure 1(b)) and the riverine DOM system becomes transport dominated.[8] In-stream sources (benthic algae) are rapidly flushed or scoured from the system, possibly only contributing to a first flush of DOM[37] (Figure 2). Similarly, biofilms are scoured from the storm drainage—sewerage system during high magnitude events, representing a highly labile source of DOM consisting of protein-like material and carbohydrates mobilized earlier in the event.[38] Deposited material on gravel bars and river banks is re-suspended and can represent a significant source of PAHs.[39] CSOs rapidly route diluted sewage to the river network consisting of highly labile DOM, diluted in proportion to the event magnitude.[38] In addition, detergents and leaf litter can be mobilized as surfaces are rewetted during storm conditions and rapid leaching of proteins and carbohydrates can

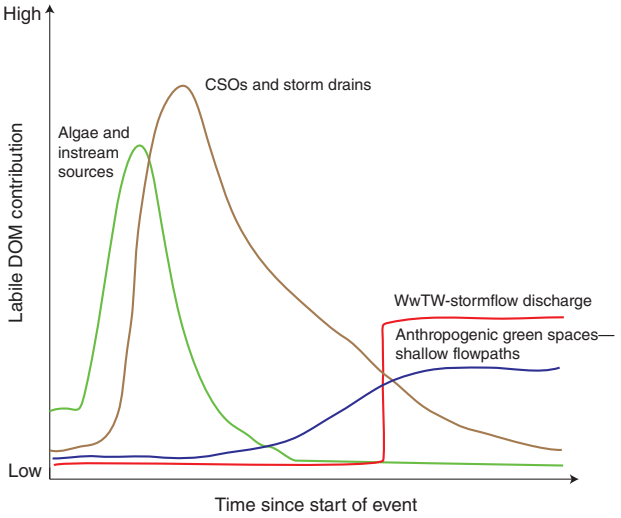

FIGURE 2 | Conceptual representation of labile dissolved organic matter (DOM) source contributions to stream to total DOM flux over storm events. CSOs, combined sewer overflows; WwTW, waste water treatment works.

occur.[40] Mobilization of dust from impervious surfaces also provides a source of PAH and low molecular weight OM.[41] In contrast, rising water tables can lead to groundwater exfiltration into the drainage and sewerage network thereby diluting labile DOM.[42] Both anthropogenic green spaces and woodland patches are likely to be characterized by the increasing predominance of shallow, sub-surface flow paths, or overland flow if the soil matrix becomes saturated or the surface is impermeable (e.g., road surfaces): the shorter residence time and change in flow path leads to 'fresher' humic-like material that is potentially more labile.[43]

Individual flow pathways differ markedly in their potential for DOM processing due to differing residence times and potential for photo-oxidation. For storm events where an initial flush of scoured biofilms occurs, or when coarse OM is transported rapidly to the river, there may be limited time for microbial processing or transformation (Figure 2). Moreover, as the drainage infrastructure is frequently below ground, there is limited potential for DOM photodegradation with implications for DOM quality and NO_3^- uptake.[27] During both low flow and high flow, sewerage networks rapidly transport a high volume of labile DOM to WwTWs for processing. The residence time within the treatment plant (usually <12 h) and biodegradability of final effluent DOM depends upon the treatment level.[44] However during high-flow events, untreated or partially treated sewage can be discharged to the river when available storage capacities are exceeded (Figure 2).[45] Natural flow paths have longer residence times;

hence, a parcel of water has increased time for microbial processing. During 'normal' flow conditions, the signal is generally more humified and less labile[43] but as the soil becomes saturated, surface flows can enable the rapid routing of fresh humic material to the channel.[46]

IN SITU ENVIRONMENTAL FLUORESCENCE

Over the last 20 years, fluorescence spectroscopy has become an increasingly popular method for rapid characterization of DOM from natural and engineered freshwater systems.[4,47–49] The physical basis for this approach has been treated in numerous texts and papers and the interested reader is directed to Lackowicz.[50] Fluorescence spectroscopy can provide quantitative and qualitative information on DOM composition and concentration.[20] Specific fluorescence peaks, that represent flurophores of similar chemical composition, can be targeted based on *a priori* knowledge (peak picking: cf. Coble et al.[51]). In urban catchments, there has been a specific focus on the protein-like peaks (tryptophan: Peak T and tyrosine: Peak B). Carstea et al.[11] highlight that in an urban catchment during base flow and low-intensity storm events, protein-like fluorescence is pronounced when compared to rural systems. However, high-magnitude rainfall events route waters via alternate flow paths and a distinct humic-like fluorescence signal (Peaks A and C) is apparent. An additional peak of interest for urban systems is associated with fluorescent whiting agents, or optical brighteners, found in household detergents that can indicate cross-connections between the sewerage and storm drainage networks.[52] However, separating this peak from the natural humic signal in urban systems can be difficult.[53,54] Fluorescence can also be used to target PAHs, and has been applied in marine environments where shortwave length peaks (<260 nm excitation) have been associated with a number of commonly occurring compounds.[55] However, further work is required to test the validity of this approach for urban rivers with rapidly changing DOM composition.

A number of excitation or emission wavelengths can be targeted and used to calculate fluorescence indices, such as the FI index and HIX. These have been used to highlight the importance of photodegradation and microbial processing of DOM in urban ponds[56] but their suitability for urban rivers has been questioned[11] with recent work highlighting nonlinear behavior when source waters are mixed.[57] One promising index for urban river systems is the ratio of protein:humic fluorescence (Peak T/Peak C[58]) that can identify wastewater in urban rivers. A full EEM (map of optical space) can be measured in minutes and the fluorophores present modeled using PARAFAC, a three-dimensional extension of factor analysis, or machine learning techniques such as self- organizing maps,.[59,60] The dominance of tryptophan-like fluorescence (TLF herein) in urban rivers has been identified using these techniques, with distinct anthropogenic humic-like fluorescence (HLF herein) peaks that may be used to track different water sources.[4] However, until relatively recently these approaches have required sample collection and transportation to a laboratory for subsequent analysis on high powered, expensive benchtop spectrofluorometers.

Recent development of *in situ*, fluorescence instruments has been facilitated by the advent of cheap and reliable UV light emitting diodes, enabling significant reductions in instrument size and power consumption.[61] These new instruments are better suited to freshwater monitoring than the original expensive submersible fluorometers developed for marine research.[62] Baker et al.[63] were the first to highlight the potential for *in situ* TLF monitoring as a rapid indication of organic pollution in human influenced rivers. However, they used a portable handheld unit focused on a single peak, with a small measurement cell and no logging capability, thus prohibiting longer-term deployment for continuous monitoring. Carstea et al.[64] adapted a laboratory instrument to enable *in situ*, full EEM measurements at sub-hourly resolution. This approach provided information on cross-connections in the urban drainage network missed by routine grab sampling but maintenance, power consumption, and fouling were major issues. More recently researchers have shown that open face submersible sensors, tuned to specific fluorescence peaks (i.e., HLF or TLF), are suitable for long-term, *in situ* monitoring.[65,66] These commercially available instruments are low powered with anti-fouling solutions and are relatively inexpensive. However, monitoring of additional parameters is needed (e.g., turbidity and temperature) to account for interferences (see below). Khamis et al.[67] used a low-cost, flow-through fluorescence sonde (bank-side, flow cell measurement system, see Figure 3(e)), to measure both HLF and TLF, alongside temperature and turbidity. This approach yielded a rich dataset but there was a significant power demand (automatic peristaltic pump) and more maintenance was required than for open face sensors.

To improve process understanding and flux estimates there is a distinct need to move from physical sampling regimes to continuous *in situ* monitoring.[16,68] While the specific benefits of using *in situ* fluorometry are clear,[16,69] solutions to the challenges of monitoring in urban systems are still being explored. During storm flow conditions, turbidity can be particularly high (>200 Nephelometric Turbidity Unit (NTU)) which can increase scattering and attenuation of excitation light and interfere with the fluorescence signal.[61] Laboratory-derived empirical correction factors based on standardized sediment particles have been successfully developed for HLF and TLF sensors.[65,66] Laboratory correction algorithms based on site-specific sediments have also been proposed.[70] In certain situations, it may be necessary to apply *in situ* calibrations either via routine field sampling and laboratory analysis[67] or via parallel *in situ* monitoring with and without filtration.[71] Fluorescence is a temperature-sensitive process and particular peaks are more sensitive to quenching at high temperature.[72] Laboratory techniques for developing corrections are well established within the research community but are yet to be implemented by sensor manufacturers.[65,66,70,73] Inner-filtering (signal saturation at high DOM concentrations) is also an issue that can either be addressed by routine parallel sampling or targeted storm sampling to highlight specific hydrograph components when issues may arise.[66] Alternatively, *in situ* monitoring of absorbance at 254 nm can be utilized but this may be cost prohibitive. Where possible additional parameters, such as pH and electrical conductivity, should be recorded to account for any potential issues associated with significant changes in ionic strength (e.g., salt spreading) or pH. With careful pre-installation laboratory calibration and compensation algorithm development, coupled with parallel sampling[74] and ancillary parameter monitoring, *in situ* fluorescence sensors can provide new insights into urban DOM dynamics.

In the following section, we illustrate the information that can potentially be obtained from an *in situ* application of a fluorescence sensor, by presenting and interpreting monitoring data collected from an urban river, the Bourn Brook, in the UK.

CASE STUDY: BOURN BROOK, BIRMINGHAM, UK

The Bourn Brook, is a tributary of the River Rea, Birmingham, UK (52°27′N, 1°54′W; Figure 3), which has been intensively studied.[9,60,61,63] Briefly, the catchment is 27.9 km^2 in area with ~80% urban/suburban land use, ~10% anthropogenic green spaces, and a small amount of woodland in the headwaters. There are no wastewater treatment works in the catchment, but an extensive, aging (>100 years) network of storm sewers and CSOs feed the main channel. These characteristics are typical of urbanized

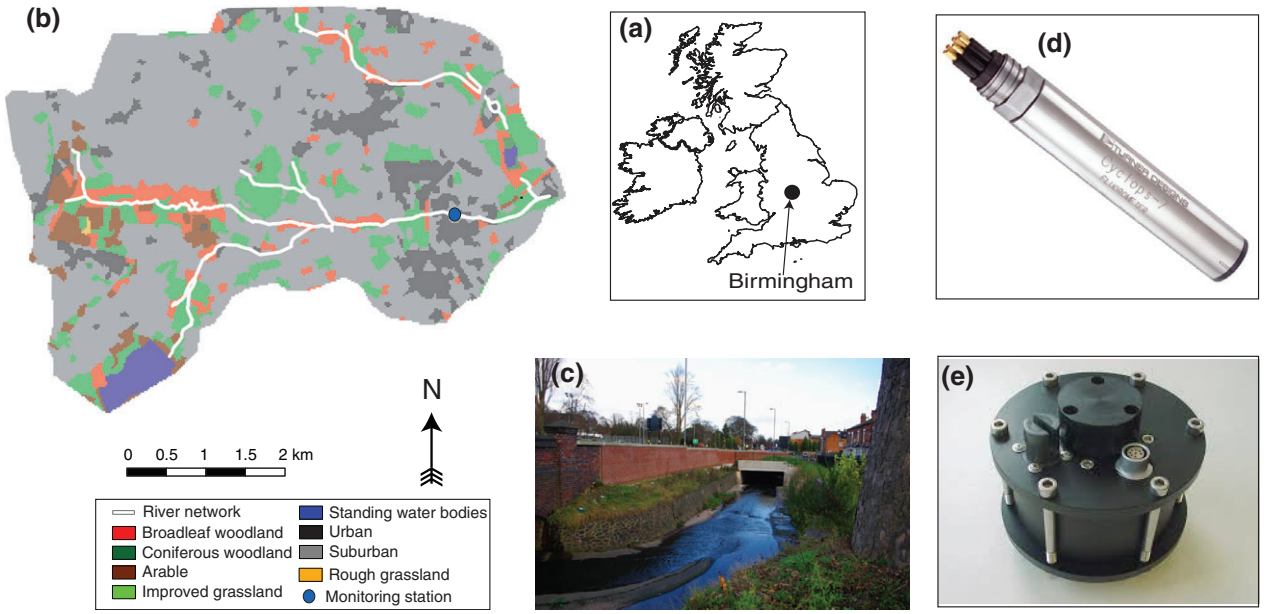

FIGURE 3 | (a) Map of UK with Birmingham highlighted, (b) land use of the Bourn Brook catchment, (c) image of the Bourn Brook directly downstream of the monitoring site. (d) Cyclops 7 (Turner Designs, San Jose, CA, USA) open face tryptophan-like fluorescence sensor, and (e) FL30 multiwavelength, flowcell fluorometer (Albillia Co, Neuchatel, Switzerland).

headwater catchments across Northern Europe. The DOM dynamics of the Bourn Brook have been characterized using both traditional indicators of quality and quantity [i.e., biochemical oxygen demand (BOD) and dissolved organic carbon (DOC)] and fluorescence spectroscopy. Carstea et al.[11] collected hourly grab samples and undertook subsequent laboratory analysis[11]; while a number of authors have utilized *in situ* sensors to measure: (1) TLF[65]; (2) TLF and HLF,[67] and (3) the full EEM.[64] The Bourn Brook displays a typical urban hydrology and is particularly responsive to rainfall events (Figure 4). During low-flow conditions, the river has a microbial DOM signal suggesting the predominance of autochthonous DOM production.[11] In storm events, the DOM flux appears to be controlled by antecedence, with exhaustion effects apparent following successive storm events as reductions in both DOC and TLF have been observed.[11,65] Diurnal variability has also been observed during low-flow periods, which is most pronounced for the HLF peak, suggesting photodegradation and microbial processing can be detected with *in situ* instrumentation.[67] In addition, stochastic events have been observed when monitoring at fine temporal resolution (5 min) including DOM pulses thought to originate from sewerage/drainage cross-connections or industrial discharges.[64] Gross pollution events have also been detected: a diesel spill was recorded *in situ* and associated with a specific fluorescence fingerprint.[64]

Recent work has highlighted the sensitivity of *in situ* fluorescence measurements to ambient environmental conditions[66] with significant work conducted on the Bourn Brook developing and refining correction factors. Khamis et al.[65] identified sediment particle size as an important control on the instrument specific response, while Khamis et al.[67] highlighted the variability of temperature compensation coefficients between sensor types and fluorescence peaks (e.g., TLF was more sensitive to temperature

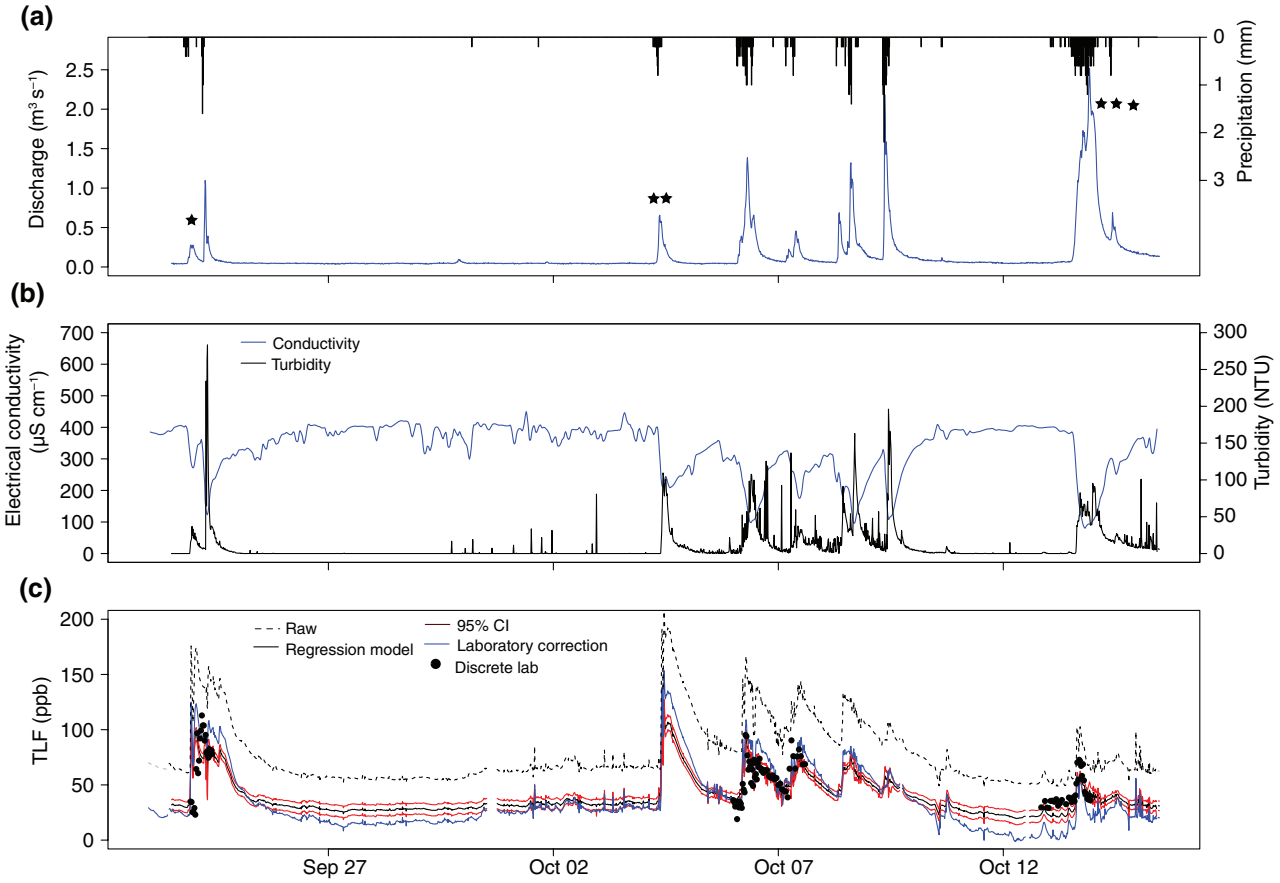

FIGURE 4 | Time series at 15 min resolution for the Bourn Brook, Birmingham, UK, autumn 2014. (a) Discharge and precipitation, (b) specific electrical conductivity (EC) and turbidity, (c) tryptophan-like fluorescence intensity (TLF), *in situ* readings are lines and laboratory measurements are dots. In (a) the number of stars denotes the event number used in Figure 5(a) and (b). The TLF sensor was a Cyclops 7 (Turner Designs, EX 285 ± 10 nm EM 350 ± 55 nm) and was integrated with a Manta 2 multi-parameter sonde (Water Probes, Austin, TX, USA) that also logged turbidity, pH, EC, and temperature (15 min resolution). CI, confidence interval.

quenching than HLF). The suitability of laboratory-derived coefficients versus field calibration was tested during a short deployment of an open face TLF sensor (Figure 3(d)). During autumn 2014, eight discrete storm events were observed with significant variability in turbidity (Figure 4). Raw TLF records appeared to systematically overestimate concentrations relative to laboratory records (Figure 4(c); Root Mean Square Error (RMSE) = 31.4 ppb). Records corrected using a laboratory derived correction factor based on a specific representative particle size (silt) did not perform as well as the field calibration (Figure 4(c), R^2 = 0.72 vs R^2 = 0.81), indicating the need for field-based calibrations in monitoring programs wherever possible.[67,71]

The utility of fluorescence as a surrogate for water quality from phosphate, nitrate ammonia, and BOD[75] through to microbial counts[76] has also been explored recently. Studies on the Bourn Brook have highlighted the utility of fluorescence as a tool to monitor widely used measures of DOM quantity (DOC) and quality (BOD). For a grab sample monitoring program, Carstea et al.[11] recorded strong correlations (r = 0.84) between Peak C, a HLF peak, and total organic carbon. Carstea et al.,[64] using an *in situ* sensor, recorded similar correlations (r = 0.83) between HLF and DOC, however, these data were not compensated for turbidity or temperature. Recent work with *in situ* sensors on the Bourn Brook suggests strong relationships with both BOD and DOC can be obtained with careful calibration (Figure 5). TLF displayed the strongest relationship with BOD (Figure 5(b) and (d)) in agreement with the literature[49] while there was more scatter in the DOC relationship. TLF is a better surrogate for the reactive component of the DOM pool while HLF is a better surrogate for the more refectory, stable pool of DOM and hence a good indicator of DOC [67,77] (Figure 5). This work indicates that when standardized calibration protocols are followed, sensors with differing designs (i.e., open face vs flow cell; Figure 3 (d) and (e)), optical configurations, and compensation coefficients can produce similar slopes for the relationship between TLF and BOD (Figure 5 (b) and (d)).

Using high-resolution fluorescence and ancillary data recorded during a field deployment,[65] we can begin to unpick event dynamics: exploring potential DOM sources and DOM pathways to the river.[69] In this case study, we examine hysteresis

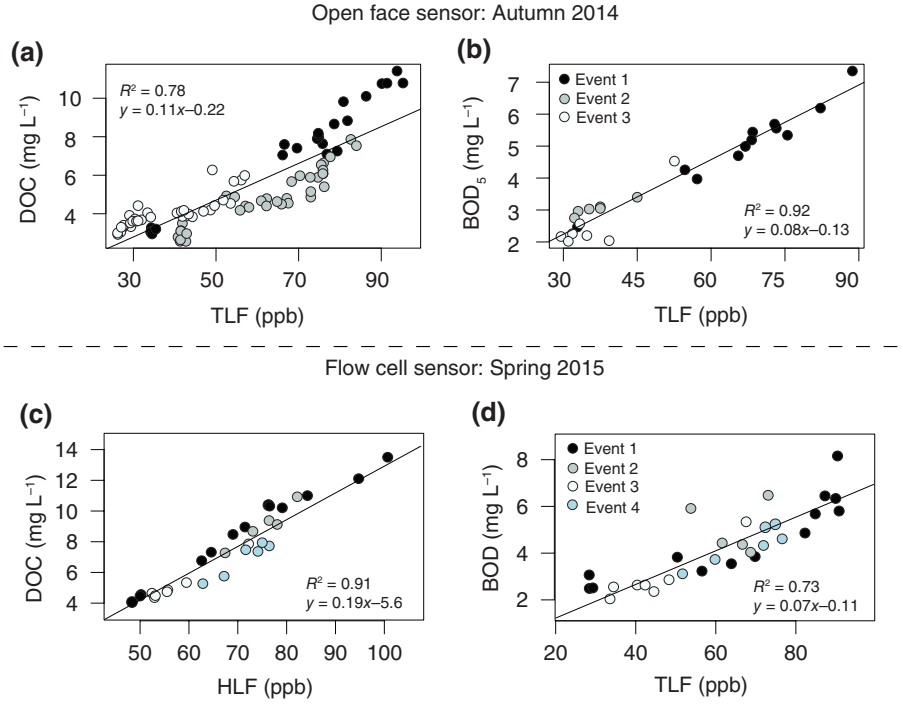

FIGURE 5 | Relationship between *in situ* fluorescence and laboratory measurements of biochemical oxygen demand (BOD) and dissolved organic carbon (DOC) for the Bourn Brook. For (a) and (b), tryptophan-like fluorescence (TLF) was measured using a Cyclops 7 corrected for turbidity and temperature following Khamis et al.[65] For (c) and (d), humic-like fluorescence (HLF) and TLF were both measured using an FL30 flow cell sensor (Albillia Co), and corrected for turbidity and temperature following Khamis et al.[67] Note that the FL30 was more sensitive to turbidity effects than the Cyclops 7 and turbidity compensation was ineffective above 200 NTU, hence these data points have been omitted.

loops for three events (see Figure 4; stars denote events 1–3) of varying magnitude and antecedent conditions. For Event 1, there is a pronounced TLF increase for a small increase in discharge and turbidity (Figure 6(a)). The steep clockwise response suggests that DOM sources were close to the river channel or in-channel (e.g., benthic algae[30]). The high TLF coupled with low turbidity suggests that the DOM was largely organic and probably scoured from the bed as increased connectivity to the wider catchment would be associated with an increase in inorganic particles and a greater increase in turbidity.[78] In addition, the low antecedent rainfall (14 days <1 mm) would have enabled the growth of in-channel periphyton and epilithic algae. For Event 2 (Figure 6(b)), a greater increase in discharge was observed but with a delayed increase in TLF (i.e., clockwise hysteresis) suggesting that sources were more distal than for Event 1. It is likely that benthic algae had not recovered from the scouring during Event 1 and the DOM increase represented material flushed from the engineered drainage system of storm drains and CSOs.[79] The increased turbidity suggests that more of the catchment was connected to the river via storm drains and thus deposited inorganic material was mobilized from impervious surfaces.[78] Event 3 (Figure 6(c)) was a prolonged storm following a series of precipitation events with high antecedent rainfall (>50 mm). A small TLF increase on the rising limb was again apparent, most probably due to CSO inputs. However, the low TLF at high flow is indicative of source limitation. This is likely an exhaustion effect as much of the source material in the catchment was mobilized and transported during previous events. Hence, the majority of the discharge comprised dilute event water that was rapidly conveyed through the fast-engineered flow paths that had been previously scoured of deposited OM and attached biofilms.

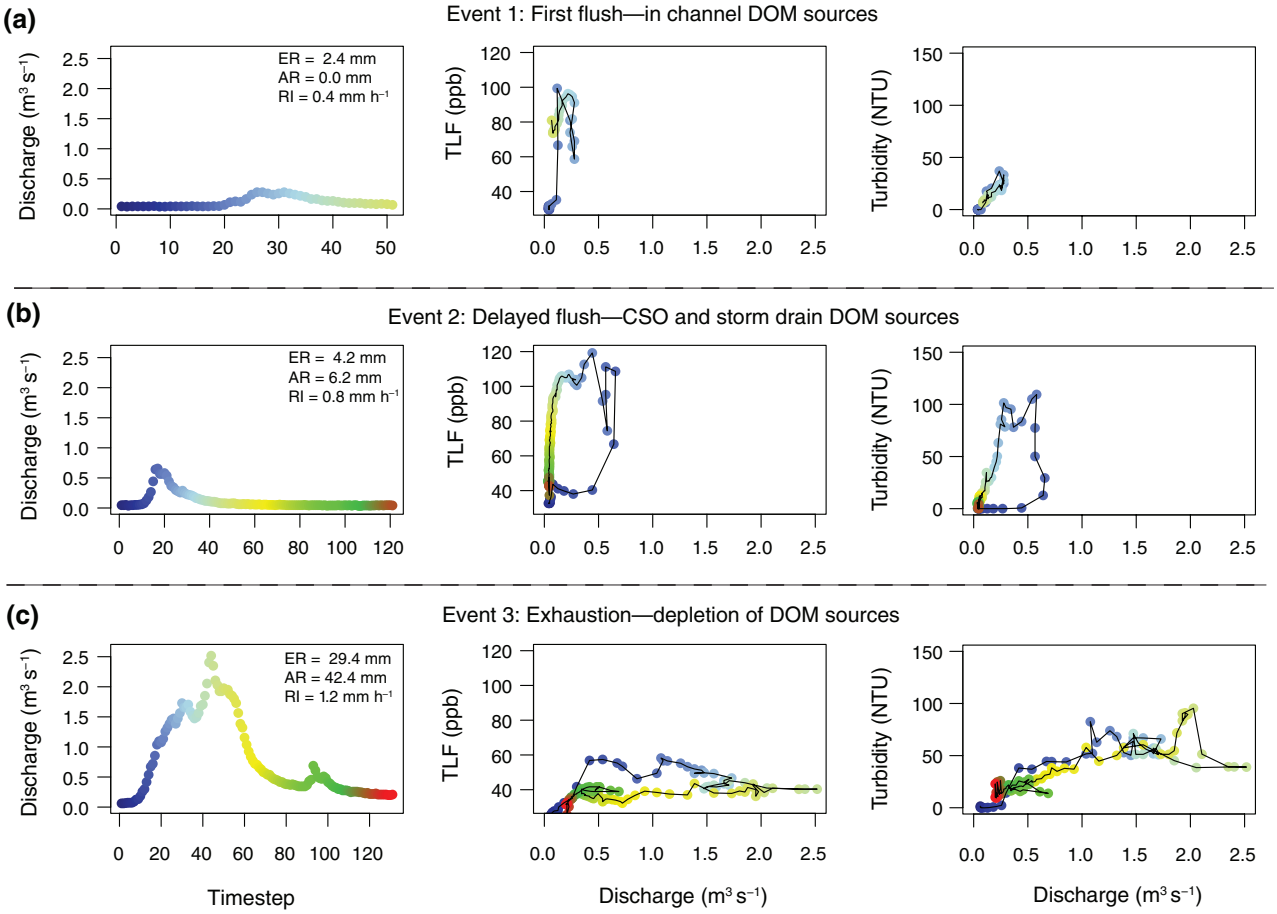

FIGURE 6 | Event discharge and hysteresis loops for tryptophan-like fluorescence (TLF) and turbidity. (a) Low magnitude event with first flush indicative of in-channel organic matter mobilization. (b) Delayed flush event with dissolved organic matter (DOM) preceding discharge and likely associated with combined sewer overflow (CSO) and storm drain sources. (c) High magnitude event with significant depletion/exhaustion of DOM sources apparent. AR, 14-day antecedent; ER, total event rainfall; rainfall; RI, maximum rainfall intensity.

CONCLUSIONS

In this *focus article*, we have advocated the enhanced process-level understanding that may be obtained using new *in situ* fluorescence sensor technology. Hitherto, our ability to unravel the complexity of urban DOM dynamics has been constrained significantly by the availability of instruments for *in situ* monitoring of urban rivers at appropriate spatial and temporal resolution. Relatively understudied urban river environments present significant challenges at several levels given the interaction between DOM sources and pathways that influence DOM processing and hence DOM availability downstream. Continuous field-based monitoring can provide invaluable additional information to supplement traditional manual water sampling and laboratory analysis. Where the challenges of *in situ* calibration are addressed successfully, applications of *in situ* fluorescence technology have enormous potential as part of a wider toolbox to aid interpretation of linkages between the hydrology and biogeochemistry of urban rivers. This can range from helping to understand the wider significance of isolated pollution events, such as oil spills to identifying specific thresholds that govern the routing of water through urban catchments (i.e., thresholds in antecedent moisture content or rainfall intensity). Recent advances in sensor technology and logging have wider applications that can further enhance our understanding of process dynamics. For example, sensor output can be used to trigger automated pump sampling, which can be regulated by prescribed thresholds, or rate of change (of river flow or water quality), thus permitting laboratory sample analysis of additional compounds of interest (e.g., emerging contaminants) throughout an event at resolutions that were previously unfeasible.[16] For urban rivers, this is important given the wider suite of (laboratory) analyses that are needed to characterize urban water quality, particularly where there are concerns over emerging contaminants[80] or potential sewer cross-connections.

However, *in situ* applications of fluorescence technology require significant attention in quality assurance and control. Regular field inspection of sensors is required given the potential for sensor fouling, and continuous quality control of sensor output is essential. Further work is also required to develop standardized calibration protocols, and there is an urgent need for simpler standards to check and correct for instrument drift. For example, the use of quinine sulfate to calibrate HLF sensors requires laboratory access and the use of sulfuric acid. The latter limits the wider use of these *in situ* sensors outside the research community.[81] In future, it may be possible to standardize calibration compounds between individual fluorescence peaks to increase confidence in sensor output. However, one of the main challenges of *in situ* applications of fluorescence sensors is understanding the degree to which sensor calibrations are site, or catchment, specific and further work is needed to develop calibration algorithms that account for nonstationarity (including the potential effects of changes in sediment particle size on sensor output, which may change during an event). In addition, the long-term stability of the photo-diodes and light emitting diodes (LEDs) used for *in situ* applications has yet to be established, emphasizing the need for regular calibration of sensor output.[82]

Where these problems are addressed, *in situ* fluorescence sensors may be used to capture multiple fluorescence peaks, providing high-frequency monitoring of fluorescence indices and enabling the routine use of peak ratios to fingerprint pollution and DOM sources. Given the need to improve our understanding of hydrological and biogeochemical process interactions (especially in urban contexts), the development and wider application of *in situ* monitoring technologies have the potential to transform our ability to quantify environmental system dynamics at different spatial and temporal scales. The possibilities are enhanced further by recent reductions in the cost of individual sensor units and components. Additional benefits may arise by tailoring the sensor design to specific freshwater applications. Given that the early fluorescence sensors were developed for marine applications, some features (e.g., operating at high pressures) are redundant and may be removed to reduce sensor costs, enabling funds to be used to build more distributed networks of sensor nodes.

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