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Increases in humic and bioavailable dissolved organic matter in a forested New England headwater stream with increasing discharge

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Abstract. Understanding the processes controlling the transfer of organic matter from terrestrial to aquatic ecosystems is of fundamental importance for the aquatic sciences. Over the course of a full year, fluorescence, absorbance and bioavailability of dissolved organic matter (DOM) were characterised in Bigelow Brook, a forested headwater stream in Massachusetts, USA. Parallel factor analysis (PARAFAC) identified a four-component model to describe observed DOM fluorescence (C1–C4). Component C2 exhibited the characteristics of a more humic-like fluorophore, with a potentially more reduced redox state and increased with discharge, whereas more fulvic-like (C1) and protein-like (C3, C4) fluorophores decreased. Under both dark and light-exposed conditions, percentage bioavailable dissolved organic carbon (%BDOC) increased with discharge ($R^2 = 0.37$ and $R^2 = 0.56$). C2 and specific absorptivity (SUVA) were reduced following BDOC incubations, whereas C1, C3 and C4 increased. These changes to DOM characteristics with increasing discharge were observed under both baseflow and stormflow conditions, indicating that with rising watertable, loading from a large riparian or hyporheic pool of organic matter is likely occurring. Other headwater streams, where loading is controlled by hillslope processes, are likely to exhibit a similar pattern of increasing export of more humic and bioavailable DOM during hydrologic events.

Additional keywords: hydrologic events, hydrology, secondary production, stream ecology.

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Introduction

Dissolved organic matter (DOM) moving from vegetation and soil into the headwater streams of forested watersheds acts as an essential source of both energy and organic nutrients to downstream aquatic ecosystems (Hedin *et al.* 1995; Aitkenhead-Peterson *et al.* 2003; Neff *et al.* 2003; Carpenter *et al.* 2005). In most aquatic systems, DOM is the largest and most bioavailable form of carbon (Battin *et al.* 2008) and influences aquatic ecosystem metabolism (Meyer and Edwards 1990; Wetzel 1992). Furthermore, its concentration and optical properties control UV light attenuation (Xenopoulos and Schindler 2001; Fasching and Battin 2012) and influence contaminant mobility (Ravichandran 2004).

The majority of DOM (measured in units of dissolved organic carbon, DOC), dissolved organic nitrogen (DON) and bioavailable organic carbon (BDOC) export from headwater streams occurs during high-flow events (Buffam *et al.* 2001; Inamdar and Mitchell 2007; Raymond and Saiers 2010; Wilson *et al.* 2013), with increases in concentration often occurring concurrently with increasing flow (Raymond and Saiers 2010; Xu *et al.* 2012; Wilson *et al.* 2013; Mei *et al.* 2014). In many small watersheds, the rise in watertable that accompanies rainfall and snowmelt events and the associated flushing of DOC from organic matter-rich soils near surface or in riparian areas have been identified as a probable mechanism controlling this phenomenon (Boyer *et al.* 1996; Xu *et al.* 2012; Mei *et al.* 2014). In watersheds with more complex topography, groundwater seeps, or heterogeneous vegetation, temporal changes of DOC concentration with flow tend to relate strongly to connectivity of carbon-rich contributing areas (e.g. wetlands; McGlynn and McDonnell 2003; Inamdar *et al.* 2011; Singh *et al.* 2014) rather than hillslope processes.

The changes in the flow rate and DOC that occur in headwater streams during rainfall or snowmelt hydrologic events have been the subject of much research and frequently exhibit predictable patterns, whereas the changes in DOM chemical composition that occur during events have received less attention (Inamdar *et al.* 2011). In streams where compositional changes have been examined during events, changes in flow path and DOM source are often accompanied by shifting DOM composition. The most commonly observed pattern across forested systems has been a shift towards DOM with higher specific absorbance and more terrestrial humic-like fluorescence characteristics (FDOM) with increasing discharge (Hood *et al.* 2006; Vidon *et al.* 2008; Fellman *et al.* 2009; Inamdar *et al.* 2011). Increases in aromaticity, and higher molecular weight are expected with these commonly observed optical changes, and where more direct measurement of molecular characteristics have been made for samples collected during storm events, this expectation generally holds true (Li *et al.* 2005; Nguyen *et al.* 2010). DOC flushed into stream systems during high-flow events is usually accompanied by an increase in the concentration of BDOC (Buffam *et al.* 2001; Fellman *et al.* 2009; McLaughlin and Kaplan 2013; Wilson *et al.* 2013); however, the proportion of the DOC pool comprising BDOC (%BDOC) has been observed to decrease with increasing concentration in some systems (Buffam *et al.* 2001; Fellman *et al.* 2009) and increase in others (Fellman *et al.* 2009; McLaughlin and Kaplan 2013; Wilson *et al.* 2013). These contrasting results indicate that DOM entering streams during hydrologic events frequently comprises differing proportions of bioavailable and recalcitrant compounds (Fellman *et al.* 2009) and recent research suggests that, during events, the different contribution of DOM from various sources in the watershed (soil water, seeps, and ground water) may be responsible for contrasting patterns of bioavailability during storms (McLaughlin and Kaplan 2013).

Although DOM characteristics do tend to shift with source, and bioavailability changes have been linked to changes in source, indicators of DOM bioavailability based on FDOM or other measures of composition are not always consistent across sites. In separate studies of temperate (Fellman *et al.* 2009), arctic (Balcarczyk *et al.* 2009) and glacial (Hood *et al.* 2009) streams, the proportion of FDOM associated with amino acid-like fluorescence has been correlated with %BDOC. In four of five coastal temperate streams studied by Fellman *et al.* (2009), increases in %amino acid-like FDOM and bioavailability were observed to correlate over the course of two major storm events; however, Cory and Kaplan (2012) suggested that the relationship between %amino acid-like FDOM and %BDOC is more nuanced than previously believed, with tyrosine-like FDOM being completely biodegraded and tryptophan-like FDOM being associated only partially with bioavailable compounds (73% being resistant to degradation) during plug-flow bioreactor experiments using temperate stream water. Also, semi-labile humic-like BDOC comprises a large proportion of the total BDOC pool and because humic-like FDOM tends to be present in larger quantities and be more resistant to biodegradation, the importance of this semi-labile contribution to %BDOC in many systems may be masked (Cory and Kaplan 2012). For example, in the Austrian brown-water streams studied by Fasching and Battin (2012), no relationship between %amino acid-like FDOM or any measure of FDOM composition and %BDOC was observed, which the authors attributed to uniformly humic-like FDOM and low %BDOC values. Although streams with a wide range of observed %amino acid-like FDOM do tend to show more consistent relationships of %amino acid-like FDOM to %BDOC, with many streams consistently exhibiting only a minor proportion of FDOM as amino acid-like components (Fasching and Battin 2012). In addition, the direct measurements of humic substances, amino acids and carbohydrates during plug-flow reactor experiments by Volk *et al.* (1997) to quantify composition of BDOC in water from White Clay Creek indicated that 75% was composed of humic substances and only 4% of BDOC was composed of amino acids (mainly bound to humic substances). The existing body of literature linking %BDOC to DOM composition indicates that source changes that control DOM composition and %BDOC may differ between watersheds with contrasting landscapes, soil types, or flow-path changes during hydrological events (Fellman *et al.* 2009).

Together, flow rate, watershed characteristics (e.g. floodplains, lakes), DOM concentration and DOM composition define how much DOM is exported from small watersheds and how reactive exported DOM is likely to be. Given the shorter transport times that characterise most headwater streams during storm and snowmelt events, there is less opportunity for changes in DOC concentration or reshaping of DOM composition by in-stream processes than in locations further down the stream continuum (Meyer and Edwards 1990; Cory and Kaplan 2012). Wherever conditions are conducive for microbial activity and photoreaction, these processes can quickly alter the DOM pool (Lowe and Likens 2005; Cole *et al.* 2007; Battin *et al.* 2008). In this sense, defining those factors controlling temporal variability in the reactivity of DOM originating from headwater streams also provides an indication of how environmental conditions may influence the susceptibility of DOM to transformation during transport.

Here, we utilise high-frequency sampling during hydrologic events, spectrofluorometric characterisation of DOM, and laboratory incubations measuring potential DOM bioavailability to examine DOM composition changes during runoff events in a temperate headwater stream over the course of a full year. Through previous research, we have observed increasing concentrations of DOC and BDOC with increasing discharge and during warmer seasons in the Upper Bigelow Brook, a first-order forested watershed in Massachusetts (Wilson *et al.* 2013). This small watershed is a forested headwater without significant wetland area, so patterns observed at this site are indicative of what DOM contributions might be expected from upland forests in the region with little interference from other landscape features. In the current study, we show that, with these increases in concentration, a greater proportion of the DOM pool exhibits peak emission intensities shifted to longer wavelengths, which is characteristic of more reduced humic matter (Zsolnay *et al.* 1999; Cory *et al.* 2011). We also test whether changes in DOM composition during hydrological events are accompanied by increasing bioavailability. Following incubation experiments to measure BDOC, changes in DOM optical characteristics were quantified to identify whether various DOM components appear to be more susceptible to microbial or photochemical transformation. In water collected from the Upper Bigelow Brook, the fluorescence of the seemingly more reduced humic-like

DOM components exported during storm events was disproportionately lost during incubation experiments, in comparison to protein-like and more oxidised humic-like material, which may indicate higher susceptibility to transformation.

Materials and methods

Site description and hydrological monitoring

Upper Bigelow Brook drains a 24-ha forested catchment of the Harvard Forest long-term ecological research (LTER) site in north-central Massachusetts, USA (42.5°N, 72°W). This first-order stream is well shaded, with eastern hemlock (*Tsuga Canadensis* L.) being common in the catchment, particularly in riparian areas (Willacker *et al.* 2009). Catchment slope tends to be highest in upland areas (up to 45%) and lower in riparian areas (as low as 15%), but most soil in the catchment can be classified as well drained, rocky and derived from glacial till. Further detail of catchment characteristics can be found in other publications (Willacker *et al.* 2009; Xu *et al.* 2012; Wilson *et al.* 2013).

Precipitation at the Harvard Forest LTER is evenly distributed throughout the year, with an annual mean near 110 cm (Harvard Forest 2011). During winter months, a snowpack typically accumulates until spring snowmelt, with monthly mean temperatures ranging from –7°C in January to 19°C in July (Currie *et al.* 1996); however, stream flow persists throughout most of the winter months and winter rain events are not uncommon (Wilson *et al.* 2013).

Stream discharge measurements are recorded at the Upper Bigelow Brook site at intervals of 15 min as a part of the Harvard Forest LTER study, utilising an established stage-rating curve and pressure transducer system (Xu *et al.* 2012; Wilson *et al.* 2013). In December of 2009, two groundwater wells were installed in the catchment to monitor watertable elevation and for the collection of water samples to characterise DOM in groundwater (Xu *et al.* 2012). An upland well was positioned 26 m from the stream with a depth of 5.4 m and a toe slope well was positioned at 13 m with a depth of 3 m. Water level in these wells was monitored at 15-min intervals using pressure transducers and verified by manual measurement.

Collection and initial processing of water samples

Water samples were collected at the outlet of the Upper Bigelow Brook between 30 October 2009 and 30 October 2010. Collection of water samples for the characterisation of DOM occurred most intensively during hydrological events ($n = 63$), but sampling also occurred during baseflow ($n = 34$). At least eight samples were collected at the Upper Bigelow Brook catchment outlet over the course of each major event in the year of sampling. Thirty-eight additional samples were collected in 2010 at a second monitoring location on the Bigelow Brook located downstream of an in-channel wetland. Samples from this downstream site were included in developing a parallel factor analysis (PARAFAC) model to describe DOM fluorescence characteristics in the catchment, but data from this location are not presented in detail in the current manuscript.

Groundwater wells were sampled eight times between March and June to characterise DOM chemistry and, because only minor variation in composition was observed over this time period, further sampling was not completed. In July of 2010, four lysimeters were installed for the collection of soil water; one ceramic cup tension lysimeter and one zero-tension lysimeter were installed at two distances from the stream (7 m and 24 m). Both types of lysimeters were installed at the base of the O-horizon to collect samples for characterisation of soil-water DOM. Because of the low frequency of precipitation between the date of installation of the lysimeters and the end date of our study (November 2010), the lysimeters yielded only eight samples. Prior to lysimeter installation, a 2-inch diameter piezometer located 3 m from the stream (depth 0.4 m) was sampled to help characterise soil water chemistry. Water was present in this piezometer in March and April and yielded two samples.

Stream-water samples were collected by hand or by using an ISCO 3700 automated sampler (Teledyne Isco, Lincoln, NE, USA) using acid-washed and deionised water-rinsed 1-L polyethylene or polycarbonate bottles. Groundwater wells, the riparian piezometer and lysimeters were sampled utilising a pre-rinsed peristaltic pump system or polyethylene syringe, and collected into 1-L polycarbonate bottles. All samples were kept on ice and filtered within 36 h of collection using pre-rinsed 0.22-μm polycarbonate membrane filters. Filtered samples were stored in the dark at 4°C until chemical analyses. Time in storage before analysis of DOC was between 15 and 47 days from filtration.

Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), NO_3, NH_4, and DON were measured using standard analytical methods (Wilson *et al.* 2013). BDOC in stream-water samples was characterised utilising 30-day, 125-mL incubations in a dark laboratory environment, as described in Wilson *et al.* (2013). All BDOC measurements were based on the difference between measurements made on Day 30 and Day 0. Samples were refiltered before measuring final concentration, providing an estimate of carbon lost through incorporation into microbial cell material and by mineralisation to CO_2. A second bioavailability assay was completed for most stream-water samples ($n = 68$) to identify the amount of DOC susceptible to degradation over the same 30-day period with exposure to both sunlight and microbial degradation (BDOC-L). For these incubations, the same methods were utilised as for BDOC, except that these incubations occurred in acid-washed clear polycarbonate bottles with daily exposure to sunlight through a west-facing window of the laboratory. The intensity of light exposure during these incubations varied seasonally up to a maximum of 5 h of direct sunlight daily during non-overcast early spring conditions and temperature was more variable than for BDOC incubations completed under dark conditions (±5°C of temperature in the dark). That light intensity and temperature were not fully controlled and may account for some unexplained variability in these measurements; however, we assume that over the course of the 30-day incubation, enough light exposure occurred to transform the most light-sensitive components of the DOM pool in each sample. BDOC-L incubations were completed primarily to identify whether certain components of the DOM pool are more susceptible to degradation under conditions of variable light and temperature than under dark conditions. For hydrological events occurring in November of 2009, December of 2009 and January of 2010, extra water was collected for BDOC experiments that were amended with nitrogen, phosphorus and potassium to measure bioavailability in the absence of potential nutrient limitation (BDOC-N; $n = 30$). During BDOC-N incubations, each 125-mL sample was supplemented with 1 mL of solution containing 0.115 g L^{-1} K_2PO_4 and 0.185 g L^{-1} NH_4NO_3 to create a ratio of nitrogen : phosphorus : potassium similar to the ratio in bacterial cell structure (60 : 7 : 1) (Cleveland and Liptzin 2007). For a subset of 23 samples collected in March and February, %BDOC and %BDOC-L were measured both with and without filtration to identify the potential for carbon loss through incorporation into microbial cell material from mineralisation to CO_2.

Spectroscopic characterisation of DOM

Spectroscopic analyses to characterise DOM were completed for all stream-water samples, soil-water samples, well-water samples, and again for stream-water samples following incubation experiments (353 samples). UV absorbance spectra were measured between 220 and 600nm (Beckman DU 520 spectrophotometer; Beckman Coulter Inc., Fullerton, CA, USA). These spectra were utilised to correct fluorescence measurements for inner filter effects and in the calculation of two measures commonly utilised as indicators of DOM aromaticity, namely, (1) the specific UV absorbance at 254 nm, which is calculated by dividing the absorbance at 254 m by DOC concentration ($SUVA_{254}$; L mg^{-1} C m^{-1}) (Weishaar *et al.* 2003), and (2) the molar absorption coefficient at 280nm, which is calculated by dividing absorbance at 280 nm by moles of DOC (ε280; L mol^{-1} m^{-1}) (Chin *et al.* 1994). Fluorescence excitation–emission matrices (EEMs) were measured using a fluorescence spectrophotometer (Varian Cary Eclipse; Palo Alto, CA, USA) over emission wavelengths from 300 to 600 nm in 2-nm steps for excitation wavelengths increasing from 240 to 450 in 5-nm steps. Scan speed for fluorescence scans was 600 nm min^{-1}, with emission and excitation slits set to 5-nm bandwidth.

Excitation–emission matrices (EEMs) were corrected for instrument-specific biases and inner filter effects (Walker *et al.* 2009). The fluorescence of an Epure water blank EEM run on the day of each scan was subtracted from each sample EEM to remove the Raman signal. Fluorescence measures were normalised to Raman units (R.U., nm^{-1}) by the area under the Raman peak at excitation wavelength 350 nm in the Epure water blank sample. The effect of Rayleigh scatter effects on EEMs were removed by deleting emission measurements in the region where emission wavelength was larger than excitation wavelength –15 nm, or less than excitation wavelength +15 nm and then interpolating through the surrounding area (Xu *et al.* 2013).

Corrected EEMs were analysed using PARAFAC analysis to identify discrete chemical components in the fluorescence dataset (Stedmon and Bro 2008). Each component identified by PARAFAC analysis represents groups of chemically similar fluorophores or groups of fluorophores with strong co-variance in the environment from which samples are collected (Cory and Kaplan 2012). The relative abundance of the components identified by the PARAFAC modelling technique can be linked to differences in chemical composition and DOM source. The procedures described in Stedmon and Bro (2008) for use of the DOMFluor tool box in MATLAB were utilised to complete PARAFAC modelling. A four-component model was validated by residual analysis and four-way split half analysis (Stedmon and Bro 2008). To allow for comparison of the relative abundance of each PARAFAC component among samples of differing DOC concentration, model scores are presented as a percentage of F_{max} for each component in each sample where: $\%F_{max} = F_{max}C_n \div \sum F_{max}C_{1\text{–}6} \times 100\%$. No outlier EEMs were identified during PARAFAC analysis; however, examination of six samples with particularly high fluorescence intensities (3 stream water samples and 2 lysimeter samples) revealed absorbance for some wavelengths high enough that inner filter effects were unlikely to have been fully removed by standard correction procedures (absorbance >0.3) (Ohno 2002; Miller *et al.* 2010) and, for this reason, these six samples were excluded from further analyses where calculation was reliant on values from fully corrected EEMs.

Three fluorescence indices commonly utilised to quantify differences in the properties of DOM were calculated using corrected EEMs, the fluorescence index (FI; Cory and McKnight 2005), the humification index (HIX; Zsolnay *et al.* 1999) and the redox index (RI; Miller *et al.* 2006). The FI has been utilised in a variety of aquatic environments to differentiate DOM of microbial origin (extra-cellular release or leaching from algae or bacteria) from that of terrestrial higher plant sources and is quantified as the ratio of emission intensity at 470 nm to that at 520 nm for excitation at 370 nm (Fellman *et al.* 2010*a*). The HIX is calculated as the ratio of the integrated emission intensities from 435 to 480 nm, divided by the integration from 300 to 345 nm at excitation wavelength of 254 nm (Zsolnay *et al.* 1999). The HIX has been used as an indicator of the extent of humification of DOM and higher HIX values are thought to indicate a shift towards longer emission wavelengths as H : C ratios decrease (Fellman *et al.* 2010*a*). Calculation of the RI is based on the pattern that humic components with red-shifted fluorescence are likely present in a more reduced oxidation state (Cory and McKnight 2005; Miller *et al.* 2006; Cory *et al.* 2011) than those that fluoresce at shorter emission wavelengths. The RI is calculated using component loadings from a PARAFAC model as the ratio of loadings from red-shifted (reduced) components to the total from all humic components (Cory *et al.* 2011).

Analyses of changes in DOM optical characteristics and bioavailability with increasing flow and by season

For the full annual dataset, the relationships among PARAFAC loading scores, fluorescence indices, bioavailability, DOC concentration and stream discharge were examined using simple linear regression and correlation (JMP Statistical Software version 11). The relationships between DOM characteristics and discharge were also examined separately for each event during which samples were collected for detailed characterisation of DOM ($n = 8$). A total of 21 events occurred between 30 October 2009 and 30 October 2010 (Wilson *et al.* 2013) and samples were collected at high frequency for DOM characterisation during events recorded in 8 of 12 months. Detectable events did not occur in April or September of 2010, and events occurring in May and June did not trigger start-up of auto samplers because of small magnitude and short duration. Only four storm-flow samples could be collected during a short duration event in July of 2010. This event was not included among those analysed by event because too few data points were available for regression analysis.

PARAFAC component loadings, fluorescence indices and absorbance characteristics of DOM were compared before and after bioavailability assays, to quantify changes in composition following microbial and photochemical action. Deviations of the measurements taken before and after incubations from a 1 : 1 relationship were utilised to quantify whether increases or decreases were observed for each parameter. The potential influence of starting DOM characteristics on %BDOC and %BDOC-L was also examined; however, significant relationships were not observed by event, so these relationships are presented only for the full annual dataset through the correlation analysis.

Results

PARAFAC model results

A four-component PARAFAC model was identified as the best fit for the EEM dataset collected for the present study (Table 1, Fig. S1, available as Supplementary material for this paper). Two components exhibited characteristics of humic-like DOM (C1, C2). Component C2 has been identified in studies at many other locations with 10 matches with other models in the OpenFluor database (Murphy *et al.* 2014). This component is generally considered to be of terrestrial origin and humic-like, correlating with biomarkers of terrestrial source such as lignin phenols (Walker *et al.* 2009). With a defined excitation maximum ~250 nm, an appreciable second excitation peak at 375 nm and a red shifted emission peak at 492 nm with high intensity relative to excitation, C2 exhibits characteristics of a potentially more reduced oxidative state (Cory and McKnight 2005; Cory *et al.* 2011). A similar C2 component identified by Santín *et al.* (2009) in soils and sediments was shown to be enriched in humic DOM fractions and associated with higher molecular weight. Component C1 shares characteristics with several commonly identified humic-like fluorophores (Table 1, with six matches in the OpenFluor database, see the Excel tables in the Supplementary material). As described in Table 1, the intense EX peak at <240 nm and EM peak at 436 nm for C1 are most similar to widespread humic-like components resembling fulvic acids that are common in forested systems (Coble 2007; Fellman *et al.* 2010*a*); however, a minor secondary peak at 320 nm may indicate co-occurrence with a more humic component not separated in the PARAFAC model and co-occurrence is commonly observed for Peaks A and C (using the traditional nomenclature to describe EEMs: Coble 2007). The blue-shifted EM peak for C1, higher-intensity EX peak near 240 nm, and lower EM intensity than that of EX are characteristics that may indicate a more oxidised state (Cory and McKnight 2005; Cory *et al.* 2011). Similar fluorophores with blue-shifted EM peaks have been shown to occur in greater

Table 1. Peak position of fluorophores identified in a parallel factor analysis (PARAFAC) model of excitation–emission matrices of samples from the Upper Bigelow Brook watershed

Comparisons are provided to other commonly utilised fluorophore groupings

	Excitation maximum (nm)	Emission maximum (nm)	Comparison to Coble (2007)	Comparison to Cory and McKnight (2005)	Comparison to Stedmon and Markager (2005)	Comparison to Fellman *et al.* (2010*a*) component name and description	Comparison to Santín *et al.* (2009)
C1	<240 (320)	436	UVA humic-like (C) UVC humic-like (A)	Q (C11[A]) Oxidised with intense lower wavelength EX and less intense, blue shifted EM peak	4[A]	No exact fit for a single commonly observed PARAFAC component summarised in Fellman *et al.* (2010*a*), but shares most characteristics with widespread UVA humic-like fluorophores, with fluorescence resembling fulvic acid, common in forested environments[A]	Fulvic acid type
C2	250 (375)	492	UVA humic-like (–)	SQ (C5) Reduced semi-quinone-like with red shifted EM peak with high intensity relative to EX	2	UVA humic-like, probable terrestrial plant or soil source, high molecular weight, aromatic, widespread	Humic acid type
C3	<240 (285)	356	Tryptophan-like, protein-like (T)	Tryptophan-like	7	Amino acids, free or bound in proteins, fluorescence resembles free tryptophan, may indicate intact proteins or less degraded peptide material	Tryptophan-like
C4	275	304	Tyrosine-like, protein-like (B)	Tyrosine-like	8	Amino acids, free or bound in proteins, fluorescence resembles free tyrosine, may indicate more degraded peptide material	Tyrosine-like

[A] Components not an exact match.

Table 2. Correlations (Pearson's *r*) among dissolved organic matter (DOM) concentration, fluorescence and bioavailability measures and with discharge in the Upper Bigelow Brook watershed

See text for definition of parameter abbreviations. The *r*-values shown in bold type indicate statistical significance of $P < 0.01$, and in italic a significance of $P < 0.05$

Parameter	RI	DOC	TON	FI	BA	SUVA	HIX	E280	C1%	C2%	C3%	C4%	BDOC %	BDOC-L%
log Q (mm)	–	**0.50**	**0.35**	**−0.51**	*−0.27*	*0.22*	**0.44**	**0.29**	**−0.34**	**0.75**	**−0.55**	−0.17	**0.37**	**0.56**
RI		**0.50**	**0.33**	**−0.57**	**−0.49**	**0.49**	**0.56**	**0.56**	**−0.48**	**0.92**	**−0.61**	−0.19	0.11	**0.46**
DOC			**0.84**	**−0.29**	**−0.51**	*0.24*	**0.59**	**0.29**	0.06	**0.59**	**−0.28**	**−0.38**	**0.38**	−0.10
TON				−0.15	**−0.42**	**0.34**	**0.50**	**0.35**	0.18	**0.46**	−0.08	**−0.43**	0.18	−0.21
FI					**0.35**	*−0.23*	*−0.21*	*−0.25*	**0.37**	**−0.48**	**0.41**	−0.01	−0.12	−0.21
BA						**−0.43**	**−0.35**	**−0.47**	0.18	**−0.48**	**0.47**	0.09	−0.04	−0.05
SUVA							0.08	**0.99**	**−0.47**	**0.38**	**−0.42**	0.14	−0.15	0.07
HIX								0.15	**0.41**	**0.81**	−0.10	**−0.82**	0.16	0.10
E280									**−0.48**	**0.45**	**−0.46**	0.10	−0.12	0.13
C1%										−0.09	**0.54**	**−0.73**	−0.07	**−0.42**
C2%											**−0.47**	**−0.54**	0.10	**0.35**
C3%												**−0.35**	−0.19	**−0.42**
C4%													0.04	0.16
BDOC%														0.39

Table 3. Regression coefficients (R^2) describing relationship between dissolved organic matter (DOM) characteristics and discharge for each hydrologic event characterised over 1 year of monitoring at the Upper Bigelow Brook

Non-significant relationships are not shown and are indicated by a hyphen. Where $P < 0.01$, values are indicated by normal text, and where $P < 0.05$, italics is used. For the large storm event on 30 September 2010, maximum absorbance for three samples exceeded 0.3 and because full correction for inner filter effects was not possible, these values were excluded from analysis for fluorescence-based metrics. For non-fluorescence-based metrics, R^2 is provided for both the subset of samples with absorbance <0.3 and for all samples collected during the event, with these values provided in parentheses below those for the subset. See text for definition of abbreviations

Event start	BA	DOC	E280	FI	HIX	C1%	C2%	C3%	C4%	RI	SUVA	%BDOC			*n*	Max Q sampled
												Dark	L	N		
20 November 2009	*−0.61*	0.84	0.83	–	0.53	–	0.76	−0.66	−0.54	0.76	0.85	–	–	–	11	0.22
2 December 2009	*−0.78*	0.92	0.87	*−0.41*	–	−0.60	0.88	−0.67	–	0.94	0.90	*0.62*	–	–	9	0.27
25 January 2010	–	0.98	0.67	–	0.83	–	0.83	–	−0.65	0.79	–	0.64	*0.46*	–	9	1.65
24 February 2010	–	0.99	0.61	–	–	–	*0.52*	*−0.45*	–	0.68	0.66	0.77	0.56	NM	8	1.05
8 March 2010	–	0.89	0.45	–	–	–	*0.37*	–	–	0.86	0.40	0.62	–	NM	20	1.48
22 August 2010	–	0.81	–	–	–	–	–	–	–	–	–	–	–	NM	9	0.05
30 September 2010	–	0.86 (0.97)	*0.59* (0.66)	–	–	–	*0.52*	–	–	*0.4*	*0.56* (0.67)	– (0.53)	– (–)	NM	9 (12)	0.08 (1.06)

abundance in soil and sediment fulvic acids with lower molecular weight (in comparison to humic acids; C1 *v.* C2 as described in Santín *et al.* 2009). Components C3 and C4 are non-humic fluorphores, with C3 exhibiting tryptophan-like and C4 exhibiting tyrosine-like fluorescence (Table 1).

Changes in DOM optical characteristics with increasing discharge

Dissolved organic carbon in Bigelow Brook consistently increases with discharge rate, with a well defined counter-clockwise hysteresis, but the rate of DOC increase with discharge differs seasonally, with the highest rates of DOC increase with Q occurring during summer and early autumn (Wilson *et al.* 2013). Although well defined changes in DOM optical characteristics with increasing Q were observed both annually (Table 2) and by event (Table 3), seasonal DOC–discharge relationships tended to be more predictable than the relationships between discharge and most optical characteristics. The dominant change in composition observed with increasing Q for all hydrologic events was an increase in the proportion of total fluorescence associated with the terrestrial humic-like C2 PARAFAC component, accompanied by a decrease in the more fulvic-like C1 and protein-like components C3 and C4 (Tables 2, 3). Over the entire year, C2 consistently increased with increasing baseflow (Fig. 1, Tables 2, 3) and during hydrologic events; however, higher variability in this relationship was observed during snowmelt when high tyrosine-like fluorescence (C4) was observed during the latter stages of snowmelt (Fig. 1, Table 3). Although a general tendency towards decreasing FI and increasing HIX with increasing discharge was observed, these relationships were quite variable (Table 2) and were rarely significant when considered separately by event (Table 3). An increase in C2 was the most predictable change in DOM character observed during events

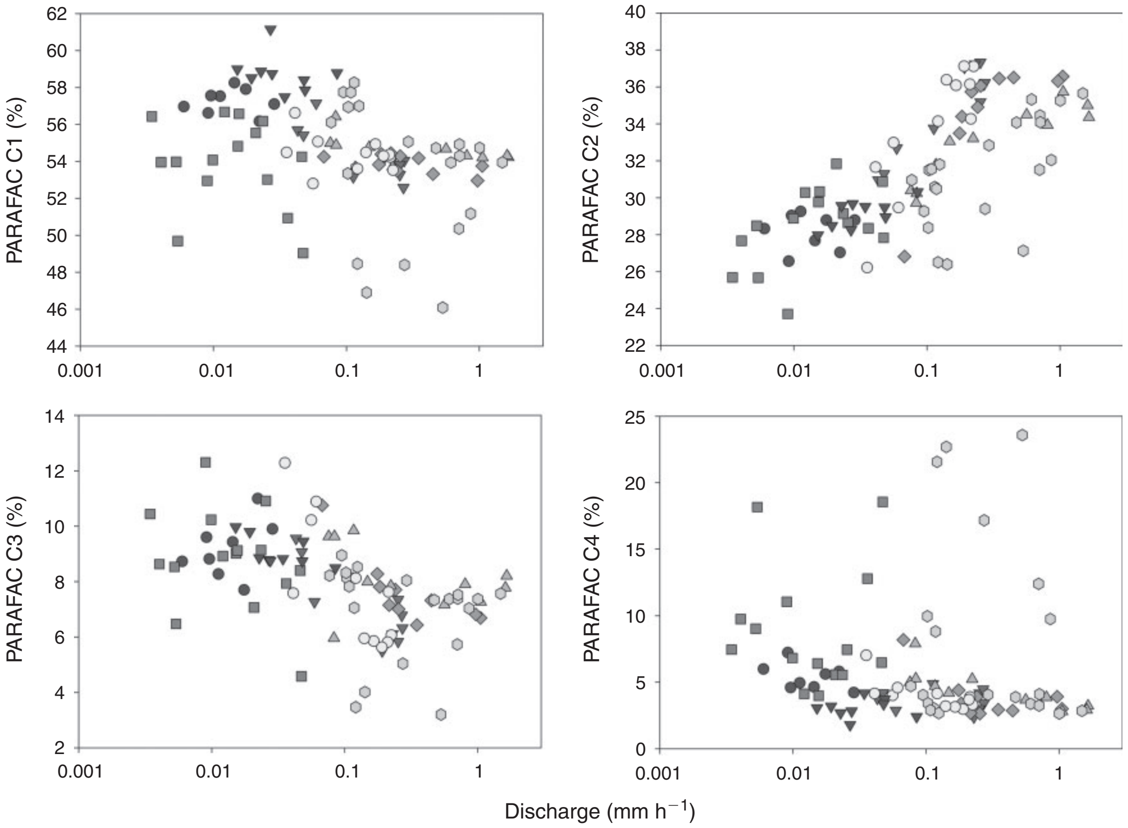

Fig. 1. The relationship between the proportion of total fluorescence associated with each parallel factor analysis (PARAFAC) component as related to discharge rate of the Upper Bigelow Brook. Marker shape and colour indicate date of sample collection: November of 2009 (white circles), December of 2009 (dark grey inverted triangles), January of 2010 (light grey triangles), February of 2010 (grey diamonds), March of 2010 (light grey octagons), May, June, July of 2010 (dark grey squares), August of 2010 (black circles), October of 2010 (inverted black triangles).

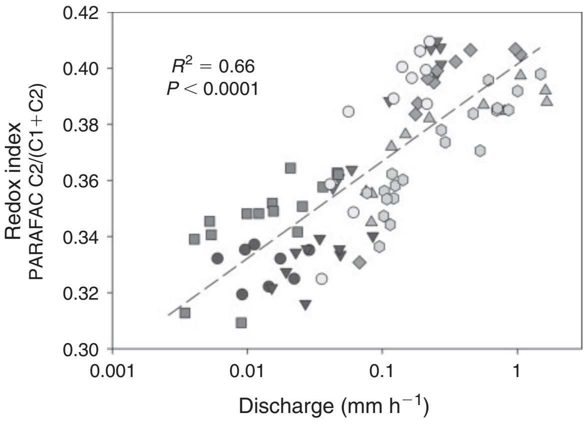

Fig. 2. Redox index of dissolved organic matter (DOM) as related to discharge rate of the Upper Bigelow Brook. Marker shape and colour indicate date of sample collection: November of 2009 (white circles), December of 2009 (dark grey inverted triangles), January of 2010 (light grey triangles), February of 2010 (grey diamonds), March of 2010 (light grey octagons), May, June, July of 2010 (dark grey squares), August of 2010 (black circles), October of 2010 (inverted black triangles).

and may be associated with an increase in the proportion of more complex humic DOM (C2) in relation to the amount of more fulvic DOM (C1). This pattern also corresponds to a potential increase in the proportion of the DOM pool composed of more reduced moieties (RI = C2/(C1 + C2), Fig. 2). The indices ε280 and SUVA_{254} generally increased with Q during events (Table 3), indicating likely increases in aromaticity; however, these relationships were much weaker when considered annually (Table 2), owing to high variability for samples collected during summer months under lower flow conditions.

Moisture conditions over the growing season following the installation of lysimeters were not conducive to the collection of a large number of samples for characterisation of soil-water DOM. Small events in spring and summer yielded only small volumes of water in zero-tension systems and concentrations in two of four tension-lysimeter samples were too high for correction of inner-filter effects before fluorescence analyses and were excluded from analysis. Despite these challenges, when considered as an indicator of soil-water chemistry, the optical characteristics of eight samples collected (two piezometer, four zero tension and two tension) do provide some insight into differences that are most likely to exist between deeper

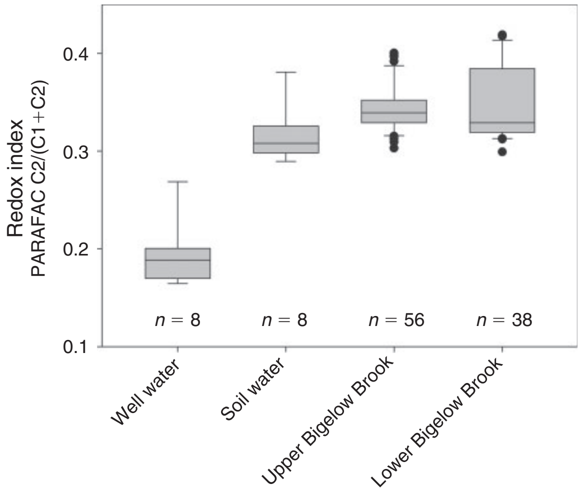

Fig. 3. Redox index of dissolved organic matter (DOM) in groundwater, soil water, and stream water of the Bigelow Brook watershed. Box plots show median, 25th and 75th percentile. Wiskers indicate the 10th and 90th percentiles. Outliers falling outside these bounds are indicated by black circles.

groundwater (well samples) and soil water or shallow groundwater (Fig. 3). The optical characteristic of soil water and shallow groundwater are similar to stream water in which a larger proportion of overall fluorescence from these sources can be attributed to C2 (17–33%). In deeper groundwater samples, very little of total fluorescence is associated with C2 (4–9%) and a large proportion is associated with the tryptophan-like C3 (42–68%). A comparison of the proportions of C1 and C2 in soil water, stream water from the Upper Bigelow Brook, and stream water collected at the Lower Bigelow Brook site downstream of a small wetland do not show statistically significant differences in the proportion of fluorescence associated with C1 and C2 (Student's *t*-test, $P > 0.10$, Fig. 3). The lack of a statistical difference among groups may be partially attributed to the low number of soil-water samples collected, but a general tendency for a higher %C2 in stream water than in soil water was observed.

Changes in DOM bioavailability with increasing discharge

For all bioavailability incubations, exposure to light and room temperature resulted in greater reduction in DOC than did nutrient amendment or incubation under standard dark conditions. In comparison to standard %BDOC, nitrogen and phosphorus addition increased %BDOC in incubations of water collected during storm events in November and December of 2009, whereas no significant response was observed for samples collected in January 2010 (Fig. 4). This trend may relate to background nitrogen availability, with a significant negative linear relationship observed between TDN concentration before nutrient addition and %BDOC in incubations with added nitrogen ($R^2 = 0.45$, $P < 0.0001$, $n = 37$). Change in %BDOC with discharge followed a statistically significant increasing pattern for all samples collected during the study (Fig. 5), and a high degree of unexplained scatter in this relationship can be related to differences in response between events and seasons (Fig. 4).

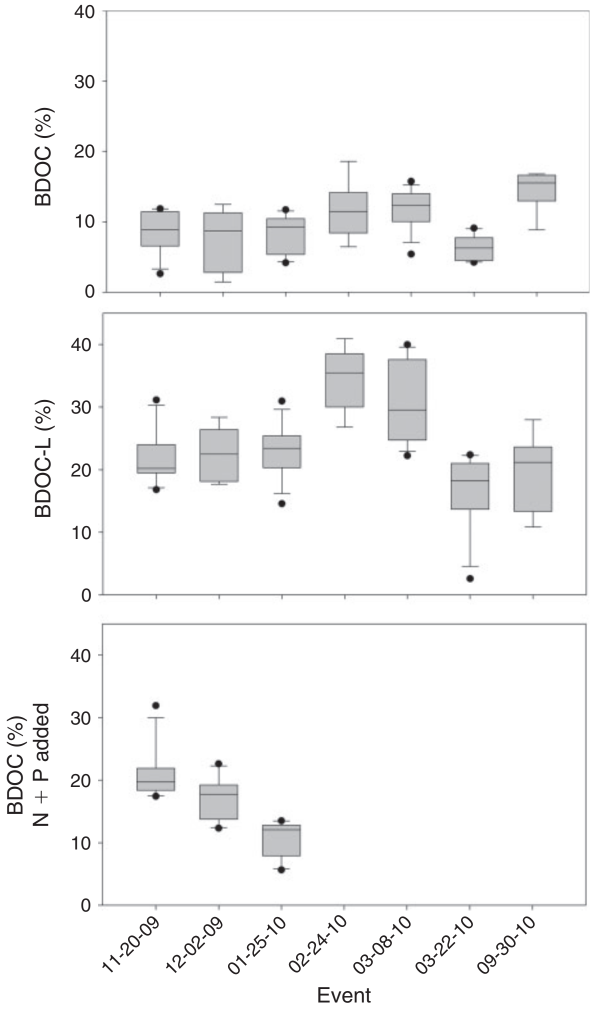

Fig. 4. Bioavailability of dissolved organic matter by hydrologic event sampled at the Upper Bigelow Brook. BDOC (%) was measured as change in concentration over a 30-day incubation under dark conditions (BDOC), with exposure to sunlight (BDOC-L), or with nitrogen, phosphorus and potassium amendment (BDOC N + P added). Box plots show median, 25th and 75th percentile. Whiskers indicate the 10th and 90th percentiles. Outliers falling outside these bounds are indicated by black circles.

No measure of change in DOM composition was correlated significantly with %BDOC (Table 3); however, over the course of each storm event during the winter months, more predictable %BDOC–discharge relationships were observed. For summer and autumn events, changes in %BDOC were less predictable (Table 3). Significant relationships between discharge and %BDOC-L were not observed for most individual events (Table 3), but response to increasing discharge was less seasonally variable, exhibiting a higher degree of correlation with discharge for the full year of data (Fig. 4). Also, %BDOC-L correlates with the RI and the associated changes in composition (Table 2) that accompany an increasing flow. This correlation of %BDOC-L with DOM composition is more seasonally consistent than the correlation with DOC observed for %BDOC (Tables 2, 3).

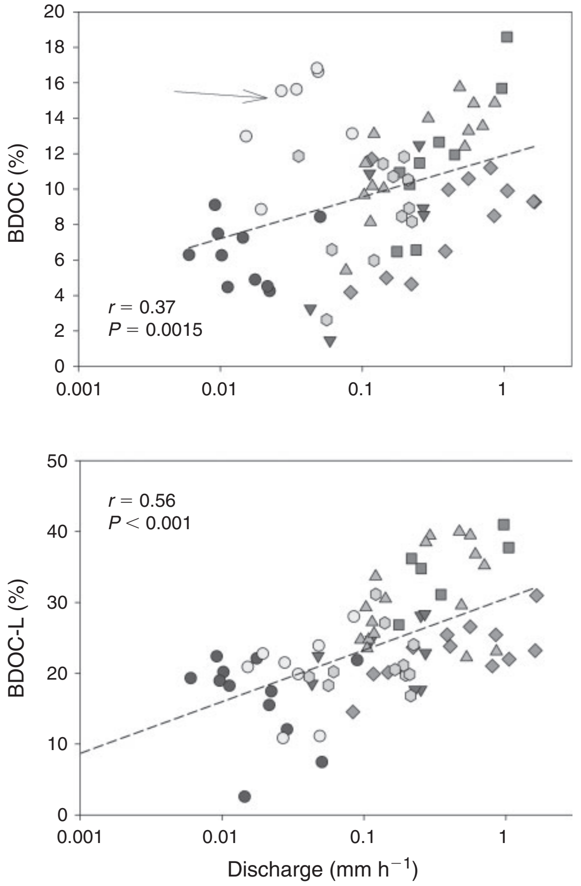

Fig. 5. Bioavailability of dissolved organic matter (BDOC) as related to discharge rate of the Upper Bigelow Brook. BDOC was measured by change in concentration over a 30-day incubation with dark (BDOC) or sunlight-exposed (BDOC-L) conditions. Marker shape and colour indicate date of sample collection: November of 2009 (white circles), December of 2009 (dark grey inverted triangles), January of 2010 (light grey triangles), February of 2010 (grey diamonds), March of 2010 (light grey octagons), May, June, July of 2010 (dark grey squares), August of 2010 (black circles), October of 2010 (inverted black triangles). An arrow points to a group of samples collected in March of 2010, with higher than expected BDOC and high tyrosine-like fluorescence ($>$7% total fluorescence).

Transformation of DOM following BDOC incubations

For 23 samples collected in March and February, %BDOC and %BDOC-L were compared for incubations with and without filtration. The slope of the relationships between filtered and unfiltered total organic carbon concentrations did not differ significantly from 1, with calculated intercepts of –2.7% for %BDOC and –2.5% for %BDOC-L. This pattern was interpreted as an indication that loss of organic carbon in BDOC experiments was primarily by conversion to CO_2.

Following 30-day incubations, similar changes in DOM optical characteristics were observed under light and dark conditions. An increase in the proportion of DOM fluorescence from tryptophan-like (C3) and tyrosine-like (C4) fluorophores was observed for most incubations (Figs 6, 7), but was more pronounced following light-exposed incubations (Fig. 7).

An increase in the proportion of fluorescence associated with fulvic acid-like and blue-shifted C1 was observed following incubations, and again, this pattern was most obvious following light-exposed incubations (Figs 6, 7). The only PARAFAC component shown to consistently decrease following incubations was the humic acid-like and red-shifted C2 (Figs 6, 7). Increases in SUVA, E280 and HIX exhibited reductions following incubations, with the greatest reductions following light exposure. Although variability in patterns of DOM composition following incubations was observed, a greater tendency for loss of humic-like DOM (potentially more reduced; C2) and an increase of both protein-like and of fulvic-like DOM (potentially more oxidised; C1) was obvious. The only significant deviation from this trend was observed for five samples collected during snowmelt that exhibited elevated tyrosine-like fluorescence ($>$7% as C4). Following incubation of these samples, a reduction in C4 was observed and, in %BDOC incubations, a concurrent increase in C1 and C2 was observed (Fig. 6).

Discussion

Potential DOM-source changes under higher-flow conditions

Previous research in the Bigelow Brook watershed has identified riparian soils in the catchment as a significant potential pool of organic matter (153 g C kg^{-1}) (Xu and Saiers 2010), but it remains to be defined what portion of the soil DOM exported from the watershed during hydrologic events originates from near stream *v.* upland sources. To fully define the mechanisms controlling shifts towards the export of humic-like and potentially more reduced DOM under higher-flow conditions in Bigelow Brook will require further research to better define links with changing source areas along hillslopes in the watershed. However, our measurements that characterise the fluorescence of potential sources of DOM in the watershed do indicate that soil water and shallow riparian groundwater are probable sources of C2-rich DOM (Fig. 3) in this forested upland stream.

The RI and C2 of soil-water samples collected in 2010 are somewhat lower than values measured in stream and indicate that the more C2-rich DOM exported at high flow may originate from another source. One possible explanation for this difference is that a significant proportion of DOM may originate from flow through organic matter-rich riparian soils or hyporheic zone rather than from upland sources or overland flow. Miller *et al.* (2006) showed higher RI in association with input of reduced fulvic acids from the hyporheic zone into mountain streams. During hydrological events in Bigelow Brook, DOM chemistry did not relate linearly to toe-slope well water level because of the presence of hysteresis between the well level and flow; however, for samples collected under baseflow conditions, a linear relationship was present between the well level and the RI ($R^2 = 0.80$, $P < 0.0001$, $n = 17$). Given that groundwater upstream of hyporheic areas exhibited low DOC and low RI and that near-surface flow does not occur in upland portions of the catchment at baseflow, this correlation between toe-slope water level and RI suggests that higher rates of loading from subsurface flow through riparian soils and hyporheic areas is likely occurring when the watertable of the catchment rises during events.

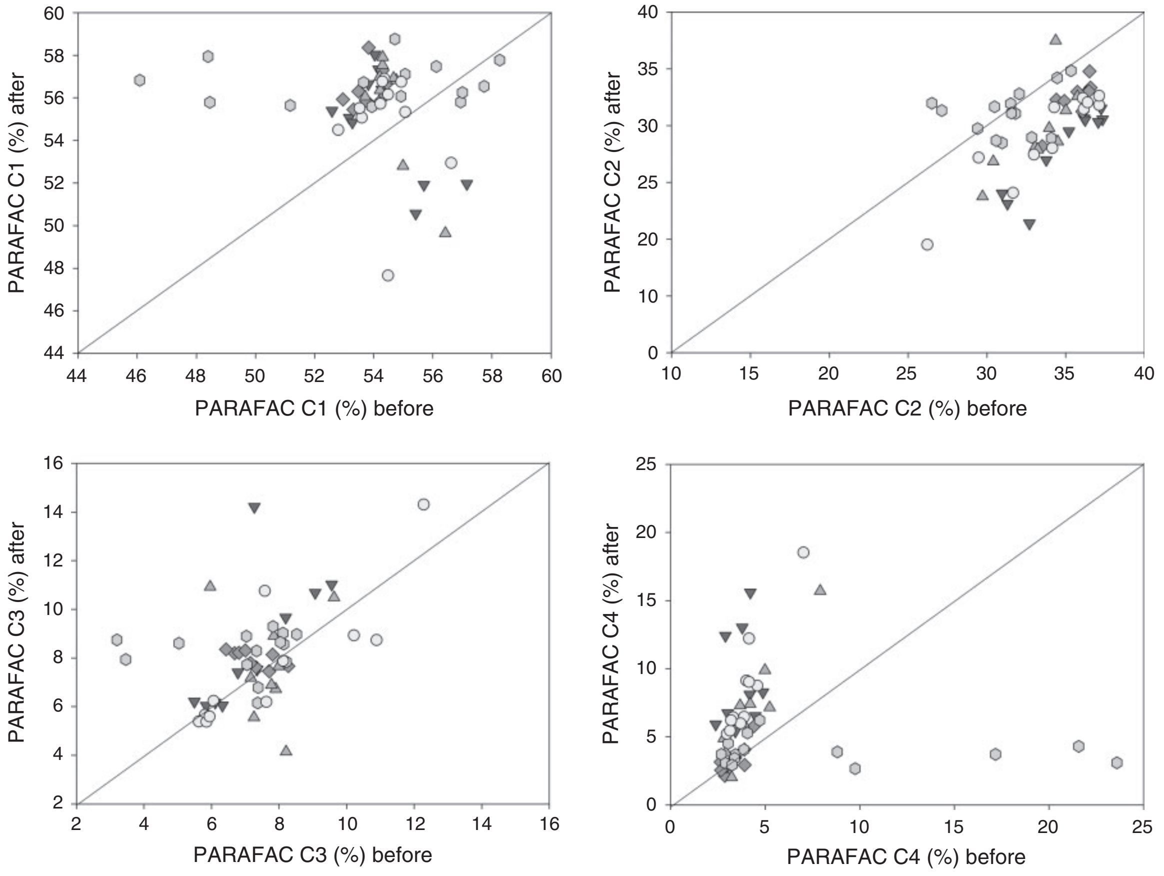

Fig. 6. Percentage of total dissolved organic matter (DOM) fluorescence associated with each parallel factor analysis (PARAFAC) component before and after 30-day incubations to measure bioavailability of dissolved organic matter (BDOC) under dark conditions. On each plot, a line indicates a 1 : 1 relationship. Points falling above the 1 : 1 line showed an increase in the percentage of total fluorescence associated with a particular component and points below the line indicate that a decrease was observed. Marker shape and colour indicate date of sample collection: November of 2009 (white circles), December of 2009 (dark grey inverted triangles), January of 2010 (light grey triangles), February of 2010 (grey diamonds), March of 2010 (light grey octagons), May, June, July of 2010 (dark grey squares), August of 2010 (black circles), October of 2010 (inverted black triangles).

Susceptibility of fluorophores exported during hydrological events to degradation

Changes in DOM composition observed following incubation experiments indicated which fluorophores appear most likely to be transformed by microbial action and photodegredation. Humic components of DOM exported from the Upper Bigelow Brook during hydrological events were disproportionately reduced following microbial processing (Figs 4, 5); however, %BDOC was not significantly correlated with measures of DOM composition (Table 2) and the magnitude of change in C2 (ΔC2) for both dark- and light-incubation experiments was poorly predicted by %BDOC or %BDOC-L respectively ($R^2 < 0.10$). More subtle differences in DOM composition than those detectable by fluorescence spectroscopy, differences in initial nutrient concentrations among incubations, and variation in the development of microbial assemblages in each incubation may all have influenced magnitude of change in each PARARAC component following incubation experiments, and likely acted to increase variability.

Strong correlations between the abundance of amino acid-like fluorescing DOM and %BDOC have been observed in a variety of stream systems (Balcarczyk *et al.* 2009; Fellman *et al.* 2009; Hood *et al.* 2009), whereas a high susceptibility of more humic-like DOM to microbial degradation has been observed in others (Fasching and Battin 2012; Ward *et al.* 2013). There is no definitive explanation for this contrasting behaviour, but in forested headwater streams such as the Upper Bigelow Brook and those studied by Fasching and Battin (2012), amino acid-like DOM tends to compose a relatively small proportion of the fluorescing DOM pool, and increases in humic-like material are associated with loading of new material to the stream from the catchment. This pattern raises at least the following two potential hypotheses:

(1) Sources or flowpaths from which amino acid-like components of the DOM pool originate may vary among stream systems and correlation of bioavailability with composition may be related to source changes rather than the compositional

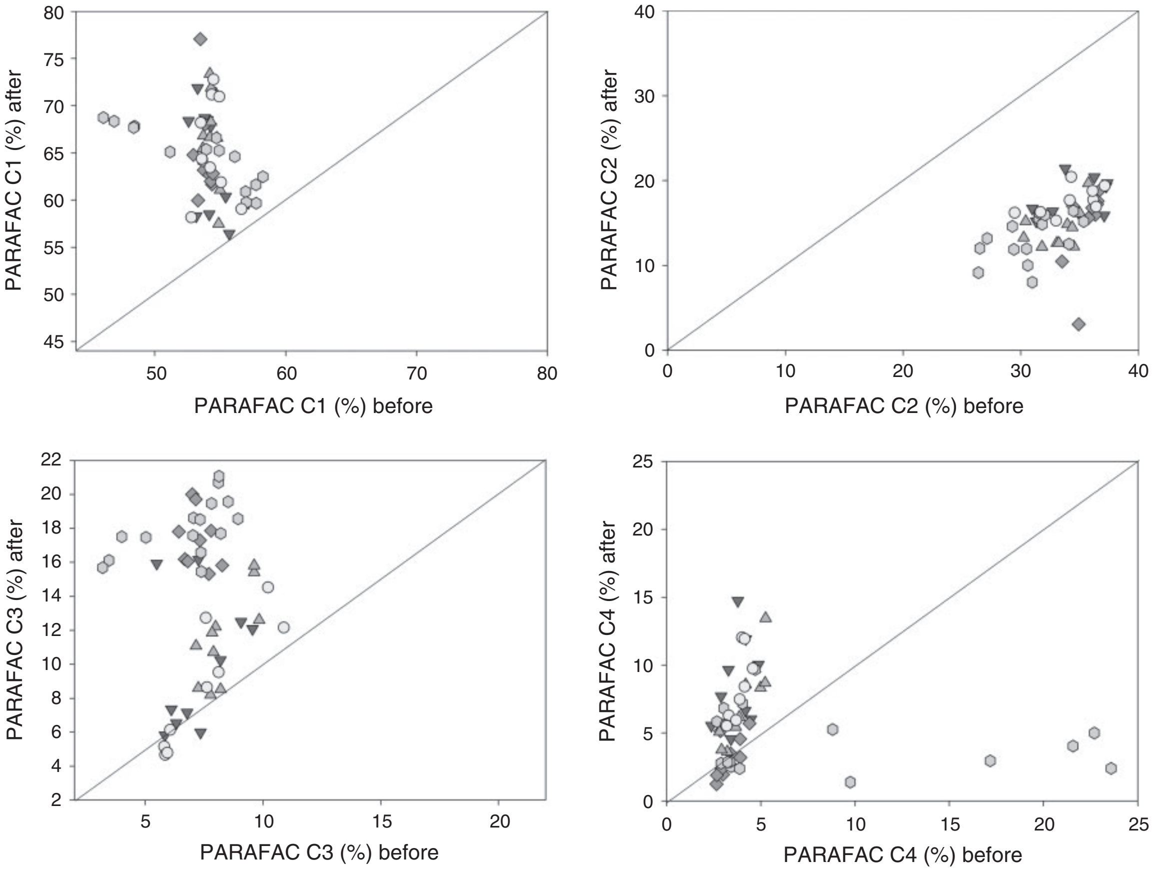

Fig. 7. Percentage of total dissolved organic matter (DOM) fluorescence associated with each parallel factor analysis (PARAFAC) component before and after 30-day incubations to measure bioavailability of dissolved organic matter (BDOC) under sunlight-exposed conditions. On each plot, a line indicates a 1 : 1 relationship. Points falling above the 1 : 1 line showed an increase in the percentage of total fluorescence associated with a particular component and points below the line indicate that a decrease was observed. Marker shape and colour indicate date of sample collection: November of 2009 (white circles), December of 2009 (dark grey inverted triangles), January of 2010 (light grey triangles), February of 2010 (grey diamonds), March of 2010 (light grey octagons), May, June, July of 2010 (dark grey squares), August of 2010 (black circles), October of 2010 (inverted black triangles).

changes detectable with fluorescence spectroscopy. In the Upper Bigelow Brook catchment, higher amino acid-like fluorescence was observed in well-water samples than in stream-water samples, so increases in amino acid-like fluorescence within the stream could be linked to deeper groundwater inputs. The conditions with highest contribution of flow from deeper flow paths are not yet well defined for Bigelow Brook, but a higher proportion from groundwater might be expected under base-flow conditions. A larger proportion of the DOM pool with amino acid-like fluorescence is likely to be associated with algal production of DOM rather than with ground-water input in less shaded stream systems, those with more wetland in the catchment, or those further down the stream continuum with higher rates of autochthonous carbon fixation (Fellman *et al.* 2009).

(2) It may also be that microbial assemblages of headwater streams exporting predominantly humic-like DOM are better adapted to take advantage of energy subsidies from humic-like DOM. In BDOC and BDOC-L incubations, a negative correlation was observed with tryptophan-like fluorescence (C3; Table 2) and a very similar weak negative trend is exhibited for tyrosine-like fluorescence (C4) when only considering samples with C4 loadings less than 7%; however, five samples collected during snowmelt with higher C4 loadings exhibit elevated %BDOC and result in a weak overall positive correlation. These same five samples are outliers in Figs 5, 6 and 7 and show a significant reduction in tyrosine-like fluorescence following incubation that contrasts samples collected throughout the rest of the year. These outlying points may relate to similar fluorescence characteristics being associated with differing source seasonally (e.g. enrichment in the snowpack in association with aerosol deposition; Mladenov *et al.* 2011), but this may also be an indication that, in bioavailability assays with a sufficiently high proportion of tyrosine-like fluorescence, the conditions are more favourable for colonisation by a microbial assemblage adapted to utilisation of tyrosine-like DOM as an energy source.

Fasching and Battin (2012) observed a shift to simpler DOM molecular structure following BDOC experiments for waters collected from Austrian headwater streams, much like the changes we observe following BDOC experiment water from the Upper Bigelow Brook (Figs 6, 7). Although the Upper Bigelow Brook is primarily a well drained and forested catchment (not a brown-water system), DOM exported from the system is primarily of terrestrial origin and the proportion of higher-complexity DOM increases during hydrological events (Fig. 1). In addition, similar increases in aromaticity of DOM have been observed in agricultural headwater streams without significant areas of wetland within the watershed (Vidon *et al.* 2008). Together, these patterns indicate that a majority of the BDOC exported from the Upper Bigelow Brook and from similar headwater systems during storm events is likely to be humic-like terrestrial derived carbon and there appears to be a high potential for transformation of this DOM by microbial metabolism and photo degradation.

Potential reactivity of DOM exported during hydrologic events

An increase in the export of bioreactive terrestrial DOM with increasing discharge has been noted in a variety of other studies (Buffam *et al.* 2001; Fellman *et al.* 2009; McLaughlin and Kaplan 2013; Wilson *et al.* 2013). Higher export of bioreactive terrestrial DOM with events is consistent with the pulse-shunt concept (Raymond *et al.* 2016), where labile terrestrial DOM is pulsed out of small headwater streams during hydrologic events and shunted through small streams because of the high velocity of waters associated with events. The positive correlations of the redox index (Fig. 2) and BDOC (Fig. 5) with discharge in Bigelow Brook indicated that events control the export of bioreactive terrestrial DOM.

For the incubation procedures used in the present study, a lack of control over starting nutrient and DOC concentrations in incubations and of temperature in light-exposed incubations likely resulted in the high degree of unexplained variability observed for relationships between %BDOC and discharge or DOM composition. Despite the shortcomings of the simple bottle experiments used to quantify DOC bioavailability in the present study, the results presented here provide some insight into how susceptibility of DOM to transformation is likely to change in forested headwater streams during hydrologic events. The results of incubation experiments both with and without nutrients (Fig. 4), the positive correlation observed between DOC and %BDOC (Table 2), and the changes we observed in composition the following incubations (Figs 6, 7) indicated the potential for %BDOC and %BDOC-L to be controlled by both availability of a suitable carbon source (DOC and DOM composition) and of limiting nutrients. These factors, along with temperature and light, are likely to vary significantly in-stream and together will influence rates of DOM processing.

Increasing complexity of sources from actively growing vegetation and not reflected in the generalised characterisation possible through fluorescence spectroscopy is one possible explanation for the reduced predictability of the %BDOC–discharge relationship during growing-season months (Table 3). Changing complexity of sources may also explain outliers observed during snowmelt, where higher than expected %BDOC was observed along with increasing tyrosine-like fluorescence (Fig. 5), particularly if this material is associated with the labile aerosols deposited in the snowpack (Fellman *et al.* 2010*b*; Mladenov *et al.* 2011) or with short-frequency release of labile C from soils and vegetation residue following freeze–thaw events (Matzner and Borken 2008).

Although it is not clear whether the relationship is correlative or causal, the presence of more humic-like (C2) and potentially reduced (RI) DOM appears to be associated with increasing susceptibility to degradation under light-exposed conditions (Table 2, Fig. 5). Reductions in humic-like C2 fluorescence following dark incubations also indicated that associated compounds may be more susceptible to transformation by microbial activity. This inference is supported by the results of Miller *et al.* (2006) who observed that the reduced state of DOM originating from hyporheic exchange was associated with increased potential for decay of DOM by microbial action in oxidised regions of the stream. The likely increased susceptibility of the more reduced C2 to photodegradation is also supported by similar observations in other aquatic systems. A shift to shorter emission wavelengths has been frequently observed to follow photo-bleaching (Coble 2007) and is consistent with the loss of C2 and gain of C1 in light-exposed incubations.

Nitrogen and phosphorus addition increased %BDOC in November and December incubations, but no significant response was observed for samples collected in January. This may be because of higher initial nitrogen availability with both mean TON and mean TDN increasing through the November, December and January events (105, 139, 164 μg L^{-1} and 130, 154, 247 μg L^{-1}), and a significant negative linear relationship was observed between TDN concentration before nutrient addition and %BDOC in the following incubation ($R^2 = 0.45$, $P < 0.0001$, $n = 37$). Research has indicated that forested watersheds in this region export appreciable amounts of unprocessed nitrate in winter months because of temperature effects on biotic processing (Barnes *et al.* 2008). Further research is needed to identify the seasonal extent of any limitation of DOC bioavailability by nitrogen or phosphorus availability, but our findings suggested that heterotrophic responses to any changes in nitrogen and phosphorus loading at Bigelow Brook are likely to vary seasonally (Fig. 4).

Conclusions

During hydrologic events, a predictable increase in the proportion of humic-like DOM in a seemingly more reduced oxidative state occurs in the Bigelow Brook. This trend of changing composition appears to be linked to increased loading from a large riparian or hyporheic pool of organic matter and shows less seasonal variability than do concurrent increases in DOC that also occur with increasing discharge. Further research is needed before connections between these composition changes and bioavailability can be fully defined; however, the more humic-like DOM exported during storm events is more susceptible to degradation by both microbial activity and photodegradation. In this sense, the higher molecular-weight and humic-like components of the DOM pool that are most likely to increase in water exported from forested headwater streams during hydrologic events will be important to the allochthony of downstream inland waters. The results from this watershed also indicated a hydrologic-induced break in the terrestrial cycle. During hydrologic events, more reduced organic matter and its associated nutrients are exported from terrestrial systems, precluding its utilisation by terrestrial microbes. These results are likely to be similar to other headwater streams where DOM loading is primarily controlled by hillslope processes.

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References

Aitkenhead-Peterson, J. A., McDowell, W. H., and Neff, J. C. (2003). Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In ‘Aquatic Ecosystems: Interactivity of Dissolved Organic Matter’. (Eds S. E. G. Findlay and R. L. Sinsabaugh.) pp. 25–70. (Academic Press: Burlington, MA.)

Balcarczyk, K., Jones, J., Jr, Jaffé, R., and Maie, N. (2009). Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry* **94**, 255–270. doi:10.1007/S10533-009-9324-x

Barnes, R., Raymond, P., and Casciotti, K. (2008). Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested watersheds in the northeastern US. *Biogeochemistry* **90**, 15–27. doi:10.1007/S10533-008-9227-2

Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Marti, E., Packman, A. I., Newbold, J. D., and Sabater, F. (2008). Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geoscience* **1**, 95–100. doi:10.1038/NGEO101

Boyer, E. W., Hornberger, G. M., Bencala, K. E., and McKnight, D. (1996). Overview of a simple model describing variation of dissolved organic carbon in an upland catchment. *Ecological Modelling* **86**, 183–188. doi:10.1016/0304-3800(95)00049-6

Buffam, I., Galloway, J. N., Blum, L. K., and McGlathery, K. J. (2001). A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. *Biogeochemistry* **53**, 269–306. doi:10.1023/A:1010643432253

Carpenter, S. R., Cole, J. J., Pace, M. L., Van de Bogert, M., Bade, D. L., Bastviken, D., Gille, C. M., Hodgson, J. R., Kitchell, J. F., and Kritzberg, E. S. (2005). Ecosystem subsidies: terrestrial support of aquatic food webs from 13c addition to contrasting lakes. *Ecology* **86**, 2737–2750. doi:10.1890/04-1282

Chin, Y. P., Aiken, G., and Oloughlin, E. (1994). Molecular-weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science & Technology* **28**, 1853–1858. doi:10.1021/ES00060A015

Cleveland, C. C., and Liptzin, D. (2007). C : N : P stoichiometry in soil: Is there a ‘redfield ratio’ for the microbial biomass? *Biogeochemistry* **85**, 235–252. doi:10.1007/S10533-007-9132-0

Coble, P. G. (2007). Marine optical biogeochemistry: the chemistry of ocean color. *Chemical Reviews* **107**(2), 402–418. doi:10.1021/CR050350+

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J. (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10**, 172–185. doi:10.1007/S10021-006-9013-8

Cory, R. M., and Kaplan, L. A. (2012). Biological lability of streamwater fluorescent dissolved organic matter. *Limnology and Oceanography* **57**, 1347–1360. doi:10.4319/LO.2012.57.5.1347

Cory, R. M., and McKnight, D. M. (2005). Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science & Technology* **39**, 8142–8149. doi:10.1021/ES0506962

Cory, R., Boyer, E., and McKnight, D. (2011). Spectral methods to advance understanding of dissolved organic carbon dynamics in forested catchments. In ‘Forest Hydrology and Biogeochemistry. Vol. 216’. (Eds D. F. Levia, D. Carlyle-Moses and T. Tanaka.) pp. 117–135. (Springer: Dordrecht, Netherlands.)

Currie, W. S., Aber, J. D., McDowell, W. H., Boone, R. D., and Magill, A. H. (1996). Vertical transport of dissolved organic c and n under long-term n amendments in pine and hardwood forests. *Biogeochemistry* **35**, 471–505. doi:10.1007/BF02183037

Fasching, C., and Battin, T. (2012). Exposure of dissolved organic matter to uv-radiation increases bacterial growth efficiency in a clear-water alpine stream and its adjacent groundwater. *Aquatic Sciences - Research Across Boundaries* **74**, 143–153. doi:10.1007/S00027-011-0205-8

Fellman, J. B., Hood, E., Edwards, R. T., and D’Amore, D. V. (2009). Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *Journal of Geophysical Research* **114**, G01021. doi:10.1029/2008JG000790

Fellman, J. B., Hood, E., and Spencer, R. G. M. (2010*a*). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. *Limnology and Oceanography* **55**, 2452–2462. doi:10.4319/LO.2010.55.6.2452

Fellman, J. B., Spencer, R. G., Hernes, P. J., Edwards, R. T., D’Amore, D. V., and Hood, E. (2010*b*). The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems. *Marine Chemistry* **121**, 112–122. doi:10.1016/J.MARCHEM.2010.03.009

Harvard Forest (2011). ‘Physiological and Biological Characteristics of Harvard Forest. Vol. 2011.’ (Faculty of Arts and Sciences of Harvard University: Petersham, MA.)

Hedin, L. O., Armesto, J. J., and Johnson, A. H. (1995). Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. *Ecology* **76**, 493–509. doi:10.2307/1941208

Hood, E., Gooseff, M. N., and Johnson, S. L. (2006). Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. *Journal of Geophysical Research* **111**, G01007. doi:10.1029/2005JG000082

Hood, E., Fellman, J., Spencer, R.G.M., Hernes, P.J., Edwards, R., D’Amore, D.,, and Scott, D. (2009). Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* **462**, 1044–1047. doi:10.1038/NATURE08580

Inamdar, S. P., and Mitchell, M. J. (2007). Storm event exports of dissolved organic nitrogen (don) across multiple catchments in a glaciated forested watershed. *Journal of Geophysical Research* **112**, G02014. doi:10.1029/2006JG000309

Inamdar, S., Singh, S., Dutta, S., Levia, D., Mitchell, M., Scott, D., Bais, H., and McHale, P. (2011). Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-atlantic watershed. *Journal of Geophysical Research* **116**, G03043. doi:10.1029/2011JG001735

Li, F., Yuasa, A., Muraki, Y., and Matsui, Y. (2005). Impacts of a heavy storm of rain upon dissolved and particulate organic c, n and p in the main river of a vegetation-rich basin area in japan. *The Science of the Total Environment* **345**, 99–113. doi:10.1016/J.SCITOTENV.2004.11.004

Lowe, W. H., and Likens, G. E. (2005). Moving headwater streams to the head of the class. *Bioscience* **55**, 196–197. doi:10.1641/0006-3568(2005)055[0196:MHSTTH]2.0.CO;2

Matzner, E., and Borken, W. (2008). Do freeze-thaw events enhance c and n losses from soils of different ecosystems? A review. *European Journal of Soil Science* **59**, 274–284. doi:10.1111/J.1365-2389.2007.00992.x

McGlynn, B. L., and McDonnell, J. J. (2003). Role of discrete landscape units in controlling catchment dissolved organic carbon dynamics. *Water Resources Research* **39**, 1090. doi:10.1029/2002WR001525

McLaughlin, C., and Kaplan, L. A. (2013). Biological lability of dissolved organic carbon in stream water and contributing terrestrial sources. *Freshwater Science* **32**, 1219–1230. doi:10.1899/12-202.1

Mei, Y., Hornberger, G. M., Kaplan, L. A., Newbold, J. D., and Aufdenkampe, A. K. (2014). The delivery of dissolved organic carbon from a forested hillslope to a headwater stream in southeastern Pennsylvania, USA. *Water Resources Research* **50**, 5774–5796. doi:10.1002/2014WR015635

Meyer, J. L., and Edwards, R. T. (1990). Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. *Ecology* **71**, 668–677. doi:10.2307/1940321

Miller, M. P., McKnight, D. M., Cory, R. M., Williams, M. W., and Runkel, R. L. (2006). Hyporheic exchange and fulvic acid redox reactions in an alpine stream/wetland ecosystem, Colorado front range. *Environmental Science & Technology* **40**, 5943–5949. doi:10.1021/ES060635J

Miller, M., Simone, B., McKnight, D., Cory, R., Williams, M., and Boyer, E. (2010). New light on a dark subject *Aquatic Sciences* **72**, 269–275. doi:10.1007/S00027-010-0130-2[Comment]

Mladenov, N., Sommaruga, R., Morales-Baquero, R., Laurion, I., Camarero, L., Dieguez, M. C., Camacho, A., Delgado, A., Torres, O., Chen, Z., Felip, M., and Reche, I. (2011). Dust inputs and bacteria influence dissolved organic matter in clear alpine lakes. *Nature Communications* **2**, 405. doi:10.1038/NCOMMS1411

Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R. (2014). Openfluor: an online spectral library of auto-fluorescence by organic compounds in the environment. *Analytical Methods* **6**, 658–661. doi:10.1039/C3AY41935E

Neff, J. C., Chapin, F. S. I., and Vitousek, P. M. (2003). Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment* **1**, 205–211. doi:10.1890/1540-9295(2003)001[0205:BITCDO]2.0.CO;2

Nguyen, H.-M., Hur, J., and Shin, H.-S. (2010). Changes in spectroscopic and molecular weight characteristics of dissolved organic matter in a river during a storm event. *Water, Air, and Soil Pollution* **212**, 395–406. doi:10.1007/S11270-010-0353-9

Ohno, T. (2002). Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science & Technology* **36**, 742–746. doi:10.1021/ES0155276

Ravichandran, M. (2004). Interactions between mercury and dissolved organic matter: a review. *Chemosphere* **55**, 319–331. doi:10.1016/J.CHEMOSPHERE.2003.11.011

Raymond, P., and Saiers, J. (2010). Event controlled doc export from forested watersheds. *Biogeochemistry* **100**, 197–209. doi:10.1007/S10533-010-9416-7

Raymond, P. A., Saiers, J. E., and Sobczak, W. V. (2016). Hydrological and biogeochemical controls on watershed dissolved organic matter transport: pulse–shunt concept. *Ecology* **97**, 5–16. doi:10.1890/14-1684.1

Santín, C., Yamashita, Y., Otero, X. L., Álvarez, M. Á., and Jaffé, R. (2009). Characterizing humic substances from estuarine soils and sediments by excitation–emission matrix spectroscopy and parallel factor analysis. *Biogeochemistry* **96**, 131–147. doi:10.1007/S10533-009-9349-1

Singh, S., Inamdar, S., Mitchell, M., and McHale, P. (2014). Seasonal pattern of dissolved organic matter (dom) in watershed sources: influence of hydrologic flow paths and autumn leaf fall. *Biogeochemistry* **118**, 321–337. doi:10.1007/S10533-013-9934-1

Stedmon, C. A., and Bro, R. (2008). Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography, Methods* **6**, 572–579. doi:10.4319/LOM.2008.6.572

Stedmon, C. A., and Markager, S. (2005). Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology and Oceanography* **50**, 686–697. doi:10.4319/LO.2005.50.2.0686

Vidon, P., Wagner, L. E., and Soyeux, E. (2008). Changes in the character of doc in streams during storms in two midwestern watersheds with contrasting land uses. *Biogeochemistry* **88**, 257–270. doi:10.1007/S10533-008-9207-6

Volk, C. J., Volk, C. B., and Kaplan, L. A. (1997). Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnology and Oceanography* **42**, 39–44. doi:10.4319/LO.1997.42.1.0039

Walker, S. A., Amon, R. M. W., Stedmon, C., Duan, S., and Louchouarn, P. (2009). The use of parafac modeling to trace terrestrial dissolved organic matter and fingerprint water masses in coastal canadian arctic surface waters. *Journal of Geophysical Research. Biogeosciences* **114** (G4), G00F06.

Ward, N. D., Keil, R. G., Medeiros, P. M., Brito, D. C., Cunha, A. C., Dittmar, T., Yager, P. L., Krusche, A. V., and Richey, J. E. (2013). Degradation of terrestrially derived macromolecules in the Amazon River. *Nature Geoscience* **6**(7), 530–533. doi:10.1038/NGEO1817

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology* **37**, 4702–4708. doi:10.1021/ES030360x

Wetzel, R. G. (1992). Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic-matter in fresh-water ecosystems. *Hydrobiologia* **229**, 181–198. doi:10.1007/BF00007000

Willacker, J. J., Sobczak, W. V., and Colburn, E. A. (2009). Stream macroinvertebrate communities in paired hemlock and deciduous watersheds. *Northeastern Naturalist* **16**, 101–112. doi:10.1656/045.016.0108

Wilson, H. F., Saiers, J. E., Raymond, P. A., and Sobczak, W. V. (2013). Hydrologic drivers and seasonality of dissolved organic carbon concentration, nitrogen content, bioavailability, and export in a forested New England stream. *Ecosystems* **16**, 604–616. doi:10.1007/S10021-013-9635-6

Xenopoulos, M. A., and Schindler, D. W. (**2001**) Physical factors determining ultraviolet radiation flux in ecosystems. In 'Ecosystems, Evolution and Ultraviolet Radiation'. (Eds C. S. Cockell and A. R. Blaustein.) pp. 36–32. (Springer: New York.)

Xu, N., and Saiers, J. E. (2010). Temperature and hydrologic controls on dissolved organic matter mobilization and transport within a forest topsoil. *Environmental Science & Technology* **44**, 5423–5429. doi:10.1021/ES1002296

Xu, N., Saiers, J. E., Wilson, H. F., and Raymond, P. A. (2012). Simulating stream flow and dissolved organic matter export from a forested watershed. *Water Resources Research* **48**, W05519. doi:10.1029/2011WR011423

Xu, N., Wilson, H. F., Saiers, J. E., and Entz, M. (2013). Effects of crop rotation and management system on water-extractable organic matter concentration, structure, and bioavailability in a chernozemic agricultural soil. *Journal of Environmental Quality* **42**, 179–190. doi:10.2134/JEQ2012.0126

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., and Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere* **38**, 45–50. doi:10.1016/S0045-6535(98)00166-0