

# Evaluation of forest disturbance legacy effects on dissolved organic matter characteristics in streams at the Hubbard Brook Experimental Forest, New Hampshire

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**Abstract** Dissolved organic matter (DOM) source and composition are critical drivers of its reactivity, impact microbial food webs and influence ecosystem functions. It is believed that DOM composition and abundance represent an integrated signal derived from the surrounding watershed. Recent studies have shown that land-use may have a long-term effect on DOM composition. Methods for characterizing DOM, such as those that measure the optical properties and size of the molecules, are increasingly recognized as valuable tools for assessing DOM sources, cycling, and reactivity. In this study we measured DOM optical properties and molecular weight determinations to evaluate whether the legacy of forest disturbance alters the amount and composition of stream DOM. Differences in DOM quantity and composition due to vegetation type and to a greater extent, wetland influence, were more pronounced than effects due to disturbance. Our results suggest that excitation-emission matrix fluorescence with parallel factor analysis is a more sensitive metric of disturbance than the other methods evaluated. Analyses showed that streams draining watersheds that have been clearcut had lower dissolved organic carbon (DOC) concentrations and higher microbially-derived and protein-like

fluorescence features compared to reference streams. DOM optical properties in a watershed amended with calcium, were not significantly different than reference watersheds, but had higher concentrations of DOC. Collectively these results improve our understanding of how the legacy of forest disturbances and natural landscape characteristics affect the quantity and chemical composition of DOM in headwater streams, having implications for stream water quality and carbon cycling.

**Keywords** Hubbard Brook Experimental Forest · Dissolved organic matter (DOM) · Dissolved organic carbon (DOC) · Stream · Watershed · Parallel factor analysis (PARAFAC) · Land use

## Introduction

Dissolved organic matter (DOM) plays an important role in surface water biogeochemistry, and a growing body of evidence indicates that anthropogenic activities have had a major impact on stream water DOM (Holbrook et al. 2006; Williams et al. 2010; Yamashita et al. 2011; Lu et al. 2013, 2014; Burrows et al. 2013). Changes in DOM dynamics following disturbances are still largely unknown due to multiple sources of DOM in headwater streams and the general lack of information regarding associated DOM composition. The advent of highly sensitive and high sample throughput optical measurements of DOM, such as UV–Vis and fluorescence, has improved the ability to characterize DOM (Fellman et al. 2010; Jaffé et al. 2014).

Using these methods, Williams et al. (2010) demonstrated effects of watershed land use on DOM composition and bacterial activity in streams; specifically, watershed disturbances increased the relative abundance of microbial

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derived DOM, while streams draining relatively undisturbed forest and wetland areas were more enriched in humic-like DOM. Data from the Coweeta Hydrologic Laboratory Long Term Ecological Research (LTER) site in North Carolina suggest that long-term effects of forest management on DOM composition in managed versus undisturbed reference watersheds exist, based on differences in DOM optical properties and molecular weight distributions (Yamashita et al. 2011). Specifically, reduced contributions of aromatic DOM were identified in watersheds that had been harvested compared to reference watersheds. Similar results were reported for other forest streams (Burrows et al. 2013), and when comparing DOM in streams from forested landscapes with that from cropland, pasture, and urban areas (Lu et al. 2014). These authors also suggested that DOC from forested environments was more photo-reactive compared to that from landscapes affected by human activities (Lu et al. 2013) implying that the reactivity and thus fate and transport of DOC exported from anthropogenically modified landscapes could be different from pristine systems.

In addition to the effects of changes in land use and forest management on stream DOM, acidic deposition may also influence DOM concentration and composition. Although  $\text{SO}_2$  and  $\text{NO}_x$  emissions regulations have been strengthened, the recovery of acidified ecosystems in the northeastern US has been somewhat muted due to the depletion of exchangeable calcium from soils (Driscoll et al. 1989, 2001). A watershed-scale calcium silicate ( $\text{CaSiO}_3$ ) addition at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, has enhanced forest health (Battles et al. 2014) and the acid neutralizing capacity of streams (Cho et al. 2012). It has been shown that an increase in the pH of base-poor soils increases the solubility of DOM, and declines in the ionic strength of soil solution can affect DOC coagulation and export (Krug and Frink 1983; Monteith et al. 2007). Thus, the addition of calcium may alter DOM concentration and composition in acid-impacted watersheds.

Although the reasons for the quantitative and qualitative differences in stream water DOM remain largely speculative, the fact that long-term disturbance effects on DOM composition have been reported is an indication of the importance of such studies to better understand carbon cycling, retention and loss in forest ecosystems. In this study, we used the unique, experimentally manipulated and undisturbed reference research watersheds at the HBEF to evaluate how watershed characteristics and the long-term effects of watershed scale landscape changes affect stream water DOM quantity and composition. We used DOM optical properties (EEM-PARAFAC; see Stedmon and Bro 2008) and size exclusion chromatography (SEC) to determine if there were differences between watersheds that had

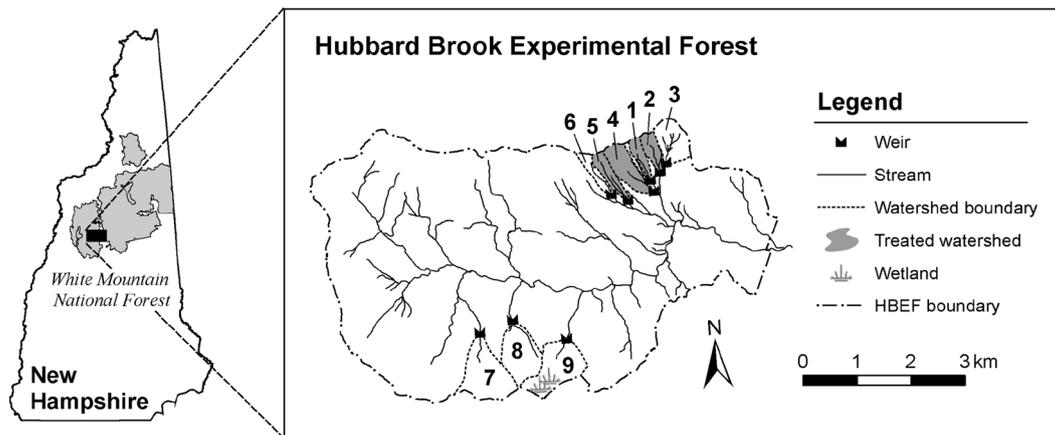
been experimentally manipulated by harvesting or by the addition of calcium, compared to undisturbed reference watersheds of similar elevation and tree species composition. In addition, we used undisturbed reference watersheds to evaluate landscape influences due to tree species composition and the presence of wetlands. A leaching study of litter, vegetation, moss, and soil was used to help identify sources of DOM. Overall, the goal of this study was to better understand how natural watershed characteristics and forest disturbances affect the quantity and composition of DOM in headwater streams.

## Methods

### Site description

The Hubbard Brook Experimental Forest (43°56'N, 71°45'W) is a 3,160-ha bowl-shaped valley ranging in elevation from 222 to 1,015 m (Fig. 1). The climate is cool, humid and continental with an average monthly low air temperature of  $-9^\circ\text{C}$  in January to a high of  $18^\circ\text{C}$  in July. Average annual precipitation is 1,400 mm and is distributed fairly evenly throughout the year. A snowpack usually persists from late December until mid-April, with a peak depth in March. Vegetation is predominantly northern hardwood, comprised mostly of American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Coniferous species, primarily red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) occur at higher elevations and on steeper slopes. Spodosols are the dominant soil type: Typic Haplorthods derived from glacial basal till. The soils have a sandy loamy texture and are well drained. Bedrock depth is highly variable, but is generally 1–2 m.

Within the HBEF Valley, there are nine gaged watersheds (Fig. 1; Table 1); six clustered on a south-facing slope (W1–W6) and three located 4 km away on a north-facing slope (W7–W9). Four whole-watershed experiments have occurred at the HBEF, and the other five watersheds remain undisturbed, including two designated reference watersheds (W3 and W6). The whole-watershed experiments consist of three cutting treatments and one calcium addition. The first manipulation was a cutting experiment in W2 that occurred from 1965–1968. The major objective was to eliminate transpiration to evaluate its influence on streamflow and nutrient cycling. All woody vegetation on the watershed was cut and herbicides were applied during the following three growing seasons to suppress regrowth and kill herbaceous vegetation. The second watershed experiment was a progressive strip cut in W4 to determine the effect of this more moderate cutting technique on forest regeneration, stream water yield, and nutrient export. The



**Fig. 1** Map of the study watersheds at the Hubbard Brook Experimental Forest in the White Mountain National Forest, New Hampshire

**Table 1** Characteristics of the study watersheds

Watershed	Area (ha)	Elevation (m)	Aspect	Deciduous/Conifer (%) <sup>a</sup>	Treatment year	Treatment description
W1	11.8	488–747	S22°E	92/8	1999	Calcium addition
W2	15.6	503–716	S31°E	92/8	1965–1968	Devegetated/herbicide
W3	42.4	527–732	S23°W	81/19	–	Reference
W4	36.1	442–747	S40°E	98/2	1970–1974	Progressive strip cut
W5	21.9	488–762	S24°E	98/2	1983–1984	Whole tree harvest
W6	13.2	549–792	S32°E	90/10	–	Reference
W7	77.4	619–899	N16°W	27/73	–	Reference
W8	59.4	610–905	N12°W	10/90	–	Reference
W9	68.4	685–910	N13°E	4/96	–	Reference (contains a wetland)

<sup>a</sup> Calculated using the National Land Cover Database (Fry et al. 2011)

watershed was divided into 25-m strips, and every third strip was cut at 2 year intervals (1970, 1972, and 1974) until the end of the treatment, at which point all merchantable wood had been removed. The third cutting experiment was a commercial whole-tree harvest on W5 during the winter of 1983–1984. Whole-tree harvesting is more intensive than conventional stem-only cuts, because most of the aboveground biomass (boles and branches) is removed. This experiment was designed to address concerns about nutrient losses associated with this management practice, and impacts on site productivity. The most recent whole-watershed experiment was a calcium addition to W1. The intent of this treatment was to replace calcium lost from soil exchange sites due to decades of exposure to acidic deposition. Calcium silicate, in the form of pelletized wollastonite ( $\text{CaSiO}_3$ ), was applied to the entire watershed by helicopter in October 1999.

Of the undisturbed watersheds, the two reference watersheds located on the low elevation, south-facing slope (i.e., W3 and W6) are dominated by northern hardwood

species (81–90 % of the cover), with some conifers occurring mostly at higher elevations within these watersheds. The undisturbed watersheds that are on the higher elevation, north-facing slope (i.e., W7, W8, and W9) are dominated by conifer species (73–96 % of the cover). In addition to the influence of conifers, W9 also has a wetland, and although it is small (0.8 ha or 1.2 % of the watershed area), it is located in the headwaters of the stream and is hydrologically connected. The forest throughout the area of the undisturbed gaged watersheds at the HBEF is largely second growth. The Hubbard Brook Valley was logged intensively between 1909 and 1917, with some additional salvage logging in the wake of the 1938 hurricane that affected much of the northeastern U.S. Some other notable natural disturbances (e.g., insect defoliations, ice storms, soil frost) have occurred since the time of the original harvest, but these have had less of an impact on stream flow and biogeochemistry than the harvesting experiments (Campbell et al. 2013; Likens and Buso 2010).

## Sample collection

From May 2010 to April 2011, stream water samples were collected for DOM analyses monthly at the outlet of each of the nine gaged watersheds (Fig. 1). At the same time samples were collected for DOM analyses, samples were also collected from W7 for analysis of dissolved silica used to partition baseflow and event flow. Stream flow is measured continuously with either a V-notch weir or a V-notch weir in tandem with a San Dimas flume (Bailey et al. 2003), which enabled evaluation of the flow conditions when the samples were collected.

Immediately after collection, samples for DOM analyses were passed through pre-combusted (450 °C for 4 h) glass-fiber filters (0.7 µm nominal pore size) into opaque high density polyethylene bottles. Prior to use, the bottles and filter holders were cleaned by soaking them in 0.5 M HCl followed by 0.1 M NaOH for 24 h each, and then rinsing with distilled, deionized water. Samples for DOM analyses were shipped on ice by overnight courier to the laboratory at Florida International University on the same day they were collected, and then refrigerated at 4 °C until analysis. Samples for dissolved silica analysis were sent to the Forest Service Laboratory in Durham, New Hampshire. Optical properties of DOM were analyzed within 14 days of collection and DOC and Si was analyzed within 30 days. Samples collected during September 2010 were inadvertently frozen and their results are not presented, as freeze/thaw has been shown to alter optical properties (Spencer et al. 2007).

Samples of leaf litter (Oi horizon), live vegetation, in-stream moss, and organic soil (Oa + Oe horizon) were collected on September 23, 2011 for use in a leaching study: mixed deciduous leaf litter from W2, mixed deciduous leaf litter from W3, conifer-dominated litter from W9, mixed deciduous tree leaves from W8, conifer tree needles (mixed red spruce and balsam fir) from W9, in-stream moss from W2, and organic soil from W2.

## Laboratory analyses

Dissolved organic carbon (DOC) concentrations were measured using a high temperature combustion method on a Shimadzu TOC-5000 equipped with a platinum catalyst and an infrared detector. Samples were acidified to pH <2 and sparged with carbon-free compressed air to remove inorganic carbon prior to analysis. Dissolved silica concentrations were measured on an Agilent Technologies 700 Series Inductively Coupled Plasma—Optical Emission Spectrometer.

Ultraviolet–visible absorption of dissolved organic matter was measured on a Varian spectrophotometer using a quartz cuvette with a 1.0 cm path length. Specific

ultraviolet absorbance (SUVA<sub>254</sub>) values were determined by dividing the ultraviolet absorbance measured at a wavelength of 254 nm by the DOC concentration and are reported in the standard units L mg C<sup>-1</sup> m<sup>-1</sup>. SUVA<sub>254</sub> is positively correlated with aromaticity and molecular weight, therefore providing insight into the composition of DOM (Chin et al. 1994; Weishaar et al. 2003). Both inorganic iron (Fe<sup>2+</sup> and Fe<sup>3+</sup>) and NO<sub>3</sub><sup>-</sup> can absorb light in the near UV, causing possible interference with the UV absorbance of DOC. We did not measure Fe in this study; however, Fuss et al. (2011) reported dissolved Fe concentrations for the outlet of W6 at the HBEF that were less than 0.05 mg Fe L<sup>-1</sup>, which would cause negligible interference (Weishaar et al. 2003). Similarly, NO<sub>3</sub><sup>-</sup> concentrations in streams at the HBEF are far less than concentrations that would cause interference (>9.0 mg N L<sup>-1</sup>; Weishaar et al. 2003; Likens and Buso 2010).

The molecular size distribution was determined by size exclusion chromatography (SEC) on a Thermo Scientific HPLC system equipped with a photodiode array (PDA Plus detector) using a method similar to Maie et al. (2004). Briefly, 100 µL of filtered water was injected onto a YMC-Pack Diol-120 column. The mobile phase, flow rate 0.7 mL min<sup>-1</sup>, was comprised of a 0.05 M tris (hydroxymethyl) aminomethane (THAM) buffer adjusted to a pH of 7 with concentrated phosphoric acid. The elution curve was recorded by measuring the absorbance of the eluate at 280 nm. Molecular weight and polydispersity were calculated according to Maie et al. (2004) from the sample chromatograms and standards of polystyrene (MW = 1,400, 4,300, 6,800, 13,000, 36,000), blue dextran, and glycine.

Excitation-emission matrices (EEMs) were obtained with a Horiba Jobin–Yvon SPEX Fluoromax-3 spectrofluorometer. EEMs were produced over an excitation wavelength range of 240–450 nm with an increment of 5 nm and an emission range of 300–550 nm with an increment of 2 nm in a 1 cm quartz cuvette. The excitation and emission slit widths were set to 5 nm. Fluorescence scans were collected in signal/reference ratio mode with an integration time of 0.25 s. EEMs were corrected for instrument optics and inner-filter effects according to Ohno (2002) and Raman normalized and blank subtracted using Matlab v2009a software.

Leaching study samples (i.e., leaves, moss, organic-rich soil) were dried in an oven at 40 °C for 24 h. A 1 g sample of each material was added to 1 L of filtered (GF/F 0.7 µm) W2 water that had been extracted using a solid phase PPL cartridge (Varian, Bond Elut) to remove most of the DOM; pH was adjusted to 6. Bottles were covered in foil to block light and leached for 24 h on a shaker table at approximately 25 °C. Samples were filtered through a GF/F filter

prior to analysis with the same methods as surface water field samples.

Data analysis

The Fluorescence Index (FI), which is indicative of DOM source (McKnight et al. 2001), was calculated as the ratio of the fluorescence emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm (Jaffé et al. 2008). A higher FI value, e.g., 1.8, indicates a microbial DOM source while a lower value, e.g., 1.2, indicates a terrestrial, humic-rich source; intermediate values indicate a mixed DOM source. A parallel factor analysis (PARAFAC) model was built with 99 EEMs from the HBEF using the N-way toolbox for Matlab (Andersson and Bro 2000; Cory and McKnight 2005; Stedmon and Bro 2008). Model components were split-half validated (Fig. 2; Murphy et al.

2013). Model fit was considered suitable if the signal in the residual EEM (modeled EEM subtracted from measured EEM) was less than 10 % of the measured EEM. PARAFAC component characteristics are described in Table 2.

Statistical analyses, including principal component analysis (PCA) and analysis of variance (ANOVA) with a Tukey’s post hoc test were performed using SPSS statistical software. The relative abundance of the PARAFAC components were used as the PCA variable inputs. The PCA analysis was run with no rotation and Eigenvalues greater than 1 were retained. A significance level of 0.05 was used for the ANOVA.

Dissolved silica was used as a conservative tracer to further evaluate flow conditions by separating the stream hydrograph into its baseflow and event water components following the method of Hooper and Shoemaker (1986). Baseflow represents the steady contribution to stream flow

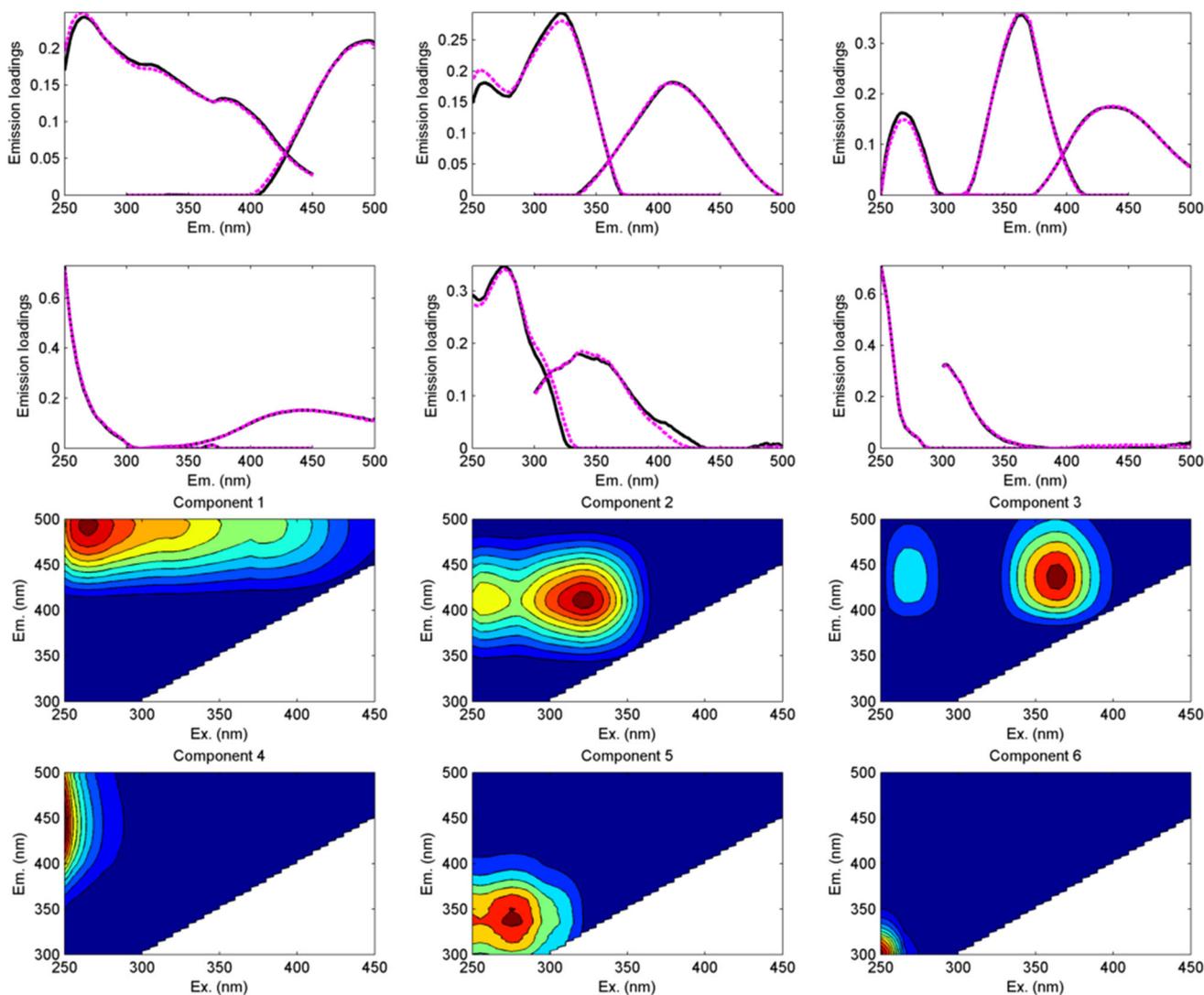


Fig. 2 Results of split-half validation of the six component PARAFAC model and 3-D contour plots of final PARAFAC model components

**Table 2** Characteristics of the six PARAFAC components identified in this study

Component	Excitation maximum (nm)	Emission maximum (nm)	Cory and McKnight (2005)	Yamashita et al. (2011)	Assignment
C1	265	494	SQ1	C3	Humic-like
C2	320 (255)	410	–	C1	Humic-like, fulvic acid-type
C3	365 (270)	436	C1 or SQ2	C2	Humic-like
C4	<250	446	Q2	–	Humic-like, oxidized quinone-like
C5	275	336	Trp	C5	Protein-like or polyphenolic
C6	<250	302	–	–	Protein-like, tyrosine-like or polyphenolic

Similar components from other studies are shown for comparison

from a groundwater source, whereas event flow represents the quick flow fraction that comes from surface runoff or interflow. These hydrologic flowpaths have been shown to influence both the quantity and composition of DOM (e.g., Inamdar et al. 2011). We used the dissolved silica concentration in precipitation to represent event or new water. The concentration of dissolved silica in precipitation is consistently below detection at the HBES; therefore a value of 0 mg L<sup>-1</sup> was assigned. The concentration of dissolved silica during low flow was used as the old water end member since it represents a groundwater source.

## Results

### PARAFAC model components

A HBEF PARAFAC model with six components was validated. On the basis of spectral characteristics, components 1, 2, 3 and 4 (C1, C2, C3 and C4) were classified as humic-like, component 5 (C5) was classified as protein-like or polyphenolic, and component 6 (C6) showed characteristics of highly blue-shifted protein-like or polyphenolic compounds (Fig. 2). Humic-like C1 and C3 are spectrally similar to C3 and C2 of a PARAFAC model validated by Yamashita et al. (2011) in a study of the effects of clear-cutting at the Coweeta LTER (Table 2). Yamashita et al. (2011) reported enrichment in the humic-like components relative to a fulvic acid-like component in the reference watersheds compared to watersheds with a clearcut forest. The highly red-shifted nature of C1 likely indicates that it is more highly conjugated, which is a characteristic of terrestrial-derived DOM (Coble 1996), while C3 is likely a ubiquitous humic-like component. Another humic-like component, C2, validated in the HBEF model is spectrally similar to a fulvic acid-like component, C1, described in Yamashita et al. (2011; Table 2). The relative blue-shift in the C2 compared to the other humic-like components in the model may be an indication that it is less conjugated and possibly derived from microbial activity (Coble 1996). The fourth humic-like component, C4, did not share any spectral

characteristics of the components validated by Yamashita et al. (2011). However, it was spectrally similar to a ubiquitous oxidized quinone-like component, Q2, in the Cory and McKnight (2005) geographically global model. The protein-like or polyphenolic component, C5, is spectrally similar to Trp from the Cory and McKnight (2005) and C5 in the Yamashita et al. (2011) PARAFAC models.

Unlike the other components in the HBEF model, C6 had a unique, highly blue-shifted, excitation/emission spectrum and was not spectrally similar to any components found in either the Yamashita et al. (2011) study of the Coweeta LTER or the geographically broad Cory and McKnight (2005) PARAFAC models. There is some evidence that highly blue-shifted fluorescence could be a result of polyphenolic-type compounds that have been reported for pine needle leachate (Beggs and Summers 2011) and mangrove tannins (Maie et al. 2007). Results from the leaching study of litter, vegetation, moss, and soil samples from the HBEF watersheds support the idea that freshly leached vegetation is a likely source of C6 (Table 3). C6, hypothesized to be protein-like or polyphenolic based on spectral properties and was only found in substantial amounts for two sample types evaluated in the leaching experiments: fresh conifer tree needles and fresh deciduous tree leaves (Table 3).

### Reference and treated watersheds (W1–W6 only)

DOC concentration was the most sensitive non-PARAFAC metric for differences between the reference and two treatment types (Fig. 3a). DOC concentration was significantly higher in the reference watersheds, W3 and W6, compared to the three clearcut watersheds, W2, W4, and W5 ( $p = 0.025$ ). The watershed treated with CaSiO<sub>2</sub> had average DOC concentrations that were significantly higher than the reference ( $p = 0.024$ ) or clearcut sites ( $p < 0.001$ ). DOM composition indicators, however, showed little difference between reference and treated watersheds. Molecular weight, as determined by SEC, did not show any significant differences between treated and reference watersheds (Fig. 3b), nor did the slope ratio (Helms et al.

2008), an absorbance-based molecular weight proxy (data not shown). Similarly, no significant differences in SUVA<sub>254</sub> (Fig. 3c), a measure of DOM aromaticity (Weishaar et al. 2003), or FI values were observed (Fig. 3d).

Effects of landscape composition (comparing all watersheds)

There were no significant differences in the average molecular weight among the five watershed type groupings (Fig. 3b). However, the stream in W9 had a significantly

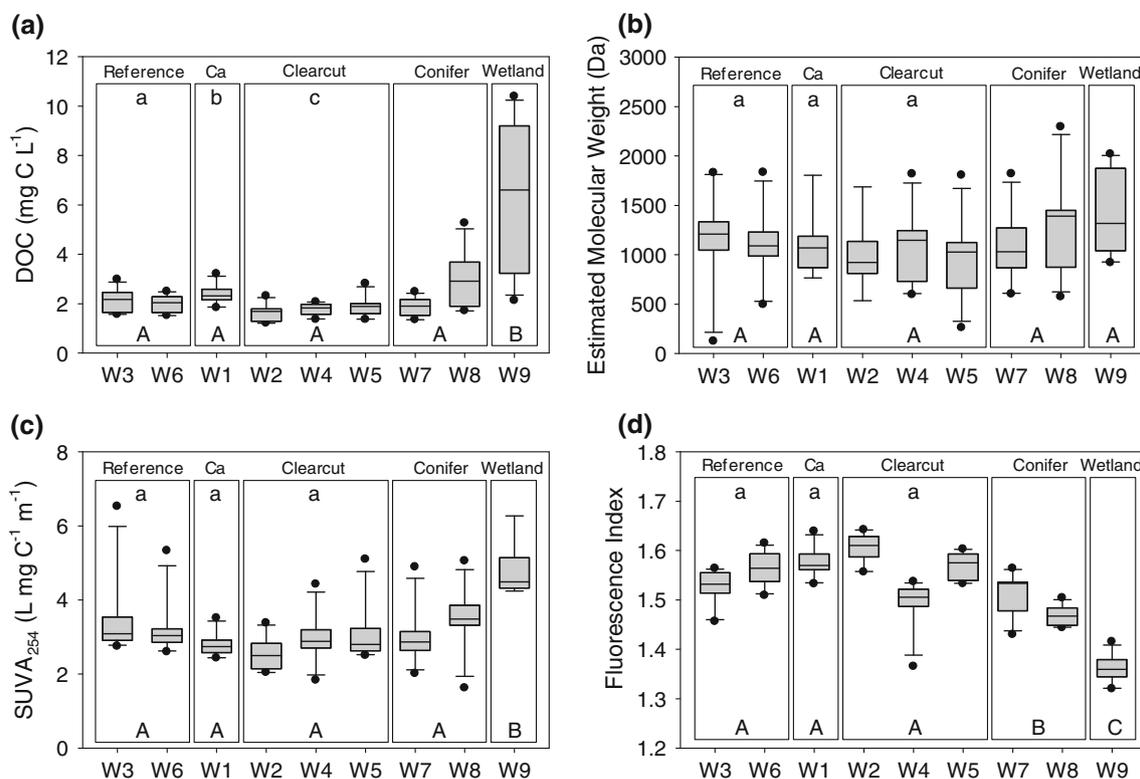
**Table 3** The percentage of component six associated with each type of material evaluated in the leaching experiment

Leached material	% C6
Mixed deciduous leaf litter from W2	1
Mixed deciduous leaf litter from W3	1
Conifer dominated litter from W9	1
Mixed deciduous tree leaves from W8	18
Conifer tree needles from W9	27
In-stream moss from W2	0
Organic soil from W2	0

higher SUVA<sub>254</sub> value compared to the other watersheds, indicating higher aromaticity for this DOM and/or the presence of higher concentrations of Fe in the samples due to increased Fe solubility under anoxic conditions in the wetland sediments (Fig. 3c; Weishaar et al. 2003). FI values also showed some significant differences among watersheds (Fig. 3d). The FI values were significantly lower in the two conifer dominated watersheds, W7 and W8, compared to the five lower elevation hardwood dominated watersheds ( $p < 0.001$ ). The conifer-dominated watershed that contains a wetland, W9, had an FI value that was significantly lower than any of the other watershed types, indicating higher inputs of more terrestrial DOM. DOC concentration was also significantly higher for the stream within W9 compared to the other watersheds (Fig. 3a) ( $p < 0.01$ ).

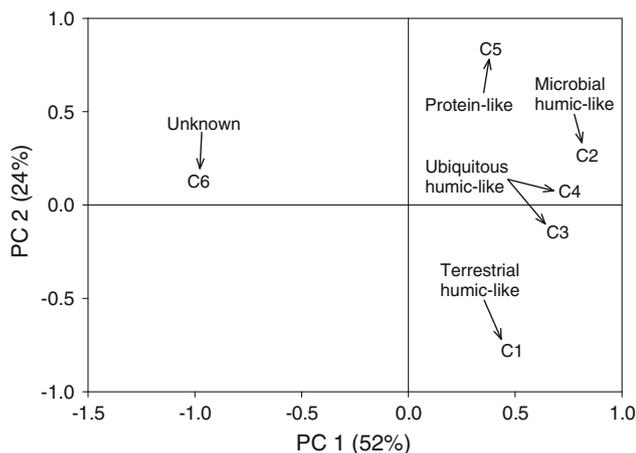
PCA results

The PCA loadings plot shows that along PC1 the PARAFAC components are distributed such that C6, the component associated with freshly leached plant material, is the only component on the negative axis, and the



**Fig. 3** Box plots of DOM characterization parameters for the watersheds at the HBEF grouped as reference (W3 and W6), Ca addition (W1), clearcut (W2, W4, and W5), conifer-dominated (W7 and W8), and conifer-dominated with a wetland in the headwaters (W9). Capital letters indicate significant differences among all

groupings determined with the ANOVA and lowercase letters indicate significant differences among the low elevation, hardwood-dominated reference and treated (i.e., Ca addition and clearcut) watersheds only



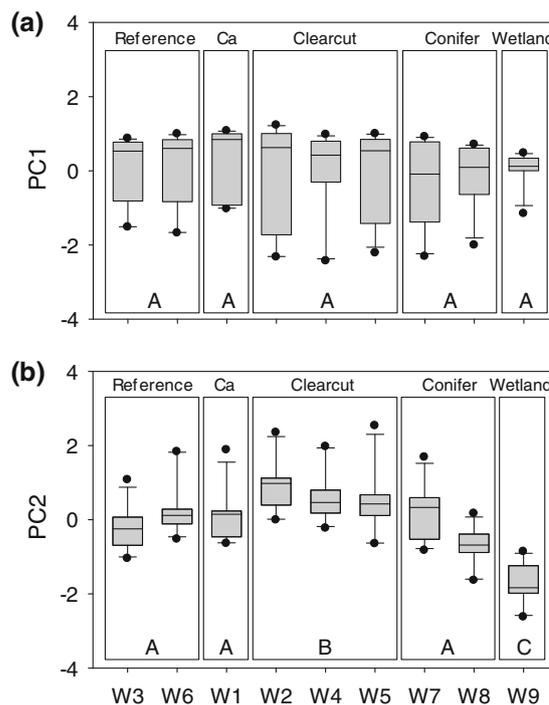
**Fig. 4** PCA loadings plot and identification of PARAFAC components

humic-like and protein-like components are all positive (Fig. 4). Along PC2 the loading for C1, a terrestrial humic-like component, is the most negative. C3 and C4, two ubiquitous humic-like components, are centered near a loading of 0 (Fig. 4). C2, the microbial humic-like component, is the most positive humic-like component and C5, the protein-like or polyphenolic component, has the most positive PC2 loading.

The PCA scores for the surface water samples show no statistical difference in PC1 across the five different types of watersheds (Fig. 5a). However, PC2 values were significantly higher for the three clearcut watersheds compared to the reference watersheds and the calcium treated watershed (Fig. 5b). The hardwood and conifer reference watersheds were not statistically different from each other or from the calcium treated watershed (Fig. 5b). W9, which has the highest composition of conifers and contains a wetland, had significantly lower PC2 values than any of the other watersheds, which is characteristic of aromatic DOM (Fig. 5b).

PC1 values showed some evidence of seasonal patterns; however the sampling period was relatively short for properly evaluating seasonal trends. Nevertheless, the highest PC1 values occurred during spring to early summer (March–July), indicating low C6 inputs during this time (Fig. 6a). In August, October, and December, PC1 values were the lowest, while for November, January, and early March significantly different intermediate PC1 values were observed. PC2 showed much less seasonal variability (Fig. 6b), with only July showing a significantly greater PC2 value compared to other months, suggesting greater microbial DOC contributions during this time.

The streamflow volume at the time the samples were collected had little effect on either PC1 or PC2 (Fig. 6c). While most samples were collected during baseflow

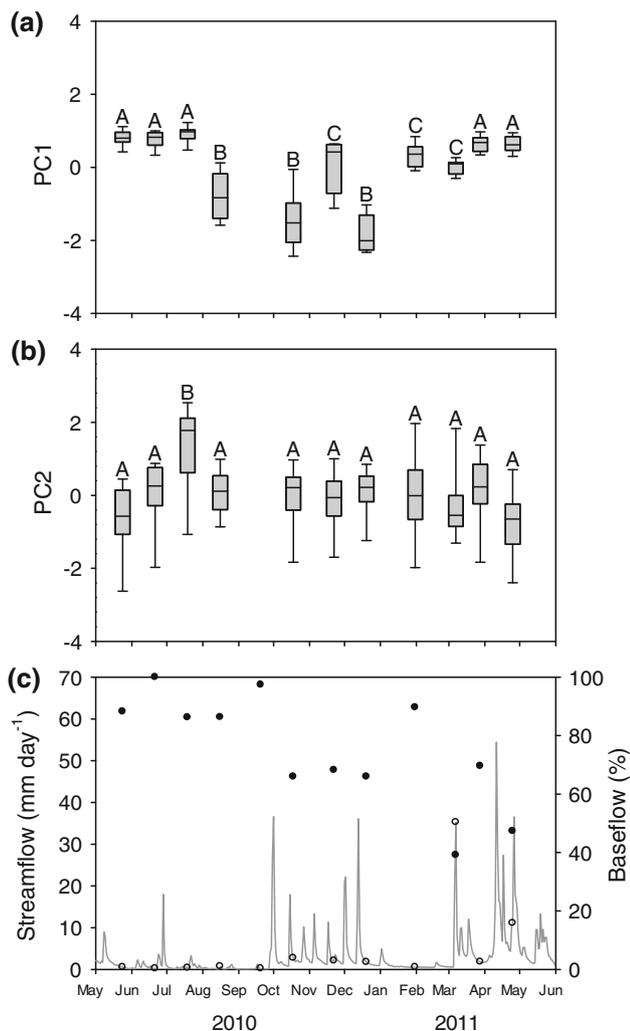


**Fig. 5** Boxplots of PCA scores for PC1 (a) and PC2 (b) for the watersheds at the HBEF grouped as reference (W3 and W6), Ca addition (W1), clearcut (W2, W4, and W5), conifer-dominated (W7 and W8), and conifer-dominated with a wetland in the headwaters (W9). Capital letters indicate significant differences among all groupings determined with the ANOVA

conditions, two events were captured (7 March and 25 April 2011), during which time more than half of the stream flow was comprised of quickflow. Even though the hydrograph separation analysis indicated that water moved laterally through the uppermost, organic soils during these events, these changes in flowpaths did not appear to influence the PCA scores.

## Discussion

The PCA data (Figs. 5, 6) provide some insights into the ecological drivers controlling DOM composition at the HBEF. While PC1 did not show any significant differences among watershed groupings, there were some seasonal differences. PC1 is largely controlled by the relative abundance of C6, which the leaching study showed is a component derived from fresh leaf and needle material. C6 appeared to have a seasonal signal, although the factors contributing to this signal are unclear. Since C6 is associated with fresh litter, throughfall would be expected to affect its relative contribution. However, the C6 signal was not evident during some of the months with leaves on (i.e., May, June, July), and there was no correlation between C6



**Fig. 6** Boxplots of PCA scores for PC1 (a) and PC2 (b) over time for all nine watersheds evaluated at the HBEF. Capital letters indicate significant differences over time. The lower panel (c) shows median streamflow (mm) for all nine watersheds during the sampling period. Hollow circles indicate the streamflow when samples were collected and the solid circles indicate the % baseflow (secondary y-axis) as calculated with hydrograph separation using Si

and total rainfall or recent rainfall amount (data source: <http://www.hubbardbrook.org/data/dataset.php?id=14>). The leaching study was done with excised leaf material so it is also possible that C6 could be associated with leaf senescence during autumn; however, the signal was evident longer than the period of observable senescence (Richardson et al. 2006) and also occurred in watersheds dominated by coniferous species, which do not have large litterfall inputs during this period. Changes in flowpaths from deeper groundwater sources, to shallow surface soils that contain fresh litter could also influence the abundance of C6. However, the dissolved silica mixing model analysis showed no apparent increase in C6 during hydrologic events, despite a greater portion of shallow flow during these periods.

Some evidence suggests that soil freeze–thaw cycles may influence the composition of DOM released during leaching, which could potentially explain the late season spike in C6 abundance observed in December. Within a week before the December sample collection, air temperatures dropped to nearly  $-15\text{ }^{\circ}\text{C}$  with less than 5 cm of snow covering the soil, resulting in a major soil freeze–thaw event. The effect of soil freeze–thaw events on DOC has been evaluated by Kiikkila et al. (2012) in a DOC decomposition experiment using Norway spruce and silver birch. They demonstrated that freeze–thaw cycles can enhance and reactivate litter leaching. These authors reported a two stage leaching pattern, where the initial, large production of leachate decreased over time, but was reactivated to higher fluxes after the freeze–thaw cycle was completed. This observation applied particularly to phenolic compounds, which would be consistent with the spectral characteristics of C6. Despite the numerous possible explanations for the seasonal pattern of the C6 component, factors controlling the pattern remain uncertain and warrant further investigation.

In contrast to PC1, PC2 showed differences among watershed groupings, with higher values in clearcut watersheds compared to reference watersheds. Differences in PC2 values among watersheds appear to be related to DOM source, with PC2 values increasing along a gradient from terrestrial, humic-rich to microbial PARAFAC components. The samples from clearcut watersheds showed significantly higher PC2 values, indicating that their fluorescence signature is enriched in microbial sources compared to the reference watersheds. These results are consistent with Yamashita et al. (2011), who reported lower humic-like fluorescence contributions from clearcut forests, even after long periods of time, compared to reference watersheds at similar elevation. While seasonal trends are not obvious, the significant enrichment in microbial DOM in July may be due to a seasonal maximum in microbial primary production during midsummer and reflects the general mixed microbial and terrestrial source of DOM in these watersheds throughout most of the year (see FI value ranges; Fig. 3d).

The calcium treated watershed (W1) had a significantly higher DOC concentration compared to the other low elevation watersheds, but surprisingly, the DOM composition was not significantly different. The higher DOC concentration in the calcium treated watershed may be explained by the higher soil pH, which enhances the solubility of soil organic matter, consequently enhancing stream water DOC concentrations (SanClements et al. 2012).

Stream water DOM concentrations and characteristics in W9 were markedly different than the other watersheds, reflecting humic-like contributions from the wetland that have been demonstrated in other studies (e.g., Williams

et al. 2010). The concentration of DOC was significantly higher at the W9 site (Fig. 3a). DOM at this location had a low FI value and a low PC2 score (Figs. 3d, 5b), consistent with a DOM source that is predominantly derived from the oxidation of terrestrial higher plant and soil organic matter.

With regards to the effect of vegetation cover, the observable differences between the conifer-dominated and deciduous-dominated watersheds were small (Figs. 3, 5). Excluding W9, where the influence of the wetland skews the comparisons, there were no significant differences between the two deciduous-dominated (W3 and W6) sites and the two conifer-dominated (W7 and W8) sites regarding DOC, SEC MW, SUVA<sub>254</sub>, PC1 or PC2; and only the FI values were observed to be lower in the conifer sites compared to the deciduous sites. Kaiser et al. (2001) reported similar trends in DOM composition between pine litter- and beech litter-derived DOM, with only some small differences in the DOM's hydrophobic to hydrophilic ratios and DOC concentration. Most of the samples in our study were collected during baseflow, and the effect of tree species on DOM might be greater during stormflow due to the influence of vegetation on DOM composition in throughfall and shallow surface flow. Nevertheless, the lack of effect of tree species composition is a surprising finding that merits further investigation, particularly with regards to potential differences in photo- and bio-reactivity of DOC sources in coniferous and deciduous forested systems.

## Conclusions

While overall landscape characteristics and associated ecological drivers seem to overshadow the effect of watershed treatment, when direct comparisons between experimentally manipulated and reference watersheds at similar elevation and land cover are performed, some long-term legacy effects were observed. This study indicates that the effects of landscape modification through clearcutting or calcium application may impact DOM quantity and composition, as reflected by DOC concentrations and some metrics of optical properties. Based on our results, EEM-PARAFAC analysis shows the most promise for evaluating these differences and appears to be more sensitive than other measurements such as molecular weight distribution, SUVA<sub>254</sub>, and FI.

Results from this study indicate that the addition of calcium, which is sometimes used to increase the pH of ecosystems to aid in recovery from acidic deposition, increased the overall concentration of DOC in forest streams draining the watershed. In general terms, the

clearcut forested watersheds had lower DOC values and enhanced relative abundances of microbially-derived humic-like and protein-like fluorescence. Since DOM with such composition has been reported to be less reactive, in particular with regard to its response to light exposure (Lu et al. 2013), forest harvesting could lead to a reduction in DOC export, and may also affect DOM composition and associated reactivity. Forest harvesting reduces the organic soil mass and alters the structure of the forest floor, which in turn reduces the concentration and fraction of humic-like DOC export. In addition, the open canopy following clear-cutting could enhance photo-degradation processes leading to the preferential destruction of more aromatic, terrestrially-derived DOM compared to less chromophoric, microbial DOM. As such, carbon dynamics may change as forest ecosystems experience human and natural disturbances. To better assess the long-term impacts of disturbance on carbon cycling, a critical aspect to consider for future studies is the linkages among litter quantity, quality, and decomposition to DOM in streams, as our results indicate an important connection.

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## References

- Andersson CA, Bro R (2000) The N-way Toolbox for MATLAB. *Chemom Intell Lab Syst* 52:1–4
- Bailey AS, Hornbeck JW, Campbell JC, Eagar C (2003) Hydrometeorological database for Hubbard Brook Experimental Forest: 1955–2000. Gen. Tech. Rep. NE-305. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station, p 36
- Battles JJ, Fahey TJ, Driscoll CT, Blum JD, Johnson CE (2014) Restoring soil calcium reverses forest decline. *Environ Sci Technol Lett* 1:15–19
- Beggs KMH, Summers RS (2011) Character and chlorine reactivity of dissolved organic matter from a mountain pine beetle impacted watershed. *Environ Sci Technol* 45:5717–5724
- Burrows RM, Fellman JB, Magierowski RH, Barmuta LA (2013) Allochthonous dissolved organic matter controls bacterial carbon production in old-growth and clearfelled headwater streams. *Freshwater Sci* 32:821–836

- Campbell JL, Bailey AS, Eagar C, Green MB, Battles JJ (2013) Vegetation treatments and hydrologic responses at the Hubbard Brook Experimental Forest, New Hampshire. In: Camp AE, Irland LC, Carroll CJW, (eds) Long-term Silvicultural and Ecological Studies: Results for Science and Management (vol 2). Research Paper 013. Yale University, Global Institute of Sustainable Forestry, pp 1–9
- Chin YP, Aiken GR, O'Loughlin E (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ Sci Technol* 28:1853–1858
- Cho Y, Driscoll CT, Johnson CE, Blum JD, Fahey TJ (2012) Watershed-level responses to calcium silicate treatment in a northern hardwood forest. *Ecosystems* 15:416–434
- Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission spectroscopy. *Mar Chem* 51:325–346
- Cory RM, McKnight DM (2005) Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environ Sci Technol* 39:8142–8149
- Driscoll CT, Likens GE, Hedin LO, Eaton JS, Bormann FH (1989) Changes in the chemistry of surface waters: 25-year results at the Hubbard Brook Experimental Forest, NH. *Environ Sci Technol* 23:137–143
- Driscoll CT, Lawrence GB, Bulger AJ, Butler TJ, Cronan CS, Eagar C, Lambert KF, Likens GE, Stoddard JL, Weathers KC (2001) Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *Bioscience* 51:180–198
- Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. *Limnol Oceanogr* 55:2452–2462
- Fry J, Xian G, Jin S, Dewitz J, Homer C, Yang L, Barnes C, Herold N, Wickham J (2011) Completion of the 2006 National Land Cover Database for the conterminous United States. *Photogramm Eng Remote Sens* 77:858–864
- Fuss CB, Driscoll CT, Johnson CE, Petras RJ, Fahey TJ (2011) Dynamics of oxidized and reduced iron in a northern hardwood forest. *Biogeochemistry* 104:103–119
- Helms JR, Stubbins A, Ritchie JD, Minor EC (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnol Oceanogr* 53:955–969
- Holbrook RD, Yen JH, Brizzard TJ (2006) Characterizing natural organic material from Occoquam Watershed (Northern Virginia, US) using fluorescence spectroscopy and PARAFAC. *Sci Total Environ* 361:249–266
- Hooper RP, Shoemaker CA (1986) A comparison of chemical and isotopic hydrograph separation. *Water Resour Res* 22:1444–1454
- Inamdar S, Singh S, Dutta S, Levia D, Mitchell M, Scott D, Bais H, McHale P (2011) Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. *J Geophys Res* 116:G03043. doi:10.1029/2011JG001735
- Jaffé R, McKnight D, Maie N, Cory R, McDowell WH, Campbell JL (2008) Spatial and temporal variations in DOM composition in ecosystems: the importance of long-term monitoring of optical properties. *J Geophys Res* 113:G04032. doi:10.1029/2008JG000683
- Jaffé R, Cawley KM, Yamashita Y (2014) Applications of excitation emission matrix fluorescence with parallel factor analysis (EEM-PARAFAC) in assessing environmental dynamics of natural dissolved organic matter (DOM) in aquatic environments: a review. In: Rosario F (ed) *Advances in the physicochemical characterization of dissolved organic matter: Impact on natural and engineered systems*, vol 1160, pp 27–73
- Kaiser K, Guggenberger G, Haumaier L, Zech W (2001) Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) stands in northeastern Bavaria Germany. *Biogeochemistry* 55:103–143
- Kiikkilä O, Kintunen V, Spetz P, Smolander A (2012) Characterization of dissolved organic matter in decomposing Norway spruce and silver birch litter. *Eur J Soil Sci* 63:476–486
- Krug EC, Frink CR (1983) Acid rain on acid soil: a new perspective. *Science* 221:520–525
- Likens GE, Buso DC (2010) Long-term changes in streamwater chemistry following disturbance in the Hubbard Brook Experimental Forest, USA. *Verh Internat Verein Limnol* 30:1577–1581
- Lu Y, Bauer JE, Canuel EA, Yamashita Y, Chambers RM, Jaffé R (2013) Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. *J Geophys Res-Biogeosci* 118:566–580
- Lu Y, Bauer JE, Canuel EA, Chambers RM, Yamashita Y, Jaffé R, Barrett A (2014) Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams. *Biogeochemistry* 119:275–292
- Maie N, Watanabe A, Kimura M (2004) Chemical characteristics and potential source of fulvic acids leached from the plow layer of paddy soil. *Geoderma* 120:309–323
- Maie N, Scully NM, Pisani O, Jaffé R (2007) Composition of a protein-like fluorophore of dissolved organic matter in coastal wetland and estuarine ecosystems. *Water Res* 41:563–570
- McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr* 46:38–48
- Monteith DT, Stoddard JL, Evans CD, de Wit HA, Forsius M, Hogasen T, Wilander A, Skjelkvale BL, Jeffries DS, Vuorenmaa J, Keller B, Kopacek J, Vesely J (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450:537–540
- Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. *PARAFAC. Anal Methods* 5:6557–6566
- Ohno T (2002) Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environ Sci Technol* 36:742–746
- Richardson AD, Bailey AS, Denny EG, Martin CW, O'Keefe J (2006) Phenology of a northern hardwood forest canopy. *Glob Change Biol* 12:1174–1188
- SanClements MD, Oelsner GP, McKnight DM, Stoddard JL, Nelson SJ (2012) New insights into the source of decadal increases of dissolved organic matter in acid-sensitive lakes of the northeastern United States. *Environ Sci Technol* 46:3212–3219
- Spencer RGM, Bolton L, Baker A (2007) Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Res* 41:2941–2950
- Stedmon CA, Bro R (2008) Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol Oceanogr Methods* 6:572–579
- Weishaar JL, Aiken GR, Bergamaschi BA, Fram MS, Fujii R, Mopper K (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ Sci Technol* 37:4702–4708

- Williams CJ, Yamashita Y, Wilson HF, Jaffé R, Xenopoulos MA (2010) Unraveling the role of land use and microbial activity in shaping chromophoric dissolved organic matter characteristics in stream ecosystems. *Limnol Oceanogr* 55:1159–1171
- Yamashita Y, Kloeppe BD, Knoepp J, Zausen GL, Jaffé R (2011) Long term effects of watershed disturbance on dissolved organic matter characteristics in headwater streams. *Ecosystems* 14:1110–1122