

# Effects of Watershed History on Dissolved Organic Matter Characteristics in Headwater Streams

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## ABSTRACT

Dissolved organic matter (DOM) is recognized as a major component in the global carbon cycle and is an important driver in aquatic ecosystem function. Climate, land use, and forest cover changes all impact stream DOM and alter biogeochemical cycles in terrestrial environments. We determined the temporal variation in DOM quantity and quality in headwater streams at a reference watershed (REF), a watershed clear-cut 30 years ago (CC), and a watershed converted to a white pine plantation 50 years ago (WP) at the US Forest Service, Coweeta Hydrologic Laboratory, in the Nantahala Mountains of western North Carolina, USA. Average stream dissolved organic carbon (DOC) concentrations in CC or WP were 60 and 80% of those in REF, respectively. Stream DOM composition showed that the difference was mainly due to changes in humic-like components in chromophoric DOM. In addition,

excitation–emission matrix fluorescence data with parallel factor analysis indicate that although the concentration of protein-like components did not differ significantly among watersheds, their relative abundance showed an enrichment in CC and WP compared to REF. The ratio of humic acid-type to fulvic acid-type components was highest and lowest at REF and WP, respectively. Our data suggest that forest ecosystem disturbance history affects the DOM quantity and quality in headwater streams over decades as a result of changes in watershed soil organic matter characteristics due to differences in organic matter inputs.

**Key words:** headwater streams; dissolved organic carbon (DOC); chromophoric dissolved organic matter (CDOM); parallel factor analysis (PARAFAC); watershed disturbance; Coweeta Hydrologic Laboratory.

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## INTRODUCTION

A large amount of carbon is processed in inland waterways and this quantity must be considered in global carbon cycle budgets (Cole and others 2007; Battin and others 2009). However, the fluxes and transport mechanisms of inland carbon processes,

including the transport from land to rivers as dissolved organic carbon (DOC), have not been well documented and we need to improve our knowledge of this subject (Battin and others 2009). Recent studies demonstrate that DOC concentration and export from land to inland waters have been affected by changes in a variety of environmental processes including atmospheric acid deposition (for example, Freeman and others 2004; Monteith and others 2007). Dissolved organic matter (DOM) chemical characteristics may also vary with changes in land use and climatic change (Hood and Scott 2008; Wilson and Xenopoulos 2009; Williams and others 2010; Yamashita and others 2010a). DOM reactivity, which is strongly related to its chemical characteristics or quality, is one of the factors controlling the microbial loop dynamics in inland waters (Balcarczyk and others 2009; Fellman and others 2008, 2009; Guillemette and del Giorgio 2011). Thus, we need a better understanding of DOM transport from terrestrial locations to inland waters in terms of quantity and quality as they relate to changes in watershed characteristics such as land use and resource management on regional and global scales.

Studies on stream DOM dynamics have mainly focused on the relationships between DOM characteristics and present day watershed land use (for example, Gergel and others 1999; Frost and others 2006; Wilson and Xenopoulos 2009). These studies demonstrate that DOC concentration is proportional to wetland coverage. Changes in DOM chemical characteristics with agricultural practices have also been well documented (Wilson and Xenopoulos 2009; Yamashita and others 2010a; Williams and others 2010). Modifications in watershed characteristics, that is, vegetation change, may result in transformation of soil organic matter characteristics over decadal time scales (Trumbore 2009). As a result, DOM characteristics in streams may reflect changes in watershed characteristics on much longer time scales. However, the long-term effects of land use and/or landscape change, in other words, effects of past land use and land-use history, on DOM quality and quantity, have not been well documented. These effects must be considered and incorporated into ecosystem modeling efforts designed to predict the environmental impacts of changes in watershed characteristics including anthropogenic influences.

Disturbances to the forest ecosystem such as clear cutting have been reported to induce changes in stream DOC concentrations (Hobbie and Likens 1973; Meyer and Tate 1983; Tate and Meyer 1983), where both increases (Johnson and others 1995)

and decreases (Meyer and Tate 1983) in stream DOC have been observed. In northern hardwood forests, Johnson and others (1995) measured increases in stream DOC for 15 years after forest cutting. Tate and Meyer (1983) sampled streams with a cutting history at Coweeta Hydrologic Laboratory and found they had decreased DOC values over periods of 10–20 years following disturbance. They suggested that differences in DOC concentration among those streams were due to soil type, water flow paths, and forest recovery. However, DOC concentration changes and dynamics after disturbance are still largely unknown due to multiple carbon sources in headwater streams and a paucity of DOM research.

Coweeta Hydrologic Laboratory, where forest management practices have been studied since 1934 (Swank and Crossley Jr 1988), is an ideal location to investigate the relationship between stream DOM dynamics and watershed history. In this study, we measured DOM quality (that is, composition) and quantity in headwater streams from a 30-year-old clear-cut, a 50-year-old white pine (*Pinus strobus*) plantation, and a hardwood reference watershed unmanaged for over 80 years. Optical measurements including excitation–emission matrix (EEM) spectra and parallel factor analysis (PARAFAC) were applied for qualitative characterization of stream DOM. This technique can evaluate the dynamics of at least two very different groups of chromophoric DOM (CDOM) in terms of their ecological role, that is, soil-derived humic-like and biologically reactive protein-like components (for example, Jaffé and others 2008; Yamashita and others 2010a). The main purpose of this study was to determine the effects (30–50 years) of land-use history on DOM quantity and quality. Our primary objective was to examine factors controlling the DOM characteristics in headwater streams with differing vegetative cover and land-use history within the Coweeta basin.

## METHODS

### Study Sites

The Coweeta Hydrologic Laboratory is a USDA Forest Service Experimental Forest located in the southern Appalachian Mountains of western North Carolina, USA. The regional climate is classified as marine humid temperate with moderate mean annual temperature (13°C) and averaging 1,800 mm of annual rainfall evenly distributed across all seasons. The dominant forest type is mixed oak hardwood. Three first- to third-order

streams from different watersheds, south-facing white pine plantation WS1 (WP), south-facing reference WS2 (REF), and 30-year-old clear-cut WS7 (CC), were selected for this study.

The history of the WP watershed included burning the watershed in 1942 while all trees and shrubs adjacent to the stream were deadened with herbicides in 1954. Remaining trees and shrubs were cut and burned in 1956–1957, and white pine seedlings were planted in 1957. Swank and Schreuder (1973) reported that leaf-area index in the pine watershed reached a maximum of 9.9 in 1969. The CC watershed was commercially harvested and cable logged in 1977. Leaf biomass and leaf-area index nearly equaled the pre-cut forest by 1984, that is, 7–8 years after clear-cutting (Elliott and others 2002). By 1997 (20 years after clear-cutting), 40, 52, and 67% of aboveground biomass was recovered for the cove-hardwood, mesic mixed-oak, and dry mixed-oak communities, respectively (Elliott and others 2002). The REF watershed is located between WP and CC and has not been managed since 1927.

In addition to the above three watersheds, samples were also collected in October 2007, December 2007, and April 2008 from undisturbed reference watersheds (WS14, WS18, WS27, WS31, WS32, WS34, WS36), a north-facing white pine plantation (WS17, planted in 1956), a clear-cut watershed in which no material was removed (WS13, clear-cut in 1962), and a watershed converted from deciduous forest to pasture (WS6, during 1958–1967), now progressing through old-field succession, returning to a mixed hardwood deciduous forest. Details of the watershed disturbance and associated management history of these watersheds can be found elsewhere (Swank and Crossley Jr 1988).

## Sample Collection

Triplicate water samples were obtained monthly from July 2007 to June 2008 just upstream of the weir in each watershed. Figure 1 shows the stream flow at REF watershed from July 2007 to June 2008. In general, sampling events were performed during base flow periods; the February 2008 sampling was conducted during a small rain event. Samples for analysis of optical properties were collected in a 140-ml plastic syringe after three consecutive rinses with stream water at each sampling site. Water samples were immediately filtered through pre-combusted GF/F filters with a polypropylene filtrate holder and poured into brown high density polyethylene bottles which had also been rinsed three consecutive times with the filtrate. Filtered samples were kept on

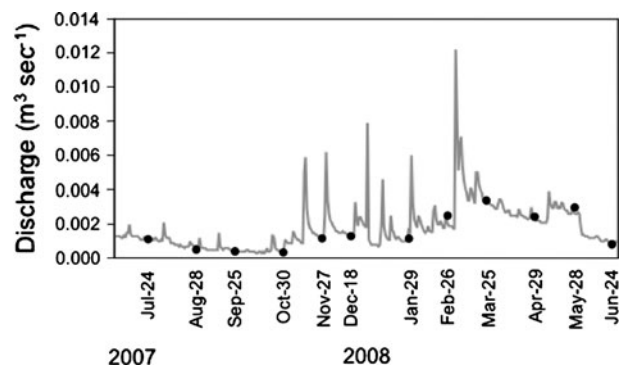


Figure 1. Stream flow ( $\text{m}^3 \text{s}^{-1}$ ) at the reference watershed (REF) from July 2007 to June 2008. Monthly sampling collection times are shown as black circles.

ice, returned to the laboratory, and stored at  $4^\circ\text{C}$  until analysis. Separate streamwater samples were collected for DOC concentration determination in 50 ml muffled glass sample bottles. Analyses of optical properties were conducted within 3–4 days after collection for monthly sampling at REF, CC, WP watersheds, and within 7 days after sampling at other watersheds. Syringes, filter holders, and brown bottles were pre-cleaned by soaking for 24 h each in 0.5 M HCl followed by 0.1 M NaOH and then rinsed with Milli-Q water.

## Analytical Methods

Dissolved organic carbon concentrations were determined following filtration through a  $0.7\text{-}\mu\text{m}$  glass fiber filter on the collection day using a Shimadzu TOC 5000 analyzer. The UV–Vis absorption spectra of each sample were measured from 200 to 800 nm in a 5-cm quartz-windowed cell using a Varian Cary 50 Bio. After this, a blank scan (Milli-Q water) was subtracted from each sample spectrum, and each spectrum was baseline-corrected by subtracting average values ranging between 700 and 800 nm, and then converted to an absorption coefficient,  $a(\lambda)$  ( $\text{m}^{-1}$ ). The absorption coefficient at 350 nm ( $a_{350}$ ) was reported as a quantitative parameter of CDOM. We also calculated the DOC-normalized absorption coefficient ( $a_{350}/\text{DOC}$ ), which has been used to qualitatively assess DOM characteristics in aquatic environments (Yamashita and others 2010b).

Excitation–emission matrix spectra were obtained using a Horiba Jovin Yvon SPEX Fluoromax-3 fluorometer using the methods of Maie and others (2006a) and Santín and others (2009). Inner-filter correction was conducted for EEM using an absorbance spectrum (McKnight and others 2001). Then, EEMs were corrected to the area under the water Raman peak at 350 nm

(measured daily) and converted to quinine sulfate units (QSU). The fluorescence index was estimated as the ratio of the fluorescence emission intensity at 470–520 nm excited at 370 nm (Cory and McKnight 2005; Maie and others 2006a). This index was originally proposed as a proxy for the assessment of sources and the aromaticity of fulvic acids (McKnight and others 2001). Since then, the fluorescence index has been applied to CDOM characterizations in a wide range of terrestrial aquatic environments and has successfully been used to assess DOM sources and quality (for example, Cory and McKnight 2005; Cory and others 2007; Jaffé and others 2008; Yamashita and others 2010a, b).

The approach of PARAFAC modeling to EEMs has been described in detail elsewhere (Stedmon and others 2003; Ohno and Bro 2006). The PARAFAC modeling was conducted in MATLAB (Mathworks, Natick, MA) with the DOMFluor toolbox (Stedmon and Bro 2008). In addition to EEMs collected from the above watersheds, EEMs collected from fourth to fifth order streams (Ball Creek and Shope Fork) at the Coweeta site were used for PARAFAC modeling. Thus, a total of 274 EEMs were used to develop the PARAFAC model. The wavelength ranges used for this purpose were 260–455 and 290–500 nm for excitation and emission, respectively. The validation of the PARAFAC model was conducted according to Stedmon and Bro (2008). Briefly, 274 EEMs were

split into two halves, and the PARAFAC algorithm was applied stepwise to both halves for 3–10 components. For determination of the appropriate number of components, the split half validation was conducted by comparing the excitation and emission loadings of the components between the two halves. After that, PARAFAC algorithms of appropriate components number were applied 10 times to 274 EEMs using random initialization to ensure that the result was not a local minimum. Then, the excitation and emission loadings of the least squared error result were compared with those obtained by split half validation.

The correlations between two parameters were determined by the non-parametric Spearman rank correlations (StatView 5.0; SAS Institute Inc). Principle component analysis using relative abundance (%) of PARAFAC components was conducted using JMP 5.0.1 software (SAS Institute, Inc.).

## RESULTS

### DOC Concentrations and Optical Characteristics in Coweeta Headwater Streams

Dissolved organic carbon concentrations ranged from 0.53 to 1.82, 0.31 to 1.02 and 0.40 to 1.20 mg L<sup>-1</sup> at REF, CC, and WP, respectively (Figure 2A). Most samples were collected during

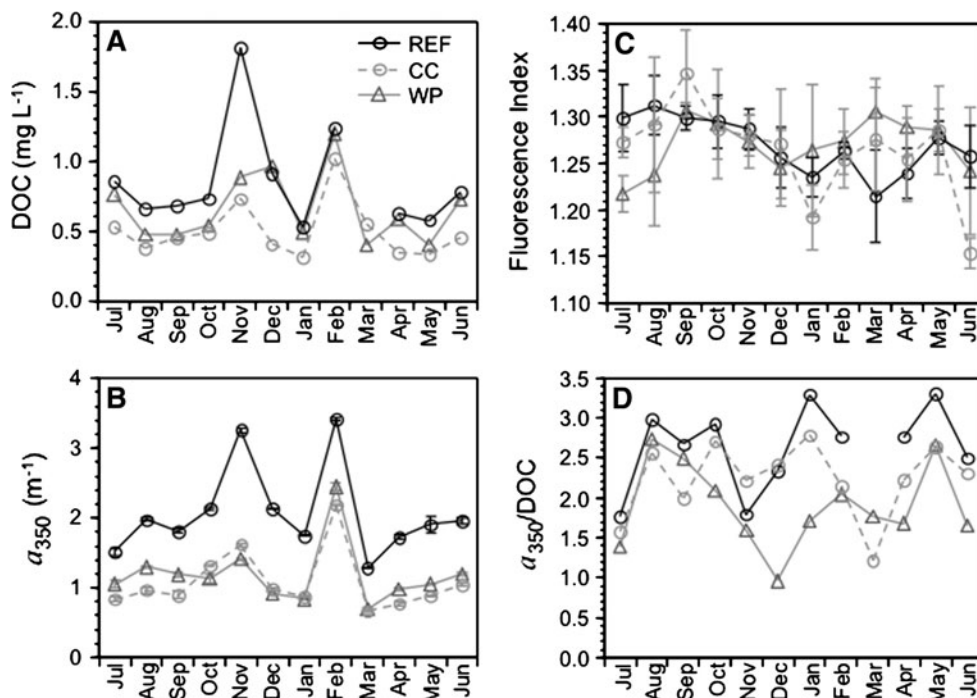


Figure 2. Temporal changes in **A** DOC, **B**  $a_{350}$ , **C** fluorescence index, and **D**  $a_{350}/\text{DOC}$  in headwater streams at the reference (REF), clear-cut (CC), and white pine (WP) watersheds.



base flow periods, except for the February 2008 sampling date, which occurred during a small rain event (Figure 1). DOC concentrations at the CC watershed were lower than those at the REF watershed throughout the year (Figure 2A). Also, DOC concentrations in the WP watershed tended to be less than those in the REF watershed (Figure 2A). The average annual DOC concentrations at the CC and WP watersheds were 60 and 80% of those at the REF watershed, respectively. Excluding February 2008, the DOC values for the samples collected during July to December were generally greater than those from January to May, with maxima at November for REF and CC and at December for WP (Figure 2A).

To quantitatively assess the humic CDOM, the absorption coefficient at 350 nm ( $a_{350}$ ) was determined. We found that  $a_{350}$  ranged from 1.28 to 3.41, 0.67 to 2.19, and 0.70 to 2.45  $\text{m}^{-1}$  at REF, CC, and WP, respectively (Figure 2B). The  $a_{350}$  levels in REF were considerably higher than CC and WP throughout the year (Figure 2B). The annual average  $a_{350}$  at CC and WP were 50 and 60% of the values at the REF watershed, respectively. Temporal variability of  $a_{350}$  was similar to those of DOC (Figure 2A, B). Excluding February 2008, CDOM levels were lowest in March, increased during spring and summer, and peaked in November, and then decreased (Figure 2B). The distinct  $a_{350}$  peak in November occurred for both REF and CC, but not for WP. DOC concentrations were correlated with levels of  $a_{350}$  among the three watersheds ( $R = 0.72$ ,  $P < 0.0001$ ). The  $a_{350}/\text{DOC}$  values, an indicator for the contribution of humic CDOM to bulk DOM, were usually higher at the REF compared to the CC and WP, but did not show temporal trends for any of the three watersheds (Figure 2D).

The fluorescence index, indicative of terrestrial/higher plant versus aquatic/microbial CDOM sources (low vs. high values, respectively), remained in a narrow range from 1.15 to 1.35 for all three headwater streams and did not differ among seasons (Figure 2C).

### PARAFAC Components in Coweeta Headwater Streams

Based on PARAFAC modeling of EEMs from water samples collected throughout Coweeta, we validated a five component model (Figure 3). Table 1 describes the characteristics of the five components. Component 1 (C1) (emission maximum 425 nm with 320 and  $<260$  nm excitations) (Figure 3) corresponds to a humic-like component and could be characterized as a fulvic acid-type component

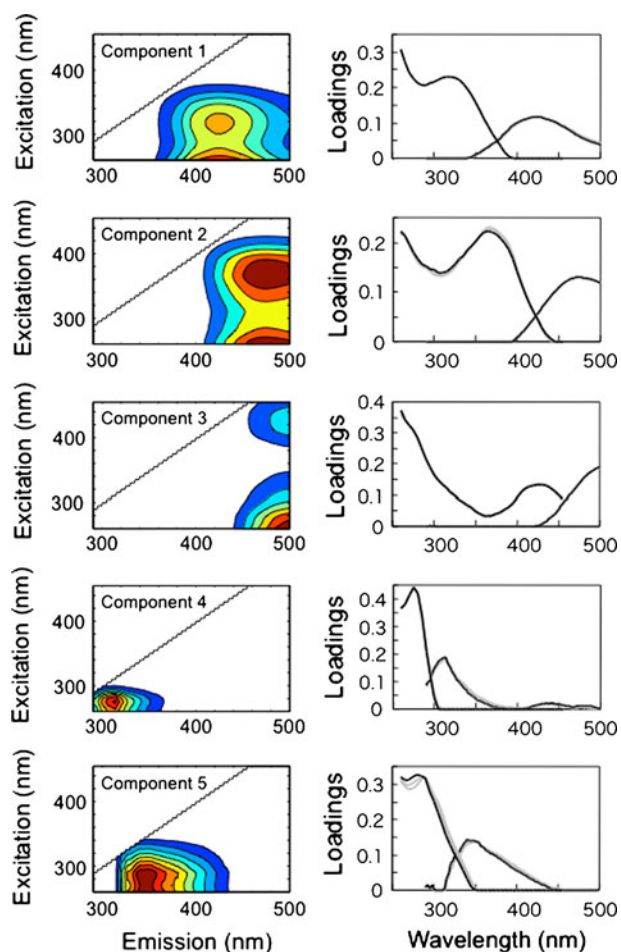


Figure 3. Fluorescence spectral characteristics and validation of the five components model by PARAFAC. The gray lines in the right column show the results of the split half validation: The loadings at shorter and longer wavelength show excitation and emission loadings, respectively.

(Table 1). Components 2 (C2) and 3 (C3) were red-shifted compared to C1 (Table 1; Figure 3); C2 has an emission maximum at 475 nm and two excitation maxima ( $<260$  and 365 nm). The two excitation maximum peaks of component C3 are at less than 260 and 430 nm with greater than 500 nm emission. Both C2 and C3 showed a peak at longer wavelength for both excitation and emission (Figure 3) and are characterized as humic acid-type components (Table 1). Components C4 (excitation/emission = 275/313) and C5 (excitation/emission = 290,  $<260/341$ ) are characterized as protein-like components, namely tyrosine-like and tryptophan-like, respectively (Table 1).

Temporal changes in the five fluorescence components obtained by PARAFAC modeling are shown in Figure 4A–E. Similar to the profiles of DOC and  $a_{350}$ , the fulvic acid-type C1 and humic acid-type C2 and C3 at REF were considerably

**Table 1.** Characteristics of the Five EEM-PARAFAC Components Compared with Previously Identified Fluorophores/Components

Component	Excitation maximum	Emission maximum	Description and possible sources (references)
1	<260 (320)	425	C peak (Coble 2007) Enriched component 1 in fulvic acid fraction (Santín and others 2009) Enriched component 3 in fulvic acid fraction (Ohno and Bro 2006)
2	365 (<260)	475	Mixture of A and C peaks (Coble 2007) Enriched component 2 in humic acid fraction (Santín and others 2009) Reduced quinone-like component SQ2 (Cory and McKnight 2005)
3	<260 (430)	> 500	Enriched component 1 in humic acid fraction (Ohno and Bro 2006) Terrestrial reduced quinone-like component SQ1 (Cory and McKnight 2005)
4	275	313	B peak (Coble 2007) Tyrosine-like fluorophore (Yamashita and Tanoue 2003a)
5	290 (<260)	341	T peak (Coble 2007) Tryptophan-like fluorophore (Yamashita and Tanoue 2003a)

higher than those at the WP and CC watersheds throughout the year. On the other hand, the protein-like C4 and C5 abundance did not differ among watersheds throughout the year.

The temporal variability in fulvic acid-type C1 and humic acid-type C2 were similar to each other and were almost identical to those of  $a_{350}$  (Figures 2, 4). Humic acid-type C3 showed similar temporal variability as  $a_{350}$ , but did not increase in November. Temporal variability of the fulvic acid-type and two humic acid-type components was also similar to DOC patterns. All three humic-like components, C1 (fulvic acid-type), C2, and C3 (humic acid-type) correlated well with DOC for all three watersheds ( $R = 0.77$ ,  $P < 0.0001$  for C1;  $R = 0.72$ ,  $P < 0.0001$  for C2;  $R = 0.58$ ,  $P = 0.0007$  for C3).

Time series analysis of the protein-like component tyrosine-like C4 showed an increasing trend from early summer, reaching a maximum in fall, followed by a decrease until spring, and a second increase into summer for all three watersheds (Figure 4D). Other quantitative parameters (DOC,  $a_{350}$ , and C1 and C2) also peaked in the fall, however, the fall peaks of tyrosine-like C4 (in September for WP; in October for REF and CC) occurred earlier than those of DOC,  $a_{350}$ , and C1 and C2, which occurred in November–December (Figures 2A, B, 4A, B). In February, tyrosine-like C4 components were higher compared to January and March; however, they did not show an

anomalous peak like the DOC,  $a_{350}$ , and humic-like components did (Figures 2, 4). The Tyrosine-like component C4 was not correlated with DOC for the three watersheds.

The levels of the protein-like component, tryptophan-like C5, were lowest among the five fluorescent components throughout the year for all three watersheds (Figure 4E). The temporal variability of tryptophan-like C5 was similar to the tyrosine-like C4 for all three watersheds, although the February peaks were more distinctive compared to C4. Fluorescence intensities of the C5 were not correlated with stream DOC for the three watersheds.

Figure 5 shows the PCA result of the PARAFAC components' relative abundance for stream samples from eight reference and six disturbed/managed watersheds at the Coweeta site collected during fall, winter, and spring. The first and second principle component explained 73 and 19% of the variability, respectively. Principle component score plots of managed/disturbed watersheds generally showed more negative first and positive second principle component scores compared to reference watersheds, especially for fall and winter samples, even though plots were scattered (Figure 5A). Spring samples showed more negative first principle component scores compared to fall and winter samples. Differences between reference and disturbed watersheds were similar for fall, winter, and

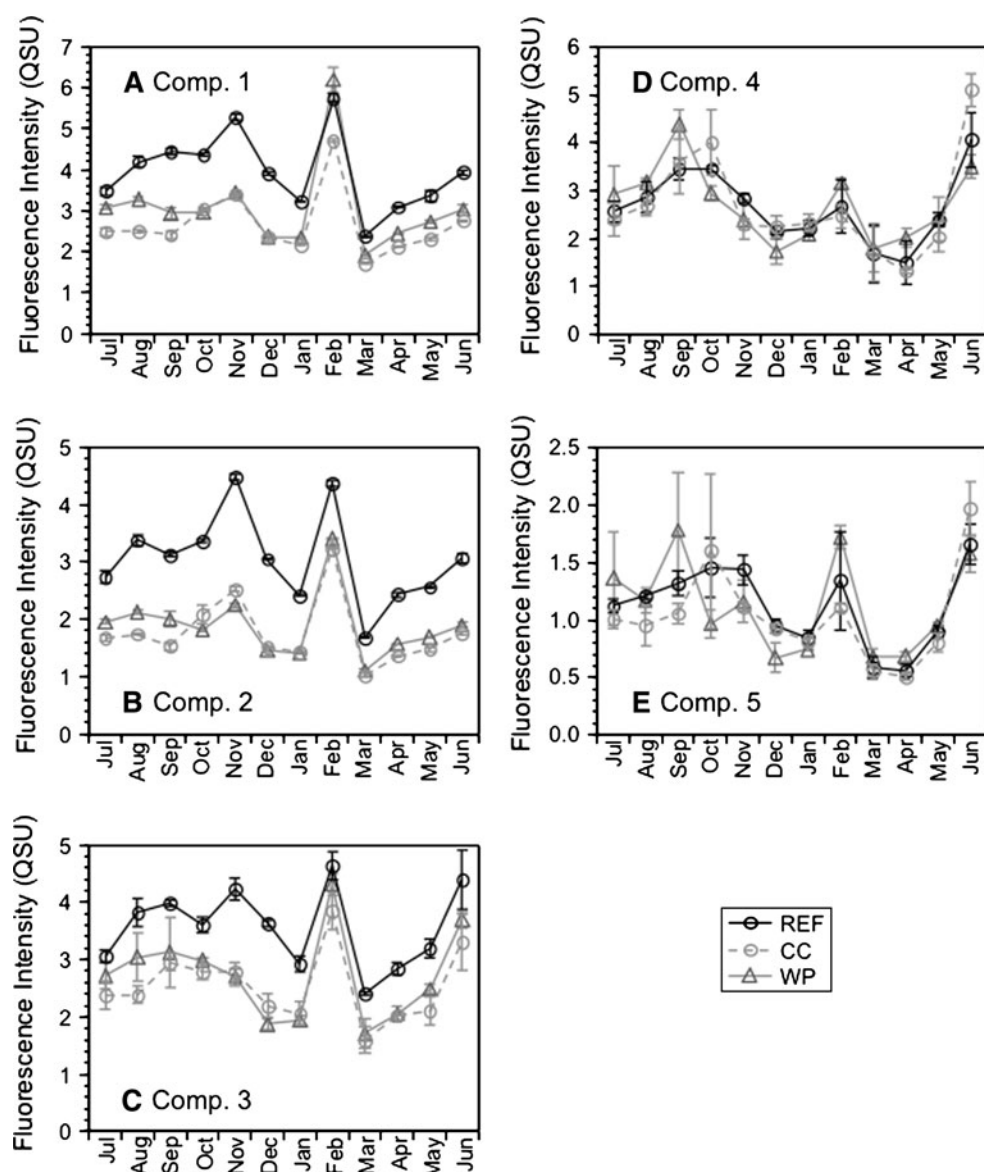


Figure 4. Temporal changes in fluorescence intensities (quinine sulfate units, QSU) of five PARAFAC components in headwater streams in reference (REF), clear-cut (CC), and white pine (WP) watersheds.

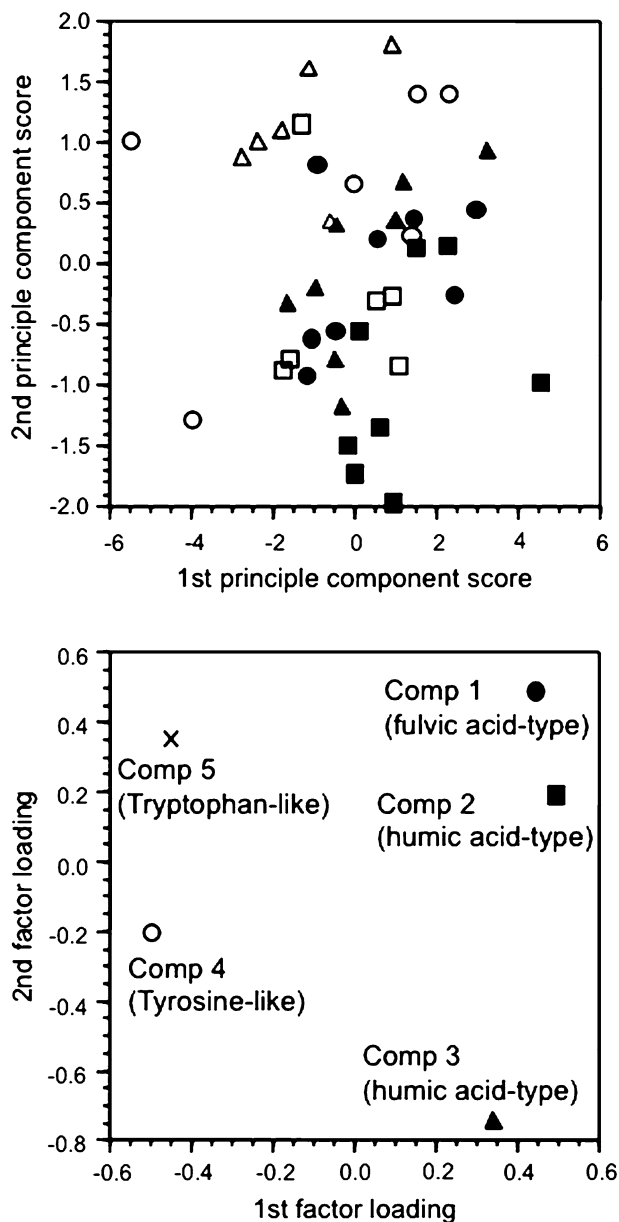
spring samples. The comparison of principle component scores (Figure 5A) with factor loadings (Figure 5B) indicated that the fluorophore composition of samples from managed/disturbed watersheds are generally more affected by protein-like C4 and C5 (and fulvic acid-type C1) compared to reference watersheds. This indicates that humic-like components C1, C2, and C3, especially, humic acid-type C3, were more abundant in reference watersheds than in managed/disturbed watersheds.

## DISCUSSION

### PARAFAC Components

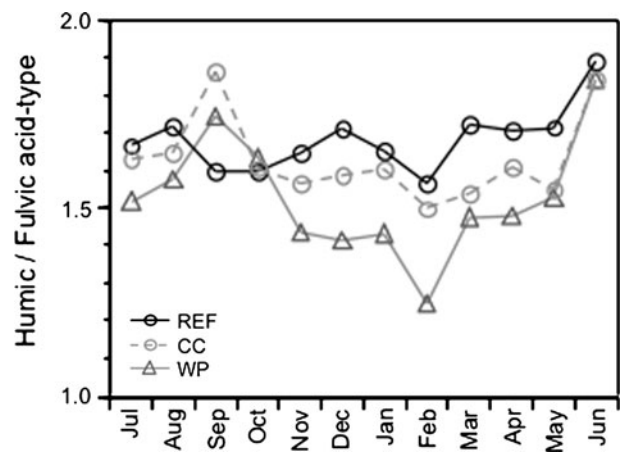
In the present study, the five DOM components identified by PARAFAC modeling (Figure 3) were

similar to PARAFAC components determined in previous studies of DOM in a wide range of aquatic environments (for example, Stedmon and Markager 2005; Cory and McKnight 2005; Yamashita and others 2010b). Three of five components (C1, C2, and C3) were categorized as humic-like components. Our fulvic acid-type C1 component was reported as enriched in the fulvic acid fractions compared to humic acid fractions in soils and sediments (Ohno and Bro 2006; Santín and others 2009), whereas the components corresponding to humic acid-type C2 and C3 were enriched in the humic acid fractions (Ohno and Bro 2006; Santín and others 2009). The emission peaks of C2 and C3 were red-shifted compared to C1 (Table 1; Figure 3), suggesting a high degree of aromatic substitution and polycondensation and higher levels of



**Figure 5.** Results of PARAFAC-PCA; **A** property–property plots between first and second principle component scores. *Closed* and *open* symbols indicate samples from undisturbed and disturbed watersheds, respectively. *Circles*, *triangles*, and *squares* indicate samples at fall (September and October), winter (December), and spring (April), respectively; **B** property–property plots between first and second factor loadings.

conjugated chromophores (Senesi 1990; Mobed and others 1996). Humic acids are usually considered older and more diagenetically altered products compared to fulvic acids (Aiken and others 1985). Thus, the humic acid-type components C2 and C3 may be derived from older soil organic matter than fulvic acid-type component C1. The ratio of humic acid-type C2 and C3 to fulvic acid-type C1 (humic/



**Figure 6.** Temporal changes in ratio of humic-acid type components 2 and 3 to fulvic acid-type component 1 (humic/fulvic acid-type) in headwater streams in reference (REF), clear-cut (CC), and white pine (WP) watersheds.

fulvic acid-type) might be a useful parameter for the assessment of the diagenetic state of DOM derived from soil organic matter (see discussion below, Figure 6).

Protein-like components C4 and C5 (Table 1) are known to be derived from aromatic amino acids, and their fluorescence signal is an indicator of total hydrolyzable amino acids concentration in DOM (Yamashita and Tanoue 2003a). These combined amino acids are considered labile or semi-labile fractions of DOM in aquatic environments (Yamashita and Tanoue 2003b; Davis and Benner 2007). Although no relationship between this protein-like fluorescence and total DOC was observed in this study, others have reported a linear relationship with the semi-labile fraction of stream water DOC (bio-degradable fraction during 30–40 day incubations) (Balcarczyk and others 2009; Fellman and others 2008). This suggests that these protein-like components may be useful indicators of freshly produced, semi-labile (and/or labile) fractions of stream DOC.

### Temporal Variability of DOM in Headwater Streams During Base Flow

We collected samples during base flow periods with the exception of the small discharge peak coinciding with the February collection (Figure 1). Therefore, we consider the temporal variability of DOM characteristics as representative of seasonal variability. DOC concentrations were greater during June to December for all three watersheds (Figure 3). Similar seasonal DOC patterns, with



higher concentrations during the growing season (May–October) compared to the dormant season (November–April), were also reported for Coweeta watersheds by Meyer and Tate (1983) and Tate and Meyer (1983). Fluorescence intensities of protein-like components were also higher during June–November. However, distinctive fall peaks of protein-like components (Figure 4D, E) appeared earlier (September to October) than the fall peaks (November–December) for DOC (Figure 2A). There was no correlation between protein-like components and DOC, suggesting that sources and degradation processes of protein-like components differ from major fractions of DOC in headwater streams. At Coweeta, Brookshire and others (2005) indicated that labile fractions of dissolved organic nitrogen (DON) are recycled rapidly within catchment boundaries, whereas less available fractions of DON as well as DOC from the channel/hyporheic zone were transported downstream. In this study, the water samples were collected at first- to third-order streams, suggesting that the high levels of protein-like components during the growing season might be related to recent biological activity and that these components are part of the less bioavailable fraction (semi-labile fraction) and, thus, are not fully recycled within the catchment boundary. Thus, protein-like components would be transported downstream due to their semi-labile character and, as such, may be important for microbial dynamics in downstream ecosystems.

Seasonal variability of humic-like components (that is,  $a_{350}$ , C1, C2, and C3) were similar to those of DOC (Figures 2, 4), suggesting that humic substances comprise a major fraction of bulk DOC in these headwater streams. Studies have shown that DOM fluorescence index values range from 1.0 to 1.8 in a wide variety of aquatic ecosystems (Cory and McKnight 2005; Jaffé and others 2008), where low and high values originate from soil- and microbial-derived DOM, respectively. Freshly leached DOM from plant tissues also feature high fluorescence index values (Jaffé and others 2004). For our study, the fluorescence index showed relatively low values (1.15–1.35) and did not vary significantly seasonally (Figure 2C), suggesting that humic-like CDOM is primarily of soil organic matter origin in headwater streams at Coweeta, and leaching from fresh leaf litter is not a major contributing factor.

Kaiser and others (2001) monitored the DOM quality and quantity in seepage waters from organic forest floor layers in Scots pine and European beech forests, and found greater DOC concentrations with more hydrophobic, aromatic and

aliphatic structures, carboxyl groups, and lignin decomposition products in summer and fall compared to winter and spring. The authors indicated that stronger oxidative transformation of the released DOM in summer and fall is due to the higher microbial activity under warm and moist conditions (Kaiser and others 2001). The seasonal variability of DOC in our study with the relatively constant low fluorescence index (Figures 2, 4) suggests that the forest floor and/or riparian zone are major contributors of DOM in these headwater streams.

### Effects of Land-Use History and Vegetation Type on DOM in Headwater Streams

For the 2 years following the harvest of CC, Meyer and Tate found lower DOC concentrations at CC compared to the REF (Meyer and Tate 1983). They suggest that such differences may be due to reduced organic matter inputs from the riparian zone, subsurface water sources, and in-stream biological activity. The present study at CC was conducted 30–31 years after clear-cut; thus, present day mechanisms regulating DOC concentrations in the associated headwater streams may differ.

At Coweeta, an abundant evergreen rhododendron understory shades many sections of the stream, resulting in less than 5% incident light (Brookshire and others 2005), suggesting minimal photodegradation of DOM and little to no in-stream primary productivity, which could potentially affect DOM quality. All watersheds had similar levels of protein-like components (Figure 4D, E) suggesting that freshly produced DOC in riparian zones did not differ significantly in absolute terms. In addition, DOC concentrations in soil solution at Coweeta decreased with soil depth indicating that major fractions of DOM derived from the organic soil layers (forest floor) were removed through adsorption processes in the mineral layer due to very high contents of Fe and Al oxyhydroxides (Qualls and Haines 1992; Qualls and others 2002). This implies that differences in DOM quality between REF, CC, and WP headwater streams were not the result of differences in subsurface water DOM supply, but differences in DOM contributions from surface soils.

Differences in leaf litter production, quality, leaching kinetics, decomposition, and organic matter incorporation into forest soils affect the DOM flux dynamics into streams (Meyer and others 1998; Park and Matzner 2003; Kalbitz and others 2007). In fact, the quantity and the environmental dynamics (bio-availability, sorption to/

leaching from soil) of DOM leachate from leaf litter vary substantially among different plant species (Yano and others 2005; Maie and others 2006b; Wallace and others 2008). Hongve (1999) compared DOM leached from different litter types and reported that the color/DOC ratio of leachate from coniferous litter was 1.7 times greater than those from deciduous litter. In this study, the  $a_{350}$ /DOC ratio at the pine watershed was lower than at the reference watershed (Figure 2D), suggesting that litter leachate from different species may be of different quality. However, from a quantitative standpoint, Elliott and others (2002) reported that although the biomass leaf-area index values in CC recovered 7–8 years after cutting, the species composition differed from pre-harvest periods. This implies that, although present litter fluxes are likely similar between reference and clear-cut watersheds, there remains a difference in composition. The leaf-area index in the WP watershed reached a maximum 12 years after planting (Swank and Schreuder 1973), with estimates of litter production ( $3253 \text{ kg ha}^{-1} \text{ y}^{-1}$ ) being slightly greater than the reference watershed ( $2773 \text{ kg ha}^{-1} \text{ y}^{-1}$ ) 14–15 years after pine planting (Cromack and Monk 1975). As such, WP and CC may feature differences in litter quality with respect to REF, whereas the litter quantity may be more similar.

In addition to litter quality, differences in stream DOM observed in this study might be due to sources of the aged organic matter from forest surface soils and riparian zones (Meyer and Tate 1983; Qualls and others 2002; McGlynn and McDonnell 2003; Hood and others 2006). Although surface soil C and N contents increased after cutting in CC (Knoepp and Swank 1997), leaf litter input was lower than REF until 7–8 years after clear-cutting (Elliott and others 2002). Similarly, litter fluxes at WP were smaller than REF for 12 years after planting (Swank and Schreuder 1973). Thus, the years of reduced leaf litter contributions during canopy recovery may have long-lasting effects on organic matter dynamics in forest soils. In addition, it was reported that the decomposition rate of pine needle litter was lower compared to oak leaf litter (Knoepp and others 2005), thus reducing the addition of OM to soil interstitial waters at the WP watershed. Therefore, our results suggest that differences in the accumulation and/or decomposition rate of organic matter in organic horizons over several decades (~40 years) coupled with the change in vegetation result in long-term effects in the quantitative and qualitative characteristics of stream water DOM.

Qualitative parameters of DOM also support our hypothesis that differences in soil organic matter

affect DOM quality in headwater streams. The humic/fulvic acid-type ratios were usually highest at REF and lowest at WP throughout the year (Figure 6). The difference between REF and CC suggests a smaller contribution of highly degraded DOM from the CC forest floor and riparian zone soils due to lower organic matter accumulation and humification. Ohno and others (2008) concluded that the relative abundance of the humic acid-type and fulvic acid-type components in water extractable soil organic matter fractions were similar between conifer and deciduous stands. However, the humic/fulvic acid-type ratio calculated from Ohno and others (2008) was much smaller for DOM extracted from the conifer stand compared to the deciduous stand. Thus, the low ratio for the WP watershed suggested that differences in organic matter characteristics and/or decomposition rate due to different litter sources in organic soil layers between pine and deciduous watersheds may affect the humic/fulvic acid-type ratio in headwater streams.

Additional evidence for long-term differences in DOM quality was obtained through PCA (Figure 5). The PCA from multiple reference and past-disturbed watersheds at Coweeta confirmed that the relative contributions of protein-like components are greater in managed/disturbed watersheds compared to reference watersheds. Consistent with the humic/fulvic acid-type ratios observed for CC, WP, and REF, PCA also indicated that the fulvic acid-type components in past-disturbed/managed watersheds were enriched compared to reference watersheds. Ultimately, the PCA data demonstrate the long-term effects on DOM quality between reference and managed/disturbed sites. The observations regarding DOM quality effects of land-use history with watershed disturbance and/or forest management practices, agree with previous observations where land-use effects due to increased agriculture showed enhancements in the more labile DOM components, including proteins and microbial humic-like DOM components (Williams and others 2010).

## CONCLUSIONS

We examined DOM characteristics in headwater streams at reference and managed watersheds (30 years after clear-cutting and 50 years after conversion to white pine) at Coweeta Hydrologic Laboratory in western North Carolina. DOC and CDOM concentrations were lower in clear-cut and pine watersheds throughout the year compared to the reference watershed. DOM characterization by optical means (EEM-PARAFAC) suggests primarily

soil-derived sources of DOC in forested headwater streams, although DOC from recent biological production was also evident. Concentrations of protein-like components were similar among watersheds, suggesting that freshly produced DOC, attributed to in-stream and riparian zone biological productivity did not differ among the three watersheds. On the other hand, levels of humic-like components and diagenetic state of humic-like fluorophores were higher at the reference watershed than those at clear-cut and pine watersheds, which showed enrichments in fulvic acid-type and protein-like components. This suggests that different soil organic matter accumulation and decomposition rates in response to litter sources and fluxes in disturbed/managed watersheds resulted in changes in DOC dynamics. The results of this study suggest that DOM quantity and quality are affected by changes in vegetation and land cover resulting from watershed scale management over a period of several decades. Such anthropogenically induced changes must be considered when assessing changes in carbon fluxes due to climate change as well as land management.

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