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Dissolved organic matter dynamics in calcium-treated and reference watersheds at Hubbard Brook Experimental Forest

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Abstract

Organic carbon makes up the molecular structure of all living organisms on Earth and is essential to understanding ecological interactions. For forest ecosystems, organic carbon pools and fluxes have been extensively quantified, while less is known about its more highly variable composition. Dissolved organic matter (DOM) is a major source of biologically available organic carbon and nitrogen and serves as an indicator of watershed carbon processing. The extent to which DOM is metabolized, before it is transported from the watershed, is highly dependent on its source molecular structure and the processes exacted upon it. Spectroscopic techniques, such as fluorescence characterization, utilize the optically active fraction of DOM to provide biochemical information such as its source, aromatic content, and molecular size. Analysis of changes to the DOM composition in watershed solutions can provide further insight to understanding its sources, dynamics and impacts on water quality.

In this study I measured DOM concentrations and optical properties from experimental watersheds at Hubbard Brook Experimental Forest, New Hampshire, USA. I report 2 years (2015-2016) of monthly soil solution and stream chemistry from watersheds 1 (W1) and 6 (W6). W6 serves as a reference, while W1 was treated with wollastonite ($CaSiO_3$) in 1999 to replenish available calcium lost due to historic leaching from acid deposition. Soil solution samples, from the Oa, Bh, and Bs horizons, were collected monthly from zero-tension lysimeters at varying elevations within the watersheds. Stream water samples were also collected at locations corresponding to lysimeter elevations. Optical properties were determined from individual excitation-emission matrices (EEMs) scanned from each sample. Parallel Factor Analysis (PARAFAC) was conducted to decompose the fluorescence matrices into independent

components. Additional Principal Component Analysis (PCA) was conducted to evaluate the relationships between DOM optical properties and quantifiable analytes.

PARAFAC results revealed three predominant DOM fractions present in soil solutions and stream water: humic, fulvic, and "blue-shift" humic components. While these fractions were not significantly different between calcium-treated and reference watersheds, additional carbon quality indicators such as molecular size and redox status suggested greater DOM processing in the calcium-treated watershed. This pattern was supported by statistically higher DOC & DON concentrations in the calcium-treated organic (Oa) soil solution. Further analysis was conducted on seasonal and elevational patterns. Organic soil solutions from the high-elevation hardwood zone experienced higher DOC and DON concentrations during fall months and elevated DIN concentrations during spring months, possibly due to fine root biomass decline. Low elevation mineral soils exhibited greater "blue-shifted" humic fractions and fluorescence indications of microbial DOM sourcing, which peaked during spring months. This pattern suggests that low-elevation mineral soils are important carbon sinks, due to greater rates of microbial DOM processing in bimodal podzols.

Dissolved organic matter dynamics in calcium-treated and reference watersheds at Hubbard Brook Experimental Forest

By

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Thesis submitted in partial fulfillment of
The requirements for the degree of

Master of Science in Environmental Engineering Science

Syracuse University

August 2019

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Introduction

Dissolved organic matter (DOM) plays a critical role in the transfer of energy and nutrients within and between terrestrial and aquatic ecosystems (Jaffé et al. 2008). Both the organic and mineral soil have the capacity to store considerably more carbon than quantities found in either the atmosphere or plant biomass (Schmidt et al. 2011). A fraction of soil carbon can be mobilized as DOM, making it one of the largest sources of organic carbon in freshwater and marine ecosystems (Battin et al. 2008).

Detrital organic matter is a complex matrix, composed of a wide variety of functional groups which can mediate the immobilization, cycling, and transport of nutrients and cations. Recently enhanced mobilization of DOM from soil to surface waters has been reported and attributed to recovery from acid deposition or changing climate (Montieth et al. 2007; Clark et al. 2006). This phenomenon is called "browning" and has enhanced interest in better understanding the function of DOM and how it is manifested under a new regime of increases in surface water concentrations. Increased mobilization of DOM and associated iron increases the attenuation of light in lakes, which increases upper water temperatures earlier in the summer season, forming shallower thermoclines and strengthening stratification (Brothers et al. 2014). Nutrients associated with DOM can enhance productivity unless light attenuation becomes so severe that systems become light limited (Solomon 2017). These changes in water clarity, temperature, stratification and productivity can alter oxygen levels causing shifts in lower trophic level zooplankton populations which could impact lacustrine food chains (Williamson et al. 2016). DOM can also complex and facilitate the transport cations from the forest into surface waters. The mobilization of some cations, such as aluminum and mercury, can have negative impacts on terrestrial and aquatic ecosystems (Chaisson-Gould et al. 2014; Li & Johnson 2016).

DOM composition is highly dependent on its source material and the biological and geochemical processes exacted upon it, as it is processed through watersheds (Catalán et al. 2014). Studies have found that when DOM is transported through soils, the lignin- like compounds tend to be retained through sorption to Al and Fe oxides-hydroxides. In contrast, more carbohydrate and protein-like organic matter more readily percolates through soil, where it is available for microbial metabolism (Kaiser et al. 2004). Soil microbes process these compounds, mineralizing organic nitrogen and contributing their own detritus to the dissolved mixture. Kaiser & Kalbitz (2012) proposed a conceptual model depicting DOM processing through the soil profile, with patterns of decreases in the C/N ratio and phenolic compounds, coupled with an increase in microbial metabolites (Appendix A).

Anthropogenic climate change could play an important role in altering how watersheds process DOM (Cawley et al. 2014). In the Northeast United States, increases in air temperature have coincided with a lengthening of the growing season (Groffman et al. 2012, USGCRP 2018). In addition, there has been an increase in annual precipitation, a decrease in the fraction of precipitation occurring as snow and an increase in the intensity of precipitation events. Intense hydrologic events can facilitate the transport of fresh, lignin-like DOM deeper into soils or to streams via bypass soil flow paths (Fröberg et al 2007; Raymond et al 2016). Recent studies linking the connectivity of soil pores to soil organic carbon suggest that enhanced wetting and drying cycles resulting from climate change can connect microbial decomposers to sources of carbon, leading to increasing CO_2 fluxes and changes in the chemical quality of DOM (Bailey et al. 2017).

An additional factor contributing to DOM transport in watersheds is the recovery from acid deposition. Long term-data has shown that volume-weighted concentrations of sulfate and

nitrate in bulk precipitation and wet deposition have significantly decreased since the Clean Air

Act and associated rules (Driscoll et al. 2001; Driscoll et al. 2016). Drought simulations on soil

cores have found an inverse relationship between sulfate concentrations and DOC, when

expressed as a fraction of the DOC concentration in the absence of sulfate (Clark et al. 2006).

Additional long-term studies on the impact of acidity on DOC have found that decreases in the

sulfate concentrations coincide with releases of DOC into surface waters (Driscoll et al. 2003).

Another key component of acid deposition is nitrate derived from NO_x emissions (Driscoll et al.

2003; Ukonmaanaho et al. 2014). Elevated inputs of anthropogenic nitrogen have been found to

significantly suppress the decomposition of organic matter in forested ecosystems (Tonitto et al.

2013), through either acidification effects or loss of biodiversity in the ecosystem (Davidson et

al. 2012). With recent decreases in atmospheric NO_3^- this mechanism may be reversed.

Furthermore, soil pH is an important regulator of DOM. pH controls the solubility of

aluminum. Laboratory studies have shown that the partitioning of DOC to forest soil decreases

with increases in soil pH (Ussiri and Johnson 2003). When mobilized under acidic conditions,

aluminum competes with hydrogen ions for binding sites on organic matter colloids, thus

enhancing coagulation of DOM and formation of soil organic matter (SOM) (Cronan &

Scholfield 1990; Li & Johnson 2016). Also, it is believed that a reduction in the ionic strength of

soil solution should lead to increased DOC export (Monteith et al. 2007).   These findings

suggest that "deacidification" could enhance the quantity of mobile DOM and alter its quality

within forested watersheds.

New evidence suggests that changes in the carbon flow to soil in response to ecosystem

recovery from acid deposition and climate change may be connected to nitrogen limitation in

forested ecosystems, through a process called "nitrogen oligotrophication" (Groffman et al.

2018). Atmospheric nitrogen deposition is the main source of "reactive nitrogen" to northeastern

forests (Driscoll et al. 2003). Recently atmospheric nitrate deposition has declined significantly

due to stronger emissions regulations (Lloret & Valeila 2016). Long-term observations of

watershed stream nitrate export have shown that declines began in the 1960s, well before Clean

Air Act, and continue today (Groffman et al. 2018). Additionally, increases in the forest floor

C/N ratio have been observed, despite no relative change to the nitrogen pool, suggesting that

nitrogen is either being retained by DOM adsorption or metabolized by microbes in lower

mineral soils (Yanai et al. 2013). The latter mechanism is supported by significant declines in

potential nitrogen mineralization and observed increases in soil microbial respiration (Durán et

al. 2016, Groffman et al. 2018). Thus, the flow of "labile" carbon (DOM) into lower mineral

soils could stimulate microbial populations to immobilize reactive nitrogen leading to an overall

tightening of available nitrogen cycling within the ecosystem (Groffman et al. 2018).

In this study, I examined the spatial and temporal patterns of DOC, nitrogen species, and

DOM quality in soil solutions and stream water in a reference and calcium silicate treated

watersheds at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New

Hampshire, USA. Patterns of nutrients along the forested landscape have been previously

examined, considering factors such as soil depth, elevation and vegetation species composition

(Johnson et al. 2000, Dittman et al. 2007). Additionally, previous studies on DOM in stream

water have not observed any significant difference in carbon quality between calcium-treated and

reference watersheds (Cawley et al. 2014).

The objectives of this study are to explore spatial and seasonal patterns in DOM quantity

and quality and their relationships with nitrogen availability at varying soil depths and in stream

export. This study also aims to examine the possible treatment effect that improvements to the

calcium status may have made on DOM in soil solution. Insights into the evolving characterization of DOM, as it is transported through soil flow paths, could be valuable to understanding how forest carbon and nitrogen sources and sinks are changing in response to acidification recovery and global climate change.

My approach included the examination of temporal, horizonal and landscape patterns of solute (DOC, DON, DIN) concentrations and their stoichiometric responses in soil and stream water from both calcium-treated and reference watersheds. I also determined chemically meaningful DOM components, using optical properties (EEM-PARAFAC), and assessed their relationships with quantitative solute measurements from stream and soil water. I hypothesized that:

(1) DOM quantity will decrease with soil depth, while DOM composition will shift from large, aromatic, plant-derived compounds to smaller, more microbial-derived compounds;

(2) $CaSiO_3$ treatment will accelerate a "deacidification" effect, resulting in an increase in carbon and organic nitrogen flow from the forest floor to the mineral horizons and eventual stream export; and

(3) DOM composition will exhibit evidence of smaller, more microbially-derived compounds within the $CaSiO_3$ treated watershed.

Methodology

Site description

Hubbard Brook Experimental Forest (HBEF) is located within the southern White Mountains of New Hampshire, USA (43º56'N, 71º45'W). The climate is humid-continental, characterized by long cold winters and short cool summers. The mean air temperature is -9º in January, and 19º in July (Campbell et al. 2007; Bailey et al. 2003). Precipitation is evenly distributed throughout the year at an annual average of 1400 mm, where approximately 30% falls as snow. The mean annual stream flow from the forest is 905 mm. 50% of the flow occurs during the months of March, April, and May (Bailey et al. 2003). Low summer stream flows of only 1 or 2 mm per month have been observed, while streamflow throughout the winter months remains relatively consistent at approximately 50 mm per day, due to snowpack melt (Bailey et al. 2003).

This study focused on two experimental watersheds found on the south-facing slope at the HBEF (Appendix B). Historical land disturbance included heavy logging from 1910 to 1919, but since then has remained relatively undisturbed except for a hurricane in 1938 and an ice storm in 1998 (Dittman et al. 2007). Watershed 6 (W6) is the biogeochemical reference watershed. It has a total area of 13.2 ha, and its elevation ranges from 540 to 800m (Cho et al. 2009). W6 receives an annual average precipitation of 1423 mm (Baily et al. 2003). Watershed 1 (W1) was treated with calcium silicate ($CaSiO_3$) to replenish calcium lost from years of leaching associated with acid deposition. The goal of the W1 experiment was to increase soil base saturation from 10% to 19% (Peters et al. 2004). The watershed has a total area of 11.8 ha ranging in elevation from 488 m to 747 m (Shao et al. 2015). It receives a mean annual precipitation of 1322 mm (Baily et al. 2003).

Both watersheds are divided into three elevational subcatchments (Figure 1). The highest is the spruce-fir-birch zone (SFB), which shows a higher density of coniferous trees: red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* L.). White birch (*B. papyrifera* var. *cordifolia* Marsh.) is also found primarily within this subcatchment. The intermediate high-elevation hardwood zone (HH) and low-elevation hardwood zone (LH) subcatchments are dominated by sugar maple (*Acer saccharum* Marsh.*)*, American beech (*Fagus grandifolia* Britt.), and yellow birch

Figure 1. Map of south-facing, experimental watersheds at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, USA.

(*Betula alleghaniensis* Britt.). Together, these tree species account for more than 95% of the forest biomass in the experimental watersheds (Battles et al. 2014).

Soils at HBEF are predominately well drained Spodosols and Inceptisols, with a sandy loam texture (Likens & Buso 2006). Mineral soil has an average depth of about 50 cm and generally decreases with increases in elevation. Conversely, the forest floor, with a mean depth of 6.9 cm, generally increases with elevation (Johnson et al. 2000). Soil pH varies with soil depth, from approximately 3.9 in the organic horizon, to 4.7 in the lower mineral horizon (Johnson et al 1991b).

Sample collection and laboratory analysis

Nine lysimeters are located immediately west of W6, grouped in three sets of triplicates along an elevation gradient corresponding with the three landscape zones. Thirteen zero-tension lysimeters are located along an elevation gradient in the calcium-treated watershed, similarly

encompassing the three elevational zones (Figure 1). The lysimeters were constructed from PVC troughs placed below the forest floor (Oa horizon) and within the mineral soil (beneath Bh and within Bs horizons). Soil solution samples were collected using a vacuum hand pump at 4-5 week intervals (Cho et al. 2010). Stream water samples were also collected at the same monthly intervals from sites along the elevational gradients of both watersheds (5 sites in W1 and 6 sites in W6). All samples were placed into acid-washed HDPE bottles, transported back to the laboratory at Syracuse University and stored below 4º C.

Soil solution and stream water samples were analyzed for total nitrogen (TN) concentrations via platinum catalyzed combustion with chemiluminescence detection, using a Teledyne Tekmar Apollo 9000 TOC Analyzer. Ammonium (NH_4) was analyzed via the Berthelot reaction, with a Seal Analytical AutoAnalyzer 3. Nitrate (NO_3) was analyzed by suppressed ion chromatography with a Dionex IonPAC-AS18 anion-exchange column. Dissolved organic nitrogen (DON) was quantitatively determined by subtracting the sum of the ammonium and nitrate concentrations (DIN) from the total nitrogen concentration from individual samples. Negative DON observations were assumed to be zero. Subsamples were filtered through a Whatman glass microfiber filter 934-AH (0.45 µm pore size), then analyzed for dissolved organic carbon concentrations (DOC) via sodium persulfate oxidation using a Teledyne Tekmar Phoenix 8000 TOC Analyzer.

Quality assurance and quality control measures were used for all sample analyses. I used an initial calibration (correlation coefficient >0.995), initial calibration verification (±10% recovery), initial calibration blank, continuing calibration verification (±10% recovery), continuing calibration blank, laboratory control sample made from secondary source (±10% recovery), duplicate sample (10% relative percent difference), and method detection limit (75-

125% recovery) for QA/QC (Cho et al. 2009). All samples that failed these criteria were reanalyzed.

Ultraviolet absorption of DOM, fluorescence scans, and excitation-emission matrices (EEMs) were conducted using a 1 cm quartz cuvette in a Horiba Aqualog Steady State Fluorometer. The excitation range was set from 240 to 550 nm at 2 nm increments, with an emission coverage of 248 to 830 nm with 2 nm increments. The excitation and emission slit widths were set to 10 nm, and the integration time was 0.1 s. EEMs were corrected for the instrument lamp signal, cuvette optics, and Raman spectra. All EEMs were also blank-subtracted using the EEM of fresh Milli-Q water.

Data analysis

Data analysis included samples collected from 2015-2016 calendar years. A parallel factor analysis (PARAFAC) model was constructed from 830 EEMs using the "drEEM" toolbox, on Matlab v2016a (Murphy et al. 2013). The EEM dataset was 3-way (excitation loading i, emission loading j, and sample k) decomposed into principal fluorescing components. Each component represents a group of dominant fluorophores that are characterized by similar properties. The optimal model was chosen through steps such as outlier identification, minimization of the sum of squares residuals, and random initialization with 10 iterations. Once the optimal number of components was chosen, the model was validated using split-half analysis, where the model is applied independently to two halves of the dataset, then compared for identical spectral properties (Stedmon & Bro 2008). The component loadings for each sample were then predicted by the model, and the fraction of each DOM component per sample was determined.

The Fluorescence Index (FIX) and the Redox Index (RIX) were calculated from fluorescence scans. Fluorescence Index was calculated as a ratio of fluorescence intensities at 470 nm and 520 nm, at an excitation wavelength of 370 nm (McKnight et al. 2001; Cory & McKnight 2005). FIX values can range from 1.2 (indication of a terrestrial humic source) to 1.8 (indication of a microbial source), and intermediate values represent a mixture of the two. The Redox index (RIX) value, an indicator of whether quinone-like components within DOM are more reduced or oxidized, was calculated as a ratio between the sum of the reduced quinone-like components and the sum of the oxidized semiquinone-like and hydroquinone-like components as well as the quinone-like components (Cory & McKnight 2005; Miller et al. 2006). Redox Index values range from 0 to 1, where 0 represents more oxidized and 1 represents more reduced components.

Specific ultraviolet absorbance and molecular size indicators were calculated from ultraviolet-visible absorption scans. Specific UV absorbance ($SUVA_{254}$), an estimate for DOM aromaticity, was calculated by dividing UV absorbance at 254 nm by the DOC concentration, reported in units of L mg C^{-1} m^{-1} (Weishaar et al. 2003). E_2:E_3, an indicator of DOM molecular size, was calculated by the ratio of absorbance at 250 nm divided by that at 365 nm. Higher values indicate smaller DOM molecular size. An E_2:E_3 value of 3.5 corresponded to a DOM diameter ranging 35-200 nm, while a value of 6.5 corresponded to a diameter of less than 5 nm (Haan & De Boer 1987). Possible UV absorbance interferences include nitrate, which has an absorbance maximum at 210 nm. Nitrate concentrations >40 mg/L can cause significant interferences as the peak shoulder may overlap with absorbance at 254 nm (Weishaar et al. 2003). The maximum nitrate concentration in this data set was 20 mg/L so I do not expect it to have caused significant interferences. Iron (II) and iron (III) have also been considered as a

possible source of UV absorbance interference (Cawley et al. 2014; Weishaar et al. 2003). Iron was not measured in this study but has been previously in W1 and W6. Mean iron (II) and (III) concentrations in soil and stream water did not exceed 0.5 mg/L and are thus considered negligible for interference (Fuss et al. 2011; Weishaar et al. 2003).

Statistical analyses

Welch's two-sample *t*-test for unequal variances was used to evaluate the response of soil solution and stream water to $CaSiO_3$ treatment compared to reference watershed conditions, and to evaluate differences between soil horizons. Statistical significance was considered by p-values based on an α-value of 0.05 and a 95% confidence interval. Principal component analysis (PCA) was used as an additional nonparametric method to evaluate the relationships among DOM PARAFAC components, spectral indices and solute concentrations. "R" statistical software was used for all statistical analyses.

Results

PARAFAC model components

The PARAFAC model with three DOM components was split-half validated. All three components were terrestrially sourced, and each had multiple excitation loadings (Appendix C). DOM component 1 (C1) represented approximately 43±1% of DOM fluoresced from soil solutions and stream water (Figure 2). C1 exhibited characteristics most strongly associated with humic acid fluorophore groups validated by Cory and McKnight (2005) (Appendix D). Component 2 (C2) represented approximately 34±3% of the DOM fluoresced in forest floor leachate in both watersheds. This fraction decreased significantly with drainage through soil horizons to approximately 28±3% in stream water. Conversely, component 3 (C3) represented only 23±3% of the DOM fluoresced in forest floor solutions from both watersheds, but increased

significantly to 29±3% in the stream (Table 4). Component 2 (ex 275 nm & 380 nm, em 504 nm) and component 3 (ex 320 nm & 275 nm, em 395 nm) were spectrally similar to components C3 (ex 280 nm & 400 nm, em 504 nm) and C2 (ex 310 nm & 250 nm, em 400 nm) respectively, as reported by Kothawala et al. (2014). C2 exhibited fluorophore peaks most associated with to terrestrial autochthonous inputs of fulvic acids, found in natural environments (Stedmon & Markager 2005). C3 was spectrally similar to humic acid fluorophores, like C1, but was "blue shifted" towards shorter wavelengths. This fluorophore group has been associated with high biological activity and used previously as an indicator

Figure 2. PARAFAC modeled DOM component fractions in soil and streamwater from watersheds 1 & 6.

of autochthonous DOM. It has been found in a wide range of aquatic environments including: marine, wetland, agricultural, and wastewaters (Coble 1996; Fellman et al. 2010).

Landscape patterns

There were distinct elevational patterns in DOM quantity and quality. In the Oa horizon, DOC, DON, and DIN concentrations (2121.4±1209.9 µmol L^{-1}; 47.6±29.6 µmol L^{-1}; 45.7±49.8 µmol L^{-1} respectively) were found to be higher in the HH zone than the SFB zone, and significantly higher than the LH zone. In the mineral horizons and streamwater, DOC concentrations decreased with elevation (Figure 3). A significant difference in DOC concentrations (689.9 µmol L^{-1}) was observed in the Bs horizon between the SFB and HH zones

Peak DOC concentrations occurred during the months of August, September, and October in the Oa, Bh and Bs soil solutions. During these months, as well as the spring months of March, April and May, HH mean concentrations exceeded SFB mean concentrations (Figure 3). DON followed the same seasonal patterns as DOC, peaking in the late summer, to fall months in all three soil horizons. DON concentrations also decreased with elevation, significantly between the HH and LH zones in the Oa horizon (HH: 47.6 ± 29.6 µmol L^{-1}; LH: 35.4 ± 32.4 µmol L^{-1}) and Bh (HH: 23.5 ± 17.1 µmol L^{-1}; LH: 13.4 ± 13.3 µmol L^{-1}) horizons, and
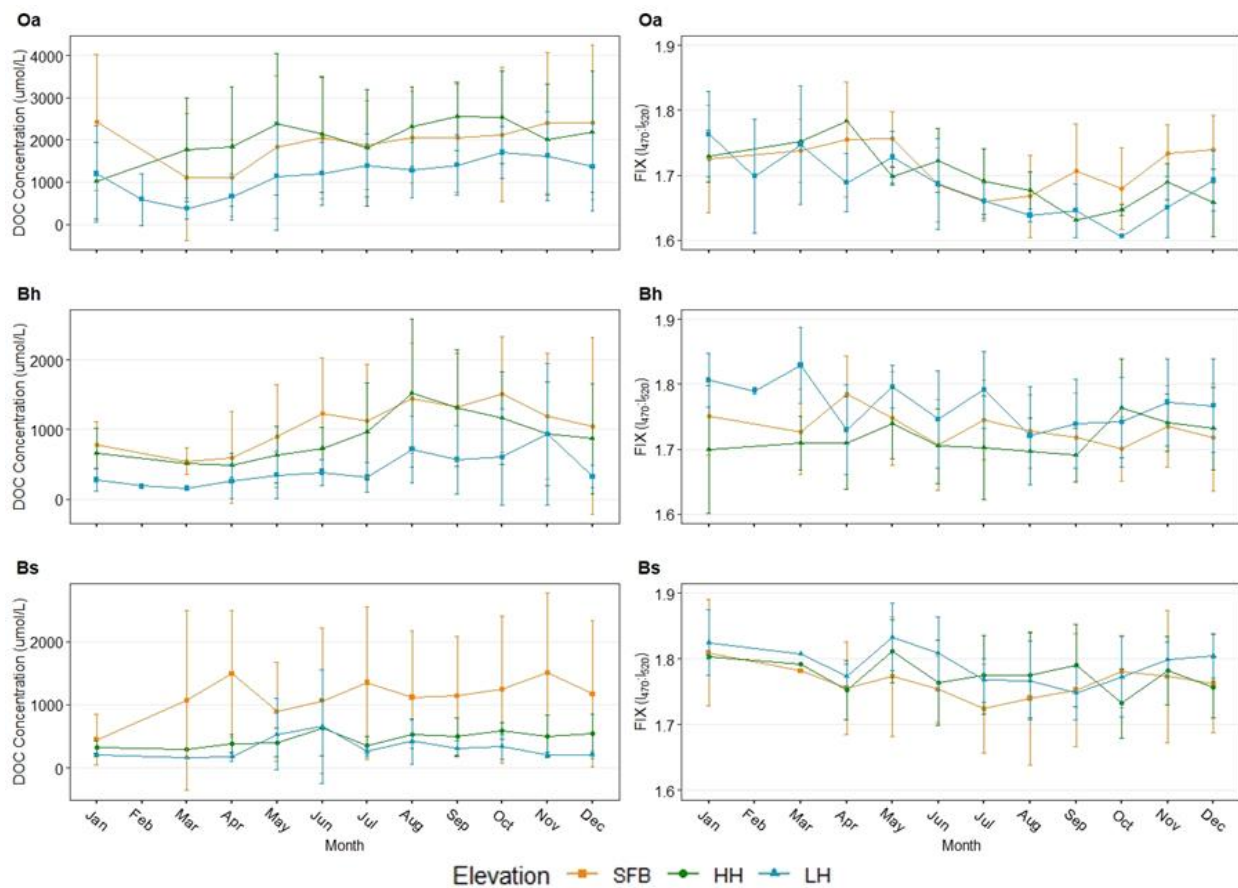
Figure 3. Average monthly DOC concentrations (µmol/L), along with fluorescence index (FIX). Collected over a period 2015-2016 (mean±SD) from three soil horizons (Oa, Bh, Bs), at each of the three subcatchments: SFB, HH, and LH.

between the SFB and HH zones in the Bs horizon (SFB: 22.1±18.4 µmol L^{-1}; HH: 11.8±6.0 µmol L^{-1}).

DIN concentrations in the HH zone were higher than both other elevational zones in all three soil horizons and in stream water. In the Bs horizon, DIN concentrations from the HH zone (29.4±31.5 µmol L^{-1}) were significantly greater than other elevations. As with DOC, peak

Figure 4. Average monthly fulvic acid component (C2 %) and "blue-shifted" humic acid component (C3) fractions. Collected over a period 2015-2016 (mean±SD) from three soil horizons (Oa, Bh, Bs), at each of the three subcatchments: SFB, HH, and LH.

DIN concentrations from the HH zone, exceeded SFB concentrations during late summer and early all months, in both the Oa and Bh horizons. In the Bs horizon, DIN concentrations from the SFB zone consistently surpassed the lower elevations throughout the year.

Table 1. *Reference (W6) mean volume-weighted concentrations (μmol/L) and standard deviations (in parentheses) for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), also DOC:DON and DOC:DIN molar ratios, DOM component fractions (%), specific ultra-violet absorbance (L mgC-1 m-1) , fluorescence index (I470:I520), redox index (Qred/[Qred+Qox]), molecular size (E2:E3). Values derived from soil solution (Oa, Bh, Bs) and streamwater at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).*

Soil Horizon	Reference solute concentration (μmol/L) and molar ratios					Reference DOM fractions & optical properties						
	DOC	DON	DIN	DOC:DON	DOC:DIN	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa	1474.7(1087.5)	28.1(18.1)	27.4(36.6)	72.4(78.8)	134.7(163.8)	42.75(1.01)	34.03(3.33)	23.22(2.66)	4.50(2.67)	1.62(0.09)	0.71(0.02)	5.02(0.55)
Bh	858.6(542.9)	19.0(15.3)	19.7(32.4)	61.5(45.3)	158.1(208.44)	42.82(1.02)	32.09(3.49)	25.10(2.94)	3.31(1.57)	1.69(0.08)	0.70(0.02)	5.60(1.15)
Bs	622.9(583.7)	13.3(12.7)	13.4(39.4)	57.8(44.2)	184.6(244.9)	43.36(0.88)	30.37(2.79)	26.26(2.41)	2.93(1.32)	1.76(0.07)	0.69(0.01)	6.20(0.82)
Stream	243.8(248.85)	5.2(2.1)	2.5(3.1)	41.0(18.1)	165.7(153.3)	43.25(0.71)	28.26(2.50)	28.49(2.66)	2.78(0.39)	1.76(0.06)	0.68(0.01)	6.93(1.38)

The humic fraction (C1) remained relatively refractory to changes in elevation and season, while strong seasonal patterns were observed in the fulv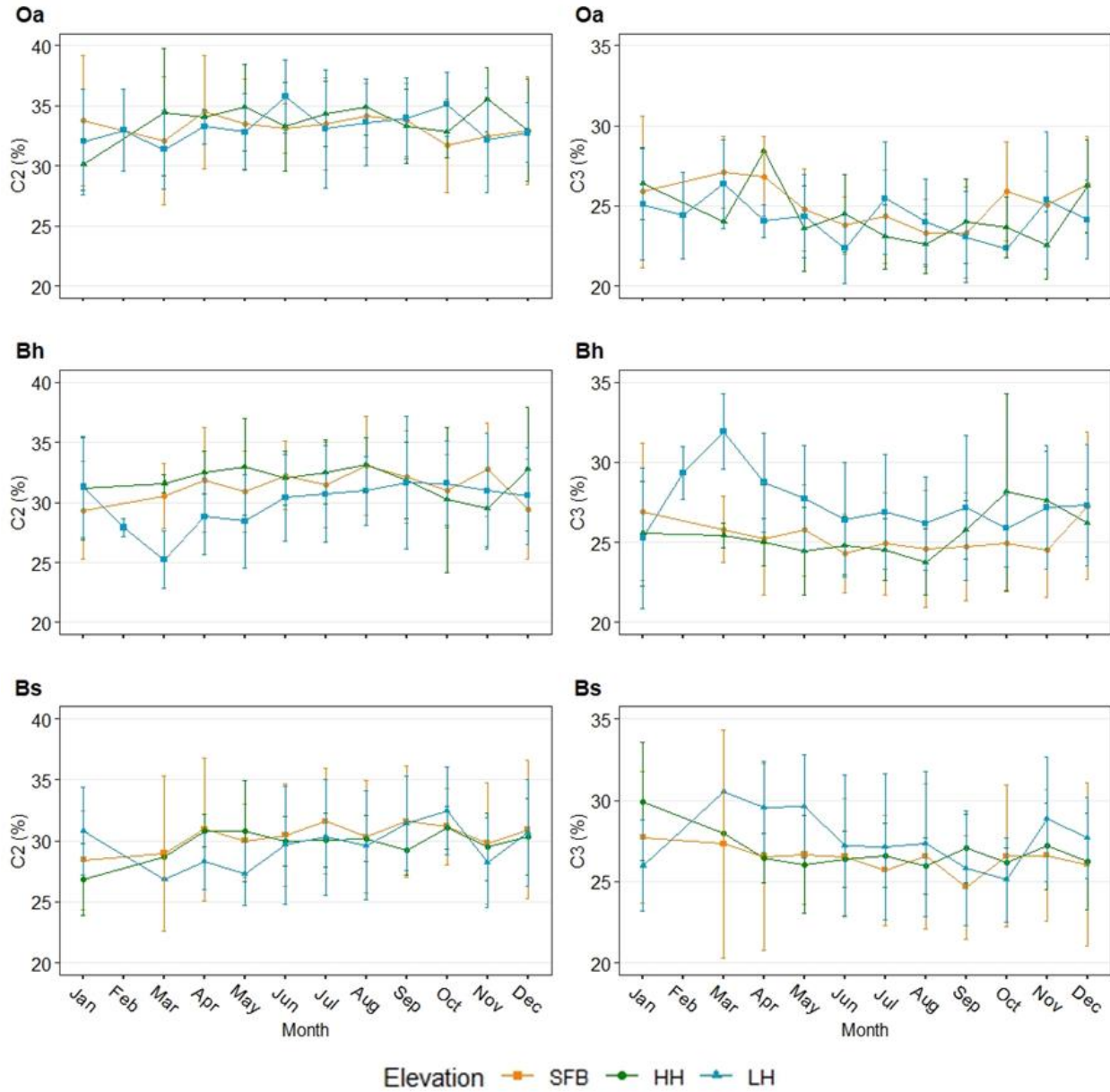ic (C2) and blue-shifted humic acid (C3) fractions (Figure 4). The fulvic acid fraction in low-hardwood mineral soil solutions followed the same seasonal pattern as DOC, increasing in late summer to fall months. Although significantly lower throughout the year, the fulvic fraction in the LH zone exceed values at the higher elevations in October. Similar to DIN, blue-shifted humic acids, peaked in winter and were significantly higher in the LH zone than in upper elevations throughout the year (Figure 4). Additional DOM properties such as FIX and E2:E3 exhibited seasonal and elevational patterns. Both properties were significantly higher in the LH zone than the upper elevational zones and peaked during the spring months with minimum values occurring during the fall (Figure 3).

Vertical patterns

The mean concentration of DOC draining the forest floor of the reference watershed was 1474.7±1087.5 μmol L^{-1}. DOC concentrations significantly decreased with transport through

each subsequent soil horizon and to streamwater (Bh: 858±81.3 µmol L^{-1}; Bs: 622.9±81.3 µmol L^{-1}; Stream: 243.8±248.9 µmol L^{-1}). The horizonal pattern of dissolved organic nitrogen (DON) was similar to that of DOC. DON in the reference watershed also decreased significantly between all successive horizons with depth and with streamwater (Oa: 28.1±18.1 µmol L^{-1}; Bh: 19.0±15.3 µmol L^{-1}; Bs: 13.3±12.7 µmol L^{-1}; Stream: 5.2±2.1 µmol L^{-1}). Concentrations of DIN from both watersheds decreased through all soil horizons to the stream, but only significantly between the Bs and streamwater. The DOC:DON ratio of drainage waters decreased through all soil horizons from forest floor to the stream in the reference watershed (Oa: 72.4±78.8; Bh: 61.5±45.3; Bs: 57.8±44.2; Stream: 41.0±18.1). However, only the difference between the Bs horizon and the stream was statistically significant. Conversely the DOC:DIN ratio in the reference watershed increased from the Oa (134.7±163.8) to the Bs horizon (184.6±244.9), then decreased slightly to 165.7±153.3 in the stream. None of the comparisons were statistically significant, due to high variability.

Optical properties also exhibited horizonal patterns in W6. FIX increased significantly from the Oa horizon solutions (1.62±0.09) to the Bs horizon solutions (1.76±0.07), but not from the Bs horizon (1.76±0.07) to the stream (1.76±0.06). E$_2$:E$_3$ decreased significantly with soil depth, while RIX decreased significantly from the Oa leachate (0.71±0.02) to the stream (0.68±0.01). Specific ultraviolet absorbance (SUVA) also decreased consistently but not significantly at depth in W6.

Calcium treatment effects

Vertical patterns shifted within calcium-treated soil solutions when compared to the reference watershed (Figure 5). Mean DOC and DON concentrations in W1 no longer significantly deceased between the Bh and Bh horizons but were similar. Like at W6, mean DIN

concentrations decreased significantly between all horizons. Concentrations of DOC

(1881.6±1300.9 µmol L^{-1}), DON (44.8±36.8 µmol L^{-1}), and DIN (56.2±60.5 µmol L^{-1}) were all

significantly higher in Oa horizon solutions in W1 than W6. DIN concentrations in the Bh

horizon (31.6±41.2 µmol L^{-1}), and streamwater (14.7±10.2 µmol L^{-1}) were also significantly

higher in W1.

Table 2. p-values for Welch's two-sample t-test comparing means of soil horizon solution concentrations and molar ratios, soil horizon DOM component fractions and optical properties between calcium-treated (W1) and reference (W6) watersheds. Table 1. p-values for Welch's two-sample t-test comparing means of soil horizon solution concentrations and molar ratios, soil horizon DOM component fractions and optical properties between calcium-treated (W1) and reference (W6) watersheds. Tested by a significance level of 0.05. Indicated by bold values.

Soil Horizon	DOC	DON	DIN	DOC:DON	DOC:DIN	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa	**0.009**	**<0.001**	**<0.001**	0.222	0.225	0.449	0.273	0.295	0.945	**0.012**	0.174	0.937
Bh	0.643	0.740	**0.039**	0.071	0.154	0.101	**0.033**	0.056	0.534	**<0.001**	**<0.001**	**0.002**
Bs	0.060	0.059	0.160	0.525	0.109	**0.011**	0.617	0.186	0.575	0.335	**0.021**	**0.003**
Streamwater	0.446	0.304	**<0.001**	0.295	**<0.001**	0.465	0.293	0.339	**<0.001**	**<0.001**	**0.023**	**0.016**

There was little change in DOC:DON between horizons in W1, however, unlike at W6,

the ratios did not consistently decrease, and fluctuated over a range from 57.5.7±101.2 in the

forest floor, to 46.6±26.1 in streamwater (Table). In W1, the DOC:DIN ratio followed the same

horizonal pattern as the reference watershed, increasing (not significantly) from the Oa to the Bs

horizons, with a significant decrease between the Bs horizon (125.7±203.0) and the stream

(47.6±85.5). There were no statistically significant differences for DOC:DON and DOC:DIN

between watershed solutions except for the DOC:DIN ratio in streamwater (Table 2). The mean

stream DOC:DIN ratio in the reference watershed was 348% higher than streamwater in the

treated watershed.

*Figure 5. Average monthly concentrations of **(A)** dissolved organic carbon, **(B)** dissolved organic nitrogen, and **(C)** dissolved inorganic nitrogen in the Oa (n=297), Bh (n=222), Bs (n=223) soil horizon solutions and corresponding stream sites (n=88) in reference and calcium-treated watersheds from 2015-2016.*

Horizonal patterns established among DOM optical properties at W6 were also evident for W1. SUVA significantly decreased from the forest floor (4.44 ± 4.03 L mg C^{-1} m^{-1}) through each soil horizon to the stream (2.51 ± 0.29 L mg C^{-1} m^{-1}). When compared to W6, FIX values at W1 were significantly lower in in the forest floor (W6: 1.62 ± 0.09; W1: 1.59 ± 0.11) but significantly higher in the Bh horizon (W6: 1.74 ± 0.09; W1: 1.69 ± 0.08) and in streamwater (W6: 1.76 ± 0.06; W1: 1.81 ± 0.05). E_2:E_3 values (indicators of molecular size) were also significantly higher in the Bh, Bs, and streamwater at W1 than W6 (Figure). The redox index (RIX) values were significantly higher in the Bh and Bs horizon solutions and streamwater in W1 than W6, but the magnitude of the difference does not suggest these results to be meaningful. SUVA in the streamwater was significantly lower at W1 (2.51 ± 0.29 L mg C^{-1} m^{-1}) than at W6 (2.78 ± 0.39 L mg C^{-1} m^{-1}). DOM fractions remained relatively consistent between both watersheds. *T*-tests revealed the humic fraction (C1) to be significantly higher in Bs solutions at W6, while the fulvic fraction (C2) was significantly lower in the Bh horizon at W1 (Figure 2).

Principal component analysis

Principal component analysis was conducted as a multivariate approach to explore relationships between PARAFAC modeled DOM fractions, their optical properties and quantitively measured analytes. Principal component 1 accounted for 51.0% of the model variance. Principal component 2 accounted for a 12.6% of the model variance. PC1 was most influenced by PARAFAC component fractions 1 & 3, as well as FIX and molecular size (E_2:E_3). PC2 was most influenced by PARAFAC component fraction 1, DOC, DON, and DIN concentrations. Plotting the PCA model scores for individual observations revealed both vertical and treatment patterns. Observations from forest floor solutions had more negative PC1 scores

coupled with a wide range of PC2 scores (Figure 6a). Bh and Bs horizon solutions were more

narrowly confined by PC2 but ranged from negative to fully positive PC1. Streamwater had a

wider range of PC2 scores, but only was found within positive PC1 quadrants. Boxplots of the
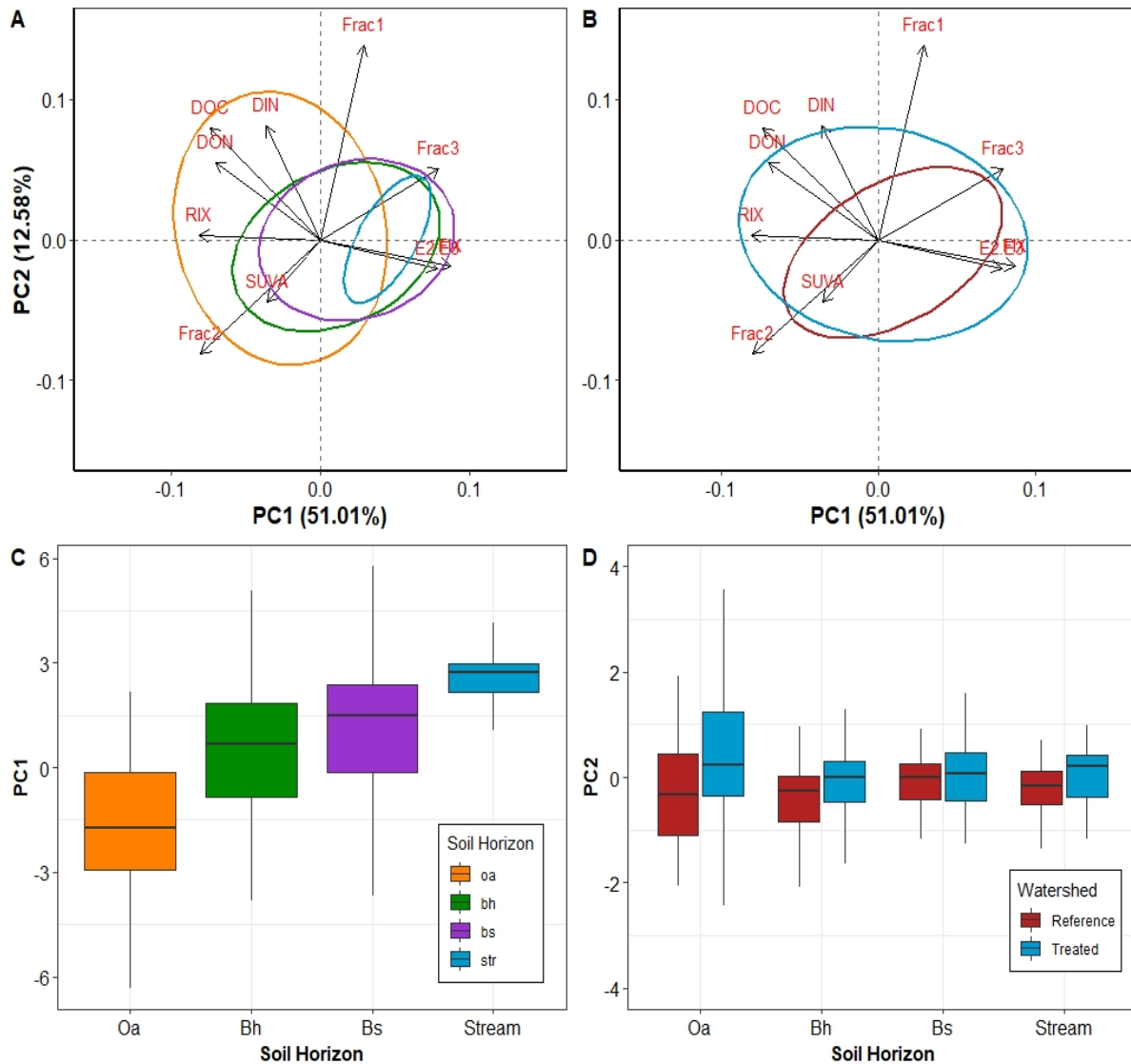
*Figure 4. (**A**) PCA loadings plot where samples scores (n=655) are grouped by soil horizon. (**B**) PCA loadings plot where samples scores (n=655) are grouped by watershed treatment. (**C**) Boxplots of PCA scores for PC1 grouped by soil horizon. (**D**) Boxplots of PCA scores for PC2 grouped by soil horizon and separated by watershed treatment.*

PC1 scores across horizons revealed significant differences among forest floor, mineral horizon

solutions and streamwater (Figure 6c). Grouping each observation by treatment also revealed

differences in the characteristics of DOM from both watersheds (Figure 6b). Boxplots of PC2

scores showed the treatment had a greater effect on Oa horizon and streamwater, rather than the mineral horizons (Figure 6d).

Discussion

Landscape patterns

DOM is highly dependent on source material. Experiments comparing carbon quality within coniferous and deciduous forests have found that coniferous forests generally have higher DOC concentrations and larger, more aromatic, plant-like structures (Thieme et al. 2019). Surprisingly, the highest mean DOC concentrations (2121.4 ± 1209.9 µmol L^{-1}), SUVA values (4.62 ± 4.87 L mg C^{-1} m^{-1}) and fulvic fraction (34.21 ± 3.65 %) in this study were found in the forest floor leachate of the transitional HH zone. Also, the lowest mean FIX value (1.58 ± 0.12) was found in these solutions, indicating a greater input of plant-like terrestrial DOM to soil solution than in the SFB zone (1.60 ± 0.12). Root litter and decaying fine root biomass could be a potential source of carbon to the soil (Persson 2012). Fine root studies in W1 have found that within the HH zone, fine root biomass has declined significantly since $CaSiO_3$ treatment in 1999. This decline has corresponded in a +5.1% change in aboveground live biomass in the HH zone, suggesting tree C allocation may be shifting in response to acid deposition recovery (Fahey et al. 2016).

Differences in hydropedological units could also play a role in explaining observed DOM elevational patterns (Figure 4). Bs soil solutions from the LH zone had the highest "blue-shift" humic fraction (27.33 ± 4.03 %) and fluorescence index value (1.79 ± 0.09). E_2:E_3 values, a measure of molecular size, suggests that DOM was the smallest in LH zone stream water (7.49 ± 1.38) and in LH zone Bs soils (6.87 ± 1.35). Mineral soils are the largest pool of carbon within a forested ecosystem (Fahey et al. 2005). At the HBEF, mineral soil thickness generally

decreases inversely with elevation, but high soil horizon variability at the catena scale has made

organic carbon pools difficult to quantify accurately (Johnson et al. 2000; Fahey et al. 2005).

Closer examination of water table fluctuations and carbon content in the Bhs and Bimodal

podzols found in HH and LH zones suggests that these soils may be important carbon sinks

(Bailey et al. 2014). Repeated wetting and drying cycles due to the landscape position of these

hydropedological units could connect labile carbon to microbial colonies within soil pores

leading to further processing (Bailey et al. 2017). The bimodal podzols which are prominent at

lower elevations have been found to be more consistently saturated (Bailey et al. 2014). This

pattern may suggest that low-elevation mineral soils have considerable potential for microbial

DOM inputs.

Vertical patterns

Vertical patterns in soil solution DOC and DON concentrations observed are similar to

those previously reported at Hubbard Brook (Dittman et al. 2007), where the highest

concentrations were found in the forest floor and values decreased consistently through the
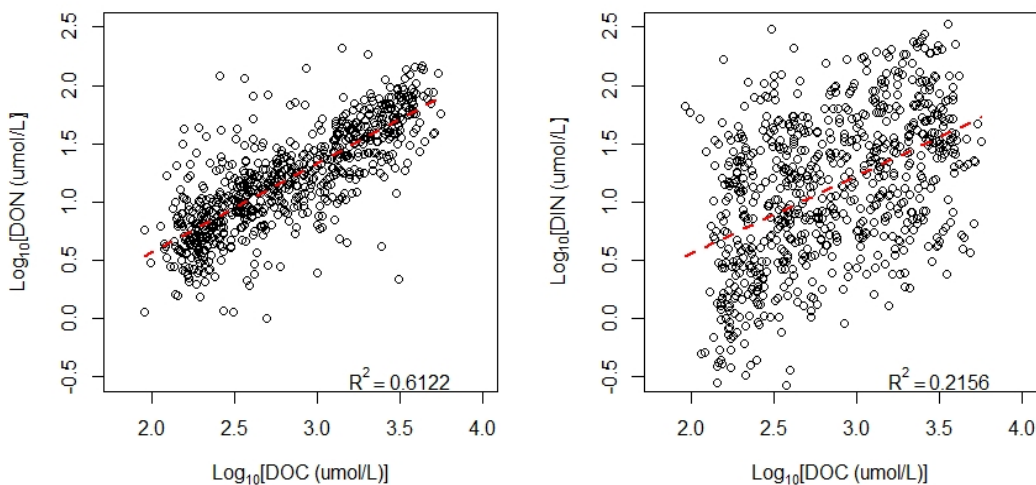
Figure 5. Log-log linear relationships between DOC, and organic, and inorganic nitrogen (p-value<0.001 for both).

mineral soil to eventual export in streamwater (Table 1). This pattern is not surprising since

DOM is mobilized from litter decomposition and fine root turnover and immobilized in the

mineral soil. Components of DON and DOC are highly correlated (Figure 8). There is also a log-log linear relationship between DOC and DIN, though less highly correlated than DON. NH_4 and NO_3^- are products of the nitrification process, beginning at organic nitrogen. It is reasonable to hypothesize that DOC exerts some control over inorganic nitrogen in soil solution and stream water. A recent nitrate tracer study concluded that soil organic matter can be an important sink for inorganic nitrogen, especially within mineral soils (Fuss et al. 2019).   Note that the DOC:DON ratio decreased vertically in the reference watershed, but these changes were not statistically significant. DIN from both watersheds also decreased with soil depth, and in streamwater leading, to increasing DOC:DIN ratios as expected.

The conceptual model for DOM processing (Kaiser & Kalbitz (2012); Appendix A) suggests that as DOM drains through the soil, microbial processing would consume carbon and release nitrogen, thus decreasing the C:N ratio. Vertical patterns of DOM quality support this model where microbial metabolites were of a greater abundance than terrestrial sources in the lower mineral soils. Additionally, DOM was observed to be less aromatic, more oxidized with a smaller molecular size, at soil depth. DOM fractions also support the processing theory. As soil depth increases, fulvic acid (C2) abundance decreased while "blue shifted" humic acids (C3) increased (Figure 2). The relative composition of the humic acid fraction (C1) did not change with soil depth suggesting there is a large pool of recalcitrant humic DOM buffering the composition of this fraction in soil solutions.   Previous ^{13}C NMR analysis of soil organic matter at HBEF found that Alkyl C dominated the proportion of carbon across the Oa, Bh, and Bs soil horizons. Additionally, O-alkyl C fractions decreased, and aromatic C fractions increase at soil depth (Ussiri and Johnson 2003). This pattern supports current observations of DOM

components and suggests dissolved organic matter is highly dependent on soil carbon composition.

Vertical patterns were also revealed through multivariate analysis of all chemical species, DOM fractions, and DOM optical properties. Observations from the Oa horizon leachate had more negative PC1 scores and were characterized by: a high fulvic acid fraction, highly reduced and aromatic DOM, and high nutrient concentrations (Figure 6a). In contrast, observations from streamwater had exclusively positive PC1 scores, indicating: a high "blue shifted" humic fraction, high microbial sourcing, low molecular size, and highly oxidized DOM. Mineral soils transitioned from negative to positive PC1 scores (Figure 6c). Note that PC1 was not strongly influenced by the C1 humic fraction. However, PC2 was and observations from the Oa horizon solutions had higher PC2 values than those from mineral soil solutions and streamwater.

Calcium treatment effects

Comparison for a "deacidification effect" on DOC and DON concentrations saw significant increases for both species, in forest floor leachate in W1. However, this pattern of increase did not result in comparable increases in mineral soil and stream solutions over the period of observations. Additionally, DIN concentrations were 205% greater in the forest floor solutions at W1, in additional to being significantly higher in the Bh horizon and stream concentrations (Figure 5). This pattern could be due in part to reduced plant N uptake in response to declining fine root biomass as trees shift to aboveground carbon allocation (Fahey et al. 2016) watershed. However, the mechanism for this short-term release in nitrate is relatively unknown and should be explored in future research.

Closer examination of DOC and DON concentrations in mineral soils indicate there might also be greater rates of nitrogen immobilization taking place in W1. DOC (W6:

858.6±542.9 µmol L^{-1}; W1: 815.1±815.8 µmol L^{-1}) and DON (W6: 19.0±15.3 µmol L^{-1}; W1: 19.9±16.4 µmol L^{-1}) concentrations in Bh horizons from both watersheds were relatively similar. Concentrations for both species decreased significantly with depth in the reference watershed, but not in the treated watershed (Figure 5b). This pattern suggests either more DOM is being retained in W6 lower mineral soils, or more SOM is being processed due to increased microbial activity in W1. Higher concentrations of DON and DIN in W1 Bs soil solutions support the latter, but the two scenarios are not mutually exclusive. Given greater DOM retention in W6 mineral soils, we would expect to see lower concentrations in streamwater there than at W1, but instead we see the opposite (W6: 243.8±248.85 µmol L^{-1}; W1: 214.3±81.3 µmol L^{-1}). This observation would appear to support theories of higher carbon flow to lower mineral soils, potentially stimulating microbial respiration and N immobilization in deacidified forests (Bailey et al. 2017; Groffman et al. 2018). However, high DIN concentrations suggest N mineralization has been stimulated also.

Further evidence is provided by comparisons of DOM quality between the watersheds. FIX was significantly lower in the forest floor at W1. This pattern suggests greater inputs of plant-like organic matter in W1, which could be driven by increased litterfall (Battles et al. 2014), increased late-stage little decomposition (Lovett et al. 2016), and fine root death (Fahey et al. 2016; Persson 2012). Also, FIX was significantly higher in Bh horizon solutions and streamwater, an indication of more microbially processed DOM in the calcium-treated watershed. RIX and E$_2$:E$_3$ are also significantly higher in W1 mineral soil solutions and streamwater, which indicate a greater degree of DOM quinone oxidation status and lower molecular size, which may be transported further into the soil profile and eventually stream export. DOM was also significantly less aromatic in the stream export of the calcium-treated

watershed, possibly due to microbial processing favoring more complex, labile compounds (Bailey et al. 2017).

These results differ from a previous study of DOM quality in HBEF stream water from disturbed watersheds (Cawley et al. 2014). While both studies found no significant difference between PARAFAC components identified in W1 and W6, the results presented in this paper found significant differences in SUVA, FIX, RIX, and molecular size. This discrepancy could be due to differences in data set sizes, or statistically analyses, but are most likely due to differences in collection. This study collected from the stream along an elevational gradient, while the other study only collected from the stream outlet (Cawley et al. 2014).

Grouping observations by treatment through multivariate analysis also showed diverging characteristics between the watersheds. Observations from the treated watershed had higher PC2 scores but relatively similar PC1 scores (Figure 6d). DOM from the calcium-treated watershed can be characterized by: a higher microbial humic fraction, higher nutrient content, and lower aromaticity than DOM than the reference watershed. This pattern suggests DOM from deacidified forests, that has been cycled through soils and microbial processed, is less likely to be colored (Kaiser & Kalbitz 2012; Kothawala et al. 2014). It also suggests that highly colored DOM contributing to lake "browning" has bypassed soil cycling before entering surface waters (Brothers et al. 2014; Kothawala et al. 2014).

Future work could include statistical analysis of seasonal patterns of DOM processing as well as how those patterns have changed over the long-term. Additionally, focus on organic carbon and nitrogen fluxes between calcium-treated and reference watersheds could determine whether differences in hydrologic or biological factors are driving differences in forest carbon transport. Additionally, collections and analysis of the DOM quality of fresh organic matter and

microbial biomass, as well as litter decomposition studies could help quantify DOM end-members in the aquatic system and provide further insight into DOM sourcing.

Conclusions

Dissolved organic matter is highly variable depending on its sourcing and the biogeochemical processes enacted upon it. PARAFAC-identified DOM components, fluorescence spectral characteristics, and analyte concentrations have revealed horizonal patterns of DOM transformation consistent with a conceptual model for DOM processing. In catenas where typical Spodosol soils gave way to podzols influenced heavily by their landscape position and dominant vegetation, DOM composition changed from more plant-like, aromatic structures in conifer-dominated high elevation zones, to more microbially sourced DOM inputs at low elevations. Unusually high DOC concentrations and characteristics of plant-like DOM inputs in the high elevation hardwood zone suggested fine root turnover contributed to soil organic matter. Improvements to the calcium status of the forest floor in W1 have potentially caused this shift. Higher concentrations of DOC, DON, and DIN along with increased microbial inputs and declines in DOM molecular size suggest there is enhanced processing within the calcium-treated watershed, but not enough to cause an ecosystem shift in reactive nitrogen uptake.

Appendices
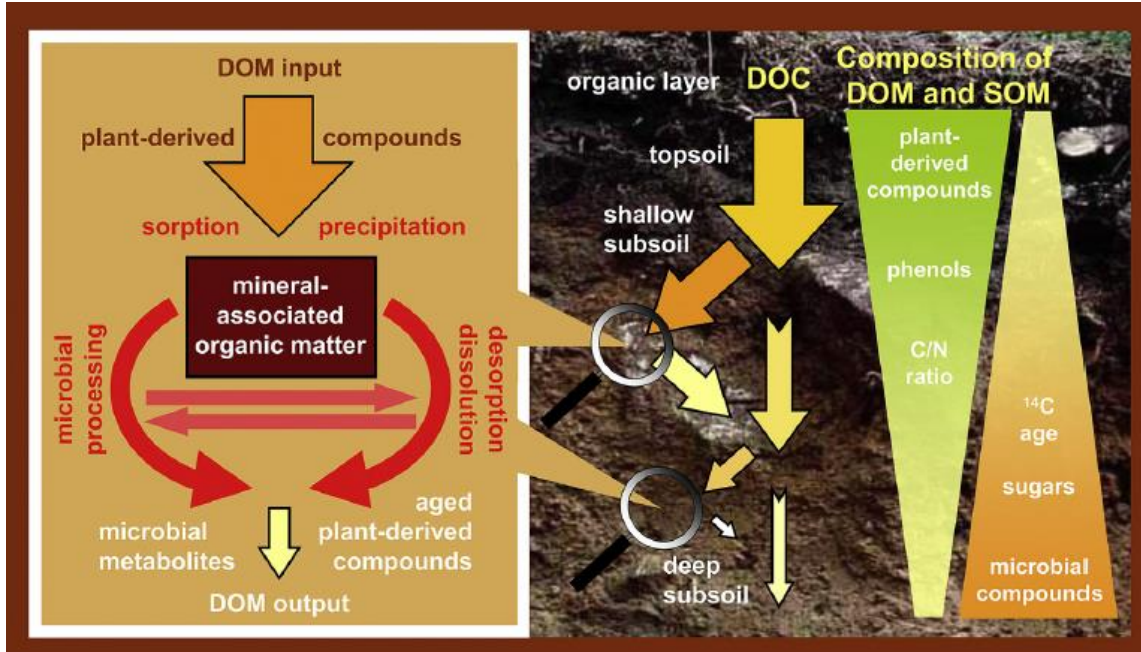
A. DOM processing in Spodosols (Kaiser & Kalbiz 2012)
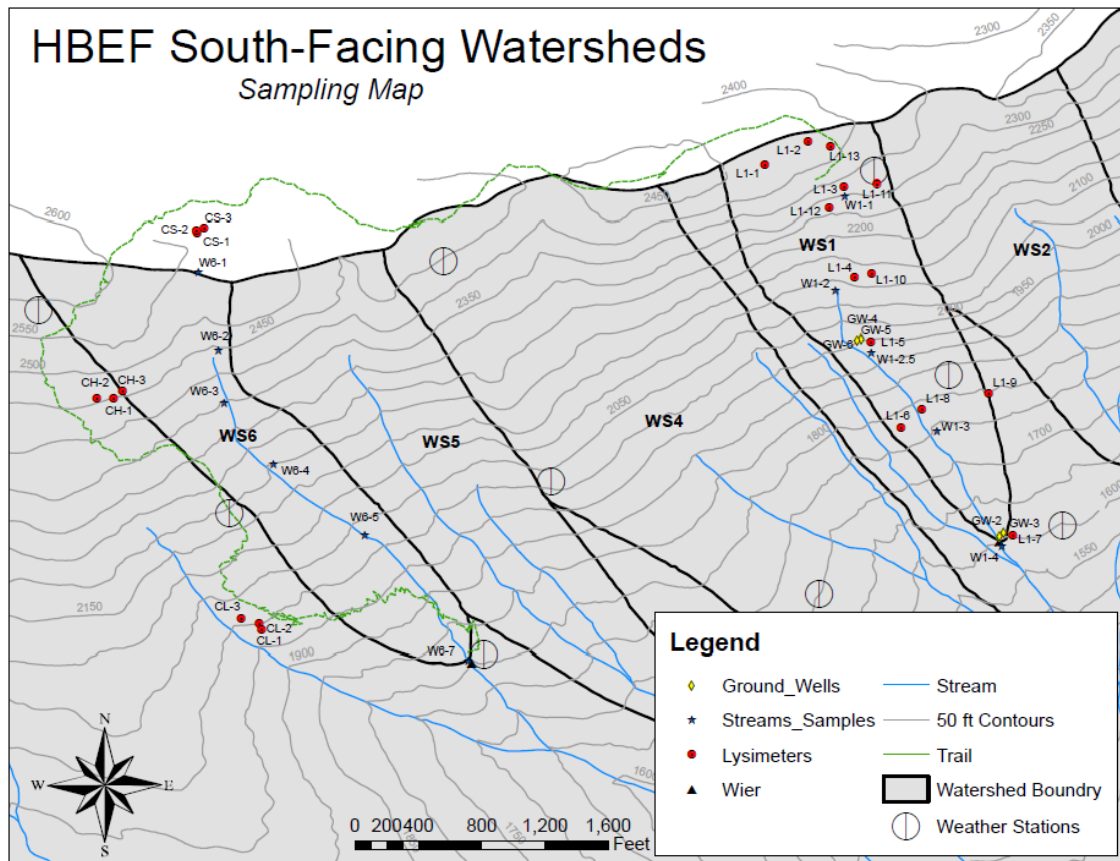
B. Soil solution, stream, and ground water sampling sites in calcium-treated (W1) and reference (W6) watersheds.
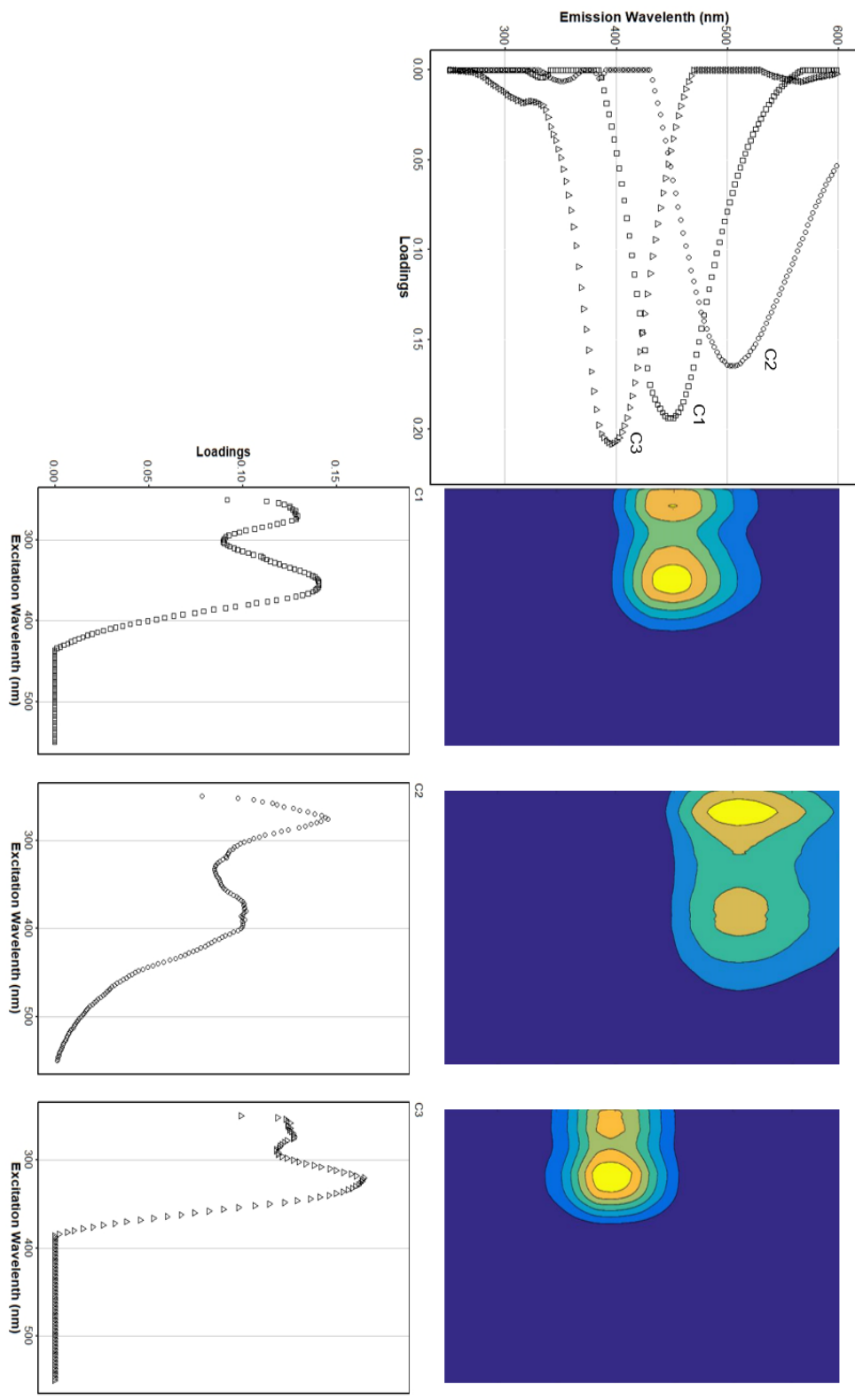
C. Three component PARAFAC model excitation & emission loadings and 3-D contour.

D. Characteristics of three PARAFAC DOM components identified in this study.

Component	Excitation maximum (nm)	Emission maximum (nm)	Kothawala et al. 2014	Cory & McKnight (2005)	Possible Sourcing	Description
C1	360 (275)	450	C4	C1	Terrestrial	Humic acid fluorophore group
C2	275 (380)	504	C3	-	Terrestrial	Fulvic acid fluorophore group
C3	320 (275)	395	C2	-	Terrestrial	Blue-shifted Humic fluorophore group

E. Mean volume-weighted concentrations (µmol/L) and standard deviations (in parentheses) for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), also DOC:DON and DOC:DIN molar ratios. Values derived from soil solution (Oa, Bh, Bs) and streamwater from spruce-fir-birch zone (SFB), high-elevation hardwood (HH) zone, and low-elevation hardwood (LH) zone, at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

Soil Horizon & Subcatchment	DOC Mean	DOC SD	DON Mean	DON SD	DIN Mean	DIN SD	DOC/DON Mean	DOC/DON SD	DOC/DIN Mean	DOC/DIN SD
Oa horizon										
SFB	2030.3	1440.1	42.1	37.1	45.6	57.5	59.1	35.9	137.6	224.6
HH	2121.4	1209.9	47.6	29.6	54.7	49.8	66.3	78.0	69.1	67.0
LH	1285.9	890.1	35.4	32.4	50.0	61.3	59.9	142.7	113.7	185.4
Bh horizon										
SFB	1150.0	815.7	24.2	15.9	34.7	48.4	55.1	37.7	158.3	306.3
HH	945.1	710.3	23.5	17.1	36.8	33.9	60.4	88.6	78.5	134.0
LH	454.6	504.6	13.4	13.3	17.1	29.0	43.9	31.8	111.5	130.9
Bs horizon										
SFB	1172.8	1043.5	22.1	18.4	17.1	26.2	63.4	50.2	205.9	50.2
HH	482.9	275.8	11.8	6.0	29.4	31.5	50.9	42.5	67.9	103.1
LH	354.9	423.4	9.5	8.0	13.9	40.0	44.5	32.9	107.6	136.1
Stream water										
SFB	470.5	289.3	7.6	4.9	10.1	7.5	56.7	23.2	89.6	113.8
HH	355.7	377.7	5.6	2.8	12.1	11.2	52.7	12.5	40.9	34.8
LH	183.0	45.8	5.3	2.0	8.0	9.7	40.8	22.8	120.0	146.8

F. Means and standard deviations (in parentheses) for DOM component fractions (%), specific ultra-violet absorbance (L mgC-1 m-1), fluorescence index (I470:I520), redox index (Qred/[Qred+Qox]), molecular size (E2:E3). Values derived from soil solution (Oa, Bh, Bs) and streamwater from spruce-fir-birch zone (SFB), high-elevation hardwood (HH) zone, and low-elevation hardwood (LH) zone, at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

Soil Horizon & Subcatchment	C1 (%)		C2 (%)		C3 (%)		SUVA		FIX		RIX		E2:E3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oa horizon														
SFB	43.05	1.21	33.39	3.84	23.56	3.50	4.54	5.44	1.60	0.12	0.70	0.02	5.13	0.80
HH	42.69	1.09	34.21	3.65	23.10	2.99	4.62	4.87	1.58	0.12	0.72	0.02	5.02	0.55
LH	42.68	1.19	33.56	3.73	23.76	3.15	4.25	4.05	1.60	0.10	0.71	0.01	4.90	0.65
Bh horizon														
SFB	43.20	1.02	31.83	3.84	24.96	3.34	4.13	4.25	1.70	0.09	0.70	0.02	5.68	0.94
HH	42.94	0.81	31.99	3.16	25.07	2.83	3.44	1.93	1.71	0.06	0.70	0.01	5.64	1.10
LH	42.82	0.92	30.27	4.13	26.91	4.02	2.83	1.19	1.77	0.09	0.69	0.02	6.52	1.46
Bs horizon														
SFB	43.19	1.28	30.61	4.37	26.21	4.23	3.02	0.91	1.74	0.11	0.69	0.02	6.27	1.57
HH	43.38	0.63	29.95	2.61	26.67	2.36	2.82	0.50	1.78	0.06	0.69	0.01	6.52	0.93
LH	42.85	0.93	29.83	4.22	27.33	4.03	2.68	1.37	1.79	0.09	0.68	0.02	6.87	1.35
Stream water														
SFB	43.24	0.19	31.03	3.82	25.73	3.77	2.95	0.29	1.75	0.08	0.68	0.02	6.11	1.00
HH	42.99	0.95	29.62	4.34	27.39	3.63	2.86	0.53	1.77	0.09	0.68	0.01	6.66	1.44
LH	43.38	0.73	27.30	2.45	29.32	2.41	2.59	0.30	1.79	0.05	0.67	0.01	7.49	1.38

G.. p-values for Welch's two-sample t-test comparing mean horizon differences from the reference (W6) watershed. Concentrations of dissolved organic carbon (DOC, dissolved organic nitrogen (DON, and dissolved inorganic nitrogen (DIN), as well as their stoichiometry, were tested by a significance level of 0.05. Values derived from soil solution (Oa, Bh, Bs) and streamwater at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

Soil Horizon	Reference solute cocentration (µmol/L) and molar ratios				
	DOC	DON	DIN	DOC:DON	DOC:DIN
Oa&Bh	**<0.001**	**0.003**	0.238	0.350	0.514
Bh&Bs	**0.016**	**0.027**	0.361	0.650	0.543
Bs &Streamwater	**<0.001**	**<0.001**	**0.040**	**0.009**	0.651

H. p-values for Welch's two-sample t-test comparing mean horizon differences from the calcium-treated (W1) watershed. Concentrations of dissolved organic carbon (DOC, dissolved organic nitrogen (DON, and dissolved inorganic nitrogen (DIN), as well as their stoichiometry, were tested by a significance level of 0.05. Values derived from soil solution (Oa, Bh, Bs) and streamwater at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

Soil Horizon	Treated solute concentration (µmol/L) & molar ratios				
	DOC	DON	DIN	DOC:DON	DOC:DIN
Oa&Bh	**<0.001**	**<0.001**	**<0.001**	0.244	0.882
Bh&Bs	0.982	0.190	**0.016**	0.345	0.494
Bs &Streamwater	**<0.001**	**<0.001**	**0.027**	0.255	**<0.001**

I. p-values for Welch's two-sample t-test comparing mean horizon differences from the reference (W6) watershed. DOM component fractions (%), specific ultra-violet absorbance (L mgC-1 m-1), fluorescence index (I470:I520), redox index (Qred/[Qred+Qox]), molecular size (E2:E3) were tested by a significance level of 0.05 . Values derived from soil solution (Oa, Bh, Bs) and streamwater at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

Soil Horizon	Reference DOM fractions & optical properties						
	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa&Bh	0.688	**0.001**	**<0.001**	0.137	**<0.001**	**0.021**	**<0.001**
Bh&Bs	**0.001**	**0.002**	**0.013**	0.138	**<0.001**	**<0.001**	**<0.001**
Bs &Streamwater	0.444	**<0.001**	**<0.001**	0.357	0.785	**<0.001**	**0.002**

J. p-values for Welch's two-sample t-test comparing mean horizon differences from the calcium-treated (W1) watershed. DOM component fractions (%), specific ultra-violet absorbance (L mgC-1 m-1), fluorescence index (I470:I520), redox index (Qred/[Qred+Qox]), molecular size (E2:E3) were tested by a significance level of 0.05 . Values derived from soil solution (Oa, Bh, Bs) and streamwater at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA (2015-2016).

| Soil Horizon	Treated DOM fractions & optical properties						
	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa&Bh	0.066	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Bh&Bs	0.667	0.099	0.071	**0.013**	**0.014**	**<0.001**	**0.002**
Bs &Streamwater	**0.022**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

K. p-values for Welch's two-sample t-test comparing means of soil horizon concentrations and molar ratios, soil horizon DOM component fractions and optical properties, between calcium-treated (W1) and reference (W6) watersheds. Tested by a significance level of 0.05.

| Soil Horizon	DOC	DON	DIN	DOC:DON	DOC:DIN	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa	**0.009**	**<0.001**	**<0.001**	0.222	0.225	0.449	0.273	0.295	0.945	**0.012**	0.174	0.937
Bh	0.643	0.740	**0.039**	0.071	0.154	0.101	**0.033**	0.056	0.534	**<0.001**	**<0.001**	**0.002**
Bs	0.060	0.059	0.160	0.525	0.109	**0.011**	0.617	0.186	0.575	0.335	**0.021**	**0.003**
Streamwater	0.446	0.304	**<0.001**	0.295	**<0.001**	0.465	0.293	0.339	**<0.001**	**<0.001**	**0.023**	**0.016**

L. p-values for Welch's two-sample t-test comparing means of soil horizon DOM component fractions and optical properties, between calcium-treated (W1) and reference (W6) watersheds. Tested by a significance level of 0.05.

Soil Horizon	C1 (%)	C2 (%)	C3 (%)	SUVA	FIX	RIX	E2:E3
Oa	0.449	0.273	0.295	0.945	**0.012**	0.174	0.937
Bh	0.101	**0.033**	0.056	0.534	**<0.001**	**<0.001**	**0.002**
Bs	**0.011**	0.617	0.186	0.575	0.335	**0.021**	**0.003**
Streamwater	0.465	0.293	0.339	**<0.001**	**<0.001**	**0.023**	**0.016**
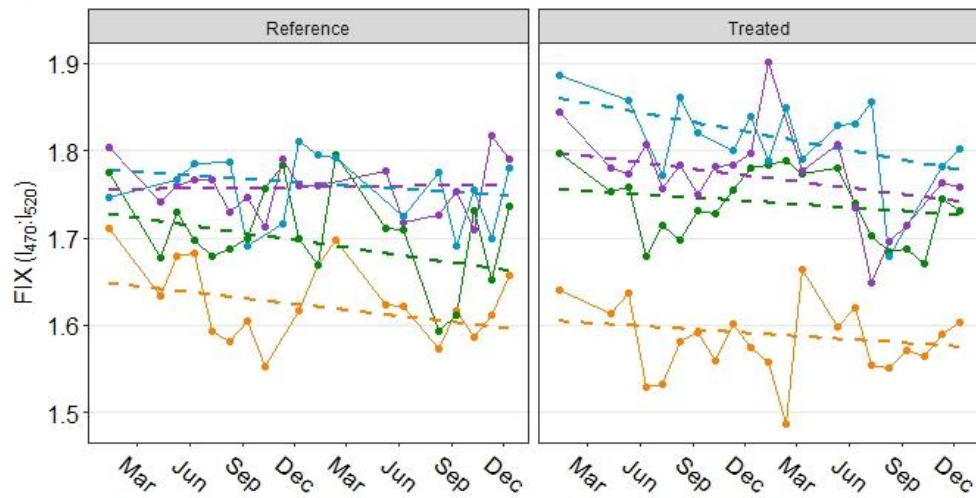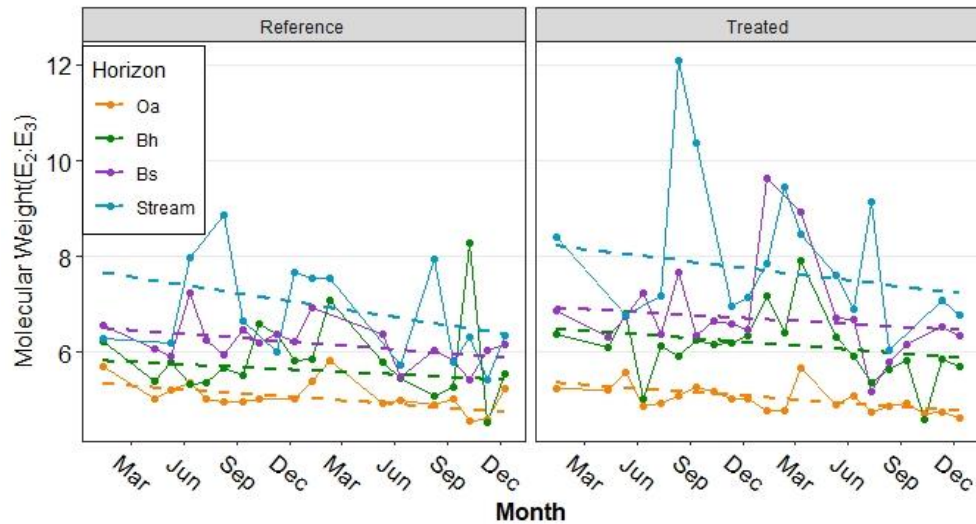
*M. Average monthly values for (**A**) Fluorescence Index and (**B**) E2:E3 (molecular size) in the Oa (n=297), Bh (n=222), Bs (n=223) soil horizons and corresponding stream sites (n=88) in calcium-treated and references watersheds from 2015-2016.*
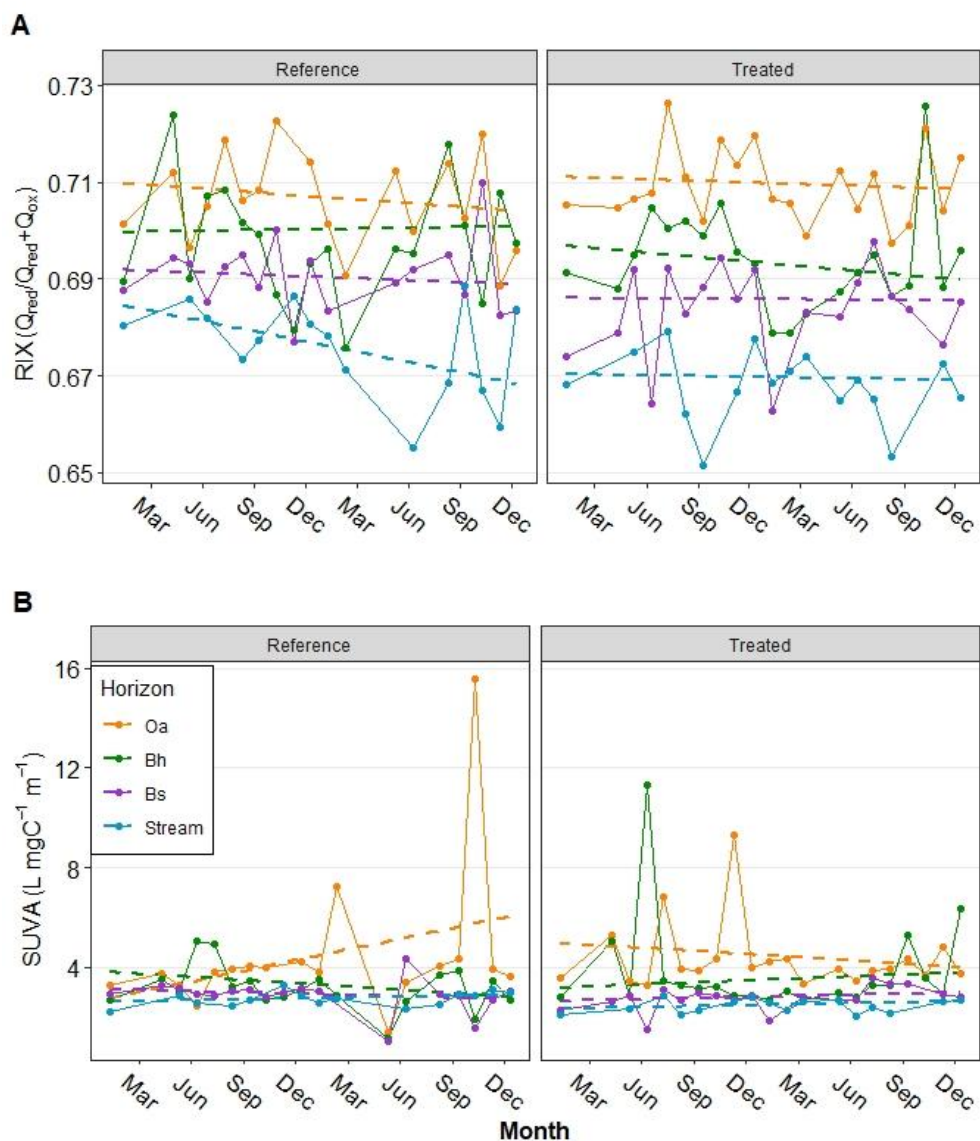
N. Average monthly values for (A) Redox Index and (B) Specific Ultraviolet Absorbance in the Oa (n=297), Bh (n=222), Bs (n=223) soil horizon solutions and corresponding stream sites (n=88) in reference and calcium-treated watersheds from 2015-2016.

References

Bailey AS, Hornbeck JW, Campbell JL, Eagar C (2003) Hydrometeorological database for Hubbard Brook Experimental Forest: 1955-2000. U.S. Department of Agriculture, Forest Service, Northeastern Research Station, Newtown Square, PA

Bailey SW, Brousseau PA, McGuire KJ, Ross DS (2014) Influence of landscape position and transient water table on soil development and carbon distribution in a steep, headwater catchment. Geoderma 226–227:279–289. doi: 10.1016/j.geoderma.2014.02.017

Bailey VL, Smith AP, Tfaily M, et al (2017) Differences in soluble organic carbon chemistry in pore waters sampled from different pore size domains. Soil Biology and Biochemistry 107:133–143. doi: 10.1016/j.soilbio.2016.11.025

Battin TJ, Kaplan LA, Findlay S, et al (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 1:95–100. doi: 10.1038/ngeo101

Battles JJ, Fahey TJ, Driscoll CT, et al (2014) Restoring Soil Calcium Reverses Forest Decline. Environmental Science & Technology Letters 1:15–19. doi: 10.1021/ez400033d

Brothers S, Köhler J, Attermeyer K, et al (2014) A feedback loop links brownification and anoxia in a temperate, shallow lake. Limnology and Oceanography 59:1388–1398. doi: 10.4319/lo.2014.59.4.1388

Campbell JL, Driscoll CT, Eagar C, et al (2007) Long-term trends from ecosystem research at the Hubbard Brook Experimental Forest. U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA

Catalán N, Obrador B, Pretus JLl (2014) Ecosystem processes drive dissolved organic matter quality in a highly dynamic water body. Hydrobiologia 728:111–124. doi: 10.1007/s10750-014-1811-y

Cawley KM, Campbell J, Zwilling M, Jaffé R (2014) Evaluation of forest disturbance legacy effects on dissolved organic matter characteristics in streams at the Hubbard Brook Experimental Forest, New Hampshire. Aquatic Sciences 76:611–622. doi: 10.1007/s00027-014-0358-3

Chiasson-Gould SA, Blais JM, Poulain AJ (2014) Dissolved organic matter kinetically controls mercury bioavailability to bacteria. Environmental Science and Technology 48:3153–3161. doi: 10.1021/es4038484

Cho Y, Driscoll CT, Blum JD (2009) The effects of a whole-watershed calcium addition on the chemistry of stream storm events at the Hubbard Brook Experimental Forest in NH, USA. Science of The Total Environment 407:5392–5401. doi: 10.1016/j.scitotenv.2009.06.030

Cho Y, Driscoll CT, Johnson CE, Siccama TG (2010) Chemical changes in soil and soil solution after calcium silicate addition to a northern hardwood forest. Biogeochemistry 100:3–20. doi: 10.1007/s10533-009-9397-6

Clark JM, Chapman PJ, Heathwaite AL, Adamson JK (2006) Suppression of Dissolved Organic Carbon by Sulfate Induced Acidification during Simulated Droughts. Environmental Science & Technology 40:1776–1783. doi: 10.1021/es051488c

Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry 51:325–346. doi: 10.1016/0304-4203(95)00062-3

Cory RM, McKnight DM (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

Cronan CS, Schofield CL (1990) Relationships between Aqueous Aluminum and Acidic Deposition in Forested Watersheds of North America and Northern Europe. Environ Sci Technol 24:1100–1105

Davidson EA, David MB, Galloway JN, et al Excess Nitrogen in the U.S. Environment: Trends, Risks, and Solutions. Issues Ecol 1–16

De Haan H, De Boer T (1987) Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic Lake Tjeukemeer. Water Research 21:731–734. doi: 10.1016/0043-1354(87)90086-8

Dittman JA, Driscoll CT, Groffman PM, Fahey TJ (2007) of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. Ecology 88:1153–1166. doi: 10.1890/06-0834

Driscoll CT, Driscoll KM, Fakhraei H, Civerolo K (2016) Long-term temporal trends and spatial patterns in the acid-base chemistry of lakes in the Adirondack region of New York in response to decreases in acidic deposition. Atmospheric Environment 146:5–14. doi: 10.1016/j.atmosenv.2016.08.034

Driscoll CT, Lawrence GB, Bulger AJ, et al (2001) Acidic Deposition in the Northeastern United States: Sources and Inputs, Ecosystem Effects, and Management Strategies. BioScience 51:180. doi: 10.1641/0006-3568

Driscoll CT, Whitall D, Aber J, et al (2003) Nitrogen Pollution in the Northeastern United States: Sources, Effects, and Management Options. BioScience 53:357. doi: 10.1641/0006-3568

Fahey TJ, Heinz AK, Battles JJ, et al (2016) Fine root biomass declined in response to restoration of soil calcium in a northern hardwood forest. Canadian Journal of Forest Research 46:738–744. doi: 10.1139/cjfr-2015-0434

Fahey TJ, Siccama TG, Driscoll CT, et al (2005) The Biogeochemistry of Carbon at Hubbard Brook. Biogeochemistry 75:109–176. doi: 10.1007/s10533-004-6321-y

Fellman JB, Hood E, Spencer RGM (2010) 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

Fröberg M, Jardine PM, Hanson PJ, et al (2007) Low dissolved organic carbon input from fresh litter to deep mineral soils. Soil Science Society of America Journal 71:347. doi: 10.2136/sssaj2006.0188

Fuss CB, Driscoll CT, Johnson CE, et al (2011) Dynamics of oxidized and reduced iron in a northern hardwood forest. Biogeochemistry 104:103-119. doi: 10.1007/s10533-010-9490-x

Fuss CB, Lovett GM, Goodale CL, et al (2019) Retention of nitrate-N in mineral soil organic matter in different forest age classes. Ecosystems. doi: 10.1007/s10021-018-0328-z

Groffman PM, Driscoll CT, Durán J, et al (2018) Nitrogen oligotrophication in northern hardwood forests. Biogeochemistry 141:523–539. doi: 10.1007/s10533-018-0445-y

Groffman PM, Rustad LE, Templer PH, et al (2012) Long-Term Integrated Studies Show Complex and Surprising Effects of Climate Change in the Northern Hardwood Forest. BioScience 62:1056–1066. doi: 10.1525/bio.2012.62.12.7

Jaffé R, McKnight D, Maie N, et al (2008) Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. Journal of Geophysical Research: Biogeosciences 113. doi: 10.1029/2008JG000683

Johnson CE, Driscoll CT, Siccama TG, Likens GE (2000) Element Fluxes and Landscape Position in a Northern Hardwood Forest Watershed Ecosystem. Ecosystems; New York 3:159–184. doi: http://dx.doi.libezproxy2.syr.edu/10.1007/s100210000017

Johnson CE, Johnson AH, Siccama TG (1991) Whole-Tree Clear-Cutting Effects on Exchangeable Cations and Soil Acidity. Soil Science Society of America Journal 55:502. doi: 10.2136/sssaj1991.03615995005500020035x

Kaiser K, Guggenberger G, Haumaier L (2004) Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. Biogeochemistry 70:135–151. doi: 10.1023/B:BIOG.0000049340.77963.18

Kaiser K, Kalbitz K (2012) Cycling downwards – dissolved organic matter in soils. Soil Biology and Biochemistry 52:29–32. doi: 10.1016/j.soilbio.2012.04.002

Kothawala DN, Stedmon CA, et al (2014) Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. Global Change Biology 20:1101-1114. Doi: 10.1111/gcb.12488

Li W, Johnson CE (2016) Relationships among pH, aluminum solubility and aluminum complexation with organic matter in acid forest soils of the Northeastern United States. Geoderma 271:234–242. doi: 10.1016/j.geoderma.2016.02.030

Likens GE, Buso DC (2006) Variation in streamwater chemistry throughout the Hubbard Brook Valley. Biogeochemistry 78:1–30

Lloret J, Valiela I (2016) Unprecedented decrease in deposition of nitrogen oxides over North America: the relative effects of emission controls and prevailing air-mass trajectories. Biogeochemistry 129:165–180. doi: 10.1007/s10533-016-0225-5

McKnight DM, Boyer EW, Westerhoff PK (2001) Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity. Limnology and Oceanography 46:38–48

Miller MP, DM McKnight, RM Cory, MW Williams, RL Runkel (2006) Hyporheic exchange and humic redox reactions in an alpine stream/wetland ecosystem, Colorado Front Range. Environmental Science and Technology 40:5943–49

Monteith DT, Stoddard JL, Evans CD, et al (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature 450:537–540. doi: 10.1038/nature06316

Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. PARAFAC. Analytical Methods 5:6557. doi: 10.1039/c3ay41160e

Peters SC, Blum JD, Driscoll CT, Likens GE (2004) Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. Biogeochemistry 67:309–329. doi: 10.1023/B:BIOG.0000015787.44175.3f

Persson HÅ (2012) The high input of soil organic matter from dead tree fine roots into the forest soil. International Journal of Forestry Research 2012:1–9. doi: 10.1155/2012/217402

Peters SC, Blum JD, Driscoll CT, Likens GE (2004) Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. Biogeochemistry 67:309–329. doi: 10.1023/B:BIOG.0000015787.44175.3f

Raymond PA, Saiers JE, Sobczak WV (2016) Hydrological and biogeochemical controls on watershed dissolved organic matter transport: pulse-shunt concept. Ecology 97:5–16. doi: 10.1890/14-1684.1

Schmidt MWI, Torn MS, Abiven S, et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. doi: 10.1038/nature10386

Shao S, Driscoll CT, Johnson CE, et al (2015) Long-term responses in soil solution and stream-water chemistry at Hubbard Brook after experimental addition of wollastonite. Environmental Chemistry. doi: 10.1071/EN15113

Solomon CT (2017) Dissolved organic matter causes genetic damage in lake zooplankton via oxidative stress. Functional Ecology 31:806-807. doi: 10.1111/1365-2435.12807

Stedmon CA, Bro R (2008) Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial: Fluorescence-PARAFAC analysis of DOM. Limnology and Oceanography: Methods 6:572–579. doi: 10.4319/lom.2008.6.572

Stedmon CA, 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(2):686-697.

Thieme L, Graeber D, Hofmann D, et al (2019) Dissolved organic matter characteristics of deciduous and coniferous forests with variable management: different at the source, aligned in the soil. Biogeosciences 16:1411–1432. doi: 10.5194/bg-16-1411-2019

Tonitto C, Goodale CL, Weiss MS, et al (2014) The effect of nitrogen addition on soil organic matter dynamics: a model analysis of the Harvard Forest Chronic Nitrogen Amendment Study and soil carbon response to anthropogenic N deposition. Biogeochemistry 11:431–454

Ukonmaanaho L, Starr M, Lindroos A-J, Nieminen TM (2014) Long-term changes in acidity and DOC in throughfall and soil water in Finnish forests. Environmental Monitoring and Assessment 186:7733–7752. doi: 10.1007/s10661-014-3963-7

USGCRP, 2018: Impacts, risks, and adaptation in the United States: Fourth National Climate Assessment, Volume II [Reidmiller, D.R., C.W. Avery, D.R. Easterling, K.E. Kunkel, K.L.M. Lewis, T.K. Maycock, and B.C. Stewart (eds.)]. U.S. Global Change Research Program, Washington, DC, USA, 1515 pp. doi: 10.7930/NCA4.2018

Ussiri DAN, Johnson CE (2003) Characterization of organic matter in a northern hardwood forest soil by 13C NMR spectroscopy and chemical methods. Geoderma 111:123–149. doi: 10.1016/S0016-7061(02)00257-4

Weishaar JL, Aiken GR, Bergamaschi BA, et al (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

Williamson CE, Overholt EP, Pilla RM, et al (2016) Ecological consequences of long-term browning in lakes. Scientific Reports 5. doi: 10.1038/srep18666

Yanai RD, Vadeboncoeur MA, Hamburg SP, et al (2013) From missing source to missing sink: long-term changes in the nitrogen budget of a northern hardwood forest. Environmental Science & Technology 47:11440–11448. doi: 10.1021/es4025723

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Education

2019   MS, Environmental Engineering Science
            Civil & Environmental Engineering, Syracuse University, Syracuse, NY

2016   BS, Environmental Science (Magna Cum Laude)
            SUNY College of Environmental Science & Forestry, Syracuse, NY

2013   AAS, Environmental Technology: Geosciences (Magna Cum Laude)
            Onondaga Community College, Syracuse, NY

Appointments

2018-2019   EMPOWER Research Fellow, EMPOWER NSF Research Traineeship Program,
            Syracuse University, Syracuse, NY

2016-2018   QA/QC Coordinator, Hubbard Brook Long Term Ecological Research Project
            Syracuse University, Syracuse, NY

2015-2016   Laboratory Technician, Department of Civil & Environmental Engineering
            Syracuse, Univeristy, Syracuse, NY

2015   Soil & Water Intern
            Cayuga County Soil & Water Conservation District, Auburn, NY

Awards

2019   Water Initiative Fellowship, Syracuse University
2018   EMPOWER Graduate Fellowship, Syracuse University
2014   ESF Presidential Scholarship
2014   Phi Theta Kappa Scholarship
2013   OCC Curriculum Honor Award: Environmental Technology

Teaching & Service

2016-2019   Academic Integrity Council Graduate Student Representative
            Syracuse University, Syracuse, NY
2016-2018   Mentor, Department of Civil & Environmental Engineering Undergraduate Research (REU)
            Program, Syracuse University
2016   Engineers without Borders
            Trees for Lights Project, Peru
2015   Teaching Assistant, SUNY ESF
            Introduction to Soils
2013-2014   Teaching Assistant, Onondaga Community College
            Intro to GIS & Advanced GIS
2011-2012   Vineyard International Ministries, Kitale, Kenya

Selected Presentations

2019   Hubbard Brook Annual Cooperator's Meeting
Dissolved organic matter dynamics in calcium-treated and reference watersheds at Hubbard Brook Experimental Forest

2019   Hubbard Brook LTER Midterm Review
Dissolved organic matter transformation in calcium-treated and reference watersheds at HBEF

2018   Hubbard Brook Annual Cooperator's Meeting
Spatial Patterns of Dissolved Organic Matter Quality

2017   Ecological Society of America National Conference
Temporal Patterns of Nitrogen Retention in Calcium-Treated and Reference Watersheds at the Hubbard Brook Experimental Forest

2017   Hubbard Brook Annual Cooperator's Meeting
Temporal Patterns of Nitrogen Retention in Calcium-Treated and Reference Watersheds

Skills & Languages

- Laboratory Data Quality Assurance/Quality Control (QA/QC) Program
- Standard Operating Procedure Writing (SOP)
- Statistical Analysis Software (SAS), R Statistical Computing Language, Visual MINTEQ, ArcGIS, ArcScene, AutoCAD
- Laboratory Techniques: Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Ion Chromatography (IC), Fluorescence Spectrophotometry, Total Carbon Analysis, and Continuous Flow Analyses