

Capturing and Characterizing Soluble Organic Matter Dynamics in Soil Formation
Processes

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ABSTRACT

Dissolved organic matter (DOM) is a highly complex, heterogeneous mix of compounds with diverse functional groups that contribute to several environmental processes such as organo-mineral complexation, nutrient bioavailability, and mineral dissolution. Because of these contributions of DOM to important ecosystem processes, it is often of interest to quantify the flux of DOM moving through different parts of ecosystems. Unfortunately, the complexity and variability of DOM makes quantification and chemical analysis of fluxes challenging. This thesis has two components, the first examines the potential of using four different resins for the purpose of quantifying time-integrated DOM fluxes across two source (e.g. Douglas fir and Yellow poplar) and concentration (30 and 5 mg C/L) leaf-extracts. The second explores how water soluble organic matter (WSOM) changes along spatial gradients of podzolization in a northeast glaciated headwater catchment. Findings from the resin study suggest that quaternary amine Cl^- resins with a cross-linked polyacrylamide matrix and gel structure have the best suitability for in-situ sampling of DOM over time. While these resins only captured and allowed for the analysis of ~ 30% of dissolved organic carbon (C) in a series of laboratory studies, it is recognized that only ~50% of natural DOM may be ionized and sorbed electrostatically. Thus, for mass balance approaches, the use of resins would require an adjustment factor to better estimate soluble loads. Though, the observed robustness across source and

concentration suggests that resins may be appropriate for indexing DOM fluxes to compare across space, time, or treatments. The second portion of this study examined chemical characteristics of water-soluble organic matter (WSOM) extracted from soils and of DOM sampled from shallow groundwater wells. Quantification of WSOM carbon content and spectroscopic analyses were used to compare samples based on genetic horizon and to compare differences along gradients of lateral and vertical podzolization. Findings show that there were significant trends in WSOM characteristics along podzolization horizon sequences which are indicative of microbial processing along the hillslope. Comparing spatial development of podzols (e.g. lateral versus vertical) found that WSOM in laterally developed E horizons are more microbial in nature when compared to vertically developed E horizons. There were also significant trends between WSOM extractions and groundwater collected from zones of soil development along a hillslope transect, which suggests some homogenization of WSOM as it is processed and transported downslope. This is evidenced by corresponding trends in fluorescence index, freshness index, and protein percent that were indicative of biogeochemical changes due to microbial processing and complexation. Characterizing WSOM can help predict trends in podzolization, and can help identify hotspots of biogeochemical processing.

Capturing and Characterizing Soluble Organic Matter Dynamics in Soil Formation Processes

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GENERAL AUDIENCE ABSTRACT

Dissolved organic matter (DOM) is made up of many different compounds that collectively contribute to several important environmental processes. The quantity and chemistry of DOM are known to vary by location on the landscape. Often, these differences are important indicators of ecosystem properties or processes. Despite the importance of DOM to ecosystem processes, sampling and analysis remain a key challenge because of DOM variability over time. This research tested a way to passively sample how much DOM moves through a location over a period of time using resins, which attract and bind a large proportion of DOM compounds so that they can be quantified. Overall, I found that one type of resin, with specific chemical and physical structure, was better at attracting and quantifying DOM and may be useful in comparing the quantity of DOM that moves through a system over time. Separately, I also looked at the chemical characteristics of DOM in different types of soils at different points along a forested hillslope in order to better understand spatial patterns of important soil processes. The results of this work identified trends in fluorescence characteristics of DOM, and indicate changes in DOM between different types of soil along the hillslope as they undergo microbial processing. There is also evidence from comparing water and soil samples that DOM from different sources become more similar as the DOM is processed and transported downslope. Characterizing DOM can help predict trends with regards to

important environmental processes and allows us to identify hotspots of nutrient sources and sinks in forests.

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Introduction

Dissolved organic matter (DOM) contributes to several environmental processes such as organo-mineral complexation and transport, nutrient bioavailability, and mineral dissolution (Chantigny, 2003; Gabor et al., 2015; T Ohno, Amirbahman, & Bro, 2007). DOM is a highly complex, heterogeneous mix of compounds with diverse functional groups including strongly acidic functional groups that deprotonate at $\text{pH} > \sim 3$, thus allowing the DOM to serve as an organic acid (Drever and Stillings 1997, Fakhraei and Driscoll, 2015; Sandron et al., 2015). The presence of DOM in low ionic strength solutions gives DOM a strong acidifying effect, often resulting in mineral dissolution and complexation, organo-metal transport, and deposition, such as is common in the process of podsolization (Drever & Stillings, 1997; Lazo, Dyer, & Alorro, 2017; Varadachari, Barman, & Ghosh, 1991).

Hydrology and soil development processes have been frequently studied at the catchment-scale. This is particularly true at the Hubbard Brook Experimental Forest (HBEF) (Bailey et al., 2014; Bourgault et al., 2015; Detty & McGuire, 2010; Gannon et al., 2015; Likens & Buso, 2006; Zimmer et al., 2013), where such studies have led to an improved understanding of how differing hydrologic flow regimes and water chemistry affect patterns of soil development along the hillslope. Quantifying DOM flux and changes in chemical characteristics as these processes occur has not been as thoroughly elucidated. Thus, this thesis has a two-fold approach to the exploration of DOM: first, to support an ongoing project focused on a whole watershed catchment analysis of weathering processes, the need arose to develop a method that could be viable in quantifying DOM flux with relation to hydrology and nutrient fluxes. As this piece of the

research developed, I began to explore a second group of questions that address how chemical characteristics of DOM change along the zones of podzolization as it undergoes complexation and biotic processing. Also, whether or not the characteristics of DOM extracted directly from soils, are reflective of DOM sampled from wells in specific zones of podsolization along a hillslope transect. DOM samples from wells likely represents water accumulating from upslope sources, but may actually be more reflective of water from the soils in the immediate area.

The first chapter examines a passive sampling technique that employs anion exchange resins for the purpose of quantifying time-integrated DOM flux. The laboratory experiment tested: (i) pretreatment, (ii) dissolved organic carbon sorption, (iii) dissolved organic carbon desorption, and (iv) overall efficacy for five quaternary anion resins. Two types of leaf-foliage extracts were tested at two concentrations of dissolved organic carbon (DOC) that are naturally occurring in forest ecosystems. Matrix and structure type in these resins were found to contribute significantly to the variance in the carbon content sorbed and extracted. These findings help guide resin selection for measuring time-integrated DOC flux in natural systems.

The second chapter builds off of findings observed from Zimmer et al. (2013), Gillin et al. (2015), and Bourgault et al. (2015, 2017) which explain spatial patterns of stream water chemistry and podzolization. While water and soil chemistry have been shown to be significantly different along transects in headwater catchments, the study of DOM quality has not been evaluated. This chapter examines chemical characteristics of water soluble organic matter (WSOM) extracted from soils and of DOM sampled from shallow groundwater wells. Measurements of carbon content and spectroscopic analyses

to determine fluorescing properties, were used to compare samples based on genetic horizon and position along a hillslope catena to compare differences along spatially distinct landscape zones. Findings suggest that WSOM characteristics were significantly distinct based on podsolization sequence, but that spatial development (e.g. lateral vs. vertical) of soil horizons matters little to WSOM composition.

Chapter 1: Evaluating the efficacy of exchange resins for the passive sampling of dissolved organic matter

1.0 Introduction

Naturally occurring dissolved organic matter (DOM) is a highly complex, heterogeneous mix of proteins, organic acids (OAs), complex carbohydrates, and other compounds (McDowell, 2003) with significance to ecosystem processes such as weathering, soil formation, plant-microbe interactions, and elemental cycling (Fakhraei & Driscoll, 2015; Sandron et al., 2015). Because of these contributions of DOM to important ecosystem processes, it is often of interest to quantify the flux of DOM moving through different parts of ecosystems. Traditionally, this has been done by pairing estimates of hydrologic flux with concentrations determined from grab samples or tension lysimeters (Susfalk and Johnson, 2006; Fröberg et al., 2007; Karavanova and Milanovski, 2015). Such approaches are particularly problematic for two reasons: first, the accuracy of estimating DOM flux is constrained by hydrologic modelling (Michalzik, Kalbitz, Park, Solinger, & Matzner, 2001). Second, instantaneous grab sampling often fails to capture the dynamics in DOM concentration over time, and efforts to collect all of the soil solution (e.g., zero-tension lysimeters), are challenged by finite collection volumes (Robertson et al., 1999). One promising method to overcome these common limitations lies in the use of passive sampling techniques, like anion exchange resins, to provide time-integrated DOM fluxes (Annable et al., 2005; Hatfield et al., 2004; Lam & Simpson, 2006; Sandron et al., 2015).

Ion exchange resins were originally designed for the removal of DOM from industrial and municipal wastewater systems (Abrams & Millar, 1997). They have been commonly used and tested for passive sampling of nutrient loads in various ecosystem

settings (Fenn et al., 2005; Fenn et al., 2002; Lam & Simpson, 2006; Langlois et al., 2003; Susfalk & Johnson, 2006). However, there is no standardized method for their selection and use in quantifying natural DOM loads and fluxes. This is due, in part, because of the multiple factors that must be considered when selecting and handling resins for such deployments, such as pretreatment, sorption capacity, desorption methods and efficacy, preferential sorption, and potential to contribute background contamination of the analyte of interest.

While many resin studies focused on inorganic nutrients, Langlois et al. (2003) was one of the first to include efficacy of resins for the absorption of organic N and P as components of DOM. They tested three brands of mixed-bed resin with and without pretreatment as suggested by (Kjønaas, 1999b). Resins were extracted with either 2 *M* KCl or 2 *M* HCl. Recovery in resins pretreated and extracted with KCl accounted for no more than 40% of initial DOP and DON, and they concluded that using resins for monitoring of N is not recommended due to large background contamination from resin amine groups. In 2006, Lam and Simpson proposed a method for passive sampling and isolation of DOM in aquatic environments using diethylaminoethyl cellulose (DEAE-C), a weak anion exchanger with a protonated amine functional group. Traditionally macroporous XAD (polymethyl methacrylate) has been used to bind DOM (Sandron et al., 2015), but one challenge of these resins is that they must be maintained at low pH for optimal functionality, whereas DEAE-C had been shown to isolate up to 90% of DOM from neutral pH waters, and after two-weeks of equilibration, DEAE-C removed 72 – 89% of DOM from laboratory solutions (Lam & Simpson, 2006). Warner et al. (2015) used the methods from Lam and Simpson to test for differences in quality of DOM

collected from DEAE-C resins deployed for in-situ sampling, and concluded that resins preferentially collected the more complex plant-like DOM compounds versus DOM from microbial sources.

Despite the suitability of resins such as DEAE-C for isolation of DOM, these resins have become increasingly difficult to source. Widespread interest in the novel use of resins to quantify DOM still exists, but in seeking alternative options, there has been little work quantitatively demonstrating resin efficacy across resin type in a systematic way that can help guide resin selection for future work. The objective of this study was to **evaluate a suite of commercially available resins with different structures and matrices for their efficacy and feasibility of use for the purpose of quantifying time-integrated DOM flux in water and soil.** Specifically, I will address the following practical considerations:

- 1) resin pretreatment approaches necessary to minimize background interference and facilitate sorption of DOM,
- 2) variability in the composition of natural DOM from two sources (e.g. hardwood and conifer),
- 3) concentration dependence of DOM sorption onto resins, and
- 4) ability to remove sorbed SOM for subsequent analysis,

in order to determine overall resin efficacy to serve as a time-integrated passive sink for mobile DOM.

2.0 Methods

2.1 Resin selection

Though the results from Lam and Simpson (2006) were promising for organic contaminants and DEAE-C is evidenced to work with a broad range of natural organic compounds, DEAE-C is increasingly difficult to source as its use in chromatography has largely been replaced by quaternary ammonium compounds for such applications.

Though, even if available, the initial preparation of DEAE-C for use in passive sampling is time intensive, with the complete process taking close to 1 month to prepare for deployment (Lam & Simpson, 2006). Thus, based on these considerations we have chosen four quaternary amines candidate resins for testing, in a 2 x 2 factorial of resin matrix (cross-linked polyacrylamide vs. cross-linked polystyrene) and resin form (microporous vs. gel) as outlined in Table 1. For additional comparison and reference, we included Amberlite IRA-400, an anion exchange resin commonly used in the quantification of inorganic N and P fluxes (Kjønnaas, 1999a; Kjønnaas, 1999b; Langlois et al., 2003; Chabani et al., 2006) as well as for water demineralization and treatment of complexed metals in waste (Rohm & Haas, 2005). According to available manufacturer literature, Lewatit S5128 is effective in adsorption and desorption of naturally occurring organic matter, especially from surface water (Lanxess Energizing Chemistry, 2016). Lewatit S5528 is suitable for decolorization of solutions of organic products and recommended for rapid removal of relatively high concentrations of organic substances (Lanxess Energizing Chemistry, 2014a). Lewatit S6268 is suitable for the removal of acid and decolorization of solutions of organic substances and is used for the treatment of waters containing high concentrations of organics (Lanxess Energizing Chemistry, 2014b). Lewatit S6368 is good at adsorption of organic substances and for partial

adsorption of organic and mineral acids, this resin also exhibits easy desorption (Lanxess Energizing Chemistry, 2014c).

Table 1: Resin attributes from manufacturer's literature.

Resin	Matrix	Structure	Form	Stable pH range	Total capacity (eq/L)	Density (g/mL)	Max temp (°C)
Lewatit S5128	Cross-linked polyacrylamide	Gel	Cl ⁻	0 – 12	1.25	1.09	30
Lewatit S5528	Cross-linked polyacrylamide	Macroporous	Cl ⁻	0 – 12	0.85	1.08	80
Lewatit S6268	Cross-linked polystyrene	Gel	Cl ⁻	0 – 12	1.2	1.08	90
Lewatit S6368	Cross-linked polystyrene	Macroporous	Cl ⁻	0 – 12	1.0	1.06	85
Amberlite IRA-400	Polystyrene divinylbenzene copolymer	Macroporous	Cl ⁻	0 – 14	1.4	N/A	76

2.2 Resin Pretreatment

The purpose of resin pretreatment is to minimize interference with analytes of interest (i.e., remove extant DOM) and facilitate sorption of DOM by covering the resin surface with an easily exchangeable counter ion (e.g., Cl⁻, OH⁻). Amberlite IRA-400 has previously been tested for optimal pre-treatment by (Kjønaas, 1999a), and was thus excluded from this portion of the analysis.

In the pretreatment phase of testing, three pretreatments solutions recommended from the manufacturer for industrial applications and other studies (Kjønaas, 1999a; Kjønaas, 1999b; Fenn et al., 2002; Langlois et al., 2003) were tested: 2 M NaCl, 1 M NaOH, and 2 M NaCl + 1 M NaOH. For each combination of resin and pretreating solution, 150 ± 1.0 g of resin was weighed into acid-washed polypropylene bottles. Then, a volume of pre-treatment solution was added to each bottle in a 20:1 pretreatment solution (mL):resin mass (g) ratio. Bottles were shaken on a fixed-speed reciprocal shaker (Eberbach Model E6010) at 180 osc min⁻¹ for one hour to equilibrate. Following

equilibration, resins were allowed to settle, the pre-treatment solution was decanted, and the resins were rinsed in triplicate with reverse osmosis deionized (RODI) water. The final rinse with RODI water was decanted through 150 μm mesh screen in order to capture the resins from the bottle for subsequent deployment.

The ideal pretreatment option would result in a low background contribution of DOC (mg L^{-1}) from the resin. So, to evaluate the effectiveness of pretreatment, pretreated resins were equilibrated with 0.01 M KCl to represent “blank” source water. To test the resins for background interference after pretreatment, 5 ± 0.1 g of each resin was weighed into 125 mL polypropylene bottles in replicates of 5, then 100 ± 1.0 mL of 0.01 M KCl was added to each bottle (20:1 KCl volume (mL): resin mass (g)). Again, bottles were shaken on a fixed-speed reciprocal shaker (Eberbach Model E6010) at 180 osc min^{-1} , this time for 24 h. Following the 24 h period, resins were allowed to settle, the solution was decanted into 20 mL scintillation vials and analyzed on a Shimadzu TOC-L Total Organic Carbon series analyzer for DOC concentration.

2.3 DOM Sorption

DOM sorption was evaluated by comparing DOC concentrations in source water solutions before and after resin equilibration. This was done as a three-way factorial experimental design with fixed effects (5x2x2 factors with 5 replications). The main factors were: resin type (see Table 1), DOM source (hardwood vs. coniferous foliage leaf-extracts), and DOC concentration (30 vs. 5 mg C L^{-1}).

To begin, DOM solutions were made from fresh tree foliage collected from either Douglas fir (*Pseudotsuga menziesii*) needles or Yellow poplar (*Liriodendron tilipifera*)

leaves. These species were chosen as different sources of DOM to test whether different foliage inputs affect DOM sorption. Litter from coniferous species often have higher percentages of waxes and lignin which are not as rapidly decomposed by microbes relative to hardwood litter (Hobbie et al., 2006). Fresh leaves were collected in August 2018 and oven dried at 60°C for 3 days or until there was a constant mass. Then, 6 g of dried biomass was added to 4 L of RODI water and extracted at 80°C for 24 hours on a magnetic stir plate. Higher temperature (90°C) and longer extraction times (48 hr) were tested, but resulted in visible microbial growth in the water. Concentrated leaf-extractions were filtered through GF/F 0.45 µm filters and then analyzed on a Shimadzu TOC-L Series Total Organic Carbon analyzer for DOC concentration. The concentrated leaf-extraction stock solutions were then diluted to high (30 mg C L⁻¹) and low (5 mg C L⁻¹) concentrations using DI water to represent concentrations that exist naturally in candidate systems for resin deployment (e.g., Bailey et al., 2019; McDowell & Likens, 1988)

Once the four stock solutions were prepared (2 sources x 2 concentrations), the candidate resins were analyzed for sorption efficacy. Each resin was pretreated using 2 M NaCl pre-treatment solution and the pre-treatment method described above (previously identified to contribute the least amount of residual DOM to the source solutions; see Results below). For each resin by solution combination, 5 ± 0.1 g of resin were weighed into to a 125 mL polypropylene bottle, then 100 ± 1.0 mL of source water was added (20:1 source volume (mL):resin mass (g)). For each resin, five blank replicates were created in the same fashion using 0.1 M KCl in order to account for any background inputs from the resins. Bottles were shaken on a fixed-speed reciprocal shaker (Eberbach Model E6010) for 24 hours on low speed (180 osc min⁻¹). After allowing resins to settle,

the source water was decanted through 150 μm mesh into 20 mL scintillation vials for analysis on a Shimadzu TOC-L series Total Organic Carbon analyzer for DOC.

Proportion of mg C sorbed was calculated as follows:

$$\text{proportion sorbed} = \frac{m_i - (m_f - (m_b * m_r))}{m_i} \quad (1)$$

where m_i is the mass of source C (mg), m_f is the final mass of C (mg), m_b is the mass of C (mg per g of resin) in blank samples, and m_r is the mass of resin (g). The resins were stored in their bottles at 4°C until the recovery phase (below) which was no longer than one week.

2.4 DOM Recovery

Following sorptive equilibrations (above), each resin by source by concentration combination was evaluated for the recovery of the sorbed DOC. Important considerations in DOC recovery are the composition and concentration of the extracting solution, the ratio of resin to extractant, and the number of extractions. In order for this study to remain tractable, we relied on previous literature to guide decisions on the composition and concentration of the extracting solution and the ratio of resin to extractant, focusing instead on the number of extractions required to efficiently maximize recovery. Common extractants are 1 and 2 M KCl (Kjønaas, 1999a; Kjønaas, 1999b; Fenn et al., 2002; Langlois et al., 2003). Due to the need to balance analytical detection limits with salt concentration for most DOC analyzers, we chose to use 1 M KCl as it has shown to be equally effective as 2 M KCl (Kjønaas, 1999a; Kjønaas, 1999b; Fenn et al., 2002; Langlois et al., 2003). At these concentrations, KCl has been shown to optimally exchange sorbed analytes at a ratio of 8-12:1 (extractant volume (mL):resin mass (g));

Kjønaas, 1999a; b; Fenn et al., 2002; Susfalk and Johnson, 2006). At these concentrations and ratios, consecutive extractions are often recommended to maximize recovery (M. E. Fenn et al., 2002; Kjønaas, 1999a; Kolberg et al., 1997; Susfalk & Johnson, 2006).

Here we quantified the increased recovery across three successive equilibrations with a 12:1 ratio of 1 M KCl to resin. Specifically, 60 ± 1.0 mL of 1 M KCl were added to a polypropylene bottle containing 5 g of resin recovered from the end of the sorption experiment (above). Bottles were shaken on a fixed-speed reciprocal shaker (Eberbach Model E6010) at 180 osc min^{-1} for one hour to equilibrate. Resins were allowed to settle, then the extracting solution was decanted into 20 mL scintillation vials and analyzed on a Shimadzu TOC-L Series Total Organic Carbon analyzer for DOC concentration.

Proportion of mg C recovered was calculated as:

$$\textit{proportion desorbed} = \frac{m_c}{m_s} \quad (2)$$

where m_s is the mass of C sorbed (mg) and m_c is the corrected mass of C (mg) extracted is calculated by:

$$m_c = m_e - m_B - m_r \quad (3)$$

where m_e is the mass of C (mg) extracted, m_B is the mass of C (mg) from blank samples, and m_r is the mass of C (mg) in residual solution after decanting in the previous step.

Total resin efficacy was then calculated from the proportion sorbed, and proportion recovered as:

$$\textit{proportion sorbed} * \textit{proportion desorbed} \quad (4)$$

Statistical Analysis

Pre-treatment analysis was done using an ANOVA two-way factorial model with fixed effects. Proportion of mg C sorbed, and mg C desorbed in the 1st, 2nd, cumulative extractions, and resin efficacy were independently analyzed using an ANOVA three-way factorial model. Mean plots and Tukey's HSD were used for comparison of interaction and main effects. All analyses were performed in JMP Pro (v. 15.0.0; SAS Institute Inc., 2019) using a significance level of $\alpha = 0.05$.

3.0 Results

3.1 Pre-treatment

A two-way ANOVA with pretreatment method and resin type as main effects showed a significant interaction effect ($p < 0.0001$) for background levels of DOC (mg C L⁻¹) (Figure 1). Resins treated with 2 M NaCl decreased the background level of DOC the most ranging from 1.1 ± 0.22 to 9.5 ± 0.99 mg C L⁻¹, while resins treated with 1 M NaOH varied in contribution of DOC, ranging from 10 ± 0.10 to 110 ± 1.6 mg C L⁻¹. The addition of NaOH to the pre-treatment process, even when mixed with NaCl, also resulted in higher background contribution of DOC, ranging from 3.3 ± 0.04 to 67 ± 9.1 mg C L⁻¹. Following this finding, all resins were pre-treated with 2 M NaCl for the remainder of the study. Amberlite resin was not included in this portion of experimentation because of well documented and recommended start up procedures (Kjønaas, 1999a).

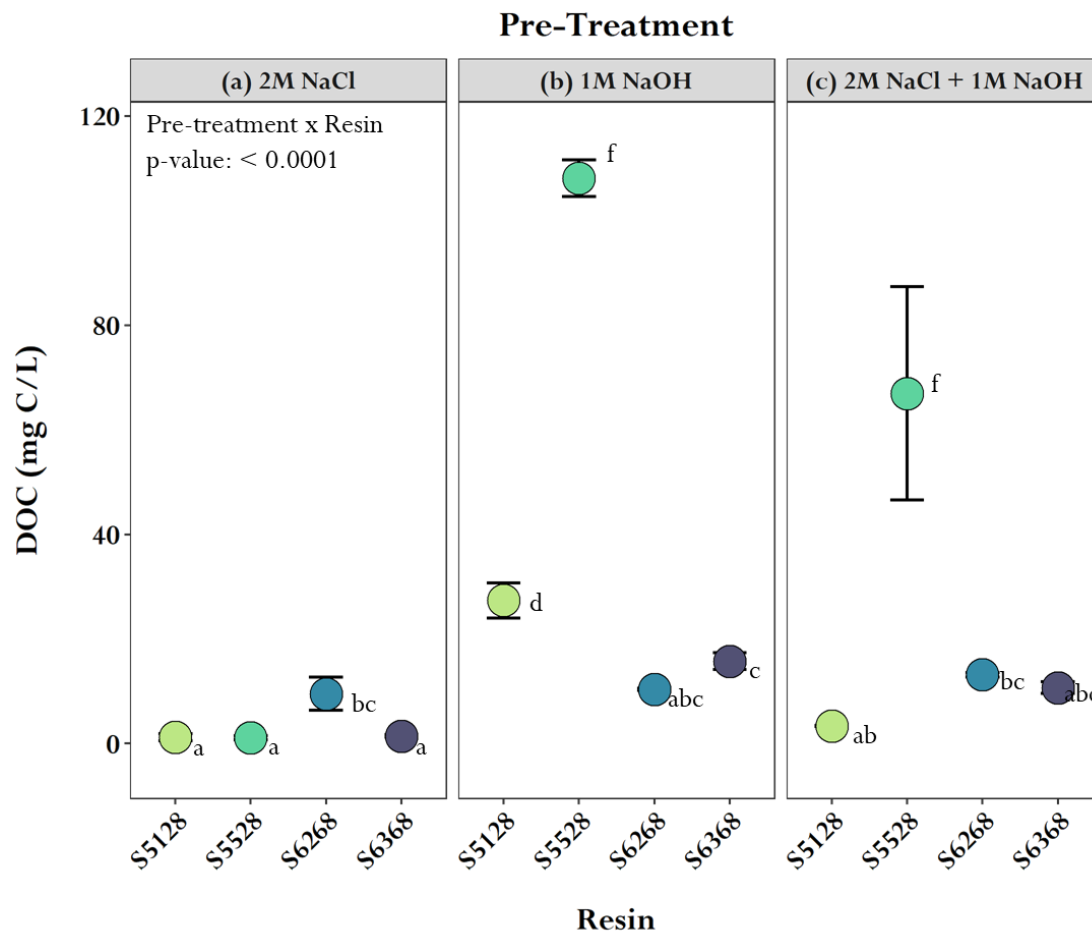


Figure 1: Mean DOC content in 0.01 M KCl extraction solution after resin pre-treatment for four Lewatit resins: (a) 2 M NaCl, (b) 1 M NaOH, (c) 2 M NaCl + 1 M NaOH.

ANOVA two-factor statistical analysis performed between factors: pretreatment and resin. Graph represents significant interaction effects between pretreatment and resin.

Letters represent Tukey HSD post-hoc testing groups.

3.2 Sorption

A three-way ANOVA showed no significant interaction effects for DOC sorption among resin by concentration by source ($p = 0.86$) or between resin by species ($p = 0.11$); however, there was a significant interaction between resin by concentration ($p < 0.0001$). Amberlite IRA-400 was significantly lower than all other resins at low concentrations (p

< 0.0001). Because of this we determined that significance in all main effect factors for sorption was meaningful (Figure 2). While Lewatit resins were similar in mean proportion of mg C sorbed, ranging from 0.52 ± 0.01 to 0.48 ± 0.01 after 24 hr of batch equilibration, Amberlite had a significantly lower mean of 0.36 ± 0.03 . Gel resins had the highest mean proportion of mg C sorbed at ~ 0.51 . Mean proportion of mg C sorbed in high concentration sources was 0.49 ± 0.01 compared to 0.45 ± 0.02 for low concentration sources. Mean proportion of mg C sorbed was higher for poplar sources (0.50 ± 0.01) versus Douglas fir sources (0.44 ± 0.01).

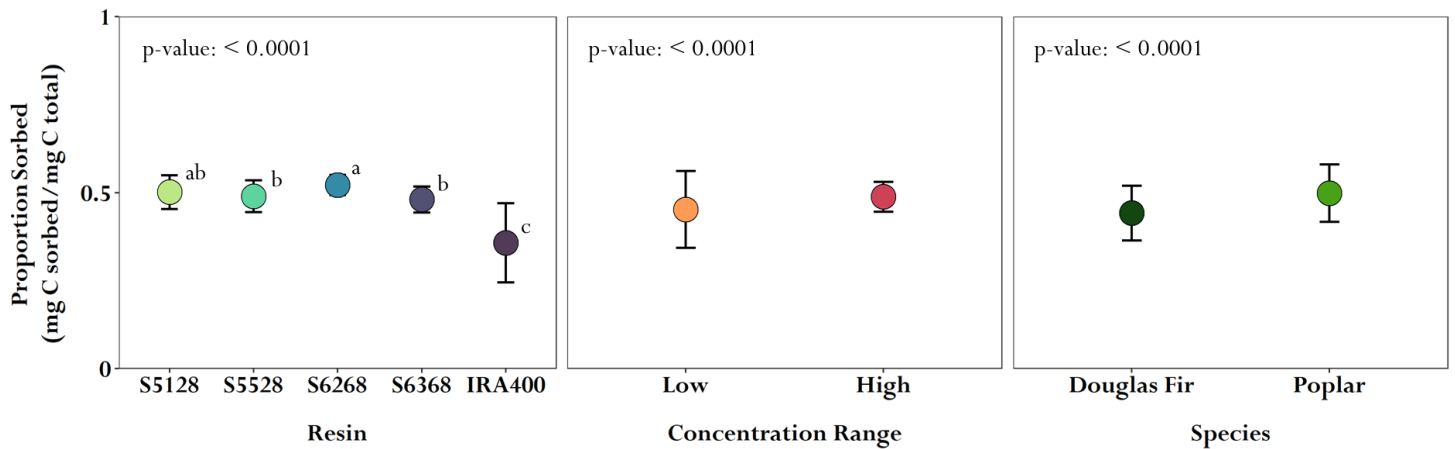


Figure 2: Mean proportion of DOC sorbed from known carbon mass after 24 hr of batch equilibration by: (a) resin type (b) source concentration, and (c) terrestrial carbon source type. For figures (b) and (c) data represents five replicate samples for each resin and source combination. ANOVA multi-factor statistical analysis confirmed meaningful main effects in all factors: resin, concentration, species. Letters represent one-way Tukey HSD post-hoc testing.

3.3 Recovery

Three-way ANOVA analysis showed no significant interaction effects in the first extraction among resin by concentration by source ($p = 0.43$) or between resin by source

($p = 0.56$). However, the interaction effect between resin by concentration was significant ($p < 0.0001$). As with the sorption results, this interaction was driven by Amberlite IRA-400 at low concentration. Amberlite IRA-400 recovered significantly more mg C than all other resins at low concentrations ($p < 0.0001$), sorbing 123% of the total carbon which suggests the resin was contributing carbon to the equilibrated solution. Because of this we determine that the only significant main effect factor for the first extraction was resin type ($p < 0.0001$). Ignoring the Amberlite due to the disproportionately high recovery at low concentration, polyacrylamide resins exhibited the highest proportion of mg C sorbed.

The second extraction (Figure 3c) had no significant interaction among resin by concentration by source ($p = 0.49$) or between resin by source ($p = 0.32$); however, there was a significant interaction between resin by concentration ($p = 0.02$). These results were consistent with the first extraction, and were driven by much higher recovery values for Amberlite IRA-400 at the low concentration. There was no significance in the main effects for any of the three factors. Samples from the third extraction were below the limit of detection when analyzed, which led to the conclusion that performing three extractions is unnecessary and not recommended.

Cumulative results (Figure 3a) were comprised of the first (Figure 3b) and second extractions (Figure 3c), and had no significant interaction among any factors, with the exception of concentration by resin ($p < 0.0001$). This is due to the disproportionate recovery of mg C at low concentration for Amberlite. Post-hoc testing of significance for the resin factor shows no significance between the cumulative amount of mg C recovered between the Lewatit resins, polyacrylamide resins had the highest mean proportions

recovered at $\sim 71\% \pm 0.02$. The majority of DOC recovery came from the first extraction ($\sim 50 - 60\%$), the second extraction recovered an additional $\sim 8-10\%$, and the third extraction, if detectable, was below $\sim 0.5\%$.

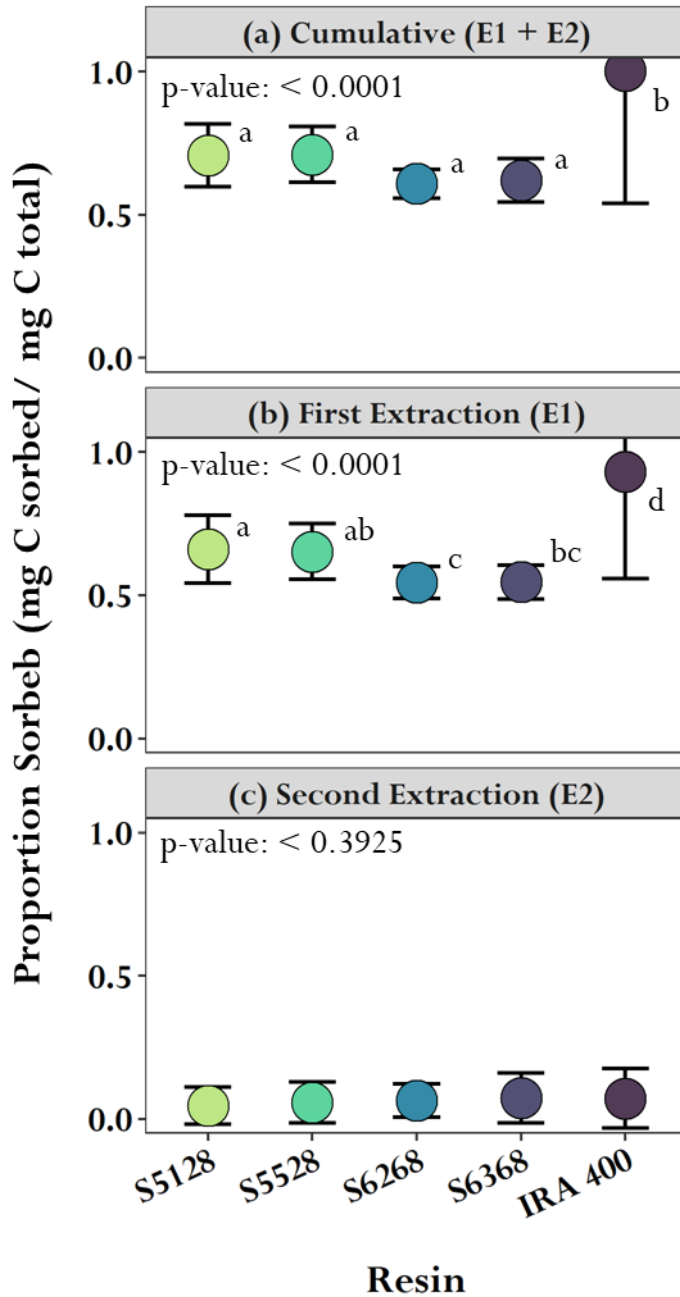


Figure 3: ANOVA three-factor statistical analysis to compare mean values for five resins in mean proportion of DOC recovered via 1M KCl extraction from a known mass of carbon sorbed onto resin: (a) cumulative: additive proportion sorbed from first and second extractions, (b) first extraction, (c) second extraction. Post-hoc testing of resin main effects were completed using one-way Tukey HSD, results indicate significance in (a) and (b), no significance found in (c).

3.4 Resin Efficacy

A three-way ANOVA showed no significant interaction effects among resin, concentration, or source, with the exception of source x concentration ($p = 0.02$) with low concentration of Poplar having significantly higher efficacy. Main effects for resin were also significant ($p < 0.0001$). Figure 4 compares the statistical main effects earlier described as mean proportion of mg C sorbed after 24 hr of equilibration (Figure 4a), mean cumulative proportion of mg C recovered (Figure 4b), and overall efficacy for each resin (Figure 4c). Overall, Lewatit S5128 (gel polyacrylamide) had the highest mean efficacy of 35.2%, followed by 34.8% for S5528 (macroporous polyacrylamide), 31.7% for S6268 (gel polyacrylamide), 31.5% for Amberlite (macroporous polystyrene divinylbenzene copolymer), and 29.8% for S6368 (macroporous polystyrene).

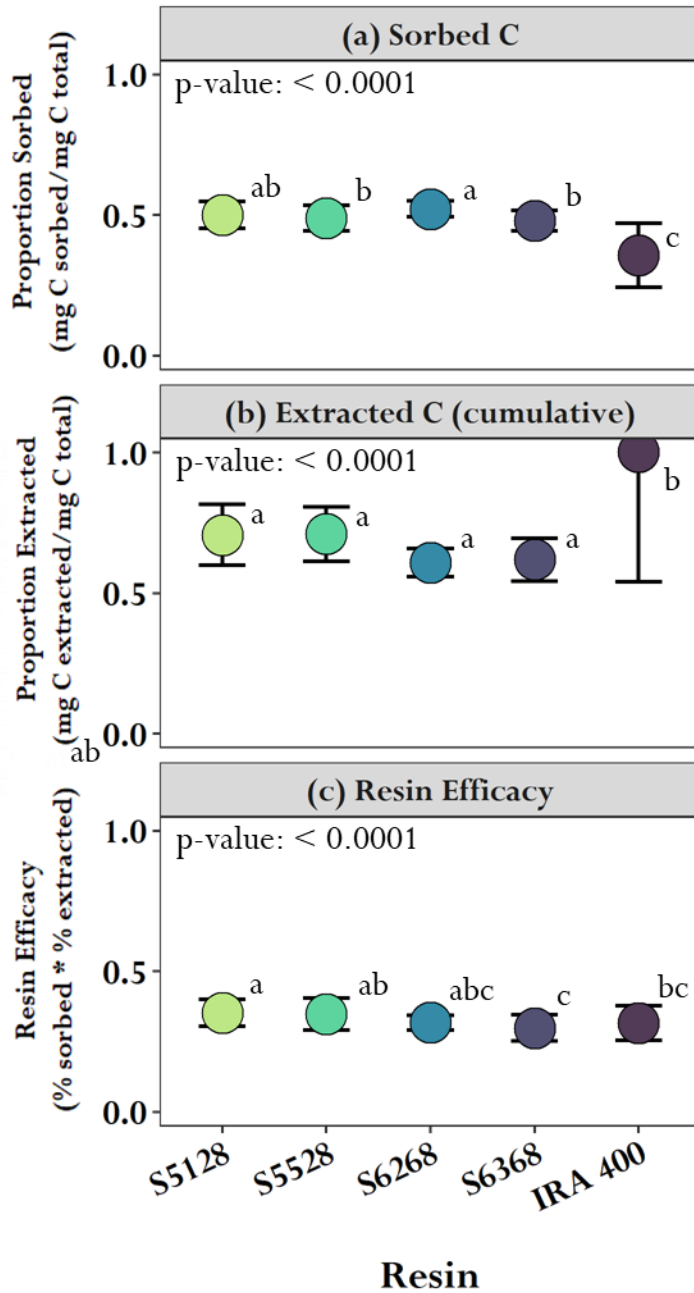


Figure 4: ANOVA three-factor analysis: significant main effects compared to (a) mean proportion mg C sorbed after 24 hr equilibration, (b) mean cumulative proportion mg C recovered, and (c) overall resin efficacy based on mean proportions sorbed and recovered from total mg C. Letters represent one-way Tukey HSD post-hoc testing.

4.0 Discussion

4.1 Resin Efficacy

The objective of this study was to evaluate a suite of commercially available resins for their efficacy and feasibility of quantifying time-integrated natural DOM flux in soils and provide suggestions for resin selection, appropriate treatment, and extraction. Taking into consideration the sorptive capacity of these resins, the differences in species and DOC concentration, while statistically significant, only varied by about ~5%, so on an ecological level, sorption is fairly robust across these factors. It is also important to keep in mind that these were stocks made from one species, where in a forest (and most ecosystems) plant species and DOC concentrations will be much more variable. The same could be said for the resins, and all resins aside from IRA-400 sorbed around 50% - which may not appear to be ideal or as high as we'd like, but complete sorption of the carbon from stocks was not expected because not all DOM is ionizable, meaning that it's not reactive so it won't sorb to the resins to begin with. Extraction was also robust across concentration and species with no statistical significance, and exhibited ~10% cumulative difference in mg C extracted between polyacrylamide and polystyrene resins.

Using sorption and extraction proportions to calculate efficacy (eq. 3), after 24 hour batch equilibrations and multiple extractions, I observed that resins only recorded ~30% of total DOC from the laboratory-created, leaf-extract source waters. Given that not all DOM compounds ionize at the pH range of natural waters, recovery values less than 100% were anticipated. Thus, for mass balance approaches, the use of resins such as those tested here would require the use of a significant weighting factor. Though, depending on the application, that may not be palatable, the small degree of variation

about the measurement and the apparent robustness across source and concentration, suggests that resins may be appropriate for indexing DOM fluxes, particularly comparing across space, time, or treatment.

This study did not find any significant differences between concentration and species, so confidence in the performance of resins regardless of concentration or litter source is appealing, because the reality is that most ecosystems are not comprised of a singular species, and there will be differences in species composition and organic matter inputs as well as concentration of DOC along spatial gradients. As there are so many variables specific to individual systems, one resin might perform better over another, or exhibit preferential absorption to certain functional groups as suggested by Warner et al. (2015), but not all studies allow for in depth resin efficacy testing prior to deployment.

4.2 Resin Selection

After weighing performance during pretreatment, sorption, and recovery phases of the study, the data suggests that gel polyacrylamide resin (Lewatit S5128) balanced all factors, and employed ease of use in handling, storage, and availability. To maximize the use of these resins in the quantification of DOC, the following treatments are recommended: to reduce interference and prime the resin for deployment pretreat via batch equilibration for 1 hour using 2 M NaCl in a 20:1 volume (mL):resin mass (g) ratio. For recovery, two extractions using 1 M KCl in a 12:1 solution volume (mL):resin mass (g), is recommended to optimize recovery of the majority of DOC without degrading the resin beads. While this study provided us with the information necessary to make a selection for future field deployment, we are mindful that resins are not always readily available or easily sourced. So, while we do recommend Lewatit S5128, more broadly,

our general recommendation is for the use of Cl⁻ gel polyacrylamide resins for passive sampling.

Chapter 2: Characterizing soluble organic matter along spatial patterns of podzolization in a glaciated headwater catchment

1.0 Introduction

Soil organic matter (SOM) contributes to several environmental processes such as organo-mineral complexation and transport, nutrient bioavailability, and mineral dissolution (Chantigny, 2003; Gabor et al., 2015; T Ohno et al., 2007). Naturally occurring dissolved organic matter (DOM) has undergone decomposition via microbial processing, oxidation and other processes, and is a highly complex, heterogeneous mix of compounds with diverse functional groups (Fakhraei and Driscoll, 2015; Sandron et al., 2015). DOM often has numerous strongly acidic functional groups that deprotonate at $\text{pH} > 3$, thus allowing the DOM to serve as an organic acid (Fakhraei & Driscoll, 2015). As a result, increases in DOM often lead to a rise in the supply of naturally occurring organic acids. The presence of DOM may accelerate dissolution rates of some minerals, and indirectly influence others, which are based largely on pH, aluminum content, and the type of geologic material (Drever & Stillings, 1997; Lazo et al., 2017; Varadachari et al., 1991). DOM has a strong acidifying effect in low ionic strength solutions, often resulting in mineral dissolution, complexation, and transport, such as is common in the process of podzolization.

Generally, soil development is viewed as a “top down” process, dominated by the vertical flow of soil water and associated solutes; however, from studies at HBEF a northeastern USA glaciated watershed, different hydrologic flow regimes have been observed to occur on steep slopes which results in significant lateral translocations which

strongly influence spatial patterns of soil development (Figure 1) (Bailey et al., 2014; Gannon et al., 2015; Gillin et al., 2015).

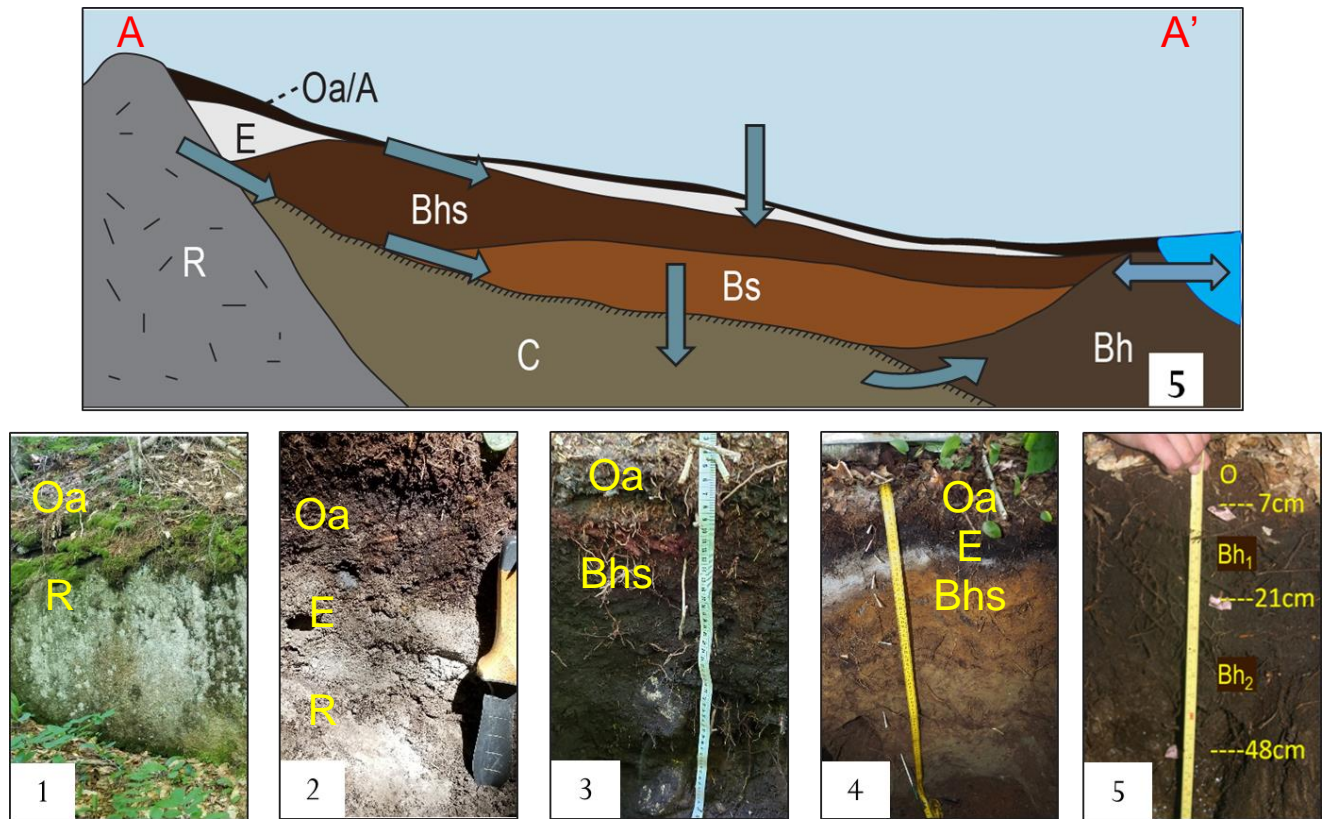


Figure 1: Conceptual model (top) showing the distribution of soil groups along a representative hillslope the Hubbard Brook Experimental Forest (modified from Bailey et al., 2014). Numbers represent landscape positions and include directional flow of water, and dominant soil horizons: organic forest floor (Oa), eluviated mineral (E), illuviated mineral (Bhs, Bs, Bh), bedrock (R). Photos depict representative soil profiles in each zone. Photo credits: Duston, S.A. & Bailey, S.W.

Research on the dynamics between water table fluctuations, groundwater chemistry, and soil development patterns in Watershed 3 (WS3) at HBEF have improved understanding of how DOM is transported along the hillslope. In ridge-top slope

positions (Fig. 1, Zones 1 and 2), groundwater is transient and shallow with low pH; saturated, thick organic soils (O) over thin mineral horizons (E) and bedrock outcrops (R) contribute high concentrations of DOM to the system (Gannon et al., 2015; Gillin et al., 2015). These zones have been identified as potential hotspots for DOM metal-ligand complexation and mobilization (Bailey et al., 2019; Bourgault et al., 2015, 2017; Gannon et al., 2015) as DOM has direct access to an abundance of primary minerals, metals, microbes and readily undergoes organo-mineral complexation and biotic processing (Sauer et al., 2007). Soils in these zones are frequently saturated and flushed, subsequently transporting newly formed complexations and remaining DOM constituents out of the upper mineral horizon, to accumulate downslope in deeper glacial deposits (Bailey et al., 2014). In the transitional zone (Fig. 1, Zone 3) between the ridge and backslope, hydrology patterns are both transient and perennial. DOM concentrations decrease, pH in surface and ground waters increase, and accumulation of spodic material coats mineral soil particles (Bailey et al., 2014, 2019; Zimmer et al., 2013). Along the backslope (Fig. 1, Zone 4) groundwater flow is perennial deeper in the C horizon. DOM concentration is low and pH is moderate. Spodosols are deep and vertically formed, with development similar to profiles reported by official soil series descriptions (Bailey et al., 2014, 2019; Zimmer et al., 2013). Soils in the near stream environment (Fig. 1, Zone 5) are dually influenced; accumulating spodic material from upslope lateral transient flow (Gannon, McGuire, Bailey, Bourgault, & Ross, 2017), and from lateral perennial flow (Bourgault et al., 2017) from near stream groundwater incursions (Bailey et al., 2014; Gillin et al., 2015).

Hydrologic controls (e.g. water residence time and rates of flushing), and topography are important factors affecting DOC concentration (Boyer et al., 1997; Gannon et al., 2015). Topography largely controls interaction between soils and water table fluctuations, contributing to spatial variations in groundwater and soil chemistry (Bourgault et al., 2017; Gannon et al., 2015, 2017; Gillin et al., 2015). These studies show that shallow hillslope soils, which receive runoff from organic matter soils over bedrock outcrops, may be a major source of DOC in headwater catchments. DOM present from organic horizons is biodegraded and transported laterally or down through mineral horizons along groundwater flow paths (Guggenberger et al., 1994). Organic acid functional groups within DOM bind with Al and Fe during mineral dissolution, and are subsequently leached deeper into the soil profile or downslope (Bailey et al., 2019; Fakhraei & Driscoll, 2015). Organic-Fe complexes precipitate out first as Al has higher solubility than Fe in low pH, so organic-Al complexes will accumulate lower in spodic horizons, which was shown by Bourgault et al., 2017.

Groundwater and stream chemistry data from HBEF show that DOC and Al concentrations are highest in O and E horizons that are shallow to bedrock at the top of watershed catchments (Zones 1 & 2) (Bailey et al., 2019; Likens & Buso, 2006; Zimmer et al., 2013). DOC and Al concentrations decrease downslope, becoming moderate in Zone 4, and are lowest in Zone 5. This pattern is coupled with pH gradients, with pH lowest in upslope Zones 2 and 3, and increasing downslope in Zones 4 and 5 (Bailey et al., 2019; Likens & Buso, 2006; Zimmer et al., 2013). Fe concentrations are highest in groundwater from Zone 2, and decrease downslope (Bailey et al., 2019; Zimmer et al., 2013). During hydrologic transport, fractionation of DOM occurs as a result of

complexation and biological degradation, which leads to different soluble organic matter properties with depth in soil profiles (Corvasce, Zsolnay, D’Orazio, Lopez, & Miano, 2006).

Spodic soil horizons at HBEF, as studied by Bourgault, were found to have significant differences in Al and Fe extracts, and organic carbon (Bourgault et al., 2015, 2017; Gannon et al., 2017). Overall, extractable Al and Fe, and spodic C (total organic C below the organic horizon (Bourgault et al., 2017)) were highest in Zones 3 and 5, with moderate levels in Zone 4, and the least amount in Zone 2. Based on differences in water table fluctuations, soils were split into “lateral” (transient, lateral saturated flow) and “vertical” (deep, perennial flow with limited transient flow in the solum) designations. Laterally developed spodic Bhs horizons (Zone 3) were twice as thick as vertical spodic Bhs horizons (Zone 4) and had higher concentrations of Al, but lower Fe and C. Vertically developed (Zone 4) spodic horizons had a higher porosity, a well-developed crumb structure, and higher ratio of organometallic complexes to mineral grains.

While water and soil chemistry has been shown to be significantly different along transects in headwater catchments, DOM quality has not been evaluated. To improve our understanding of DOM influence on soil development and ecosystem processes in these spatially distinct areas of podzolization, this study evaluated carbon content and chemical characteristics in water soluble organic matter (WSOM) in order to answer the following:

1. Are the chemical characteristics of WSOM significantly different across genetic soil horizons?

2. Does WSOM extracted from soil horizons of same genetic designation, but developed under different water table regimes, have similar chemical characteristics?
3. Do WSOM chemical characteristics reflect those of subsurface groundwater flow collected in specific zones of soil development?

2.0 Methods

2.1 Site description

This study was conducted at HBEF in Watershed 3, located within the White Mountains in New Hampshire (43°56'N, 71°45'W). The climate at HBEF is humid-continental with mean temperatures of -9°C and 18°C in January and July, respectively. The annual precipitation averages 1400 mm, a third of which comes from snowfall (Likens & Bormann, 2013). HBEF is a second-growth forest dominated mainly with red spruce (*Picea rubens*), balsam fir (*Abies balsamea*), and white birch (*Betula cordifolia*) in shallower and wetter soils. Northern hardwood species sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula allegheniensis*) dominate deeper and better drained soils (Likens & Bormann, 2013).

The underlying bedrock consists of sillimanite-grade pelitic schist and calc-silicate granulite of the Silurian Rangeley Formation which is overlain by glacial deposits of varying thickness from Wisconsinan glacial retreat (Bailey et al., 2014). The soils are predominantly sandy loam to loamy sand textured Spodosols, with a range of drainage class. Average depth to the C horizon is 0.7 m and depth to bedrock is highly variable (Bailey et al., 2014).

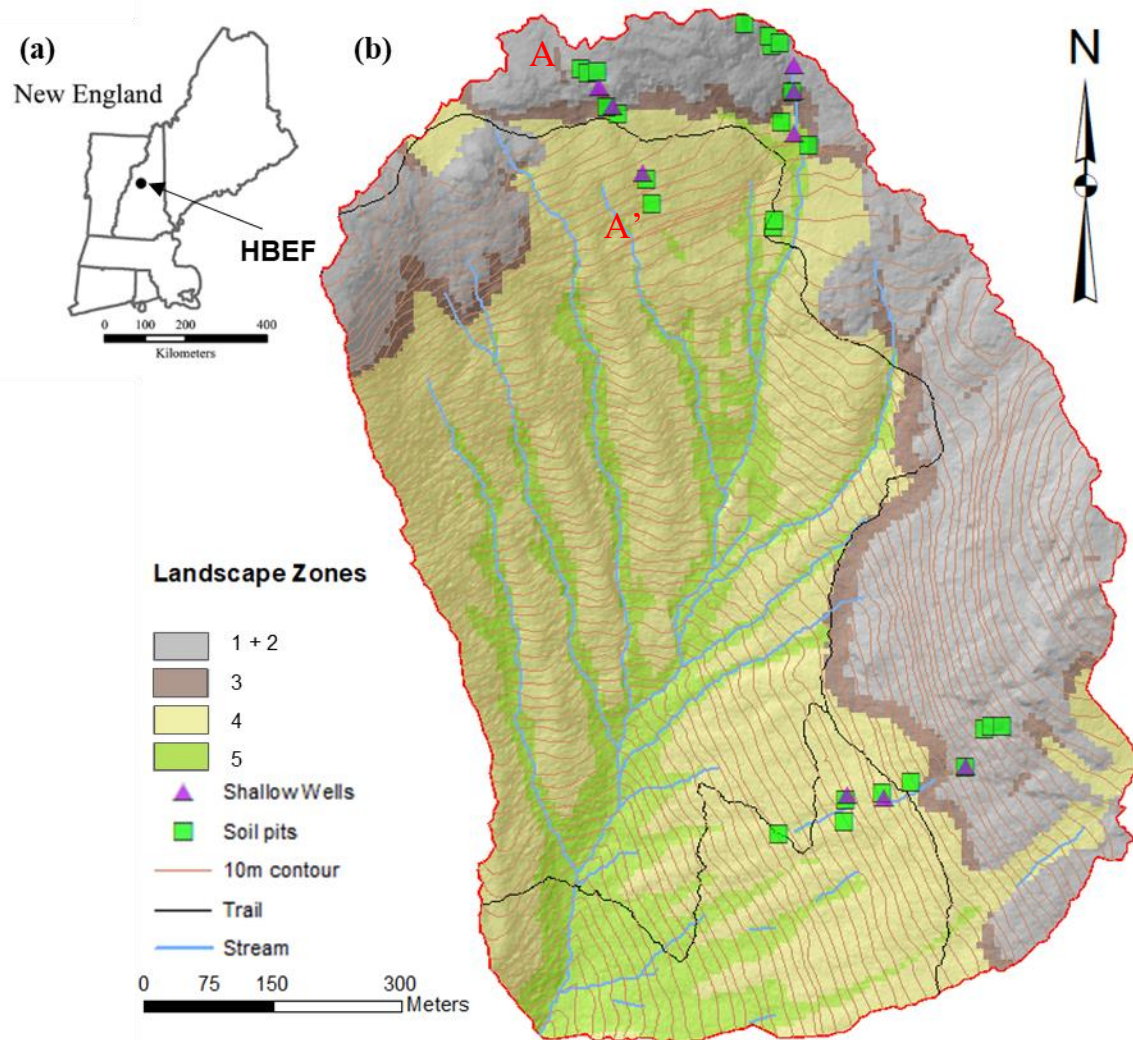


Figure 2: (a) Map showing the location of HBEF in New England. (b) Map of WS3 within HBEF. Lidar-derived hillshade DEM overlain by digital soil map (Bailey et al., 2019; Gillin et al., 2015).

2.2 Field collection

Within three study sub-catchments, soils were sampled from five zones as described in Fig. 1. One soil pit per zone 1, 2, and 3, and three soil pits per landscape positions 4 and 5 per sub-catchment were sampled. Soils were field described following NRCS field description protocols (Schoeneberger et al., 2012). In each pit, samples were

collected from the Oa horizon , and for each genetic mineral horizon. Horizons were composited during sampling throughout the horizon, and horizons with the same genetic designation (i.e. Bhs, Bh) but with significant different morphological attributes (i.e., coarse fragment percentage, structure, texture, color) were split into separate horizons (i.e., Bhs1, Bhs2) as necessary. Soils were transported to the lab and immediately stored at 4°C until the extraction process, which occurred within a week following field sampling.

Water levels in shallow screened PVC wells were recorded and then the wells were pumped dry with a peristaltic pump and Tyvek tubing and then allowed to recharge. A 500 mL Nalgene bottle was rinsed with the sample water, and then the bottle was filled for sample collection. Water samples from the weir were collected in a similar manner, with the bottle first being rinsed with sample water, and then filled. Water samples were stored at 4°C until filtering. After filtration, water samples are brought to room temperature for pH and EC analysis. Samples were filtered through ashed 0.45 µm GF/F filters using a vacuum pump. Filtrate was collected in 20 mL scintillation vials; one subset of samples was analyzed for DOC at the USFS Forestry Sciences Laboratory in Durham, NH on a Shimadzu TOC-5000A series TOC analyzer, another subset of samples was stored at 4°C until spectral analysis.

2.3 WSOM as a proxy for DOM

Given the importance of DOM to processes of weathering and soil formation, quantifying DOM fluxes and identifying potential hotspots of DOM production is of great interest. One commonly used proxy is the analysis of water soluble organic matter

(WSOM) extracted from soil samples (Kalbitz, et al., 2000; Rennert et al., 2007; Zsolnay et al., 1999). Like DOM, WSOM represents the extractable and potentially mobile portion of SOM. And though WSOM is not an exact match for DOM extracted from lysimeters or wells, it provides the opportunity to index potentially mobile C at places and times where solute collection is not feasible. It is important to note that while DOM and WSOM generally reflect the composition of total soil organic matter, DOM collected from lysimeters is mostly located in soil macropores and represents DOM accumulating from upslope soils, whereas WSOM extraction results in the disturbance of soil structure and is considered to include DOM from macropores and from smaller pores and represent DOM to a specific sampling point. Because of the structural disturbance of soil during the extraction process, magnitudes of WSOM can be higher than DOM in natural systems (Zsolnay et al., 1999). WSOM is also more representative of organic matter derived from new C sources versus older decomposed organic matter (Rennert et al., 2007). To minimize the negative effects of aggregate breakdown and cell lysis, it is recommended to extract field moist samples (Jones & Willett, 2006; Zsolnay et al., 1999). Despite these differences, WSOM is commonly extracted for analysis with the understanding that may not be representative of natural DOM in soils (Gabor et al., 2015; Gabor et al., 2014; Tsutomu Ohno et al., 2007; Provenzano et al., 2010; Rousk & Jones, 2010). Obtaining samples for analysis is critical, and a demand for rapid methods to characterize DOM is necessary; thus, installation of lysimeters and waiting for equilibration to occur after disturbance, is not always feasible. (Chantigny, 2003; Kalbitz et al., 2000; Rennert et al., 2007). Analyzing the WSOM fraction provides an alternative option for characterizing the potentially mobile fraction of soil organic matter in lieu of available soil pore water.

2.4 WSOM extraction

Field moist samples were homogenized in their collection bag after removal of coarse fragments and organic matter (roots, stems, leaves). 30 ± 0.1 g of soil was added to an acid washed 250 mL Erlenmeyer flask and mixed with 150 ± 1.0 mL of 0.01 M CaCl₂ (Gabor et al., 2015) on a wrist action shaker (Burrell Model DD, Burrell Corp, Pittsburg, PA) for 1 hr at room temperature. Samples were centrifuged for 15 minutes at 2000 g then filtered through ashed 0.42 μ GF/F filters using a 60 mL syringe. Filtrate was collected in 20 mL scintillation vials. Samples were analyzed on a Shimadzu TOC-L series TOC analyzer for DOC and stored at 4°C until spectral analysis.

2.5 Fluorescence characterization of WSOM

As DOM is heterogeneously complex, fluorescence analysis generates quantitative data that can be used to determine how chemical characteristics of DOM varies in surface and subsurface waters and soils (Burns et al., 2016; Cory & McKnight, 2005; Fellman et al., 2008; Karavanova & Milanovskiy, 2105). Fluorescence spectroscopy is used to determine optical properties of DOM through excitation-emission matrices and Parallel Factor Analysis (PARAFAC) modeling. It is a commonly used technique for studying the chemical characteristics of DOM because it is a more sensitive indicator of organic matter source when compared to absorbance analysis, with an additional benefit of a decrease in potential interference from other fluorescing components such as Fe and Al.

There are several indices utilized in this form of analysis, and it is important to note that not all indices are appropriate for all applications. For this study, six commonly used spectral indices were selected for use in the analysis of WSOM and subsurface waters based on reference literature and other studies (Table 1).

Table 2: Fluorescence indices examined in this study, with definitions, calculation of parameters, and relevant references. Note: em = emission wavelengths, ex = excitation wavelengths.

Index	Definition and Interpretation	Parameters	Citation
Fluorescence Index (FI)	Identifies relative contribution of terrestrial (plant) and microbial sources in DOM pool. Higher values (~1.8) indicate microbial DOM; lower values (~1.2) indicate plant DOM.	Ratio of em 450 nm and em 500 nm at ex 370 nm	(Cory and McKnight, 2005)
SUVA ₂₅₄ (L mg ⁻¹ m ⁻¹)	Absorbance measured at 254 nm normalized to DOC concentration of sample. Measurement of aromatic carbon content; higher values indicate higher degree of aromatic character.	A = absorbance D = cuvette diam. UVA = A * d ⁻¹ SUVA = UVA * (DOC*100) ⁻¹	(Chin, Alken, & O'Loughlin, 1994; Weishaar et al., 2003)
Humification Index (HIX)	A measure of the complexity and aromatic nature of DOM. Higher values indicate lower H/C ratios attributed to higher degrees of aromaticity.	Peak area under em 435-480 nm divided by the peak area under em 300-345 nm at ex 254 nm	(Tsutomu Ohno, 2002; Zsolnay et al., 1999)
Freshness Index (BIX)	Proportion of DOM recently derived from microbial processes. Higher values indicate a greater proportion of recently derived microbial DOM; lower values indicate a greater proportion of older decomposed DOM.	Ratio of β peak (max intensity within ex 290-310 nm/em 370-410 nm) and α peak (max intensity within ex 320-360 nm/em 420-460 nm)	(Coble, 1996; Parlanti, Wörz, Geoffroy, & Lamotte, 2000; Wilson & Xenopoulos, 2009)
Redox Index (RI)	Characterizes the redox state of dissolved fulvic acids; typical values are ~0.42. High proportion of reduced carbon (closer to 1); high proportion of oxidized carbon (closer to 0).	R/(R + O) Reduced (R): (HQ+SQ1+SQ2+SQ3) Oxidized (O): (Q1+Q2+Q3)	(Miller, McKnight, Cory, Williams, & Runkel, 2006)
Protein %	Tryptophan and tyrosine like fluorophores found in DOM from degraded plant matter and microbial byproducts. Higher values correspond to a higher percentage of protein-like fluorophores.	%C13 (tyrosine) + %C8 (tryptophan)	(Cory and McKnight, 2005)

2.6 Spectroscopic analysis and PARAFAC modeling

UV-vis analysis was performed using a Shimadzu Series UV-1800 UV Spectrophotometer with measurements taken every 1 nm from 190 to 850 nm. For

analysis of WSOM extractions, a reference blank of 0.01 *M* CaCl₂ was used to match the matrix of the extraction, while RODI water was used as a reference blank for water sample analysis. To correct for inner-filter effects for fluorescence analysis, samples measuring greater than 0.2 cm⁻¹ at 254 nm were diluted to between 0.02 and 0.2 cm⁻¹ (Cox et al., 2000; Kalbitz et al., 2000; Ohno, 2002).

Fluorescence analysis was completed on a Fluoromax-4 (Horiba Jobin Yvon Inc.). A matrix blank of 0.1 *M* CaCl₂ was analyzed to use as a blank for EEM correction during post-processing of soil samples, while a blank of RODI water was analyzed to use for correction of water samples. Excitation Emission Matrices (EEMs) were collected at excitation wavelengths 240 – 450 nm in 5 nm increments and at emission 300 – 600 nm in 2 nm increments with a 5 nm slit width and 0.1 s integration time. EEMs were raman-normalized and blank-subtracted in post-processing. FI, HIX, and BIX were calculated during post-processing in using MATLAB ver. 2019a (Mathworks, Natick, MA) and SUVA₂₅₄ was calculated by normalizing the absorbance at 254 nm by DOC concentration (Table 1).

A thirteen component PARAFAC model (Cory & McKnight, 2005) was used to calculate redox index and protein percentage using MATLAB ver. 2019a (Mathworks, Natick, MA). Four reduced Quinone components (e.g. HQ, SQ1, SQ2, and SQ3) were identified and divided by total reduced and oxidized (e.g. Q1, Q2, and Q3) components (Miller et al., 2006). Two protein-like components that resemble tyrosine (C13) and

tryptophan (C8) derived from degraded plant matter and microbial products were summed to calculate % protein in the sample (Cory & McKnight, 2005).

2.7 Principal Component Analysis (PCA)

To visually interpret the chemical characteristics of WSOM and water samples along the landscape, PCA was utilized for exploratory analysis and to determine correlation coefficients between the components along dimensions 1 and 2 (PC1 = x-axis; PC2 = y-axis). PCA analysis of components and calculation of correlations was completed using the FactoMineR package (Lê & Husson, 2008). All PCA analyses were completed using RStudio (ver. 1.2.1335, RStudio, Inc.).

2.8 Statistical analysis

Analysis for significance of fluorescence parameters between genetic soil horizons of landscape position was done using the one-way ANOVA test. Tukey's HSD was used for post-hoc testing. For the analysis of horizons and development, the data were not normally distributed, so the non-parametric analysis Wilcoxon signed rank test was used to test pairwise differences. To determine significant variation of all factors between WSOM and water sample groups, non-parametric permutation ANOVA and pairwise post-hoc testing were performed using `vegan` (ver. 2.4-6) (Oksanen, et al., 2019) and `pairwise.adonis` (ver. 0.4) (Martinez, 2020) in RStudio (ver. 1.2.1335, RStudio, Inc.). ANOVA and Wilcoxon analyses and post-hoc testing were performed in JMP Pro (v. 15.0.0; SAS Institute Inc., 2019). All statistical analysis reflects a significance level of $\alpha = 0.05$.

3.0 Results

3.1 Comparison of genetic soil horizons

The results of a one-way ANOVA analysis showed significant differences in WSOM characteristics along the hillslope in carbon content (Figure 3) and fluorescent characteristics (Figure 4). WSOM in Oa horizons, which represents highly decomposed organic soil, is comprised of plant-like material (low FI). Progressing along podzolization sequence from the eluvial (E), into the illuvial (Bhs, Bs), and then near stream (Bh) horizons, fluorescing WSOM groups progressively resemble material derived from microbial processing (increasing FI). This trend of change from plant-like to microbial in Fluorescence Index is supported by similar trends in BIX, RI, and Protein %. As plant-materials are biodegraded and complexed, proteins are consumed, and freshly produced microbial exudates are added back to the soluble pool (increasing BIX, decreasing Protein). Higher proportions of reduced fulvic acids (high RI) in the Oa are readily consumed by microbes, but also undergo complexation with metal ions (e.g. Fe, Al) and correspond to the oxidation of carbon (decreasing RI). Though there was no significant trend observed for aromatic character (HIX and SUVA₂₅₄), results showed much lower values for Bh horizons, an indication that WSOM in these soil horizons have the lowest degree of aromaticity.

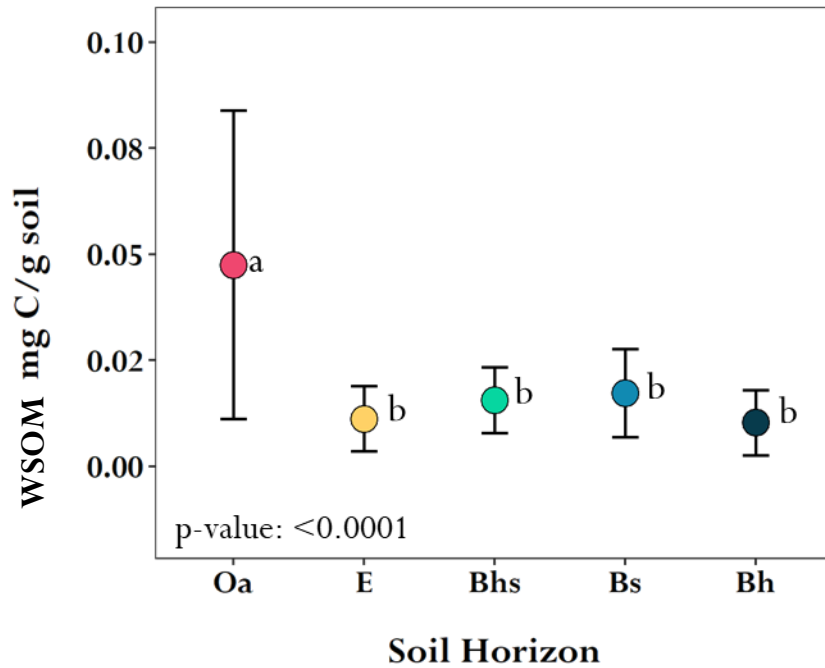


Figure 3: Mean carbon content per gram of extracted field moist soil in WSOM samples. Samples account for all horizons regardless of landscape position (1-5). ANOVA one-way analysis performed, Tukey HSD post-hoc testing was used for significance between means. Error bars represent standard error.

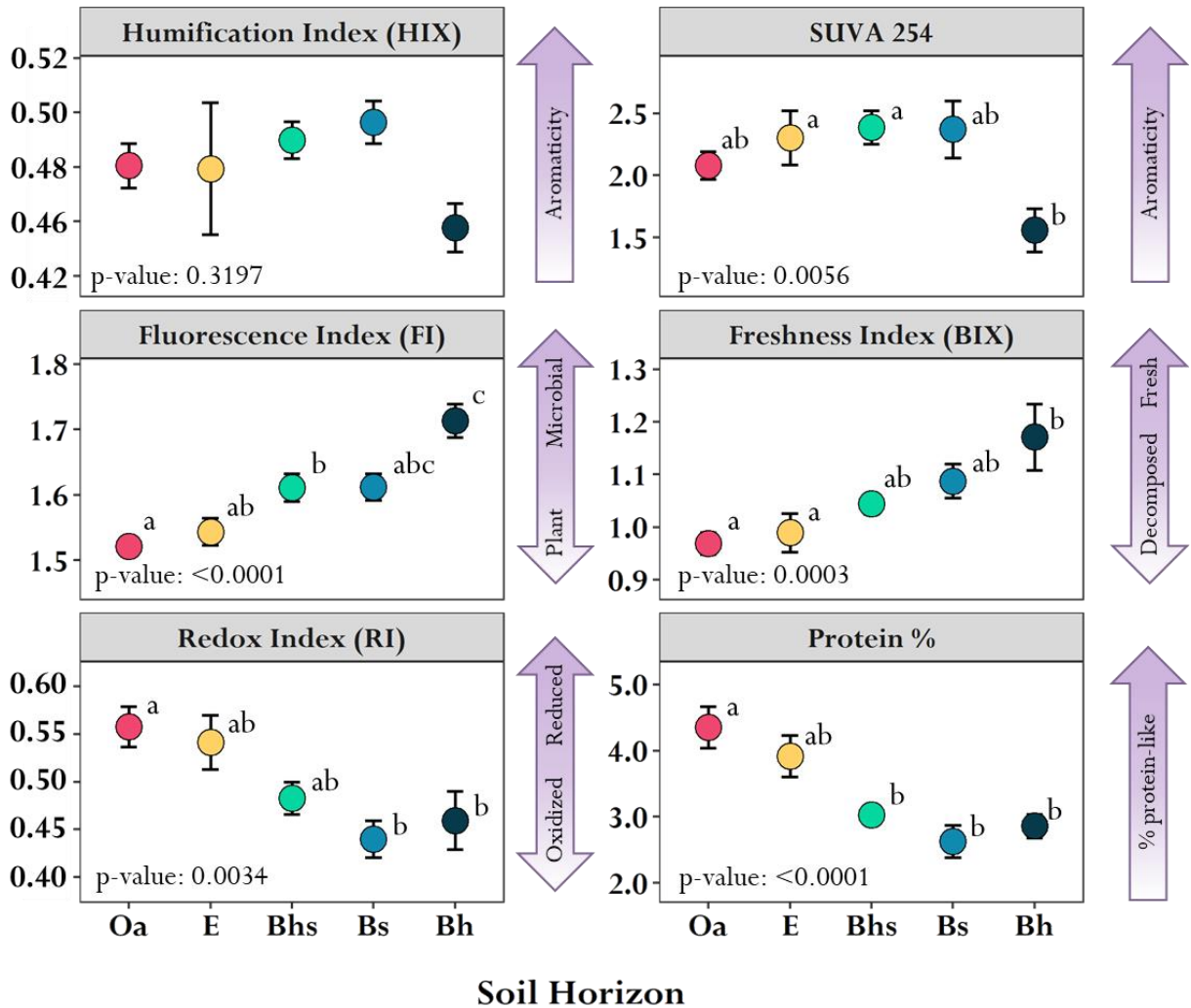


Figure 4: Mean values for fluorescence indices in WSOM samples. Samples account for all horizons regardless of landscape position (1-5). ANOVA one-way analysis performed and Tukey HSD post-hoc testing was used for significance between means. Error bars represent standard error.

3.2 Comparison of DOM in Lateral versus Vertical pedogenic sequences

Soils from Zones 1-4 were assigned a development designation based on hydrologic flow regime: “lateral” is represented by a common podzolic pedogenic

sequence of O-E-Bhs horizons as water flows in a predominantly lateral direction through soil Zones 1, 2, and 3, and “vertical” is represented by common podzolic pedogenic sequence of O-E-Bhs horizons as water flows in a predominantly vertical direction through Zone 4. Since the data was not normally distributed, the Wilcoxon rank sum analysis was used to analyze the difference between lateral and vertical development patterns of genetic horizons. Results show that vertical E and Bhs horizons were significantly higher ($p < 0.05$) in mg C/g soil (Figure 5), and that lateral E horizons had a significantly higher FI ($p < 0.05$) compared to vertical E horizons (Figure 6).

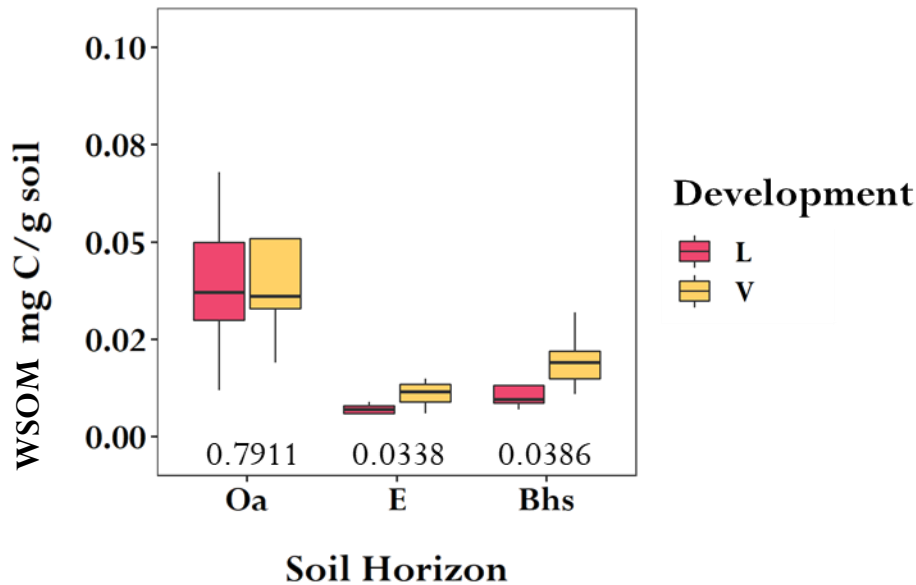


Figure 5: Comparison of mean carbon content per gram of extracted soil in WSOM samples between lateral and vertical soil development. Two-tailed Wilcoxon rank sum testing performed with corresponding p-values shown above each horizon.

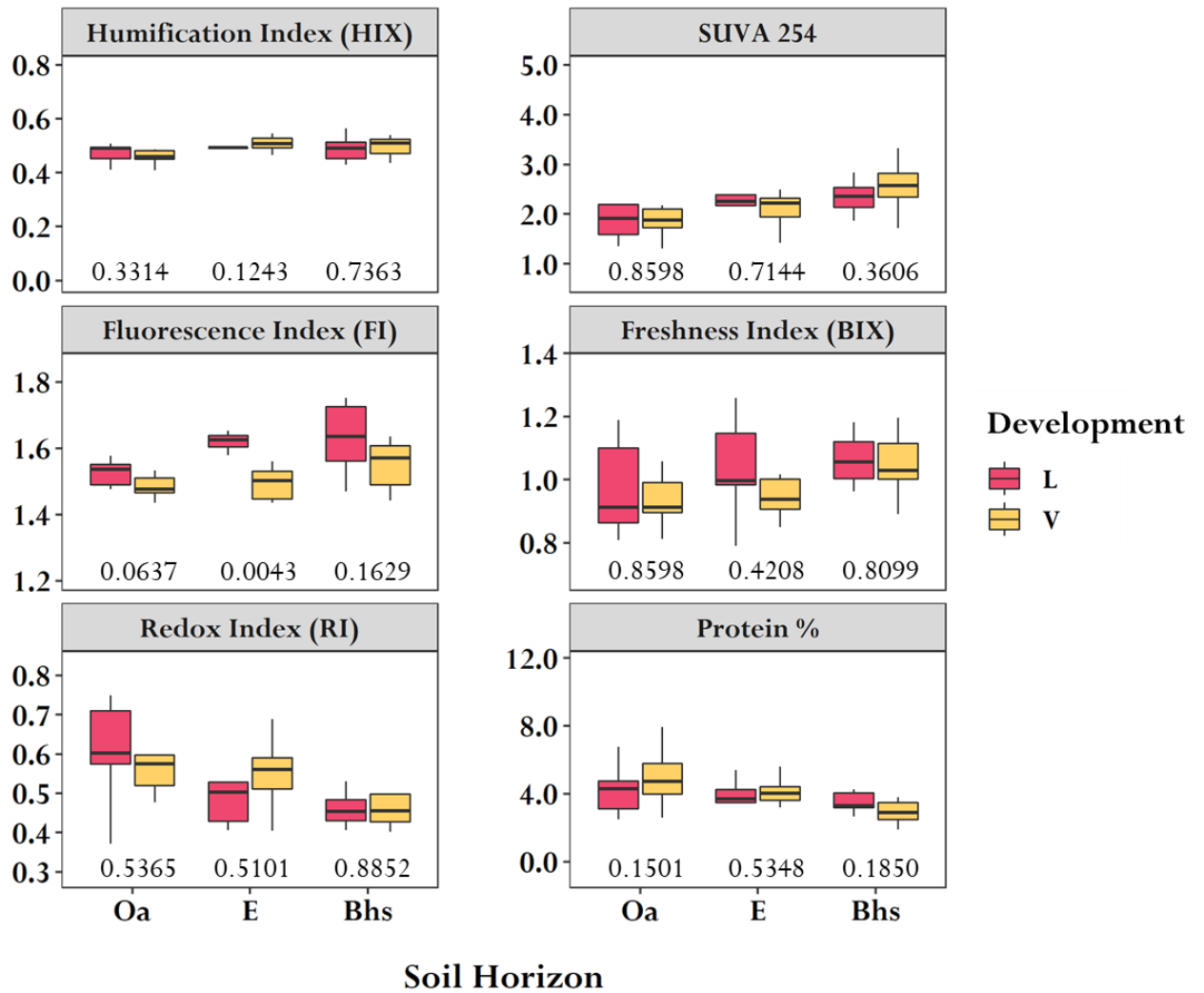


Figure 6: Mean values for fluorescence indices of WSOM extracted from genetic soil horizons between lateral and vertical soil development. Two-tailed Wilcoxon rank sum testing performed with corresponding p-values shown above each horizon.

3.3 Water and soil along the landscape

PCA was utilized for an exploratory analysis of the chemical characteristics of WSOM and water samples along the landscape (Figure 7). Table 2 describes the correlation coefficient between the components and dimensions 1 and 2 (PC1 = x-axis;

PC2 = y-axis). For PC1 the components driving the variance were BIX, SUVA₂₅₄, Protein and RI and account for 41.95% of the total variation in the data. For PC2 the driving components were DOC and FI, explaining 26.13% of the total variation. Values in close proximity to one another are positively correlated (e.g. RI and Protein), and values on opposing extremes are negatively correlated (e.g. BIX and RI).

Table 2: Correlation coefficient summary for PC1 and PC2. Components greater than absolute value of 0.60 shown in parentheses.

<i>PC1 (41.95%)</i>		<i>PC2 (26.13%)</i>	
<i>Factor</i>	<i>Correlation</i>	<i>Factor</i>	<i>Correlation</i>
BIX	(0.93)	DOC	(0.76)
SUVA ₂₅₄	(0.62)	HIX	0.58
FI	0.29	SUVA ₂₅₄	0.38
HIX	0.22	FI	(-0.87)
DOC	-0.26		
Protein	(-0.77)		
RI	(-0.95)		

WSOM and water samples were distinguished by genetic horizon and water sampling type. Shallow groundwater represents all grab samples taken from wells screened at the B/C interface during weekly sampling throughout the summer of 2019, while Weir samples represent weekly sampling from the weir outlet of WS3 (Figure 7). Visual interpretation of the PCA plot shows variation between WSOM samples by horizon type along the x-axis, but no variation is observed between water samples. Water samples most resemble the B horizons, and have a distinct separation from Oa samples. Statistical PERMANOVA analysis confirms significant differences by sampling location ($p = 0.001$); post-hoc pairwise testing shows that all pairs are significantly different ($p <$

0.05), with the exception of Bhs vs Bs horizons ($p = 0.56$) and Shallow vs Weir samples ($p = 0.07$).

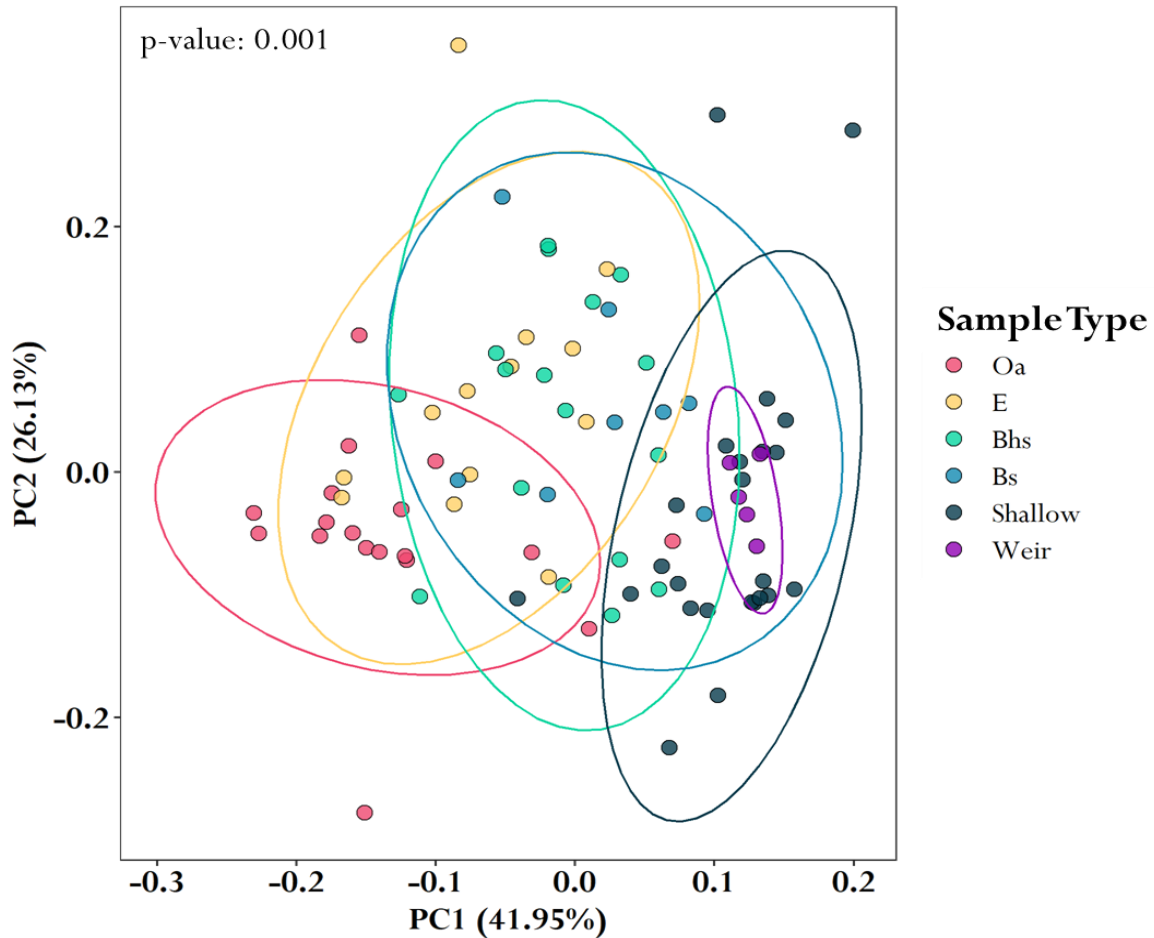


Figure 7: PCA plot of WSOM samples collected from soil pits in Zones 1-4 categorized by genetic horizon, and water samples categorized by sampling type: Shallow represents grab samples from screened PVC wells installed at the B/C (or R) interface, Weir represents samples collected from the weir output in WS3. Ellipses represent 95% CI that a sample will appear within the clustered area. Note: Zone 5 was left out of PCA and PERMANOVA analyses because there were no wells sampled in this position for comparison.

While there are significant differences between sample types, we pose the question of whether groundwater and WSOM samples from the same location (see Fig 1. For description of zones) have similar chemical characteristics of DOM. Based on landscape position, PCA analysis (Figure 8) shows a nested pattern of sample clusters becoming less dispersed in PCA space along the hydrologic flow path from Zone 1 to the weir. PERMANOVA analysis on the data shows no significant differences between landscape positions ($p = 0.054$), which confirms the visual pattern observed in the PCA. Next, data centroids were tested for dispersion homogeneity which showed significant differences between centroid groups ($p = 0.004$). Pairwise t-tests show significant differences between each zone and weir samples ($p < 0.04$) and between Zones 2 and 4 ($p = 0.048$).

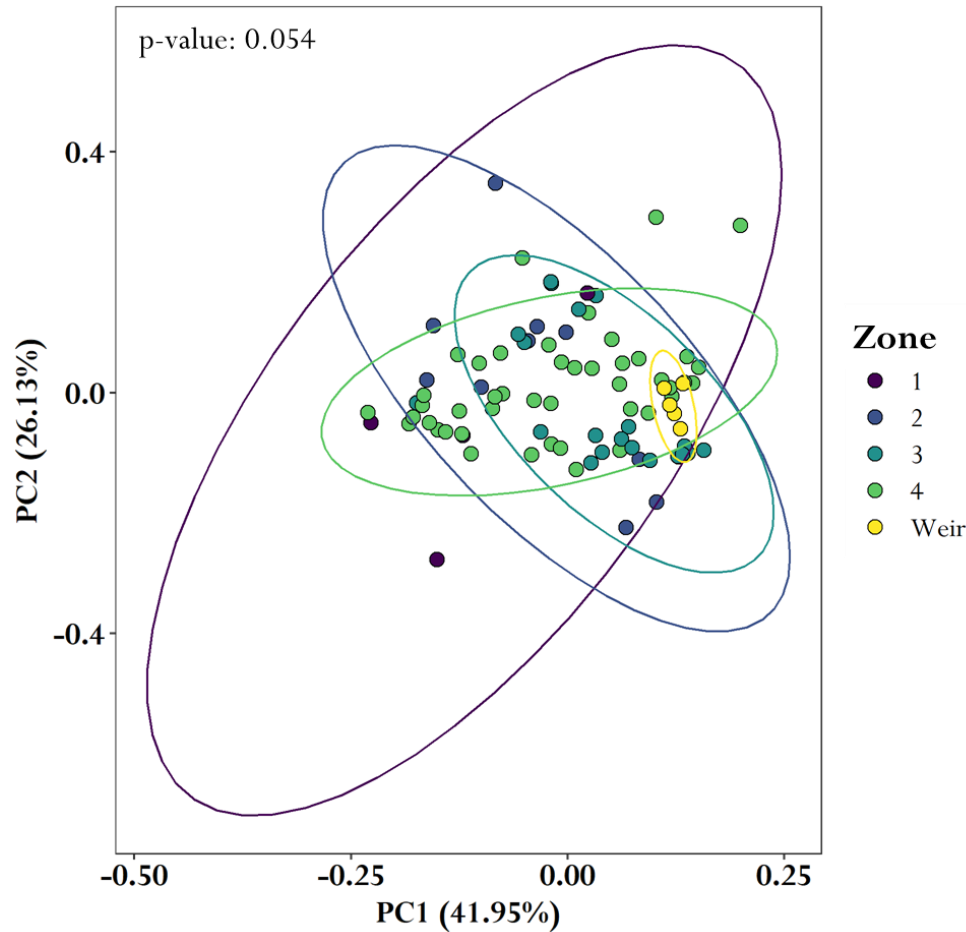


Figure 8: PCA plot of WSOM and groundwater samples from Zones 1-4 categorized by Zone. Ellipses represent 95% CI that a sample will appear within the clustered area. Note: Zone 5 was left out of PCA and PERMANOVA analyses because there were no wells sampled in this position for comparison.

4.0 Discussion

The first aim of this research was to determine if the **chemical characteristics of WSOM are significantly different as determined by genetic soil horizon?** Based on our data observations, some clear patterns arose between genetic soils which provide insight to WSOM with regards to ecosystem processes. First, WSOM from organic soil

horizons contribute large quantities of carbon to the system, which corresponds to patterns found in water chemistry from Zimmer et al. (2013) and Bailey et al. (2019). WSOM in organic soils was comprised mostly of decomposed plant-like material (high FI) with a higher abundance of reduced C-bonded functional groups (high RI). These characteristics of functional groups in WSOM gradually change along hydrologic flow paths, which is likely attributed to microbial processes and organo-mineral complexation exerting more and more influence on the DOM as it moves down the hillslope. The decrease in RI indicates that C-bonded functional groups are undergoing oxidation, likely through increased microbial processing and decomposition (Miller et al., 2006; Mladenov et al., 2008). This is further supported by the observation that WSOM becomes less plant-like and more microbially derived (e.g., FI and BIX indices) as it is transported downslope. An increase in microbial processing corresponds to the observed decrease in protein, which are a class of DOM compounds that are rapidly decomposed by microbes (Brady & Weil, 2008).

We found no significance in HIX between soil horizons, though Bh soils were the least aromatic. Aromaticity measured from $SUVA_{254}$ only showed significance in the near-stream Bh horizons. Findings from Ohno (2007) observed a significant decrease in HIX with soil depth in deciduous forests from emission analysis, but did not observe that same pattern in coniferous forests, or when HIX was calculated with PARAFAC modeling. It is possible that we did not see any significance due to clustering soil horizons by type, which could indicate that some chemical properties remain similar in a genetic horizon regardless of location. Though, the significant decrease in aromatic

character for Bh soils is meaningful as these horizons were only found in Zone 5 for this study, which is in the footslope and near stream riparian area.

Now that we recognize that there are significant differences between genetic soil type, our next question was to determine whether or not **WSOM extracted from soils of same genetic designation, but developed under different hydrologic flow regimes, have similar chemical characteristics?** In this study, extractable carbon in WSOM samples was higher in vertically developed E and Bhs horizons versus lateral. These results are similar to previous research by Bourgault et al. (2015, 2017), which found that vertically developed Bhs horizons had higher total carbon and carbon associated with spodic materials, which is attributed to different dynamics in subsurface groundwater flow. For fluorescence characteristics between development type, the only observed difference was in FI, with lateral E horizons exhibiting a higher degree of microbial character. Despite this, our results suggest that chemical characteristics of WSOM are largely similar by horizon, despite podzolization development sequence (e.g. lateral versus vertical). On one hand, since organic horizons represent the forest floor and might be the same regardless of hydrologic flow, we would not expect differences in WSOM fluorescence. But, in our study sites, the litter quality of organic matter input varies among zones. Zones 1 and 2 are largely comprised of conifer vegetation (e.g., spruce), while Zones 3, 4 and 5 are dominated by hardwood species (e.g., list species), which determines differences in litter quality. For example, lignin percentages in conifers are much higher than in hardwood species and are slower to undergo microbial decomposition (Brady & Weil, 2008), this would imply that WSOM from conifer dominated regions would have different composition than hardwood areas. Referencing

back to Ohno (2007), there were clear differences in how some fluorescing parameters behaved in conifer dominant vs. deciduous dominant forests. So, the lack of difference among content or characteristic is striking. Another factor could be if the WSOM extraction preferentially extracts DOM that is more representative of organic matter derived from new C sources versus older decomposed organic matter (Rennert et al., 2007).

Bourgault et al. (2015, 2017), showed significant differences between extractable Fe, Al, and carbon content between laterally and vertically developed spodic horizons. We find a similar result with regards carbon content. Our results observed that carbon content in WSOM extracted vertical E and B horizons was significantly greater than carbon content in laterally developed horizons, which corresponds to findings of Bourgault et al. (2015, 2017) for carbon when comparing vertical and lateral B horizons. The lack of significance between genetic horizons formed under differing development regimes was unexpected. We know that as DOM moves through soil, it is undergoing complexation with metals, and is utilized in microbial processing. In podzolization, organic-mineral complexes accumulate in the B horizons through illuviation, which essentially removes certain functional groups out of the available DOM pool. Since the functional groups that we extract with WSOM are considered the most labile and available DOM in the soil, we are likely not accounting for the organics that have already been complexed, and the fraction that we are extracting, which has already undergone complexation, likely represents the same types of functional groups, which explains the lack of difference in characteristics.

Finding little significance of WSOM characteristics when comparing same horizons by soil development pattern, is WSOM instead, reflective of the characteristics of groundwater along the same landscape zones? On a visual interpretation of the PCA plots, two key points stand out: first, WSOM samples by horizon group vary across the first axis, and water groups do not exhibit a similar variation. Since well and weir samples were collected over a five-week period in the summer of 2019, they may not be representative of the potential variation in DOM chemistry observable over a longer sampling range, and under differing flow conditions. That the shallow water samples most resemble the B horizons (Figure 7), is likely due to the fact that wells where grab sampling occurred, were screened at the interface of B and C horizons. Second, the trend observed in the PCA data by landscape zone (Figure 8) progresses from a broad grouping at the top of the hillslope, and slowly becomes more condensed within each other down-slope. One interpretation of this data corresponds well with the ecosystem processes discussed throughout this study: DOM starts out with a broad mix of heterogeneous groups at the top of the hillslope, as it is processed, it becomes more homogenous.

5.0 Conclusion

Water and soil chemistry has been well studied at HBEF, which is a site that is representative of northeast glaciated temperate forests. While our general understanding of chemistry, hydrology, and soil forming processes have vastly improved, a need still exists to understand the intricacies of DOM and the role it plays in these systems. This thesis explores differences in fluorescence WSOM composition as it is transported and

transformed along spatial gradients. Understanding the composition of WSOM provides insight to differentiate soil formation processes and to track patterns in nutrient flux cycling in critical zone processes.

To improve our understanding of DOM influence on the soil development and ecosystem processes in these spatially distinct areas of podzolization, this thesis addressed three key questions about WSOM: (i) does it vary by genetic horizon, (ii) are there differences based on soil development under different hydrologic flow regimes, and (iii) is it similar to water along the same point on the landscape? From this research, we have provided additional evidence that carbon distribution and fluorescence characteristics of WSOM varies between genetic horizons along a hillslope, and that while there is significance in the amount of carbon in different development patterns of podzolization, fluorescing chemical attributes of WSOM from same horizons but in differing development regimes are similar. This poses that while hydrologic controls on podzolization processes result in significant morphological and chemical differences for metals and other soil nutrients, the same might not be said for WSOM.

There is evidence that WSOM extractions are preferential to specific types of DOM (e.g. labile, mobile, freshly processed) (Zsolnay, 1999; Rennert, 2007), but it could also be an indication that WSOM is homogenous between genetic spodic horizon regardless of hydrologic controls and biogeochemistry involved in lateral and vertical formation. This was a pattern that was also observed when comparing water samples to WSOM samples across the landscape. Progressing from the ridge, down elevation to the stream, and weir, samples became clustered tighter together, becoming more homogenized. While this thesis has answered some questions on DOM chemistry and

patterns in a northeast glaciated forest, additional work is recommended to elucidate on these patterns of homogeneity in the fluorescing characteristics of WSOM. This thesis contributes robust methods and data to help better understand processes dynamics in these systems. Looking at the composition of DOM between water and soil sheds insight on nutrient movement from soil to stream and will help identify connectedness of soil and water flow paths to identify seasonal or spatial hotspots of organic nutrient cycling and allow for an improved understanding of forest ecosystem dynamics.

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