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Malayika Cincotta
University of Vermont

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SOIL AGGREGATES: THE MECHANISTIC LINK TO INCREASED DISSOLVED ORGANIC CARBON IN SURFACE WATERS?

A Thesis Presented

by

Malayika M. Cincotta

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Thesis Examination Committee:

Julia N. Perdrial, Ph.D., Advisor
James Shanley, Ph.D., Chairperson
Nicolas Perdrial, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

ABSTRACT

Dissolved organic carbon (DOC) plays an important role in the global carbon (C) cycle because increases in aqueous C potentially contribute to rising atmospheric CO_2 levels. Over the past few decades, headwater streams of the northern hemisphere have shown increased amounts of DOC coinciding with decreased acid deposition. Although the issue is widely discussed in the literature, a mechanistic link between precipitation composition and stream water DOC has not yet been proposed.

In this study, the breakup of soil aggregates is hypothesized as the mechanistic link between reduced acid deposition and DOC increases in surface waters. Specific hypotheses state that soil aggregate dispersion (and the ensuing release of DOC from these aggregates) is driven by a decrease in soil solution ionic strength (IS, decreasing the tendency of flocculation) as well as a shift from divalent to monovalent cations (reducing the propensity for cation bridging) in soil solution.

These hypotheses were tested on soil samples collected from several riparian zone and hillslope positions along three flagged transects in the acid-impacted Sleepers River Research Watershed in northeastern Vermont. To determine soil C content by landscape position, samples from transects spanning hilltop to hillslope and riparian area, as well as replicated hillslope and riparian samples (n=40) were analyzed. Aqueous soil extracts simulate the flushing of soils during hydrologic events (e.g. rain or snowmelt) and were used to test the effect of soil solution chemistry on DOC release. Extracts were prepared with solutions of varying IS (0-0.005M) and composition ($CaCl_2$ and NaCl) on replicated soil samples (n=54) and changes in DOC release and aggregate size were monitored. As IS of the extraction solution increased, the amount of DOC in solution decreased, and aggregate size increased. This was presumably due to cations bridging and diffuse double layer effects. This effect was reversed in low ionic strength solutions where DOC release was significantly higher and average aggregate size was smaller. While extraction solution controlled the amount of C liberated, landscape position impacted the quality, but not quantity, of released DOC.

This study is the first to propose a mechanistic link observed changes in DOC in surface waters and recovery from acidification and provides initial experimental evidence that soil aggregates indeed play a role in the generation of DOC.

CITATION

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CHAPTER 1: INTRODUCTION

1.1. From soils to streams: a carbon flux

Carbon (C) is one of the building blocks of life and moves between organic and inorganic reservoirs on land, in water, and in the air. This movement is known as the C cycle (Cory et al., 2011) and is highly sensitive to changes in the hydrologic cycle, land management, and changes in temperature (Worrall et al., 2004). Even though soils represent the largest terrestrial C reservoir in this cycle (Scharlemann et al., 2014), changes in frequency and intensity of hydrologic events (i.e. rain storms and snowmelt) can transfer soil C from the relatively stable solid phase into the more bioavailable dissolved phase by, for example, flushing the soils.

1.2. Global trends of DOC

The dissolved organic C (DOC) represents the most mobile and bioavailable form of C (Zou et al., 2005) and has therefore been monitored across the globe since the 1960s (Clark et al., 2010; Monteith et al., 2007). Microbial processes and photo-bleaching readily transform DOC into dissolved inorganic C forms. If the partial pressure in the water is higher than that of the atmosphere (Doctor et al., 2008), carbon dioxide (CO_2), a potent greenhouse gas, will outgas into the atmosphere.

Over the past few decades DOC increased significantly in many surface waters in forested, northern hemisphere systems (Monteith et al., 2007; Wu et al., 2010). Records of

DOC are available for countries north of the equator, including Canada, the Czech Republic, Scandinavia, the United Kingdom, and the United States. A selection of these countries has seen a decrease in sulfate and an increase in DOC (Monteith et al., 2007).

A multitude of possible drivers for this increase have been proposed and are debated in the literature, including climate change, changes in land use, or reduction in acid deposition (Worrall et al., 2004). A change in acid deposition is put forth as a main potential driver (Driscoll et al., 2001b; Löfgren and Zetterberg, 2011), however, a mechanistic understanding of the connection between the decrease in acid deposition and DOC increase has not been established.

1.3. Research objectives and Hypotheses

My research objective was therefore to investigate such a potential mechanistic link by simulating recovery from acid deposition and monitoring DOC dynamics experimentally.

I hypothesized that the destabilization of soil aggregates due to changes in precipitation chemistry is the cause for DOC release into soil solution and streams because i) surface water DOC is largely sourced from soils, ii) soil carbon is typically stabilized in aggregates, iii) aggregate stability is impacted by soil solution, and iv) soil solution is impacted by precipitation chemistry.

To test this overarching hypothesis, I designed a set of experiments on soils from the acid-impacted Sleepers River Research Watershed in Vermont (VT). For this, I characterized soils from several locations of the two main landscape positions (i.e. the riparian zone close to the stream and hillslope locations) by soil aggregate size, shape, and physical stability. To test specific hypothesized processes on aggregate dispersion, I designed experiments where soils were reacted with solutions of variable composition to test the impact of ionic strength and solution composition (monovalent vs. divalent cations) on aggregate stability. Please see specific hypotheses in Chapter 2.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

How can changes in precipitation chemistry (system input) lead to changes in DOC in surface waters (system output)? Investigation of this question requires "opening the environmental black box" to examine characteristics and processes in the space between input and output, which is mostly occupied by soils (Figure 1). In this literature review, I will lay out what is known about each piece of the puzzle, specifically: acid deposition and recovery, soil dynamics and C storage as well as stream water DOC. The objective of my research is to put these pieces together.

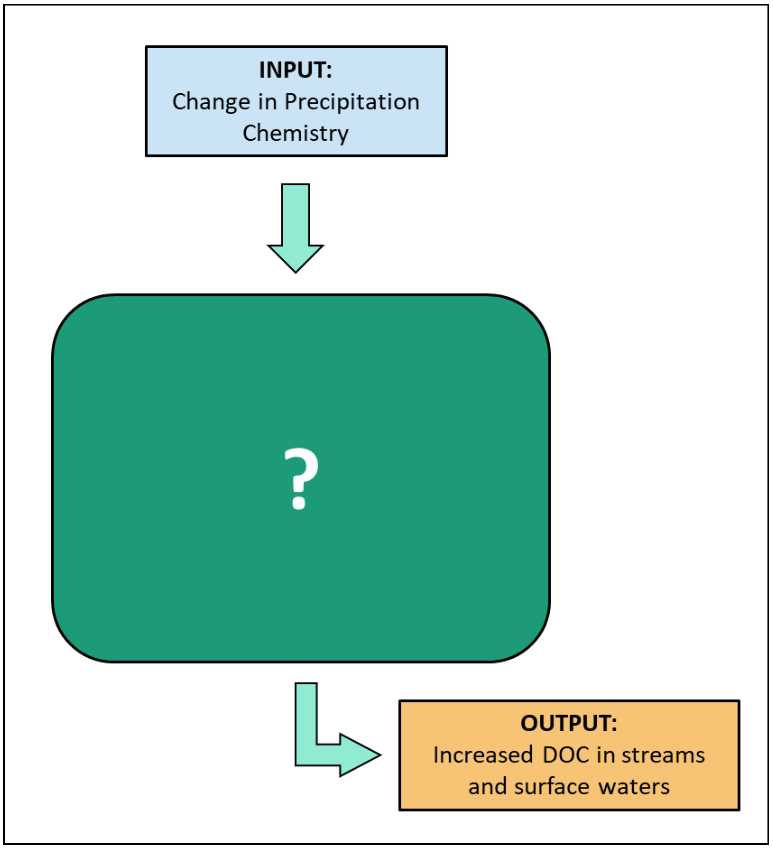

Figure 1. This diagram shows the "Environmental Black Box," where a change in an environmental driver might impact what is in the stream and surface waters through a series of processes within the black box, such as soil dynamics.

2.1. Chemical Driver: Change in precipitation chemistry

2.1.1. Acidic wet deposition

The northeastern United States was impacted by acidic wet deposition, which is the transfer of strong acids and acid-forming substances, such as sulfur dioxide (SO_2) and nitrogen oxides (NO_X), from the atmosphere to the surface of the Earth (Driscoll et al., 2001b). The highest sulfur dioxide emissions were generated from Midwestern states within the Ohio River Valley (Driscoll et al., 2001b). SO_2 and NO_X emissions enter the atmosphere and can be transported great distances until they are deposited. When these gases dissolve in water (either while in the atmosphere or on the ground), strong sulfuric and nitric acid form (Driscoll et al., 2001a). Both acids readily dissociate, releasing large amounts of protons as well as sulfate and nitrate into ecosystems, resulting in widespread detrimental effects. The ensuing low pH leads to the leaching of base cations, such as Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Potassium (K^+), and Sodium (Na^+), diminishing soil health. The low pH also leads to a mobilization of Al, which is toxic to plants and aquatic wildlife (Driscoll et al., 2001a; Rice et al., 2014). Widespread die-off of forests and aquatic wildlife was the result of this continued acid deposition across the northeast (NE) United States.

To counter these impacts, the Clean Air Act was legislated in 1970 to control emissions and since then, the atmospheric deposition of SO_2 has declined more than 80% in the eastern United States (Rice et al., 2014). Further amendments in 1977 and 1990 focused on the continued decrease of SO_2 emissions and initiated controls on NO_X, leading to a slow but steady recovery of these acid-impacted areas (Driscoll et al., 2001b) .

2.1.2. Recovery

We recognize two main stages of ecosystem recovery: i) chemical and ii) biological recovery. Because chemical recovery is the basis for biological recovery, it must occur first (Driscoll et al., 2001a). Chemical recovery encompasses the decrease in sulfate, nitrate, and aluminum concentrations in soils and surface waters (Driscoll et al., 2001a).

Importantly, precipitation is slowly transitioning **from high ionic strength and low pH (acidification) to low ionic strength and higher pH (recovery)**. The NE is still recovering as base-cations accumulate in soils and the pH of soils and water bodies is increasing. At the same time, biological recovery is a slow but ongoing process towards sustained plant and wildlife health (Driscoll et al., 2001a).

2.2. Soils

2.2.1. Soil organo-mineral associations

Soils represent an important interface between the atmosphere and the lithosphere. Soils integrate inorganic products from weathering (bottom up) as well as organic inputs from soil organisms and plants (top down) (Figure 2). The combination of inorganic and organic constituents is often found in the form of aggregates, which are the basic unit of soil structure. Such aggregates are made of clusters containing mineral particles (e.g. clay minerals and oxides) and organic matter (OM) in various stages of decay, as well as living organisms (Frey, 2005). Most aggregation occurs in the top layers of the soil profile (Jarvis et al., 2012).

Aggregates have been studied for quite a few decades and are thought to play an important part in stabilizing C (Jastrow, 1996; Lehmann et al., 2007; Perdrial et al., 2010). For example, clay minerals can physically shield the organic matter from microbial attack, slowing the rate of decomposition (Chorover et al., 2007; Fernández-Ugalde et al., 2013; Lehmann et al., 2007). Different classes of aggregates will form depending on the soil composition. This ultimately determines the type of binding agents present. The most stable aggregates are small (sized less than 250 µm) and are composed of a mixture of clay particles, bacterial and fungal debris, and amorphous aluminosilicates, fundamentally held together by organic glue (Frey, 2005). These microaggregates have a relatively large surface area and are very dynamic. For example, they can **coagulate or disperse based on solution chemistry where both composition and ionic strength play a role** (Schneider et al., 2013; Six et al., 2004).

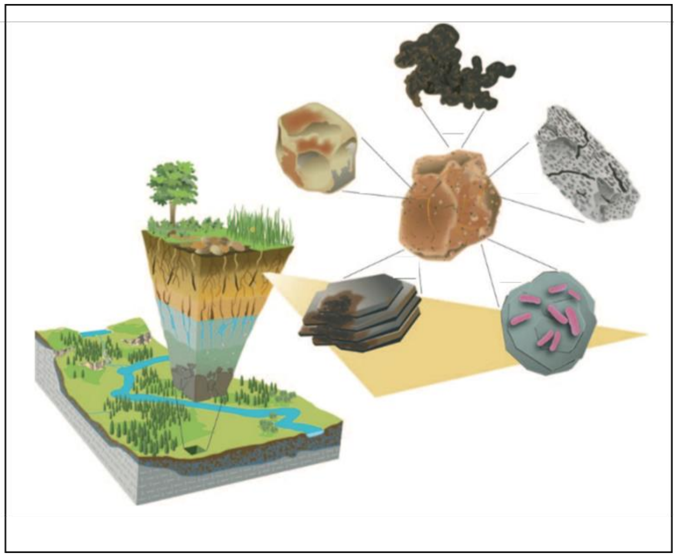

Figure 2. The components and interactions of aggregates within the soil profile. (Modified from (Chorover et al., 2007))

2.3. Lessons from colloidal and clay sciences

Colloidal sciences focus on understanding the interaction between nanoparticles and metals or other constituents (Pfeiffer et al., 2014). These colloids can be either organic or inorganic and are quite complex due to their very small size (Perdrial et al., 2010). Colloidal and clay science provides us with a template for understanding how aggregation might be impacted by solution chemistry and is therefore very relevant for the study of aggregates.

Colloids are constituents in a solution that share properties of a particle as well as a liquid. Colloids are therefore very dynamic (Kanti Sen and Khilar, 2006). Depending on solution chemistry, colloids can readily coagulate (i.e. aggregate) or disperse. Important colloidal effects are i) diffuse-double layer effects varying with ionic strength and ii) cation-bridging between negatively-charged moieties. Both mechanisms are discussed in more detail in the following sections.

2.3.1. Diffuse-double layer

The DLVO (Derjaguin-Landan-Verwey-Overbeek) theory describes the net effect of both attractive and repulsive forces between colloidal particles and grain surfaces (Kanti Sen and Khilar, 2006). The Diffuse-Double layer (DDL) governs the behavior of colloids in solution. It is based on ionic composition and it can be used to explain flocculation and dispersion dynamics of suspended clay minerals (Moore and Reynolds, 1989). The first layer of the DDL is represented by charges near the surface of a particle, such as a negatively-charged clay mineral, and comprises the oppositely-charged ions (positive). These ions are tightly held and immobile in the so-called Stern layer (Lagaly, 2006a; Moore and Reynolds, 1989). The second part of the DDL, the diffuse layer, is generated to dissipate the charge of the Stern layer into the bulk solution by more loosely held anions. Together, the Stern layer and the diffuse layer form the DDL (Derjaguin and Landau, 1941; Moore and Reynolds, 1989; Verwey, 1947).

The thickness of the DDL is dependent on the ionic composition of the solution as univalent charges result in a thicker immobile layer compared to the thinner immobile layer that is generated when divalent or trivalent charges are in solution (Moore and Reynolds, 1989). In some instances, increased ionic strength leads to the compression of the layers as they are held in place by a balance between repulsive and attractive forces, to the extent that repulsive forces are overcome by the weak attractive Van der Waals forces. **The result is flocculation and aggregate formation in high ionic strength solutions** (Lagaly, 2006a). In turn, if the ionic strength of a solution is lowered and the DDL is extended, dispersion will occur because repulsive forces dominate.

2.3.2. Cation bridging

Cation-bridging is another charge-related process that aids aggregation, which is particularly effective for divalent cations such as Ca^{2+}. Such cations can bridge two negatively charged particles (such as clays and often bacteria) (Perdrial and Warr, 2011). Solutions dominated by divalent cations promote aggregation more than those dominated by monovalent cations and can foster aggregation in addition to DDL effects.

2.4. Aggregate dispersion and DOC in soils

Dissolved Organic Carbon (DOC) is operationally defined as any material that can pass through a µm-sized filter (e.g. 0.7 or 0.45 µm) (Creed et al., 2018a; Søndergaard and Middelboe, 1995). During aggregation, DOC and other soil constituents can become sequestered in larger particles, operationally removing them from the dissolved pool. Coagulation and dispersion based on changes in solution chemistry can therefore strongly impact DOC concentrations in soils.

The DDL effect has been described in general for soils with a high clay content but not specifically for acid-impacted soils. However, the impact of divalent cations in both the mineral soil and the soil solution has been observed for such systems (Kerr and Eimers, 2012). The concentration of divalent cations in the mineral soil and soil water was found to influence DOC adsorption through increased flocculation due to cation bridging, resulting in the increased sorption of DOC onto particle surfaces (Kerr and Eimers, 2012).

2.4.1. Dissolved Organic Carbon from soils to streams

DOC is very mobile and can be liberated from the terrestrial systems (i.e. soils) into the water pathways (i.e. streams). For upland surface waters, DOC is primarily sourced from organic soils in the riparian zones (RZ) near the stream as well as from the more distant hillslopes (HS) (Aitkenhead-Peterson et al., 2003; Boyer et al., 1997; Yallop and Clutterbuck, 2009). The RZ, defined as semiterrestrial areas lying at the interface of the terrestrial and aquatic environment, are the direct sources of organic matter to adjacent streams and wetlands (Vidon et al., 2010). Virtually all water entering the stream traverses the RZ (Bishop et al., 1994), which is typically so C-rich that they present near-infinite sources of DOC to streams (Ledesma et al., 2015).

Even though HS soils are farther from the streams, steep slopes can contribute C during times of high hydrologic connectivity. Significant quantities of water can flush HS soils on their way to the stream network during such events (e.g. snowmelt) and can transport solutes, including DOC and nutrients, downslope (Jencso et al., 2009; Raymond et al., 2000).

Because upland headwater catchments often have steep topography, it is important to investigate the role of topography. For example, accumulation of organic matter and debris is greatest at the base of steep slopes compared to gentle slopes. This is likely due to the lateral transfer of materials through the flushing of surface soils during wet events (Jencso et al., 2009). **Overall, this means that soil processes can have a direct influence on stream water composition, especially with respect to C**.

2.4.2. Characteristics of Carbon: bioavailability and fluorescence spectroscopy

Bioavailability describes the potential of microorganisms to interact with dissolved organic C. Stream water C is more readily available for microbial processing than soil C (Marschner and Kalbitz, 2003). A direct way of assessing DOC bioavailability involves measuring the decrease in organic C within, or the increase in CO_2 emitted from a sample over time in incubation experiments (McDowell et al., 2006). The results are expressed as % of respired C and give a reliable estimate of how readily C is transformed.

General characteristics of aqueous C (some of them co-variant with bioavailability) can also be assessed indirectly through the interpretation of fluorescence spectroscopy, a rapid method to analyze dissolved organic matter (DOM) in natural waters. DOM includes other constituents of organic molecules (such as oxygen, hydrogen, nitrogen or phosphorus) and is comprised of a heterogeneous mixture of organic compounds derived from plant and soil decomposition and leachate, degradation of microbial biomass and microbial exudates (Gabor et al., 2014b). The fluorescence spectroscopic analyses are based on the interaction between organic molecules and light (Osburn et al., 2012; Pellerin et al., 2011; Stedmon et al., 2003; Xie et al., 2017). Because the measurements are quick, very large fluorescence datasets are now available. Typical data products include maps that show the relationship between light excitation wavelength and the intensity of the light emitted after passing through the sample. These Excitation-Emission Matrices (EEMs) contain information on the fluorescence intensity for each excitation-emission pair and provide qualitative information on the nature of DOM. Empirical indices can be calculated from these 3-dimensional data arrays such as: the fluorescence index (FI), a measure that suggests the terrestrial vs. microbial source type for DOM (McKnight et al., 2001); or the humification index (HIX), a measure indicating the degree of organic matter decomposition (Zsolnay et al., 1999). Additionally, parallel factor analysis (PARAFAC) applied to these EEMs decomposes the multi-variate dataset to identify fluorescing components that can be identified as either terrestrial humic-like, terrestrial fulvic-like, or microbial protein-like, and provide further insights on DOM reactivity (Fellman et al., 2008; Fellman et al., 2009).

In summary, from prior research we know that there has been a decrease in sulfate deposition (Rice et al., 2014) and we know that soils are a large store of carbon (Lehmann et al., 2007) that could source DOC to streams (Raymond et al., 2000; Yallop and Clutterbuck, 2009). We also know that soil C is mostly stabilized in soil aggregates (Jastrow, 1996; Lehmann et al., 2007; Perdrial et al., 2010) and that solution chemisty affects aggregate stability (Jarvis et al., 2012). As these aggregates disperse, previously protected C can be released into the environment and could, if bioavailable, become respired to contribute to rising CO_2 levels (Doctor et al., 2008; Raymond et al., 2000).

2.5. Objectives and specific hypotheses

The objective of this study was therefore to systematically test how changes in ionic strength and composition of soil solution (monovalent vs. divalent cations) impact soil aggregates and DOC release in a combined lab and field study. The sensitivity of soil aggregates to changes in solution chemistry is the basis for a release of DOC as a result of changes in precipitation chemistry.

Soil aggregate dispersion is expected to result in an increased concentration of DOC in aqueous solution, reflecting the effects of both the shifting diffuse-double layer and the mechanism for cation bridging. I hypothesized that as both ionic strength and the concentration of divalent cations in solution decrease (simulating recovery), soil aggregate size decreases and concentration of DOC in solution increases.

Additionally, I hypothesized that soil type, covariant with landscape position, exerts a strong control on the amount and type of released DOC. Specifically, I hypothesized organic-rich soils from riparian zones release higher amounts of DOC than hillslope soils, and lastly, DOC released from protected aggregates is terrestrial and very bioavailable.

CHAPTER 3: MATERIALS AND METHODS

3.1. Field Site

3.1.1. Description

My field site is located within the headwaters of the Sleepers River Research Watershed (SRRW), a USGS site in northeastern Vermont (Figure 3a). This site has over 25 years of data on stream water composition and has shown increased DOC stream water effluxes similar to the trend observed in many northern hemisphere catchments (Monteith et al., 2007; Worrall et al., 2004; Wu et al., 2010; Yallop and Clutterbuck, 2009). SRRW is a series of nested basins including both agricultural and forested land covers. Each basin has ample above and below-ground installations to monitor soil and stream water composition. The headwater catchment W-9 is the focus of this study, covering an area of 40.5 ha with elevations ranging from 524 to 672 m (Figure 3b, Shanley et al. (2004)).

The watershed is underlain by the Waits River formation, a quartz-mica phyllite with beds of calcareous granulite (Shanley et al., 2004). At the end of the Wisconsinan glaciation (10,000 years before present), a layer of calcareous glacial till was deposited over the bedrock with varying thickness (1 – 4 m). Weathering of the glacial till results in the release of calcium-bicarbonate water that is enriched in sulfate from acid deposition and oxidation of Pyrite present in the till and bedrock (Shanley et al., 2004). The presence of calcium carbonates in the till can buffer pH at depth, helping to mitigate acidification. Groundwater is in equilibrium with these carbonates and exhibits high pH and calcium content. Upwelling of this ground water is typical for low-lying landscape positions close to the streams in SRRW.

All soils were sampled in the W-9 forested catchment which is drained by several tributaries to the main stream (A, B, and C, Figure 3c). Within this small catchment, the RZs are mostly composed of thick Histosols and the HSs are a mixture of both Inceptisols and Spodosols (Shanley et al., 2015). I selected stream B because it has been intensely studied and previous data is available (McGlynn et al., 1999; Sebestyen et al., 2008). Stream B has two main forks (B_X and B_Y) that join in the midsection of the headwaters.

3.1.2. Soil Sampling Zones and Methods

In order to assess the impact of topography on soil composition adjacent to the stream, I separated stream B into three main zones that were sampled in two key sampling campaigns in 2016 and 2017. The perennial stream B_X is surrounded by relatively steep HSs (averaging ~16%, hereafter *steep slope*) while the ephemeral steam B_Y is surrounded by a bed of organic-rich muck that transitions into a wetland during periods of low flow. The HSs bordering B_Y are ~5% (hereafter the *intermediate slope*). The third topographic zone has gently dipping HSs averaging ~3% (hereafter the *gentle slope*) and is located several meters close to the confluence of both streams B_X and B_Y. The forest floor at this field site is typically covered with a thick blanket of leaf litter and decomposing organic matter forming a wetland.

The goal of the first sampling campaign was to capture variation in soil composition along transects - from hilltop to riparian area back to hilltop - for each of the different zones (i.e. Steep, Intermediate and Gentle, hereafter referred to as *Full Transect* sampling, Figure 3d). Each transect includes low-lying landscape positions (RZs, spanning ~ 2 meters on either side of the channels) and several HS locations. Samples were taken in replicate every two meters along the HSs and every meter in the RZ to capture variability in the narrower zone.

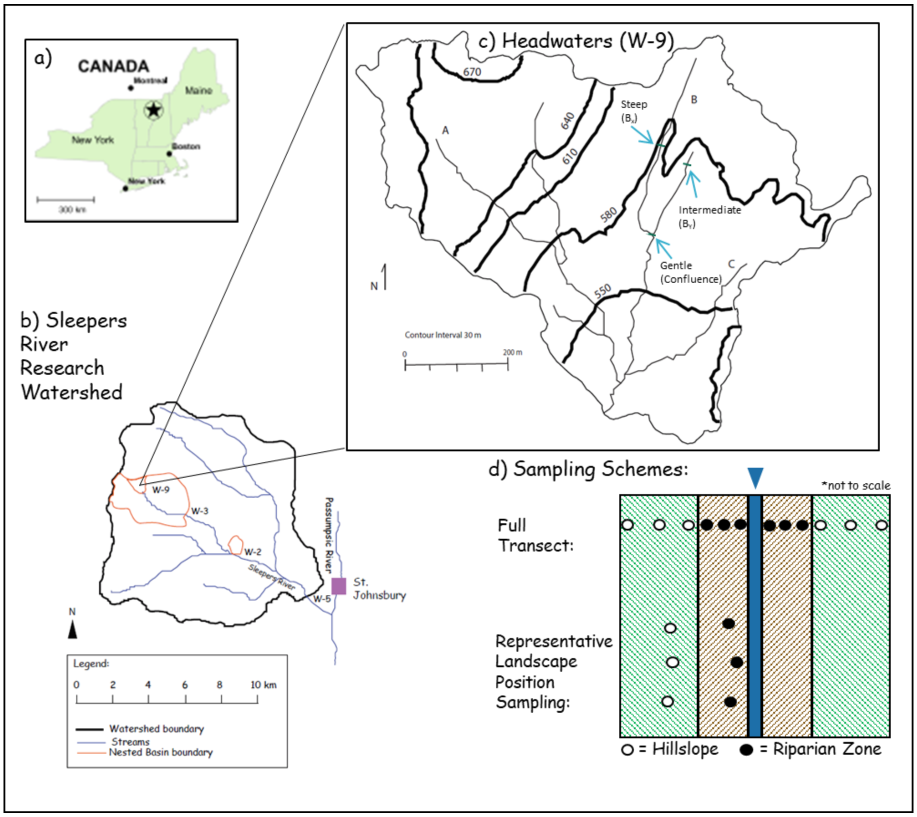

Figure 3. (a) The location of the study site in northeastern Vermont. (b) The Sleepers River Research Watershed is located west of St. Johnsbury, VT (modified after (Shanley et al., 2015). (c) The headwaters (W-9) with stream B is the focus of my study (modified after (Shanley et al., 2015). (d) Contour map with an interval of 30 m for W-9 (modified after Kendall et al. (1999)). (e) Schematic representation of my sampling design in map view (not to scale) that included samples from full transects from HSs (green shaded area) and RZs (brown shaded areas) to the stream (blue). Representative zone samples included replicated samples for each landscape position (each dot represents 3 samples).

The second sampling campaign was based on results of the Full Transect sampling, which showed distinct differences in soil composition for HS vs. RZ landscape positions for all zones. To better capture the signature of soils for these two landscape positions, I resampled representative RZ and HS soils for further analysis (hereafter referred to as "Representative Landscape Position" [LSP] sampling) in replicate from all zones (steep, intermediate and gentle slopes, Figure 3e). A total of 18 soil samples were collected, six were from each of the three zones. Half of these six samples were collected from the RZ, the other half from the HS locations.

Samples for all campaigns were collected in a way to avoid decaying trees, heavily rooted areas, and other sources of excess organic matter. Using a pre-cleaned 15-cm bucket auger, samples were obtained from the top 15 cm of the soil profile after the leaf litter was removed. The samples were handled gently to preserve soil aggregates. In addition to single samples, I also prepared composite samples for selected analyses where analytical replicates were time- and cost-prohibitive (Density separation and Scanning Electron Microscopy (SEM)). For this, one scoop of each individual, field-moist sample was taken and gently mixed on a tray, air-dried for 3-4 days, and sieved through a 2-mm mesh (Figure 4).

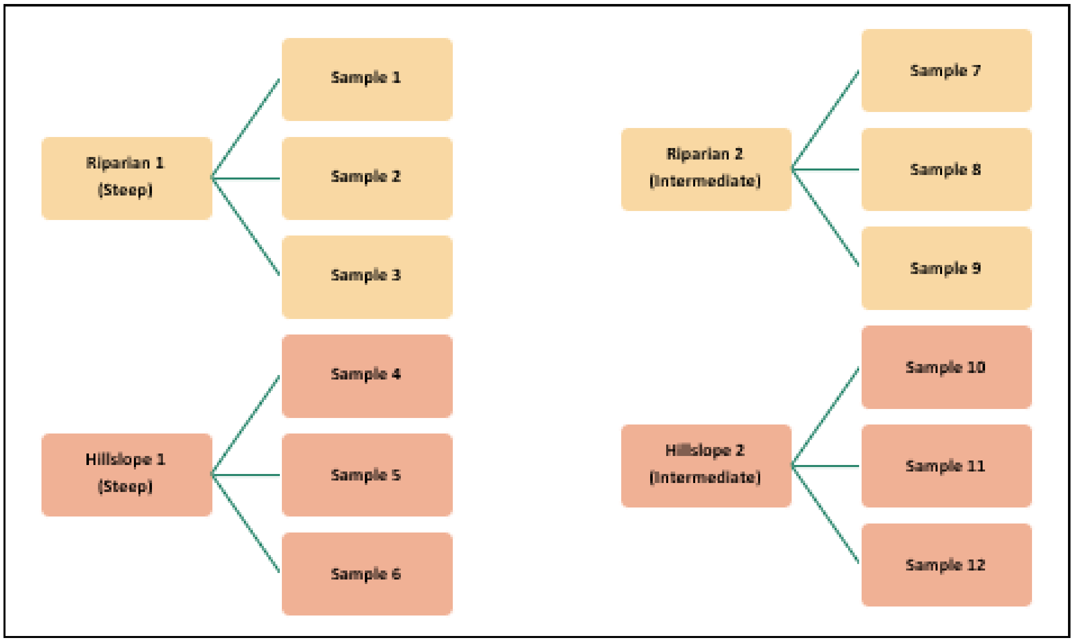

Figure 4. Composite samples combine the characteristics for three representative landscape positions and two zones (steep vs. intermediate) leading to four composite samples.

3.2. Laboratory procedures and analytical techniques

3.2.1. Elemental Analysis

All soil samples were dried on trays (4-5 days), sieved through a 2-mm mesh, and homogenized using a ball mill. Duplicates of each sample were weighed using a Mettler-Toledo XP26 balance and pressed into 5x9 tin capsules (Costech Analytical Technologies, Inc.) that were analyzed using a combustion-based elemental analyzer in the University of Vermont (UVM) Geology Stable Isotope Lab, where the samples were combusted in a chamber and Helium gas transported the sample to the detector. The Full Transect samples were individual soil samples along a transect and the Representative Landscape Position samples were composite soils. These were made up of three samples (as shown in the tree above, Figure 4) from each of the three transects and were analyzed as lab triplicates.

Resulting percent C values are compared to standards (B2150 for High organic content sediment standard and B2152 for Low organic content soil standard) provided by Elemental Microanalysis Limited and were converted to mg/kg.

3.2.2. Scanning Electron Microscopy

To visualize small aggregate architecture and determine aggregate composition, I used the *VEGA3 TESCAN* Scanning Electron Microscope (SEM) and *AZtec* Elemental Mapping software at Middlebury College. Air-dried samples were first sieved through a 2-mm mesh to exclude larger particles (i.e. roots and small gravel) and then sieved through a 250-μm mesh that matched the maximum particle size observed with laser particle size analysis. Three replicates of each composite sample were mounted using double-sided carbon tape on separate metal stubs and sputter-coated with carbon before analysis. Observations were performed in BSE mode at 5 keV acceleration voltage and EDS maps were acquired for 5 minutes with a probe resolution of 15 mm.

Two solutions that simulated acidification and recovery (calcium chloride ($CaCl_2$) and Nanopure water (NW), respectively) were added to each sample from both locations, (HS and RZ) to investigate the effect of extraction solution on aggregate size, shape, and composition. For this, several drops of the respective solutions were added with a micro-pipette directly onto the aggregates and allowed to react with the aggregates for one hour. To simulate the conditions of the Aqueous soil extraction procedure (see below), the pipette was used to carefully mix the solution around the aggregate without touching it. We refrained from aspiring the reaction solution but allowed the solution to dry in place to avoid removal of the aggregates, a process that was completed within 90 minutes.

3.2.3. Particle Size Analysis

Unfiltered extracts were analyzed for particle size using the Micro Volume Module on a *Beckman Coulter* LS230 Laser Particle Analyzer to assess the impact of extraction solution on aggregate size. This instrument measures the size distribution of particles and aggregates (from 0.4 μm to 2000 μm) suspended in liquid using the principles of light scattering. Each sample was analyzed in its respective extraction solution right after shaking.

3.2.4. Aqueous Soil Extracts

I simulated the flushing of soils during hydrologic events (such as snowmelt) in a laboratory setting by preparing Aqueous Soil Extracts (ASE) on all representative LSP samples. The ASE were conducted on field moist soils (existing water content was accounted for) by mixing soil and aqueous solutions at a 1:5 ratio and by shaking the mixture on a reciprocal shaker (Eberbach, Ann Arbor, MI, USA) for 1 hour (Perdrial et al., 2012). Separation of solids from colloidal and dissolved matter was performed by centrifugation (30 minutes, 3000 rpm) and filtration using combusted 0.7 μm glass fiber filters (Whatman GF/F, Buckinghamshire, UK). Extraction solutions of NW as well as salt solutions (0.005 mol L^{-1} sodium chloride (NaCl), and 0.005 mol L^{-1} $CaCl_2$) were used to simulate changes in soil solution composition due to changes in precipitation chemistry. Filtrate was transferred to combusted amber glass bottles for analyses that were completed immediately after extraction.

3.2.5. Aqueous Phase Analysis

I analyzed all ASE samples in duplicate for concentrations of DOC and Total Dissolved Nitrogen (TDN) *via* combustion using a *Shimadzu* Total Organic Carbon Analyzer (Columbia, MD, USA). Results are reported normalized to the exact amount of solution and soil used for each extract (mg/kg) to allow for comparison between samples.

All ASE were also analyzed to determine spectral characteristics of dissolved organic matter (DOM) in solution using the *Horiba* Aqualog Fluorescence and Absorbance Spectrometer (Horiba, Irvine, CA, USA). The Excitation range spanned from 250 to 600 nm and Emission ranged from 212 to 599 nm. All Excitation-Emission Matrices (EEMs) were blank-subtracted (NW, resistivity 18MΩ cm^{-1}), corrected for inner filter effects, and Raman normalized.

From these data, a couple indices were used to provide insight on the type and characteristic of carbon released from dispersed aggregates. The Fluorescence Index (FI) is calculated as the intensity at Em 470 nm divided by the intensity at Em 520 nm for Excitation at 370 nm (Cory and McKnight, 2005). FI provides information on precursor DOM sources. Strictly microbial DOM includes extracellular release and leachate from bacteria and algae (Hansen et al., 2016) and has FI values of 1.8 and higher (Gabor et al., 2014b). Terrestrially-derived DOM include degraded plant and soil organic matter and has lower FI values (FI ~1.2) (Gabor et al., 2014b). The humification index (HIX) is correlated to changes in H/C ratios typical for humification and is calculated as a ratio of the area under Em 435 – 480 nm divided by the quantity of both areas under Em 300 - 345 and Em 435 – 480 nm, at an Excitation of 254 nm (Gabor et al., 2014b).

EEMs were further processed using *Matlab R2017b* and the drEEM_4_0_(Ext.) toolbox (Murphy et al., 2013) to perform a Parallel Factor Analysis (PARAFAC). This n-way statistical analysis decomposes the fluorescence spectra of DOM into independent components whose abundance can be related to differences in composition and source material (Fellman et al., 2008). The samples in my study were described by a validated 3-component PARAFAC model (Figure 5). The three different peak pairs (Excitation-Emission) were identified by comparison to a few other published studies (Fellman et al., 2008; Singh et al., 2013; Yamashita et al., 2010) as terrestrial fulvic-like (C-1) which is of a lower molecular weight, terrestrial humic-like (C-2) which is of a higher molecular weight, and microbial protein-like fluorescence (C-3, Table 1). The peak loadings as Excitation and Emission for each of the three components are as follows C1: Ex 252, Em 482 (fulvic-like); C2: Ex 250, Em 413 (humic-like); C3: Ex 278, Em 331 (protein-like).

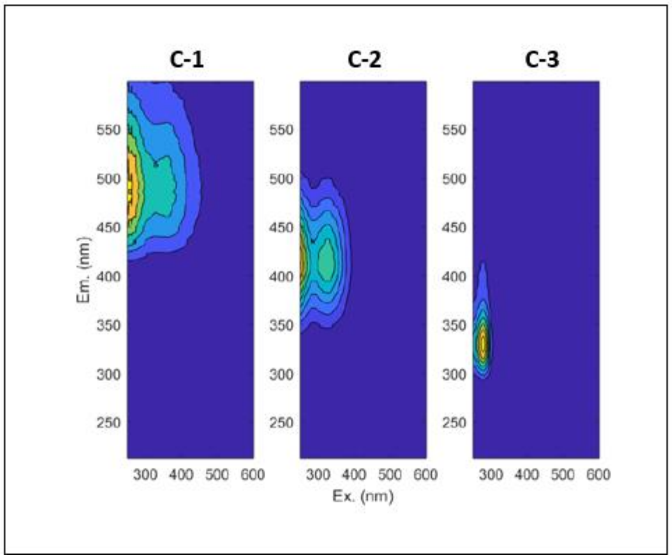

Figure 5. Contour plots for the three components validated by PARAFAC, where C-1 is terrestrial fulvic-like, C-2 is terrestrial humic-like, and C-3 is microbial protein-like.

Table 1. The published PARAFAC models used to confirm my 3-component model.

My Component	Literature Components	Designation
C1: Terrestrial, fulvic-like EX: 250- 300; EM: 425-575	Singh et al., 2013 (FH3) Fellman et al., 2008 (C3) Yamashita et al., 2010 (C1)	Fulvic-like Fulvic-like Fulvic-acid like
C2: Terrestrial, humic-like EX: 250-325; EM: 340-500	Singh et al., 2013 (FH1) Fellman et al., 2008 (C1) Yamashita et al., 2010 (C2)	Humic-like Humic-like Humic-like
C3: Microbial, protein-like EX: 280; EM: 300-420	Singh et al., 2013 (FH3) Fellman et al., 2008 (C3)	Protein-like Tryptophan

3.2.6. Incubations

To determine the bioavailability of dissolved carbon in ASE, an aliquot of each filtered ASE was tented in aluminum foil to avoid photo-degradation and incubated for the duration of 14 days on a reciprocal shaker table (Eberbach, Ann Arbor, MI, USA). Because the filtered samples did not contain microbes, each ASE was inoculated with an unfiltered slurry containing O-horizon microbes from all 18 soils before shaking.

After the incubation period was complete, samples were re-analyzed for DOC to determine % of C loss through respiration (evaporation losses were accounted for by tracking sample mass and adding lost solution). Blanks of each extraction solution were incubated for the same duration. The samples were re-filtered over a combusted (475 °C, 4h) 0.7 μm glass fiber filter (Whatman GF/F, Buckinghamshire, UK) and analyzed for DOC. Particles were not observed on the filters, indicating minimal loss of C through this process.

3.2.7. Statistical Analyses

The JMP Pro 13.0.0 software by *SAS* was used to conduct the statistical analyses. An Analysis of Variance (ANOVA) and the Tukey Multiple Comparison Test were performed to determine significance of variations by extraction solution and landscape position. The average DOC concentrations for each of the representative landscape positions (RZ and HS) were compared, using a T-test to determine if the means were significantly different.

CHAPTER 4: RESULTS

4.1. Solid Phase

4.1.1. Full transect samples: Effect of landscape position on soil C

Total Organic Carbon (TOC) analysis on full transect samples revealed that C content is highest in the RZ, especially adjacent to the steep HS (3500 mg/kg, Figure 6). RZ C contents for the intermediate and the gentle transect were lower than that for the steep transect (intermediate= 847 mg/kg, gentle = 2076 mg/kg) but significantly elevated relative to average HS soils (averaging 570 ± 200 mg/kg for steep, averaging 485 ± 73 mg/kg for intermediate and 550 ± 80 mg/kg for gentle).

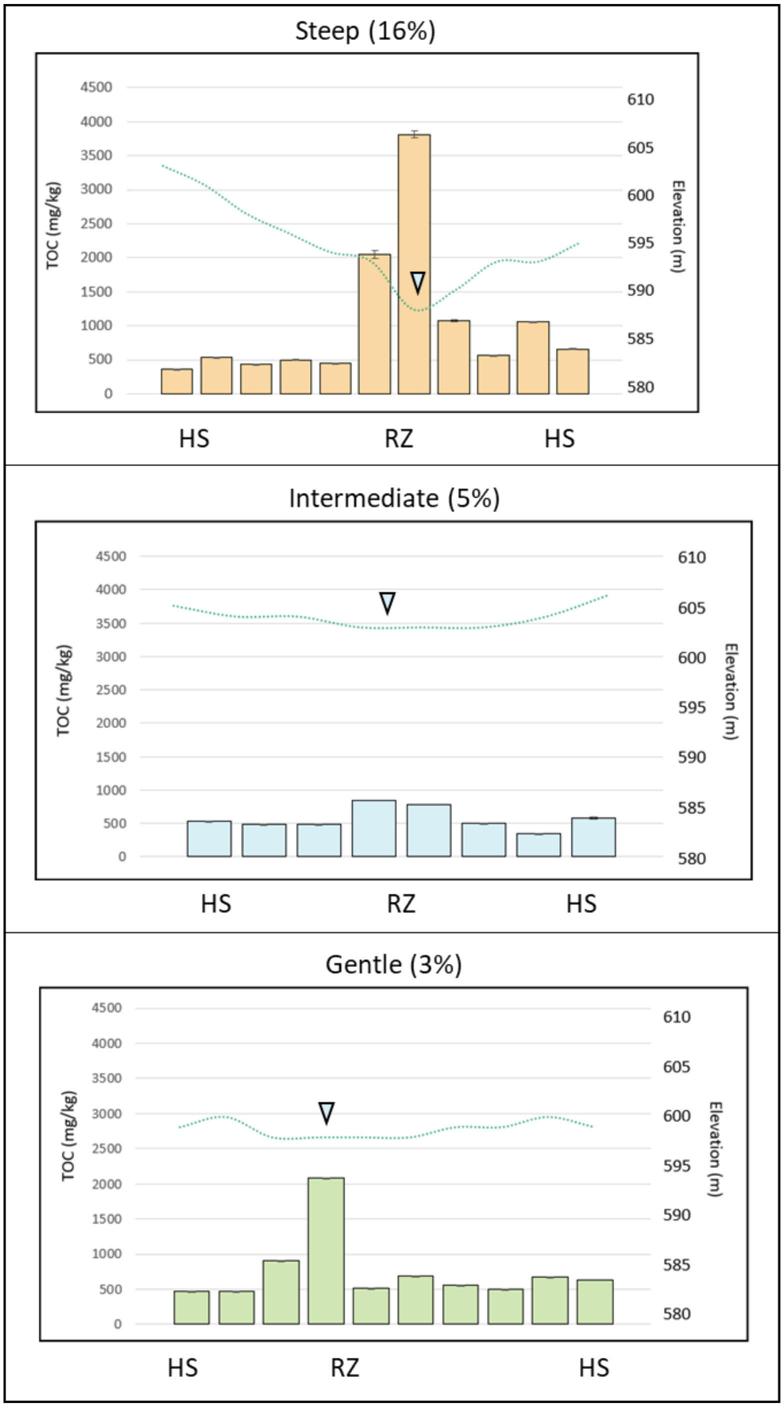

Figure 6. Solid-phase analysis on soils from the Full Transect with TOC in mg/kg (columns with error bars), Elevation in meters (dotted line), and varying slope (%) for all three transects; Riparian zone = RZ and hillslope = HS. Location of the stream is indicated by an inverted triangle.

4.1.2. Representative LSP: Effect of landscape position on soil C

Analyses for Total Organic Carbon (TOC) on LSP samples were conducted on composite samples (Figure 24). The RZ for all three transects had the greatest amount of soil C compared to the HSs and once again, the steep transect had the maximum soil C at 3344 mg/kg. The least amount of C content was on the gentle HS (384 mg/kg).

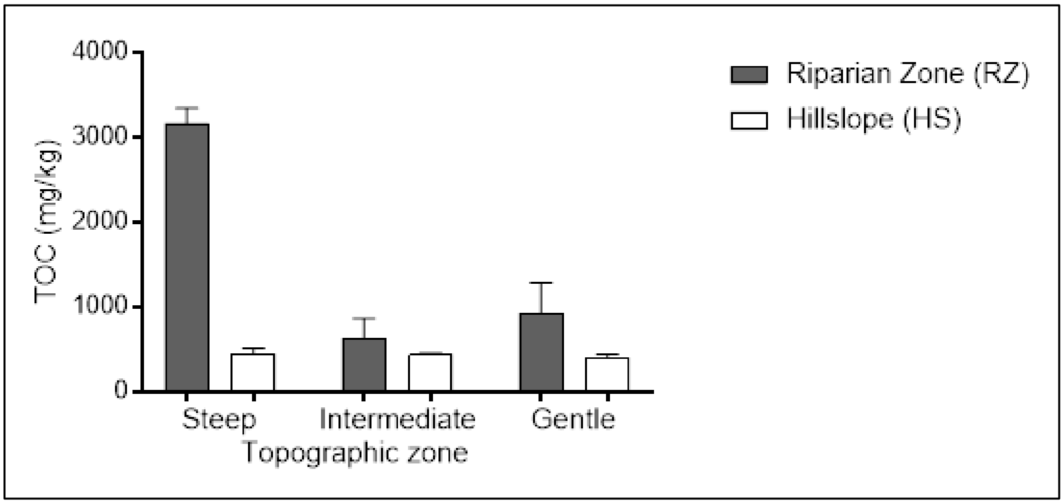

Figure 7. Total organic carbon (TOC) content of soils from representative landscape position samples for all three topographic zones (steep, intermediate, and gentle, expressed as average with standard error) as composite samples.

4.1.3. Visualization of Aggregates

Soil aggregate morphology and size varies with dominant landscape position. Examples shown for typical untreated RZ aggregates are elliptical and appear to contain smaller amounts of angular mineral fragments (Figure 8). The matrix is mostly organic matter visible as fine-grained material, fine roots, and possibly fungal hyphae. In contrast, examples of the untreated HS soil aggregates are smaller and have a greater proportion of mineral grains (Figure 9).

Elemental distribution on the aggregate surface also varies with landscape position. The RZ aggregates have a consistent coating of Ca compared to the HS samples (Figure 8). In the HS, there is a greater distribution of Al and the quartz grains tend to be larger compared to the RZ (Figure 9). The results from the modified Aqueous soil extraction illustrate dispersion of particles for both landscape positions with the NW treatment compared to the $CaCl_2$ treatment. The liberated organic matter moved to another part of the carbon tape (used to prep the sample for analysis).

Using qualitative XRD results from another study by Armfield et al. (in prep) and knowledge from morphology, some minerals were identified. The spectra indicate the presence of clinochlore, hornblende, and quartz mixed in with the organic matter in the RZ (Figure 10) along with anorthite and orthoclase (Appendix 2). Amphibole, quartz, and biotite were identified using the spectra in the HS aggregates (Figure 11) as well as albite (Appendix 2). Powder X-Ray Diffractometry is required to accurately determine the minerals.

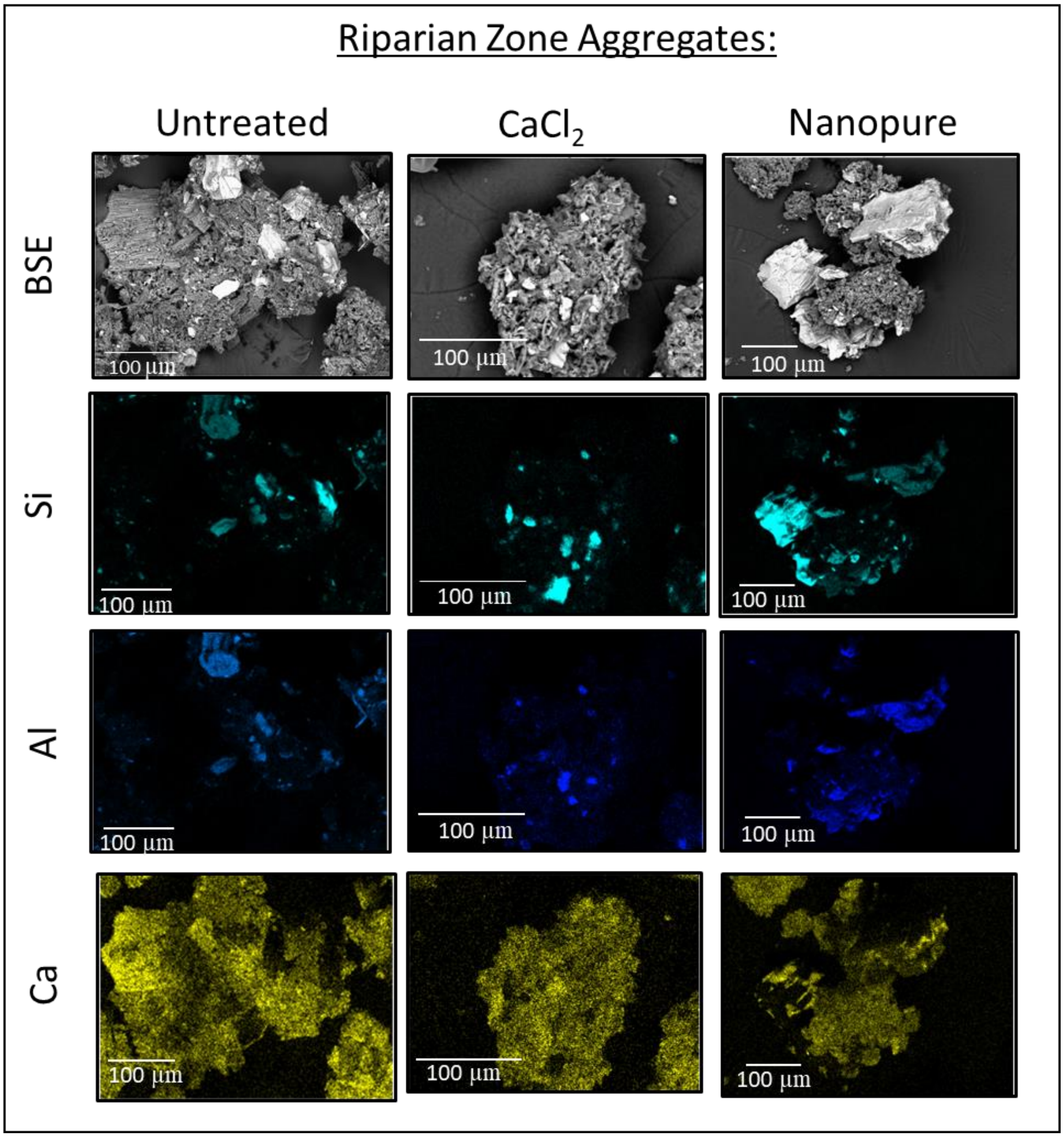

Figure 8. Representative riparian zone (RZ) aggregate as a backscatter electron (BSE) image. Maps show the distribution of specific elements in untreated air-dried samples (left panel), treated with high ionic strength $CaCl_2$ solution (center panel), and low ionic strength Nanopure water (right panel).

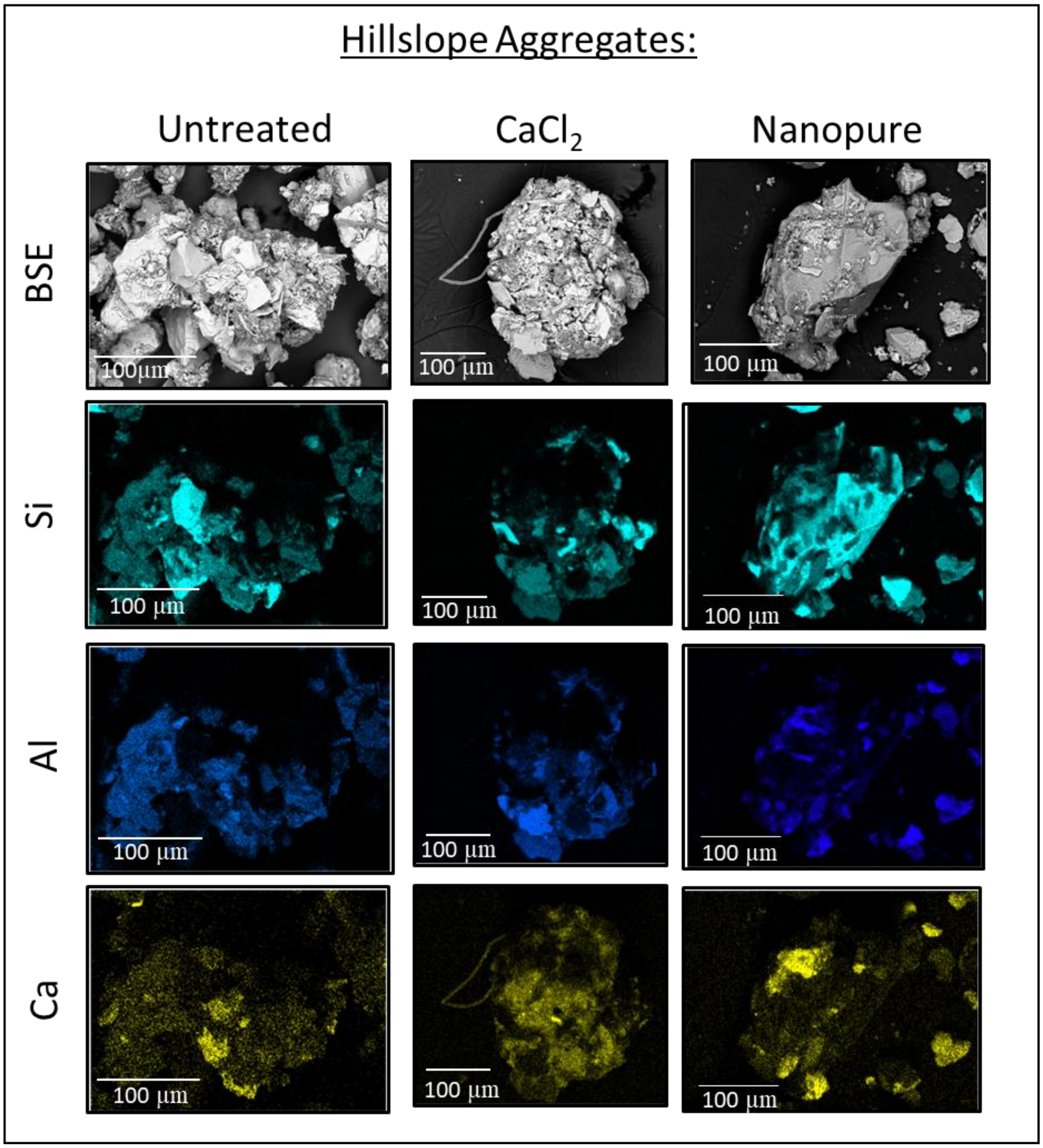

Figure 9. Representative hillslope (HS) aggregate as a backscatter electron (BSE) image. Maps show the distribution of specific elements in untreated air-dried samples (left panel), treated with high ionic strength $CaCl_2$ solution (center panel), and low ionic strength Nanopure water (right panel).

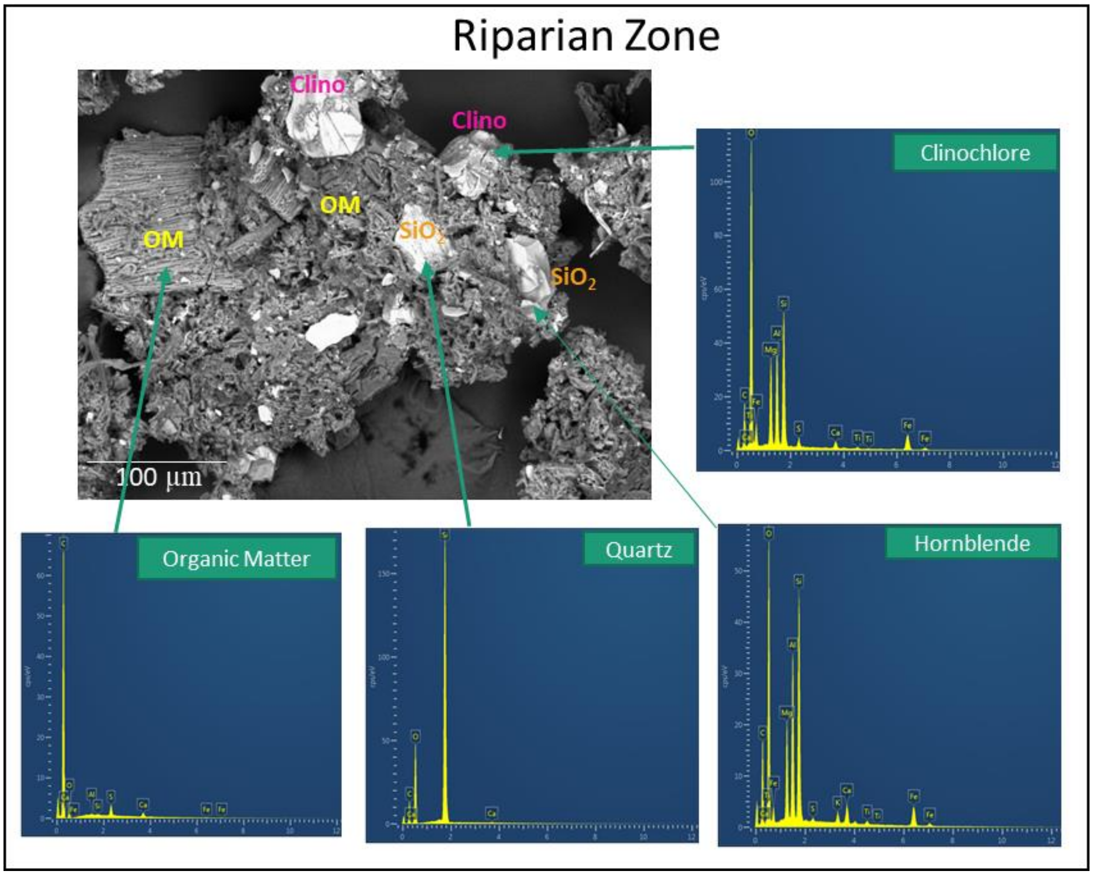

Figure 10. Secondary electron scanning microscopy (SEM) image of soil aggregates present in composite soils from the riparian zone (RZ) of the steep transect. OM = organic matter, clino = clinochlore.

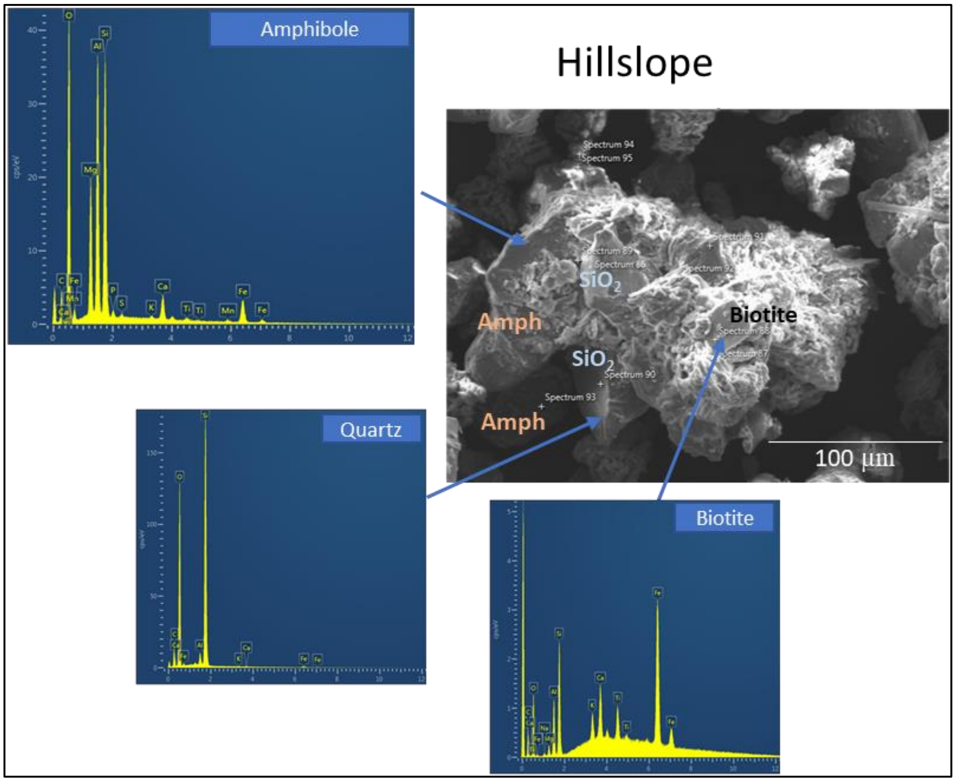

Figure 11. Secondary electron scanning microscopy (SEM) image of soil aggregates present in composite soils from the hillslope (HS) of the steep transect. Amph = Amphibole, SiO_2 = Quartz

4.1.4. Particle Size Analysis

Particle size distribution changes with solution chemistry and landscape position. The shift is visible more so in the RZ soils (Figure 12a) compared to the HS soils which, in the latter, is minimal (Figure 12b). Average particle-sizes are generally larger in the RZ samples compared to the HS and decrease as ionic composition changes (Table 2). Results of typical samples from the RZ and the HS illustrate the distinct change in particle size for each solution (Appendix 3).

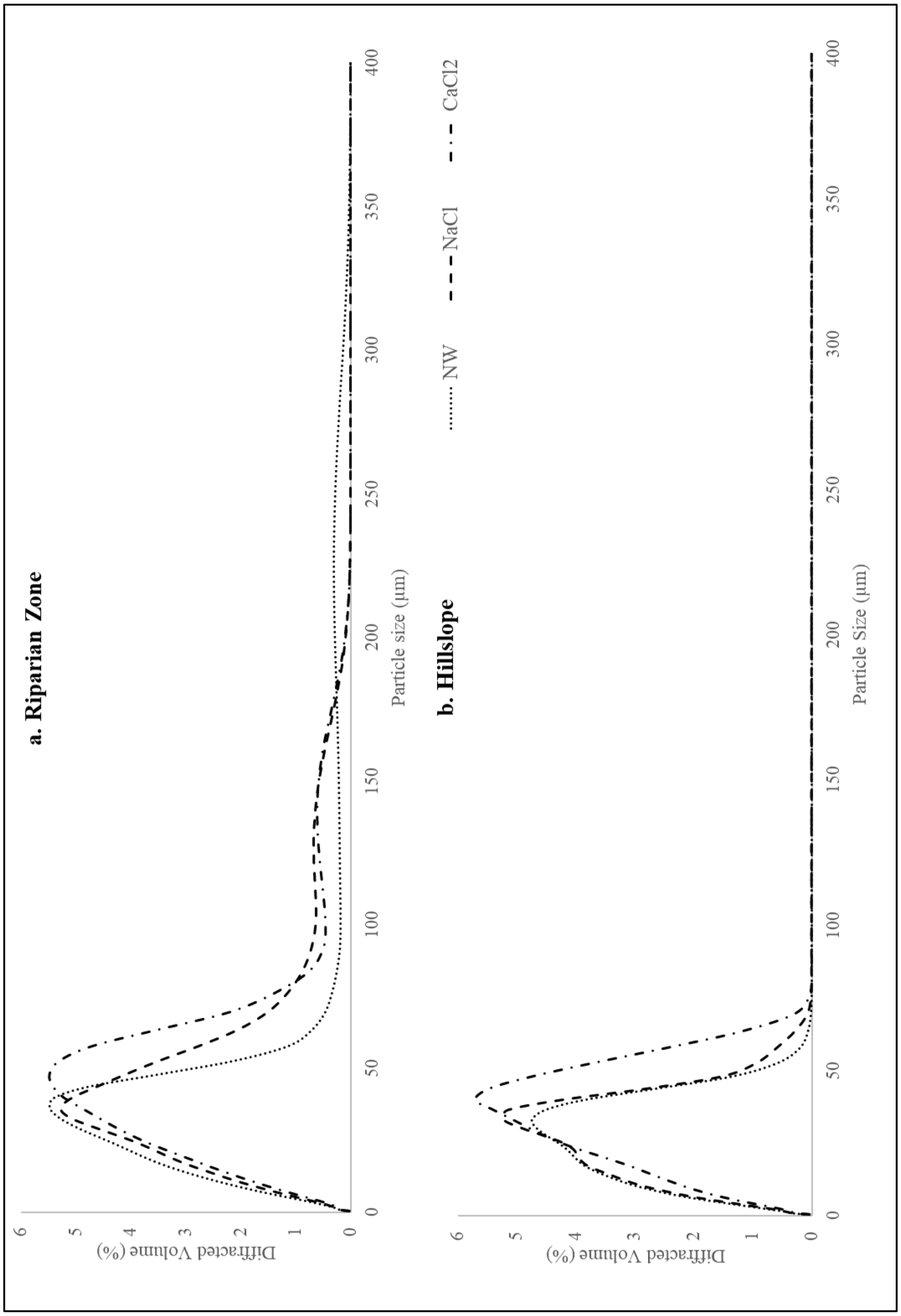

Figure 12. Distribution of average particle size by treatment for landscape positions, RZ (a) and the HS (b). There is a shift to the left in peaks from higher ionic strength to lower ionic strength solution. (NW = Nanopure Water, NaCl = sodium chloride, $CaCl_2$ = calcium chloride)

Table 2. Mean particle sizes (n = 3) in µm ± standard deviation for solids in the different extraction solutions.

Location:	**NW:**	**NaCl:**	**$CaCl_2$:**
RZ (Steep)	19.1 ± 1.97	32.0 ± 14.8	34.2 ± 9.69
HS (Steep)	13.9 ± 1.27	-	-
RZ (Intermediate)	15.5 ± 2.13	16.7 ± 3.59	19.6 ± 1.68
HS (Intermediate)	11.6 ± 2.29	-	-
RZ (Gentle)	19.5 ± 3.96	23.9 ± 2.21	26.6 ± 4.13
HS (Gentle)	14.1 ± 1.52	-	-

4.2. Aqueous Phase

4.2.1. Representative LSP: Effect of extraction solution

Dissolved carbon content in aqueous soil extracts from LSP samples was strongly impacted by the composition of the extraction solution but did not vary by HS vs. RZ LSP (Figure 13). Independent of representative zone, the amount of DOC extracted with NW showed the highest values (over 55 mg/kg) but also the greatest variation (SD = 12.2 mg/kg). The salt solutions extracted significantly lower DOC concentrations and showed lower variability (averaging 17.7 ± 7.18 mg/kg and 11.6 ± 3.02 mg/kg for NaCl and $CaCl_2$, respectively).

The TDN content leached into the various extraction solutions followed the same pattern as DOC and was not affected by landscape position (Figure 14). The greatest amount of TDN was extracted in the Nanopure treatment (3.37 mg/kg) and this solution had the highest average (2.12 ± 0.816 mg/kg). The $CaCl_2$ treatment extracted the least (0.515 mg/kg) with an average of 1.35 ± 0.515 mg/kg and the NaCl had an average that overlapped with the two other solutions (1.70 ± 0.85 mg/kg).

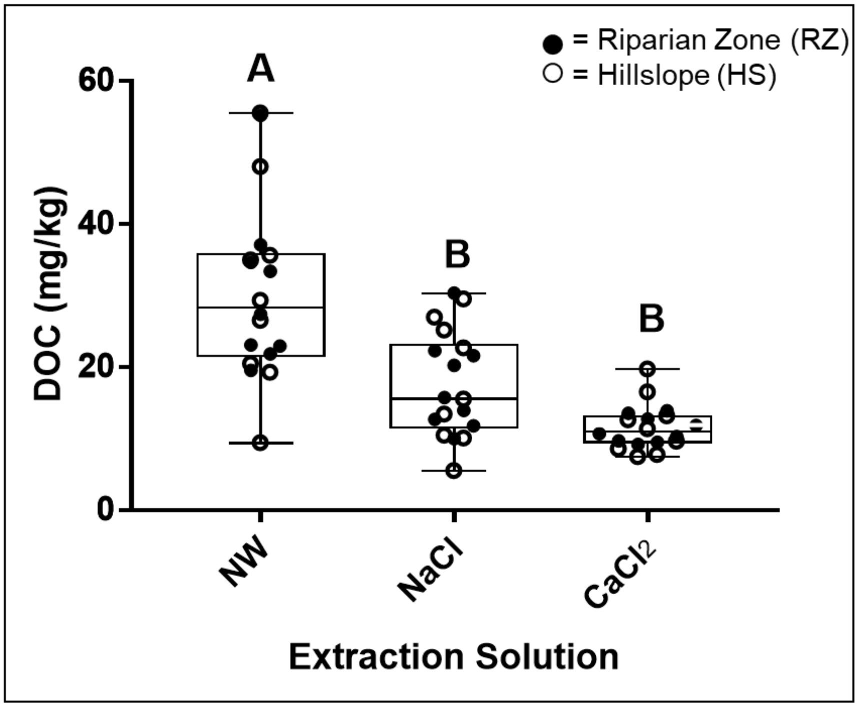

Figure 13. Boxplots showing the mean and range of DOC concentrations (mg/kg) determined by treatment with three aqueous soil extraction solutions (Nanopure Water (NW), sodium chloride (NaCl), and calcium chloride ($CaCl_2$)). Treatments (n = 18) with different letters indicate pairs of means that are significantly different ($p < 0.05$).

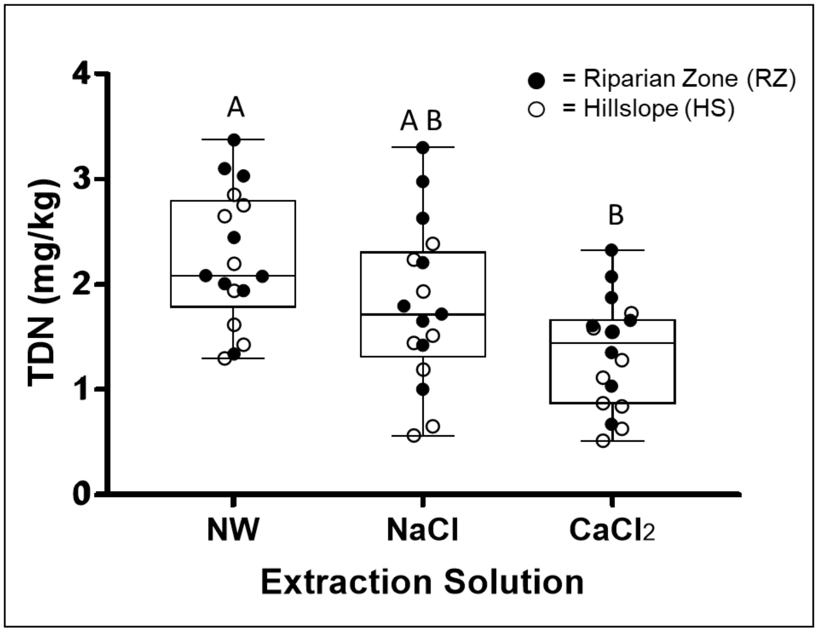

Figure 14. Boxplots of the mean and range of extraction solution (NW = Nanopure water, NaCl = sodium chloride, $CaCl_2$= calcium chloride) illustrate TDN is not affected by landscape position (RZ or HS). Different letters indicate pairs of means that are significantly different at alpha = 0.05 (each treatment n=18).

The Fluorescence Index (FI) values differed with extraction solution. Highest (most microbial) FI values were observed for $CaCl_2$ extracts (ranging from 1.50 to 2.04). FI values for NW and NaCl extracts varied but were generally lower (ranging from 1.24 to 1.77 for Nanopure and 1.30 – 1.57 for NaCl, respectively, Figure 15a). For all extraction solutions, HS soils had higher FI values than RZ soils (Figure 15b).

The values for the Humification Index (HIX) varied greatly in all extraction solutions but Nanopure extracts had highest HIX values (up to 6.49) and $CaCl_2$ extracts had lowest HIX values (as low as 1.84, Figure 15c). Generally, HS soil extract HIX values were greater than RZ extracts (averaging 3.73 ± 1.16 compared to 3.19 ± 0.79, respectively) but the difference is not significant ($p > 0.05$) (Figure 15d).

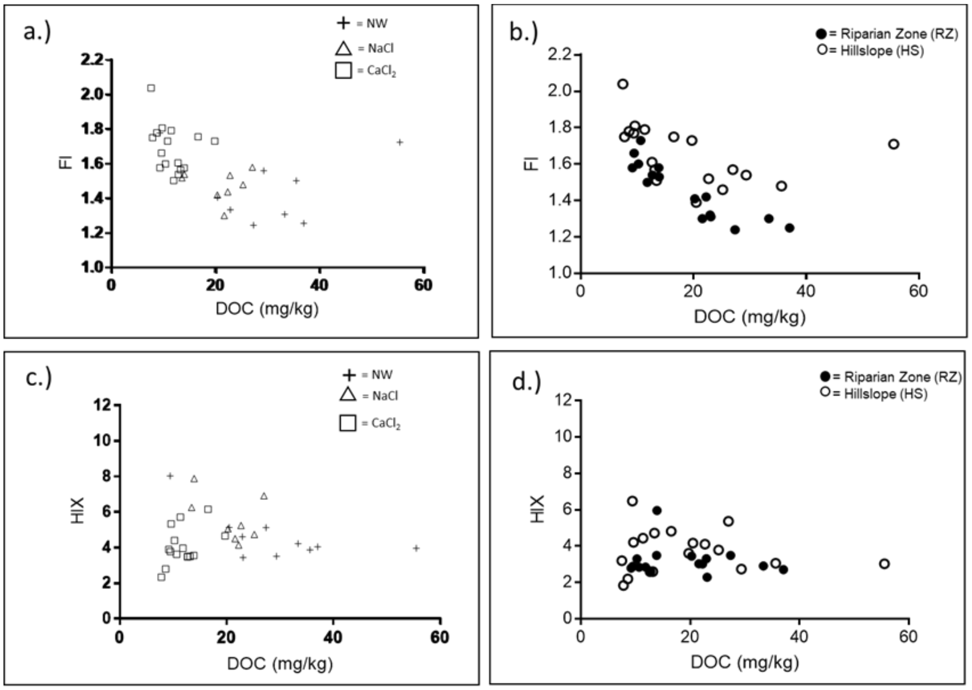

Figure 15. Spectral Indices: Fluorescence Index (FI) (a, b) and Humification Index (HIX) (c, d) by extraction solution (Nanopure water = NW, sodium chloride = NaCl, calcium chloride = $CaCl_2$) and landscape position.

PARAFAC results indicate that the abundance of both %C-2 (terrestrial, humic-like) and %C-3 (microbial, protein-like) are similar in all three extraction solutions. However, the abundance of %C-1 (terrestrial, fulvic-like) in the $CaCl_2$ extracted DOM was significantly lower than in other solutions ($p < 0.05$).

HS soil extracts generally show a greater variability in PARAFAC component abundance than RZ soil extracts. The latter show lower abundance of C-2 (humic-like) and higher abundance of C-3 (protein-like), which are significant (both $p < 0.005$ at alpha = 0.05, Figure 16).

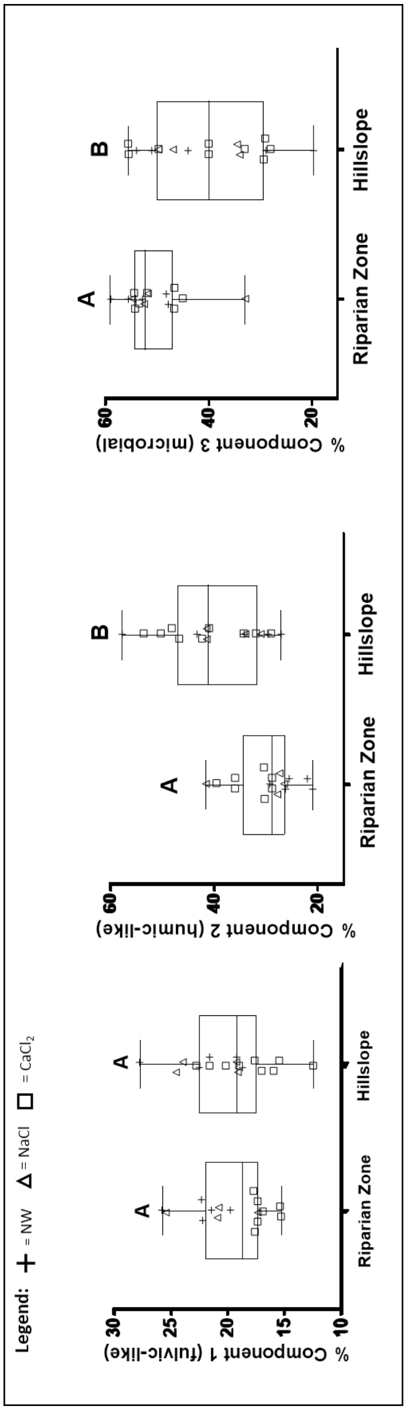

Figure 16. The relative abundance of each PARAFAC component by extraction solution (Nanopure water = NW, sodium chloride = NaCl, and calcium chloride = $CaCl_2$) and landscape position. Letters above the boxplots indicate significant differences according to the Tukey Multiple Comparison test at $p < 0.05$.

4.2.2. Representative LSP: Bioavailability of extracted carbon

The bioavailability of the extracted C, assessed as the %DOC respired over two weeks in the liquid phase, ranged from minimal to large losses (below 1% to almost 60%, Figure 17). Generally, extracted DOC in the NaCl treatment decreased very little over two weeks while the decrease in extracted C for both the $CaCl_2$ and the NW treatments was more pronounced. However, differences between extraction solutions were not significant ($p > 0.05$).

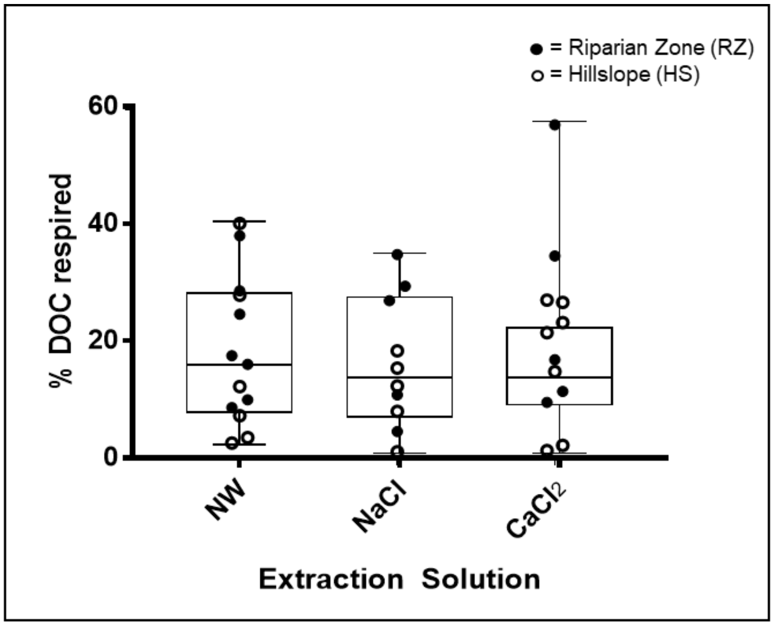

Figure 17. The distribution for the bioavailability of carbon by extraction solution (Nanopure Water = NW, sodium chloride = NaCl, and calcium chloride = $CaCl_2$) and representative landscape position reported as %DOC respired (loss). None of the treatments or representative landscape positions were significantly different.

CHAPTER 5: DISCUSSION

5.1. Aggregate dispersion: a mechanistic link between changes in solution composition and DOC increases?

The Clean Air Act was legislated in 1970 and further amended in 1990 to reduce the emissions of sulfur dioxide and nitrogen oxides into our environment, resulting in substantial reductions of acidic wet deposition (Driscoll et al., 2001b). At the same time, an increase in surface water DOC was observed in many forested, northern hemisphere catchments. The issue is widely debated in the literature (De Wit et al., 2007; Eimers et al., 2008; Evans and Monteith, 2001; Freeman et al., 2001; Hruška et al., 2009; Lepistö et al., 2008; Monteith et al., 2007; Porcal et al., 2009; Stuart, 2005; Worrall and Burt, 2007; Yallop and Clutterbuck, 2009), but a mechanistic link has not yet been confirmed (Monteith et al., 2007). A few other drivers in addition to a recovery from prior acidification that attempt to explain the trend of increased DOC are changes in climate, variations in the hydrologic cycle, and changes in land use (Clark et al., 2010; Worrall et al., 2004).

Surface water DOC is largely sourced from organic soils (Aitkenhead-Peterson et al., 2003; Yallop and Clutterbuck, 2009), soil carbon is typically stabilized in aggregates (Six et al., 2002), and aggregate stability is in part a function of solution chemistry (Jarvis et al., 2012; Six et al., 2004). I therefore hypothesized soil aggregate dispersion as a response to changes in precipitation and soil water chemistry as the mechanistic link between decreases in sulfate inputs and increases in DOC outputs. Specifically, I hypothesized that soil aggregate dispersion (and the ensuing release of DOC from these aggregates) is driven by a decrease in ionic strength (decreasing the tendency of flocculation) and a shift from divalent to monovalent cations (reducing the propensity for cation bridging).

Based on these hypotheses, I expected to see the highest amounts of DOC (per kg soil) released into low ionic strength solution (NW), followed by NaCl solution and finally the lowest DOC amounts in $CaCl_2$ solution. Indeed, the highest amount of DOC was leached into NW and significantly lower amounts for the high ionic strength solutions (NaCl and $CaCl_2$, Figure 13). This change in DOC concentration was accompanied by a systematic shift in particle size distribution where decreases in ionic strength led to losses in the particle size range of 40-100 µm and gains in the smaller size range below 30 µm (Figure 12). This could either suggest i) large aggregates break up to produce smaller aggregates, ii) aggregates of all sizes lose volume, or a combination of both.

The SEM analysis showed that treatment with NW led to a separation of organic materials from larger silicate grains (e.g. quartz, feldspars, pyroxenes and clays, Figures 8 and 9), creating smaller aggregates. Because I did not separate solution from solids in the case of the SEM samples, the organic materials were still present as smaller aggregates. It is possible that some of the organic material dispersed and would have passed a filter (i.e. increasing DOC) but re-coagulated during the drying process. In contrast, aggregates treated with the higher ionic strength $CaCl_2$ solution did not show this disaggregation. Together, these results suggest that indeed aggregate dispersion is more pronounced in low ionic strength solution and could explain the higher amounts of DOC released in these cases.

These results are in agreement with findings from a field study of the Pluhuv Bor catchment in the Czech Republic, where the concentration of both stream water and soil-water DOC showed large increases between WY1994 and 2007 (Hruška et al., 2009). Of all the other assessed parameters (pH, ionic strength, and DOC) under different flow conditions (high flows, low flows, and all flows) changes in DOC concentration mirrored changes in ionic strength (Hruška et al., 2009). The negative correlation was attributed to recovery from acidification, however the underlying mechanism was not addressed.

In an experimental study where the fluorescence signature of extractable DOM was assessed using different extraction solutions, a direct relationship was observed between both an increase in concentration of the extraction solution and DOC release but the specific mechanism for the said DOC increase with increasing concentration could not be identified (Xie et al., 2017). Data from my experiment show the exact opposite trend; as the concentration of the solution decreased, more DOC was released. However, soil composition, including mineralogy, strongly affect aggregate composition. This might result in the main stabilizing mechanisms varying with location; pH might have a greater effect on aggregate stability compared to ionic strength in soils that are rich in metal oxides.

Because divalent cations are more effective in bridging negative charges (Mikutta et al., 2007), potentially promoting aggregation and DOC sequestration, I had also expected that DOC release will be lower for $CaCl_2$ vs. NaCl solutions of the same ionic strength. My results show a general trend of lower DOC release into $CaCl_2$ solutions, but these differences were not significant (Figure 13). Particle size analysis showed that size was generally larger in $CaCl_2$ vs. NaCl solution, suggesting that cation bridging might play a role even though ionic strength effects dominate disaggregation and DOC release (Figure 12).

These results contrast with the outcome from a recent study conducted in southern Ontario where the effect of mono vs. divalent cations on DOC adsorption in soil water of an upland catchment was investigated (Kerr and Eimers, 2012). In this combined laboratory and field study, both single and multi-point adsorption experiments were conducted and data on DOC adsorption from the B-horizon mineral soils were collected. DOC increases were more strongly driven by declines in one or more divalent cation species and not so much by an overall change in ionic strength. The authors explained their results by both a reduction of DOC sorption at low Ca^{2+} concentrations and an observed linear relationship between adsorbed Ca^{2+} and adsorbed DOC, which is most likely the result of cation bridging (Kerr and Eimers, 2012).

Not only did total amounts of DOC fluctuate with extraction solution, the type of DOM varied too. For example, NW extracts had lowest values for both FI (i.e. terrestrial) and the humic-like PARAFAC component C-2, while the $CaCl_2$ extract showed the opposite, with high values for both. This suggests that a change in extraction solution composition and ionic strength (as an analogue for changes in soil solution composition) has significant effects on the type of released DOM.

The importance of extraction solution composition for DOM characteristics has already been investigated in detail by Gabor et al. (2015). Similar to my study, they found that the $CaCl_2$-extracted DOM had the highest FI, water-extracted DOM had the lowest values, and DOM extracted with monovalent cation solutions had intermediate values.

These results suggest that microbially produced organic matter is preferentially released into higher ionic strength solutions (Gabor et al., 2015), or, in reverse, that less microbial DOM is released into more dilute solutions. This could have important implications for ecosystem dynamics (See section 5.3.).

Despite the trend of decreasing aggregate size with decreasing ionic strength (modulated by the presence of mono vs. divalent cations), my observations also show that these aggregates are remarkably stable. For example, my experimental treatment involved one hour of vigorous shaking with the different extraction solutions, and yet, mean particle size only decreased by 13 to 17 % (Table 2). Any larger macroaggregates were destroyed yet the microaggregates resisted destruction due to their small size and the binding agents holding them together (Frey, 2005). The images of untreated soil show that at 100 μm there is visible aggregation of soil particles (Figures 8 and 9). In no case did I observe a complete destruction of aggregates despite the intense physical and chemical stress imposed by the treatment.

The targeted laboratory experiment tested the effects of both mechanisms, the diffuse-double layer and cation bridging. As the ionic strength of my solutions decreased from 0.005 mol L^{-1} to 0 mol L^{-1}, more DOC was released into solution, likely from the extension of the diffuse-double layer. This allowed any protected organic matter to be liberated into solution as the number of ions in solution declined. The two salt solutions tested whether the presence of monovalent vs. divalent cations influenced coagulation. When the divalent cations were not available to bridge the particles in the soil solution, such as in the NaCl treatment, more DOC was released into solution.

5.2. Does landscape position modulate the response to solution chemistry?

Soil composition is a function of a variety of factors including parent material, climate, time, vegetation, and slope (Jenny, 1941). In my study system all soil forming factors were the same except slope. I therefore included testing the role of general landscape position on DOC dynamics in my study design. Because low-lying landscape positions, especially RZs, are considered the main source of DOC entering streams (Ledesma et al., 2015), a differential response of RZ vs. HS soils to shifts in environmental parameters is significant.

I hypothesized that soils from low-lying landscape positions contain more TOC than those from the HS locations and therefore, would release more DOC. I also hypothesized that cycling and lateral transfer of materials and processors (microbes) from HSs to the RZ would result in a greater terrestrial fluorescence signature and greater DOM bioavailability in RZ soil extracts.

My results confirm that TOC is consistently higher in RZ soils (Figures 6 & 7). SEM analysis show a larger proportion of organic matrix vs. inorganic mineral fragments in RZ soils than in HS soil aggregates (Figures 8 & 9), which would suggest a greater potential for DOC release. However, RZ soil extracts did not contain significantly higher amounts of DOC or TN (Figures 13 & 14), which is remarkable, given that certain locations in the RZ had up to 6 times higher TOC content compared to the HS locations. One explanation for the relatively small releases of DOC could be the generally larger aggregate size in RZ soils (Figures 8 and 12) that results in smaller surface areas. This difference in surface area could be significant for DOC desorbing from aggregate surfaces instead of aggregate break up (Figure 18) potentially due to the carboxylic acid functional group on the organic matter (David et al., 1992). Another important factor is that RZ soil aggregates show a large amount of Ca^{2+} (Figure 8) that could further help to stabilize these aggregates through cation bridging or increased sorption capacity. In this specific study, the RZs at Sleepers River receive upwelling ground water (Armfield et al., 2018 (in prep); Shanley et al. (2004)), which provide a constant supply of Ca in high concentrations.

Despite that fact that extractable DOC amount did not differ significantly between RZ and HS positions, the type of extracted C did vary. For example, extracts from RZ soils consistently had lower values for FI, HIX and the humic-like PARAFAC component C-2, while the protein-like PARAFAC component C-3 was significantly more abundant compared to HS extracts (Figures 15b, 15d, and 16). These results paint a complex picture of the molecular make up of DOM extracted from RZ soils and likely reflect high amounts of fresh and terrestrial organic matter such as roots and decomposing leaf litter (indicated by FI, HIX and C-2) as well as abundant microbial DOM fueled by a wet and nutrient-rich environment (indicated by C-3). In contrast, HS extracts had higher values for HIX, the humic–like C-2 and FI (Figure 15b and Figure 15d), suggesting the presence of typical humified soil DOM with active microbial processing during the wet and warm sampling season. Together, these results suggest that landscape position presents an important control on the type of DOM that is washed out from soils into surface waters.

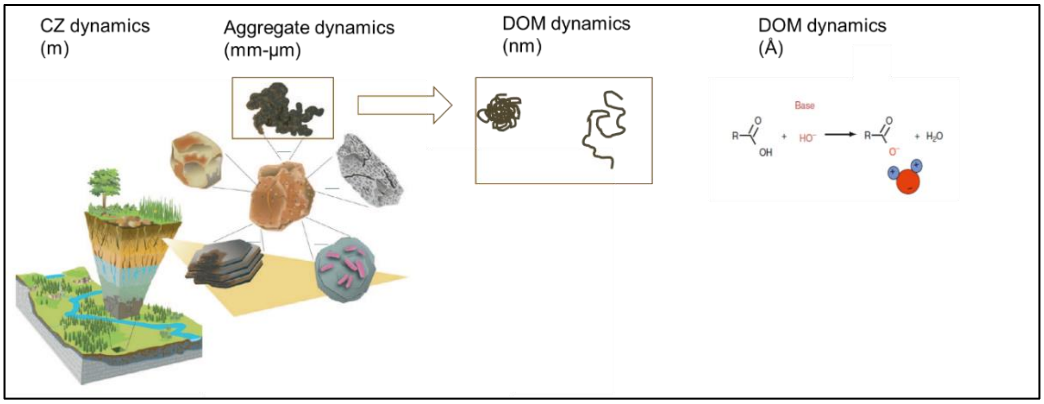

Figure 18. Conceptualization of DOC release at varying scales. Solution composition could lead to aggregate dispersion and release of dissolved organic matter (DOM, mm- µm scale). Solution chemistry also changes DOM conformation (nm-scale) and DOM solubility (Å-scale). These smaller scale processes would not lead to significant changes in aggregate size. Figure modified after (Chorover et al., 2007; Kleber and Johnson, 2010).

5.3. Implications for recovering ecosystems

DOM serves as a source of energy at low trophic levels, hence the success of all aquatic organisms, including microorganisms and macrophages, ultimately depend on it. For this, the type of DOM including its bioavailability matters greatly (Creed et al., 2018a). My results suggest that either aggregate break up or desorption of DOM from aggregate surfaces leads to changes in DOM released from soils. With solutions simulating recovery (NW), DOM shifts from microbial and less humified to terrestrial and more humified (indicated by greater HIX values). Typically, DOM with larger HIX values is less bioavailable because the ensuing molecular makeup including polyphenols and double bonds tend to be harder to degrade (Gabor et al., 2015). If this pattern holds true, we might expect that the increasingly released recovery DOM is less bioavailable than acidification DOM. This has potential impacts to aquatic ecology. For example, high amounts of unavailable DOM in water could lead to browning of water without feeding aquatic ecosystems (Creed et al., 2018a). At the same time, my results suggest that N release into the low ionic strength solution is higher (Figure 14). This might have implications for downstream aquatic ecosystems where N is a limiting nutrient.

My study also indicates that DOM with very different spectral characteristics was released from HS vs. RZ soils (Figure 15), despite that fact that total amounts and bioavailability did not differ. This especially impacts the NE, where we are expecting more extreme precipitation events (IPCC) with increased advective transfer of soil materials into surface waters (Aitkenhead-Peterson et al., 2003). The timing of such extreme hydrological events might shift as, for example, snowmelt is observed earlier (Dudley et al., 2010; Zia et al., 2016) and summer rains are increasing in intensity.

These regional changes might affect the relative proportion and timing of RZ vs. HS soil flushing. For example, connectivity is highest during snowmelt where the proportion of HS signature might be relatively high, whereas short-lived intense summer precipitation might mostly affect RZ inputs. My results suggest that N in extracts from RZ soils was generally higher, which would lead to an additional pulse of nutrients into surface waters during precipitation events. Further research is necessary to unravel these potential effects of differential response of landscape position to ecosystem recovery while responding to changes to the climate system.

Lastly, DOM is readily processed by microorganisms that can transfer aquatic C into the greenhouse gas CO_2 (Gougoulias et al., 2014; Søndergaard and Middelboe, 1995). My results hint that more DOC could be released during recovery due to the break-up of aggregates or the desorption of DOM from aggregate surfaces. The DOM in my experiments had variable bioavailability, but up to 40% of the recovery extract was respired within two weeks. Together with increases in total amounts, this represents a high contribution of CO_2 to the atmosphere and should be monitored carefully.

5.4. Limitations

My results indicate that precipitation chemistry can indeed impact DOC in streams by impacting soil aggregates (Figure 19).

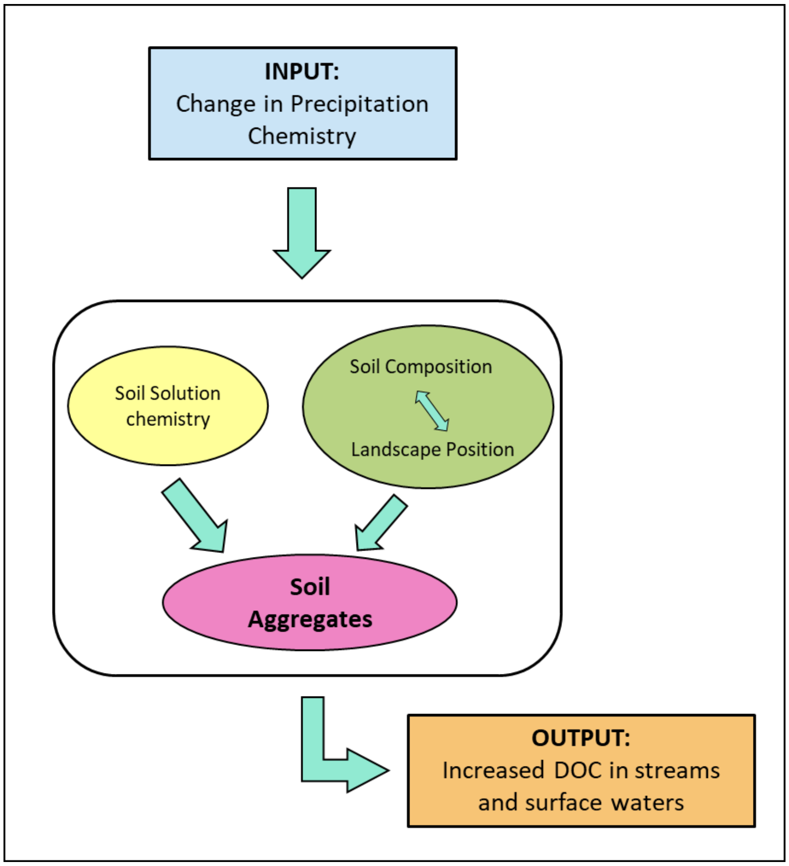

Figure 19. The environmental black box is revealed, illustrating that soil aggregates are affected by modulating factors can be influenced by changes in the chemistry of precipitation and result in the release of DOC into waterways.

However, the most important shortcoming of such experiments is that the transfer of experimental results to the larger scale is difficult. For my study, I faced this issue because my experiments clearly showed that ionic strength and type of extraction solution mattered as did the landscape position of collected soils. However, can I extrapolate these results to the bigger scale of an ecosystem recovering from acidification?

A few aspects limit the extent to which a study such as this one can be applied to the larger scale. Firstly, my sampling design included only a fraction of the W-9 catchment within Sleepers River and variability between sample sites is likely quite great. Therefore, more research regarding DOC release is necessary within this small headwater basin alone. Secondly, and relatedly, the response to an external driver such as changes in precipitation chemistry, is likely system specific and cannot be applied to other watersheds without careful investigation. This is because my results indicate DOC and nutrient release are influenced by soil type, which varies by location. And finally, my samples were collected in both summer and autumn, where the labile, extractable fraction likely reflects seasonal characteristics as well as general soil characteristics.

CHAPTER 6: CONCLUSION AND FUTURE RESEARCH

At Sleepers River, the observable DOC increase in streams and surface waters can be explained by a change in precipitation composition. The results reported here, from my targeted experiments, provide initial evidence that soil aggregates do play a role in the generation of DOC as the effects of both the diffuse double layer and cation bridging were observed. The reduced ionic strength and composition drive the breakup of aggregates, yet the type of DOM released is dependent on landscape position.

Continued research is important, however, as our understanding of the observed DOC release mechanisms is still minimal and aggregate stabilizing mechanisms likely vary by soil type. For example, I tested the Density Separation analysis using Sodium Polytungstate as it is a common procedure for aggregate analysis (Crow et al., 2007; Glochin et al., 1994; Osipov, 2012; Sohi et al., 2001; Sollins et al., 2006). However, I did not conduct the analysis in replicate as it was time-prohibitive (Appendix 1) and I could not investigate the large variability in the amount of free-light fraction by landscape position in detail. Another future experiment could compare the three streams across seasons or years to add a temporal component.

CHAPTER 7: SOIL AGGREGATES AS A SOURCE OF DISSOLVED ORGANIC CARBON TO STREAMS: AN EXPERIMENTAL STUDY ON THE EFFECT OF SOLUTION CHEMISTRY ON WATER EXTRACTABLE CARBON

(Submitted to *Frontiers in Earth Science: Biogeosciences)*

Abstract

Over the past two decades, headwater streams of the northern hemisphere have shown increased amounts of dissolved organic carbon (DOC), coinciding with decreased acid deposition. The issue is widely debated in the literature but a mechanistic link between precipitation composition and stream water DOC has not been proposed. We hypothesize that soil aggregates are the main source of stream water DOC and that DOC release is greater in organic rich, riparian soils vs. hillslope soils. To test these hypotheses, we collected soils from two main landscape positions (hillslope and riparian zones) from the acid-impacted Sleepers River Research Watershed in northeastern Vermont. We performed aqueous soil extracts with solutions of different ionic strength (IS) and composition to simulate changes in soil solution, and then we monitored dynamic changes in soil particle size, aggregate architecture and composition, leachate DOC concentrations, dissolved organic matter (DOM) characteristics with fluorescence spectroscopy and trends in bioavailability. In low IS solutions, extractable DOC concentrations were significantly higher, particle size (by laser diffraction) was significantly smaller and organic material was separated from mineral particles in scanning electron microscope observations. Furthermore, higher DOC concentrations were found in Na^+ compared to Ca^{2+} solutions of the same IS. These effects are attributed to aggregate dispersion due to expanding diffuse double layers in decreased IS solutions and due to decreased bridging by divalent cations. Landscape position impacted quality but not quantity of released DOC. Overall, these results indicate that soil aggregates might be one important link between Critical Zone inputs (i.e. precipitation) and exports in streams.

1 Introduction

Over the last few decades increased levels of dissolved organic carbon (DOC) have been observed in forested streams across the northern hemisphere (Monteith et al., 2007; Porcal et al., 2009) and several processes have been proposed to explain observed patterns. For example, some studies indicate that increases in DOC are consistent with a climate change driver including changing temperature or hydroclimatic conditions (Eimers et al., 2008; Freeman et al., 2001; Lepistö et al., 2008; Worrall and Burt, 2007). Other suggested drivers include changes in nitrogen deposition (Stuart, 2005) or land management practices (Yallop and Clutterbuck, 2009). Lastly, recovery from acidification is put forth as a general driver for observed changes in stream water DOC (De Wit et al., 2007; Evans and Monteith, 2001; Hruška et al., 2009; Monteith et al., 2007). The latter is in agreement with both the observation of significant decreases in acid anions (i.e. sulfate) in many catchments that show increases in DOC (Monteith et al., 2007) and observations of DOC increases with decrease in ionic strength (IS, Hruška et al. (2009)) . However, little focus has been given to the precise mechanisms of DOC release, making it difficult to investigate the link between critical zone input (i.e. precipitation) and stream water export.

Streams integrate complex and coupled hydrological and biogeochemical processes at hillslopes, riparian areas, in the hyporheic zone and the stream itself (Lohse et al., 2009). In forested catchments, stream DOC is mostly allochthonous (produced elsewhere) and often sourced from organic-rich soils of hillslopes and riparian areas (Aitkenhead-Peterson et al., 2003; Boyer et al., 1997; McDowell et al., 2006) where carbon (C) is typically associated with organo-mineral aggregates (Kaiser and Guggenberger, 2000; Lehmann et al., 2007; Six et al., 2000). These aggregates protect C from microbial attack often by physically shielding it with soil minerals including clays (Angst et al., 2017; Mikutta et al., 2006).

DOC can be stabilized on mineral surfaces *via* electrostatic interactions but desorb if solution chemistry changes. For example, the change in type and amount of solutes in soil solution can cause competition for sorption sites leading to the release of certain aggregate constituents (Sokolova and Alekseeva, 2008). Changes in IS and pH fundamentally impact colloidal interactions and aggregation: increased IS such as during acidification, leads to the compression of the charged layer around an ion (i.e. diffuse double layer) so that attractive forces overcome repulsive forces (Derjaguin and Landau, 1941; Verwey, 1947). As a result, increases in IS lead to coagulation and the stabilization of larger colloidal associations and aggregates (Lagaly, 2006b). Furthermore, increased proton concentration and the presence of divalent cations supply positive charges that can bridge between negatively charged constituents, such as clays and organic matter, equally aiding aggregation. The surface charge on organic matter can change depending on pH where the functional groups, such as carboxylic acids, are protonated at lower pH (David et al., 1992). In acid impacted soils, a reduction in IS and an increase of pH could therefore lead to the dispersion of such colloidal associations and aggregates and result in a release of associated DOC.

The objective of this study was to systematically test how changes in IS and composition of soil solution (monovalent vs. divalent cations) impact soil aggregates and DOC release in a combined lab and field study. We hypothesized that, as IS and the concentration of divalent cations decreases (simulating recovery from acid deposition), soil aggregate size decreases as a result of aggregate breakup, and concentration of DOC in soil solution increases. We furthermore hypothesized that organic-rich soils from riparian zones release larger amounts of DOC than hillslope soils, and that DOC released from riparian aggregates is terrestrial and very bioavailable.

To test these hypotheses, we collected soils from two main landscape positions (hillslope and riparian zones) from the acid-impacted Sleepers River Research Watershed in eastern Vermont. We performed aqueous soil extracts with solutions of different IS and composition to simulate changes in soil solution chemistry. We also monitored dynamic changes in soil particle size, aggregate architecture and composition, leachate DOC concentrations, dissolved organic matter (DOM) characteristics using fluorescence spectroscopy, and trends in bioavailability.

2 Materials and Methods

2.1 Field Site

The Sleepers River Research Watershed (SRRW) is a USGS site in northeastern Vermont (Fig. 1a). This site has over 25 years of data on stream water composition and has shown increased DOC stream water effluxes similar to the general trend in many northern hemisphere catchments (supplementary materials). The headwater catchment W-9 is the focus of this study and covers an area of 40.5 ha with elevation ranging from 524 to 672 m (Fig. 1b , Shanley et al. (2004)). The watershed is underlain by the Waits River formation, a quartz-mica phyllite with beds of calcareous granulite that is covered with layer of varying thickness (1 - 4m) of calcareous glacial till (Shanley et al., 2004). Weathering of the glacial till results in the release of calcium-bicarbonate rich water with a well-buffered pH. Ground water in equilibrium with these carbonates exhibits high pH and calcium content in ground water in riparian zones (RZs) close to the stream.

All soils were sampled in the W-9 forest along stream B, a 12.9-ha tributary that has been most intensely studied (Fig. 1c, Kendall et al. (1999)). The RZs are mostly composed of thick Histosols and the hillslopes (HSs) are dominated by a mixture of both Inceptisols and Spodosols (Shanley et al., 2015). In order to assess the impact of topography on soil composition adjacent to the stream, we separated the area around stream B into three main topographic zones (i.e. *steep slope (16%*), *intermediate slope (5%*) and *gentle slope* (3%)) and sampled along transects perpendicular to the channel. For this we collected field replicates every 2 meters along the HSs and, to capture variability in the narrower RZ, every meter in the RZ for a total of ~10 samples per transect. We also collected replicates from the center of each HS and RZ of each topographic zone (Fig. 1d).

Because there were distinct differences between the soils of RZ and HS in the full transect, we resampled representative RZ and HS soils for further analysis. A total of 18 soil samples (3 for each topographical zone in HS and RZ) as well as composite samples for analyses where replication was time- and cost-prohibitive (e.g. Scanning Electron Microscopy (SEM) analysis), were extracted. All samples were collected from the top 15 cm after the leaf litter was removed with a pre-cleaned bucket auger (15 cm in length, 10 cm diameter) and handled gently to preserve soil aggregates. Composite samples were prepared by gently combining subsets of each individual, field-moist sample (composite n = 6).

2.3 Elemental Analysis

For elemental analyses, soil samples were air-dried, sieved through a 2-mm mesh, and homogenized using a ball mill. Duplicates of each sample were analyzed for C content using a combustion-based elemental analyzer in the UVM Geology Stable Isotope Lab. Resulting percent C values were compared to standards (B2150 for high organic content sediment standard and B2152 for low organic content soil standard) provided by Elemental Microanalysis Limited.

2.4 Scanning Electron Microscopy

To visualize aggregate architecture and determine aggregate composition, we used a *VEGA3 TESCAN* Scanning Electron Microscope (SEM) and *AZtec* Elemental Mapping software in the Geology Department at Middlebury College. Air-dried samples that were first sieved through a 2-mm mesh to exclude larger particles (i.e. roots and small gravel) were sieved a second time to isolate the aggregate fraction (250-µm mesh) and match the maximum particle size observed with laser particle size analysis (see below). Replicates of each composite sample were mounted using double-sided C tape on separate metal stubs and sputter-coated with C before analysis. To investigate the effect of simulated soil solution on aggregate size, shape, and composition solution, we added several drops of solutions that simulated acidification (calcium chloride ($CaCl_2$)) and recovery (Nanopure water (NW)), to one replicate from both locations (HS and RZ). Using a micro-pipette, solutions were carefully dropped directly onto the aggregates and carefully mixed around the aggregates. The solutions were allowed to dry in place to avoid removal of the aggregates, a process completed within 90 minutes. SEM observations were carried out in

BSE mode at 5 keV acceleration voltage and energy-dispersive spectroscopy (EDS) maps were acquired for 5 minutes with a probe resolution of 15 mm.

2.5 Aqueous Soil Extractions and Analyses

To simulate the higher IS of solutions during acidification and to test the effect of monovalent vs. divalent solutions, we used aqueous soil extracts (ASE) as an analogue for soil solution. Acidification solutions consisted of salt solutions (0.005M sodium chloride [NaCl], and 0.005M $CaCl_2$). To simulate the more dilute solution of recovery, we used Nanopure water (NW). We also tested pH effects but, presumably because SRRW is well buffered, we saw no effects (see supplementary materials). All ASE were prepared from field moist soils (existing water content was accounted for, supplementary materials) by mixing soil and aqueous solutions at a 1:5 ratio and shaking the mixture on a reciprocal shaker (Eberbach, Ann Arbor, MI, USA) for 1 hour (Perdrial et al., 2012).

To monitor changes in particle size (from 0.4 µm to 2000 µm) unfiltered extracts were analyzed using the Micro Volume Module on a *Beckman Coulter* LS230 Laser Particle Analyzer. To determine the amount and type of the DOC in solution, the solids were separated from the colloidal and dissolved matter by centrifugation (30 minutes, 3000 rpm) and filtration using combusted 0.7µm glass fiber filters (Whatman GF/F, Buckinghamshire, UK). Filtrates were transferred into combusted amber glass bottles for immediate analyses of C quality and quantity. We analyzed all ASE samples in duplicate for concentrations of DOC and Total Dissolved Nitrogen (TDN) *via* combustion using a Total Organic Carbon Analyzer (*Shimadzu*, Columbia, MD, USA). Results are reported normalized to the exact amount of solution and soil used for each extract (mg/kg) to allow for comparison among samples.

Spectral characteristics of DOM were determined using the Aqualog Fluorescence and Absorbance Spectrometer (Horiba, Irvine, CA, USA). The Excitation wavelength range spanned from 250 to 600 nm and Emission ranged from 212 to 599 nm. All excitation emission matrices (EEMs) were blank-subtracted (NW, resistivity 18MΩ cm^{-1}), corrected for inner filter effects, and Raman normalized. From these data, we computed a couple indices including the Fluorescence Index (FI, calculated as the intensity at Emission 470 nm divided by the intensity at Emission 520 nm for Excitation at 370 nm (Cory and McKnight, 2005) and the humification index (HIX, calculated as a ratio of the area under Em 435 – 480 nm divided by the quantity of the both areas under Em 300 - 345 nm and Em 435 – 480 nm, at an Excitation of 254 nm, Gabor et al. (2014a)).

We performed a Parallel Factor Analysis (PARAFAC) using *Matlab R2017b* and the drEEM_4_0_(Ext.) toolbox (Murphy et al., 2013) to validate a 3-component PARAFAC model (Fig. 2). The three components are terrestrial fulvic-like (C-1), terrestrial humic-like (C-2), and microbial protein-like fluorescence (C-3, Table 1).

2.6 Bioavailability of carbon

To determine the bioavailability of dissolved organic C in ASE, an aliquot of each filtered ASE was inoculated with an unfiltered slurry (containing O-horizon microbes from all 18 soils) and incubated shaking for 14 days in the dark. Samples were re-analyzed for DOC to determine the fraction of C loss through respiration (evaporation losses were accounted for by tracking sample mass and adding lost solution).

2.7 Statistical Analyses

We used JMP Pro 13.0.0 software by *SAS* for statistical analyses. An Analysis of Variance (ANOVA) and the Tukey's Paired Test were performed to determine significance of variations by extraction solution and landscape position. The average DOC concentrations for each of the representative landscape position (RZ and HS) were compared using a T-test to determine if the means were significantly different.

3 Results

3.1 Effect of Landscape Position on soil carbon

The highest C contents were found in the RZ samples of both the transect samples (ranging from 850-3500 mg/kg in RZ and 490-570 mg/kg for HS, see supplementary materials) and representative soil samples (Fig. 3). The RZ adjacent to the steep slope of the representative zones had the greatest amount of soil C with greater than 3340 mg/kg. The least amount of C was found in HS of the gentle slope (380 mg/kg).

3.2 Particle size analysis

Particle size distribution in ASE varied as a function of position (HS vs. RZ) and solution chemistry. Particle size in RZ extracts had a slightly greater range (up to 80 µm, Fig. 4a), while HS soil extract particle size did not exceed 60 µm (Fig. 4b). When extracted with higher IS solution, particle size distribution in extracts of soils from both landscape positions shifted towards larger sizes. In contrast, the low IS extracts showed a larger proportion of smaller particles and a smaller proportion of larger particles. The change in particle size varies with extraction solution (supplementary materials). Results of typical samples from the RZ and the HS illustrate the distinct change in particle size for each solution (supplementary materials).

3.3 Visualization of aggregates

SEM analyses of soil aggregates showed that aggregate morphology and size differed with dominant landscape position (RZ vs. HS, typical aggregates are exemplified in Fig. 5 and 6). Typical RZ aggregates were rounded and contained large amounts of organic rich materials with only small amounts of angular mineral fragments (Fig. 5, left panel). Elemental distribution confirmed mineral fragments to be aluminosilicates. The RZ spectra indicate the presence of clinochlore, hornblende, and quartz mixed in with the organic matter (Fig. 7) and HS spectra for aggregates show amphibole, quartz, and biotite (Fig. 8). The organic-rich matrix was devoid of Al and Si but enriched in Ca. Typical HS aggregates contained larger amounts of Al- and Si-rich mineral fragments and relative smaller amounts of organics (Fig. 6, left panel).

Aggregates from both RZ and HS treated with $CaCl_2$ solution (simulating the high IS of rain during acidification) showed no change in overall morphology. The main difference was the removal of scattered fragments besides aggregates (Fig. 5 and 6, center panels). In contrast, when aggregates were treated with NW (simulating the low IS solution during recovery), organic rich materials were separated from the mineral fragments (Fig. 5 and 6, right panels). Because the solution was dried in place, the organic material was, however, not removed.

3.4 Effect of extraction solution on soils from different landscape positions

DOC content in aqueous soil extracts was strongly impacted by the composition of the extraction solution but did not vary by position (HS vs. RZ, Fig. 9). Extractions with NW showed the highest DOC (over 55 mg/kg) but also largest variation (SD = 12.2

mg/kg). The high IS solutions extracted significantly lower DOC concentrations and showed lower variability (averaging 17.7 ± 7.18 mg/kg and 11.6 ± 3.02 mg/kg for NaCl and $CaCl_2$, respectively). The total dissolved nitrogen content leached into the various extraction solutions followed the same pattern as DOC and was not affected by landscape position (supplementary materials).

The value of fluorescence-derived indices (FI and HIX) varied with extraction solution and landscape position. Highest (most microbial) FI values were observed for $CaCl_2$ extracts (ranging from 1.50 up to 2.04). FI values for NW and NaCl extracts varied but were generally lower (ranging from 1.24 to 1.77 for NW and 1.30 – 1.57 for NaCl, respectively (Fig. 10a)). HS soils had higher FI values than RZ soils in all extraction solutions (Fig. 10b). HIX values varied greatly in all extraction solutions but were highest in NW (up to 6.49) while $CaCl_2$ extracts had lowest values (as low as 1.84, Fig. 10c). Differences in HIX values by landscape position were not significant ($p > 0.05$).

PARAFAC results indicate that the abundance of both the terrestrial, humic-like (C-2) and microbial, protein-like (C-3) components were similar in all three extraction solutions. However, the abundance of the terrestrial, fulvic-like C-1 in the $CaCl_2$ extracted DOM was significantly lower than in other solutions ($p < 0.05$, Fig. 11). HS soil extracts generally showed a greater variability in PARAFAC component abundance than RZ extracts. HS soils show a significantly higher abundance of the humic-like C-2 and lower abundance of the protein-like C-3 (both $p < 0.005$ at alpha = 0.05, Fig. 11).

3.5 Bioavailability of extracted carbon

The bioavailability of the extracted organic C, assessed as the %DOC respired over two weeks in the ASE, ranged from minimal to large losses (below 1 to almost 60%, Fig. 12). Generally, extracted DOC in the NaCl treatment decreased very little over the two-week incubation while decreases in both the $CaCl_2$ and the NW treatments were more pronounced, but differences were not significant ($p > 0.05$).

4 Discussion

4.1 Aggregate dispersion: a mechanistic link between changes in solution composition and DOC increases?

We hypothesized that soil aggregate dispersion is the mechanistic link between reduced acid deposition and DOC increases in soil solution and streams because i) surface water DOC is largely sourced from organic soils (Yallop and Clutterbuck, 2009), ii) soil C is typically stabilized in aggregates (Six et al., 2002), and iii) aggregate stability is partially controlled by solution chemistry (Six et al., 2004; Tisdall and Oades, 1982). Specifically, we hypothesized that soil aggregate dispersion (and the ensuing release of DOC from these aggregates) is driven by a decrease in soil solution IS (decreasing the tendency of flocculation) and a shift from divalent to monovalent cations (reducing the propensity for cation bridging).

Based on these hypotheses, we expected to see the highest amounts of DOC (per kg soil) released into low IS solution (NW), followed by NaCl solution and finally the lowest DOC amounts in $CaCl_2$ solution. Indeed, highest amounts of DOC were leached into NW and significantly lower amounts were released into the high IS solutions (NaCl and $CaCl_2$, Fig. 9). This change in DOC concentration was accompanied by a systematic shift in particle size distribution where decrease in IS led to losses in the particle size range of 40-100 µm and gains in the smaller size range below 30 µm (Fig. 4). This could either suggest that large aggregates break up to produce smaller aggregates and/or that aggregates of all sizes lose volume.

The SEM analysis showed that treatment with NW led to a separation of organic materials from larger silicate grains (Fig. 5 and 6), creating smaller aggregates. Because we did not remove the treatment solution (but allowed it to dry in place), the organic materials were still present as smaller aggregates. Based on these data, it is impossible to tell how much of this organic material dispersed and coagulated again during the drying process, however it is likely that some of the organics would have passed a 0.7 µm filter (i.e. increasing DOC concentrations in the solution). In contrast, aggregates treated with higher IS $CaCl_2$ solution did not show the separation into organics vs. mineral fragments. Together with the DOC data these results suggest that indeed aggregate dispersion is more pronounced in low IS solution and could explain the higher amounts of DOC released in these cases.

These results are in agreement with results from a field study of the Pluhuv Bor catchment in the Czech Republic (Hruška et al., 2009) where the concentration of both stream water and soil-water DOC showed large increases between WY1994 and 2007. Of the assessed water quality parameters (pH and IS) under different flow conditions changes in stream DOC concentration were inversely correlated to changes in IS (Hruška et al., 2009). This pattern was attributed to recovery from acidification, however the underlying mechanism was not addressed. In contrast, a recent study on water-extractable DOC showed a positive correlation between concentration of monovalent cations in the extraction solution and DOC (Xie et al., 2017). The specific mechanism producing this correlation was not identified; however these results do not necessarily contradict our hypotheses of aggregate dispersion as DOC generator. Soil composition, including mineralogy, strongly affect aggregate composition and, as a result, the main mechanism stabilizing aggregates differs; for example, in soils rich in metal oxides, pH might have a larger effect on aggregate stability than IS.

Because divalent cations are more effective in bridging negative charges (Mikutta et al., 2007), potentially promoting aggregation and DOC sequestration, we had also expected lower DOC release for $CaCl_2$ vs. NaCl solutions of the same IS. Particle size analysis showed that aggregate size was generally larger in $CaCl_2$ vs. NaCl solution, suggesting that cation bridging might play a role in preserving aggregates. DOC release into $CaCl_2$ was indeed lower than into NaCl solution, but the effect was not significant (Fig. 9). However, the trend is in agreement with results from Kerr and Eimers (2012), who conducted a combined laboratory and field study on the effect of monovalent vs. divalent cations on DOC release into soil water. They found that DOC release was driven by declines in one or more divalent cation species (not so much by an overall change in IS). The authors explained their results by reduced DOC sorption at low Ca^{2+} concentrations which is most likely the result of cation bridging (Kerr and Eimers, 2012).

4.2 Landscape position modulated the response to solution chemistry

We had hypothesized that soils from low-lying landscape positions contain more total C than those from the HS locations and therefore would release more DOC. Our results confirm that TOC is consistently higher in RZ soils (Fig. 3) and SEM analyses show a larger proportion of organic matrix vs. inorganic mineral fragments than HS soil aggregates (Fig. 4 and 5), which would suggest a greater potential for DOC release. However, RZ soil extracts did not contain significantly higher amounts of DOC or TN (Fig. 9, supplementary materials), which is remarkable, given that certain locations in the RZ had up to 6 times higher TOC content compared to the HS locations (Fig. 3). One explanation for the relatively small releases of DOC could be the generally larger aggregate size in riparian soils (Fig. 4 and 6) resulting in smaller surface areas. This difference in surface area could be significant for DOC desorbing from aggregate surfaces instead of from complete aggregate break up (Fig. 13, Chorover et al. (2007)), e.g. when increases in solution pH increases organic matter solubility (David et al., 1992; Kleber and Johnson, 2010; SanClements et al., 2018). Another important factor is that RZ at our sites naturally contain large amounts of Ca^{2+} from upwelling ground water (Armfield et al. 2018 (this issue); Shanley et al. (2004)), which could further help to stabilize RZ aggregates through increased sorption capacity or cation bridging (Fig. 5).

Landscape position strongly impacted fluorescent DOM characteristics. For example, extracts from RZ soils consistently had lower values for FI, HIX and the humic-like PARAFAC component C-2, while the protein-like PARAFAC component C-3 was significantly more abundant compared to HS extracts (Fig. 10b, Fig. 10d, and Fig. 11). These results paint a complex picture of the molecular make up of DOM extracted from RZ soils and likely reflect high amounts of fresh and terrestrial organic matter such as roots and decomposing leaf litter (indicated by FI, HIX, and C-2) as well as abundant microbial DOM fueled by a wet and nutrient-rich environment (indicated by C-3). In contrast, HS extracts had higher values for HIX, the humic –like C-2 and FI (Fig. 10b and Fig. 10d), suggesting the presence of typical humified soil DOM with active microbial processing during the wet and warm sampling season. Together, these results suggest that landscape position presents an important control on the type of DOM that could be flushed from soils into surface waters.

4.3 Aggregate-derived DOC, a significant factor for ecosystems?

We had also hypothesized that DOC released from aggregates would be mostly terrestrial and very bioavailable because it would have been shielded from microbial processing. NW extracts (presumably containing more DOC from aggregates than the other extracts) had indeed lowest, most terrestrial values for FI and lowest humic-like PARAFAC component C-2, results that are consistent with other studies (Gabor et al., 2015). Our results are also in agreement with another study where most of the released C into solution was plant-derived (terrestrial) in addition to a mixture of compounds that could be either plant- and/or microbially-derived (Angst et al., 2017).

DOM serves as a source of energy at low trophic levels, hence the success of all aquatic organisms depend on it and type of DOM matters greatly (Creed et al., 2018b). Our results suggest that either aggregate break up or desorption of DOM from aggregate surfaces leads to changes in DOM released from soils. With solutions simulating recovery (NW), DOM shifts from microbial and less humified to terrestrial and more humified (indicated by greater HIX values). Typically, DOM with larger HIX values is less bioavailable because the ensuing molecular makeup including polyphenols and double bonds tend to be harder to degrade (Gabor et al., 2015). However, our study showed that, other than hypothesized, NW extracted DOC was not more bioavailable than DOC in higher IS extracts. If this pattern holds true beyond simple laboratory experiments, high amounts of somewhat recalcitrant DOM could be released into surface waters, water browning could decrease photosynthetic activity (Creed et al., 2018b) while N might increase downstream aquatic productivity.

Despite the trend of decreasing aggregate size with decreasing IS (modulated by the presence of mono vs. divalent cations), our observations also show that these aggregates are remarkably stable. For example, the experimental treatment involved one hour of vigorous shaking with the different extraction solutions, and yet, mean particle size did only decrease by 13 to 17 % (supplementary materials). These results indicate that DOC release *via* this mechanism is likely gradual and limited by overall aggregate stability.

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APPENDIX 1: DENSITY SEPARATION

Procedure: I used the Density Separation Procedure (DSP) from Glochin et al. (1994) and Sohi et al. (2001) to physically separate soil fractions related to variations in aggregate stability. Three different fractions are considered: the free-light fraction (FLF), the occluded light-fraction (OLF), and the heavy occluded fraction (HOF). The FLF is described as the active organic matter pool (Crow et al., 2007) and is composed of plant derived debris (leaves, branches, roots), some animal residue, seeds, pollen, and some microorganisms colonizing organic residues (Cerli et al., 2012). The Occluded light-fraction (OLF) is the organic matter that floats to the top of the solution after sonication and centrifugation. The HOF material has a density greater than 1.65 g/cm^3 and remains at the base of the centrifuge tube. This fraction consists of organo-mineral associations with clay minerals, whose density ranges from 2.3 to 2.98 g/cm^3 (Osipov, 2012).

DSP was conducted using a method from Glochin et al. (1994) and Sohi et al. (2001, Table 1). For each composite sample, 150 mL of Sodium Polytungstate (SPT; ρ = 1.65 g/cm^3) was added to 250 mL polycarbonate centrifuge tubes containing about 25 g of air-dried, sieved soil. After the samples were left to sit overnight, the FLF was aspirated off each sample using a vacuum. They were rinsed with five 200 mL aliquots of 0.01 M $CaCl_2$ and 1 L of NW over a 0.8 μm Isopore^{TM} Membrane filter and set out to dry. The samples in the centrifuge tubes were sonicated in an ultrasonic bath for 20 minutes and centrifuged for 30 minutes to release the OLF. This fraction was aspirated off the top of the solution, rinsed with both 1 L of 0.01M $CaCl_2$ and 1 L of NW, and set out to dry. After removing the SPT from the centrifuge tube, the last fraction (HOF) was dispersed by shaking for 5 minutes with 0.01 M $CaCl_2$ and then filtered and rinsed with 1 L of NW over a 0.8 µm Isopore^{TM} membrane filter.

Table A1. Summary of the Density Separation Procedure; Modified from Glochin et al., 1994 and Sohi et al., 2001.

STEP	**Procedure**	**Goal:**
1	Soil air-dried and sieved gently through 2 mm mesh	
2	Weighed to 20 g and transferred to 250 mL centrifuge tube	
3	Add 150 mL Sodium Polytungstate; left to sit overnight	Separates two fractions
4	Free-Light Fraction (FLF) aspirated off *via* vacuum	Isolate FLF
5	Rinsed with 1 L NW over 0.8µm filter; dried and weighed	
6	Remaining soil treated ultrasonic bath; centrifuged at 3240 rpm for 30 minutes	
7	Occluded Light Fraction (OLF) aspirated off (same as FLF), rinsed, dried, and weighed	Isolate OLF
8	Heavy Occluded Fraction dispersed with 200 mL 0.01M $CaCl_2$	
9	HOF rinsed with 1 L NW, dried, and weighed	Isolate HOF

Results: The physical fractionation of the soil by density showed that most soil samples had highest amounts (up to 98.8%) in the heavy occluded fraction (HOF, Table 2). The exception was a sample from the RZ adjacent to the steep transect (S RZ) that was mostly made up of the unstable Free-Light Fraction (FLF). The occluded light fraction showed less variation between samples but was generally higher in riparian samples compared to HS samples.

After the Sodium Polytungstate (SPT) solution was added to the composite soil samples, it was clear that a great proportion of the S RZ was composed of the FLF because much of the sample floated to the surface of the SPT (Figure 27) and this trend is displayed as a percentage in Table 2.

Table A2. Density Separation results where Free-Light Fraction, Occluded-Light Fraction, and Heavy-Occluded Fraction recovery are recorded as a percent of the total mass recovered, values total 1.

Sample ID	*Free-Light Fraction*	*Occluded-Light Fraction*	*Heavy Occluded Fraction*
S RZ	**0.8**	0.08	0.12
S HS	0.01	0.01	**0.99**
I RZ	0.01	0.03	**0.96**
I HS	0.01	0.01	**0.98**

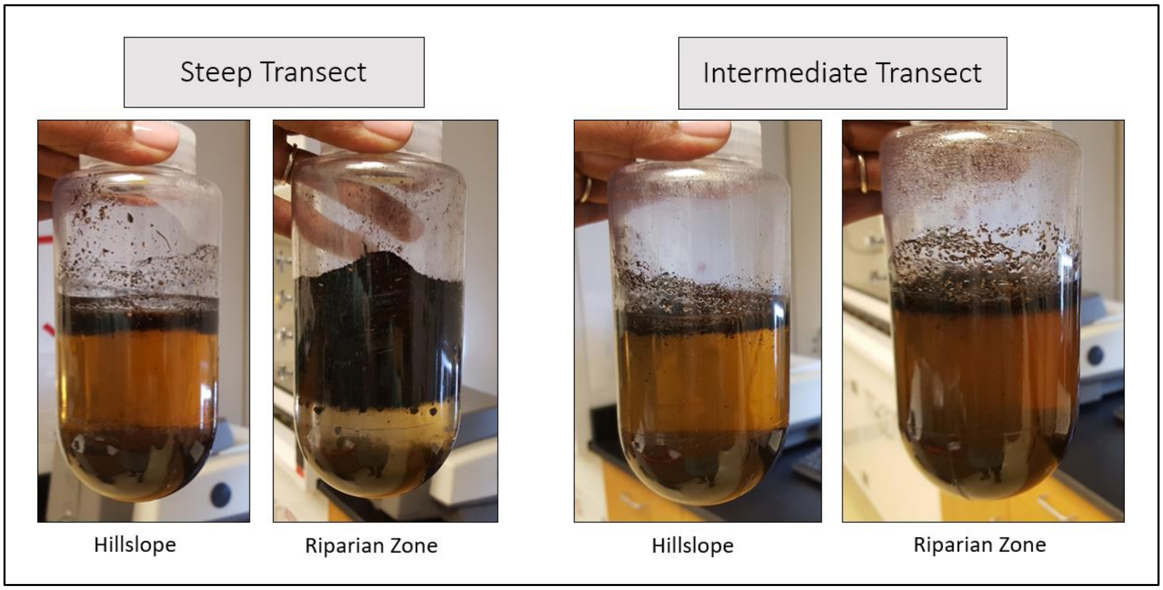

Figure A1. Soil samples mixed with Sodium Polytungstate ($\rho = 1.65$ g/cm^{-3}) show separation into various fractions based on density

APPENDIX 2: MINERAL IDENTIFICATION

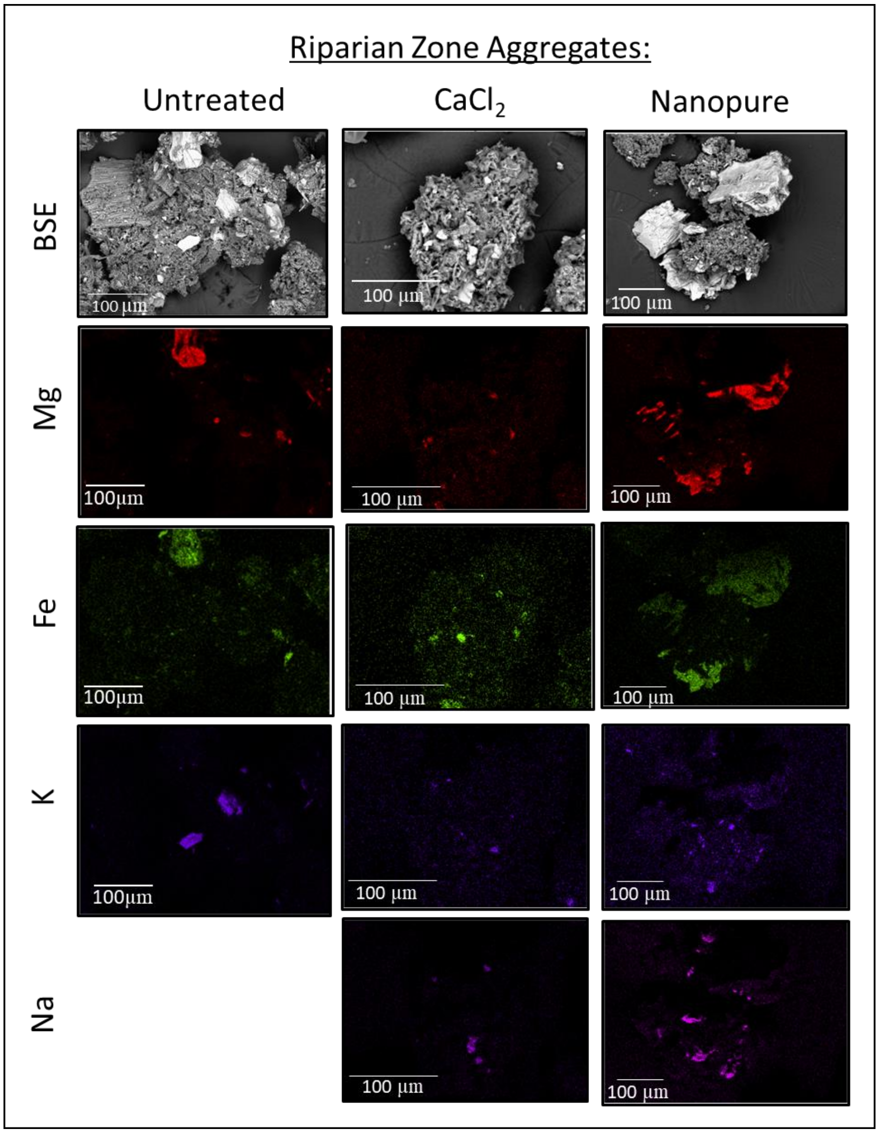

Figure A2. RZ Aggregates with maps of elemental distributions.

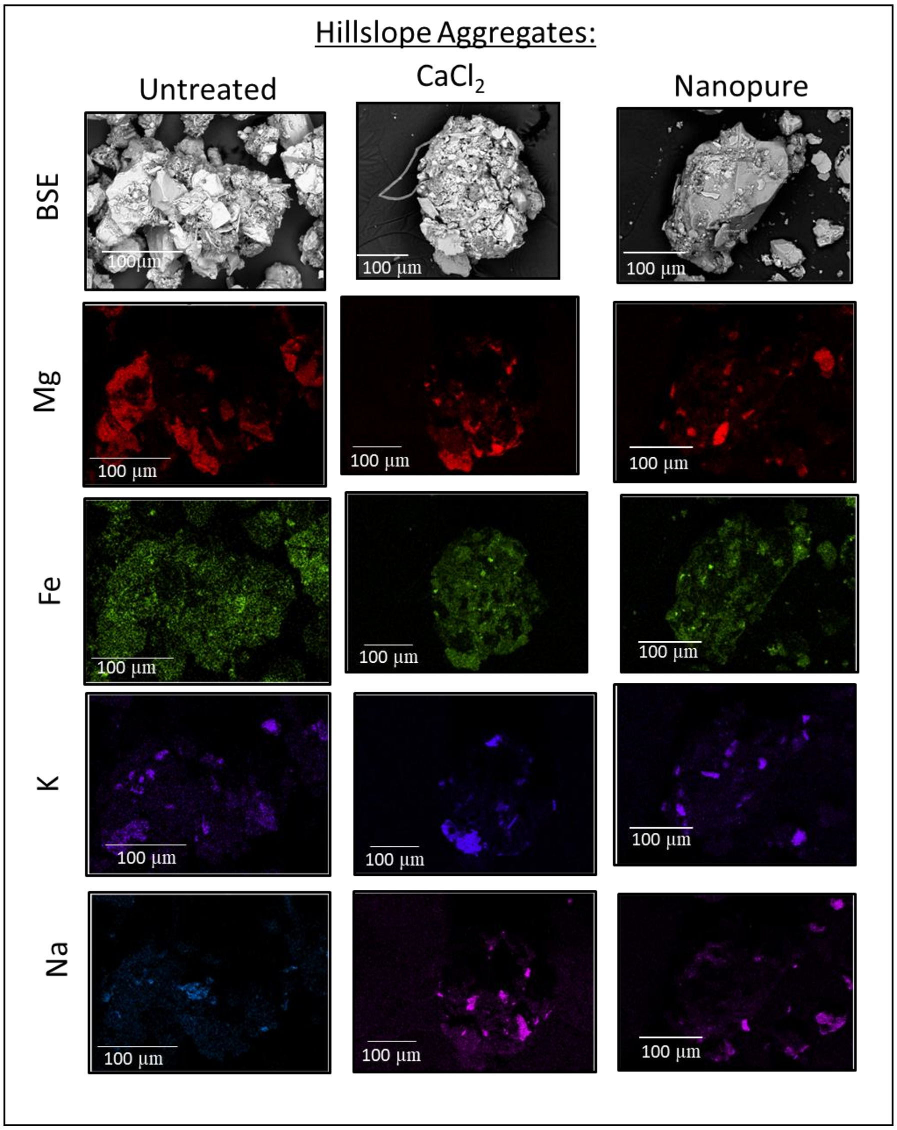

Figure A3. HS aggregates with maps of elemental distributions.

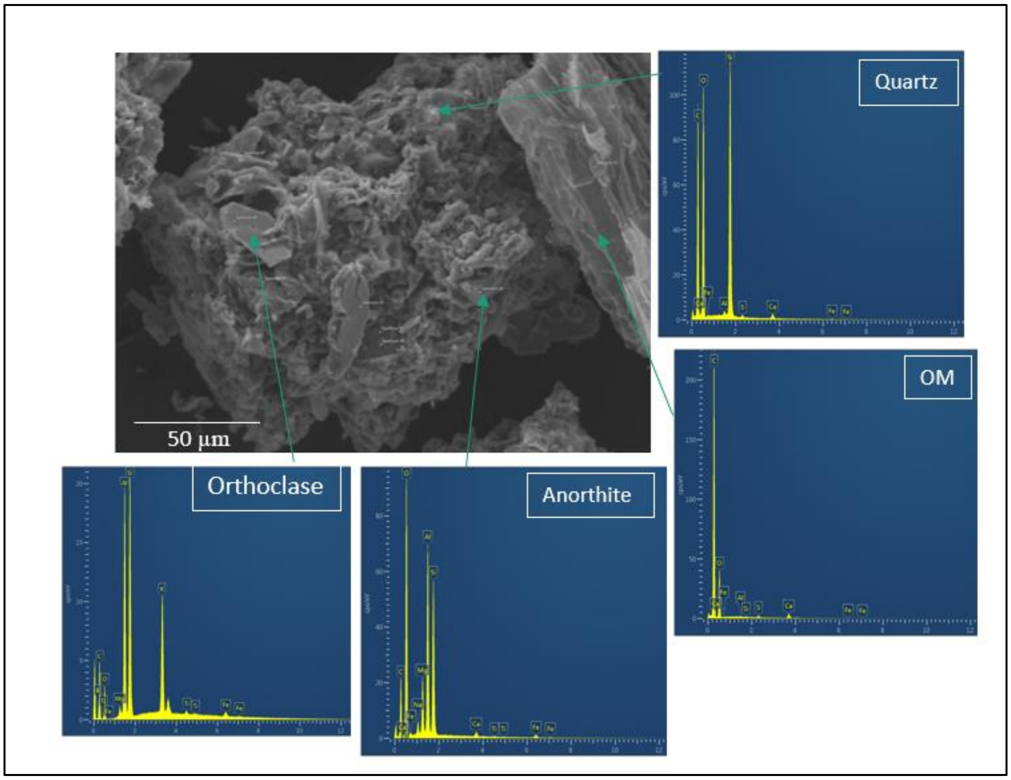

Figure A4. A secondary electron image of an RZ aggregate and elemental spectra.

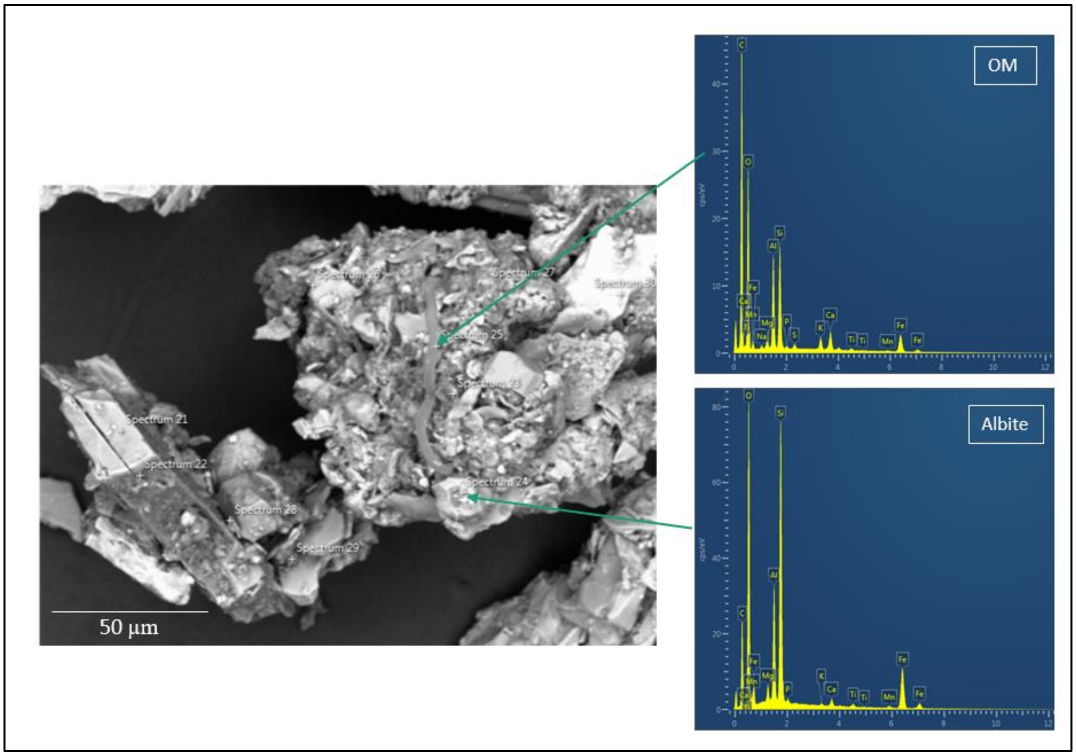

Figure A5. A backscatter electron image of an HS aggregate and the elemental spectra.

APPENDIX 3: PARTICLE SIZE

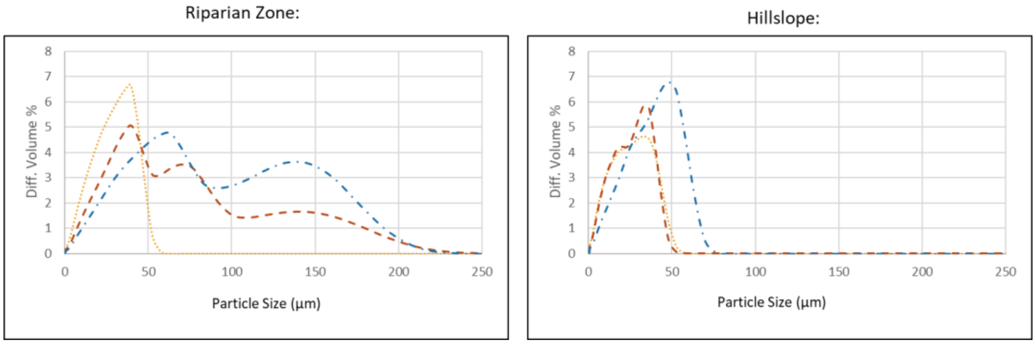

Figure A6. The shift in peaks illustrates the change in particle size distribution of diffracted volume (%) as a function of particle size (μm) for Riparian zone and Hillslope samples. Values were determined using three aqueous extraction treatments: NW (dotted line), NaCl (long dashes), and $CaCl_2$ (dash-dot).

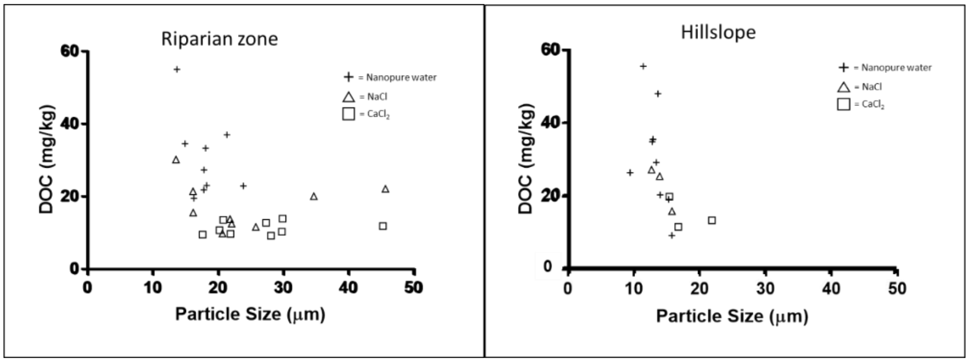

Figure A7. DOC release (mg/kg) plotted against average particle size (μm) for RZ and HS samples.