

Use of Liquid Chromatography – Organic  
Carbon Detection to Characterize  
Dissolved Organic Matter from a Variety  
of Environments

by

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## **AUTHOR'S DECLARATION**

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

## Abstract

Dissolved organic matter (DOM) is a heterogeneous mixture of organic compounds comprised mostly of carbon, oxygen, nitrogen, and hydrogen, but is often operationally defined as the concentration of dissolved organic carbon that passes through a 0.45  $\mu\text{m}$  filter. Derived from the degradation of both plant and animal organic matter, DOM can act as an important redox constituent within groundwater, or form carcinogenic disinfection-by-products during water treatment. It is important to understand the quality of DOM in order to be able to understand how it will react within its environment. A number of different techniques are used to characterize DOM (such as resin fractionation, ultraviolet and visible light absorption, and fluorescence) but these techniques can be both laborious and time consuming, in addition to requiring large amounts of sample. Recently, a new technique has been developed, Liquid Chromatography – Organic Carbon Detection (LC-OCD), that provides a fast and reproducible technique. LC-OCD can group components of DOM into six categories based upon molecular weight: hydrophobics (HPho), humic substances (HS), building blocks (BB), low molecular weight neutrals (LMW-N), and acids (LMW-A). Furthermore, the molecular weight and aromaticity of HS can be determined. The primary goal of this thesis was to use the LC-OCD to better understand characteristics of DOM, with special attention to how the quality of DOM can be defined.

A laboratory decomposition experiment was set up to identify the composition of soluble leaf leachate and observe the changes to DOM during microbial degradation. In addition, other widely used characterization techniques were used to compare with LC-OCD results. Soluble leaf leachate consisted of nitrogen poor, high molecular weight (HMW) aromatic molecules with some low molecular weight neutrals (LMW-N). The DOM degraded by 44% over 150 days, with only 24% of the total DOM rapidly degrading within 22 days. Degradation resulted in an increase in the proportion of HS while LMW-N decreased. Furthermore, comparison of specific ultraviolet absorption (SUVA: an indicator of aromaticity) from HS and total DOM showed an increase in aromatic LMW compounds. Generally, LC-OCD analysis compared well with other methods, allowing for a quantitative measure into the microbial changes to DOM quality.

Septic-impacted groundwater from Long Point, ON, was used as a case study to observe the changes in lability of DOM over the length of the septic plume. It was hypothesized that the septic tiles would introduce highly-labile DOM into the groundwater, which would degrade into a recalcitrant form as it was subjected to subsurface processes. Two distinct flow paths were observed under the tile bed: a vertical (shorter time period) and horizontal (longer time period) dominated flow. The concentration and SUVA of DOM decreased in both flow paths, with aromatic LMW-N slightly increasing in the horizontal flow path. The decrease of DOM and nitrate, and the presence of nitrous oxide all indicate

the presence of denitrification, indicating that active microbial degradation would be heterotrophic, thus requiring DOM. Furthermore, changes to the molecular weight and SUVA (both of the DOC and HS) while other proportions remain relatively constant indicate HS may be preferential for microbial degradation. LC-OCD analysis found sorption of DOM to be unlikely. The LC-OCD was also able to identify dissolved organic nitrogen (DON) bound to HS, which decreased in concentration as groundwater aged. These results indicate that the active microbial degradation of DOM created a more recalcitrant form along the plume.

A variety of surface and ground water environments were sampled in order to better understand the different compositions of DOM within the natural environment. Samples were taken from lakes, rivers, and streams, as well as from agriculturally impacted aquifers and an un-impacted aquifer. In most cases, HS and BB comprised the majority of DOM, with LMW-N comprising the rest. Hydrologic environments appeared to control the composition of DOM, with surface waters having higher DOC concentrations than ground waters. Furthermore, streams and rivers contained higher proportions of HS, while lakes contained higher proportions of BP (indicative of microbial interaction). Ground water can be distinguished from surface waters by having little BP or LMW-A. Furthermore, ground water compositions indicate a higher amount of reworking in the subsurface. In addition, agriculturally impacted aquifers contained lower HS molecular weights and HS SUVAs, while a large range was found from the un-impacted watershed. These results indicate that the LC-OCD is able to identify differences between different hydrological environments, providing a quantitative tool to measure DOM character.

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## CHAPTER 1

### INTRODUCTION AND CHARACTERIZATION OF DISSOLVED ORGANIC MATTER

#### 1.1 INTRODUCTION

##### 1.1.1 DISSOLVED ORGANIC MATTER

Dissolved organic matter (DOM) is an important, yet complex, part of the carbon cycle. The term DOM encompasses organic compounds comprised of carbon, oxygen, nitrogen, and hydrogen within solution (Thurman, 1985). This group of heterogeneous molecules is derived from the degradation of organic matter. Studies commonly use the concentration of dissolved organic carbon (DOC) as a proxy for the concentration of DOM, since carbon comprises around 50% of the total DOM, and that measurement of the total organic matter (and other elements) is difficult. This study will use concentrations of DOC to discuss the amount of DOM. In the environment, DOC concentrations typically range between 2 – 25 mg/L in surface waters, to 0.1 – 17 mg/L in ground waters (Aiken et al., 1985; Thurman, 1985; Bourbonniere, 1989; Artinger et al., 2000; Wilson & Xenopoulos, 2008). Highest concentrations are often found in waters originating from wetlands and bogs, where large amounts of organic debris are found (Chin et al., 1998). Alternatively, lowest concentrations are found in ground waters, where DOM is often depleted within the first few meters of the subsurface (Marmonier et al., 1995).

Importantly, DOM plays a key environmental role by transferring carbon, nutrients, and contaminants within the terrestrial system, as well as between terrestrial and aquatic ecosystems (Thurman, 1985; Jaffé et al., 2008). The importance of this link allows for carbon to be cycled among the entire ecosystem, using hydrologic pathways as a transporting mechanism between carbon pools. For example, it was found that terrestrial and aquatic ecosystems within the Hubbard Brook Experimental Forest were closely linked due to the flow of water (McDowell & Likens, 1988). Not only does DOM cycle carbon throughout the ecosystem, but it also plays an important role within the ecosystem by acting as a source of energy.

DOM can be used by various biological organisms as a source of energy. For instance, one study observed DOM as an important food resource for zooplankton in a highly humic lake (Salonen & Hammar, 1986). Conversely, DOM in the surface of a lake or river can absorb light, hindering the availability of light for photosynthesis but also protecting aquatic organisms from ultraviolet (UV) exposure (Schindler et al., 1996; Williamson et al., 1999). In ground water environments, DOM also plays a key role by acting as an important redox constituent. In ground water, DOM may be oxidized during natural attenuation of nitrate by heterotrophic bacteria (Starr & Gillham, 1993; Robertson & Cherry, 1995; Fenton et al., 2011), as well as act as an electron acceptor for the anaerobic oxidation of organic compounds (Lovley et al., 1996; Heitmann & Blodau, 2006; Blodau et al., 2009). This illustrates one instance of the importance of DOM in relation to the quality of drinking water.

Drinking water treatment applications seek to reduce the concentration of DOM in order to lower the possibility of potential microbial growth, and remove colour, taste, and odour (World Health Organization, 2011). It has also been observed that DOM reacts during the chlorination of water (a disinfection technique), forming carcinogenic disinfection-by-products (DBP) (Marhaba & Van, 2000). Furthermore, the presence of DOM and potential of DBP formation make it the single most important determinant for drinking water treatment cost (Dr. S. Schiff, *personal communication 2012*). Furthermore, DOM has the capability of mobilizing organic pollutants and heavy metals within waters, which is a major concern for the overall quality (Reza et al., 2010; Neale et al., 2011). It is evident that understanding the characteristics of DOM is important when dealing with the quality of drinking water.

Dissolved organic matter has numerous ways in which it can interact with its environment, both positively and negatively. The difficulty in characterizing such a parameter, however, is caused by its intermolecular heterogeneity. Comprised of thousands of organic compounds with varying structures and weights, each mixture of compounds can differ among different environments. DOM can consist of hard to break down, or recalcitrant, forms of carbon, whereas other sources are more easily degraded, or labile,

form of DOM. For instance, DOM formed *in-situ* (referred to as autochthonous) in lakes and rivers is considered relatively labile compared to DOM originating from outside sources (termed allochthonous). The range in reactivity can be attributed to the variety of source materials, as well as the amount of reworking or processing upon the DOM. The rate of or extent to which these reactions occur, depends upon the reactivity or lability of the carbon. Thus in the environment, there is a wide variety of DOM compositions and types, thus advanced methods are required in order to aid in the characterization of DOM.

### **1.1.2 CHARACTERIZATION OF DISSOLVED ORGANIC MATTER**

The difficulty in characterizing DOM results from its complexity. This can be seen by the number of definitions used to describe organic matter. Thurman (1985) observed thirteen different definitions that can all be used to describe aqueous organic matter. As previously mentioned, many aqueous studies refer to DOM as the concentration of organic carbon passing through a 0.45  $\mu\text{m}$  filter. However, a concentration does not give any indication to whether the sample contains labile or recalcitrant forms of carbon. Lapworth et al. (2008) observed samples with identical DOM amounts to contain different fluorescent characteristics. This illustrates how characterizing DOM beyond a concentration is necessary to understand its potential within the environment.

Despite the thousands of compounds that make up DOM, it is important to determine whether the DOM is easily degradable or not. Microbially available or labile portions can be considered the fraction of ‘useful’ carbon, determined as that which biodegrades in a certain amount of time (ranging from hours to weeks), whereas the remaining DOM can be termed refractory (Servais et al., 1987; Marmonier et al., 1995). However, such a determination is based upon a loss in overall DOM concentration, as well as the amount of time left for it to degrade, and doesn’t address the components that make up DOM. In order to address this, other studies have attempted to isolate and chemically characterize the constituents of DOM using a variety of techniques, in order to determine the quality of DOM at hand.

One common technique to characterize DOM is the separation of different components using XAD resin fractionation. These resins are comprised of non-ionic,

macroporous co-polymers with large surface areas, enabling different components to elute at different times. Macroreticular Amberlite XAD resins have been widely used to classify different components of DOM (Aiken et al., 1979; Leenheer, 1981; Bourbonniere, 1989; Drewes & Croue, 2002). It has been found that the majority of DOM is comprised of humic substances (Aiken et al., 1985; Thurman 1985; Wassenaar et al., 1990; Grøn et al., 1996; Vetter et al., 2010). The term ‘humic substances’ encompasses both humic and fulvic acids, consisting of numerous organic compounds that have similar properties. Humic acids are defined as the fraction not soluble below pH 2 but soluble at greater pH levels, while fulvic acids are soluble at any pH (Aiken et al., 1985; Thurman, 1985). However, it has been found that DOM cannot be described by humic and fulvic acids alone. Bourbonniere (1989) was one of the earlier studies to further divide fulvic acids based on physio-chemical characteristics, characterizing hydrophilic components as well. Although XAD resins allow for component characterization, this technique is laborious and requires large amounts of sample (liters) in order to obtain a result (Aiken et al., 1992).

Another method to characterize DOM is through the use of ultraviolet (UV) absorption. As UV is absorbed by aromatic structures and unsaturated carbon atoms, using specific ratios determine the relative aromaticity or molecular size of the DOM. For instance, the  $E_2:E_3$  ratio uses the absorbance at 250nm ( $E_2$ ) and 365nm ( $E_3$ ) as an indicator of DOM aromaticity and molecular weight (De Haan & De Boer, 1987; Dahlén et al., 1996; Ågren et al., 2008). Furthermore, it has been found that the specific UV absorbance (SUVA; absorbance at 254nm standardized to DOC concentration) is well correlated with aromaticity (Weishaar et al., 2003). The limit of UV techniques is that only chromophore-containing constituents absorb UV, not necessarily all the molecules that comprise DOM. Her et al. (2002b) used an on-line UV-detector with an organic carbon detector to observe the signal obtained from DOM. It was found that using UV-absorbance alone failed to pick up fractions detected by the organic-carbon detector (Her et al., 2002b). Although UV methods do not require a large amount of time, they should be paired with other techniques in order to obtain a reliable characterization of DOM (Kawasaki et al., 2011).

Fluorescence spectroscopy, another optical technique to characterize DOM, uses fluorescence excitation emission of the sample. This technique is becoming more common and can provide information on the structure and humification (formation of humus) of DOM (McKnight et al., 2001; Hunt & Ohno, 2007). However, computation of the data may involve more complex modelling, such as parallel factor analysis (PARAFAC), and can be affected by interferences (such as nitrate or iron) that change the fluorescence of the sample (Hunt & Ohno, 2007). Furthermore, like UV-absorbance, only fluorophore-containing DOM constituents will respond to this method, indicating the lack of characterization of non-fluorophore fractions.

Pairing the changes of DOM with changes of other compounds can provide information on the character of the DOM. For instance, the concentrations of dissolved oxygen and DOC within an aquifer can lead to an inference about its bioavailability (Chapelle et al., 2012). Another study used carbon dioxide (CO<sub>2</sub>) measurements from a microbial incubation study to quantify DOC biodegradation (Kalbitz et al., 2003), thus in turn determining the lability of DOM. Elemental compositions (such as N:C, H:C, and O:C ratios of humics) can also be used as indicators of bioavailability (Sun et al., 1997; Hunt et al., 2000). More complex techniques such as <sup>13</sup>C-NMR or pyrolysis-GC/MS require technical analysis, yet provide detailed information on the specific chemical features and structures of the organic matter on a molecular level (Conte et al., 2004; Kalscheur et al., 2012).

A common technique to characterize DOM has been to use size exclusion chromatography (SEC), where molecules are separated by their physical and chemical differences. The basis of analysis stems from larger molecules eluting before smaller ones, allowing for the determination of different size fractions (Huber & Frimmel, 1991; Pelekani et al., 1999; Her et al., 2002; Lankes et al., 2009; Szabo & Tuhkanen, 2010; Huber et al., 2011; Kawasaki et al., 2011; Ruhl & Jekel, 2012). These size fractions are comparable to other molecular-weight methods (Zhou et al., 2000). Furthermore, SEC can be paired with UV-absorbance detection to give quantitative information of UV-absorbing fractions (Lankes et al., 2009). An advantage of these systems is the ability to use small amounts of sample (from µL to mL), as well as receive a quantitative index into the composition of DOM.

This study used a relatively new method where SEC is paired with an organic carbon detector (UV-thin film reactor developed by Huber & Frimmel (1991)) and termed the Liquid Chromatography – Organic Carbon Detection (LC-OCD). The LC-OCD has previously been used to determine changes in DOM composition from wastewater effluent, as well as its effect upon membrane fouling (Batsch et al., 2005; Grünheid et al., 2005; Kennedy et al., 2005; Haberkamp et al., 2007; Ciputra et al., 2010; Neale et al., 2011). The DOM classification system explained by Huber et al. (2011) is based on SEC, where fractions are defined by the elution time. Although the groupings are arbitrarily defined, physio-chemical characteristics assigned to each fraction are comparable with other studies (Zhou et al., 2000; Lankes et al., 2009; Müller et al., 2009). Importantly, the organic carbon reactor provides sufficient exposure and residence time to allow for excellent oxidation yields of organic molecules (Huber et al., 2011). The advantage of such a method is the ease of sampling and analysis, providing a highly informative result. Furthermore, other than filtering, samples are analyzed without any pretreatment, reducing the possibility of alteration or contamination.

## **1.2 THESIS OUTLINE**

The objective of this thesis was to assess the use of a new and logistically expedient method (Liquid Chromatography – Organic Carbon Detection) in understanding characteristics of DOM. Special attention is paid to how the quality of DOM can be defined. This will be explored through four chapters.

The first chapter provides an introduction to DOM, focusing upon definitions and common characterization techniques. This introduces the background and basic properties of DOM from which the other chapters will build upon with data collected from the LC-OCD.

The second chapter uses the LC-OCD to analyze the composition of DOM from a laboratory leaf litter decomposition experiment. Here, different methods of DOM characterization will be compared with LC-OCD analysis, in order to observe changes to DOM during microbial degradation of fresh labile material. This will provide an indication

to what components are preferentially utilized over others, as well as the ability of the LC-OCD to identify such changes.

The third chapter presents a case study of the change in DOM lability over the length of a septic effluent-impacted plume. DOM from groundwater at Long Point, ON, provided an environment where the change in DOM along the flow path could be paired with the age of groundwater. This was done by comparing the changes in LC-OCD and SUVA to changes in quality, and whether these were similar to microbial or sorption processes found in literature.

Finally, the fourth chapter looks at DOM from a variety of surface and ground water environments, illustrating what the range in composition of natural DOM. Here it will be determined whether or not DOM composition varies among different hydrological environments, and whether the LC-OCD can be used to distinguish between such environments.

## CHAPTER 2

### USING THE LC-OCD TO ASSESS COMPOSITIONAL CHANGES TO DOM DURING BIODEGRADATION OF LEAF LEACHATE

#### 2.1 INTRODUCTION

Dissolved organic matter (DOM) plays an important part within the carbon cycle by moving carbon through terrestrial and aquatic ecosystems. The accumulation and subsequent degradation of surface organic litter can create high concentrations of DOM within only a few centimeters. For instance, soluble components originating from degrading leaf litter can create DOM concentrations up to 100 mg/L, but when leached into the subsurface, can be rapidly degraded or sorbed to concentrations that are an order of magnitude lower (Wetzel & Manny, 1972; Thurman, 1985; Qualls et al., 1991; Hongve et al., 2000; Cleveland et al., 2004). However, not all DOM degrades at the same rate since various organic components comprising DOM differ in biodegradability (Qualls, 2005). It can be difficult to determine which components comprise the labile or recalcitrant portions of DOM, especially in an environment where a suite of organisms and degradation rates exist. Hence, estimating the biodegradability of DOM from the environment most likely underestimates the 'true' value (Cleveland et al., 2004). For this reason, it is important to have a method that can determine what is labile without having to do conventional biodegradability techniques that are laborious and time consuming. Understanding the composition of fresh, soluble organic matter may provide a better indication of which organic components are more bio-available than others.

##### 2.1.1 LEAF LEACHATE: A SOURCE OF DOM

To better understand the availability of labile substrates, previous studies have observed qualities of young, labile DOM through decomposing leaf litter. It has long been known that organic matter can be solubilized rather quickly from organic litter (Nykqvist, 1963; Wetzel & Manny, 1972; Petersen & Cummings, 1974). In addition, leachate provides an important source of both carbon and nitrogen, and is known to be available to the

microbial population (Benner et al., 1986; Qualls et al., 1991). DOC leached from leaf litter produces a large amount of DOC for the watershed and is rapidly taken up near the surface through biotic and abiotic mechanisms (McDowell & Likens, 1988; Fröberg et al., 2007). The type of litter and bacterial community present can affect both the total amount of biodegradation and rate at which it degrades (McNamara & Leff, 2004; Wallace et al., 2008). In order to better understand specific changes to the leachate, incubation studies can be used to specifically examine the changing character of DOM during microbial degradation.

Organic molecules leaching from litter can lead to a number of dissolved organic constituents. Some studies have found leached DOM from partially degraded leaf litter to consist of low molecular weight (LMW) organic molecules (Wetzel & Manny, 1972; Hur et al., 2009), whereas leachate from decomposed leaf litter found the majority to consist of fulvic and hydrophilic acids (Qualls, 2005). Regardless of the organic source, DOC formed from degradation has generally been found to consist of certain components, such as polysaccharides, degraded lignins, lipids, sterols and proteins (McKnight & Aiken, 1998). Incubation studies have determined that DOM concentrations resulting from leaching decrease rapidly within the first 15 days, and small, non-humic components were preferentially lost over that time (Cleveland et al., 2004; Hur et al., 2009). These findings illustrate that readily leached DOM from leaf litter provides an array of labile components that are available for the surrounding ecosystem.

### **2.1.2 CHARACTERIZING DOM**

The quality of DOM can be associated with certain parameters. Ultraviolet (UV) and visible spectrum (VIS) light absorption occurs as a result of absorption from the aromatic structures and unsaturated structures within DOM. It is possible to achieve an indication of relative molecular weight, aromaticity, and biodegradability from ratios of different UV-VIS wavelengths. For instance, the ratio of absorbances at 255nm ( $E_2$ ) and 345nm ( $E_3$ ) is negatively correlated with molecular size of the UV-absorbing components (Dahlén et al., 1996), while the absorbance ratio of 465nm ( $E_4$ ) to 665nm ( $E_6$ ) correlates with humic substance molecular weight and size (Chen et al., 1977; Chin et al., 1994). In addition, the

specific UV-absorbance (SUVA) at 255nm has been documented to positively correlate with aromatic components, and in turn, the bioavailability of the DOM (Weishaar et al., 2003).

Decomposition of carbon has been linked with the availability of nitrogen, allowing the C:N ratio to provide information of the biodegradability of DOM (Cleveland et al., 2004). A high ratio in leaf litter has been found to correspond with slow decomposition (Enríquez et al., 1993). For microbes (with a C:N around 8:1), their ~40% growth efficiency prefers a substrate C:N ratio of 25:1, meaning decomposition of substrates with higher ratios leads to nitrogen being taken up from the surroundings, while lower ratios leads to microbial mineralization of nitrogen (Fagerbakke et al., 1996; Hunt et al., 2000; Chapin III et al., 2002). A high C:N ratio within the leachate indicates a nitrogen-poor composition (Qualls et al., 1991). Specifically, bacteria can grow more efficiently on nitrogen-rich DOM, thus a low DOC:DON ratio within DOM can indicate a better biodegradable fraction (Wiegner & Seitzinger, 2004; Fellman et al., 2008).

The Liquid Chromatography - Organic Carbon Detection (LC-OCD) method uses size exclusion chromatography (SEC) to group organic molecules based upon their molecular size. This method provides a promising approach to characterize the composition of DOM (Huber et al., 2011).

### **2.1.3 RESEARCH OBJECTIVES**

Based on published studies, I hypothesized that DOM leached from fresh litter will consist of labile, low molecular weight (LMW) molecules due to the absence of external processes removing DOM from solution. Over time, fresh DOM will be rapidly degraded, leaving a recalcitrant form that will consist of humic substances (HS) and some LMW molecules. These hypotheses will be tested through a series of objectives. The first objective will be to use different methods to quantify components that make up the majority of initial leachate composition, and to compare these results to other leachate experiments. The second objective will be to see if changes from the various methods over time capture a change in the labile components of DOC. Finally, the third objective will be to compare changes observed from the LC-OCD with other DOM-quality parameters in order to determine the sensitivity of the LC-OCD with other widely used methods.

## 2.2 METHODS

### 2.2.1 EXPERIMENTAL DESIGN – LEAF LEACHATE

A number of senesced leaves from sugar maple trees (*Acer saccharum*) were collected from the surficial litter layer located on a treed lot in Waterloo, ON, in October 2011. Leaves were brushed gently to remove attached soil and other detritus and stored in a cold room ( $>4^{\circ}\text{C}$ ) for a week before starting the experiment. All leaves were then placed in an acid-washed 10L glass jar. Leaves were not washed so the microbial community collected on the leaves remained attached. The jar was then filled with Nanopure DI water, submerging all leaves, and left for 48 hours. In addition, the jar was lightly shaken to ensure complete mixing of the water.

Leachate was then poured through a coffee filter to remove large particulates that may have formed during shaking (Figure 2.1). The leachate was then filtered through a  $2.7\mu\text{m}$  filter, where a sub-sample was collected to be used for inoculation. The rest of the leachate was then filtered through pre-washed  $1.2\mu\text{m}$  and  $0.45\mu\text{m}$  filters. The leachate was then diluted with Nanopure DI within a 15-L acid-washed jug to a concentration of 25 mg/L and then poured into ten 1L aliquots within 2L-media bottles. The aliquots included five sacrificial time points and a replicate for each time point (T1-T5). The remaining diluted leachate was used as time zero (T0) to test for initial chemical parameters. Each media bottle contained 1L of the diluted leachate and a stir-bar, at which point 5mL of the inoculant was added (corresponding to 10% of the total carbon by mass). No attempt at characterizing the microbial community was made since each bottle contained the same source of inoculant. Each bottle was then capped with a rubber stopper and lid and 60mL of lab air was injected into each bottle to ensure no oxygen limitation with time. Bottles were kept sealed until sampled, covered with aluminum foil, and stored at in the dark at room temperature ( $\sim 22^{\circ}\text{C}$ ), and stirred once each day at a sufficient velocity to induce a small vortex to ensure complete mixing with the headspace. Two bottles were opened after each of 8, 22, 62, 152, and 159 days. The leachates were immediately sampled for concentrations

of DOC, total dissolved nitrogen (TN), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), UV-VIS absorbance, and SEC analysis of DOC.

After 152 days, the last time point (T5) was further divided into four aliquots of 300mL. Nutrients ( $\text{KNO}_3$  and  $\text{Na}_2\text{HPO}_4$ ) were added at concentrations of 92.6 mg/L and 30 mg/L (respectively) in order to reduce nutrient limitation within the diluted leachate. The same parameters were measured after 7 and 38 days of nutrient addition.

In order to test the changes to composition as a result of storage, and not microbial degradation, a separate storage experiment was run. The reader is referred to the Appendix for a full description and analysis of changes to DOM during storage.

### **2.2.2 SPECTRAL ANALYSIS**

Absorbance was measured for each sample using a Beckman DU<sup>®</sup> Series 500 Spectrophotometer with a 1cm quartz cuvette. Samples were warmed to room temperature and scanned at 5nm intervals between 200nm and 700nm. Absorbance ratios of  $E_2:E_3$  and  $E_4:E_6$  were calculated by dividing the absorbance at 255nm ( $E_2$ ) and 465nm ( $E_4$ ) by the absorbance at 365nm ( $E_3$ ) and 665nm ( $E_6$ ), respectively. Furthermore, specific UV-absorbance (SUVA,  $\text{L}/(\text{mg}\cdot\text{m})$ ) was calculated by dividing the measured absorbance at 255nm by the concentration of DOC (mg/L).

### **2.2.3 CHEMICAL ANALYSIS**

All chemical analysis was completed at the Environmental Geochemistry Laboratory at the University of Waterloo. Ammonium ( $\text{NH}_4^+$ ) and nitrite ( $\text{NO}_2^-$ ) concentrations were determined using colourimetric methods, using a Beckman DU<sup>®</sup> Series 500 Spectrophotometer. Samples were analyzed for nitrate ( $\text{NO}_3^-$ ) using a Dionex ICS-90 ion chromatograph. Calibration curves were created using Dionex brand standards. TDN was measured using a Tekmar Dohrman Apollo 9000 High Temperature catalytic oxidation TOC analyzer with total nitrogen add-on equipped with an autosampler. Standards were prepared using potassium nitrate ( $\text{KNO}_3$ ).

DOC concentrations and a set of standards (created using potassium hydrogen phthalate) were analyzed on a using a Dohrman DC-190 High Temperature Total Carbon

analyzer. Here, samples and standards were acidified using 20% phosphoric acid and sparged to remove inorganic carbon. Samples were then injected, combusted at 680°C to convert DOC into CO<sub>2</sub>, and measured by a non-dispersive infrared (NDIR) spectrum. Samples were measured in triplicate, with the standard deviation being less than 3% of the overall concentration.

#### **2.2.4 LC-OCD ANALYSIS**

Dissolved organic carbon characterization was completed using a LC-OCD at the University of Waterloo (Figure 2.2). Samples were first diluted within a range of 1-5 mg/L in order to obtain optimal results. The LC-OCD used a Toyopearl HW-50S (Tosoh, Japan) size-exclusion column. The mobile phase is created using a pH 6.85 phosphate buffer comprised of potassium dihydrogen phosphate and sodium hydrogen phosphate dehydrate and purified of any organics by passing through UV-irradiation. The sample is then injected into the mobile phase and passed through an in-line 0.45µm filter into either a bypass or the size-exclusion column. The bypass allows for the measurement of the overall DOC concentration and UV-absorbance at 254nm. Once through the column, the sample enters the UV Detector (set at 254nm). After this, the sample is acidified using phosphoric acid. The mobile phase enters the organic carbon detector (OCD), a thin film UV-reactor (Grüntzel Thin-film reactor, Huber & Frimmel, 1991), which spreads the sample thinly over a UV-lamp where irradiation causes the organic carbon to oxidize into CO<sub>2</sub>. The CO<sub>2</sub> is then measured by a highly-sensitive infrared detector, and the data are collected over time as the sample elutes from the SEC column.

Calibration for the OCD was completed using a potassium hydrogen phthalate (KHP) stock solution of 1000 mg-C/L and diluted to concentrations of 0.10, 1.00, 2.50, 3.75, and 5.00 mg/L. Calibration of the humic substances weight was completed using Suwannee River Standard II for humic and fulvic acids. Approximately 4 mg of each standard were dissolved in 100 mL of distilled lab water and diluted by a factor of ten. Two measurements were used to calibrate molecular mass, whereas five measurements were used to test detector sensitivity.

Results were analyzed using customized software (ChromCALC, DOC-LABOR, Karlsruhe, Germany). The LC-OCD initially determines the DOC concentration from the bypass peak. It then divides the DOC into hydrophobic and hydrophilic components. The hydrophilic portion is defined as the total sample that elutes from the column, whereas the hydrophobic portion is defined as the fraction that remains on the column. The hydrophilic portion is then further subdivided into five categories based upon retention time: biopolymers (BP), humic substances (HS; which includes both humic and fulvic acids), building blocks (BB), and low-molecular-weight neutrals (LMW-N) and acids (LMW-A). Boundaries of each fraction are determined relative to the elution of the humic substances peak. Biopolymers elute first and are thus the largest molecules. These are comprised up of polysaccharides, proteins, and aliphatic hydrocarbons (Grünheid et al., 2005; Lankes et al., 2009). Next to elute are humic substances, which are comprised of a heterogeneous mixture of large, complex molecules. The third fraction to elute is termed ‘building blocks’, which are essentially degraded humic substances with humic-like characteristics, but of lower molecular weight. The final two fractions to elute are the LMW acid and neutral fractions, which contain monoprotic acids, amino sugars, ketones, and aldehydes (Kennedy et al., 2005; Huber et al., 2011).

Additionally, the LC-OCD measures the SUVA and nominal average molecular weight of the HS. Plotting these parameters allows for the determination to whether it consists more of humic or fulvic acids. The reader is referred to Huber et al. (2011) for a more detailed account of the determination in HS characteristics. Briefly, as a result of the larger size of humic acids they elute earlier than fulvic acids, allowing for the calculation of molecular weight. Humics that are more aromatic and higher molecular weight are similar to allochthonous humics formed from pedogenic environments. This can be seen by the location of the IHSS-HA and IHSS-FA standards at the upper right and upper center of the HS-Diagram. Alternatively, lower weight and lower aromatic HS are more like autochthonous, microbial-derived humics. Between these end-points, a wide range of humic characteristics may exist, dependent upon the source of DOM. This differentiation

provides more of a continuum of humics, rather than an operational distinction between humic and fulvic acids.

During analysis, the large response between 20 and 40 minutes made it difficult to use the standard automated integration that was part of the instrumental software. Normally, a *Poisson* distribution is fitted to the steepest slope off the main humic substances (HS) peak, and integrated to obtain a concentration. However, the initial sample contained enough HMW that this main peak would encompass the biopolymer (BP) in the HS determination. In order to integrate a more realistic interval, a manual integration for OCD was completed at the boundary where humics would typically elute (>30 min; Figure 2.3). With the current column setup, fulvic and humic acids are known to peak between 43-45 minutes (Huber et al., 2011), indicating that the manual integration provides the best representation of the data. Since BP do not normally absorb UV, the integration was not manually altered for UV.

## **2.3 RESULTS**

### **2.3.1 LEACHATE COMPOSITION AND DEGRADATION**

Over 150 days (before nutrient addition), DOC concentration decreased by 11.6 mg/L (or 44% of the initial concentration) (Figure 2.4a). In the initial leachate, the majority of leached DOM to consist of HS ( $16.3 \pm 0.9$  mg/L; Figure 2.4b), with the remaining portion consisting of LMW-neutrals (LMW-N;  $3.8 \pm 0.1$  mg/L), building blocks (BB;  $2.5 \pm 0.1$  mg/L), hydrophobics (HPho;  $1.6 \pm 1.0$  mg/L), and BP ( $1.4 \pm 0.2$  mg/L), along with a small amount of LMW-acids (LMW-A;  $1.0 \pm 0.1$  mg/L).

The majority of changes occurred within the first 22 days, with the concentration of DOC decreasing by 6.3 mg/L. The proportion and concentration of BP and LMW-N both decreased, while LMW-A disappeared within the first week (Figure 2.4c). Alternatively, while concentrations of HS decreased, its proportion increased to an asymptotic value of 75% of the total DOM. Both HPho and BB show little change throughout the experiment. On day 8, a notable decrease in the concentration of HS was observed with a concomitant sharp increase in HPho. After the first two time points, a film-like substance was observed

within the bottles that remained after stirring. In addition, the concentration of BP increased by approximately 0.4 mg/L (2.3% of the overall DOM) between 62 and 152 days.

Humic substance characteristics did not exhibit much change as a result of microbial degradation (Figure 2.5). Initially, leached HS was comprised of moderate weight (727 g/mol), aromatic structures (5.84 L/(mg·m)). During the incubation, both the aromaticity and molecular weight increased only slightly. Comparison of HS-SUVA to DOC SUVA was a linear relationship ( $r^2=0.65$ ,  $n=7$ ) but the slope was much smaller than a 1:1 line (slope= 0.33) indicating a larger proportion of LMW aromatic compounds present.

### **2.3.2 ADDITIONAL LEACHATE PARAMETERS**

The leaf leachate was acidic ( $\text{pH}<5$ ) and well oxygenated ( $\text{DO}>7.90$  mg/L) throughout the incubation (Figure 2.6). Inorganic nitrogen was not found in any considerable concentration on any sampling date, thus the majority of TDN could be considered as organic nitrogen (Figure 2.7). Although the LC-OCD measured DON, the response from the samples was too low to integrate and could not be used in this study. High molar ratios of DOC:DON illustrate soluble components were low in nitrogen (Figure 2.8). The ratio illustrated sharp decrease (from 96 to 56), followed by an increase to 85 after 62 days, and a final ratio of 51 at 150 days. Ratios were not calculated after the addition of nutrients due to the high concentration of inorganic nitrogen species.

The majority of change to spectral-based measurements occurred within the first 22 days (Figure 2.9). The  $E_2:E_3$  ratio decreased over the incubation, which has been interpreted to indicate an increasing total molecular weight. Conversely, the  $E_4:E_6$  ratio increased between 8 and 62 days, after which the ratio decreased. In addition, SUVA increased over the length of the experiment, illustrating either a preferential loss of non-absorbing components or increasing aromaticity of the remaining organic compounds.

### **2.3.3 ADDITION OF NUTRIENTS**

The addition of nutrients increased the concentration of DOC by 2 mg/L (outside of analytical error indicating a notable increase), but did little to further degrade the DOM. Although slight decreases in the proportions of HPho (-2.5%) and BP (-1.7%) and an

increase in HS (2.8%) were observed, other components did not vary in proportion. No net difference was found between the HS molecular weight before and after the addition of nutrients. Furthermore, the overall pH of the solution increased towards neutral, yet DO and inorganic nitrogen (excluding nitrate) did not show any observable differences. Both ratios ( $E_2:E_3$  and  $E_4:E_6$ ) decreased by 0.2 and 2.0 respectively, whereas SUVA and HS-aromaticity were found to increase by 0.4 and 0.2 L/(mg·m), respectively.

## **2.4 DISCUSSION**

### **2.4.1 CHARACTERISTICS OF SOLUBLE LEAF LITTER**

The results of this study provide insight into the composition of soluble organic matter. Many of the parameters showed that the leached DOM had similar characteristics to previous studies. The proportion of HS (~60%) and DOC:DON (~100) within DOM was similar to leachate from a variety of litter species (Qualls et al., 1991; Cleveland et al., 2004). Furthermore, low DOC:DON ratios indicate that soluble organic matter from litter is poor in organic nitrogen, possibly requiring an inorganic source of nitrogen to aid with further humification or degradation (Qualls et al., 1991). Hur et al. (2009) observed a low aromaticity in DOC from freshly degraded leaf litter, indicating a composition of non-aromatic compounds. Contrary to those results, in this study the  $E_4:E_6$  ratio and SUVA, along with the LC-OCD composition, show that soluble components from fresh litter consisted mainly of high molecular weight (HMW), aromatic HS molecules.

Similar characteristics are seen when comparing leaf litter leachate to other environments. The measured  $E_2:E_3$  ratio and SUVA were similar to other studies measured from streams and humic lakes (Dahlén et al., 1996; Peuravuori & Pihlaja, 1997; Ågren et al., 2008), indicating leached DOM contained a similar bulk weight to that of surface water environments. Although leached DOM from this study does not contain as much LMW compounds as previous leachate studies, it is representative of DOM that can be found within the environment.

The LC-OCD provided a fast method for characterizing DOM from leached litter. Sachse et al. (2001) found high concentrations of BP to be a result of autochthonous

formation of DOM. Interestingly, the BP fraction at the start of this study was lower than other LC-OCD studies of DOM (Fischer et al., 2002; Rauch & Drewes, 2005; Neale et al., 2011). Although, the chromatogram had a very wide HS peak not observed in other LC-OCD studies, comparison with SUVA and  $E_4:E_6$  values suggest the majority of the broad peak are HMW HS. This further illustrates that BP may appear as a product of microbial production rather than from physical leaching of large molecules, explaining why the original leachate did not contain a high concentration of BP. Interestingly, other measured parameters did not distinguish anything unique, illustrating the sensitivity of the LC-OCD for distinguishing defining characteristics.

#### **2.4.2 CHANGES DUE TO MICROBIAL DEGRADATION**

The change in concentration of DOC gives a direct measurement of biodegradability, which can be paired with changes to the lability of DOC. A high DOC:DON ratio suggests the DOM is poor in nitrogen, which has been found to be linked with low bioavailability and bacterial growth efficiency (Kroer, 1993; Hunt et al., 2000; Fellman et al., 2008). In addition, oxic conditions have been found to maintain the stability of non-biodegradable components (Grünheid et al., 2005). Leaching of fresh litter did not result in a composition susceptible to rapid degradation. Instead, only 44% was lost over the course of the experiment, with only 24% rapidly degraded in 22 days. After the addition of nutrients, no subsequent degradation was observed, illustrating a relatively non-labile composition. Regardless of the amount of DOM lost, results indicated that labile DOM was assimilated as a result of microbial degradation.

As indicated by the increasing SUVA, the decomposition of DOM leaves behind a more aromatic and a lower quality (less labile) form (Kalbitz et al., 2003; Ågren et al., 2008). This can also be observed from the LC-OCD data since analysis revealed different DOM compositions at the beginning and end of the experiment. From this, labile components can be considered as those that decreased relatively quickly. Since proportions of BP, LMW-N, and LMW-A decreased within the first 22 days, it can be concluded that microbes preferentially consume these components. The BP fraction is known to encompass polysaccharides and proteins, which are both considered labile compounds (Fischer et al.,

2002; Grünheid et al., 2005). Rauch and Drewes (2005) found the degradation of simple sugars led towards formation of HMW polysaccharides. The decrease of LMW-N may account for the slight increase in BP over time. Furthermore, concentrations of each component are found to decrease, likely resulting from the loss of DOM into respired CO<sub>2</sub>. Overall, these results indicate that simpler compounds were utilized by microbes and may even allow for the formation of larger, biosynthesized molecules.

The final composition likely represents recalcitrant DOM, characterized by high proportions of HS and a small amount of LMW molecules. This is similar to an incubation study by Fischer et al. (2002), who observed a narrower spectrum of organic components after degradation of river DOC through sediment cores, consisting mainly of HS. Studies have found non-humic components to be more bioavailable than humics, leading towards an increase of humic-like components (Tranvik, 1993; Sun et al., 1997; Hunt & Ohno, 2007; Hur et al., 2009). Conversely, Cleveland et al. (2004) believed that lack of soil mechanisms (such as preferential adsorption of HS) within an incubation experiment allowed for labile humics to remain in solution, while Hunt et al. (2000) observed the uptake of HS by microbes. The chromatograms from the LC-OCD support the former, where the composition becomes dominantly HS (Figure 2.10). Thus, it could be said over time, the increase in the proportion of HS (and relative constant BB proportion) indicates that humics may not be rapidly degraded by microbes. Interestingly, although DOC continues to decrease, the proportion of HS remains relatively stable, stemming from the decline of HS at a similar rate to that of DOC. These observations lead to the idea that DOC undergoes a rapid loss of labile substrates, followed by a longer degradation of recalcitrant compounds; in this case HS. Furthermore, since HS characteristics (molecular weight and aromaticity) remained relatively consistent, it is likely that HS were generally not altered into other forms during microbial degradation.

A film-like substance was found inside the bottles and is most likely the result of the growth of microbial biomass, further contributing to the decline in DOC concentration (Lush & Hynes, 1973; Paerl, 1978; Mulholland, 1981). This can be further supported by the increase in BP, which likely consists of structurally similar soluble microbial products

(Rauch & Drewes, 2005). Although not characterized, the growth of biofilm with abundant bacteria likely sustains continual degradation of DOM.

### **2.4.3 COMPARING THE LC-OCD WITH OTHER MEASURED PARAMETERS**

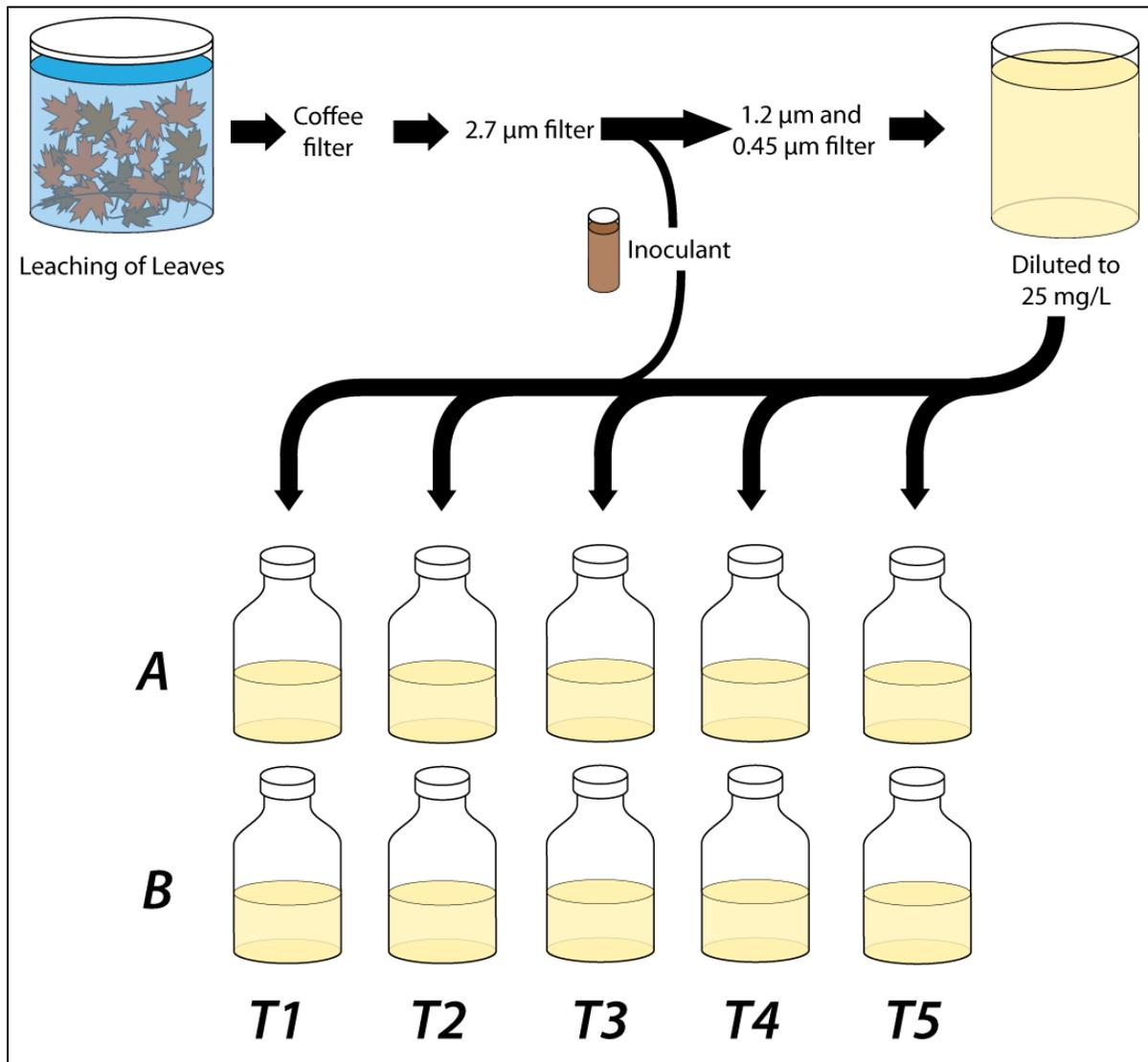
Overall, the LC-OCD provided valuable information on the compositional changes during microbial degradation. Importantly, it allowed for the observation that an overall loss in DOC concentration is the result of the preferential loss of certain components over others. Changes to the composition were supported by both LC-OCD analysis and spectral information. Unfortunately, the LC-OCD was unable to accurately measure the amount of organic nitrogen, which is known to be linked with the biodegradability of both HS and DOC overall (Hunt et al., 2000; Cleveland et al., 2004). However, calculation of DON proved difficult using wet chemistry, due to the low concentrations found in leached litter.

Spectral data compared well with data supplied by the LC-OCD. For instance, the decreasing  $E_2:E_3$  ratio is indicative of a loss in LMW compounds (Dahlén et al., 1996), which supports the compositional changes measured from the LC-OCD. Independent measures of the molecular weight of DOM also indicated a composition comprised mainly of HMW aromatic molecules, which LC-OCD analysis determined to be HS. Conversely,  $E_4:E_6$  ratios did not agree with data from the LC-OCD. The  $E_4:E_6$  ratios from this study were characteristic of fulvic acids, rather than humic acids (Chen et al., 1977), which is not supported by the HS molecular weight and HS SUVA (Huber et al., 2011). Furthermore, Peuravuori and Pihlaja (1997) and Osborne et al. (2007) did not find  $E_4:E_6$  to be a useful parameter in characterizing DOM, which is in agreement with the results from this study. Finally, comparison of bulk SUVA and HS-SUVA illustrate that degradation of DOM caused a decrease in the HS SUVA, but formed LMW aromatic components. These results show that LC-OCD analysis is comparable to other DOM characterization methods.

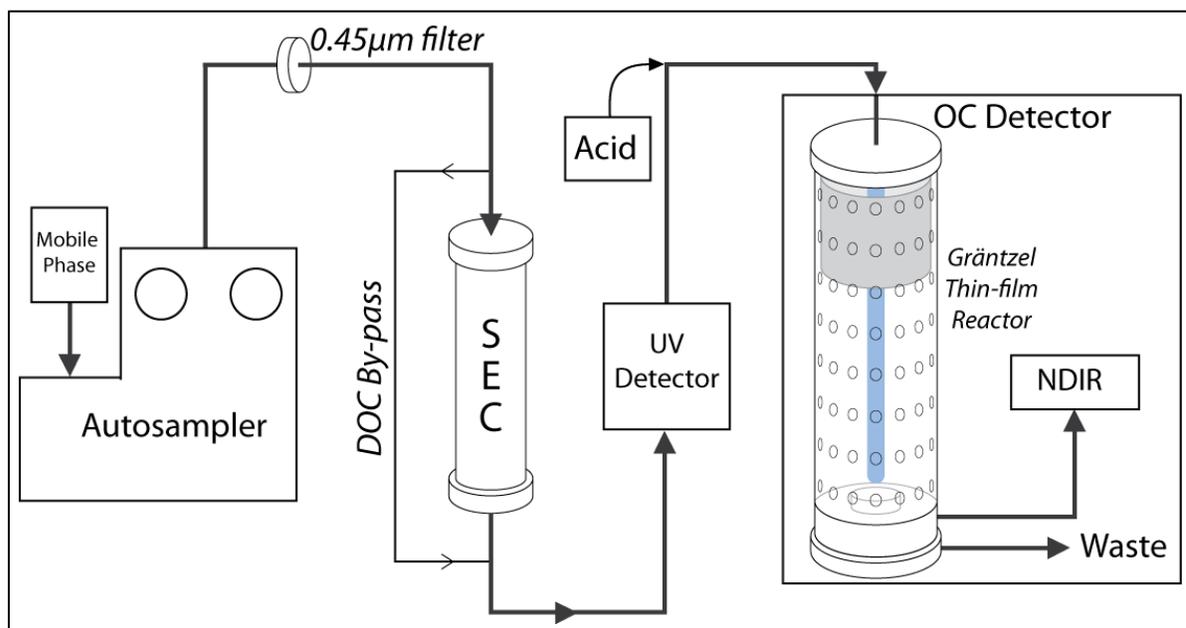
## **2.5 CONCLUSION**

The observations from this study further the understanding of changes to DOM as a result of microbial degradation. From this experiment, soluble components of leaf litter were found to consist of HMW, nitrogen poor, aromatic organic components. However, the LC-

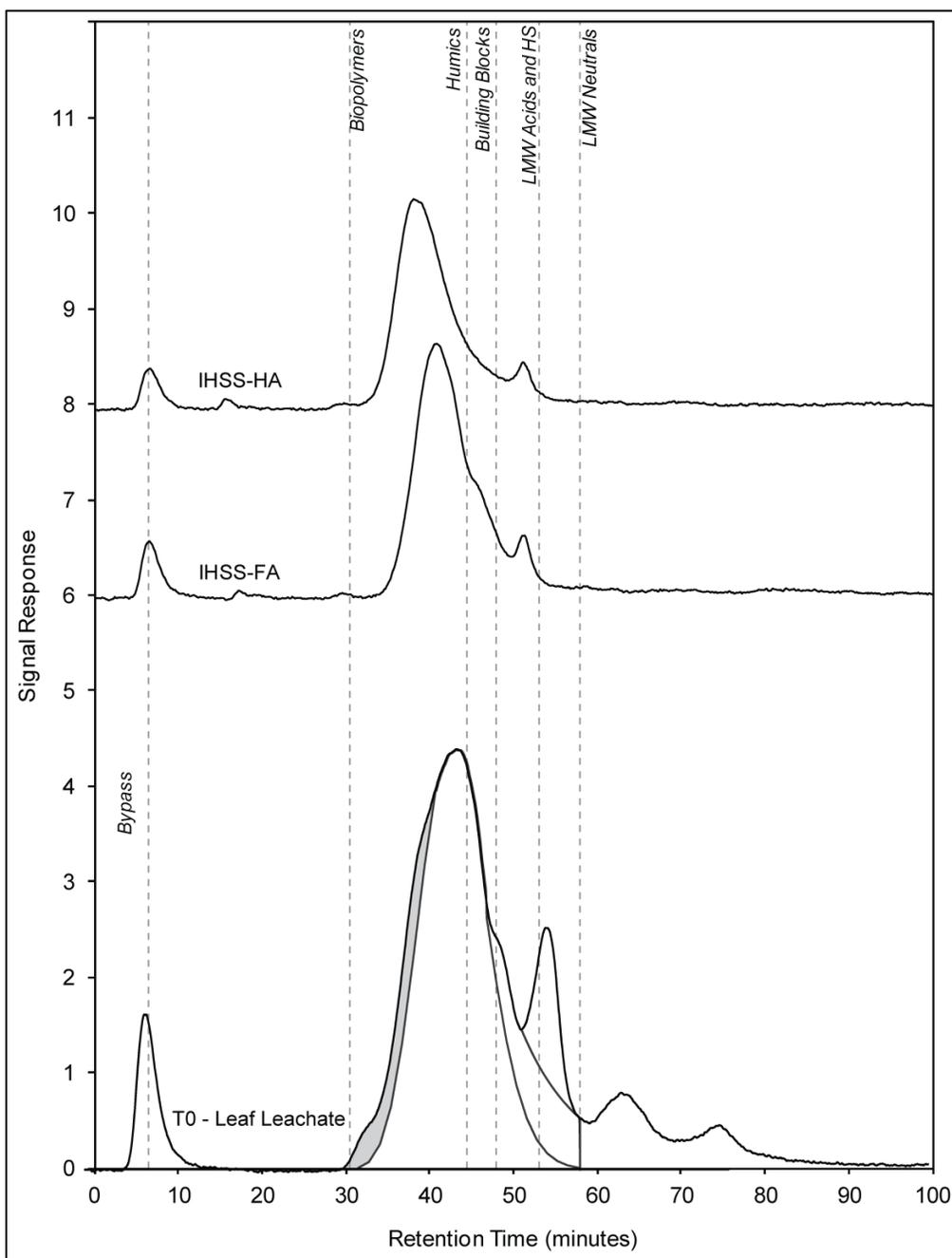
OCD was able to identify a group of HMW molecules that contained both BP and HS characteristics, not previously observed in other LC-OCD studies. The decrease in DOC concentration provided a direct measurement of biodegradation, illustrating that only 44% of the leached DOC could be degraded over the course of the experiment, with only 24% rapidly degraded in 22 days. Components rapidly utilized were LMW molecules, while humic-like components (HS, BB, and HPho) either remained proportionally similar or became enriched within the DOM. Furthermore, degradation of DOM formed more aromatic LMW molecules. Although HS remained proportionally constant at later times, its concentration decreased concomitantly with the decrease in DOC, illustrating a pool of slower biodegradable carbon. Importantly, most methods were able to support observations obtained from the LC-OCD, while  $E_4:E_6$  ratio was found to be not as useful for characterizing DOM. Although both spectral and LC-OCD analysis gave a comprehensive look into the change of quality during microbial degradation of DOM, LC-OCD provides information on all fractions within DOM and not only the UV-absorbing components. These results give an indication of components that comprise freshly soluble organic matter, as well as the microbial utilization of certain fractions, which ultimately defines the character of DOM within the environment.



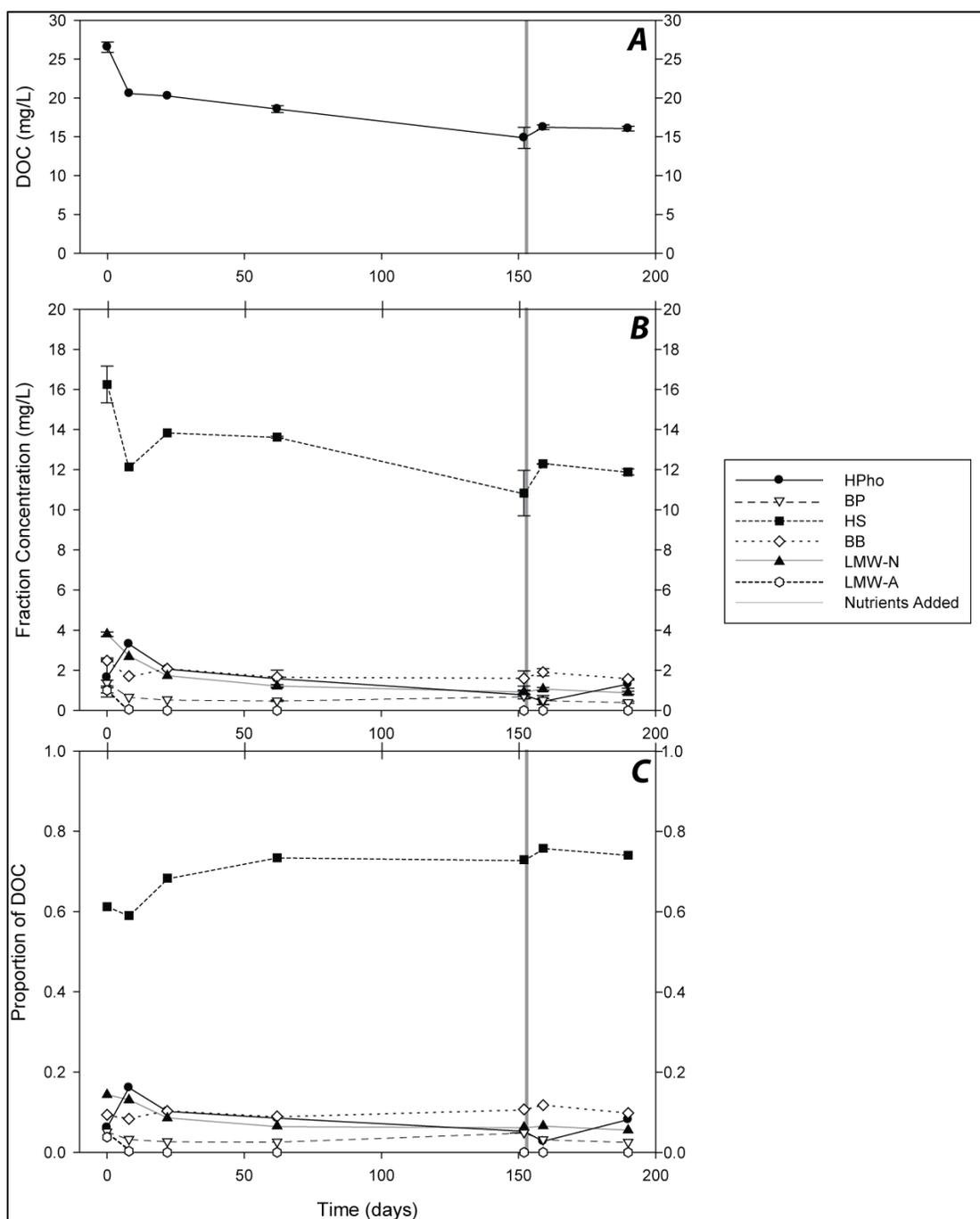
**Figure 2.1:** A visual representation of the experimental setup for leaching leaf litter, filtering, dilution to 25 mg/L, and separating aliquots into sacrificial bottles. In addition, the schematic illustrates when the inoculant was taken.



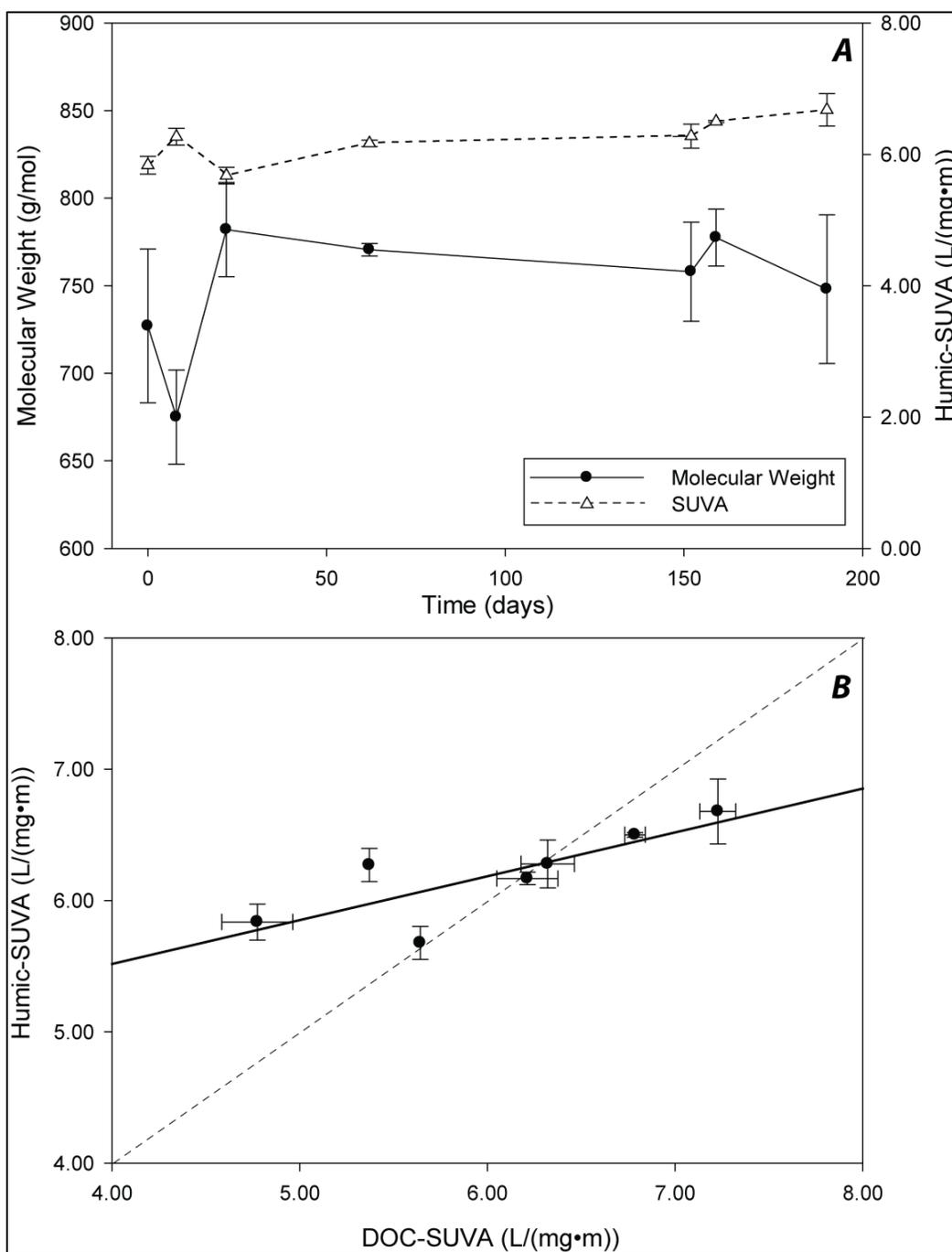
**Figure 2.2:** Schematic of the LC-OCD. The DOC by-pass allows for measurement of the total DOC concentration and SUVA by skipping the size-exclusion chromatography (SEC) column. The UV-Detector is run at 254nm. Adapted from Huber et al. (2011).



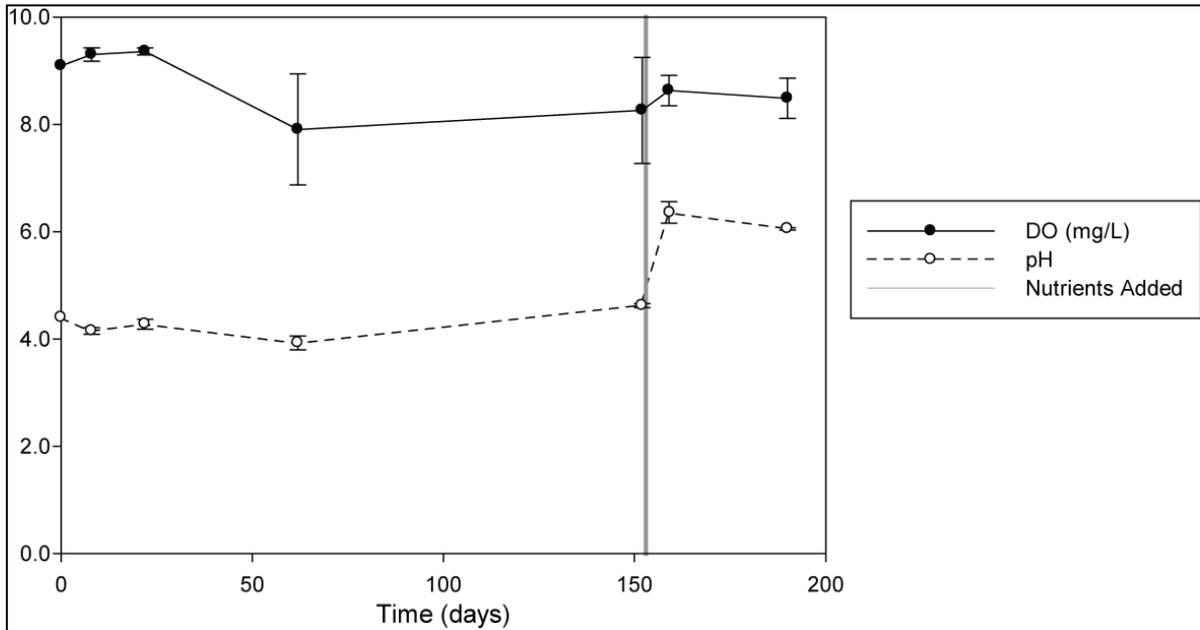
**Figure 2.3:** Manual integration of the HS left slope. Dotted lines represent the elution time of different organic fractions. The shaded area depicts the HMW molecules between the BP and HS peak. The above two chromatograms are International Humic Substances Society (IHSS) humic (-HA) and fulvic (-FA) acid standards. Although the HS begin to elute at the time of IHSS-HA, the peak is closer to the IHSS-FA, indicating the molecular weight of the humics are closer to the fulvic acids.



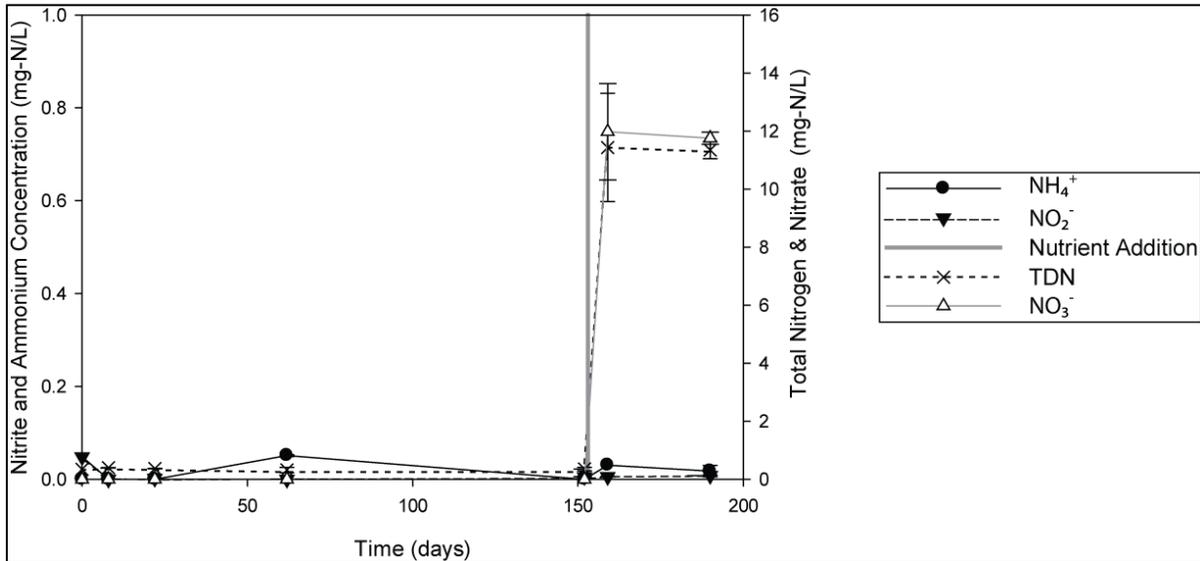
**Figure 2.4:** Results from the LC-OCD analysis (with standard deviations from analysis on duplicate bottles). **A:** change in DOC concentration with time. **B:** changes to hydrophobics (HPho; closed circle), biopolymers (BP; open triangle), humic substances (HS; closed square), building blocks (BB; open diamond), low molecular weight neutrals (LMW-N; closed triangle) and acids (LMW-A; open circle). **C:** changes to the proportion of these components. The solid grey line at 152 represents when additional nutrients were added.



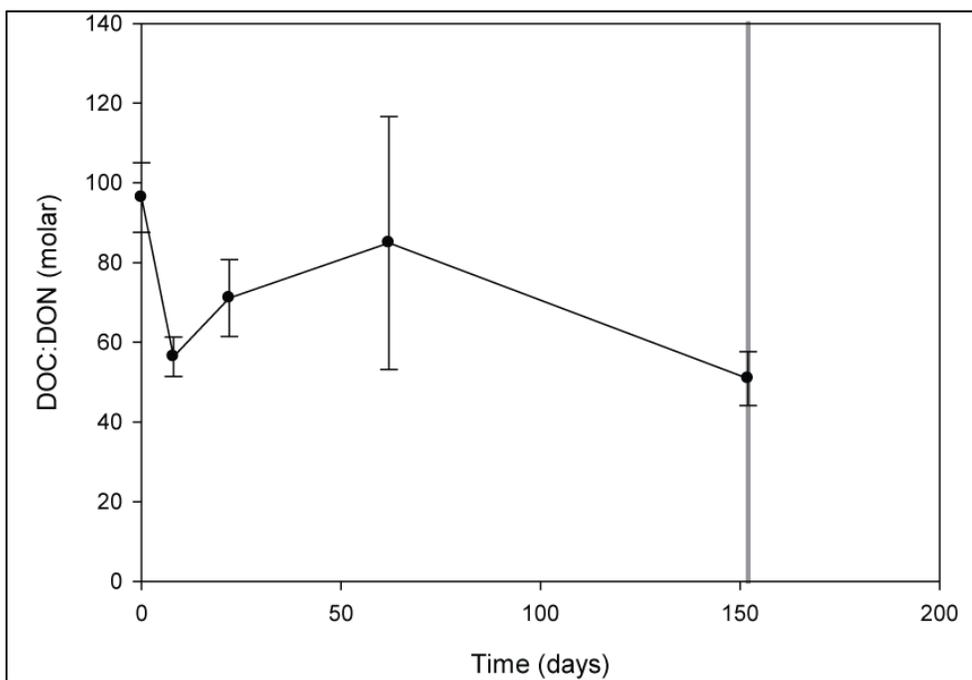
**Figure 2.5:** HS characteristics determined by LC-OCD analysis. **A:** HS molecular weight and aromaticity over time with standard deviations determined from duplicates. Duplicates were not run on days 8 and 22, thus the average of all standard deviations was used for those time points. **B:** HS-SUVA versus overall DOC SUVA with standard deviations (determined from duplicates). In graph B, a 1:1 line is represented by the dotted grey line while a regression line is fitted with the solid line ( $R^2=0.65$ , slope=0.33).



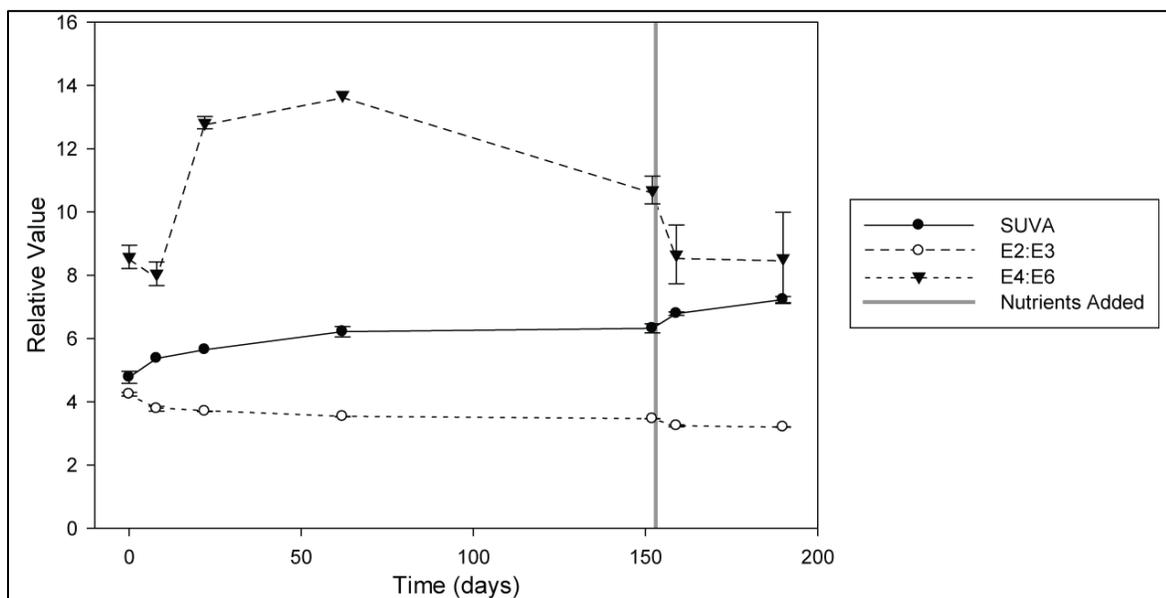
**Figure 2.6:** Dissolved oxygen (DO, mg/L) and pH in the leachate solution over time with standard deviations. The solid gray line at 152 days represents when nutrients were added.



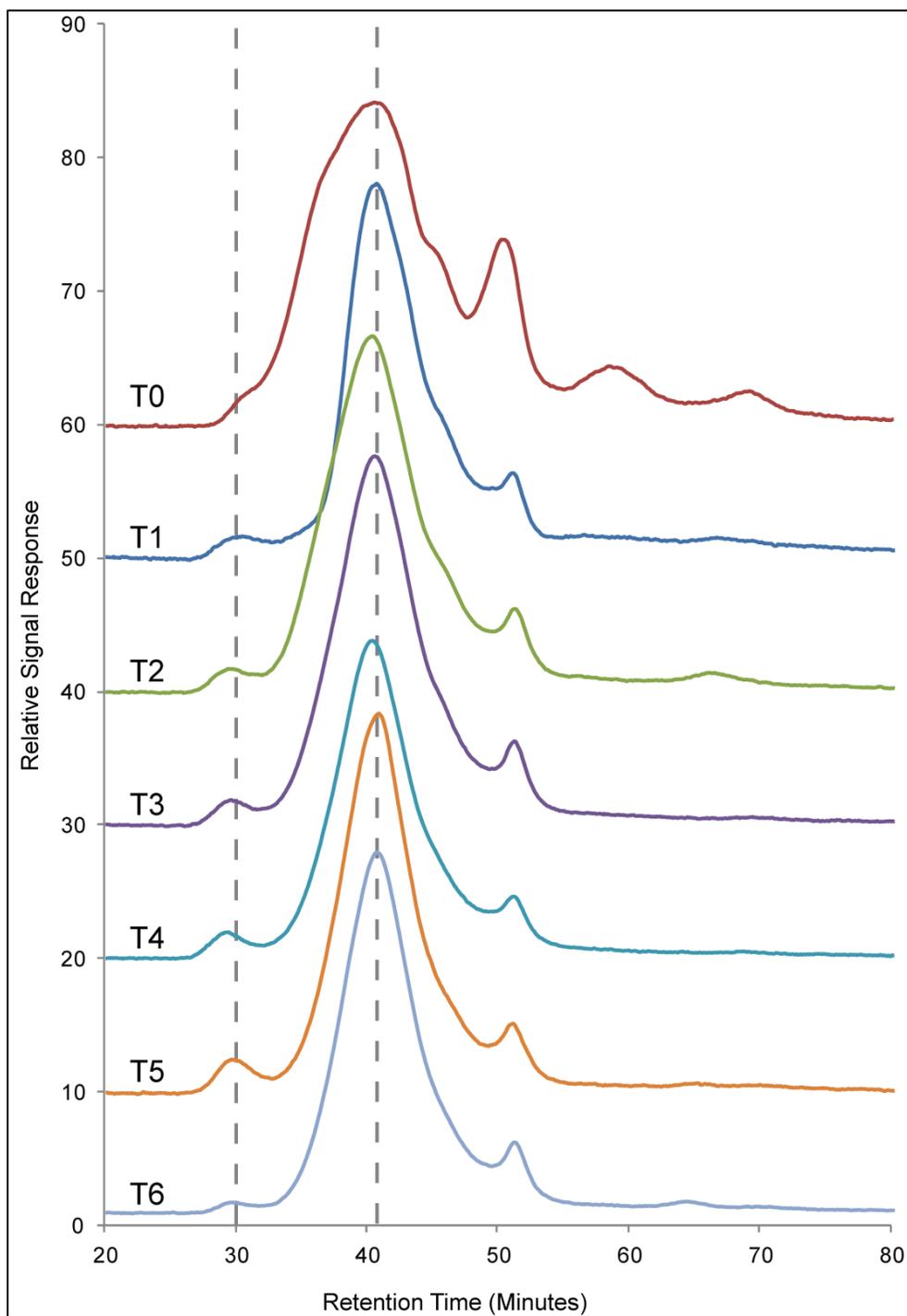
**Figure 2.7:** Measured ammonium ( $\text{NH}_4^+$ ; closed circle) and nitrite ( $\text{NO}_2^-$ ; closed triangle) on the left axis (mg-N/L), as well as nitrate ( $\text{NO}_3^-$ ; open triangle) and total nitrogen (TN; cross) on the right axis (mg-N/L) over the length of the experiment with standard deviations. The grey line at 152 days represents when nutrients were added. It can be seen by the higher  $\text{NO}_3^-$  than TN concentration at 159 and 190 days that calculation of TN from wet chemistry did not provide the best method for calculating dissolved organic nitrogen (DON).



**Figure 2.8:** Calculated molar ratio of DOC:DON (with standard deviations calculated from duplicates) over time. The grey line represents when nutrients were added. No DOC:DON ratios were calculated after nutrient addition.



**Figure 2.9:** The ratios of absorbance at 255nm and 365nm (E2:E3; open circle) and 465nm and 665nm (E4:E6; closed triangle) are illustrated over time (days) with standard deviations. Measured SUVA (L/(mg·m); closed circle) is also plotted against time. The solid grey line at 152 days represents when nutrients were added.



**Figure 2.10:** Changes to the DOM composition as illustrated by the chromatograms for each time point. Chromatograms run from top to bottom: 0 days, 8 days, 22 days, 62 days, 152 days, 159 days, and 190 days. Nutrients were added in-between T4 and T5. Dotted lines represent the elution peak for BP (~30 minutes) and HS (~42 minutes).

## CHAPTER 3

### EVOLUTION IN THE QUALITY OF SEPTIC-DERIVED DOM

#### 3.1 INTRODUCTION

Dissolved organic matter (DOM) is a heterogeneous and ubiquitous component of the aquatic ecosystem. Derived from the degradation of organic materials (plant, animal, or microbial origin), natural DOM exists in a variety of compositions and concentrations. Dissolved organic carbon (DOC) is used as a quantitative measure of DOM, which is often operationally defined as the organic molecules that can pass through a 0.45 $\mu$ m filter. Typically, groundwaters contain concentrations less than 5 mg/L, but it is not unreasonable to find concentrations higher than 10 mg/L (Thurman, 1985; Wassenaar et al., 1989; Lapworth et al., 2008). However, groundwaters impacted by septic system effluent can contain much higher concentrations. For instance, DOC concentrations greater than 50 mg/L were measured in a septic tank, while groundwater impacted by the septic-system ranged between 4 – 14 mg/L (Robertson et al., 2012). Although differences in concentrations are easily measured, it is more difficult to determine changes in the lability of DOC. A septic plume hypothetically should provide a wide spectrum of DOC qualities from freshly degraded, labile components originating close to the septic tank to more recalcitrant forms further along the flow path.

##### 3.1.1 SEPTIC TANKS AND GROUNDWATER

Septic tanks are known to be a point-source for groundwater contamination, contributing pathogens, nutrients, and pharmaceuticals to groundwater (Robertson & Cherry, 1992; Harman et al., 1996; Wilhelm et al., 1996; Carrara et al., 2008). The addition of these compounds to groundwater is of concern due to the possibility of contamination to nearby wells. Approximately 87% of households not connected to a municipal water source use a private septic system (Statistics Canada, 2009). With such a high percentage, there is a potential risk of contaminating drinking water.

Septic tank effluent can alter the quality of groundwater in several ways. One case study described a Norovirus outbreak as a consequence of a leaking septic system contaminating down-gradient drinking water wells (Borchardt et al., 2011). Furthermore, septic tanks may contribute to a decreasing pH, which becomes important for the mobility of metals (Robertson & Blowes, 1995). High loading of organic matter also contributes to the mobilization of heavy metals (Christensen et al., 1996; Christensen & Christensen, 1999). More commonly, loading of nitrogen-rich species into groundwater is of great concern. Nitrate, a ubiquitous groundwater contaminant, is formed above the water table through the oxidation of ammonium, and is often found in concentrations higher than the drinking water limit of 10 mg-N/L (Robertson & Cherry, 1992). One way to mitigate groundwater nitrate contamination is through the process of denitrification, where labile DOC can be used by heterotrophic bacteria to convert nitrate into inert nitrogenous gases (such as N<sub>2</sub> or N<sub>2</sub>O). In groundwaters with high amounts of nitrate, denitrification is generally dependent upon the availability of both labile organic carbon and pyrite in older sediments (Aravena & Robertson, 1998; Fenton et al., 2011). Such a parameter cannot only be defined by a concentration since different carbon substrates were found to lead to differing rates of denitrification (Greenan et al., 2006). Thus, the lability (quality) needs to be understood in order to predict such attenuation rates.

### **3.1.2 LABILE CARBON ENVIRONMENTS**

Labile organic carbon can be considered as compounds that are readily lost or altered. Studies have shown that environments of freshly degraded organic matter (like decaying leaf litter on forest floor) provides a source of labile carbon that can be distributed throughout the watershed via leaching, streams, or groundwater transport (Thurman, 1985; Amon & Benner, 1996; Schiff et al., 1997b; Schlieff & Mutz, 2007). The continual processing of marine DOC by micro-organisms has been found to decrease its bioavailability, resulting in a more recalcitrant form (Ogawa et al., 2001). A similar situation may also be found within aquifers, where relatively high residence times of groundwater potentially allow for substantial degradation by microbes within the subsurface. However, increased loading of organic matter into the subsurface (such as from septic systems) may allow for highly labile

DOC to be transported within the aquifer. It is apparent there may be a wide range of DOC qualities present in groundwaters, thus a method to characterize the quality of DOC is needed.

### **3.1.3 QUALITY OF DISSOLVED ORGANIC CARBON**

Different terms can be used to describe the quality of DOC, dependent upon the changes that may occur to the DOC. For instance, the ability for DOC to complex with surrounding dissolved metals can be referred to as its 'reactivity', whereas the ease for microbial degradation can be referred to as its 'biological availability'. Differences in terminology may result from the fact there is no generally accepted standard method to determine the biodegradability of DOC (Marschner & Kalbitz, 2003). Since the term 'DOC' encompasses a wide variety of organic molecules, no one molecule is responsible for the overall quality. Hence, different groups of molecules may be responsible for interacting either biologically, chemically, or physically with the surrounding environment.

Constituents of DOC consist of thousands of different molecules, thus grouping components of DOC based on their structural or chemical characteristics is an efficient way of characterizing DOM. The DOC pool can be sub-divided into different groups depending upon the characterization method. Resin fractionation (such as XAD columns or size-exclusion chromatography) allows for the determination of different components based upon size or reactivity of the molecules. For example, determination of the composition using XAD resins found DOC to consist of hydrophobic and hydrophilic acids, termed humic and fulvic acids, respectively (Leenheer, 1981; Bourbonniere, 1989). Bourbonniere (1989) further classified fulvic acids into components based upon their physico-chemical characteristics. Ultraviolet (UV) absorption can be used as an index of the amount of aromatic components with DOC (through specific UV-absorbance at 255nm) or the relative contribution of high and low molecular weight compounds (through absorbance ratios such as  $E_2:E_3$ ). The division of DOC into certain fractions allows for a more efficient method to understand changes or alterations to DOC. Furthermore, such a classification allows for the determination of components that comprise high-quality (labile) or low quality (non-labile) components, instead of using an overall difference in DOC concentration.

Different components of DOC can react differently within the subsurface. Humic and fulvic acids (generally considered to be high molecular weight (HMW) molecules), can form complexes with heavy metals (Christensen & Christensen, 1999). Furthermore, it was found that the extracellular polymeric substances ('housing' structures for microbes) can absorb heavy metals (Liu et al., 2001). Studies have found polysaccharides and low molecular weight (LMW) molecules to not only be preferential for biological demand, but also increase the bacterial growth efficiency (Amon & Benner, 1996; Gardner et al., 1996; Fischer et al., 2002). Another study hypothesized that biodegradability of DOC is controlled by the accessibility of chemical functional groups rather than molecular size (Fischer et al., 2002). Specifically, the ratio of DOC to dissolved organic nitrogen (DON) can be used to predict its lability, with low ratios indicating a higher lability (Hunt et al., 2000; Fellman et al., 2008). Overall, the reworking of DOC by bacteria has been found to result in a refractory product (Ogawa et al., 2001). In summary, it appears that biological degradation may occur to all dissolved fractions, whereas complexing with metals is driven by the larger molecular weight fractions (such as humic substances or biopolymers).

For the purpose of this study, high quality DOC will be defined as the ability to interact with its environment physically, chemically, and biologically. High quality DOC will consist of components rapidly lost (by either microbial or sorption processes), while low quality will refer to components that remain over time. Characterization of DOC using a Liquid Chromatography – Organic Carbon Detection (LC-OCD; a size-exclusion chromatography based method) identifies concentrations of different organic fractions based upon its molecular mass distribution. Wastewater has been found to consist mainly of colloids, polysaccharides, and LMW compounds (Brinkmann et al., 2004). Literature has identified labile components to consist of polysaccharides, acidic functional groups, and low weight compounds (Amon & Benner, 1994; Brinkmann et al., 2004), which correspond to the LC-OCD fractions of biopolymers, LMW-acids, and LMW-neutrals, respectively, in Huber et al. (2011). Degradation of DOC has been found to result in a narrower range of compounds, consisting mainly of humics (Fischer et al., 2002; Hunt et al., 2007; Hur, 2011).

These studies lead to the idea that it may be possible to observe a change in quality of DOC through a change in the distribution of different DOC fractions.

### **3.1.4 RESEARCH OBJECTIVES**

Characterization of DOC has previously been considered difficult and laborious. However, the LC-OCD allows for a rapid assay of DOC size based on molecular weight. The key objective of this study will be to gain a better understanding of the components that correspond to labile, high quality DOC. By focusing upon an extensively studied septic plume in Long Point, Ontario, it will be possible to pair DOC changes to the processes found within the aquifer (Robertson & Cherry, 1992; Robertson & Cherry, 1995; Aravena & Robertson, 1998, Robertson et al., 2012). I hypothesize that DOC located closest to the septic tile consists of high quality components due to high concentrations and rate of loading, allowing only minimal degradation of labile components that reach the water table. This DOC should consist primarily of biopolymers and LMW-neutrals as a result of the freshly decomposed labile carbon from the tank (Neale et al., 2011). As the plume ages, DOC quality will steadily change towards a recalcitrant form (consisting of humic substances) due to physical, chemical, and biological influences. This will be accomplished through three objectives. The first objective will be to see if LC-OCD analysis and changes to SUVA are able to determine changes to the quality as DOC ages. This will be completed by comparing LC-OCD analysis and SUVA values over the length of the plume. The second objective will be to see whether changes to the composition or SUVA relate to microbial or sorption processes as described within literature. Finally, the third objective will be to see if septic system DOC differs from other groundwater DOC.

### **3.2 SITE DESCRIPTION**

The Long Point Provincial Park campground on the northern shore of Lake Erie is approximately 83km south-east of London, Ontario (Figure 3.1). The campground has 256 campsites and is open seasonally between the months of May to October. The campsite offers a comfort station, which is connected to two septic system tile beds (approximately 290m<sup>2</sup>). These tile beds are made of perforated PVC pipe (10cm diameter) approximately 2m

above the water table (Robertson & Cherry, 1992). The tile bed used in this study (Tile Bed 2) has been used since 1990 except for a 2-year period (1995-1996) when flow was diverted to another tile bed (Robertson, 2008).

Physical hydrogeology and instrumentation at the Long Point site has been previously characterized by Robertson & Cherry (1992), and is briefly summarized here. The septic plume is found in a relatively homogenous unconfined sand aquifer (5 to 6 m thick,  $K=2 \times 10^{-2}$  cm/s), underlain sharply by a silt aquitard. The groundwater has an estimated horizontal velocity of 28 m/year near Tile Bed 2 (Robertson, 2008), has minimal dispersion, and flows southward towards Lake Erie. Bromide tracer tests showed that the effluent spends one to two days in the septic tank, followed by approximately seven days during heavy-use periods when the effluent percolates through the 2 m-thick unsaturated zone. Furthermore, the increased loading rate below the tile bed causes the ground water table to mound directly beneath the tiles.

The septic plume can be easily distinguished within the calcareous sandy aquifer by its geochemical composition. Importantly, the effluent penetrates the entire thickness of the aquifer (Robertson et al., 2012). As septic effluent begins to load early in the season, it first blankets the shallow water table zone under the tile, and subsequently flows horizontally at the groundwater velocity (Robertson, 2008). The plume can be identified by high concentrations of chloride, nitrate, and phosphorus, as well as elevated electrical conductivity levels (Robertson & Cherry, 1992). In addition, the aquifer contains acid-extractable metals (specifically Al, Fe, and Mn), which may indicate the presence of hydroxides (Robertson, 2008). Decreasing concentrations of nitrate along the plume have been attributed to denitrification, anammox, and pyrite ( $\text{FeS}_2$ ) oxidation occurring within the plume (Aravena & Robertson, 1998; Robertson et al., 2012). The aquifer contains a high amount of solid organic carbon (organic carbon content (foc) of 0.15 wt %), which has been thought to be the carbon source for the denitrifying bacteria (Robertson & Cherry, 1992). DOC values have also been observed to decrease with depth, attributed to DOC oxidation (Robertson & Cherry, 1992). In addition, a high DOC (10 to 15 mg/L) and high  $\text{NH}_4^+$  (>30 mg-N/L) pulse has been observed in the plume, resulting from a period of heavy loading

from the septic tiles after the Victoria Day holiday (“May 24” long weekend) (Li, 2010; Robertson et al., 2012, in prep). Essentially, since nitrifiers were not active to oxidize the  $\text{NH}_4^+$  early in the season, it was able to pass through into the groundwater along with high concentrations of DOC (Li, 2010). Over the course of the summer, the  $\text{NH}_4^+$  is replaced by high concentrations of  $\text{NO}_3^-$  (>50 mg/L), but can still be distinguished by an elevated- $\text{NH}_4^+$  zone (~10 to 20 mg-N/L) (Robertson et al., in prep).

### **3.3 METHODS**

#### **3.3.1 FIELD METHODS**

Details of the installation of instrumentation can be found in earlier studies (Robertson & Cherry, 1992). Multi-level piezometers were purged for several minutes, using a peristaltic pump, until the electrical conductivity and pH reached a stable value. Electrical conductivity (EC), dissolved oxygen (DO), pH, and temperature were all measured using a Barnant 20 digital meter. A flow-through cell was used in order to inhibit contact with the atmosphere. In addition, samples were filtered inline using a 0.45 $\mu\text{m}$  Supor membrane filter (Pall Corporation).

Samples for ammonium, nitrite, total dissolved nitrogen (TDN) and anions (nitrate, chloride, sulphate) were collected in 30mL plastic containers, whereas DOC was collected in 40mL glass vials. Ammonium samples were immediately acidified with sulphuric acid ( $\text{H}_2\text{SO}_4$ ) to a pH of 3-4, while sodium hydroxide (NaOH) was added to nitrite samples to a pH of 10. Both DOC and anion samples were filtered but not preserved. After collection in the field, samples were immediately kept cool and in the dark until they were able to be stored in a cold room (<4°C). Analysis was completed within three days of sampling.

Gas samples were collected using 60mL glass serum bottles. Unfiltered groundwater was filled from the bottom of the bottle to overflowing to ensure no atmospheric contact with the sample. A serum rubber stopper and needle were used to cap the bottle, minimizing any headspace. Samples were then preserved with 0.2mL of mercuric chloride ( $\text{HgCl}_2$ ) in order to inhibit further microbial action.

### 3.3.2 LABORATORY METHODS

All chemical analysis was completed at the Environmental Geochemistry Laboratory at the University of Waterloo. Ammonium ( $\text{NH}_4^+$ ) and nitrite ( $\text{NO}_2^-$ ) concentrations were determined using colourimetric methods, using a Beckman DU<sup>®</sup> Series 500 Spectrophotometer.

Samples were analysed for chloride ( $\text{Cl}^-$ ), nitrate ( $\text{NO}_3^-$ ), and sulphate ( $\text{SO}_4^{2-}$ ) using a Dionex ICS-90 ion chromatograph at Environmental Geochemistry Laboratory (EGL) at the University of Waterloo. Calibration curves were created using Dionex brand standards.

TDN was measured using a Tekmar Dohrman Apollo 9000 High Temperature catalytic oxidation TOC analyzer with total nitrogen add-on equipped with an autosampler. Standards were prepared using potassium nitrate ( $\text{KNO}_3$ ).

Gas samples were analysed for  $\text{N}_2\text{O}$  via a headspace equilibrium technique and gas chromatograph (Thuss, 2008). Briefly, a pressurized headspace was created by injecting 10mL of helium while withdrawing 5mL of sample. Samples were then shaken for 90 minutes in order to allow for all the  $\text{N}_2\text{O}$  to equilibrate with the headspace. Concentrations were then determined using a Varian CP 3800 gas chromatograph and calculated according to Henry's Law.

A detailed description of the LC-OCD and analytical techniques can be found in Chapter II. Duplicates were run from six locations in order to obtain the precision for each fraction. Precision of the LC-OCD was less than  $\pm 0.09$  mg/L for all fractions, with HS SUVA and molecular weight at  $\pm 0.11$  L/(mg·m) and  $\pm 24$  g/mol, respectively. Overall DOC SUVA precision was  $\pm 0.17$  L/(mg·m).

In addition, concentrations of dissolved organic nitrogen (DON) were measured upon the BP and HS fractions. As the sample exits the UV-Detector, a small subsample is diverted to a helical UV-detector where organically and inorganically bound nitrogen is converted to nitrate and subsequently measured at 220nm. Based on duplicate analysis, the precision was determined to be  $\pm 6.4$   $\mu\text{g-N/L}$ .

DOC concentrations and a set of standards (created using potassium hydrogen phthalate) were also independently run on a using a Dohrman DC-190 High Temperature

Total Carbon analyzer. Here, samples and standards were acidified using 20% phosphoric acid and sparged to remove inorganic carbon. Samples were then injected, combusted at 680°C to convert DOC into CO<sub>2</sub>, and measured by a non-dispersive infrared (NDIR) spectrum. Samples were measured in triplicate, with the standard deviation being less than 3% of the overall concentration.

Ultraviolet absorbance was also independently measured using a Beckman DU<sup>®</sup> Series 500 Spectrophotometer with a 1cm path length. From this, Specific Ultra-violet Absorbance at 255nm (SUVA; L/mg·m) was calculated by:

$$SUVA = \frac{A/l}{C} \quad (1)$$

where  $A$  is the absorbance at 255nm,  $l$  is the path length of the quartz cuvette (m), and  $C$  is the DOC concentration (mg/L). In addition, Nanopure water was used as a blank to subtract background absorbance. Furthermore,  $E_2:E_3$  ratios were calculated by dividing the measured absorbance at 255nm by the absorbance at 365nm. It has been found that NO<sub>3</sub><sup>-</sup> may absorb UV at similar wavelengths as DOC (Edwards et al., 2001). However, Weishaar et al. (2003) determined it would take more than 100 mg/L of NO<sub>3</sub><sup>-</sup> to increase the absorbance at 254nm by 0.01. Since the aquifer does not contain NO<sub>3</sub><sup>-</sup> concentrations above 100mg/L, it can be said that there is no significant absorbance interfering with the overall DOC absorbance.

### 3.3.3 GROUNDWATER AGES

Groundwater ages in the proximal plume zone were calculated using two NaBr tracer tests and the seasonal breakthrough of elevated EC (Robertson et al., 2012). In this study, the same multi-level piezometers were used, allowing for a calculation based on the contours of the previous study. Specifically, the position of the measured port between two age contours was used to extrapolate the age of the groundwater at that location (Figure 3.2). Furthermore, the ages of certain piezometers in the distal plume zone (20 - 200m down gradient from the tile bed) were further supported with tritium-helium dating (Robertson et al., in press).

## 3.4 RESULTS

### 3.4.1 HYDROGEOLOGICAL AND GEOCHEMICAL PROPERTIES OF THE AQUIFER

Assumptions on the groundwater flow path need to be established in order to better understand how DOC is transported within the subsurface. First, it is assumed that groundwater flow directly under the tile bed is predominately vertical, whereas the rest of the aquifer has a dominant horizontal flow towards the south, both at relatively constant velocities. In addition, it is assumed there is no flow into or out of the till aquitard. Based on groundwater dating, the samples in this study had a wide range of different ages, ranging from 5 days to 11.5 years. However the majority of samples was from the proximal zone and found to be between 5-600 days.

Measured conductivity of the samples ranged from 453 to 1322  $\mu\text{S}/\text{cm}$ , while  $\text{Cl}^-$  ranged from 9.8 to 61.0 mg/L (Table 3.1). The sampling scheme may have included samples that were not directly in the effluent plume. In order to correct for this, samples within the plume were characterized as having an EC above 600  $\mu\text{S}/\text{cm}$  and  $\text{Cl}^-$  concentrations above 20 mg/L (Robertson et al., 2012) (Figure 3.3). This separates the data into two groups: non-plume samples ( $n=3$ ; average EC:  $519 \pm 58 \mu\text{S}/\text{cm}$ ; average  $\text{Cl}^-$ :  $11.8 \pm 2.1 \text{ mg}/\text{L}$ ) and plume samples ( $n=20$ ,  $1071 \pm 142 \mu\text{S}/\text{cm}$ ;  $\text{Cl}^-$ :  $46.3 \pm 9.0 \text{ mg}/\text{L}$ ). Non-plume samples can be found near the water table, approximately 10 meters from the septic tiles (Figure 3.4).

Non-plume samples have relatively low concentrations of DO ( $<2 \text{ mg}/\text{L}$ ) and  $\text{NO}_3^-$  ( $>0.1$  to  $14.2 \text{ mg-N}/\text{L}$ ) (Figure 3.5). Furthermore, non-plume samples contained low saturations of both  $\text{N}_2\text{O}$  ( $>312 \%$ ) and  $\text{CH}_4$  ( $>1.0 \times 10^3 \%$ ) (Figure 3.5). The concentration of DOC is relatively high (3.54 to 5.53 mg/L), and were generally higher than plume DOC.

Almost all plume samples also contained DO concentrations below 2 mg/L (Figure 3.5). Oxygenated groundwater was found in young groundwaters (5.66 and 6.09 mg/L), as well as in one sample of groundwater 83 days old (14 m away from the septic tiles, 5.35 mg/L). Nitrate was found only in high concentrations ( $>40 \text{ mg-N}/\text{L}$ ) in young groundwater, and followed two distinct trends towards lower concentrations with groundwater age. The first trend of  $\text{NO}_3^-$  can be seen between 5 to 182 days ( $-81.7 \text{ mg-N}/\text{L}$ ), while the second

trend is between 250 to 985 days (-52.8 mg-N/L) (Figure 3.5). A similar division is seen in CH<sub>4</sub> data, which increases by  $3.1 \times 10^4$  %-saturation between 5 and 182 days, as well as between 250 to 985 ( $+6.4 \times 10^3$  %-saturation). A high saturation of N<sub>2</sub>O is found among younger groundwaters ( $4.3 \times 10^5$  to  $1.2 \times 10^6$  %-saturation at 0 to 99 days), but then decreases with age. Concentrations of DOC within the plume are highest in young groundwaters (4.63 and 8.02 mg/L in 5 and 9 day old groundwaters, respectively), and decrease to 1.97 mg/L at groundwater aged 182 days (Figure 3.6). Similar to NO<sub>3</sub><sup>-</sup>, DOC illustrates a second decreasing trend in concentration between 250 and 985 days, with concentrations decreasing from 3.75 mg/L to 1.68 mg/L.

Nitrate and DOC concentrations both suggest that two distinct groundwater paths with different travel times are captured in the data. Robertson et al. (2012) found a strong vertical component directly under the septic tiles due to heavy sewage loading rates. This is seen in the data collected from this study in the decreasing DOC, SUVA, and NO<sub>3</sub><sup>-</sup> values within the first 200 days, occurring predominantly beneath the septic tiles. Secondly, higher NO<sub>3</sub><sup>-</sup> and DOC values further along the plume (starting at 250 days) steadily decrease with age, indicating a more horizontally-dominated flow path. The plume maps presented in Robertson et al. (in prep) illustrate the horizontal flow of plume tracers along the aquifer, illustrating the overall distribution of the plume. Seasonal changes in septic use may be responsible for this pattern, since the septic tank is inactive from October to May.

### **3.4.2 PLUME AND NON-PLUME CHARACTERISTICS**

The majority of non-plume DOC can be characterized as having a high proportion of HS ( $64.4 \pm 5.3\%$  of total DOC) (Figure 3.7). Two samples have different DOC concentrations (173 days: 5.40 mg/L; 181 days: 3.92 mg/L), but contain very similar compositions. With increasing groundwater age, the proportion of HS decreases from a maximum of 67.9% at 173 days to 58.3 % at 285 days, while BB increase from 15.6 % to 22.6 %. The proportion of LMW-N is relatively low ( $8.26 \pm 1.4$  %), with less than 1% of BP or LMW-A. HS SUVA from non-plume samples range from 2.85 to 3.77 L/(mg·m) (Figure 3.8), while the molecular weight of HS range from 575 to 630 g/mol (Figure 3.9).

Plume DOC within the vertical flow path area generally has a lower proportion of HS than non-plume groundwaters (Figure 3.10). One exception is found from 83 day old groundwater with the anomalous DO concentration, where HS comprise 70.6% of the total DOC. The rest of the DOC from this flow path is comprised mainly of HS (average:  $53.9 \pm 6.4$  %), with BB (average:  $20.6 \pm 2.2$ %), LMW-N ( $14.5 \pm 2.9$  %), and HPho ( $9.4 \pm 3.7$  %), with small proportions of LMW-A ( $1.3 \pm 1.0$  %) and BP ( $0.7 \pm 0.4$ %). Furthermore, LMW-A are found only in groundwaters between 5 and 99 days. Although concentrations of each fraction decrease as DOC decreases along the flow path, proportions remain relatively stable. However, humic substances from the vertical flow path decrease in HS SUVA and molecular weight with age (Figure 3.8; Figure 3.9).

Plume DOC within the horizontal flow path (days 250 to 604) is similar in composition to the vertical flow path, with HS comprising the majority ( $58.1 \pm 9.0$ %), with BB ( $16.0 \pm 6.8$ %), LMW-N ( $16.8 \pm 3.4$ %), HPho ( $7.3 \pm 2.4$ ), and LMW-A ( $0.7 \pm 0.6$ %) comprising the rest (Figure 3.11). Again, BP do not comprise more than 3.6 % of the total DOC. Humic substances increase by 24.7 % between 9 and 503 days, then decrease to a final proportion of 45.3 % at 985 days. The proportion of BB is relatively constant (around 22%) to 389 days, where it decreases to 7.4 % and increases back to its former percentage. A similar trend is seen in the concentration of BB. The proportion of LMW-N increases with time after 250 days. Concentrations of HS linearly decrease with time, while initial LMW-N concentrations slightly decrease from 1.38 mg/L to 0.38 mg/L at 503 days, where it remains relatively constant as groundwater ages. The SUVA and molecular weight of humic substances within the horizontal flow path exhibited a negative relationship with groundwater age (Figure 3.8; Figure 3.9). The molecular weight of HS decreased by 286 g/mol, while HS SUVA decreased by 3.94 L/(mg·m).

DOC SUVA values from non-plume samples were between 2.42 to 4.54 L/(mg·m) (Figure 3.12). The highest values are found with younger groundwater, while the lowest value is found in the oldest aged sample. However, SUVA does not decrease linearly with age. Instead, a similar trend to DOC and  $\text{NO}_3^-$  is seen, indicating SUVA decreases along both flow paths. Furthermore, measured non-plume SUVAs from high DOC are lower than

low DOC samples (Figure 3.13). The  $E_2:E_3$  ratio (an indication of bulk molecular weight) finds the oldest samples to have the lowest ratio (1.22), while the younger samples contain the highest values (5.56 to 9.91) (Figure 3.14).

From the overall plume, highest SUVA was found from the youngest samples (Figure 3.12). Similar to  $\text{NO}_3^-$  and DOC, different flow paths can be determined by decreasing SUVA values between 0 and 182 days and 250 to 985 days. The vertically-dominated flow path (5 to 182 days) has an overall decrease of 2.40 L/(mg·m), while the horizontally-dominated flow path (250 to 985 days) decreased in SUVA by 1.43 L/(mg·m). In addition, a positive relationship is found between all plume SUVA and DOC samples (Figure 3.13). The  $E_2:E_3$  ratio of plume DOC does not show such a distinction from different flow paths. Instead, only general observations can be made. From this study, younger groundwater generally contains ratios between 5.00 and 8.00, increasing until 604 days (a maximum of 9.00), where the ratio then decreases to 1.20 (Figure 3.14).

### **3.4.3 LC-OCD ANALYSIS OF DISSOLVED ORGANIC NITROGEN**

The LC-OCD could measure DON within the HS and BP component in all samples. However, the BP fraction did not generate a sufficient response to accurately integrate the peak, unlike HS which had a clear peak. For this reason, only DON bound to HS was measured. Concentrations for non-plume HS-bound DON range from 59 to 206  $\mu\text{g-N/L}$ , with an average value of  $146 \pm 58.8 \mu\text{g-N/L}$  (Figure 3.15). Molar ratios of HS DOC to HS-bound DON (hereafter referred to as HS DOC:DON) are generally low, ranging from 14.0 to 26.4 (Figure 3.16).

The vertical flow path contained HS-bound DON from 77 to 375  $\mu\text{g-N/L}$ , and generally decreased with age (Figure 3.15). The HS DOC:DON molar ratio was found to range between 11.0 and 12.0 in young groundwaters, to a ratio of 14.0 at 189 days (Figure 3.16). The horizontal flow path illustrates a similar trend in DON, decreasing from 188 to 41  $\mu\text{g-N/L}$  over 735 days. Conversely, the DOC:DON ratio increases from 13 to 22 over the same time period.

## 3.5 DISCUSSION

### 3.5.1 CHANGES TO DOC QUALITY FROM SUVA AND LC-OCD PARAMETERS

The aquifer at Long Point is impacted by the septic tiles, which are contributing a high concentration of labile (high quality) DOC into the aquifer. It has been found that the plume exhibits very low dispersivities and has a core zone that shows only minor dilution (Robertson & Cherry, 1992; Robertson et al., 2012). The concentrations of  $\text{Cl}^-$  (a conservative tracer) and values of EC within the plume (along both flow paths) imply there is likely little dilution or mixing of plume DOC with background DOC, thus the decrease in DOC is altered by internal processes rather than by dilution. Although extensive precipitation can dilute concentrations in the most shallow wells (van Stempvoort et al., 2011), days leading up to the sampling had no significant precipitation events (Canadian Historical Weather Radar, Environment Canada). Hence, as the DOC is transported, alterations to its quality must be a result of the internal processes within the aquifer.

Non-plume groundwater generally contains DOC concentrations higher than plume groundwater. Previous studies have found that the high rate of  $\text{NO}_3^-$  loading, anoxic conditions and oxidation of carbon within the plume allows denitrification to occur (Robertson & Cherry, 1992; Aravena & Robertson, 1998). In addition, the presence of  $\text{N}_2\text{O}$  supports the presence of this process since it is an intermediate product of denitrification. Thus, if there are active microbial processes occurring, it would likely be heterotrophic, requiring labile carbon, and would likely follow the denitrification reaction in an anaerobic environment:

(1)

Looking at the vertical flow path only, a mass balance calculation indicates that around 49 mg/L of  $\text{NO}_3^-$  is lost (or 3.50 mmol), meaning that through Equation 1, a total of 53 mg/L of DOC (4.38 mmol of  $\text{CH}_2\text{O}$ ) would be required for the observed loss in  $\text{NO}_3^-$ . A similar situation is found in the horizontal flow path, where the loss of 51 mg/L of  $\text{NO}_3^-$  (3.64 mmol) would require 55 mg/L of DOC (or 4.55 mmol  $\text{CH}_2\text{O}$ ). The mass balance indicates that the loss of DOC is not sufficient to act as a sole source of carbon for denitrification,

indicating that a combination of the sediment organic matter (foc) and possibly FeS<sub>2</sub> oxidation are used for denitrification. Although DOC is not the main source of energy, it is likely involved with denitrification and that heterotrophic degradation likely alters the quality of DOC.

The high concentrations of NH<sub>4</sub><sup>+</sup> found within the shallow plume is similar to previous studies, attributed to heavy sewage loading early in the season ('May 24' event) (Li, 2010; Robertson et al., 2012). This 'slug' represents relatively young DOC that was slow to degrade and would have undergone different reactions than the surrounding groundwater (Li, 2010). Specifically, this area would have undergone denitrification for over 20 years, making it possible that all labile foc has been consumed, and is too shallow to interact with available pyrite deeper in the aquifer. From this, it can be argued that the NH<sub>4</sub><sup>+</sup>-rich plume represents a location where DOC is predominantly used for denitrification.

The decrease in DOC concentration occurs concomitantly with a decrease of SUVA values, indicating a change in the character of DOC. Aromaticity and biodegradability of bulk DOC can be compared through SUVA. An increase in SUVA is correlated to an overall increase in the aromaticity of DOC (Weishaar et al., 2003), which has been found to be inversely related to the amount of biodegradation (Kalbitz et al., 2003). Interestingly, as the DOC concentration decreases, SUVA values indicate an overall decrease in aromatic components along both flow paths. This is supported in both flow paths by LC-OCD chromatograms and E<sub>2</sub>:E<sub>3</sub> ratios. In addition, there is a decrease in the concentration and SUVA of HS, as well as a shift towards lower molecular sized components. Furthermore, comparison of HS SUVA to the overall DOC SUVA illustrates a positive linear relationship from both flow paths, indicating that the loss of aromatic HS may influence the overall SUVA (Figure 3.17). In addition, since the data does not follow a 1:1 relationship, this shows that there is a preferential loss of HS, indicating a change to the character of DOC.

By comparing the two flow paths in the plume, a slight difference exists when observing changes to the composition of DOC. Although the vertical flow path illustrates a decreasing SUVA, very little change among fraction proportions are observed with time,

whereas the horizontal flow path shows an increase in the proportion of LMW-N. As DOC concentrations decrease along the horizontal flow path, concentrations of LMW-N remain constant, thus increasing in proportion. Qualls and Haines (1992) observed a portion of organic matter that degrades rapidly, as well as a portion that degrades much slower. It is possible that the longer residence time of DOC in the horizontal flow path allows for the accumulation of LMW-N, not possible in the shorter vertical flow path. Integrating these results shows a decrease of DOC concentration from the preferential loss of aromatic components, forming LMW-N as possible products of degradation. Furthermore, the lower HS SUVA than DOC SUVA indicates some products of degradation are aromatic. In both flow paths, HS contain the highest variability in proportions, leading to the idea that these constituents may dictate the quality of DOC.

In both flow paths, decreasing aromaticity and molecular weight of HS with increasing groundwater age illustrated active degradation of humics. Differences in HS characteristics have been attributed to different sources (Artinger et al., 2000). Within this plume, DOC can originate from either the septic tile or from the high sediment organic carbon (SOC) content within the aquifer. Studies have observed the *in-situ* generation of DOC through the degradation of SOC in the presence of an oxidizing agent (such as oxygen, nitrate, or sulphate) within the aquifer (Aravena & Wassenaar, 1993; Buckau et al., 2000). Thus, changes to the DOC will result from processes occurring within the plume. Although HS have been considered to be relatively recalcitrant (Frimmel, 2003; Cleveland et al., 2004; Qualls, 2005), this may not always be the case. Volk et al. (1997) determined humic substances to be an important part of biodegradable DOC, while Camper (2004) demonstrated that humics can act as a sole energy source for biofilms. Degradation could be accounted for by the continual reworking of the HS structure. For instance, large molecular weight molecules may break down into lower weight and lower aromatic molecules, but still be the appropriate size to be classified as HS, which can be seen in the data. Furthermore, younger groundwater found near the septic tiles had similar HS SUVAs and molecular weights as sewage-impacted humics, while older groundwater were similar

to *in-situ*, autochthonous HS (Huber et al., 2011). These changes within the DOC illustrate that HS may act as a labile portion of DOC.

The LC-OCD provides valuable data on the organic nitrogen bound to the HS fraction. When inorganic nitrogen is low, organic nitrogen provides a major nutrient to support bacterial growth, and is generally found at very low concentrations (<0.2 mg/L) (Nolan & Stoner, 2000; Chapin III et al., 2002; Wiegner et al., 2006). Studies have found that an increase of inorganic nitrogen to a system increases the amount of DON release, decreasing the overall C:N ratio (Williams & Silcock, 1997; Williams et al., 1999; Neff et al., 2000). High loads of inorganic nitrogen are introduced into the groundwater from the septic tiles, forming higher amounts of DON and contributing to the very low HS DOC:DON ratio. Results from this study are similar to groundwater samples collected from a moorland (average molar DOC:DON=6.95, n=18; Lapworth et al., 2008) and a degraded and intact peatland (average DOC:DON=9.4 and 17.5 (respectively), n=41; Kalbitz & Geyer, 2002). The ratio of DOC:DON can be used to observe the lability of organic matter. Biodegradability of DOC was found to be higher with a low DOC:DON ratio (Fellman et al., 2008). The horizontal flow path illustrates a greater change in the DOC:DON ratio with age than the vertical flow path, showing more loss of DON with time. The greater amount of inorganic nitrogen in the vertical flow path may allow DON to persist throughout the flow path, while lower concentrations of inorganic nitrogen along the horizontal flow path may reduce the formation of DON, thus increasing the DOC:DON ratio. The decrease in both DON and DOC along both flow paths further supports the degradation of labile DOC into a recalcitrant form. In addition, the distal plume as been found to contain low levels of  $\text{NH}_4^+$  (1 to 3 mg/L) that contain a lighter isotopic signature than that of wastewater  $\text{NH}_4^+$ , indicating a different source (Robertson et al., in prep). It is possible the degradation of HS DON provides a secondary formation for this small amount of  $\text{NH}_4^+$  mineralization away from the septic tiles.

Both SUVA and LC-OCD analysis illustrated that DOC decreases over the length of the plume and was becoming a reworked and less labile form. Specifically, changes to the DOC occurred to HMW, aromatic HS, possibly producing LMW-N as degradation products.

In order to better understand such changes to DOC character, it is best to compare to other DOC degradation studies and determine if similar changes are found.

### **3.5.2 PROCESSES AFFECTING DOC COMPOSITION**

DOC from the plume changes in quality as a result of internal processes within the aquifer from four potential mechanisms: changes from the unsaturated zone, sorption, mineralization of SOC, or microbial degradation along both flow paths.

#### *3.5.2.1 THE UNSATURATED ZONE*

The unsaturated zone provides opportunity for DOC to be altered before it reaches the groundwater table. Foulquier et al. (2011) found most labile DOC from a storm-water recharge zone to be retained in the vadose zone. Among the natural environment, studies have determined soils to be responsible for most DOC degradation within the first few meters of the subsurface (Ludwig et al., 2000). However, Long Point is situated upon a sand spit and contains little amounts of soil at the surface, thus water (and DOC) may move relatively quickly, which may contribute towards the high concentrations seen in this study. Although concentrations within the plume (~5 mg/L) are high for groundwaters, the source effluent contains much higher concentrations (~32 mg/L; Robertson & Cherry, 1992; Robertson et al., 2012), indicating that the DOC has, in fact, been subject to considerable alterations before reaching the water table. It has been observed that aerobic soil passage results in preferential degradation of chained (aliphatic) carbon, whereas anaerobic infiltration degrades more aromatic sources (Grünheid et al., 2005). Furthermore, Huber et al. (2011) determined that microbial-influenced HS contain low molecular weights (<500 g/mol) and HS SUVAs (<2.00 L/(mg·m)). The data corroborate this idea since the composition of DOC beneath the septic tiles consists mainly of humics (HS and BB), in addition to containing highly aromatic molecules (determined from the high SUVA), which degrade over time into microbially influenced HS. These findings suggest the potential for biological degradation of DOC above the water table, which should be taken into account when assessing the overall quality along the plume.

### 3.5.2.2 SORPTION AND DOC QUALITY

Adsorption to sediments has been found to alter the overall composition of DOC (Meier et al., 1999; Hur & Schlautman, 2003; Meier et al., 2004). Specifically, the presence of hydroxides results in fractionation, where HMW and aromatic components are preferentially adsorbed (Zhou et al., 2001; Maurice et al., 2002). Robertson (2008) found ‘substantial amounts’ of acid-extractable aluminum, iron, and manganese within the aquifer, indicating the presence of hydroxide minerals as well as the potential for DOC fractionation. McIntyre et al. (2005) found adsorption of humic acids to lower both concentration and aromaticity of bulk DOC. However, the data do not support preferential adsorption since proportions of most fractions remain relatively constant in both flow paths as concentrations decrease. This indicates that most fractions are decreasing at the same rate, which is against the idea of preferential retention of HS.

### 3.5.2.3 MINERALIZATION OF SEDIMENT ORGANIC CARBON

Mineralization of SOC within aquitards and deep aquifers has been found to increase DOC and dissolved inorganic carbon concentrations. Furthermore, humic substances are preferentially released during this process (Artinger et al., 2000; Buckau et al., 2000; Aravena et al., 2004; Hendry & Wassenaar, 2005). Furthermore, the mineralization of SOC can be observed by low concentrations of sulphate ( $\text{SO}_4^{2-}$ , an oxidizing agent) and increased concentrations of  $\text{CH}_4$  gas (Buckau et al., 2000; Aravena et al., 2004). It is unlikely that mineralization of SOC influences the quality of DOC within either flow path due to the relatively unchanging proportion of HS. In addition, both flow paths contain detectable amounts of  $\text{SO}_4^{2-}$  (5.5 to 47.7 mg/L), furthering the idea that mineralization of SOC does not change the quality of DOC.

### 3.5.2.4 MICROBIAL DEGRADATION OF DOC

Different fractions of DOC may provide better substrates for microbial degradation, illustrating the importance of LC-OCD analysis. Bacterial degradation of inorganic nitrogen was found to occur simultaneously with the degradation of HMW DOC (Amon & Benner, 1996). The loss of  $\text{NO}_3^-$  and DOC, in addition to the decrease in SUVA, may support these

findings. Furthermore, the size-reactivity continuum model states that microbial degradation of DOC leads to lower weight, refractory compounds (Amon & Benner, 1996), supporting the relative increase in LMW-N proportion. These results go against the findings of Grøn et al. (1992) and Qualls (2005), both who observed LMW compounds to be preferentially utilized. Conversely, Brinkmann et al. (2004) found LMW acids to be highly bioavailable, which can be seen in the data by the rapidly decreasing concentration of LMW-A. It is plausible there are processes occurring where the uptake of labile LMW molecules occurs at the same rate as the formation of recalcitrant LMW compounds from HMW degradation.

Pairing the degradation of DOC with groundwater age allows for calculation of a degradation rate. Studies have used an exponential decay model to account for labile and recalcitrant portions (Scully et al., 2004; Cuss & Guéguen, 2012), while Worrall et al. (2006) found a zero-order degradation rate was indicative of an environment with excess substrate. A variety of degradation rates are found within the environment, ranging from 0.03 to 23 mg-C/L per day (Table 3.2). The data from this study is best fit through a simple linear regression. By fitting a linear regression to the vertical flow path area (day 0 to 182;  $R^2 = 0.45$ ), the degradation rate is found to be  $2.2 \times 10^{-2}$  mg-C/L per day (or 7.99 mg-C/L per year). For the horizontal flow path area, the initial concentration below the septic tiles is used, as well as data between 250 to 1000 days ( $R^2: 0.68$ ), giving a degradation rate of  $4.8 \times 10^{-3}$  mg-C/L per day (or 1.75 mg-C/L per year). These calculations indicate a slower degradation than other environments; however offer an initial estimate to the rate of DOC loss within the aquifer.

The BP fraction is of particular interest since it can be associated with microbial degradation of DOC. Biopolymers are found to be more abundant under areas of high organic matter loading (Foulquier et al., 2011; Neale et al., 2011), thus it would be expected to find a high concentration under the septic tiles. In addition, it has been found that aquifer DOC contains a significant microbial component (Birdwell & Engel, 2009). Within both flow paths, heterotrophic bacteria (likely housed in biofilms) would use the DOC during oxidation or denitrification, increasing the proportion of proteins and

polysaccharides (Hesse et al., 1999). The data indicate that both DOC loss and denitrification to occur, creating conditions that should produce high amounts of BP. However, there must be a process either hindering the formation and transport or causing rapid recycling of these large biomolecules, since very low amounts of BP are found in the dissolved fraction. One study of artificial recharge observed the removal of larger fractions by adsorption and mechanical filtering (Lindroos et al., 2002). It is possible that the larger DOC fractions were filtered out from the unsaturated zone before entering the groundwater, possibly contributing to biological mats found directly under septic tanks (Wilhelm et al., 1994). In addition, BP may coat the grains within the unsaturated zone, rather than be found in solution (Marshall, 1988; Griebler et al., 2002). Regardless, groundwaters may not be effective conduits for BP transport, but that does not indicate they are not present within the environment.

Low DOC:DON ratios can be considered to be characteristic of autochthonous (formed *in-situ*) organic matter (Westerhoff & Mash, 2002). Amon and Benner (1996) observed bioreactive HMW compounds to be depleted in organic nitrogen, requiring an inorganic nitrogen source to allow microbes to degrade it into LMW compounds. Within the vertical flow path, HS bound DOC:DON ratios remain constant, even with decreasing DON concentrations, illustrating that equal amounts of carbon and nitrogen are being lost under the septic tiles. However, the increasing DOC:DON ratio in the horizontal flow path indicates that nitrogenous species are depleted along the plume, creating more carbon-enriched molecules. This may illustrate that labile DOC from groundwater can be considered to contain relatively higher nitrogen moieties. In addition, Fellman et al. (2008) observed an increasing DOC:DON ratio with a decrease in humic:fulvic ratio. Although the LC-OCD provides a range of HS characteristics (from high values of molecular weights and SUVAs to low values), a decreasing humic:fulvic ratio would indicate HS becoming less aromatic and of lower molecular weight. This result is supported through findings from both LC-OCD data and HS SUVA data, as well as from the increase in DOC:DON, furthering the idea that HS may be an important constituent in the determination of DOC quality.

### **3.5.3 COMPARISON OF SEPTIC DERIVED DOC TO NATURAL ENVIRONMENTS**

Due to the high load of anthropogenic DOC from the septic tiles, it is thought labile components of carbon found here may not be found within the natural environment, and thus would have a different composition. Humic substances comprise the majority of the DOC in groundwater, both in natural systems and within this impacted septic system plume (Thurman, 1985; Grøn et al., 1996). Furthermore, the overall composition appears not to differ greatly from other groundwaters (Figure 3.18), with humics (HS and BB) forming the majority, and the rest comprised of LMW compounds. However, the proportion of HPho is indicative of DOC from effluent-impacted rivers, which usually contain 10% of the DOC as HPho (Huber et al., 2011). Although the average proportions differ from other environments, it can be seen the general composition is similar to other groundwater environments.

The stability of DOC composition in both flow paths leads to the idea that labile DOC cannot be properly defined by the abundance of a single component. Wickland et al. (2007) suggested a microbial ‘filter’ conformed DOC into a specific composition. Results from this study did not show this, since the loss of DOC within the plume did not change the overall proportion of the DOC composition. Furthermore, comparison of DOC to a variety of natural environments illustrates that although the compositions are generally similar, septic-derived DOC contains not only higher proportions of HS, but also more aromatic and higher molecular weight HS (Figure 3.19). If DOC from Long Point represents a labile form, these results show degradation of DOC would lead toward a lower aromatic and molecular weight HS, as well as proportionally within the DOC.

### **3.5.4 FUTURE RESEARCH**

By grouping DOC fractions based on molecular size, it is difficult to determine structural differences within a fraction (such as labile LMW-N or reworked LMW-N). Pairing the characterization of DOC with a more qualitative method on individual separated fractions (such as <sup>13</sup>C-NMR) would likely add further insight to whether the increasing proportion of LMW-N is due to the recalcitrance of this fraction, or the breakdown of HMW molecules.

Regardless, data show septic-influenced plume DOC to contain higher proportions of LMW-N than non-plume DOC, leading to the idea that active microbial degradation occurs, with this fraction indicative of microbial reworking.

The mineralization of isotopically-lighter  $\text{NH}_4^+$  from the distal plume illustrates an area for further research. Although the mineralization of HS DON may account for some of this, concentrations are too low ( $<100 \mu\text{g-N/L}$ ) to completely account for all the  $\text{NH}_4^+$  (1 to 3 mg-N/L). Being attributed to organic-rich zones within the aquifer (Robertson et al., in prep), focusing LC-OCD characterization on this area would allow for a better understanding to the sources of DOC.

By focusing only upon the DOC within groundwater, this study was able to identify changes to the DOC character along the plume. However, future research could focus on using the LC-OCD to measure changes within the unsaturated portion of the aquifer, since this may play a key role in determining which fractions enter the groundwater and which are retained above the water table. In addition, an attempt at the composition of organic matter on the solid grains of the aquifer would provide worthwhile results since it is possible that large molecules of DOC are found in these locations.

### **3.6 CONCLUSIONS**

The septic plume at Long Point, distinguished by its high concentrations of  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and high EC, introduced a large concentration of HMW DOC with elevated aromatic character into the groundwater. Two distinct flow paths were determined within the plume: a relatively short vertical flow path, and a longer, horizontal flow path; both exhibiting a decrease in  $\text{NO}_3^-$  and DOC with increased age. As DOC decreased, proportions remained relatively the same, but SUVA and HS SUVA, molecular weight, and DOC:DON illustrated a clear decrease with increasing age. In addition, a slight increase of LMW-N and increase in aromatic character possibly illustrated the products of HMW degradation. Changes to the lability of DOC were found to be similar to that of other microbial degradation studies, while preferential sorption of HS was not found to occur within either flow path. Comparison of septic-influenced DOC to other groundwater environments illustrates that although the relative proportions of each fraction within DOC are similar, labile DOC from

the septic tiles contains a higher proportion of HS. These HS are of higher molecular weight and more aromatic than other groundwater environments.

The changes to molecular weight and aromaticity of HS lead to an inference to DOC quality. Data from this study suggest that HS may play an important role in providing an energy source for microbes, leading to the idea that HMW and aromatic HS provide high quality components. Furthermore, since HS comprise the majority of DOC, it can be thought that they dictate the overall quality of DOC. For example, the decrease in HS aromaticity corresponds with the decrease in SUVA (a measure of the bulk DOC), suggesting that the decreasing SUVA may be a result of aromatic, UV-absorbing HS degrading into non-absorbing components. These results allow for a better understanding into the components that help make up the labile portion of DOC, furthering our understanding of DOC dynamics within the subsurface.

**Table 3-1:** Chemical parameters of groundwaters collected at Long Point, Ontario (n=23). Parameters for EC, DO, and pH were measured in the field, whereas Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and DOC were measured at the University of Waterloo. Included are values from the effluent measured in the septic tank, piezometer nests directly below the tiles, and 40 to 70m down gradient of tiles, all from previous studies measured at the same location as this study.

	EC	DO	pH	Cl <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	DOC
	<i>μS/cm</i>	<i>mg/L</i>		<i>mg/L</i>	<i>mg-N/L</i>	<i>mg/L</i>	<i>mg/L</i>
Max	1322	6.09	8.27	61.0	83.6	47.7	8.02
Min	453	0.34	6.05	9.8	1.9	5.5	1.68
Average	999	1.20	6.65	41.8	43.2	19.4	3.29
Std Dev	232	1.84	-	14.6	27.4	12.6	1.54
Effluent Measured in Tank <sup>a,b,c</sup>	-	-	7.25	60.7	0.54	37.5	32.1
Directly Under Septic Tile Beds, (depth between 2 to 6 m) <sup>a,b</sup>	1390	0 - 7	6.6	46.0	51.0	34.0	5.0
Downgradient of Septic Tiles (40 to 70m from septic tiles) <sup>a</sup>	1200	1 - 3	6.7	44.0	5.0	78.0	3.2

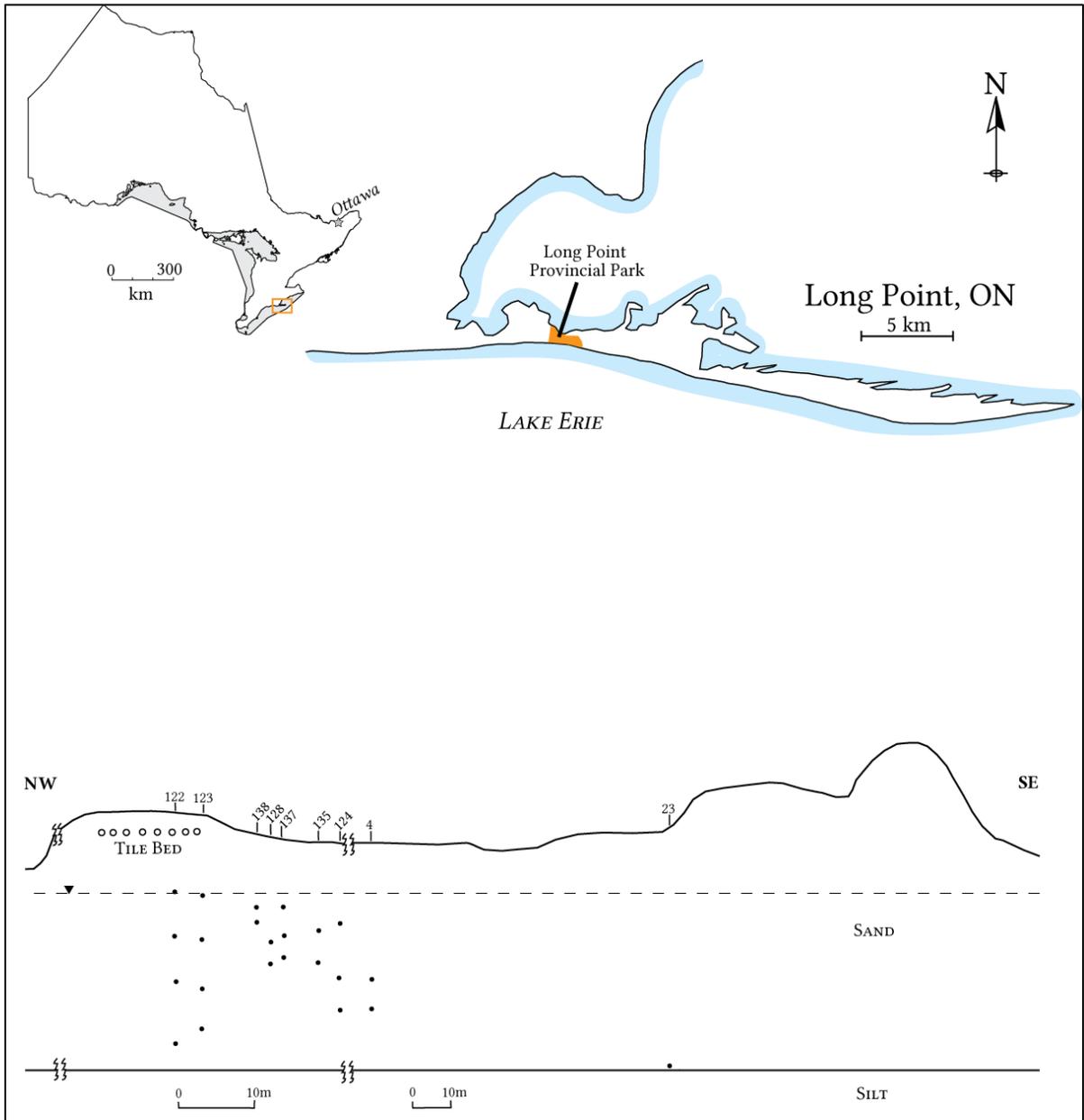
*a: Robertson & Cherry, 1992: Tile Bed 1*

*b: Robertson et al., 2012: Tile Bed 2*

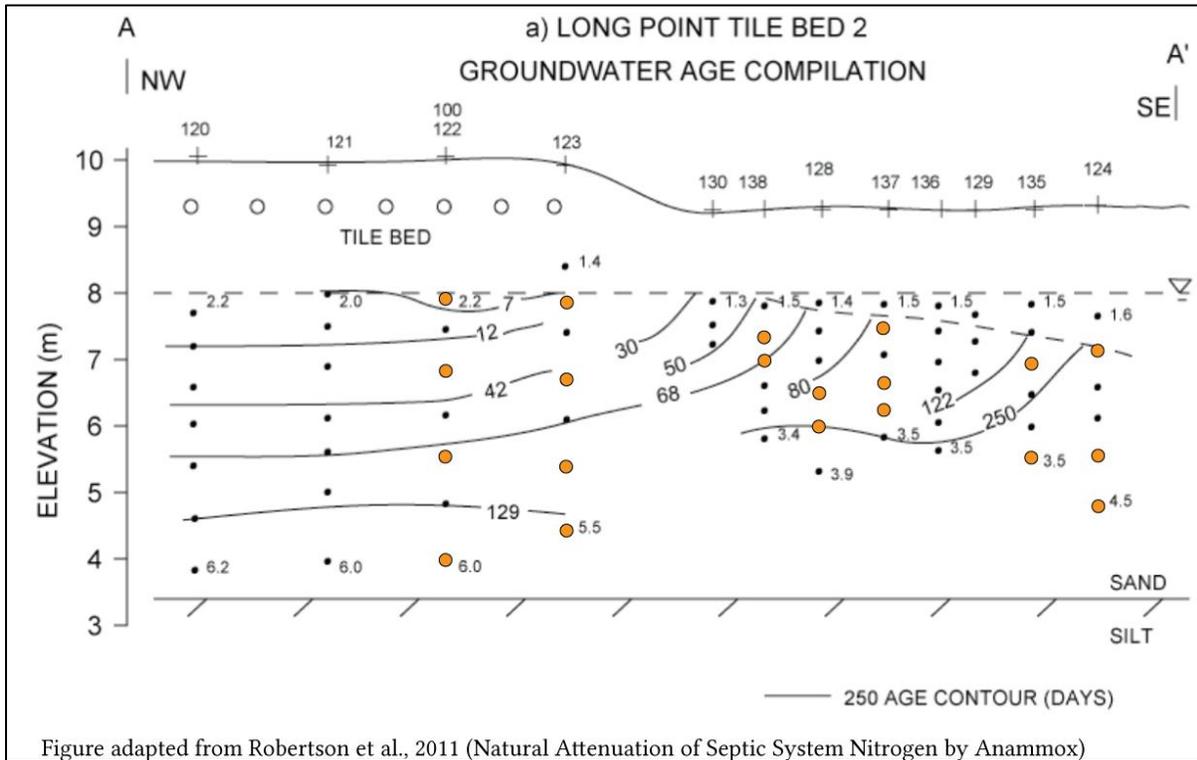
*c: Robertson, 2008: Tile Bed 2*

**Table 3-2:** Comparison of DOC degradation rates from a variety of environments.

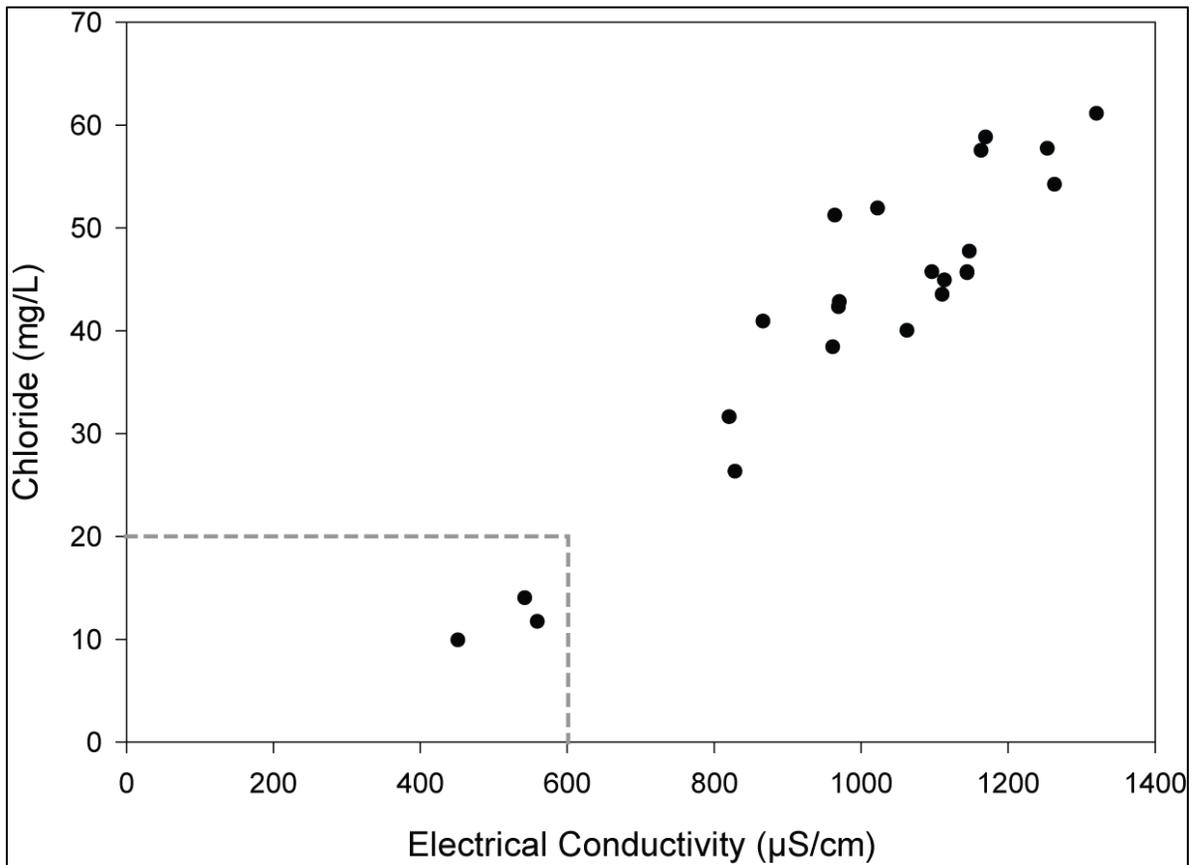
Environment	Degradation Rate	Length of Time	Source
	<i>mg-C/L per day</i>	<i>days</i>	
Leaf Litter	1.29	14	Hur et al., 2009
Stream	0.03 to 23 Average: 3	Continual monitoring	Worrall et al., 2006
Soil from fen	0.21	30	Fellman et al., 2008
Soil from bog	0.24	30	Fellman et al., 2008
Soil from upland wetland	0.09	30	Fellman et al., 2008
Directly Beneath Tile Bed 2	0.02	186	This study
Along length of plume	0.0048	985	This study



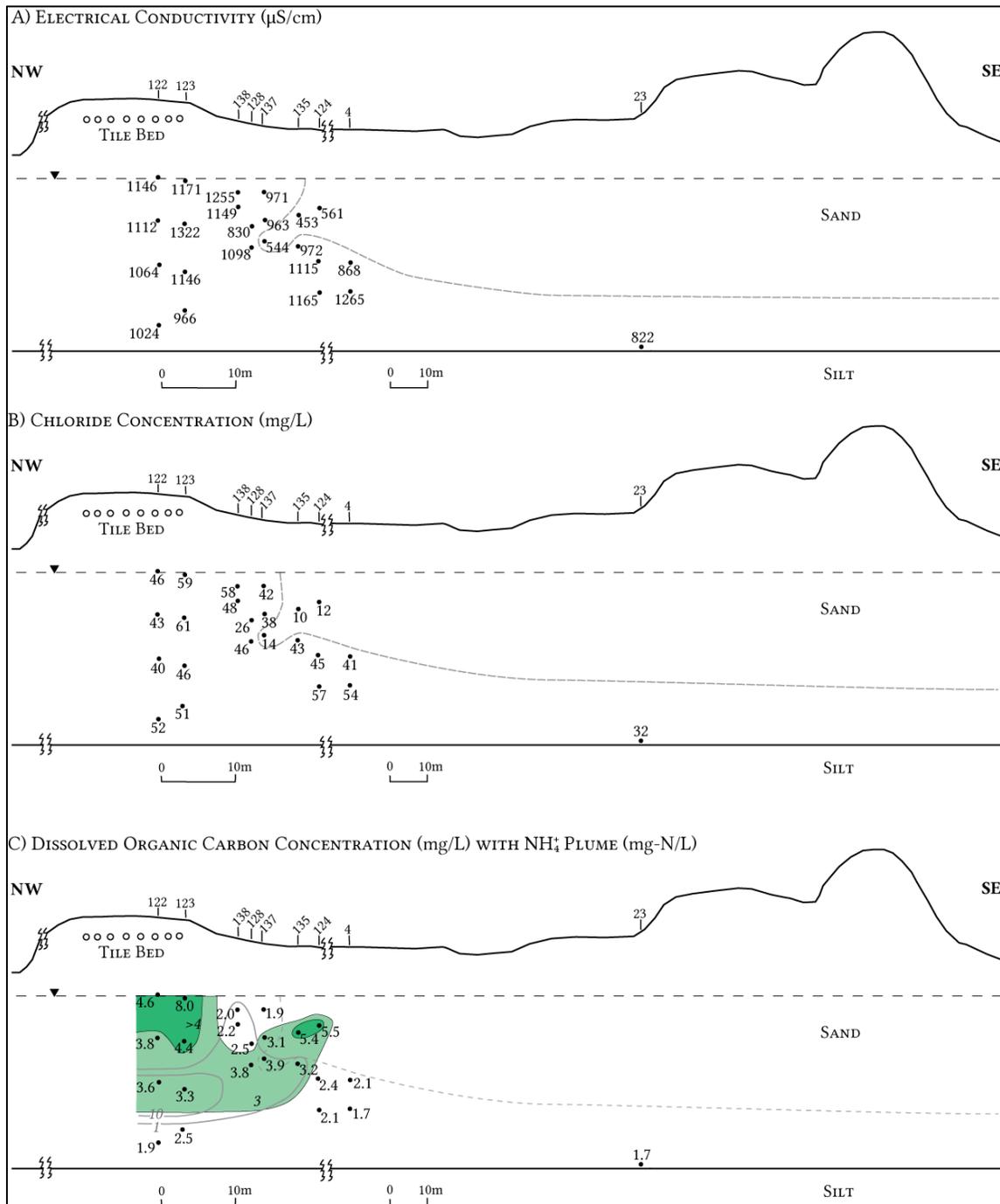
**Figure 3.1:** Site location of Long Point Provincial Park in Ontario (upper diagrams, outlined in orange), as well as a cross-sectional view of Tile Bed 2 and aquifer (below). Black dots represent ports in the multi-level piezometers, whereas numbers represent the piezometer nest. The dotted line represents the water table.



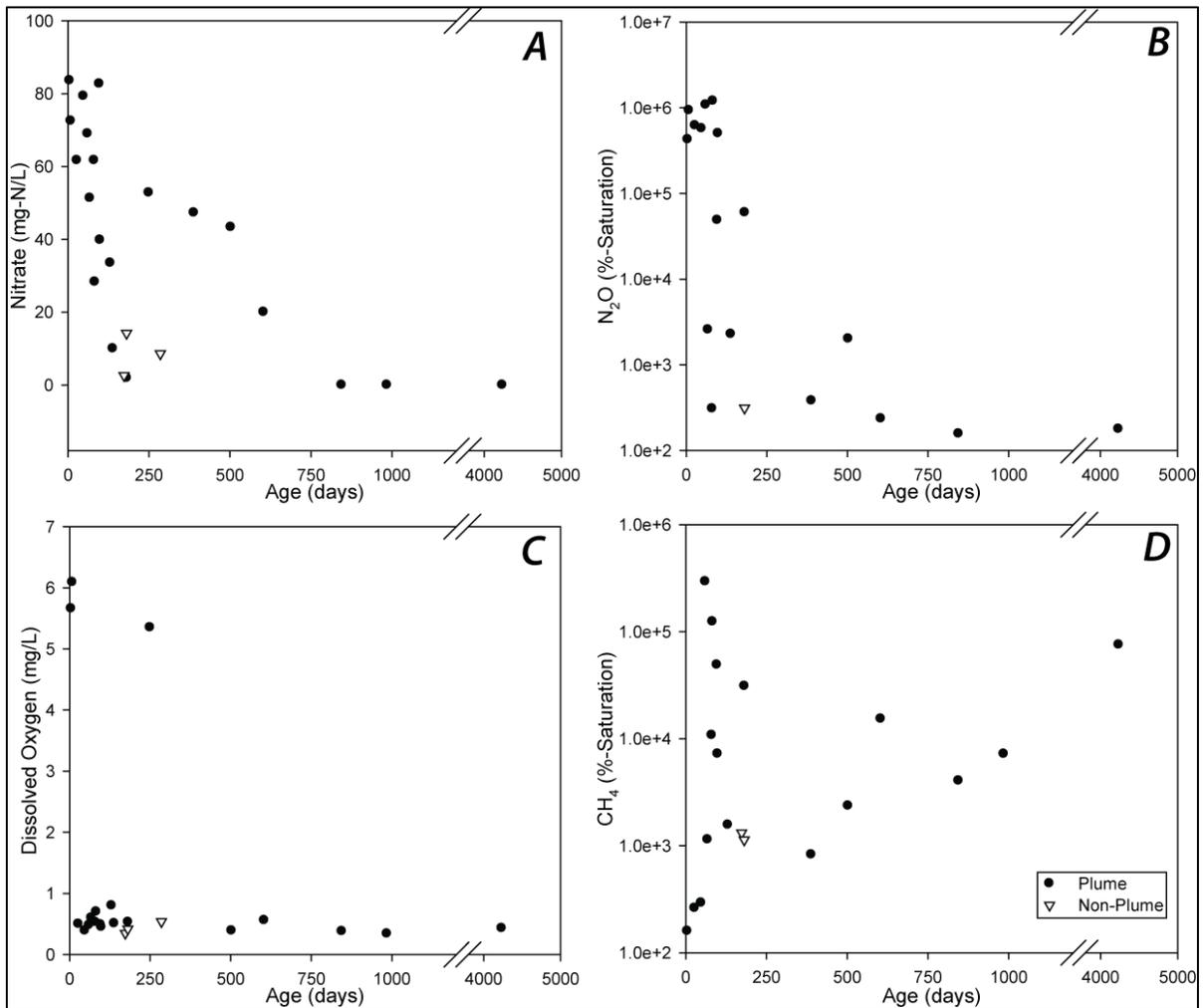
**Figure 3.2:** Groundwater age contours within the Long Point septic plume (days). Figure was taken from Robertson et al., (2011). The dotted line represents the water table, while ports with the large shaded dot indicate ports sampled in this study. Numbers next to ports indicate depth below surface (m).



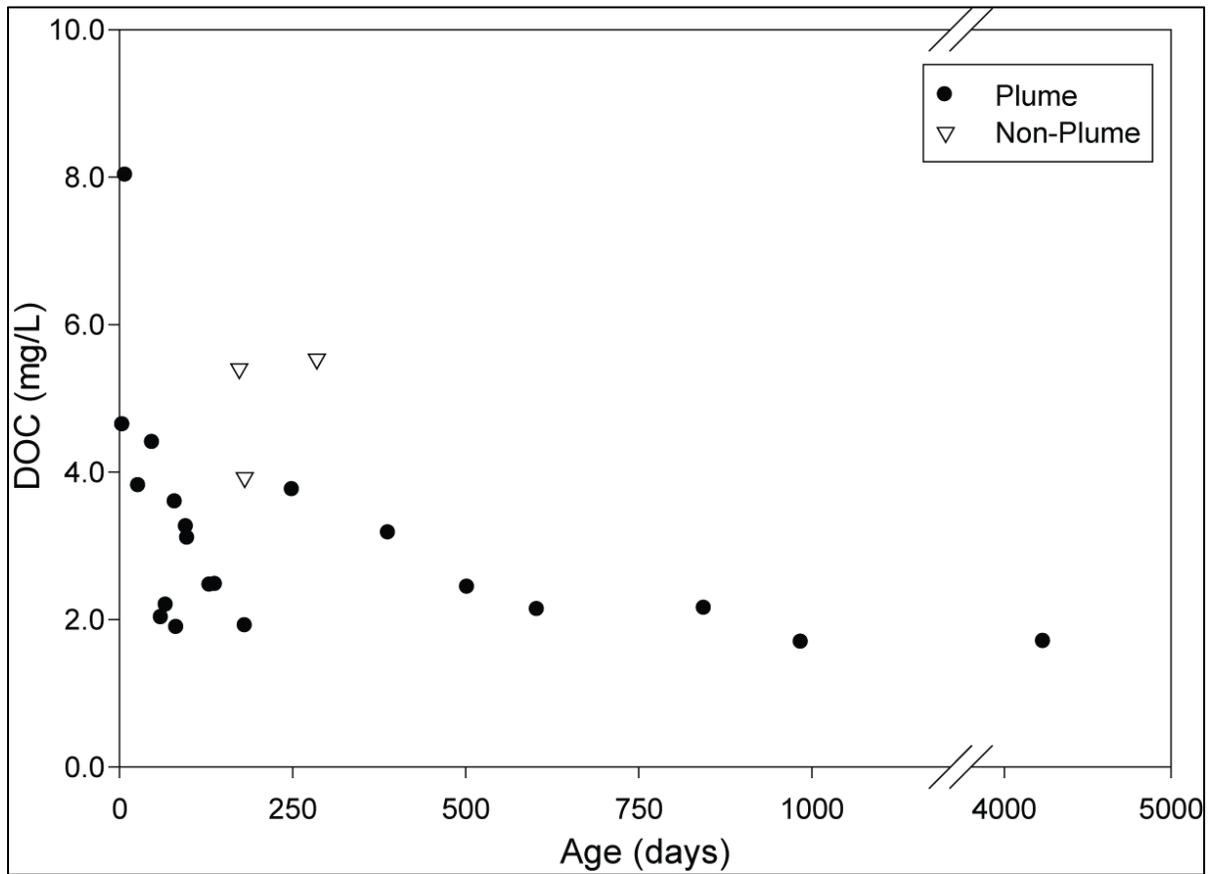
**Figure 3.3:** Chloride (mg/L) versus electrical conductivity (µS/cm) for all samples. Dotted line represents the boundary between plume samples (>20 mg/L Cl<sup>-</sup>; >600 µS/cm) and non-plume samples.



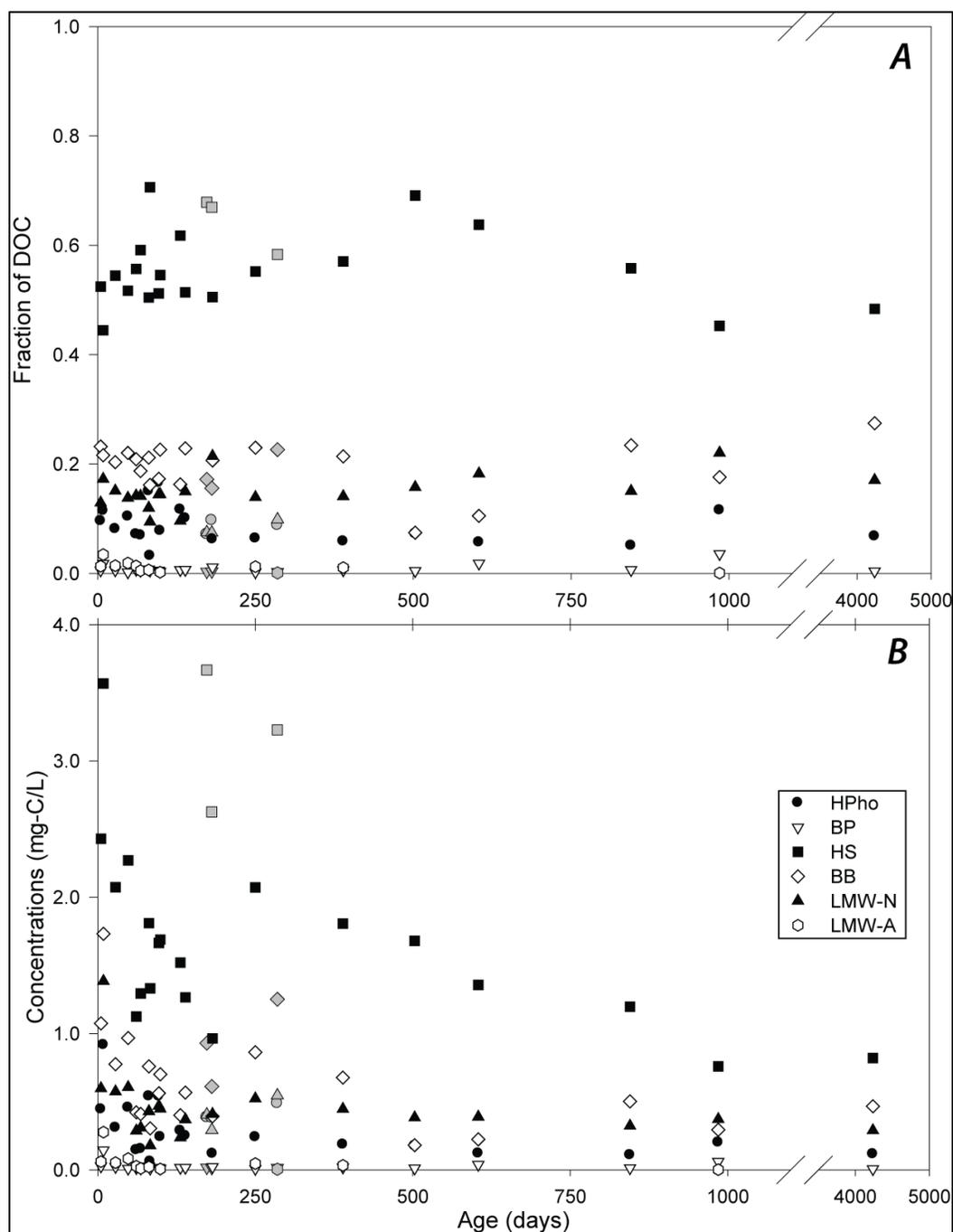
**Figure 3.4:** Cross-sectional diagrams of electrical conductivity (A;  $\mu\text{S}/\text{cm}$ ), chloride concentrations (B; mg/L), and dissolved organic carbon concentration (C: mg/L) illustrating piezometer nests within or out of the plume. Figure C also includes the boundary of the plume (dotted grey line), as well as the early season heavy sewage event contributing high  $\text{NH}_4^+$  ('May 24' event, solid grey lines). Samples represent a 'snapshot' in time, taken from a single sampling trip on October 10<sup>th</sup>, 2011.



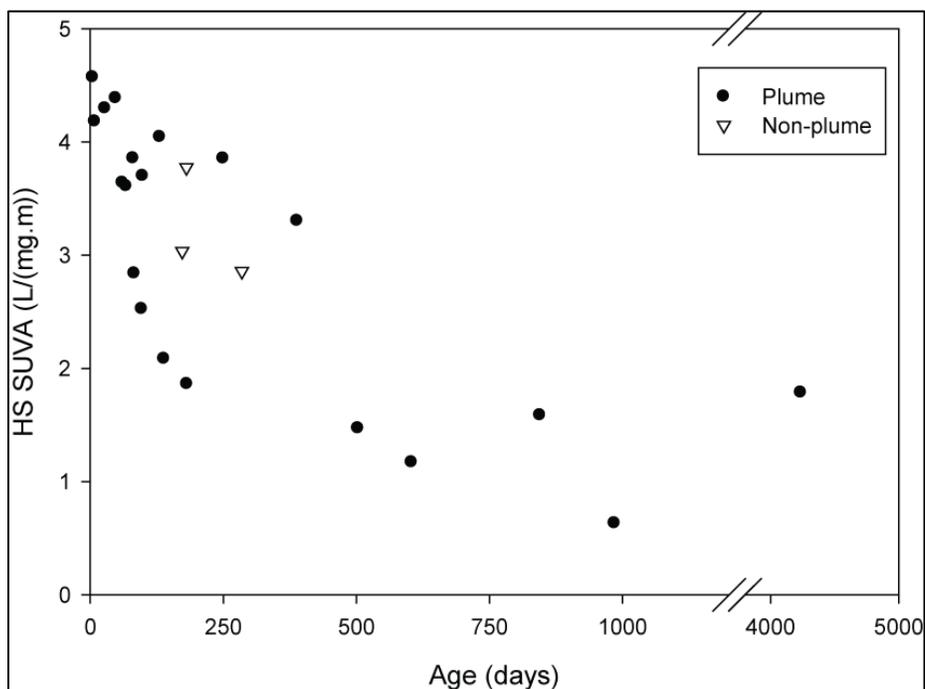
**Figure 3.5:** Concentrations of nitrate (Graph A: mg-N/L) and dissolved oxygen (Graph C: mg/L), along with percent saturations of nitrous oxide (Graph B;  $N_2O$ ) and methane (Graph D;  $CH_4$ ) versus age of groundwater (days). Solid circle symbols represent samples within the plume, while open triangles represent samples not in plume.



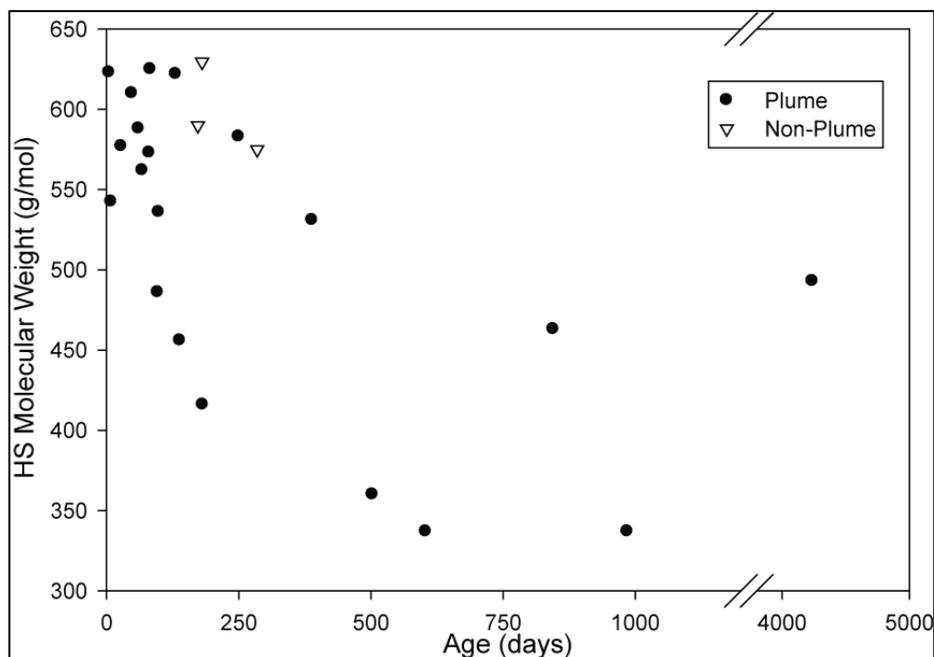
**Figure 3.6:** Dissolved organic carbon concentration (mg/L) over age (days) for groundwater samples. Closed circles represent samples within the plume, while open triangles represent samples not in the plume.



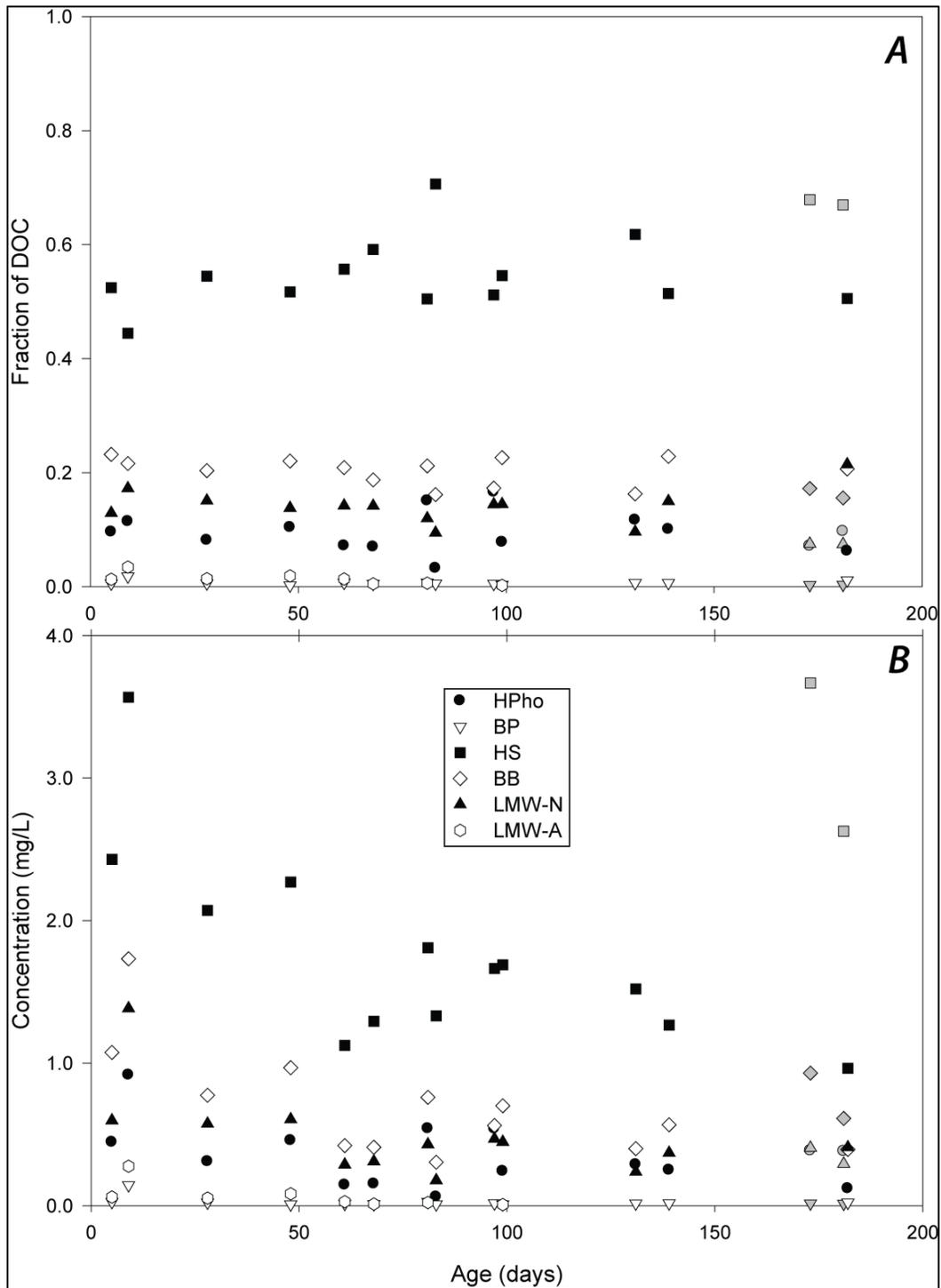
**Figure 3.7:** DOC fraction proportions (Figure A) and concentrations (Figure B; mg/L) versus age (days) along the septic plume. LC-OCD fractions include hydrophobics (HPho, closed circle), biopolymers (BP, open triangle), humic substances (HS, closed square), building blocks (BB, open diamond), low molecular weight neutrals (LMW-N, closed triangles) and acids (LMW-A, open hexagon). Black and white symbols represent samples within the plume, while grey symbols represent samples out of the plume.



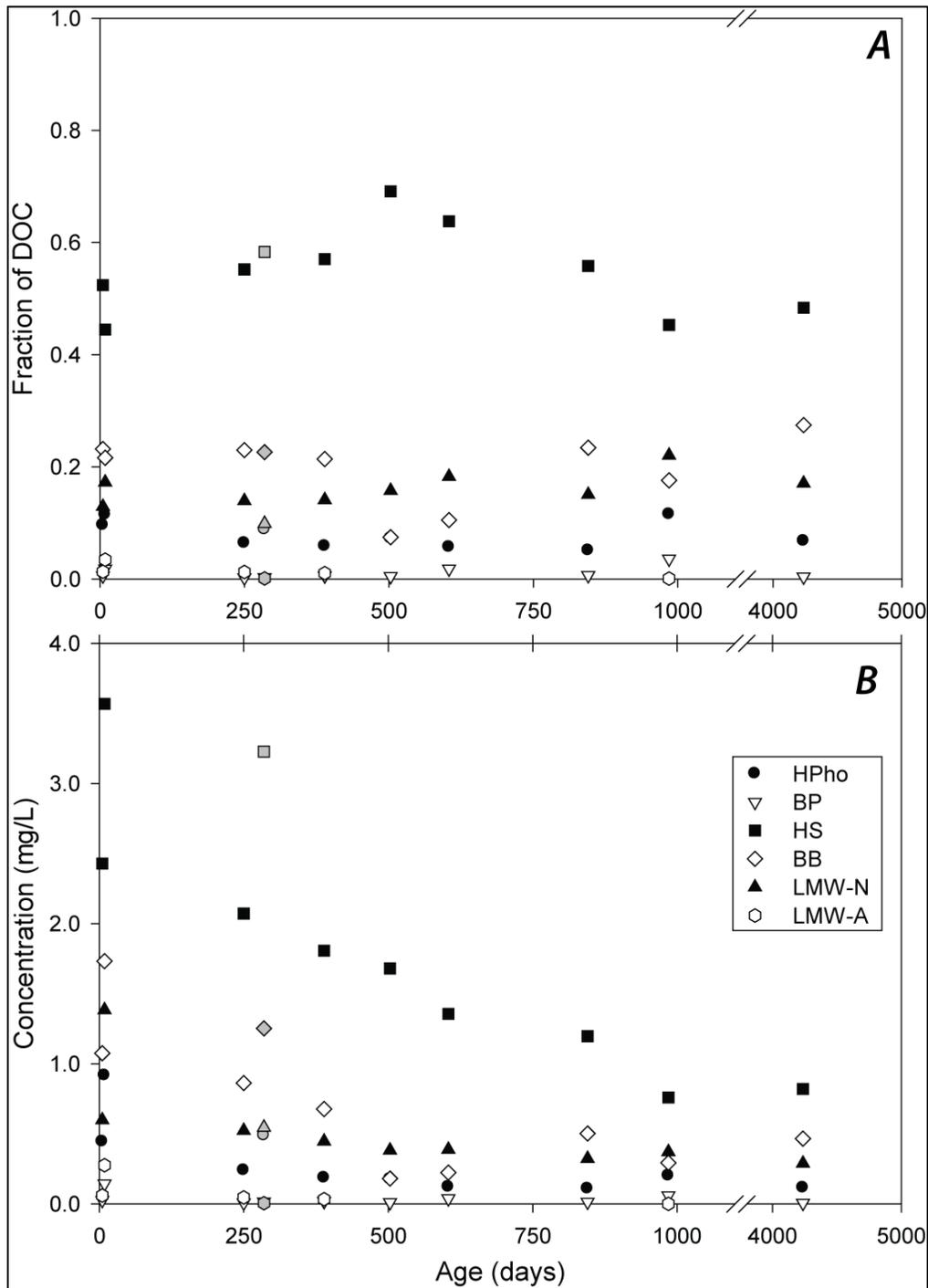
**Figure 3.8:** Humic substance SUVA (L/(mg·m)) versus groundwater age (days). Closed circles represent samples within the plume, while open triangles represent samples not in the plume.



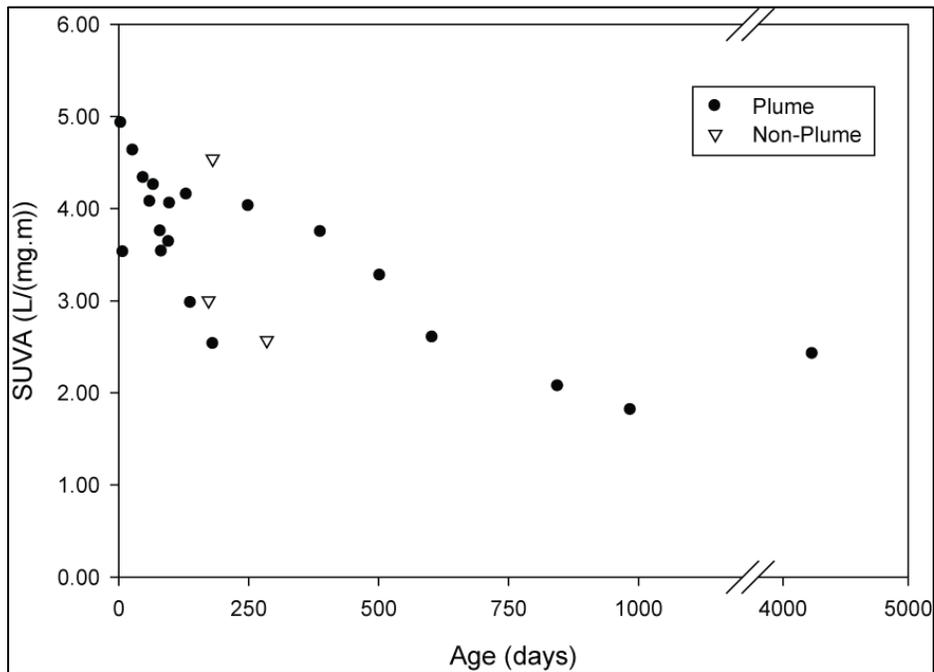
**Figure 3.9:** Humic substance molecular weight (g/mol) versus groundwater age (days). Closed circles represent samples within the plume, while open triangles represent samples not in the plume.



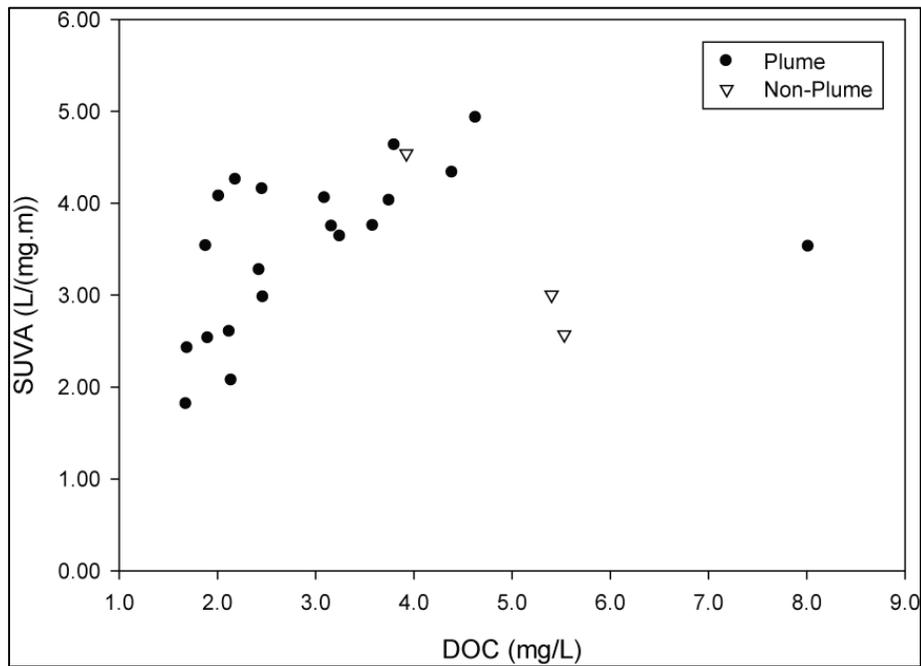
**Figure 3.10:** Vertical flow path area (0 to 182 days) LC-OCD fractions of DOC (Graph A) and concentrations (Graph B, mg/L) versus age (days). Black and white symbols represent samples within the vertical flow path of the plume, while grey symbols represent samples not in the plume.



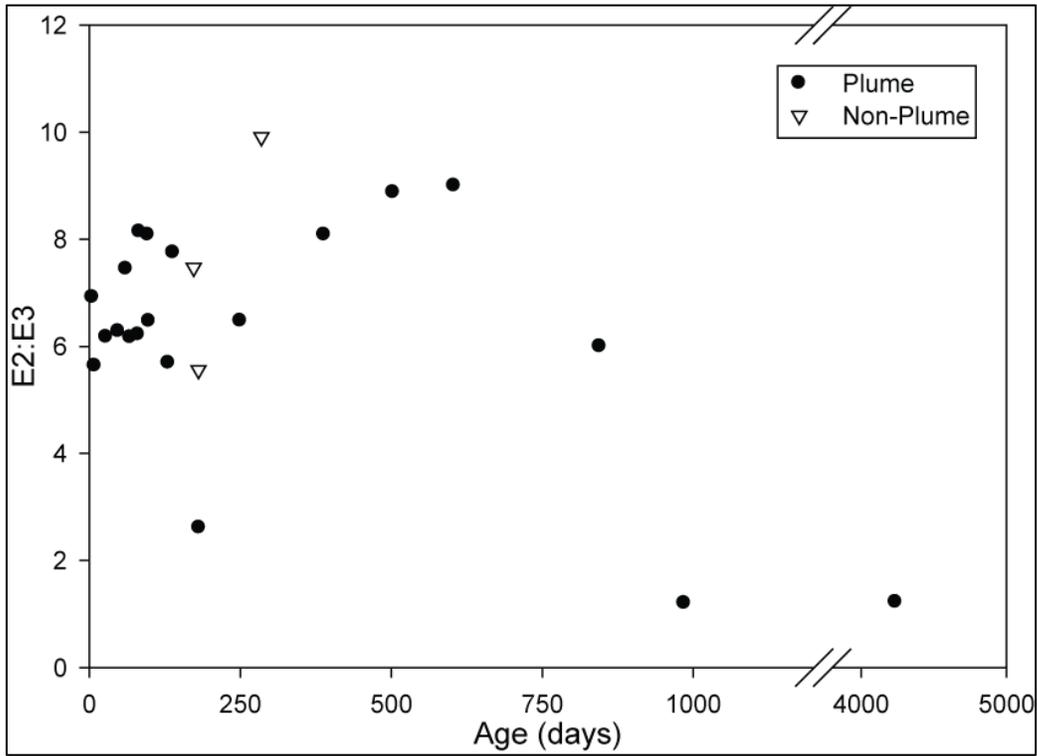
**Figure 3.11:** Horizontal flow path (0 to 985 days) LC-OCD fractions of DOC (Graph A) and concentrations (Graph B, mg/L) versus age (days). Black and white symbols represent samples within the horizontal flow path of the plume, while grey symbols represent samples not in the plume.



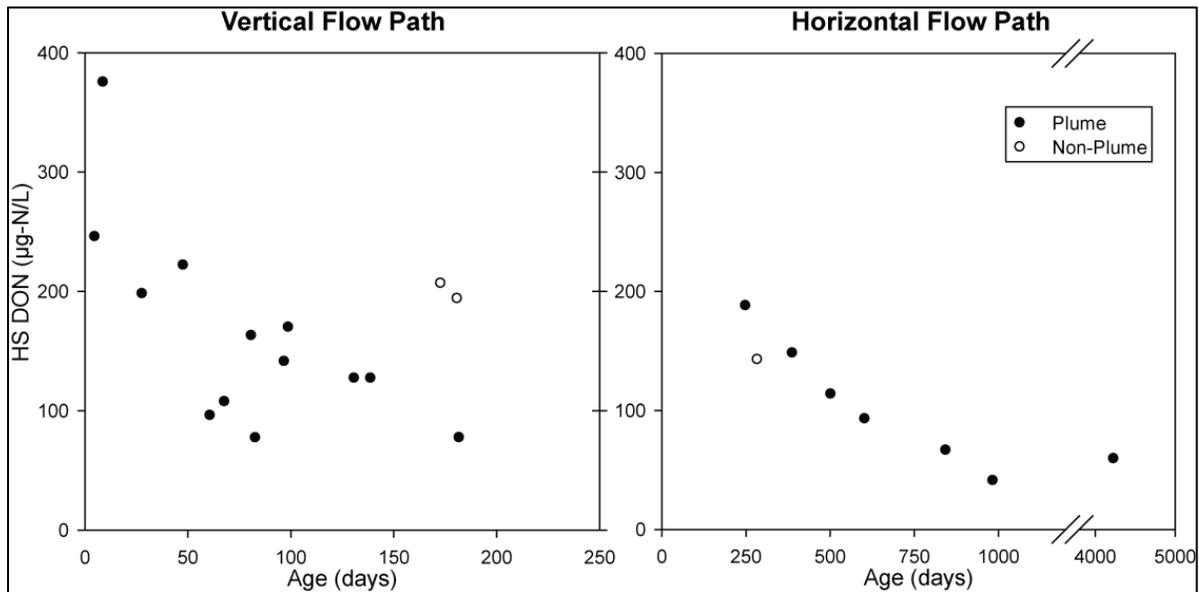
**Figure 3.12:** SUVA values (L/(mg·m)) versus groundwater age (days) for samples within the plume (both flow paths illustrated; closed circles) and not in the plume (open triangles).



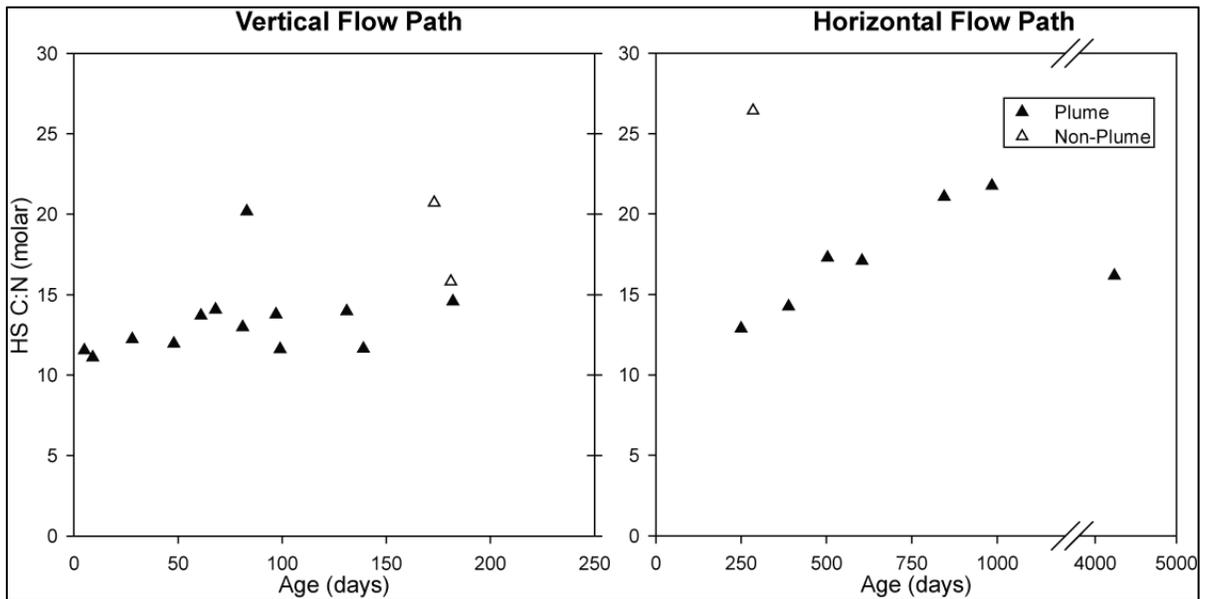
**Figure 3.13:** SUVA values (L/(mg·m)) versus dissolved organic carbon concentration (mg/L) from both flow paths within the plume. Closed circles represent samples within the plume, while open triangles represent samples not in the plume.



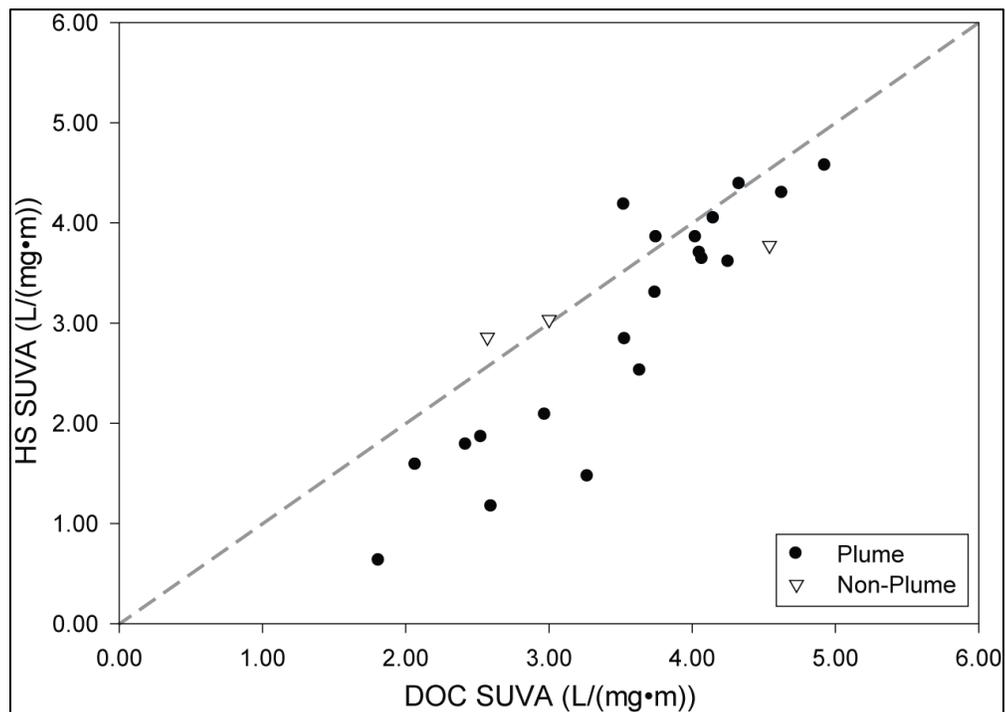
**Figure 3.14:** Spectral ratio of E<sub>2</sub>:E<sub>3</sub> versus groundwater age (days) for plume (both flowpaths; closed circles) and out of plume samples (open triangles).



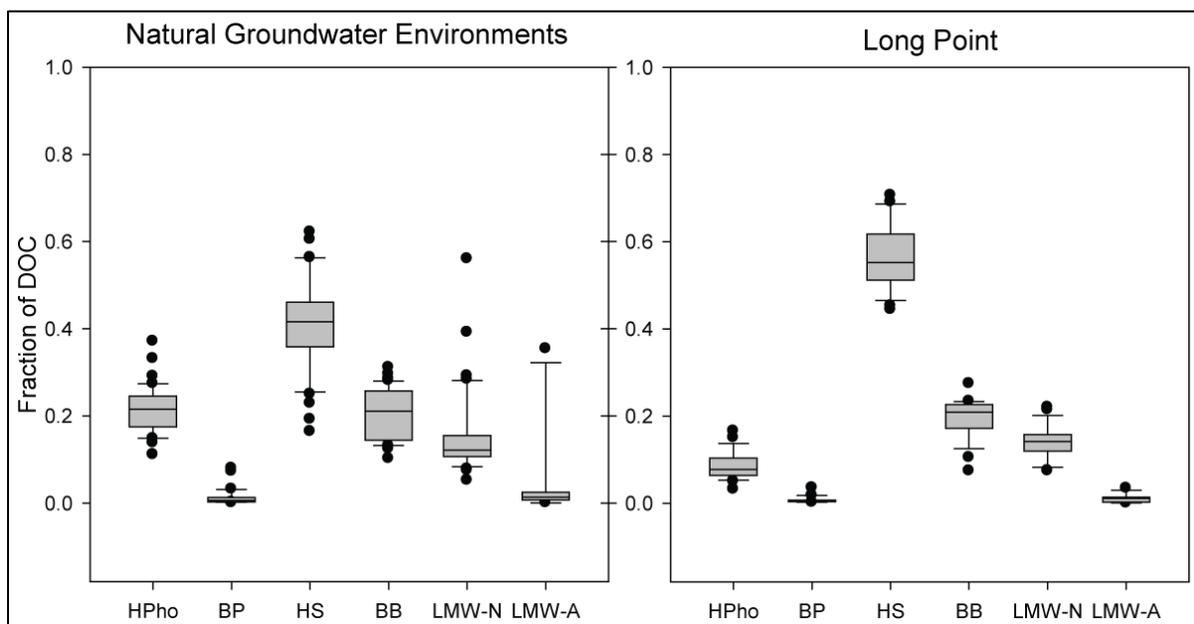
**Figure 3.15:** Humic substances bound dissolved organic nitrogen (µg-N/L) versus groundwater age (days) for the vertical flow path (left) and horizontal flow path (right). Closed circles represent samples in the plume, while open circles represent samples out of plume.



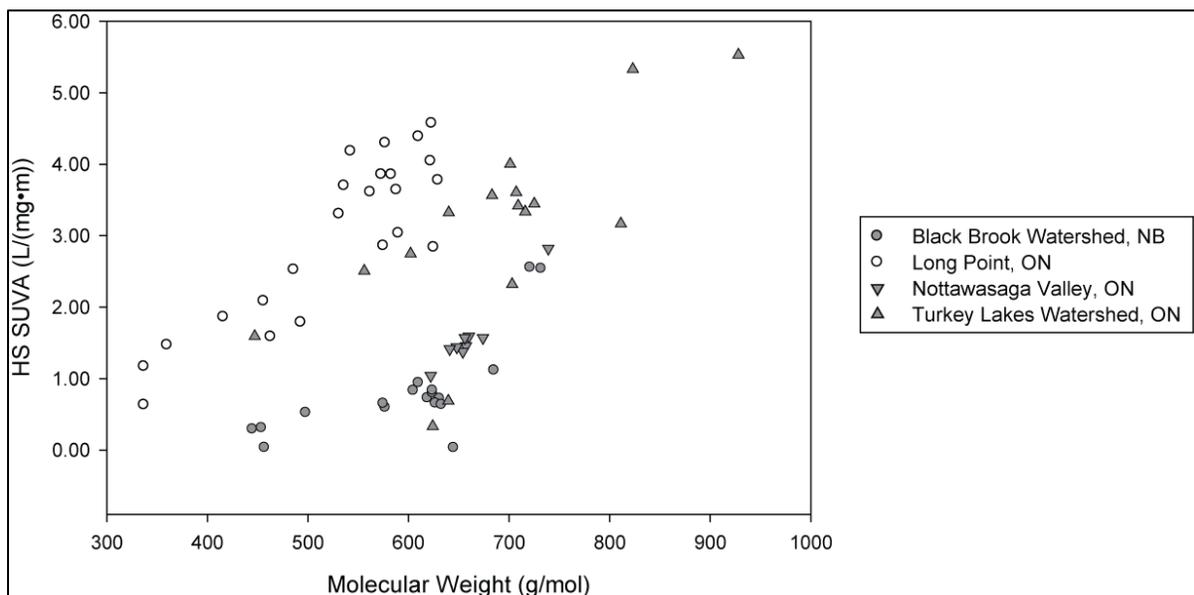
**Figure 3.16:** Ratio of humic DOC:DON versus groundwater age (days) for the vertical flow path (left) and horizontal flow path (right). Closed triangles represent samples in the plume, while open triangles represent samples out of plume.



**Figure 3.17:** Comparison of humic substances SUVA to dissolved organic carbon SUVA from samples in the plume (including both flow paths; closed circles) and samples out of the plume (open triangles). A 1:1 line is represented by the dotted grey line.



**Figure 3.18:** Boxplots illustrating the relative similarity of DOC compositions from other natural groundwater environments (n=43) to Long Point (n=23). Natural groundwater environments include samples from Nottawasaga Valley (ON; shallow aquifer), Black Brook Watershed (NB; deep aquifer), and Turkey Lakes Watershed (ON; variety of hydrological environments), depicting a variety of different environments. Each fraction is represented by the median, 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles, while dots represent outliers.



**Figure 3.19:** Comparison of humic substances SUVA (L/(mg·m)) and molecular weight (g/mol) from Long Point to a variety of other environments.

## **CHAPTER 4**

### **USE OF THE LC-OCD TO CHARACTERIZE DOM FROM A NUMBER OF SURFACE AND GROUND WATER ENVIRONMENTS**

#### **4.1 INTRODUCTION**

Although DOM has been characterized by the LC-OCD in wastewater treatment processes, it has not been applied as a method to characterize organic matter quality in the natural environment. A wide range of DOM characteristics have been observed when DOM is sampled from a variety of environments (Bourbonniere, 1989; Jaffé et al., 2008; Mueller et al., 2012) (Figure 4.1). The purpose of this study is to characterize natural DOM from a variety of both surface and ground waters, using the LC-OCD classification scheme. The comprehensive sampling of this study will provide a detailed look at natural DOM, as well as offer a basis for future studies to quantify long-term changes to the character of DOM.

##### **4.1.1 RESEARCH OBJECTIVES**

It is hypothesized that different environments will have different compositions due to the variety of sources and physical, chemical, and biological processes encountered in the environment. This will be explored through a four objectives:

- 1) To observe whether different surface water environments contain similar DOM compositions, by comparing LC-OCD chromatograms from three different surface water environments: streams, lakes, and rivers
- 2) To see whether different ground water environments contain similar compositions, by examining the changes to DOM with depth in a shallow aquifer, followed by an overall comparison of DOM at an aquifer scale, and finally observing the composition of DOM from an entire watershed
- 3) To see whether the aromaticity and molecular weight of HS can be used to distinguish different environments, determined by comparing the SUVA of HS (an indicator of its aromaticity) to its molecular weight

- 4) To see whether DOM from surface waters are compositionally different than ground waters, determined by comparing compositions of different hydrologic environments and observing whether certain fractions are more dominant than others.

## **4.2 SITE DESCRIPTIONS**

### **4.2.1 SURFACE WATERS**

#### *4.2.1.1 EXPERIMENTAL LAKES AREA, ONTARIO*

The Experimental Lakes Area (ELA; 49° 39' 40"N, 93° 43' 48"W, Figure 4.2) is situated in north-western Ontario, approximately 55km southeast from Kenora. Located within the Boreal forest, ELA has been extensively studied during whole-lake and long-term scientific experiments. The geology of the area consists of Precambrian bedrock (granite) with a discontinuous surficial layer of sandy-gravel till (Davies et al., 1965; Zoltai, 1965). Samples from both lakes and streams were collected between May and August in 2010 and 2011 (Table 4.1).

#### *4.2.1.2 GRAND RIVER, ONTARIO*

The Grand River Watershed (6,700 km<sup>2</sup>; 43° 30' 41"N, 80° 29' 43"W) is located in southern Ontario. The surrounding land is predominately of agricultural use. The Grand River (GR) flows ovetop Silurian limestone and dolomite (Ayers et al., 1971), with surficial deposits of glaciofluvial outwash and till (Barnett et al., 1991). Samples were taken along a 90km stretch beginning north of the City of Waterloo and ending just before the City of Brantford. Within this stretch, six wastewater treatment plants are located along the river. Samples were taken between February and November, 2011.

## **4.2.2 GROUND WATERS**

### *4.2.2.1 NOTTAWASAGA VALLEY, ONTARIO*

The Nottawasaga Valley (NW) was sampled approximately 5 km southeast of Alliston in southern Ontario (44° 7' 26"N, 79° 49' 12"W). The regional bedrock of the area is Middle Ordovician limestone and shale (Ayers et al., 1979), while surficial deposits are comprised of glaciolacustrine deposits (sand and gravel) (Barnett et al., 1991). Land use of the area is dominantly agricultural. Samples were collected in April 2011 from a single multi-level piezometer in the unconfined surficial sand aquifer. The depths sampled were at 4.35 m, 5.13 m, 5.94 m, 6.68 m, 9.90 m, and 11.26 m below surface.

### *4.2.2.2 BLACK BROOK WATERSHED, NEW BRUNSWICK*

The Black Brook Watershed (BBK) is found near St. André, New Brunswick (47° 6' 11"N, 67° 45' 40"W). The watershed covers an area of 14.5km<sup>2</sup> with land cover consisting predominantly of agricultural activities, especially potato production (Valentin, 2002, p. 6). The bedrock of the area consists of sedimentary rock from Ordovician deep marine clastics to Early Silurian carbonates (New Brunswick Department of Natural Resources and Energy, 2000). Surficial geology consists of till and small deposits of glacial outwash (Rampton, 1984). Samples were collected from twelve domestic wells and three multilevel piezometers between the months of May and June in 2011. Depths ranged from 6.1 m to 29.9 m below surface.

### *4.2.2.3 TURKEY LAKES WATERSHED, ONTARIO*

The Turkey Lakes Watershed (TLW; 47° 2' 54"N, 84° 24' 25"W) is a relatively undisturbed area found approximately 60km north of Sault Ste. Marie, Ontario. The watershed has been extensively studied by Environment Canada and the Canadian Forest Service for the effects of acid rain upon an undisturbed environment. The watershed is approximately 10.5 km<sup>2</sup>, and is located within the Great Lakes-St. Lawrence forest region (Rowe, 1972). The geology of the area consists of a Precambrian Bedrock (undifferentiated igneous and metamorphic rock) with small areas of surficial glacial deposits of glaciofluvial outwash and ice-contact

deposits (gravel, sand, till, and eskers) (Barnett et al., 1991b). A more comprehensive description of the geochemistry and instrumentation can be found within the literature (Craig & Johnston, 1988; Jeffries et al., 1988; [www.tlws.ca](http://www.tlws.ca)). Samples were taken in May of 2011 from a variety of ground water locations to capture a comprehensive representation of the watershed. Specifically, these areas can be classified into five groups: 1) lake shore, 2) headwater (near watershed boundary), 3) wetland, 4) upland wetland (near watershed boundary), and 5) slope draining the wetland. Depths ranged from 0.90 m to 6.89 m below surface.

### **4.3 METHODS**

#### **4.3.1 FIELD METHODS**

Surface water samples were collected approximately 30 cm under the surface, while deeper samples required the use of a Greylor PQ-12 gear pump. Ground water samples were taken from wells that were pumped into a flow-through cell until a stable electrical conductivity and temperature reading were measured. Samples were filtered using a 0.45  $\mu\text{m}$  filter and collected in 40 mL glass vials. In some instances, duplicates were taken in order to test the reproducibility of the LC-OCD. Samples were stored in the dark at  $<4^{\circ}\text{C}$  and measured no more than a week after sampling.

#### **4.3.2 LC-OCD ANALYSIS**

A full description of the LC-OCD and its technical analysis can be found in Chapter II. Duplicates from numerous samples were used to obtain a precision of the LC-OCD. Here, standard deviations for all fractions were less than  $\pm 0.20$  mg/L and were less than  $\pm 0.08$  L/(mg·m) and  $\pm 10$  g/mol for HS SUVA and molecular weight, respectively.

## 4.4 RESULTS

### 4.4.1 SURFACE WATERS

Overall, surface waters contain DOC concentrations from 3.54 to 38.7 mg/L (n=70, Figure 4.3). Surface waters can be divided into three categories by location sampled: ELA lakes, ELA streams, and the Grand River. Concentrations of DOC are highest from ELA streams (n= 12, average: 16.7 mg/L, standard deviation:  $\pm 9.28$  mg/L), but relatively similar between ELA lakes (n=11,  $8.70 \pm 2.69$  mg/L) and the GR (n=47,  $6.70 \pm 2.01$  mg/L). Among all three types, the majority of the DOM is made up of HS (45 to 62 %), with BP, BB, LMW-N making up the other 30%. Low molecular weight acids rarely comprise more than 2% of the total DOM. Furthermore, a wide range of nominal average molecular weights (545 to 844 g/mol) and aromaticities (2.57 to 6.22 L/(mg·m)) of HS are found (Figure 4.4).

DOM from ELA lakes are mainly comprised of larger molecular-weight compounds (HS:  $46 \pm 9\%$ ; BB:  $19 \pm 3\%$ ) (Figure 4.5). The DOM from ELA lakes not only varies among lakes, but also with depth (Figure 4.6). Profiles from L227 and L442 indicate an increasing DOC concentration (increasing by 3.47 mg/L and 0.24 mg/L, respectively) and proportion of BP (17% and 6%, respectively). This occurred concomitantly with a decreasing proportion of HS (-12% and -1%, respectively).

Differences in DOM composition can be seen between ELA streams flowing through different environments: wetland (surrounded by a high amount of organics) or upland bedrock (little organics). Generally, wetland streams that have similar concentrations to that of the upland stream are compositionally similar (Figure 4.7). Furthermore, HS from upland streams have higher aromaticities ( $5.62 \pm 0.57$  L/(mg·m)) than wetland HS ( $4.77 \pm 0.92$  L/(mg·m)) (Figure 4.8). Alternatively, the molecular weights of HS are relatively similar (upland:  $764 \pm 54$  g/mol; wetland:  $769 \pm 50$  g/mol).

Samples from multiple trips (five trips throughout the year) were averaged for each site along the Grand River. (n=47; Figure 4.9). Although the concentration of DOC decreases from  $8.12 \pm 1.21$  mg/L upstream to  $5.41 \pm 1.17$  mg/L downstream, no change is seen in the overall composition. The highest proportion belongs to HS (59 to 66%) and BB

(12 to 14%), with relatively similar proportions of and BP (7 to 10 %) and LMW-N (8 to 10%). No change is seen in the HS aromaticity; however, although within the standard deviation, the HS molecular weight decreases from  $710 \pm 117$  g/mol to  $659 \pm 100$  g/mol along the entire flow path (Figure 4.10). An elevated DOC concentration ( $6.89 \pm 1.56$  mg/L) is found at the Blair site, which also has higher proportions of BP (10%) and BB (13%), and the lowest proportion of HS (59%).

#### 4.4.2 GROUND WATERS

Ground water data collected from this study (n=43) can be separated into three groups by location: un-impacted watershed (TLW), shallow agriculturally-impacted aquifer (NW), and deep agriculturally-impacted aquifer (BBK). The highest DOC concentration is found from TLW (n=16;  $2.87 \pm 3.75$  mg/L) and is followed by NW (n=9;  $2.00 \pm 0.46$  mg/L), while BBK contains the lowest concentrations (n=18;  $0.79 \pm 0.57$  mg/L) (Figure 4.11). Together, HPho (19 to 22%) and HS (39 to 43%) generally comprise the largest portion among all three groups, followed by BB (18 to 24%) and LMW-N (13 to 18%). Furthermore, BP (0 to 2%) and LMW-A (0 to 6%) are rarely found in ground water environments (Figure 4.12). The HS found from TLW are more aromatic ( $3.05 \pm 1.40$  L/(mg·m)) than either NW ( $1.59 \pm 0.49$  L/(mg·m)) or BBK ( $0.82 \pm 0.69$  L/(mg·m)), but the wide range in molecular weights makes it difficult when comparing sample locations (Figure 4.13).

The data from NW are best described when compared with depth. The DOC concentration decreases ( $-0.89$  mg/L) in the upper portion of the aquifer (~4 to 6 m) (Figure 4.14). In addition, the deepest point has an elevated DOC concentration (3.04 mg/L), as well as the highest aromaticity ( $2.82$  L/(mg·m)) and molecular weight (739 g/mol). Both the aromaticity and molecular weight of HS decreases within the first six meters ( $-0.54$  L/(mg·m) and  $-45$  g/mol, respectively), at which point they both increase the following three meters ( $0.45$  L/(mg·m) and  $26.5$  g/mol, respectively).

Although having the lowest DOC concentration, there exists a wide range of compositions from the BBK data. There is little overlap among different fractions, with the majority of BBK DOM consisting of HS ( $50 \pm 9\%$ ), BB ( $21 \pm 7\%$ ) and HPho ( $22 \pm 4\%$ ), with a small amount of LMW-N ( $13 \pm 5\%$ ). Like other ground water environments, there is little BP

(1 ±2%) or LMW-A (1%) (Figure 4.11). Furthermore, depth does not appear to affect the composition (Figure 4.15). However, HS contain a low aromaticity ( $0.81 \pm 0.69$  L/(mg·m)), but moderate SUVA ( $2.29 \pm 0.88$  L/(mg·m)), indicating most of the UV-absorbing components are not found from the HS (Figure 4.13).

Ground water samples from TLW were collected from a variety of different environments, which can be seen from the wide DOC range (0.94 to 16.4 mg/L) (Figure 4.16). The highest concentration is found from a shallow well near the shore of the lake, containing a high proportion of HS (56%) and HPho (26%), and less than 10% of any other fraction. The lowest concentration originated from the upland wetland, which contained low HS (35%) and high LMW-A (35%).

#### **4.4.3 AROMATICITY AND SUVA**

The comparison of HS aromaticity and DOC SUVA provides additional details of DOM. If the HS SUVA is equal to the DOC SUVA, it can be thought that HS are the main UV-absorbing component of the DOM. If the HS SUVA is higher than the DOC SUVA, then the DOM contains less UV-absorbing structures, while a lower HS aromaticity would indicate that the DOM contains more UV-absorbing structures.

Surface water HS contain a positive relationship with the DOC SUVA (Figure 4.17). Furthermore, HS SUVA values are higher than the overall SUVA at lower concentrations. ELA lakes fall above the 1:1 line, while GR and ELA streams fall relatively closer. Ground water samples illustrate less of a relationship between HS SUVA and DOC SUVA than surface water samples (Figure 4.17). In addition, most of the HS SUVA values are lower than the DOC SUVA.

#### **4.4.4 HYDROPHOBIC PARAMETER**

The hydrophobic (HPho) portion is not determined through SEC elution of the sample. This fraction is defined as the DOM that remains on the column as a result of hydrophobic interactions (non-chromatographable DOC), which can be speculated to include lipids, lipoids, and hopanoids (Batsch et al., 2005; Ciputra et al., 2010; Huber et al., 2011). At the sites, natural DOM consisted of up to 30% HPho, indicating a large portion is relatively

unclassified by the LC-OCD (Figure 4.3; Figure 4.11). Furthermore, no relationship exists between relative proportion and concentration. It is possible that due to the nature of which HPho are calculated, noise in the baseline may result in a small residual error (possibly integrating  $\pm 0.1$  mg/L to the overall DOC concentration). However, this error would not affect the overall composition of other fractions. Overall, the concentration of HPho determined from the LC-OCD should be taken with caution since it does not represent a fraction that has been eluted through the column.

## **4.5 DISCUSSION**

### **4.5.1 QUALITY OF SURFACE WATER DOM**

Among these surface water sites, there is a wide range of DOM concentrations (3.5 to 38.7 mg/L) and a difference in the LC-OCD chromatograms, indicating that the composition of DOM is variable among hydrological environments. Generally, the high proportion of humics is not surprising since the majority of DOM is known to consist of humics (Thurman, 1985). However, samples that contain similar concentrations can be compositionally different. The difference in compositions may arise from a number of processes that sorbed or degraded DOM. To better understand the different compositions, it is best to explore each environment from which the sample was taken.

#### *4.5.1.1 STREAMS – CLOSER TO THE TERRIGENOUS DOM SOURCE*

Decomposing organic matter creates DOM that either leach through the soil or be transported by surface runoff into streams, lakes, or rivers. Streams contain the highest proportion of HS, but lowest proportion of all other components. Rapid transportation of DOM may not allow much degradation time, which may account narrow spectrum of DOM components. Furthermore, differences between the flow paths of each stream may contribute to different compositions. For example, the higher DOC concentration of upland streams (flowing directly on bedrock) indicates a composition different than wetland streams (flowing through soil layers) with lower concentrations. Furthermore, the decreasing of HS SUVA and molecular weight in wetland samples further illustrates

characteristic changes to the DOM, likely from microbial interactions. Humics with a low SUVA and molecular weight generally indicate *in-situ* microbial activity (Huber et al., 2011). Similarly, wetland streams with the same concentration as upland streams are compositionally (and HS characteristically) similar. These trends indicate a continuum of DOM composition, where the degradation of DOM would likely lead to degraded HS and increased amounts of BP, BB, and LMW-N.

#### *4.5.1.2 IMPACTED RIVERS – TRANSPORTATION AND AUTOCHTHONOUS PRODUCTION OF DOM*

Samples from the Grand River contained different proportions of HS and BB than other surface water environments. Samples from different seasons did not show any difference in composition, thus GR data illustrates a year-averaged composition. The higher proportion of BP than streams indicates there must be a higher microbial influence within the river. However, little change is seen in composition over the flow path. This was not expected since samples were taken both above (WM, BR, VIC) and below (BL, FB, GM, BCA) a wastewater treatment plant (Figure 4.9). Although there is a slight increase in DOC concentration and BP proportion, composition remained relatively similar. Furthermore, Hutchins (2011) found an increase in autochthonous characteristics along the Grand River, yet this was not apparent in the LC-OCD data. A possible explanation may be that the autochthonous production may not affect DOM composition, which means the LC-OCD analysis may not be well suited for this determination. Alternatively, since samples from this study did not extensively study the upper reaches of the Grand River, it may be possible that the autochthonous signature is found both above and below the wastewater treatment plant. Instead, the HS-Diagram would be able to distinguish differences in HS character. In support, the HS molecular weight decreased along the flow path, which indicates a change in the character of HS.

#### *4.5.1.3 LAKES – A CONVERSION PLACE FOR DOM*

The wide range of concentrations and compositions was expected when comparing different lakes. The inter-variability in DOM composition among different lakes can result

from watershed or local lake and catchment characteristics, as well as differences in residence time (Curtis & Schindler, 1997; Sobek et al., 2007; Mueller et al., 2012). All lakes are located within the same environment, which is illustrated by relatively similar compositions. For example, proportions remained within 10% for the majority of DOM fractions. However, local conditions may influence certain components, which can be illustrated by one lake containing a high proportion of BP (~35%) whereas all other lakes contained less than 15% BP.

Lakes were expected to have a different DOM composition than rivers and streams as they represent a sink for DOC (Schiff et al., 1990). Although lakes represent a location for DOM accumulation, the recalcitrant HS were found in lower proportion than the other environments. This could be explained by the presence of processes that alter DOM, such as UV irradiation, microbial transformation, and flocculation (Frimmel, 1998; Bertilsson & Tranvik, 2000; Tranvik & Bertilsson, 2001; von Wachenfeldt et al., 2008). Studies have determined that photochemical transformations of humics degrade DOM into more labile components (de Haan, 1993; Moran & Zepp, 1997; Bertilsson & Tranvik, 2000; Sulzberger & Durisch-Kaiser, 2009). Furthermore, irradiation of natural organic matter (NOM) increased LMW substances (both neutrals and organic acids), which paralleled an increase in biodegradability of NOM (Allard et al., 1994; Wetzel et al., 1995; Dahlén et al., 1996; Frimmel, 1998; Espinoza et al., 2009). The photodegradation of HS and BB in lakes (and possibly all surface waters) may allow increased DOM degradation by microbes, which may also increase the proportion of BP. The photodegradation of HS is also observed from the difference in HS SUVA and molecular weight, where higher molecular weights and SUVAs of stream HS could be attributed to the lower amount of UV degradation when compared to lakes. However, these results only illustrate differences among lakes, and do not address differences within a lake.

Lake profiles illustrate different DOM compositions between the surface and bottom of lakes. As mentioned earlier, the surface of the lake is affected by UV-irradiation, contributing to the difference in composition. However, the relative decrease of HS at the bottom of both lakes indicates that HS may be used for microbial degradation. The

anaerobic environment found at the bottom of lakes (occurring at a depth of 4 m for L227 and 13 m for L442) likely allows for the degradation of humic matter (Tranvik, 1998). In addition, the elevated DOC concentration indicates an accumulation of BP molecules, which would likely result from the biosynthesis of organic matter within the water column.

## **4.5.2 QUALITY OF GROUND WATER DOM**

Although ground waters do not have UV-irradiation to break down DOM, the subsurface provides plenty of opportunity for a number of physical, chemical, and biological processes, which can have a significant effect upon the concentration and composition of DOM (Thurman in Aiken et al., 1985; Schiff et al., 1990, 1997; Sun et al., 1997; Ludwig et al., 2000; Guo et al., 2010). Thus one would expect higher proportions of lower weight DOM (Maurice et al., 2002). For instance, Amon et al. (1996) determined that biodegradation of DOM formed smaller and less bioavailable molecules. Conversely, Westerhoff and Pinney (2000) found LMW to be preferentially removed during soil column experiments. From the data, one is able to see a large range of compositions, thus looking at each site individually allows for a better understanding to the differences in composition.

### *4.5.2.1 NOTTAWASAGA – DOM WITH DEPTH*

The NW site allows for a detailed examination of DOM with depth in an unconfined, shallow aquifer with mostly horizontal flow. Interestingly, DOM composition varied as a function of depth. Most of the DOM is sorbed within the first meter of the subsurface (Wassenaar et al., 1989; Marmonier et al., 1995), illustrating the effectiveness of subsurface environments in causing DOM loss. Unexpectedly, the lowest depth not only contained the highest concentration of DOM, but also was comprised of a different composition than the DOM above. Specifically, the high amount of HS (~60%) indicates this may be a different source of degrading organic matter, representing DOM from either a different ground water flow path or stagnant area.

Throughout the rest of the vertical profile, the decrease in DOM altered the overall composition, which led to an increased proportion of HS. The concomitant decrease in both LMW-N concentration and proportion indicates this fraction is preferentially removed. It

has been suggested that DOC infiltrating through the soil is subjected towards a microbial ‘filter’ that conforms it into a certain composition (Wickland et al., 2007). This diagenetically altered DOM becomes less bioavailable as a result of the reworking by soil microbes (Sun et al., 1997). Hence, the decrease in DOM concentration leads towards a more recalcitrant composition, which is observed in the data.

The low amount of BP may be attributed to the fast biodegradation of this fraction as it infiltrates towards the ground water (Grünheid et al., 2005). Alternatively, it may be that the BP are present, but not found in the water sampled. Biofilms may coat the grains within the subsurface, and thus may not be sampled from the collected water (Marshall, 1988; Griebler et al., 2002; Flemming et al., 2007). Furthermore, it is possible that the relatively large size mechanically inhibits it from moving easily within the subsurface (Lindroos et al., 2002).

This site illustrates the range of DOM compositions from a single multi-piezometer reach; thus on a larger scale it is expected that a larger range of compositions and concentrations would be found, since different sources and processes would inherently be sampled.

#### *4.5.2.2 BLACK BROOK WATERSHED – DOM AT THE AQUIFER SCALE*

The data from BBK illustrates a range of DOM compositions within an aquifer. Furthermore, BBK provides samples of deep, low concentration DOM. Deep samples would have longer residence times than shallower depths, allowing for longer interaction within the subsurface. Surprisingly, a number of different compositions are still found. Similar to the deepest sample at NW, DOM throughout the aquifer mainly consists of HMW (HS and BB) molecules. However, depth does not constrain the range in compositions, which can be seen by the spread in HS proportion (30 – 56 %). Combining all HMW molecules (HS, BB, and BP), it can be seen that HMW molecules decreased with depth while LMW molecules slightly increased (Figure 4.18). The larger amount of HMW fractions to LMW fractions may be due to the relative stability of larger, aromatic molecules (Sun et al., 1997).

By looking at both agriculturally-impacted aquifers (NW and BBK), HS SUVA from these environments contained the lowest of all the sampled environments. Two scenarios

may attribute towards such low aromaticities from these locations. First, both environments are agriculturally-impacted, thus it is possible that the high input of inorganic nutrients (such as nitrate) increases the degradation of DOC. In the presence of labile carbon, denitrifying bacteria are able to reduce the amount of nitrate in a reducing environment (Knowles, 1982), which means the uptake and reworking of DOC may account for the low aromaticities of HS. Heavily reworked DOM from deep marine waters was also found to have low aromaticities (Dittmar & Kattner, 2003). The alternative scenario is the sorption of aromatic HS within the subsurface, leaving DOM with lower aromatic constituents. Lower aromaticities have been linked to preferential adsorption of HMW substances (Meier et al., 1999; Westerhoff & Pinney, 2000).

#### *4.5.2.3 TURKEY LAKES WATERSHED – WATERSHED INFLUENCES ON DOM COMPOSITION*

Data collected from the TLW allows for the characterization of ground water DOM from a number of different local environments. Typically, ground water concentrations are expected to be lower than 2 mg/L (Thurman, 1985). However, one sample from TLW contained a DOC concentration of 16.7 mg/L, supporting the idea of recently formed DOM. In addition, the high HS and low LMW-N composition suggests a relatively young composition (Amon & Benner, 1996). Furthermore, the HS characteristics contained high SUVA and molecular weights, which are characteristic of pedogenic origins rather than microbial-derived HS (Huber et al., 2011), furthering the idea of recently degraded material.

As expected, the variety of different environments sampled led to a wide range in both DOM composition and HS characteristics within TLW. Fellman et al. (2008) observed the lability of DOM to be related with the quality of the precursor material. In addition, O'Donnell et al. (2010) found the concentration and composition of DOM within a stream to be strongly dependent upon the source of water. It is likely that the various environments within the watershed provide differing qualities of DOM, in addition to the high amount of heterogeneity associated with DOM.

Among ground waters, samples from TLW contained the highest proportion of BP and LMW-N. Specifically, wetlands were responsible for most of the high values. For example, the highest proportion of BP (~10%) was found to originate only from wetlands

near the edge of the watershed. Furthermore, wetlands also contributed a large amount of LMW molecules, which has been previously observed and related to a recalcitrant form of 'polymeric LMW molecules' that remains in solution as more labile, HMW molecules are degraded (Chin et al., 1998). These results suggest that wetlands may be responsible for contributing a recalcitrant form of DOM into the environment.

#### **4.5.3 CHARACTERISTICS OF HUMIC SUBSTANCES**

The HS-Diagram may give an indication to either the source or the degree to which the HS have been reworked. The fact that no samples are found near the IHSS-HA standard indicates that such molecule arrangements are rarely found within the natural environment. Instead, surface and ground water HS can be grouped based upon their environment, leading to the idea that the LC-OCD can be used to comparatively group different environments based upon the molecular weight and aromaticity of HS.

The differences between surface and ground water HS aromaticity versus DOC SUVA were unexpected. Surface water HS are in general agreement with the DOC SUVA, illustrating that HS may dominate the UV-absorbing components within DOM. However, ground water HS are lower than the DOC SUVA, which may result from the subsurface sorption and degradation of HMW molecules. In addition, lower HS molecular weights can be found in groundwaters, indicating a higher degree of reworking. Regardless, the data illustrates that the subsurface environment exerts different controls upon the DOM than surface waters, allowing the LC-OCD to distinguish between surface and ground water HS.

#### **4.5.4 DOM AMONG HYDROLOGICAL ENVIRONMENTS**

The large range in compositions can be attributed to the number of different sources and processes that are found among different hydrological environments (Figure 4.19). Humics substances comprise the largest portion of aquatic DOM, which is similar to other studies (Aiken et al., 1985; Thurman, 1985; Grøn et al., 1996; Sachse et al., 2001). The combination of being comprised of hard to break down compounds, products of microbial decay, and sorption-desorption processes, may account for HS persisting within the environment (McKnight & Aiken, 1998; Frimmel, 2003; Hur & Schlautman, 2003). However, it is possible

for HS to be utilized by bacteria (Hunt et al., 2000; Kalbitz et al., 2003b). Photolytic degradation of humics results in a slight increase in the BB fraction (Espinoza et al., 2009). In addition, microbial degradation has been found to enhance HS-like structures (Hur, 2011), which would be comparable to the BB fraction from the LC-OCD. The presence of BB could then indicate that HS are being actively degraded within the natural environment. The LC-OCD allows for quantification of relatively lower weight humics (BB), which illustrates the degradation of large humic substances.

The largest size fraction (BP) comprises relatively little of the total DOM, which may be due to the relative lability of its components: polysaccharides, proteins, and sugars (Ciputra et al., 2010; Huber et al., 2011). These molecules are mainly found in surface waters (particularly lakes and the GR), while in ground waters, only found in wetland-like environments. It has been found that two contradictory trajectories exist for organic matter decomposition in aquatic ecosystems. The first is the degradation of HMW substances into LMW substances, whereas the other is the microbial formation of HMW substances and biomass (Frimmel, 1998; Tranvik & Bertilsson, 2001). The availability of biodegradable, LMW molecules is what allows for the synthesis of larger, organic molecules. In this case, the abundance of the BP fraction would be most representative of such a process occurring, unless BP are rapidly consumed at an equal rate of formation. Furthermore, proteins and polysaccharides (included within BP) make up the housing materials for bacteria (termed biofilms or extracellular polymeric substances) (Costerton et al., 1995; Flemming et al., 2007). Thus, a higher proportion of this fraction, which appears to be an attribute of some surface waters while possibly sorbed within ground waters, can indicate microbial re-working of DOM.

The extremely low appearance of LMW-A suggests that this fraction is altered (biologically, chemically, or physically) or is never produced within the natural environment. Conversely, LMW-N appears to be found in greater proportions, possibly indicating a higher degree of recalcitrance than acids. In support, recalcitrant marine DOM has been found to consist of high amounts of LMW neutral molecules (Dittmar and Kattner, 2003). Whether or not LMW-N comprise a labile portion of DOM is likely a

function of the source material from which the DOM is derived, in addition to the amount of reworking upon the DOM.

The wide range of HS characteristics from both surface and ground water environments further illustrates the amount of heterogeneity associated with characterizing DOM. In this study, the data illustrate that surface waters generally have a higher aromaticity than ground waters. Furthermore, the IHSS-FA standard appears to be the limit of the data set. The higher aromatic structures in surface water may indicate a lower degree of reworking than ground waters, due to the relative stability of aromatic compounds (Kalbitz et al., 2003; 2003b). However, low aromaticities do not necessarily imply the unavailability of HS. UV-induced transformations have also been observed at the interface between streams and ground water, where microbial utilization of ground water DOM was aided by its photodegradation (Fasching & Battin, 2012).

Interestingly, proportions of each fraction remain relatively constrained across a range of DOM concentrations. For instance, BB rarely comprise more than 30%, while the majority of HS are found above 40% of the total DOM. Such a result indicates that, overall, there are certain limits to each fraction within the environment. Regressions of the fraction proportions to DOM concentrations gave  $R^2$ -values above 0.70 for HS, BB, and LMW-N (Figure 4.20). The relationship indicates higher concentrations of DOM are dominantly comprised of those components. However, as concentrations increase, HS are the only fraction to increase in proportion within the DOM. The fractions not influenced by DOM concentrations are BP and LMW-A, leading to the idea that these are dependent upon the source or external processes reworking the DOM. From these results, it can be thought that organic matter degrades into three main classes (HS, BB, and LMW-N), which are then subsequently altered, consumed, or synthesized to possibly form BP or other LMW-N molecules. Furthermore, LMW-A components are likely unstable within the natural environment, thus an external process is needed to form these constituents.

#### **4.5.5 USEFULNESS OF THE LC-OCD**

The LC-OCD provides a fast, in-depth, and reproducible characterization of natural DOM. Pairing OCD and UVD analysis allowed for the complete characterization of DOM, instead

of a subfraction or surrogate (such as UV absorbance alone). Although the LC-OCD cannot give concentrations of specific functional groups, it can quantify six size fractions and their relative proportions within DOM. The LC-OCD is still an empirical method, yet in order to characterize the thousands of molecules that comprise DOM, one would likely end up using an empirical method. Furthermore, except for dilution before analysis, little preparation needs to be done on the sample. However, using a similar setup as the LC-OCD, Ruhl & Jekel (2012) determined some LMW-N molecules to elute much earlier than the designated time. This illustrates that the defined fractions are not absolute, but offer a simple method that can be used to compare among environments.

Since the LC-OCD uses UV-absorbance to characterize the HS, it is important to understand that inorganic species within the sample may affect the overall UV absorbance. Studies have found that nitrate ( $\text{NO}_3^-$ ) and iron (as either  $\text{Fe}^{2+}$  alone or  $\text{Fe}^{3+}$  complexing with humics) can effectively absorb UV, possibly skewing the results (Dilling & Kaiser, 2002; Weishaar et al., 2003; Maloney et al., 2005). However, nitrate has a peak at 220nm (whereas UVD is at 254nm), indicating that only large concentrations (>40mg/L) of  $\text{NO}_3^-$  would influence the signal (Weishaar et al., 2003). Thus, samples containing high concentrations of nitrate or iron will not have a 'true' UV-absorbance for DOM, which must be taken into account when comparing HS characteristics to other environments.

It is possible to see the LC-OCD proved useful in quantifying natural DOM within a variety of environments from the data collected in this chapter. Differences among hydrological environments were quantified, illustrating that the LC-OCD can be a powerful tool to quickly and easily determine specific changes to the DOM. Furthermore, the simplicity and ease of sampling in the field further supports the use of such a method in future characterization of natural DOM.

#### **4.5.6 FUTURE RESEARCH**

Although this study provides an extensive DOM data set, it does not address changes with seasonality. This study focused upon sampling a wide range of hydrological environments, since localized conditions may have a greater influence towards the overall character (Bourbonniere, 1989). It has been observed that DOM changes with season in concentration

and quality (Fellman et al., 2008). In addition, Sachse et al. (2001) determined differences in DOM composition as a result of variations in seasonal allochthonous input. It would be worthwhile to explore these compositional differences, in order to determine the annual variability in DOM character.

Variability of DOM characteristics in surface waters can be attributed to the differences in watershed characteristics. Mueller et al. (2012) observed such differences among a set of lakes, which was attributed to the relative percentages of surface water, rock outcrops, and vegetative cover of the watershed. Natural cover was not measured in this study, but already the high proportion of LMW-N at TLW suggests high occurrences of wetlands may create LMW-enriched DOM. Furthermore, it would prove interesting to further explore whether HS contain unique signatures (such as specific molecular weights or SUVA values) as a result of the surrounding watershed characteristics.

The difference in discharge within a river can also affect the quantity and quality of DOM. For instance, changes to bacterial abundance were observed during increased discharge, which affected the uptake of certain sizes of DOM (Sabater et al., 1993). This is not seen in the data from the Grand River, where concentrations and proportions of each fraction remain relatively similar between sampling sites.

## **4.6 CONCLUSION**

The LC-OCD provides a fast, reproducible, and detailed determination of DOM components. Surface and ground waters contain a wide variety of DOM compositions, with surface waters having an overall higher DOC concentration than ground waters. Interestingly, regardless of the composition, proportions of each component appear to be constrained within certain intervals. The majority of DOM is made up of HS, with the remainder consisting of degraded humics (BB) and LMW-N molecules. Furthermore, a wide range of humic characteristics are found within the environment, yet can be grouped according to the environment from which it was found. Surface water DOM can be distinguished from ground water by containing detectable amounts of BP and LMW-A. Streams provide rapid transportation of DOM, allowing for little degradation to occur, whereas in lakes, UV irradiation and microbial decomposition appears to play an important

role in breaking down and transforming HS. Furthermore, autochthonous production of DOC within an impacted river illustrates an increased amount of BP compared to other environments. Ground waters appear to exert a higher amount of reworking upon the DOM, due to infiltration through the upper subsurface. Here, physical, chemical, and biological influences create a more mixed DOM composition, compared with surface waters. Although the ground water contains higher HPho than surface water, there is little data or literature over what this fraction may be comprised of, or the influence it may have over the character of DOM. Overall, the LC-OCD proves to be a useful tool in characterizing DOM. Use of this classification scheme provides a quantitative tool of natural DOM, yet still allowing for a measure of the amount of heterogeneity that is inherent with natural DOM.

**Table 4-1:** Site descriptions for both surface and ground waters. Includes sample ID, number of samples, and a brief description of the surrounding environment where the sample was taken.

<b>SURFACE WATERS</b>		
<b>Sample</b>	<b>n</b>	<b>Environment Description</b>
<i>Experimental Lakes Area (ELA)</i>		
L303, L304, L373, L239, L114, L661, L373, L227, L239, L626, L442	11	Lakes through-out ELA
L227 - 0.5m, 4m, 6m, 8m, 10m	5	Lake, profile with depth
L442 - 0.5m, 5m, 9m, 11m, 13m, 16.5m	6	Lake, profile with depth
L626 Outflow, L626 Wetland A+B Outflow, L626 Wetland B Inflow, L239 EIF, L239 NWIF, L239 NEIF	9	Streams running through wetlands, surrounded by organic matter
U1, U3, U8	3	Upland Streams; running atop bedrock
<i>Grand River (GR)</i>		
West Montrose	5	Most upstream location (0km)
Bridgeport	4	River sample
Victoria	3	River sample
Blair	5	After Wastewater Treatment Plant
Footbridge	4	River sample
Glen Morris	4	River sample
Brant Conservation Authority	5	Most downstream location (90km)
<b>GROUND WATERS</b>		
<b>Sample</b>		<b>Environment Description</b>
<i>Turkey Lakes Watershed (TLW)</i>		
BT3 - 2.34m, 5.24m	2	Lake shore, boreal lake,
H10 - 1.67	1	Wetland, edge of lake
W0 - 0.74m, 2.90m; W1 - 5.34m, 6.89m; W3 - 3.74m	5	Upland wetland, near watershed boundary
H1 - 0.90m, 1.81m	2	Headwater, near watershed boundary, steep slopes
PZ 95, 98, 100	3	Wetland
PZ 022, 023, 045	3	Slope draining wetland
<i>Black Brook Watershed (BBK)</i>		
DW18 - 1; DW18 - 2	4	Domestic well; near stream
DW - 19	2	Domestic well; opposite of DW 18
MW 1; MW 2	2	Municipal water well
PZ 1 - 3; PZ 1 - 4	2	Upstream transect perpendicular to Black Brook
PZ 2 - 3; PZ 2 - 4	2	Upstream transect perpendicular to Black Brook
PZ 3 - 1; PZ 3 - 2; PZ 3 - 3	3	Downstream transect perpendicular to Black Brook

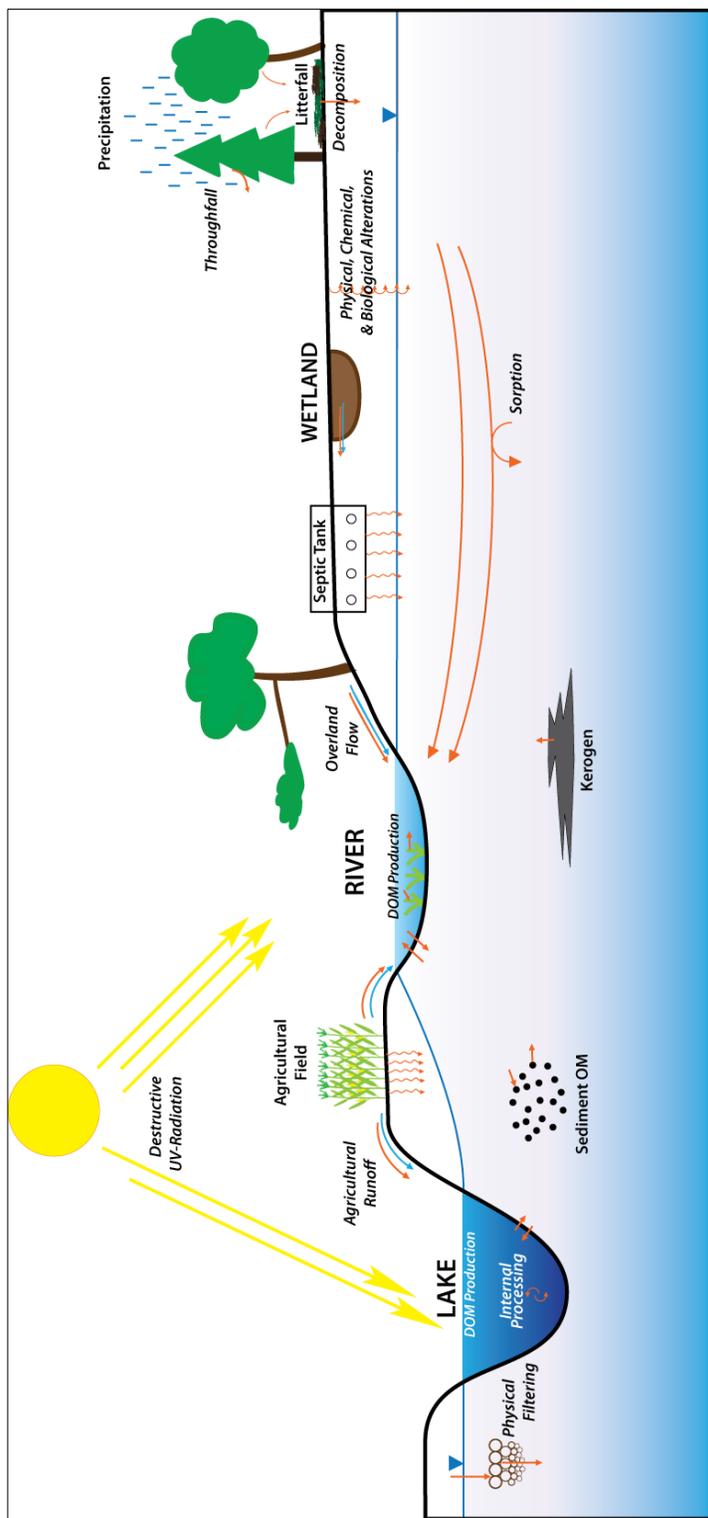
DW - 09	1	Edge of watershed boundary
DW - 01	1	Domestic well; end of watershed
DW - 03	1	Outside of watershed; upgradient stream which Black Brook connects with

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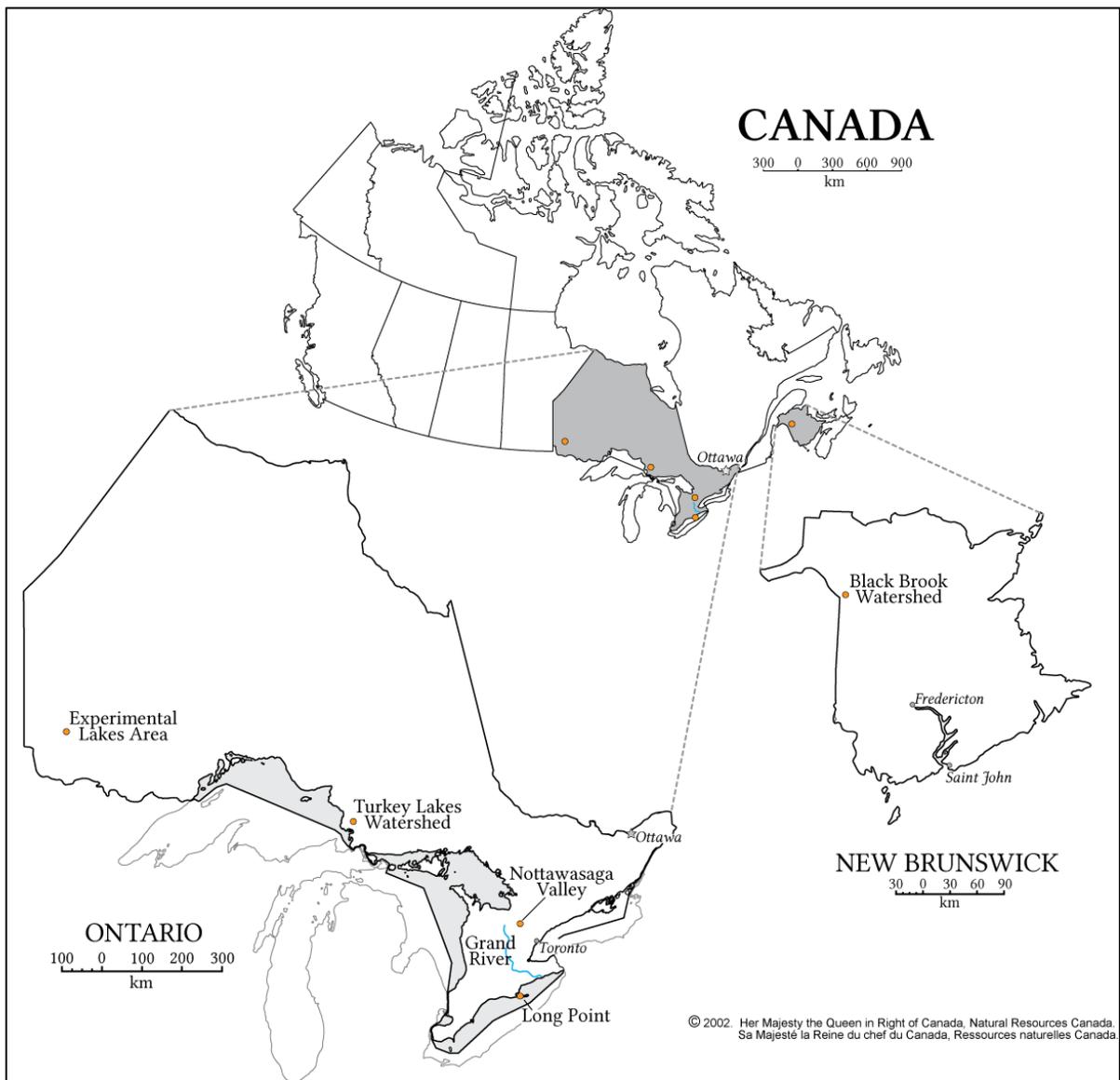
*Nottawasaga Valley (NW)*

MC2 - 4.34m, 5.13m, 5.94m, 6.68m, 9.90m, 11.26m	6	Multilevel piezometer; unconfined sand aquifer
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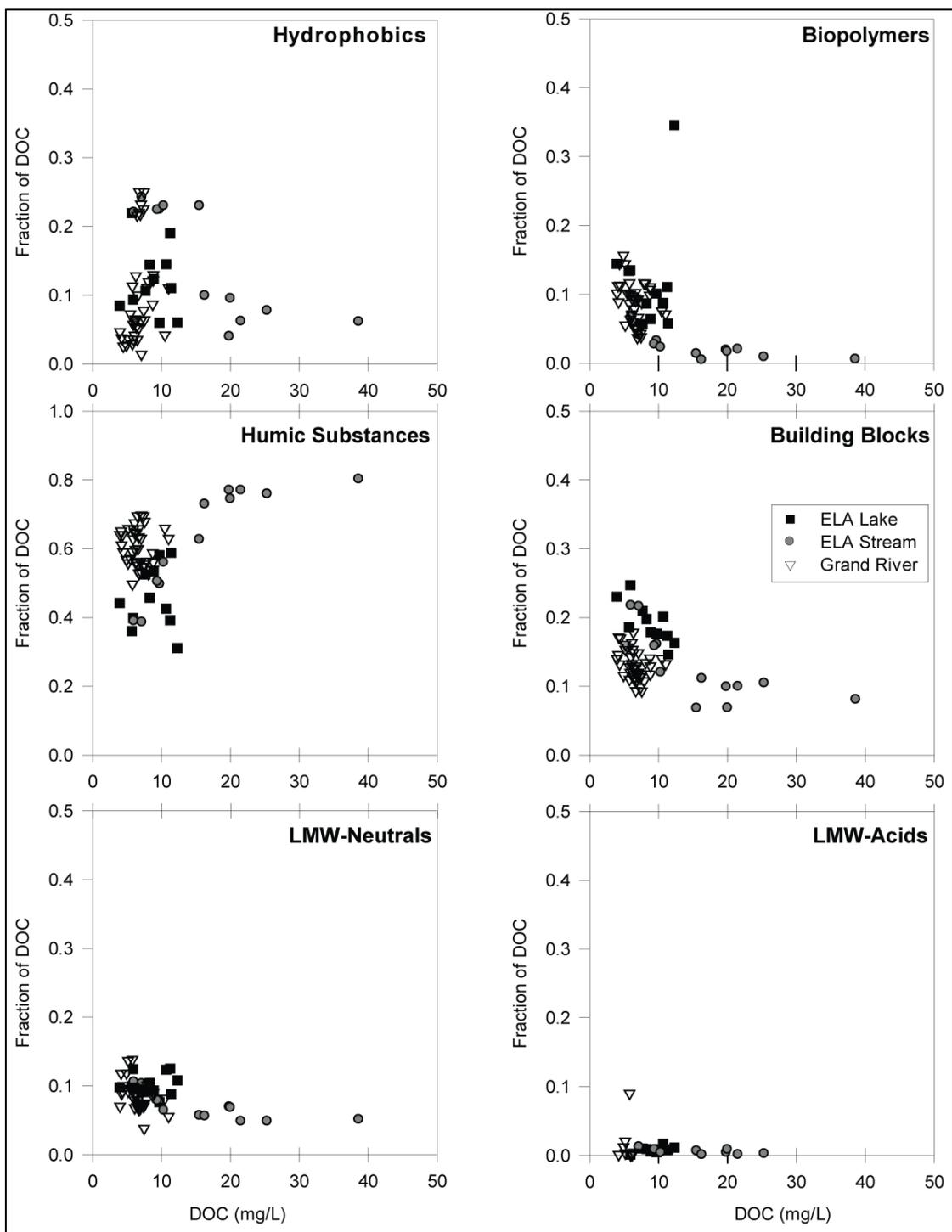
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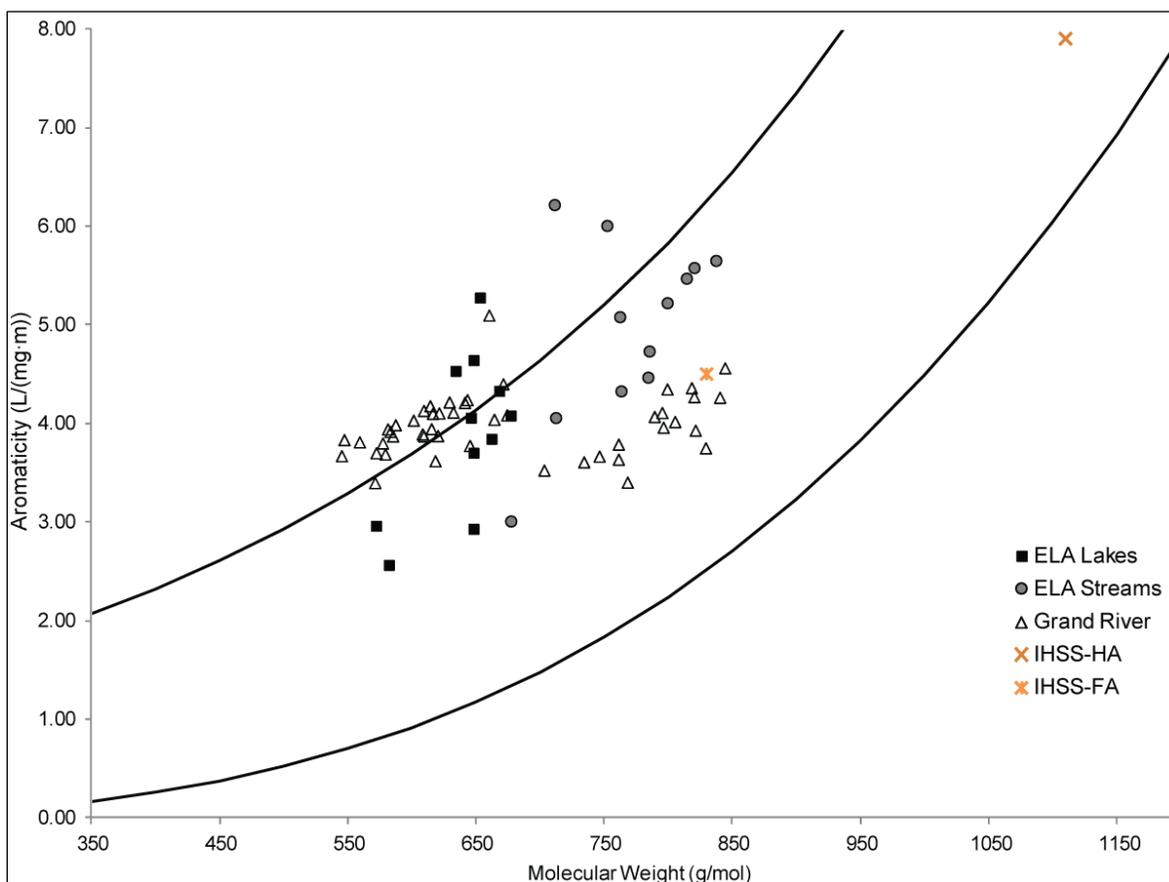
**Figure 4.1:** Conceptual model of the variety of sources, processes, and influences that can affect the characteristics of dissolved organic matter (DOM) in both surface and ground water environments.



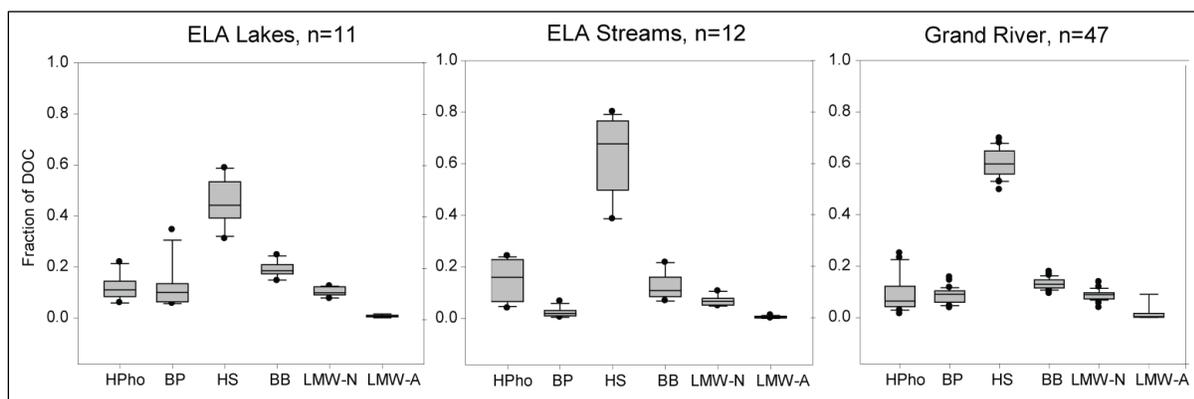
**Figure 4.2:** Map illustrating the sampling locations in Ontario and New Brunswick. Surface water sites include Experimental Lakes Area and the Grand River (blue line). Ground water locations include Turkey Lakes Watershed, Nottawasaga Valley, Long Point, and the Black Brook Watershed. Maps adapted from Natural Resources Canada, Government of Canada (<http://atlas.gc.ca>).



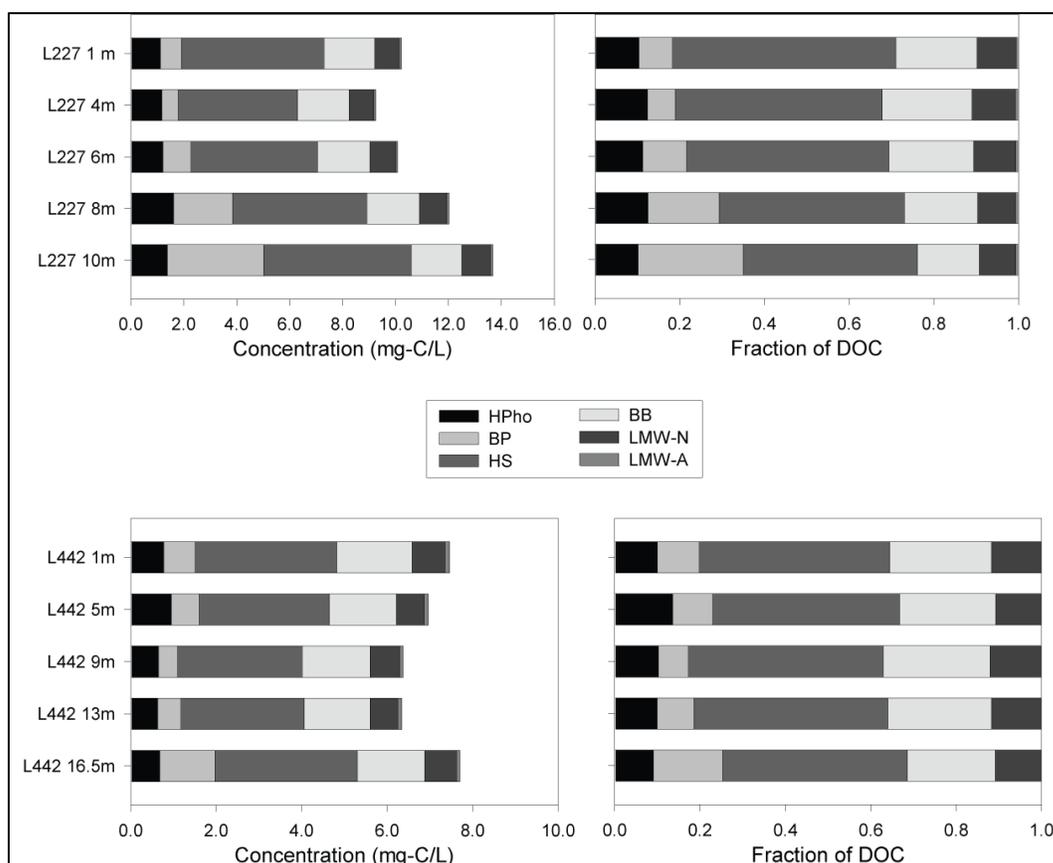
**Figure 4.3:** Fraction proportion of the total dissolved organic carbon (DOC) versus the overall DOC concentration for surface water environments. Data represents all surface waters sampled: ELA lakes (closed squares), ELA streams (closed circles), and the Grand River (open triangles).



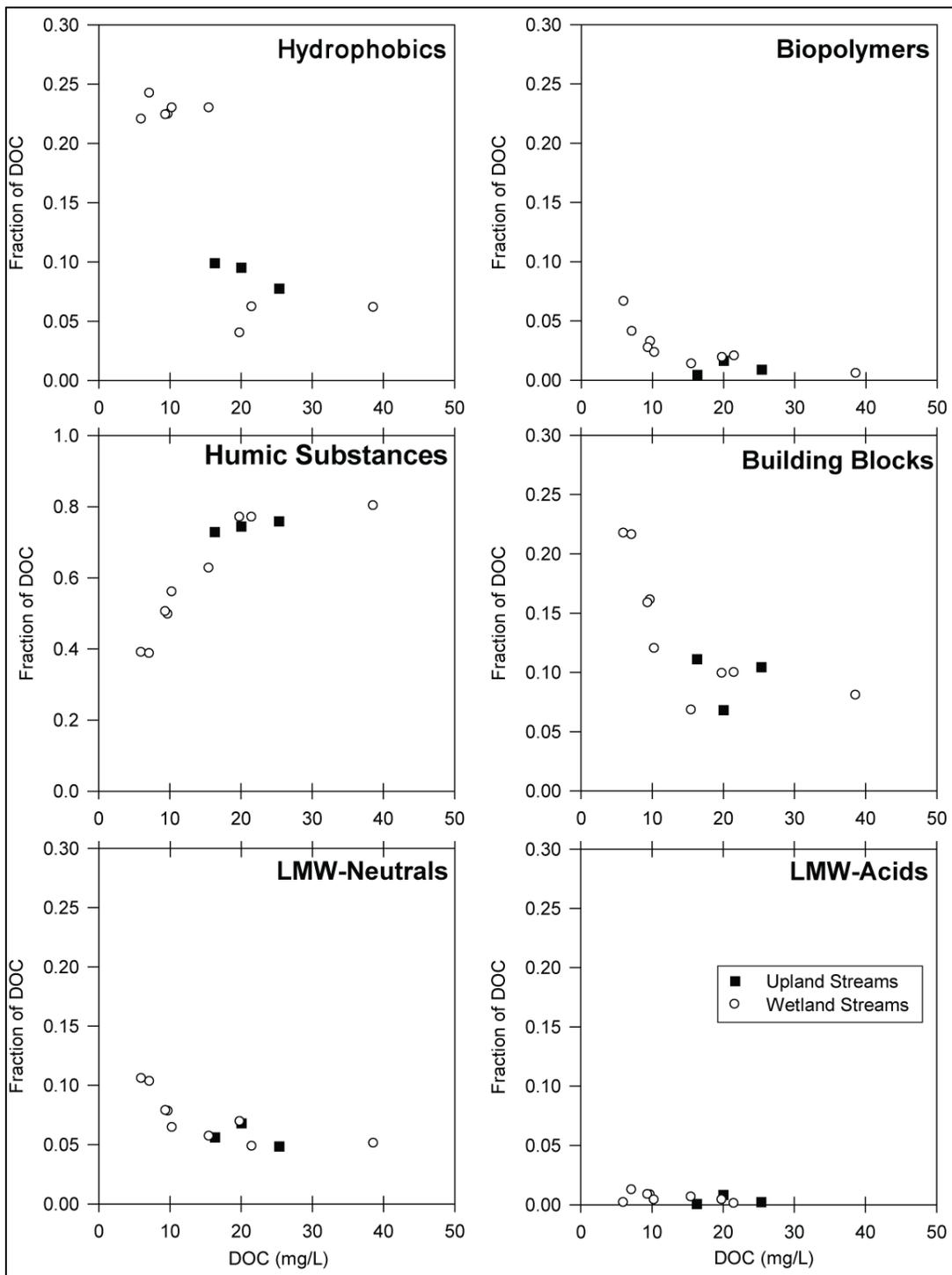
**Figure 4.4:** Humic substances diagrams illustrating the aromaticity (L/(mg·m)) versus molecular weight (g/mol) of humics from surface water environments. Environments sampled include ELA lakes (closed squares), ELA streams (closed circles), and the Grand River (open triangles). Included are the positions of the International Humic Substance Society Humic acid (IHSS-HA; cross) and Fulvic acid (IHSS-FA; star) standards. Black lines indicate the boundaries of natural waters, as suggested by Huber et al. (2011).



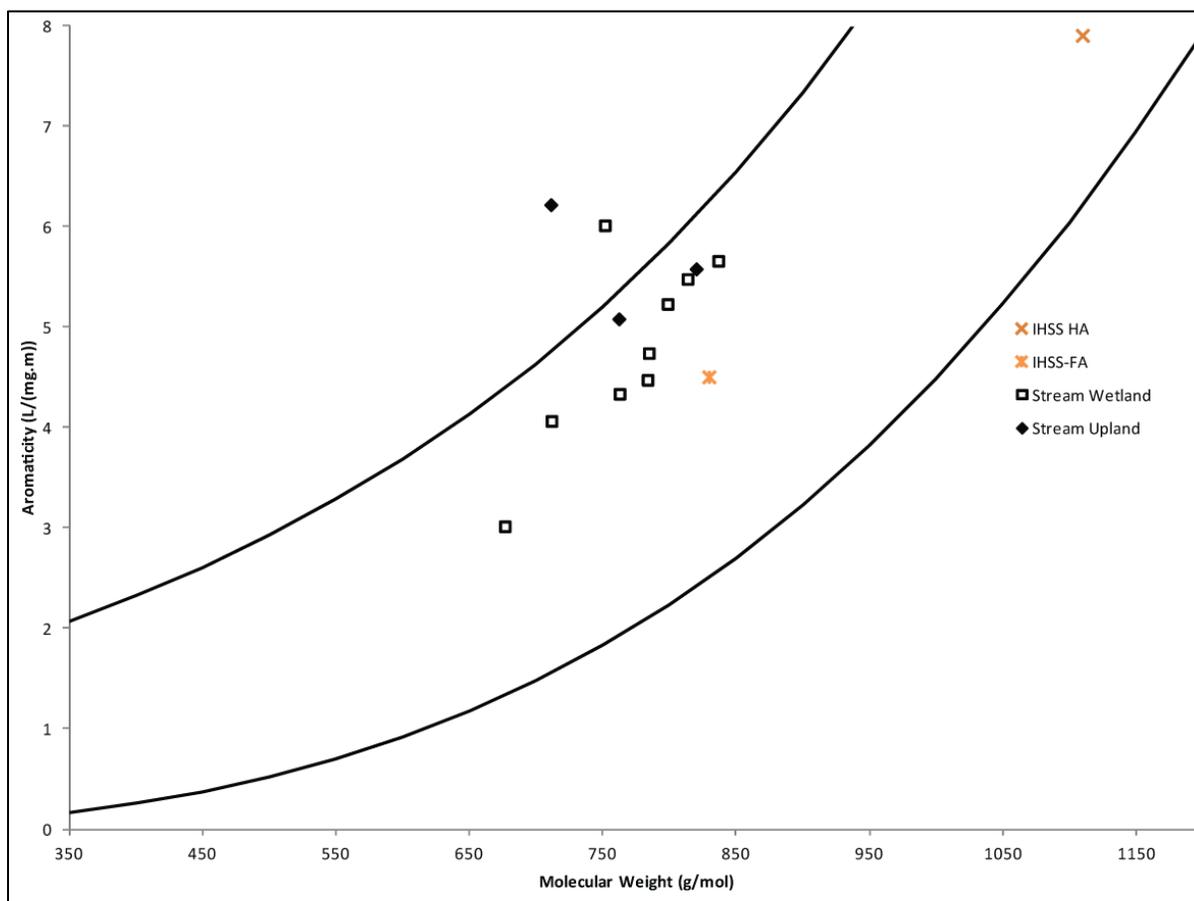
**Figure 4.5:** Boxplots representing the proportion of hydrophobics (HPho), biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight neutrals (LMW-N) and acids (LMW-A) within the dissolved organic matter for each surface water environment. Each fraction is illustrated by the median, 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles, while dots represent outliers.



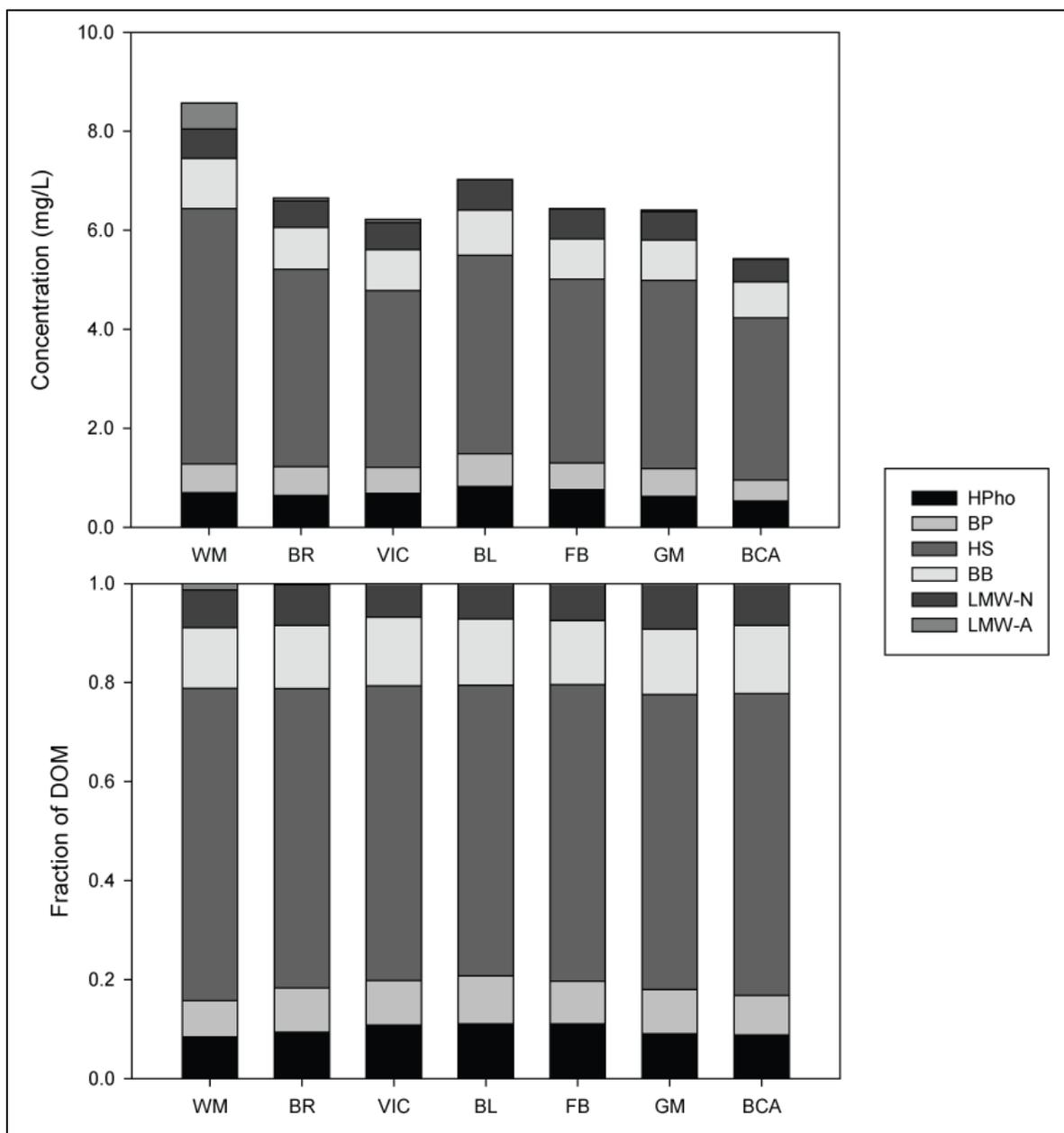
**Figure 4.6:** Lake profiles for ELA lakes L227 and L442, illustrating the change in concentration (left) and proportion (right) of the components that comprise dissolved organic matter.



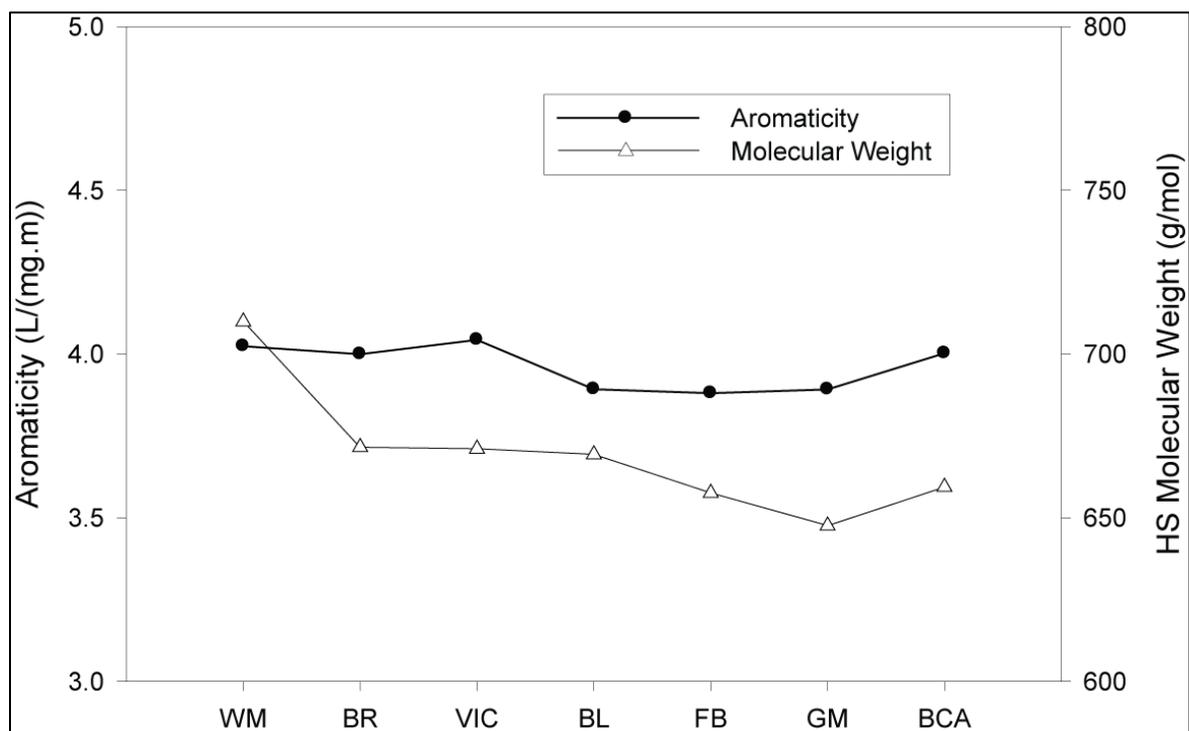
**Figure 4.7:** Fraction proportion versus total dissolved organic carbon concentration (mg/L) for ELA streams. Upland streams are represented through closed squares, while wetland streams are represented by open circles.



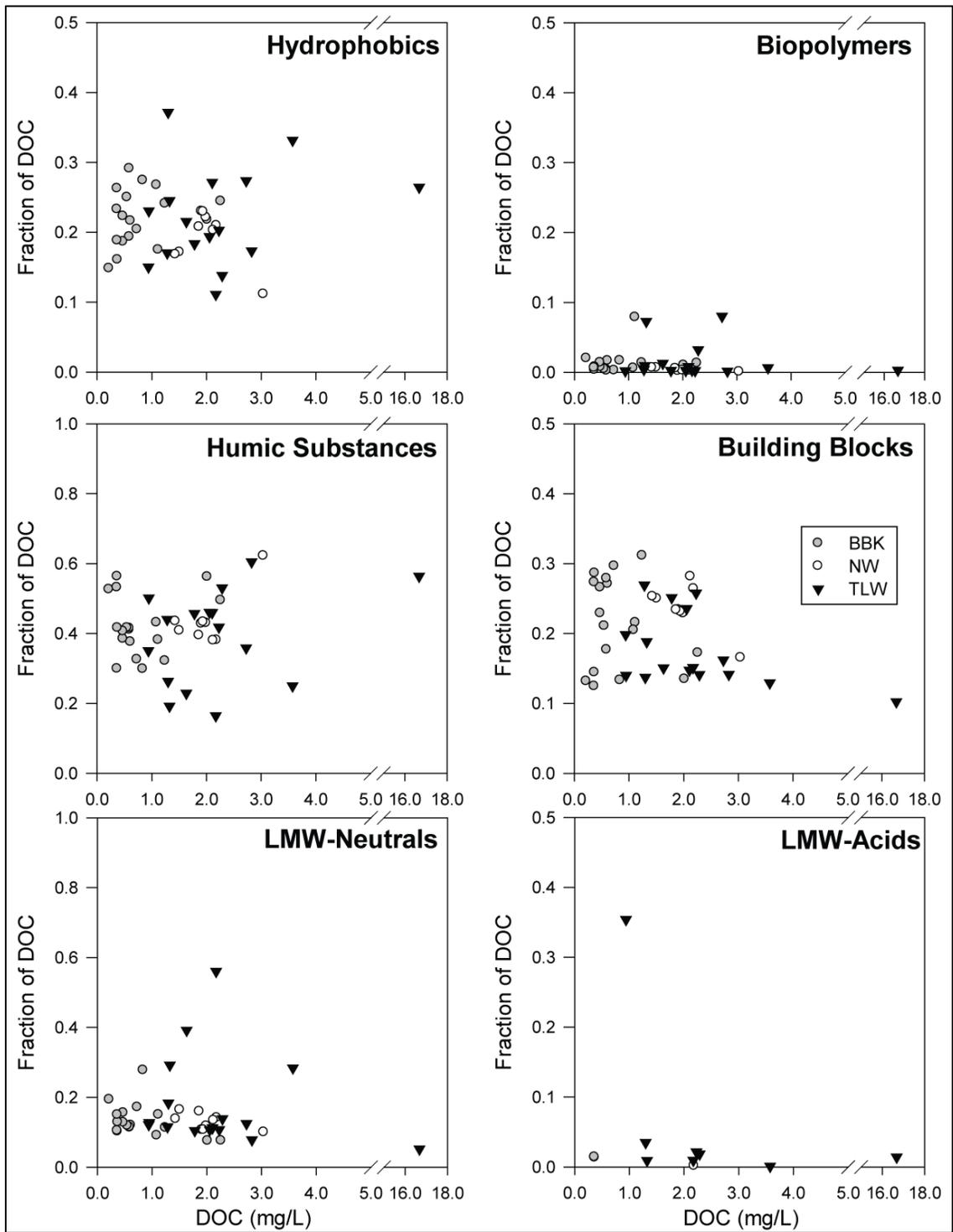
**Figure 4.8:** Humic substances diagrams illustrating the aromaticity (L/(mg·m)) versus molecular weight (g/mol) of humics from ELA streams. Upland streams are represented by a closed diamond, while wetland streams are represented by an open square. Included are the positions of the International Humic Substance Society Humic acid (IHSS-HA; cross) and Fulvic acid (IHSS-FA; star) standards. Black lines indicate the boundaries of natural waters, as suggested by Huber et al. (2011).



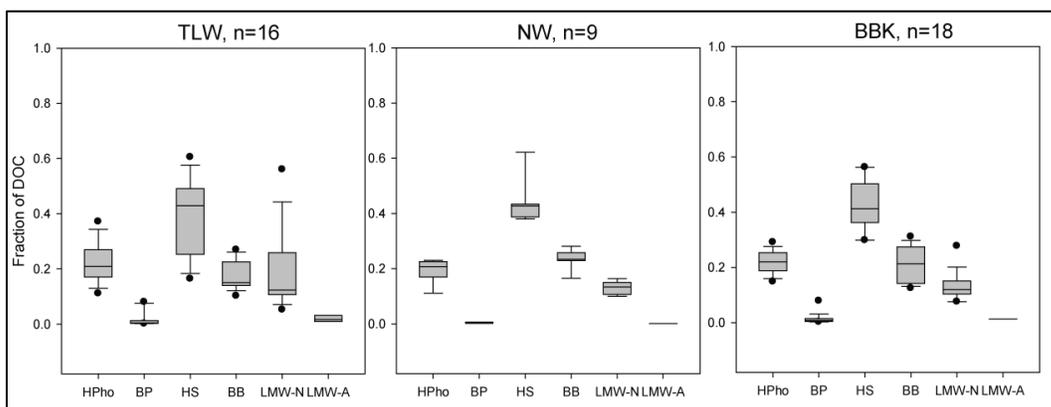
**Figure 4.9:** Concentration for each component (top, mg/L) and proportion of the total dissolved organic matter (bottom) for sites along the Grand River. Sites range from upstream (0km) to downstream (90km) in the following order: West Montrose (WM), Bridgeport (BR), Victoria Street (VIC), Blair (BL), Footbridge (FB), Glen Morris (GM), and Brant Conservation Authority (BCA). Each location represents an average from five sampling trips between January and December 2011.



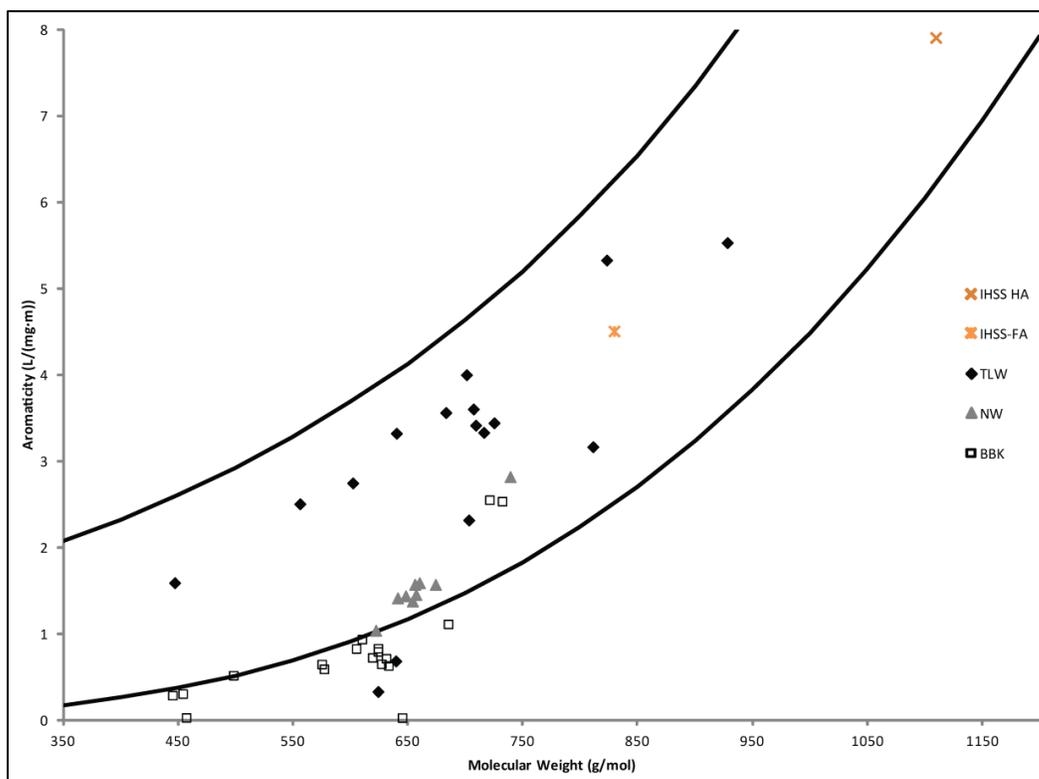
**Figure 4.10:** Humic substance aromaticity (left y-axis, closed circle; L/(mg.m)) and molecular weight (right y-axis, open triangle; g/mol) along the Grand River. Sites range from upstream (0km) to downstream (90km) in the following order: West Montrose (WM), Bridgeport (BR), Victoria Street (VIC), Blair (BL), Footbridge (FB), Glen Morris (GM), and Brant Conservation Authority (BCA). Each location represents an average from five sampling trips between January and December 2011.



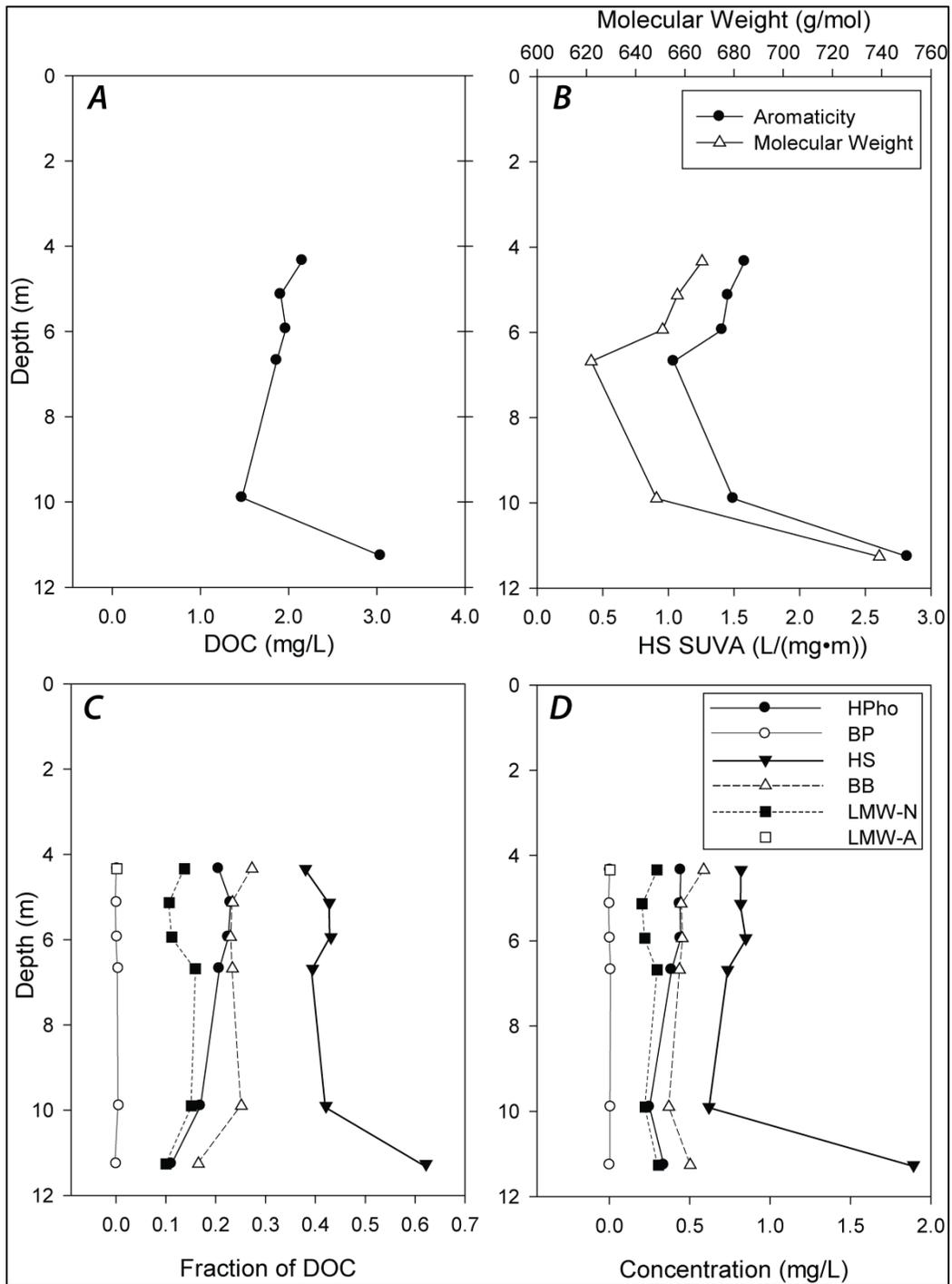
**Figure 4.11:** Fraction proportion versus the total DOC concentration for all ground water environments. Data represents Black Brook Watershed (BBK, closed circle), Nottawasaga Valley (NW, open circle), and Turkey Lakes Watershed (TLW, closed triangle).



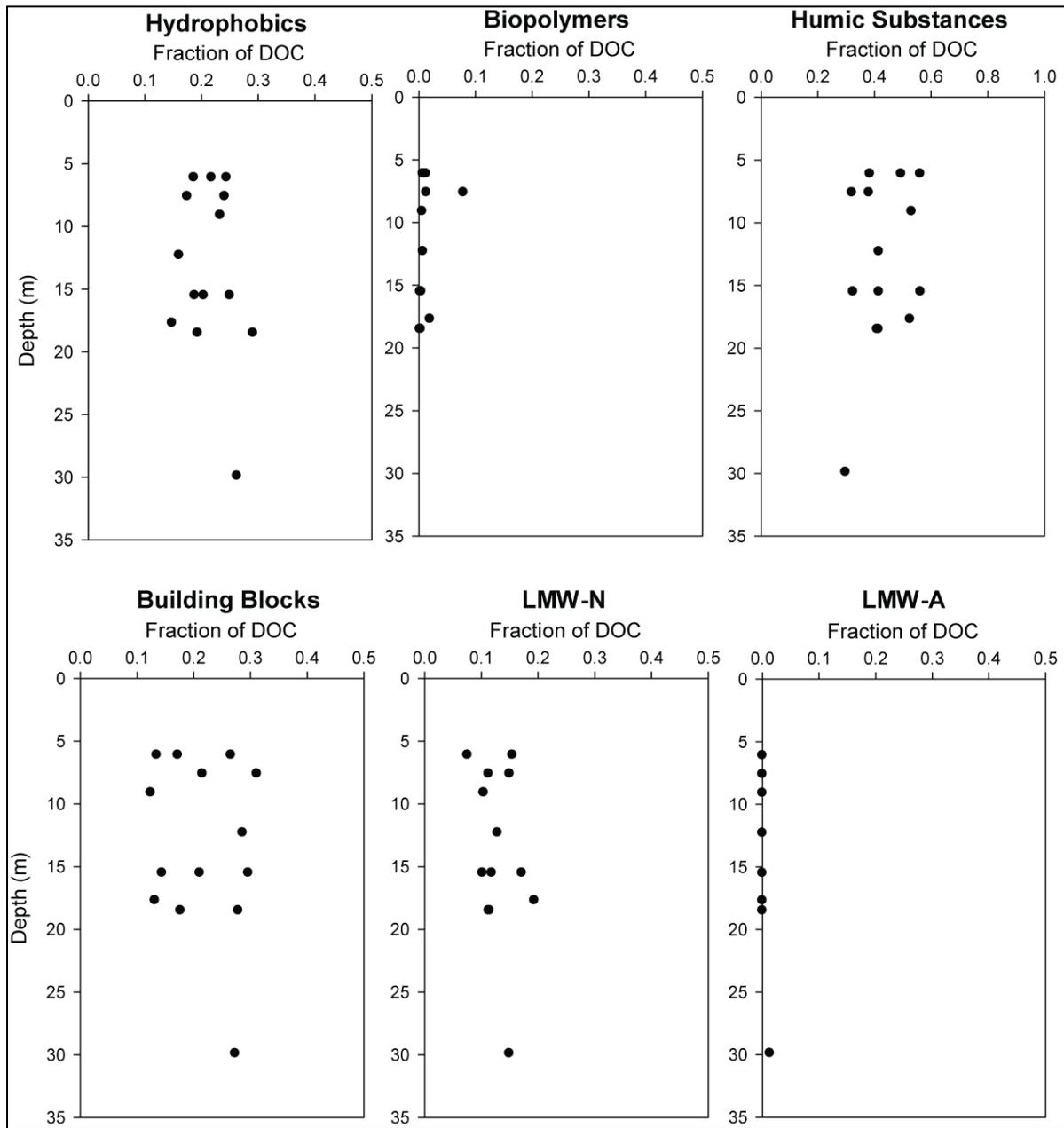
**Figure 4.12:** Boxplots representing the proportion of hydrophobics (HPho), biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight neutrals (LMW-N) and acids (LMW-A) within the dissolved organic matter for each ground water environment. Each fraction is illustrated by the median, 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles, while dots represent outliers.



**Figure 4.13:** Humic substances diagrams illustrating the aromaticity (L/(mg·m)) versus molecular weight (g/mol) of humics from ground waters: Black Brook Watershed (BBK, open squares), Nottawasaga valley (NW, closed triangle), and Turkey Lakes Watershed (TLW, closed diamonds). Included are the positions of the International Humic Substance Society Humic acid (IHSS-HA; cross) and Fulvic acid (IHSS-FA; star) standards. Black lines indicate the boundaries of natural waters, as suggested by Huber et al. (2011).

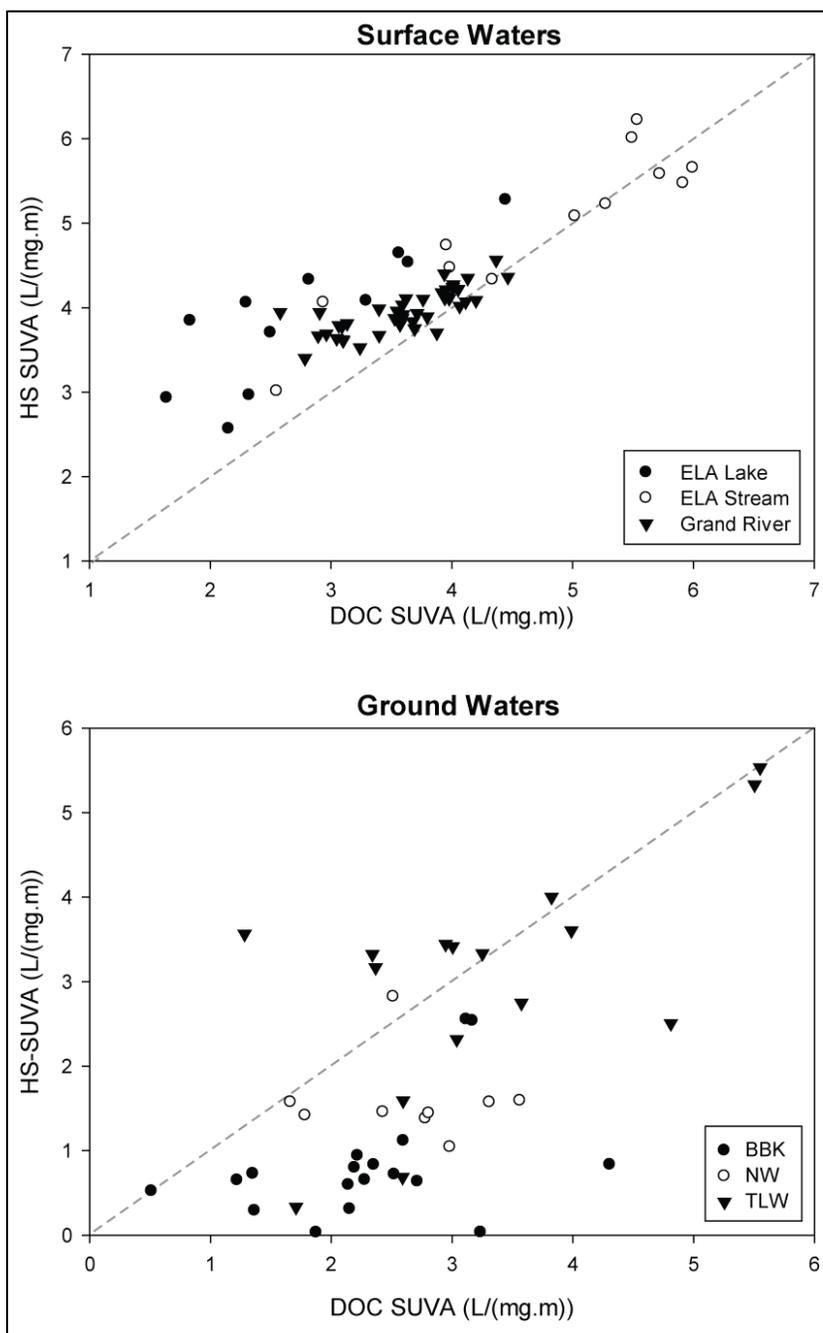


**Figure 4.14:** Depth profiles from a multi-piezometer at Nottawasaga Valley, illustrating changes with depth (m) to dissolved organic concentration (Graph A; mg/L), humic substance characteristics (Graph B; aromaticity (bottom axis, closed circle) and molecular weight (top axis, open triangle)), proportion of each fraction (Graph C), and concentration of each fraction (Graph D; mg/L).

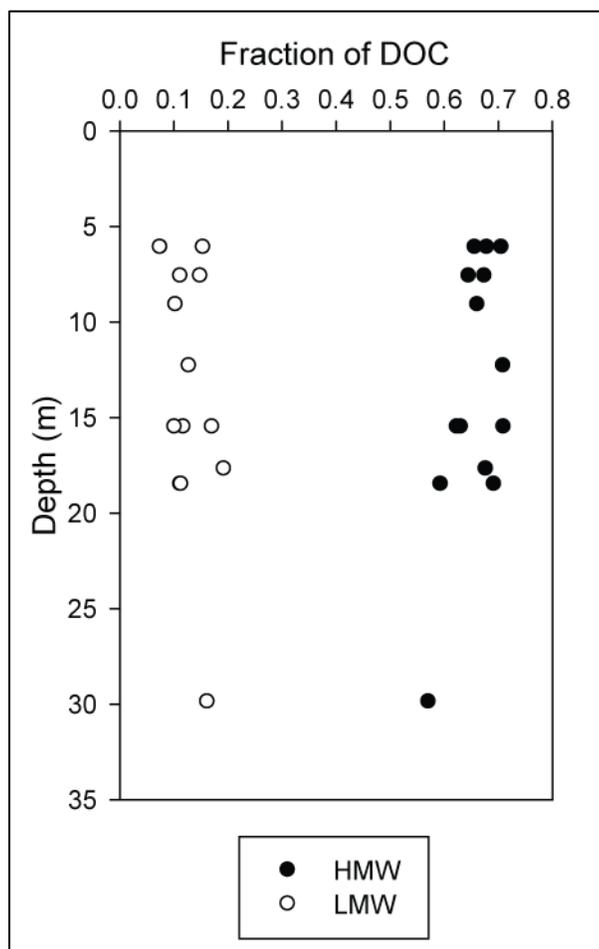


**Figure 4.15:** Black Brook Watershed LC-OCD fraction proportions with depth (m).

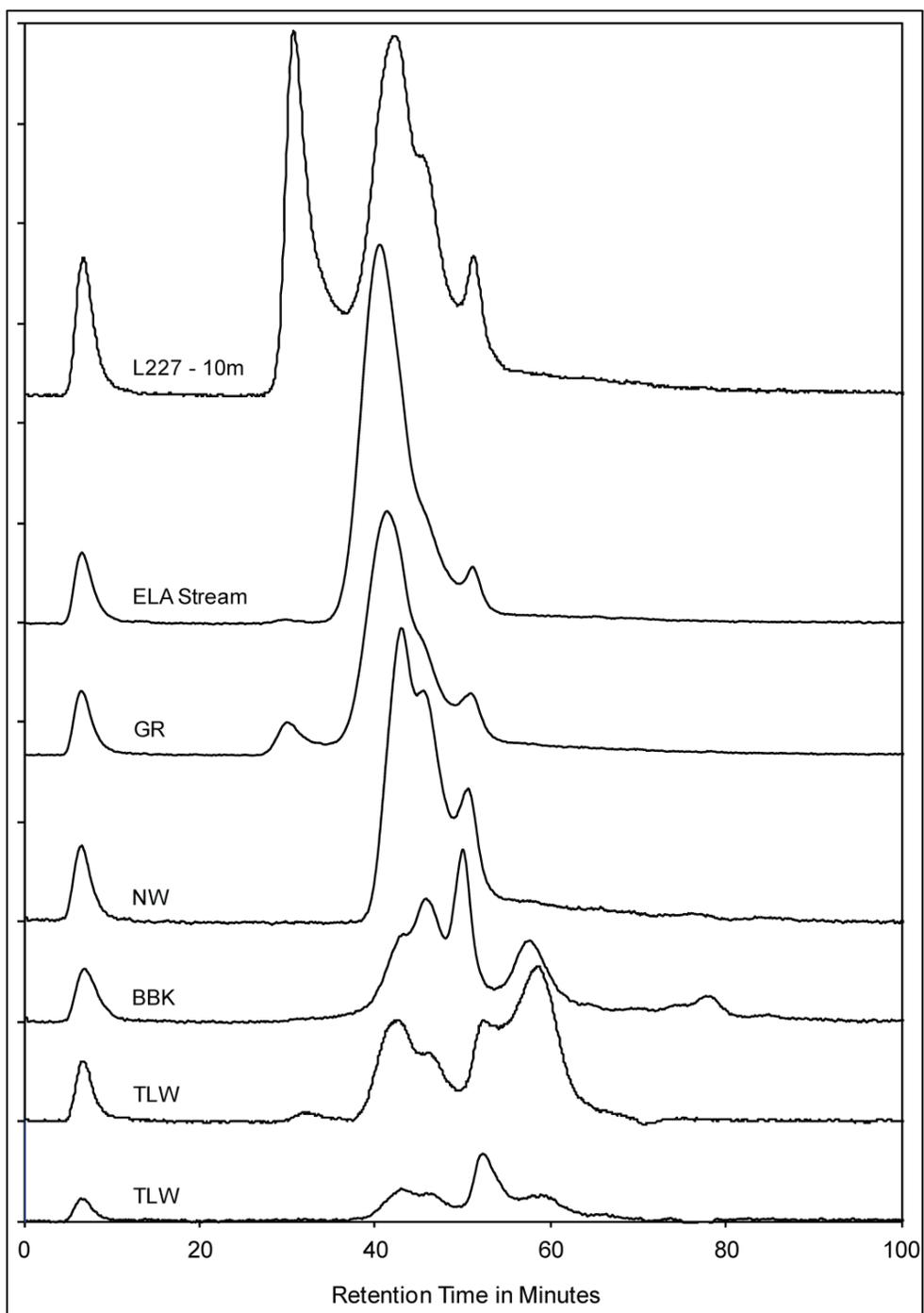




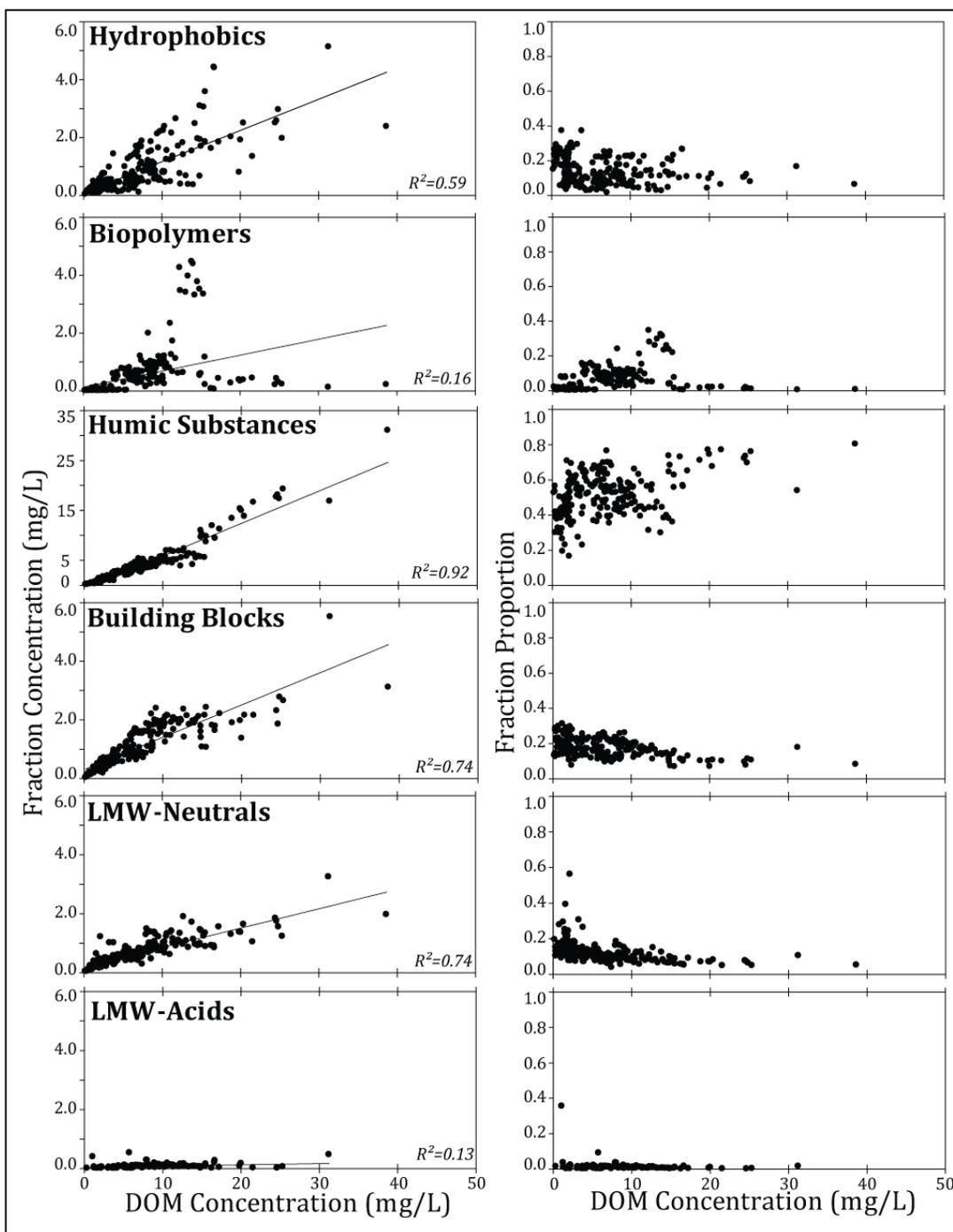
**Figure 4.17:** Comparison of humic substance aromaticity (or SUVA; L/(mg·m)) versus overall dissolved organic carbon SUVA (L/(mg·m)) for both surface waters (top graph) and ground waters (bottom graph). Included are the individual sites for surface water environments (ELA lakes: closed circle; ELA Streams: open circle; Grand River: closed triangle) and ground water environments (Black Brook Watershed (BBK): closed circle; Nottawasaga Valley (NW): open circle; Turkey Lakes Watershed (TLW): closed triangle). Include in both graphs is a 1:1 line, depicted by a dotted grey line.



**Figure 4.18:** Comparison of depth (m) to the fraction of high molecular weight DOM (HMW= HS, BB, and BP; closed circle) and low molecular weight DOM (LMW= LMW-N and -A; open circle) from Black Brook Watershed data.



**Figure 4.19:** LC-OCD chromatograms of different environments.



**Figure 4.20:** The relationship between fraction concentration and proportion are illustrated with varying DOM concentrations. The left depicts regressions of the fraction concentration (mg/L) versus overall DOM concentration (mg/L) for all hydrological environments. R-squared values are included in the bottom right corner. The right illustrates corresponding fraction proportion versus DOM concentration (mg/L).

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**APPENDIX A**  
**CHANGES TO DISSOLVED ORGANIC MATTER DURING STORAGE AS DETERMINED BY**  
**LC-OCD ANALYSIS**

**INTRODUCTION**

The LC-OCD provides a reproducible method to analyze changes to different DOM fractions, and has mainly been used during wastewater treatment applications (Batsch et al., 2005; Ciputra et al., 2010; Huber et al., 2011; Neale et al., 2011). Other than filtering through a 0.45 $\mu$ m filter, LC-OCD requires no sample pre-treatment. No attempt has been made to observe whether DOM undergoes compositional changes during storage, raising concern over whether the amount of time between sampling and analysis has a significant effect upon the composition of DOM. It is hypothesized that storage will have little effect on the concentration and composition of DOM due to the cold, dark environment (hindering further degradation) in which samples are stored. The objective of this short study will be to see whether untreated DOM changes composition over time during storage, using LC-OCD analysis to compare changes in concentration and composition.

**METHODS**

One lake sample (L239) and a stream sample (U8) were collected in 1L-volumes from the Experimental Lakes Area (ELA; Ontario, Canada) in June 2011. Samples were collected near the surface, untreated, and stored in the dark at 4°C until analysis at the University of Waterloo. Each sample was filtered in the laboratory, shaken well, and divided into five 40mL glass vials. All vials were stored in a cold room (>4°C) until analysis at 1, 3, 5, and 7 weeks after the dilutions were made.

DOM characterization was completed using the LC-OCD. A full description of the LC-OCD system and analytical technique is outlined in Chapter 2. Duplicates were run on the initial sample (time zero, T<sub>0</sub>) in order to determine the reproducibility of the LC-OCD and calculate a standard error for each component and used over the entire experiment.

## RESULTS

### CONCENTRATION AND PROPORTION OF DOM COMPONENTS

Stream DOC concentration (average: 18.76 mg/L, standard error:  $\pm 0.25$  mg/L) was found to be higher than the lake sample ( $7.54 \pm 0.07$  mg/L), and did change more than the standard error throughout the length of the storage experiment (Figure A.1). Although no net difference in concentration was observed after seven weeks of either hydrophobics (HPho) or building blocks (BB) in the stream sample, the concentrations of these components fluctuated during the course of the experiment (Figure A.2). In addition, these parameters appeared to have an inverse relationship to each other. Concentrations of low molecular weight neutrals (LMW-N;  $1.09 \pm 0.00$  mg/L) and acids (LMW-A;  $0.04 \pm 0.02$  mg/L), as well as humic substances (HS;  $4.06 \pm 0.00$  mg/L), did not change over the seven weeks.

Overall, the lake DOM appeared to be more stable than stream DOM. The DOC from L239 did not decrease over the length of the experiment, which is also reflected through unchanging proportions of the fractions (Figure A.3). However, the HPho fraction fluctuated more than the other components.

### CHANGES TO ABSORBANCE CHARACTERISTICS

Ultraviolet-absorbance data changed during storage. The overall SUVA of the stream sample increased slightly from  $4.96 \pm 0.06$  L/(mg·m) by 0.2 L/(mg·m) over the first week, but then remained constant ( $5.24 \pm 0.06$  L/(mg·m)) (Figure A.4). The HS SUVA ( $4.83 \pm 0.03$  L/(mg·m)) was lower than DOM SUVA, and also decreased from the initial time point. Furthermore, the initial molecular weight of HS ( $733 \pm 5$  g/mol) had decreased slightly during storage ( $-17$  g/mol). Lake SUVA ( $3.43 \pm 0.14$  L/(mg·m)) had a lower value than the stream, but displayed a similar trend. In addition, HS SUVA did not change, while it was found that the molecular weight of HS decreased from 638 to 612 g/mol ( $\pm 4$  g/mol).

## DISCUSSION

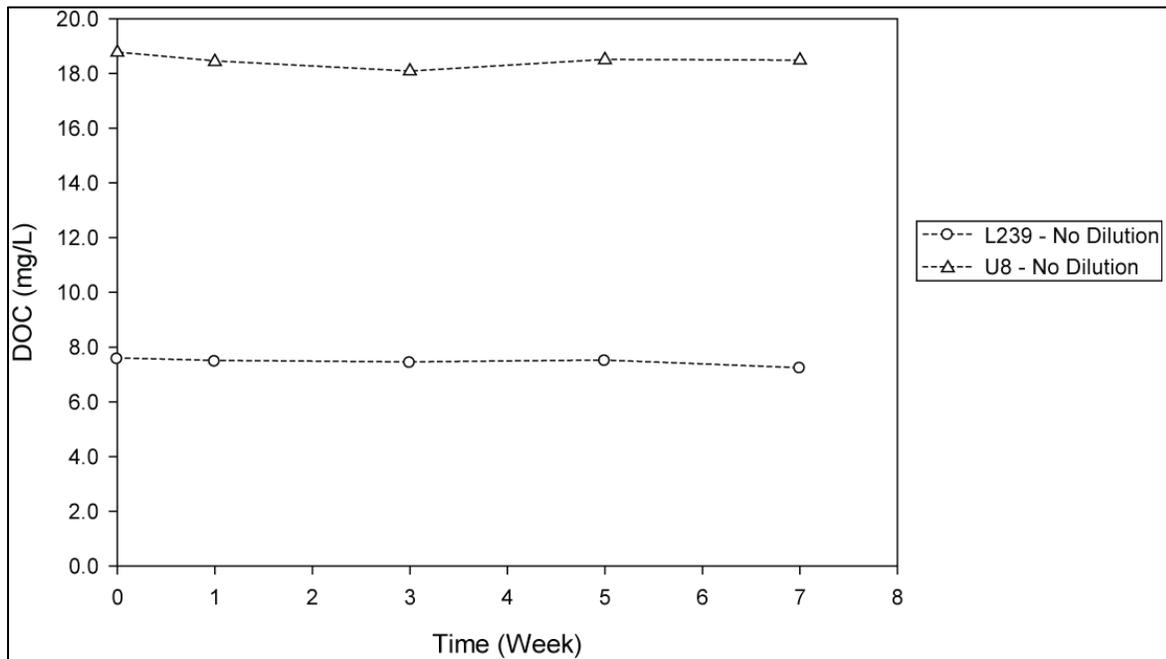
Storage did not greatly affect the overall composition or concentration of DOM determined with the LC-OCD. The lake sample fluctuated in concentrations of fractions more than the

stream sample. Lake DOM has the potential to be more reworked due to external processes such as UV-irradiation and microbial reworking (de Haan, 1993; Moran & Zepp, 1997), leading to the paradigm that DOM would be more recalcitrant and would be more likely to resist further change. However, exploring differences based upon different sampling environments was not in the scope of this project, but would prove interesting to pursue elsewhere. Regardless, the overall increase in stream DOM rather than lake DOM indicates that samples taken from environments containing labile carbon should be analyzed within two weeks to minimize potential changes during storage.

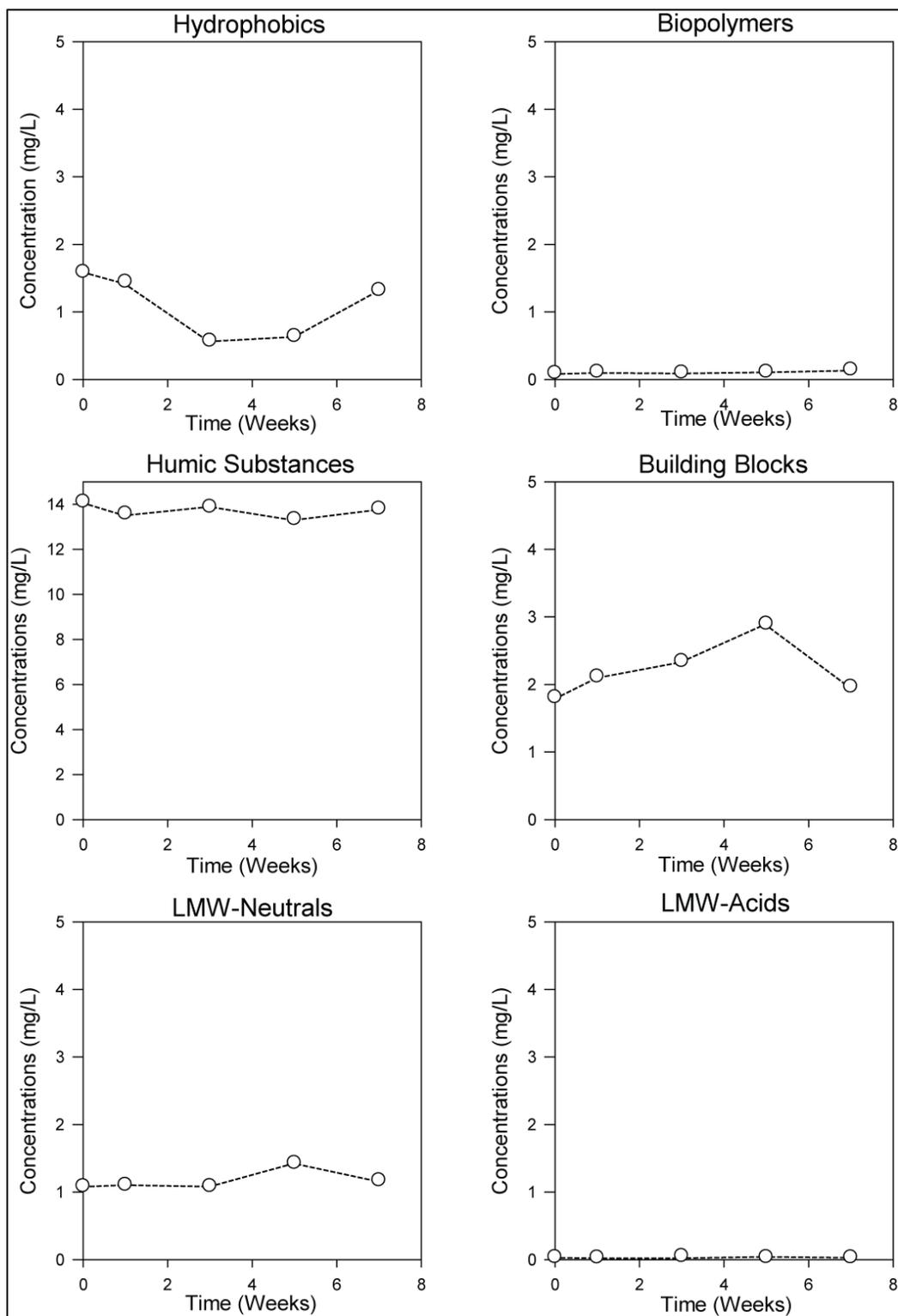
Both samples changed in the molecular weight of HS to smaller values. Although no change was seen in either the aromaticity or concentration, this result indicates that storage can alter the characteristics of HS. Although HS are generally regarded to be recalcitrant and difficult to further break down (Frimmel, 2003), studies have found HS to act as an important bioavailable source of carbon (Volk et al., 1997). Results from this study indicate larger HS break apart into lower weight HS, lowering the average molecular weight of the HS while maintaining the same concentration. Samples were filtered to reduce further degradation, making it unclear what would cause such a result. However, it is seen in both samples, illustrating that LC-OCD analysis should be run as early as possible in order to have a more realistic molecular weight HS of the sample.

## **CONCLUSION**

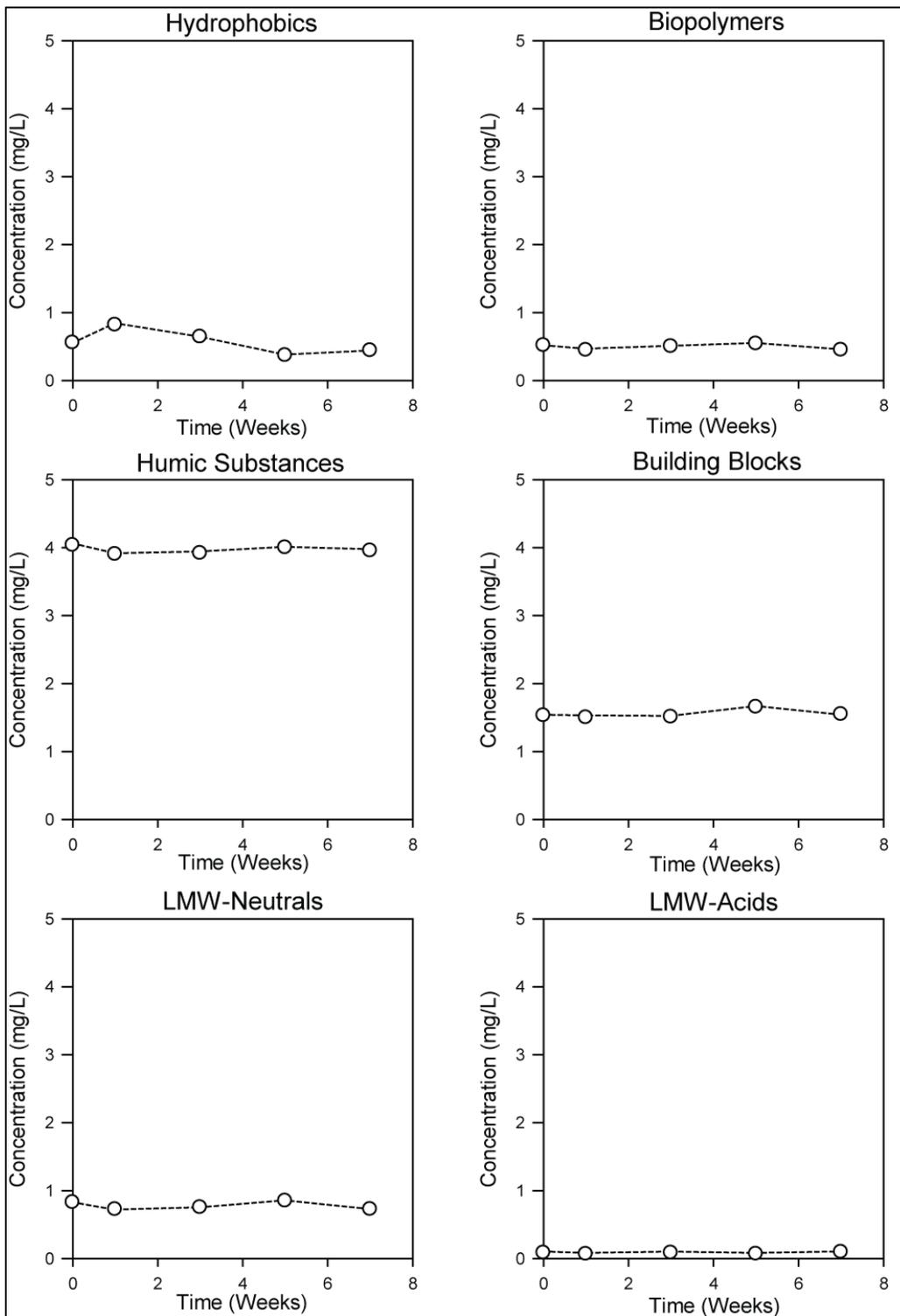
The results from this study find that the concentration of DOM does not greatly decrease during storage. Importantly, LC-OCD analysis illustrated that the overall composition does not greatly change. The only fraction that may be affected during storage is a decrease in molecular weight of HS, as well as a loss in LMW-A components. In addition, samples that may contain labile forms of DOM (such as from freshly degraded organic matter) would benefit from having the least amount of storage time possible. It can be concluded that changes during storage are minimal, and should not affect the overall 'signature' of the DOM, however it is recommended that samples are stored no longer than two weeks to minimize changes to the molecular weight of HS.



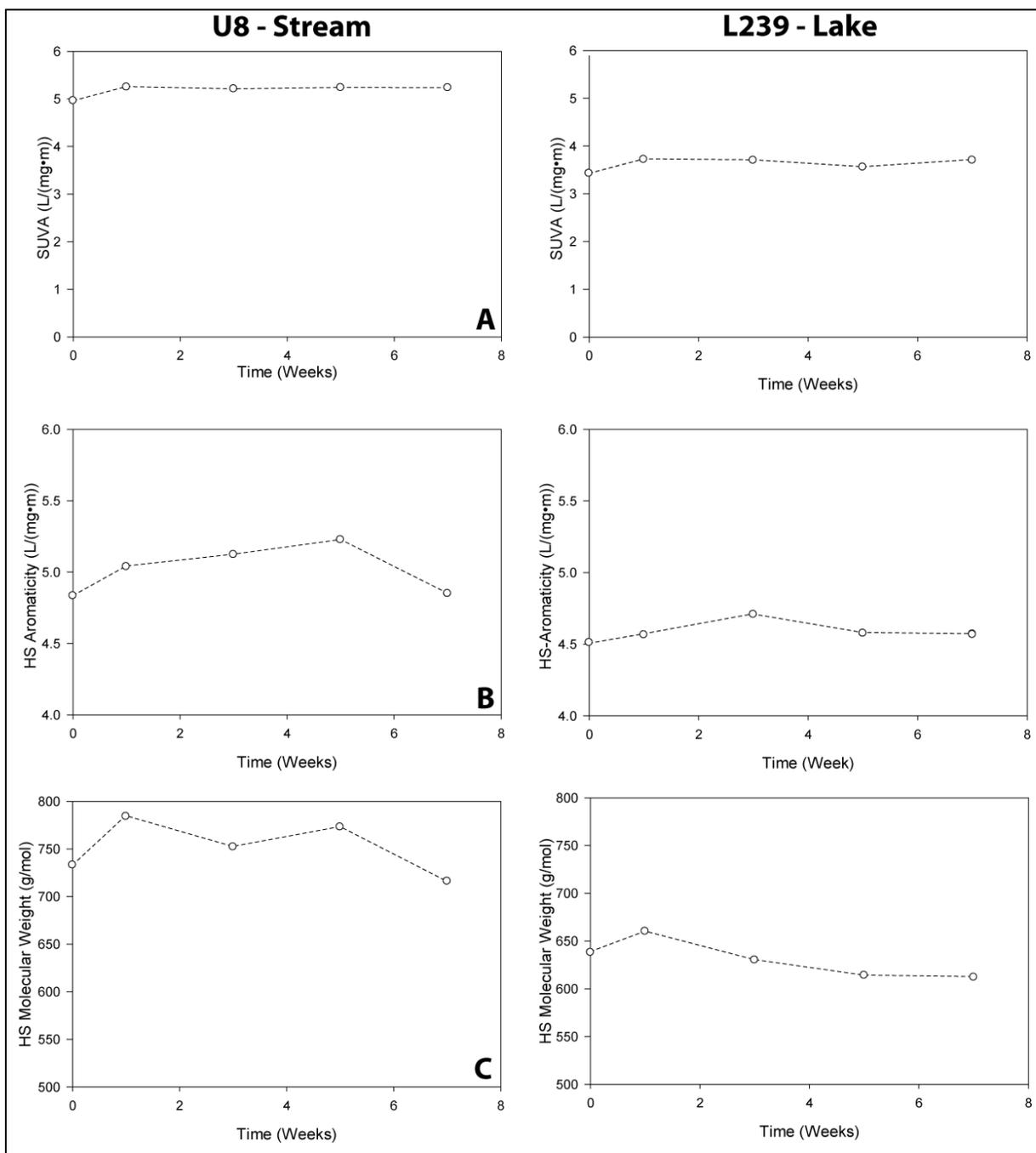
**Figure A.1:** Dissolved organic carbon concentration (mg/L) versus time (weeks) for the lake sample (circles) and the stream sample (triangles). Standard error for each sample was smaller than symbol size.



**Figure A.2:** Stream DOM fraction concentrations (mg/L) versus time (weeks). Standard error for each fraction was smaller than the symbol size.



**Figure A.3:** Lake DOM fraction concentrations (mg/L) versus time (weeks). Standard error for each fraction was smaller than the symbol size.

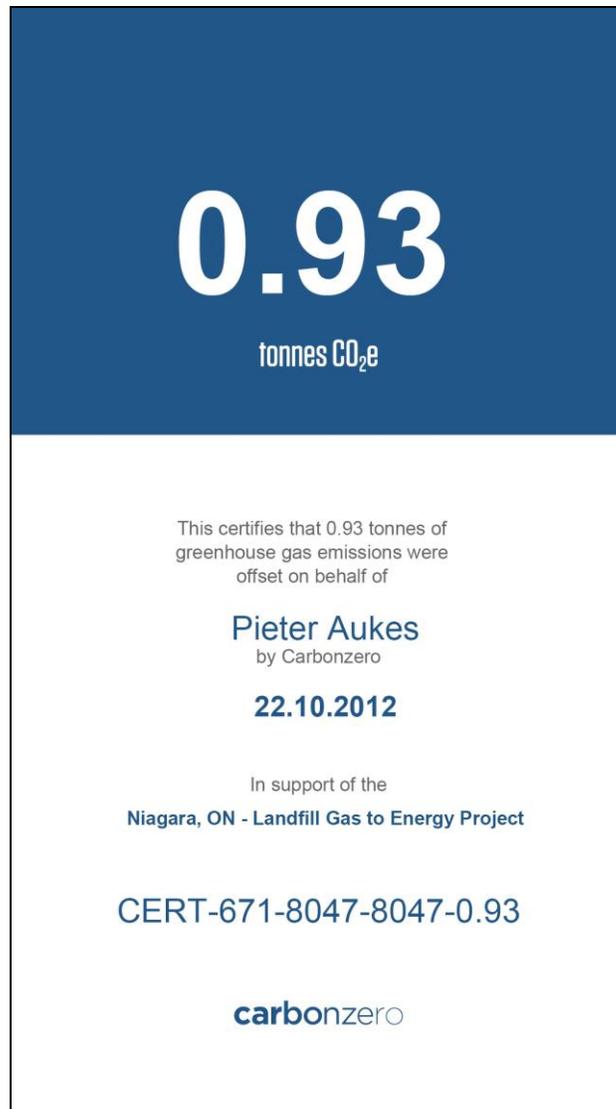


**Figure A.4:** Stream (left side) and lake (right side) data illustrating overall SUVA (Graphs A; L/(mg·m)), HS aromaticity (Graphs B; L/(mg·m)), and HS molecular weight (Graphs C; g/mol) versus time (weeks). Standard error was smaller than the symbol size.

## ENVIRONMENTAL CONSIDERATIONS

This thesis is carbon neutral.

All transportation and electricity that was put into making this thesis has been offset using carbonzero ([www.carbonzero.ca](http://www.carbonzero.ca)).



**0.93**  
tonnes CO<sub>2</sub>e

This certifies that 0.93 tonnes of  
greenhouse gas emissions were  
offset on behalf of

**Pieter Aukes**  
by Carbonzero

**22.10.2012**

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