The Control of Fe and pH on the Photodegradation and Characterization of Dissolved Organic Matter in Small, Oligotrophic Canadian Shield Freshwaters

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.

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Abstract

Carbon is an essential building block for life and is involved in many biotic and abiotic processes. Thus it is imperative to have a comprehensive understanding of carbon cycling. Recent research has established that inland lakes are important contributors to the regional carbon cycle because they store, process and emit large masses of carbon over relatively short timescales (ie. days to years). Lakes receive 1.9 Pg C $y^{-}1$ of terrestrially-derived carbon but only export approximately half of that to oceans while the remainder is either transferred to the sediment as particulate organic carbon (POC) or evaded to the atmosphere as carbon dioxide (CO_2; Molot and Dillon, 1996; Cole et al. 2007). Increased atmospheric CO_2 has caused global environmental problems including ocean acidification, temperature increases leading to melting glacial ice and sea level rise, and changes in precipitation patterns including extreme weather resulting in flooding and droughts. Investigating the mechanisms that affect these processes is important for understanding the global carbon cycle and predicting future changes to the lake systems under the stress of climate change.

As the largest input of terrestrial carbon to lakes, dissolved organic carbon (DOC) (composed of a complex mixture of thousands of different organic molecules less than 0.1-0.7μm) is important to lake ecosystem function. Large quantities of DOC are converted into other forms of carbon within lakes and some of the transport mechanisms between sources and sinks do not add up. Photodegradation of DOC is an important abiotic process for DOC loss. Products of DOC photodegradation including POC, dissolved organic carbon (DIC; can evade to the atmosphere as CO_2), and photolytically altered DOC affect the size of carbon pools in lakes. Concomitantly, pH and Fe can influence the rates of carbon transformation, yet these influences are poorly understood. The goal of my research was to explain an important gap in our understanding of why some carbon goes to the atmosphere as CO_2 and some goes into the lake sediments as POC. This has implications for contaminant transport, lakes recovering from acidification, increasing lake water temperatures, and climate change modeling.

In laboratory experiments, pH and Fe of three boreal streams (two from the Experimental Lakes Area, Kenora, ON and one near Dorset, ON) were manipulated to observe the impact on DOC photodegradation and POC formation. Measurement of carbon pools during the photolysis experiment allowed for the determination of DOC loss (production of DIC and POC formation) and carbon and isotopic mass balances. A novel size exclusion chromatography method, liquid chromatography organic carbon detection (LC-OCD), was used with other measures of quality to identify changes in DOC composition resulting from photodegradation. While photodegradation was an important process that transformed stream organic carbon (rates decreased at pH 6.5 and increased with increased Fe concentrations), no significant POC was generated from this process. Thus it is still unknown as to how large portions of DOC in oligotrophic boreal freshwater lakes ends up in the sediments as POC.

Experiments were also conducted to study the influence of pH, Fe(II) and Fe(III) on measures of DOC quality (specifically absorbance and LC-OCD). This work has demonstrated that certain measures of DOC quality are influenced by pH, Fe(II) and Fe(III) and thus, these parameters must be considered when making conclusions about DOC quality when using such measures.

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Chapter 1 Introduction

1.1 The Carbon Cycle

All living organisms affect the chemical conditions of Earth, but today one species is altering Earth's chemistry at rates that threaten the stability of the environment (Schlesinger and Bernhardt, 2013). There is little scientific doubt that the global carbon cycle, in particular, is changing as a result of human activities as the world population has doubled since the first Earth Day in 1970 from 3.7 billion to 7.4 billion and increased by almost 900% since pre-industrial times (UN Population Division, 2016). In this time, the concentrations of carbon dioxide (CO_2), an important greenhouse gas, has risen dramatically due to the increased combustion of fossil fuels and clearing of land for agriculture and urban development (Cox et al. 2000). In the past few years, for the first time in recent Earth's history, atmospheric CO_2 concentrations have exceeded 400ppm, providing scientists with an even greater need to understand the changing carbon cycle and its implications for our planet (Showstack, 2013).

Carbon is an essential building block for life and is involved in many biotic and abiotic processes. Although, carbon in the form of greenhouse gases, specifically CO_2 and methane (CH_4), are essential to all living organisms and their surrounding environment, they can also be harmful at high atmospheric concentrations. CO_2 in particular, has caused environmental problems globally including temperature increases, ocean acidification, melting glacial ice, sea level rise and extreme weather resulting in flooding and droughts (Cox et al. 2000).

Recent research has established that inland lakes are important contributors to the regional carbon cycle because they store, process and emit large masses of carbon over relatively short timescales (Cole et al. 2007; Juutinen et al. 2013). Lakes receive large quantities of terrestrially-derived carbon (1.9 Pg C y^{-1}) but only export approximately half of that to oceans while the remaining carbon stock is either transferred to the sediment as POC or evaded to the atmosphere as CO_2 (Molot and Dillon, 1996; Cole et al. 2007). Investigating the mechanisms affecting these processes is important in understanding the global carbon cycle and predicting future changes to the lake systems under the stress of climate change.

1.2 Dissolved Organic Carbon

As the largest input of terrestrial carbon to lakes, DOC is essential to lake ecosystem function (Schindler et al. 1997). DOC is a complex mixture of thousands of different organic molecules with molecular weights ranging from less than 100 to over 300,000 Daltons (Thurman, 1985) and can vary significantly between aquatic environments. As a significant microbial food source, DOC provides energy to aquatic organisms, has the ability to maintain acidity and alkalinity and can shuttle electrons and play an important role in redox and photo-Fenton reactions (Kalff, 2002). DOC also absorbs solar radiation, affecting the attenuation of light, stratification and thermal heat budgets, and is an important transport mechanism for metals and nutrients (Schindler, 1971; Schindler and Curtis, 1997; Schindler, 2001; Driscoll et al. 1994).

DOC is composed of degraded plant and animal organic carbon and other biological by-products that are functionally defined to be less than 0.1 – 0.7 μm (Mostofa et al. 2013). With such a broad definition, the term DOC can cover a large range of carbon molecules and be difficult to categorize as it can have very different chemical compositions depending on its origin and the processes affecting it. Using basic categorization, DOC can be either allochthonous, which is DOC that originates from the terrestrial landscape, or autochthonous which is produced within a body of water (Mostofa et al. 2013; Kent et al. 2014). Determining whether DOC is solely allochthonous or autochthonous is difficult because most aquatic DOC initially comes from the landscape, however, certain measures of DOC can aid in determining the composition of DOC which is useful in understanding the productivity and carbon cycling of aquatic systems. These measures can be obtained using analytical techniques including absorbance and fluorescence spectroscopies, as well as liquid chromatography organic carbon detection (LC-OCD) (Huber et al. 2011; Poulin et al. 2014; Weishaar et al. 2003).

The concentration and composition of DOC are important considerations when investigating its role in aquatic environments. One approach is to measure the amount of solar radiation that DOC absorbs. DOC contains variable concentrations of photon absorbing

chromophores depending on the concentration and composition of DOC and this can influence how much solar radiation is absorbed by DOC. Therefore DOC can affect how deep the radiation penetrates, which can influence thermal regimes as well as where chemical reactions such as photosynthesis and photolysis can occur. If DOC concentration is low, then solar radiation can penetrate deeper into an aquatic system, providing more energy and influencing primary and secondary productivity, and potentially lowering the depth of the thermocline and influencing heat budgets (Kalff, 2002; Karlsson et al. 2009; Wetzel, 2001).

Although high DOC concentrations may prevent the penetration of solar radiation, it provides abundant substrate for microbes. DOC also acts as a microbial food source, contributing to the biological productivity in aquatic ecosystems. DOC plays a large role in redox reactions and commonly complexes with nutrients and trace metals such as iron (Fe), aluminum (Al), and manganese (Mn), which can affect metal transport and solubility, water toxicity and nutrient availability. DOC has a large binding capacity for Fe (Koening and Hooper, 1976; Sojo and de Haan, 1991), which can increase the bioavailability of Fe, an essential micronutrient for phytoplankton growth (Murphy and Yesaki, 1983).

1.3 Aquatic Carbon Cycle

The global carbon budget cannot be balanced because the known global amount of CO_2 produced is larger than the amount of carbon stored in known sinks (Smith et al. 1993). This large missing sink of carbon has been suggested to be located in the trees, soils, and peatlands in the forest biome of boreal ecosystems in the Northern Hemisphere (Tans et al. 1990, Kauppi et al. 1992; Sedjo, 1992; Quay et al. 1992; Sellers et al. 1997; Apps et al. 1993; Kurz and Apps, 1993; Sampson et al. 1993).

Catchments of boreal ecosystems are important to the global carbon cycle because they fix a lot of carbon, as CO_2, into biomass. Some of this carbon then enters surface water straight from soils while some takes longer and enters through groundwater. There are often higher carbon concentrations entering the system by water from soils because organic-rich soils leach organic matter (Cole et al. 2007). Carbon can enter a lake in many different forms, as organic or inorganic carbon and either dissolved or non-dissolved. Once carbon is in the

lake, both biotic and abiotic processes can transform it. The major forms of carbon in lakes are organic matter (OM; a mixture of thousands of different carbon molecules), CO_2, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). OM in water passing through a 0.45μm filter is operationally defined as dissolved organic matter (DOM) and is generally measured as the concentration of dissolved organic carbon (DOC; the dominant organic fraction of DOM), while OM remaining on the filter is particulate organic matter (POM) (Danielsson, 1982; Kennedy et al. 1974; Liu et al. 2007; Mostofa et al. 2009). POM is composed of organic matter, including plant debris, algae, phytoplankton cell, bacteria etc. (Mostofa et al. 2009).

DOC represents the largest input of terrestrial carbon to lakes (Schindler et al. 1997) and plays a large role in aquatic and regional carbon cycles. Molot and Dillon (1996) estimated that during the export of 66 Tg C y^{-1} from catchments to lakes of boreal forest biomes, 30-52 Tg C y^{-1} is either evaded to the atmosphere as CO_2 or sequestered to the sediment as POC. Cole et al. (2007) use a simplified mass balance equation to track the fate of carbon in aquatic systems:

Equation 1.1 $$I = G + S + E$$

where the imported carbon (I) is the sum of the net carbon gas balance (G), storage (S) and export (E) (Figure 1.1, Table 1.1).

Lakes accumulate significant quantities of POC in sediment each year (Kortelainen et al. 2004) and although it is largely unknown how this POC is formed, the settling rates have been found to be proportional to DOC concentration (von Wachenfeldt and Tranvik, 2008). Lake metabolism studies suggest that primary production rates are similar to respiration rates (del Giorgio and Peters 1994; del Giorgio et al. 1997; Prairie et al. 2002), and respiration rates exceed gross primary productivity in oligotrophic lakes (Duarte and Prairie, 2005). Thus, for oligotrophic lakes, significant sediment accumulation of POC requires another source of carbon in addition to phytoplanktonic debris. It is likely that an abiotic transfer mechanism such as photodegradation is important for the formation of POC from DOC,

bypassing mineralization to CO_2 followed by photosynthetic fixation and burial (Porcal et al. 2013)

The annual transfer of organic carbon to lake sediments is very small in terms of the global carbon cycle; however, sediments store large quantities of carbon. DOC plays an important role in lake function and the global carbon cycle because DOC can be transported and transitioned into other carbon fractions in aquatic systems. Increases in atmospheric CO_2 concentrations due to anthropogenic activities, (Houghton et al. 1995), and the associated climate change predictions resulting from these increases, provide the need for a greater understanding of the processes that affect carbon cycling in aquatic systems. There are approximately 800,000 boreal lakes in Canada and many others in the Northern Hemisphere and are thus important in the global carbon cycle and the changing climate (Sellers et al. 1997).

1.4 Carbon Transfer Mechanisms

To understand the aquatic carbon cycle, it is important to understand the in-lake mechanisms that convert and redistribute POC, DOC, and dissolved inorganic carbon (DIC) in lakes. These mechanisms include biological processes, redox reactions, photodegradation, mixing, gas exchange and sedimentation (Figure 1.2).

1.4.1 Biological Processes

DOM can influence biological productivity in lakes as it plays an important role in aquatic food webs and can be transformed by aquatic organisms between particulate matter (PM), DIC, and different compositions of DOM (Tranvik, 1992). Initially, allochthonous DOM enters a lake through runoff however, autochthonous DOM can be produced by algae and macrophytes, respired by heterotrophic bacteria, released during herbivore grazing, during the active growth of cells, and after death and decay (Baines and Pace, 1991; Bertilsson and Jones, 2003; Lampert, 1978). Predominantly biologically labile and low molecular weight (LMW) compounds are produced by algal and macrophyte communities and in addition, DOM, DIC, and PM can also be produced through respiration and after cell death and decay

(Bertilsson and Jones, 2003). Both allochthonous and autochthonous DOM provide metabolic substrates available for uptake by heterotrophic microorganisms in the photic zone (Pomeroy, 1974; Azam and Cho, 1987).

1.4.2 Redox Reactions

Organic carbon is the most common reducing agent in natural waters, driving many redox reactions in lakes. It can be produced through the reduction of CO_2 during photosynthesis using light energy or oxidized to CO_2 by the reduction of O_2 during respiration (Morel and Hering, 1993). Organic matter also accumulates at the bottom of lakes during sedimentation, producing steep redox gradients due to decomposition at the sediment-water interface (Wehrli, 1990). Intermediate zones are created in lakes by mixing, diffusion, and biological activity occurring between highly oxygenated surface waters and lake sediments creating a highly variable redox environment (Stumm and Morgan, 1996).

1.4.3 Photodegradation

During photodegradation, the aromatic rings and unsaturated carbon skeletons in DOM absorb ultraviolet (UV) radiation, breaking the bonds in the presence of a catalyst (Sinsabaugh and Findlay, 2003). Dissolved oxygen (DO) acts as an electron acceptor in this reaction and can convert DOM into more labile photoproducts including carboxylic acids, small organic compounds, and DIC, lowering the average molecular weight of carbon compounds (Opsahl and Benner, 1998; Zepp et al. 1998). These smaller organic products can then be consumed by and stimulate microbial activity (Miller and Moran, 1997; Tranvik et al. 2000).

The photodegradation of DOM also induces PM formation, especially in the presence of Fe as a catalyst (Gao and Zepp, 1998). Ferric Fe (Fe(III)) that is photolytically reduced to ferrous Fe (Fe(II)), and reoxidized in oxygenated waters to form ferric hydrous oxides, can bind with DOM and form PM. Although PM can form in both the light and the dark, Gao and Zepp (1998) suggest that photo-oxidation products of DOM complex more readily with Fe.

1.4.4 Mixing

When PM enters a lake, it can settle to the sediment, while DOM remains in suspension and is subjected to the physics and chemistry of the water and surrounding organisms. There is often significant mixing in the epilimnion (little mixing between the epilimnion and hypolimnion). When DOM molecules collide during mixing, they adhere due to the attractive van der Waals forces and flocculation of DOM can occur (Wetzel, 2001). The number of collisions and the adhesiveness of particles determine whether these collisions create larger DOM molecules or PM. These factors can be affected by Brownian motion, shear from laminar or turbulent flow, differential settling caused by the collision of larger particles, the capture of smaller particles within the boundary layer of larger particles, surface coagulation at the gas-water interface, and bacterial collision with colloids (O'Melia and Tiller, 1993; Kepkay, 1994).

1.4.5 Gas Exchange

Gas exchange is often very difficult to quantify. The amount of CO_2 that invades or evades from a lake is affected by the mineralization of DOM through metabolism or photodegradation. Photosynthesis can decrease CO_2 concentrations in the photic zones of lakes while respiration and photodegradation can increase CO_2 concentrations (Anesio and Granéli, 2003; Granéli et al. 1998; Bertilsson and Tranvik, 2000). The balance between these rates affects the release or resupply of CO_2 by gas exchange at the atmosphere-water interface.

In a study by Dillon and Molot (1997), net CO_2 evasion to the atmosphere occurred in most of their northern temperate study lakes. Thus there was in-lake mineralization of DOM to CO_2 because the CO_2 evasion was greater than DIC loading and precipitation. Photodegradation experiments were performed to study this in-lake mineralization and to compare the photodecay constants from these experiments to the mass balance rate constants. This comparison revealed that although photolysis could not account for all DOC losses to the atmosphere and to the sediments in lakes with DOC > 4mg/L, it could be responsible for

CO_2 evasion and carbon sedimentation in lower DOC lakes (<4mg/L) (Molot and Dillon, 1997).

1.4.6 Sedimentation

DOM can be lost to sedimentation after forming PM and settling out of suspension. This can occur through a number of processes discussed above including biological processes, photodegradation and mixing. During the biological consumption of DOM, living organisms mineralize carbon but also incorporate carbon into their biomass, increasing in size. When these organisms die, they decompose to DOM and DIC but can also end up in the sediment as PM. When DOM particles collide during mixing they adhere and flocculate according to the processes outlined in section 1.4.4 (O'Melia and Tiller, 1993; Kepkay, 1994; Wetzel, 2001).

1.5 Analytical Methods for Characterizing DOM

DOM is difficult to characterize because it is composed of many thousands of organic molecules that are present at different abundances depending on the sources of the DOM and processes influencing it. Two DOM samples could have the same concentration but different compositions (Lapworth et al. 2008), due to differences in colour, carbon lability, molecular weight, aromaticity, chromophore, or fluorophore concentrations. These are important characteristics to analyze in order to better understand the role of DOM in an aquatic system.

In attempts to characterize the function of DOM in an aquatic system, it is important to determine whether the DOM is labile (microbially available and easily degradable) or recalcitrant (more difficult to degrade). Fractions of DOM that are labile are determined as biodegradable within hours to weeks depending on the analytical procedure used. The remaining DOM fraction is considered refractory (Servais et al. 1987; Marmonier et al. 1995). In addition to investigating lability, other methods of characterization aim to examine DOM components that determine its function in the aquatic system.

Many methods may be used to measure either the quantity or the composition, or both, of DOM; however, spectral analyses are a fast and cost effective method to provide

estimates of the aromaticity, lability and potential sources of DOM (Weishaar et al. 2003) and thus are often used. Absorbance determines how much visible and UV radiation a sample can absorb and has been used to measure changes in DOM concentration and/or composition. The absorbance of certain wavelengths of UV or visible radiation has been associated with DOM composition, degree of degradation and aromaticity and relative quantification of hydrophobicity (Dilling et al. 2002; Traina et al. 1990). Specific UV absorbance (SUVA) uses the absorbance at 254nm normalized to the DOC concentration as a surrogate for aromaticity (recalcitrant aromatic functional groups that absorb UV) (Weishaar et al. 2003). The specific absorption coefficient at 350nm normalized to the DOC concentration (SAC_{350}) is used as an index of coloured DOM (CDOM; Moran et al. 2000). Specific ratios of absorption coefficients provide insight into the relative aromaticity or molecular size (De Haan and De Boer, 1987; Dahlén et al. 1996; Ågren et al. 2008) and water colour is often measured using absorbance at wavelengths around 400nm (Pace et al. 2012). However, only portions of the DOM pool contain chromophores that absorb UV, which may not comprise the entire DOM sample. Therefore, Kawasaki et al. (2011) suggests pairing absorbance measures with other DOM characterization techniques.

Fluorescence measures the excitation emission wavelengths where the fluorescence of a sample occurs and can provide information on the structure and humification of the DOM (McKnight et al. 2001; Hunt and Ohno, 2007; Fellman et al. 2010). Complex modeling using techniques such as parallel factor analysis (PARAFAC) is often required to manage the large datasets composed of fluorescence measurements to decompose these datasets into underlying fluorescent components (Murphy et al. 2013; Hunt and Ohno, 2007). However, only portions of the DOM pool contain fluorophores that absorb UV, which may not comprise the entire DOM sample (Hunt and Ohno, 2007).

Liquid chromatography organic carbon detection (LC-OCD) pairs size exclusion chromatography (SEC) with an organic carbon detector to separate groups of DOC molecules based on their hydrodynamic radii, allowing inferences about physical and chemical compositions (Huber and Frimmel, 1991; Aukes, 2012). Changes in the fractions of DOC suggest changes to the concentration and/or composition of the DOC and allows for the

comparison of DOC fractions between different samples (Huber and Frimmel, 1991; Huber et al. 2011). LC-OCD also allows fractions of DOC to be compared spatially, seasonally and annually and provides information on the size and type of DOC molecules while using only small amounts of sample.

There are many limitations to using absorbance, fluorescence, and LC-OCD techniques to measure DOM quality because they are affected by other parameters (e.g. pH and Fe concentration) as well. It is therefore important to have as much supporting data as possible including pH, DOC and trace metal concentrations when using these techniques to infer DOM composition.

1.6 Thesis Objectives

The overall objective of this thesis is to investigate the effect of dissolved Fe and pH on measures of DOM quality and on the quantity and quality of DOM during photodegradation in Canadian freshwaters.

This thesis is composed of an introductory chapter that reviews and outlines the scope and objectives of the thesis. The second chapter provides site descriptions and methods of data analysis. The objective of the third chapter is to quantify the influence of pH, Fe(II), and Fe(III) concentrations on measures of DOM quality and to compare these influences between a range of naturally occurring DOM qualities. The use of a large range of natural river, stream, and subsurface samples in pH and dissolved Fe titration experiments and the investigation of the influence of pH, Fe(II), and Fe(III) on LC-OCD results were unique to this study. The objective of the fourth chapter is to quantify the effect of increased pH and Fe concentrations on DOM quantity and quality during photodegradation and POC formation and to characterize the changes in $\delta^{13}C$ during DOM photolysis in three stream waters from Ontario, Canada. This study was the first, to my knowledge, to collect and analyze subsamples during the experiment to observe changes in carbon transformation rates and $\delta^{13}C\text{-}CO_2$ values.

Table 1.1. Annual global transport of carbon (Pg) through inland waters (Cole et al. 2007).

Water type	Form of C	CO_2 to atm	Sediment storage	Ocean export
Lakes	Inorganic C	0.7-0.15	NA	
	Organic C	0	0.3-0.7	
Reservoirs	Inorganic C	0.28	NA	
	Organic C	0	0.16-0.2	
Rivers	Inorganic C	0.15-0.30	NA	0.21-0.30
	Organic C	0	NA	0.38-0.53
Groundwater	Inorganic C	0.003-0.03	0	0.13-0.25
	Organic C	0	<0.016	
Total		**0.75**	**0.23**	**0.9**

NA: no estimate was made.

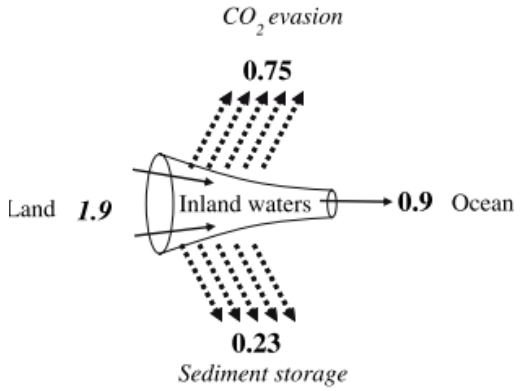

Figure 1.1. Aquatic carbon balance (Cole et al. 2007).

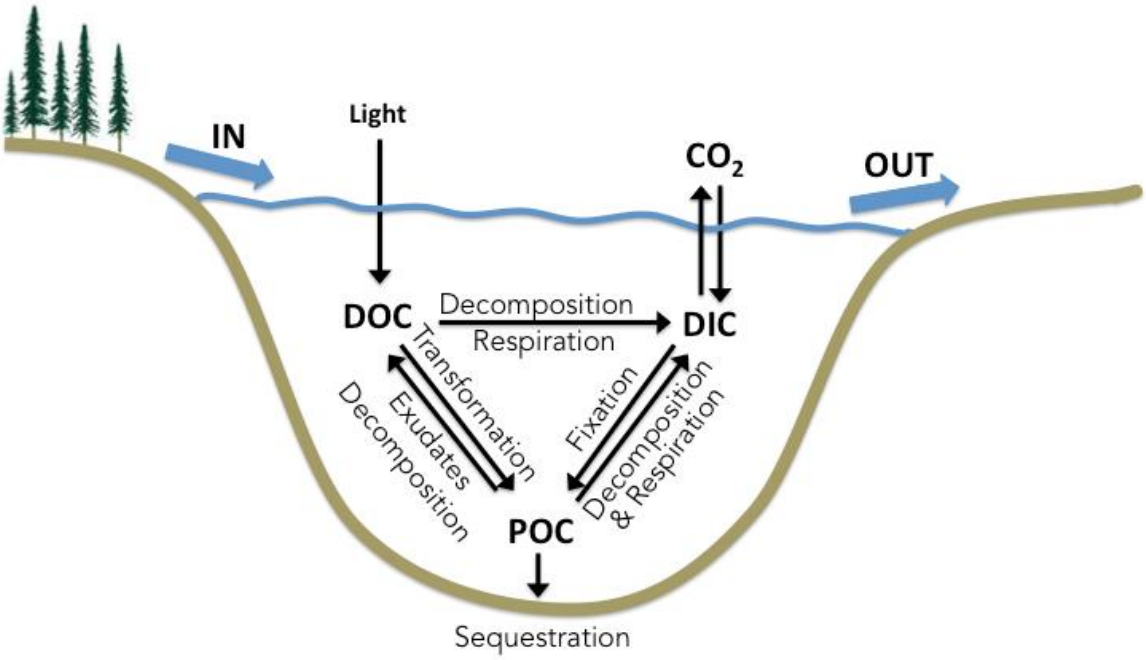

Figure 1.2. Carbon cycling processes in freshwater lakes (Schiff, unpublished figure).

Chapter 2 Site Descriptions and Sample Characterization

2.1 Site Descriptions

A range of freshwaters from Northwest Territories (NWT) and Ontario, Canada (Table 2.1) were used in the titration experiments and three of these samples (U8, NEIF and DE10) were used in the photolysis experiment. These samples were selected because longterm historical databases are kept for these sites and previous work shows DOM quality is very different in these sites. Also, the samples covered a large range of natural pH, Fe and DOC concentrations and thus they were ideal for these experiments. The three sites used in photolysis experiment were selected to cover a range of Fe and DOC concentrations from the historically monitored and previously studied (Chomicki, 2009) sites.

Two sites, Airport Pond (Pond) and Airport P2 (P2), were located in the subarctic on the Taiga Shield approximately 5km west of Yellowknife, NWT, Canada (62° 27' N, 114° 31' W). Pond samples were collected from the surface water of the pond and P2 samples were collected from a piezometer at a depth of approximately 1.4m. The mean annual air temperature of this region is -4.9°C with a mean annual total precipitation of 299mm (MSC, 2014). These sites are part of a hummocky peatland with approximately 5m of organic layer underlain by mineral soil and sporadic discontinuous permafrost. The sites underlie a thin canopy of black spruce (*Picea mariana*), as well as Labrador tea (*Rhododendron groenlandicum*), lichens (*Cladina* spp. and *Cladonia* spp.), cloudberry (*Rubus chamaemorus*), mosses (*Sphagnum* spp.), Tamarack (*Larix laricina*) and cotton grass (*Eriophorum angustifolium*) (Hickman, 2016).

Stream waters were collected from three streams located within the Experimental Lakes Area (ELA) near Kenora, Ontario, Canada: Lake 302 Upland 8 (U8), Rawson Lake (L239) Northeast Inflow (NEIF), and Northwest Inflow (NWIF), and four streams located in the Muskoka and Haliburton region 200 km north of Toronto, Ontario, Canada: Dickie Lake Inflow 10 (DE10), Harp Lake Inflow (H4 1.0 from 2014 and H4 2.0 from 2015 and H4-21), and Plastic Lake Inflow 1-08 (P1-08). River water was collected from the Grand River at two

sites, one just below Belwood Lake (Belwood) and the other at the Brant Conservation Area (BCA). Samples from these sites were collected at weirs or directly from the stream or river.

The ELA watersheds (49°30'N-49°45'N, 93°30'W-94°00'W) lie on the Precambrian Shield, are composed of thin tills, and contain *Sphagnum* peatlands dominated by Labrador tea and leather leaf (*Chamaedaphne calyculata*). Jack pine (*Pinus banksiana* Lamb.), trembling aspen (*Populus tremuloides*), white birch (*Betula papyrifera*), black spruce, balsam fir (*Abies balsamea*), and red pine (*Pinus resinosa*) are also common at the ELA. The ELA has a humid continental climate with an annual mean air temperature of 2.2°C and a mean annual total precipitation of 689mm (McCullough and Campbell, 1993). Detailed descriptions of the streams and forested catchments can be found in Brunskill and Schindler (1971).

The Lake 302 Upland 8 (U8) catchment is 7.2ha of granodiorite bedrock covered by thin mineral soils composed largely of silt loam (Lamontagne and Schiff, 1999; McCullough and Campbell, 1993). There is partial lichen cover and a young forest of dense stands of jack pine on thinner soils, while black spruce, and white pine (*Pinus strobus* L.) occur on deeper soils. Common juniper (*Juniper communis* L.) shrubs, common bracken (*Pteridium aqualinum*) ferns, feathermoss (*Pleurozium schreberi*), and fork mosses (*Dicranum* spp.) were also common (Lamontagne and Schiff, 1999; St. Louis et al. 1996).

Lake 239 Northwest Inflow (NWIF) catchment (49°40'N, 93°44'W) is 56.4ha of thin discontinuous deposits of granitic, sandy till overlying granite and granodiorite bedrock. Approximately 72% of the watershed is upland with shallow mineral soil where deposits are generally less than 1m thick on valley slopes with deeper deposits in the lowlands. Approximately 21% of the watershed is barren rock outcrop, and 3% is wetlands. Jack pine, black spruce, trembling aspen, white birch, black alder (*Alnus nigra*), balsam poplar (Populus balsamifera), and pin cherry (Prunus pennsylvanica) are the dominant vegetation. Herbaceous vegetation includes Fireweed (*Epilobium angustifolium*), black bindweed (*Polygonum convolutus*), raspberry (*Rubus strigosus*), and blueberry (*Vaccinium* spp.).

Lake 239 Northeast Inflow (NEIF) (49°40'N, 93°44'W) drains 10.6ha of thin discontinuous deposits of granitic, sandy till overlying granite and granodiorite bedrock. Approximately 62% of the catchment is upland with shallow mineral soil where deposits are generally less than 1m thick on valley slopes with deeper deposits in the lowlands. Approximately 3% of the watershed is barren rock outcrop, and 35% consists of wetlands (Bayley and Schindler, 1987). Jack pine, black spruce, trembling aspen (*Populus tremuloides*), white birch (*Betula papyrifera*), black alder (*Alnus nigra*), balsam poplar (*Populus balsamifera*), and pin cherry (Prunus pennsylvanica) are the dominant vegetation. Herbaceous vegetation includes Fireweed (*Epilobium angustifolium*), black bindweed (*Polygonum convolutus*), raspberry (*Rubus strigosus*), and blueberry (*Vaccinium* spp.).

The Dorset watersheds lie on the southern tip of the Precambrian Shield and the boundary of the Boreal ecozone (Porcal et al. 2014), are composed of thin till deposits (<1m thick), and contain peatlands. The mean annual air temperature of this region is 4.9°C with a mean annual total precipitation of 1010mm (Dillon et al. 1991). Detailed descriptions of the streams, forested catchments and meteorological features are given in Dillon et al. (1991).

The Dickie 10 (DE10) stream catchment is 78.9ha and is underlain with thin till (< 1m thick) and peat over hornblende migmatite bedrock (Jeffries and Snyder, 1983). Approximately 78% of the catchment consists of shallow surficial deposits, generally less than 1m in depth. Of the remaining area, approximately 3% consists of deep surficial deposits (> 1m deep), and 17% consists of peatlands (Yao, 2009). DE10 discharges from a dark coloured bog with a baseflow of 0.25m/year, is a major water and carbon contributor to Dickie Lake and is surrounded by deciduous trees.

The Harp 4 (H4) stream catchment (45°22'N, 79°08'W) is 119.1ha underlain with gneissic bedrock of the Canadian Shield with mixed forests of sugar maple (*Acer saccharum*), beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), white pine, and aspen (*Populous tremuloides*) (Schiff et al. 1997). H4 is a second order stream to Harp Lake with a catchment composed of 5% peatland (Dillon and Molot, 1997). This stream discharges from a wetland. H4-21 is a first order upland stream that discharges from a beaver pond into

H4. The H4-21 catchment size is 3.7ha and overlies up to 15m of glacial till (Hinton, 1998). Groundwater flows through the glacial till to maintain perennial stream discharge (Hinton et al. 1993) and the total annual precipitation ranges from 741mm to 1246mm (Mueller, 2008).

Plastic 1-08 (P1-08) is a small stream within a 3.4ha catchment (45°11'N, 78°50'W) that discharges into Plastic Lake through the second order stream, Plastic 1 (Mueller, 2008). White pine and eastern hemlock (*Tsuga canadensis*) are the most abundant trees in the watershed. The Plastic Lake catchment is underlain with thin (< 0.5m thick) deposits of Pleistocene glacial till with exposed bedrock (10% of the catchment) of Precambrian metamorphic silicate bedrock (Devito and Dillon, 1993; Mueller, 2008). Annual precipitation ranges from 786mm to 1213mm (Mueller, 2008).

The Grand River sites are located near Fergus (Belwood) and Brantford (BCA) in the Grand River Watershed, Southern Ontario, Canada that drains an area of 6800km^2 into Lake Erie (Rosamond, 2013). The catchment is underlain with Paleozoic limestone and shale overlain by calcite-rich glacial drift. The Grand River is seventh-order and is well buffered by dissolved carbonate (Rosamond, 2013).

2.2 Sample Characterization

Stream water samples were collected in pre-rinsed carboys and filtered through 2 and 0.9µm Balston cartridge filters then 0.45µm Whatman filters. Wotton (1994), among others, suggests filtering water to 0.2µm to limit microbial activity. However, a filter size of 0.45µm was chosen to compare these samples to a large body of literature that measures DOC concentration and quality in samples filtered to 0.45µm. Once filtered, bulk samples were kept in the dark and cold (4°C) until experiments were conducted. After experiments were conducted, all samples were filtered to 0.45µm prior to analysis except for samples used for pH, POC, and CO_2 measurements. Unless otherwise noted, all analyses were completed at the Environmental Geochemistry Laboratory, University of Waterloo, Waterloo, Ontario.

2.2.1 Spectral Analysis

Absorbance

Spectral absorbance (A_λ) samples were collected in 20mL glass vials, and measured using a Cary 100 UV-Vis spectrophotometer. Samples were scanned at 5nm intervals from 200 to 800nm in a 1cm quartz cuvette and corrected based on the absorbance of NANOpure water (≥18.2mΩ-cm). Absorbance spectra were normalized to absorbance coefficient (a_λ) units (m^{-1}) using the following equation (Xiao et al. 2013):

$$a_\lambda = \frac{2.303\, A_\lambda}{0.01}$$

Specific ultraviolet absorbance ($SUVA_{255}$, L/(mg·m)) and specific absorption coefficients (SAC; L/(mg·m)) were calculated using the ratio of UV absorption at λ = 255nm (m^{-1}) and visible absorption at λ = 350nm and 410nm (m^{-1}), respectively, to DOC concentration (mg/L) (Weishaar et al. 2003; Moran et al. 2000).

2.2.2 Chemical Analysis

pH samples were collected in 15mL PET containers and measured with a Hach HQ40d meter and IntelliCAL™ PH301 probe. DOC concentration samples were collected in the same 20mL glass vials as spectral absorbance samples and measured using a Shimadzu Total Organic Carbon (TOC-L) analyzer with a precision of ± 0.3mg-C/L.

In titration experiments, cation concentration samples were collected in 15mL Celltreat® Scientific Centrifuge Tubes, acidified with 0.3mL of Omni*Trace* Ultra® Nitric Acid (67-70%), and analyzed using a Perkin Elmer Optima 8000 ICP-EOS at the Centre for Cold Regions and Water Science (CCRWS) at Wilfrid Laurier University with a precision of ± 0.1mg-C/L. In the photolysis experiment, cation concentration samples were collected in 20mL Starplex containers, acidified with Omni*Trace* Ultra® Nitric Acid (67-70%) to a pH of approximately 2, and analyzed using a Horiba Jobin Yvon Ultima 2 ICP at the Canadian Centre for Inland Waters, Environment Canada, Burlington, Ontario with a precision of ± 0.1mg-C/L.

2.2.3 Photolysis Chemical Analysis

Samples for DIC concentrations were collected in 12mL Labco Exetainer® vials, capped with baked Labco Exetainer® caps with no headspace. Immediately prior to analysis, 6mL of helium was added to the vials while 6mL of sample was simultaneously removed. Samples were acidified with 0.05mL H_2SO_4, injected with another 6mL of helium and placed on a shaker for 2 hours to equilibrate aqueous and gaseous phases. DIC concentrations were measured using a Varian CP-3800 Gas Chromatograph.

CO_2 concentrations were analyzed on a Varian CP-3800 Gas Chromatograph. The initial headspace of the Tedlar bags were composed of laboratory air from the CCRWS at Wilfrid Laurier University, so initial CO_2 concentrations were measured using 12mL Labco Exetainer® vials over-pressurized with 25mL of laboratory air and presented as an average of two samples. The subsampled CO_2 concentration data collected during the photolysis experiment consisted of one 5mL Labco Exetainer® vial containing approximately 9mL of sample taken from the second set of duplicates. The final CO_2 concentration data presented is an average of two samples from one duplicate bag from each treatment.

Samples of POC were collected on precombusted and weighed QMA filters by filtering approximately 2L of water from each bag after thorough manual shaking. The filters were then combusted at 60°C and weighed again. The mass of the POC was insufficient to be measured accurately in all samples.

2.2.4 LC-OCD Analysis

DOC composition analysis was completed using a liquid chromatography organic carbon detection (LC-OCD) method. Samples were collected in 40mL glass vials and analyzed using a Toyopearl HW-50S (Tosoh, Japan) size-exclusion column at the University of Waterloo. This method separates DOC into 5 fractions based on their molecular size: biopolymers, humic substances, building blocks, LMW-acids and LMW-neutrals. All treatments (except dark) were run before the photolysis experiment began in order to obtain initial conditions. One set of final duplicates from each site was run for 3 of the treatments (Light, Light + Fe and Light + pH) and one final dark treatment was run for U8 to show minimal change in LC-

OCD results. Initial samples were taken before the titration experiment. One set of final duplicates for the Fe(II) titration experiment and two samples from the pH titration experiment were also run.

2.2.5 Isotope Analysis of Photolysis Experiment Samples

CO_2

Initial $\delta^{13}C$-CO_2 values were obtained from two 12mL Labco Exetainer® vials over-pressurized with 25mL of laboratory air from the CCRWS building at Wilfrid Laurier University. Subsamples during the photolysis experiment were collected from the headspace of the second set of Tedlar bag duplicates by injecting 15mL of sample into an evacuated 12mL Labco Exetainer® vial and final samples were collected from the headspace of all Tedlar bags by injecting 25mL of sample into an evacuated 12mL Labco Exetainer® vial. The samples were analyzed on a Micromass Isochrom Gas Chromatograph Combustion Isotope Ratio Mass Spectrometer (GC-C-IRMS) at the Environmental Isotope Laboratory (EIL), Waterloo, Ontario, with a precision of ± 0.3‰. In order to insert enough CO_2 for accurate isotopic measurements, sample injection volume was adjusted for each sample depending on concentration. Duplicates were run every 5 samples and all isotope results were reported in standard δ notation relative to a Pee Dee Belemnite (PDB) standard as shown in Equation 2.1 where R is ^{13}C:^{12}C.

Equation 2.1 $$\delta^{13}C = \left(\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right) \times 10^3 \text{ ‰}$$

DIC

$\delta^{13}C$-DIC samples were collected in 12mL Labco Exetainer® vials, capped with baked Labco Exetainer® caps with no headspace before and after the photolysis experiment. 6mL of helium was added to the vials while 6mL of sample was simultaneously removed. Samples were acidified with 0.05mL H_2SO_4, injected with another 6mL of helium and placed on a shaker for 2 hours to equilibrate aqueous and gaseous phases.

Gas from the headspace was analyzed on a Micromass Isochrom Gas Chromatograph Combustion Isotope Ratio Mass Spectrometer (GC-C-IRMS) at the EIL, Waterloo, Ontario, with a precision of ± 0.3‰. Duplicates were run approximately every 5 samples.

DOC

δ^{13}C-DOC samples were collected before and after the photolysis experiment. Samples were concentrated by heating at 60°C in glass beakers on a hotplate, freeze dried and analyzed using a Thermo Finnigan-Delta^{plus}XL coupled to a Costech 4010 continuous flow isotope ratio mass spectrometer (CF-IRMS) with a precision of ± 0.2‰ by the EIL, Waterloo, Ontario. Duplicates were run approximately every 8 samples.

POC

δ^{13}C-POC samples were collected after the photolysis experiment by filtering water through pre-combusted QMA filters. The filter was then dried at 60°C in an oven for a minimum of 4 hours and analyzed by the EIL, Waterloo, Ontario using a Thermo Finnigan-Delta^{plus}XL coupled to a Costech 4010 continuous flow isotope ratio mass spectrometer (CF-IRMS) with a precision of ± 0.2‰. Duplicates were run approximately every 3 samples.

2.3 Statistics

Linear regression, p-values, and R^2 values were calculated using R, version 3.3.0 (R, 2016) and significance was assigned a *p*-value of 0.05. If sample concentrations changed by 1.7 times the standard deviation (1.7SD) or greater, then a change in concentration was said to have occurred. Standard deviation was calculated from three replicates of the same sample

Table 2.1. Summary of selected characteristics of sampling sites (Dillon et al. 1991; Hinton et al. 1997; Mueller, 2008).

Site name	Location	Site Description	Vegetation	Water Type	Catchment Percent Wetland
Pond	Yellowknife	Hummocky peatland, subarctic, 5m of organic layer underlain by mineral soil and sporadic discontinuous permafrost	Taiga	Pond Surface Water	-
P2	Yellowknife	From a 1.4m deep piezometer located in the subarctic, 5m of organic layer underlain by mineral soil and sporadic discontinuous permafrost	Taiga	Groundwater	-
U8	ELA	Discharging into L302, surrounded by coniferous trees and *Sphagnum* mosses, high elevation and low vegetation cover	Boreal	Headwater Stream	0
NEIF	ELA	Discharging into L239, flows through moss and swamp valley bottom wetlands	Boreal	Headwater Stream	35
NWIF	ELA	Discharging into L239	Boreal	Headwater Stream	3
Belwood	Fergus	Grand River just below the Belwood reservoir	Deciduous	7^{th} Order River	0
BCA	Brantford	Grand River located in the Brant Conservation Area	Deciduous	7^{th} Order River	0
DE10 or DE10-ND	Dorset	Discharges from a dark coloured bog into Dickie Lake, surrounded by deciduous trees	Boreal	Headwater Stream	17.1
H4 1.0 or H4 2.0	Dorset	Discharges from a wetland into Harp Lake	Boreal	2^{nd} Order Stream	5
H4-21	Dorset	Upland stream that discharges from a beaver pond into H4	Boreal	Headwater Stream	0
P1-08	Dorset	Discharges into Plastic Lake	Boreal	Headwater Stream	0
IHSS	N/A	International Humic Standard made in the UW Environmental Geochemistry Laboratory	N/A	International Humic Standard	N/A
DI	N/A	From UW Environmental Geochemistry Laboratory	N/A	NANOpure Water	N/A

Chapter 3 Do pH, Fe, and Fe Valence Influence Measures of DOM Quality?

3.1 Introduction

In recent decades, brownification, a term used to describe the increase in water colour observed in Europe and eastern North America, has been a topic of scientific interest (Roulet and Moore, 2006; Graneli, 2012). Increases in water colour can directly impact ecosystem health and function by increasing solar radiation absorption and thus, altering lake photochemistry, thermal stratification and heat budgets (Ekström, 2013; Graneli, 2012; Mostofa et al. 2013; Litved et al. 2001). Although brownification has commonly been attributed to increases in dissolved organic matter (DOM) concentrations (estimated using dissolved organic carbon (DOC) concentrations), other parameters such as trace metal concentration, pH and DOM composition also influence water colour (Weishaar et al. 2003; Poulin et al. 2014; Xiao et al. 2013). It is important to determine if increasing DOM concentration is the cause of brownification because DOM has implications for aquatic metabolism, trace metal transport, and drinking water treatment (Mostofa et al. 2013). Although absorbance data has commonly been used to link increases in DOM concentrations to increases in water colour, it is inconclusive to analyze absorbance data or any other DOM analyses data without supporting information on other important aquatic parameters such as trace metal concentration, pH and DOM composition (Graneli, 2012). As these parameters influence water colour, they have important, but poorly defined relationships with analytical techniques used for DOM quantification and characterization and should be considered when investigating brownification or making conclusions about changes in DOM quantity or composition (Ekström, 2013; Kritzberg and Ekström 2012).

3.1.1 Analytical Methods for Characterizing DOM

DOM is composed of a mixture of organic compounds but is often operationally defined as DOC that can pass through a filter; where filter sizes cited in literature range from 0.1μm to 0.7μm (Chin et al. 1998; Mostofa et al. 2013). DOC is one of the major forms of carbon

transported throughout aquatic systems and plays an important role in the metabolism of lake ecosystems, protection of aquatic organisms, drinking water treatment, thermal stratification and heat budgets (Mostofa et al. 2013).

DOC is difficult to characterize because it is composed of many thousands of organic molecules that are present at different abundances depending on the sources of the DOC and processes influencing it. Two DOC samples could have the same concentration but different compositions (Lapworth et al. 2008), due to differences in colour, carbon lability, molecular weight, aromaticity, chromophore or fluorophore concentrations and thus function differently in aquatic ecosystems. These are thus important characteristics to analyze in order to better understand the role of DOC in an aquatic system.

In attempts to characterize the function of DOC in an aquatic system, it is important to determine whether the DOC is labile (microbially available and easily degradable) or recalcitrant (more difficult to degrade). Fractions of DOC that are labile are determined as biodegradable within hours to weeks depending on the analytical procedure used. The remaining DOC fraction is considered refractory (Servais et al. 1987; Marmonier et al. 1995). In addition to investigating lability, other methods of characterization aim to examine DOC components that determine its function in the aquatic system.

Many methods may be used to measure either the quantity or the composition, or both, of DOC; however, spectral analyses are a fast and cost effective method to provide estimates of the aromaticity, lability and potential sources of DOC (Weishaar et al. 2003) and thus are often used. Absorbance determines how much visible and UV radiation a sample can absorb and has been used to measure changes in DOC concentration and/or composition. The absorbance of certain wavelengths of UV or visible radiation has been associated with DOC composition, degree of degradation and aromaticity and relative quantification of hydrophobicity (Dilling and Kaiser, 2002; Traina et al. 1990). Specific UV absorbance (SUVA) uses the absorbance at 254nm normalized to the DOC concentration as a surrogate for aromaticity (recalcitrant aromatic functional groups that absorb UV) or molecular weight (Weishaar et al. 2003). The specific absorption coefficient at 350nm normalized to the DOC concentration (SAC_{350}) is used as an index of coloured DOM (CDOM; Moran et al. 2000).

Specific ratios of absorption coefficients provide insight into the relative aromaticity or molecular size (De Haan and De Boer, 1987; Dahlén et al. 1996; Ågren et al. 2008) and water colour is often measured using absorbance at wavelengths around 400nm (Pace et al. 2012). However, only portions of the DOC pool contain chromophores that absorb UV, which may not comprise the entire DOC sample. Therefore, Kawasaki et al. (2011) suggests pairing absorbance measures with other DOC characterization techniques.

Fluorescence measures the excitation emission wavelengths where the fluorescence of a sample occurs and can provide information on the structure and humification of the DOC (McKnight et al. 2001; Hunt and Ohno, 2007; Fellman et al. 2010). Complex modeling using techniques such as parallel factor analysis (PARAFAC) is often required to manage the large datasets composed of fluorescence measurements to decompose these datasets into underlying fluorescent components (Murphy et al. 2013; Hunt and Ohno, 2007). However, only portions of the DOC pool contain fluorophores that absorb radiation, which may not comprise the entire DOC sample (Hunt and Ohno, 2007).

Liquid chromatography organic carbon detection (LC-OCD) pairs size exclusion chromatography (SEC) with an organic carbon detector to separate groups of DOC molecules based on their hydrodynamic radii and elution time, allowing inferences about physical and chemical compositions (Huber and Frimmel, 1991; Aukes, 2012). Changes in the fractions of DOC suggest changes to the concentration and/or composition of the DOC and allows for the comparison of DOC fractions between different samples (Huber and Frimmel, 1991; Huber et al. 2011). LC-OCD also allows fractions of DOC to be compared spatially, seasonally and annually and provides information on the size and type of DOC molecules while using only small amounts of sample.

There are many limitations to using absorbance, fluorescence and LC-OCD techniques because they measure a bulk sample instead of looking at each parameter (e.g. DOC concentration or composition) individually. It is therefore important to have as much supporting data as possible including pH, DOC and trace metal concentrations when using these techniques to infer DOM composition.

3.1.2 Effect of Dissolved Fe on Quality Measures of DOM

Dissolved Fe absorbs UV and visible light and can influence the spectral absorbance of natural waters (Kelton et al. 2007; Kritzberg and Ekström, 2012; Sarkkola et al. 2013). Fe that passes through a 0.45μm filter can be a) dissolved monomeric or polymeric inorganic Fe complexes, b) ferrihydrate, or c) Fe bound to OM (Jensen et al. 2003; Lofts et al. 2008). Regardless of DOM composition, researchers using DOM from international humic substances society (IHSS) standards, have shown that UV and visible absorption increases linearly with increasing Fe(III) concentrations (Poulin et al. 2014, Weishaar et al. 2003, Xiao et al. 2013). This relationship is observed because Fe(III) in natural water can complex with DOM, form Fe hydroxides, or exist as free dissolved Fe, all of which absorb UV and visible light (Doane and Horwáth, 2010; Maloney et al. 2005) depending on the pH. The pH, Eh, temperature, DOC concentration and composition primarily determine Fe solubility, its species and the rate of oxidation of Fe(II) to Fe(III) in oxygenated water (Wetzel, 2001). However, in the presence of DOC, Fe(III) exhibits enhanced aqueous solubility compared to inorganic solubility (Gaffney et al. 2008; Pédrot et al. 2011; Pullin and Cabaniss, 2003). Weyhenmeyer et al. (2014) showed that the absorbance of filtered water at 420nm (a_{420}) normalized by DOC concentration is increased by the presence of dissolved Fe while Poulin et al. (2014) similarly found that Fe(III) addition increased $SUVA_{254}$. Both Fe(II) and Fe(III) have been found to quench DOC fluorescence; both the degree and region of the fluorescence quenching were influenced by the dissolved Fe:DOC ratio, DOC composition and pH (Poulin et al. 2014). Fluorescence changes in natural waters due to the wide range of metals that form complexes with fulvic acids (Green et al. 1992). Poulin et al. (2014) also found that there was a low PARAFAC sensitivity to dissolved Fe addition when using a 7- and 13-component PARAFAC model. Although researchers provide evidence for the influence of dissolved Fe on spectral measurements, more research is needed to better understand the influence of Fe on spectral measurements with different DOM qualities and how this may change spatially and temporally, especially in natural waters (Weyhenmeyer et al. 2014).

3.1.3 Effect of pH on Quality Measures of DOM

pH is an important parameter to consider in spectral analysis because it affects the solubility and structure of DOM molecules and the solubility of trace metals, such as Fe (Chin et al. 1998; Pace et al. 2012; Porcal et al. 2014). The increased solubility of trace metals at low pH can increase metal complexation to DOM and thus change DOM composition. At low pH, DOM molecules are condensed as a result of the protonation of functional groups, reducing the overall number of negative charges per DOM molecule and limiting the exposure of chromophores to radiation (Chin et al. 1998; Myneni et al. 1999; Baalousha et al. 2006; Pace et al. 2012). At high pH, deprotonation promotes the expansion of DOM molecules, exposing more chromophores. Ionic strength also affects the structure of DOM molecules with structural changes at higher ionic strength. With more exposed chromophores to absorb solar radiation, absorbance is often higher (Pace et al. 2012).

Increasing pH also causes an increase in fluorescence intensities (Patel-Sorrentino et al. 2002; Pullin and Cabaniss, 1995). When measuring fluorescence, spectral properties may change due to chemical reactions, quenching, interactions between fluorophores, and changes in the electronic environment of the fluorophores (e.g. changes in pH) and can make PARAFAC modeling difficult or impossible (Murphy et al. 2013).

The pH and dissolved Fe concentration can change dramatically within one aquatic system and between different aquatic systems over space and time. If pH changes spatially or temporally, it is likely to influence DOM solubility, structure, and complexation with trace metals, which also influences spectral measurements. In order to compare spatial and temporal changes in DOM, it is important to first quantify the effects of pH and Fe on measures of DOC concentration and quality. This can avoid falsely attributing spectral changes to changes in DOM quantity or quality.

3.1.4 Brownification

There are several proposed drivers of brownification (increases in water "colour") including a) climate change causing changes in temperature, precipitation, runoff, frequency of severe events, hydrological flow paths, and resultant water quality, b) changes in land use causing

changes in water quality and runoff and c) the recovery of freshwater systems from acidic deposition (Graneli, 2012; Monteith et al. 2007). All of the potential drivers can be connected to human influence; however, natural fluctuations within aquatic environments complicate the interpretation. Understanding which parameters (DOM, dissolved Fe or pH) are increasing water colour in freshwaters will help identify which potential drivers are causing brownification. This information is important for a) predicting how brownification trends will respond in the future with projected changes in different regions around the world (Lapierre et al. 2013), b) for the management of landscapes to reduce brownification, and c) for the management of drinking water treatment.

Climate change has caused increased temperature and precipitation during the last century in Scandinavian countries (Hyvärinen, 2003). Scenarios developed by the Regional Climate Development Under Global Warming (REGCLIM) predict further long-term increases in temperature and changes in precipitation, including increases in the frequency and severity of extreme events such as floods and storms (Hongve et al. 2004). These extreme precipitation events cause saturated soils and increased flow through upper organic soil horizons (Steinberg, 2003), leading to increases in the fluxes and changes to the chemical composition of DOM compared to low flow conditions (Schiff et al. 1998). This could also lead to increased acidification as DOM can contribute to the acidity of surface waters (Brakke et al. 1987).

As a result of the industrial revolution, toxic emissions including sulfur and nitrous oxides have polluted and acidified the natural environment. These aerosols can provide a nucleus for moisture accumulation, causing acidic precipitation. Acid rain caused the acidification of lakes of glaciated landscapes across eastern North America and northern and central Europe, decreasing the pH and increasing the solubility of metals in solution (Monteith et al. 2007). Today, acidic aerosol release is regulated and these lakes continue to recover from this acidification, which can change metal solubility and DOM properties (Graneli, 2012; Pace et al. 2012).

Researchers often use spectral analyses to infer changes in DOC concentration and composition in freshwater ecosystems to help explain brownification trends (Graneli, 2012).

Often, historically, spectral analyses are all that is available. Although some researchers discuss the relationship between water colour, pH, dissolved Fe and DOC concentration and DOC composition, often metal concentrations and pH are ignored when calculating trends (Poulin et al. 2014; Weishaar et al. 2003; Xiao et al. 2013). It is difficult to solely use spectral analyses to determine which parameter (DOC, Fe or pH), or combination of parameters, contribute to the brownification of a freshwater environment. Instead, a more complex data set, including data on pH, trace metal and DOC concentrations and DOC composition is required.

3.1.5 Objectives

The objective of this study was to determine which measures of DOM quantity and/or composition were affected by changes in pH, Fe(II), and Fe(III) concentrations and to what extent for a variety of different types of naturally occurring DOM samples. This was done using a series of titration experiments to adjust the pH and dissolved Fe concentrations. A wide range of river, stream and subsurface samples from the Northwest Territories and Ontario, Canada were used to cover a large natural range of dissolved Fe and pH interactions with DOM. The use of a large range of natural samples and the investigation of the influence of pH, Fe(II) and Fe(III) on LC-OCD results are novel to this study.

3.2 Methods

3.2.1 Experimental Design

IHSS is a Suwannee River fulvic acid (SRFA) standard purchased from the International Humic Substances Society and was prepared at a concentration of 5.8mg-C/L in NANOpure water. It represents primarily wetland-derived aquatic humic substances comparable to the natural sites selected and can be used to compare to the SRFA commonly used in other published studies (Xiao et al. 2013). Subsamples were taken from each sample for initial DOC concentrations and pH. Samples with DOC concentrations above 7 mg-C/L were diluted to 6-7 mg-C/L with NANOpure water (Table 5.1) to achieve similar concentrations across all sites. To assess possible dilution effects, one site with a DOC concentration greater

than 7 mg/L (DE10) was left undiluted (DE10-ND). DOC, dissolved oxygen (DO), cation and anion concentrations, pH, absorbance, fluorescence and LC-OCD were analyzed following dilutions.

pH was adjusted to cover a pH range of natural systems (3, 4.5, 6, 7.5, 9). Aliquots of initial samples were left unaltered and assigned to target pH values near their initial pH (Table 3.1). $FeCl_2$ and $FeCl_3$ were added to cover a dissolved Fe range of natural systems (1, 2, 3, and 4 mg/L and 0.5, 1.0, 1.5 and 2.0 mg/L, respectively). Both $FeCl_2$ and $FeCl_3$ were chosen because Fe(II) and Fe(III) are known to influence measures of DOM differently (Poulin et al. 2014, Xiao et al. 2013).

pH Titration Experiment

Samples were distributed into 250mL glass vials and titrated to the remaining four target pH values using < 1mL of 0.1M or 1M HCl or NaOH depending on the buffering capacity of the sample. pH was checked to ensure that it was in the desired range (within 0.85 pH units) and remained stable overnight.

Fe(II) Titration Experiment

All samples and the NANOpure water used for the $FeCl_2$ solution were bubbled with N_2 gas to remove oxygen and were titrated to a pH of 3.5 ± 0.4 using HCl to prevent oxidation of Fe(II). A 250 mg-Fe/L solution of $FeCl_2$ was made using Alfa Aesar Iron (II) chloride (ultra dry) mixed into NANOpure water (solution pH ~ 3.5) and subsequently filtered to 0.45µm to ensure no particulate Fe was added to samples. Samples were distributed into glass vials and the $FeCl_2$ solution was added to reach the four target dissolved Fe concentrations (

Table 5.2). The samples were subsequently placed in an anoxic glovebox from July 22, 2015 until July 27, 2015 (5 days) to allow the Fe time to complex with the DOM. Once removed from the glovebox, all vials were titrated with NaOH to their starting pH (Table 3.1) and checked the following day to ensure that the pH was stable overnight.

Fe(III) Titration Experiment

A 250 mg-Fe/L solution of $FeCl_3$ was made using Alfa Aesar Iron (III) chloride mixed into NANOpure water (solution pH ~ 2.6) and subsequently filtered to 0.45µm to ensure no particulate Fe was added to samples. Samples were distributed into glass vials and the $FeCl_3$ solution was added to reach the four target dissolved Fe concentrations (Table 5.3) (acidifying samples by a maximum of 1.5 pH units) and left overnight to allow the Fe time to complex with the DOM. All vials were titrated with NaOH to their initial pH (Table 3.1) and checked the following day to ensure pH was stable overnight.

Once all samples were stable overnight, they were filtered to 0.45µm before analysis of DOC, cation, and anion concentration, pH, absorbance, fluorescence, and LC-OCD to characterize the starting conditions.

3.3 Results

3.3.1 Initial pH, Fe, DOC, and DOC quality of samples from different sites

Study sites covered a wide range of pH values, Fe and DOC concentrations (Figure 3.1, Figure 3.2) and DOC compositions (Figure 3.3, Figure 3.4, Figure 3.5). Although there was a positive relationship between Fe and DOC concentrations (Figure 3.2) and between $SUVA_{255}$ and Fe:DOC (Figure 3.3) in the wide range of samples, there were also differences between samples (over 50% in DOC with Fe and over 20% in $SUVA_{255}$ and Fe:DOC) in some cases.

Overall, there were similar DOC fractions (determined by LC-OCD) in samples from a shared location, but differences between locations. As outliers from this trend, the Dorset Plastic stream (P1-08) (the sub-boreal upland stream) and the Grand River sample (BCA) had the lowest proportion of humic substances and the largest proportions of building blocks and LMW neutrals. BCA also had the highest proportion of biopolymers. The two subarctic sites (Pond and peat groundwater) also had different DOC fractions. DOC fractions displayed a significant positive relationship between Fe:DOC and biopolymer concentration (Figure 3.6).

3.3.2 Summary of titrations on sample DOC concentration and quality

After titrations, some significant changes were observed in measures of DOC concentration and quality (Table 3.2).

3.3.3 Influence of Fe and pH on cations

pH Titration Experiment

Although there were decreases (1.7SD) (not significant in most samples) in Fe, Al, and Mn concentrations with increased pH, there were only significant decreases in Al concentration in the ELA wetland (NWIF) and Mn concentration in the Muskoka Plastic stream (P1-08) (Figure 3.7). Samples with higher initial concentrations of Al or Mn had larger negative slopes although most changes were not statistically significant.

Fe(II) Titration Experiment

$FeCl_2$ additions increased Fe and Mn concentrations significantly in most samples but caused no significant changes in Al concentrations.

Although none of the samples reached the maximum Fe target of 4 mg-Fe/L, the increase in Fe concentration with increased dose of $FeCl_2$ was different between samples (Figure 3.8). The Grand River sites did not increase in dissolved Fe when $FeCl_2$ was added and thus were unable to retain Fe(II) in solution, the Plastic stream complexed less Fe(II) compared to the other sites, only reaching a maximum of 1.3 mg-Fe/L, and the DI sample had a similar slope as P1-08 but a very low DOC concentration (0.4 mg-C/L; within analytical precision of 0 mg-C/L). There were no significant changes in Al concentrations with additions of $FeCl_2$ but there were significant increases in Mn concentrations in most sites. Samples covering a large range of natural pH values (4.5 to 8.5) were able to retain the added Fe(II) in solution.

Fe(III) Titration Experiment

Additions of $FeCl_3$ resulted in significant increases in Fe concentrations in most samples, significant decreases in Al concentrations in some samples and no significant changes in Mn concentrations. Similar to the additions of $FeCl_2$, there was no increase in Fe concentration in

Belwood, BCA, or DI (Figure 3.8); however, there were fewer significant increases in Fe concentration with $FeCl_3$ additions compared to $FeCl_2$.

The increase in Fe concentration in response to added Fe(III) was different between most sites. This is likely because particulate matter (PM) was formed in many samples (Table 3.3) and filtered out before analysis of dissolved Fe. PM formation thus prevented Fe concentrations from increasing linearly with additions of $FeCl_3$. Some samples had threshold Fe concentrations: any additions above this concentration resulted in PM formation. The Grand River sites (Belwood and BCA) showed no increase in Fe concentration after visible PM was observed. There were significant decreases in Al concentrations in some sites with Fe(III) additions and thus, must have been removed with the PM (Figure 3.7). This PM formation was not observed with additions of $FeCl_2$ but the loss of Al suggests that in a few samples, PM formation may have occurred.

3.3.4 DOC Concentration

There were decreases (1.7SD) in DOC concentrations with increased Fe(II) and Fe(III), but not many decreases were significant (Figure 3.9). There were small but significant decreases in DOC concentrations in two sites (P2, 21% and U8, 7%) after $FeCl_2$ additions and in three sites (NWIF, 31%, H4 2.0, 19% and IHSS, 45%) after $FeCl_3$ additions. However, DOC concentrations were lower (1.7SD) in five sites after additions of $FeCl_2$ (losing up to 22% DOC) and eleven sites after additions of $FeCl_3$ (losing up to 48% DOC) indicating that Fe additions influenced DOC concentrations and that PM formation removed DOC. Therefore, if DOC is lost to PM formation, then the composition of the DOC is changing in samples with PM formation. To my knowledge, this is the first study to demonstrate significant decreases in DOC concentration after additions of either Fe(II) or Fe(III). Xiao et al. (2013) filtered samples to 0.2µm before the final analysis of Fe and DOC concentrations and noted a 2% decrease in DOC concentration, but others did not document a decrease in DOC concentration (Poulin et al. 2014; Weishaar et al. 2003).

3.3.5 Effects of pH and Fe on Absorbance

pH Titration Experiment

Increases in pH caused some significant increases in $SUVA_{255}$, SAC_{350}, and SAC_{410} but had more influence at higher wavelengths (SAC_{410}). There were significant increases in $SUVA_{255}$ in two samples (P2 and H4-21), SAC_{350} in seven samples (H4 2.0, H4-21, IHSS, NWIF, Pond, P2, and U8), and SAC_{410} in almost all samples (except Belwood, BCA, P1-08 and DI) (Figure 3.10). At high pH (pH ~ 9), $SUVA_{255}$, SAC_{350}, and SAC_{410} were up to 20%, 37%, and 63% different than low pH samples (pH ~ 3), respectively (Table 3.4). At low pH (pH ~ 3), $SUVA_{255}$, SAC_{350}, and SAC_{410} values are a maximum of 71%, 111%, and 126% different between samples, respectively. The range in $SUVA_{255}$, SAC_{350}, and SAC_{410} between natural samples was higher than the range between the same sample after changes in pH.

Fe(II) Titration Experiment

Additions of $FeCl_2$ significantly increased $SUVA_{255}$, SAC_{350}, and SAC_{410} in most samples (Figure 3.10, Table 3.2). After additions of $FeCl_2$, $SUVA_{255}$, SAC_{350}, and SAC_{410} were up to 86%, 116%, and 124% different from initial conditions, respectively (Table 3.5). Initial $SUVA_{255}$, SAC_{350}, and SAC_{410} values ranged between samples by a maximum of 87%, 123%, and 142%, respectively. The range in $SUVA_{255}$, SAC_{350}, and SAC_{410} between natural samples was similar to the range between the same sample before and after increases in Fe(II).

Fe(III) Titration Experiment

Additions of $FeCl_3$ significantly increased $SUVA_{255}$, SAC_{350}, and SAC_{410} in some samples (Figure 3.10, Table 3.2). $SUVA_{255}$, SAC_{350}, and SAC_{410} significantly increased in one subarctic and two Harp sites (Pond, H4 1.0, and H4-21). $SUVA_{255}$ also increased in P2 and H4 2.0, SAC_{350} in U8 and H4 2.0, and SAC_{410} in U8. Several sites (Belwood, BCA, DE10, DE10-ND, P1-08 and IHSS) that did not show a significant increase in $SUVA_{255}$, SAC_{350}, or SAC_{410} had visible PM formation (Table 3.3). After additions of $FeCl_3$, $SUVA_{255}$, SAC_{350}, and SAC_{410} were up to 78%, 104%, and 104% different from initial conditions, respectively (Table 3.6). Initial $SUVA_{255}$, SAC_{350}, and SAC_{410} values between samples are a maximum of 78%, 116%, and 134% different, respectively. There was a similar range in $SUVA_{255}$,

SAC_{350}, and SAC_{410} values between natural samples as there was before and after increases in Fe(III).

3.3.6 Effects of pH and Fe on LC-OCD Results

DOC fraction concentrations determined by LC-OCD did not change with changing pH (Figure 3.11) but increasing Fe(II) concentrations caused an increase in the biopolymer concentration and a decrease in humic substance concentration in most samples (Figure 3.12, Figure 3.13). Most samples had low biopolymer concentrations in the original sample. The biopolymer concentration of the Muskoka Plastic stream (P1-08) increased the least and the Dickie stream (DE10) increased the most. There was a statistically significant positive relationship between Fe concentration and biopolymer:DOC (Figure 3.14) but there is considerable variability so that predicting the effect or correcting for Fe concentrations is unlikely to be successful. Thus, similar to spectral analysis ($SUVA_{255}$, SAC), DOC quality measures by LC-OCD are affected by Fe(II) concentration.

3.4 Discussion

3.4.1 Range of DOC, Fe, pH, and DOC quality from different sites

Differences in geology, vegetation, hydrology, land use, and climate at different sample locations resulted in a large natural range of DOC and Fe concentration, pH, and DOC quality in the samples. Since these parameters have a range of influences on measures of DOM quality, it is important to correlate the physical and chemical site parameters to predict how measures of DOM quality may change temporally and spatially in natural environments.

Greater areal wetland coverage in stream catchments often cause increased Fe and DOC concentrations due to leaching from peat and can also influence pH, DOC composition, $SUVA_{254}$ and aromaticity (Chomicki, 2009; Weishaar et al. 2003; Poulin et al, 2014; Table 2.1). Samples collected from areas dominated by peatlands and/or wetlands were those with the highest Fe and DOC concentrations (Table 2.1, Figure 3.2). Conversely, ELA NEIF had greater percent peatland coverage than Muskoka DE10, however, DE10 had higher Fe concentrations and thus higher $SUVA_{255}$, indicating that the type of peatlands, vegetation,

hydrogeologic setting, and the residence time of source water in wetlands may also be important factors. The positive relationship between $SUVA_{255}$ and Fe:DOC (R^2=0.58) suggests that although geology, vegetation, and climate influence DOC composition, Fe concentration may influence $SUVA_{255}$. Geology and hydrologic flowpaths are therefore important to consider in $SUVA_{255}$ analyses because Fe concentrations correlate with soil type and flowpaths (Björkvald et al. 2008).

There were often similar characteristics in samples from a shared location (Dorset, ELA, Yellowknife, Grand River), but differences between locations. The Grand River samples were the most different from the other samples as they were from a seventh-order river underlain with Paleozoic limestone, shale and calcite-rich glacial drift and impacted by agricultural and urban runoff. This environment produces largely autochthonous DOM and well-buffered samples from high productivity and high concentrations of dissolved carbonate (Rosamond, 2013; Hutchins, 2011) leading to higher pH, moderate DOC, and low trace metal concentrations and $SUVA_{255}$. It appears that the autochthonous DOM and trace metals do not complex as readily as allochthonous DOM and trace metals do. The remainder of the samples are from sites underlain by silicic Precambrian metamorphic and igneous rocks, resulting in shallow subsurface or surface waters with low calcium concentrations, low buffering capacity, and low pH. Although the ELA upland has higher slope and lower vegetation and soil cover than the ELA wetlands, all ELA sites have similar Fe:DOC but different $SUVA_{255}$ values indicating differences in DOC composition. H4-21 is mostly upland, has steeper slopes, and greater volumes of groundwater discharge (Hinton et al. 1997) compared to the Harp 4 stream which flows through wetlands causing lower Fe:DOC and lower $SUVA_{255}$. The subarctic samples (Pond and P2) are susceptible to different biotic and abiotic processes influencing the pH, DOC composition and DOC and Fe concentration. Although some samples were collected from similar locations, samples can be naturally very different depending on site characteristics and samples from different locations can also be more similar than samples from different settings within the same location.

Changes in climate and seasons also elicit changes in aquatic parameters spatially and temporally. Temperature drives rates of biotic and abiotic processes while precipitation

increases the loading of chemical species (including DOC and Fe) to streams and lakes. Pre-diluted, natural samples may have large ranges in DOC and Fe concentrations, pH and DOC quality temporally and spatially leading to difficulties in using measures of absorbance and LC-OCD to compare DOC quality. When characterizing DOC, it is important to consider that different geology, vegetation, climate, hydrology, residence time and land cover influence pH, Fe and DOC concentrations and DOC composition and thus their role in the aquatic setting.

3.4.2 pH affects measures of DOM quality

Adjusting pH influenced absorbance but did not significantly influence trace metal concentrations or LC-OCD results in most samples. The significant increase in SAC_{410} with increased pH could indicate that pH influenced the protonation of DOC molecules similar to results by Pace et al. (2012). Although researchers (Pace et al. 2012; Poulin et al. 2014, Xiao et al. 2013) have published that pH has an influence on $SUVA_{254}$, a significant influence was only observed in two samples in this experiment. This suggests that the reported relationship between $SUVA_{254}$ and percent DOC aromaticity (Weishaar et al. 2003) may not be influenced by changes in pH in all types of samples.

Increased pH, alone, in the same waters can increase SAC_{410} by a similar percent as natural differences between samples. For example, near pH 3, the SAC_{410} of H4-21 was 85% different than P1-08 suggesting the sample composition was different between the two upland samples (Table 3.7). However, the SAC_{410} of H4-21 at pH 3.1 was 63% different than at pH 9.1 suggesting that pH has the potential to influence spectral measurements similarly to differences in sample composition. As the influence of changing pH was less in $SUVA_{255}$ than SAC_{410}, the influence of pH on $SUVA_{255}$, SAC_{350}, and SAC_{410} is different depending on the wavelengths selected. Therefore, it is important to acknowledge that differences in absorbance between two samples could be caused by differences in sample pH, rather than the difference in DOC quality, especially at higher absorbance wavelengths.

Contrary to published results on the effects of pH on metal solubility, there was no significant decrease in most Fe and Al concentrations with increased pH. Most Al and Fe

remained in solution and likely complexed by DOC. This suggests that changing pH (between pH 3 and pH 9) in these samples does not influence the trace metal concentrations and cannot influence measures of DOM quality except for the protonation of the DOM molecules.

LC-OCD is a useful measure of DOC composition using samples with different pH values. Changing pH did not significantly influence LC-OCD results because a phosphate buffer solution adjusts the pH of the sample before analysis (Huber et al. 2011). This demonstrates that at natural Fe concentrations, DOC-complexation is not altered at the pH of the buffer (pH of 6.85) and LC-OCD analysis is not affected. To my knowledge, this is the first study to investigate the influence of changing pH on LC-OCD results.

Although pH influences $SUVA_{255}$, SAC_{350}, and SAC_{410}, these influences are rarely addressed in studies of brownification or DOC quality (Kritzberg and Ekström, 2012). pH influences DOC structure and composition and could affect the spectral and size analyses of DOC quality (Pace et al. 2012; Poulin et al. 2014; Xiao et al. 2013). It is important to know that pH affects measures of DOM in order to use caution when comparing the DOM quality of samples with different pH values.

3.4.3 Fe(II) concentration affects measures of DOC quality

Natural and manipulated Fe concentrations influenced measures of DOC concentration and quality. The initial positive relationship between Fe:DOC and $SUVA_{255}$ ($R^2 = 0.58$) suggests that the reported relationship between $SUVA_{254}$ and percent DOC aromaticity (Weishaar et al. 2003) may be influenced by Fe(II). Further, $FeCl_2$ additions caused significant increases in $SUVA_{255}$, SAC_{350}, and SAC_{410}, suggesting that increased Fe(II) concentrations can influence all DOC quality inferences made by UV and visible absorbance. This is inconsistent with previous studies that report Fe(II) has negligible effects on measures of absorbance (Poulin et al. 2014; Doane and Horwáth, 2010; these references do not provide data). As samples can retain Fe(II) in a complexed form at the natural pH range used in this experiment, natural increases in Fe concentration could influence measures of DOC in a large range of aquatic settings and is not limited by pH.

Increased Fe(II) concentrations, alone, in the same waters can increase $SUVA_{255}$, SAC_{350}, and SAC_{410} more than differences between natural samples (Table 3.8). This likely suggests that Fe(II) has the potential to influence spectral measurements more than differences in DOC composition. Therefore, it is important to acknowledge that differences in absorbance between two samples could be caused by differences in Fe(II) concentration, especially at higher absorbance wavelengths.

A significant positive relationship between diluted Fe and biopolymer concentration ($R^2 = 0.6$) suggests that the presence of Fe can influence LC-OCD results. Biopolymers are often defined as polymers produced by living organisms, but the LC-OCD biopolymer designation is the fraction containing the largest DOC molecules (>20 000 Da) eluting first from the size exclusion column. LC-OCD biopolymers are also reported to be composed of polysaccharides, proteins, aliphatic hydrocarbons (Grünheid et al. 2005; Lankes et al. 2009), that could be associated with the microbial degradation of DOC (Aukes, 2012) and be more abundant in areas of high OM loading (Foulquier et al. 2011; Neale et al. 2011). Although part of this LC-OCD biopolymer definition implies biological origin, the technical definition is based on the hydrodynamic radii of the DOC molecules and could be influenced by parameters such as pH and Fe that have been found to affect the composition and structure of DOC molecules (Pace et al. 2012).

High molecular weight (HMW) DOC (humic acids and biopolymers) have many cation binding sites and are thus highly reactive with trace metals and affect metal distribution and speciation in freshwaters (Worms et al. 2010; Christensen and Christensen, 1999). As Fe(II) is added to a sample, it likely complexes with HMW DOC and increases the molecular size of humic substances into the size class of biopolymers. Thus a certain concentration of biopolymers as reported by LC-OCD analysis also encompasses the concentration of HMW DOC molecules bound to trace metals such as Fe. LC-OCD analysis likely overestimates true biopolymer concentrations and underestimates humic substance concentrations because it is based on the hydrodynamic radii of organic molecules. If the sample matrix (e.g. metal concentration) changes the size of the DOC molecules, then LC-OCD results will not be representative of the true DOC fraction concentrations in the sample.

$SUVA_{255}$, SAC_{350}, SAC_{410}, and LC-OCD are useful measures of DOC composition using samples with similar pH and trace metal concentrations. However, when comparing samples with different pH and Fe concentrations, results may be different due to these parameters and it is therefore difficult to compare absorbance and LC-OCD results between such samples, or caution should be used when doing so. As this is often difficult, there is a need to know trace metal concentrations and pH when using LC-OCD or absorbance data to characterize DOC.

3.4.4 Effects of Fe(III) are different than Fe(II)

Additions of $FeCl_3$ had different but significant influences on measures of DOM concentration and quality than additions of $FeCl_2$. The DOC concentrations decreased (1.7SD) in some samples with additions of $FeCl_3$ likely due to the complexation of Fe(III) by HMW DOM, forming larger PM molecules that were filtered out of the sample. Thus, Fe concentrations increased more with additions of $FeCl_2$ than with $FeCl_3$ as most Fe(II) and DOM complexes remained in the dissolved phase. However, the larger losses in DOC concentrations in the Fe(III) experiment suggest that Fe(III) additions had a greater influence on overall DOM quantity. This was likely caused by both the lower solubility of Fe(III) and stronger complexation of Fe(III) with HMW DOM. Further, Fe(III) complexed with DOM only precipitated in some samples, resulting in different slopes of increasing Fe concentrations between samples.

Similar to the findings of Poulin et al. (2014), the mass of Fe(III) added exceeded the Fe(III) binding capacity of the DOM for some samples and thus, these samples appeared to have maximum Fe concentrations. Since Fe likely preferentially binds with the colloids and HMW DOM, Fe did not increase higher than the threshold concentration and DOC concentrations decreased.

As the influence of Fe(III) on DOM is different than Fe(II), different Fe species would likely have different measures of DOM quantity and quality results. This suggests that it is important to measure the Fe species (often been difficult, or would need specialized equipment) when determining its role in aquatic systems and interpreting and comparing

measures of DOM concentration and quality. This is especially important where Fe is added to DOM in soils or wetlands, etc. because it can be complexed under oxic or anoxic conditions.

In other studies, increases in Fe(II) have been reported to have negligible effects on absorbance (Poulin et al. 2014; Doane and Horwáth, 2010) while increases of Fe(III) have caused linear increases in absorbance and $SUVA_{254}$ (Poulin et al. 2014; Doane and Horwáth, 2010). Conversely, this experiment found samples with increased Fe(III) concentration to have fewer significant increases in absorbance due to the formation and filtration of PM compared to samples with the same Fe(II) concentrations. However, similar to Fe(II), increased Fe(III) concentration, alone, in the same waters can still increase $SUVA_{255}$, SAC_{350}, and SAC_{410} more than natural differences between samples (Table 3.9). This indicates that Fe has the potential to influence/magnify spectral measurements more than differences in DOM composition alone. However, this is only true for a few samples because the Fe(III) concentrations did not increase past a certain maximum threshold, that was likely determined by the binding capacity of DOM. Instead, added Fe(III) likely bound to HMW DOM and was filtered out in the other samples, resulting in lower $SUVA_{255}$, SAC_{350}, and SAC_{410}. Therefore, it is important to acknowledge that differences in absorbance between two samples could be caused by differences in Fe concentrations but that there are also differences between Fe(II) and Fe(III) at the same concentration.

Although Fe can influence $SUVA_{255}$, SAC_{350}, and SAC_{410}, and LC-OCD results, these influences are rarely addressed in studies of brownification or DOM quality (Kritzberg and Ekström, 2012). It is important to know that Fe(II) and Fe(III) both affect measures of DOM quantity and quality and that they affect measures differently depending on the Fe oxidation state. Thus, one should be cautious when comparing the DOM quality of samples with different Fe(II), and Fe(III) concentrations.

3.4.5 Corrections for the effects of pH and Fe are problematic

Correction of DOM quality measures for the effects of pH and Fe differs between samples of different DOM quality. Several researchers have proposed corrections, including using the

slopes of linear regressions between Fe concentration and $SUVA_{254}$ to calculate $SUVA_{254}$ without the interference of Fe (Table 3.10) (Poulin et al. 2014; Doane and Horwáth, 2010; McKnight et al. 2001). However, this correction would not work for the natural samples in this experiment because of the natural range in pH, Fe and DOC concentrations and DOM quality producing a large range of linear regression slopes (Table 3.11). As DOM complexes Fe differently at different concentrations and with different DOM compositions, the interference of Fe on absorbance cannot be easily determined when DOM quality differs (USEPA, 2005; Doane and Horwáth, 2010). Furthermore, there is no uniform pattern of changes in measures of DOM quality between different DOM qualities with changed pH and Fe concentrations and there is a large range in DOM qualities in the natural world.

Although some samples followed similar trends to those noted in published literature (Poulin et al. 2014; Xiao et al. 2013), the slopes are not consistent between samples. Further, changes in pH and Fe affects all quality measures: UV and visible absorbance, LC-OCD and other size exclusion methods. Therefore, we need to be aware of these effects and use supporting data (pH, Fe concentrations, Fe species, DOC concentration, DOC composition) as often as possible when comparing DOM samples.

3.4.6 Implications for understanding the trends and causes of brownification

Water colour is increasing in lakes and rivers in Europe and eastern North America and the influence of pH and Fe on measures of absorbance suggests that both pH and Fe could play an important role in the brownification story. Further, changes in pH and Fe can cause a change in brownification even when DOM is not changing, and thus, brownification does not necessarily imply increasing DOM concentrations or changing DOM quality. The contribution of Fe and/or pH to brownification can often depend more on processes affecting DOM quality and Fe availability and binding, and thus these factors are also implicit in the assessment of brownification.

Certain types of lakes and rivers with different pH, trace metal and DOM concentrations, and DOM qualities are more susceptible to brownification. As these parameters all influence water colour and the binding capacity of Fe and DOM, the extent of

brownification of a lake or river depends on these parameters and the processes that influence them. Further, understanding which parameters (DOM, Fe or pH) are affecting water colour increase in freshwaters will help determine which potential drivers are causing brownification.

pH changes (from pH ~ 3 to pH ~ 9) in these samples do not cause as much change in water colour as changes in Fe. However, increases in Fe(II) or Fe(III), alone, in the same waters can increase SAC_{410} by a greater percent than natural differences between samples and are therefore especially important parameters to monitor when investigating brownification trends. Further, environmental changes that influence Fe cycling and Fe export (e.g. redox and hydrologic flowpaths), and the availability of Fe that can bind to DOM will also affect brownification. This information is important when predicting how brownification trends will respond in the future with projected changes in different regions around the world (Lapierre et al. 2013).

Increases in SAC_{410} were larger due to increases in Fe(II) concentrations versus Fe(III) concentrations, however, Fe(II) has a low solubility in oxic waters. Therefore, Fe(II) is likely only important in the brownification of natural waters if it was previously bound to DOM under anoxic conditions such as anoxic layers in wetlands or soils, aquatic environments where groundwater discharges into surface waters, or where there is high organic loading and decomposition, etc.

Although some researchers discuss the relationship between water colour, Fe concentrations, pH and DOC concentration and composition, often metal concentrations and pH are left out of the discussion of brownification trends and causes (Poulin et al. 2014; Weishaar et al, 2003; Xiao et al. 2013). It is difficult to solely use spectral analysis or LC-OCD to determine which parameter (DOC, Fe or pH) or combination of parameters, contribute to the brownification of a freshwater environment. Instead, a more comprehensive analysis to address causes of brownification will need to include pH, trace metal and DOC concentrations and DOC composition.

3.5 Summary and Implications

The pH and Fe concentrations were manipulated in samples with natural DOM and differing quality to investigate their influence on measures of DOM quantity and composition. Changing pH affects absorbance measurements while natural and manipulated Fe concentrations influence measures of DOC concentration and quality determined by spectral absorbance and LC-OCD. However, since the responses of samples were different depending on sample composition, it is difficult to predict how differences in pH and/or Fe concentration affect measures of DOM. Single, simple relationships between $SUVA_{255}$ and pH/Fe concentrations cannot be obtained for natural samples unless the relationship has been previously studied because the slopes of linear regressions greatly differ. Although some researchers have attempted to correct for pH and/or Fe concentration changes (Poulin et al. 2014; Doane and Horwáth, 2010; McKnight et al. 2001), this study suggests that corrections would be difficult to apply to natural systems with differing Fe and pH. The best method for interpreting measures of DOM is to compile a complete dataset including pH, cation and DOC concentration and DOM composition in order to supplement DOM characterization results. Although some researchers have suggested that increases in absorbance measures indicate an increase in DOM concentration or change in DOM composition, obviously one measure cannot be used to determine both of these or confirm that pH or Fe are not also influencing spectral measures. However, if DOC and cation concentrations and pH are also known, they can aid in eliminating whether a change in pH or Fe concentration is the cause for the differences in absorbance. We must include the effect of combinations of pH, Fe, and/or DOC concentrations, and/or DOM quality causing brownification in order to make accurate predictions about and management decisions for the future of aquatic systems.

Table 3.1. Diluted starting DOC, dissolved Fe, Al, and Mn concentrations and starting and assigned pH.

Sample Name	Date Sampled	[DOC] (mg/L)	Starting pH	Assigned pH	[Fe] (mg/L)	[Al] (mg/L)	[Mn] (mg/L)
Pond	July, 2014	6.3	6.6	6	0.06	0.04	0.01
P2	July, 2014	6.7	6.7	6	0.23	0.04	0.01
U8	July, 2014	6.6	4.8	4.5	0.10	0.34	0.01
NEIF	July, 2014	6.6	4.5	4.5	0.10	0.08	0.01
NWIF	July, 2014	6.7	5.0	4.5	0.10	0.18	0.01
Belwood	June 12, 2015	6.5	8.2	7.5	0.03	0.07	0.05
BCA	June 12, 2015	6.8	8.2	7.5	0.04	0.08	0.01
DE10	June 15, 2015	6.6	5.3	4.5	0.20	0.09	0.01
DE10-ND	June 15, 2015	19.1	4.7	4.5	0.60	0.21	0.03
H4 1.0	August, 2014	6.6	6.7	6	0.17	0.13	0.01
H4 2.0	June 15, 2015	6.8	6.4	6	0.17	0.15	0.01
H4-21	June 15, 2015	3.5	6.7	6	0.06	0.09	0.01
P1-08	June 15, 2015	2.8	5.2	4.5	0.06	0.22	0.11
IHSS	June, 2015	5.8	4.5	4.5	0.11	0.13	0.01
DI	June, 2015	0.2	6.3	6	0.03	0.04	0.01

Table 3.2. Summary of significant ($p < 0.05$) changes to measures of DOM.

Site	Treatment	[DOC]	$SUVA_{255}$	SAC_{350}	SAC_{410}	LC-OCD
Pond	↑ pH	↑	-	↑	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	↑	↑	↑	N/A
P2	↑ pH	-	↑	↑	↑	N/A
	↑ [Fe(II)]	↓	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	↑	-	-	N/A
U8	↑ pH	-	-	↑	↑	N/A
	↑ [Fe(II)]	↓	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	-	↑	↑	N/A
NEIF	↑ pH	↑	-	-	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	-	-	-	N/A
NWIF	↑ pH	↑	-	↑	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	↓	-	-	-	N/A
Belwood	↑ pH	-	-	-	-	N/A
	↑ [Fe(II)]	-	-	-	-	N/A
	↑ [Fe(III)]	-	↑	-	-	N/A
BCA	↑ pH	-	-	-	-	N/A
	↑ [Fe(II)]	-	-	-	-	N/A
	↑ [Fe(III)]	-	↑	-	-	N/A
DE10	↑ pH	-	-	-	↑	-
	↑ [Fe(II)]	-	-	-	-	BP↑; HS↓
	↑ [Fe(III)]	-	-	-	-	N/A
DE10-ND	↑ pH	-	-	-	↑	-
	↑ [Fe(II)]	-	-	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	-	-	-	N/A
H4 1.0	↑ pH	↑	-	-	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	-	↑	↑	↑	N/A
H4 2.0	↑ pH	-	-	↑	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	BP↑; HS↓
	↑ [Fe(III)]	↓	↑	↑	-	N/A
H4-21	↑ pH	-	↑	↑	↑	N/A
	↑ [Fe(II)]	-	↑	↑	↑	HS↓
	↑ [Fe(III)]	-	↑	↑	↑	N/A
P1-08	↑ pH	-	-	-	-	N/A
	↑ [Fe(II)]	-	-	-	-	HS↓
	↑ [Fe(III)]	-	-	-	-	N/A
IHSS	↑ pH	-	-	↑	↑	N/A
	↑ [Fe(II)]	-	-	-	-	BP↑; HS↓
	↑ [Fe(III)]	↓	-	-	-	N/A

"-" = no significant change ($p > 0.05$); N/A = sample not analyzed; BP = Biopolymers; HS = Humic substances. The DOC concentrations of DI were below detection limits.

Table 3.3. Samples from the Fe(III) titration experiment that had visible signs of PM formation (denoted "Y") before filtering.

Site Name	PM Formation			
	0.5 mg-Fe/L	1 mg-Fe/L	1.5 mg-Fe/L	2 mg-Fe/L
Pond	-	-	-	-
P2	-	-	-	-
U8	-	-	-	-
NEIF	-	-	-	-
NWIF	-	-	-	-
Belwood	Y	Y	Y	Y
BCA	Y	Y	Y	Y
DE10	-	-	Y	Y
DE10-ND	Y	Y	Y	Y
H4 1.0	-	Y	Y	Y
H4 2.0	-	-	Y	-
H4-21	-	-	-	-
P1-08	-	-	-	Y
IHSS	-	-	-	Y
DI	-	-	-	-

Table 3.4. Change in spectral measurements (L mgC^{-1} m^{-1}) due to increases in pH. Percent change is calculated as high-low/(average of high and low).

Sample	Low pH	High pH	Low $SUVA_{255}$	High $SUVA_{255}$	% Change	Low SAC_{350}	High SAC_{350}	% Change	Low SAC_{410}	High SAC_{410}	% Change
Pond	3.1	9.3	7.5	7.9	5	1.6	1.9	17	0.5	0.7	30
P2	3.1	9.0	8.0	9.7	20	2.2	3.2	36	0.8	1.4	49
U8	3.1	8.8	8.7	9.0	4	2.2	2.5	15	0.7	0.9	29
NEIF	3.1	9.1	9.9	10.3	3	3.0	3.3	11	1.1	1.4	18
NWIF	3.1	8.7	9.3	9.9	6	2.6	3.1	17	1.0	1.3	30
Belwood	3.0	8.9	7.1	7.3	3	1.4	1.6	9	0.4	0.5	23
BCA	3.1	9.0	7.4	7.3	-1	1.6	1.7	5	0.5	0.6	10
DE10	3.1	8.8	11.7	12.6	8	3.6	4.3	19	1.3	1.8	29
DE10-ND	3.0	8.9	11.2	12.5	12	3.4	4.2	21	1.3	1.7	30
H4 1.0	3.0	9.0	10.1	10.7	5	2.7	3.2	18	0.9	1.3	30
H4 2.0	3.1	9.1	11.0	12.1	10	3.0	3.9	24	1.1	1.6	40
H4-21	3.1	9.1	7.9	9.0	14	2.0	2.7	31	0.6	1.1	63
P1-08	2.8	9.3	5.6	5.6	0	1.0	1.3	28	0.3	0.5	44
IHSS	3.1	8.7	9.2	9.6	4	2.4	2.8	14	0.7	1.0	30

Table 3.5. Change in spectral measurements (L mgC^{-1} m^{-1}) due to increases in Fe(II) concentration. Initial Fe is post dilution concentrations.

Sample	Initial Fe	Final Fe	% Change	Initial $SUVA_{255}$	Final $SUVA_{255}$	% Change	Initial SAC_{350}	Final SAC_{350}	% Change	Initial SAC_{410}	Final SAC_{410}	% Change
Pond	0.06	2.27	190	7.9	17.6	75	2.0	6.4	106	0.7	2.4	112
P2	0.23	3.36	174	9.0	21.6	83	2.8	8.6	101	1.1	3.4	101
U8	0.10	3.50	189	8.8	17.3	65	2.2	6.4	96	0.7	2.3	104
NEIF	0.10	3.12	188	10.3	16.4	46	3.1	6.1	64	1.2	2.4	67
NWIF	0.10	3.35	189	9.7	17.3	56	2.7	6.6	83	1.0	2.5	86
Belwood	0.03	0.03	9	7.8	6.4	-19	1.6	1.2	-28	0.5	0.4	-22
BCA	0.04	0.02	-40	7.5	7.3	-3	1.8	1.6	-9	0.6	0.5	-16
DE10	0.20	2.57	171	12.2	17.9	38	3.9	6.8	54	1.5	2.7	56
DE10-ND	0.60	3.35	139	13.4	13.9	3	4.3	4.8	12	1.7	2.0	16
H4 1.0	0.17	3.43	181	10.4	18.6	57	3.1	7.1	78	1.1	2.7	80
H4 2.0	0.17	2.95	178	11.3	19.1	52	3.4	7.4	75	1.4	2.9	73
H4-21	0.06	2.39	190	8.0	21.8	93	2.1	8.8	122	0.8	3.3	124
P1-08	0.06	1.26	181	5.3	9.0	52	1.0	3.3	105	0.3	1.2	118
IHSS	0.11	1.62	174	8.8	11.2	24	2.4	3.5	38	0.8	1.3	48

Table 3.6. Change in spectral measurements (L mgC^{-1} m^{-1}) due to increases in Fe(III) concentration. Initial Fe is post dilution concentrations.

Sample	Initial Fe	Final Fe	% Change	Initial $SUVA_{255}$	Final $SUVA_{255}$	% Change	Initial SAC_{350}	Final SAC_{350}	% Change	Initial SAC_{410}	Final SAC_{410}	% Change
Pond	0.06	0.88	176	7.9	9.3	16	2.0	2.6	28	0.7	0.9	27
P2	0.23	0.85	114	8.5	9.5	11	2.7	3.0	13	1.1	1.1	6
U8	0.10	0.94	162	7.8	8.9	13	2.0	2.7	30	0.7	1.0	38
NEIF	0.10	1.36	174	10.3	12.1	16	3.1	4.3	31	1.2	1.7	37
NWIF	0.10	1.13	169	9.7	9.6	-1	2.7	3.0	10	1.0	1.1	10
Belwood	0.03	0.00	-200	7.8	7.3	-6	1.6	1.5	-7	0.5	-	-
BCA	0.04	0.00	-200	7.9	7.4	-6	1.9	1.7	-7	0.6	-	-
DE10	0.20	0.72	112	12.1	10.8	-11	3.9	3.5	-10	1.5	1.3	-16
DE10-ND	0.60	2.09	110	11.6	11.1	-4	3.7	3.7	1	1.5	1.5	3
H4 1.0	0.17	1.83	165	9.3	12.3	28	2.8	4.1	39	1.0	1.6	43
H4 2.0	0.17	1.00	141	11.4	12.5	9	3.4	4.0	16	1.4	1.6	13
H4-21	0.06	2.25	190	8.4	19.0	78	2.2	7.1	104	0.8	2.5	104
P1-08	0.06	0.11	56	5.3	6.0	13	1.0	1.7	51	0.3	0.5	57
IHSS	0.11	1.40	170	8.8	11.0	22	2.4	3.8	46	0.8	1.5	60

Table 3.7. SAC_{410} values are 85% different between different sites of similar pH and Fe concentrations (H4-21 and P1-08) and 63% different between the same site of different pH values (H4-21).

	Similar pH	Different pH
Same Sample Location	H4-21 Fe = 0.1 mg/L pH = 3.1 **SAC_{410} = 0.56 L mg C^{-1} m^{-1}**	H4-21 Fe = 0.1 mg/L pH = 9.1 **SAC_{410} = 1.06 L mg C^{-1} m^{-1}**
Different Sample Location	P1-08 Fe = 0.1 mg/L pH = 2.8 **SAC_{410} = 0.30 L mg C^{-1} m^{-1}**	

Table 3.8. $SUVA_{255}$ values are 40% different between different sites of similar Fe concentrations (P1-08 and Pond) and 52% different between the same site of different Fe concentrations (P1-08 and P1-08 + Fe).

	Similar Fe Concentration	Different Fe Concentration
Same Sample Location	P1-08 Fe = 0.1 mg/L **$SUVA_{255}$ = 5.3 L mg C^{-1} m^{-1}**	P1-08 + Fe Fe = 1.3 mg/L **$SUVA_{255}$ = 9.0 L mg C^{-1} m^{-1}**
Different Sample Location	Pond Fe = 0.1 mg/L **$SUVA_{255}$ = 7.9 L mg C^{-1} m^{-1}**	

Table 3.9. $SUVA_{255}$ values are 45% between different sites of similar Fe concentrations (H4-21 and P1-08) and 78% different between the same site of different Fe concentrations (H4-21 and H4-21 + Fe).

	Similar Fe Concentration	Different Fe Concentration
Same Sample Location	H4-21 Fe = 0.1 mg/L **$SUVA_{255}$ = 8.4 L mg C^{-1} m^{-1}**	H4-21 + Fe Fe = 2.3 mg/L **$SUVA_{255}$ = 19.0 L mg C^{-1} m^{-1}**
Different Sample Location	P1-08 Fe = 0.1 mg/L **$SUVA_{255}$ = 5.3 L mg C^{-1} m^{-1}**	

Table 3.10. Correction slopes of Fe interference with absorbance.

Reference	Sample Name	Spectral Measure	Units	Correction Equation
Weishaar et al. 2003	Williams Lake HPOA	a_{254}	cm^{-1}	$a_{254} = 0.080 * [Fe(III)] + 0.210$
		$SUVA_{254}$	L mg C^{-1} m^{-1}	$SUVA_{254} = 0.842 * [Fe(III)] + 2.211$
	Suwannee River Fulvic Acid	a_{254}	cm^{-1}	$a_{254} = 0.081 * [Fe(III)] + 0.407$
		$SUVA_{254}$	L mg C^{-1} m^{-1}	$SUVA_{254} = 0.853 * [Fe(III))] + 4.284$
Poulin et al. 2014	Everglades F1 HPoA	a_{254}	cm^{-1}	$a_{254} = 0.061 * [Fe(III)] + 0.104$
		$SUVA_{254}$	L mg C^{-1} m^{-1}	$SUVA_{254} = 2.652 * [Fe(III)] + 4.522$
	Suwannee River HPoA	a_{254}	cm^{-1}	$a_{254} = 0.066 * [Fe(III)] + 0.117$
		$SUVA_{254}$	L mg C^{-1} m^{-1}	$SUVA_{254} = 2.640 * [Fe(III)] + 4.680$
Xiao et al. 2013	Suwannee River Humic Acid	a_{410}	m^{-1}	$a_{410} = 0.029 * [Fe(III)] + 0.092$
		SAC_{410}	L mg C^{-1} m^{-1}	$SAC_{410} = 0.592 * [Fe(III)] + 1.891$
	Suwannee River Fulvic Acid	a_{410}	m^{-1}	$a_{410} = 0.029 * [Fe(III)] + 0.036$
		SAC_{410}	L mg C^{-1} m^{-1}	$SAC_{410} = 0.597 * [Fe(III)] + 0.749$

Table 3.11. Slopes of significant ($p < 0.05$) changes in measures of DOM.

Site	Treatment	[DOC]	$SUVA_{255}$		SAC_{350}		SAC_{410}	
			Slope	±	Slope	±	Slope	±
Pond	↑ pH	-	-	-	0.051	0.014	0.032	0.004
	↑ $[Fe^{2+}]$	-	3.741	0.840	1.738	0.347	0.680	0.136
	↑ $[Fe^{3+}]$	-	1.498	0.350	0.712	0.177	0.246	0.076
P2	↑ pH	-	0.256	0.062	0.148	0.028	0.080	0.017
	↑ $[Fe^{2+}]$	-	4.086	0.465	1.883	0.199	0.742	0.087
	↑ $[Fe^{3+}]$	-	1.509	0.436	-	-	-	-
U8	↑ pH	-	-	-	0.066	0.017	0.044	0.012
	↑ $[Fe^{2+}]$	-	2.499	0.031	1.222	0.027	0.470	0.016
	↑ $[Fe^{3+}]$	-	-	-	1.041	0.205	0.391	0.057
NEIF	↑ pH	-	-	-	-	-	0.047	0.015
	↑ $[Fe^{2+}]$	-	1.905	0.175	0.928	0.087	0.378	0.034
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
NWIF	↑ pH	-	-	-	0.084	0.010	0.062	0.014
	↑ $[Fe^{2+}]$	-	2.082	0.289	1.100	0.108	0.424	0.048
	↑ $[Fe^{3+}]$	↓	-	-	-	-		
Belwood	↑ pH	-	-	-	-	-	-	-
	↑ $[Fe^{2+}]$	-	-	-	-	-	-	-
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
BCA	↑ pH	-	-	-	-	-	-	-
	↑ $[Fe^{2+}]$	↓	-	-	-	-	-	-
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
DE10	↑ pH	-	-	-	-	-	0.086	0.026
	↑ $[Fe^{2+}]$	-	-	-	-	-	-	-
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
DE10-ND	↑ pH	-	-	-	-	-	0.071	0.022
	↑ $[Fe^{2+}]$	-	-	-	0.205	0.033	0.105	0.017
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
H4 1.0	↑ pH	-	-	-	-	-	0.048	0.012
	↑ $[Fe^{2+}]$	-	2.751	0.442	1.303	0.180	0.489	0.066
	↑ $[Fe^{3+}]$	-	1.774	0.274	0.777	0.111	0.336	0.028
H4 2.0	↑ pH	-	-	-	0.110	0.034	0.076	0.017
	↑ $[Fe^{2+}]$	-	2.541	0.271	1.363	0.230	0.501	0.053
	↑ $[Fe^{3+}]$	↓	1.410	0.287	0.704	0.119	-	-
H4-21	↑ pH	-	0.182	0.028	0.111	0.017	0.078	0.010
	↑ $[Fe^{2+}]$	-	5.911	0.044	2.867	0.024	1.072	0.002
	↑ $[Fe^{3+}]$	-	4.090	1.011	1.866	0.440	0.675	0.140
P1-08	↑ pH	-	-	-	-	-	-	-
	↑ $[Fe^{2+}]$	-	-	-	-	-	-	-
	↑ $[Fe^{3+}]$	-	-	-	-	-	-	-
IHSS	↑ pH	-	-	-	0.057	0.016	0.041	0.010
	↑ $[Fe^{2+}]$	-	-	-	-	-	-	-
	↑ $[Fe^{3+}]$	↓	-	-	-	-	-	-

"-" = no significant change ($p > 0.05$).

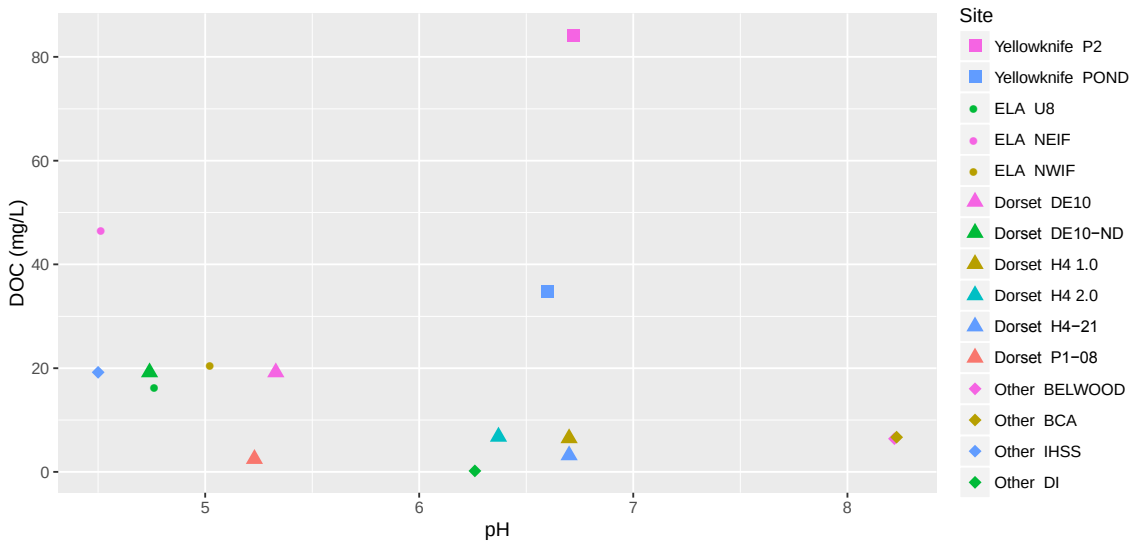

Figure 3.1. Natural pH and DOC concentrations before dilution for all sites. Data points encompass analytical precision.

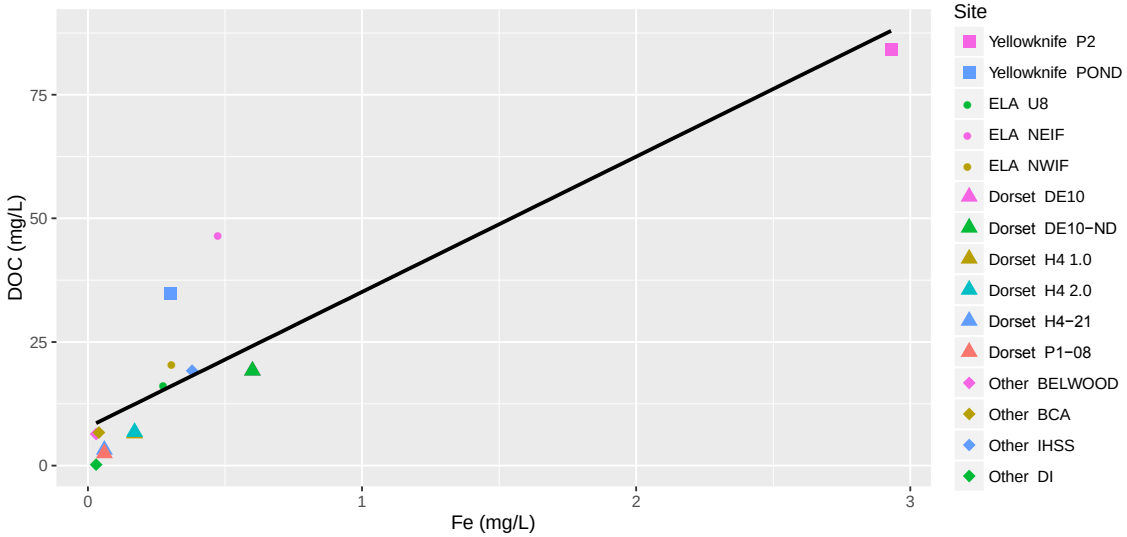

Figure 3.2. Natural Fe and DOC concentrations before dilution. Data points encompass analytical precision. Slope y=27.36x+7.77, $p < 0.05$; $R^2 = 0.80$.

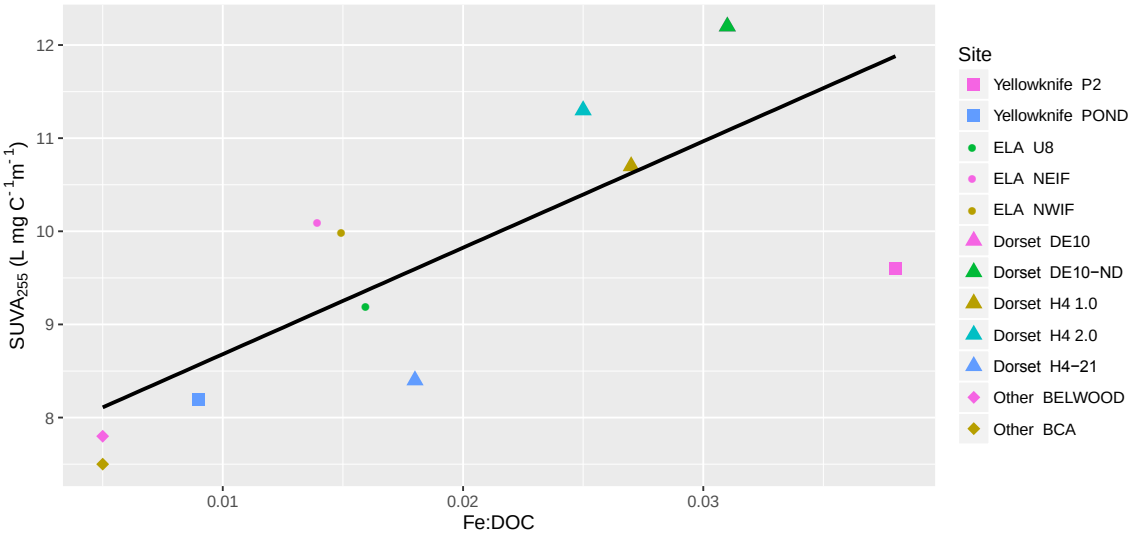

Figure 3.3. The relationship between initial Fe:DOC and $SUVA_{255}$ after dilutions. DE10 data point is located behind DE10-ND. Data points encompass analytical precision. DI, IHSS, and P1-08 were not included because there was either no Fe or no DOC present. Y = (114.27)X + 7.54 ($p < 0.05$; $R^2 = 0.58$).

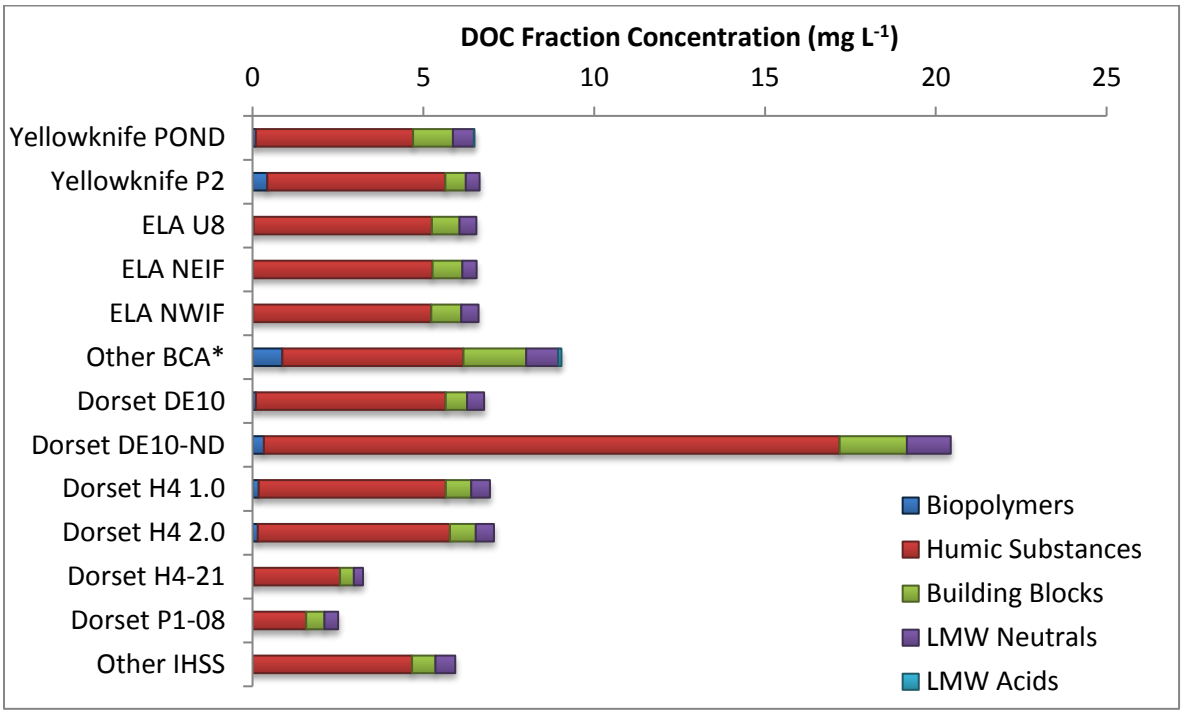

Figure 3.4. Diluted DOC fraction concentrations determined by LC-OCD at natural sample pH. *BCA sample is from a different experiment because GR samples were not analyzed for LC-OCD due to low Fe concentrations in experimental samples.

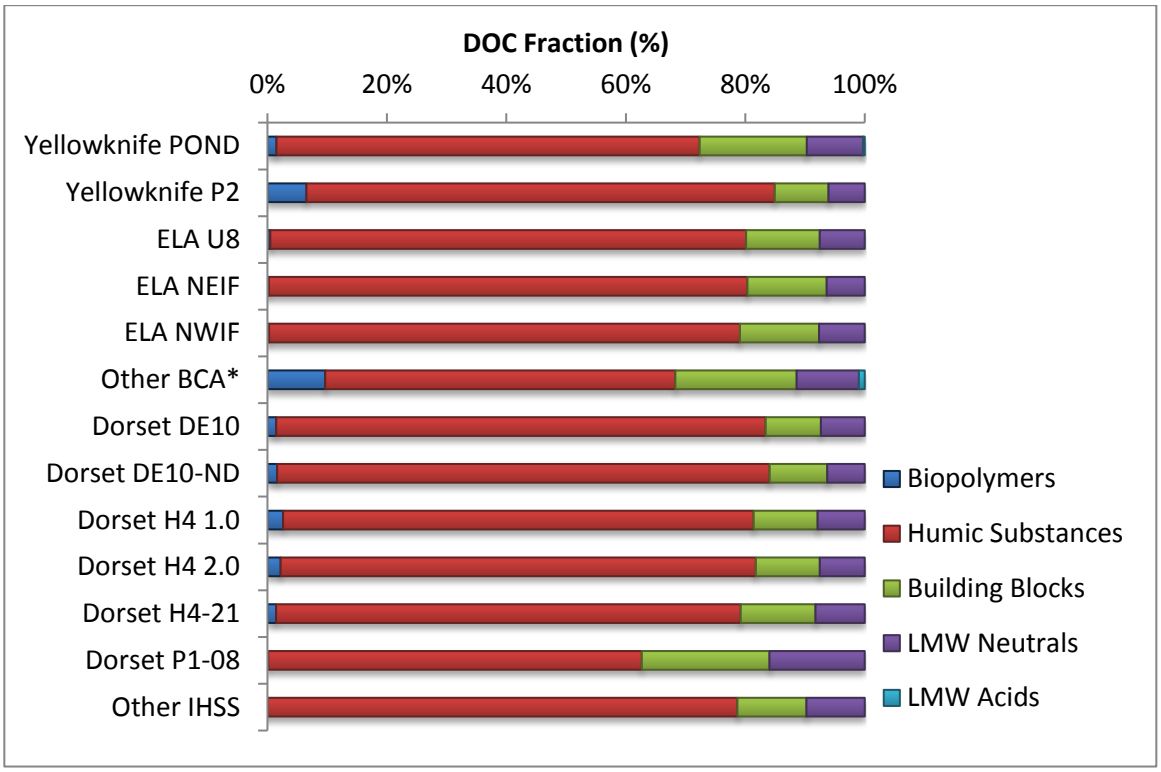

Figure 3.5. Diluted DOC fraction percent compositions determined by LC-OCD at natural sample pH. *BCA sample is from a different experiment because GR samples were not analyzed for LC-OCD due to low Fe concentrations in experimental samples.

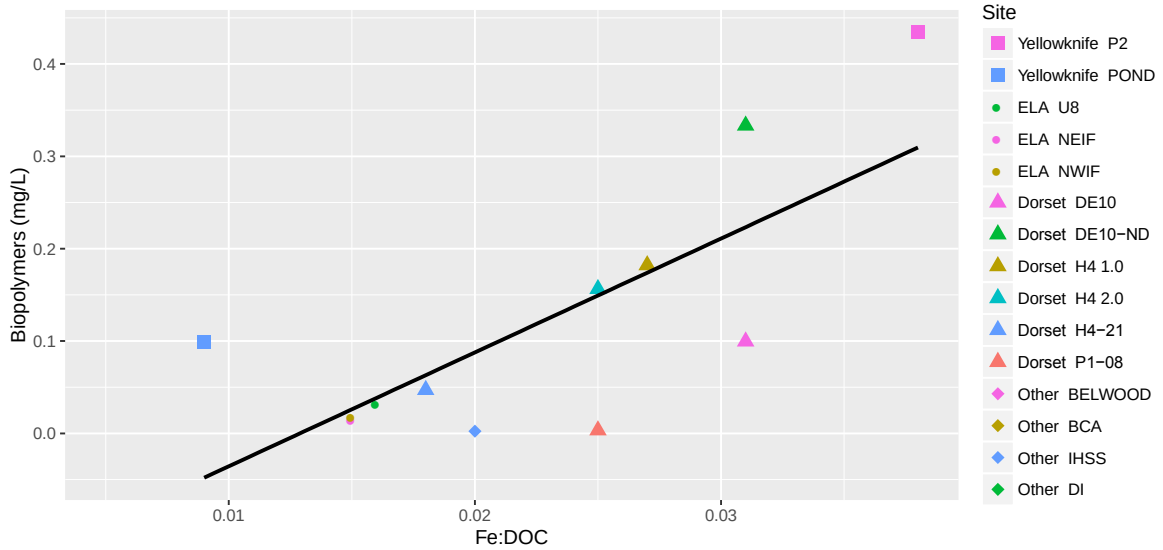

Figure 3.6. Initial diluted BP concentration and Fe:DOC. y=(12.33)x-0.16 (p<0.05; R^2=0.56).

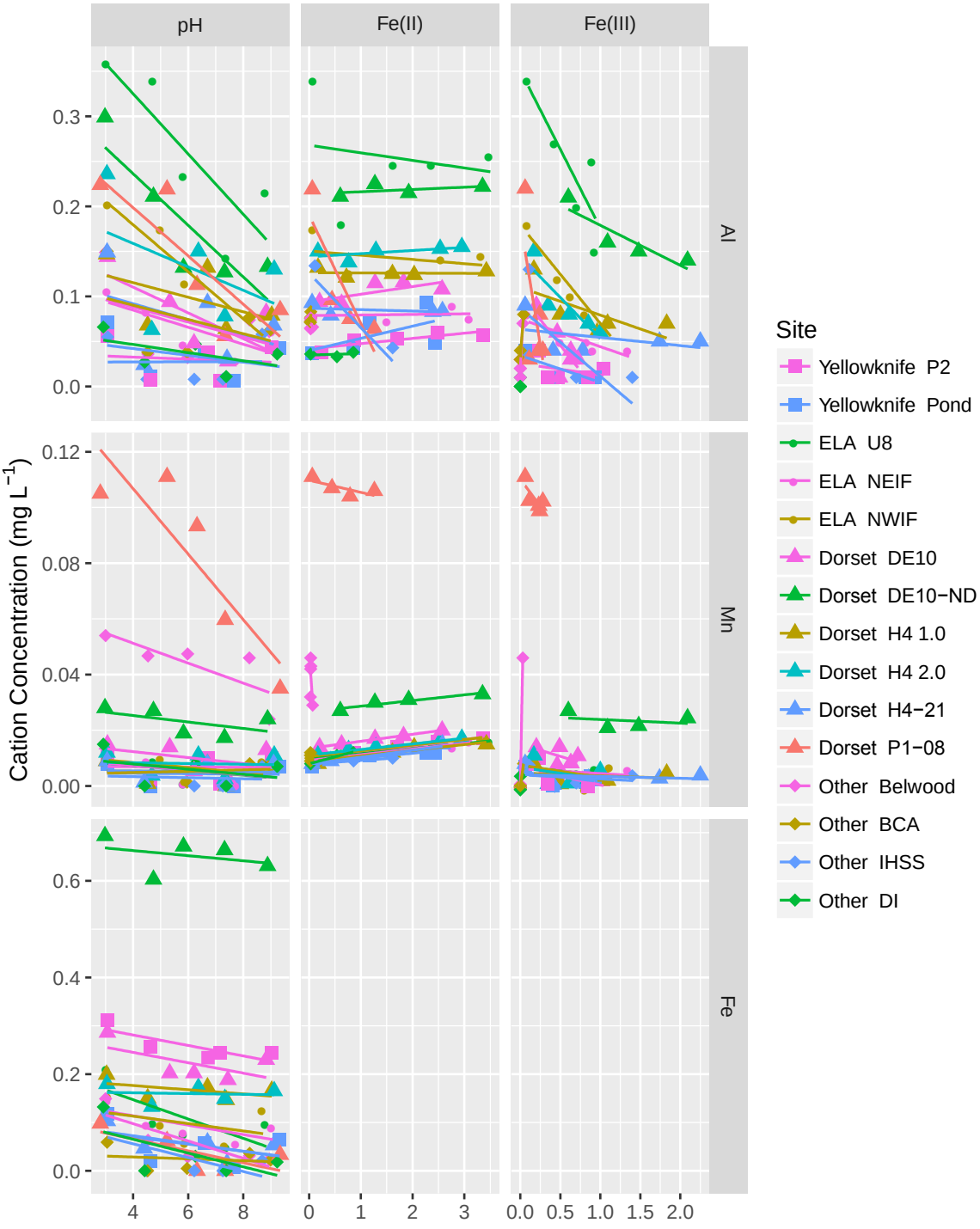

Figure 3.7. Dissolved Al, Mn, and Fe concentrations (mg L^{-1}) for pH, Fe(II) (mg L^{-1}), and Fe(III) (mg L^{-1}) experiments. Slope significance listed in Table 3.2.

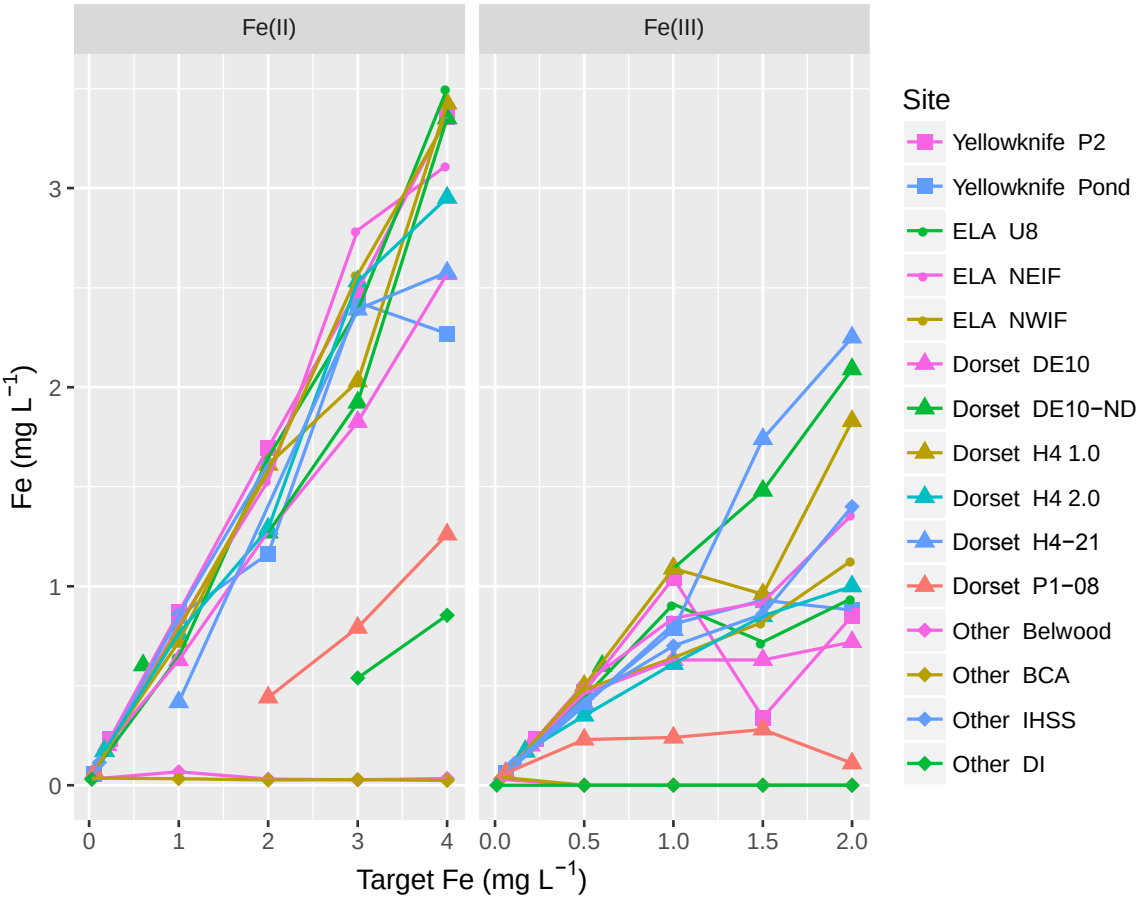

Figure 3.8. Fe concentration after $FeCl_2$ and $FeCl_3$ addition.

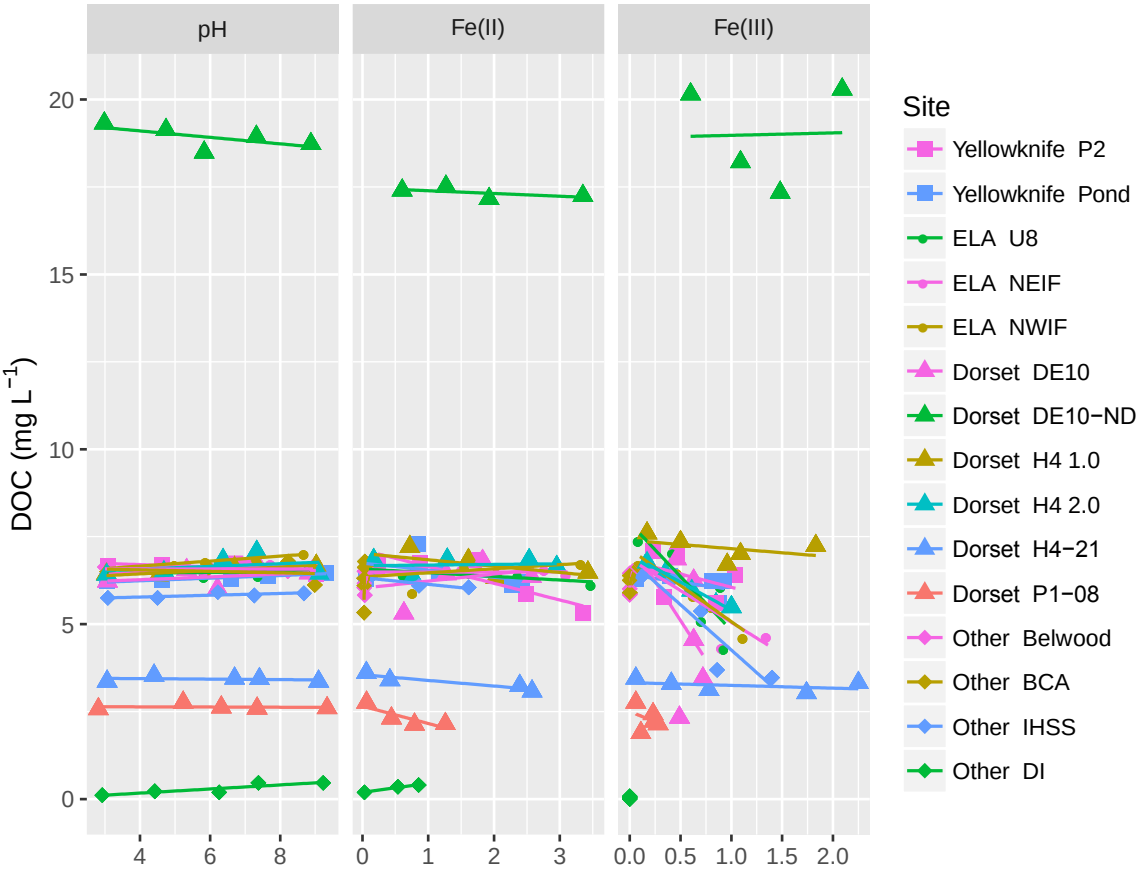

Figure 3.9. DOC concentrations after adjusted pH, Fe(II) (mg L^{-1}), and Fe(III) (mg L^{-1}). Slope significance listed in Table 3.2.

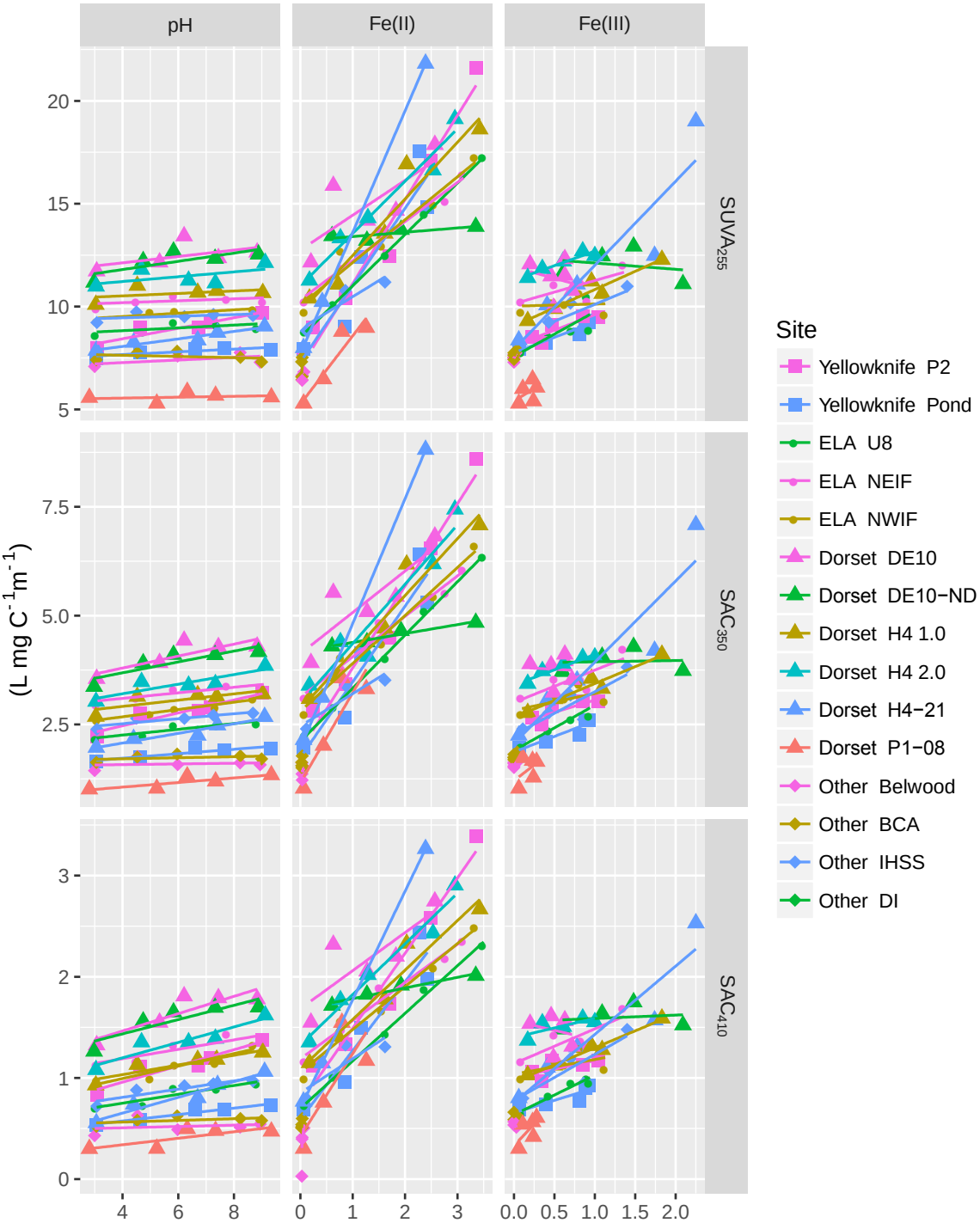

Figure 3.10. The relationship between $SUVA_{255}$, SAC_{350}, and SAC_{410} (L mgC^{-1} m^{-1}) and actual pH, Fe(II) (mg L^{-1}), and Fe(III) (mg L^{-1}) values in the titrations. Slope significance listed in Table 3.2.

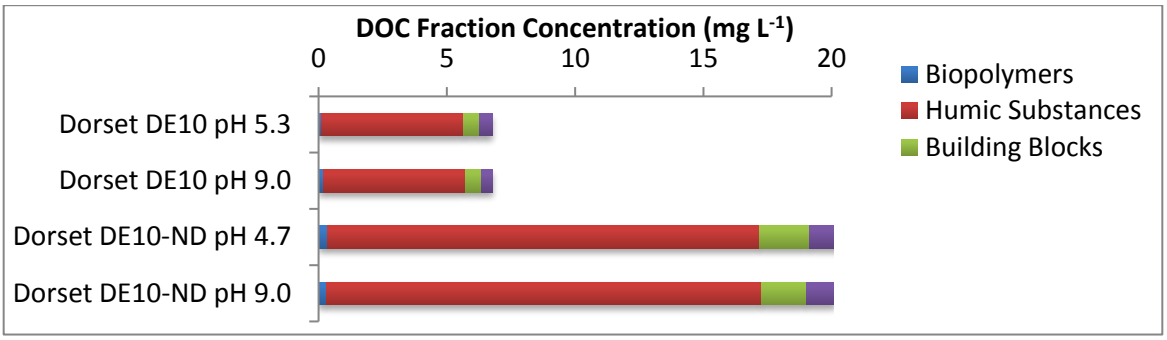

Figure 3.11. LC-OCD fraction concentrations in samples from pH titration experiment.

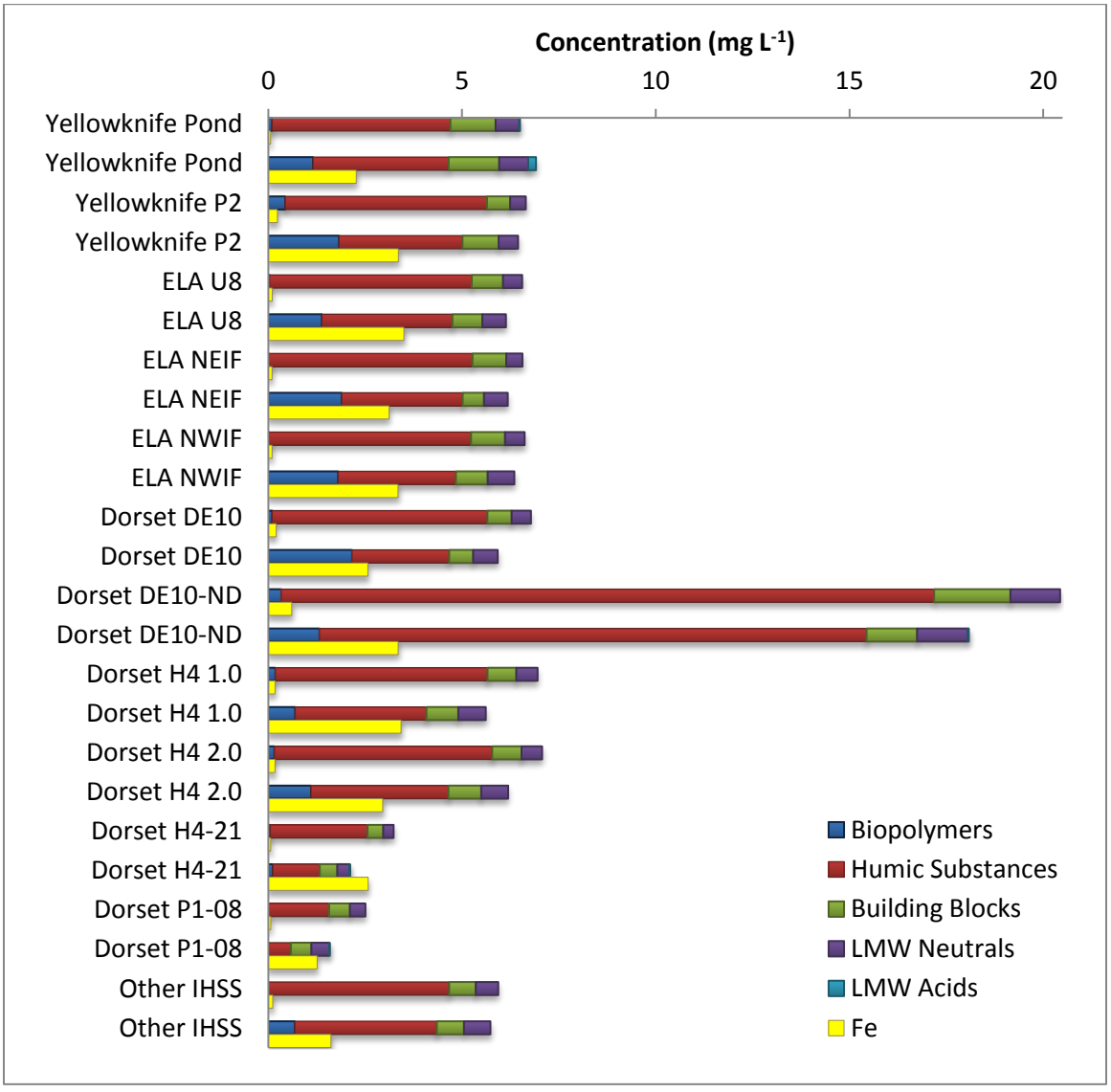

Figure 3.12. LC-OCD fraction concentrations before and after $FeCl_2$ addition.

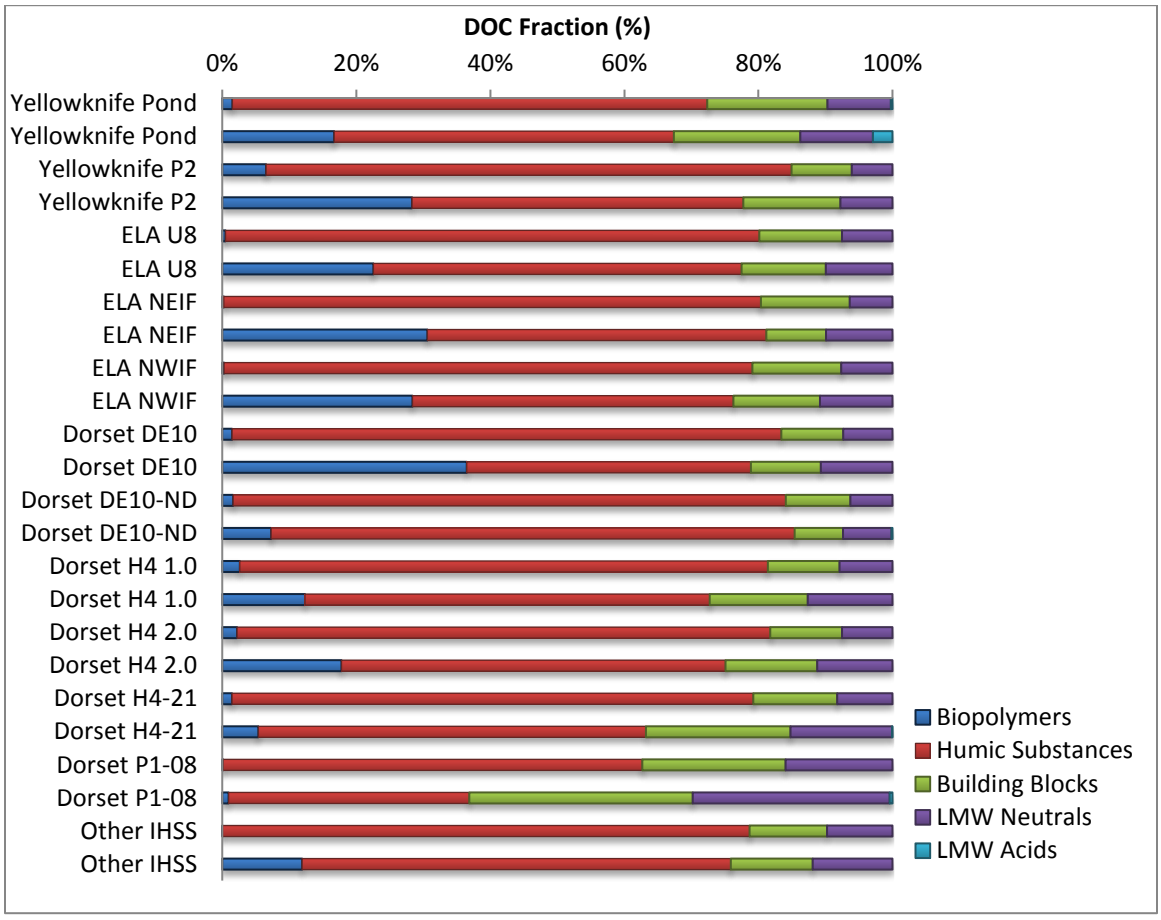

Figure 3.13. LC-OCD DOC fraction percent composition before and after $FeCl_2$ addition.

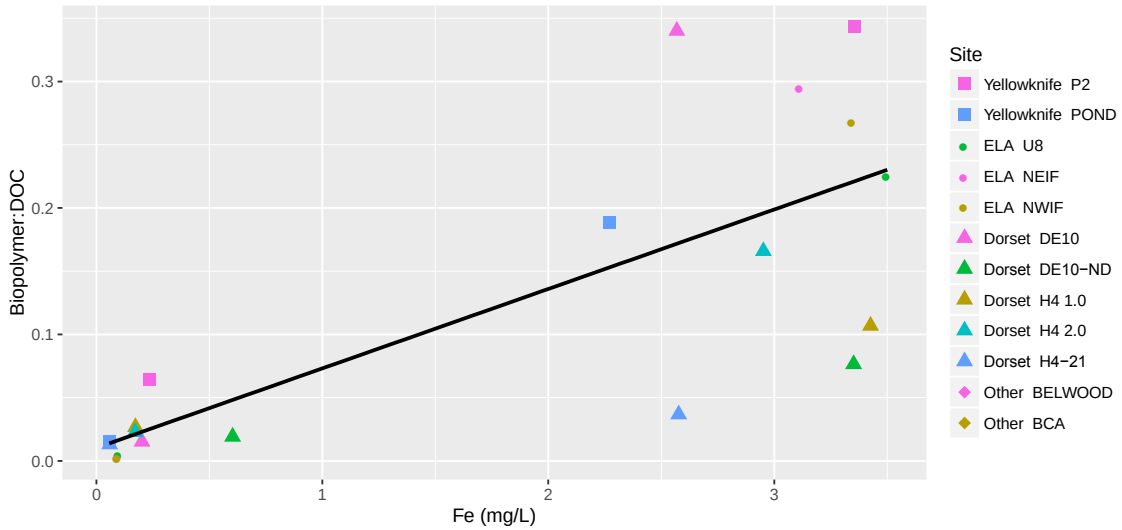

Figure 3.14. Fe(II) experiment biopolymer:DOC and Fe. y=(0.063±0.012)x+0.010±0.026 (p<0.05; R^2=0.603).

Chapter 4 The Effect of Fe and pH on the Quantity and Quality of DOM during Photodegradation

4.1 Introduction

To better predict the future influence of climate change on the aquatic carbon cycle, it is essential to first improve the understanding of the cycle and factors that influence it. Cole et al. (2007) estimated that inland waters receive 1.9 Pg C annually from terrestrial landscapes. Of this amount, 0.2 Pg C is buried in aquatic sediments, 0.8 Pg C evades to the atmosphere as CO_2, and 0.9 Pg C ends up in the oceans. Inland aquatic systems thus play a large role in modifying the fate of aquatic carbon, especially in northern boreal environments where large quantities of carbon are stored in soil, peatlands, and the biomass of forests. Although the regional carbon cycles involving boreal lakes are on a much smaller scale than the global carbon cycle, these cycles are especially important in the Northern Hemisphere where there are approximately 800,000 boreal lakes in Canada alone. Thus they are relatively small but important parts of the global carbon cycle and the changing climate (Sellers et al. 1997).

Dissolved organic matter (DOM; generally measured as the concentration of dissolved organic carbon (DOC)) is the largest input of terrestrial carbon to lakes (Schindler et al. 1997) and plays a large role in the aquatic and regional carbon cycles. Molot and Dillon (1996) estimated that 66 Tg terrestrial C y^{-1} is exported to lakes of boreal forest biomes and 30-52 Tg C y^{-1} is retained in northern surface waters and either evaded to the atmosphere as carbon dioxide (CO_2) or sequestered in the sediment as particulate matter (PM). However, the mechanisms by which large quantities of dissolved carbon end up as sediment in boreal systems have not been well studied and are largely unknown.

The transfer of organic carbon to lake sediments is small in terms of the global carbon fluxes. However, as DOM can be transformed into other carbon fractions in aquatic systems, it has an important role in lake function and thus the global carbon cycle. Atmospheric CO_2 concentrations are increasing due to anthropogenic activities and climate change predictions

determined from these increases provide a need for a greater understanding of the processes that affect carbon cycling in aquatic systems (Houghton et al. 1995).

4.1.1 Carbon Sequestration

DOM can contribute to carbon sedimentation by particulate matter (PM) formation and subsequent deposition. This can occur through a number of processes including biological processes, mixing, and photodegradation. During the biological consumption of DOM, living organisms mineralize carbon but also incorporate carbon into their biomass, increasing in size. When these organisms die, they decompose to DOM and DIC but can also end up in the sediment as PM. When DOM particles collide during mixing they adhere and flocculate according to the processes outlined in section 1.4.4 (O'Melia and Tiller, 1993; Kepkay, 1994; Wetzel, 2001).

Lakes annually accumulate significant quantities of PM in their sediment (Kortelainen et al. 2004) and although it is largely unknown how this PM is formed, the settling rates have been found to be proportional to DOC concentrations (von Wachenfeldt and Tranvik 2008). Primary production rates are found to be similar to respiration rates (del Giorgio and Peters 1993; del Giorgio et al. 1997; Prairie et al. 2002), and respiration rates even exceed gross primary productivity in oligotrophic lakes (Duarte and Prairie 2005). If these rates are similar, then significant sediment accumulation of PM requires another source of carbon in addition to phytoplanktonic debris. It is likely that an abiotic mechanism that would bypass DOM mineralization to CO_2 followed by photosynthetic fixation and burial is important for the formation of PM from DOM (Porcal et al. 2013). There are two possible abiotic pathways: 1) the irradiation of DOM that induces PM formation (not well understood) (Gao and Zepp, 1998; Porcal et al. 2004; von Wachenfedlt et al. 2008; Porcal et al. 2009, 2010) or 2) the complexation between soluble metal species and anionic sites on DOM or DOM by-products, neutralizing their charge and reducing DOM colloidal stability and thus its solubility (reduced solubility → PM formation) (Duan and Gregory, 2003). However, the mechanisms involved in photochemical PM formation have not been sufficiently described

and its relative importance to sediment accumulation of PM in lakes is unknown (Porcal et al. 2013).

4.1.2 Photodegradation of DOM

When calculating lake carbon budgets, a large portion of carbon in lake sediments originates from an unknown source. As DOM is a large carbon pool in lakes, it is hypothesized that the carbon in lake sediments may come from the photodegradation of DOM (Porcal et al. 2013; von Wachenfeldt and Tranvik, 2008).

Photodegradation of DOM occurs when UV radiation, in the presence of a catalyst, is absorbed and breaks the bonds of the aromatic rings and unsaturated carbon skeletons in DOM (Sinsabaugh and Findlay, 2003). Dissolved oxygen (DO) acts an electron acceptor in the oxidation of DOM and can convert recalcitrant DOM (and labile DOM) into more labile photoproducts including carboxylic acids, small organic compounds, and DIC; lowering the average molecular weight of the carbon compounds (Opsahl and Benner, 1998; Zepp et al. 1998). These smaller organic products can then stimulate microbial activity (Miller and Moran, 1997; Tranvik et al. 2000).

The photodegradation of DOM also induces PM formation, especially in the presence of Fe as a catalyst (Gao and Zepp, 1998). Fe(III) that is initially bound to DOM can be liberated, photolytically reduced to Fe(II), and reoxidized in oxygenated waters to form ferric hydrous oxides. It can then bind with DOM and form PM. Although PM can form in both the light and the dark, Gao and Zepp (1998) suggest that when DOM is oxidized in the light, it complexes more readily with Fe.

Photodegradation can create a major sink for DOM in the surface layer of lakes (Bertilsson and Tranvik, 2000), an important part of regional carbon cycles (Miller and Zepp, 1995; Granéli et al. 1996; Bertilsson and Tranvik, 2000) and a potentially important mechanism in the production of PM in lakes (Figure 4.1; Porcal et al. 2013). Photodegradation of DOM also influences water chemistry and transparency affecting both the photic zone depth and the region of photosynthetic activity (Andrews et al. 2000; Anesio

and Granéli, 2003), and can affect aquatic organisms by altering UV penetration, and the lability of carbon sustenance in the food web (Gao and Zepp, 1998).

The importance of O_2 consumption during DOM photodegradation and microbial respiration has been compared to that of primary production (Lindell and Rai, 1994). Photodegradation of DOM influences O_2 consumption in surface water (Amon and Benner, 1996; Miles and Brezonik, 1981; Chomicki, 2009) and transparency (Andrews et al. 2000; Anesio and Granéli, 2003) and thus affects aquatic organisms and their extent of UV exposure (Gao and Zepp, 1998). The decomposition of DOM to DIC during photolysis is an important mechanism in CO_2 production and influences the carbon balance of aquatic ecosystems (Miller and Zepp, 1995; Granéli et al. 1996; Bertilsson and Tranvik, 2000), and the $\delta^{13}C$ of DOC and DIC from photolysis (Opsahl and Zepp, 2001; Osburn et al. 2001; Vähätalo and Wetzel, 2008; Chomicki, 2009). However, only a few researchers have investigated the influence of increased Fe concentration and pH on photodegradation (Gao and Zepp, 1998; Molot et al. 2005). To my knowledge, there are only two studies which reported over 20% POC formation from the photodegradation of DOC (von Wachenfeldt et al. 2008; Porcal et al. 2013) demonstrating that photodegradation could be the mechanism for the unaccounted for POC pool. As samples with high Fe:DOC were used in these studies (Porcal et al. 2013; von Wachenfeldt et al. 2008), it was hypothesized that the missing link in determining the source of the unaccounted for sedimented carbon could be the interaction of Fe or pH or both on the photodegradation of DOM. Understanding this process and the parameters that influence it is important in understanding the aquatic carbon cycle.

4.1.3 The Influence of Photolysis on Carbon Isotopes

The influence of photolysis on dissolved and particulate carbon isotopes in aquatic systems are often not considered (Chomicki, 2009, Quay et al. 1986). Stable carbon isotopes have been used to investigate carbon cycling in lakes and the incorporation of carbon into food webs (Cole et al. 2006), track the uptake and transfer of carbon to POC and aquatic species (Cole et al. 2002; Pace et al. 2004; Carpenter et al. 2005), and investigate the source of POC (Pace et al. 2004; von Wachenfeldt and Tranvik, 2008). However, to effectively use stable

isotopes as a tool to interpret and understand the processes occurring in aquatic systems, it is first important to understand how these processes can affect the isotopes. During photolysis, the more negative isotopes of DOM are cleaved first, producing DIC and $CO_{2(g)}$ with more negative $\delta^{13}C$ values and thus $\delta^{13}C$-DOC values become more positive (Chomicki, 2009). $\delta^{13}C$-POC values are also influenced by photolysis as the POC formed originates from the degraded DOC pool with photolytically altered $\delta^{13}C$-DOC values (Figure 4.2; Chomicki, 2009). Therefore, as photolysis changes the size and the isotope composition of the aquatic and atmospheric carbon pools, it is essential to understand the influence of photolysis on carbon isotopes used in coupled mass and isotope balances.

4.1.4 The Role of Fe and pH in Carbon Sequestration

Iron

Iron plays an important role in determining the concentration, solubility, and flux rates of trace metals, and DOM in aquatic environments. It can complex with DOM, is an essential micronutrient for biota, and generates alkalinity in acidified lakes with the microbial reduction of Fe(III) and sulfate buffering aquatic systems against acidification (Kalff, 2002). Fe can affect both the cycling and analysis of DOM due to its large binding capacity with DOM and ability to absorb solar radiation (Xiao et al. 2013).

The two species of Fe, Fe(II) and Fe(III), are among the most electroactive redox reactants in natural waters. Although Fe(II) is more soluble than Fe(III) at typical lake pHs, Fe(III) is the oxidized form and thus the most common form in oxygenated waters (Wetzel, 2001). pH, Eh, temperature, and DOC concentration and quality primarily determine the solubility of Fe in solution and the rate of oxidation of Fe(II) to Fe(III) in oxygenated water.

Softwater lakes with low concentrations of bicarbonate (HCO_3^-), like those found in boreal environments, contain higher concentrations of Fe, most of which is oxidized to Fe(III). Theoretically, Fe(III) may be regarded as insoluble in natural waters because the solubility of Fe in the ionic form in well-oxygenated waters with pH above 4.8 is less than 10 µg/L (Mill, 1980). Despite this, Fe concentrations measured in 0.45 µm filtered river water are about 1,000 times higher, averaging 670 µg/L (Riley and Chester, 1971). This occurs

when Fe is complexed to DOM in aquatic environments, greatly affecting Fe and DOM cycling and concentrations (Kalff, 2002). Fe(III) exhibits enhanced aqueous solubility in the presence of DOM (Pullin and Cabaniss, 2003; Gaffney et al. 2008; Pédrot et al. 2011) and Fe has elevated concentrations in acidic waters (Stefánsson, 2007). Lake systems containing high concentrations of dissolved humic compounds, especially those derived from the *Sphagnum* moss of bogs and bog lakes, have lower redox potentials, around 350mV (Kjensmo, 1970; Visser 1964). These higher reducing conditions lead to metal enrichment by complexation and adsorption to the acidic molecules of DOM (Szilágyi, 1973; Zimmerman, 1981).

Fe can complex with DOM in natural waters through several mechanisms including the mixing of acidic water (containing dissolved free Fe(III) ions) from the pore water of acidic soil with DOM in nonacidic waters (Peiffer et al. 1999). Another mechanism is anoxic water mixing with oxic surface water, oxidizing Fe(II) to Fe(III), and subsequent complexation with DOM (Maloney et al. 2005). Fe can also bind with DOM through microbial redox reactions, siderophores, or light-assisted reduction and dissolution of Fe (Barbeau et al. 2001; Gledhill et al. 2004).

pH

pH is one of the most important variables in aquatic systems because it influences alkalinity, acidity, acid neutralizing capacity, carbon solubility (CO_2, HCO_3^-, pCO_2, DIC), trace metal solubility, and DOM structure and composition (Kalff, 2002; Wetzel, 2001). pH also affects the complexation between trace metals (such as Fe, Al and Mn) and DOM, further influencing the structure of DOM molecules and trace metal solubility (Chin et al. 1998; Mokma and Buurman, 1982; Pace et al. 2012; Porcal et al. 2014).

At low pH, DOM molecules are condensed as a result of the protonation of functional groups reducing overall number of negative charges per DOM and limiting the exposure of chromophores to radiation. At high pH, deprotonation promotes the expansion of DOM molecules, exposing more chromophores (Chin et al. 1998; Myneni et al. 1999; Baalousha et al. 2006; Pace et al. 2012). Ionic strength also affects the structure of DOM molecules with structural changes occurring at higher ionic strength. With more exposed chromophores to

absorb solar radiation, absorbance is often higher (Pace et al. 2012) and thus has implications for light attenuation, thermocline depths, and lake heat budgets. This limits the benthic primary and secondary productivity in nutrient-poor lakes (Karlsson et al. 2009).

Aquatic systems have been experiencing changes in pH, trace metal concentration, and DOM concentration and composition due to climate change, land use change, and recovery from acidification (Cox et al. 2000). These changing conditions influence a large suite of parameters and processes due to the significance of pH, trace metal concentration, and cycling and DOM quantity and composition in aquatic settings (Pace et al. 2012; Poulin et al. 2014; Xiao et al. 2013). Increases in pH and Fe concentrations can change the structure of DOM molecules and increase absorption. This can influence the rate of photolysis and thus the rates of formation of DIC, DOM, and PM in lakes but these influences are poorly understood.

4.1.5 Chapter Objectives

This study examined the affect of increased pH and Fe concentrations on the quantity and quality of DOM during photodegradation in three stream waters from Ontario, Canada. This study is the first, to my knowledge, to collect and analyze subsamples during the experiment to observe changes in carbon transformation rates and $\delta^{13}C$-CO_2 values. The objectives were to: 1) quantify the role of Fe and pH on DOM quantity and quality changes during photodegradation and PM formation; and 2) characterize the changes in $\delta^{13}C$ of DOC, DIC, CO_2, and POC during DOM photolysis.

4.2 Methods

4.2.1 Site Descriptions

Incubation experiments were performed using water from three small inflows to three oligotrophic lakes. Stream waters were collected from two streams, L302 Upland 8 (U8) and Rawson Lake (L239) North-East Inflow (NEIF) located within the Experimental Lakes Area (ELA) near Kenora, Ontario, Canada and one stream, Dickie Lake Inflow 10 (DE10) located in the Muskoka region 200 km north of Toronto, Ontario, Canada (Figure 4.3; Table 4.1).

These streams are major DOC contributors to their respective lakes and DE10 has been the focus of previous studies on the photodegradation of DOM (e.g. Chomicki, 2009; Molot and Dillon, 1997; Gennings et al. 2001; Porcal et al. 2014; etc.).

4.2.2 Experimental Design

To assess the effects of photolytic processes, there were 3 light treatments per site; one exposed to light (Light), one exposed to light with added Fe (Light + Fe), and one exposed to light with increased pH (Light + pH). Light + Fe treatments contained 54.9 mL, 38.3 mL and 44.5 mL of 1M ferric chloride ($FeCl_3$) solution in approximately 4L of 0.45µm-filtered water of U8, NEIF and DE10, respectively. Light + pH treatments contained the 0.45µm-filtered water and an addition of 1M NaOH to bring the pH of the samples to the higher end of the natural range, approximately 6.5. To assess the effects of non-photolytic processes on each site, a dark treatment containing 0.45µm-filtered water was wrapped in aluminum foil to prevent photodegradation.

After Fe and pH modifications, samples were left to sit overnight before they were put into 5L Tedlar bags. Tedlar bags act as a closed system micro-environment, have a gas tight seal, an attached septa from which subsamples can be taken and do not leach DOM (Figure 4.14; Chomicki et al. 2009). Once the samples sat overnight, 4L from each site and treatment combination were split between two Tedlar bags (2L per bag, 4 treatments and 3 sites, 24 Tedlar bags in total). Following this, 2L of laboratory air from the Centre for Cold Regions and Water Science (CCRWS) at Wilfrid Laurier University was added to each bag using a 1L gas syringe in order to provide oxygenated headspace. The bags were then placed in shallow water baths to regulate bag temperature, and placed on the roof of the Centre for Environmental and Information Technology (EIT) at the University of Waterloo (43°28’25.6” N and 80°33’27.5” W; elevation approximately 335m). The water baths of duplicate treatments were arranged side by side to minimize differences in exposure and temperature and a Hoskin Scientific Ltd. photosynthetically active radiation (PAR) sensor was set up next to the water baths to measure cumulative PAR. A total of 24 bags were incubated beginning on August 19, 2014 for 29 days until September 17, 2014.

4.2.3 Subsample Collection

All subsamples were filtered using a Whatman 0.45µm syringe tip filter except those taken for pH, DIC and POC analyses. Aliquots of samples were taken before and after the photolysis experiment for initial and final characterization of pH, absorbance, fluorescence, LC-OCD, $\delta^{13}C$ of DOC, CO_2, and DIC, and the concentrations of cations, anions, DOC, CO_2, and DIC. Samples were taken at the end of the photolysis experiment for the analysis of $\delta^{13}C$-POC and during the photolysis experiment for pH, absorbance, $\delta^{13}C$-CO_2, and concentrations of anions, DOC, and CO_2.

One bag of each duplicate was subsampled during the experiment (every 2-5 days) to measure changes in CO_2 and DOC concentration, pH, absorbance, and $\delta^{13}C$-CO_2. The other set of duplicates remained undisturbed. A three-way valve, tubing, needles, and separate syringes for gas and water were used to collect samples to minimize CO_2 contamination from the atmosphere.

4.3 Results

4.3.1 Initial sample characterization

The percent peatland coverage in stream catchments correlated ($R^2 = 0.956$, n=3) with DOC concentration (Table 4.1, Figure 4.4) and initial Fe concentration correlated with $SUVA_{255}$ ($R^2 = 0.992$; Figure 4.5) and biopolymer:DOC ($R^2 = 0.778$). Fe concentrations of U8, NEIF, and DE10 were increased by 0.8, 0.7 and 0.7 mg-Fe/L, respectively, in the Light + Fe treatments and the pH was increased to approximately 6.5 in the Light + pH treatments (Table 4.2). Although there was a range of sites with different $SUVA_{255}$, Fe, and DOC concentrations, LC-OCD results showed similar percent composition of DOC fractions (Figure 4.6).

4.3.2 Fe change with photolysis

Fe concentrations decreased (1.7SD) by an average of 80% in most of the light treatments while dark treatments did not change (1.7SD) with photolysis (Figure 4.7; Table 4.3). Fe concentrations in the Fe spiked ELA upland (U8) and Dorset bog (DE10) samples decreased

to 0.1mg/L before 50 MJ/m^2 and 200 MJ/m^2 of PAR, respectively. Thus the U8 Light + Fe treatment had similar sample parameters to the U8 Light treatment due to rapid initial loss of Fe. The Fe spiked ELA wetland (NEIF Light + Fe) treatment was the only light treatment where the Fe concentration did not decrease (1.7 SD). Overall, there were three different changes in Fe concentrations between the three Light + Fe treated samples

4.3.3 DOC loss during photolysis

DOC loss was significant in all of the light treatments and was, in most samples, degraded more rapidly early in the experiment and in samples with higher initial DOC concentrations (Figure 4.9). As a result of different site characteristics in the U8 catchment, the original DOC concentration of U8 (17.8 mg/L) was less than DE10 (26.4 mg/L; Table 4.2) and thus DOC degraded in the U8 Light treatment was approximately 20% higher than in DE10. Although the decrease in DOC concentrations was less in Light + pH treatments (Table 4.4; Figure 4.8), DOC loss was similar between Light and Light + Fe treatments, especially where the Fe:DOC decreased during photolysis (U8 and DE10; Figure 4.10).

4.3.4 ΣCO_2 and POC production from photolysis

ΣCO_2 ($DIC_{(aq)} + CO_{2(g)}$) gain was significant in all of the light treatments, was caused by the loss of at least 50% of the DOC, and between the same treatments, was higher with higher initial DOC concentrations (Figure 4.11; Figure 4.12). The DOC degradation and thus the change in ΣCO_2 were greatest in the Light and Light + Fe treatments (Table 4.5) and the percent ΣCO_2 increases in the Light + pH treatments were approximately half of those in the other light treatments. The DOC lost during photolysis in most samples was converted to ΣCO_2 with less than 10% POC production (Figure 4.13). Although POC formed during photolysis in both the light and dark treatments, there was not enough POC collected for mass analysis (Table 4.6). The final mass of POC was calculated using carbon mass balances (Figure 4.13). Although the NEIF sample had the highest mass of POC (close to 20% of the DOC lost) and the highest initial DOC concentration, increased Fe and pH increased the POC formation relative to the Light treatments in all samples.

4.3.5 pH change during photolysis

Although the pH of all light treatments changed (1.7SD) with photolysis, the pH of the dark treatments did not change significantly during photolysis (Figure 4.15, Table 4.3). The pH of the Light + pH treatments decreased and the pH of the Light and Light + Fe treatments increased to a pH between 4.8 and 5.8. The pH of Light and Light + Fe treatments were similar after photolysis but the pH of Light + pH treatments remained higher.

4.3.6 Effects of photolysis on $\delta^{13}C$ of DOC, DIC, CO_2 and POC

With photolysis, the production of LMW DOC, DIC, and POC increased the remaining (residual) $\delta^{13}C$-DOC, decreased the $\delta^{13}C$-DIC and $\delta^{13}C$-CO_2, and produced $\delta^{13}C$-POC values similar to the post-photolysis $\delta^{13}C$-DOC values. The $\delta^{13}C$-DOC and $\delta^{13}C$-DIC of the dark treatments did not change and compared to the light treatments, the final $\delta^{13}C$-CO_2 values were more positive and the $\delta^{13}C$-POC values were more negative.

In the light treatments, the $\delta^{13}C$-DOC of the Light + pH treatments changed the least while the Light + Fe treatments changed the most (Figure 4.16). There was thus a strong positive correlation between the percent DOC loss (Table 4.4) and the increase in $\delta^{13}C$-DOC (R^2= 0.77; Figure 4.17). After photolysis, the Light + pH treatments had the most positive $\delta^{13}C$-DIC values of the light treatments, while the Light and Light + Fe treatments had the most negative values. Changes in $\delta^{13}C$-DIC were lower in U8 treatments where less DIC was produced and higher in NEIF and DE10 (Table 4.5). The $\delta^{13}C$-ΣCO_2 became more positive with the cumulative production of ΣCO_2 and thus with photolysis (Figure 4.18). The $\delta^{13}C$-POC values from light treatments were between the initial and final $\delta^{13}C$-DOC values (and were less than 1‰ more negative than the final $\delta^{13}C$-DOC values) except for NEIF, where $\delta^{13}C$-POC values were 1.7‰ to 3.0‰ more positive than the final $\delta^{13}C$-DOC values. The NEIF samples were the only samples where the $\delta^{13}C$-POC values were more positive than the final $\delta^{13}C$-DOC values and also the only samples to have greater than 10% POC production.

4.3.7 Change in absorbance during photolysis

There were significant decreases in a_{410}, $SUVA_{255}$, and SAC_{350} in light treatments but no significant changes were observed in dark treatments (Table 4.8; Figure 4.19; Figure 4.20; Figure 4.22). Among the light treatments, the decreases in DOC concentration, and thus, a_{410}, $SUVA_{255}$, and SAC_{350} were typically smallest in the Light + pH treatments (Figure 4.21). The decrease in $SUVA_{255}$ and SAC_{350} is greatest in U8 samples and the U8 Light and Light + Fe treatments (both with similar Fe concentrations after 50 MJ/m^2 of PAR) have the highest rate of change of $SUVA_{255}$ and SAC_{350} during the first 100 MJ/m^2 of PAR. There was a large range in the percent decrease of $SUVA_{255}$ in all light treatments (15-91% decrease), indicating that the influence of photodegradation on DOM composition is different between sites.

4.3.8 Change in LC-OCD after photolysis

With photolysis, there was a decrease in humic substances and increase in LMW neutrals and acids, shifting the DOC fractions (determined by LC-OCD) towards LMW DOC. Due to budget constraints, the U8 dark treatment was the only dark sample analyzed because it had similar results to the pre-photolysis sample (Figure 4.23 and Figure 4.24). The humic substance concentration decreased the most in the Light + Fe treatments, the least in the Light + pH treatments and all of the humic substances were degraded in the U8 Light and Light + Fe treatments. Increased Fe concentrations increased both DOC and humic substance decomposition while increased pH decreased it (Figure 4.25). Thus, similar to spectral analysis ($SUVA_{255}$, SAC_{350}; Figure 4.26), DOM quality measured by LC-OCD before and after photolysis is different between sites and treatments.

4.4 Discussion

4.4.1 Photolysis changes DOM concentration and composition

DOM concentration and composition can change during photolysis, forming constituents for PM production and reducing water colour. However, the use of LC-OCD analysis to observe

how much certain fractions of DOC are degraded and the changes in the size of carbon pools, a_{410}, $SUVA_{255}$, and SAC_{350} during the experiment are unique to this photodegradation study.

Photolysis is likely the main mechanism responsible for DOC degradation and DIC production in these experiments because solar radiation degraded 50-85% of the DOC in the light treatments and DOC degradation and DIC production was much lower in the dark treatments. Concomitantly, the decrease in humic substances and increase in proportion of LMW neutrals and acids as shown by LC-OCD indicates that photolysis shifts DOM to LMW DOM. As previously reported (Chomicki, 2009; von Wachenfeldt et al. 2008; Porcal et al. 2013; etc.), photolysis changes the DOM lability, aromaticity, size of the carbon pools, and together with decreased DOC concentrations, leads to decreases in water colour (a_{410}). Photolysis can thus increase water transparency, increasing the penetration depth of solar radiation, and influence aquatic heat budgets, as well as biotic and abiotic processes. However, the novel use of LC-OCD determined that the humic substance size molecules could be completely degraded in some samples by photolysis in a short amount of time. This may influence the results of DOM quality analysis and thus the interpretation of results. For instance, DOM quality results of highly humic, allochthonous DOM that as been photolytically altered may have similarities to autochthonous DOM even though the DOM was not biologically altered.

DOC degradation was not linear with cumulative PAR. DOC was degraded more rapidly early in the experiment (before 75 MJ/m^2 of PAR; approximately 9 days), consistent with the photolytic degradation of larger, more labile DOC molecules, especially humic substance size molecules. The decrease in specific absorbance parameters ($SUVA_{255}$ and SAC_{350}) in light treatments also suggests larger, UV-absorbing components are degraded first, ultimately resulting in smaller molecules. As photolysis cleaves DOM molecules, DOM composition changes and more recalcitrant DOM is produced. This indicates that photobleaching, water transparency, and the change in aromaticity and UV absorption of the DOM does not change linearly with solar radiation. Further, as certain fractions of DOM (humic substances size molecules) are more easily degraded by photolysis, the DOM

composition changes during photolysis and thus the isotopes of the DOC, DIC, and CO_2 also do not change linearly.

Fe and pH both influence water colour and the rates of photolysis. The decrease in specific absorbance parameters (a_{410}, $SUVA_{255}$, and SAC_{350}) during photolysis also encompasses the change in Fe concentrations and pH, which have been found to influence a_{410}, $SUVA_{255}$, and SAC_{350} (Chapter 3). There was a large range in the percent decrease of $SUVA_{255}$ in all light treatments (15-91% decrease), indicating that the influence of photodegradation on DOC composition changes with site and treatment. This suggests that water colour would likely decrease the most in samples with low/no starting Fe concentrations.

4.4.2 DOM concentration and composition influences photolysis

The large range in photolysis results (final DOC concentration, quality, etc.) likely reflect the natural range in pH, DOC and Fe concentrations, and DOM quality that are caused by differences in geology, vegetation, hydrology, land use and climate. These parameters provide a range of influences on the rates and photoproducts of photolysis. In particular, initial DOM concentration and composition influenced the rates of DOM degradation, DIC production, change in water colour and final DOC composition, POC production and the $\delta^{13}C$ of the DOC and POC. It is therefore important to understand the effect of the physical and chemical site parameters on photolysis rates and products to predict how photolysis may change (carbon cycling, DOM quality, etc.) temporally and spatially in natural environments.

As samples with the highest initial DOC concentrations (NEIF) had the highest rates of DOC degradation and DIC production, initial DOC concentration influences the size of the carbon pools during photolysis. Therefore, aquatic systems with high initial DOC concentrations are more likely to have high initial rates of DOC degradation and DIC production. However, the percent DOC degradation was greater in samples with lower initial DOC concentrations (Table 4.4) and lower a_{410}. The resulting highest percent decrease of DOC, lowest final a_{410}, $SUVA_{255}$, SAC_{350}, and the complete decomposition of humic

substance size fractions as determined by LC-OCD, indicates that water transparency plays a large role in the final DOC concentration and composition of water post-photolysis.

Most samples (except NEIF) produced less than 10% POC indicating that the photolytic degradation of DOC alone did not produce enough POC to account for the large unknown source of sedimented carbon in some lakes. However, Porcal et al. (2013) observed increases in POC followed by decreases and suggested this was caused by the formation of an intermediate compound, that was degraded by further photolysis into LMW DOC (Kieber et al. 2006) or DIC (Vähätalo et al. 1998; Anesio et al. 1999; Mayer et al. 2006). In natural systems, this intermediate compound may fall out of suspension before it could be further degraded in surface waters and contribute to larger POC masses than estimated in my experiment. In my photolysis study, the calculation of POC concentration during photolysis was impossible, however, similar processes may have led to the underestimation of total POC production masses. Both Fe and pH increased contributed to greater POC production in the NEIF samples and as the only site to plot below the 10% POC production line, the DOC composition is therefore important to the production of POC, especially with pH and Fe concentration manipulations.

δ^{13}C-DOC values changed with photolysis relative to percent loss of DOC (R^2=0.771), and were thus different between different sites, suggesting sample source and composition are also important to changes in isotope values with photolysis. δ^{13}C-POC values of the light samples were more positive than the δ^{13}C-DOC values in the NEIF samples, suggesting that samples with the most POC formation will have the lowest $\alpha_{DOC\text{-}POC}$ (Table 4.7). It is therefore difficult to estimate how photolysis will affect the isotope balance of a freshwater system without knowing the percent loss of DOC or final POC mass.

Aquatic systems with high initial DOC concentrations and a_{410} will likely have high initial rates of DOC degradation and higher POC formation, but lower transparency, less DOC molecules exposed to solar radiation, slower changes in the δ^{13}C of DOC and more positive δ^{13}C-POC values. This could lead to greater sediment formation and possibly the

ability to track past lake DOC concentrations using the δ^{13}C-POC values of the sediment record.

4.4.3 Increased pH protects DOM from some effects of photolysis

Increased pH appears to protect DOM from some effects of photolysis and is likely contributing to the brownification of some waters. After photolysis, Light + pH samples had more POC production, higher a_{410}, $SUVA_{255}$, and SAC_{350}, and thus darker water. Increased pH also slowed the rate of DOC degradation and DIC production, influencing the DOC composition and producing more negative δ^{13}C values for the post photolysis DOC, DIC and CO_2 relative to other light treatments.

An increase in pH slows DOC loss, especially humic substance and HMW DOC degradation, leading to higher a_{410}, $SUVA_{255}$, and SAC_{350} values and thus darker water colour. This suggests that higher pH protects the photobleaching of freshwaters. Thus, as lakes recover from acidification, increasing pH, the rate of photolysis will be influenced and fractions of DOM, especially humic substance size molecules will not be degraded as quickly. This could contribute to brownification and also influence the ability to trace DOM sources as measures of DOM may indicate photodegraded DOM is similar to autochthonous DOM. Further, darker water increases absorption of solar radiation, preventing the deep penetration of solar radiation, affecting heat budgets and photo-processes. However, as the DOC of other light treatments was photolyzed (and likely, acidic carboxylic acids were cleaved), the pH increased. Porcal et al. (2013) also observed similar increases in pH from photolysis (pH 4.5 to 5 to pH 5.5 to 6). Similar to high Fe concentrations, these pH increases resulted in higher a_{410}, $SUVA_{255}$, SAC_{350}, and thus, water colour than would be predicted if the same sample was diluted to a lower DOC concentration (Chapter 3). Although $SUVA_{255}$, SAC_{350}, and water colour decrease overall with photolysis, pH also increases and, in turn, protects DOC from photodegradation.

Although Light + pH treatments did not lose as much DOC as other light treatments, they had higher percent POC formation, indicating that increasing pH increases POC formation. This trend was also observed in previous experiments (Porcal et al. 2013) and has

implications for carbon balances, as increasing pH is important for decreasing the CO_2 evaded to the atmosphere and increasing the carbon sink.

pH also influences the rate at which carbon isotopes change. Photolysis degrades the more negative isotopes of DOC first and thus the $\delta^{13}C$ of the CO_2 added to the headspace is initially more negative and becomes more positive during photolysis. As less DOC is degraded in samples with higher pH, less CO_2 evades to the headspace and only the more negative $\delta^{13}C$-DOC is cleaved and added to the headspace as $\delta^{13}C$-CO_2. Therefore, $\delta^{13}C$ of the DOC, DIC, and CO_2 produced by photolysis will change more gradually which has implications for freshwater isotope balances, as pH is an important determinant in $\delta^{13}C$ values. Thus understanding the influence of pH on the $\delta^{13}C$ of the DOC, DIC, and CO_2 produced by photolysis can be used as a tool to determine how recovery from acidification will affect carbon balances and CO_2 release from lakes. Further, the sediment record could possibly be used to trace the past pH of lakes.

4.4.4 Increased Fe concentration alters the photolysis of DOM

The photodegradation of DOM in samples with increased Fe appears to preferentially degrade certain fractions of DOM and have higher rates of DOM consumption and PM production. There was greater DOC degradation in samples with increased Fe and thus, those treatments lost the most humic substance size molecules. As Fe plays an important role in the photolyzed DOM composition, specific spectral absorbances would be expected to decrease. However, dissolved Fe also absorbs solar radiation, and thus Light + Fe treatments had similar $SUVA_{255}$, SAC_{350}, and water colour to the Light treatments. This suggests there could be similar photobleaching of freshwaters even with higher Fe concentrations. Further, as Fe concentrations increase in some lakes, the rates of DOC degradation from photolysis could increase and some fractions of DOM, especially humic substance size molecules could be degraded more quickly.

As increased Fe concentrations did not increase the production of CO_2, the addition of Fe aided in the formation of POC from DOC degradation. However, the DOC of different sites had different binding capacities with Fe during photolysis. Depending on DOM

composition, the dissolved concentrations of Al and Fe in some samples decreased with photolysis as the concentration of POC increased due to the preferential binding of trace metals with POC and subsequent filtration out of solution. However, the high DOC concentration in the ELA wetland (NEIF) likely provided more Fe binding locations and was the only sample to have constant Fe concentrations during photolysis. Thus, aquatic environments with similar characteristics to the NEIF Light + Fe sample (high % peatland and DOC concentrations, and low pH, $SUVA_{255}$ and Fe:DOC ratio) and higher binding capacities for Fe, might create conditions for greater POC formation during photolytic degradation. Therefore, the influence of DOM concentration and composition on DOM binding capacity for trace metals can play a large role in the photoproducts of photolysis.

As the rate of DOC degradation was higher in samples with increased Fe concentrations, there were less HMW DOC molecules, and thus the $\delta^{13}C$ of the post photolysis DOC, DIC, and CO_2 were affected. More DOC is degraded in samples with higher Fe, however, the same concentration of CO_2 as the Light treatments evades to the headspace producing similar $\delta^{13}C$-CO_2 values. This is important to know in order to determine how changes in Fe concentrations driven by climate change and human interference will affect freshwater carbon balances and CO_2 release from lakes.

4.4.5 Photolysis alters the aquatic carbon isotope balance

Changes in DOC, DIC, CO_2, and POC concentrations due to photolysis alter the carbon isotopes of these pools and thus influence isotope mass balances involving carbon species. As increases in Fe concentration and pH influence the percent of DOC loss and DIC production, they also change the $\delta^{13}C$ signatures of DIC, CO_2, DOC, and POC. Several studies (e.g. Quay et al. 1986; Cole et al. 2002; Pace et al. 2004; Carpenter et al. 2005; Cole et al. 2006; von Wachenfedlt and Tranvik, 2008) document isotope balances that do not consider the change in isotope values caused by photolysis. However, it is difficult to estimate how the $\delta^{13}C$ of DIC, CO_2, DOC, and POC will change in freshwaters as DOM is photolytically degraded.

Increases in δ^{13}C-DOC with photolysis suggest that functional groups (usually carboxylic acids) that were more negative than the rest of the DOC were preferentially cleaved (Kavitha and Palanivelu, 2004). Therefore, adding Fe increased DOC degradation and likely caused more cleaving, while pH decreased photolysis, likely reducing the number of functional groups cleaved. Although there is a poor negative correlation ($R^2 = 0.278$) between the δ^{13}C-DOC change and percent peatland that has previously been suggested to have a strong positive correlation (Chomicki, 2009), there is a positive correlation with percent DOC loss and change in δ^{13}C-DOC (0.771). As δ^{13}C-DOC values relate to percent DOC loss (Figure 4.17), percent DOC loss due to photodegradation can be used to estimate the change in δ^{13}C-DOC (Equation 4.1). However, DOC concentration and composition also play a role in this relationship and make this estimation more difficult. In this experiment, there was low analytical precision (±2.4 ‰) associated with estimating the change in δ^{13}C-DOC (Equation 4.1).

Equation 4.1 $y=0.050\pm0.009x - 0.807\pm0.518$ ($p<0.05$, $R^2=0.771$)

The decreases in δ^{13}C-DIC values (by approximately 3‰ to 6‰) in the light treatments were similar to changes observed in previous photolysis experiments (Chomicki, 2009). Changes in δ^{13}C-DIC were lower in U8 treatments and higher in NEIF and DE10 because there was less DIC produced in the photodegradation of all U8 samples (Table 4.5). The cumulative δ^{13}C-DIC values became more negative with photodegradation because the DIC was produced from the more negative isotopes of the functional groups (e.g. carboxylic acids) cleaved from the DOC, however, the value of the DIC added became more positive. The DOC concentration of the U8 Light treatment decreased by approximately 80%, changing the δ^{13}C-DOC by the most of all the sites and treatments and suggesting that the fractionation factor between δ^{13}C-DOC and δ^{13}C-DIC decreases with photolysis.

The light treatments had more positive δ^{13}C-POC values than the dark treatments suggesting that photolysis changes the isotopic values of the DOC contributing to POC production. All of the δ^{13}C-POC values from the dark treatments were within 0.5‰ of each other across all sites suggesting that regardless of DOC composition, or isotopic signature,

the POC formed during processes in the dark could be very similar. As the POC formed in the light treatments originates from the photolytically degraded DOC, the δ^{13}C-POC is similar to the δ^{13}C-DOC values. This is the case for all sites but NEIF, which has δ^{13}C-POC values 1.7‰ to 3.0‰ more positive than the final δ^{13}C-DOC values, likely due to greater POM formation.

4.5 Summary and Conclusions

This study determined that increased pH and Fe concentrations influenced the photodegradation of DOM differently and that the response of between sites was different. Increased Fe concentrations increased the rate of photolysis and POC production and led to the greatest shift towards LMW DOC concentrations. Although increased pH also increased POC production (the most of all treatments), it decreased the rate of photolysis leading to relatively less LMW DOC molecules, darker water colour, and less change in δ^{13}C-DOC. Overall, photodegradation, producing mostly less than 10% POC, was not found to be an important mechanism for POC production in experiments continuously exposed to light. This suggests that there must be more factors involved in the large portions of natural aquatic carbon sedimentation of which the source is currently unknown. However, POC is susceptible to further photodegradation so the exposure length is critical to the final mass of POC. Further, increasing both pH and Fe together, could have produced the largest mass of POC. This is especially likely in natural systems where it is possible that higher masses of POC are produced, fall out of suspension, and cannot be later degraded by solar radiation and reconverted into LMW DOC and DIC. Therefore, natural increases in pH in lakes recovering from acidification may increase sediment accumulation, especially if the Fe supply, already bound to DOM when delivered from the catchments, is not decreased. Photolysis also influenced the aquatic isotope balance differently between different sites, pH, and Fe concentrations. In general, photolysis increased the δ^{13}C of the DOC, decreased the δ^{13}C of the DIC and CO_2, and the ΣCO_2 produced became more positive with photolysis. As the δ^{13}C of DOC, DIC, CO_2, and POC change with photolysis, this has implications for determining DOC, DIC, CO_2, and POC sources in aquatic systems and interpreting the sediment record.

Table 4.1. General characteristics of stream water samples and catchments.

Inflow	% Peatland	Area (ha)	DOC (mg/L)	Fe (mg/L)	Mn (mg/L)	Al (mg/L)	pH	DIC (mg/L)
U8	0[1]	7.2[1]	17.8	0.27	0.01	0.70	4.4	1.33
NEIF	35[2]	10.6[2]	46.5	0.46	0.01	0.27	4.0	1.62
DE10	17[3]	78.9[3]	26.4	1.26	0.03	0.06	4.7	1.14

[1]Lamontagne & Schiff, 1999; [2]Bayley & Schindler, 1987; [3]Dillon & Molot, 1997.

Table 4.2. Initial sample characterization after Fe and pH additions and prior to photolysis.

Site	DOC (mg/L)	Fe (mg/L)	Fe:DOC	Al (mg/L)	pH	DIC (mg/L)
U8 Light	17.8	0.27	0.02	0.70	4.4	1.3
U8 Light + Fe	17.9	1.09	0.06	0.70	4.4	2.0
U8 Light + pH	17.9	0.29	0.02	0.72	6.5	2.7
NEIF Light	46.5	0.46	0.01	0.27	4	1.6
NEIF Light + Fe	47.2	1.15	0.02	0.17	4	1.8
NEIF Light + pH	47.1	0.49	0.01	0.20	6.6	3.1
DE10 Light	26.4	1.26	0.05	0.06	4.7	1.1
DE10 Light + Fe	28.3	1.93	0.07	0.04	4.3	2.0
DE10 Light + pH	30.0	1.25	0.04	0.04	6.6	3.0

Table 4.3. Initial and final pH, Fe and Al concentrations.

Site	Initial pH	Final pH	Initial Fe (mg/L)	Final Fe (mg/L)	Initial Al (mg/L)	Final Al (mg/L)	Initial Al:Fe	Final Al:Fe
U8 Light	4.4	4.9	0.27	0.04	0.70	0.09	2.59	2.25
U8 Light + Fe	4.4	4.9	1.09	0.02	0.70	0.11	0.64	5.20
U8 Light + pH	6.5	5.7	0.29	0.02	0.72	0.01	2.48	0.40
U8 Dark	4.4	4.3	0.27	0.08	0.70	0.00	2.59	0.00
NEIF Light	4.0	4.8	0.46	0.17	0.27	0.60	0.59	3.54
NEIF Light + Fe	4.0	5.0	1.15	0.92	0.17	0.16	0.15	0.18
NEIF Light + pH	6.6	5.8	0.49	N/A	0.20	0.13	0.41	N/A
NEIF Dark	4.0	4.0	0.46	0.52	0.27	0.04	0.59	0.07
DE10 Light	4.7	5.1	1.26	0.38	0.06	0.16	0.05	0.43
DE10 Light + Fe	4.3	5.0	1.93	0.12	0.04	0.17	0.02	1.38
DE10 Light + pH	6.6	5.8	1.25	0.06	0.04	0.00	0.03	0.00
DE10 Dark	4.7	4.7	1.26	0.95	0.06	0.05	0.05	0.05

Table 4.4. Change in DOC concentrations and Fe:DOC by photolysis.

Site	DOC initial (mg/L)	DOC final (mg/L)	Change in DOC (mg/L)	% DOC Loss	SD ±	Initial Fe:DOC	Final Fe:DOC
U8-Light	17.8	2.9	14.9	84%	5%	0.02	0.01
U8-Light + Fe	17.9	3.0	14.9	83%	5%	0.06	0.01
U8-Light + pH	17.9	7.3	10.7	60%	4%	0.02	0.00
U8-Dark	17.8	16.1	1.7	10%	4%	0.02	0.01
NEIF-Light	46.5	13.5	33.0	71%	2%	0.01	0.01
NEIF-Light + Fe	47.2	11.4	35.8	76%	2%	0.02	0.08
NEIF-Light + pH	47.1	21.7	25.4	54%	2%	0.01	N/A
NEIF-Dark	46.5	40.9	5.6	12%	1%	0.01	0.02
DE10-Light	26.4	9.5	16.9	64%	3%	0.05	0.04
DE10-Light + Fe	28.3	8.2	20.2	71%	3%	0.07	0.01
DE10-Light + pH	30.0	13.0	15.1	57%	3%	0.04	0.00
DE10-Dark	26.4	26.7	-0.3	-1%		0.05	0.04

Table 4.5. pH, ΣCO_2, and POC before and after photolysis.

Site	Initial pH	Final pH	Initial ΣCO_2 (mmol)	Final ΣCO_2 (mmol)	% ΣCO_2 Increase	% POC Formation*
U8 Light	4.4	4.9	0.26	2.60	912	3
U8 Light + Fe	4.4	4.9	0.35	2.62	637	6
U8 Light + pH	6.5	5.7	0.48	2.00	320	9
U8 Dark	4.4	4.3	0.25	0.51	101	-
NEIF Light	4	4.8	0.30	4.69	1459	11
NEIF Light + Fe	4	5.0	0.33	5.01	1428	17
NEIF Light + pH	6.6	5.8	0.54	3.79	600	19
NEIF Dark	4	4	0.30	0.63	110	-
DE10 Light	4.7	5.1	0.22	3.39	1406	-23
DE10 Light + Fe	4.3	5.0	0.36	3.77	952	-3
DE10 Light + pH	6.6	5.8	0.53	2.94	459	0
DE10 Dark	4.7	4.7	0.22	0.67	197	-

*% POC formation is the percent of DOC loss that contributed to POC formation.

Table 4.6. Visual sample descriptions after photolysis (relative to the three treatments for each site).

	ELA Upland (U8)	**ELA Wetland (NEIF)**	**Dorset Bog (DE10)**
Light	Most PM; lightest coloured water	Most PM; clearest coloured water	Most PM; clearest coloured water
Light + Fe	Less PM; darkest coloured water	Most PM; moderate coloured water	Most PM; moderate coloured water
Light + pH	Least PM; moderate coloured water	Least PM; darkest coloured water	Least PM; darkest coloured water

Table 4.7. $\delta^{13}C$ of DIC, CO_2, DOC, and POC (‰) before and after photolysis and the $\alpha_{DOC(final)\text{-}POC(final)}$ and $\varepsilon_{DOC(final)\text{-}POC(final)}$.

Site	**Initial $\delta^{13}C$-DOC**	**Final $\delta^{13}C$-DOC**	**Final $\delta^{13}C$-POC**	**Initial $\delta^{13}C$-DIC**	**Final $\delta^{13}C$-DIC**	***Initial $\delta^{13}C$-CO_2**	**Final $\delta^{13}C$-CO_2**	**$\alpha_{DOC(final)\text{-}POC(final)}$**	**$\varepsilon_{DOC(final)\text{-}POC(final)}$**
U8 Light	-26.5	-22.1	-23.4	-17.6	-21.5	-16.2	-25.9	1.0013	1.3
U8 Light + Fe	-	-21.6	-22.2	-18.3	-21.6	-17.1	-25.9	1.0006	0.6
U8 Light + pH	-	-24.2	-25.4	-18.0	-20.8	-20.2	-27.0	1.0012	1.2
U8 Dark	-26.5	-26.4	-29.1	-17.6	-16.8	-16.2	-21.3	1.0028	2.8
NEIF Light	-28.1	-25.4	-23.7	-19.2	-22.7	-17.7	-28.0	0.9982	-1.8
NEIF Light + Fe	-	-25.1	-22.8	-16.8	-23.0	-15.7	-27.4	0.9977	-2.3
NEIF Light + pH	-	-26.3	-23.3	-16.6	21.7	-19.1	-28.7	0.9969	-3.1
NEIF Dark	-28.1	-28.2	-29.5	-19.2	-19.4	-17.6	-22.0	1.0013	1.3
DE10 Light	-27.3	-26.1	-26.9	-15.6	-21.8	-14.6	-26.9	1.0008	0.8
DE10 Light + Fe	-	-25.7	-26.3	-16.9	-21.7	-15.9	-26.7	1.0007	0.7
DE10 Light + pH	-	-26.7	-26.6	-16.5	-21.0	-19.2	-27.6	0.9999	-0.1
DE10 Dark	-27.3	-27.4	-29.5	-15.6	-17.7	-14.5	-21.5	1.0022	2.2

"-" indicates no measurement. *Initial $\delta^{13}C$-CO_2 is calculated, not measured.

Table 4.8. Initial and final $SUVA_{255}$ and SAC_{350}.

Site	Initial $SUVA_{255}$	Final $SUVA_{255}$	% $SUVA_{255}$ Decrease	Initial SAC_{350}	Final SAC_{350}	% SAC_{350} Decrease	Final [Fe] (mg/L)
U8-Light	8.2	0.8	90%	2.1	-	-	0.04
U8-Light + Fe	-	0.8	-	-	-	-	0.11
U8-Light + pH	-	2.5	69%	-	0.4	84%	0.02
U8-Dark	8.2	8.9	-8%	2.1	2.3	-6%	0.08
NEIF-Light	8.5	6.2	27%	2.5	1.5	40%	0.17
NEIF-Light + Fe	-	7.1	-	-	2.1	14%	0.34
NEIF-Light + pH	-	7.8	9%	-	2.0	18%	0.77
NEIF-Dark	8.5	9.6	-12%	2.5	2.8	-12%	0.52
DE10-Light	11.3	5.9	48%	3.7	1.3	47%	0.38
DE10-Light + Fe	-	4.0	-	-	0.8	78%	0.41
DE10-Light + pH	-	9.6	15%	-	2.7	28%	0.06
DE10-Dark	11.3	10.8	4%	3.7	3.5	5%	0.95

"-" indicates no measurement. Values are from the set of duplicates that were subsampled during the experiment.

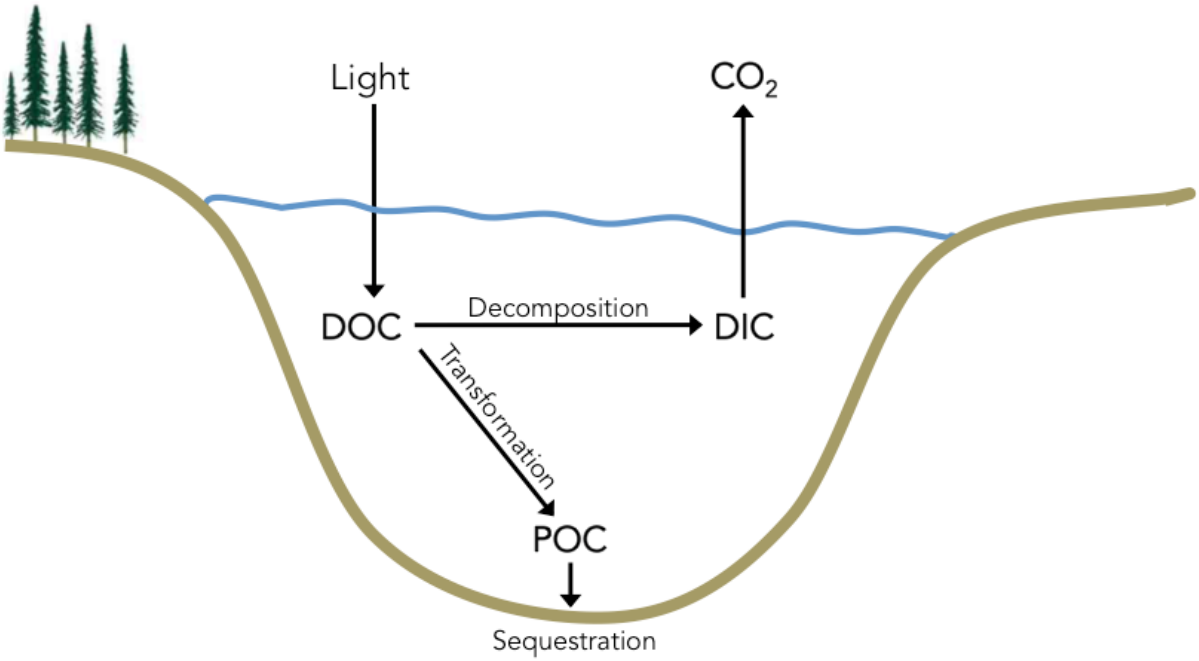

Figure 4.1. The photodegradation of DOC in aquatic systems (Schiff, unpublished figure).

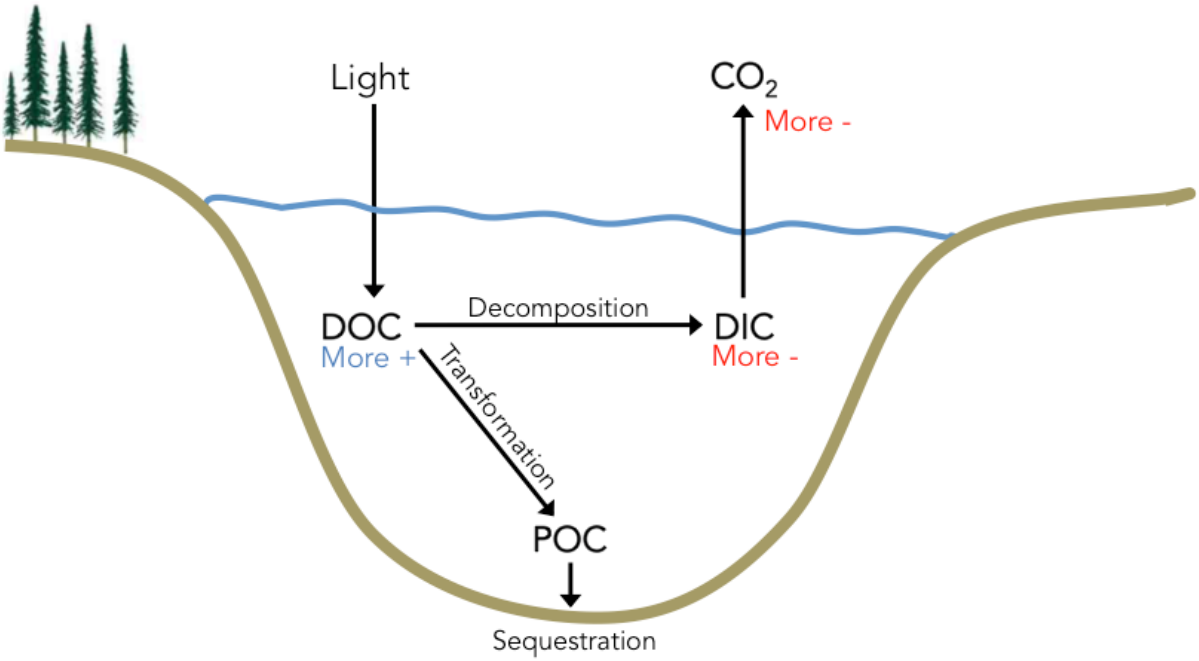

Figure 4.2. Influence of photodegradation of DOC on the $\delta^{13}C$ values of DOC, POC, DIC, and CO_2 (Schiff, unpublished figure).

U8	NEIF	DE10
		
17 mg-C L^{-1}	42 mg-C L^{-1}	26 mg-C L^{-1}

Figure 4.3. Images and DOC concentrations of samples from the three study sites.

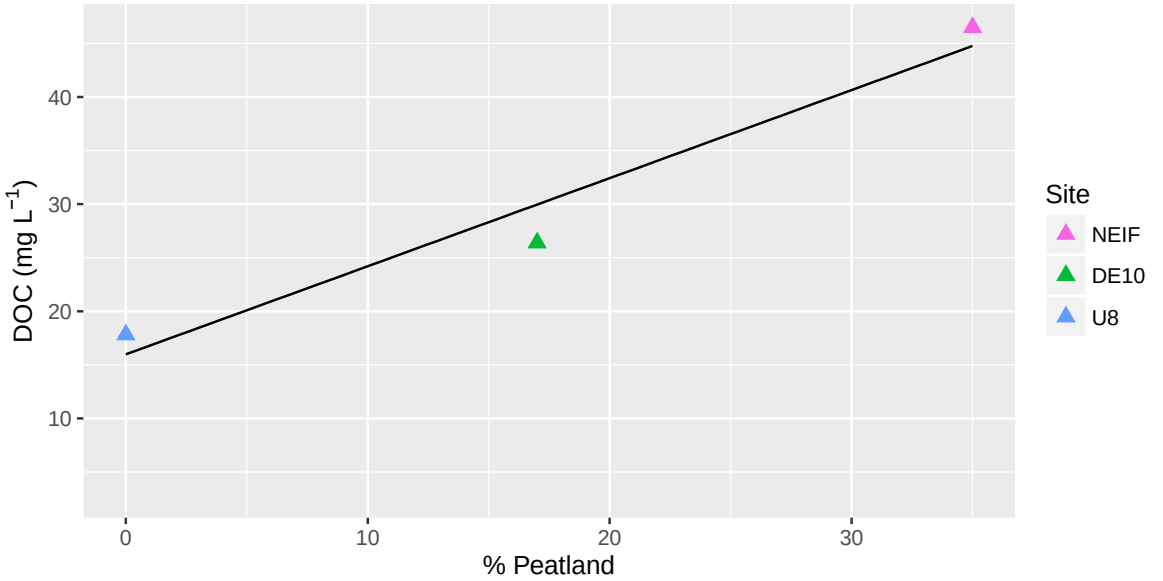

Figure 4.4. The relationship between initial DOC concentration and wetland coverage within the catchment. Data points encompass analytical precision. Y=0.822±0.176X+15.986±3.964 (R^2=0.956, p>0.05).

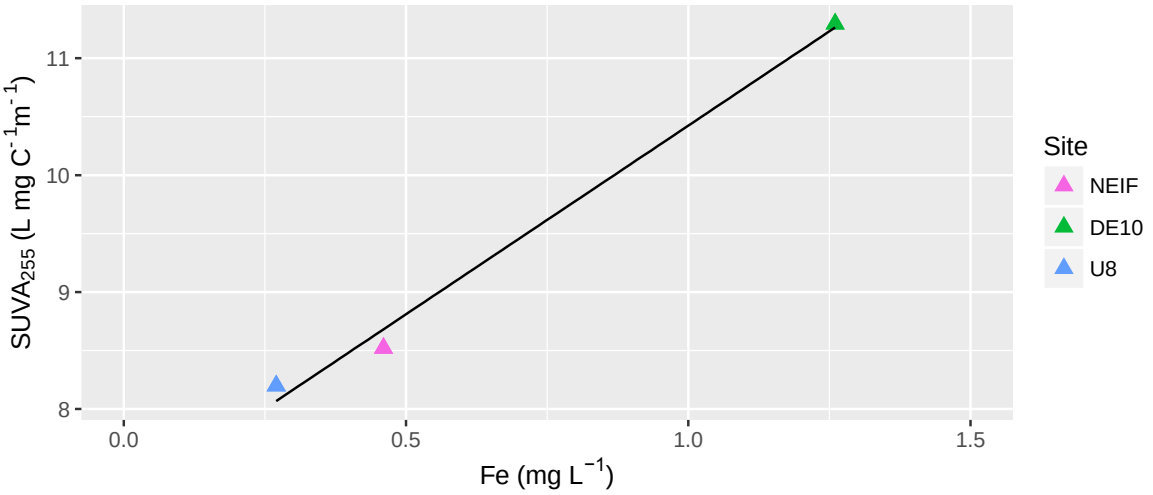

Figure 4.5. The relationship between $SUVA_{255}$ and Fe concentrations. Data points encompass analytical precision. $Y=3.2256\pm0.141X+7.1978\pm0.112$ ($R^2=0.9924$, $p>0.05$).

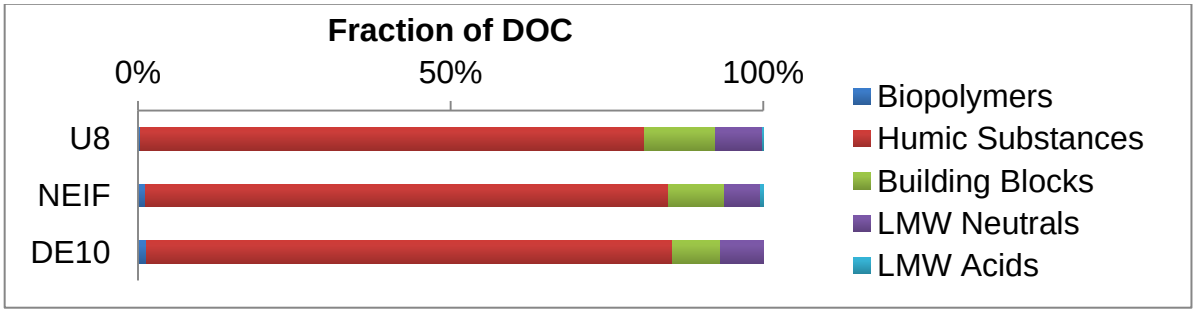

Figure 4.6. Natural DOC fraction percent compositions determined by LC-OCD.

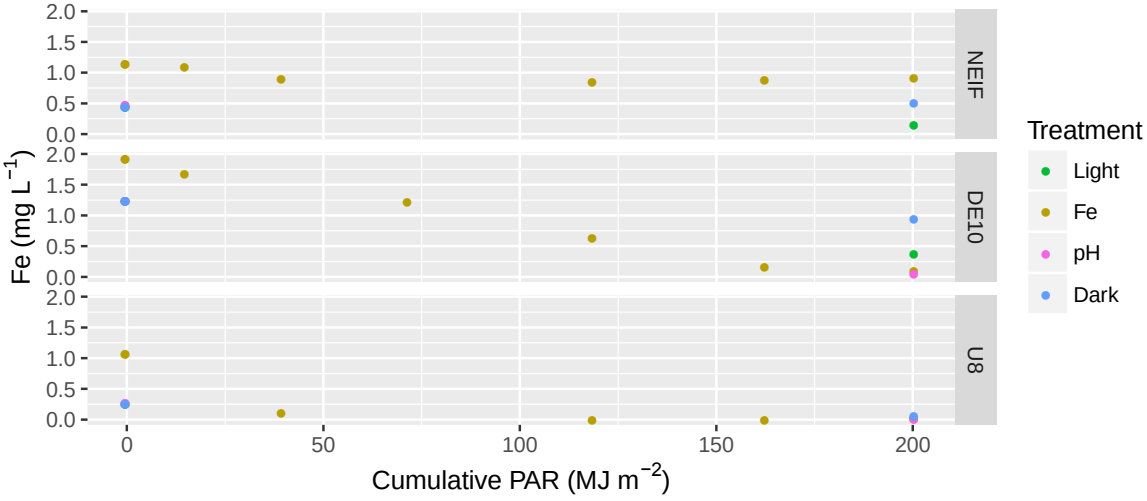

Figure 4.7. Fe concentrations during photolysis experiment. Data points encompass analytical precision.

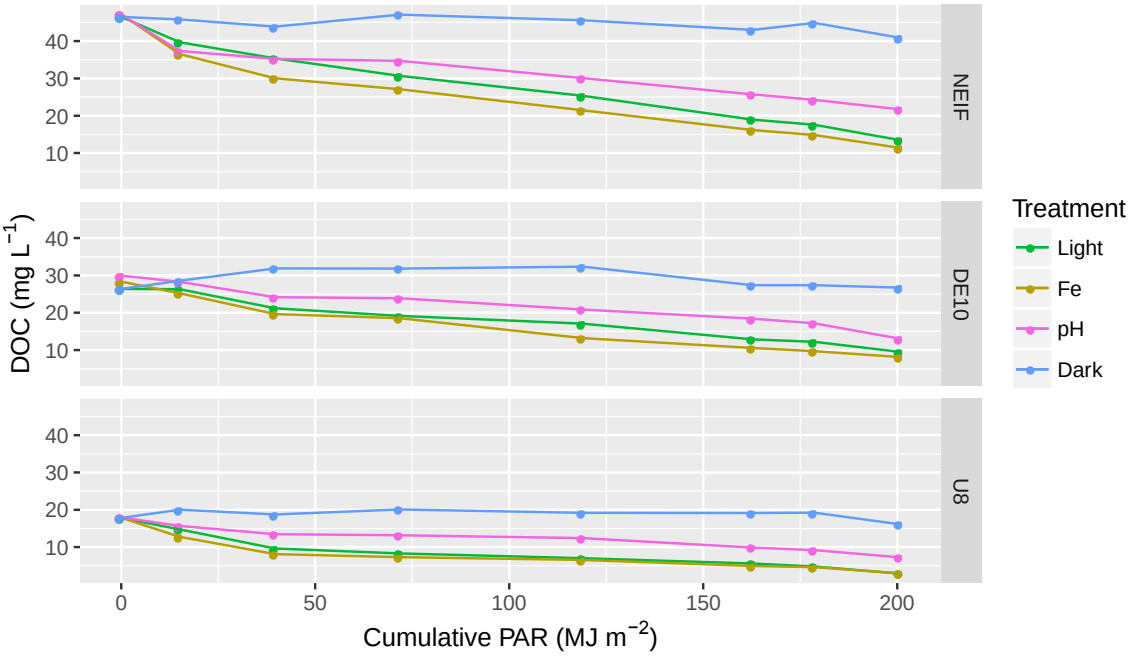

Figure 4.8. Change in DOC concentrations during photolysis experiment for a) Light, b) Light + Fe (Fe), c) Light + pH (pH) and d) Dark treatments. Data points encompass analytical precision.

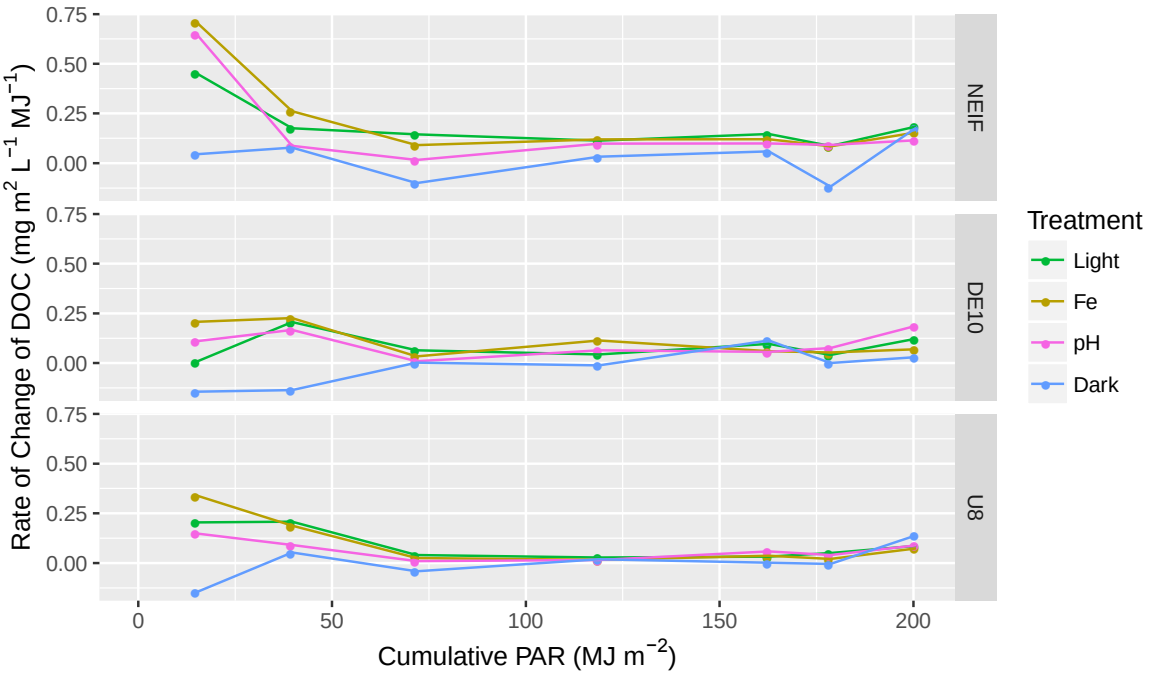

Figure 4.9. Rate of change of DOC during photolysis. Data points encompass analytical precision.

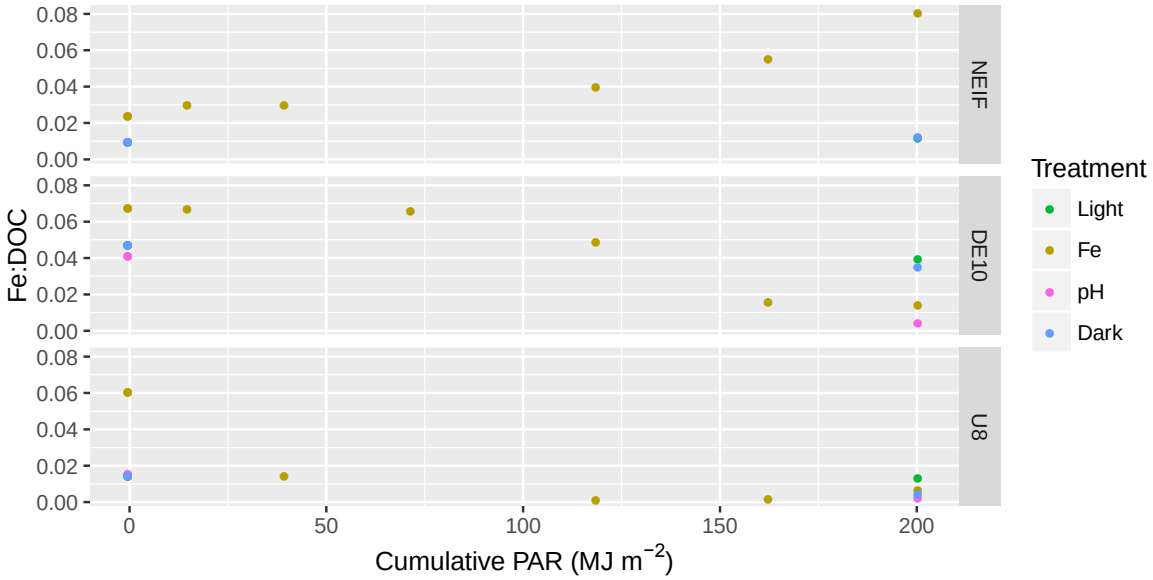

Figure 4.10. Fe:DOC change during photolysis. Data points encompass analytical precision.

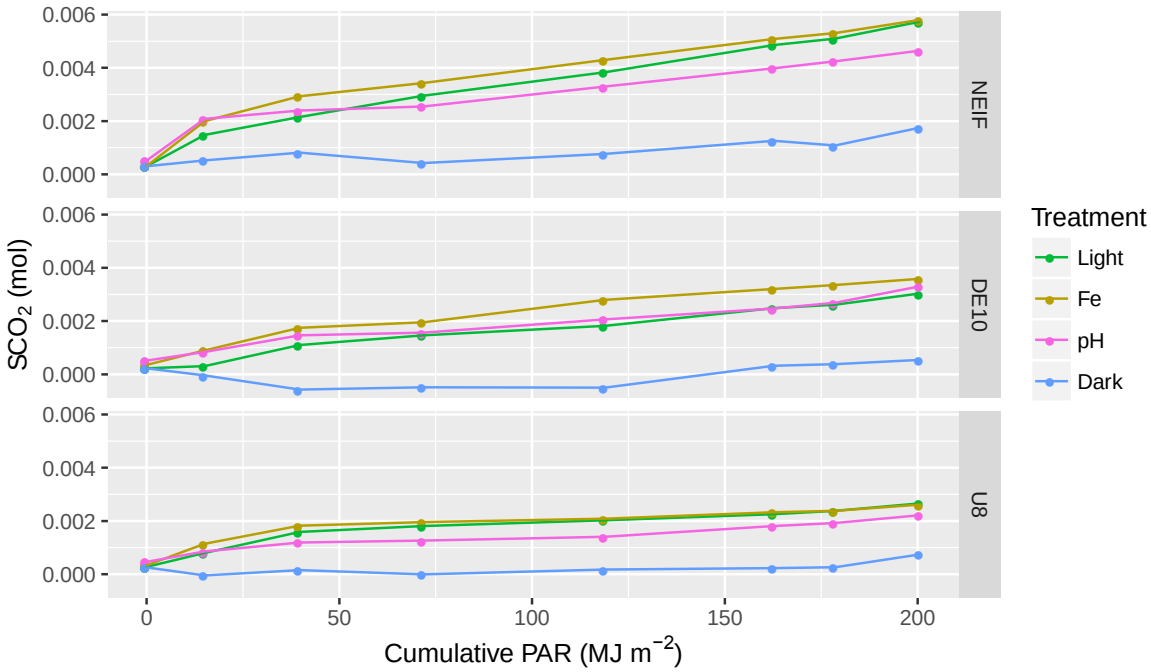

Figure 4.11. ΣCO_2 ($DIC_{(aq)}$ + $CO_{2(g)}$) concentrations during photolysis. Data points encompass analytical precision.

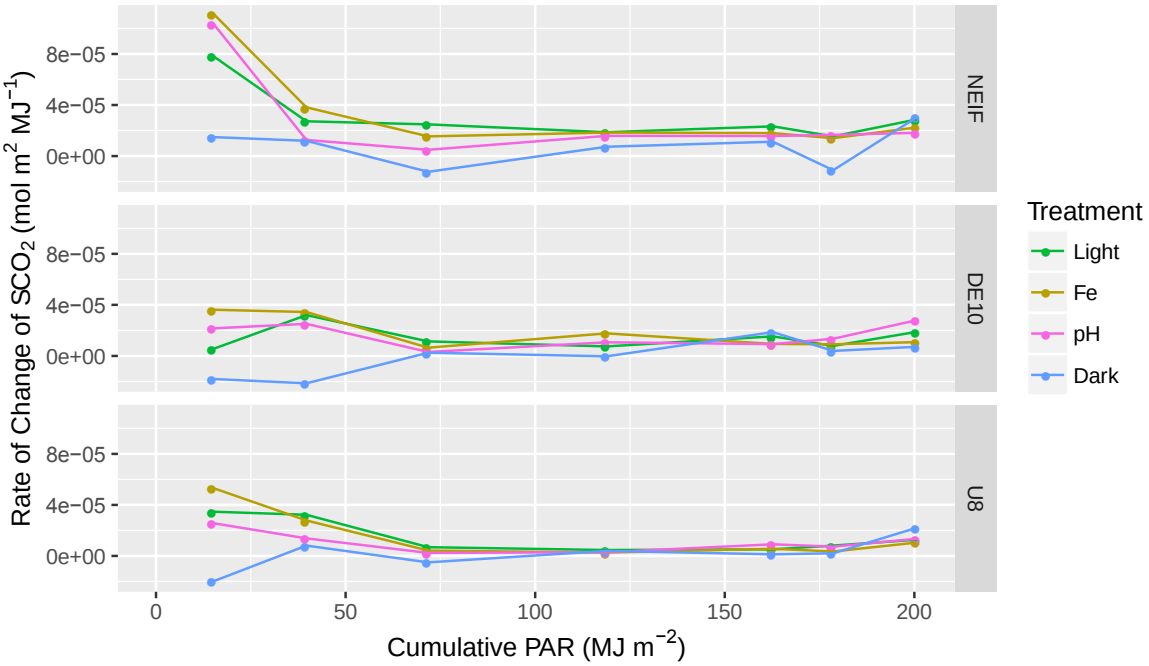

Figure 4.12. Rate of change of ΣCO_2 ($DIC_{(aq)}$ + $CO_{2(g)}$) during photolysis. Data points encompass analytical precision.

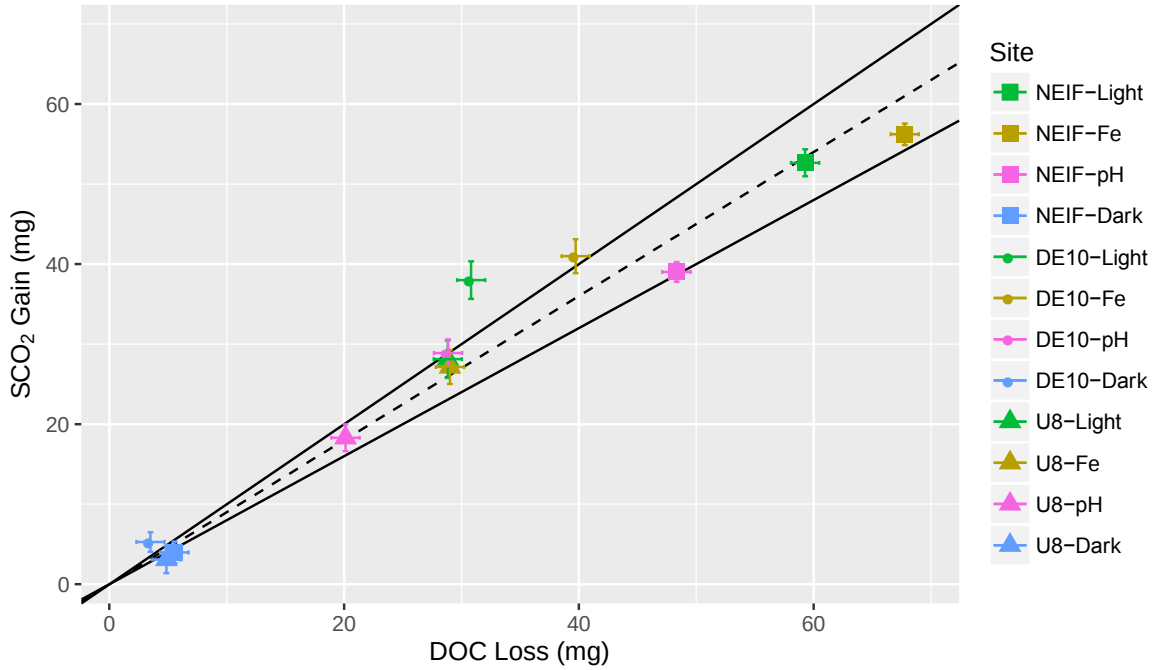

Figure 4.13. The relationship between DOC lost and ΣCO_2 produced during photolysis. The upper solid line represents 0% POC formation, the dotted line represents 10% POC formation, and the lower solid line represents 20% POC formation.

Figure 4.14. Tedlar bags after photolysis experiment.

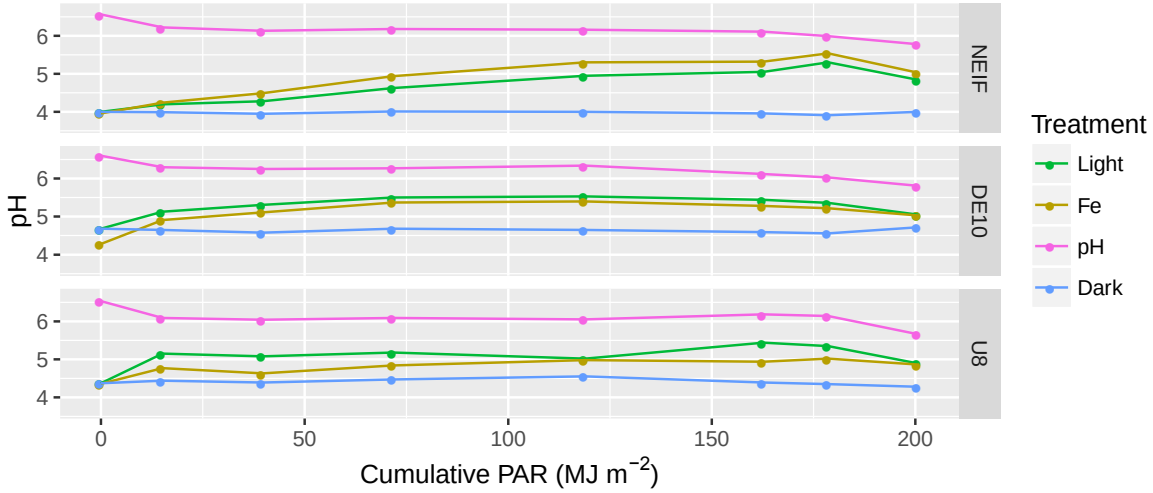

Figure 4.15. pH change during photolysis experiment. Data points encompass analytical precision.

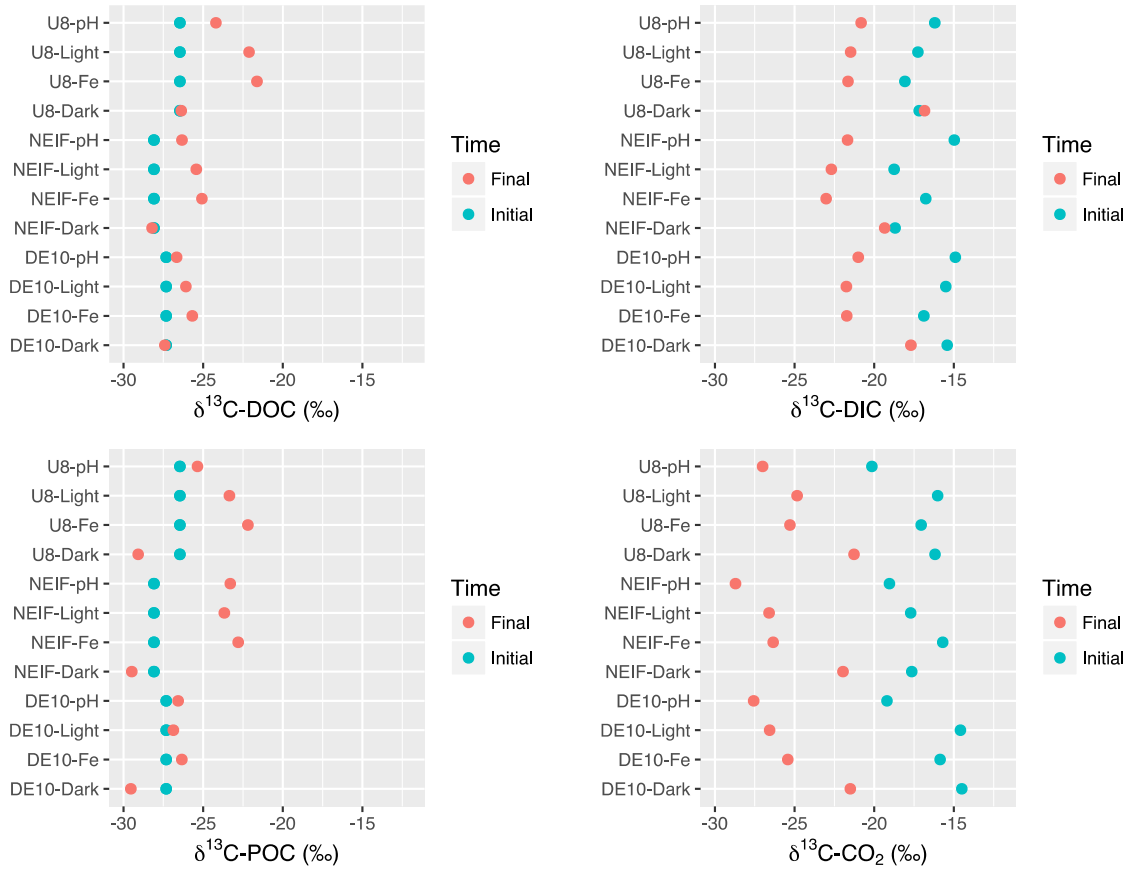

Figure 4.16. $\delta^{13}C$ of DOC, POC, DIC, and CO_2 before and after photolysis. The initial $\delta^{13}C$-POC numbers are the initial $\delta^{13}C$-DOC values. Data points encompass analytical precision.

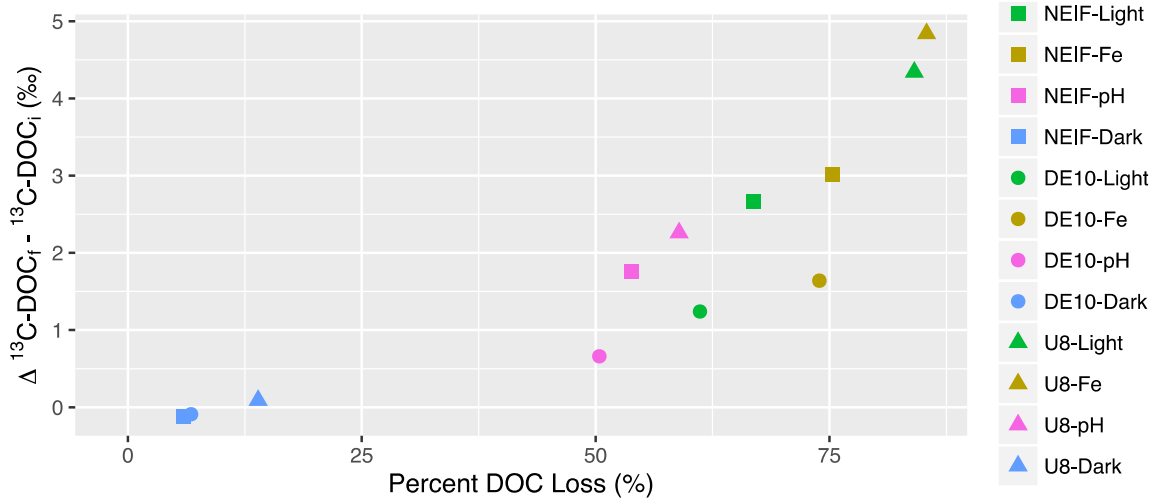

Figure 4.17. The relationship between total change in $\delta^{13}C$-DOC (relative to initial $\delta^{13}C$-DOC) and total % of DOC lost by photolysis. Data points encompass analytical precision.

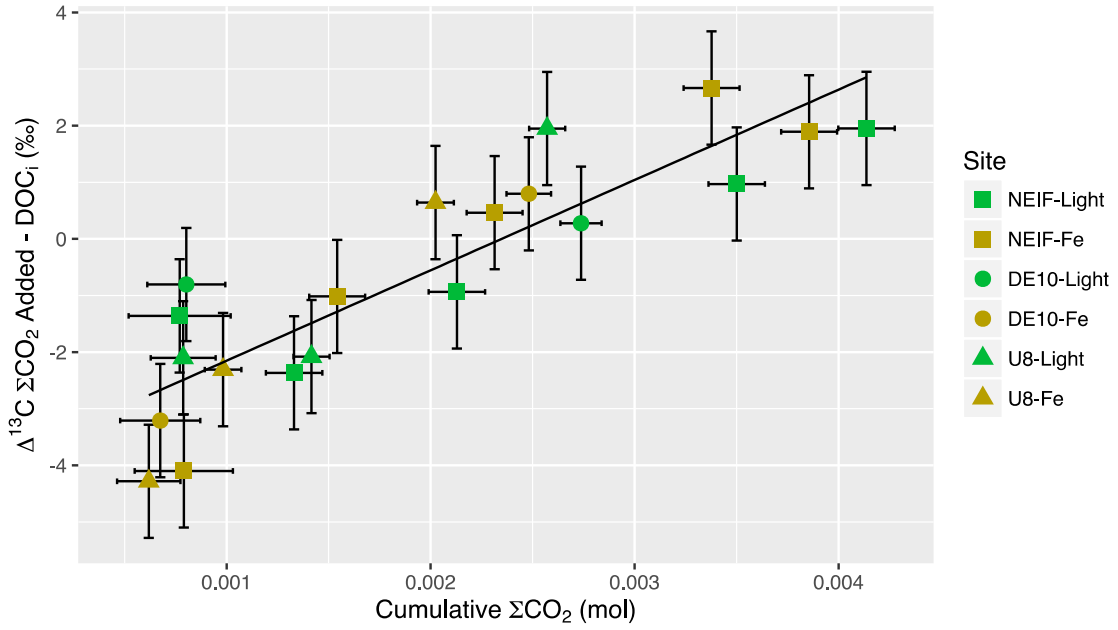

Figure 4.18. The relationship between $\Delta^{13}C$ ΣCO_2 Added – DOC_i (Initial $\delta^{13}C$-DOC) and cumulative ΣCO_2. Y=1596.041±202.028X-3.748±0.452, $p<0.05$, $R^2=0.776$. Data points encompass analytical precision.

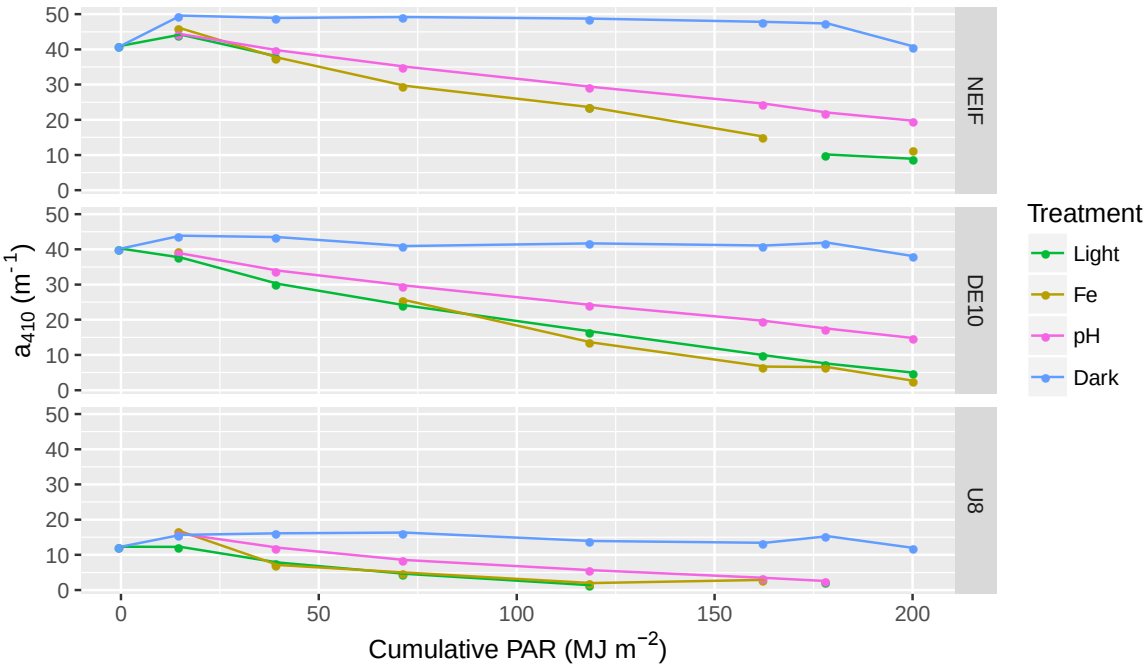

Figure 4.19. Change in a_{410} during photolysis. Data points encompass analytical precision.

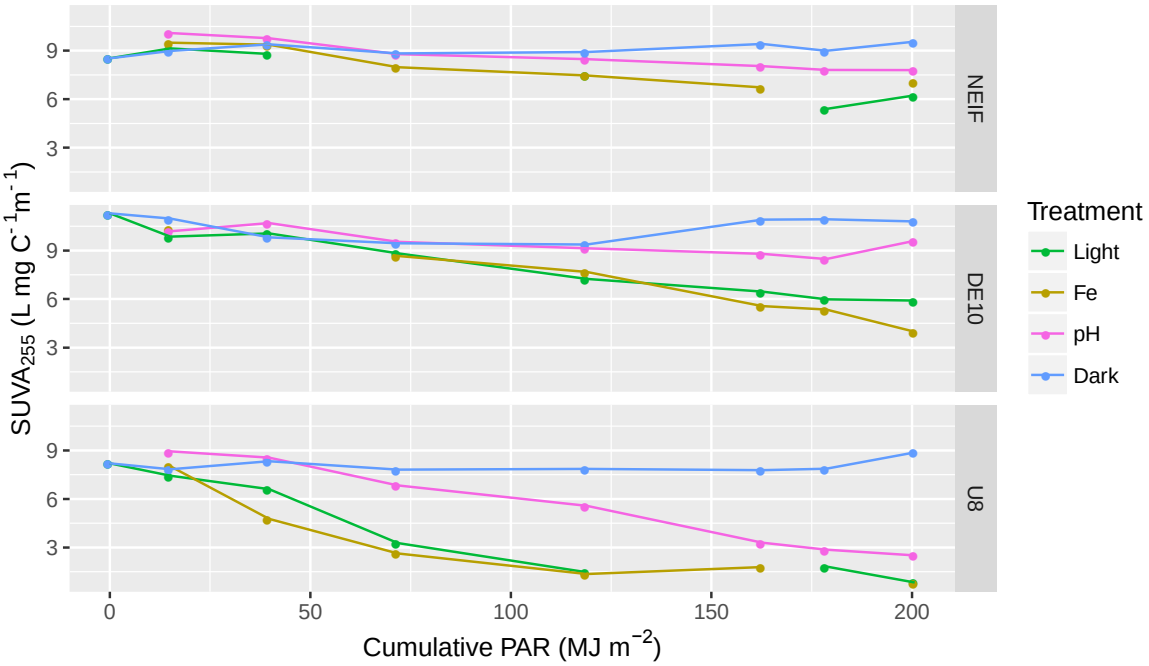

Figure 4.20. Change in $SUVA_{255}$ during photolysis. Data points encompass analytical precision.

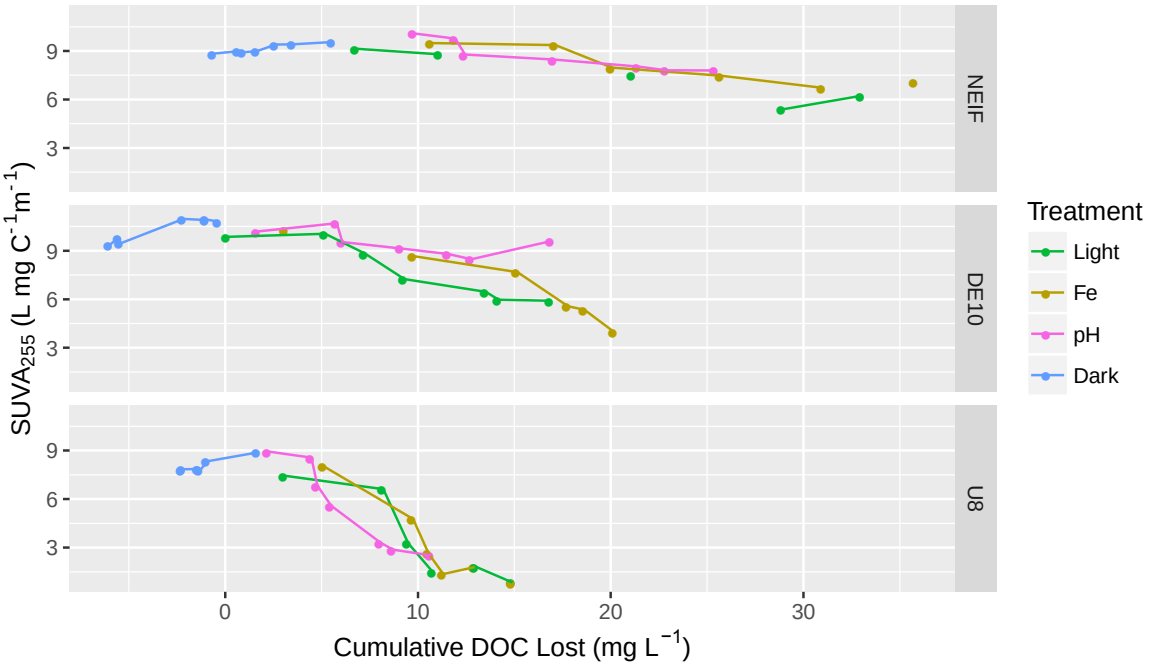

Figure 4.21. Change in $SUVA_{255}$ with cumulative DOC loss. Data points encompass analytical precision.

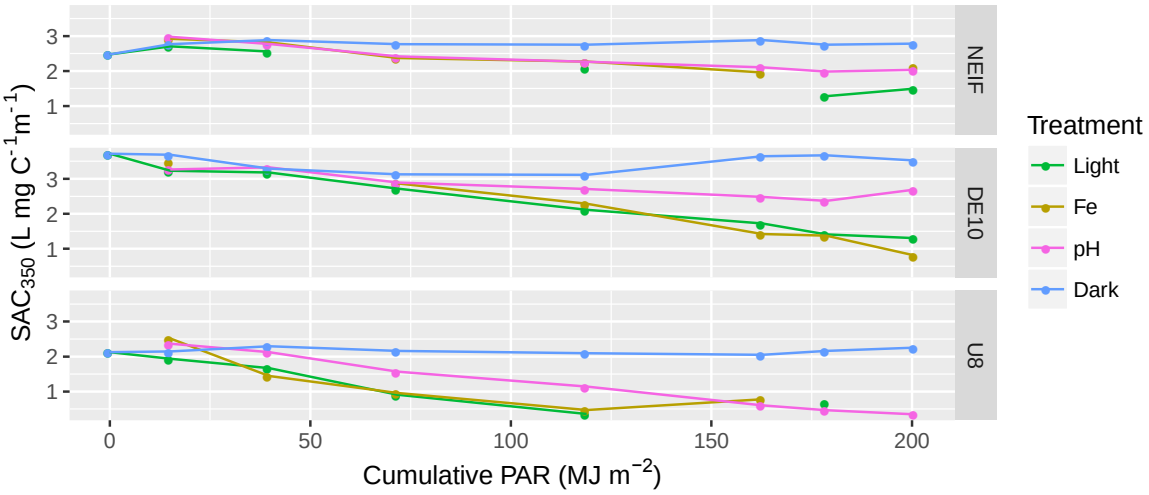

Figure 4.22. Change in SAC_{350} during photolysis. Data points encompass analytical precision.

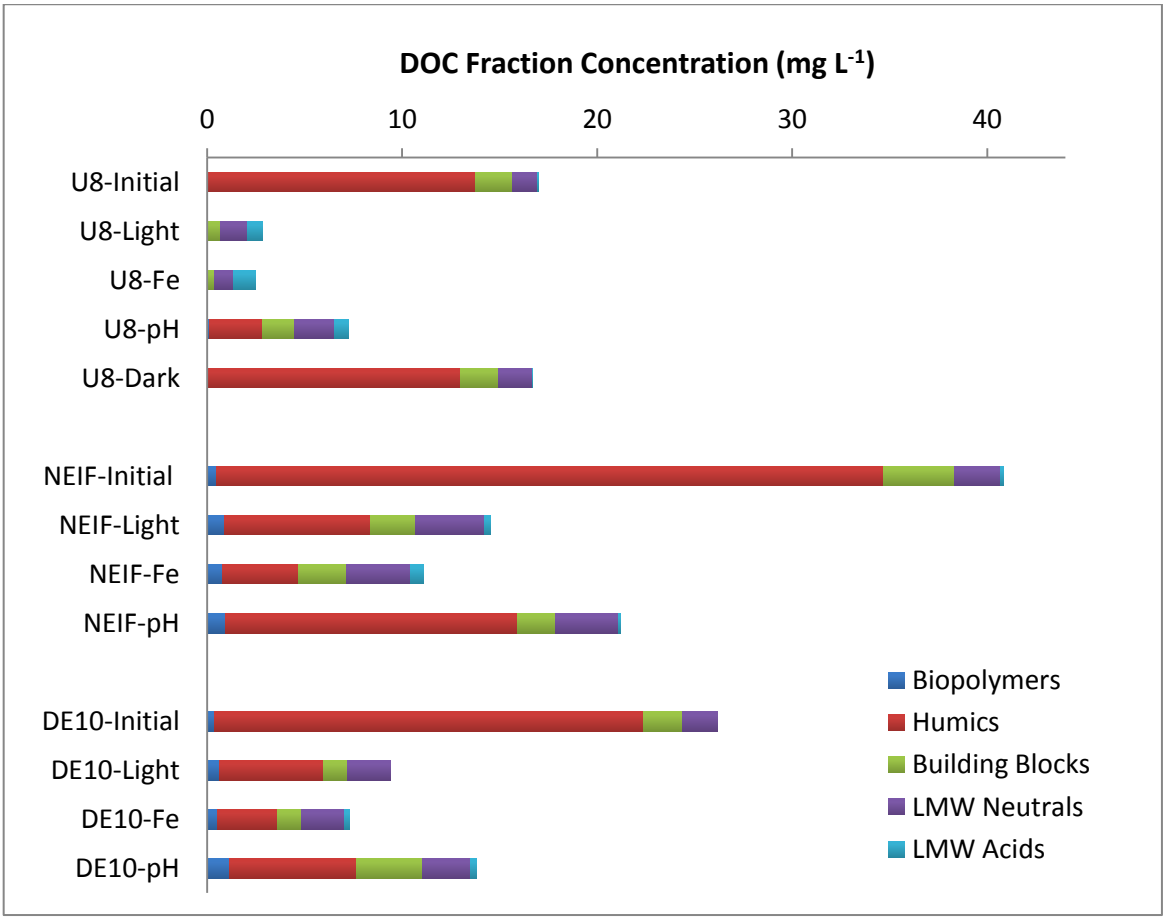

Figure 4.23. LC-OCD DOC fraction concentrations before and after photolysis.

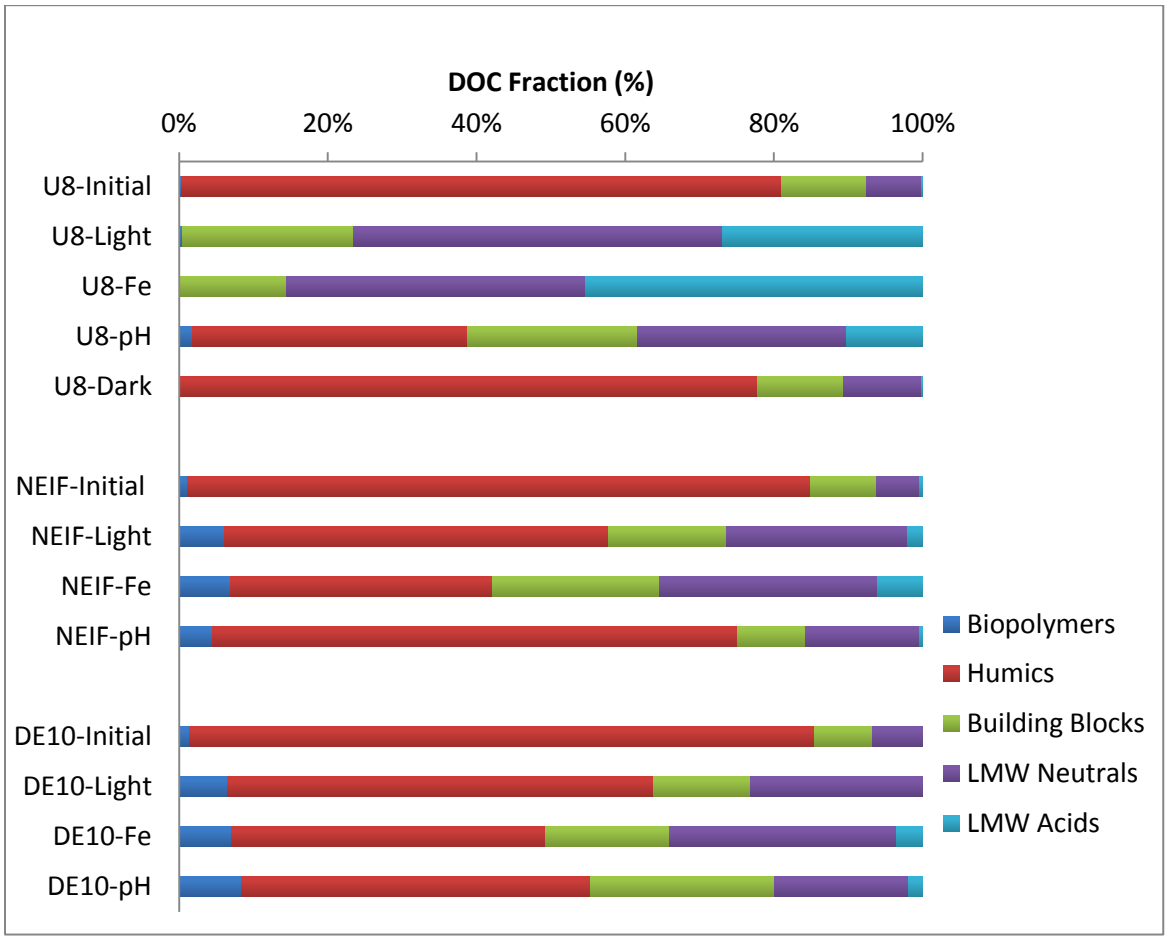

Figure 4.24. LC-OCD DOC fraction percent before and after photolysis.

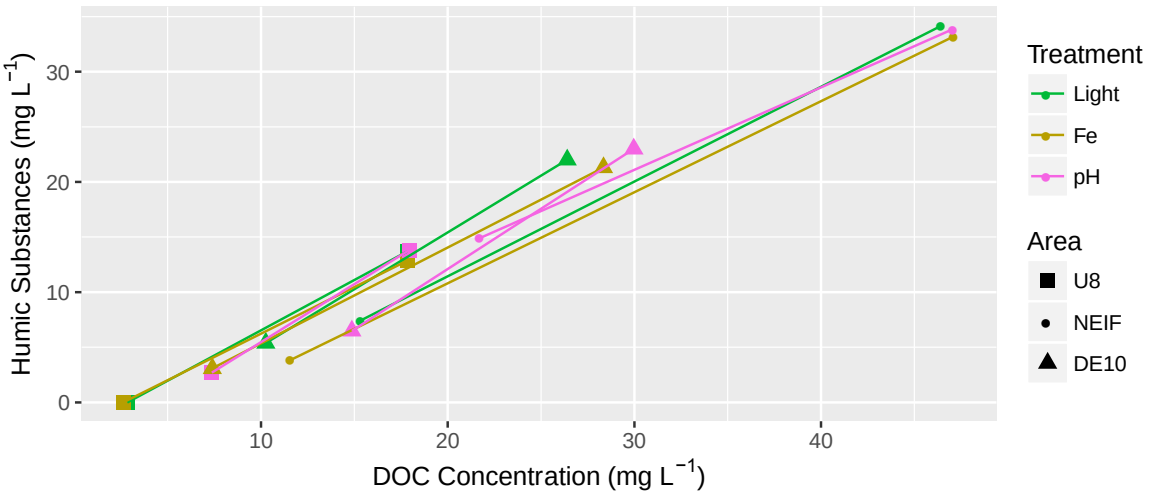

Figure 4.25. The relationship between humic substance concentration and DOC concentration. Data points encompass analytical precision.

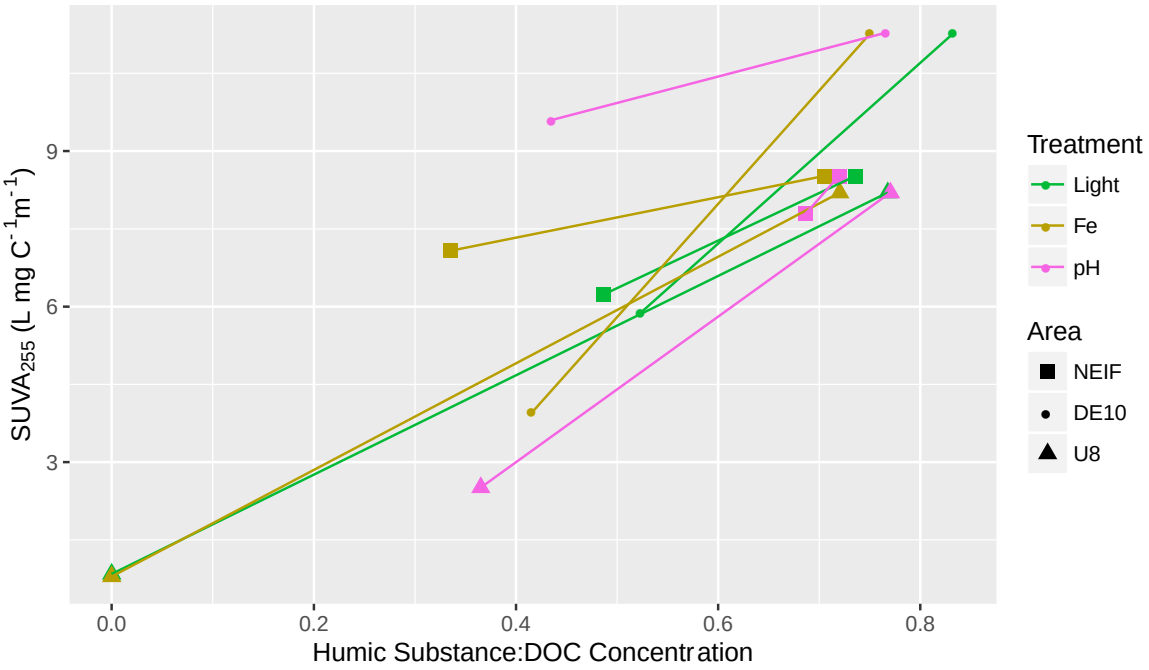

Figure 4.26. The relationship between $SUVA_{255}$ and humic substance:DOC concentration. Data points encompass analytical precision.

Chapter 5 Conclusions and Implications

5.1 Conclusions and Implications

The objective of this thesis was to investigate the role of dissolved Fe and pH on the measures of DOM quality and on the quantity and quality of DOM during photodegradation in small, oligotrophic Canadian Shield freshwaters. It is difficult to measure the quantity and quality of DOM using any single analytical method. However, a suite of characterization techniques allows for a holistic and comprehensive look at DOM and how it changes temporally and spatially. Although characterization can provide information on the role of DOM in the aquatic system, some aquatic parameters including pH and Fe concentrations interfered with some measures of DOM quantity and quality including absorbance, fluorescence, and LC-OCD. Fe absorbs UV and visible light (Kelton et al. 2007; Kritzberg and Ekström, 2012; Sarkkola et al. 2013) and pH affects the structure of the DOM molecule as well as the solubility of trace metals, such as Fe (Chin et al. 1998; Pace et al. 2012; Porcal et al. 2014). These parameters also influenced the quantity and quality of DOM during photodegradation, a potentially important mechanism in the formation of PM and carbon sequestration.

5.1.1 Influence of pH and Fe on measures of DOM quantity and quality

One objective of this thesis was to determine which measures of DOM quantity and/or quality are affected by changes in pH, Fe(II), and Fe(III) concentrations and to what extent for a variety of different types of naturally occurring DOM samples. The use of a large range of natural river, stream and subsurface samples in pH and dissolved Fe titration experiments and the investigation of the influence of pH, Fe(II) and Fe(III) on LC-OCD results were unique to this study. The goal was to determine how these parameters affect the measures of DOM quantity and quality and if results vary by water source.

This study manipulated pH and Fe concentrations of samples with a range of natural DOM qualities to investigate their influence on measures of DOM quantity and composition.

Changing pH influenced absorbance measurements while natural and manipulated Fe concentrations influence measures of DOC concentration and quality determined by spectral absorbance and LC-OCD. There was a significant positive relationship between Fe and the LC-OCD designated biopolymer concentration ($R^2 = 0.6$), suggesting that the LC-OCD designation of biopolymer likely also encompasses smaller DOM molecules bound to trace metals such as Fe. Thus LC-OCD can only provide information on the size of the DOM molecules.

Since the response of samples (spectral absorbance, LC-OCD results) were different depending on sample composition, it is difficult to predict how differences in pH and/or Fe concentration affect measures of DOM. For instance, increased Fe in most samples was found to influence $SUVA_{255}$ while increased pH did not. This suggests that the reported relationship between $SUVA_{254}$ and percent DOC aromaticity (Weishaar et al. 2003) may not be influenced by changes in pH in all types of samples but is likely influenced by Fe. Further, increased Fe concentrations can influence all DOC quality inferences made by UV and visible absorbance. This is inconsistent with previous studies that report Fe(II) has negligible effects on measures of absorbance (Poulin et al. 2014; Doane and Horwáth, 2010). At higher wavelengths, increased pH can significantly increase measures of absorbance. Thus, along with Fe, differences in absorbance between two natural samples could be caused by differences in sample pH or Fe concentration. Therefore pH and trace metal concentration must be taken into account when analyzing the spectral absorbances of natural samples.

Fe(II) and Fe(III) had different but significant influences on measures of DOC concentration and quality. The DOC concentrations decreased in some samples with additions of $FeCl_3$ likely due to the binding of Fe(III) by HMW DOM, forming larger POM molecules that were filtered out of the sample. As the influence of Fe(III) on DOM is different than Fe(II), different Fe species would likely have different measures of DOM quantity and quality results. This suggests that it is important to measure the Fe species (often been difficult, or would need specialized equipment) when determining its role in aquatic systems and interpreting and comparing measures of DOC concentration and quality. This is especially important where Fe is added to DOM in soils or wetlands, etc. because it can be

complexed under oxic or anoxic conditions. It is important to know that Fe(II) and Fe(III) affect measures of DOM quantity and quality and that they affect measures differently depending on the oxidation state. Thus, one should be cautious when comparing the DOM quality of samples with different Fe(II) and Fe(III) concentrations.

Although some researchers have attempted to correct for pH and/or Fe concentration changes (Poulin et al. 2014; Doane and Horwáth, 2010; McKnight et al. 2001), this study suggests that corrections would be difficult to apply to natural systems with differing Fe and pH. Single, simple relationships between $SUVA_{255}$ and pH/Fe concentrations cannot be obtained for natural samples because regressions greatly differ. The best method for interpreting measures of DOM is to compile a complete dataset including pH, cation and DOC concentration and DOM composition in order to supplement DOM characterization results. Some researchers have suggested that increases in absorbance measures indicate an increase in DOM concentration or change in DOM composition, but obviously one measure cannot be used to determine both of these or confirm that pH or Fe are not also influencing spectral measures. However, if DOC and cation concentrations and pH are also known, they can aid in eliminating whether a change in pH or Fe concentration is the cause for the differences in absorbance. We must include the effect of combinations of pH, Fe, and/or DOC concentrations, and/or DOM quality causing brownification in order to make accurate predictions about and management decisions for the future of aquatic systems.

5.1.2 The role of Fe and pH on the quantity and quality of DOM during photodegradation

Lakes accumulate significant quantities of POC in sediment each year (Kortelainen et al. 2004) and although it is largely unknown how this POC is formed, the settling rates have been found to be proportional to DOC concentrations (von Wachenfeldt and Tranvik, 2008). If primary production rates are similar to respiration rates, as they often are (Duarte and Prairie 2005), then it is likely that an abiotic transfer mechanism such as photodegradation is important for the formation of POC from DOC. Further, as increasing Fe and pH have been found to increase the rate of photodegradation, they can be manipulated to determine if they

contribute to its importance as a mechanism for POC formation. However, less than 20% of the DOC degraded contributed to POC formation in this study and was thus insufficient to be a significant mechanism for sediment formation.

The affect of increased Fe concentrations and pH on the quantity and quality of DOM during photodegradation was investigated using three stream waters from Ontario, Canada. This study was the first, to my knowledge, to collect and analyze subsamples during the experiment to observe changes in carbon transformation rates and $\delta^{13}C$-CO_2 values. The objectives were to: 1) quantify the role of Fe and pH on DOM quantity and quality changes during photodegradation and PM formation; and 2) characterize the changes in $\delta^{13}C$ during DOM photolysis. Methods of DOM characterization and stable carbon isotope analysis were used to characterize photoproducts of the photodegradation of DOM.

This study determined that increased pH and Fe concentrations influenced the photodegradation of DOM differently between sites with different natural and manipulated aquatic parameters. Increased Fe concentrations increased the rate of photolysis and POC production and led to the greatest shift towards LMW DOC concentrations. Although increased pH also increased POC production (the most of all treatments), it decreased the rate of photolysis leading to relatively less LMW DOC molecules, darker water colour, and less change in $\delta^{13}C$-DOC.

Overall, photodegradation, producing less than 20% POC, was not found to be an important mechanism for POC production, and suggests that there must be more factors involved in the large portions of natural aquatic carbon sedimentation of which the source is currently unknown. However, increasing both pH and Fe together, could have produced the largest mass of POC. This is especially likely in natural systems where it is possible that higher masses of POC produced, fall out of suspension, and cannot be later degraded by solar radiation and reconverted into LMW DOC and DIC. Therefore, natural increases in pH in lakes recovering from acidification may increase sediment accumulation.

Photolysis also influenced the aquatic isotope balance differently between different sites, pH and Fe concentrations. In general, photolysis increased the $\delta^{13}C$ of the DOC,

decreased the $\delta^{13}C$ of the DIC and CO_2, and the ΣCO_2 produced became more positive with photolysis. As the $\delta^{13}C$ of DOC, DIC, CO_2, and POC change with photolysis, this has implications for determining DOC, DIC, CO_2, and POC sources in aquatic systems. Further, to understand the impacts of DOM on the sediment record, it is necessary to understand how the photodegradation of DOM affects stable carbon isotopes. Thus, as rates of carbon transformations and isotopic fractionation factors change with cumulative PAR, photolysis should be included in both carbon and isotope mass balances.

Understanding the role of photodegradation in POC formation has substantial implications for understanding the aquatic carbon cycle and accounting for large portions of sedimented carbon of which the source is currently unknown. Future work should focus on increasing both Fe and pH and using *in situ* experiments that allow for the sedimentation of POC, to avoid degradation after formation.

5.2 Recommendations

5.2.1 pH and Fe Titration Experiments

Some of the samples used in the titration experiments had been kept in the cold room for over a year but were still used in the experiment to a) compare the results of year old samples to fresh samples and b) reduce the amount of time and money spent on resampling. Ideally, all samples would be freshly collected, filtered, titrated and run immediately but this would have been difficult due to time and money constraints and available assistance. It is also difficult to compare results between samples because they were collected at varying times of the year, which influences the DOC concentration and composition.

Perhaps an additional method such as ^{13}C nuclear magnetic resonance (^{13}C-NMR) should have been used to characterize DOC composition because dissolved Fe influenced absorbance, fluorescence, and LC-OCD analysis. ^{13}C-NMR provides more detailed information on the specific chemical structures and features of the DOC (Conte et al. 2004; Kalscheur et al. 2012) and would be interesting to measure to investigate if these results were also influenced by Fe additions. Additionally, when using LC-OCD and thus grouping DOC

fractions based on molecular size, it is difficult to determine structural differences within a fraction. Pairing LC-OCD and/or spectral absorbance measures with ^{13}C-NMR could add further insight to the structure and function of the DOM in aquatic systems.

This experiment should also be duplicated to investigate whether the calculated slopes between spectral measures and dissolved Fe concentrations are reproducible. If so, then those slopes could be used to predict the influence of pH and/or Fe on absorbance measures on similar samples. However, if the slopes change significantly as the chemical parameters change at a site, then this possible method of correction for pH and/or Fe interference would not be useful. This would also help determine whether these slopes could be used as indicators of quality.

5.2.2 Fe and pH Photolysis Experiments

The experimental results indicate that DOM degradation is the main process affecting the concentrations of DOC, DIC and CO_2 and influencing δ^{13}C-DOC, δ^{13}C-POC, δ^{13}C-DIC and δ^{13}C-CO_2 values. However, future work could attempt to distinguish between other carbon transfer mechanisms, which are likely also occurring such as respiration and other abiotic reactions.

Samples were collected within a month of each other; however, several parameters such as pH, Fe and DOC concentration, and DOC composition vary over time and can influence concentrations of DOC, DIC, and CO_2 and changes in δ^{13}C of DOC, POC, DIC, and CO_2 values. Samples could be collected during different times during the year or in different years to provide information on the influence of these changing parameters on the results of photolysis experiments.

Although LC-OCD provides information on the size of the DOM fractions degraded by photolysis, ^{13}C-NMR analysis would have been useful to investigate more specific changes to DOC composition. This method could provide more information on the functional groups cleaved and LMW DOC created.

DOC and Fe concentration, pH and DOC composition varied between samples and likely differences in both the DOC photolability and the influences of sample self-shading. Samples could have been diluted to the same DOC concentration in order to better compare results between samples, but Fe concentration, pH and DOC composition would still vary and self-shading would still be an issue because although NEIF had a higher DOC concentration, DE10 had higher measures of absorbance.

In attempts to measure POC concentrations, the total volume of water from Tedlar bags after photolysis were filtered through pre-combusted QMA filters, which have a pore size of 1μm. Since DOC concentrations were measured as organic carbon passing through a 0.45μm filter, theoretically, POC should be all organic carbon greater than 0.45μm meaning that the organic carbon between the size of 0.45μm and 1μm were not collected. This leads to an underestimation of POC formation had it been possible to measure the weight of POC formed and also leads to a bias in the δ^{13}C-POC values measured. Future experiments could use Sterlitech Advantec's grade GB140 Glass Fiber Filter with a 0.4μm pore size, which could be combusted in breakseals to gain a better estimate of POC concentrations and δ^{13}C-POC values.

As increasing the pH and Fe concentration increased the formation of POC, increasing the pH and Fe concentration in the sample may lead to more POC formation. Further, if $FeCl_2$ is used instead of $FeCl_3$ and added to the samples anoxically, Chapter 3 suggests that the binding capacity between Fe(II) and DOC is higher and more Fe-DOC bonds will be made. Thus, future experiments could combine $FeCl_2$ additions and pH increases to investigate if POC formation would be increased.

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Appendix A

Table 5.1. Sample dilution factors and resulting DOC concentrations.

Site Name	Initial [DOC] (mg-C/L)	Sample Volume (mL)	NANOpure Volume (mL)	Dilution Factor	Final [DOC] (mg-C/L)
Pond	34.8	1216.3	5284.8	0.19	6.1
P2	84.2	537.5	5963.7	0.08	6.2
U8	16.4	2570.1	3930.3	0.40	6.3
NEIF	46.7	1117.3	5595.7	0.17	6.6
NWIF	20.7	3058.4	6699.1	0.31	6.5
Belwood	6.4*	-	-	-	6.4
BCA	6.7*	-	-	-	6.7
DE10	19.2	2202.4	4300.65	0.34	6.5
DE10-ND	19.2*	-	-	-	19.2
H4 1.0	6.5*	-	-	-	6.5
H4 2.0	6.8*	-	-	-	6.8
H4-21	3.2*	-	-	-	3.2
P1-08	2.5*	-	-	-	2.5
IHSS		1000	2323.2	0.30	5.8
DI	0.2*	-	-	-	0.2

*samples were not diluted

Table 5.2. Volume of $FeCl_2$ solution added to samples.

Site Name	Initial [Fe] (mg-Fe/L)	$FeCl_2$ Solution Added to Sample (mL)			
		1 mg-Fe/L	2 mg-Fe/L	3 mg-Fe/L	4 mg-Fe/L
Pond	0.06	0.96	1.97	2.99	4.02
P2	0.23	0.74	1.75	2.77	3.80
U8	0.10	0.82	1.84	2.86	3.89
NEIF	0.10	0.91	1.92	2.94	3.97
NWIF	0.10	0.87	1.88	2.90	3.93
Belwood	0.03	1.00	2.02	3.04	4.07
BCA	0.04	1.00	2.02	3.04	4.07
DE10	0.20	0.36	1.37	2.38	3.41
DE10-ND	0.60	-	0.75	1.76	2.79
H4 1.0	0.17	0.75	1.76	2.78	3.81
H4 2.0	0.17	0.75	1.76	2.78	3.81
H4-21	0.06	1.00	2.02	3.04	4.07
P1-08	0.06	0.03	1.04	2.06	3.08
IHSS	0.11	0.97	1.99	3.01	4.04
DI	0.03	1.00	2.02	3.04	4.07

Table 5.3. Volume of $FeCl_3$ solution added to samples.

Site Name	Initial [Fe] (mg-Fe/L)	$FeCl_3$ Solution Added to Sample (mL)			
		0.5 mg-Fe/L	1 mg-Fe/L	1.5 mg-Fe/L	2 mg-Fe/L
Pond	0.06	0.44	0.94	1.45	1.96
P2	0.23	0.27	0.77	1.28	1.78
U8	0.10	0.40	0.90	1.41	1.92
NEIF	0.10	0.40	0.90	1.41	1.92
NWIF	0.10	0.40	0.90	1.41	1.92
Belwood	0.03	0.47	0.97	1.48	1.99
BCA	0.04	0.46	0.96	1.47	1.98
DE10	0.20	0.30	0.80	1.31	1.81
DE10-ND	0.60	-	0.40	0.91	1.41
H4 1.0	0.17	0.33	0.83	1.34	1.85
H4 2.0	0.17	0.33	0.83	1.34	1.85
H4-21	0.06	0.44	0.94	1.45	1.96
P1-08	0.06	0.44	0.94	1.45	1.96
IHSS	0.11	0.45	0.95	1.46	1.97
DI	0.03	0.50	1.00	1.51	2.02

5.2.3 Effect of Storage and Dilution on Samples

The storage of samples in the cold room for one year did not significantly change sample parameters and the dilution of samples did not affect the initial DOC composition but it influenced the complexation of added Fe with DOC.

Similar pH, DOC, Fe, Al, and Mn concentrations and DOC fractions between the Harp streams (H4 1.0 and H4 2.0) suggested that samples were not changed after they were filtered to 0.45μm and left in the cold room at 4°C for one year. There were some differences between the $SUVA_{255}$ of H4 1.0 and H4 2.0 but since these samples were collected at different times of the year, some variation in $SUVA_{255}$ was expected due to seasonality.

Prior to titrations, the diluted Dickie stream (DE10) had the same Fe:DOC and $SUVA_{255}$ as the undiluted Dickie stream (DE10-ND). The samples also had almost identical DOC fractions in LC-OCD results suggesting that dilution did not affect DOC composition (Figure 3.5). With added $FeCl_3$, DE10-ND formed more complexes with Fe and the DOC concentration did not change significantly compared to the lower Fe concentration and significant decrease in DOC concentration in DE10. This suggests that the samples did not bind with Fe in a similar manner causing different results in measures of DOC. When the Dickie stream was diluted, the number of available DOC binding sites decreased because the DOC concentration decreased. When Fe was added, there were not enough DOC binding sites and PM was formed and filtered out before analysis. This demonstrates that diluting samples changed the number of binding sites and consequently some results of this experiment.