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Dissolved Organic Carbon Dynamics in Constructed and Natural Fens in Athabasca Oil Sands Development Region near Fort McMurray, Alberta

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Development Region near Fort McMurray, Alberta

by

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ABSTRACT

Peatlands, mainly fens, are largely disturbed in order to recover bitumen below the surface in the Athabasca oil sands development region, Alberta. Mine closure plans require ecosystem reclamation: hence fen construction method is being investigated. In this study, dissolved organic carbon (DOC) dynamics in a constructed fen were compared with three other diverse natural fens in the region. The constructed fen had lower soil DOC concentration than all natural fens. Based on E2/E3, E4/E6 and $SUVA_{254}$ of the DOC, the constructed fen had DOC with significantly greater humic content, aromatic nature and larger molecular size than the natural fens. A laboratory DOC production study revealed that these patterns are likely due to the limited DOC contribution from newly planted vegetation at the constructed fen resulting in DOC largely derived from humified peat. These preliminary results suggest that DOC dynamics in the constructed system could be useful for evaluating reclamation success through time.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CF	Constructed fen
DI	Deionized
DOC	Dissolved organic carbon
EC	Electric conductivity
FA	Fulvic acid
GEE	Generalized estimating equation
GHG	Greenhouse gas
HA	Humic acid
HCl	Hydrochloric acid
IC	Inorganic carbon
NA	Naphthenic acid
NPOC	Non-purgeable organic carbon
NPP	Net primary production
OSPW	Oil sands process-affected water
PF	Poor fen
PPT	Precipitation
PVC	Polyvinyl carbon
RF	Rich fen
SF	Saline fen
SUVA	Specific ultraviolet absorbance
TC	Total carbon
TOC	Total organic carbon
UV/VIS	Ultraviolet/Visible
WT	Water table

CHAPTER 1: INTRODUCTION AND STUDY MOTIVATION

1.1 Study Motivation

In the Fort McMurray Athabasca oil sands region nearly 65% of the landscape is covered by fens (Vitt and Chee, 1989), which are largely affected by oil sands mining to recover bitumen below the surface. Hence, post oil sands mining reclamation is a part of the mine closure process in order to return functioning ecosystems. Though fen creation is not extensively studied yet, Price *et al.* (2010) have suggested its feasibility and the concept has been adapted by the Alberta Environmental Protection and Enhancement Act subject to a test of the concept by Suncor Energy. Therefore, this study was conducted at Suncor's pilot fen reclamation project (also called the Nikanotee fen) using three other diverse natural fens in the region as reference ecosystems. In this research, ecosystem functioning of the constructed fen (CF) was evaluated with reference to natural fens (Figure 1.1) based on soil pore water dissolved organic carbon (DOC) concentration and chemistry. In addition, DOC production from available substrates at each study site and its bioavailability was investigated using dominant vegetation and peat from all study fens and other substrates (*e.g*., tailings sand, petroleum coke, upland soil) used in the constructed fen.

1.2 DOC and Peatlands

DOC is carbon contained in organic molecules that pass through a 0.45 µm filter (Thurman, 1985), which is an important component in peatland carbon balances (Moore, 1998; Billett *et al*., 2004). It is produced by partial decomposition of organic matter and subsequent dissolution in pore water. It consists of a variety of molecules with various chemical forms and functional groups (Schnitzer and Khan, 1972; Thurman, 1985) derived from plant growth and decomposition processes. Therefore, evaluating DOC dynamics in peatlands should be a useful method for evaluating ecosystem function.

Peatlands are ecosystems formed by the accumulation of partially decomposed organic matter, called peat, due to net primary production (NPP) that exceeds decomposition in saturated conditions over thousands of years (Vitt, 2006). According to National Wetlands Working Group (1997) in Canada, wetlands containing more than 40 cm thickness of organic soil or peat, are

called peatlands. Peatlands comprise nearly 3% of the Earth's land surface, mainly in the northern hemisphere, covering large areas of North America, Russia and Europe (Joosten and Clarke, 2002). More than 12% of Canada's and 16% of Alberta's land area is occupied by peatlands.

According to Ingram (1978), the vertical peat profile is commonly divided into two layers: acrotelm and catotelm. The upper portion of peat, in which living plants occupy the top few centimetres and partially degraded plant material remains underneath, is known as the acrotelm. It is sometimes known as the active layer because most of the growth and oxic decomposition occur in this layer. The layer below the acrotelm, that is permanently saturated with water and is anoxic, is called catotelm. The peat mass in this layer is denser and microbial activities are comparatively slow.

Peatlands are broadly differentiated as bogs and fens based on water source. Bogs are isolated from groundwater; they receive most of their water and nutrition from atmospheric sources and thus they are referred to as ombrotrophic (Bourbonniere, 2009). These bogs are acidic and nutrient-deficient, therefore, favouring the establishment of dwarf ericaceous shrubs and *Sphagnum* moss (Bourbonniere, 2009). Fens interact with groundwater; hence, they contain higher concentrations of mineral components (Mitsch and Gosselink, 2000). They are thus called minerotrophic (rich fens) and those with less groundwater contact are transitional and are called "poor fens" (Mitsch and Gosselink, 2000). Minerotrophic fens are influenced by more alkaline, nutrient-rich groundwater and are dominated by sedges, forbs and bryophytes (Bourbonniere, 2009). The water level in fens is generally closer to the surface than bogs (Pastor *et al*., 2002) resulting in a thick anoxic layer, which slows down the decomposition process and produces more DOC (Freeman *et al*., 2001). Therefore, DOC concentration in fens is generally higher than bogs (Moore and Dalva, 2001). In addition, both bogs and fens are strongly influenced by local climate, regional substrate and geomorphology, prevalent regional vegetation and flora, and water chemistry (Vitt *et al.,* 2003).

Within a peatland, microtopography exists with various microforms characterized as hummocks and hollows (Belyea and Baird, 2006). Hummocks are elevated whereas hollows are depressed land surfaces found in peatlands. Microforms also control the peatland's vegetation gradient due

to variation in microclimate, especially due to water table position (Malmer, 1986). Variation of vegetation community types between microforms results in corresponding differences in gross ecosystem productivity (Laine *et al*., 2007; Riutta *et al*., 2007) with greatest productivity generally at an intermediate water table level (Waddington *et al*., 1998). Several studies suggest that carbon accumulation is higher at hummocks than hollows (Moore, 1989; Belyea and Clymo, 2001). However, these studies generally ignore hydrologic fluxes of carbon, particularly between microforms. Strack *et al.* (2008) found that hummocks tend to lose carbon as DOC while hollows gain it.

Northern peatlands play an important role in the global carbon cycle by sequestering carbon in organic matter, releasing significant amounts of greenhouse gases (GHGs) and supplying DOC to downstream ecosystems (Moore*, 1998*). Drainage of peatlands for agriculture, forestry and peat extraction for fuel and horticultural use results in an increase of DOC concentration in both surface water runoff and peat pore water, consequently increasing the DOC export from peatlands (Van Seters and Prince, 2002; Strack *et al.,* 2008; Limpens *et al.,* 2008; Waldron *et al.,* 2008; Waddington *et al*., 2008). DOC plays important ecological and geochemical roles in peatlands and downstream ecosystems, affecting acidity, nutrient availability, metal mobility, and light penetration in aquatic habitats (Steinberg, 2003).

DOC also represents a potential source of energy and nutrients to the soil microflora both within the peatland and in downstream ecosystems. Microbial consumption of DOC can regulate the production of GHG both by reducing the oxygen content of soils and by providing the electrons required for methanogenesis and denitrification (Yavitt, 1997; Zsolnay, 1997; Lu *et al.*, 2000). Furthermore, knowledge about the biodegradability of DOC is also crucial in assessing its contribution to soil organic matter build up (Kalbitz *et al.,* 2003), fate of the exported DOC and contribution to GHG emissions in downstream ecosystems (Marschner and Kalbitz, 2003). The biodegradation of DOC is defined as the metabolic breakdown of organic matter by soil microorganisms, which is quantified by the disappearance of DOC or O_2 or by the evolution of CO_2. Bioavailability describes the potential of microorganisms to interact with DOC.

Ecological restoration is one of several activities that attempts to change the biota and physical conditions at a site, which includes reclamation, rehabilitation, mitigation, ecological engineering

and various kinds of resource management (Society for Ecological Restoration, 2004). According to Wheeler and Shaw (1995) restoration of a disturbed peatland aims to bring back a naturally functioning self-sustaining ecosystem as quickly as possible. However, disturbance may often lead to irreversible changes in structure, composition, hydrology and position in the landscape of the peat (Schouwenaars, 1993). This makes it difficult to re-establish the conditions that are essential for the formation and growth of the peatland. On the other hand, the term reclamation is commonly used in the context of mined lands in North America and the UK (Society for Ecological Restoration, 2004). The main objectives of reclamation include the stabilization of terrain, assurance of public safety, aesthetic improvement and usually a return of the land to what is considered to be a useful purpose. Furthermore, reclamation projects that are more ecologically based can qualify as rehabilitation or even restoration (Society for Ecological Restoration, 2004).

The basic problem for peatland reclamation is to design a groundwater system that can support the inflow of water required to sustain the hydrological, biogeochemical and ecological processes and functions of the peatland and minimize the adverse effect of poor water quality derived from tailings sand (Jacobs and Timmer, 2005; Timmer and Teng, 2004; Trites and Bayley, 2008). Furthermore, the extraction of bitumen from oil sands produce process affected tailings, which contain sand, silts and clays in suspension, soluble organic chemicals (*e.g*., naphthenic acids), ammonia, heavy metals and salts (Bott, 2007). These chemicals adversely affect the ecology of the system being reclaimed; *i.e.,* they act as a significant barrier to the establishment of peatland vegetation (Trites and Bayley, 2008; Apostol *et al.,* 2004).

1.3 Study Objectives

The main objective of this study was to evaluate the ecosystem functioning of CF in comparison to reference fens based on DOC dynamics. The spatial and temporal variation of DOC concentration and chemistry of CF was analyzed and compared with reference fens. In addition, the environmental factors affecting the DOC concentration and chemistry were also investigated. Furthermore, production and bioavailability of DOC produced from dominant vegetation and other substrates (peat, tailings sand, petroleum coke and upland soil) were studied in different treatment conditions (temperature and salinity) in the laboratory. We were anticipating that CF would develop in line with RF due to vegetation similar to RF planted in the fen and geographic proximity. In addition, environmental factors like pH, temperature, EC and WT were expected to

play a vital role in determining DOC concentration and chemistry. Moreover, substrate, salinity and temperature should have an effect on DOC production and bioavailability.

This research project has a complementary and interdisciplinary research team involved in investigating the different aspects of constructed fen function including hydrology, ecology, greenhouse gas exchange, microbiology and biogeochemistry. This project investigates DOC dynamics in the system. The fen construction project was started in 2010 in order to validate the fen model recommendations (Price *et al*., 2010) and to meet the terms and conditions of the Alberta Environmental Protection and Enhancement Act. Monitoring of the constructed fen will continue until at least 2016.

This study is divided into two main parts and presented in a manuscript format. Chapter 2 presents the results from a field investigation of DOC concentration and chemistry of a constructed fen and three reference undisturbed peatlands near Fort McMurray, Alberta. Chapter 3 is focused on laboratory production of DOC from dominant vegetation, peat and other materials used in the constructed fen at different temperatures and salinities, and the evaluation of the bioavailability of the produced DOC.

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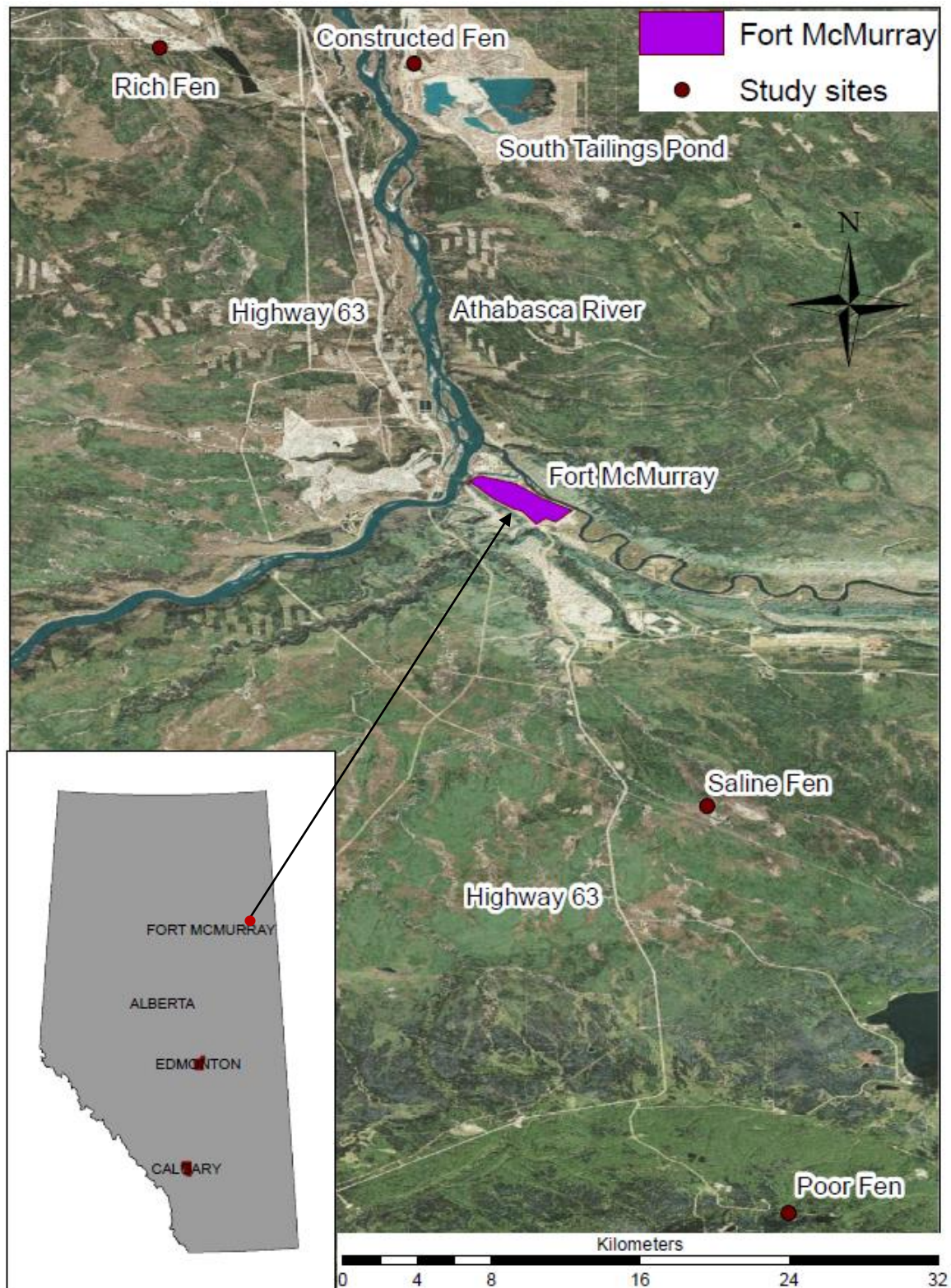
Figure 1.1: Map showing the location of study sites. There are altogether four fen sites: two north and two south of Fort McMurray, Alberta

CHAPTER 2: DISSOLVED ORGANIC CARBON DYNAMICS IN CONSTRUCTED AND NATURAL FENS IN ATHABASCA OIL SANDS DEVELOPMENT REGION NEAR FORT MCMURRAY, ALBERTA

Abstract

In the Athabasca oil sands region near Fort McMurray, Alberta, peatlands are disturbed extensively in order to recover bitumen below the surface. Hence, post oil sands mining landscape reclamation is a part of the mine closure process in order to return functioning ecosystems to the region. This study was conducted at Suncor's pilot fen reclamation project and three other diverse natural fens in the region during the growing season of 2013. Ecosystem functioning of the constructed fen (CF) was evaluated with reference to natural fens based on pore water DOC concentration and chemistry. Environmental factors (pH, temperature, electric conductivity and water table) and rate of water discharge were recorded during each sampling period. Water samples collected biweekly from piezometer nests installed in hummocks and hollows at 50, 75 and 100 cm depths were analyzed for DOC concentration and chemistry (E2/E3, E4/E6 and specific UV absorbance).

Significant variation of DOC concentrations among the reference sites was observed, varying from average of 34.5 mg/L at the rich fen (RF) to 70.9 mg/L at the saline fen (SF). DOC concentration at CF (29.5 mg/L) was similar to the rich fen. Seasonal variation of DOC concentration was also observed in each site with concentrations increasing over the growing season. The study suggested that there was no significant difference in DOC concentration between microforms or at different depths. It was also identified that DOC in CF was comprised of more humic and complex aromatic compounds than reference fens based on E2/E3, E4/E6 and specific UV absorbance ($SUVA_{254}$). Site hydrology, in association with biogeochemistry and environmental factors, appear to be linked to DOC concentration and chemistry. Based on this preliminary analysis, CF will potentially develop in line with the RF in terms of DOC dynamics.

2.1 Introduction

In the Athabasca oil sands development areas near Fort McMurray 65% of the landscape is covered by fens (Vitt and Chee, 1989) and over 250 km^2 is under active mining (Woynillowicz *et al.*, 2005), which is expected to cover 1400 km^2 by 2023 (Alberta Environment, 1999). Open pit mining causes the removal of large tracts of undisturbed peatland to recover bitumen below the surface. Hence, reclamation is a part of the mine closure process required by the government of Alberta in an effort to recover natural ecosystem functions. Fen reclamation is a new concept and its design has not been extensively tested yet at the field-scale. One study by Amon *et al.* (2005) succeeded in establishing fen plants and a thin deposit of organic matter on small plots lined with gravel and fed by calcareous artesian water.

In 2010, Suncor initiated a new approach for fen reclamation (Nikanotee Fen) in the post oil sands landscape in order to validate fen model recommendations (Price *et al*., 2010) and to meet the terms and conditions of the Alberta Environmental Protection and Enhancement Act. The goal of the fen reclamation is to create a self-sustaining ecosystem that is carbon accumulating, capable of supporting peat forming plant species and resilient to normal periodic stresses (Price *et al.,* 2010). A variety of revegetation strategies are being tested on the fen based on the North American peatland restoration approach (Rochefort *et al*., 2003) and other North American fen restoration studies (Cooper and Macdonald, 2000; Vitt *et al*., 2011). This research is focused on the evaluation of ecosystem functioning of the constructed fen (CF) compared to natural fens in the region based on dissolved organic carbon (DOC) dynamics.

Dissolved organic carbon is operationally defined as carbon contained in organic molecules that pass through a 0.45 μm filter (Thurman, 1985). DOC is an important component in peatland carbon balances (Moore, 1998; Billett *et al*., 2004), which is produced by partial decomposition of organic matter and subsequent dissolution in pore water. High net production of DOC in peatlands is mainly due to slow microbial decomposition of plant material because of anoxic soil conditions (Moore and Dalva, 2001). Increased primary production can also result in increased DOC concentrations because exudates from plants contribute DOC (Freeman *et al.*, 2004). In the boreal region, peatlands supply most of the DOC found in downstream ecosystems (Mulholland and Kuenzler, 1979; Maybeck, 1982; Urban *et al.,* 1989; Dalva and Moore, 1991; Molot and Dillon, 1996), representing significant regional redistribution of terrestrial carbon. Moreover, the

export of carbon from aquatic ecosystems to the atmosphere (*i.e*., release of CO_2 from exported DOC by microbial decomposition) may have profound influences on the regional carbon budget and the ecology of the region (Dalva and Moore, 1991; Schiff *et al.* 1998). Furthermore, DOC is also a major problem in water treatment for human consumption because of the formation of carcinogenic compounds during chlorination (Hsu *et al.,* 2001).

DOC represents a relatively small fraction (0.04% - 0.2%) of the total organic matter in soil (Zsolnay, 1996); however, it is often perceived as the most active fraction of soil organic matter due to its mobility and presumed labile nature (Zsolnay, 1996). According to Steinberg (2003), DOC is a complex mixture of organic compounds varying widely in molecular weight, from small molecules like organic acids and sugars to intermediate polymers like hemicellulose and large polycondensates usually called humic matter (fulvic acids, humic acids and humin). In general, fulvic acid in peatland pore water ranges from 58 to 97% of the total DOC. This contributes substantially to peatland acidity (Moore *et al.,* 2003). Humic acids are dark brown to black in colour, whereas fulvic acids are light yellow to yellow-brown in colour and have a much lower molecular weight (Thurman, 1985).

Spectroscopy in the ultraviolet and visible spectral ranges has been used for the characterization of DOC in peatlands. Absorbance ratios (E2/E3=250/365 and E4/E6=465/665 nm) and specific UV absorbances (SUVA) have been used to investigate DOC chemistry. A high value for E2/E3 ratio is indicative of low aromaticity and low molecular size of aquatic humic solutes (Richard and Joseph, 2012). The absorbance ratio E4/E6 indicates the fulvic (FA) vs. humic (HA) nature of DOC (Hautala *et al*., 2000; Spencer *et al*., 2007). The ratio <5 and >6 indicates humic and fulvic acids, respectively, which in turn gives the molecular weight and aromaticity (Christopher *et al.*, 2006). In addition, SUVA is determined by normalizing absorbance at 254 or 250 nm with DOC concentration and used to measure aromatic character of DOC in soils, with higher SUVA indicating higher aromaticity. Evaluating differences in absorbance ratios between the constructed fen and reference fens in the region may be useful in evaluating sources and processes contributing to net DOC production post-reclamation.

2.1.1 Controls on DOC concentration and export

A number of studies have reported increasing DOC export over the past several decades due to an increase in temperature resulting from climate change, roughly doubling DOC production with each 10ºC increase in temperature (Cole *et al.*, 2002; Moore *et al.*, 2008; Worrall *et al.*, 2008). Increased temperature leads to greater microbial activity and enhanced decomposition of peat and thus increased production and consumption of DOC. Increases in temperature can also result in deeper WT, leading to changes of DOC concentration, due to the exposure of different portions of the peat profile for aerobic and/or anaerobic decomposition. However, Lumsdon *et al.* (2005) have proposed a model of DOC runoff from high-organic matter soils based upon changes in DOC solubility. With increasing temperature, the hydrophilicity of the DOC increases leading to increased DOC solubility and increased runoff of DOC. The concentration of DOC also generally increases upon rewetting of the peatland after a period of drought (Kalbitz *et al.*, 2000) suggesting that WT fluctuation is an important control for net DOC production.

Several studies have examined DOC distribution patterns, export and quality from natural peatland ecosystems and noted that hydrology is the major control on DOC production, distribution and export in these ecosystems (Buffam *et al.,* 2001; Freeman *et al*., 2001; Waddington *et al.,* 2008). This is largely controlled by climatic variables affecting the water budget of the site and peatland hydrologic characteristics such as WT position, hydraulic conductivity through the peat profile, hydraulic gradient across the peatland (Fraser *et al.,* 2001) and the composition and distribution of microforms (Belyea and Clymo, 2001). DOC export is generally greater from peatlands with higher measured discharge (Fraser *et al*., 2001; Freeman *et al*., 2001). DOC fluxes in freshwater systems increase during storm events. Studies have shown that a large proportion of the annual DOC export (36 to >50%) occurs during short-duration high-intensity rainfall events (Hinton *et al.,* 1997; Buffam *et al.,* 2001).

Vegetation community, composition and productivity are also important for controlling pore water DOC concentration since the production of DOC from various litter types is different (Kuiters, 1993; Currie *et al.,* 1996). Enhanced vegetation productivity is correlated to higher DOC concentrations in soil solution (Lu *et al.,* 2000; Kang *et al.,* 2001; Freeman *et al.,* 2004). Moreover, the rate of carbon cycling can vary greatly between microforms within a peatland due

to varying environmental conditions. Several studies suggest that carbon accumulation is higher at hummocks than hollows (Moore, 1989; Belyea and Clymo, 2001). However, these studies generally ignore hydrologic fluxes of carbon, particularly between microforms. It has been found that hummocks tend to lose carbon as DOC while hollows gain it (Strack *et al.,* 2008).

2.1.2 Fen reclamation and DOC dynamics

The basic problem for fen reclamation is to design a groundwater system that can support the inflow of water required to sustain hydrological and biogeochemical functions within the peatland and minimize the adverse effect of poor water quality derived from tailings sand. Tailings materials contain oil sand process-affected water (OSPW) including sodium (Na) and naphthenic acids (NAs), which can be toxic to wetland plants (Trites and Bayley, 2008; Apostol *et al.,* 2004) and alter microbial communities (Nyman, 1999). In addition, elevated levels of salinity cause toxic accumulation of ions in plants, which inhibit plant growth (Jacobs and Timmer, 2005; Timmer and Teng, 2004). All of these adverse hydrological and biogeochemical conditions may affect the establishment of plant and microbial communities, ultimately limiting the DOC concentration in constructed peatlands. However, greenhouse experiments suggest that some fen species can survive conditions present in OSPW (Federico *et al.,* 2012; Pouliot *et al*., 2012).

This research aims to improve our understanding of the effect of fen reclamation on DOC dynamics. It also provides baseline data on DOC concentration and chemistry in natural fens useful for future DOC studies in the region and for the evaluation of reclamation success. Hence, it will help to improve management strategies to maximize carbon sequestration in reclaimed systems. The primary objectives of this study are to: 1) compare DOC concentrations and chemistry between the constructed fen (CF) and reference fens and 2) evaluate the environmental factors (pH, temperature, EC and WT) affecting the concentration of DOC in CF and reference fens.

2.2 Study Sites

2.2.1 Natural Fens

Three diverse natural fens near Fort McMurray, Alberta were used in this study. They are referred as poor fen (PF), rich fen (RF) and saline fen (SF) throughout the paper.

PF is located ~40 km south of Fort McMurray (56° 22.610 N, 111°14.164 W). This fen features a forested upland surrounding the fen and also receives discharge from bogs within its catchment boundaries. Plant species at the site included *Sphagnum* spp*., Chamaedaphne calyculata, Carex* spp*., Picea mariana* and *Betula pumila.* The PF was dominated by *Sphagnum* moss species (*Sphagnum fuscum* and *Sphagnum angustifolium*); however, distinct plant communities were observed in the north and south ends of the fen basin. The central part with lower WT had more trees than the northern and southern wetter parts, which were shrubbier. The peat depth was 4 m on average; however, thickness varied widely ranging from 1 m near the margins to up to 10 m in the centre of the northern portion. The fen had an outflow through a culvert under a road at the west end of the fen. The area of PF was 7.29 ha.

RF was located ~20 km north of Fort McMurray (56° 56.330 N, 111° 32.934 W). It was a treed moderate-rich fen. This site was disturbed due to cutlines and dirt roads passing through the broader fen boundaries, although the actual study area has not been directly impacted. The site was dominated by *Larix laricina*, *Betula pumila*, *Equisetum fluviatile*, *Smilcina trifolia*, *Carex* spp., and brown mosses, largely *Tomenthypnum nitens*. The peat is about 1 m thick. This site had two culverts for discharge, which pass under a dirt road on the west side of the site.

SF was located 10 km south of Fort McMurray (56° 34.398 N, 111° 16.518 W). This fen was dominated by *Juncus balticus, Calamagrostis stricta* and *Triglochin maritima*. It was an extremely saline site due to its geological setting that resulted in discharge of saline groundwater (Wells, 2014). The site was surrounded by forested peatland but there were no trees in the study area. This site was less disturbed by human activities than the other reference fens. There was no culvert for water discharge but excess water runoff was moving towards the west into the forested portion of the peatland.

2.2.2 Constructed (Nikanotee) Fen

The constructed fen (CF) is ~30 km north of Fort McMurray (56° 55.8701 N, 111° 25.0166 W) built on an area abandoned by Suncor after oil sands extraction (Figure 1.1). A low permeability geotextile liner was placed over the target site; petroleum coke forms the aquifer system, overlying the liner and extending partway up the upland slope. At the lower end of the system, peat from a moderate-rich fen (2 m thick) from a new lease area was placed over the petroleum coke aquifer to form the fen (Figure 2.3). Information about the specific depth of peat collected and how the peat was mixed during placement was not available from the consulting company that completed the peat placement, but measurements of peat hydrophysical properties is an ongoing component of the larger project. The upland area, which contributes groundwater to the fen, was covered with boreal forest soils and revegetated primarily with ericaceous shrubs and a sparse cover of trees including from *Larix laricina* and *Picea mariana* near the fen, and *Pinus banksiana* (Jack pine) at the uppermost part of the upland in fall 2013. Reclaimed slopes on the east and west sides of the upland and fen form the remainder of the basin. Finally, plant species (dominant in nearby peatlands) were introduced in the last week of June 2013 in CF. A variety of planting treatments were spread across the fen including moss transfer (Quinty and Rochefort, 2003), planted sedge and shrub seedlings (including *Carex aquatilis, Juncus balticus, Betula glandulosa, Calamagrostis inexpansa, Triglochin maritima*) and seeding with native peatland species. The land surface was covered with wood mulch on half of all treatments so that loss of water by evaporation could be reduced. A spill-box was also installed in the fen basin in order to prevent flooding during snow melt and storm events. The area of CF is 2.9 ha.

2.3 Methods

2.3.1 Instrumentation

In May 2012, three pairs of piezometer nests were installed in each reference fen, with one nest of the pair in a hummock and the other in a neighbouring hollow (Figure 2.1). Each nest had three piezometers at 50 cm, 75 cm and 100 cm depth, and a well (Figure 2.2). For CF, six piezometer nests were installed in two transects on July 4, 2013 immediately after planting vegetation. Each nest had three piezometers at 50 cm, 75 cm and 90 cm depth and a well. The

culvert installed for excess water discharge in PF and a weir just upstream of the spill-box at CF were used to collect water samples and discharge measurements.

Wells were constructed of PVC pipe (2.8 cm inner diameter) having 5 mm holes over the length of a 1 m long well. The pipe was wrapped from outside with a nylon stocking to prevent clogging. Each piezometer was slotted with 5 mm holes over a 17 cm intake that was installed so that it was centered at the desired sampling depth. There was a 100 ml capacity reservoir below the piezometer intake to collect water samples. The slotted length of each piezometer was wrapped with 1.5 μm polypropylene mesh net (Nitex) to prevent movement of peat into the piezometer. A hand-auger of the same diameter as the pipes was used to make holes through the peat at each site for installation of the wells and piezometers.

2.3.2 Field Sampling

Piezometers were flushed and a freshly recharged water sample was collected in clean 70 ml Nalgene bottle after first rinsing it with a small amount of sample. Sampling was conducted every two weeks starting from May until September 2013. The soil was wet enough so that all the water samples could be collected on the same day as flushing. In addition, discharge water samples were also collected from PF and CF from the culvert/spillbox at every occasion of pore water sampling if discharge occurred. Environmental factors such as pH, temperature and EC were measured using YSI^{TM}-63 Multi-Parameter Probe, whereas WT was recorded using blow pipe for each sampling location and date.

2.3.3 DOC concentration and chemistry analysis

All water samples collected from the field were immediately pre-filtered with a 1.5 μm glass fibre filter (Sterlitech 934-AH), followed by filtration with a 0.4 μm glass fibre filter (Sterlitech GB-140) and were transported in a cooler to the Physical Geography Laboratory at University of Calgary for analysis. These samples were stored at 4°C until analysis.

All the water samples collected were analyzed using a PerkinElmer UV/VIS Spectrophotometer Lambda 35 to measure absorbance at 250 nm, 254 nm, 365 nm, 400 nm, 465 nm and 665 nm wavelengths. The absorbance ratios (E2/E3, E4/E6) and SUVA$_{254}$ were determined for DOC

chemistry analysis. These absorbance ratios are commonly used to investigate DOC chemistry in peatland studies (Moore, 1987). The absorbance by DOC of each sample was measured in three different ways: natural sample, diluted sample with deionized (DI) water (1:1 ratio) and neutralized samples using $NaHCO_3$. The absorbance ratios were calculated from neutralized samples whereas $SUVA_{254}$ was measured from natural samples (Weishaar *et al.,* 2003). For the calculation of $SUVA_{254}$ absorbance at 254 nm was normalized by DOC concentration (Weishaar *et al.,* 2003). Diluted samples were measured in case high concentrations of DOC resulted in saturation of absorbance; however, as this was not observed, the values from the diluted samples were not used in the study.

A 20% subset of the total samples was randomly selected for Total Organic Carbon (TOC) analysis. These samples were diluted at 1:10 ratio in DI water and then acidified to lower the pH to ~2 by using concentrated HCl. The concentration of TOC was determined using a Shimadzu TOC analyzer (Environmental Sciences Program, University of Calgary) by Non-Purgeable Organic Carbon (NPOC) method. The sample was sparged with gas, which converts all inorganic carbon (IC) in the sample to CO_2 and drives the CO_2 out of the sample solution. The TOC concentration was determined by measuring the total carbon (TC) in the sample after the IC was eliminated. Given that TOC was determined on filtered samples, this represents DOC. These values were regressed against absorbance at 254 nm to produce an equation ($y = 21.72x + 5.02$, $R^2 = 0.83$, $F = 491.51$, $p < 0.01$) to estimate DOC concentration in all samples (Appendix A Figure A1).

2.3.4 Data analysis

Regression lines between DOC concentrations and environmental variables such as pH, temperature, EC and WT were fitted using generalized estimating equation (GEE) repeated measures method to assess the strength and direction of the relationship and were considered significant if $p < 0.05$. All models were validated to ensure that assumptions were met (*e.g.*, normality of residuals). In addition, GEE ANOVA was used to assess the differences of DOC concentration between sampling locations considering site, depth and microform as factor. All statistical analyses were performed using IBM SPSS statistics version 22 and excel 2013.

2.4 Results

Altogether 615 water samples collected from the sites were analyzed for this study (Appendix 2.A). Samples comprised pore water from hummocks and hollows and discharge water. There were no microforms developed at CF and no discharge collected from SF and RF.

2.4.1 Environmental conditions

Monthly mean environmental conditions (temperature and precipitation) for the study period were collected from meteorological stations installed in PF, RF and SF, which were verified with the data from Environment Canada, Fort McMurray, Alberta. This provided important local weather information because the study sites were in the region surrounding Fort McMurray and were geographically diverse in nature.

Total precipitation for the growing season (May-September) was 304.2 mm, 379.6 mm and 317.5 mm for PF, RF, and SF, respectively (Table 2.1). Sites received most of the precipitation in June when approximately half of the total growing season precipitation fell. The constructed fen was within 15 km north of RF and thus is assumed to have similar weather conditions during the period. Mean temperature during the growing season across the sites was 17°C.

There was a variation of WT position in each site over the growing season (Figure 2.4). However, the mean WT position across all sites was generally above the surface to within 20 cm below the surface throughout the study period. Mean WT position in SF was above the surface until late-July and there was high fluctuation of WT observed over the study period in this fen. Mean WT position of PF and RF was similar, though the variation was very high in RF. The mean WT position of PF was -12.5±7.7 cm, whereas the range was 41.0 cm. The mean WT position of RF (-13.2±14.2 cm) was slightly lower than PF; however, the range was higher (*i.e.,* 55.0 cm change over the growing season). In contrast, mean WT position of both SF (-7.4±11.2 cm) and CF (-6.7±9.3 cm) were similar. However, the range of WT was higher in SF (52.0 cm) than CF (33.0 cm).

2.4.2 DOC concentration

As stated in the methods, DOC concentration of a 20% subset of samples was used to develop a regression line with DOC absorbance at 254 nm, which showed strong correlation (R^2=0.83; Appendix A, Figure A1). The regression line equation was used to calculate DOC concentration of the remaining samples. Similar correlations between DOC absorbance and concentration have been used previously to estimate DOC when a large number of samples are collected (*e.g.* Baker *et al.*, 2008; Chow *et al.*, 2008; Her *et al.*, 2008; Korshin *et al.*, 2009; Leenheer and Croue, 2003; Spencer *et al.*, 2007).

The concentration of DOC varied across the sites with individual samples ranging from 9.8 to 88.9 mg/L (Figure 2.5). The mean (±standard deviation) concentration of DOC at SF was the highest at 70.9±13.0 mg/L followed by PF at 48.0±13.1 mg/L and RF at 34.5±12.7 mg/L. Mean DOC concentration of CF (29.5±5.7 mg/L) was similar to RF when comparing to all reference fens. The range of DOC concentrations was the highest in PF (62.8 mg/L) and lowest in CF (23.7 mg/L) over the study period. Pairwise comparisons indicate that DOC concentrations of all the reference sites were significantly different from each other at $p < 0.01$. Similarly, CF had significantly lower DOC concentrations than PF and SF ($p < 0.01$) but not RF ($p = 0.19$).

All the sites exhibited temporal variation of DOC concentration throughout the growing season starting from May until September (Figure 2.6). DOC concentration was the lowest in May, which then increased to the highest at the end of the growing season (September). There was not much temporal variation in pH and EC over the study period; however, both showed slight increases towards the end of the season in each fen (Table 2.2).

2.4.3 Controls on DOC concentration

2.4.3.1 Spatial variation of DOC within sites: microforms and depth

Mean DOC concentrations across the sites at 50, 75 and 100 cm depths were 44.1 mg/L, 44.5 mg/L and 44.8 mg/L, respectively. However, the pattern of change of DOC concentration with depth was different in each fen (Figure 2.7). For example, mean DOC concentration decreased consistently with depth in CF and RF. In contrast, mean DOC concentration was the highest at

100 cm (46.9 mg/L) depth but similar at 50 (43.4 mg/L) and 75 cm (43.7 mg/L) depth in PF. SF had the highest mean DOC concentration at 75 cm depth (75.6 mg/L) and the lowest at 50 cm depth (69.1 mg/L).

To analyze the variation of DOC concentrations between microforms (hummocks and hollows) and depths (50, 75 and 100 cm) a GEE model with repeated measures sampling was used. In the model, DOC concentration was the dependent variable whereas microform, depth, site and interactions among these factors were explanatory variables. Table 2.3 shows the result, indicating that there were no significant variations in DOC concentrations among microforms, depths or interacting with microforms/depths at $p < 0.05$ across all sites. However, as stated above, it was observed that DOC concentration varied significantly between sites. Similarly, DOC concentrations at 50, 75 and 100 cm depths did not vary significantly even within any fens except in the CF at 50 and 75 cm depth, at 95% confidence interval.

2.4.3.2 Environmental factors: pH, Temperature, EC and WT

A GEE model was created with environmental variables (pH, temperature, EC and WT), as explanatory variables and DOC concentration as the dependent variable. All the environmental factors included in the model were found to have a significant positive correlation with DOC concentration, except pH that was negatively correlated at 95% confidence interval (Table 2.4). Furthermore, EC had a stronger correlation with DOC concentration than temperature and WT based on chi square values (Table 2.4).

Within individual sites, all reference fens (PF, RF and SF) showed similar positive correlations between DOC concentration and pH; however, pH varied widely across these sites (Figure 2.8A). For instance, pH in PF was acidic (4.7) but other fens had nearly neutral pH. In contrast, CF exhibited a negative correlation between DOC and pH. In addition, the correlation between pH and DOC across the sites, represented by the family line was also negative.

All the sites showed positive correlations between DOC concentrations and $\log_{10}$ (EC) (Figure 2.8B), although the salinity among the sites also varied widely. SF had the highest mean EC (13,979.1±5,245.4 µS/cm), followed by CF (1,679.7±479.4 µS/cm) and RF (313.4±165.1 µS/cm). The mean EC of PF was the lowest (35.1±24.9 µS/cm; Table 2.2).

2.4.4 DOC chemistry

Figure 2.9 shows the comparison of absorbance ratios (E2/E3=250/365nm, E4/E6=465/665nm) and SUVA$_{254}$ of DOC among sites. All the reference fens had similar mean E2/E3, whereas CF was significantly lower (p<0.01). In contrast, CF had significantly lower E4/E6 than PF and SF (p<0.01) but was similar to RF (p = 0.65) based on pairwise comparisons. Among the reference fens, E4/E6 of PF was similar to SF (p = 0.11) but higher than RF (p < 0.01). In addition, the mean SUVA$_{254}$ of CF, PF, RF and SF were 0.093, 0.042, 0.039 and 0.045 L mg C^{-1} cm^{-1} respectively. SUVA$_{254}$ for all the sites were significantly different from each other (p<0.01). However, CF had the highest SUVA$_{254}$ among all sites.

2.4.5 Temporal variation of DOC chemistry

Figure 2.10A shows the temporal variation of E2/E3. There was a common pattern of change for E2/E3 at all sites over the growing season with a lower value at the beginning, which increased to nearly double by the end of June and then continued to decline for the remainder of the summer, with the exception of a few slight increases. For example, E2/E3 of RF in mid-May was 2.5, which went up to 4.2 by late June and then declined to 2.0 at the end of the summer. At SF, E2/E3 started to drop sharply from late June (4.6) until mid-July (3.2) and then went up by the end of July. In contrast, E2/E3 of PF started with a higher value of 3.5 and declined slightly to 3.2 until mid-July, followed by sharp decrease in mid-August. At CF, temporal variation at the beginning of the season is missing due to lack of data as the site was still being completed, though the pattern later in the season was in line with other sites.

The mean E4/E6 of DOC showed a very similar pattern of change over the study period across all sites (Figure 2.10B). There was no major variation of E4/E6 throughout the growing season except from June 4 to July 10, when all the sites had their highest value, especially PF, which increased more than eight times on comparing to the initial value. Similarly, E4/E6 of RF and SF also exhibited increases to almost 3 and 5 times higher than the initial value, respectively.

The specific UV absorbance ($SUVA_{254}$) of DOC in all the natural fens had a similar value (nearly 0.04 L mg C^{-1} cm^{-1}) throughout the season, but DOC from CF had double the $SUVA_{254}$ value (0.09 L mg C^{-1} cm^{-1}) than all the reference fens (Figure 2.10C).

The result of the GEE model with repeated measures shows that E2/E3 in DOC was significantly controlled by pH, temperature and WT but not EC at 95% confidence interval (Table 2.5). Furthermore, pH and WT exhibited a negative relationship with E2/E3, whereas there was a positive correlation with temperature. Among these environmental factors pH was found to be the most influential according to the Chi Square value (Table 2.5). Similarly, the effect of environmental factors on E4/E6 of DOC was analyzed using GEE repeated measures at 95% confidence interval. It was found that pH and WT were both significantly negatively correlated to E4/E6 (Table 2.6). On investigating the impact of environmental factors on $SUVA_{254}$, it was found that pH and EC significantly explained the variation in $SUVA_{254}$ (Table 2.7). According to Chi Square value, pH controlled $SUVA_{254}$ more than EC. Furthermore, pH had a positive correlation with $SUVA_{254}$, whereas EC was negative.

2.5 Discussion

Suncor has designed a constructed fen (Nikanotee fen) aiming to create a self-sustaining ecosystem that is carbon accumulating, capable of supporting peat-forming plant species, and resilient to normal periodic stresses (Price *et al.,* 2010). In evaluating the success of the design, a number of diverse natural fens within the same region as the constructed fen were assessed for comparison to the constructed system. However, the first year following construction represents a very short duration to create a typical peatland condition. Therefore, the present study helps to evaluate the initial ecosystem functioning of the constructed fen but cannot explain how DOC concentrations and chemistry will change as the system develops in future.

2.5.1 DOC concentration and chemistry

In this study, DOC concentration was found to vary considerably among the reference fens; however, DOC chemistry was similar to some extent. The range of DOC concentration (10-89 mg/L) found in these sites was within the concentration range observed in other peatlands (McKnight *et al.* 1985; Moore 1997; Fraser *et al.* 2001; Kane *et al.* 2010).

While the DOC concentration at CF was found to be close to RF, we observed a higher DOC concentration at the PF site. The higher concentration of DOC at PF is likely due to input from its watershed and biogeochemical conditions within the peatland. PF was surrounded by upland slopes with shallow organic soils. The runoff from these uplands likely contributed both water and DOC to the fen basin. Koprivnjak and Moore (1992) found that DOC concentrations in peatland generally increase with increasing catchment size. In addition, dominance of *Sphagnum* moss and high water table probably reduced the efficiency of decomposition resulting in more DOC production (Moore and Dalva, 2001). Furthermore, peat depth at the PF site, especially on the north side was nearly 10 times thicker than any other reference fens, which may have affected the DOC production by providing more peat volume to contribute to more DOC production (Sachse *et al.,* 2001).

Unlike the PF site, DOC in RF was produced mainly within the system, which was rich with a variety of plant species. Freeman *et al*. (2004) state that increases in primary production leads to higher DOC concentrations. However, lower DOC concentration observed in RF site may be attributed to the possibility of photolysis and microbial uptake of DOC. Kelley *et al.,* (1997) reported increased microbial activity in nutrient rich peatland compared to poor sites suggesting that RF may have a microbial community that more efficiently consumes DOC. Moreover, RF site had pH close to neutral, conditions resulting in higher DOC solubility (Fenner *et al.,* 2009), which likely contributed to greater DOC export along with discharge water.

SF site has extremely high salt concentration, neutral pH, comparatively warmer soil temperatures, higher WT, and vegetation dominated by sedges, which was considered the optimum condition for DOC production. Salinity provided nutrients to enhance microbial activities causing faster decomposition of organic matter resulting in higher DOC concentration. The correlation between DOC and EC also supports this fact. According to Marschner and Kalbitz (2003) the enhanced biodegradability observed in the presence of salts may be due to flocculation, as larger structures will provide better attachment for microbial colonies. As biodegradation is dependent on microbial activity, the composition and density of the microbial population used in the degradation studies also influence biodegradation. Absence of trees at SF was probably the reason for warmer pore water temperature, which is a favourable condition for

microbial growth and higher solubility of DOC (Clark *et al.,* 2012). In addition, litter from sedges is easier to decompose in comparison to moss and tree litters (Marschnera and Kalbitz, 2002), *i.e.,* the easier the decomposition, the higher the DOC production.

In the SF site, the higher WT oscillation over the season than any other studied fen is probably also one of the reasons for higher DOC concentration. Increased DOC concentration occurring after the drop of the WT has been explained by the oxidation of peat material (Blodau *et al.*, 2004) or by what is known as the enzymatic 'latch' mechanism (Freeman *et al.*, 2001). Oxidative enzymes that are normally repressed in anoxic (wet) conditions become active in oxic (dry) conditions leading to the degradation of organic matter. DOC produced during dry periods can then be dissolved in soil water as the WT rises during wet periods. Furthermore, there was no regular channel for water discharge from this site, which may increase water residence time in soils and slow the loss of DOC.

CF had the lowest DOC concentration among the sites but was similar to RF site, even though the biogeochemical conditions were completely different between these sites. The newly planted vegetation community at CF was in the process of establishment, so fresh plant litters were not expected to contribute much in DOC production, especially in July as the vegetation was just planted. In addition, construction materials used in the site (petroleum coke, tailings sand and upland soil) were poor sources of DOC (< 1 mg/L per gram of dry weight as reported in Chapter 3). Furthermore, contamination of the fen by organic acids derived from tailings materials, especially naphthenic acid, likely contributed little to near surface measured DOC concentration given that measured concentration was less than 1 mg/L at 100 cm depth (Price, unpublished). In fact, naphthenic acid and salts were expected to have a negative impact on vegetation and microbial community establishment resulting in lower DOC concentration, but did not exhibit any remarkable effect within the first growing season. Plants and microbes probably adapted to new environmental conditions and/or low concentrations of toxins in the first season have yet to have any substantial impact on growth. Peat used in CF site construction was similar to RF site with double the thickness and was considered the main source of measured DOC. Thus, the source of DOC in CF was likely peat only, whereas peat and fresh plant litters were the sources of DOC in RF.

The absorbance ratios (E2/E3 and E4/E6) and SUVA$_{254}$ of DOC provided additional evidence for little plant litter contribution to DOC at CF. The absorbance ratios suggested that DOC in reference fens had more fulvic, less aromatic and low molecular size of humic solutes than CF site. Variation in DOC aromaticity (CF>SF>PF>RF) among the study sites was also observed. In contrast to reference fens, CF had DOC with more humic, aromatic nature and high molecular size. Peat has more mature humic substances than fresh plant litters (Strack *et al.,* 2011) resulting in higher aromatic content in DOC in the CF site. Hence, hydrology in association with biogeochemistry could be the main reason for the differences in DOC chemistry. Input of fresh plant material in reference fens were the reason for more fulvic and less aromatic humic solutes, whereas lack of vegetation resulted in more humic and aromatic content in DOC in CF. Higher WT at CF created anoxic condition favorable for DOC production.

2.5.2 Seasonal variation of DOC concentration and chemistry

An increase in DOC concentration as the soil warms-up throughout the growing season was supported by the seasonal DOC concentration patterns in this study. This is consistent with findings by other authors (Christ and David, 1996; Bonnett *et al.,* 2006; Bossio *et al.,* 2006) who reported lower DOC concentrations in winter than in summer. There was a common trend of temporal variation in all fens: lower DOC concentration at the beginning of the growing season and higher concentration towards the end. Snowmelt and precipitation were considered as the main reason for lower DOC concentration early in the season due to dilution, whereas warmer, drier conditions and fresh vegetation inputs caused higher DOC concentration later in the growing season. This is further supported by the fact that the absorbance ratios in each reference site were found to be the highest during peak growing season and large precipitation events indicating high fulvic, low aromatic and low molecular size of humic solutes. Previous research (e.g. Austnes *et al*., 2010) has also reported a similar result. The decline of absorbance ratios at the end of the growing season is due to lack of fresh plant inputs. The presence of aromatic and high molecular humic content of DOC in the CF site further support the role of fresh plant litters in DOC chemistry because the plant community at CF was in the process of establishment and likely contributed little to the soil DOC pool. Significantly higher SUVA$_{254}$ in the CF site than reference fens is further evidence of the fact that DOC in the CF site contains high aromatic content consistent with peat sources (see also Chapter 3).

In the PF site, DOC concentration was the lowest during early season and precipitation events. However, mean DOC concentration slightly increased in June in spite of precipitation events, probably due to fresh plant material input. In addition, warmer temperatures escalated the microbial activities and decomposition processes, affecting the DOC chemistry *i.e.,* addition of fulvic, less aromatic and low molecular weight humic content in the system. Similar to this study, Moore *et al*. (2003) found a considerable variation in monthly precipitation and mean temperature over the study period was related to the changes in DOC concentration and chemistry. In turn, increased microbial activity at higher temperatures can lead to an increase in DOC production, which is followed by enhanced C mineralization (Kalbitz *et al.,* 2004). The changes in hydrological inputs in association with biological and environmental conditions at the PF site explain the lower DOC concentration on May and June. In contrast, the drier period (August-September) had the highest DOC concentration of the season. Moore *et al.* (2003) also observed higher DOC concentrations during the dry summer months. Elevated DOC concentrations during the summer were accompanied by the accumulation of aromatic compounds that do not easily degrade (Figure 2.10A, B). Similar temporal variation of DOC concentration was observed in the RF, SF and CF sites.

The trend of temporal variation of absorbance ratios in all reference fens were similar, though their values were markedly different especially during the peak growing season. The temporal variation of absorbance ratios in CF from July onwards was in line with reference fens. However, the initial growing season DOC chemistry variation trend was missed in CF due to lack of data as the site was still being prepared. However, we would expect a similar pattern of lower absorbance ratios and higher $SUVA_{254}$ than reference fens due to the absence of vegetation. Moore (1987) reported similar seasonal differences in absorbance ratios (E4/E6) for a boreal peatland, with higher values in the early growing season than at the end, suggesting that more humic acid character is dominant in the late summer.

Overall, production, absorption and consumption of DOC are factors within peatlands that could contribute to the differences in DOC concentration and chemistry among the sites. These factors along with the water pathways through the peatlands impact DOC concentrations and chemistry (Moore and Dalva, 2001). However, the critical factors in determining DOC production rates are

still not well understood because isolating variables in field conditions is very difficult (Moore and Dalva, 2001). Even though the sites were hydrologically and ecologically diverse and DOC concentrations were quite different, the seasonal patterns of DOC concentration and chemistry changes were similar suggesting a broader control on seasonal patterns linked to regional weather patterns (*i.e.,* temperature and WT shifts driven by timing of warming and precipitation events).

2.5.3 Spatial variation of DOC within sites: microforms and depth

The rate of carbon cycling can vary greatly between microforms within a peatland due to varying environmental conditions. Several studies suggest that carbon accumulation is highest at hummocks, whereas hollows or pools are generally sources of carbon to the atmosphere (Moore, 1989; Belyea and Clymo, 2001). However, these studies generally ignore hydrologic fluxes of carbon, particularly between microforms. According to Strack *et al.* (2008) hummocks tend to lose carbon as DOC, while hollows/pools gain DOC. In the current study, DOC concentration at microforms among reference fens was investigated. It was observed that changes in environmental conditions due to microtopography had little impact on DOC concentration.

Similarly, DOC concentrations at depths 50 cm, 75 cm and 100 cm did not exhibit any significant differences. This is similar to the finding of a previous study carried out in northern boreal fens (Wyatt *et al.,* 2012). Small changes below the subsurface may not have an impact on DOC production because WT was close to the ground surface (within 20 cm) throughout the season in all sites, which created similar hydrochemical conditions within these shallow depths. In contrast, Bettina *et al.,* (2009) found significantly different DOC concentrations even at soil depths of 40 and 60 cm in similar water saturation conditions; however, these horizons differed significantly in the redox conditions, underlining the fact that water saturation does not always represent the actual redox conditions. Furthermore, Moore *et al.* (2003) observed a decrease of mean pore water DOC concentration with depth ranging from 20-60 mg/L.

2.5.4 Environmental controls on DOC concentration across boreal fens

The impact on DOC concentrations of environmental factors such as temperature, pH, EC and WT were investigated. All of the environmental factors tested showed significant impact on

DOC concentration. EC showed profound impact on DOC concentration, which was exhibited by high concentrations at SF. Salinity likely provided nutrients to microbes and enhanced the net production of DOC (see also Chapter 3). CF had mean DOC concentration close to RF, though RF was rich in vegetation diversity. Higher salt concentration in CF than RF (> 5 times) could have played a role to some extent to compensate for the lack of DOC from fresh vegetation litters at the former. PF, though it had the lowest EC among the sites, exhibited higher DOC concentration than CF and RF likely due to other factors such as *Sphagnum* dominance as explained previously (section 2.5.2).

DOC concentrations in all reference fens increased with increasing pH (Figure 2.8). Similar to this study, Worrall *et al.,* (2008) also found a positive correlation between pH and DOC concentration. This correlation is due to increases in DOC solubility as pH increases (Worrall *et al.* 2008). In contrast, DOC concentrations in CF showed a negative relationship with pH, which might be due to lack of data at the beginning of season. Since there was a temporal variation of DOC concentration in all reference sites, this also could be expected in CF. Emergence of vegetation likely also drives DOC concentrations and may mask correlations with chemical conditions such as pH. Therefore, further investigation with a complete seasonal dataset is required to confirm the relationship between DOC and pH at CF as the site develops and vegetation community stabilizes.

Higher WT generally results in greater net DOC production due to anoxic conditions (Moore and Dalva, 2001). Hence, SF with the highest WT among the study sites had the highest DOC concentration, whereas RF with the lowest WT had lower DOC concentration. Likewise, warmer temperature enhances microbial activity resulting in faster decomposition of organic matter and producing higher soil DOC concentration (Kalbitz *et al.,* 2004). In addition, warmer temperature increases the solubility of DOC. As a result, warmer temperatures at SF likely also contributed to it having the highest DOC concentration among the sites. Seasonal patterns of increasing DOC concentration across all sites may also be linked to, among other controls, increasing soil temperatures over the study period.

Environmental factors also had a significant impact on DOC chemistry, though the variability of DOC chemistry explained by the model was 16% only. In all reference fens, absorbance ratios

(E2/E3 and E4/E6) were higher during peak growing season when the temperature was warmer. This suggests that fresh plant litters and higher temperature resulted the smaller molecular size and fulvic content in DOC. Decomposition of organic matter may increase at higher temperatures due to enhanced microbial breakdown of larger insoluble compounds to soluble entities, as observed by Christ and David (1996). In addition, aromaticity of DOC was increased with the increase of pH and WT, whereas decreased with higher temperature and EC. Similarly, higher WT creates anoxic conditions, which slows down the decomposition and results in high molecular size humic substances dominant in DOC.

2.5.5 DOC export

The amount of DOC exported from peatlands may depend on interactions between the flow of water through the peatland and the production and consumption of DOC within the peatland, which in turn may depend on the diverse chemical nature of peat derived from different plant communities. Although detailed hydrologic measurements were beyond the scope of this study, total export of DOC from PF and CF was calculated based on mean discharge and DOC concentrations to get rough estimation for comparison between the sites. Accordingly, rough DOC export calculated from PF and CF was estimated to be 11.06 gC/m^2 and 3.3 gC/m^2 respectively over the growing season. In this study, PF exhibited the higher DOC export among the sites due to higher discharge relative to area and higher DOC concentrations. The catchment of PF was bigger than CF, so was the discharge. Therefore, DOC export was higher in PF than CF. DOC export is controlled by site hydrology (Hinton *et al*., 1997; Fraser *et al*., 2001; Pastor *et al*., 2003) and is generally greater from peatlands with higher measured discharge (Fraser *et al*., 2001; Freeman *et al*., 2001). In future, further investigation with continuous discharge measurement and more frequent DOC concentration measurements capturing storm events would be required for better DOC export estimation. Moreover, the majority of DOC may be lost during snowmelt (e.g., Waddington *et al.,* 2008) and monitoring export during this period will be critical to accurately describe DOC dynamics of these fens.

2.6 Conclusion

This study compared the concentration and chemistry of DOC in a constructed fen (CF) with natural fens in boreal Alberta. Significantly different DOC concentrations were observed among reference fens: the saline fen (SF) had the highest DOC concentration followed by the poor fen (PF), while the rich fen (RF) had the lowest DOC concentration among the reference fens but was similar to CF. In addition, temporal variations of DOC concentration with similar trends in each fen were observed. In all sites DOC concentration was lower in May and rose throughout the growing season to be highest in September. Differences in hydrological inputs, in association with environmental conditions in the system explain the variation in DOC concentration. Dominant vegetation likely determines the composition of DOC. Therefore, reference fens had DOC composed of less aromatic and lower molecular weight organic compounds than DOC in CF. There was a difference in dominant vegetation community among the reference fens whereas CF had newly planted vegetation. The peat, which was considered as a main source of DOC in CF, contained more mature humic content. Even the seasonal variation of DOC chemistry was similar across the reference fens with fulvic content more dominant at the peak growing season and humic content more dominant in the remaining period. Using a rough estimation of DOC export, PF was found to export more DOC in comparison to CF due to both higher discharge and DOC concentration.

Potential factors controlling DOC concentration were also investigated during the study. Changes in microclimate due to microforms and depths did not lead to significant variation in DOC concentration. Other environmental factors such as EC, pH, temperature and WT were correlated to DOC concentration.

This study indicated that CF may be developing similar conditions to the RF ecosystem based on DOC concentration; however, the study period was very short to come to any conclusion, as conditions at CF are expected to change as the site develops. Therefore, long term study is recommended for better understanding of the DOC dynamics of CF. As vegetation becomes established on site we expect that DOC concentration at CF will rise and its chemistry will reflect a more fulvic, less aromatic nature similar to the reference fens. Monitoring DOC dynamics should thus provide a good measure of ecosystem recovery over time.

2.7 References

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Table 2.1: Monthly mean air temperature and precipitation of PF, RF and SF in 2013.

	Poor Fen		Rich Fen		Saline Fen	
Month	PPT (mm)	Temp (°C)	PPT (mm)	Temp (°C)	PPT (mm)	Temp (°C)
May	12.3	14.0	5.2	16.7	11.7	14.0
June	165.9	19.1	165.6	16.4	136.6	19.1
July	87.1	21.5	119.6	16.9	72.5	21.5
August	3.9	19.4	34.8	16.4	4.9	19.4
Sept	35.0	14.9	54.4	11.3	30.1	14.9
Total	304.2	-	379.6	-	317.5	-
Average	-	17.8	-	15.5	-	17.8

Note: PPT = precipitation

Table 2.2: Monthly mean pH and EC of each fen during study period.

	Poor Fen		Rich Fen		Saline Fen		Constructed Fen	
Month	pH	EC(μS/cm)	pH	EC(μS/cm)	pH	EC(μS/cm)	pH	EC(μS/cm)
May	4.1	22	5.8	220	-	-	-	-
June	4.9	26	5.9	100	6.2	14300	-	-
July	4.9	30	7.3	380	6.4	10900	7.7	1,650
August	4.7	32	7.2	390	6.8	15800	7.4	1,700
September	4.8	32	7.3	410	6.7	15800	-	-
Average	4.7	28	6.8	300	6.5	14200	7.5	1,675

Table 2.3: GEE result showing the impact of microforms, depths and sites on DOC concentrations.

Source	Type III Wald χ^2	df	P
Intercept	5651.32	1	<0.01
Microforms	0.48	1	0.49
Depth	2.82	2	0.24
Site	543.72	2	<0.01
Microforms*Site	0.74	2	0.69
Microforms*Depth	1.13	2	0.57
Site*Depth	7.64	4	0.11
Microforms*Site*Depth	3.20	4	0.52

Table 2.4: Result of environmental factors controlling DOC concentration using GEE model with repeated measures of sampling.

Parameter	Slope	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald χ^2	df	P
Intercept	46.284	6.6929	33.166	59.402	47.823	1	<0.01
pH	-3.531	0.8309	-5.159	-1.902	18.058	1	<0.01
Temp	1.103	0.1850	0.740	1.466	35.545	1	<0.01
EC	0.002	0.0002	0.002	0.003	223.174	1	<0.01
WT	0.123	0.0353	0.054	0.192	12.096	1	<0.01

Table 2.5: Result of GEE model explaining environmental factors controlling E2/E3 in DOC.

Parameter	Slope	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald χ^2	df	P
Intercept	3.52	0.23	3.06	3.98	225.57	1	<0.01
pH	-0.18	0.03	-0.25	-0.12	31.04	1	<0.01
Temp	0.03	0.01	0.01	0.06	9.40	1	<0.01
EC	9.66E-06	8.20E-06	-6.41E-06	2.57E-05	1.39	1	0.24
WT	-0.01	0.002	-0.02	-0.007	25.61	1	<0.01

Table 2.6: Result of GEE model explaining environmental factors controlling E4/E6 in DOC.

Parameter	Slope	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald χ^2	df	P
Intercept	3.78	0.46	2.88	4.67	68.50	1	<0.01
pH	-0.31	0.06	-0.42	-0.19	27.99	1	<0.01
Temp	0.04	0.02	-0.003	0.08	3.30	1	0.07
EC	-1.35E-05	1.13E-05	-3.57E-05	8.58E-06	1.44	1	0.23
WT	-0.022	0.0045	-0.03	-0.01	25.02	1	<0.01

Table 2.7: Result of GEE model explaining environmental factors controlling $SUVA_{254}$ in DOC.

Parameter	Slope	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald χ^2	df	P
Intercept	0.0135	0.0090	-0.004	0.03	2.25	1	0.13
pH	0.0079	0.0016	0.004	0.01	20.79	1	<0.01
Temp	0.0004	0.0004	-0.001	0.00	1.43	1	0.23
EC	-4.64E-07	2.01E-07	-8.58E-07	-6.98E-08	5.32	1	0.02
WT	0.0001	7.43E-05	0.00	2.22E-05	2.76	1	0.10
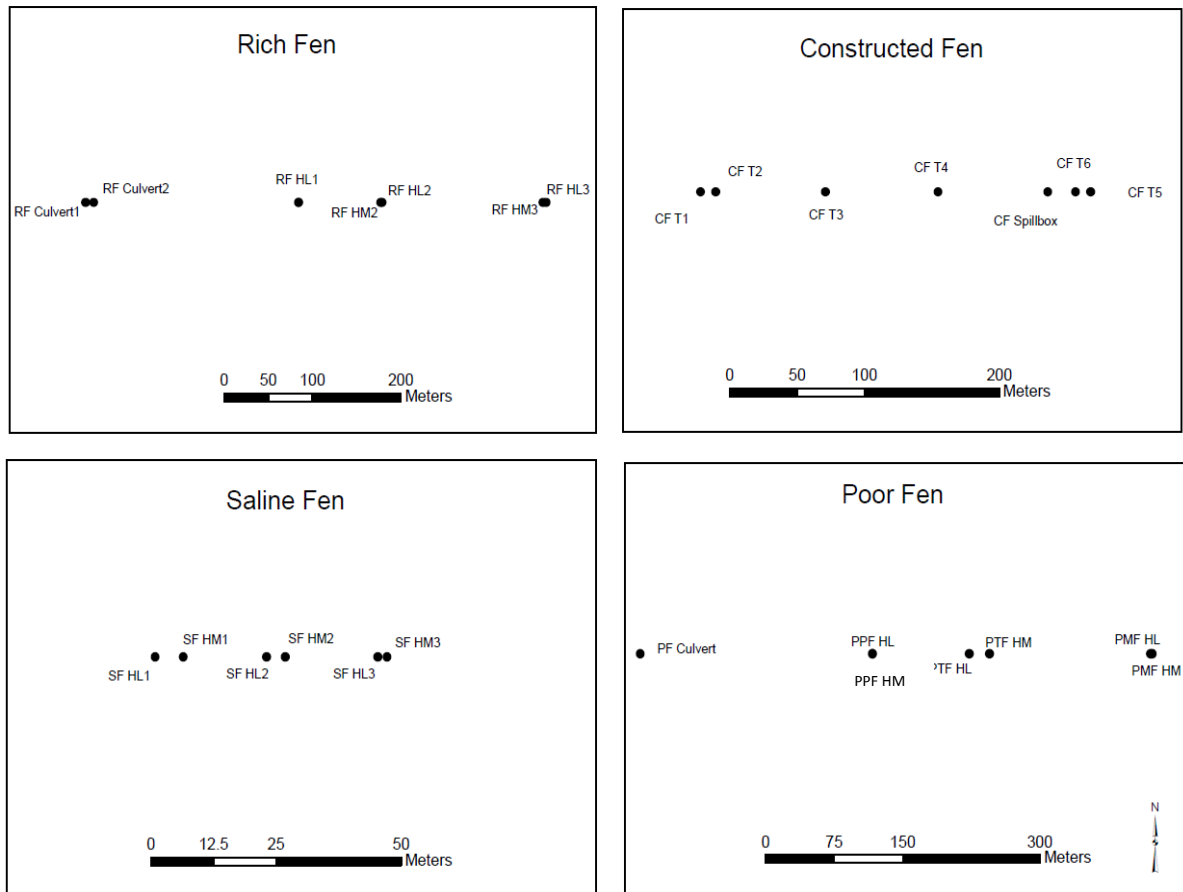
Figure 2.1: Location of piezometer nests, culverts and spillbox in study sites. (Note: HM = hummock and HL = hollow).
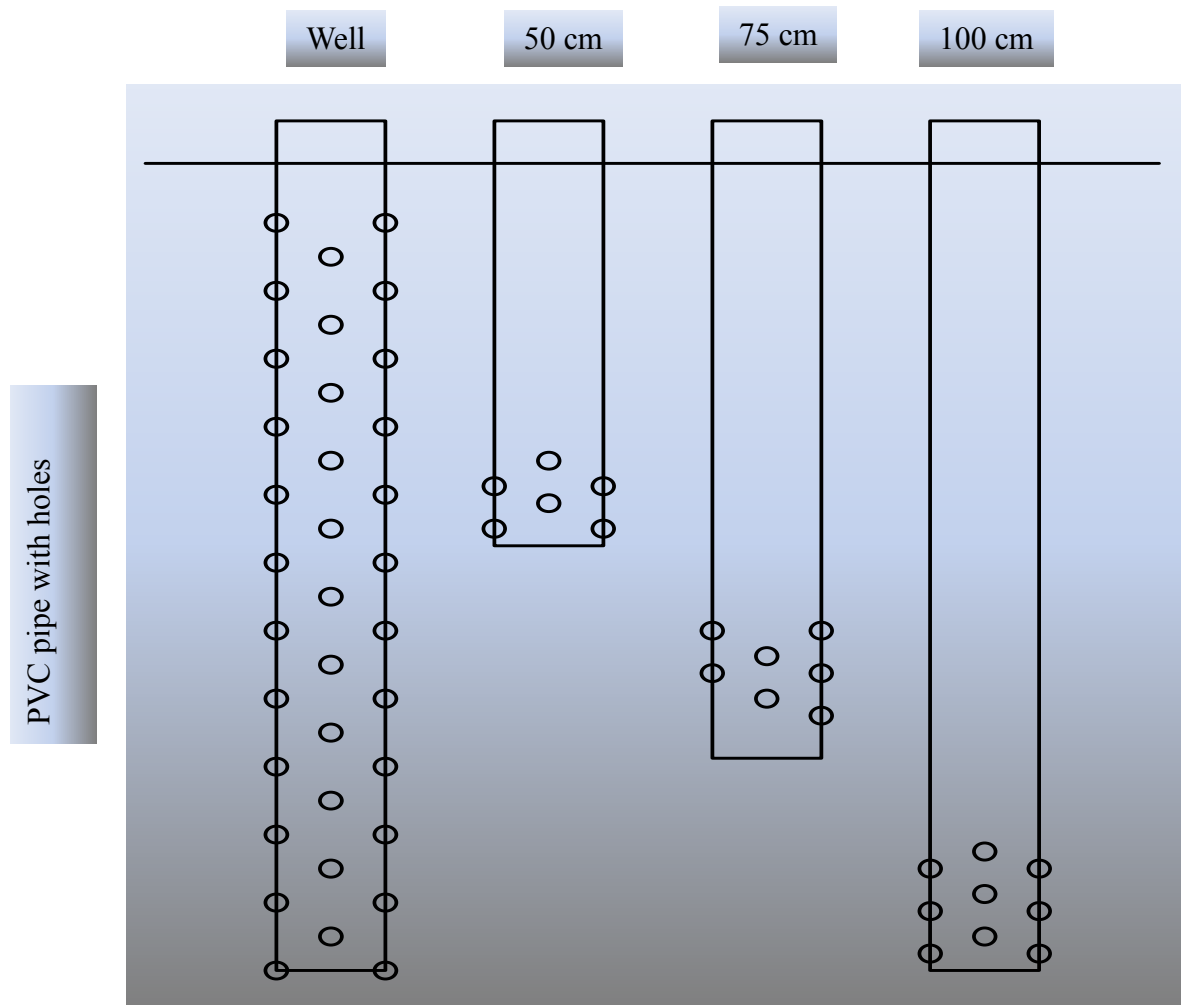
Figure 2.2: Piezometer nest installed in each fen for water sampling. In one nest, there were three piezometers installed at 50, 75 and 100 cm depth and a well.
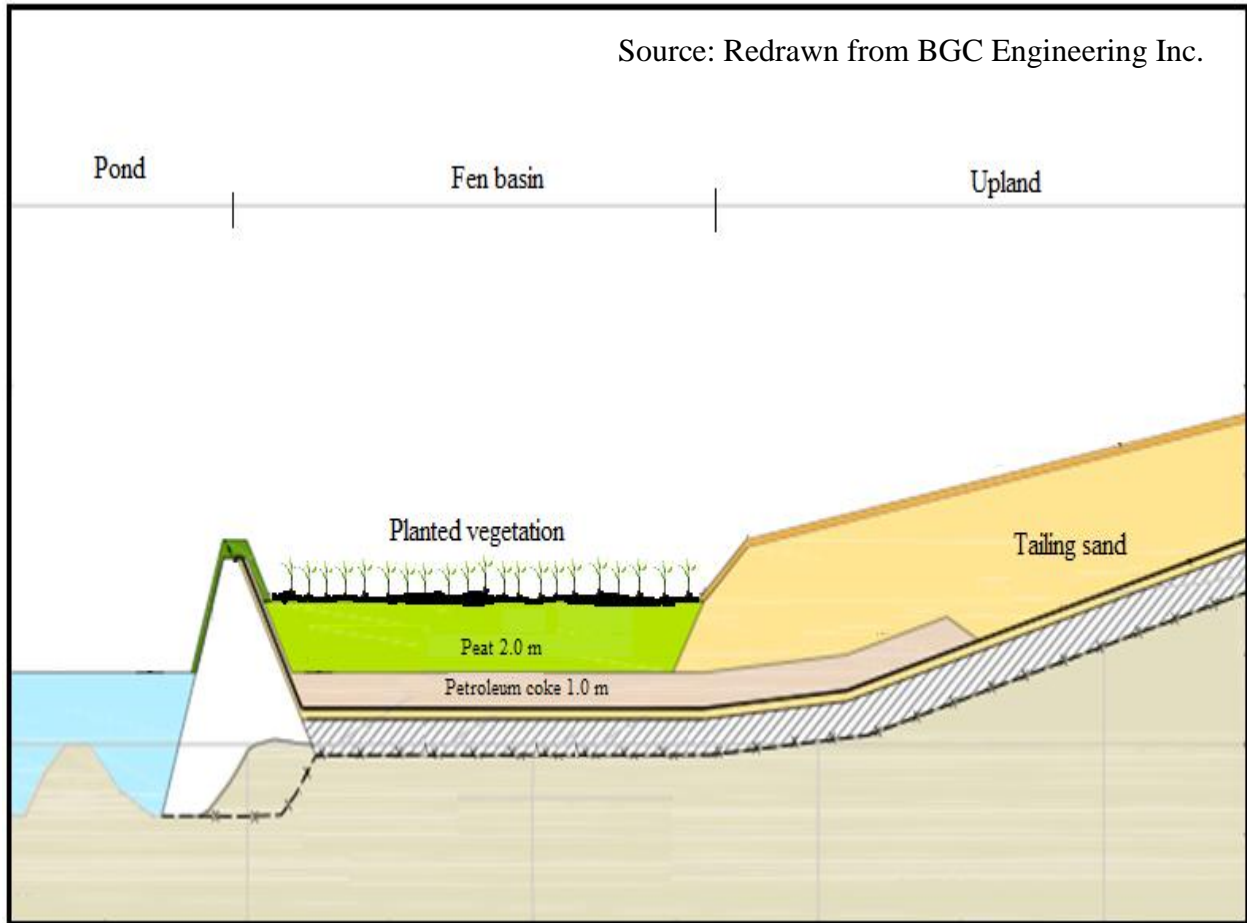
Figure 2.3: Cross sectional view of the reclaimed fen and adjacent upland.
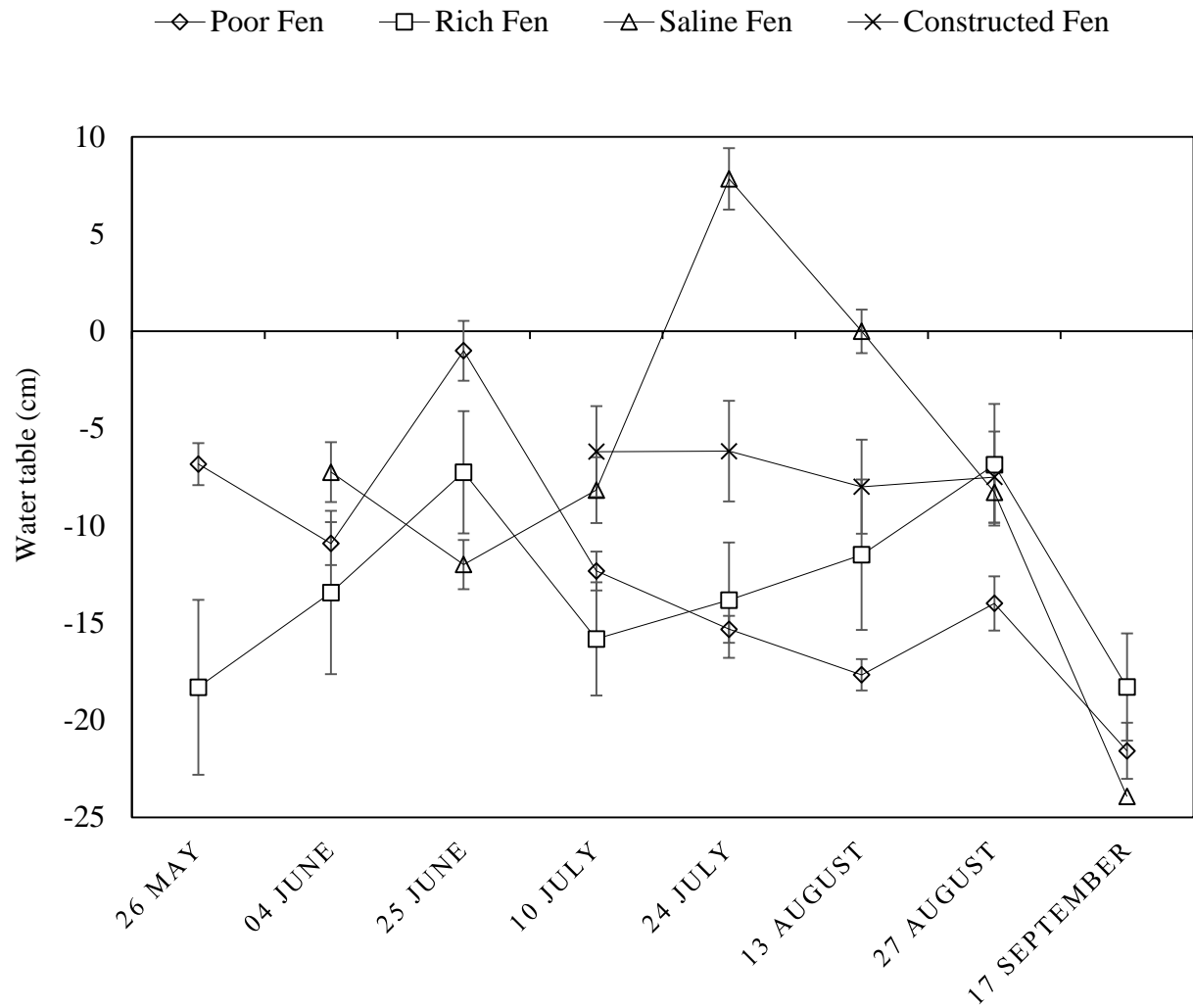
Figure 2.4: Temporal variation of mean WT position in each site. The mean was calculated from six wells from each site at each sampling period separately. Error bars show the standard error of the mean. Negative values indicate depth below the surface.
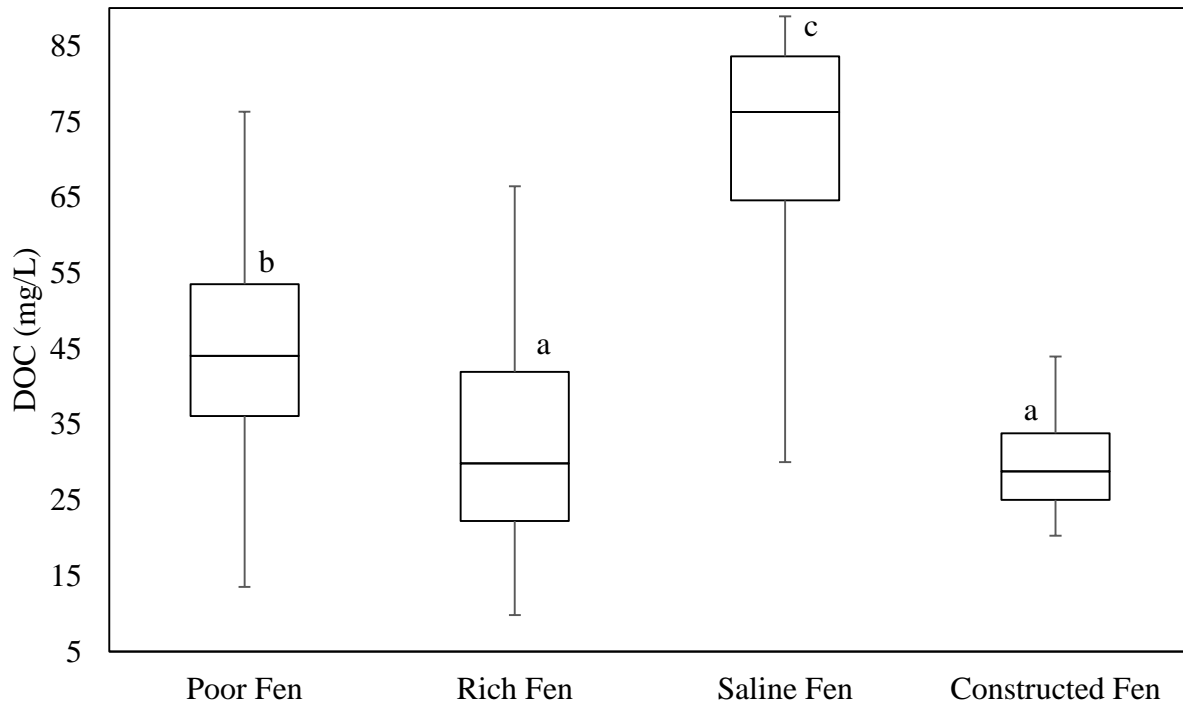
Figure 2.5: Box plots showing the comparison of DOC concentration among sites. Values are significantly different (ANOVA, p<0.05) if they do not have letters in common. Boxplot of each site display variation of DOC in samples. The horizontal line in the middle of the box represents the median. Minimum and maximum whiskers represent the range of the data, whereas lower and upper end of the boxes are first and third quartiles of the data respectively.
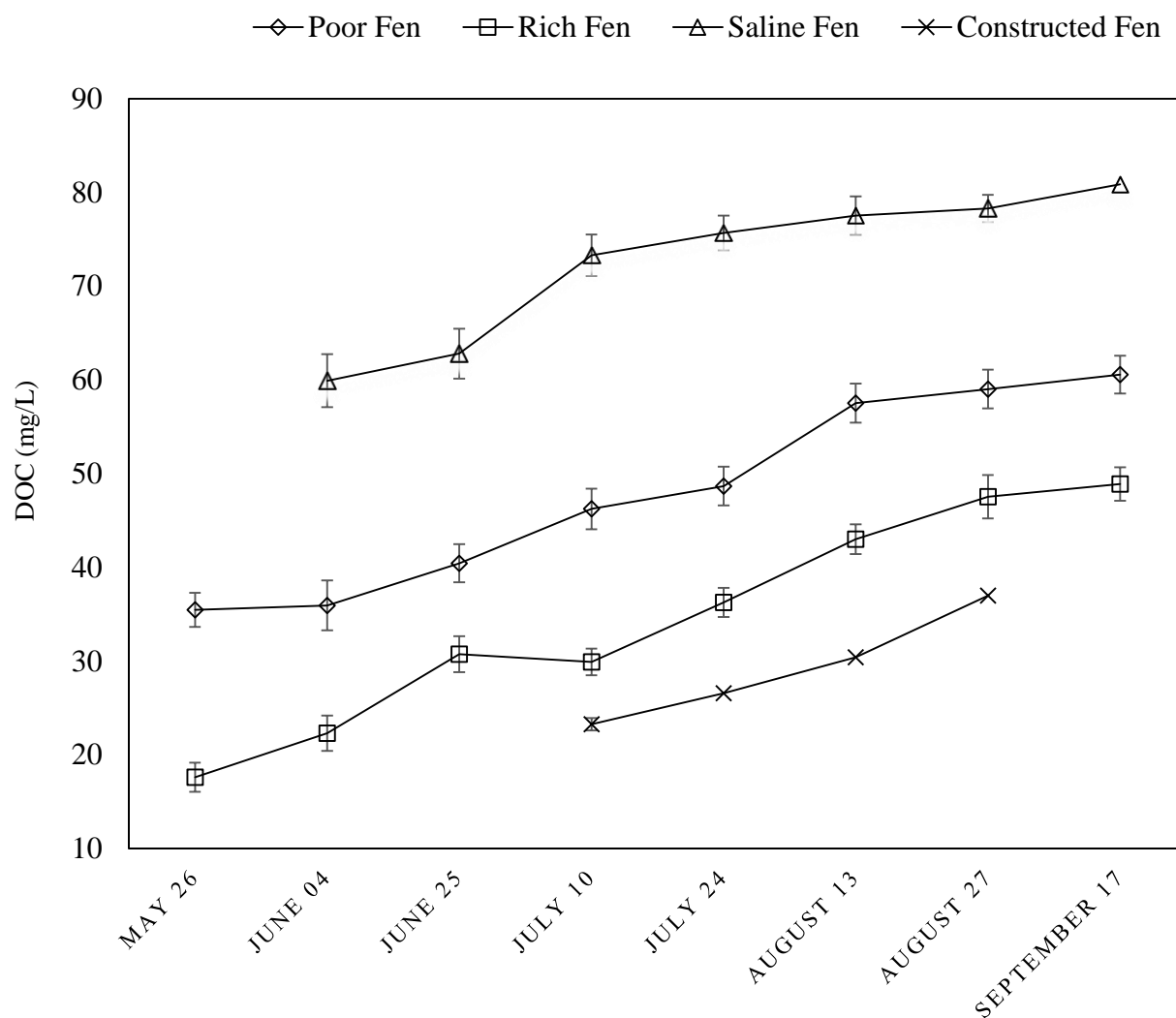
Figure 2.6: Temporal variation of mean DOC concentration in each site. Values are based on the mean DOC concentration calculated from all microforms and depths on each sampling period. Error bars give standard error of the mean.
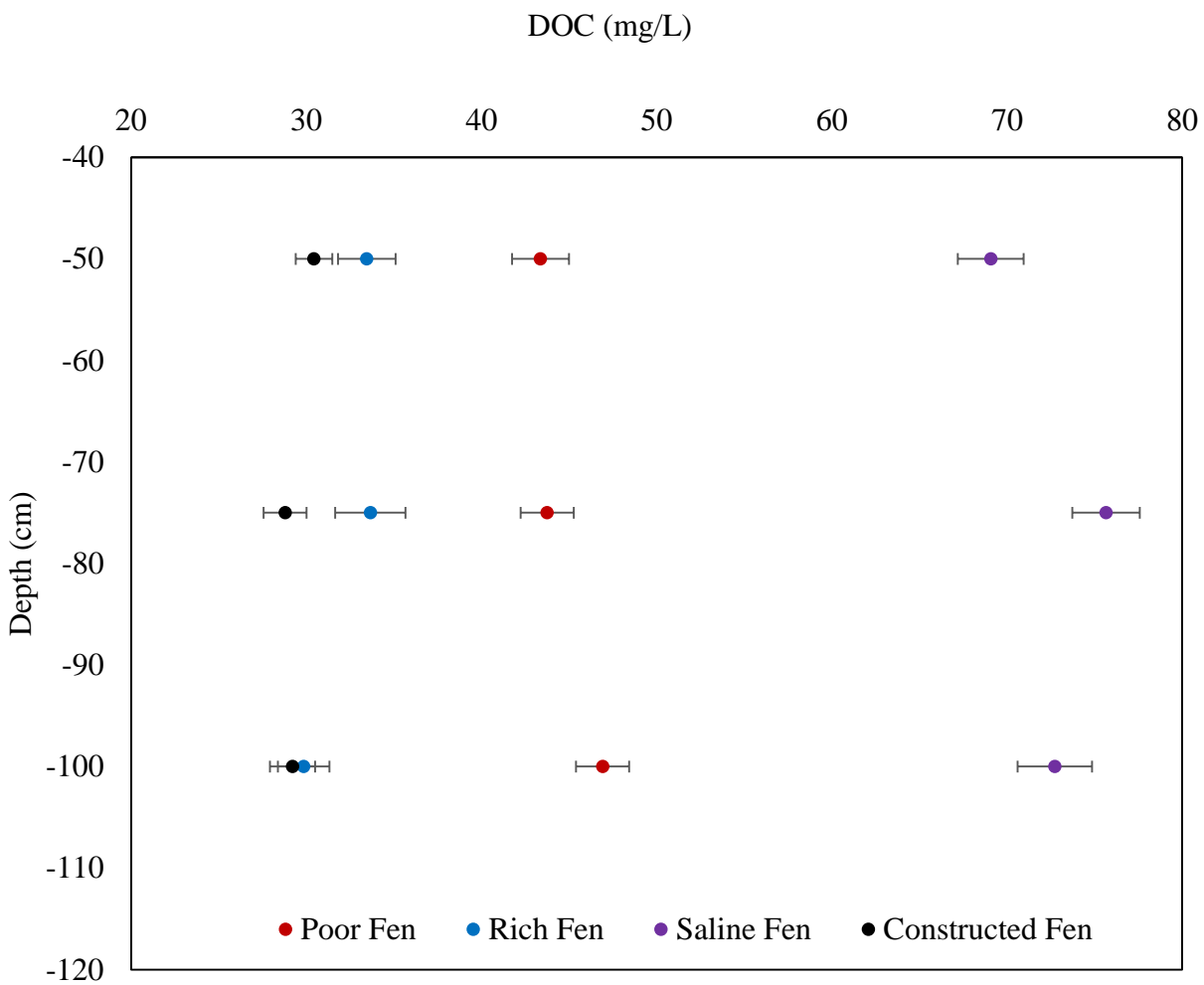
Figure 2.7: Mean DOC concentration of each site at different depths. Each value is based on all samples from both hummocks and hollows (except at constructed fen where no microforms existed) collected on all sampling days for that depth at each site. Error bars give standard error of the mean.
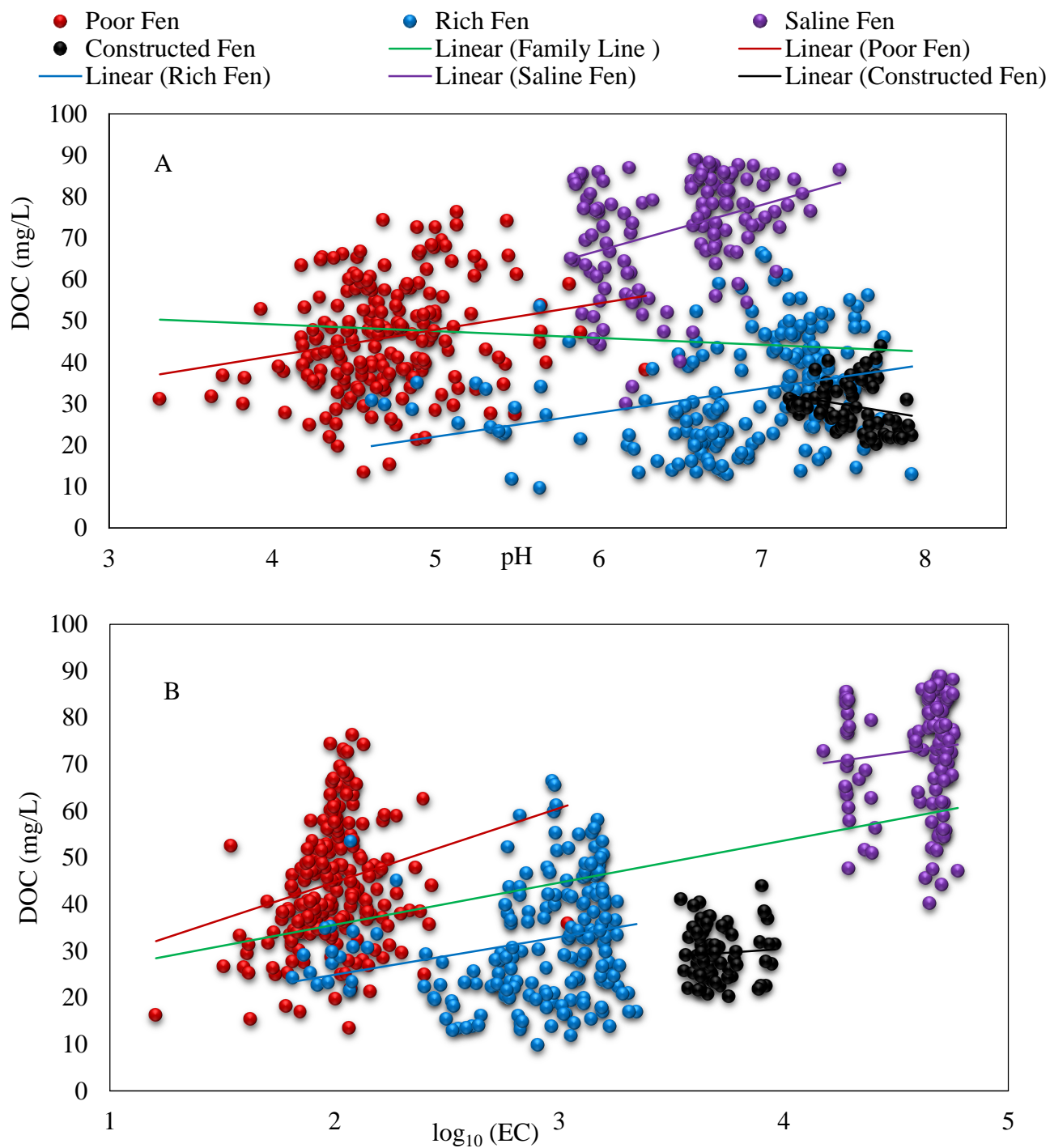
Figure 2.8: Relationship between DOC with (A) pH and (B) $\log_{10}$ (EC) for RF, PF, SF and CF. The coloured circles represent DOC value in relation to pH (A) and $\log_{10}$ (EC) (B) for different sites. The regression lines indicate the relationships between DOC concentrations with pH and $\log_{10}$ (EC).
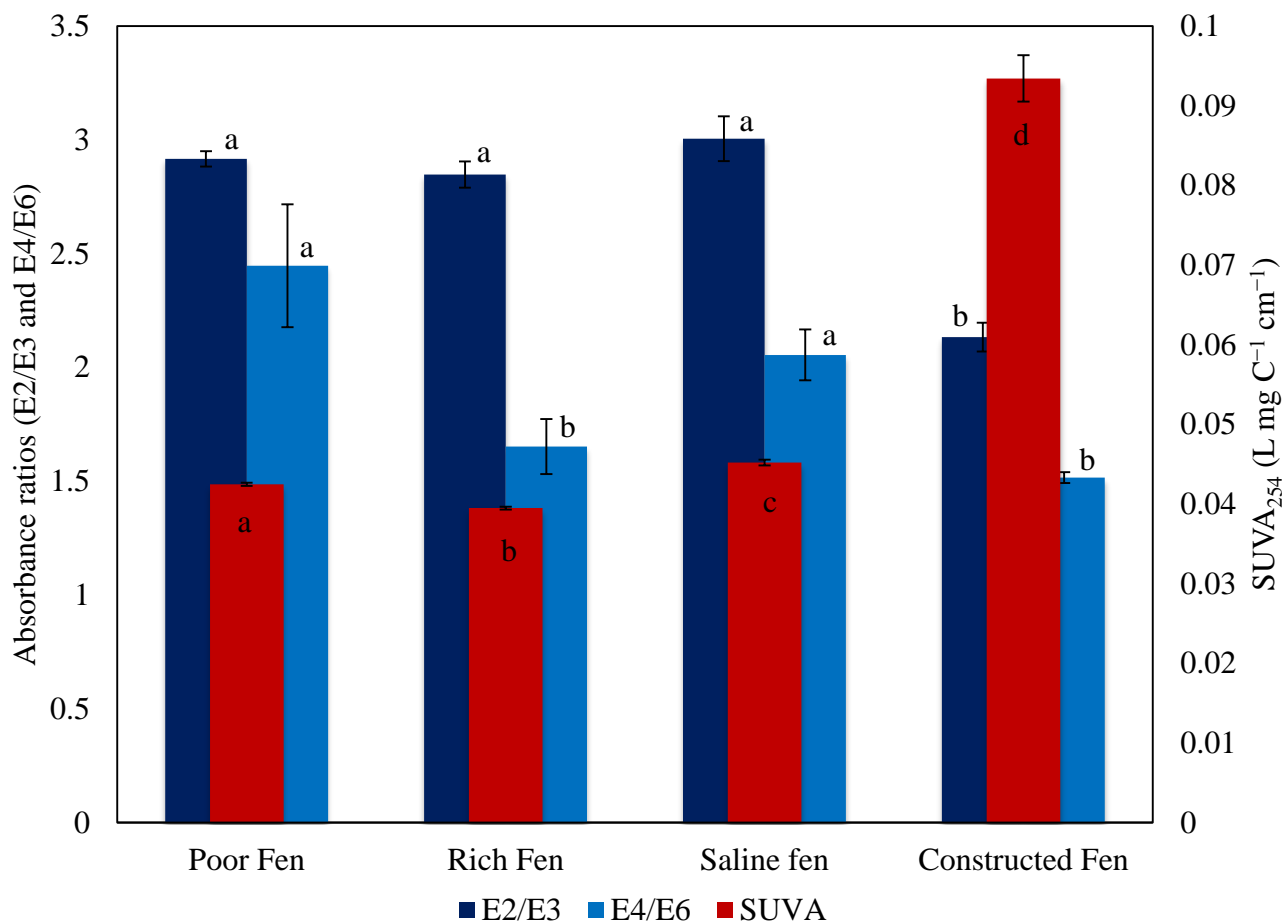
Figure 2.9: Comparison of mean absorbance ratios (E2/E3 and E4/E6) and SUVA$_{254}$ among the sites. Error bars on the graph shows the variation from the mean. Letters should be compared separately for E2/E3, E4/E6 and SUVA$_{254}$ between the sites. Values are significantly different (α = 0.05) if they do not have letters in common.
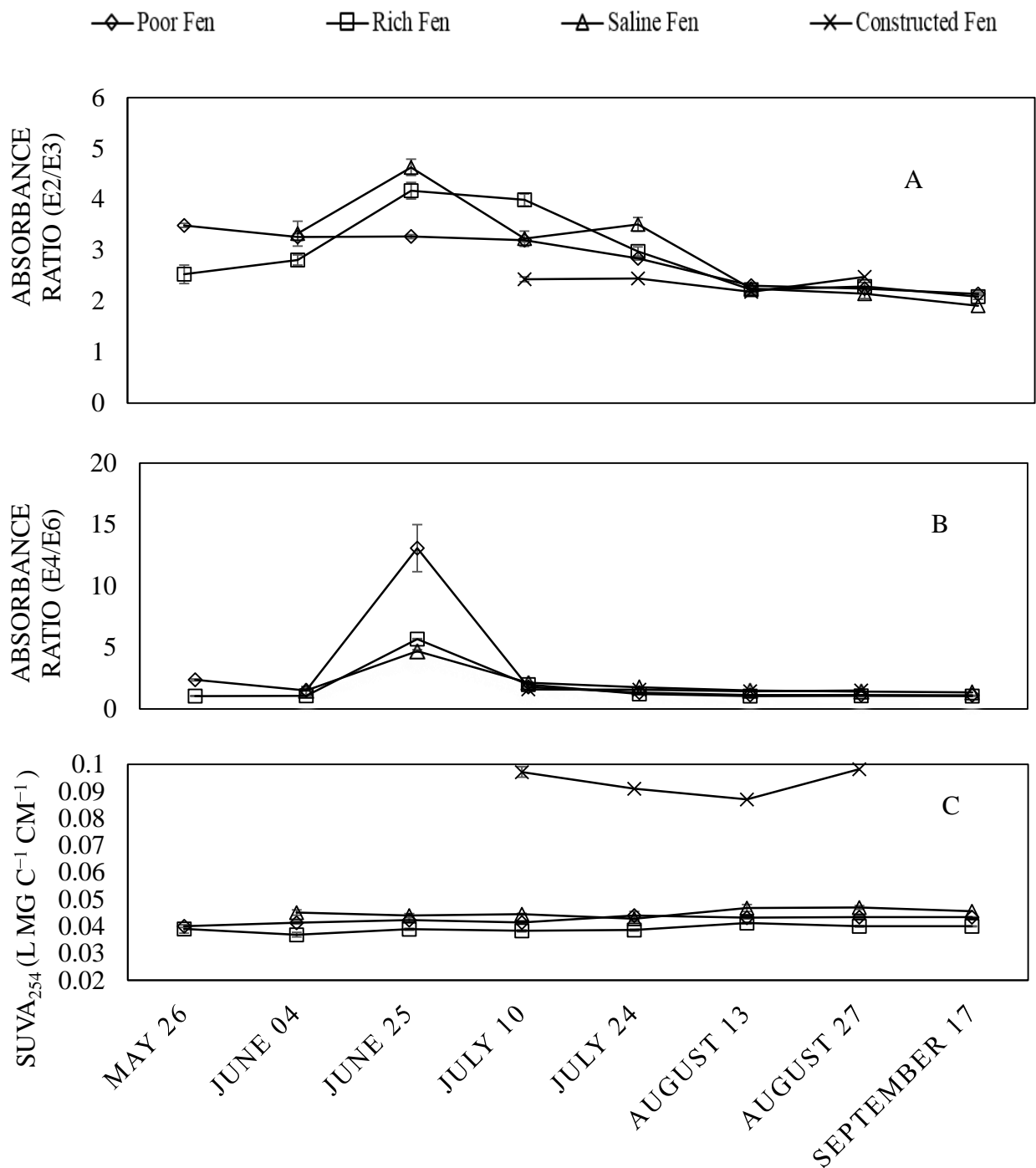
Figure 2.10: Temporal variation of absorbance ratios (A) E2/E3, (B) E4/E6 and (C) SUVA$_{254}$. The measurement were taken biweekly from mid-May until mid-September 2013 but the CF started from mid-July until August 2013. Values are a mean of all samples collected at each site (all depths and microforms) on each sampling date. Error bars give the standard error of the mean.

APPENDIX A: Supplemental information from Chapter 2

Table A1: Samples collected from individual sites and analyzed in this study.

Sites	Hollow	Hummock	Discharge	CF[a]
PF	109	107	8	
RF	97	94	0	
SF	63	63	0	
CF	NA	NA	4	70
Total	269	264	12	70
Grand Total	615			

NA: Not applicable as there was no microforms developed at the CF

   a. CF = CF pore water samples
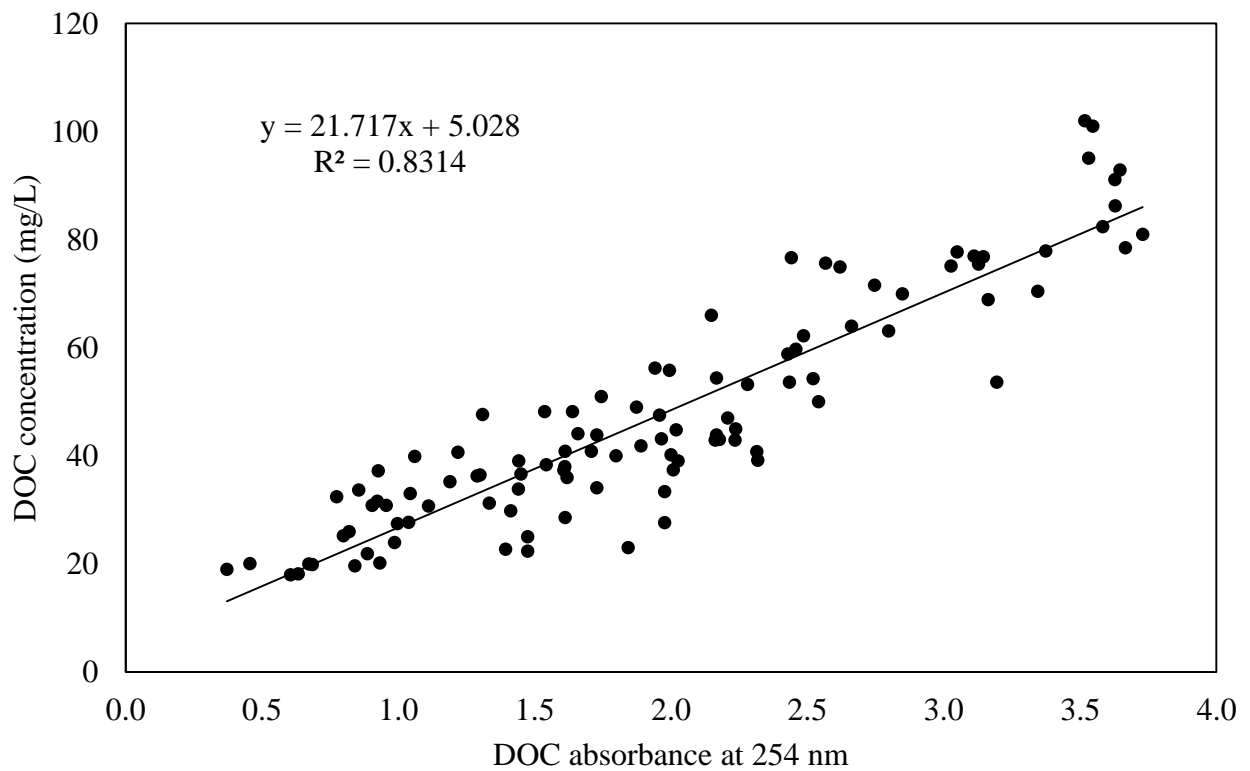
Figure A1: Correlation between DOC concentration and DOC absorbance at 254 nm.

CHAPTER 3: SOURCES OF DISSOLVED ORGANIC CARBON AND BIOAVAILABILITY IN CONSTRUCTED AND NATURAL FENS IN ATHABASCA OIL SANDS DEVELOPMENT REGION NEAR FORT MCMURRAY, ALBERTA

Abstract

Dissolved organic carbon (DOC) production and its bioavailability were investigated from substrates collected from constructed and natural fens in the Athabasca oil sands region near Fort McMurray, Alberta. Dominant vegetation and other substrates used in fen construction, like peat, petroleum coke, tailings sand and upland soil, were collected for the study. The effect of substrate type, salinity and temperature on DOC production and bioavailability were analyzed. Sedges and trees produced the largest amount of DOC, followed by peat and moss in all the fens, whereas the substrates from the CF such as petroleum coke, tailings sand and upland soil produced very little DOC per unit dry mass. In addition, temperature and salinity were also significant explanatory variables for both initial (30 day) and final (60 day) net DOC production rates. Calculated Q_{10} values (effect of temperature on DOC production) were mostly between 1.0 and 2.0, except higher values for moss and *Carex aquatilis*. Moss had higher initial (4.2) and final (3.2) values, particularly in deionized water. It was also observed that production of DOC had a profound influence on the incubation water pH and EC such that higher production of DOC resulted in lower pH and higher EC. In addition, the quality of DOC depended upon the type of substrate. Sedges produced DOC having lower molecular size and less aromatic humic substances than peat, tailings sand, upland soil and petroleum coke, which was reflected in $SUVA_{254}$. Substrate, EC, temperature and interaction among these factors were found to significantly explain variation in DOC bioavailability. In addition, molecular size and aromaticity of DOC produced from these substrates had a large effect on DOC bioavailability, captured by E2/E3 and E4/E6. These results help explain observations of low DOC concentration and higher aromaticity at CF relative to the reference sites. As various substrates produced different amounts and quality of DOC, it is likely that the DOC production and quality will continue to change as the new vegetation community develops at CF. Hence, establishment of the vegetation community and its composition will play an important role in DOC dynamics at the constructed fen in future.

3.1 Introduction

Boreal peatlands store a large amount of carbon in the form of organic matter due to saturated soil conditions and cool temperatures. Peatlands, mainly fens, in the Athabasca oil sands region have been extensively exploited in order to extract bitumen. Suncor has initiated a fen reclamation project based on the design by Price *et al.* (2010) in order to meet the terms and conditions of the Alberta Environmental Protection and Enhancement Act.

DOC is produced by partial decomposition of organic matter and subsequent dissolution in pore water (Moore and Dalva, 2001) and is an important component in peatland carbon balances (Moore, 1998; Billett *et al*., 2004). It plays a key role in a wide variety of chemical, physical and biological processes occurring in soils (Kalbitz *et al*., 2000; Zsolnay, 1996, 2003). In addition, DOC is a potential source of energy and nutrients to the soil microflora and downstream aquatic communities. Microbial consumption of DOC can regulate the production of greenhouse gases (GHGs) both by reducing the oxygen content of soils and by providing the electrons required for methanogenesis and denitrification (Lu *et al.*, 2000; Yavitt, 1997; Zsolnay, 1997). Furthermore, knowledge about the biodegradability of DOC is crucial in assessing its contribution to soil organic matter build up (Kalbitz *et al.,* 2003), the fate of exported DOC, and its contribution to GHG emissions in downstream ecosystems (Marschner and Kalbitz, 2002). The biodegradation of DOC is defined as the metabolic breakdown of organic matter by soil microorganisms, which is quantified by the disappearance of DOC or O_2 or by the evolution of CO_2. Bioavailability describes the potential of microorganisms to interact with DOC.

Generally, decomposition of organic matter depends on four factors: substrate quality, environmental conditions, decomposer type and nutrient availability (Laiho, 2006). All these four factors have interactive effects: environmental conditions and nutrient availability regulate the vegetation composition (Økland *et al.,* 2001; Wheeler and Proctor, 2000), which in turn largely determines substrate quality (Hobbie, 1996), which then, together with environmental conditions and possibly nutrient availability, regulates the composition of the decomposing community (Borga *et al.,* 1994; Panikov, 1999). The release of DOC in incubation experiments represents the balance of production, desorption, adsorption and microbial utilization (Moore and Dalva, 2001).

Vegetation community composition and productivity are important factors in controlling soil DOC concentration since the production of DOC from various litter types is different (Kuiters, 1993; Currie *et al.,* 1996). Generally, enhanced vegetation productivity is correlated to higher DOC concentrations in soil solution (Lu *et al.,* 2000; Kang *et al.,* 2001; Freeman *et al.,* 2004). In addition, minerals present in the peat can have inhibitory effects on microorganisms due to toxicity, and consequently influence decomposition. In contrast, the enhanced degradability observed in the presence of minerals may be due to flocculation, as larger structures will provide better attachment for microbial colonies (Marschnera and Kalbitz, 2003). Furthermore, DOC production may increase at higher temperatures due to enhanced microbial breakdown of larger insoluble compounds to soluble entities (Christ and David, 1996). If this process dominates over the enhanced production, the net effect of a temperature-stimulated microbial activity will be DOC depletion (Marschner and Bredow, 2002).

Spectroscopy in the ultraviolet and visible spectral ranges has been used for the characterization of DOC. Absorbance ratios (E2/E3=250/365 nm and E4/E6=465/665 nm) and specific UV absorbance (SUVA) have been used to investigate DOC chemistry. A high value for E2/E3 ratio is indicative of low aromaticity and molecular size of aquatic humic solutes (Richard and Joseph, 2012). The absorbance ratio E4/E6 indicates the fulvic (FA) vs. humic (HA) nature of DOC (Hautala *et al*., 2000; Spencer *et al*., 2007). The ratio <5 and >6 indicates humic and fulvic acids respectively, which in turn gives the molecular weight and aromaticity (Christopher *et al.*, 2006). In addition, SUVA is determined by normalizing absorbance at 254 or 250 nm with DOC concentration and is used to measure aromatic character of DOC in soils, with higher SUVA indicating higher aromaticity. Evaluating differences in absorbance ratios of DOC produced from substrates collected from the constructed fen and reference fens in the region may be useful in evaluating sources and processes contributing to net DOC production post-reclamation. DOC having high content of carbohydrates, organic acids and proteins is easily biodegradable, whereas aromatic and hydrophobic structures have lower biodegradability due to resistance to microbial enzymes (Marschner and Kalbitz, 2003).

The effects of environmental factors on DOC production can be studied by incubating substrates under varying conditions. Laboratory studies may yield single effects of different environmental factors, while field studies yield the interactive effect of all the factors. The aim of this research

was to improve our understanding of the effect of substrate type, salinity and temperature on DOC dynamics, which can be used to evaluate the reclamation success and better understand the sources contributing to soil pore water DOC in the field. The specific objectives of this study were to: (1) determine the effect of substrate, temperature and salinity on DOC production and chemistry, and (3) determine the bioavailability of DOC produced from these substrates under various conditions.

3.2 Study Sites

3.2.1 Natural Fens

Three diverse natural fens near Fort McMurray, Alberta were used in this study. They are referred as poor fen (PF), rich fen (RF) and saline fen (SF) throughout the paper.

PF is located ~40 km south of Fort McMurray (56° 22.610 N, 111°14.164 W). This fen features a forested upland surrounding the fen and also receives discharge from bogs within its boundaries. Plant species at the site included *Sphagnum* spp*., Chamaedaphne calyculata, Carex* spp*., Picea mariana* and *Betula pumila.* The PF basin was dominated by *Sphagnum* moss species (*Sphagnum fuscum* and *Sphagnum angustifolium*); however, distinct plant communities were observed in the north and south of the fen basin. The central part with lower WT had more trees than the northern and southern wetter parts, which were shrubbier. The peat depth was 4 m deep on average; however, thickness varied widely ranging from 1 m up to 10 m. The fen had an outflow through a culvert under a road at the west end of the fen. The area of PF was 7.29 ha.

RF was located ~20 km north of Fort McMurray (56° 56.330 N, 111° 32.934 W). It was a treed moderate-rich fen. This site was disturbed due to cutlines and dirt roads passing through the broader fen boundaries although the actual study area has not been directly impacted. The site was dominated by *Larix laricina, Betula pumila*, *Equisetum fluviatile*, *Smilcina trifolia*, *Carex* spp. and brown mosses, largely *Tomenthypnum nitens*. The peat is about 1 m thick. This site had two culverts for discharge, which pass under a dirt road on the west side of the site.

SF was located 10 km south of Fort McMurray (56° 34.398 N, 111° 16.518 W). This fen was dominated by *Juncus balticus, Calamagrostis stricta* and *Triglochin maritima*. It was an

extremely saline site due to its geological setting that resulted in discharge of saline groundwater (Wells, 2014). The site was surrounded by forested peatland but there were no trees in the study area. This site was less disturbed by human activities than the other reference fens. There was no culvert for water discharge but excess water runoff was moving towards the west into the forested portion of the peatland.

3.2.2 Constructed (Nikanotee) Fen

The constructed fen (CF) is ~30 km north of Fort McMurray (56° 55.8701 N, 111° 25.0166 W) built on an area abandoned by Suncor after oil sands extraction. A low permeability geotextile liner was placed over the target site; petroleum coke forms the aquifer system, overlying the liner and extending partway up the slope. At the lower end of the system, peat from a moderate-rich fen (2 m thick) was placed over the petroleum coke aquifer to form the fen. The upland area, which contributes groundwater to the fen, was covered with boreal forest soils and revegetated primarily with ericaceous shrubs and a sparse cover of trees including from *Larix laricina* and *Picea mariana* near the fen and *Pinus banksiana* (Jack pine) at the uppermost part of the upland in fall 2013. Finally, plant species (dominant in nearby peatlands) were introduced in the last week of June 2013 in CF. A variety of planting treatments were spread across the fen including moss transfer (Quinty and Rochefort, 2003), planted sedge and shrub seedlings (including *Carex aquatilis, Juncus balticus, Betula glandulosa, Calamagrostis inexpansa, Triglochin maritima*), and seeding with native peatland species. The land surface was covered with wood mulch on half of all treatments so that loss of water by evaporation could be reduced. A spill-box was also installed in the fen basin in order to prevent flooding during snow melt and storm events. The area of CF is 2.9 ha.

3.3 Methods

3.3.1 Sampling

For the DOC production and bioavailability study, dominant plants/peat from the reference fens and other substrates from CF were collected on July 11, 2013 as listed in Table 3.1 and stored in plastic bags at -10°C until analysis.

3.3.2 DOC production by incubation

From each field sample, ~10 g of fresh substrate was placed in a clear 500 ml mason jar filled with 250 ml of deionized water (DI) in triplicate (Strack *et al.,* 2011). Similarly, a second set of incubation jars in triplicate were prepared for each substrate but in saline water. An additional two sets of mason jars, one set containing only distilled water and the other set with saline water, in triplicate, were used as blanks. Then, 20 ml of nutrient solution (Appendix B, Table B3) was added in each jar (McDowell *et al.,* 2006). Saline solution used for incubation was prepared based on the mean concentration of cations and anions present in the pore water collected from CF at different depths in the peat (Appendix B, Table B1). The ion concentrations were determined using ion chromatography and titration technique in Environmental Sciences Laboratory, University of Calgary. The mean of the ion concentrations from the different samples were considered for saline water preparation. The salts used to create the saline solution were $CaCO_3$, $MgSO_4$, KCl and $NaHCO_3$ (Appendix B, Table B2).

All samples were capped and incubated, with one set at 10°C and the other set at 25°C (Figure 3.1), for 60 days inside growing chambers in dark conditions at the Department of Biological Sciences, University of Calgary. Using a plastic syringe, 50 ml of water was collected from all the jars on day 30 of incubation and 50 ml of DI water was added at the same time immediately after sampling. The jars were further incubated until day 60 and 100 ml of water sample was collected. Out of the 100 ml, 50 ml was used for DOC concentration and chemistry analysis, whereas the remaining 50 ml was used for the bioavailability study following filtration.

3.3.3 DOC analysis

All the water samples were prefiltered with a 1.5 μm glass fibre filter (Sterlitech 934-AH), followed by 0.4 μm glass fibre filter (Sterlitech GB-140) and pH and EC were recorded on filtered samples using YSI^{TM}-63 Multi-Parameter Probe. These samples were stored at 4°C until further analysis.

For absorbance measurement, all samples were analyzed using a PerkinElmer UV/VIS Spectrophotometer Lambda 35 to measure absorbance at 250 nm, 254 nm, 365 nm, 400 nm, 465 nm and 665 nm wavelengths. The absorbance ratios (E2/E3, E4/E6) and SUVA$_{254}$ were

determined for DOC chemistry analysis. The absorbance by DOC of each sample was measured in three different ways: natural sample, diluted sample with DI water (1:1 ratio) and neutralized samples using $NaHCO_3$. The absorbance ratios (E2/E3 and E4/E6) were calculated from neutralized samples whereas $SUVA_{254}$ was measured from diluted samples due to very high DOC concentrations in some of the natural samples.

For DOC measurement, all the samples were first diluted at 1:10 ratio in DI water and then acidified using concentrated HCl to lower the pH to ~2. The samples with higher DOC concentration (>100 mg/L) were reanalyzed with further dilution at 1:100 ratio in DI water. The concentration of DOC was determined using a Shimadzu TOC (Total Organic Carbon) analyzer (Environmental Sciences Program, University of Calgary) by Non-Purgeable Organic Carbon (NPOC) method. The sample was sparged with gas, which converts all inorganic carbon (IC) in the sample to carbon dioxide and drives the CO_2 out of the sample solution. The TOC concentration was determined by measuring the total carbon (TC) of the sample after the IC is eliminated.

3.3.5 DOC bioavailability analysis

After filtration, a 50 ml water sample, collected on day 60 of incubation, was transferred to a 125 ml incubation jar with a septum cap. A microbial inoculum was made by mixing 1 g of peat with 10 mL of DI water (Appendix B, Table B4), then 315 µl of inoculum was added to each jar and the mass of DOC added to each sample was corrected for by adding the mean DOC of triplicate controls made from DI mixed with 315 µl of inoculum to each sample's initial DOC mass (McDowell *et al.,* 2006).

The jars were incubated at room temperature (22ºC) in dark condition for 7 days to investigate DOC bioavailability. From each jar 20 ml of gas was collected in an evacuated vial from the headspace on day 0, 1, 2, 4 and 7 using a syringe, with subsequent addition of 20 ml nitrogen to maintain pressure inside the incubation jar. The concentrations of CO_2 and CH_4 in collected gas samples were measured using gas chromatography on a Varian 3800 gas chromatograph equipped with thermal conductivity detector and flame ionization detector. The rate of CO_2 or CH_4 release for each jar was determined from the linear increase in CO_2/CH_4 concentration in

the jar over time after correcting for dilution with N_2 during each sampling. DOC samples after seven days incubation were further analyzed for DOC concentration and chemistry using the same method as described above. The mass of DOC depleted and CO_2 released during incubation were used to estimate the bioavailability of DOC.

3.3.6 Data analysis

The effect of substrate type, salinity and temperature on DOC production was analyzed using an estimating simple main effects model ANOVA. The data sets were found heterogeneous using the Bonferroni test. According to Zar (1999), the analysis of variance is robust, operating well even with considerable heterogeneity of variances, as long as all sample sizes are equal or nearly equal. Therefore, *Juncus balticus* and *Carex aquatilis* (from CF), which did not have enough sample available to incubate in all treatment conditions, were not included in the DOC production model. The effect of substrate, salinity and temperature on DOC chemistry was investigated using estimating simple main effects model ANOVA. All two-way and three-way interactions among variables were also included in the models. The effect of DOC chemistry on bioavailability was investigated using a general linear model (GLM) regression. Moreover, the effect of substrate, temperature and salinity on DOC bioavailability was analyzed using ANOVA, where substrate and temperature were categorical variables. In all cases, the relationship between dependent and explanatory variables was considered significant if $p<0.05$. All statistical analyses were performed using IBM SPSS statistics version 22 and excel 2013.

In order to investigate the effect of temperature on DOC production from each substrate, Q_{10} was calculated according to:

$$Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)}$$

Where, R_1 and R_2 are the rates of net DOC production at the temperatures T_1 and T_2, respectively and T_1 and T_2 represent 10 °C and 25 °C in this study.

3.4 Results

Altogether 402 samples were analyzed in this study that were produced from 18 different substrates in triplicate in various treatment conditions (in DI and saline water at 10 °C and 25 °C). Due to limited availability of substrate, *Juncus balticus* from CF was incubated at 25 °C in saline water only, whereas *Carex aquatilis* from CF was incubated in both DI and saline water conditions at 25°C only.

3.4.1 DOC Production

All of the explanatory variables (substrates, temperature and salinity), including interactions among them, showed highly significant effects on net DOC production, except the interaction between temperature and salinity (Table 3.2). Furthermore, substrate type explained the most variation in net DOC production, followed by temperature and salinity. Significant interactions between substrate type and temperature, and substrate type and salinity indicate that the effect of local conditions on net DOC production rate is substrate dependent.

The large range of net DOC production rate among the various substrates tested is evident in Figure 3.2. Sedges and trees had large net DOC production per unit mass, whereas peat and moss produced very little DOC. Other substrates like petroleum coke, tailings sand and upland soil produced almost no DOC on a per mass basis. In CF, there were a wide range of substrates in terms of net DOC production including both the highest and the lowest producers. SF also had abundant DOC-producing vegetation such as *Calamagrostis stricta* and *Triglochin maritima*.

Figure 3.2 illustrates that the higher incubation temperature (25 °C) was more favourable for producing DOC than the lower temperature (10 °C). Furthermore, salinity showed mixed effect on DOC production depending on substrate; however, there seems to be a stronger increasing effect of salinity on net DOC production at the higher temperature especially for reference fen vegetation. This resulted in the significant substrate-temperature-salinity interaction (Table 3.2) in the ANOVA. For example, *Picea mariana, Carex aquatilis, Calamagrostis stricta* and *Triglochin maritima* have higher net DOC production at 25 °C in saline condition than at 10 °C in DI water. In contrast, substrates from CF like *Carex aquatilis*, moss and peat produced more DOC in DI water than saline water at 25 °C.

Table 3.3 shows that the temperature effect on net production of DOC (Q_{10}) from most of the substrates was higher on 30 day than 60 day production rates in both DI and saline water. In contrast, petroleum coke and peat from CF had higher Q_{10} considering data after 60 days in DI water. *Chamaedaphne calyculata* had a steady temperature effect on net DOC production in all conditions and duration. In addition, peat (CF), tailings sand, *Calamagrostis stricta* and trees had higher Q_{10} in saline water, whereas moss, sedges, tailings sand, upland soil and peat (RF/PF) showed higher Q_{10} in DI water on day 30. Peat (SF) had similar Q_{10} in both DI and saline water on day 30. On the other hand, Q_{10} on day 60 was lower in DI water in comparison to saline water from moss (RF), trees, *Calamagrostis stricta* and peat (SF).

Figure 3.3 shows that DOC production is positively and negatively related to EC and pH, respectively. There is a cluster of points at the origin in both cases. However, the relationship still seems quite strong in each case ($R^2 = 78.5\%$ and 89% for DOC vs. EC and DOC vs. pH respectively, $p < 0.05$). EC was found to increase with DOC production (Figure 3.3A). For example, EC in *Juncus balticus* jars after 60 days incubation in saline water at 25 °C increased to 3520 μS/cm from an initial value of 650 μS/cm. During this period the mass of DOC produced was 100.3 mg. In contrast, other substrates like *Sphagnum*, peat, petroleum coke, tailings sand and upland soil produced DOC mass <0.5 mg, so the EC changed little. On the other hand, production of DOC resulted in reduced pH (Figure 3.3B). For instance, substrates like *Juncus balticus* incubated at 25 °C in saline water resulted in mass DOC production of 102.0 mg which reduced the pH from 8.0 to 5.5. Substrates that resulted in DOC mass production <0.5 mg did not affect pH, so clustered along the x-axis.

Substrate, salinity, and the interaction between temperature and substrate had significant effects on E2/E3 variation (Table 3.4). Furthermore, substrate type had more impact than any other variable. Temperature and other interactions remained insignificant in determining E2/E3. This model was able to explain 57.8% of the variability of E2/E3. In addition, all explanatory variables (substrate, temperature and salinity) including the interaction between temperature and substrate were significant in describing variation in E4/E6 (Table 3.5). Substrate had the highest impact followed by salinity and temperature. This model was able to explain 70.8% of the variability in E4/E6. Moreover, substrate, salinity and interactions among variables (except temperature and salinity) significantly explained the variation in SUVA$_{254}$ (Table 3.6). In

contrast, temperature did not show any effect on $SUVA_{254}$. This model was able to explain 52.2% variability of $SUVA_{254}$.

Considering absorbance ratios (E2/E3 and E4/E6) of DOC produced from different substrates in various treatments (at different temperature and salinity), no remarkably distinguishable pattern was observed (Figure 3.4A, B). However, $SUVA_{254}$ showed a very interesting result of DOC chemistry with regard to different substrates (Figure 3.4C). It is evident that higher net DOC producers such as *Carex aquatilis, Juncus balticus, Larix laricina, Picea mariana, Chamaedaphne calyculata, Calamagrostis stricta* and *Triglochin maritima* had lower $SUVA_{254}$ values, whereas lower DOC producers like moss, petroleum coke, tailings sand, upland soil, peat and *Sphagnum* had higher $SUVA_{254}$ values. Furthermore, petroleum coke and tailings sand, which produced very little DOC, had the highest $SUVA_{254}$ among the substrates. In addition, $SUVA_{254}$ values were lower at 25 °C but higher at 10 °C in both DI and saline water. There was a negative correlation between E2/E3 and $SUVA_{254}$ (Figure 3.5). Similarly, the relationship between DOC production and $SUVA_{254}$ was also negative (Figure 3.6).

3.4.2 Bioavailability

Bioavailability of DOC from various substrates produced under different treatment conditions was analyzed using ANOVA. Substrate, EC and temperature were independent variables, whereas percentage of DOC depleted was dependent variable. All the variables including their interactions were found to significantly explain the variation in bioavailability of DOC (Table 3.7). Substrates had the most effect in determining DOC bioavailability followed by EC and temperature. Here, temperature represents the temperature at the time of DOC production as all DOC samples were incubated at room temperature for the bioavailability study.

Bioavailability of DOC produced from various substrates under different treatment conditions was also analyzed using GLM regression model, where E2/E3, E4/E6 and $SUVA_{254}$ were the explanatory variables and mass of DOC depleted during the seven-day incubation was the dependent variable. Among the explanatory variables, E2/E3 and E4/E6 showed a significant impact on bioavailability of DOC with both having a positive correlation to DOC depletion (Table 3.8).

Figure 3.7 shows how the DOC produced from different substrates were consumed in different treatment conditions. In addition to the effect of substrate type on the DOC bioavailability, temperature in both saline and DI water showed positive impact on DOC bioavailability. Interestingly, poor substrates for DOC production like petroleum coke, tailings sand, upland soil, peat appears to be as bioavailable as high-DOC producing substrates like *Carex aquatilis, Juncus balticus, Larix laricina, Picea mariana, Chamaedaphne calyculata, Calamagrostis stricta* and *Triglochin maritima*.

The relationship between the DOC depleted and CO_2 released shows that CO_2 release increases with the consumption of DOC (Figure 3.8). DOC consumed from different substrates shows that when more DOC was consumed, larger amounts of CO_2 were also released in both DI and saline water treatments. This is likely due to the much larger amount of DOC available for consumption in these water samples. Only 9% of the DOC consumed was converted to CO_2 and <1% was converted to CH_4 suggesting that the remainder was utilized in microbial growth or converted to insoluble compounds.

3.5 Discussion

In evaluating the success of the constructed fen design at Suncor, three diverse natural fens around the constructed fen region were assessed to compare the ecosystem functioning of the constructed fen. Since vegetation community and environmental factors play a vital role in the development of peatland, dominant vegetation and other substrates including peat collected from constructed and reference fens were studied for DOC production and bioavailability in various environmental conditions. Even though the plant community composition might change in future, the current study can help understand how that will impact the DOC dynamics by providing information on the potential for DOC production and the chemistry arising from different sources.

3.5.1 Controls on DOC production

In the present study, the effect of substrate quality on decomposition leading to DOC production was studied by incubating different substrates in various environmental conditions in the laboratory. Substrate type, temperature and salinity were all important factors in determining net DOC production. Substrate quality has usually been described as being dependent on the

substrate type. Generally, litters with high concentrations of soluble carbon compounds, low concentrations of lignin or lignin-like phenolic polymers and high nutrient concentrations decompose relatively quickly (Bartsch and Moore, 1985; Limpens and Berendse, 2003; Preston and Trofymow, 2000; Scheffer *et al.,* 2001; Szumigalski and Bayley, 1996; Thormann *et al.,* 2001). In addition, microbes are active and grow faster in warmer environmental conditions consequently increasing the decomposition process resulting in higher DOC production (Kalbitz *et al.,* 2004). Furthermore, higher DOC production observed in the presence of salts may be due to flocculation, which provide better attachment for microbial colonies enhancing microbial activities leading to faster decomposition (Marschner and Kalbitz, 2003).

The type of vegetation community dominant on a peatland, which is the major source of organic matter, plays a vital role in DOC production. The three reference fens were diverse in terms of biogeochemical properties and so were the dominant vegetation species. Sedges and trees produced a greater concentration of DOC, followed by peat and moss in all the fens, whereas the substrates from CF such as petroleum coke, tailings sand and upland soil produced almost nothing. Hence, vascular vegetation is the major contributor to DOC production in these fens while construction materials at CF are likely only small sources. Since the composition and proportion of substrates dominant in each fen is one of the important factors in determining the production of DOC, the concentration of DOC in the CF was the lowest among all the sites (see Chapter 2) because most of the substrates in CF are poor sources of DOC except for a few of the newly planted vegetation species like *Juncus balticus* and *Carex aquatilis*. Similarly, SF and PF had dominant vegetation with high rates of DOC production, hence their soil pore water DOC concentration was higher (see Chapter 2). In addition, plant litter decomposition rate varies not only among plant types but also between different parts of the same plant. For example, leaf and root litters from herbs and sedges decompose relatively fast (51–75% mass loss during 2 years), moss litters decompose slowly (9–32% mass loss during 2 years) and arboreal litters stand in between (Scheffer *et al*., 2001; Szumigalski and Bayley, 1996; Thormann *et al*., 2001). We investigated DOC production only above ground components of plants in this study although incubated peat likely included some belowground biomass components.

While microbial production and consumption of DOC are temperature dependent, absorption is temperature independent (Kalbitz *et al.,* 2000). Thus, the effect of temperature on DOC

production has varied between studies. Moore and Dalva (2001) and Strack *et al.,* (2011) reported Q_{10} values of 1.0 –1.5 and Christ and David (1996) found a constant Q_{10} close to 2 was appropriate over a wide range of temperatures. In contrast, MacDonald *et al.* (1999) reported little change in DOC production with incubation temperature. In the present study, temperature and salinity were significant explanatory variables for both initial and final net DOC production rates. Calculated Q_{10} values were similar to those previously reported by Strack *et al.,* (2011) except moss, which had Q_{10} of 4.2 and 3.5 for initial and final incubations in DI water, respectively.

The current study showed that production of DOC had a profound influence on pH and EC of the incubation water. As the DOC production increased, pH was lowered due to the release of various types of organic acids (fulvic and humic acids). This is one of the reasons why peatlands are acidic in nature (Charman, 2002). In addition, the change of pH in DI water was higher than saline water likely because cations present in the saline water neutralized the free hydrogen ions produced from organic acids thereby buffering the changes in pH. This is representative of what happens in bogs and fens. Fens interact with groundwater rich in minerals, which controls the change of pH resulting in more neutral conditions. On the other hand, bogs receives precipitation water only at their surface, which is low in mineral content and results in a lower pH due to lack of buffering mechanisms. On the other hand, production of DOC increased EC. This change of EC is probably due to charges on functional groups of produced DOC and release of minerals from substrates after decomposition. Therefore, production of DOC also affects the soil solution conditions including pH and EC. This supports the idea that peatland ecosystem characteristics are determined by the interaction of different biogeochemical and environmental factors (Laiho, 2006; Wheeler and Proctor, 2000; Økland *et al.,* 2001; Hobbie, 1996; Borga *et al.,* 1994; Panikov, 1999).

3.5.2 Controls on DOC quality

The quality of DOC is determined by its source and environmental factors. Molecular size and aromaticity of humic substances in DOC are controlled primarily by substrate type and salinity. Sedges produced DOC with lower molecular size and less aromatic humic substances than peat, tailings sand, and petroleum coke based on absorbance ratios (E2/E3 and E4/E6). For example,

the E2/E3 of *Carex aquatilis, Calamagrostis stricta, and Triglochin maritima* was > 2.0 but E2/E3 of upland soil and petroleum coke was nearly 1.5. In addition, higher $SUVA_{254}$ values found in the DOC produced from substrates collected from CF (except *Juncus* and *Carex*) reveals the presence of more aromatic DOC, which is harder to mineralize by microbes. This was likely one of the reasons why DOC concentration in CF was the lowest among the fens (see Chapter 2). Salinity affects the chemical composition of DOC because it activates microbes by providing nutrients, which escalates the decomposition of organic matter resulting in simpler molecules due to cleavage of complex compounds. In addition to substrate type and salinity, temperature controls the process of humification *i.e.* warmer temperature tends to result in production of simpler molecules like fulvic acids, whereas colder temperature results in complex molecules like humic acids (Bourbonniere, 1989; Guggenberger *et al.,* 1994). Microbial activity can certainly be the cause of this shift as at higher temperature microbes are more active and can degrade large molecules to smaller ones (Bourbonniere, 1989; Guggenberger *et al.,* 1994).

The quality of the substrate affects its decomposition rate. The more aromatic components that make up the DOC, the slower the decomposition process. This is evidence from the negative relationship between $SUVA_{254}$ and DOC production. A high $SUVA_{254}$ value indicates more aromatic DOC, which is more recalcitrant and may reflect a substrate that is more difficult to decompose to produce DOC. The differences in $SUVA_{254}$ of DOC produced from various CF substrates can be clearly observed. Substrates like petroleum coke, tailings sand, upland soil and peat had higher $SUVA_{254}$, and consequently lower overall production of DOC. In contrast, lower $SUVA_{254}$ values reflect less aromatic DOC resulting from easily decomposable substrates and thus leading to greater production of DOC. For instance, *Carex aquatilis* and *Juncus balticus* in CF, which produced large amounts of DOC, had very low $SUVA_{254}$.

3.5.3 Bioavailability of DOC

DOC represents a potential supply of energy and organic nutrients to soil microflora. Microbial degradation of DOC can regulate the production of greenhouse gases such as CO_2, CH_4 and N_2O (Yavitt, 1997; Zsolnay, 1996; Lu *et al.,* 2000). Researchers have focused on production of CO_2 or consumption of DOC as measures of DOC bioavailability. The most widely used method appears to be the measurement of DOC disappearance. However, both the methods were used in this study.

The present study showed that substrate type, salinity and temperature at which the DOC was produced, and interactions among these variables had significant effects on DOC bioavailability. As described in the previous section (3.5.1 Controls on DOC production), these factors help to convert complex organic molecules to simpler ones making them easier for microbes to interact with and resulting in the increase of bioavailability.

Interestingly, the present study indicated that salinity and temperature under which the DOC was produced had a significant interaction with substrate for DOC bioavailability. Therefore, some substrates showed positive, whereas others negative, effects on DOC bioavailability in the presence of higher salinity and temperature. For example, salinity had a positive effect on peat (CF) and *Sphagnum angustifolium* (PF) DOC bioavailability, whereas all other substrates showed a negative effect. Similarly, the effect of increasing temperature was positive for all substrates except SF peat. This suggests that substrate itself did not have the sole control on DOC bioavailability but depended on the environmental factors under which the DOC was produced.

Molecular size and aromaticity of DOC produced from these substrates appear to be correlated to DOC bioavailability, since E2/E3 and E4/E6 were statistically significant in determining DOC bioavailability. Similarly, SUVA has been used as a surrogate for aromatic carbon in soil and aquatic humic substances (Kalbitz *et al.,* 2003; Traina *et al.,* 1990). Higher $SUVA_{254}$ values indicate an increase in the relative proportion of aromatic carbon in the DOC. Furthermore, the negative relationship between E2/E3 and $SUVA_{254}$ indicated that smaller molecules in DOC were more bioavailable. A number of researchers have also found that carbohydrates and amino acids are highly decomposable in soils and are utilized preferentially by microorganisms during degradation of different compounds in DOC solutions (Amon *et al.,* 2001; Haider, 1992; Kalbitz *et al.,* 2003; Volk *et al.,* 1997). However, Amon and Benner (1996) illustrated the complexity of the issue by reporting that low-molecular DOC was less degradable than high-molecular DOC. Therefore, Volk *et al.* (1997) stated that the often-used classification of carbohydrates as labile DOC components should be seen with caution, as carbohydrates can also be bound to stable DOC compounds. In their extensive review on DOC dynamics in soils, Kalbitz *et al.* (2000) also point out that the mechanisms and controls of DOC degradation in soils are still poorly understood.

In the present study, bioavailability analysis was based on the percentage of DOC depleted during incubation, whereas DOC production widely varied among the samples. Therefore, even though percentage of DOC depletion was similar in two different samples having different DOC concentrations, the total mass of DOC consumed was hugely different *i.e.,* higher total mass consumed in concentrated DOC samples. For instance, higher DOC producing substrates like *Carex aquatilis, Calamagrostis stricta* and *Triglochin maritima* had almost similar bioavailability (percentage of DOC depletion) as the very low DOC producing substrates like petroleum coke, tailings sand, upland soil and peat. Therefore, using a uniform concentration of DOC produced from different substrates may give more reliable results for DOC bioavailability studies in the future.

Laboratory experiments have shown that within the range of environmental conditions found in peatlands, CO_2 evolution acts as an indicator of organic matter decomposition (Blodau *et al.,* 2004). In the current study also, the release of CO_2 increased with the increased consumption of DOC indicating the relationship between CO_2 evolution and DOC decomposition. It was also observed that a large proportion of DOC (nearly 90% average ranging from 50 – 98% depending upon substrate) was likely utilized for microbial growth. Remaining DOC was mostly released in the form of CO_2 (average 9%) and very little in the form of CH_4 (<1%) as a by-product, probably due to oxic incubation condition. This indicates that when the DOC is exported to the downstream ecosystem, CO_2 would be the major GHG released if it is decomposed in a largely oxic aquatic environment.

The major obstacle for a better understanding of the controls of DOC biodegradability is lack of a standardized methodology or at least systematic comparisons between various methods used to assess DOC biodegradability. The high variability in incubation durations, inoculum and/or nutrient additions and the different measures for the quantification of DOC degradation greatly hinder comparisons between studies (Marschnera and Kalbitz, 2003). Therefore, efforts should be put in place for the development of a standardized protocol for DOC degradation studies. With this in mind, the consistent method used in this study across all substrate types suggests that DOC produced at the constructed fen will have similar bioavailability as that produced in natural systems. If more saline conditions develop at the site due to the availability of salts in

tailings sand, results from these incubations suggest that DOC may be more rapidly degraded than in a freshwater system.

3.6 Conclusion

This study compared the production and bioavailability of DOC released from different substrates collected from constructed and natural fens in boreal Alberta. Sedges and trees produced the most DOC followed by peat and moss in all the fens, whereas the substrates from the CF such as petroleum coke, tailings sand and upland soil produced very little DOC. In addition, temperature and salinity were also significant explanatory variables for both initial (30 day) and final (60 day) net DOC production rates. Calculated Q_{10} values were mostly in between 1.0 - 2.0 except for moss, which had higher initial and final values especially in deionized water. It was also observed that greater production of DOC resulted in lower pH and higher EC in the incubating water.

The chemistry of DOC produced depended upon the type of substrate. Sedges had lower molecular size and lower aromatic humic substances in DOC than peat, tailings sand, upland soil and petroleum coke, which was reflected in $SUVA_{254}$. This was further supported by the negative relationship between $SUVA_{254}$ and DOC production. Salinity resulted in greater DOC production likely by providing nutrients that activated the microbial community and escalated the decomposition of organic matter. In addition, temperature also controlled the process of humification *i.e.* higher temperature resulted in simpler molecules like fulvic acids, whereas colder temperature resulted in complex molecules like humic acids. Substrate, salinity, temperature and interaction among these factors were found to influence the bioavailability of DOC. In addition, chemistry of the DOC produced from these substrates had a significant effect on DOC bioavailability, as expressed by E2/E3 and E4/E6. The relationship between the release of CO_2 and depletion of DOC was clearly observed in this study, but CO_2 release accounted for only 9% of the total DOC consumed. Very little CH_4 was produced from the DOC (<1% of DOC depleted), likely because incubations were conducted in oxic conditions.

This study showed that vegetation type, in association with temperature and salinity, play important roles in determining the DOC dynamics in peatlands. Most of the substrates from the

constructed fen resulted in production of DOC that had high molecular weight and aromatic components. However, the newly planted vegetation in CF had greater DOC production and bioavailability due to higher fulvic content in DOC. Since field sampled DOC from the constructed fen had lower E2/E3 than reference fens in the region (see Chapter 2), it is likely that the newly planted vegetation made a minimal contribution to DOC in the first year post-construction. Therefore, establishment of vegetation community and its composition will determine future DOC dynamics at the constructed fen.

3.7 References

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Table 3.1: List of dominant vegetation and other substrates used for this study.

Site No.	Site	Substrate No.	Vegetation and other Substrates
1	Constructed (Nikanotee) Fen	1	Peat
		2	*Carex aquatilis*
		3	A mix of moss and vascular plants from donor site
		4	*Juncus balticus*
		5	Petroleum coke
		6	Tailings sand
		7	Upland cover soil
2	Rich Fen	8	Mix of brown mosses, mostly *Tomenthypnum nitens*
		9	*Larix laricina*
		10	Peat
3	Poor Fen	11	*Picea mariana*
		12	*Sphagnum angustifolium*
		13	*Carex aquatilis*
		14	Peat
		15	*Chamaedaphne calyculata*
4	Saline Fen	16	Peat
		17	*Calamagrostis stricta*
		18	*Triglochin maritima*

Table 3.2: Result of the model using estimating simple main effects ANOVA evaluating the impact of temperature, substrates and salinity in DOC production.

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	0.330[a]	63	0.0052	222.27	<0.01
Intercept	0.1176	1	0.1176	4990.00	<0.01
Temperature	0.010	1	0.0101	428.12	<0.01
Substrates	0.2762	15	0.0184	781.10	<0.01
Salinity	0.0001	1	0.0001	6.10	<0.01
Temperature * Substrates	0.0394	15	0.0026	111.56	<0.01
Temperature * Salinity	0.0001	1	0.0001	2.36	0.12
Substrates * Salinity	0.0031	15	0.0002	8.84	<0.01
Temperature * Substrates * Salinity	0.0010	15	0.0001	2.92	<0.01
Error	0.0075	320	0.00002		
Total	0.4554	384			
Corrected Total	0.3377	383			

Table 3.3: Q_{10} for net DOC production rate determined from substrate incubation.

Site	Substrate type	Initial 30 day net DOC Production rate		Final 60 day net DOC production rate	
		DI	SW	DI	SW
	Moss	4.19	1.87	3.45	1.64
	Petroleum coke	0.59	1.48	1.35	1.06
	Peat	1.33	1.40	1.60	1.33
	Tailings sand	1.19	0.91	1.68	0.99
CF	Upland soil	1.48	1.28	1.21	1.24
	Moss	1.41	2.45	1.21	1.34
	Peat	1.53	1.44	1.24	1.05
RF	*Larix laricina*	1.34	1.89	1.18	1.30
	Picea mariana	1.14	1.25	1.02	1.12
	Carex aquatilis	3.01	2.63	2.02	1.72
	Chamaedaphne calyculata	0.98	0.87	0.98	0.88
	Peat	1.01	0.88	1.25	0.92
PF	*Sphagnum angustifolium*	1.22	1.04	1.14	0.83
	Calamagrostis stricta	1.28	1.72	1.33	1.79
	Peat	1.51	1.50	1.35	1.43
SF	*Triglochin maritima*	1.74	1.95	1.74	1.77

Table 3.4: Results of the model using estimating simple main effects ANOVA evaluating the impact of temperature, substrates and salinity in E2/E3.

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	31.14[a]	66	0.47	6.95	<0.01
Intercept	1038.20	1	1038.20	15294.27	<0.01
Temperature	0.14	1	0.14	2.07	0.15
Substrates	21.44	17	1.26	18.58	<0.01
Salinity	3.97	1	3.97	58.43	<0.01
Temperature * Substrates	2.65	15	0.17	2.60	<0.01
Temperature * Salinity	0.09	1	0.09	1.37	0.24
Substrates * Salinity	1.62	16	0.10	1.49	0.10
Temperature * Substrates * Salinity	0.95	15	0.06	0.93	0.53
Error	22.74	335	0.07		
Total	1120.93	402			
Corrected Total	53.88	401			

a. R^2 = 0.58 (Adjusted R^2 = 0.50)

Table 3.5: Results of the model using estimating simple main effects ANOVA evaluating the impact of temperature, substrates and salinity in E4/E6.

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	78.63[a]	66	1.19	12.29	<0.01
Intercept	866.70	1	866.70	8937.64	<0.01
Temperature	0.59	1	0.59	6.12	<0.01
Substrates	68.63	17	4.04	41.63	<0.01
Salinity	0.94	1	0.94	9.67	<0.01
Temperature * Substrates	6.11	15	0.41	4.19	<0.01
Temperature * Salinity	0.05	1	0.05	0.52	0.47
Substrates * Salinity	1.06	16	0.07	0.68	0.81
Temperature * Substrates * Salinity	1.21	15	0.08	0.83	0.64
Error	32.49	335	0.1		
Total	997.82	402			
Corrected Total	111.12	401			
a. $R^2 = 0.71$ (Adjusted $R^2 = 0.65$)					

Table 3.6: Results of the model using estimating simple main effects evaluating the impact of temperature, substrates and salinity in $SUVA_{254}$.

	Tests of Between-Subjects Effects				
Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	371.34[a]	66	5.63	5.55	<0.01
Intercept	42.07	1	42.07	41.51	<0.01
Temperature	3.03	1	3.03	2.99	0.08
Substrates	222.73	17	13.10	12.93	<0.01
Salinity	4.97	1	4.97	4.90	0.03
Temperature * Substrates	26.87	15	1.79	1.77	0.04
Temperature * Salinity	3.32	1	3.32	3.28	0.07
Substrates * Salinity	55.55	16	3.47	3.43	<0.01
Temperature * Substrates * Salinity	53.96	15	3.59	3.55	<0.01
Error	339.44	335	1.01		
Total	755.31	402			
Corrected Total	710.78	401			
a. $R^2 = 0.52$ (Adjusted $R^2 = 0.43$)					

Table 3.7: Results of ANOVA model evaluating the relationship between substrate, EC and temperature with percentage of DOC depleted.

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	65645.55[a]	47	1396.71	18.03	<0.01
Intercept	56326.62	1	56326.62	727.32	<0.01
EC	1825.79	1	1825.79	23.58	<0.01
Temperature	435.03	1	435.03	5.61	<0.01
Substrates	8375.27	15	558.35	7.21	<0.01
Substrates * EC	4817.62	15	321.17	4.15	<0.01
Substrates * Temperature	3090.72	15	206.05	2.66	<0.01
Error	11151.93	144	77.44		
Total	772558.98	192			
Corrected Total	76797.48	191			

a. $R^2 = 0.85$ (Adjusted $R^2 = 0.81$)

Table 3.8: Result of GLM regression model showing the relationship and strength of absorbance ratios (E2/E3 and E4/E6) and $SUVA_{254}$ on mass DOC depleted ($F = 48.14$, $P < 0.01$, $R^2 = 0.42$).

Coefficients

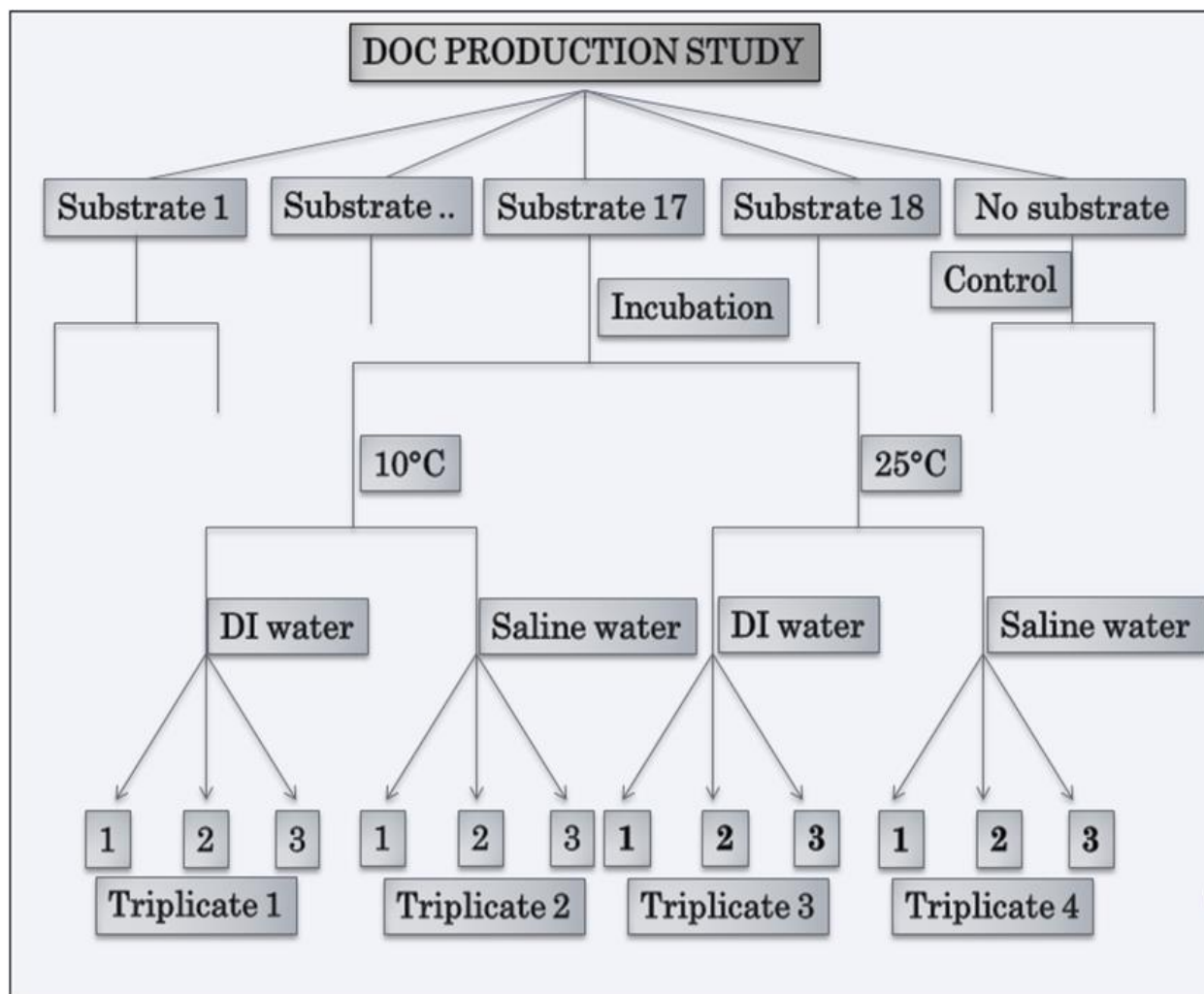|	B	Std. Error	T	P
(Constant)	-26.26	4.89	-5.38	<0.01
E2/E3	27.96	3.18	8.80	<0.01
E4/E6	21.39	6.09	3.51	<0.01
$SUVA_{254}$	-18.82	10.71	-1.76	0.08

Dependent Variable: DOC

Figure 3.1: Flowchart showing the DOC production experiment using 18 different types of substrates.
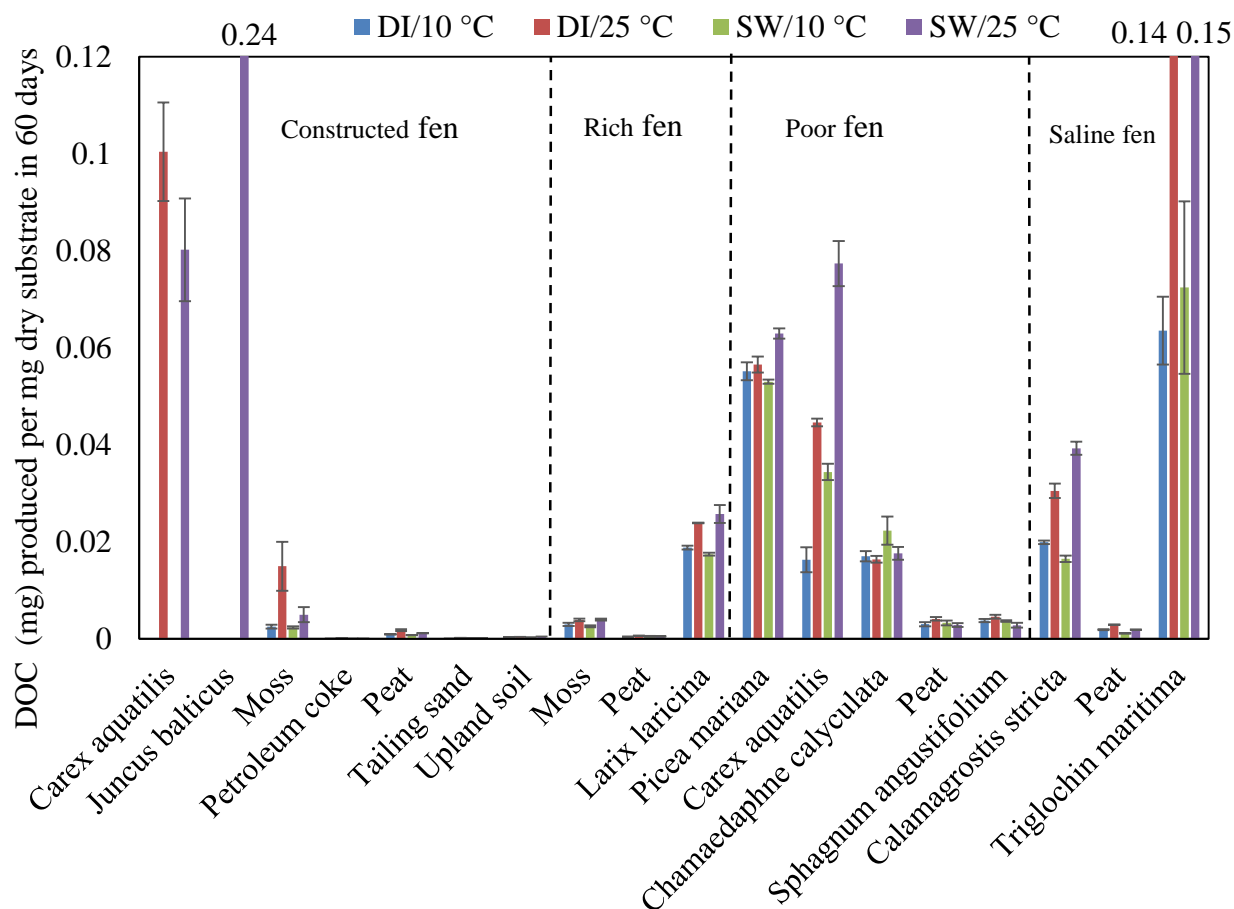
Figure 3.2: Comparison of DOC production per mg dry weight of each substrate at different treatments (DI/saline water and 10 °C/25 °C). The error bars give standard errors. *Juncus balticus* was studied at 25 °C saline water, whereas *Carex aquatilis* from CF was studied in both DI and saline water conditions but at 25°C only. Amount of DOC produced by *Juncus balticus* and *Triglochin maritima* is given at the top of the bar, as the bar is cut off to magnify the smaller bars.
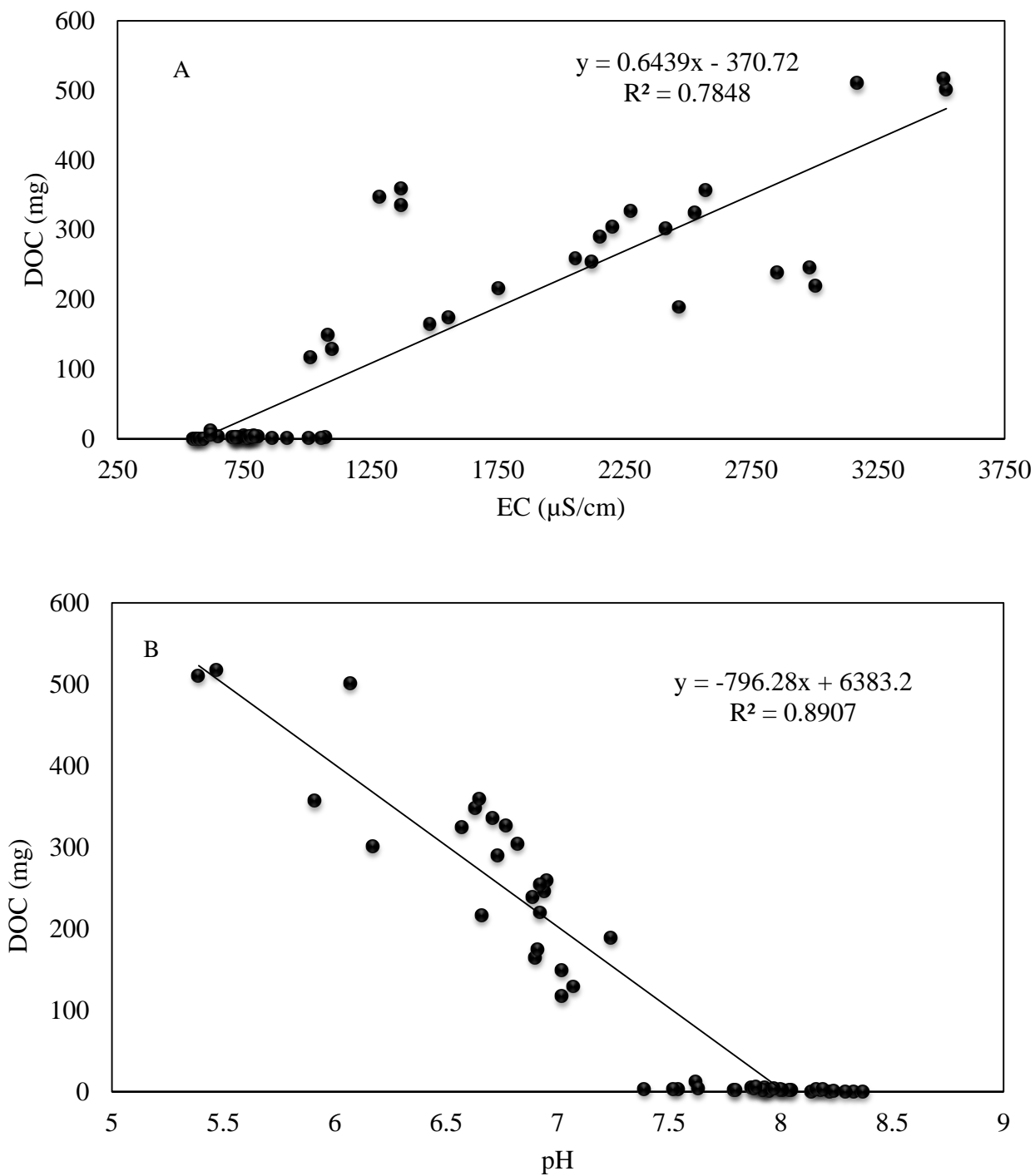
Figure 3.3: Correlation of DOC concentration with EC (A) and pH (B). DOC production is positively correlated with EC but negatively with pH. The data are clustered at the bottom in both the figures.
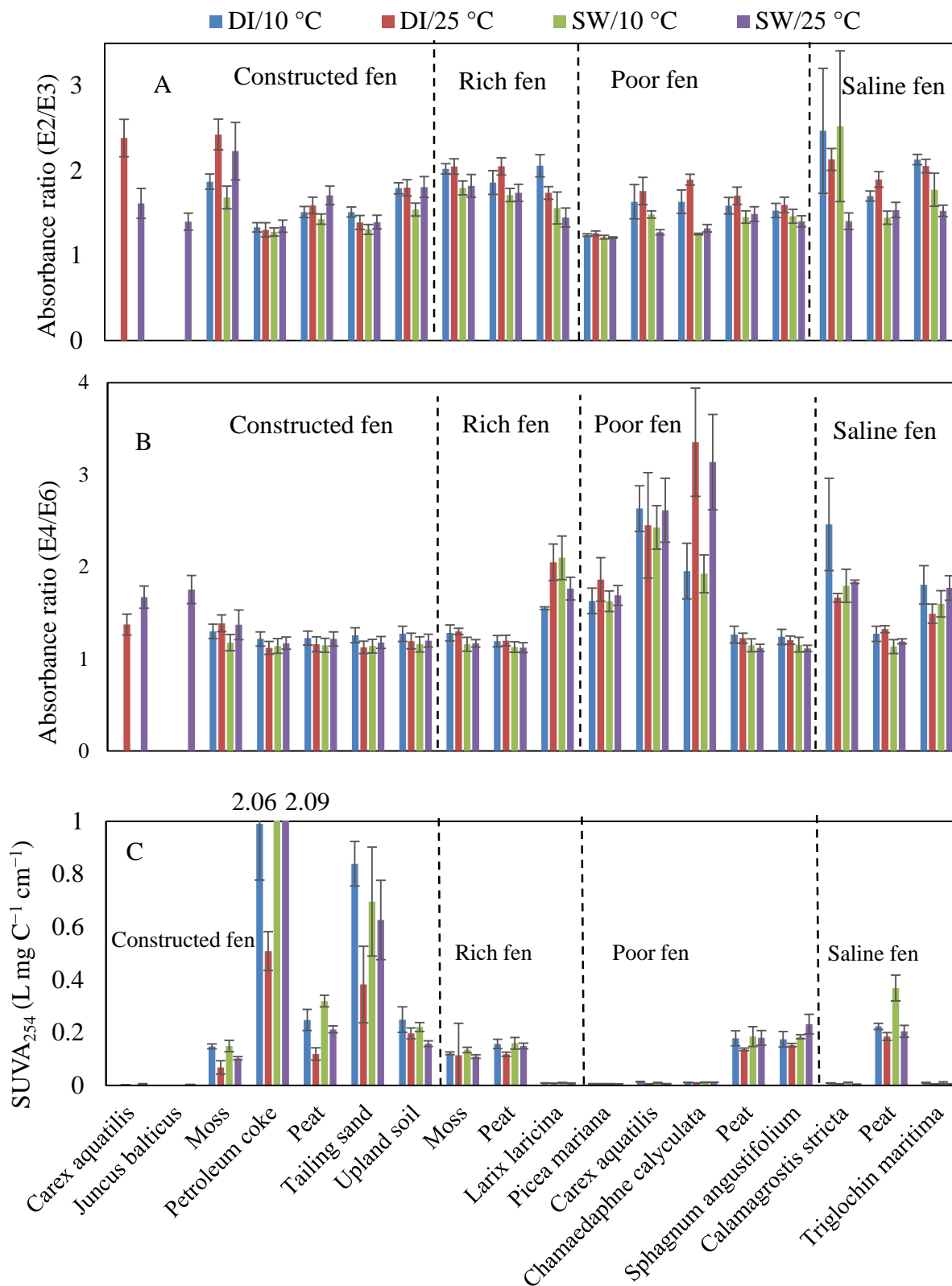
Figure 3.4: Comparison of E2/E3 (A), E4/E6 (B) and SUVA$_{254}$ (C) of DOC from different substrates. The error bars give standard errors *i.e.* deviation from the mean.
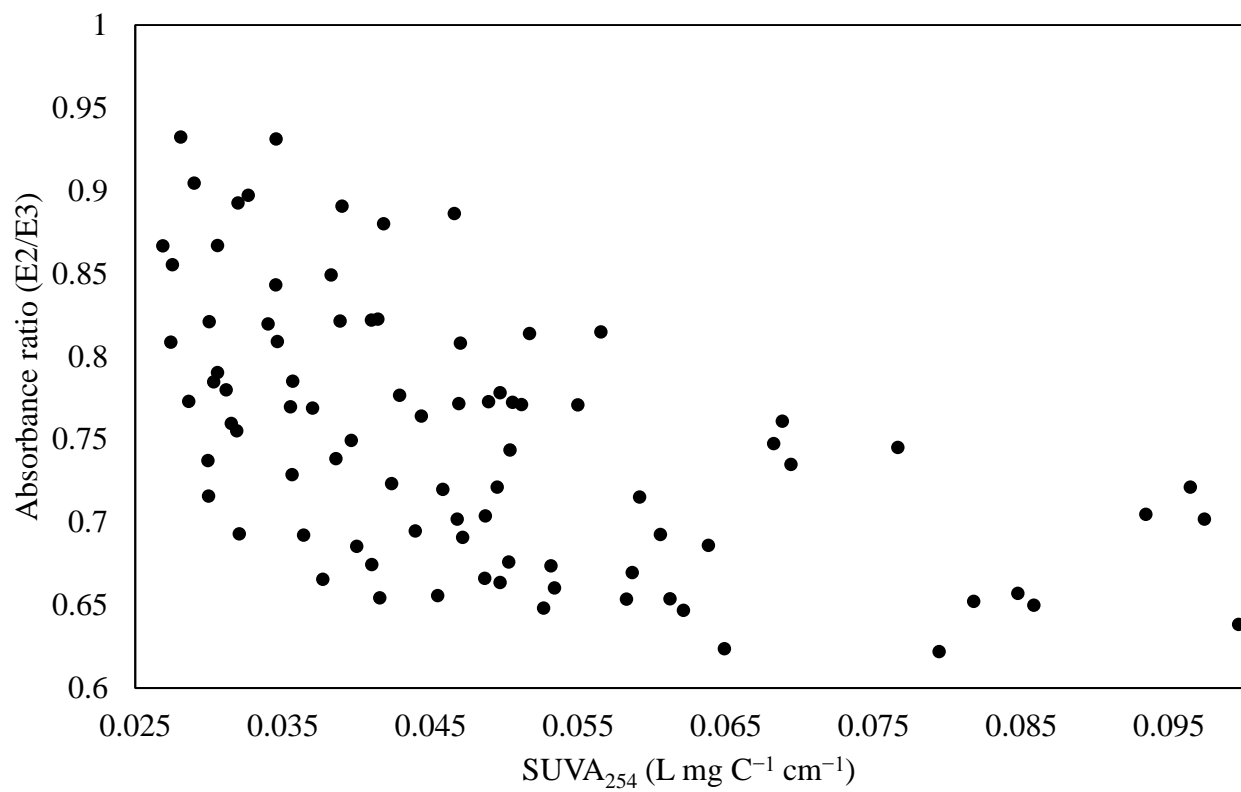
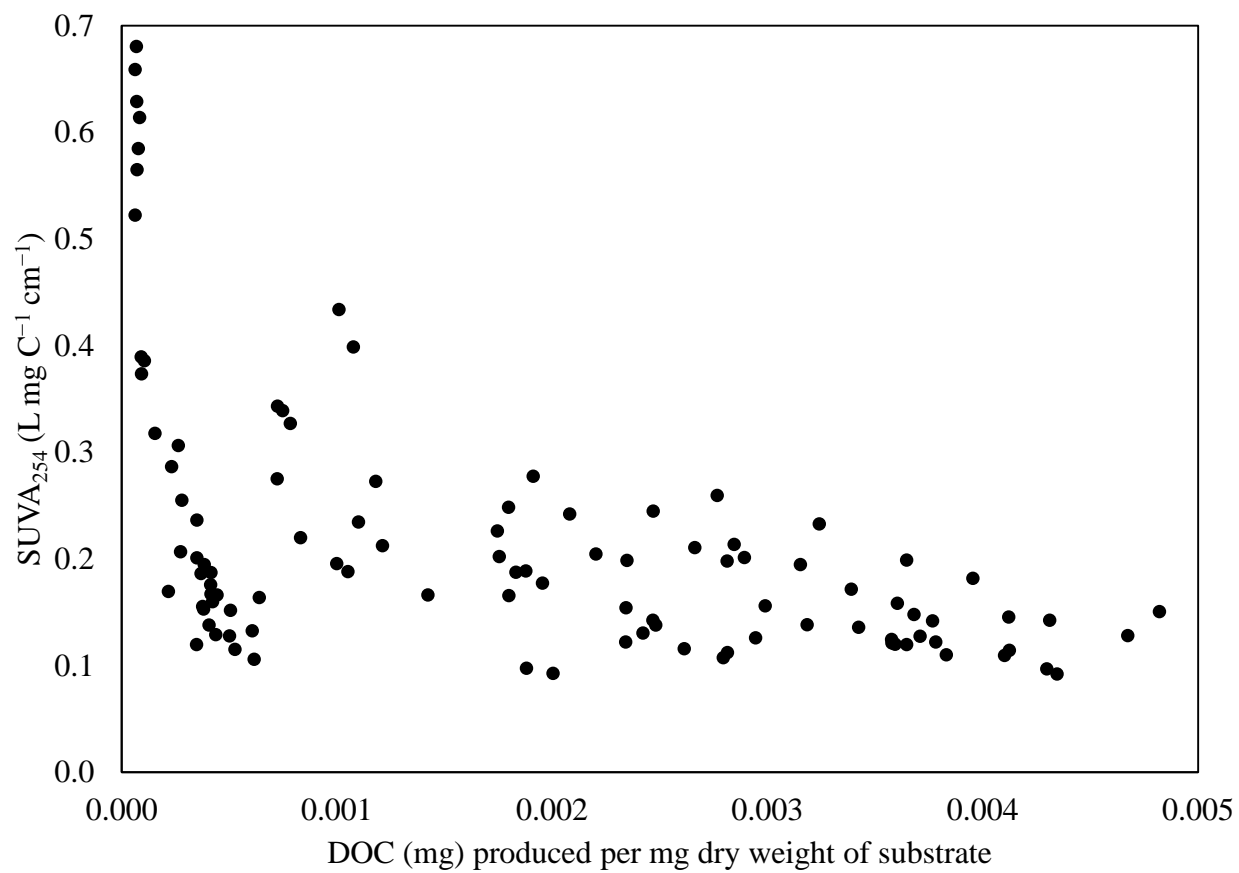Figure 3.5: Negative correlation between E2/E3 and SUVA$_{254}$.

Figure 3.6: Correlation between DOC production and SUVA$_{254}$. There is a negative relationship between SUVA$_{254}$ and DOC produced per mg dry weight of substrate.
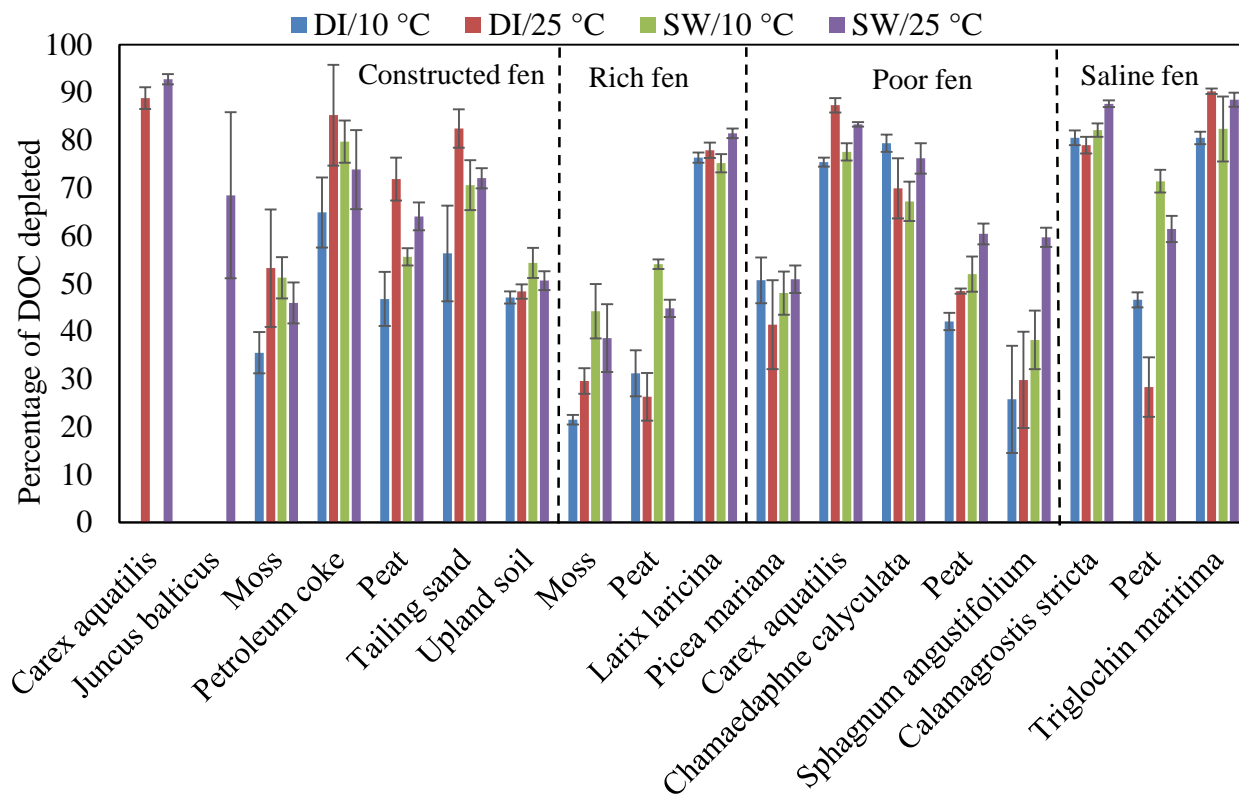
Figure 3.7: Comparison of bioavailability of DOC produced from different substrates. All the DOC samples were incubated at room temperature (22 °C) for 7 days but they were produced from different substrates in saline/DI water at 10 °C and 25 °C, which are represented by different colour bars. The temperature on the legend represents the temperature at the time of DOC production but not the incubation. The error bars give standard errors.
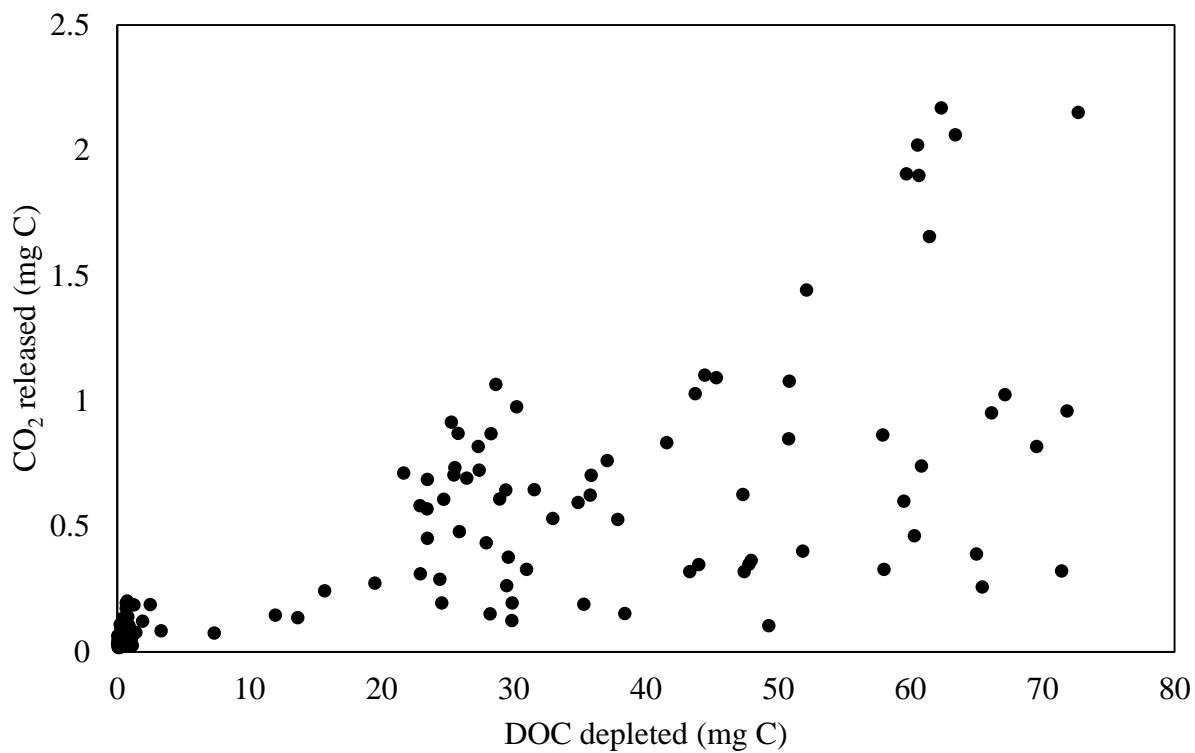
Figure 3.8: Correlation between DOC depletion and cumulative CO_2 release in seven days incubation of DOC at room temperature. All the DOC samples produced from various substrates in different treatment conditions were incubated to investigate the relationship between DOC depletion and CO_2 release.

Appendix B: Supplemental material from Chapter 3

Table B1: Concentration of ions (mg/L) present in CF pore water samples. The water sample was collected from 50, 75 and 90 cm deep piezometers at different sampling period during the growing season.

Piezo Depth	Cl^-	NO_3^-	SO_4^{2-}	HCO_3^-	Na^+	K^+	Mg^{2+}	Ca^{2+}
50 cm	9.39	<0.10	449.00	709.13	65.40	7.79	66.86	271.00
75 cm	9.58	3.21	538.00	549.00	56.51	6.15	63.72	275.00
90 cm	13.49	0.76	540.00	534.00	54.93	5.94	65.27	267.00
Average (mg/L)	10.82	1.32	509.00	597.38	58.95	6.63	65.28	271.00

Table B2: Saline water preparation (32 litres)

S. No.	Salts	Amount (gm)
1.	Calcium Carbonate ($CaCO_3$)	35.08
2.	Magnesium Sulphate ($MgSO_4$)	10.72
3.	Potassium Chloride (KCl)	0.40
4.	Sodium Bicarbonate ($NaHCO_3$)	6.89

Table B3: Nutrient solution preparation

S. No.	Salts	Amount
1.	Ammonium Nitrate (NH_4NO_3)	0.1%
2.	Dipotassium hydrogen phosphate (K_2HPO_4)	0.1%

Table B4: Inoculum solution preparation

S. No.	Materials used	Amount
1.	Donor peat	1 g
2.	DI water	10 ml

CHAPTER 4: SUMMARY, FUTURE RESEARCH AND CONTRIBUTION

4.1 Summary

This two-part research highlights the utility of dissolved organic carbon (DOC) dynamics for evaluating the function of a constructed fen (CF) with reference to natural fens. Concentration and chemistry of DOC in CF was compared with reference fens considering physical and environmental factors affecting pore water DOC concentration and chemistry (Chapter 2). In addition, the production, chemistry and bioavailability of DOC released from different potential organic substrates dominant in the fens were analyzed in various environmental conditions in the laboratory (Chapter 3).

The field study showed that DOC concentration varied significantly among reference fens, the highest being in the saline fen (SF) followed by the poor fen (PF) and the rich fen (RF). Interestingly, DOC concentration at CF was similar to RF, which were geographically closest to each other. In addition, both seasonal variation of DOC concentration and chemistry was observed at each site. DOC concentration consistently increased over the growing season, as did the aromaticity (captured by $SUVA_{254}$) of pore water DOC. $SUVA_{254}$ has been used as a surrogate for aromatic carbon in soil and aquatic humic substances (Kalbitz *et al.,* 2003; Traina *et al.,* 1990). In contrast, E4/E6 indicated that the fulvic content in DOC was dominant during peak growing season. It was also identified that CF had a more humic and aromatic DOC fraction in comparison to reference fens, which was likely due to limited fresh vegetation input into the system. Site hydrology, vegetation community and composition, environmental factors (temperature, salinity, pH and water table), microbial community and interactions among all these factors are thought to be the main driving forces to determine the concentration, chemistry and export of DOC from these fens.

The laboratory incubation study illustrated the important effect of temperature and salinity acting on substrate type in determining DOC production, quality and bioavailability. There were clear differences in the net DOC production and quality from different substrates across all sites. Sedges and trees (vascular plants) produced the largest amount of DOC per unit dry mass followed by peat and moss, whereas the substrates used in construction at CF, such as petroleum coke, tailings sand and upland soil, produced very little DOC. This reveals the importance of

organic matter composition and litter type in DOC production. There was no significant difference in the initial and final net DOC production for the majority of substrates except for higher initial (*e.g.*, moss, *Carex aquatilis*) and lower initial (*e.g.*, petroleum coke, tailings sand) net DOC production in some cases. This suggests that there are differences in readily soluble DOC available in some substrates. DOC produced from sedges had lower $SUVA_{254}$ suggesting lower molecular size and lower aromatic humic substance content than in DOC produced from peat, tailings sand, upland soil and petroleum coke. In addition, temperature and salinity were found to explain variation in the release of DOC from substrates, which is most likely due to the activation of microbes in saline conditions and at warmer temperature. It was also observed that not only did the environmental factors control DOC production, but also DOC production affected the environmental factors. For instance, production of DOC had a profound influence on pH and EC, with higher production of DOC resulting in lower pH and higher EC.

Moreover, DOC produced from various substrates had different bioavailability resulting from variability in the chemical composition of DOC. E4/E6 indicate the fulvic (FA) vs. humic (HA) nature of DOC reflecting the molecular weight and consequently the positive correlation with bioavailability. This suggests that absorbance ratios may be easily measurable parameters that can be used to infer bioavailability of DOC in the field. It was also observed that a large proportion of DOC was utilized for microbial growth. Most of the remaining DOC was converted to CO_2 whereas very little converted to CH_4 as a by-product, probably due to oxic incubation conditions.

In summary, environmental factors controlling the concentration and chemistry of DOC in both laboratory incubations and the field study helped to investigate their impact individually and collectively. Laboratory studies may yield information on single effects of different environmental factors, while field studies yield the interactive effect of all the environmental factors. These two different approaches complement each other to improve our understanding about the potential environmental controls on DOC dynamics in peatlands. Since the DOC concentration and chemistry in CF appears to reflect the initial low productivity of newly planted vegetation, it is significantly different from reference systems. From this study, it can be concluded that DOC dynamics can provide important information for evaluating ecosystem function in constructed fens.

4.2 Future research

Given the timing of the present study, CF did not have a complete growing season of DOC data. This limited the comparison of temporal variation of DOC concentration, chemistry and export with reference fens. Therefore, a complete growing season dataset for all the fens should be evaluated in future comparisons. In addition, as the vegetation community continues to develop on CF, hydrologic pathways and DOC production rate, chemistry and bioavailability may also be altered. Continued study is required to understand the DOC dynamics with time following peatland ecosystem reclamation.

Microorganisms play a significant role in peatland ecosystem stability and sustainability (Andersen *et al.*, 2010). Therefore, different aspects of microbial community should always be considered and used together in long-term monitoring programs following reclamation. The interactions between the microbial community and DOC dynamics would add further insight into ecosystem function at CF.

As the production and bioavailability of DOC is determined by its chemical composition, a future study should include the chemical composition analysis of DOC. Specific measurements could include DOC fractionation (Strack *et al.,* 2011), high performance liquid chromatography (Yan *et al.,* 2012) and fluorescence spectroscopy (Zaccone *et al.,* 2009).

DOC export from the fens was roughly estimated using biweekly discharge rate and DOC concentration. Frequent sampling for DOC concentration and continuous discharge measurement should be used for better estimation of DOC export. Since DOC export can account for a significant portion of the peatland carbon balance (e.g., Billett *et al.,* 2004) and can impact the chemistry and biology of downstream ecosystems, better quantification of both the quantity and chemistry of DOC exported from reclaimed peatlands is critical for understanding both ecosystem and regional C balances (Koprivnjak and Moore, 1992; Urban *et al.,* 1989; Fraser *et al.*, 2001). Moreover, the loss of carbon as particulate organic carbon (POC) can also play an important role in peatland carbon balance (Fiedler *et al.,* 2008). The loss of POC with discharge water should also be considered in future carbon balance studies for better estimation of ecosystem carbon budget.

4.3 Contribution to Understanding of Peatland Reclamation Post Oil Sands Extraction

This research helps to improve our understanding of the effect of peatland reclamation on DOC dynamics and potential sources of DOC at both constructed and reference fens. As this will likely be an important component of the constructed site's carbon balance, these results provide information to help assess whether the constructed site is a carbon sink. It also provides baseline data on DOC concentration and chemistry in reference fens useful for future DOC studies in the region and for comparison to other reclaimed peatlands. Hence, these results can be used to improve management strategies in the post oil sands landscape to maximize carbon sequestration in reclaimed systems.

According to Price *et al.* (2010) fen systems can be replaced with fen peat materials supported by ground water inflow from a constructed watershed. After one growing season, vegetation appears to be establishing on site and water table is near the surface of the peat. These observations suggest that the constructed fen may be successful, although longer study of ecosystem function will be required. In terms of DOC dynamics, the pore water DOC at the constructed fen remains significantly different than the reference fens within the first year *i.e.,* constructed fen had DOC having high molecular weight and more humic and aromatic character than reference fens. We expect that establishment of vegetation on site will increase DOC concentration and change chemistry (more fulvic, less aromatic) similar to the reference fens. Therefore, monitoring DOC dynamics should provide a good measure of ecosystem recovery over time.

4.4 References

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