Salinity Effects on Dissolved Organic Carbon Concentration and Quality in a Constructed Fen Peatland, Fort McMurray, AB

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Geography (Water)

Waterloo, Ontario, Canada, 2020

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

In northern Alberta, oil sands mining disturbs the landscape; reclamation to an "equivalent land capability" is required, and industry is testing peatland construction as part of landscape reclamation. To determine if these constructed peatlands can be self-sustaining, an understanding of the cycling of solutes in peat pore water and their interactions with dissolved organic carbon (DOC) is needed. DOC is a component of pore water that is of interest due to its biotic relevance and its impact as a component of the carbon budget. Additionally, salinity as a control on DOC quantity and quality may be important in reclaimed systems due to the likelihood of elevated sodium (Na^+) from saline groundwater input derived from the tailings used to construct reclaimed catchments. As part of post-mining oil sands reclamation, a pilot fen (Constructed Fen) was constructed to test reclamation techniques for peatland establishment. Previously, DOC in the Constructed Fen was found to be largely internally produced, suggesting rhizodeposition, decomposition and solubility govern DOC dynamics.

For this research DOC concentration and quality, and Na^+ concentration were measured in the rooting zone of the Constructed Fen to evaluate the role of Na^+ in DOC dynamics. DOC concentration and quality throughout the fen revealed that DOC was largely sourced from vegetation inputs. Increases in vegetative inputs between years was indicated by the annual increase in DOC lability. Elevated Na^+ at 30 cm below ground surface (bgs) corresponded with high concentrations of labile DOC. This relationship suggests increased rhizodeposition contributing labile carbon to DOC and decreased decomposition preserving labile DOC. At 10 cm bgs, spatial variability and temperature were the largest predictors of DOC quantity and quality. With expected increases in Na^+ at this site, increased production of a mobile and microbially active fraction of DOC may lead to higher rates of carbon export.

Due to the identification of rhizodeposition as a potential mechanism of the influence of Na^+ on DOC and its suggested importance in previous years, a hydroponic study characterizing the rhizodeposits of the dominant plant species of the Constructed Fen (*C. aquatilis* and *J. balticus*) under different Na^+ concentrations was performed. Rhizodeposition was similar between species with no difference in rate and similar proportions of the fractions of rhizodeposits investigated. Both species exhibited potential tolerance mechanisms to Na^+ accumulation through suspected Na^+/H^+ antiporter activity and increases in membrane stabilizing aromatics. Elevated Na^+ did not significantly change rates of rhizodeposition but there was a general increase in aromatic and polyphenol rhizodeposition and decrease in biopolymer and organic acid rhizodeposition. This represents a shift to lower molecular weight more aromatic rhizodeposits; however, as this study did not consider the contribution of low molecular weight neutrals to rhizodeposition, the results could not be applied directly to field results. Given the large contribution of aromatics and biopolymers to rhizodeposition (average 63% and 25% of measured rhizodeposits, respectively), it is recommended that these fractions be considered in rhizodeposition studies. The findings of this work thus provide a foundation for understanding the effects of Na^+ concentration on rhizodeposition and identify Na^+ as playing a significant role in peatland DOC dynamics.

Acknowledgements

First, I would like to thank my supervisors Maria Strack and Jonathan Price for the invaluable advice and guidance they provided throughout my graduate studies. I am so glad that I had the opportunity to work with you and be a part of the Wetland Soils and GHG Exchange Lab and the Wetland's Hydrology Research Lab. Thanks also to Scott Liddycoat for his help in the Waterloo greenhouse, and Sigrid Peldszus and her lab for their help with the LC-OCD, I know my samples weren't easy to process.

Within the Wetland Soils and GHG Exchange Lab and the Wetland's Hydrology Research Lab, I would like to thank everyone who helped me over these past two years. Thanks to Dryden Miller, Kimberly Tran and Suyuan Yang for all their help in the field. A big thank you also to Scott Davidson who was also willing to help with statistics, writing or anything else. I would also like to thank Nicole Balliston, Dr. Helen Jarvie and Dr. David Cooper for contributing edits to the final thesis draft. To everyone else who helped me over the past two years with field work, analyses or advice, I really appreciate all your help.

Thank you to Suncor's Field Services and Reclamation team and Bear Scare for all their help in the field and for helping us to work safely. Funding for this project was provided by an NSERC CRD grant and co-funded by Suncor Energy Inc., Imperial Oil Resources Limited and Teck Resources Limited. Additional funding was provided by the Northern Scientific Training Program for field work expenses.

Lastly, I would like to thank my friends and family for their support. To both my new friends in Waterloo and my friends from everywhere else, I appreciate you always uplifting me and making me smile. To my brother, Mum and Dad, thank you for always listening to me talk about my thesis and for doing your best to provide me with advice even though you generally didn't understand what I was ranting about. I appreciate you for encouraging me and helping me work through any problems I had along the way.

Land Acknowledgments

I would like to acknowledge that my project’s lab work and desk work takes place on the traditional territory of the Neutral, Anishnaabeg, and Haudenosaunee Peoples. The University of Waterloo is situated on the Haldimand Tract, land promised to Six Nations, which includes six miles on each side of the Grand River.

I would like to acknowledge that my project’s field research takes place within the boundaries of Treaty 8, traditional lands of the Dene and Cree, as well as the traditional lands of the Métis of northeastern Alberta.

I understand that in both these regions Indigenous Peoples have been stewards of the land long before I began my research and have thus accumulated knowledge of the lands and waters on which they have lived and currently live. I am committed to learning about the history of the land where I work and live, to consider the Indigenous Peoples in these territories and to actively work towards reconciliation.

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List of Abbreviations

AIC	Akaike information criterion
AOSR	Athabasca Oil Sands Region
BB	Building blocks
BP	Biopolymers
C	*C. aquatilis* plot
Ca^{2+}	Calcium ion
CH_4	Methane
$C_{10}H_{16}N_2O_8$	Ethylenediaminetetraacetic acid
C:N	Carbon to nitrogen ratio
CO_2	Carbon dioxide
$CO(NH_2)_2$	Urea
CS	*C. aquatilis* early senescence plot
DI	Distilled water
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
E2/E3	Ratio of absorbance at 250 and 365 nm
E4/E6	Ratio of absorbance at 465 and 665 nm
EC	Electrical Conductivity

EDTA	Ethylenediaminetetraacetic acid
g_dwt	Grams of dry weight root biomass
GHG	Greenhouse gas
H^+	Hydrogen ion
HMW	High molecular weight
HOC	Hydrophobic organic carbon
HS	Humic substances
J	*J. balticus* plot
LC-OCD	Liquid chromatography organic carbon detection
LME	Linear mixed effects model
LMW	Low molecular weight
LMWA	Low molecular weight acids
LMWN	Low molecular weight neutrals
M	*C. aquatilis* and *J. balticus* plot
Na^+	Sodium ion
Na_2SO_4	Sodium sulphate
POC	Particulate organic carbon
RDA	Redundancy Analysis

SO_4^{2-}	Sulphate ion
$SUVA_{254}$	Specific Ultraviolet Absorbance at 254 nm
T	*Typha* spp. plot
UPDI	Ultrapure deionized water
T_{avg}	Biweekly average temperature
WL	Depth to water table

Chapter 1: Introduction

Peatlands are globally significant ecosystems due to their roles in carbon storage (Gorham 1991; Limpens et al. 2008), water regulation, nutrient cycling and biodiversity (Kimmel and Mander 2010). Boreal and subarctic peatlands alone are expected to store between 15 and 30% of the world's soil carbon in the form of peat (Limpens et al. 2008). In Canada, peatlands are defined as wetlands that have accumulated over 40 cm of organic matter in peat (Alberta Environment 2017). This peat and carbon storage potential is possible due to a high water table creating anoxic conditions, slowing decomposition and allowing primary production to exceed rates of respiration (Evans et al. 2016). Fens are one type of peatland that occur in the boreal region and are characterized by seasonal connections to ground and surface water (Vitt 2006). Due to this connection, fens are minerotrophic, having inputs of water elevated in dissolved solids when compared to peatlands that are fed by precipitation alone (bogs). Fens are subcategorized based on their water chemistry and vegetation into rich and poor fens, where poor fens have lower concentrations of dissolved solids and are dominated by *Sphagnum* spp. over vascular plants (Vitt 2006).

In northern Alberta approximately 50% of the pre-disturbance landscape was classified broadly as peatlands with fens comprising approximately 95% of these (Vitt et al. 1996). With 953 km^2 of land having been disturbed by surface oil sands mining before December 2016, some of the important functions of peatlands have been removed from the landscape (Alberta Environment and Parks 2020). Furthermore, oil and gas exploration through seismic lines has disturbed an additional 1 900 km^2 in Alberta, with most of this disturbance occurring in northern Alberta (Strack et al. 2019a). For surface mines, where restoration is not possible, fen construction as a form of reclamation has been suggested to return the ecosystem services of peatlands to the landscape (Price et al. 2010; Daly et al. 2012) and as a way for industry to meet requirements of mandatory replacement (Alberta Government 2013).

In Alberta, the Environmental Protection and Enhancement Act requires companies to return the land to “equivalent land capability” following mine closure (Alberta Environment 2017). Wetland reclamation has generally been focused on non-peatlands due to the complexities of supporting peat forming vegetation (Alberta Government 2007), maintaining appropriate hydrological functioning, especially in drought years, (Price et al. 2010) and the long time expected for peat accumulation (Clymo 1983). However, in focusing reclamation on wetlands that are often unconnected to existing hydrological flow paths, the important functions of fens like high carbon storage and water distribution are not restored (Ketcheson et al. 2016). Therefore, to test fen construction as a reclamation technique, Nikanotee Fen (Constructed Fen) was developed on an oil sands lease following the concept of Price et al. (2010). The goal of this project is to construct a peatland that restores fen ecosystem function, such as carbon accumulation and growing conditions suitable for fen species, while being self-sustaining (Daly et al. 2012). Furthermore, through study of this site, techniques used in the construction of Nikanotee Fen can be refined and implemented in future reclamation projects (Daly et al. 2012).

The constructed 2.9 ha fen is a part of a larger 32.1 ha watershed, composed of three reclaimed slopes and an upland that drives water movement into the fen through a tailings sand aquifer. Construction of the Constructed Fen was initially completed in January 2013 but there have since been modifications to improve system function, with ridges and furrows dug in the upland in fall 2013 to promote infiltration and a raised peat bed placed in the northwestern corner of the fen in spring 2019, to exaggerate hydrological variability and alter sodium (Na^+) migration rates and patterns. The watershed is underlain by a geosynthetic clay liner that forces lateral water flow into the fen and prevents seepage into the underlying groundwater (Price et al. 2010; Ketcheson et al. 2017). The peatland itself comprises a 2 m peat layer connected laterally to the tailing sand aquifer and underlain by a high permeability layer of petroleum coke to evenly distribute hydraulic pressure beneath the fen (Ketcheson et al. 2017). Tailings sand was used as the aquifer material as it is readily available in the region and requires reclamation itself. However, due to the use of extraction and tailings process aids, as well as process water recycling (E. Hollander, personal communication, July 20, 2020) and the high initial Na^+ concentration due to oil sands being marine sediments (Sui et al. 2016; Simhayov et al. 2017), the tailings sand used in the constructed watershed has a large leachable pool of Na^+ that has mobilized into the Constructed Fen (Kessel et al. 2018). Though high sorption to the peat matrix is expected to delay Na^+ movement to the rooting zone (Rezanezhad et al. 2012b; Simhayov et al. 2017), high evapotranspiration can be expected to lead to evapoconcentration of Na^+ in the rooting zone (Simhayov et al. 2018; Kessel et al. 2018). Indeed, Na^+ migration into the fen has led to the average Na^+ concentration in the rooting zone of the fen being ~200 mg L^{-1} by 2016 (Ketcheson et al. 2017; Kessel et al. 2018).

High Na^+ concentrations can be toxic to plants by competing for binding sites with nutrients like potassium, and by causing osmotic stress (Bartels and Sunkar 2005). In anticipation of the expected high solute concentrations in the fen, planting included species characteristic of rich and saline fens. These species were planted under a randomized split plot design from mid-June to mid-July 2013 (Borkenhagen and Cooper 2019). Though moss, *Carex aquatilis*, *Juncus balticus* and lower densities of other vascular species were all used in the split plot design, *C. aquatilis* currently dominates most plots with *J. balticus* and *Typha* spp. also being abundant in the fen. With projected continued increases in Na^+ concentrations in the Constructed Fen, the effect of Na^+ on plant and fen carbon dynamics should be investigated.

When primary production exceeds carbon losses, fens store carbon leading to an accumulation of organic matter and peat production. Losses of carbon in fens include respiration of carbon dioxide (CO_2) from plant roots and in organic matter decomposition, methane (CH_4) emissions as a product of anaerobic decomposition, and aqueous losses of carbon through dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and particulate organic carbon (POC) (Limpens et al. 2008). DOC is generally a relatively small flux of carbon compared to other respiration products (Limpens et al. 2008). However, for fens that are found to be close to carbon neutral considering gaseous fluxes alone, DOC fluxes may be sufficient to shift what was thought to be a carbon sink into a source (Strack et al. 2008; Limpens et al. 2008; Evans et al. 2016). DOC transformed into CO_2 and CH_4 in the fen are considered in the greenhouse gas (GHG) emissions of the site, but without considering DOC fluxes downstream total fen GHG emissions may be underestimated as most DOC released from fens is converted into CO_2 and CH_4 before reaching the ocean (Marschner and Kalbitz 2003; Evans et al. 2016). Therefore, characterizing DOC is important to determining the net carbon budget of fens. In addition to its significance in carbon storage, DOC can bind to heavy metals (e.g. manganese and mercury) and organic contaminants (e.g. naphthenic acids) abundant at reclaimed mine sites and transport them to downstream ecosystems (Kalbitz et al. 1997; Kalbitz and Wennrich 1998). Furthermore, DOC, especially that sourced from plants, can play a large role in priming the microbial community for decomposition, leading to nutrient and organic matter turnover, which further supports plant and microbial communities (Neumann and Romheld 2000). This may be especially important at the Constructed Fen where highly humified peat may prevent activation of microbial processes without vegetative carbon inputs (Nwaishi et al. 2016). Understanding DOC quantity and quality in the Constructed Fen can help to evaluate the fen's role in nutrient and organic matter turnover, carbon sequestration and the fen's expected effects downstream.

Since 2013, DOC at the Constructed Fen has been described in relation to nearby natural fens. Studies by Khadka et al. (2016) and Irvine (2018), found that initial DOC concentration was low with higher proportions of recalcitrant compounds, but by four years post-construction concentration and lability had increased to be comparable to natural sites. This increase was attributed to the increase in vegetative inputs to fen DOC as the plant community became established (Irvine 2018). Electrical conductivity (EC) and temperature were the most consistent controls on DOC concentration with increases in EC and temperature leading to higher DOC concentrations (Khadka et al. 2016; Irvine 2018). EC was also a positive control of DOC quality, with higher EC associated with DOC of lower molecular weight (Irvine 2018). Additionally, DOC was largely produced within the fen and export was insignificant relative to production (Irvine 2018). These previous characterizations provide insight on the initial trajectory of deeper peat, but they did not focus on the near surface rooting zone, the region where DOC production and removal are most dynamic. Furthermore, these studies failed to address the impact of Na^+, the influence of which is expected to be large in reclaimed systems.

Beyond reclamation projects, Na^+ concentrations are expected to increase in coastal peatlands due to sea level rise (Church et al. 2013; Guêné Nanchen et al. 2020), thus understanding the role of Na^+ in DOC dynamics at the Constructed Fen has applications to other peatlands. Na^+, like other geochemical factors can influence DOC through rhizodeposition, decomposition and changes in solubility (Kalbitz et al. 2000; Mavi et al. 2012; Moore 2013; Vranova et al. 2013). These processes are expected to be dominant in controlling DOC dynamics in the Constructed Fen as DOC has been largely internally produced and DOC export has been low (Irvine 2018).

Rhizodeposits originate from plant roots, where root cells, mucilage, lysates and exudates enter pore water and contribute to DOC (Dennis et al. 2010). These compounds can be released by damaged root cells (lysates), or by intact root cells (exudates) through passive diffusion (Farrar et al. 2003) or active transport (Jones et al. 2009). While rhizodeposits may sometimes be expanded to also refer to root symbionts and particulate carbon (Oburger and Jones 2018), in this thesis rhizodeposits refer to the dissolved fraction specifically. Decomposition by the microbial community can both produce and remove DOC from fens. DOC is produced through decomposition of peat and litter, but is removed when DOC itself is decomposed (Kane et al. 2014). Generally, aerobic decomposition favours DOC conversion into CO_2, while anaerobic decomposition favours the production of intermediate decomposition products contributing to DOC (Moore and Dalva 2001). Changes in DOC solubility through aggregation, dissolution, or sorption/desorption from the peat matrix can remove or introduce DOC into the measurable pore water DOC pool. While sorbed and aggregated DOC may not be measured in the DOC fraction, it can still be microbially active with the larger surface area generally leading to easier microbial attachment facilitating decomposition (Marschner and Kalbitz 2003). Due to the interactions between these DOC production and removal processes, it is difficult to disentangle individual process effects.

Of these mechanisms, increased rhizodeposition at the Constructed Fen is thought to be the mechanism driving increases in labile DOC (Irvine 2018). Despite the expected contribution of rhizodeposits, rhizodeposition in general (Dennis et al. 2010) and specifically for peatland plants (Proctor and He 2017) is not well understood. Contaminant induced changes in general rhizodeposition and passively rhizodeposited root exudates have also been identified as an important area for research as they may impact the ability of plants to withstand contaminant toxicity (Vranova et al. 2013). At the Constructed Fen where elevated Na^+ is expected to influence long-term fen persistence, especially due to its influence on plant health and the plant community, there is a need to understand the effect of Na^+ on dominant plant species rhizodeposition.

1.1 Objectives

Given the knowledge gap of the influence of Na^+ on DOC and rhizodeposition, and the relative importance of Na^+ in post-oil sands mining landscapes, this study looked at the role of Na^+ on DOC concentration and quality with a focus on the process of rhizodeposition. While DOC has been studied at the Constructed Fen from 2013 to 2016 by Khadka et al. (2016) and Irvine (2018), these studies focused on comparisons of the Constructed Fen to natural fens to determine if DOC concentration and quality were similar. However, given the accumulation of Na^+ in the Constructed Fen and the expected high Na^+ concentration in reclamation projects in the Athabasca Oil Sands Region (AOSR), this site provides a unique opportunity to better evaluate the influence of Na^+ on DOC concentration and quality and rhizodeposition, topics that are little studied to date but may be crucial for understanding carbon budgets and plant health in reclaimed systems and peatlands influenced by sea level rise. This thesis will also contribute to rhizodeposit literature by characterizing the rhizodeposits of *C. aquatilis* and *J. balticus*, which appears to be novel for these species. The objectives of this study are below and will be addresses in Chapter 2 and 3, respectively.

1) Identify the environmental drivers of DOC quantity and quality in the rooting zone of a Constructed Fen, with a focus on the influence of Na^+

2) Quantify and characterize the rhizodeposits of *C. aquatilis* and *J. balticus*, two species dominant in the Constructed Fen, under different sodium sulphate concentrations

1.2 Format and Author Contributions

This thesis is written in a manuscript style with two complementary chapters comprising two manuscripts intended for publication. As the manuscripts are intended for individual publication there is repetition throughout the thesis. The first manuscript (Chapter 2) considers environmental drivers of DOC quantity and quality in the rooting zone of the Constructed Fen, focusing on the specific role of Na^+. The second manuscript (Chapter 3) characterizes rhizodeposition of the two dominant vascular species in the Constructed Fen grown hydroponically under different sodium sulphate concentrations. I conducted the experiments, collected data and wrote the first draft of all chapters of this thesis with contributions from Dr. Maria Strack, Dr. Jonathan Price and Dr. Scott J. Davidson on experimental design, synthesis and editing to complete the final thesis.

Chapter 2: Response of Dissolved Organic Carbon Dynamics to Salinity in a Constructed Fen Peatland in the Athabasca Oil Sands Region

2.1 Introduction

Oil sands extraction has resulted in ~953 km^2 of land disturbance in the AOSR of northeastern Alberta (Alberta Environment and Parks 2020). Oil sands companies are mandated to return the landscape disturbed by oil extraction activities to an "equivalent land capability" after closure (Alberta Environment 2017). Since peatlands comprise ~50% of this region (Vitt et al. 1996), the importance of including peatlands in the post-mining landscape has been recently recognized and efforts to construct these systems have been undertaken with projects such as Nikanotee Fen (Price et al. 2010; Ketcheson et al. 2016). Fens are minerotrophic peatlands and have been selected for reclamation attempts due to their prevalence in the pre-mined landscape (Vitt et al. 1996) and their connection to surface and groundwater sources that can maintain sufficient water levels for fen functioning when precipitation inputs are low (Price et al. 2010). Construction of fens as part of reclamation is considered to be difficult as the design must account for their complex hydrology (Price et al. 2010), and peat formation generally occurs over thousands of years (Clymo 1983). The Nikanotee Fen was thus constructed to test fen reclamation and will henceforth be referred to as the Constructed Fen. The goal of the project is to restore self-sustaining ecosystem functions, including carbon accumulation, support for peat-forming vegetation and resilience to climatic stresses (Price et al. 2010; Daly et al. 2012). Another goal of the Constructed Fen is to study and monitor the site to determine factors that can optimize future reclamation designs.

The Constructed Fen was created as part of a larger constructed watershed that consists of slopes and an upland that feed water into the fen (Price et al. 2010). To facilitate water movement toward the fen, a tailings sand aquifer was constructed to function as the upland recharge zone (Price et al. 2010). As water passes through the aquifer into the fen, solutes present in the tailings sand can be mobilized and transported to the fen. One such solute that is present at high concentrations in the tailings sand (Daly et al. 2012; Simhayov et al. 2017), and was identified as a solute of concern (Kessel et al. 2018), is Na^+. Though the 2 m peat layer present in the fen is expected to slow Na^+ migration into the rooting zone (Price et al. 2010; Simhayov et al. 2017), Na^+ from the upland tailings sand has already begun to migrate to the surface of the fen (Kessel et al. 2018). Na^+ entering the rooting zone of the fen poses a concern as it can alter carbon cycling and affect the ability of the fen to support vegetation (Trites and Bayley 2009; Rezanezhad et al. 2012a; Pouliot et al. 2012; Brouns et al. 2014). Since fens can store large amounts of carbon (Limpens et al. 2008), it is important to understand how salinity will influence carbon cycling.

DOC is generally defined as the carbon in organic molecules passing through a 0.45 μm filter (Thurman 1985). DOC plays an important role in carbon budgets, generally accounting for 10% of total carbon released from boreal peatlands (Limpens et al. 2008). While some of this exported DOC may be stored through sedimentation, it is expected that around 90% is converted to CO_2 downstream, thus influencing downstream GHG emissions (Evans et al. 2016). In addition to its influence on carbon budgets, DOC also influences peatland and downstream chemistry by altering redox conditions and affecting the mobility of other compounds, such as organic contaminants (Vitale and Di Guardo 2019) and heavy metals (Kalbitz and Wennrich 1998). Thus, understanding DOC and its environmental controls can help to determine the fate of carbon and post-mining contaminants.

To better determine the origins, transformations and fate of DOC, information is needed on the specific carbon compounds in the DOC pool. However, due to the complexity and quantity of these compounds, it is generally not feasible to characterize every compound contributing to DOC; instead, measures of DOC quality are often used to generalize the overall pool (Strack et al. 2011; Peacock et al. 2014). Specific Ultraviolet Absorbance at 254 nm ($SUVA_{254}$), E2/E3 and E4/E6 are parameters that are used to indicate DOC quality. $SUVA_{254}$ is positively correlated with aromaticity, whereas E2/E3 is negatively correlated with molecular weight (Peacock et al. 2014). E4/E6 is related to the degree of humification of organic matter, with ratios between 2 and 5 indicating mature humic acids and less mature fulvic acids having ratios between 5.5 and 17 (Thurman 1985; Grayson and Holden 2012). DOC with lower molecular weight and less aromatic character is generally more available for decomposition and more mobile (Strack et al. 2015; Khadka et al. 2016).

As the Constructed Fen has been extensively studied, it is already known that DOC has been largely internally produced (Irvine 2018). Internal biochemical processes regulating peatland DOC include rhizodeposition, decomposition, and solubility (Moore 2013). While DOC export is largely controlled by peatland discharge (Limpens et al. 2008), these internal biochemical processes along with internal hydrological processes are responsible for determining DOC quality and quantity within the fen. Hydrological processes like evapoconcentration, within fen water movement, and dilution from precipitation, will alter DOC concentration at locations within the fen, but will not directly change DOC quality. The biochemical processes, however, can influence both concentration and quality of DOC.

Increases in rhizodeposition, decomposition and solubility differ in their effects on DOC quantity and quality. Higher rhizodeposition is expected to increase DOC concentration, yet lead to less aromatic and lower molecular weight DOC through a direct input of labile DOC from plant roots (Jones et al. 2009; Kane et al. 2014). Products of decomposition are more complex than rhizodeposits or DOC leached directly from litter (Dieleman et al. 2016), and thus increased decomposition should lead to an increase in complex DOC entering pore water. The effect of decomposition on DOC concentration is more difficult to disentangle. Under aerobic conditions decomposition will favour CO_2 as the metabolic end product and likely decrease DOC concentration (Strack et al. 2008), while anaerobic decomposition is less efficient and will lead to the production of soluble intermediate metabolites contributing to increased DOC concentration (Kalbitz et al. 1997). Due to the complexity of decomposition simultaneously removing and producing DOC, it is difficult to infer its effect from field data. Finally, factors that lead to an increase in DOC solubility allow larger and more aromatic DOC, which is preferentially adsorbed and less soluble, to be introduced into solution (Kalbitz et al. 2000), increasing DOC concentration. Environmental factors like soil temperature, water table, vegetation type and salinity, among other factors, can all influence DOC concentration and quality through their influence on these biochemical mechanisms.

Of the environmental factors above, one of the least studied for its effect on DOC in Canadian peatlands is salinity (Moore et al. 2008; Moore 2013), potentially due to saline peatlands being rare in North America’s boreal forests (Trites and Bayley 2009). Due to the high input of solutes (especially Na^+) in the constructed Nikanotee Fen, this site provides an opportunity to better determine the effects of salinity on DOC quantity and quality. In 2015 and 2016, salinity inferred from EC, was a significant control on DOC quantity of the Constructed Fen (Irvine 2018). However, studies considering the effect of Na^+ specifically on DOC dynamics have not yet been undertaken at Nikanotee Fen.

Salinity can influence DOC quantity and quality in peat soils by affecting its adsorption and desorption from the peat matrix. Specifically, ions may saturate sorption sites forcing DOC into solution and bind to DOC directly, increasing its solubility (Kalbitz et al. 2000). Or, ions may reduce charge density allowing for aggregation and precipitation of DOC (Tamamura et al. 2013). Salinity may also influence DOC production and removal by microbes (Khadka et al. 2015), increasing activity by providing nutrients or decreasing activity from salt stress (Marschner and Kalbitz 2003). Rhizodeposition may also be affected by salinity. Rhizodeposits may be actively exuded from plant roots or released by passive diffusion as root exudates, the latter process being vulnerable to changes in root permeability (Farrar et al. 2003). As elevated salinity is expected to increase root membrane permeability (Vranova et al. 2013), salinity is expected to be positively correlated with root exudation. As DOC present in the fen has been shown to be recently produced, small and labile, it is expected that rhizodeposition plays a dominant role in determining DOC concentration and quality in the fen (Khadka et al. 2016; Irvine 2018). Despite these expected relationships, it is unclear what the cumulative effect of these processes will be in the Constructed Fen.

To test how Na^+ and other environmental factors influence DOC in the rooting zone of the Constructed Fen, pore water samples were collected and analyzed over the summer of 2019. The objective of this study is to identify the environmental drivers of DOC quantity and quality in the rooting zone of the Constructed Fen, with a focus on the influence of Na^+. It is hypothesized that salinity will be an important control on DOC quantity and quality, with higher salinity leading to higher concentrations and more labile DOC due to increased input from rhizodeposits.

2.2 Methods

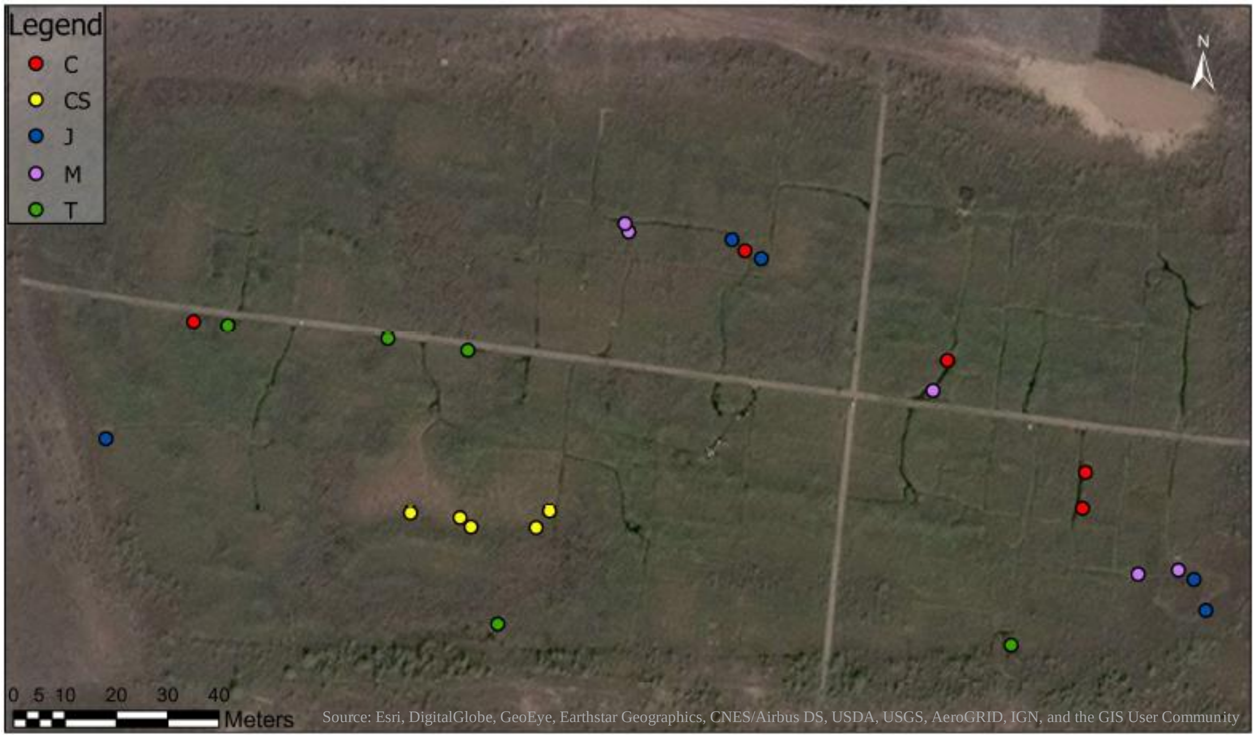
2.2.1. Study Site

This study was conducted on a constructed fen located on an oil sands lease, 40 km north of Fort McMurray (56°55.944’N 111°25.035’W). The Constructed Fen has an area of 2.9 ha and is part of a 32.1 ha reclaimed watershed (Ketcheson and Price 2016). To facilitate water movement through the watershed into the fen, an upland aquifer was created from tailings; a more permeable petroleum coke underdrain was placed beneath the fen and keyed into the adjacent section of the upland to more evenly distribute hydraulic head below the fen (Ketcheson et al. 2016). The Constructed Fen was originally vegetated in June and July 2013 using a randomized split-plot design, where a variety of vascular plants and moss species were introduced, with *C. aquatilis* and *J. balticus* introduced at the highest density (Borkenhagen and Cooper 2019). At the time of this study (2019) the plant cover was dominated by *C. aquatilis*, with *J. balticus* and *Typha* spp. also being common throughout the site.

2.2.2. Instrumentation

To sample porewater from the Constructed Fen, suction lysimeters (Model 1900 Soil Water Sampler, SoilMoisture Equipment Corp., Santa Barbara, California and retrofitted tensiometers) were installed on May 27th, 2019. These lysimeters are made with a high fire silica body ceramic cup that does not significantly absorb DOC (Lilienfein et al. 2004). Lysimeters were installed next to existing collars that were used to measure GHG fluxes, to allow for consideration of plant productivity in the analysis. However, due to equipment failure, there were insufficient carbon dioxide fluxes to consider productivity in the analysis with sufficient statistical power. Fluxing collars were selected based on achieving a balanced design of different types of vegetation cover, hereinafter referred to as vegetation types. The vegetation types targeted were *J. balticus* (J), *C. aquatilis* (C), *Typha* spp. (T), mixed *J. balticus* and *C. aquatilis*

(M) and *C. aquatilis* that showed early senescence in 2018, suggesting potential salinity stress (CS). After selection there were 5 plots within each vegetation type. The location of these collars and installed lysimeters within the Constructed Fen is shown in **Fig 2-1**. Suction lysimeters were installed at 10 and 30 cm depths within 50 cm of the GHG fluxing collars, to obtain samples representative of the rooting zone of the fen. Though root depths extended to at least 0.75 m in 2018, over 90% of root biomass was in the top 30 cm with 67% found in the first 10 cm alone (Messner et al. in prep.). Thus, the top 30 cm was considered the rooting zone. This study also used half-hourly precipitation measurements taken from a Texas Electronics TE525MM/TE525M Tipping Rain Gauge connected to a Campbell Scientific CR1000 set up in the upland approximately 150 m south of the fen.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Fig 2-1 Locations of fluxing collars and suction lysimeters in the Constructed Fen. Colours of points indicate the vegetation type: C- *C. aquatilis*, CS- *C. aquatilis* early senescence in 2018, J- *J. balticus*, M- mixed *C. aquatilis* and *J. balticus*, T- *Typha* spp.

2.2.3. Water Sampling

Water sampling from suction lysimeters was conducted on May 30th, June 4th, June 18th, July 2nd, July 17th, July 26th, August 1st and August 14th of 2019. The day prior to sampling, lysimeters were suctioned using a vacuum pump to -75 kPa. All lysimeters were suctioned within a 2-hour period to reduce the impact of temporal variability in samples. Initial samples were collected the morning of sampling days by connecting a three-way valve to the Tygon™ tubing of the lysimeter and attaching a 60 mL syringe to extract the sample. Samples were then placed into a 120 mL sample bottle and the lysimeters were suctioned again to -75 kPa. The depth to water table (WL) at each location was then measured from a 50 cm depth well using the blowstick method. After the first round of sampling, all lysimeters were sampled approximately 4 hours later the same day with the new sample being added to the same 120 mL bottle as the first sample.

Immediately after samples were placed in the 120 mL bottle, pH and EC were measured using an Orion Star™ A325 pH/Conductivity multiparameter meter with an Orion 8107UWMMD Ultra pH/ATC Triode and a Thermo Scientific™ Orion™ Conductivity and Temperature Probe. This was done after the first round of sampling with the initial sample and repeated on the combined sample in the afternoon. The combined sample values were used in analysis but were compared to previous values to test for significant changes in pore water chemistry between rounds of sampling. Probes were calibrated for pH with pH 4, 7 and 10 standards and calibrated for EC with 1413 $\mu S\ cm^{-1}$ and 12 880 $\mu S\ cm^{-1}$ standard solutions. pH values were higher than those measured at 30 cm piezometers. This was attributed to the applied vacuum shifting the CO_2 equilibrium of the water, degassing CO_2 and raising the pH of the sample. As the measured pH was not representative of the water in situ, pH was not considered in analysis. While the multiparameter probe did provide temperature, the measured temperature was not representative of pore water as samples were pulled into the lysimeter hours before sampling and thus could equilibrate with surface water temperature. Temperature of samples was thus taken as a biweekly average temperature (T_{avg}) using a thermocouple temperature probe at 10 and 30 cm depths during GHG flux measurements. With each collar being measured for CH_4 and CO_2 fluxes weekly, this corresponded to the use of approximately four measurements to calculate the average for each DOC sampling date.

Samples were stored in a cooler during the day and were placed in a 4 °C fridge within 10 hours of sampling. Within 24 hours of sampling, samples were filtered using vacuum filtration through 0.45 µm nitrocellulose filters. Between 3 to 10 mL of the filtered sample was then used to measure the Na^+ concentration of the water using an Orion Star^{TM} A324 pH/ISE multiparameter meter with a ROSS Sodium Sure Flow Electrode (model 8611BNWP). The probe was calibrated before use and after every 2 hours of use using 10, 100 and 1000 ppm standards. The electrode slope was also confirmed to be between 54 and 60 mV dec^{-1} after each calibration ensuring proper function of the probe.

2.2.4. Vegetation Survey

A vegetation survey of the GHG flux collars was completed on August 9th, 2019. This survey used the 60 cm by 60 cm collar as the survey area and recorded percentage cover of *J. balticus*, *C. aquatilis*, *Typha* spp., forbs, shrubs, moss, litter and water. Total sedge and rush cover including the presence of the species considered individually, was also recorded for each collar. Since suction lysimeters were installed into peat with similar vegetation coverage to collars, these vegetation surveys are representative of vegetation cover over suction lysimeters.

2.2.5. Sulphate (SO_4^{2-}) Concentration

Due to the effect of SO_4^{2-} on redox and DOC solubility shown in other peatlands (Clark et al. 2005; Fenner et al. 2011), SO_4^{2-} concentrations was also determined for consideration in models. Samples for SO_4^{2-} concentration were measured in the field to minimize oxidation of sulfide gas, that could contribute to measured SO_4^{2-}. This required SO_4^{2-} sampling and DOC sampling to occur on separate days, due to time constraints and retrieving sufficient volume for analysis. SO_4^{2-} samples were collected six times throughout the summer from 30 cm suction lysimeters on June 11th, 21st and 28th, July 9th and 23rd and August 7th. Immediately after sample collection 0.25 mL of sample was pipetted into a sample cell and mixed with one Hach SulfaVer 4 powder pillow, stoppered and allowed to react for 5 minutes. After 5 minutes, 9.75 mL of Ultrapure deionized (UPDI) water was pipetted into the sample cell to bring the volume to the 10 mL needed for measurement. During reaction, a blank was prepared where 0.25 mL of sample and 9.75 mL of UPDI were pipetted into an additional sample cell and stoppered. This led to a 40 times sample dilution of the blank and sample which was required to ensure that SO_4^{2-} concentration remained below the 70 mg/L detection maximum. The blank and sample were then run on a Hach DR2800 spectrophotometer using method 680, where the output concentration was multiplied by 40 to obtain the sample concentration. Given the temporal difference between SO_4^{2-} and DOC sample results, values were averaged over the summer within a plot for analysis.

2.2.6. DOC Concentration and Quality

Samples were analyzed for DOC concentration with a Shimadzou TOC analyzer using the Non-Purgeable Organic Carbon method at the University of Calgary. Though this analyzer measures total organic carbon, since the samples were filtered prior to analysis this represents the dissolved fraction of organic carbon. Samples were also analyzed for absorbance using a Thermo

Scientific^{TM} GENESYS^{TM} 10S UV-Vis Spectrophotometer at 250, 254, 365, 400, 465 and 665 nm wavelengths. Using absorbance data, $SUVA_{254}$, E2/E3 and E4/E6 were calculated using the equations below:

$$SUVA_{254} = \frac{Absorbance\ at\ 254\ nm\ (cm^{-1})}{[DOC]\ (mg\ L^{-1})} \times 100 (cm\ m^{-1})$$

$$\frac{E2}{E3} = \frac{Absorbance\ at\ 250\ nm}{Absorbance\ at\ 365\ nm}$$

$$\frac{E4}{E6} = \frac{Absorbance\ at\ 465\ nm}{Absorbance\ at\ 665\ nm}$$

These measures were selected as they are useful measures for indicating lability of carbon and are often used for DOC quality comparisons (Peacock et al. 2014). While E4/E6 was initially considered in analysis, due to the low absorbance of samples at 665 nm (0.004±0.004 cm^{-1}) the ratio is extremely sensitive to small changes in absorbance at 665 nm. Where in previous studies using E4/E6, larger variation in absorbance at 665 nm (~0.1 cm^{-1}) led to observed lower E4/E6 ratios (Grayson and Holden 2012; Peacock et al. 2014), in this study variation in E4/E6 was largely due to smaller changes in absorbance at 665 nm. Due to the debate on the usefulness of applying this metric in freshwater systems (O'Driscoll et al. 2006; Peacock et al. 2014) and the sensitivity and uncertainty of using this metric with low absorbance, it was excluded from cumulative analyzes. E4/E6 values are reported in this study for completeness, with E4/E6 linear mixed effects models included in the Appendix for reference (**Table A 4**).

2.2.7. Data Analysis

Data were analyzed using R (R Core Team 2019) and R studio (RStudio team 2016). The lme function from the *nlme* package (Pinheiro et al. 2018) was used to create linear mixed effect models explaining variation in DOC concentration, $SUVA_{254}$, E2/E3 and E4/E6. Models were created separately for 10 and 30 cm depth as the processes contributing to DOC variability at these depths were expected to differ. Vegetation type, Na^+ concentration, WL and T_{avg} and all two-way interactions were considered in linear mixed effects (LME) models and models were blocked by plot to account for repeated measures. Stepwise GLM selection was used to select models with the lowest Akaike information criterion (AIC). EC data was not included in these LME models as Na^+ and EC are correlated with Na^+ being a contributor to EC. A separate LME model revealed that EC was a significant predictor of Na^+ concentration (LME, $F_{1,365}=328.28$, $p<0.001$), but that the correlation was moderate between these variables with an R^2 of 0.46. Thus, EC was considered in separate LME models with EC as a fixed effect and vegetation type and plot as nested random effects once interactions between EC and vegetation type were confirmed to be non-significant. Since percent sedge and rush cover and SO_4^{2-} concentration were not available with the same temporal resolution as DOC data, they were compared as averages by plot in additional LME models. For comparison of percent sedge and rush cover, DOC concentration, $SUVA_{254}$ and E2/E3 averaged from August 1st and August 14th were used as over this period sedge and rush cover was fairly stable at the surveyed value. All LME models were visually assessed for normality and homogeneity of variance using residuals and were log transformed to meet test assumptions when necessary (DOC concentration and EC were log transformed for models where they were the response variable). LME models were tested for significance using a type III ANOVA to account for unequal sample sizes due to missing data and significance was determined at $p<0.05$. The ghlt function with method ‘Tukey’ from the *multcomp* package (Bretz et al. 2020) and the emtrends function from the *emmeans* package (Lenth et al. 2018) were used to evaluate which vegetation types were significantly different and to compare slopes between categories when interactions were significant, respectively. R^2 for models was determined using the r.squaredGLMM function from package *MuMIn* (Barton 2019) and for fixed effects using the r2beta function from package *r2glmm* (Jaeger 2017).

To consider the dominant environmental influences on DOC concentration, $SUVA_{254}$ and E2/E3, redundancy analyses (RDA) were performed on data averaged over the summer by plot for 10 and 30 cm using the *vegan* package (Oksanen et al. 2019). Environmental parameters considered were Na^+ concentration, WL, T_{avg}, percent sedge and rush cover, SO_4^{2-} concentration and vegetation type. Prior to the RDA all variables were standardized using the scale function. The final model was selected using the ordistep function with forward selection. For a parameter to enter the model, a p-value below 0.05 was needed, whereas a p-value above 0.10 allowed the parameter to be removed from the model.

2.3 Results

2.3.1 Environmental Conditions

While most vegetation types had similar percent sedge and rush cover at peak season, T plots had lower cover than all vegetation types but J (LME, $F_{4,20}$=4.27, p=0.01). *C. aquatilis* grows with higher biomass density than *J. balticus* and *Typha* spp. allowing for a higher percent cover. There were significant differences in WL between vegetation types (LME, $F_{4,20}$=6.60, p=0.001), with T plots having a higher water table relative to the peat surface than C, J and M, and J plots having a lower water table than CS and T (**Table 2-1**). Average plot temperature also differed between vegetation types at both 10 and 30 cm (LME, $F_{4,20}$=7.09, p=0.001; LME, $F_{4,20}$=7.60, $p<0.001$; respectively). At 10 cm, C plots had a lower T_{avg} than CS and J, with CS having a higher T_{avg} than all plots other than J (**Table 2-1**). At 30 cm CS and J plots had a higher T_{avg} than C and T plots (**Table 2-1**). Generally, CS and J plots had higher soil temperature than other plots. SO_4^{2-} concentration was only measured at 30 cm and was similar between most plot types, with the exception of J plots, which had significantly higher concentration than other vegetation types (**Table 2-1**). Neither EC nor Na^+ were significantly different among vegetation types at both 10 cm (LME, $F_{4,20}$=1.30, p=0.305; LME, $F_{4,20}$=0.19, p=0.940; respectively) and 30 cm (LME, $F_{4,20}$=2.28, p=0.096; LME, $F_{4,20}$=0.87, p=0.499; respectively).

Table 2-1 Mean (standard deviation) of environmental parameters at 10 cm and 30 cm depth for vegetation type categories over the summer of 2019 at the Constructed Fen. The letters representing vegetation types are: C- *C. aquatilis*, CS- *C. aquatilis* early senescence in 2018, J- *J. balticus*, M- mixed *C. aquatilis* and *J. balticus*, T- *Typha* spp. Significance letters are indicated in superscript and indicate differences between vegetation type categories within an environmental parameter and within depths. Vegetation types sharing a letter indicates that there is no significant difference.

	EC (μS cm^{-1})		**Na^{+} (mg L^{-1})**		**T$_{avg}$ (°C)**		**SO$_4^{2-}$ (mg L^{-1})**	**WL (cm)**
	10 cm	30 cm	10 cm	30 cm	10 cm	30 cm	30 cm	
C	3379 (1040)^{a}	2982 (851)^{a}	240 (52)^{a}	183 (42)^{a}	13.8 (3.6)^{a}	10.6 (4.5)^{a}	1525 (610)^{a}	-5.63 (6.66)^{ab}
CS	3543 (661)^{a}	3085 (529)^{a}	258 (97)^{a}	219 (98)^{a}	16.9 (2.3)^{c}	14.4 (2.5)^{b}	1372 (382)^{a}	-1.91 (5.01)^{ac}
J	4262 (1124)^{a}	4329 (767)^{a}	290 (150)^{a}	270 (122)^{a}	16.2 (2.8)^{bc}	13.9 (3.1)^{b}	2168 (565)^{b}	-15.26 (12.59)^{b}
M	4203 (801)^{a}	3163 (875)^{a}	279 (92)^{a}	175 (56)^{a}	14.5 (3.5)^{ab}	12.3 (4.2)^{ab}	1487 (590)^{a}	-7.82 (7.63)^{ab}
T	3554 (641)^{a}	2912 (538)^{a}	282 (97)^{a}	212 (69)^{a}	13.6 (3.7)^{a}	10 (4.5)^{a}	1600 (746)^{a}	7.97 (10.12)^{c}

While Na^+ and EC were not significantly different between vegetation types, they did exhibit depth dependency with 10 cm having higher EC and Na^+ than 30 cm (LME, $F_{1,369}$=106.16, p<0.001; LME, $F_{1,369}$=198.23, p<0.001; respectively). Na^+ at 10 cm was higher under all vegetation types, while EC did not significantly differ between depths under J plots. Average temperature was also shown to be depth dependent with 10 cm having higher temperature than 30 cm under all vegetation types (LME, $F_{1,342}$=58.10, p<0.001).

2.3.2 DOC Concentration and Quality

DOC concentration, $SUVA_{254}$ and E2/E3 were all found to vary over the summer of 2019 with 10 cm and 30 cm values varying similarly (**Fig 2-2**). The largest difference in DOC between 10 and 30 cm was on May 30th with 10 cm DOC having a higher concentration, lower aromaticity and higher molecular weight. DOC concentrations remained higher at 10 cm for the remainder of the summer, but quality parameters were comparable between 10 cm and 30 cm from June to August. DOC concentration increased over the summer with a steep increase in concentration between July 2nd and 17th, coinciding with a period of low precipitation (**Fig 2-2**) during peak growing season. Between July 2nd and July 17th there was also a steep decrease in $SUVA_{254}$, but no significant change in the E2/E3 temporal trend was observed over this period (**Fig 2-2**). $SUVA_{254}$ generally increased over the summer, while E2/E3 decreased throughout the summer. Overall, while DOC concentration increased over the summer, lability decreased.

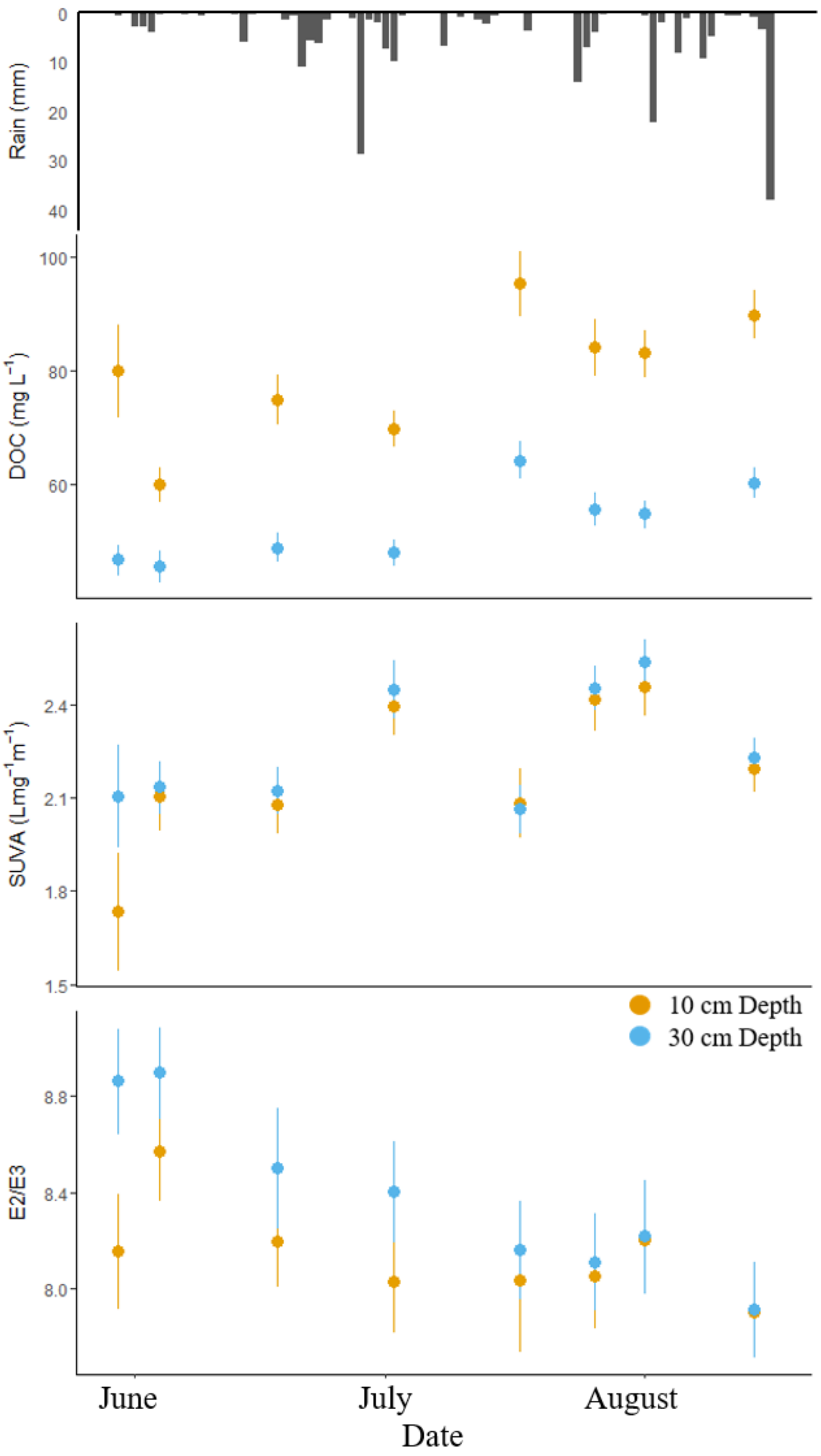


Fig 2-2 Average DOC concentration, $SUVA_{254}$ and E2/E3 and their confidence intervals of standard error for each DOC sampling event at the Constructed Fen in 2019. Cumulative daily precipitation is shown above.

Post hoc Tukey HSD tests revealed which vegetation types differed in DOC concentration, $SUVA_{254}$, E2/E3 and E4/E6. Vegetation type was not a significant control on DOC concentration and $SUVA_{254}$ at 10 cm, but was a significant control on E2/E3 and E4/E6 (**Table 2-2**; **Table 2-3**; **Table 2-4**; **Table A 4**). Specifically, only T had higher E2/E3 than C at 10 cm ($p=0.040$) and M had higher E4/E6 than T (**Fig 2-3**). At 30 cm there was significant variability in all DOC parameters with vegetation types. DOC concentration was higher under M than CS ($p<0.001$) and $SUVA_{254}$ was higher at C than at CS ($p=0.018$). E2/E3 again showed more overall variability than other DOC measures with J having higher E2/E3 than C and M, and M also having significantly lower E2/E3 than CS (**Fig 2-3**).

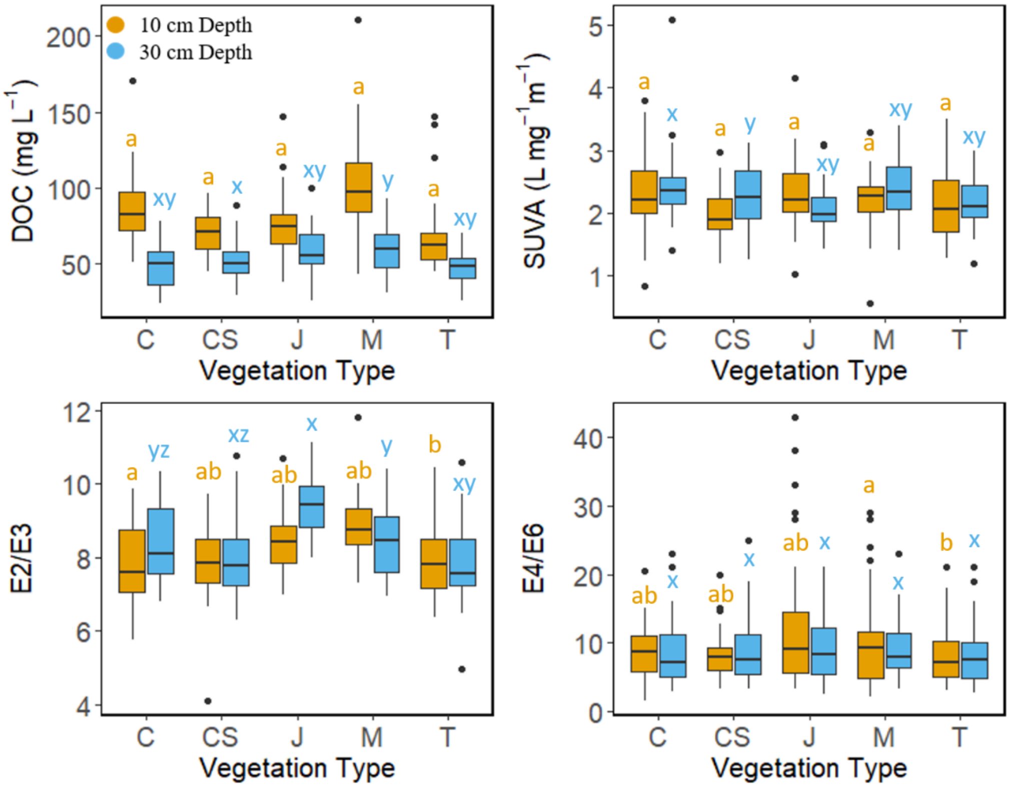


Fig 2-3 DOC, $SUVA_{254}$, E2/E3 and E4/E6 under the different vegetation types of the Constructed Fen 2019. Pairwise comparison significance letters are shown above plots with ‘ab’ used for 10 cm and ‘xyz’ used for 30 cm. The letters representing vegetation types are: C- *C. aquatilis*, CS- *C. aquatilis* early senescence in 2018, J- *J. balticus*, M- mixed *C. aquatilis* and *J. balticus*, T- *Typha* spp. Vegetation types sharing a letter indicates no significant difference.

DOC concentration differed significantly with depth, with higher concentrations at 10 cm than 30 cm (LME, $F_{1,372}$=275.01, $p<0.001$). This relationship was significant under all vegetation types. Differences in $SUVA_{254}$ with depth were only significant under certain vegetation types. Specifically, CS and M had higher $SUVA_{254}$ at 30 cm (LME, $F_{1,74}$=13.86, $p<0.001$; LME, $F_{1,67}$=4.65, p=0.035; respectively), where J had higher $SUVA_{254}$ at 10 cm (LME, $F_{1,73}$=5.59, p=0.021) and $SUVA_{254}$ did not vary significantly with depth at C and T (LME, $F_{1,73}$=0.73, p=0.394; LME, $F_{1,74}$=0.01, p=0.904; respectively). The relationship between depth and E2/E3 also differed with vegetation type, where C and J had higher E2/E3 at 30 cm (LME, $F_{1,73}$=31.55, $p<0.001$; LME, $F_{1,73}$=48.86, $p<0.001$; respectively), M had lower E2/E3 at 30 cm (LME, $F_{1,67}$=15.08, $p<0.001$) and CS and T were not significantly different between depths (LME, $F_{1,74}$=0.001, p=0.973; LME, $F_{1,74}$=0.28, p=0.600; respectively). E4/E6 again had little variability with no significant differences between depths (LME, $F_{1,365}$=1.26, p=0.263). Thus, DOC quality differences between depths were dependent on vegetation cover type but were not consistent between measures.

2.3.3 Controls on DOC Concentration and Quality

The results of LME models for DOC concentration, $SUVA_{254}$ and E2/E3 are shown in **Table 2-2**, **Table 2-3** and **Table 2-4**, respectively. Na^+ significantly explained variation in DOC concentration and quality at 30 cm, but only variation in $SUVA_{254}$ at 10 cm (**Table 2-2**; **Table 2-3**; **Table 2-4**; **Fig 2-4**). The relationship of $SUVA_{254}$ and E2/E3 with Na^+ was dependent on vegetation type (**Table 2-2**; **Table 2-3**). While most vegetation types showed negative $SUVA_{254}$ and Na^+ relationships, CS had a positive slope despite this slope not significantly differing from other vegetation type slopes. Despite the overall positive trend of E2/E3 with Na^+, this relationship was driven by the positive slopes of M and C plots, as CS, J and T had negative slopes that significantly differed from the positive slopes. At 30 cm depth in the Constructed Fen, elevated salinity plots tended to have higher DOC concentrations and DOC was characterized by lower aromaticity and lower molecular weight.

Table 2-2 Results of linear mixed effect model for environmental parameters correlated to DOC concentration at 10 and 30 cm in the Constructed Fen over the summer of 2019. DOC concentration was log transformed to achieve normality. Only significant parameters and parameters with a significant interaction are shown.

Model	Effect	F	p	R^2_m	R^2_c
log([DOC]) 10 cm	Na^+	$F_{1,135}=0.24$	0.625	0.50	0.67
	WL	$F_{1,135}=5.46$	0.021		
	$Na^+ \times$ WL	$F_{1,135}=5.50$	0.020		
	Intercept	$F_{1,135}=105.61$	<0.001		
log([DOC]) 30 cm	Vegetation Type	$F_{4,20}=4.03$	0.015	0.41	0.84
	Na^+	$F_{1,150}=27.93$	<0.001		
	T_{avg}	$F_{1,150}=12.27$	<0.001		
	Vegetation Type × T_{avg}	$F_{4,150}=3.56$	<0.001		
	Intercept	$F_{1,150}=493.73$	<0.001		

Table 2-3 Results of linear mixed effect model for environmental parameters correlated to $SUVA_{254}$ at 10 and 30 cm in the Constructed Fen over the summer of 2019. Only significant parameters and parameters with a significant interaction are shown.

Model	Effect	F	p	R^2_m	R^2_c
$SUVA_{254}$ 10 cm	Na^+	$F_{1,138}=4.10$	0.045	0.25	0.71
	T_{avg}	$F_{1,138}=17.15$	<0.001		
	Intercept	$F_{1,138}=25.00$	<0.001		
$SUVA_{254}$ 30 cm	Vegetation Type	$F_{4,20}=3.17$	0.036	0.32	0.56
	Na^+	$F_{1,146}=8.57$	0.004		
	Vegetation Type × Na^+	$F_{4,146}=2.79$	0.028		
	Intercept	$F_{1,146}=75.07$	<0.001		

Table 2-4 Results of linear mixed effect model for environmental parameters correlated to E2/E3 at 10 and 30 cm in the Constructed Fen over the summer of 2019. Only significant parameters and parameters with a significant interaction are shown.

Model	Effect	F	p	R^2_m	R^2_c
E2/E3 10 cm	Vegetation Type	$F_{4,20}$=2.50	0.075	0.20	0.81
	WL	$F_{1,138}$=5.79	0.017		
	T_{avg}	$F_{1,138}$=0.66	0.418		
	Vegetation Type × T_{avg}	$F_{4,138}$=4.46	0.002		
	Intercept	$F_{1,138}$=119.54	<0.001		
E2/E3 30 cm	Vegetation Type	$F_{4,20}$=4.92	0.006	0.47	0.74
	Na^+	$F_{1,150}$=5.03	0.026		
	T_{avg}	$F_{1,150}$=25.59	<0.001		
	Vegetation Type × Na^+	$F_{4,150}$=5.49	<0.001		
	Intercept	$F_{1,150}$=83.03	<0.001		

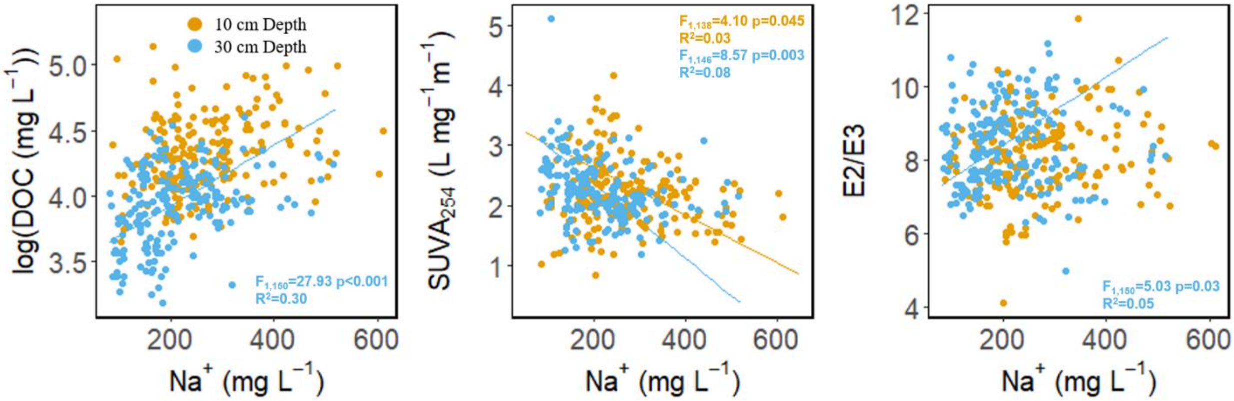


Fig 2-4 Significant relationships between DOC concentration, $SUVA_{254}$ and E2/E3 with Na^+ concentration at the Constructed Fen over the summer of 2019. Relationships were extracted from LME models with F statistic, p-value and the R^2 attributed to variation in Na^+ are shown. DOC concentrations were log transformed to meet normality.

Though EC and Na^+ are closely related, they did exhibit differences in their relationships with DOC quantity and quality. DOC concentration was weakly and positively correlated with EC at both 10 and 30 cm (LME, $F_{1,169}$=39.73, p<0.001; LME, $F_{1,174}$=42.35, p<0.001). Where $SUVA_{254}$ was negatively correlated to EC at 30 cm (LME, $F_{1,174}$=5.92, p=0.016), E2/E3 was positively correlated with EC at 10 cm (LME, $F_{1,163}$=6.25, p=0.013). Despite the differences in significance between Na^+ and EC, both show overall trends of increases in DOC quantity and quality with increasing salinity.

WL was weakly and negatively correlated to both DOC concentration and E2/E3 at 10 cm (**Table 2-2**; **Table 2-4**). With WL typically varying between 5 and 10 cm below surface (**Table 2-1**), 10 cm samples were subject to periods above and below the water table. DOC concentration was weakly and positively correlated to T_{avg} at 30 cm under all vegetation types (**Table 2-2**), with a post hoc Tukey HSD test revealing that CS had a significantly steeper slope than C, M and T. E2/E3 was weakly and negatively correlated to T_{avg} at 30 cm (**Table 2-4**). At 10 cm, $SUVA_{254}$ was weakly and positively correlated with T_{avg} (**Table 2-3**), while E2/E3's relationship with T_{avg} was dependent on the vegetation type with C and J having positive slopes and CS, M and T having negative slopes. The overall E2/E3 correlation with T_{avg} at 10 cm was not significant.

Plots with high SO_4^{2-} had high DOC lability, with $SUVA_{254}$ being negatively and E2/E3 being positively correlated to SO_4^{2-} concentration (LME, $F_{1,19}=14.36$, $p=0.001$; LME, $F_{1,19}=18.12$, $p<0.001$; respectively). However, there was no significant correlation between SO_4^{2-} and DOC concentration (LME, $F_{1,19}=2.93$, $p=0.103$). Percent sedge and rush cover did not significantly correlate with any DOC parameters at 10 cm, though DOC concentration at 30 cm positively correlated with percent cover (LME, $F_{1,19}=7.54$, $p=0.012$). Correlations were largely weak suggesting a variety of factors influenced DOC quantity and quality at the Constructed Fen.

DOC concentration was moderately explained by the fixed effects of the LME models at both 10 and 30 cm (**Table 2-2**). DOC aromaticity and molecular weight, while moderately explained by environmental variables at 30 cm depth, were only weakly explained by models at

10 cm (**Table 2-3**; **Table 2-4**). DOC concentration showed the highest spatial variability at 30 cm, with 43% of variability in concentration explained by plot location, compared to 17% at 10 cm. In contrast, $SUVA_{254}$ and E2/E3 showed the greatest spatial variability at 10 cm with 46% vs 24% and 61% vs 27% of variability being explained at 10 cm vs 30 cm for $SUVA_{254}$ and E2/E3, respectively. While DOC concentration is explained more by measured variables at 10 cm and spatial variability at 30 cm, this relationship was reversed for DOC quality.

The effect of environmental factors on DOC quantity and quality was further evaluated with redundancy analysis for 10 and 30 cm. At 10 cm the RDA explained 42.3% of variation over three RDA axes (RDA, $F_{5,19}$=2.79, p=0.003), where at 30 cm 47.6% was explained over three axes (RDA, $F_{3,21}$=6.35, p<0.001). Temperature was correlated with RDA1 at 10 cm, with increases in temperature correlated to lower DOC concentration and higher molecular weight DOC (**Fig 2-5**). Vegetation types were correlated with different RDA axes and showed a large degree of overlap between types (**Fig 2-5**). SO_4^{2-} was strongly correlated with RDA1 at 30 cm, with higher concentrations associated with higher E2/E3 and lower $SUVA_{254}$. Both Na^+ and percent sedge and rush cover were moderately correlated with RDA2 at 30 cm with higher Na^+ concentration and percent sedge and rush cover being associated with higher DOC concentration. The RDAs thus support the results found from LME models, while also reflecting the relative importance of SO_4^{2-} and percent sedge and rush cover along with Na^+ on DOC quantity and quality at 30 cm depth.

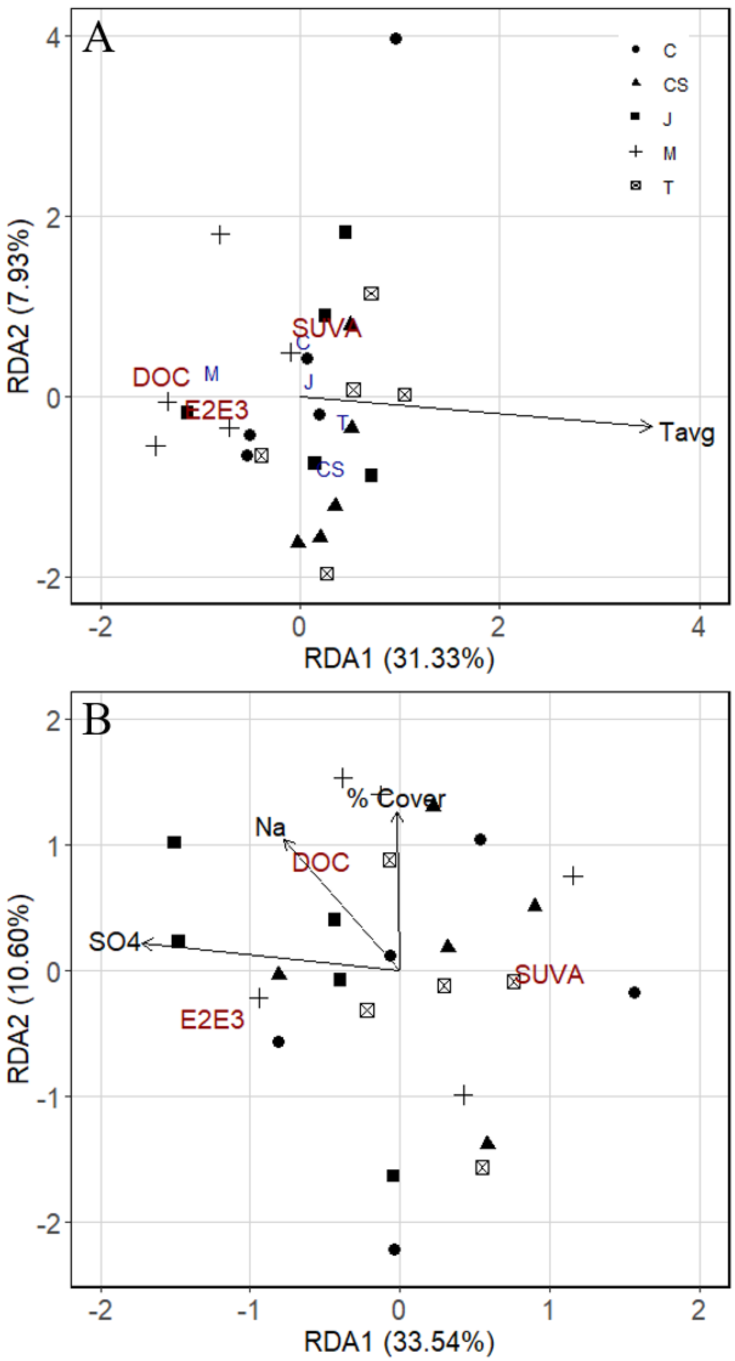


Fig 2-5 RDAs for 10 (A) and 30 (B) cm investigating environmental controls (black text and arrows) on DOC concentration, SUVA$_{254}$ and E2/E3 (red text). Plot positions on the RDA are shown as points, with the symbol indicating vegetation type. Centroids for categorical variables are shown in blue text with the letters following Cat indicating the vegetation type as C- *C. aquatilis*, CS- *C. aquatilis* early senescence in 2018,, J- *J. balticus*, M- mixed *C. aquatilis* and *J. balticus*, T- *Typha* spp. RDAs were scaled into two dimensions to account for the third RDA axis. Na represents Na$^+$, SO4 represents SO$_4^{2-}$ concentration and % Cover represents percent sedge and rush cover.

2.4 Discussion

2.4.1 Rhizodeposition Influence on DOC Quantity and Quality

Both LME models and the RDA revealed that Na^+ concentration was a control on DOC concentration and quality at the Constructed Fen. This relationship in the Constructed Fen can in part be explained by the influence of Na^+ on rhizodeposition. Monovalent cations like Na^+ can influence DOC concentration and quality by increasing the permeability of root membranes, leading to an increase in root exudation (Vranova et al. 2013). Root exudation releases small molecular weight compounds, which are thought to have low aromaticity (Farrar et al. 2003; Kane et al. 2014). Additionally, as the compounds released from plant roots are generally highly available to the microbial community, in solution they will be decomposed to smaller and less aromatic products, and may prime the microbial community for increased decomposition of other sources (Jones et al. 2004; Basiliko et al. 2012). Thus, a Na^+-induced increase in rhizodeposition is supported as a mechanism for the influence of Na^+ on DOC quantity and quality at 30 cm depth (**Fig 2-4**). The importance of rhizodeposition is further supported by the significant positive correlation between DOC concentration and percent sedge and rush cover (**Fig 2-5B**), with higher sedge and rush cover leading to more root biomass available for rhizodeposition. The significance of vegetation type in determining DOC concentration and quality may also support rhizodeposition as a mechanism, as distinct plant species will have different exudation rates and compounds exuded, and have varying tolerance to salinity (Jones 1998; Bartels and Sunkar 2005; Isayenkov 2012; Proctor and He 2017).

From 2013 to 2016 DOC concentrations increased through time in the near surface (Khadka et al. 2016; Irvine 2018); however, this study found similar DOC concentrations to those recorded in 2016 (average ~55-60 mg/L at 30 cm in 2016; **Fig 2-2**). Aromaticity and molecular weight of DOC at the Constructed Fen, however, continued the trend of increasing lability with average $SUVA_{254}$ decreasing from ~2.6 to 2.3 L mg^{-1} m^{-1} and E2/E3 increasing from ~6.5 to 8.4 from 2016 to 2019. This suggests that the mechanisms of DOC production and removal may not have stabilized yet. Specifically, the lower $SUVA_{254}$ and higher E2/E3 reflect continued increases in labile carbon contributions to DOC, potentially through the impact of rhizodeposition. E4/E6 ratios largely above 5, indicating low humification of DOC, further support the high contribution of plant-derived DOC. With vascular plant cover having become established at the Constructed Fen (Borkenhagen and Cooper 2019), this increase in DOC lability may reflect the continued increase in Na^+ concentration leading to higher rhizodeposition, instead of increases in vascular plant cover as found in previous years at the Constructed Fen (Khadka et al. 2016) and at other peatland restoration projects (Strack et al. 2015). With Na^+ concentrations continuing to rise in the Constructed Fen (Kessel et al. 2018), increases in Na^+-induced rhizodeposition may continue to contribute to interannual increases in DOC lability.

Overall, the trend of increasing lability of DOC at the fen, as well as continued increases in DOC concentration and lability due to Na^+-induced rhizodeposition, is expected to increase the role of DOC in the carbon budget of the Constructed Fen. Having higher concentrations of smaller and less aromatic DOC may lead to enhanced decomposition of DOC and thus higher gaseous carbon fluxes from the fen (Khadka et al. 2015). This labile carbon source may also prime the microbial community allowing for enhanced microbial degradation of peat and recalcitrant DOC (Basiliko et al. 2012) further increasing carbon loss from the Constructed Fen. Additionally, with labile DOC being more mobile (Strack et al. 2015), increased aqueous DOC export from the site can be expected, leading to a greater export of carbon with discharge, a loss not currently considered at the site. With less complex DOC compounds generally forming organometallic complexes, mobilizing heavy metals (Steinberg 2003), increased DOC lability may also mobilize contaminants, making them bioavailable for the microbial and vegetation community at the fen. This presents an area for future research into the mobility of DOC-bound contaminants. Furthermore, when reclaimed systems are hydrologically connected to the landscape, this mobilization of contaminants may impact downstream ecosystems unless appropriate measures are taken to reduce efflux.

2.4.2 Decomposition Influence on DOC Quantity and Quality

Though Na-induced rhizodeposition can explain the Na-DOC relationship at 30 cm and interannual changes in DOC concentration and quality at the Constructed Fen, changes in decomposition may also explain these relationships. Na^+ can decrease decomposition through salt stress or increase decomposition due to flocculation promoting microbial attachment (Marschner and Kalbitz 2003; Mavi et al. 2012; Chambers et al. 2014). The balance of these processes will determine the net effect on DOC in the field. In Mavi et al. (2012), the authors found that $SUVA_{254}$ decreased as salinity increased, which they attributed to preservation of labile aliphatic compounds from decomposition at high salinity. Therefore, enhanced preservation of labile DOC under high Na^+ concentration may have led to the increases in DOC concentration and decreases in $SUVA_{254}$ and molecular weight observed at 30 cm (**Fig 2-4**). Additionally, continued increases in Na^+ concentration interannually may contribute to interannual decreases in molecular weight and $SUVA_{254}$, while shifts in the balance of flocculation and salt stress may have led to a lack of change in DOC concentration since 2016.

While the effects of Na^+ could explain variation in DOC concentration and quality at 30 cm depth, this effect was not observed at 10 cm (**Fig 2-4**; **Fig 2-5**). DOC concentrations were significantly higher at 10 cm than 30 cm, with quality generally being similar between depths.

The higher concentrations at 10 cm can be explained by fluctuations in water table above and below this depth occurring more frequently, likely leading to increases in DOC release upon rewetting. In their literature review, Kalbitz et al. (2000) found substantial evidence that DOC concentrations increased following rewetting and suggest that dissolution of built up microbial products from reduced decomposition during dry periods and cell lysis following rewetting contribute to this increase. Additionally, a greater presence of labile litter available for decomposition at the surface and higher EC and temperature may contribute to the higher DOC concentrations at 10 cm. Though these mechanisms would also coincide with more labile DOC at 10 cm, DOC had similar lability at 10 and 30 cm depth (**Fig 2-3**). This may be due to anaerobic conditions at 30 cm which preserve labile DOC (Moore and Dalva 2001) and lead to DOC production being favoured over complete decomposition to carbon dioxide (Freeman et al. 2004; Strack et al. 2008) resulting in abundant labile DOC at both depths.

The different mechanisms explaining the labile quality of DOC at the Constructed Fen between depths may explain why there was higher spatial variability in DOC quality at 10 cm (**Table 2-3**; **Table 2-4**). While anoxic conditions were consistently present at most plots at 30 cm creating uniform conditions for preservation of labile DOC, litter quality and fluctuations in water table are plot dependent. Though some aspect of litter quality may be explained by vegetation type, due to variation in vegetation cover through time, the current plant cover often will not represent litter quality. This lack of connection between current vegetation type further supports decomposition over rhizodeposition as a dominant influence at 10 cm as rhizodeposition would directly relate to the current vegetation cover. Additionally, local drainage conditions may have led to the high degree of spatial variability at 10 cm. Fenner et al. (2011) found decomposition "hotspots" in a rewetted peatland where areas with low initial soil moisture were characterized with elevated DOC concentration and molecular weight as well as soluble phenolics upon rewetting. These “hotspots” are produced by anaerobic decomposition producing DOC and increasing pH, where increasing pH creates a positive–feedback, allowing for increased DOC and phenolic solubilization (Fenner et al. 2011). pH was not measured due to the use of suction lysimeters, so we were unable to consider it to further strengthen evidence for this mechanism.

Temperature has generally been identified as a positive control on DOC concentration due to stimulation of the microbial community at higher temperature (Moore and Dalva 2001; Kane et al. 2014; Khadka et al. 2015; Dieleman et al. 2016; Irvine 2018), and this was supported at 30 cm depth. However, at 10 cm, DOC concentration correlated negatively with temperature in the RDA (**Fig 2-5A**). While this may seem to contradict the higher DOC concentrations being found at 10 cm due to temperature, the temperature range within 10 cm depth is much narrower than that between 10 and 30 cm (**Table 2-1**). Additionally, the RDA used temperature averaged by plot over the entire season instead of changes in temperature through time. Higher temperature increases microbial activity, and thus DOC production and removal (Marschner and Kalbitz 2003), suggesting the net effect of these processes may differ based on confounding factors. Therefore, under a narrower temperature range, temperature effects may be harder to discern.

Decomposition may also explain the seasonal increase in DOC concentration and $SUVA_{254}$ and decrease in E2/E3 (**Fig 2-2**). Though E4/E6 has been suggested as a better metric for looking at temporal variability (Peacock et al. 2014), given the uncertainty in using this metric for this study, it was not considered. A similar pattern of DOC concentrations generally increasing over the summer has been previously found in peatlands (Clark et al. 2005; Koehler et al. 2009; Strack et al. 2015; Rosset et al. 2019). While this trend may be due to evapoconcentration of

DOC at the surface (Clark et al. 2012), the corresponding changes in DOC quality do not reflect this mechanism. Instead, increasing concentrations of DOC over the summer may reflect the pattern of decomposition temporally, which increases DOC concentration while preferentially removing labile carbon and contributing to a larger proportion of recalcitrant DOC remaining in pore water. This pattern of decomposition governing seasonal patterns in DOC has been previously suggested and has been attributed to increases in microbially-mediated decomposition with increasing temperature (Clark et al. 2005; Koehler et al. 2009). Under high temperatures, as were present at the end of the summer, it has been found that DOC production outweighs consumption leading to increases in concentration (Moore and Dalva 2001; Freeman et al. 2001).

Another area of note in the temporal DOC concentration and $SUVA_{254}$ pattern is the large increase in DOC concentration and decrease in $SUVA_{254}$ between July 2nd and 17th. This change coincides with a period of low precipitation (**Fig 2-2**), where water table dropped and thus the upper peat was aerated. The associated increase in oxygen availability may have then stimulated the microbial community leading to an increase in decomposition and greater production of DOC (Hribljan et al. 2014). Strack et al. (2019b) also found increased DOC concentration and decreased aromaticity with lower water tables in natural and experimentally drained peatlands in Alberta and attributed this to increased decomposition. However, this mechanism is not supported by the results of the LME, where shallow water table coincided with lower concentrations of generally large DOC molecules compared to deeper water tables. Furthermore, while this relationship was observed at both 10 and 30 cm depth, 30 cm samples were still well below the water table at most plots during this time, so while plants may supply some oxygen at 30 cm, substantial oxidation at this depth was unlikely. With many conflicting studies on the influence of water table on plot scale DOC dynamics and further debate on the mechanism

(Clark et al. 2005, 2012; Fenner et al. 2011; Strack et al. 2015; Khadka et al. 2015; Dieleman et al. 2016; Davidson et al. 2019), this presents an area for further research.

Though DOC is becoming more labile interannually, the seasonal impacts of decomposition may create conditions where DOC is least labile when DOC export is highest. Specifically, decomposition leads to more recalcitrant DOC in the late summer when there is higher precipitation and thus DOC export is likely to be higher (Limpens et al. 2008). While the DOC pool at the Constructed Fen is still trending towards becoming more labile through time, slightly more recalcitrant DOC when export is highest may help contain DOC and the compounds it complexes within the fen. However, with DOC concentrations also being highest at the end of the summer the net effect may still lead to higher export of carbon. As climate change is expected to increase temperature and lead to drier and longer summers in the AOSR (Welham and Seely 2012) and water table and temperature were identified as controls on decomposition at 10 cm, the role of decomposition in the fen may evolve through time.

2.4.3 Solubility Influence on DOC Quantity and Quality

SO_4^{2-} concentration at 30 cm was a dominant control on DOC quality in the Constructed Fen (**Fig 2-5B**). Increasing SO_4^{2-} concentration may increase DOC lability by operating as a terminal electron acceptor for decomposition (Fenner et al. 2011); however, given the high concentration of SO_4^{2-} at the Constructed Fen it is unlikely to be the limiting factor in decomposition. Instead, SO_4^{2-} is likely to influence DOC through its influence on solubility where SO_4^{2-} facilitates DOC precipitation (Clark et al. 2005; Jager et al. 2009; Brouns et al. 2014) as well as decreases pH, further decreasing DOC solubility (Clark et al. 2012). This mechanism may also explain the temporal increases in DOC quality interannually at the Constructed Fen. Between 2017 and 2019 average SO_4^{2-} concentration increased from ~1000 mg L^{-1} (Osman 2018) to ~1600 mg L^{-1}. As already stated, with larger DOC compounds being preferentially adsorbed and precipitated, increasing SO_4^{2-} concentration interannually likely contributed to the interannual increases in DOC lability found in this study.

While this study found DOC to be more labile at higher SO_4^{2-} concentrations, there was no corresponding decrease in DOC concentration. The removal of DOC compounds from solution by SO_4^{2-} may allow for enhanced decomposition which will reintroduce flocculated or absorbed carbon into solution as smaller and potentially less aromatic products of decomposition. While this effect will further increase the effect of SO_4^{2-} on molecular weight and aromaticity, it will counteract the effect on DOC concentration, potentially leading the lack of a significant effect on concentration. Additionally, with microbial activity increasing following adsorption or flocculation of DOC (Marschner and Kalbitz 2003), this removed DOC will contribute to gaseous losses of carbon. Future studies should thus consider sorbed DOC and its potential for decomposition. Furthermore, pH should be considered in future studies as it is known to be a strong control on DOC solubility (Kalbitz et al. 2003) and thus, may explain some of the unexplained variation in this study.

2.5 Conclusion

This study has presented results on the DOC dynamics of the rooting zone of the Constructed Fen, representing the net impacts of rhizodeposition, decomposition and solubility. DOC at the Constructed Fen had spectrophotometric properties indicative of low molecular weight, low aromaticity and low humification, suggesting largely inputs from vegetation to the DOC pool. Within season DOC dynamics appeared to be governed by decomposition, increasing DOC concentrations while decreasing lability on a seasonal basis. However, this effect may change with expected changes in temperature and precipitation with climate change affecting the role of decomposition at the Constructed Fen. Interannually, vegetation inputs to DOC through processes like Na^+-induced rhizodeposition and decreased decomposition leading to preservation of labile plant litter may govern DOC dynamics, contributing to the continued increase in DOC concentration between years. With Na^+ concentrations expected to continue to rise in the fen, this may lead to greater export of carbon from the Constructed Fen and mobilization of contaminants, suggesting that aqueous carbon fluxes should be monitored and accounted for when completing the site level carbon budget for the Constructed Fen. Currently, concentrations of DOC expected in outflow are unlikely to significantly impact downstream ecosystems; however, if DOC production continues to rise this may change. Therefore, ecosystems constructed downstream should be tolerant to a potential for salt and metal contamination and high DOC concentration when peatlands are constructed into reclaimed systems. Overall, high Na^+ concentrations led to higher concentrations of more labile DOC.

Chapter 3: The Effect of Sodium Concentration on Rhizodeposition of *Juncus balticus* and *Carex aquatilis* taken from a Constructed Fen Peatland

3.1 Introduction

Rhizodeposition refers to the root derived carbon that is lost to the soil, including that lost from live roots, root symbionts and root turnover (Oburger and Jones 2018); it is often simplified to reflect just the dissolved organic compounds released from roots as these are thought to have the largest influence on the rhizosphere (Dennis et al. 2010; Hassan et al. 2019). Rhizodeposition by plant roots can represent a large loss of carbon from plants into the rhizosphere, with root exudate derived CO_2 accounting for approximately 24% of total CO_2 emissions of a sedge dominated peat (Crow and Wieder 2005). Despite being a significant carbon flux, rhizodeposition is understudied due to the complexity of achieving representative sampling (Vranova et al. 2013). Studies characterizing rhizodeposits in peatlands have recently been undertaken (Koelbener et al. 2010; Proctor and He 2017; Edwards et al. 2018), but these have yet to characterize the effects of contaminants on rhizodeposition in peatland species. Na^+, as a potential contaminant, is expected to become an increasingly important control on terrestrial plant health and survival with increasing Na^+ in soils from sea level rise (Montemayor et al. 2008; Church et al. 2013; Guêné Nanchen et al. 2020), salinization of agricultural soils (Isayenkov 2012) and construction of ecosystems with materials elevated in Na^+ as part of reclamation in the AOSR (Daly et al. 2012; Rezanezhad et al. 2012a). Therefore, this study aims to assess the influence of Na^+ on the rhizodeposition of peatland species.

Organic carbon can be released into the rhizosphere through the loss of root cap and border cells, cell lysis (lysates), passive diffusion from living cells (exudates) and the active secretion of organic compounds such as mucilage (Jones et al. 2009). Once released these compounds play a variety of roles in the rhizosphere, signaling to plants or microorganisms, priming decomposition, mobilizing nutrients, minimizing stress and contributing to the DOC pool and carbon fluxes (Nardi et al. 2000; Dakora and Phillips 2002; Jones et al. 2004; Bartels and Sunkar 2005; Basiliko et al. 2012; Hassan et al. 2019). Rhizodeposition is affected by light intensity, temperature, nutrient deficiency and heavy metal toxicity (Neumann and Romheld 2000; Fan et al. 2001; Vranova et al. 2013); however, the effects of Na^+ on rhizodeposition are not well understood.

Na^+ is expected to increase rhizodeposition by increasing root membrane permeability and allowing for higher rates of the passive release of root exudates (Vranova et al. 2013). Production of root mucilage may also increase, as was found in a halophytic salt marsh species in response to high Na^+ concentration, with mucilage potentially aiding in water transport due to its water holding capacity (Edmond Ghanem et al. 2010). In addition, plants may actively release lower molecular weight compounds from their roots, known as compatible solutes, under salt stress to prevent ion uptake by altering electrochemical gradients or by immobilizing ions (Bartels and Sunkar 2005; Touchette 2006; Munns et al. 2020). Changes in rhizodeposition from elevated Na^+ can influence food security through its effect on the health of agricultural plants (Bartels and Sunkar 2005; Munns et al. 2020) and may alter carbon fluxes of coastal and constructed ecosystems, including peatlands, leading to changes in carbon cycling and storage (Chambers et al. 2013; Harpenslager et al. 2015; Ardón et al. 2016).

In peatlands, where carbon storage is high, it is critical to understand carbon fluxes to project their continued function in the carbon cycle. The contribution of rhizodeposits to carbon cycling has recently been studied for peatlands with the characterization and quantification of rhizodeposits for some typical peatland species and their role in peat (Trinder et al. 2008; Koelbener et al. 2010; Proctor and He 2017; Edwards et al. 2018). These studies have revealed that rhizodeposits may contribute significantly to the DOC pool of peatlands, with Edwards et al. (2018) reporting that vascular plant rhizodeposits may account for up to 5% of DOC in peatlands. Beyond this direct contribution to DOC, rhizodeposits can also influence the carbon cycle by priming the microbial community for decomposition (Basiliko et al. 2012). This in turn can lead to increases in decomposition contribution to DOC, CH_4 and CO_2 fluxes from peat (Hopple et al. 2019). In a constructed peatland in northern Alberta, rhizodeposits have been suggested as a significant contributor to rising DOC concentrations at this site (Khadka et al. 2016; Irvine 2018; Chapter 2), and continued increases in Na^+ concentration are expected to affect long term peatland functioning (Kessel et al. 2018).

Nikanotee Fen (Constructed Fen) was constructed in northern Alberta to test peatland reclamation techniques (Ketcheson et al. 2016). As peatlands occupied approximately 50% of the pre-mined landscape (Vitt et al. 1996), peatland reclamation is important to return peatland ecosystem functions such as carbon storage to the landscape (Daly et al. 2012). Tailings sand was used in the construction of the upland section of the watershed due to its abundance in the post-mining landscape. However, the elevated Na^+ concentration in tailings sand is transported to the Constructed Fen as water flows through the watershed (Kessel et al. 2018). Na^+ has the potential to influence of variety of peatland processes including the production and removal of DOC (Kalbitz et al. 2000; Farrar et al. 2003; Vranova et al. 2013). EC, a variable related to Na^+ concentration, is a significant control on DOC quantity and quality at the Constructed Fen (Khadka et al. 2016; Irvine 2018; Chapter 2). Thus, the Constructed Fen provides an opportunity to evaluate the effect of Na^+ on peatland plant rhizodeposition in a system expected to be largely influenced by Na^+.

Previous studies have generally focused on characterizing the low molecular weight (LMW) root exudate component of rhizodeposition (Trinder et al. 2008; Proctor and He 2017) and have not focused on high molecular weight (HMW) compounds. LMW compounds typically considered are organic acids, amino acids and simple sugars, which generally have a molecular weight below 1000 Da (Oburger and Jones 2018). However, there is no apparent basis for only considering LMW root exudates in these studies, as HMW compounds (>1000 Da) and lysates can also serve important functions in the rhizosphere (Dennis et al. 2010) and are contributors to DOC. For *Eriophorum vaginatum*, a peatland sedge commonly known as cottongrass, between 77 to 94% of rhizodeposits were higher molecular weight than the amino acids, simple sugars and organic acids typically studied, depending on the month when rhizodeposits were measured (Edwards et al. 2018). However, there is still debate on whether LMW or HMW compounds typically dominate rhizodeposition in most species (Walker et al. 2003; Bais et al. 2006; Oburger et al. 2013). As the effect of Na^+ on rhizodeposition has, to our knowledge, not been tested before this study, characterization of its effect on LMW and HMW compounds is necessary.

Therefore, the aim of this study was to use hydroponics to characterize the dissolved rhizodeposits of the dominant plant species extracted from the Constructed Fen. Specifically, the objective is to quantify and characterize the rhizodeposits of *C. aquatilis* and *J. balticus,* two vascular plant species dominant in the Constructed Fen, under different sodium sulphate (Na_2SO_4) concentrations. Na_2SO_4 was selected over sodium chloride due to relative importance of SO_4^{2-} compared to chloride in the Constructed Fen (Kessel et al. 2018) and the importance of SO_4^{2-} in DOC dynamics, especially in coastal peatlands (Clark et al. 2012; Gosch et al. 2019; Chapter 2). While Na^+ is thought to increase the rhizodeposition of LMW compounds into the rhizosphere through increased root permeability, leading to an expected increase in LMW relative to HMW compounds entering pore water, this has yet to be confirmed. This study will not only help determine the role of rhizodeposition as a contributor to DOC at the Constructed Fen but will also provide baseline information on how Na^+ affects rhizodeposition.

3.2 Methods

3.2.1 Study Site

Cores were collected from the Constructed Fen, 40 km north of Fort McMurray (56°55.944’N 111°25.035’W). This site was constructed to evaluate the effectiveness of peatland reclamation in a post-oil sands mine site. The fen was constructed with 2 m of peat underlain by 0.5 m petroleum coke layer and covers 2.9 ha of the 32.1 ha constructed watershed (Ketcheson and Price 2016). Site construction was finished in January 2013, and the site was planted with moss layer transfer and vascular planting dominated by *J. balticus* and *C. aquatilis* by July 2013 using a split plot design (Borkenhagen and Cooper 2019). At the time of core collection in June 2019, the fen’s vegetation cover was very different from the original planted distribution, with *C. aquatilis* dominating most of the fen area and *Typha* spp. and *J. balticus* being present to a lesser extent. Cores were collected at two representative locations for *C. aquatilis* and *J. balticus* from plots originally planted with *C. aquatilis* and *J. balticus*, respectively, so the root network of both species would be approximately the same age.

3.2.2 Core Collection

A total of sixteen 6-inch (~15 cm) diameter by 45 cm length peat cores were collected from the south east corner of the Constructed Fen on June 6, 2019. Eight *J. balticus* and eight *C. aquatilis* cores were collected from the southeast corner of the fen where frost table was first recorded below 45 cm and where a low water table facilitated the collection of intact cores. As both species are clonal, core collection would have severed rhizome networks leading to the collection of rhizome sections over plant individuals. Cores were collected by hammering 45 cm length 15 cm diameter PVC pipe into the peat until the ground surface was even with the top of the PVC pipe. The core extraction process may have led to some compression of the cores, leading to some variability in the depth of root collection and damage to roots. However, as many plant roots did not extend the full 45 cm, plants roots continued to grow prior to treatment and root biomass was accounted for in analysis, the compression consequences from core collection were expected to have a minor effect on rhizodeposition. A hole directly next to the core was then dug to provide space to remove the core from the ground. A flat spade was used to separate the bottom of the core from the peat and help lift the core to the surface. Cores from each vegetation type were taken adjacent to one another, so the hole created by the previous core could be used to remove the next, thus minimizing disturbance to the fen. Once removed, plant shoots were left unbroken and collected on the top of the core. Then, the cores were wrapped in plastic wrap and sealed with tape for their protection.

Cores were shipped to the University of Waterloo in mid-June where the plastic wrap was removed, then mesh screen was applied to the bottom of each core. Cores were placed in sets of four from the same vegetative cover into large plastic bins, where the water table was maintained at 40 cm above the bottom of the core with distilled water (DI) ponded in the tub. The intent was to establish a water table 5 cm below the surface, but due to the compression of some of the *C. aquatilis* cores, the water table was between 2 and 5 cm below the surface. All plants survived removal and shipping and both roots and shoots continued to grow once at the University of Waterloo greenhouse. Plants were grown from the date of their arrival in Waterloo in mid-June until early September 2019 (**Fig 3-1A**).

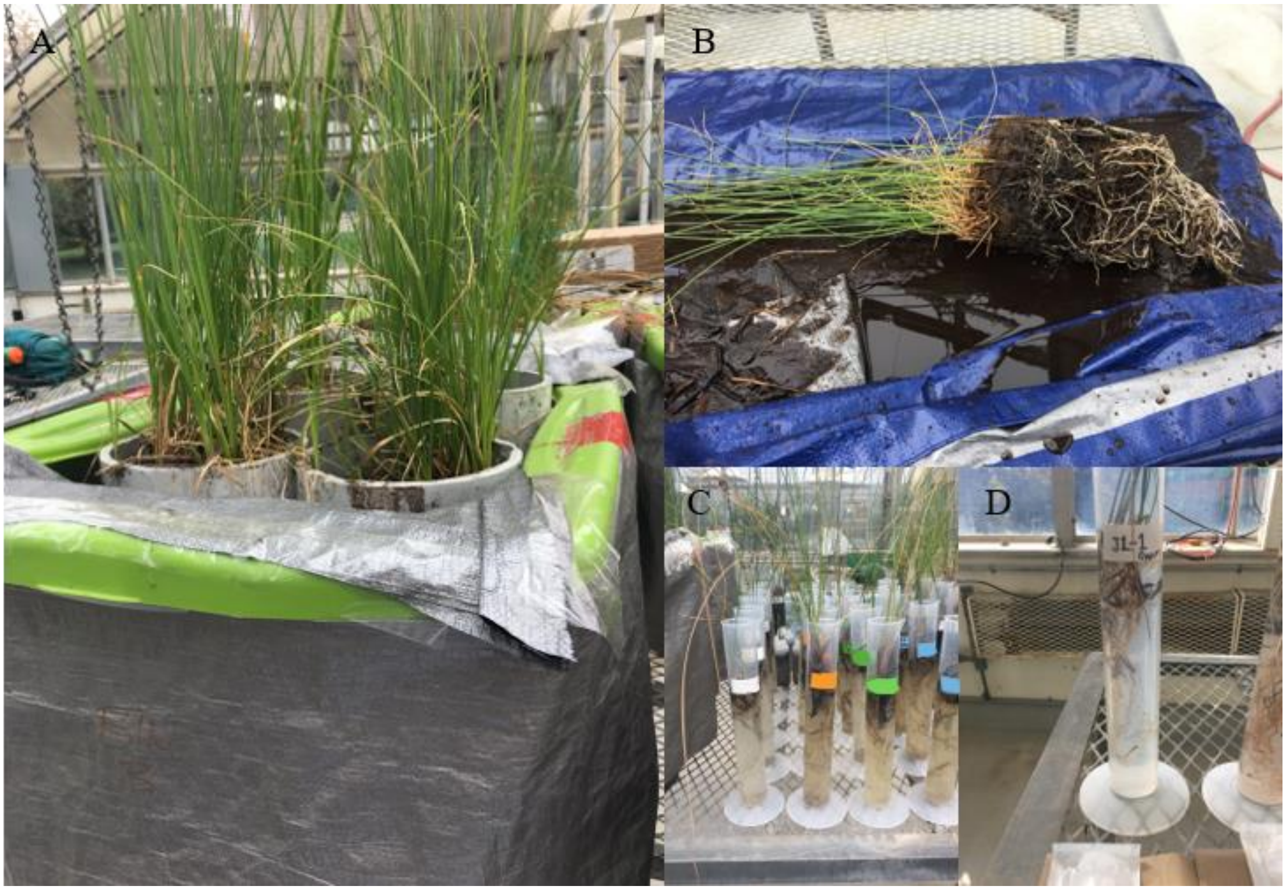


Fig 3-1 Setup for rhizodeposition experiment of *Carex aquatilis* and *Juncus balticus*. A- Core setup in the greenhouse with plant cores grown in DI water. B- Removal of rhizome sections plants from cores. C- Plants grown hydroponically under different Na^+ concentrations. D- *J. balticus* growing hydroponically in 0 ppm Na^+ treatment.

3.2.3 Plant Removal

On September 10 – 12, 2019 rhizome sections were removed from peat cores. As both species grow clonally, rhizome sections could include multiple shoot growths. Cores were first removed from the PVC pipe by pushing the cores from the shoot end out through the bottom. The core was then delicately rinsed with DI water to separate the roots from the peat starting with the bottom of the core and moving upward (**Fig 3-1B**). Each core required between 2 to 5 hours to isolate all the plants. Care was taken to preserve as many fine roots as possible during plant removal, though damage to some fine roots was expected. If there was visible damage to large roots the plant was discarded. To compensate for the damage to fine roots caused by removing the plants from cores, plants were grown hydroponically for three weeks before samples were collected, to allow for plant recovery. This recovery length was selected based on the three-week recovery used in both Koelbener et al. (2010) and Proctor and He (2017). Once removed, rhizome sections were placed in 500 mL graduated cylinders containing 450 mL of the assigned Na^+ concentration solution.

3.2.4 Experimental Design

Rhizome sections were grown in hydroponic solutions of different Na^+ concentrations, forming the treatments for the study. The Na^+ concentrations used were 0, 200, 600 and 2000 mg L^{-1} of Na^+. These solutions were made using Na_2SO_4, as SO_4^{2-} is a dominant anion in the Constructed Fen (Kessel et al. 2018). The first three concentrations were selected to represent the range of Na^+ concentrations in the fen (80 to 613 mg L^{-1}; Chapter 2) and the average Na^+ concentration (240 mg L^{-1}; Chapter 2). A 2000 mg L^{-1} solution was used to test the upper limits of Na^+ resiliency and as it represents a SO_4^{2-} concentration of approximately 4200 mg L^{-1}, which is near the upper limit of SO_4^{2-} concentration in the fen (4960 mg L^{-1}; Chapter 2). The concentrations selected, therefore, span the range of concentrations observed in the fen and allow for projection of future exudation rates, as salt concentrations are expected to increase (Kessel et al. 2018). In addition to the concentrations of Na_2SO_4, 40 mg L^{-1} of 24-8-16 nitrogen-phosphorus-potassium nutrient fertilizer was added to each solution. The nutrient concentrations, beyond the addition of Na_2SO_4, consisted of 9.6 mg L^{-1} nitrogen, 3.2 mg L^{-1} phosphorus, 6.4 mg L^{-1} potassium, 1.6 mg L^{-1} sulfur, 0.008 mg L^{-1} boron, 0.028 mg L^{-1} copper, 0.06 mg L^{-1} chelated iron, 0.02 mg L^{-1} chelated manganese, 0.0002 mg L^{-1} molybdenum, 0.024 mg L^{-1} zinc and 0.48 mg L^{-1} ethylenediaminetetraacetic acid (EDTA) as the chelating agent dissolved in UPDI.

This study used hydroponics to analyze rhizodeposition, recognizing the limitation of not replicating natural plant growth conditions. This was done as the goal of the experiment was not to determine the magnitude of exudation rates that would be seen in the fen, but instead to examine the effect of Na^+ on rhizodeposit contribution to DOC by fen plants. Plants were initially grown in peat, so their main root structures were developed with the mechanical impedance of peat, a factor known to influence exudation (Vranova et al. 2013; Oburger and Jones 2018). Furthermore, using plants from the Constructed Fen that have been subject to increasing Na^+ concentrations since its construction, allows for evaluation of rhizodeposition for species that are tolerant to current Constructed Fen Na^+ concentrations. Vascular plants can gradually acclimate to salt stress, leading to some degree of tolerance (Bartels and Sunkar 2005); using plants grown under different conditions would thus not consider the tolerance level of plants grown at the constructed site.

Eight replicates were used for each treatment. Therefore, with two plant species, four treatments and eight replicates, a total of 64 plants were extracted. Within each core, plants were randomly assigned to a treatment solution. Once plants were placed in their treatment solution, they were left to grow for three weeks with the hydroponic solution being replaced weekly (**Fig 3-1C-D**). At the time of solution replacement, the final volume in each cylinder was recorded without the plant, and cylinders were triple rinsed with DI. After the third solution replacement, plants were left to grow for three days before water and plant samples were collected. All plants from both species had green shoots and exhibited signs of root growth until the end of the experiment. The whole plant was placed in a paper bag and dried at 60 °C for 48 h and then weighed for root and shoot biomass. Prior to sampling, filters and syringes were rinsed with UPDI and a blank of filtered UPDI and DI was collected to consider any background DOC present. Water samples were collected directly from the graduated cylinders with plants removed using a 60 mL syringe and were lightly shaken prior to extracting the sample to reduce solute stratification. The sample was then filtered through 0.45 µm PES syringe filters into 50 mL centrifuge vials. Two samples were collected from each plant using a new filter with each sample. Samples were stored in a cooler until all samples were collected and then stored in a fridge at 4 °C until analysis.

3.2.5 Sample Analysis

Due to algal growth in some of the solutions, only six samples from each treatment-species combination were analyzed for rhizodeposit concentration and composition using Liquid Chromatography Organic Carbon Detection (LC-OCD). This method is typically used to determine the composition of organic carbon compounds in surface waters for water treatment (Huber et al. 2011), but is used in this study to characterize the organic carbon released as rhizodeposits. The LC-OCD system (DOC Labor Huber, Stuttgart, Germany) consists of an autosampler, injection unit and high pressure liquid chromatography pump that injects the sample carried by a phosphate buffer eluent into a size exclusion column (Toyopearl HW 50S, 250 mm x 20 mm, 30 µm from TOSOH Bioscience), the outflow is analyzed for UV absorbance at 254 nm, then by organic carbon and organic nitrogen detectors to determine concentration. The size exclusion column fractionates organic carbon based on its binding strength with the column, with larger compounds eluting from the column before smaller compounds. Plotting detection through time produces a chromatogram which is integrated to determine the concentration of different fractions of organic matter. Peaks of the chromatograph have been identified as representing five groups of organic compounds, listed from shortest to longest elution time: biopolymers (BP), humic substances (HS), building blocks (BB), LMW acids

(LMWA) and LMW neutrals (LMWN). The fraction of organic carbon retained in the column can be calculated by subtracting the fraction passing through the column from the bypass peak, this fraction is known as hydrophobic organic carbon (HOC).

LC-OCD analysis of the bulk hydroponic solutions for each treatment revealed initial DOC concentrations in the HS to BB range, the LMWN range and in HOC. The HS to BB peak in the bulk hydroponic solutions was identified as the EDTA ($C_{10}H_6N_2O_8$), which was added with the nutrient solution as a chelating agent to aid the absorption of iron and manganese. As EDTA is not retained by plants and is resistant to microbial degradation (Oviedo and Rodríguez 2003), this initial peak was accounted for by subtracting the contribution from the bulk hydroponic solution from quantities in samples to obtain the rhizodeposited fraction. Two *J. balticus* samples had slightly negative rhizodeposition rates (-0.02 and -0.17 µgC g_dwt^{-1} hr^{-1}) after this subtraction, so were assumed to be, and were replaced with, 0 µgC g_dwt^{-1} hr^{-1}. The LMWN peak was also introduced from the fertilizer as urea (CH_4N_2O), one of the nitrogen sources for plants. As the nitrogen derived from urea is taken up by plant roots it is not conservative, thus for final publication the urea concentration of each sample will be quantified and subtracted from the LMWN concentration to obtain the rhizodeposition of LMWN. In this thesis, however, LMWN are excluded from analysis. The initial peak of HOC was not identified and was thus excluded from analysis. After subtracting the initial peaks, results provided by the LC-OCD were converted into mass of carbon of rhizodeposits released per gram of dry weight root biomass (g_dwt) per hour to account for differences in root biomass and volumes of water in each cylinder. As BP, HS, BB and LMWA were the only fractions considered for the rate of rhizodeposition, total carbon rhizodeposition for this study was calculated as the sum of these components.

The additional sample was used to measure pH and EC. pH and EC were measured using an Orion Star^{TM} A325 pH/Conductivity multiparameter meter with an Orion 8107UWMMD Ultra pH/ATC Triode and a Thermo Scientific^{TM} Orion^{TM} Conductivity and Temperature Probe. Probes were calibrated for pH with pH 4, 7 and 10 standards and calibrated for EC with 1413 µS cm^{-1} and 12 880 µS cm^{-1} standard solutions. Both EC and pH were normalized for root biomass and solution volume for analysis.

3.2.6 Data Analysis

Data was analyzed using R (R Core Team 2019) and R studio (RStudio team 2016). Significant differences between change in pH, change in EC, total rhizodeposition, rhizodeposition of DOC fractions, and the proportional rhizodeposition of DOC fractions between species and Na^+ concentrations was done using two-factor ANOVAs on linear models in R. Additional models evaluating controls on rhizodeposition rate were developed adding shoot to root ratio (calculated through biomass) or pH as additional fixed effects to evaluate the variability in rhizodeposition introduced by these variables. Na^+ concentration was considered as both numeric and categorical and results were similar between models. Models considering Na^+ concentration as numeric are presented. Due to missing data in the carbon to nitrogen ratios (C:N) of BP and HS, and the average molecular weight of humic substances a type III ANOVA was performed on linear models for these response variables to account for unequal sample sizes. Statistical significance was determined when $p<0.05$. The R^2 of linear models was determined using the r.squaredGLMM function from package *MuMIn* (Barton 2019). All models were visually assessed for normality and homogeneity of variance with BP, HS and BB for both rhizodeposition rates and proportion, total rhizodeposition and HS C:N log transformed to meet the ANOVA assumptions. To account for rhizodeposition rates of 0 μgC g_dwt^{-1} hr^{-1} for BB, a value of 1 was added to rates prior to log transformation in models.

3.3 Results

3.3.1 Treatment effect on pH and EC

Plant influence on the pH and EC of their hydroponic solutions was dependent on Na^+ concentration (**Fig 3-2**). Prior to plant addition, the pH of hydroponic solutions was 6.73, 5.90, 5.97 and 6.21, while EC was 33.29, 1030, 2898 and 8710 μS cm^{-1}, with both measurements listed by increasing Na^+ concentration. While *C. aquatilis* tended to increase the pH of the solution, *J. balticus* decreased the pH (two-factor ANOVA, $F_{1,44}$=18.00, p<0.001). However, as Na^+ concentration increased the solution pH for both species increased (two-factor ANOVA, $F_{1,44}$=6.69, p=0.013), with no significant difference in the rates of increase between species (two-factor ANOVA, $F_{1,44}$=0.017, p=0.900). At a Na^+ concentration of 0 mg L^{-1}, plants from both species increased the EC of the solution, but change in EC correlated linearly and negatively with Na^+ concentration (two-factor ANOVA, $F_{1,44}$=8.77, p=0.005), leading to plants decreasing EC at 2000 mg L^{-1} Na^+ (**Fig 3-2**).

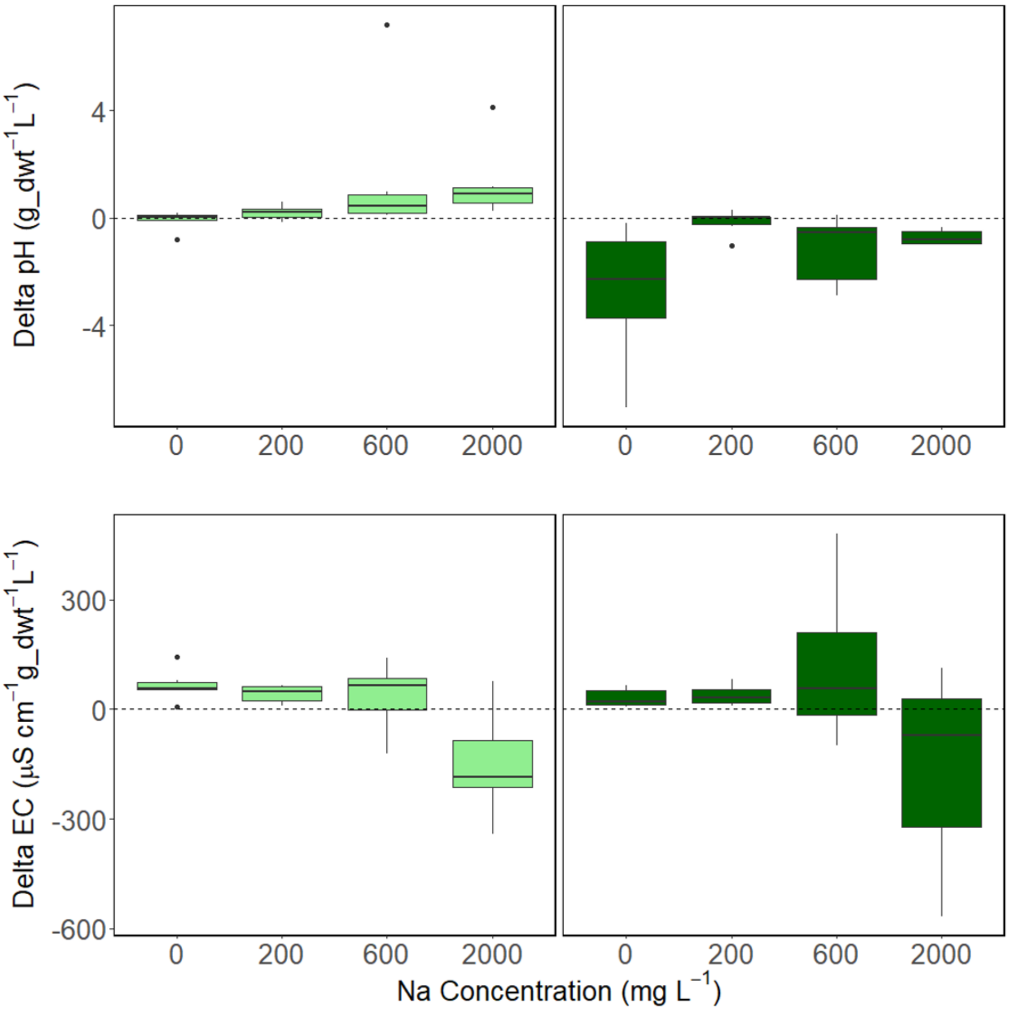


Fig 3-2 Change in EC and pH normalized to root biomass and water volume of hydroponic solutions where *Carex aquatilis* (light green) and *Juncus balticus* (dark green) were grown for 3 days. Values are normalized to dry root biomass (g_dwt) and the volume of the solution remaining at the end of the experiment. The dashed lines indicate no change in pH or EC.

3.3.2 Treatment effect on rhizodeposition rate

Total rhizodeposition rates ranged from 3.51 to 11.24 µgC g_dwt^{-1} hr^{-1} for *C. aquatilis* and 1.03 to 44.94 µgC g_dwt^{-1} hr^{-1} for *J. balticus* with no significant differences between species (two-factor ANOVA, $F_{1,44}$=0.53, p=0.209) or with changes in Na$^+$ concentration (two-factor ANOVA, $F_{1,44}$=1.07, p=0.307). While the change in total rhizodeposition rate with Na$^+$ concentration was not significant, a general increase in total rhizodeposition rate with increasing Na$^+$ concentration was observed (**Fig 3-3**). These rates do not include the contribution of LMWN which could increase average rhizodeposition rates by up to 29% or 51% for *C. aquatilis* and *J. balticus*, respectively. However, these maximum increases were evaluated using the concentration without removal of urea, so actual increases by LMWN contribution are expected to be much lower and more similar between species. There were also no significant differences in BP, HS, BB or LMWA rhizodeposition rates with species or Na^+ concentration (**Table A 5**). Despite the lack of significance, the rhizodeposition rate of BP and LMWA decreased with increasing Na^+ concentration while the rhizodeposition rate of HS increased, with BB rhizodeposition also increasing with increasing Na^+ concentration for *C. aquatilis* (**Fig 3-4**).

Rhizodeposition rate was highly variable between rhizome sections, with *J. balticus* having the highest variability between rhizome segments within a species and 600 and 2000 mg L^{-1} having the highest variability among treatments (**Fig 3-4**). This was reflected in the low R^2 described by linear models when only species and Na^+ concentration were considered as fixed effects (**Table A 5**). Solution pH and shoot to root ratio were able to explain some of this variability. pH and its interactions were able to explain an additional 24% of total rhizodeposition rate variability where shoot-to-root ratio and its interactions explained an additional 13%. Within DOC fractions, shoot to root ratio explained more additional variability than pH for BP and BB, where the reverse was true for HS and LMWA.

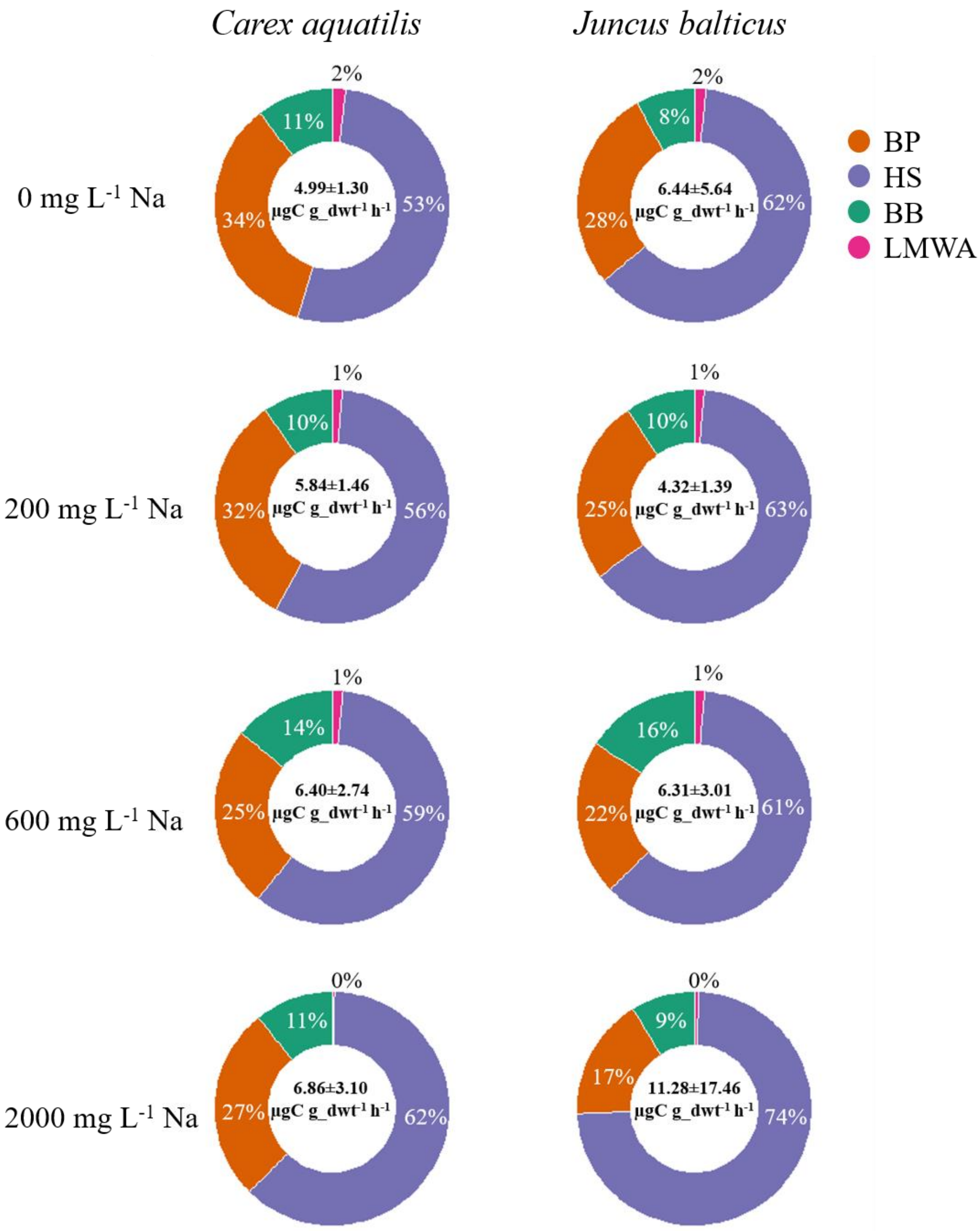


Fig 3-3 Total Rhizodeposition rate and standard deviation for *Carex aquatilis* and *Juncus balticus* grown hydroponically under different Na^+ concentrations. Average percentages of each organic carbon fraction are shown (BP- biopolymers, HS- humic substances, BB- building blocks and LMWA- low molecular weight acids).

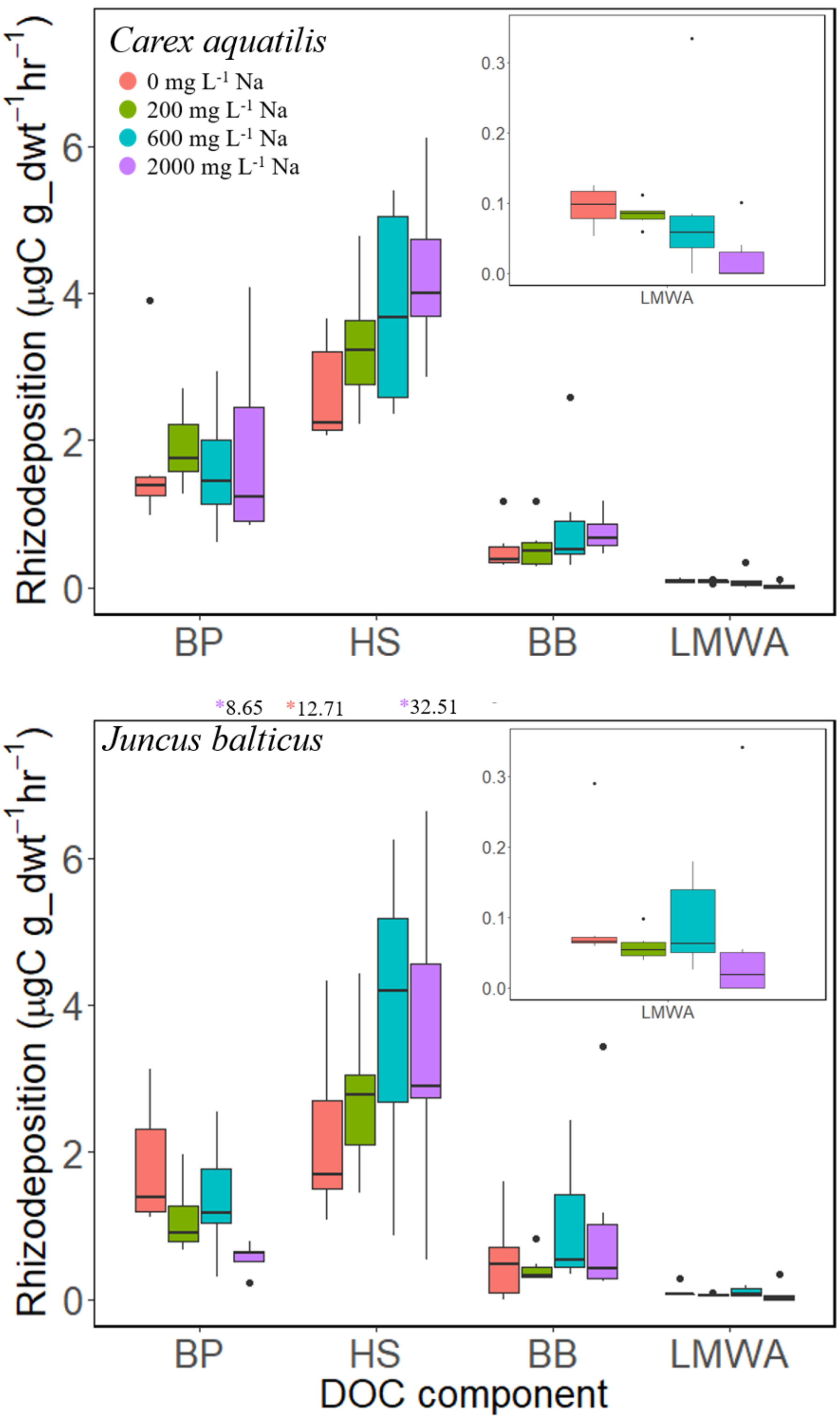


Fig 3-4 Rhizodeposition rate of each organic carbon fraction (BP- biopolymers, HS- humic substances, BB- building blocks and LMWA- low molecular weight acids) for *Carex aquatilis* and *Juncus balticus*. The top right images provided a zoomed in scale for LMWA. Asterixis above the plot indicate the rhizodeposition rate for extreme outliers.

3.3.3 Treatment effect on rhizodeposit proportions and quality

Though there were no significant differences between species and Na^+ concentrations for the rate of rhizodeposition, there were significant changes in the proportion of DOC fractions (**Fig 3-3**). The proportion of rhizodeposition in each fraction was similar between species, with the exception of BP, where *C. aquatilis* rhizodeposits had a higher proportion of BP than *J. balticus* (two-factor ANOVA, $F_{1,44}$=5.74, p=0.021; **Fig 3-3**). Na^+ concentration significantly affected the proportion of BP, HS and LMWA, with the proportion of BP and LMWA decreasing with increasing Na^+ concentration (two-factor ANOVA, $F_{1,44}$=21.00, p<0.001; two-factor ANOVA, $F_{1,44}$=39.63, p<0.001; respectively), and the proportion of HS increasing with increasing Na^+ concentration (two-factor ANOVA, $F_{1,44}$=6.84, p=0.012). These changes in proportion appeared to be largely from the increase in rhizodeposition rate of HS (**Fig 3-4**), with the combination of increases in some fractions and decreases in others leading to the significance of results. The proportion of BB did not significantly differ with Na^+ concentration (two-factor ANOVA, $F_{1,44}$=2.52, p=0.120). Despite the significance of these changes in proportion, most variability was due to the rhizome section with Na^+ concentration, species and their interaction explaining 38, 14, 6 and 46% of variability for the proportion of BP, HS, BB and LMWA, respectively.

For HS the C:N, molecular weight and $SUVA_{254}$ for this fraction was determined with the C:N for BP also calculated. Rhizodeposit quality reflected in these parameters was not significantly different between species or Na^+ concentrations (**Table A 6**). Though not significant, HS rhizodeposits generally increased in $SUVA_{254}$ and C:N for both species, while the molecular weight of HS in *J. balticus* rhizodeposits increased as Na^+ concentration increased

(**Fig 3-5**). C:N for BP was higher than that of HS (two-tailed t-test, t=6.88, df=58, p<0.001), but exhibited little change under different Na^+ concentrations (**Fig 3-5**).

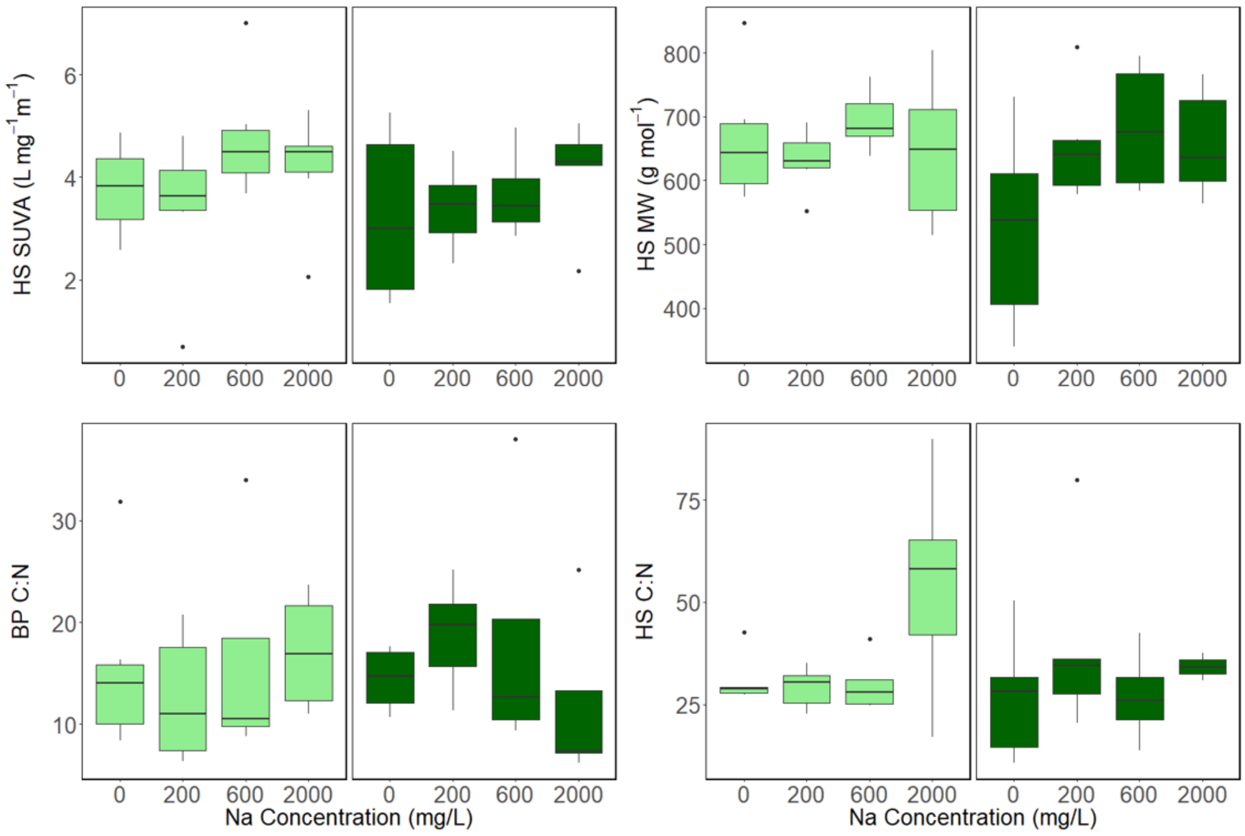


Fig 3-5 SUVA$_{254}$, average molecular weight (MW) and carbon to nitrogen ration (C:N) for humic substances (HS) and C:N for biopolymers (BP) for rhizodeposits of *Carex aquatilis* (light green) and *Juncus balticus* (dark green). Plants were grown hydroponically with rhizodeposits collected 3 days after solution replacement.

3.4 Discussion

As peatlands with elevated Na^+ concentrations are expected to become more abundant, due to sea level rise and reclamation (Kessel et al. 2018; Guêné Nanchen et al. 2020), the current study provides information on the effects of Na^+ on rhizodeposition of two peatland species. While currently the contribution of LMWN to rhizodeposition could not be assessed, the results presented still provide valuable information on *C. aquatilis* and *J. balticus* rhizodeposits and influence on rhizosphere chemistry under different Na^+ concentrations.

3.4.1 Root-mediated changes to pH and EC

The plant species studied had differing effects on hydroponic solution pH at 0 mg L^{-1} Na^+, with *C. aquatilis* having little effect on pH while *J. balticus* decreased pH (**Fig 3-2**). Roots are known to alter rhizosphere pH primarily though the release of OH^- or H^+ to compensate for the uptake of anions and cations, respectively (Hinsinger et al. 2003). This suggests that *C. aquatilis* uptake had roughly equal proportions of cations and anions while *J. balticus* primarily took up cations. The release of low molecular weight organic acids may also acidify the rhizosphere, largely through root uptake of OH^- to maintain charge balance (Hinsinger et al. 2003; Jones et al. 2004). While rhizodeposition of low molecular weight organic acids was low (**Fig 3-3**) other rhizodeposit fractions may be charged or have acidic functional groups and thus their export may have contributed to the shifts in solution pH observed.

Na^+ concentration affected root-mediated changes in solution pH with both species having an increase in solution pH as Na^+ concentration increased (**Fig 3-2**). Na^+ and SO_4^{2-} do not behave as buffers, due to the strength of the conjugate base and acid, respectively. Therefore, changes in pH will not be buffered differently based on the Na^+ treatment. One strategy by which plants prevent Na^+ from accumulating to toxic levels is to actively transport Na^+ out of root cells using Na^+/H^+ antiporters (Bartels and Sunkar 2005; Isayenkov 2012; Munns et al. 2020). In actively exporting Na^+ from cells these antiporters must uptake H^+, thus increasing the pH of solution outside of the cell. The increase in solution pH as Na^+ concentration increases could thus be explained by the action of Na^+/H^+ antiporters and indicates that their activity can change to account for different levels of Na^+ uptake over the concentration range of this study. However, based on the trend of decreasing change in EC as Na^+ concentration increases, plant mechanisms to exclude or remove Na^+ do not appear sufficient to prevent accumulation of Na^+. At Na^+

concentrations of 2000 mg L^{-1}, the change in EC decreased despite a similar rhizodeposition rate, with rhizodeposits contributing to increased EC (**Fig 3-2**; **Fig 3-3**), indicating net Na^+ uptake by plants. While *C. aquatilis* and *J. balticus* may be unable to prevent net uptake of Na^+ at 2000 mg L^{-1} Na^+, accumulation of Na^+ in vacuoles and transport of Na^+ within the plant may still reduce salt stress (Isayenkov 2012).

3.4.2 C. aquatilis and J. balticus rhizodeposits

As there is no known way to measure rhizodeposits under field conditions without introducing confounding factors, a variety of methods can be employed based on the purpose of study (Vranova et al. 2013; Oburger and Jones 2018). It is therefore difficult to compare rhizodeposition rates between studies. For example, for *E. vaginatum* Proctor and He (2017) measured average rhizodeposition rates of 1.24 to 2.55 µg C g_dwt^{-1} hr^{-1}, depending on root class, while Edwards et al. (2018) measured rates between 1.47 to 50.48 mg C g_dwt^{-1} hr^{-1}. This represents a difference in measured rhizodeposition of 3 to 4 orders of magnitude for the same species. Yet comparisons between treatments can still be made within studies, with this study finding similar rhizodeposition rates overall and within DOC fractions between *C. aquatilis* and *J. balticus*.

Though the fraction names provided by LC-OCD are useful for surface water, these names may not accurately describe the fractions of carbon represented for rhizodeposits. BP and LMWA are still appropriate to refer to plant derived compounds with BP being composed of polysaccharides and proteins over 10 kDa and LMWA composed of LMW organic acids (Huber et al. 2011). However, as humic substances and building blocks are the product of decomposition, these names may be misleading for representing rhizodeposits. It is likely that HS and BB represent plant phenolic and aromatic compounds that are the precursors to humic substances (Stevenson 1994; Wershaw 2004; Cesco et al. 2012), with BB being distinguished by a lower molecular weight. HS were still relatively small in this study (644 ± 102 g mol^{-1}), placing this fraction around the molecular weight of glycosylated flavonoids (Cesco et al. 2010; Mierziak et al. 2014), where BB represent smaller compounds such as flavonoids. However, both fractions are likely to represent a variety of plant-derived aromatic compounds.

Previous studies on peatland species rhizodeposits have focused on characterizing organic acids, amino acids and sugars (Saarnio et al. 2004; Koelbener et al. 2010; Proctor and He 2017; Edwards et al. 2018). Though some of these studies placed these LMW compounds in the context of total rhizodeposition, characterization of compounds in LC-OCD classifications of BP, HS and BB have not been considered. This study found that for both *C. aquatilis* and *J. balticus*, rhizodeposits primarily consist of compounds in the HS fraction, with this fraction generally accounting for over 50% of rhizodeposited carbon (**Fig 3-3**). Though HS may have been overestimated in this study due to the use of a 3-day sampling period, which may overestimate rhizodeposition of flavonoids (Cesco et al. 2010), HS would still represent a large fraction of rhizodeposit contribution to DOC. Biopolymers represented a larger proportion of rhizodeposits for *C. aquatilis* over *J. balticus*, but still generally represented over 20% of characterized rhizodeposits for both species, reflecting the importance of measuring HMW compounds. LMWA rhizodeposition was low for both species, with LMWA accounting for below 2% of total rhizodeposition. Given the importance of plant-derived organic acids in the rhizosphere (Jones 1998; Jones et al. 2004) and the focus on their characterization in many rhizodeposition studies (Vranova et al. 2013; Oburger and Jones 2018), the low concentration and proportion of organic acids is unexpected. Similar to the effect of sampling methodology on rhizodeposition rates, differences in sampling procedure may lead to differences in the proportion of rhizodeposit components. Using *E. vaginatum* as an example again, both Edwards et al. (2018) and Saarnio et al. (2004) found the dominant sugars and organic acids to vary over the growing season, with the dominance of individual compounds varying differently between studies. For example, in July while organic acids, namely ascorbic and oxalic acid, dominated characterized rhizodeposits in Edwards et al. (2018), in Saarnio et al. (2004) mannose was the most abundant characterized rhizodeposit with acetic acid being the most abundant organic acid. The specific methodology employed during this study may thus explain the low contribution of organic acids.

3.4.3 Influence of species and Na^+ concentration on rhizodeposition rate and quality

Though this study found no significant differences in the rhizodeposition rate between Na^+ concentrations or species for any DOC fraction, the trends may have been masked by high interplant variability and low sample size. Total rhizodeposition increased under elevated Na^+ concentrations due to an increase in HS rhizodeposits, despite the decreases in BP and LMWA (**Fig 3-3**; **Fig 3-4**). When proportions of DOC fractions were considered, relationships were significant due to the interacting effects of increases in rhizodeposition rates for some fractions (HS) and decreases in other fractions (BP and LMWA) and results agreed with trends identified in rates.

The similar responses of *C. aquatilis* and *J. balticus* to elevated Na^+, as reflected by the lack of interaction between species and Na^+ concentration, suggests a similar mechanism for changes in rhizodeposition with Na^+ concentration. As Na^+ concentration increases, the permeability of the plasma membrane increases, and this effect is largest when calcium (Ca^{2+}) is not present to stabilize the plasma membrane (Nedjimi and Daoud 2009; Vranova et al. 2013). Flavanoids, a type of polyphenol, can enter the hydrophobic core of phospholipid membranes, reducing their fluidity and thus reducing diffusion across the cell membrane (Arora et al. 2000; Mierziak et al. 2014). Therefore, the increase in polyphenol rhizodeposition (expressed through HS) as Na^+ concentrations increased could be explained by increasing production of polyphenols to stabilize the cell membrane. Stabilization of the cell membrane, to reduce toxic Na^+ concentration, may explain the corresponding decreases in LMWA rhizodeposition as organic acids generally passively diffuse across the plasma membrane (Jones et al. 2009). Due to the high molecular weight of BP, however, compounds in this class must be actively released by roots and thus would not respond directly to changes in membrane permeability. Mucilage, a HMW rhizodeposit composed predominately of polysaccharides with proteins and phospholipids, (Jones et al. 2009), likely contributed to measured BP. Mucilage was found to increase in roots under higher Na^+ concentrations in *Kosteletzkya virginica*; however, the authors suggest that Na^+ may facilitate mucilage gelling (Edmond Ghanem et al. 2010). This gelling effect could lead to decreases in mucilage solubility with increased Na^+ concentration, contributing to the lower proportion of BP under high Na^+ concentration in this study. Alternatively, as Na^+ and SO_4^{2-} reduce the solubility of DOC (Clark et al. 2005, 2012; Tamamura et al. 2013; Brouns et al. 2014), which has a larger effect on HMW compounds, precipitation of BP as Na^+ and SO_4^{2-} concentration increased may have led to the decreasing BP rhizodeposition measured. While the observed decrease in BP as Na^+ concentrations increased likely did not reflect a direct change in rhizodeposition rate, it still reflects a change in the rate of contribution of rhizodeposits to solution DOC.

Shifts in the proportions of compounds rhizodeposited under different Na^+ concentrations may contribute to shifts in DOC quality in the rhizosphere. Though the MW of HS increased as Na^+ concentration increased (**Fig 3-5**), as BP have much higher MW (> 10 kDa) compared to HS

(~650 Da), molecular weight of rhizodeposits decreased as Na^+ concentrations increased. Additionally, given the aromatic character of HS ($SUVA_{254}$ = 3.82 ± 1.16 L mg^{-1} m^{-1}) compared to BP, which exhibit little UV absorbance, and the increase in aromaticity of HS with increasing Na^+ concentration (**Fig 3-5**), aromaticity of rhizodeposits would be expected to increase as Na^+ concentration increased. It is unclear how this relates to the overall lability of rhizodeposit DOC as while HS are smaller than BP they are generally more recalcitrant due to their aromaticity and higher C:N (**Fig 3-5**). Without considering the contribution of LMWN to rhizodeposition under different Na^+ concentrations, the overall effect of Na^+ on rhizodeposit quality and contribution to DOC quality cannot be determined.

3.4.4 Limitations

This study has revealed the importance of considering higher molecular weight compounds than organic acids, amino acids and simple sugars in rhizodeposit analysis, and shifts in the rhizodeposition of different fractions of rhizodeposits under elevated Na^+ concentration. However, further research is needed to better evaluate the effects of Na^+ on rhizodeposition. One limitation to this study is the lack of consideration for compounds in the LMWN range of the LC-OCD, which would include compounds like simple sugars and amino acids. As these compounds are generally considered to be abundant rhizodeposits (Dakora and Phillips 2002; Walker et al. 2003), their inclusion in analysis may shift the trends of overall rhizodeposition observed in this study.

Another finding of this study was the high variability between individual rhizome sections in rhizodeposition, with species and Na^+ concentration weakly explaining rhizodeposition rates in each fraction. Some additional variability was related to the shoot to root ratio and solution pH. The shoot to root ratio determines the amount of carbon available to roots and thus, the amount available for rhizodeposition (Farrar et al. 2003), where pH effects the solubility of rhizodeposits once they enter solution (Kalbitz et al. 2003). However, unexplained variation was still high and adding these factors in models did not lead to Na^+ concentration and species being significant in shifting rhizodeposition rates. Only when the interacting effects of decreases in some fractions and increases in other fractions, as revealed by proportions, were these effects significant (**Fig 3-3**). As roots of different classes and ages are known to have differing rhizodeposition rates (Proctor and He 2017), some of the variability associated with rhizome sections may be due to the composition of its root types. Future studies examining Na^+ effects on rhizodeposition should include a larger sample size to better account for variability between rhizome sections and consider biomass associated with root classes in analysis.

Though this study has provided information on rhizodeposition of the dominant plant species at the Constructed Fen (*C. aquatilis* and *J. balticus*), the results are not directly applicable to a field setting. Specifically, the use of hydroponics, which would shift root growth after hydroponics had begun, and removal from peat cores leading to some root damage would lead to differences in rhizodeposition as compared to the field (Oburger and Jones 2018). Additionally, the exclusion of Ca^{2+} to facilitate determination of the effect of Na^+ on rhizodeposition in thus study, ignored the high concentrations of Ca^{2+} found at the Constructed Fen (Simhayov et al. 2017), which may significantly influence rhizodeposition (Oburger et al. 2013; Vranova et al. 2013; Oburger and Jones 2018). Therefore, studies attempting to apply rhizodeposition results to the Constructed Fen should consider varying Ca^{2+} concentration and Ca^{2+} to Na^+ ratios.

3.5 Conclusion

Although total rhizodeposition of *C. aquatilis* and *J. balticus* generally increased with increasing Na^+ concentration, rate did not vary significantly in this study due to the high variability between rhizome sections in rhizodeposition. However, when considering proportions of rhizodeposition components, the underlying changes in rhizodeposition rate led to significant differences. Specifically, overall rhizodeposition increased with increasing Na^+ concentration due to an increase in HS despite the decreases in BP and LMWA. *C. aquatilis* and *J. balticus* had similar rhizodeposition rates of each fraction with *C. aquatilis* having a slightly higher rate of BP rhizodeposition. Implications of these results should be taken with caution due to the exclusion of LWMN from this study. Both species displayed evidence of mechanisms used to prevent Na^+ accumulation, with increasing Na^+ concentration leading to higher pH, suggesting the activity of Na^+/H^+ antiporters, and a higher proportion of aromatics in rhizodeposits, potentially to reduce membrane fluidity. Furthermore, this study has revealed the high contribution of aromatics and biopolymers to rhizodeposition, fractions that are generally excluded from rhizodeposition studies. While providing initial results on the effect of Na^+ concentration on rhizodeposition, this study also revealed several knowledge gaps in understanding the effects of Na^+ on rhizodeposition. Specifically, factors expected to influence variability in rhizodeposition between rhizome sections and the effect of Ca^{2+} on rhizodeposition were identified as areas for further study.

Chapter 4: Conclusions and Recommendations for Industry

Under the global effects of climate change, the importance of peatland carbon stores has become increasingly relevant. It is thus useful to better understand carbon dynamics in peatlands and what factors may affect carbon storage. Elevated Na^+ concentrations in peatlands constructed in post-mined landscapes and peatlands influenced by sea level rise may lead to shifts in carbon dynamics. Elevated Na^+ in the Constructed Fen provides an opportunity to study the effects of Na^+ on carbon dynamics, with this study evaluating the effects of Na^+ on DOC specifically. As a mobile and relatively bioavailable source of carbon in peatlands, DOC can provide insight on peatland carbon dynamics and expected peatland effects downstream. Furthermore, the results of this study provide insight on how constructed peatlands should be designed and monitored during reclamation and how reclamation decisions pertaining to solute release into constructed peatlands may affect carbon dynamics.

DOC concentrations in the Constructed Fen were high with low molecular weight, aromaticity and humification, suggesting a plant-derived origin over peat decomposition. Na^+, SO_4^{2-} and percent sedge and rush cover controlled DOC quantity and quality at 30 cm below the peat surface. Elevated Na^+ and SO_4^{2-} led to more labile DOC, with elevated Na^+ and higher percent sedge and rush cover contributing to higher DOC concentrations. Rhizodeposition, decomposition and solubility all played a role in controlling DOC concentration and quality at the Constructed Fen, with Na^+ exerting its dominant influence on rhizodeposition and decomposition. Closer to the surface, at 10 cm depth, models explained little variability in DOC suggesting more complex processes at this depth and identifying this region as an area for further study. With higher DOC concentrations at 10 cm over 30 cm, DOC dynamics at the near surface may exert a stronger influence on carbon dynamics at the Constructed Fen.

Due to the high concentrations of DOC at the Constructed Fen and the inferred mobility of DOC from its lability, it is recommended that industry monitor DOC outflow for consideration in carbon budgets. DOC quality in the Constructed Fen should also continue to be monitored to assess any changes in carbon dynamics such as shifts from plant-derived DOC to peat-derived DOC, which would indicate changes in peatland processes and function. Additionally, with DOC concentration and lability rising at the Constructed Fen and expected to continue as Na^+ concentrations continue to rise, monitoring of metal transport is suggested. DOC is known to bind and transport metals, which may be abundant in reclaimed systems, with labile DOC having a higher binding affinity and mobility than recalcitrant compounds. As connectivity between reclaimed systems will be implemented in final reclamation plans, ecosystems constructed downstream of peatlands should be tolerant to high DOC concentrations and the potential for elevated metal concentrations.

To facilitate better understanding of rhizodeposition at the Constructed Fen, rhizodeposition of *C. aquatilis* and *J. balticus* under various Na^+ concentrations was evaluated. Although this study was unable to consider the contribution of LMWN to rhizodeposition, it found a large proportion of rhizodeposits from these species to be aromatic, suggesting high rhizodeposition of polyphenolics. Furthermore, the proportion of HMW species such as proteins and polysaccharides revealed that the contribution of these compounds to rhizodeposition is not insignificant. While biopolymers may be relatively labile, the contribution of rhizodeposits to lability defined by lower $SUVA_{254}$ and higher E2/E3 may be overestimated in the Constructed Fen, due to the abundance of biopolymers and aromatics in rhizodeposits. As Na^+ concentration increased rhizodeposition of aromatics increased, while the rhizodeposition of biopolymers and LMW organic acids decreased. This may indicate a shift to lower molecular weight and higher aromaticity for rhizodeposition contributions to DOC in the Constructed Fen as Na^+ concentrations increase. Despite these results, without consideration for LMWN and the concentration of Ca^{2+}, known to stabilize root membranes, the applicability of results to the field is questionable.

Based on the results of this study thus far, the planting of *C. aquatilis* and *J. balticus* at the Constructed Fen appears appropriate to limit losses of carbon from plants to DOC under elevated Na^+. Furthermore, both species exhibited signs of root growth and live shoots at the end of the study suggesting resilience to elevated Na^+. *C. aquatilis* and *J. balticus* also displayed potential mechanisms to limit uptake of Na^+ to toxic levels with the effects of Na^+/H^+ antiporters and increases in membrane stabilizing polyphenols observed. Overall, this suggests that the planting of these species is appropriate given the observed effects of elevated Na^+ on rhizodeposition carbon loss. However, this study only considered the effects of Na^+ on one source of carbon loss and thus more insight into the survival of these species under field conditions at the Constructed Fen and on other carbon fluxes (e.g., CO_2 and CH_4) from plants is needed.

This study has illustrated the role of Na^+ in determining DOC concentration and quality at the Constructed Fen, as well as determined Na^+ concentration's effect on the rhizodeposition of the two dominant plant species at the Constructed Fen. This work has implications for peatland construction as a part of reclamation and for the effects of sea level rise on coastal peatlands. Furthermore, areas for future research have been identified to better understand the effects of Na^+ in peatlands and in the Constructed Fen specifically. Namely, work to better understand the effects of Na^+ on DOC decomposition and solubility should be undertaken to better predict the long-term trajectory of the Constructed Fen, with a focus on controls in the near surface. The important contribution of aromatics to rhizodeposition also suggests that future studies should continue to characterize rhizodeposits other than organic acids, amino acids and simple sugars to total rhizodeposition. Large variability in rhizome sections rhizodeposition suggests that research should be undertaken to better evaluate the effects of Na^+ on rhizodeposition, employing larger sample sizes and varying Ca^{2+} concentration and Ca^{2+} to Na^+ ratio.

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Appendix

Table A 1 Complete results of linear mixed effect model for environmental parameters correlated to DOC concentration at 10 and 30 cm in the Constructed Fen over the summer of 2019. DOC concentration was log transformed to achieve normality.

Model	Effect	F	P	R^2_m	R^2_c
log([DOC]) 10 cm	Vegetation Type	$F_{4,20}=0.23$	0.920	0.50	0.67
	Na^+	$F_{1,135}=0.24$	0.625		
	WL	$F_{1,135}=5.46$	0.021		
	T_{avg}	$F_{1,135}=0.01$	0.934		
	Vegetation Type × Na^+	$F_{4,135}=1.76$	0.141		
	Vegetation Type × WL	$F_{4,135}=1.51$	0.204		
	Vegetation Type × T_{avg}	$F_{4,135}=2.39$	0.054		
	Na^+ × WL	$F_{1,135}=5.50$	0.020		
	Na^+ × T_{avg}	$F_{1,135}=2.66$	0.105		
	Intercept	$F_{1,135}=105.61$	<0.001		
log([DOC]) 30 cm	Vegetation Type	$F_{4,20}=4.03$	0.015	0.41	0.84
	Na^+	$F_{1,150}=27.93$	<0.001		
	WL	$F_{1,150}=1.46$	0.228		
	T_{avg}	$F_{1,150}=12.27$	<0.001		
	Vegetation Type × T_{avg}	$F_{4,150}=3.56$	<0.001		
	Intercept	$F_{1,150}=493.73$	<0.001		

Table A 2 Complete results of linear mixed effect model for environmental parameters correlated to $SUVA_{254}$ at 10 and 30 cm in the Constructed Fen over the summer of 2019.

Model	Effect	F	P	R^2_m	R^2_c
$SUVA_{254}$ 10 cm	Vegetation Type	$F_{4,20}=0.54$	0.711	0.25	0.71
	Na^+	$F_{1,138}=4.10$	0.045		
	WL	$F_{1,138}=3.15$	0.080		
	T_{avg}	$F_{1,138}=17.15$	<0.001		
	Vegetation Type × Na^+	$F_{4,138}=0.28$	0.891		
	WL × T_{avg}	$F_{1,138}=2.49$	0.117		
	Intercept	$F_{1,138}=25.00$	<0.001		
$SUVA_{254}$ 30 cm	Vegetation Type	$F_{4,20}=3.17$	0.036	0.32	0.56
	Na^+	$F_{1,146}=8.57$	0.004		
	WL	$F_{1,146}=2.03$	0.157		
	T_{avg}	$F_{1,146}=0.003$	0.955		
	Vegetation Type × Na^+	$F_{4,146}=2.79$	0.028		
	Vegetation Type × T_{avg}	$F_{4,146}=1.47$	0.213		
	Intercept	$F_{1,146}=75.07$	<0.001		

Table A 3 Complete results of linear mixed effect model for environmental parameters correlated to E2/E3 at 10 and 30 cm in the Constructed Fen over the summer of 2019.

Model	Effect	F	P	R^2_m	R^2_c
E2/E3 10 cm	Vegetation Type	$F_{4,20}=2.50$	0.075	0.20	0.81
	Na^+	$F_{1,138}=3.18$	0.077		
	WL	$F_{1,138}=5.79$	0.017		
	T_{avg}	$F_{1,138}=0.66$	0.418		
	Vegetation Type × T_{avg}	$F_{4,138}=4.46$	0.002		
	Intercept	$F_{1,138}=119.54$	<0.001		
E2/E3 30 cm	Vegetation Type	$F_{4,20}=4.92$	0.006	0.47	0.74
	Na^+	$F_{1,150}=5.03$	0.026		
	WL	$F_{1,150}=2.55$	0.112		
	T_{avg}	$F_{1,150}=25.59$	<0.001		
	Vegetation Type × Na^+	$F_{4,150}=5.49$	<0.001		
	Intercept	$F_{1,150}=83.03$	<0.001		

Table A 4 Complete results of linear mixed effect model for environmental parameters correlated to E4/E6 at 10 and 30 cm in the Constructed Fen over the summer of 2019.

Model	Effect	F	P	R^2_m	R^2_c
E4/E6 10 cm	Vegetation Type	$F_{4,20}=3.07$	0.040	0.27	0.35
	Na^+	$F_{1,137}=2.23$	0.138		
	WL	$F_{1,137}=9.42$	0.003		
	T_{avg}	$F_{1,137}=4.15$	0.043		
	Vegetation Type × Na^+	$F_{4,137}=3.34$	0.012		
	Na^+ × WL	$F_{1,137}=8.42$	0.004		
	Na^+ × T_{avg}	$F_{1,137}=1.48$	0.226		
	Intercept	$F_{1,137}=1.60$	0.208		
E4/E6 30 cm	Na^+	$F_{1,152}=3.68$	0.057	0.24	0.31
	WL	$F_{1,152}=9.89$	0.002		
	T_{avg}	$F_{1,152}=0.27$	0.602		
	Na^+ × T_{avg}	$F_{1,152}=4.17$	0.043		
	WL × T_{avg}	$F_{1,152}=8.30$	0.005		
	Intercept	$F_{1,150}=10.53$	0.001		

Table A 5 Two-factor ANOVA results for the influence of Na^+ concentration and plant species on rhizodeposition of BP- biopolymers, HS- humic substances, BB- building blocks and LMWA- low molecular weight acids. The species investigated were *Carex aquatilis* and *Juncus balticus* at Na^+ concentrations of 0, 200, 600 and 2000 ppm. Parameters were log transformed to meet normality requirements.

Model	**Effect**	**$F_{1,44}$**	**p**	**R^2**
log(BP)	Na^+	1.95	0.169	0.12
	Species	3.47	0.069	
	Na^+ × Species	1.04	0.314	
log(HS)	Na^+	3.16	0.082	0.07
	Species	0.14	0.709	
	Na^+ × Species	0.01	0.931	
log(BB)	Na^+	3.17	0.082	0.07
	Species	0.02	0.903	
	Na^+ × Species	0.13	0.720	
LMWA	Na^+	1.79	0.188	0.05
	Species	0.09	0.762	
	Na^+ × Species	0.52	0.476	
log(Total Rhizodeposition)	Na^+	1.04	0.314	0.03
	Species	0.52	0.475	
	Na^+ × Species	0.06	0.802	

Table A 6 Two-factor ANOVA results for the influence of Na^+ concentration and plant species on rhizodeposit quality of BP- biopolymers and HS- humic substances. The species investigated were *Carex aquatilis* and *Juncus balticus* at Na^+ concentrations of 0, 200, 600 and 2000 ppm. N:C- nitrogen to carbon ratio, MW- molecular weight and $SUVA_{254}$- specific ultraviolet absorbance at 254 nm were investigated. Parameters were log transformed to meet normality requirements.

Model	**Effect**	**$F_{1,44}$**	**p**	**R2**
BP C:N	Na^+	0.23	0.478	0.10
	Species	0.77	0.387	
	Na^+ × Species	0.68	0.415	
log(HS C:N)	Na^+	3.07	0.089	0.13
	Species	0.00	0.998	
	Na^+ × Species	0.21	0.651	
HS MW	Na^+	2.93	0.094	0.13
	Species	1.08	0.304	
	Na^+ × Species	2.89	0.096	
HS $SUVA_{254}$	Na^+	3.51	0.067	0.10
	Species	1.63	0.209	
	Na^+ × Species	0.01	0.904	