ORGANIC MATTER CYCLING IN A RESTORED WETLAND RECEIVING COMPLEX EFFLUENT

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ABSTRACT

Wetlands are used to treat anthropogenic effluents due to their capacity for intense biogeochemical processing of nutrients and other pollutants, yet their role in processing effluent dissolved organic matter (DOM) is unclear. Here, I quantified the cycling of DOM in Frank Lake, an economically-important wetland restored using multiple effluent sources. Optical analyses and microbial incubations showed DOM changed from protein-like and bioavailable at the effluent inflow site to more aromatic and less bioavailable at the outflow, due to the processing and replacement of effluent DOM with that from marsh plants and wetland soils. My mass balance showed Frank Lake was a net long-term source of DOM, but shifted from a source to sink among wet and drought periods that respectively shortened or lengthened the water residence and DOM processing times. Overall, Frank Lake appears to effectively process effluent DOM, despite being a longer-term source of DOM to downstream environments.

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LIST OF ABBREVIATIONS

a_{254}	Absorbance coefficient at 254 nm
AFETUW	Alberta Flow Estimation Tool for Ungauged Watersheds
ANOVA	Analysis of variance
BL	Blackie Creek
B1	Basin 1
B1O	Basin 1 outflow
B2	Basin 2
B2O	Basin 2 outflow
B3O	Basin 3 outflow
BDOC	Biodegradable dissolved organic carbon
C	Carbon
CO_2	Carbon dioxide
CDOM	Chromophoric dissolved organic matter
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOC_{IN}	Input of dissolved organic carbon
DOC_{OUT}	Output of dissolved organic carbon
DOC_{conc}	Dissolved organic carbon concentration
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EDTA	Ethylene diamine tetraacetic acid
FI	Fluorescence index
HMW	High molecular weight
k	Decay coefficients
LAS	Linear alkylbenzene sulfonate
LMW	Low molecular weight
MA	Mazeppa Creek
N	Nitrogen
OM	Organic matter
P	Phosphorus
PARAFAC	Parallel factor analysis
Q	Daily discharge
Q_{month}	Monthly discharge
Q_{yr}	Annual discharge
S_R	Spectra slope ratio
$SUVA_{254}$	Specific UV absorbance at 254 nm
$t_{1/2}$	Half-life
WRT	Water residence time

CHAPTER 1: INTRODUCTION

1.1 Background

Wetlands contribute disproportionately to the services that humans obtain from natural ecosystems, as they account for about 40% of global ecological services despite occupying only 4 to 6% of total land area (Zedler and Kercher, 2005). Wetlands have been described as a transition between aquatic and terrestrial ecosystems that have water cover on land for at least part of the year (Orimoloye et al., 2020). Wetlands are important for biodiversity conservation as they provide a diverse range of habitats to animals, especially waterfowl and amphibians (Knight et al., 2001; Orimoloye et al., 2020; Verones et al., 2013). They also provide economic value to humans through their capacity to process nutrients and improve water quality, and to store water and mitigate flooding (Euliss et al., 2008; Gren et al., 1994; Mitsch and Gosselink, 2000). Wetlands also play an important role in the global carbon (C) cycle, as they can sequester large quantities of carbon dioxide (CO_2) from the atmosphere through intense primary production by emergent vegetation, phytoplankton, and macrophytes (Bogard et al., 2020; Mitra et al., 2005; Rhee and Iamchaturapatr, 2009). Anaerobic conditions in wetland sediments can enhance C storage, such that wetlands store up to 71% of the global organic C pool (Mitra et al., 2005; Zedler and Kercher, 2005). However, the functioning of individual wetlands can vary, and depends on important factors such as human modifications of wetlands (e.g., drainage) or natural hydrologic cycles (e.g., drought versus wet periods), which have major impacts on biogeochemical process (Mitra et al., 2005; Zedler and Kercher, 2005).

Remediation of wastewater is one of the most important ecosystem services that wetlands provide. Globally, annual effluent release is ~360 km^3 yr^{-1} and up to 58.6% is transferred to aquatic ecosystems with or without treatment (Ehalt Macedo et al., 2022). Wetlands are often used to process nutrient rich effluents (Fisher and Acreman, 2004; Vymazal, 2007) and there are more than 1000 treatment wetlands in North America (Knight et al., 2001). Intense biogeochemical cycling in wetland sediments and in productive littoral vegetation can make wetlands efficient sites for the removal of C, nutrients, and organic contaminants (e.g., pesticides, linear alkylbenzene sulfonates (LAS), and ethylene diamine tetraacetic acid (EDTA)) (Barber et al., 2001; Mitra et al., 2005; Supowit et al., 2016). Constructed treatment wetlands are highly efficient in removing organic contaminants and can remove up to 44% of pesticides and up to 99% of LAS and EDTA, despite short water residence time (WRT; generally less than 14 days) (Barber et al., 2001; Supowit et al., 2016). Wetland vegetation and phytoplankton uptake nutrients from the water column (Stottmeister et al., 2003). Intense microbial activities enhance nutrient and organic matter removal through processes such as denitrification (Euliss et al., 2008; Werker et al., 2002; White and Bayley, 2001) and sediment burial and sorption (Fisher and Acreman, 2004; Vymazal, 2007; White and Bayley, 2001). While most research to date has focused on the processing of bulk or inorganic nutrients (phosphorus (P) and nitrogen (N)), less is known about the capacity for wetlands to process effluent-derived organic materials.

The cycling and composition of dissolved organic matter (DOM) plays a central role in regulating the functioning of aquatic ecosystems (Williamson et al., 1999), including wetlands. Measurements of DOM quantity and composition provide insights

into the physical and chemical drivers of ecosystem functioning (Casas-Ruiz et al., 2020; Zhang et al., 2019). For instance, DOM provides C and nutrients that support food-webs (Amaral et al., 2016; Asmala et al., 2013; Findlay and Sinsabaugh, 2003), and can be transferred to higher trophic-levels or released to downstream aquatic ecosystems (Asmala et al., 2013; Vanni, 2002). DOM content and composition influence light penetration and the photochemical reactions that take place in water (Williamson et al., 1999). Specifically, the content of chromophoric dissolved organic matter (CDOM) influences the aquatic light climate through the absorption of solar radiation over a broad range of wavelengths, which has implications for the transfer energy within aquatic ecosystems (Clark et al., 2008; Leenheer and Croué, 2003). Light penetration regulates rates of photosynthesis (Karlsson et al., 2009), and determines the exposure of aquatic species to ultra-violet light-that can cause cell and DNA damage (Leenheer and Croué, 2003). Photochemical reactions that mineralize DOM also supply inorganic matter (e.g., carbon dioxide) and bioavailable N (e.g., ammonium) to the food web (Bushaw et al., 1996). Due to its high cation exchange capacity, molecules adhere to DOM, and the content and composition of DOM can regulate the transport and bioavailability of nutrients, metals, and contaminants such as pesticides (Camino-Serrano et al., 2014; Findlay and Sinsabaugh, 2003). Understanding DOM biogeochemistry also has important ramifications for human health. The concentration and characteristics of DOM can influence the development of disinfection by-products (DBPs) after chlorination in water treatment plants, which can be toxic or carcinogenic to humans (Krasner, 2009; Xu et al., 2021). Despite decades of research, I still lack a clear understanding of the mechanisms controlling the composition and cycling of DOM in wetlands. A better understanding of

DOM cycling will shed light on the mechanisms that regulate many critical wetland ecosystem services.

DOM is categorized into two types: autochthonous (internally-derived in an ecosystem) and allochthonous (externally-derived) DOM. Natural allochthonous DOM (e.g., from peatlands, soils, and forests) typically contains a high degree of aromaticity and more complex compounds (high molecular weight DOM, HMW) with higher C to N ratios, and tends to be less bioavailable (Asmala et al., 2013; Findlay and Sinsabaugh, 2003). Terrestrial DOM inputs provide a large diversity of DOM compounds, but a large fraction of this DOM is resistant to microbial consumption over shorter (monthly to annual) timescales and can accumulate through time and space in aquatic ecosystems (Guillemette and del Giorgio, 2011; Nebbioso and Piccolo, 2013; Roebuck et al., 2020). Bioavailable DOM (e.g., proteins and carbohydrates) is often consumed by soil microbes and less bioavailable DOM (e.g., lignin, tannins, and lipids) can accumulate in surface soils or be exported to aquatic systems (Kindler et al., 2011; Thieme et al., 2019). Therefore, changes to inputs of DOM from individual terrestrial sources may affect the physicochemical characteristics and functioning of downstream aquatic ecosystems.

Many factors affect the composition of terrestrial DOM that reaches aquatic systems, including the presence of distinct terrestrial plant species (Camino-Serrano et al., 2014; Thieme et al., 2019) and dominant soil types (Tank et al., 2018; van den Berg et al., 2012). The residence time of water on the landscape determines the extent that DOM is exposed to bio- and photo-degradation processes, and the relative contribution of individual hydrological flow paths (e.g., overland, versus soil- or groundwater inputs) with distinct DOM endmembers further changes the composition and content of DOM reaching aquatic ecosystems (Fellman et al., 2013; Kaiser and Kalbitz, 2012; Singh et al.,

2014). Other factors such as soil temperature, pH, and dryness and seasonality also affect the composition and concentration of terrestrial DOM (Singh et al., 2014; van den Berg et al., 2012). Collectively, these factors interact to shape the diversity of DOM, and therefore bioavailability, in headwater systems adjacent to land (Camino-Serrano et al., 2014; Singh et al., 2014).

On the other hand, autochthonous DOM is produced by phytoplankton, macrophytes, emergent or submergent plants, microbes, and animals. This DOM is generally less aromatic (more aliphatic), with relatively lower molecular weight (LMW) and greater N content that are more biolabile (Asmala et al., 2013; Findlay and Sinsabaugh, 2003; Weigelhofer et al., 2020). For example, half of the phytoplankton-derived DOM pool can be made up of dissolved organic N (DON)-rich proteins and nucleic acids, and the other half by carbohydrates, lipids, and other compounds (Thornton, 2014; Weigelhofer et al., 2020). Similar to phytoplankton, macrophyte derived DOM is also DON-rich and can provide N and energy that supports bacterial metabolism (Lapierre and Frenette, 2009). Aquatic animals also provide highly biolabile DOM to aquatic ecosystems that is rich in N and P, and more aliphatic relative to the bulk aquatic DOM pool (Johnston et al., 2022; Parr et al., 2018; Schmitz et al., 2014). Beyond external factors that control terrestrial DOM export to aquatic systems, local properties of aquatic systems are also important in controlling aquatic DOM composition and concentrations. For instance, WRT and the position of an aquatic ecosystem in the landscape can impact DOM cycling through process such as primary production and bio-and photo-degradation (Hosen et al., 2021; Vachon et al., 2021). Higher WRT leads to enhanced retention and processing of allochthonous DOM via flocculation and burial in sediments, and mineralization in the water column (Vachon et al., 2021). Autochthonous DOM

production can offset these losses (Vachon et al., 2021), and, in ecosystems with relatively long WRT, can shift the ecosystem balance toward net export of DOM (Evans et al., 2017).

Human activities have multiple and complex effects on aquatic DOM cycling; both directly through increased terrestrial or anthropogenic DOM inputs, and indirectly through nutrient inputs or hydrologic manipulations that alter internal DOM cycling (Xenopoulos et al., 2021). The composition of anthropogenic DOM is complex, but is usually rich in unsaturated aliphatic and LMW compounds with high concentrations of N (Osburn et al., 2016; Roebuck et al., 2020; Seitzinger et al., 2002; Wilson and Xenopoulos, 2008b). Urban and agricultural land use enhances soil disturbance and erosion, which can increase terrestrial nutrient and DOM loading to aquatic ecosystems (Regnier et al., 2013; Wilson and Xenopoulos, 2008b; Xenopoulos et al., 2021). Nutrient pollution increases primary production and further shifts DOM pools toward a greater content of autochthonous DOM (Mesfioui et al., 2015; Seitzinger et al., 2002; Xenopoulos et al., 2021). Wilson and Xenopoulos (2008b) showed that the importance of autochthonous DOM in aquatic ecosystems is positively related to agricultural land use and N load, and negatively related to wetland extent. Therefore, landscapes that experience intense human land use can undergo dramatic shifts in the quantity and composition of the DOM pool. These effects can have ramifications for society. For example, increased DOM production linked to discharging of nutrient-rich effluent from wastewater treatment plants can lead to the formation of nitrogenous DBPs (Krasner et al., 2009) that are more toxic than other DBPs, and can cause chronic cytotoxicity and acute genomic DNA damage (Muellner et al., 2007).

1.2 Thesis rationale

Although wetlands are often used to mitigate the effects of chemically-complex effluents on downstream ecosystems, the net outcome (i.e., net removal or production, and compositional changes) of DOM processing in treatment wetlands is unclear. Without an understanding of the net balance of DOM cycling, it is impossible to determine the exact role that a treatment wetland plays within a given aquatic network. The net processing (addition versus removal) of DOM may vary among wetlands as a function of the source and composition of DOM inputs, environmental conditions (e.g., vegetation and soil type, hydrology, temperature, pH, etc.) and capacity to sustain DOM processing via microbial mineralization and photodegradation (Hertkorn et al., 2016; Lu et al., 2003; Stottmeister et al., 2003). In general, the addition of untreated wastewater to constructed wetlands should lead to net removal of DOM, while the treatment of wastewater prior to release may lead to variable patterns of DOM processing dependent on specific wetland characteristics and the properties of effluent (e.g., dairy effluent versus municipal sewage) (Barber et al., 2001; Pinney et al., 2000). Therefore, individual wetlands can have different capacities to improve water quality, depending on regional environmental conditions and land use settings, and implications for DOM compositional changes are not well defined. This variability in DOM processing is not unique to wetlands. A synthesis of lake and reservoir DOC budgets showed that net DOC cycling ranged widely, with the ratio of DOC export to import spanning 0.10 to 2.55 (Evans et al., 2017) (where values above or below 1 represent a net source or sink, respectively). To clearly define the role of treatment wetlands in the landscape and their downstream effects via DOM export, we require detailed investigation as to how effluent DOM cycles while in transit through individual treatment wetlands.

1.3 Thesis objective

Here I define the composition and cycling of DOM within one of Canada's largest restored treatment wetlands, and I identify the functional role of the wetland as a sink or source of DOM in the watershed. I used a combination of routine sampling over an annual cycle to measure field-based water quality and hydrological conditions, laboratory-based measurements of water characteristics and incubations, a mass balance using new and existing datasets, plus comparisons to available literature data. Chapter 2 details the findings from these approaches as well as their implications. Chapter 3 expands the discussion and places my findings from chapter 2 in the broader context of wetland functioning and the role of wetland DOM export for downstream ecosystems. I summarize the implications of wetland effluent cycling for aquatic resource management and ecosystem services, then discuss the potential avenues for future research.

CHAPTER 2. ORGANIC MATTER CYCLING IN ONE OF CANADA'S LARGEST RESTORED WETLANDS RECEIVING COMPLEX EFFLUENT

2.1 Introduction

Wetlands serve multiple functions in society, providing many economically valuable services including the conservation of biodiversity, flood water storage and management, and water treatment (Euliss et al., 2008; Gren et al., 1994; Mitsch and Gosselink, 2000). For decades, the use of natural and constructed wetlands as sites of treatment and processing of anthropogenic effluents has been widespread (Cole, 1998; Euliss et al., 2008; Werker et al., 2002). Wetlands cycle organic matter (OM) and nutrients from effluents via both aerobic and anaerobic process (Kayranli et al., 2010). Intense biogeochemical cycling in wetland sediments, in littoral vegetation, and in suspended plankton (microbes, phytoplankton) can make wetlands efficient sites for the processing of OM and nutrients (Mitra et al., 2005; Werker et al., 2002; Zhang et al., 2014). In the global summary of 57 natural wetlands from Fisher and Acreman (2004), about 80% of natural wetlands displayed strong nutrient retention, with 67 ± 27% retention of loaded N and 58 ± 23% retention of loaded P. Constructed wetlands are often smaller in area (< 10 ha) and have shorter WRT (often ~2 to 14 days) than natural wetlands, though the retention capacity for N (40 to 50%) and P (40 to 60%) is still relatively high, depending on wetland construction and hydrologic regulation (Vymazal, 2007). For OM, on the other hand, net processing (production versus consumption) can be variable among wetlands, and depends on the source and composition of inputs, wetland conditions (e.g., vegetation types, hydrology, soil types, temperature, and pH, etc.) and dominant processes (e.g., microbial mineralization, photodegradation) (Hertkorn et al.,

2016; Li et al., 2008; Lu et al., 2003; Stottmeister et al., 2003). Anaerobic conditions in wetland sediments can restrict the efficiency of OM mineralization, but can also enhance OM removal through process such as denitrification (Kayranli et al., 2010; Werker et al., 2002; Zhang et al., 2014) and sorption to sediments (Fisher and Acreman, 2004; Vymazal, 2007). Taken together, wetland restoration is a potential mechanism to improve water quality through increased inorganic nutrient processing (Cheng et al., 2020), yet the role of wetlands, especially treatment wetlands, in OM cycling and remediation is not clear.

Overall, DOM, the largest fraction of bulk OM (i.e., dissolved and particulate OM combined) represents a complex mixture of organic molecules including, macro- and micro-nutrients that support both auto- and heterotrophic activities, which can influence environmental properties of aquatic ecosystems (Amaral et al., 2016; Asmala et al., 2013; Findlay and Sinsabaugh, 2003). The concentration and composition of DOM can also influence the development of DBPs after chlorination and bromination in drinking water treatment plants, which can be toxic or carcinogenic to humans (Chen et al., 2008; Krasner, 2009; Xu et al., 2021). Broadly-speaking, DOM is categorized into two types: autochthonous (internally-derived in an ecosystem) and allochthonous (externally-derived) DOM. Internally-derived, autochthonous DOM is generally less aromatic (more aliphatic), with LMW and greater bioavailability (Findlay and Sinsabaugh, 2003). Natural allochthonous DOM (e.g., from terrestrial soils and forests) is transported into aquatic ecosystems and typically contains more aromatic, complex compounds (HMW DOM) with higher C to N ratios, that tend to be less bioavailable (Asmala et al., 2013; Findlay and Sinsabaugh, 2003; van den Berg et al., 2012). Terrestrial DOM inputs provide a large diversity of DOM compounds that vary with vegetation type (Camino-Serrano et al.,

2014; Thieme et al., 2019), soil type (Tank et al., 2018; van den Berg et al., 2012), exposure to bio-and photo-degradation processes (Fellman et al., 2013; Hernes and Benner, 2003), residence time on land (Fellman et al., 2013), and hydrological characteristics (Kaiser and Kalbitz, 2012; Singh et al., 2014; Tank et al., 2018). A large fraction of this DOM is resistant to microbial consumption over shorter (monthly to annual) timescales and can accumulate through time and space in aquatic networks (Guillemette and del Giorgio, 2011; Nebbioso and Piccolo, 2013). These processes interact to shape the diversity of DOM and its chemical properties and bioavailability (Camino-Serrano et al., 2014; Singh et al., 2014; Thieme et al., 2019). Therefore, changes to inputs of DOM from individual sources will impact the composition of DOM and may affect both the functioning of aquatic ecosystems and human health through changes in drinking water quality.

Human activities affect the composition of DOM through diverse mechanisms (Wilson and Xenopoulos, 2008a; Xenopoulos et al., 2021) that can have complex impacts on wetland DOM cycling, making the net processing of DOM variable and hard to predict. Allochthonous OM inputs are enhanced by effluent loading and soil disturbance and erosion by agricultural and other land uses (Drake et al., 2018; Regnier et al., 2013; Xenopoulos et al., 2021). Conversely, nutrient pollution linked to agricultural land use and wastewater loading stimulates autochthonous DOM production via aquatic primary production (Mesfioui et al., 2015; Seitzinger et al., 2002; Xenopoulos et al., 2021). Impacts on both allo- and autochthonous DOM cycles can influence the overall quality and quantity of DOM in inland waters. These effects can have ramifications for society, since the release of effluent from wastewater treatment plants can increase DOM concentrations, and in turn lead to DBP formation (Krasner et al., 2009). The composition

of DOM also impacts the potential toxicity in waterbody, since DOM has high cation exchange capacity which increases the affinity of molecules to adhere to DOM, including metals and contaminants such as pesticides (Supowit et al., 2016; Wood et al., 2011). Depending on the types of wastewater sources and their chemical composition (e.g., industrial, municipal, or livestock waste), the same treatment processes in constructed wetlands could lead to different effects on effluent DOM composition (Barber et al., 2001). Given the multitude of factors involved, it remains difficult to predict how individual wetlands (natural or constructed) cycle and transform effluent DOM, and what effect this has on the net production or consumption of DOM at the ecosystem scale.

Here, my goal was to define how DOM is cycled in one of Canada's largest mineral wetland complexes (Fig. 1) restored using effluent inputs from municipal and agro-industrial sources. To achieve my goal, I aimed to address the following questions: 1) What is the compositional change in the DOM pool along the hydrologic continuum from distinct inputs (effluent and tributaries) to the outflow below the wetland complex? 2) Does the capacity for microbial DOM processing shift in a predictable way along the hydrologic continuum from sources to outflow? 3) Is the wetland complex acting as a net source or sink of DOM? By answering these questions, I provide new knowledge to better understand DOM cycling within this model treatment wetland, and the role that the wetland plays in the watershed DOC budget.

2.2 Methods

2.2.1 Study Site

Frank Lake (55°53'N, 116° 56'W; Figure 1) is a restored wetland that has received treated wastewater from the Cargill meat processing plant and treated municipal sewage (town of High River) since 1989 (White and Bayley, 1999; 2001; Zhu et al., 2019). Hydrologic inputs from both sources average 1,651,038 m^3 yr^{-1} and 2,132,711 m^3 yr^{-1} (the town of High River and Cargill Foods Ltd), accounting for 44% and 56% of total effluent inputs, respectively. Briefly, the Cargill plant processes 4,500 cattle per day and the wastewater is treated through filtering suspended large particles, anaerobic and aerobic treatment processes, and UV disinfection before export to Frank Lake (Blue Source Canada ULC, 2017). The Town of High River has a population of 14,000, and wastewater receives secondary treatment using aeration treatment processes, but without UV disinfection (www.highriver.ca). Wastewater effluent discharge to Frank Lake is 10,821 m^3 day^{-1} (based on the 3,950,000 m^3 yr^{-1} discharge reported in Zhu et al. (2019)). Two ephemeral creeks (Blackie and Mazeppa Creeks) discharge water to Frank Lake during the spring (Zhu et al., 2019). Blackie Creek also receives untreated municipal wastewater from an upstream lagoon in the town of Blackie (population ~300).

Frank Lake is a multi-basin wetland complex that has four basins and is divided at the outflow of Basins 1, 2, and 3 by steel weirs (Fig. 1). Basin 1 has a surface area of 5.01 km^2 and mean depth of 0.67 m (White and Bayley, 2001; Zhu et al., 2019). Basin 2 has 3.6 km^2 surface area and a similar depth to Basin 1. Basin 3 has a surface of area of 1.4 km^2 with 0.3 m depth. Basin 4 is only used for back flooding from Basin 3 during flooding seasons (not shown in Fig.1) (White and Bayley, 1999). The wetland is fringed by emergent vegetation, primarily bulrush (*Schoenoplectus acutus* Muhl.), and contains submerged vegetation including sago pondweed (*Stuckenia pectinata*), northern water

milfoil (*Myriophyllum exalbescens* Fern.) and Richardson's pondweed (*Potamogeton richardsonii* (Benn.) Rydb.) (White and Bayley, 2001).

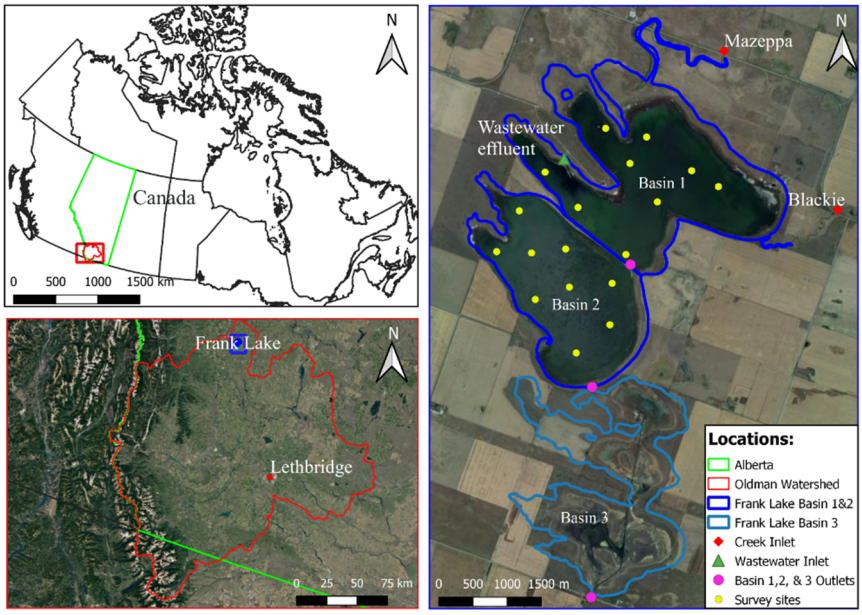

Figure 1. Location of Frank Lake in the Oldman Watershed, Alberta, Canada. Top left: The Oldman Watershed (red outline; ~27,500 km^2) in Canada. Bottom left: Study site in the Oldman Watershed (blue box). Right: sampling site for inlets including Blackie and Mazeppa Creeks (red diamonds) and wastewater effluent (green triangle) and Frank Lake Basin 1, 2, and 3 outflow sites (pink circles) and survey sites (yellow circles). Google satellite reference: EPSG:3857 - WGS 84 / Pseudo-Mercator – Projected.

The mean annual air temperature near Frank Lake was estimated as 2.3 °C (Zhu et al., 2019), with a monthly average mean temperature ranging from -11 to 15 °C (White et al., 2000). From 2013 to 2015, the mean annual precipitation in the region encompassing Frank Lake was 450 mm and mean evaporation was 782.5 mm, as reported by Zhu et al. (2019) with total water loss of 332.5 mm yr^{-1}. In contrast to the relatively wet 2013 to 2015 period, the annual precipitation for 2021 in the region was 245 mm (Blackie AGCM; Alberta Agriculture and Forestry), which was about half the amount of precipitation received annually from 2013 to 2015, but the evaporation in 2021 was 990.4 mm (Blackie AGCM; Alberta Agriculture and Forestry), which was 1.27 times higher than in 2013 to 2015. By difference, the net vertical water loss rate was 745.4 mm yr^{-1}, which was roughly double the amount of regional water loss compared to 2013 to 2015. Due to this extreme difference, I have broadly categorized these distinct hydroclimatic periods as wet (2013 to 2015) and drought (2021) periods.

Like other aquatic ecosystems in semi-arid regions, Frank Lake experiences high rates of evaporation (7.9×10^6 m^3 yr^{-1} (Zhu et al., 2019)), which concentrates solutes as water masses move through the wetland complex. I have reported general features from existing data, including DOC concentrations and mean discharge from 2012 to 2018 (Table 1) for all sampling sites using publicly available published data from (Zhu et al., 2019) and unpublished data from Alberta Environment and Parks (Wendell Koning, personal communication). Based on data from 2012 to 2018, Blackie and Mazeppa Creeks had mean discharge of 0.02 to 0.03 m^3 s^{-1} from March to June, and no flow after June. Effluent discharged year-round, with a mean of 0.12 ± 0.03 m^3 s^{-1}. Basin 3 outflow

had a high flow peak in April and May and decreasing discharge rates through the year, with lowest flow in September, and a mean annual discharge rate 0.23 ± 0.32 m^3 s^{-1}.

Table 1. Physical and chemical features of sampling sites in Frank Lake. Mean of measurements are included and standard deviation is shown (in brackets) if applicable*.

Site	Discharge ($m^3 s^{-1}$) [b]	Alkalinity CaCO3 ($mg L^{-1}$) [a]	Salinity ($mg L^{-1}$) [a]	pH [a]	Specific conductivity ($\mu S cm^{-1}$) [a]	TOC ($mg L^{-1}$) [a]	TN ($mg L^{-1}$) [a]	TP ($mg L^{-1}$) [a]	DOC ($mg L^{-1}$) [a]
Inlet									
Blackie	0.02 (0.07)	178.0 (88.7)	9 (6)	8.0 (0.4)	1499 (1020)	26.0 (4.8)	2.2 (0.6)	0.9 (0.3)	24.4 (4.8)
Mazeppa	0.03 (0.08)	188.5 (94.9)	11 (5)	8.0 (0.2)	1129 (624)	22.0 (6.9)	1.9 (0.8)	0.4 (0.2)	21.9 (6.0)
Effluent	0.12 (0.03)	310.3 (103.8)	274 (147)	7.1 (0.2)	2300 (429)	12.7 (3.7)	55.5 (19.8)	4.3 (1.1)	11.7 (3.1)
Outlet									
Basin 1 outflow	0.29 (0.47)	307.5 (88.0)	159 (85)	8.7 (0.6)	1703 (538)	23.6 (6.3)	7.2 (4.0)	2.1 (0.9)	23.5 (5.4)
Basin 2 outflow	0.34 (0.50)	404.3 (87.1)	180 (46)	8.7 (0.5)	2011 (416)	24.9 (4.4)	4.9 (2.6)	2.4 (0.9)	25.7 (4.8)
Basin 3 outflow	0.28 (0.35)	479.9 (152.4)	201 (75)	8.3 (0.4)	2441 (786)	32.4 (8.8)	3.9 (1.6)	2.7 (1.0)	31.6 (8.4)

* Mean concentration calculated by using all data from 2013 to 2015 that are available.

[a] Data calculated from Alberta Environment and Parks.

[b] Data calculated from a and Zhu et al. 2019.

2.2.2 Water sample collection

Routine samples were collected bi-weekly during Spring and Summer (March 12, 2021 to Aug 23, 2021) and monthly in November and December 2020 and September and October 2021. Where possible, I sampled during both periods with and without flow to understand in situ DOM processing and flux, even during periods of hydrologic stagnation in the wetland and its tributaries. Surface water samples were collected from Blackie and Mazeppa Creeks, from the mouth of the pipe delivering effluent to Frank Lake, and from the outlets of Basin 1 to 3 (Fig. 1). Given that 2021 was a drought year with limited precipitation, discharge in creeks and at the outflow were limited. For Blackie Creek (BL), samples were collected in spring when the creek was briefly flowing, and thus are limited to one data point. This flow period at BL represented wastewater discharge from the Town of Blackie lagoon (observed on May 10; Fig. 1). For Mazeppa Creek (MA), I observed no flow, and the samples were collected from a stagnant pool of water that remained in the creek. For the outflow from Basin 3 (B3O), samples were collected from April 9 to July 26, however, flows were only observed from April 9 to May 19, with samples representing both the period of flow, and the period when flow ceased. The outflow at Basin 3 had a consistent water height from April 9 to May 29 (see below) and water height consistently decreased after that time (Fig. 1). Detailed spatial surveys of Frank Lake Basins 1 and 2 (B1 and B2) were conducted by motorized boat in June and August 2021 (Fig. 1). Water was collected at a consistent depth of about 0.25 m and filtered through pre-rinsed 0.45 µm capsule filters (FHT-45, Waterra) or 0.45µm filters (Cellulose nitrate membrane filter, Whatman) into acid-washed bottles or pre-combusted (450 °C, 4h) amber glass vials within 6 hours of water collection. Whole

water samples were either filtered on site or transported in the dark on ice to the laboratory for filtration.

2.2.3 Hydrologic measurements

To better constrain the period of flow, I monitored water height at each site (BL, MA, B3O) visually and using water level data loggers (HOBO® U20L) from April 9, 2021 to September 26, 2021 (Fig. 1). Water height was measured manually at the site where loggers were moored in the creeks with a metal stake. The loggers recorded ambient pressure at 30 min intervals, and water level was calculated using standard HOBO software. The reference air pressure needed to convert absolute pressure into water level was downloaded from the nearest weather station at the CALGARY INT'L CS Station (51° 06’ N, 114° 00’ W; Government of Canada). Local precipitation and evaporation data were obtained at station Blackie AGCM (November 2020 to October 2021).

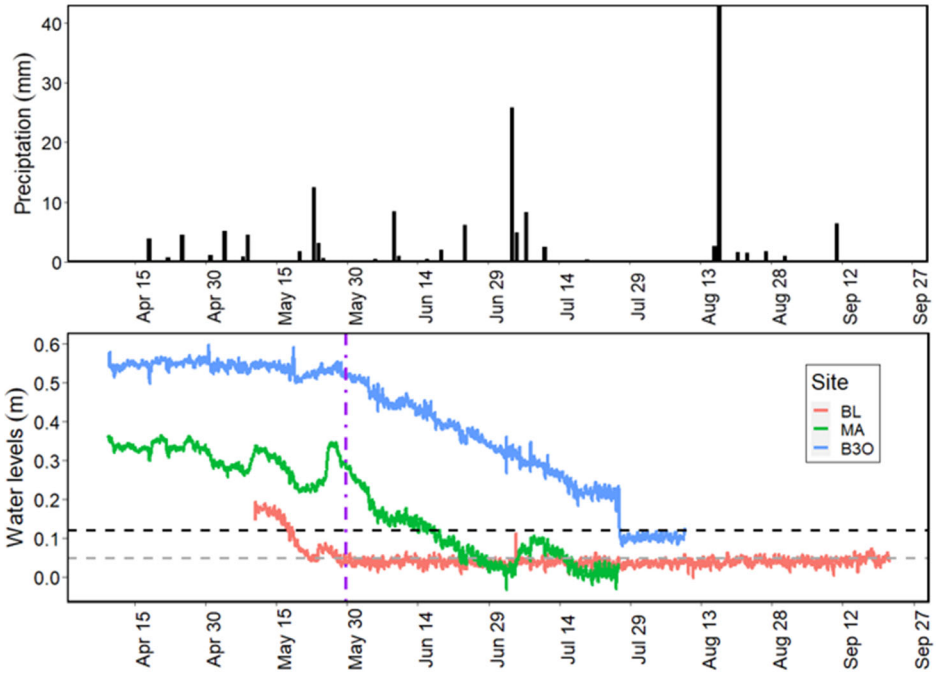

Figure 2. Daily regional precipitation (top panel) and water levels at Frank Lake (bottom panel), including Blackie (BL; red) and Mazeppa Creeks (MA; green) and Basin 3 outflow (B3O; blue). The black dashed line (0.12 m) and grey dashed line (0.05 m) denotes the water depth at which flow stopped at B3O and BL, respectively. MA had no flow since the beginning of the measurement time (April 9), and BL and B3O had no flow beyond May 19 and B3O had no flow beyond May 29 based on water depth (shown in vertical purple dashed-dotted line).

2.2.4 BDOC experiment set up

To evaluate the capacity for the ambient microbial communities to process DOM, I conducted standardized, 28-day BDOC incubations for water samples from all routine monitoring sites in July and October. Incubations were set up in triplicate following standard protocols (Vonk et al., 2015) but with the filter pore size changed from 0.7 µm to 0.2 µm to ensure no microbial activity in sub-samples from the incubation bottles. All bulk water samples were filtered to 0.2 µm (PALL Supor 200) into pre-combusted (450 °C, 4h), 1L amber bottles and stored in the refrigerator at 4°C for less than 24 hrs while setting up experiments. To begin the incubation, I added a 1% microbial inoculation (water filtered using pre-combusted 1.2 µm Whatman GF/D filters) from corresponding sample sites to each sample. I stored all sample bottles in the dark at room temperature during incubations (21 to 23.5 °C). After inoculation, I collected microbe-free, filtered (0.2 µm) subsamples on days 0, 2, 7, 14, 21, and 28 for DOC concentration and optical (absorbance and fluorescence spectra) measurements.

2.2.5 Measurement of DOC concentrations

I measured DOC concentrations and absorbance and fluorescence spectra following established methods (Johnston et al.2018). I determined DOC concentrations (mg L^{-1}) using a Shimadzu TOC-L CPH high temperature catalytic oxidation total organic carbon analyzer calibrated with a six-point standard curve ($R^2 = 0.999$) based on the estimated concentration range of DOC in these samples. Each sample (20 to 40 ml of 0.45 µm filtered water for routine and survey samples, and 0.2 µm filtered water for BDOC samples) was acidified to pH 2 (1 µl 12N HCl per 1 ml sample water) and run on the

TOC analyzer. I used acidified ultrapure lab water (pH = 2) as a blank, and blank concentrations did not exceed 0.05 mg L^{-1}. The concentration of each sample was determined by averaging 3 of the 7 injections with the lowest coefficient of variance (C.V. < 0.02) and standard deviation (S.D. +/- 0.1). The samples were run in either duplicates or triplicates and results were averaged to determine final concentrations.

2.2.6 Optical properties of DOM and analysis

The measurements of absorbance (Biochrom Ultrospec 3100 pro UV-visible spectrophotometer) and fluorescence (Shimadzu RF-6000 fluorometer) were completed at room temperature using a 1 cm quartz cuvette within 2 weeks of collection with few exceptions. Filtered water samples were typically run in triplicate. Absorbance spectra for each sample were measured from wavelengths of 230 to 800 nm. Fluorescence spectra of each sample were measured between the wavelengths of 230 to 500 nm, in 5 nm intervals for excitation and 250 to 700 nm, in 2 nm intervals for emission. I used a scan speed of either 2000 nm s^{-1} or 6000 nm s^{-1}, as determined by DOC concentration and fluorescence intensity. Fluorescence spectra were blank corrected, inner filter effect corrected, Raman normalized, and remove of scattering in R (R Core Team 2021) using the StaRdom script (Pucher et al., 2019).

To characterize bulk optical properties of DOM in each sample, I used the StaRdom package (Pucher et al., 2019) in R (R Core Team 2021) to process fluorescence and absorbance data and calculate fluorescence and absorbance parameters that have been defined in previous studies (Dobbs et al., 1972; Helms et al., 2008; McKnight et al., 2001; Murphy et al., 2013). Absorbance at 254 nm (a_{254}) is an indicator of chromophoric DOM

and typically has a linear relationship with DOC concentration in natural aquatic systems. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$; L mg C^{-1} m^{-1}), the DOC normalized absorbance at 254 nm, is commonly used as an indicator of aromaticity in bulk DOM, for which higher $SUVA_{254}$ indicates relatively more aromatic-, and lower $SUVA_{254}$ values indicates relatively less aromatic DOM in a given sample (Weishaar et al., 2003). Spectral slope ratio (S_R) is defined as the ratio of the exponential regression slope from 275 to 295 nm ($S_{275\text{-}295}$) to that from 350 to 400 nm ($S_{350\text{-}400}$), where S_R is inversely related to the average molecular weight of the DOM pool (Coble, 2007; Cory and McKnight, 2005; Helms et al., 2008; Spencer et al., 2012), with lower values (~ 0.7) indicating more terrestrial, and higher values (~ 1.1) indicating more autochthonous DOM (Helms et al., 2008). Similar to $SUVA_{254}$, fluorescence index (FI) is an indicator of DOM aromaticity but has an inverse relationship to aromaticity (Cory and McKnight, 2005; Findlay and Sinsabaugh, 2003). The lower FI value (about 1.2 to 1.4) indicates relatively more aromatic DOM in a sample and higher FI value (1.8 to 1.9) indicates lower aromaticity.

I calculated the widely reported fluorescence peaks using the excitation emission matrices for each sample, B (ex/em=270 nm/310 nm), T (ex/em=275 nm/340 nm), A (ex/em=260 nm/380 to 410 nm), M (ex/em=312 nm/380 to 420 nm), and C (ex/em=350 nm/420 to 480 nm), which generally correspond to protein-like (B and T, more bio-available) and humic-like (A, C, and M, less bio-available) DOM availability (Fellman et al., 2010). I also calculated the A:T peak ratio, which is an indicator of the relative amount of less bio-available to more bio-available fluorescent DOM in a sample (Hansen et al., 2016).

Fluorescence data from routine samples were analyzed using parallel factor analysis (PARAFAC) (Murphy et al., 2010; Pucher et al., 2019; Stedmon and Bro, 2008)

in R (R Core Team 2021) to determine the best fit number of components in the model, and compared with previously published results in the OpenFluor database (Murphy et al., 2014). After fluorescence spectra were corrected, a total of 50 samples were used for PARAFAC model development, and five outliers were removed for validation of the model using split-half analysis (> 95%). The five outliers were effluent (EF) on July 13 and 26 and Aug 23, Basin 2 outflow (B2O) on July 26, and BL on May 10.

2.2.7 Mass balance construction and relation to WRT

To understand the ecosystem-level effect of effluent inputs on DOM cycling and fluxes through the wetland complex, I built a DOC mass balance for Frank Lake following the general methods of Finlay et al. (2010) and Evans et al. (2017), and compared the wet period (2013-2015) to the drought period during which I collected our samples (2021), then combined both to determine the long-term budget. To construct the mass balance, I estimated the annual mass of DOC entering and leaving Frank Lake (Mg DOC yr^{-1}):

$$\text{Net DOC flux} = DOC_{OUT} - \Sigma\, DOC_{IN} \quad \text{(eq. 1)}$$

Where the total DOC input ($\Sigma\, DOC_{IN}$) was the sum of inputs from BL and MA, and EF. Output (DOC_{OUT}) includes only the flux of DOC at the B3O (Fig. 1). Positive flux indicates that Frank Lake is a net DOC source (enhancing downstream output), and a negative flux represents a net DOC sink (lowering downstream output).

The mass of DOC output or input at each site was calculated as the sum of bi-weekly to monthly estimates of the product of water flux (Q_{month}) and measured DOC concentrations (DOC_{conc}):

$$DOC_{OUT} \text{ or } DOC_{IN} = \Sigma (Q_{month} \times DOC_{conc}) \quad \text{(eq. 2)}$$

DOC concentrations from 2013 to 2015 were obtained from Alberta Environment and Parks, and for 2021 from our own measurements.

For Q_{month} in the drought period, monthly effluent release volumes (Nov. 2020 to Oct. 2021) were obtained from the town of High River and Cargill Foods Ltd. In 2021, I observed no discharge in MA. For BL site, I observed a brief period of discharge (~5 days) associated with the annual release from the upstream Blackie lagoon (Fig. 1, 2), with a total discharge volume of 15,000 m^3 (Jeff Edgington, Foothills County, personal communication). This total volume was averaged to determine daily discharge in May 2021. For the B3O site, flows were below detection limits for our flow meter, so I used the estimated daily average discharge values (Q; $m^3\ s^{-1}$) from the Government of Alberta Flow Estimation Tool for Ungauged Watersheds (AFETUW) (https://afetuw.alberta.ca/). These mean daily estimates from the model were constrained to the period of observed flow based on visual observations and estimates of water height (from the logger) at the B3O. As my goal was to use discharge data to construct a first order budget of DOC flux for the wetland, these estimates of discharge are more than sufficient, because all estimates of inflow (<1%) and outflow (<18%) presented here for 2021 were minor relative to annual discharge from the effluent inflow, and small errors associated with these flux calculations are minor relative to the comparatively well-constrained estimates of effluent discharge and DOC concentrations.

For the wet period of 2013 to 2015, mean annual effluent discharge values at EF were taken from Zhu et al. (2019), and DOC data from unpublished Government of Alberta datasets. To calculate discharge at BL, MA, and B3O sites, I used published,

directly measured discharge from Zhu et al. (2019) to calculate Q_{month} for a number of reasons: Estimates of annual discharge (Q_{yr}, m^3 yr^{-1}) from the AFETUW model for 2013 to 2015 for BL and MA sites were 40 to 60% higher than direct estimates, and were beyond reasonable expectations since they included numerous periods of flow when none were observed (Zhu et al. 2019). Ultimately, mean daily Q (m^3 s^{-1}) observations (Table 1) from the inlets (BL and MA) and B3O were averaged into monthly mean values when flow was observed (Q_{month}). The same monthly averaging was applied to DOC concentration data. To overcome gaps in the dataset, for the 2013 to 2015 wet period, I averaged Q_{month} and DOC concentrations across all three years for each month.

For the overall (all years) average DOC flux values, I used the same method as for the 2013 to 2015 period but included data from 2021. Annual discharge (Q_{yr}, m^3 yr^{-1}) and annual DOC fluxes (Mg yr^{-1}) were determined by summing monthly mean values. All final flux estimates are reported as annual rates.

I have made several assumptions in calculating DOC outputs and inputs: 1) The flux of DOC is based on discharge data available at each gauging site. If discharge is not observed for a certain month, I assumed no flux of DOC (e.g., I assumed no outflow at B3O in winter due to ice formation). 2) I assumed the amount of DOC input from birds is negligible. According to Andersen et al. (2003), the maximum estimate of bird density (12,000 birds present at their study wetland) would add 2.6% and 7% of N and P, respectively compared to input from wastewater inflows in a constructed wastewater wetland. For the Frank Lake wetland, such inputs would be of much less importance, given our higher rates of effluent inflow and higher concentrations of N and P in wastewater, and lower bird population density (Andersen et al., 2003; Himer, 2001; Zhu et al., 2019).

I determined annual WRT for Frank Lake using the mean total volume of the wetland complex from Zhu et al. (2019) divided by the mean Q_{yr} from B3O (calculated for 2013 to 2015, 2021, and as a long-term average). To evaluate the DOC removal efficiency at Frank Lake for each measurement period, I reconstructed the relationship between the ratio of DOC_{OUT} to DOC_{IN} (DOC_{OUT}/DOC_{IN}) and WRT following Evans et al. (2017). To remain consistent with this past work, I did not consider evaporation in calculations of WRT, and therefore only present the ratio of lateral exchanges of DOC.

2.2.8 Modelling DOC consumption in BDOC experiments

I modelled the changes in DOC content during the BDOC experiment and reactivity of DOM pools following existing methods of Catalán et al. (2016) and Guillemette and del Giorgio (2011). The decay rate k for each site was calculated as $k = \ln(t/i) / T$, where the rate of decomposition is defined as $\ln(t/i)$, with t indicating DOC concentration on day 28 of incubations, i indicating initial DOC concentration (on day 0), and T the duration of the incubation. I estimated the half-life of the DOC pool as: $t_{1/2} = \ln(2) / k$.

2.2.9 Statistical analyses

All analyses were performed in R version 4.1.2 (R Core Team 2021). I used analysis of variance (ANOVA) with Tukey's honestly significant difference *post hoc* tests (Kao and Green, 2008) (*Tukey_hsd* function) to compare DOC concentrations and BDOC concentrations, and optical parameters across sites. BL and MA sites had one data point, and no flow, respectively, so were excluded from analyses. Data were transformed to

meet assumptions of normality only where data violated these assumptions as identified using the Shapiro-Wilks test (*Shapiro* function). If a dataset failed the test of normality, a non-parametric analysis (Kruskall-Wallis) (*Kruskal.test*) with Wilcoxon signed-rank test (*Wilcox.text*) was used to evaluate inter-group differences. I used the *lm* function for all linear regression calculations.

2.3 Results

2.3.1 General properties of the Frank Lake wetland complex

Estimated discharge for B3O derived from the AFETUW model was 0.116 ± 0.05 $m^3 s^{-1}$ (mean ± S.D.) for the flow period. During the 2021 sampling period, daily precipitation was low (Fig. S1) with mean daily averages of 1 ± 4 mm between April 9 to September 22 and a maximum daily precipitation rate of 43 mm on August 17.

2.3.2 DOC concentration and DOM composition

The concentration of DOC varied among sites (Fig. 3a; ANOVA: $p < 0.01$, $n = 78$) more widely than within sites (see our open water surveys). EF and B3O had significantly different DOC concentrations from other sites (lower, and higher, respectively). Although MA was stagnant and did not flow during the entire sampling period, I sampled the ponded water that was disconnected from Basin 1, and included this water mass for comparison to EF and BL sites. On average, MA had an intermediate concentration compared to both sites (15.5 ± 3.3, 32.0, and 19.4 ± 11.6 mg L^{-1} respectively for the EF, BL, and MA). At each of the outflows of Basins 1 to 3, DOC

concentrations increased from 33.7 ± 4.7 mg L^{-1} at B1O to 81.0 ± 47.4 mg L^{-1} at B3O (Fig. 3a). The DOC concentration ranges that I observed in spatial surveys of B1 and B2 were not statistically different from those at the seasonally sampled outflow locations (Fig. 3a). Optical absorbance (a_{254}; m^{-1}) followed generally consistent patterns with DOC concentrations, with EF and B3O having significantly lower and higher absorbance values, respectively, than the other sites (Fig. 4).

Overall, the DOM pool at EF appeared to be unique, with more bio-labile fluorescent DOM (FDOM) relative to all other sites (Fig. 3b-f). Although excluded from statistical analyses due to the fact I could only gather one sample for BL site, the DOM at BL had intermediate FDOM intensity for all peaks. Across the Basins, fluorescence peak intensities showed the same pattern as for DOC concentrations, increasing from B1 to B2O, with a dramatic increase at B3O (Fig. 3b-f). Fluorescence peaks A, M, and C (Kruskal-Wallis: $p < 0.001$, $n = 78$) were consistently more intense at B3O than other sites, and lower at B2 than other sites (except for peak A). B and T peak intensity at EF was greater than B1O and moderately greater than at other sites (Kruskal-Wallis: $p < 0.001$, $n = 78$), while at B3O not significantly different from all sites except B2. Generally, the FDOM peak intensities were higher in EF and B3O sites and had no clear shift from inflow to outflow locations.

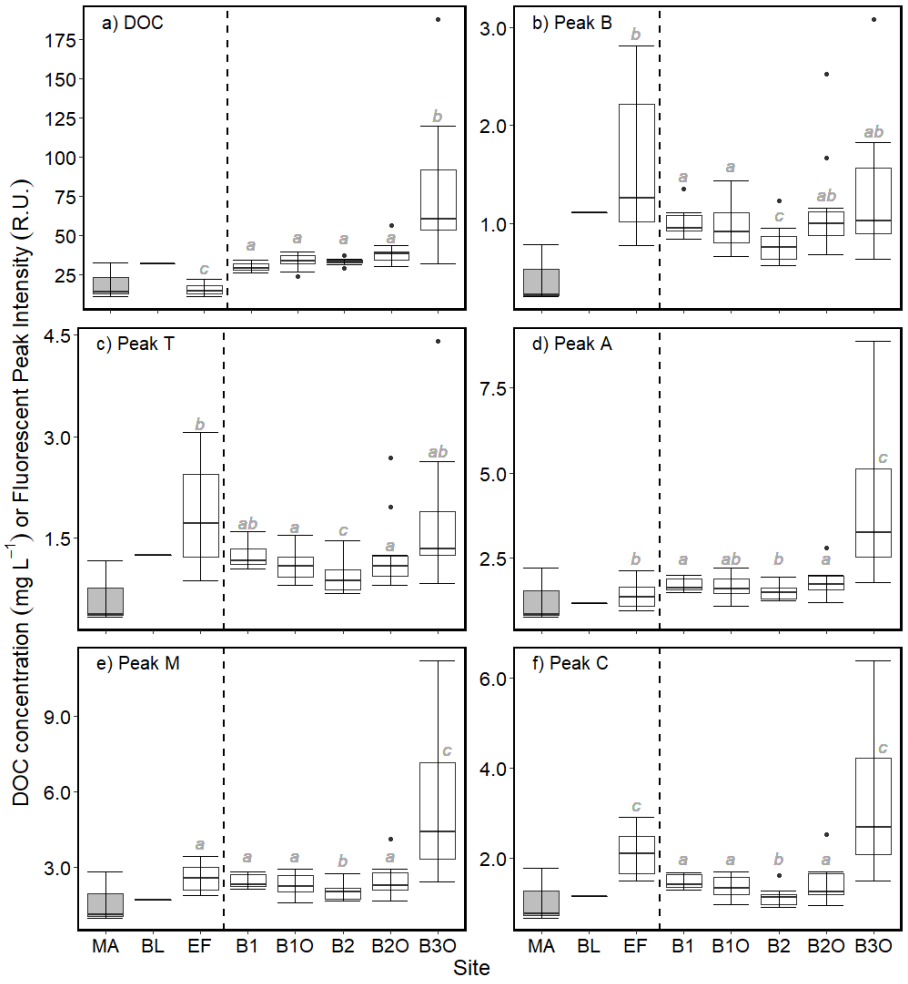

Figure 3. Differences in DOC concentration (mg L^{-1}; panel a) and fluorescent DOM composition (R.U.; B, T, A, M, and C peaks; panels b to f) among sites at the Frank Lake wetland complex. The dashed lines separate inlet sites, MA, BL, and EF (left), from Frank Lake Basin outflows (B1O, B2O, B3O) and spatial surveys (B1 and B2) (right). All ANOVA (DOC) and Kruskal-Wallis (B, T, A, M, and C peaks) results showed significant group differences ($p < 0.001$) with statistically significant post hoc comparisons ($p < 0.05$) denoted by grey, italicized, lower case letters. Note that results for the stagnant pool of water in MA (grey boxes) and limited data point for BL are shown, but not included in analyses.

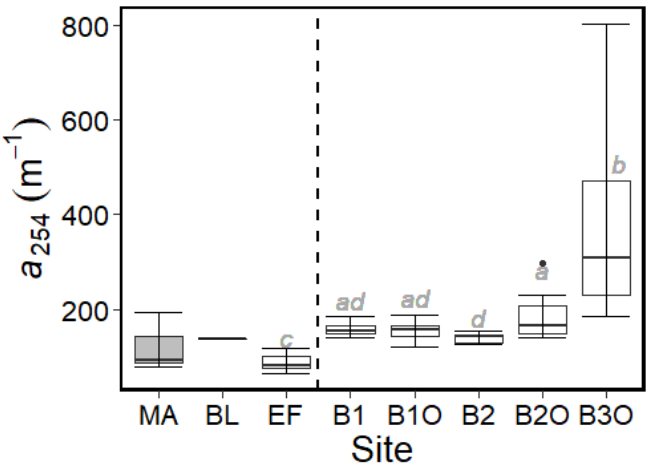

Figure 4. Box plot of absorbance coefficients at 254 nm (a_{254}) among sites at the Frank Lake wetland complex. All inlets (MA, BL, and EF), are to the left of the dashed line and Frank Lake Basins outflows (B1O, B2O, and B3O) and spatial survey (B1 and B2) are to the right of the dashed line. ANOVA ($p < 0.001$) indicated significant differences between groups, with groupings denoted by grey, italic, lower case letters summarizing post hoc comparisons. BL and MA (grey box) were not included in the ANOVA due to only one data point being available for BL, and the fact that water from MA was stagnant and not contributing to Frank Lake.

Optical properties of DOM at the EF inlet site indicated that the relative composition of this DOM pool was quite distinct from that at other inlets (BL or MA) and the Basin sites (Fig. 5). $SUVA_{254}$ was lowest at inlet BL and Frank Lake B2 (1.8 - 1.9 L mg C^{-1} m^{-1}) and highest at inlet MA (2.8 ± 0.2 L mg C^{-1} m^{-1} as shown in Fig. 5a). $SUVA_{254}$ values at EF (2.4 ± 0.1 L mg C^{-1} m^{-1}) were intermediate to those at BL or MA, but greater than values from the downstream Basins (Kruskal-Wallis, $p < 0.001$, $n = 78$). $SUVA_{254}$ values increased at B2O and B3O (2.1 ± 0.2 and 2.0 ± 0.3 L mg C^{-1} m^{-1}, respectively). The water from EF had the lowest S_R values (0.60 ± 0.17) compared to all other sites (Kruskal-Wallis, $p < 0.001$, $n = 78$), while inlets BL (0.85) and MA (0.97 ± 0.08) had intermediate values (Fig. 5b). S_R values were relatively consistent among basins, and not statistically different from one another. Across sites, FI values had similar but opposite patterns compared to S_R values, and only samples from EF and B3O were significantly different from other sites (Kruskal-Wallis, $p < 0.001$, $n = 78$; Fig. 5c). Water at the EF site had the highest FI values (1.86 ± 0.05), while MA and BL had intermediate values of 1.39 ± 0.04 and 1.50, respectively. Among the basins, FI values from B1 to B2O ranged from 1.47 to 1.49, but decreased to 1.43 ± 0.01 at B3O. The A:T ratio showed a similar pattern to S_R values with the lowest ratio observed at site EF (0.83 ± 0.19) and BL (0.94) (Fig. 5d). Only EF and B3O had A:T ratios that were statistically different from all other sites (lower and greater respectively; Kruskal-Wallis, $p < 0.001$, $n = 78$; Fig. 5d). Ratios of A:T increased from B1 (1.41 ± 0.12) to B3O (2.25 ± 0.36). The inlet MA had a ratio of 2.20 ± 0.24, similar to B3O.

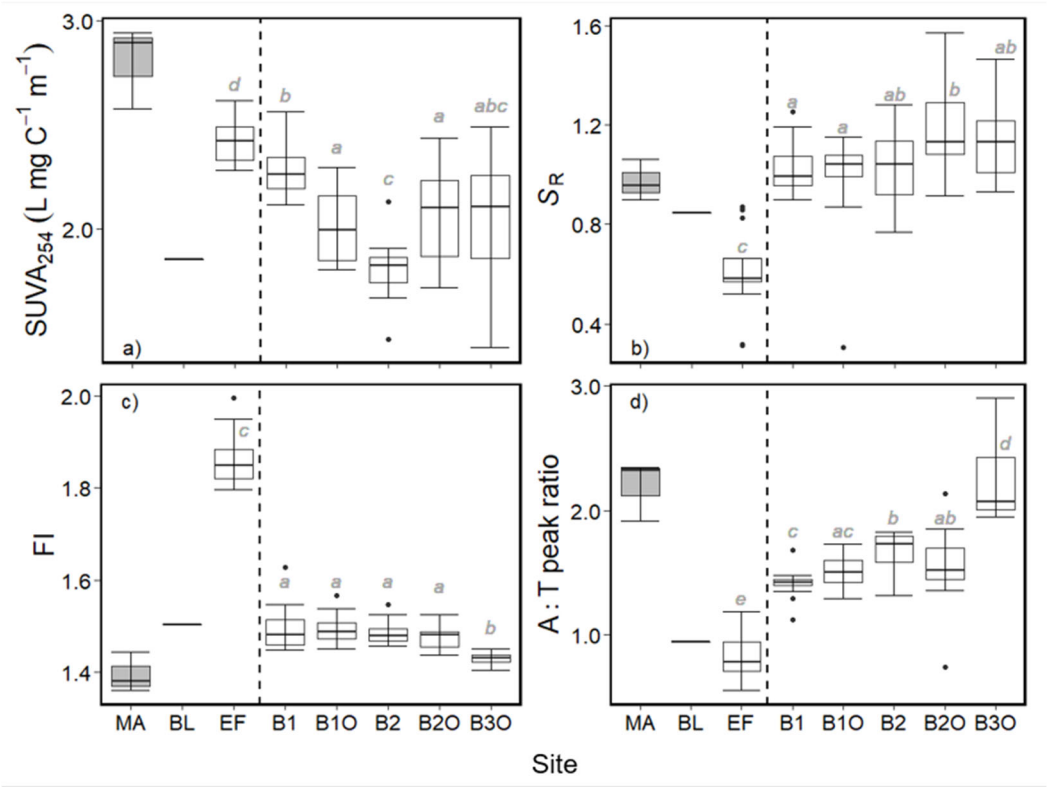

Figure 5. Compositional differences in DOM pools across sites. Significant differences among groups were observed (Kruskal-Wallis test; $p < 0.001$ for panels a to d, $n = 78$) and inter-group differences are summarized by gray italicized letters (Wilcoxon test; $p < 0.05$). Specific UV absorbance at 254 nm ($SUVA_{254}$; panel a), spectral slope ratio (S_R; panel b), fluorescence index (FI; panel c) and Peak A to T intensity ratio (A:T; panel d) results are shown.

2.3.3 PARAFAC model results

My PARAFAC model contained five components (C1 to C5), and DOM from EF showed a unique sequence of components compared to other sites (Fig. 6; Table 2). Component C1 had the highest percent contribution at all sites except EF (ANOVA, $p < 0.001$, $n = 45$), and represents humic-like DOM common in terrestrial environments (Coble, 2007; Wünsch et al., 2017). The percent contribution of C1 increased from EF to B3O and showed no significant difference between B1O and B2O (ANOVA, $p < 0.001$, $n = 45$). Unlike other sites, the EF site had DOM with the highest percent contribution of C2 (ANOVA, $p < 0.001$, $n = 45$), a component that has previously been related to wastewater or nutrient-rich surface waters (Jutaporn et al., 2020; Murphy et al., 2011). For C2 and C3, I saw a decrease in percent contribution moving through the wetland complex from EF to B2O, then an increase from B2O to B3O. C3 is a mix of peak A and C (Yamashita et al., 2011). MA had a high percent contribution of C3 compared to other sites (ANOVA, $p < 0.001$, $n = 45$). EF samples had relatively high amounts of C4, which represents microbially derived, humic-like DOM (DeFrancesco and Guéguen, 2021), compared to other sites, and I saw a decrease in relative contributions of C4 and C5 from EF to B3O (ANOVA, $p < 0.001$, $n = 45$). C5 is often associated with autochthonous sources of protein-like DOM (Osburn et al., 2011), possibly tryptophan-like DOM ((Coble, 2007). The abundance of C4 and C5 were lower at MA and B3O sites relative to other sites (ANOVA, $p < 0.001$, $n = 45$). The percent contribution to F_{max} scaled rank for EF was C2 > C5 > C4 > C3 > C1 while B1O and B2O had the same ranking in order of C1 > C5 > C4 > C2 > C3 (Fig. 7). B3O had a similar rank compared to MA, which had the same order for the first three components C1 > C2 > C3.

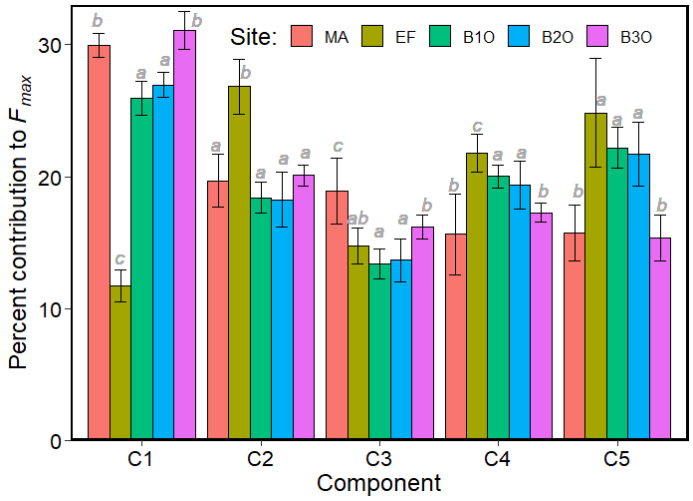

Figure 6. Percent contribution to F_{max} for C1 to C5 (see details in Table 2) for all sites except for BL, which was excluded to generate a significant PARAFAC model. Significant differences (ANOVA) are summarized with grey italicized letters that denote inter-group differences as defined by Tukey HSD post hoc comparisons ($p < 0.001$, n =45). Error bars represent +/- 1 S.D.

Table 2. The characteristics of the five components in the PARAFAC model and their relative abundance at each site.

Component	Ex max (nm)	Em max (nm)	Potential sources	Relative contribution at each site (%)				
				MA	EF	B1O	B2O	B3O
C1	265	422	Terrestrial humic-like [a, b]	30.0	11.7	25.9	27.0	31.1
C2	335	416	Wastewater related or from nutrient rich environment [c, d]	19.7	26.8	18.4	18.3	20.1
C3	365	478	Mixture of peak A and C [e]	18.9	14.8	13.4	13.7	16.2
C4	305	372	Microbial humic-like [f]	15.6	21.8	20.0	19.4	17.3
C5	280	328	Autochthonous protein-like, tryptophan-like (peak T) [a, g]	15.8	24.9	22.2	21.7	15.4

Literature sources: [a] Coble (2007), [b] Wünsch et al. (2017), [c] Jutaporn et al. (2020), [d] Murphy et al. (2011), [e] Yamashita et al. (2011), [f] DeFrancesco and Guéguen (2021), and [g] Osburn et al. (2011).

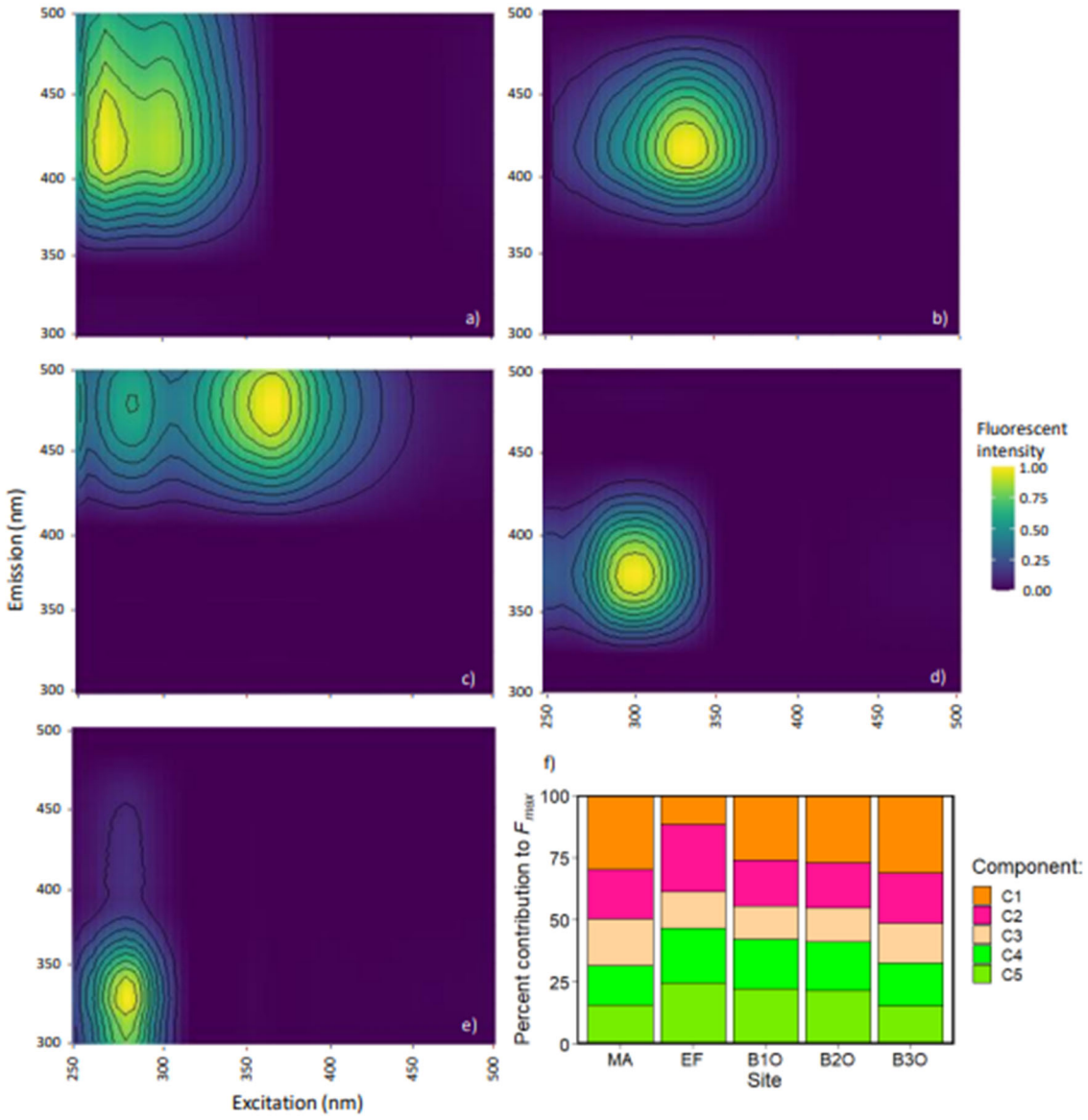

Figure 7. Exitation-emission matrices for the five fluorescence components (C1 to C5; panel a to e; respectively) assigned using PARAFAC. Each component in panel f is scaled to F_{max}, and components for each site sum to 100%.

2.3.4 BDOC experiments

Total DOC consumption during incubations was greatest for the EF site in July (8.4 ± 0.58 mg L^{-1}), while no difference was found across the rest of the sites that all had lower total DOC removal in July (between 2.0 to 3.3 mg L^{-1}) and in October (between 1.1 to 4.2 mg L^{-1}) (ANOVA: $p < 0.001$, $n = 30$; fig. 5a). The percent BDOC consumption (% BDOC) for EF decreased from 40.4 ± 3.0 % in July to 8.9 ± 1.9 % in October but remained consistent for B1O and B2O among seasons (6.9 to 5.3 % and 8.4 to 10.1 %, respectively). % BDOC for MA and B3O were 6.8 ± 2.3 % and 1.9 ± 2.7 % in July. All mean optical properties during incubations are shown in Table 3, but not discussed in detail. Based on BDOC consumption over 28 days for each site in July (Fig. 8; Table 3), EF had the highest decay rate (k) at 0.02 day^{-1}, compared to lower rates for the stagnant water in MA (0.0023 day^{-1}), B1O and B2O (~0.003 day^{-1}), and B3O (0.0015 day^{-1}) (Table 3; Fig. 9b). In October, the EF and B2O had similar k values (0.0034 and 0.0033 day^{-1}, respectively). Both EF and B1O showed a decrease of k from July to October. The half-life of DOC ($t_{1/2}$) in incubations in July was the lowest for EF (35 days), and highest at B3O (462 days), while both B1O (248 days) and B2O (210 days) had intermediate values (Fig. 9c, Table 3). In October, the $t_{1/2}$ for EF increased to 204 days, and B1O increased to 315 days, while B2O was unchanged. The ratio of A:T fluorescence peaks were strongly positively correlated with $t_{1/2}$ ($R^2 = 0.71$; $p = 0.009$; Fig 9c).

Table 3. Summary of DOC concentration and optical properties for the 28 day BDOC experiments. Standard deviation in brackets if applicable.

Site	Initial DOC (mg L^{-1})	Final DOC (mg L^{-1})	a_{254} (m^{-1})	S_R	$SUVA_{254}$ (L mg C^{-1} m^{-1})	FI	A:T	k (day^{-1})	$t_{1/2}$ (days)
July									
MA	31.3 (0.6)	29.2 (0.4)	192 (5)	0.98 (0.05)	2.73 (0.05)	1.42 (0.01)	2.27 (0.13)	0.0023	301
EF	20.8 (0.6)	12.4 (0.1)	90 (5)	0.43 (0.10)	2.41 (0.40)	1.91 (0.04)	0.86 (0.06)	0.0200	35
B1O	32.7 (0.3)	30.4 (0.2)	136 (2)	1.00 (0.18)	1.85 (0.05)	1.48 (0.01)	1.67 (0.06)	0.0028	248
B2O	39.5 (0.7)	36.2 (0.1)	191 (6)	1.07 (0.09)	2.17 (0.03)	1.48 (0.01)	1.84 (0.18)	0.0033	210
B3O	109.3 (0.9)	107.3 (2.8)	498 (12)	1.04 (0.05)	1.95 (0.02)	1.44 (0.01)	2.42 (0.17)	0.0015	462
October									
EF	12.4 (0.1)	11.3 (0.2)	76 (1)	0.41 (0.06)	2.76 (0.07)	1.87 (0.02)	1.02 (0.05)	0.0034	204
B1O	37.9 (0.4)	35.9 (0.2)	160 (2)	0.89 (0.09)	1.87 (0.05)	1.47 (0.02)	1.62 (0.11)	0.0022	315
B2O	41.6 (2.8)	37.4 (0.3)	155 (2)	1.04 (0.15)	1.71 (0.07)	1.47 (0.02)	1.58 (0.11)	0.0033	210

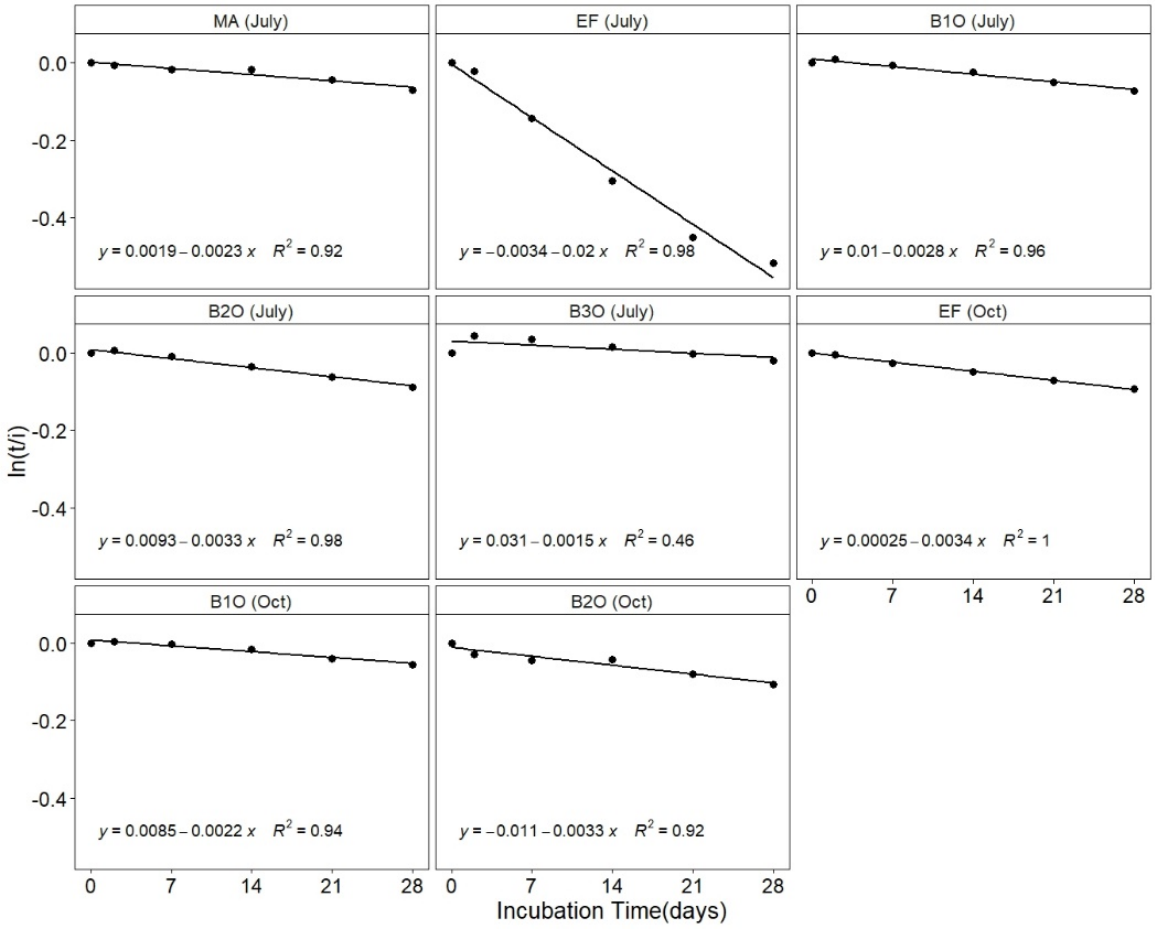

Figure 8. Loss of DOC through time in BDOC incubations. The decomposition rate of DOC (ln(*t*/*i*)) is plotted for all sites for the July 14 sampling period (five sites for July) and the October 21 sampling period (three sites for Oct.). Each point represents the mean of three subsamples.

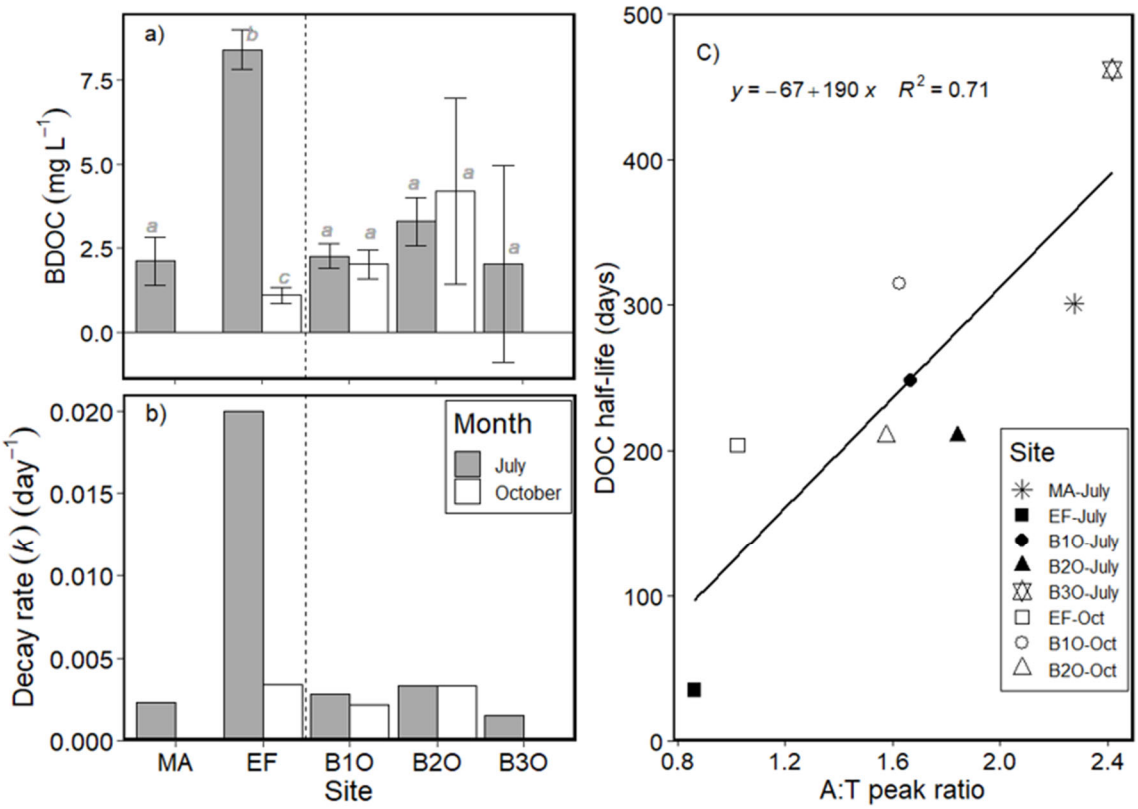

Figure 9. Microbial incubations showed distinct DOC processing through the wetland and through time. Biodegradable DOC values (BDOC; day 0 minus day 28 DOC concentrations; panel a) with clear inter-group differences (ANOVA, $p < 0.001$, $n = 30$; error bars represent +/- 1 S.D). DOC decay rates (panel b) were calculated from DOC concentration change (Fig. 8) for each site and sample period. The relationship between the A:T fluorescence peak ratio at the onset of incubations, and the half-life ($t_{1/2}$) of the DOC pool (panel c) from July (labeled as site name – July, e.g., MA-July) and October (labeled as site name - Oct).

Over the course of BDOC incubations, I observed large changes in the composition of the effluent, but not in water from the other sites. From day 0 to 28 in the EF incubation, I saw a large shift (~0.09) in FI values toward more aromatic DOM composition, and a large decrease in all individual fluorescence peak intensities (0.4 to 0.7 R.U.) in the EF incubation in July (Fig. 10). Among the incubations from the other sites in July, I observed consistent decreases in the intensity of B and T peaks, little change for A peaks, and increased intensity in M and C peaks (Fig. 10). In October, I observed small decreases or no changes in peaks B and T intensities and increases in peaks A, M, and C for all the sites during the incubation.

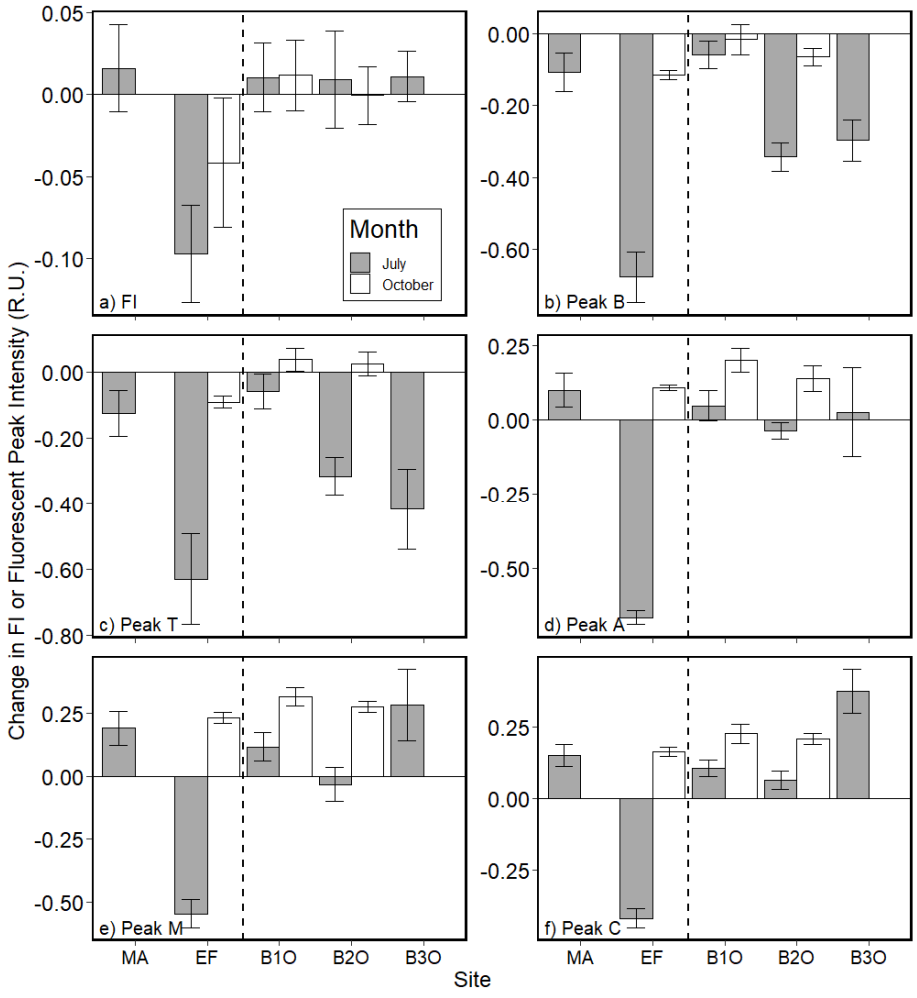

Figure 10. The change in fluorescence index (FI) (a) and fluorescence peak intensities (B, T, A, M, and C peaks) (b to f) in BDOC incubations for July (dark grey) and October (white), with inlets (left of dashed line) separated from outlets (right of dashed line). These changes represent the difference between day 0 and 28. Note that MA and B3O were not flowing, and these incubations were conducted with stagnant water. Error bars represent +/- 1 S.D.

2.3.5 Mass balance of lateral DOC flux across distinct hydrologic conditions

The averaged lateral water flux (output at B3O minus the sum of inputs) at Frank Lake was -1.44 ± 0.61 x 10^6 m^3 yr^{-1}. Water added to Frank Lake from the EF inlet totalled 3.82 ± 0.31 x 10^6 m^3 yr^{-1} (Table 4) and accounted for 82 to 99.5% of all hydrologic inputs to Frank Lake among all years. During the wet period (2013 to 2015), the inlets BL and MA imported 0.43 to 0.45 x 10^6 m^3 yr^{-1} water and EF imported 3.94 ± 0.30 x 10^6 m^3 yr^{-1} water while water exported at B3O was 3.91 ± 0.60 x 10^6 m^3 yr^{-1}, with a net water flux of -0.92 ± 0.74 x 10^6 m^3 yr^{-1}. During the drought period (2021), the net water flux was -2.88 ± 0.61 x 10^6 m^3 yr^{-1}. The negative net water flux in both periods indicated water loss from evaporation, assuming no groundwater recharge. The inlet BL imported 0.015 x 10^6 m^3 yr^{-1} and I observed no flow at the MA inlet for the entire year of 2021 (Table 1), so the MA inlet was excluded from the mass balance for the drought period. The WRT was 3.27 years averaged across all four years but increased from the 2013 to 2015 wet period (WRT average of 2.56 years) to the 2021 drought period (16.40 years).

The import of DOC to Frank Lake from the EF inlet ranged in importance from 71% (45.9 ± 12.7 Mg DOC yr^{-1}) of total inputs in the wet period to 99% (53.7 ± 11.3 Mg DOC yr^{-1}) in the drought period (Table 4). This variability was largely due to higher DOC concentrations in 2021 since the water fluxes were comparatively stable. Total inputs from BL and MA were lower (7.3 ± 1.5 and 7.2 ± 4.3 Mg DOC yr^{-1}, respectively; Table 4) than EF and contributed 23% of total inputs. In the drought period, the BL inlet contributed 0.48 Mg DOC yr^{-1}, and had a higher DOC concentration (32.03 mg L^{-1}). During the wet period when both creeks flowed for at least part of the year, both MA and

BL contributed 9.4 ± 5.7 and 9.5 ± 2.0 Mg DOC yr^{-1}, respectively. The output of DOC at B3O averaged 103.1 ± 19.7 Mg DOC yr^{-1} across all years, ranging from 117.9 ± 22.9 Mg DOC yr^{-1} (mean from 2013 to 2015) to 29.8 ± 13.5 Mg DOC yr^{-1} (2021). When averaged over the four full years of available data, the Frank Lake wetland complex was a net source of DOC, exporting 39.7 ± 24.7 Mg DOC yr^{-1} (Table 4). Among years, I observed dramatic differences in net DOC flux, with net export in the wet period (53.0 ± 26.9 Mg DOC yr^{-1}) and net DOC consumption in the drought period (-24.4 ± 17.6 Mg DOC yr^{-1}). The mean ratio of DOC output at B3O to total DOC inputs (DOC_{OUT}/DOC_{IN}) was 1.63 across all years (1.82 and 0.55 in during wet and drought periods).

Table 4. Mean annual discharge and DOC flux for wet and drought periods, and all years averaged. Standard deviation in parentheses if applicable.

Hydrologic Phase (Year(s))	Site	Discharge (10^6 m^3 yr^{-1})	DOC (mg L^{-1})	DOC flux (Mg C yr^{-1})	Net DOC flux (Mg C yr^{-1})
Wet period (2013 to 2015)	BL	0.43 (0.07)	24.4 (4.8)	9.5 (2.0)	
	MA	0.45 (0.26)	21.9 (6.0)	9.4 (5.7)	
	EF	3.94 (0.30)	11.7 (3.1)	45.9 (12.7)	
	B3O	3.91 (0.60)	31.6 (8.4)	117.9 (22.9)	
	Total				53.0 (26.9)
Drought period (2021)	BL	0.015	32.03	0.48	
	EF	3.47	15.5 (3.3)	53.7 (11.3)	
	B3O	0.61 (0.24)	49.4 (12.2)	29.8 (13.5)	
	Total				-24.4 (17.6)
All years	BL	0.33 (0.05)	25.4 (5.2)	7.3 (1.5)	
	MA	0.34 (0.02)	21.9 (6.0)	7.2 (4.3)	
	EF	3.82 (0.31)	12.8 (3.6)	48.9 (14.3)	
	B3O	3.06 (0.50)	33.2 (10.2)	103.1 (19.7)	
	Total				39.7 (24.7)

2.4 Discussion

I showed that the Frank Lake wetland complex effectively mineralizes and modifies effluent DOM, despite being a long-term (multi year) net source of DOM to downstream environments (Table 4). Within the wetland complex, DOC concentrations increased, and the DOM composition shifted from more protein-like at EF, towards more humic-like at the outlet of the wetland at B3O, which provides a clear indication of active DOM processing in transit (Fig. 3). Consistent with this interpretation of spatial surveys, our incubations showed that DOM is mineralized by wetland microbes. The half-life of the DOC pool was shortest in water masses entering the wetland but increased through the basins, tracking the shift toward more humic-like and less bioavailable DOM (Fig. 9). Despite being a net source of DOM over a longer, multi-year timescale, the source or sink strength of Frank Lake shifted between wet and drought periods that are common in the Prairie region (DOM source versus sink, respectively, Table 4, Fig. 11). This indicates that the role of the wetland complex in the regional aquatic network shifts over interannual timescales depending on climatic conditions. Together, my findings help characterize the capacity for mineral wetlands like Frank Lake to act as systems for organic effluent processing. They also demonstrate the importance of including multi-year observations across representative hydrologic conditions when attempting to determine the net role of wetlands as sites for effluent organic matter processing.

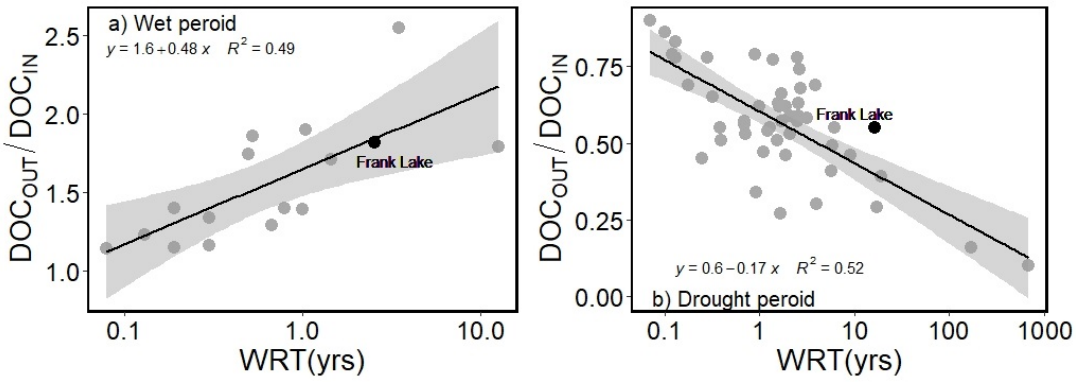

Figure 11. The relationship between the ratio of DOC_{OUT} to DOC_{IN} (DOC_{OUT}/DOC_{IN}) and water residence time (WRT) for lakes in Evans et al. (2017) (grey points) relative to Frank Lake (labelled, black points) during the wet period (2013-2015, panel a) and the most recent drought period (2021, panel b). As done in Evans et al. (2017), I present separate relationships for systems where $DOC_{OUT}/DOC_{IN} \geq 1.1$ (a) and those with $DOC_{OUT}/DOC_{IN} \leq 0.9$ (b). Linear regression (best fit) models are represented by the black line, with the 95% confidence interval represented in grey shade for relationship between DOC_{OUT}/DOC_{IN} and $\log_{10}$(WRT) lakes.

2.4.1 Frank Lake wetland is an overall source of DOC

Frank Lake is an overall net DOC source, with a long-term ratio of export to import (DOC_{OUT}/DOC_{IN}) of 1.63. This is consistent with empirical predictions based on WRT (Evans et al., 2017) (Fig. 12), suggesting Frank Lake functions similar to other inland water systems, in terms of DOM processing. Evans et al. (2017) showed a positive relationship between $\log_{10}$ (WRT) and the ratio of DOC_{OUT}/DOC_{IN} for lentic systems that act as net sources of DOC. There, the ratio of DOC_{OUT}/DOC_{IN} was not clearly controlled by the nutrient status of inland waters, but by WRT. Therefore, while the extreme nutrient content (White et al. 1999; Zhu et al. 2019) likely enhances autotrophic production and DOM transformation in Frank Lake, this had less impact on the net balance of DOC processing. Although Frank Lake exports a large amount of DOC annually (103.1 ± 19.7 Mg DOC yr^{-1}), this is equivalent to 10.3 ± 2.0 g C m^{-2} yr^{-1} when scaled to the wetland surface area (10.1 km^2, Zhu et al. 2019). This value is comparable to, and on the low end of DOC export rates from other natural wetland systems including tidal marsh wetlands (9.7 ± 2.2 g C m^{-2} yr^{-1}) (Bogard et al., 2020), mangrove systems (12 g C m^{-2} yr^{-1}) (Dittmar et al., 2006), and temperate wetlands (36 ± 12 g C m^{-2} yr^{-1}) (Clair et al., 2002). To my knowledge, few mass balance estimates exist for DOC flux in treatment wetlands that explicitly consider both input and output in a way that provides a net DOC flux value. Thus, comparing Frank Lake to other inland water systems (Fig. 12) and linking the export rate to hydrologic residence time provides empirical evidence that, along with existing experimental work (Pinney et al., 2000; Vähätalo and Wetzel, 2008), helps to explain the role of treatment wetlands in overall DOM processing.

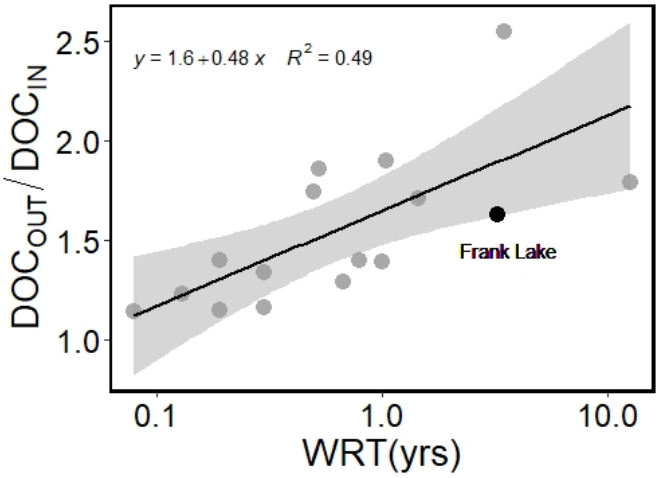

Figure 12. DOC export ratio of Frank Lake relative to other lentic systems that are net exporters of DOC. The long-term (2013 to 2015 and 2021 combined) ratio of DOC output to input (DOC_{OUT}/DOC_{IN}) and water residence time (WRT) for Frank Lake was compared to lake and reservoir data from Evans et al. (2017), including their reported linear regression relationship (black line with the 95% confidence interval in grey).

Frank Lake appears to switch from a net DOC source to sink between wet and drought periods, respectively (Table 4, Fig. 11). This difference in DOC processing is linked to hydrological changes that shifted WRT from 2.56 to 16.40 years (Fig. 11). The shorter WRT in the wet period can limit the capacity for DOM to be exposed to mineralization and photodegradation processes on site (Granéli et al., 1996; Vachon et al., 2021). In contrast, during the drought period when the wetland was a net DOC sink (Table 4, Fig. 11b), water loss through evaporation was 7.53×10^6 m^3, which was double that in the wet period (Zhu et al., 2019). This resulted in the hydrological disconnection between Basins 2 and 3, and Basin 3 to downstream, which increased WRT and the potential for in-situ DOM removal by multiple mechanisms (e.g., respiration, photodegradation, burial, or assimilation) (Tranvik et al., 2009; Vachon et al., 2021; Ward et al., 2017). It is likely that photodegradation would preferentially remove aromatic DOM (Clark et al., 2008; Vähätalo and Wetzel, 2008; Waiser and Robarts, 2004) while biodegradation would tend to mineralize protein-like or LMW DOM (Findlay and Sinsabaugh, 2003; Hutchins et al., 2017). More work is needed to identify the exact mechanisms driving the DOM sink in Frank Lake. However, my findings demonstrate that caution is needed when attempting to attribute a net DOM sink or source status to effluent treatment wetlands (Barber et al., 2001), particularly when datasets do not cover the full range of representative interannual hydrologic conditions that a given wetland may experience.

2.4.2 Frank Lake efficiently processes effluent-derived DOM

The half-life of the DOC pool at each site (35 to 462 days; Fig. 9) is shorter than overall WRT (mean of 3.27 years; Fig. 12), suggesting the wetland rapidly mineralizes most effluent-derived DOM. DOC decay coefficients (k) decreased from 0.02 day^{-1} to 0.0015 day^{-1} moving downstream from the EF inlet site to B3O outflow (Fig. 9b), and these values are within the range of comparable incubations that ranged from 0.0066 ± 0.0109 day^{-1} in small wetlands with 0.06 ± 0.25 years WRT (Catalán et al., 2016), and 0.0293 day^{-1} in small marsh wetlands with WRT of 0.77 years (Guillemette and del Giorgio, 2011). The rates of microbial DOC processing varied with DOM composition (as A:T peak ratios; Fig. 9c), reflecting the role that DOM bioavailability plays in controlling metabolism of the microbial community (Kritzberg et al., 2006; Li et al., 2008; Logue et al., 2016). Seasonal BDOC differences were minor for all but the EF for July, and generally BDOC processing decreased with the downstream shift toward more humic-like DOM and relatively less protein-like DOM. This trend is consistent with expectations for longitudinal DOM processing patterns in another wetland system (Pinney et al., 2000). These incubations were conducted under controlled laboratory conditions that excluded photochemical or sediment processes, and therefore do not necessarily reflect ecosystem-level DOM processing. While laboratory BDOC incubations do not encompass all ecosystem-level DOM processing, BDOC incubations do capture general DOM cycling patterns in aquatic systems (Kelso et al., 2020), so I am confident that the intense mineralization of effluent DOM observed in incubations extends to the wetland ecosystem, especially since this conclusion is supported by the 2021 mass balance that showed intense net DOM consumption.

2.4.3 Compositional shifts in DOM along the hydrological continuum

As in other treatment wetlands (Barber et al., 2001), I observed a compositional shift in the DOM pool from the effluent-derived signature that is more bioavailable but higher in molecular weight, toward more aromatic, humic-like wetland-derived DOM that reflected mixed potential inputs that may diversify the composition of the DOM pool. Effluent derived DOM had the lowest DOC concentration (~15.5 mg L^{-1}) and highest FI values among sites (~1.86), with relatively high $SUVA_{254}$ values (~2.4 L mg C^{-1} m^{-1}), and higher molecular weight based on S_R (~0.6; Fig. 3,5). This is comparable to previous work showing treated municipal wastewater also had $SUVA_{254}$ values around 2.2 L mg C^{-1} m^{-1}, with higher molecular weight DOM after secondary treatment, indicating the presence of microbially-derived DOM, proteins and polysaccharides (Maizel and Remucal, 2017). However, these values depend on effluent sources and treatment processes, such that effluent $SUVA_{254}$ values have been shown to vary from 0.7 to 2.9 L mg C^{-1} m^{-1} (Wang and Chen, 2018), with the EF site at Frank Lake on the upper end of this reported range. Consistent with $SUVA_{254}$ values that suggest an abundance of more aromatic DOM in effluent, PARAFAC results showed effluent also had a large relative contribution of C2, indicating an abundance of humic-like DOM, consistent with wastewater and other nutrient rich environments (Jutaporn et al., 2020; Murphy et al., 2011). DOM from the EF site also had a relatively greater contribution of microbial humic-like DOM (C4) (DeFrancesco and Guéguen, 2021) and tryptophan-like DOM (C5) (Osburn et al., 2011), similar to municipal and domestic sewage from other studies, likely

due to leaching of DOM from microbes during biological treatment in secondary wastewater processing (Wang and Chen, 2018). Effluent DOM was likely replaced via DOM leaching from emergent vegetation and wetland soils (Clark et al., 2008; Pinney et al., 2000), especially below the outflow of Basin 2. PARAFAC component C2 decreased, while C1 (humic-like terrestrial DOM; Wunsch et al. 2017) and C3 increased significantly at B3O (Fig. 6). Further, FI values decreased to those characteristic of other natural wetlands (1.30 to 1.58; (Hertkorn et al., 2016; Lu et al., 2003)). The decrease of C4 and C5 across Basins are consistent with photodegradation and biodegradation processes removing significant portions of this DOM pool (discussed above). Taken together, Frank Lake not only removes effluent DOM (Fig. 9), but it modifies the composition of the DOM pool (Fig. 5, 6), and this study helps to pinpoint the zone of intense DOM modification (below Basin 2). This zone is more terrestrial-like and shallow (see methods and Fig. 1), so water likely interacts with soils and emergent vegetation more effectively, leading to the observed compositional shifts in DOM. Further work is needed to determine whether this compositional shift has water quality and toxicological implications.

2.5 Conclusions

Here, I provide new information regarding the role of an economically-important, model treatment wetland that receives multiple sources of complex effluent. Consistent with numerous other wetlands and inland water systems, Frank Lake appears to be a net source of DOM to downstream ecosystems, though effluent is efficiently mineralized, and a large fraction appears to be replaced with internally-derived DOM, thereby shifting

DOM quantity and quality to that the wetland prior to export. Further, by quantifying net DOC flux individually between wet and drought periods, I show that treatment wetlands can switch from net sources to sinks of DOM across distinct hydrologic regimes, underscoring the importance of long-term monitoring. The processing of effluent DOM by treatment wetlands represents an important, but underappreciated global service to society. Collectively, my findings will help to develop a general understanding of this important service.

CHAPTER 3. CONCLUSIONS

3. 1 Major research findings

My combination of routine sampling, multiple historical datasets, an ecosystem mass balance, and incubation experiments provides new information about treatment wetland functioning and DOM cycling. Multi-year datasets showed that Frank Lake is a net source of DOM to downstream ecosystems, with a DOC export rate (10.3 ± 2.0 g C m^{-2} yr^{-1}) that is low, but in the range of other wetlands and inland water systems (Bogard et al., 2020; Clair et al., 2002; Dittmar et al., 2006; Evans et al., 2017). Although Frank Lake receives massive amounts of DOC from effluent (48.9 ± 14.3 Mg DOC yr^{-1}), a large fraction of this DOM is highly bioavailable, and is removed or transformed (Fig. 3, Fig. 6, and Fig. 7). The replacement of effluent DOM with wetland-derived materials results in a shift toward more humic-like DOM characteristic of natural wetland sources at the outflow of Frank Lake. Further, I determined the lateral DOC budget for both wet and drought periods and showed that under variable hydro-climatic conditions, treatment wetlands can switch from net sources to sinks of DOM as a function of the shortening or lengthening of water residence time (WRT; Fig. 11). While Frank Lake is a long-term net DOC source to downstream ecosystems, it appears to be an efficient site for effluent DOM removal and replacement, which may have important implications for downstream ecosystems.

3. 2 Implications for ecosystem management

3.2.1 Assessing ecosystem function based on net DOC budgets

Many studies have explored wetland DOC concentrations and export rates, yet I present one of the few complete mass-balance assessments of treatment wetland DOC flux. Our general understanding is that treatment wetlands receiving raw effluent are net sinks of DOM, and wetlands receiving treated effluent are a net source of DOM (Barber et al., 2001; Pinney et al., 2000). Most treatment wetlands are constructed, and the only water source is effluent water, thus the change of DOC concentration from in- to outflow is considered its removal efficiency (Li et al., 2008; Pinney et al., 2000). In wetlands with multiple water sources (natural or restored treatment wetlands), or considerable evaporative losses of water in transit, these simple techniques to determine DOC removal or input are not suitable, and mass balance construction is more complicated.

By considering all DOC inputs and the output simultaneously in my mass balance, I showed that the Frank Lake wetland complex is a net DOC source due to in situ production and wetland inputs from plants and soils, a finding that has important implications for the broader watershed C budget. Inland waters (rivers, lakes, reservoirs) are reactors that mineralize, transform, store, and export C as water flows from land to the ocean (Cole et al., 2007; Drake et al., 2018). Wetlands are a transition between terrestrial systems and inland waters, and DOC concentration in inland water systems is often positively related to the presence of wetlands in a watershed (Dillon and Molot, 1997; Gergel et al., 1999; Xenopoulos et al., 2003). Yet wetlands are often excluded from aquatic C budgets, despite this influence. Therefore, defining the net lateral DOC flux

from wetlands, as I have here for Frank Lake, is the first step toward the construction of a more complete watershed C budget for the upper Little Bow River network.

This mass balance approach has limitations, and without a complete net ecosystem C budget (NECB) that includes rates of internal cycling of DOC (Finlay et al., 2010), my ability to explain the net flux of C in Frank Lake is limited. I can infer ecosystem heterotrophy and the dominance of respiration in 2021 because of the large discrepancy between DOC input (54.2 ± 11.3 Mg DOC yr^{-1}; Table 4) and output (29.8 ± 13.5 Mg DOC yr^{-1}). This is not surprising, because high rates of external DOC inputs result in higher rates of respiration with increased microbial mineralization (Duarte and Prairie, 2005). At the same time, Frank Lake also appears to have high rates of DOC production. The compositional changes of DOM through the wetland system provide another line of evidence that there is addition of DOM to Frank Lake from wetland sources (plants, soils). Therefore, the addition of DOM from gross primary production could potentially be important, despite the ecosystem being heterotrophic in 2021. It is likely that gross primary production exceeds respiration in wet years (e.g., 2013 to 2015) when the ecosystem is a net source of DOM. To better understand the cycling of DOM in Frank Lake, a complete NECB as shown in Finlay et al. (2010), which accounts for rates of primary production, respiration, and sedimentation, should be considered in future work.

3.2.2 The effects of hydro-climatic variability on wetland performance

In this study I highlight the importance of having multiple years of ecosystem mass balance data when inferring wetland functioning. The Frank Lake wetland changed its functional role from a DOC source to sink as a function of hydrological conditions that

shifted WRT by up to an order of magnitude (2.56 to 16.40 years). During the drought period, Frank Lake had a longer WRT that led to clear compositional shifts in DOM transiting through the wetland. The transformation of DOM during wet periods is unclear, as shorter WRT may restrict DOM processing. Further research is needed to determine whether this leads to the release of more effluent DOM and larger quantities of DOM downstream compared to the drought period. Overall, accounting for both wet or drought periods in ecosystem mass balances will minimize the potential for mischaracterization of wetland function and the supply of DOC to downstream ecosystems. My study was consistent with other work in a peatland ecosystem that showed drought periods lowered water levels and resulted in lower DOC export compared to other years over a ten year timeframe (Clark et al., 2005). The effects of drought periods on DOC fluxes can be complex, either lengthening WRT and favoring heterotrophy and reducing DOC fluxes (as seen here), or favoring increased DOC production and fluxes due to particulate organic matter decomposition and increased DOC supply (Evans et al., 2005). Therefore, long-term studies of DOC flux are necessary to understand overall wetland functioning, especially in regions like the Canadian Prairies that have inconsistent climatic conditions and widely varying ecosystem hydrology and WRT.

The multi-year mass balance that I constructed for DOC can be extended to other elements that are processed in Frank Lake. While similar budgets exist for both N and P (Zhu et al., 2019), this approach would help build an understanding of the cycling of effluent-derived metals that may be liberated following extensive ecosystem desiccation during the 2021 drought. Wetland soils have a strong affinity to absorb heavy metals such as cadmium, zinc, and lead, and can remove up to 85% of heavy metals from the water

column (Gambrell, 1994; Sinicrope et al., 1992). Extreme drought periods caused directly by human wetland drainage, or indirectly through hydro-climatic changes, lengthen WRT and increase evaporation, and lead to lower water levels that expose littoral soils. Low water levels and sediment exposure to the atmosphere can increase sulphur oxidation, leading to increased acidification, thereby reducing the affinity of heavy metals bound to sediment organic matter (Adkinson et al., 2008; Szkokan-Emilson et al., 2013). This process can liberate metals from sediments (Adkinson et al., 2008; Zedler and Kercher, 2005). Re-wetting can increase the export of liberated heavy metals (Szkokan-Emilson et al., 2013) and impact downstream drinking water sources. Whether similar outcomes will occur at Frank Lake following the drought of 2021 are unclear. To account for these effects, a continuous, multi-element (metals, nutrients, and DOC) study across multiple years is required that accounts for biogeochemical processing during the post-drought re-wetting period. Such knowledge will help to guide watershed managers in efforts to reduce the impact of releasing of heavy metals to human health. Globally, about 35% of wetland area has been lost in the past five decades, with most having been lost through the drainage of wetlands for agricultural land and drying related to climate change (Gardner and Finlayson, 2018; Xu et al., 2020; Zedler and Kercher, 2005). Therefore, desiccation of wetland soils due to human activity or natural climate cycles can have major and complex (e.g., lagged) effects on the capacity of wetlands to treat wastewater, with global scale implications for water quality.

3.2.3 Importance of treatment wetlands in processing effluent DOM

Consistent with other studies (Barber et al., 2001; Supowit et al., 2016), Frank Lake appears to efficiently remove and modify effluent organic matter. This observation has major consequences for aquatic ecosystem health and functioning. Multiple absorbance and fluorescence metrics demonstrated a shift in DOM composition through the wetland network from that characteristic of effluent (e.g., protein-rich and extremely biolabile) toward more natural, wetland-derived DOM. Water leaving Frank Lake had values of FI that were lower than in the effluent, and more characteristic of natural wetland DOM composition (Hertkorn et al., 2016; Lu et al., 2003). DOM exported from Frank Lake also had intermediate aromaticity ($SUVA_{254}$ values of 2.0 ± 0.3 L mg C^{-1} m^{-1}) in the mid-range of values observed in other natural systems (0.6 to 5.3 L mg C^{-1} m^{-1}), and on the low end of values for terrestrially-dominated lakes that have $SUVA_{254}$ values from 4 to 5 L mg C^{-1} m^{-1} (Massicotte et al., 2017). On one hand, high amounts of colored, aromatic DOM in water can be beneficial to aquatic species as it provides shade, thereby reducing cell and DNA damage by ultra-violet light (Leenheer and Croué, 2003). However, aromatic compounds also have higher affinity to sorb heavy metals and contaminants (Camino-Serrano et al., 2014; Findlay and Sinsabaugh, 2003; Leenheer and Croué, 2003), therefore, intermediate aromaticity seen here may limit the transportation of heavy metals and contaminants to downstream ecosystems, while providing an moderate level of protection via shade to downstream aquatic species.

The shifts in DOM composition from effluent-derived DOM to wetland-derived DOM that I observed may also reflect changes in water quality linked to organic toxin content, which may have important implications for downstream ecosystems including

the TVR. Supowit et al. (2016) showed wastewater treatment plants can decompose and lower the toxicity of pesticides and related compounds, but are not able to remove them from the water column, while wetlands can remove both pesticides and related compounds by up to 47% from the water column (Supowit et al., 2016). Whether Frank Lake DOM transformations reflect the processing of organo-toxins including pesticides is unclear, but should be explicitly considered in future work. Furthermore, the decrease in protein-like DOM observed in my study may indicate that Frank Lake may reduce the potential for export of nitrogenous materials that can lead to DBP formation during drinking water treatment (Krasner et al., 2009). Protein-like DOM is a precursor in the formation of nitrogenous DBPs such as haloacetonitriles (HANs), which are more toxic than other carbon based DBPs (Muellner et al., 2007). My study did not directly determine the quantity of organic N removed in Frank Lake. However, a previous mass balance (Zhu et al., 2019) showed that 95% of N entering Frank Lake is retained or mineralized prior to downstream export, and this aligns with the decrease in protein-like fluorescence that I observed. The reduction of organic N exported from Frank Lake may also help to restrict the formation of harmful algal blooms and associated problems downstream in the TVR (e.g., algal toxin production, anoxia, fish kills) (Chen et al., 2008; Gonsior et al., 2019; Graham et al., 1998; Sellner et al., 2003). Therefore, treatment wetlands like Frank Lake are likely an important tool for improving water quality by removing nutrients and toxins before being transported downstream.

While fluorescence spectroscopy is a technique that characterizes bulk DOM characteristics, many studies have shown that these properties are linked to the molecular-level composition of DOM (Kellerman et al., 2015; Stubbins et al., 2014). Thus, the shifts

in optical characteristics that I observed indicate that the Frank Lake wetland modifies the molecular composition of effluent-derived DOM. Future applications of higher resolution molecular techniques are needed to fully identify the molecular level changes in DOM composition (Kellerman et al., 2015) and removal of effluent related compounds in Frank Lake.

3.2.4 Appreciation of wetland DOM cycling as an ecosystem service

My study highlights that wetlands provide important ecosystem services, particularly with regards to treatment wetlands such as Frank Lake. I identified that Frank Lake is an important wetland for water quality improvements in the upper Little Bow watershed, because it processes effluent DOM before entering the downstream Little Bow river network and influencing drinking water supplies. The net DOC budget that I constructed provides foundational knowledge about treatment wetland functioning that spans multiple years and accounts for extreme interannual variation. Overlooking this variation, it would be easy to mis-characterize the overall functioning of Frank Lake. Although wetlands provide important ecosystem services, they are relatively understudied in Canada. According to the global-scale study of Xu et al. (2020), 1711 studies of wetlands were conducted between 1995 and 2018, but Canadian wetlands accounted for less than 50 studies (< 2.9%), despite accounting for 14% of total Canadian land cover (Kennedy and Mayer, 2002). My work therefore adds important data and knowledge to our understanding of treatment wetland functioning and services in Canada. Further, it highlights the overlooked value of wetland processing of effluent DOM as an ecosystem service, which is important for downstream ecosystems and ultimately human health. The

study of Frank Lake that I present here also provides knowledge and serves as a model for future assessments and management of treatment wetlands.

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