# EXPLORING SULFATE CYCLING IN A MINERAL-SOIL WETLAND RESTORED WITH WASTEWATER

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# ABSTRACT

Sulfate ($SO_4^{2-}$) pollution is contributing to the salinization of surface waters worldwide. Wetlands are natural filters on the landscape that remediate surface water by retaining and processing pollutants. However, the capacity for wetlands to process excess $SO_4^{2-}$ from wastewater is poorly understood, especially for natural (as opposed to constructed) wetlands. Here, I explore the $SO_4^{2-}$ remediation capacity of Frank Lake, a restored, multi-basin wetland complex in southern Alberta, Canada, that is used to treat effluent from municipal and beef slaughterhouse sources. Using a combination of approaches, I show that there was limited $SO_4^{2-}$ processing throughout the wetland. Mass balances constructed for two distinct hydrologic periods showed that Frank Lake shifted from a $SO_4^{2-}$ source during wet years (2013 - 2015) to a sink during drought years (2021 - 2022). Yet I found little evidence of active $SO_4^{2-}$ processing in surveys conducted during drought years. $SO_4^{2-}$ remained the dominant form of sulfur (S) among all three basins (>95% of total S), implying little net change in the S pool. Similarly, dual stable isotope ($\delta^{34}S$ and $\delta^{18}O$) analysis showed limited isotopic enrichment among wetland basins, implying limited transformation of $SO_4^{2-}$ via microbial reduction. Sediment incubations confirmed the patterns observed with stable isotopes, showing little net removal of $SO_4^{2-}$ throughout the wetland. The preferential reduction of nitrate ($NO_3^-$) and other more energetically favourable constituents of the effluent may restrict the extent of microbial $SO_4^{2-}$ reduction throughout Frank Lake. The limited extent of emergent and submerged vegetation may also limit $SO_4^{2-}$ uptake by plants. Given the limited $SO_4^{2-}$ processing in Frank Lake, and the headwater position of this wetland complex in the broader aquatic network, my work provides context for previous reports of increasing salt concentrations documented in rivers of the South Saskatchewan River watershed.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| Al | Aluminium |
| ANOVA | Analysis of variance |
| ASR | Assimilatory sulfate reduction |
| B1O | Basin 1 outflow |
| B2O | Basin 2 outflow |
| B3O | Basin 3 outflow |
| BaCO3 | Barium carbonate |
| $BaCl_2$ | Barium chloride |
| $BaSO_4$ | Barium sulfate |
| BK | Blackie Creek |
| C | Carbon |
| $Cl^-$ | Chloride |
| CO | Carbon monoxide |
| COD | Chemical oxygen demand |
| Del ($\delta$) | Stable isotope composition |
| DO | Dissolved oxygen |
| DOC | Dissolved organic carbon |
| DOM | Dissolved organic matter |
| DSR | Dissimilatory sulfate reduction |
| EFF | Effluent outlet/discharge point |
| Fe | Iron |
| FL | Frank Lake |
| $H_2O$ | Water |
| $H_2S$ | Hydrogen sulfide |
| HCl | Hydrochloric acid |
| HRT | Hydraulic residence time |
| IRMS | Isotopic ratio mass spectrometry |
| Mn | Manganese |
| MZ | Mazeppa Creek |
| N | Nitrogen |
| $NO_2^-$ | Nitrite |
| $NO_3^{2-}$ | Nitrate |
| O | Oxygen |
| $O_2$ | Oxygen molecule |
| OC | Organic carbon |
| P | Phosphorus |
| S | Sulfur |
| $SO_2$ | Sulfur dioxide |
| $SO_4^{2-}$ | Sulfate |
| $SO_4^{2-}{}_{in}$ | Input of sulfate into the wetland |
| $SO_4^{2-}{}_{out}$ | Output of sulfate from the wetland |
| $SO_x$ | Oxides of sulfur |
| SRB | Sulfate reducing bacteria |
| U.S. | The United States |
| V-CDT | Vienna-Canyon Diablo Troilite |
| V-SMOW | Vienna Standard Mean Ocean Water |
| WRT | Water residence time |

# CHAPTER 1. INTRODUCTION

## 1.1 Background

Sulfur (S) is an important limiting nutrient for the growth and development of organisms including plants (Li et al., 2020; Nazar et al., 2011). Therefore, S plays crucial biochemical and physiological roles and shapes ecosystem functioning. Sulfur is required for the synthesis of amino acids such as methionine and cysteine, vitamins (biotin and thiamine), some pigments, and numerous other secondary metabolites and phytohormones (Anderson and Spencer, 1950; Narayan et al., 2022; Shah et al., 2022). The role of S is irreplaceable in sustaining life, as its deficiency negatively impacts the growth, disease resistance, development and productivity of plants, and lowers their nutritional quality (Kopriva et al., 2019). Thus, an understanding of the biogeochemical cycling of S in natural environments is needed to better appreciate the health and functioning of communities and ecosystems.

### *1.1.1 The global S cycle*

While S primarily cycles between the lithosphere, atmosphere and hydrosphere (Lavelle et al., 2005), only a small amount of S enters the biosphere (Charlson et al., 1992; Schlesinger and Bernhardt, 2013). The early deposition of S on Earth as pyrite in the Earth's crust represents the largest global S reservoir ($2.4\times10^{10}$ Tg S). Geologically bound S is released to the land, air, and water by natural and anthropogenic activities. Weathering of minerals (72 Tg S $yr^{-1}$), volcanic activity (10 Tg S $yr^{-1}$), fires (3 Tg S $yr^{-1}$), and dust deposition (8 Tg S $yr^{-1}$) constitute the natural fluxes of S, while the key anthropogenic activities mobilizing S include mining (160 Tg S $yr^{-1}$), fossil fuel burning (70-100 Tg $yr^{-1}$) and agricultural inputs (70 Tg S $yr^{-1}$) (Hinckley et al., 2020; Ivanov and Freney, 1983; Lavelle et al., 2005; Schlesinger and Bernhardt, 2013). The ocean is the second largest pool of S, with water and sediments containing around $1.3\times10^9$ Tg S, and $3\times10^8$ Tg S respectively (Schlesinger and Bernhardt, 2013). Marine sea salt sprays (140 Tg S $yr^{-1}$) and biogenic organic gases (15 - 30 Tg S $yr^{-1}$) represent important flux pathways that remove S from the ocean (Charlson et al., 1992; Lavelle et al., 2005). Gaseous forms of S are unstable, have short residence times in the atmosphere, and are a comparatively small pool (4.8 Tg S; residence time of 0.1 yr (Ivanov and Freney, 1983)). Gaseous S combines with other elements to form stable compounds, mainly S oxides ($SO_x$), which are deposited on land and water as wet or dry sprays (Charlson et al., 1992; Zak et al., 2021).

Inland waterbodies such as rivers and lakes act as channels connecting the land to the coastal ocean, hence, they are significant conduits of S between major planetary reservoirs (Ivanov and Freney, 1983). The S flux through inland waterbodies is now around 213 Tg S $yr^{-1}$, now double preindustrial levels (Charlson et al., 1992; Lavelle et al., 2005; Schlesinger and Bernhardt, 2013), with anthropogenic sources contributing around 109 Tg S $yr^{-1}$ (Ivanov and Freney, 1983). Thus, increased human perturbations have dramatically modified the biogeochemical cycling of S by mobilizing excess amounts of geologically bound S and thus causing severe S pollution in ecosystems worldwide (Hinckley et al., 2020).

### *1.1.2 Sources of S pollution*

The S cycle is one of the most anthropogenically altered biogeochemical cycles, as ecosystems receive large amounts of S from various human activities (Galloway, 1996). Historically, industrialization and urbanization were made possible through intense fossil fuel mining and combustion, yet this emits reactive S into the atmosphere

(Cowling, 1982; Galloway, 1996) at rates of ~113 Tg S $yr^{-1}$ (Ivanov and Freney, 1983). Excess S aerosols reduce air quality (Goyer et al., 1985; Smil, 1996), and S deposition on land and water as 'acid rain' has caused dramatic declines in water and soil quality and the functioning of ecosystems (Likens and Bormann, 1974; Likens et al., 1996).

Measures to reduce S pollution have lowered the annual rate of atmospheric S emissions since the mid-1970s, especially in North America and Europe (Mitchell and Likens, 2011). As of 2006, sulfur dioxide ($SO_2$) emissions in Canada and the United States (U.S.) were ~14 million tonnes, which is about half of the total emissions that occurred in 1980 (Feinberg et al., 2021). Consequently, agricultural soils of the Northern Hemisphere have become deficient in S by 70-90% (Feinberg et al., 2021). Hence, S requirements of crops are now met through additions of fertilizers, pesticides, and soil amendments that can be lost through leaching into surface and ground water (Kirchmann et al., 1996; Zhao et al., 2006). Thus, while atmospheric S emissions have declined, anthropogenic manipulation of the S cycle has continued, largely through agricultural inputs.

Rates of S transfer from land to inland waters have increased due to human activities. While mineral weathering is a natural pathway of S transfer to waterbodies, human activities including agriculture have enhanced the rate of soil weathering and S transfers from land to water (Schlesinger and Bernhardt, 2013). Such additions are prevalent in the Prairie region of North America where the glacial tills are rich in sulfide minerals (Rock and Mayer, 2006). Apart from such natural additions, wastewater is another important anthropogenic point source of S pollution. Effluent water from both domestic and industrial sources often contains elevated concentrations of $SO_4^{2-}$ as a common constituent (Wu et al., 2013) at levels that can have negative effects on aquatic ecosystem health and functioning (Wiessner et al., 2010). Combined, these point- and non-point sources of S pollution have major impacts on aquatic ecosystems. In recent decades, the rivers throughout the South Saskatchewan watershed have increased in $SO_4^{2-}$ content and the concentration of other salts due to salinization linked to human activities (Kerr, 2017). Similar trends of increased salinity and alkalinity (due to $SO_4^{2-}$ and other ions) are observed in rivers throughout the U.S. (Kaushal et al., 2021).

### *1.1.3 Importance of $SO_4^{2-}$ regulation in freshwater systems*

Sulfur exhibits various oxidation states from -2 to +6, which facilitates the formation of a variety of compounds relevant in biogeochemical reactions and nutrient cycling (Solomon et al., 2005). Among the forms of S, $SO_4^{2-}$ is a relatively stable entity, is one of the most abundant anions in rivers (Charlson et al., 1992), and it is the major form of S transported through aquatic networks (Mackenzie et al., 1993). Excess $SO_4^{2-}$ in water has toxicological impacts on both macro and microorganisms, as it acidifies ecosystems and leads to both increased sulfide toxicity and heavy metal mobilization from sediments through altered sediment redox conditions (Zak et al., 2021). Such redox shifts also mobilize nutrients into the water column including previously bound phosphorus (P) (Wang and Zhang, 2019) and nitrogen (N) (Wiessner et al., 2005). This mobilization of nutrients can promote algal blooms, eutrophication, and depletion of dissolved oxygen (DO) (Qin et al., 2022).

The concentration of $SO_4^{2-}$ in global freshwater ranges from 0 to 630 ppm, 0 to 250 ppm and 0 to 230 ppm, in rivers, lakes and ground water respectively (Zak et al., 2021 and references therein), while the concentrations in surface water of industrial and non-industrial areas of China have been measured at 410 and 258 ppm, respectively (Qin et al., 2022). The permissible limit of $SO_4^{2-}$ in potable water is below 250 ppm (Sharma and Kumar, 2020). Consumption of water containing high $SO_4^{2-}$ causes diarrhoea, dehydration, gastrointestinal and kidney troubles, increased methaemoglobin and sulfhemoglobin in humans (Sharma and Kumar, 2020). Taken together, both direct, and indirect (due to metabolites like sulfide) toxic effects of $SO_4^{2-}$ pollution in waterbodies have negative impacts on aquatic and human life. Thus, quantification and management of $SO_4^{2-}$ is a necessity to minimise the occurrence and intensity of such negative impacts.

### *1.1.4 Using wetlands to treat S rich wastewater*

Wetlands contribute roughly 40% of planetary ecosystem services to society, while only occupying a marginal fraction of the global land surface (around 2.4% out of 11.6%) (Schlesinger and Bernhardt, 2013). They provide food, fuel, and medicines, support wildlife and biodiversity, and regulate flood and drought events. Wetlands also improve water quality through the retention and transformation of nutrients and sequester atmospheric carbon (C) (Costanza et al., 1997; Dinsa and Gemeda, 2019; Mitsch et al., 2015). Historically, wetlands have been the most undervalued ecosystems, often considered wastelands, and subjected to extensive degradation, drainage, and conversion to croplands and urban landscapes (Zedler and Kercher, 2005). Efforts and policies to conserve and restore wetlands are underway worldwide, as humankind increasingly appreciates the numerous benefits that wetlands provide (Yang et al., 2010; Mitsch et al., 2015).

Wetlands are effective sites for wastewater treatment owing to their unique capacity to process nutrients via mineralization and off-gassing to the atmosphere, or through storage in plant biomass and sediments (Yang et al., 2010). Thus, these wetland ecosystems act as a barrier or filter in a watershed, by removing nutrients and pollutants from incoming water and protecting downstream waterbodies (Mitsch and Gosselink, 2015), thereby supporting societal activities including agricultural, industrial, and recreational purposes. Moreover, relative to conventional methods, wetlands provide water quality improvements and effluent processing with lower energy inputs and costs of operation (Werker et al., 2002). In addition to being used to treat domestic sewage and diffuse (i.e., non-point) agricultural runoff, wetlands are now also used to treat many different sources of chemically complex effluent, including wastewater from mining operations, metallurgical refineries, tanneries, agricultural operations, and pharmaceutical and textile industries (Vymazal, 2005; Werker et al., 2002). A study assessing $SO_4^{2-}$ removal in a constructed wetland found that 21% of $SO_4^{2-}$ inputs were removed by the wetland, through significant biogeochemical processing of $SO_4^{2-}$ in the sediments (Wu et al., 2011). Therefore, there is potential for wetland processes to remediate effluent-based S pollution, though the extent of this ecosystem service in different wetland habitats remain unclear.

Spatial gradients of redox conditions throughout a given ecosystem (Wu et al., 2011) support the coexistence of different forms of organic, inorganic, and elemental S (Wang and Zhang, 2019). These circumstances, together with elevated organic matter content that sustains high rates of microbial activity, make S a key component of wetland biogeochemical processes (Deng et al., 2019). The cycling of S is closely interconnected with the cycles of bio-reactive elements including C, N, and P (Guo et al., 2020). Different species of S can shape food web energetics by acting as electron acceptors or donors during redox reactions, mediating ecosystem electron storage (Deng et al., 2019). Additional factors that influence the fate of $SO_4^{2-}$ in wetlands include dilution due to rainfall inputs, increased concentration due to gradients in evapotranspiration, matrix effects, and mineral precipitation and removal from the water column (Wu et al., 2011). Owing to the number of factors that can potentially affect the processing of S in wetlands, more detailed research in diverse wetland types is needed to better explain the mechanisms driving effluent S removal.

### 1.2 Research objectives

Given the rise in $SO_4^{2-}$ concentrations in southern Alberta surface waters (Kerr, 2017), and the potential water quality implications of this concerning trend, my project tracks the output and transformation of effluent $SO_4^{2-}$ from one of the major sources entering the headwaters of the Oldman River in southern Alberta, a sub-basin of the broader South Saskatchewan River watershed. My work explores the role that the Frank Lake wetland plays in transforming effluent-derived $SO_4^{2-}$ before entering the downstream river network. My study has the following three objectives:

1) Quantification of the $SO_4^{2-}$ budget of Frank Lake during two distinct hydrological periods (a wet versus a drought period, 2013 to 2015 and 2021 to 2022, respectively) to determine whether the wetland acts as a source or sink of $SO_4^{2-}$ under different hydrological conditions.
2) Use stable isotopes to trace the transformations of $SO_4^{2-}$ that occur in the three main basins of the Frank Lake wetland complex.
3) Identify the potential for sediment $SO_4^{2-}$ removal using lab-based incubation experiments that track the rate of sediment $SO_4^{2-}$ removal or addition.

### 1.3 Research implications

Although there are many studies detailing the cycling of other bio-reactive elements including C, P and N, the quantitative description, and dynamics of S transformations in treatment wetlands are relatively understudied (Wu et al., 2011). Most of the research on wetland S cycling is limited to constructed wetlands (Chen et al., 2016; Pester et al., 2012), with little emphasis on natural and restored ecosystems. Given the complexity of S biogeochemical cycling, that is linked to both biotic and abiotic transformations, a mass balance model paired with stable isotope analyses can help to address this knowledge gap.

# CHAPTER 2. EXPLORING SULFATE CYCLING IN A MINERAL-SOIL WETLAND RESTORED WITH WASTEWATER

## 2.1 Introduction

Since the onset of the industrial revolution, sulfur (S) availability in the biosphere has dramatically increased due to the intensification of mining and agricultural activities, as well as increasing wastewater effluent release. Point and non-point S pollution largely enters the hydrosphere, where inland waterbodies act as channels for S transport (around 213 Mg S $yr^{-1}$; (Ivanov and Freney, 1983; Lavelle et al., 2005). For instance, ground water sulfate ($SO_4^{2-}$) content in some parts of the Netherlands increased six-fold between 1966 and 1991 due to increased aerial deposition and agricultural additions (Jansen and Roelofs, 1996). Agricultural watersheds across the U.S. act as net S exporters (Hinckley et al., 2020). Moreover, $SO_4^{2-}$ is ubiquitous in domestic and industrial effluents (Wiessner et al., 2005; Wu et al., 2013). Domestic effluent often has $SO_4^{2-}$ concentrations from 20 to 500 ppm, while acid mine drainage (Batty et al., 2005; Nyquist and Greger, 2009) and other industrial effluents have concentrations reaching several thousand ppm (Wu et al., 2013). Industrial organic peroxide production generates effluent with 12,000 to 35,000 ppm of $SO_4^{2-}$ (Silva et al., 2002), while food (2,500 to 4,300 ppm) and paper industries that use sulfuric acid in their production processes generate effluent with high $SO_4^{2-}$ concentrations (O'Flaherty et al., 1998). Thus, while the reuse of treated wastewater is widely considered a sustainable approach to overcome global water shortages (Almuktar et al., 2018), excessive $SO_4^{2-}$ pollution exceeding 250 ppm can acidify aquatic habitats and negatively impact aquatic life (Wiessner et al., 2005), while also altering the biogeochemical cycling of nutrients, especially N and P, resulting in ecosystem eutrophication (Feng and Hsieh, 1998;

Lamers et al., 1998). Hence, improved management of $SO_4^{2-}$ pollution can have many environmental benefits (Wiessner et al., 2005).

Wetlands provide a wide array of unique ecosystem services that sustain and balance the diversity of life on Earth (Costanza et al., 1997; Mitsch et al., 2015; Zedler and Kercher, 2005). Flooded soil conditions, together with submerged or emergent vegetation, creates strong redox gradients that promote the biogeochemical processing of nutrients and pollutants (Chen et al., 2016). Therefore, wetland protection, restoration, and construction are widely seen as cost-effective approaches for treating wastewater from domestic and complex industrial sources (Vymazal, 2005). Wetland nutrient processing improves water quality, while water retention also regulates water quantity on the landscape (Hammer, 1989; Whigham, 1995). Pollutant or nutrient removal is mediated through multiple mechanisms that include precipitation and storage in sediment or soil, microbial transformations, and litter decomposition (Allinson et al., 2000; Johnston, 1991; Moshiri, 2020). For $SO_4^{2-}$ in particular, wetland processing capacity has been shown to vary. A recent study demonstrated up to 21% removal of $SO_4^{2-}$ from contaminated groundwater entering a constructed wetland (Wu et al., 2011). Yet earlier work showed $SO_4^{2-}$ retention in natural temperate and boreal bogs and wetlands ranged from 25 to 77% (Bayley et al., 1986). Thus, wetland restoration, construction, and protection can provide enhanced management of aquatic $SO_4^{2-}$ pollution, but the generalizability and magnitude of this ecosystem service remains unclear.

The major transformations of S in wetlands include biologically mediated $SO_4^{2-}$ reduction, sulfide oxidation, and the disproportionation of reduced compounds (Whitmire and Hamilton, 2005). Rhizosphere conditions, oxygen (O) availability, and labile C availability, along with its ionic composition, exert important controls over the major S transformations (Dalcin Martins et al., 2017; Wiessner et al., 2005). Wetland S cycling is highly interlinked with that of C, N, and P, by means of common substrates, intermediates, and products (Wu et al., 2013). Wastewater treatment wetlands principally remove $SO_4^{2-}$ through reduction mediated by $SO_4^{2-}$ reducing bacteria (SRB) (Qian et al., 2019). Major factors influencing the performance and survival of SRB are temperature, pH, redox potential and the presence of alternate electron acceptors, chemical oxygen demand (COD)/$SO_4^{2-}$ ratio, the availability of organic matter and electron donors, and other competitive groups of bacteria (Zhang et al., 2022). Different strains of SRB thrive well under varying pH (pH 2.0 to 9.0) and temperature ranges (7°C to 60°C) (Zhang et al., 2022). The optimum growth and performance of SRB can occur under both acidic (pH = 2.0 to 4.0) (Zhao et al., 2017) and alkaline conditions (pH = 7.0 to 7.8) (Sharma et al., 2014) and at temperatures of 28°C to 30°C (Zhang et al., 2022). Of relevance to wetland effluent processing, excess nutrients in effluent, especially nitrate ($NO_3^-$) (or other more energetically favourable terminal electron acceptors like O, nitrite ($NO_2^-$), manganese ($Mn^{4+}$), iron ($Fe^{3+}$)) can limit the extent of $SO_4^{2-}$ reduction by promoting $NO_3^-$ reduction. Further, the occurrence of $NO_3^-$ reduction simultaneously favours sulfide oxidation, which increases the availability of $SO_4^{2-}$ (Mitchell and Baldwin, 1999). Thus, while $SO_4^{2-}$ removal may be more common in natural wetlands (e.g., Bayley et al. 1986), the chemically-complex nature of many effluents (Kaushal et al., 2019) may alter the $SO_4^{2-}$ removal capability of effluent treatment wetlands in unknown ways.

The role of S cycling in effluent-receiving wetlands is a relatively underexplored area (Wu et al., 2013). To address this knowledge gap, I explore $SO_4^{2-}$ cycling in the Frank Lake wetland complex (southern Alberta, Canada). Frank Lake is a restored wetland that currently receives, and processes treated wastewater from a local municipality and beef slaughterhouse (White and Bayley, 1999). The wetland plays a crucial role in processing effluent derived nutrients (Flanagan et al., 2022; White and Bayley, 2001; White et al., 1999; Zhu et al., 2019) and organic matter (Zhou et al., 2023), thereby improving the downstream water quality in the Little Bow River. While effluent-$SO_4^{2-}$ concentrations have been documented (Zhu et al., 2019), the fate of $SO_4^{2-}$ entering Frank Lake is unclear. Here, I develop a mass budget during two distinct hydrological periods, use stable isotope surveys, and incubation analyses to quantify $SO_4^{2-}$ transformation and retention in Frank Lake. For the mass balance, I compared the budget of $SO_4^{2-}$ to that of chloride ($Cl^-$). As $Cl^-$ possesses conservative characteristics such as high mobility, solubility, and limited adsorption or desorption, it is useful as a non-reactive tracer (England and Maier-Reimer, 2001). The mass balance of $Cl^-$ can therefore help to distinguish between biotic and abiotic $SO_4^{2-}$ cycling. Overall, my hypotheses were: 1) that the wetland acts as a passive transporter of $Cl^-$ (i.e., no net retention or export) but a net $SO_4^{2-}$ sink similar to other treatment (Wu et al., 2011) and non-treatment (Bayley et al., 1986) wetlands; and 2) that isotopic surveys and incubations will reveal intense microbial S reduction coupled with previously documented organic matter cycling (Zhou et al., 2023) and microbial respiration (Bogard et al., 2023).

## 2.2 Methods

### *2.2.1 Study site*

Frank Lake (FL) is a wetland in the prairie pothole region of southern Alberta (50°33'N; 113°42'W) (Fig. 1; see Appendix A for photos of each location sampled in Frank Lake). The wetland was restored to hydrologic permanence by Ducks Unlimited Canada in 1989, using secondary treated wastewater from the Town of High River municipal wastewater treatment plant, and effluent from the Cargill Foods beef processing plant in High River (White and Bayley, 1999, 2001). The wetland provides critical habitat for waterfowl and migratory birds, including endangered species and hence, is crucial in supporting regional biodiversity (White and Bayley, 1999). The wetland complex has a total area of 10.1 $km^2$, with three basins separated from one another by control weirs. Basins 1, 2 and 3 have surface areas of 5.1, 3.6 and 1.4 $km^2$ respectively, with Basins 1 and 2 having average depths of 0.67 m and Basin 3 depth averaging 0.3 m (White and Bayley, 2001; Zhu et al., 2019). Blackie and Mazeppa are two ephemeral creeks (Fig. 1) flowing in from the East and North of the wetland, respectively, during the spring, resulting in higher water levels in Frank Lake compared to the normal levels during summer (Zhu et al., 2019). Water leaving the wetland via an outflow in Basin 3 drains into the Little Bow River, which is a tributary of the Oldman River and part of the larger South Saskatchewan River watershed. The soil in the watershed is characterised by low hydraulic conductivity due to clay rich glacial deposits, limiting subsurface flow (Conly et al., 2004).

The average annual air temperature of the area is 2.3°C (Zhu et al., 2019) and the monthly mean temperature varies from -11 to 15°C (White et al., 1999) (Blackie AGCM; Government of Alberta). The average annual precipitation in the area was 450 mm and mean evaporation was 782.5 mm during the 2013-15 hydrological years (Zhu et al., 2019). In 2021, however, the average precipitation was 245 mm and mean evaporation increased to 1.27 times (i.e., 990.4 mm) that of the 2013-15 period (Zhou et al., 2023). The annual precipitation during 2022 was 312.8 mm (Blackie AGCM; Government of Alberta). Hence in this study, I am considering the years 2013-15 as a hydrologically wet period and that of 2021-2022 as a dry (drought) period.

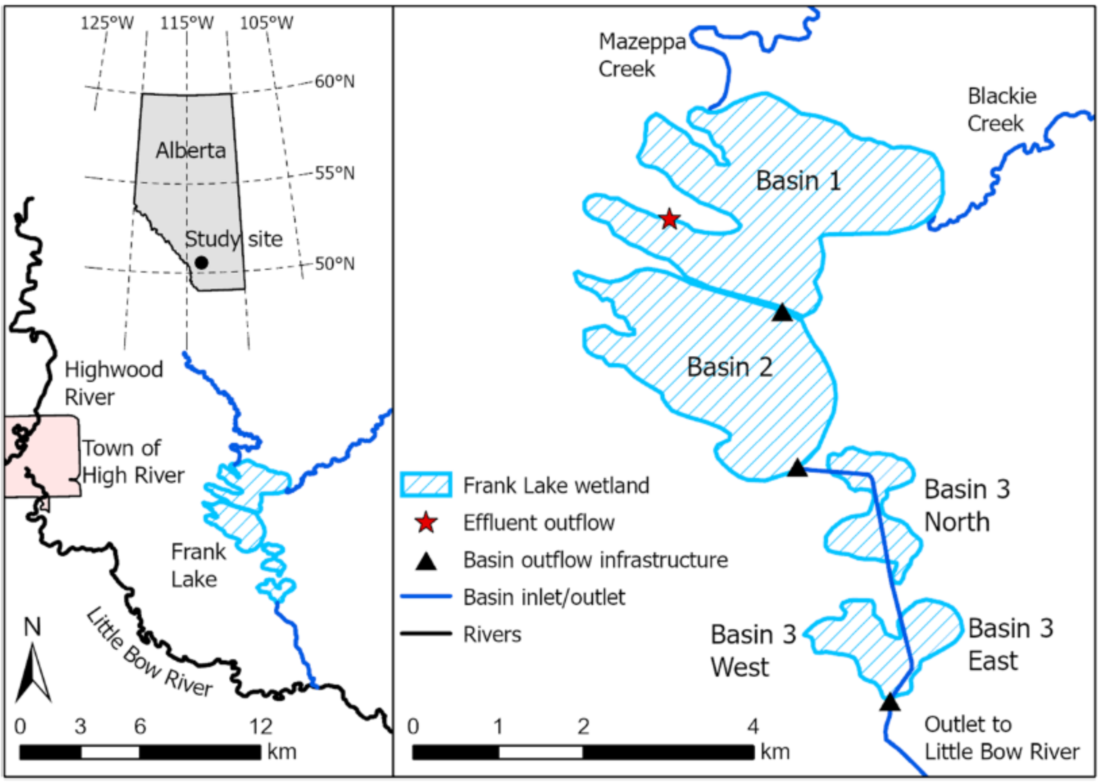


Figure 1. Map of Frank Lake, Alberta (taken from Flanagan et al., 2022). See Appendix A for photos of each location sampled.

### *2.2.2 Water sample collection and analysis*

Water samples were collected at weekly to monthly intervals, mostly in spring and summer (April - October) between April 2021 to October 2022. Surface water samples were taken from the effluent (EFF) ($n$ = 25) discharge point into Basin 1, outlets of Basin 1 (B1O) ($n$ = 20), Basin 2 (B2O) ($n$ = 20), Basin 3 (B3O) ($n$ = 8), and at the ephemeral creeks above where they flow into the wetland (Fig. 1). No flow was observed in Blackie (BK) or Mazeppa (MZ) creeks during the dry period, yet samples were collected from the stagnant pool of water ($n$ = 1 and $n$ = 3 for BK and MZ, respectively). Limited flow occurred from Basin 3 outflow in spring 2021 but not 2022. All water samples were collected in acid washed polyethylene bottles, transported on ice in coolers, filtered using 0.45 µm capsule filters (Whatman) in the lab and archived for analysis by freezing at -20°C. Samples were analysed for stable isotope ratios of $SO_4^{2-}$ and water ($H_2O$) (methods described below). The concentrations of $SO_4^{2-}$ and $Cl^-$ were analysed by colorimetric ion analysis (Barium chloride turbidimetric method (Roy et al., 2011) and the Ferrithiocyanate method (Center et al., 1974) using a Colorimetric Autoanalyzer (Thermo Gallery Beermaster Autoanalyzer). Total S (particulate and dissolved) concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Prietzel et al., 1996).

### *2.2.3 Mass balance construction*

The ability of a wetland to retain nutrients can be better understood by estimating the mass of a specific nutrient flowing in and out of the ecosystem (Bayley et al., 1986; Wu et al., 2011). This approach was used to construct a mass balance for dissolved organic carbon (DOC) in Frank Lake and was explained in detail in Zhou et al. (2023). Following this published approach, $SO_4^{2-}$ and $Cl^-$ mass balances were constructed for both wet and drought periods. The flux was defined as the mass of solute exported downstream per second, which was calculated by multiplying the discharge by the individual ion concentrations at a specific site. The amount of $SO_4^{2-}$ and $Cl^-$ entering [$SO_4^{2-}$ or $Cl^-_{(in)}$] and leaving [$SO_4^{2-}$ or $Cl^-_{(out)}$] the wetland was defined as follows:

$$[SO_4^{2-} \text{ or } Cl^-_{(in)}] = [(C_{EFF} \times Q_{EFF}) + (C_{BK} \times Q_{BK}) + (C_{MZ} \times Q_{MZ})]$$

$$[SO_4^{2-} \text{ or } Cl^-_{(out)}] = (C_{B3O} \times Q_{B3O})$$

where $C$ and $Q$ are the average values over the specific hydrologic period (i.e., wet, and dry) of ionic concentrations (mg $L^{-1}$) and discharge ($m^3$ $s^{-1}$), respectively, over the different sites. Wetland inflows included here were effluent discharge (EFF), Blackie Creek (BK) and Mazeppa Creek (MZ), while the Basin 3 outflow (B3O) was the sole wetland outflow.

Ultimately, if the ratio between [$SO_4^{2-}$ or $Cl^-_{(out)}$] and [$SO_4^{2-}$ or $Cl^-_{(in)}$] was equal to 1, it denoted a lack of net solute processing in the wetland. A value > 1 indicated greater export than input (the system is a net downstream source). If the ratio was < 1, the wetland retained more mass of the solute than it exported (the system was a net sink).

The $SO_4^{2-}$ and $Cl^-$ concentration and discharge data for the wet period were taken from Zhu et.al., (2019), while data collected by the Bogard Lab were used for calculations during the dry period. Monthly discharge values for effluent release during dry years were obtained from the town of High River and Cargill Industries Ltd. (Zhou et al., 2023). There was no discharge from both inflowing creeks during dry years. I calculated the annual flux of $SO_4^{2-}$ and $Cl^-$ using monthly to twice-monthly measurements of concentration, with paired discharge measurements (for the basins and creeks). The total effluent input into Basin 1 was reported as monthly discharge, which was converted to $m^3$ $s^{-1}$. I first calculated the mean discharge and mean concentration for each month within each year (2013-2015 and 2021-2022). Then, I averaged discharge and concentration for each month within each period (averaging over the years). At this point, I calculated the flux by multiplying the mean discharge by the mean concentration for each month within wet and dry periods, separately. Finally, I calculated the annual flux for each period, by summing the monthly flux estimates from March to October for the basins and creeks (in either period), but all 12 months for the effluent (for which there is flow year-round).

I made a number of assumptions when constructing the mass balance, including: 1) The absence of discharge during a period was assumed to equate to an absence of $SO_4^{2-}$ flux, especially during the winter months while the basins remained frozen; 2) all $SO_4^{2-}$ runoff from nearby agricultural fields entered the wetland through the inflowing creeks, which had no flow during the dry period and hence was considered a negligible input; 3) the major vegetation of the wetland were Bulrush (*Schoenoplectus acutus*) and Cattail (*Typha latifolia*), which were restricted to the water edge boundaries in Basin 1 and 2 and throughout Basin 3. Influence of these plants on the S cycle was assumed to be minimal as they were not found to directly affect patterns of S deposition or absorption (Bansal et al., 2019); 4) atmospheric deposition of S was considered negligible as the study site was not in close proximity to marine environments; and 5) the small volume of upstream lagoon wastewater discharged into Blackie creek in 2021 (Zhou et al., 2023) was considered negligible and not included as an input term in the mass balance.

### *2.2.4 Isotope analysis*

The filtered water collected in 2021 and 2022 throughout Frank Lake was processed for stable isotope analysis at the Isotope Science Lab at the University of Calgary, using standard protocols. There, samples were brought to a $pH < 2$ by adding 6M hydrochloric acid (HCl). A 10% barium chloride ($BaCl_2$) solution was added to samples to bind $SO_4^{2-}$ as barium sulfate ($BaSO_4$) and boiled to minimize production of barium carbonate ($BaCO_3$). Samples were filtered and the clean residue of $BaSO_4$ was combusted prior to the analysis of sulfur dioxide ($SO_2$) and carbon monoxide (CO) gases that were analysed on an Isotope ratio mass spectrometry (IRMS; Vathi (Thermo Finnigan Delta V) (Hosono et al., 2014). As defined below, the values of $\delta^{34}S$-$SO_4^{2-}$ and $\delta^{18}O$-$SO_4^{2-}$ were respectively expressed relative to international standard values for Vienna-Canyon Diablo Troilite (V-CDT) and Vienna Standard Mean Ocean Water (V-SMOW) with an estimated precision of ±0.3‰ and ±0.5‰, respectively. To measure the isotopic composition of water ($\delta^{18}O$-$H_2O$), filtered and archived samples were analysed using a Los Gatos Research (LGR) DLT 100 instrument following standard methods (Lis et al., 2008). The value of $\delta^{18}O$-$H_2O$ was expressed relative to the international standard value of Vienna Standard Mean Ocean Water (V-SMOW) with a precision of ±0.2‰.

The stable isotope composition (δ, 'delta') of individual elements was reported as the fractional difference in isotope ratio of sample, relative to the isotope ratio within a known standard, and expressed in parts per thousand (‰, per mil).

$$\delta\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right)$$

Here, $R$ is the ratio of heavy to light isotope, which in my study is [$^{34}S/^{32}S$] and [$^{18}O/^{16}O$] for S and O, respectively. Positive δ values refer to sample materials with higher isotopic ratios than the standard, while negative δ values refers to isotopic ratios in a sample that were lower than the standard (Kendall and Caldwell, 1998; Kendall and Doctor, 2003).

### *2.2.5 Isotope applications for wetland S cycling*

Physical, chemical, and biological processes modify the isotopic composition (ratio of heavy to light isotope) of two interacting compounds or phases, and this phenomenon is termed isotopic fractionation (Thode, 1991). Understanding fractionation is critical for differentiating the sources or processes of formation of water and solutes (Kendall and Caldwell, 1998; Kendall and Doctor, 2003) (Appendix B, Fig. B1). An isotopic fractionation factor is the ratio of isotopic composition between substrate and product, expressed as, $\alpha = R_p/R_s$, where $R_p$ and $R_s$ are the ratio of heavy to light isotope in a product and substrate, respectively. The Rayleigh fractionation model was used to explain the isotope partitioning among two interacting reservoirs, as one of them decreased in size. The model was used to describe isotopic fractionation under the continuous removal of material in a system with two or more isotopic species and there was a constant fractionation factor ($\alpha$) throughout. The Rayleigh equation was expressed as,

$$R = R_0 f^{(\alpha-1)}$$

where, $R_0$ was the isotopic ratio of the original substrate, $f$ was the fraction of substrate remaining after certain time $t$, $R$ was the isotopic ratio of the residual substrate after certain time $t$ (Kendall and Doctor, 2003; Thode, 1991).

As detailed in previous research (Canfield, 2001; Canfield et al., 2010; Canfield et al., 2006; Kamyshny et al., 2011; Kendall and Doctor, 2003; Knossow et al., 2015) key processes can alter the isotopic composition of $SO_4^{2-}$ (Appendix B; Fig. B1). Briefly, microbial processes cause large associated isotopic fractionation (Kendall and Doctor, 2003; Thode, 1991) compared to abiotic processes (i.e., weathering or adsorption-desorption), such that biologically mediated transformations result in S fractionation in aquatic environments (Kendall and Doctor, 2003). Microbial $SO_4^{2-}$ reduction may be either assimilatory or dissimilatory (Thode, 1991). Assimilatory $SO_4^{2-}$

reduction (ASR) refers to the incorporation of S into biomass as part of plant or animal metabolism, resulting in minimal isotope fractionation ($\delta^{34}S$ of organic S product ranges between +0.5‰ and -4.4‰, relative to the $SO_4^{2-}$ substrate (Ishii, 1953; Kaplan et al., 1960)). Dissimilatory $SO_4^{2-}$ reduction (DSR) to hydrogen sulfide ($H_2S$) by anaerobic bacteria (Chambers and Trudinger, 1979; Thode, 1991) was accompanied by higher magnitude of isotopic fractionation (Chambers and Trudinger, 1979; Kemp and Thode, 1968; Thode, 1991) on the order of 25‰ for $\delta^{34}S$ on average (Clark and Fritz, 1997; Kendall and Doctor, 2003), but can be as high as 72‰ (Canfield, 2001; Sim et al., 2011; Wortmann et al., 2001). However, the fractionation of $^{18}O$ during microbial $SO_4^{2-}$ reduction increased until reaching equilibrium with the water, due to the isotope exchange between water and the intermediate compounds in the reduction process (Antler et al., 2013). In an experiment carrying out DSR using cultures of *Desulfovibrio desulfuricans* in water with different isotopic composition, the $\delta^{18}O$-$SO_4^{2-}$ was observed to reach a steady-state value as that of $\delta^{18}O$-$H_2O$, with temperature dependent fractionation factors (25‰ at 30°C) (Fritz et al., 1989). Meanwhile, during oxidation of organically bound S, three of the four O atoms in $SO_4^{2-}$ were derived from water molecules (Thode, 1991). Hence, measuring the $\delta^{18}O$ of $SO_4^{2-}$ and $H_2O$ provides additional understanding of the transformations mediating the S cycling in aquatic systems (Appendix B, Fig. B1), because it may be partially independent from the isotopic changes in $\delta^{34}S$ of $SO_4^{2-}$ (Sim et al., 2023).

In Frank Lake, application of the above isotopic theory to patterns across the three basins can help to explain whether the wetland effectively recycles $SO_4^{2-}$ via DSR or ASR. When bacterial DSR dominates the S cycle, residual $SO_4^{2-}$ should become enriched in $\delta^{34}S$ and $\delta^{18}O$ (Massmann et al., 2003; Tuttle et al., 2009). Here, I assume this enrichment follows a hypothetical fractionation of 25‰ for both $\delta^{34}S$-$SO_4^{2-}$ (Antler et al., 2013; Clark and Fritz, 1997) and $\delta^{18}O\text{-}SO_4^{2-}$ (Fritz et al., 1989), based on fractionation values applied to the Rayleigh Distillation equation (see above). Exploring potential isotopic discrepancies among each basin sequentially was used to pinpoint the locations of intense S processing (if present) within the wetland complex.

### *2.2.6 Sediment incubation experiment*

Sediment and overlying water samples were collected from the three basins of Frank Lake during September 2023. Sampling was done at six locations three locations per basin, see Appendix C; Table. C1 and Fig. C1) along a transect from inflow to the outflow to cover the range of geochemical properties throughout the wetland complex. Sediments were collected using an Ekman grab sampler and transferred into pre-baked 1-litre glass jars in equal volumes to fill the bottom 5 cm of each jar (0.35 $m^3$ sediment). Surface water collected (0.5 L) from the same site was gently poured over the sediment to minimize disturbance and resuspension, and to limit atmospheric air exposure (Baldwin and Mitchell, 2012). The jars were covered with aluminium foil and transported to the lab in the dark in a cooler. In the lab, sediments were incubated in an incubation chamber under light (photoperiod: 14h light, 10h dark; temperature: 24°C day, 18°C night) for a period of 7 days. Water samples were gently collected from each jar on Days 0, 3, and 7 using a 60 ml syringe causing minimal disturbance and filtered through Whatman GF/F filter paper. These water samples were analysed for the concentration of $SO_4^{2-}$ as described above. These incubations were intended to provide a first order understanding of the potential for $SO_4^{2-}$ reduction at various locations within the wetland complex, as indicated by declines in $SO_4^{2-}$ concentration over time, the small subsamples collected on day 3 and day 7 from each jar would not substantially modify concentrations. Further while many sediment incubations were conducted under anoxic conditions in other studies, our primary objective was to replicate conditions observed in Frank Lake, which remains oxygenated in surface waters (Bogard et al., 2023). We therefore left incubations open to the atmosphere.

### *2.2.7 Statistical analysis*

All statistical analyses and figure generation was conducted in R version 4.2.1 (v4.2.1; R Core Team 2022). Where data are presented as boxplots using default settings in ggplot2, the range of boxes represent the first to third quartile, or interquartile range (IQR), the thick midline is the median value, and the vertical lines extend to the largest and smallest values within 1.5 times above or below the IQR. Points beyond these ranges are shown individually as outliers.

To explore whether data were normally distributed or violated assumptions of regression or group comparison tests, I visually inspected histograms and used the Shapiro-Wilks Test (*shapiro.test*). Both $SO_4^{2-}$ and $Cl^-$ concentration data did not meet assumptions of normality, so I square root and $\log_{10}$ transformed $SO_4^{2-}$ and $Cl^-$ concentration data, respectively. To compare ion concentration patterns among locations and among hydrologic periods, I used a two-way analysis of variance (ANOVA) (*aov*) followed by Tukey's Honestly Significant Difference (*Tukeys's HSD*) post hoc test. I excluded Blackie and Mazeppa creeks due to the absence of flow at each location (see above). To compare isotopic values among sites only in the dry period, I used a Kruskal-Wallis (*kruskal.test*) test followed by the Dunn post hoc test (*dunn.test*), applied to untransformed data.

All regression analyses were conducted using ordinary least squares regression (*lm*) on untransformed, or $\log_{10}$ transformed data, after visual inspection (as above), and inspection of regression residuals and Quantile-Quantile plots (*qqplot*).

## 2.3 Results

### *2.3.1 Hydrologic conditions in wet and drought periods*

Effluent remained the major water source to the wetland irrespective of the hydrological period, contributing ~94% and ~99% of the total inflow water during wet and dry periods respectively (Table 1). There was no flow observed in the ephemeral creeks Blackie and Mazeppa during the dry period. Limited outflow from Basin 3 was observed during spring 2021. The averaged lateral water flux (Basin 3 outflow minus the sum of inputs) during wet period was $-0.31 \pm 1.47 \times 10^6$ $m^3yr^{-1}$ and $-2.94 \pm 0.24 \times 10^6$ $m^3yr^{-1}$ during the drought period. The negative water flux implies evaporative loss of water, as the ground water recharge is assumed to be zero. Frank Lake remained mostly hydrologically disconnected from the Little Bow River during the dry period due to lack of flow from the creeks, that limited Basin 3 outflow (Table 1).

### *2.3.2 The composition of S in the water column*

The major form of S in the wetland during the dry period was $SO_4^{2-}$, accounting for the major share of total S (TS) throughout the system. On average, $SO_4^{2-}$-S made up 94% of the TS in the effluent water, 91%, and 92% in that of Basins 1 and 2 respectively (Fig. 2).

Table 1. Mean annual discharge at different locations of Frank Lake for wet and drought periods, and all years averaged (S.D. values in parentheses).

| Site | Discharge ($10^6$ $m^3$ $yr^{-1}$) | |
|---|---|---|
| | Wet period (2013 to 2015) | Drought period (2021 and 2022) |
| BK | 0.50 (0.27) | 0 |
| MZ | 0.52 (0.33) | 0 |
| EFF | 3.80 (0.18) | 3.55 (0.05) |
| B3O | 5.12 (1.40) | 0.61 (0.24) |

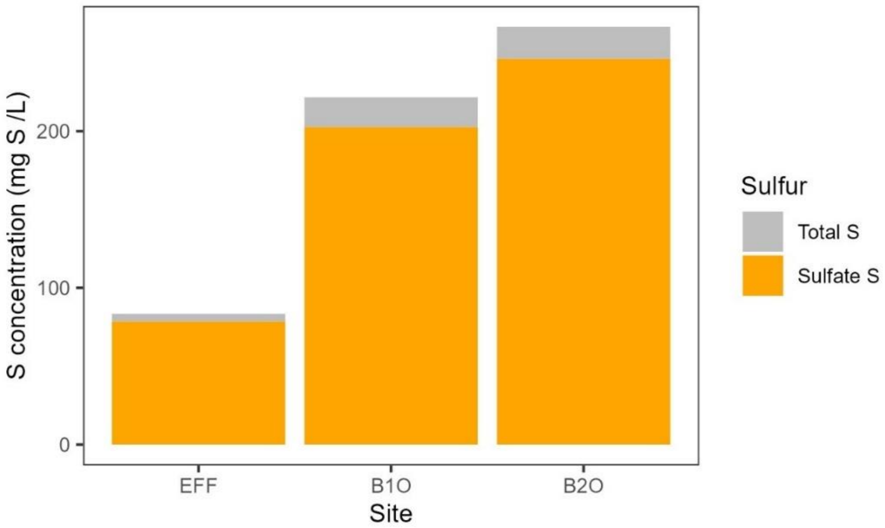


Figure 2. The concentration of total S and the proportion of total S that is $SO_4^{2-}$ at three sites, effluent (EFF), Basin 1 outflow (B1O), and Basin 2 outflow (B2O), within the Frank Lake wetland complex. In this figure, concentrations of $SO_4^{2-}$ are reported in mg S $L^{-1}$, as opposed to mg $SO_4^{2-}$ $L^{-1}$ in the rest of the thesis.

### *2.3.3 Concentrations of $SO_4^{2-}$ and $Cl^-$*

Concentrations of $SO_4^{2-}$ ($n = 144$) differed significantly among sites (Fig. 3a; two-way ANOVA, $p < 0.001$, $F = 28.99$, excluding both inflowing creeks), but not among the wet and dry periods ($p = 0.45$, $F = 0.56$), with a marginally significant interaction effect ($p < 0.05$, $F = 3.00$). During both the wet and dry periods, the $SO_4^{2-}$ concentrations significantly increased by 112 and 254% from the effluent site (mean ± 1.S.D. = 246 ± 42 and 195 ± 76 mg $L^{-1}$, respectively) to the Basin 3 outflow (Fig. 3a; 523 ± 208 and 692 ± 493 mg $L^{-1}$; *Tukey's* HSD test: $p < 0.001$ for both periods). The observed $SO_4^{2-}$ concentrations in Blackie and Mazeppa Creeks (548 ± 15 and 353 ± 254 mg $L^{-1}$, respectively) were higher during the wet than dry period, during which no flow was observed (standing water in Mazeppa Creek, and no natural flow in Blackie Creek). The effluent $SO_4^{2-}$ concentrations did not vary significantly between the two periods (*Tukey's* HSD test, $p = 0.67$). During the wet years, the $SO_4^{2-}$ concentrations in Basin 3 outflow were greater than in Basin 1 outflow (*Tukey's* HSD test, $p < 0.001$). In dry years, there were no significant differences in concentrations between Basin 1 and Basin 2 outflows versus those in Basin 3 outflow (*Tukey's* HSD test, $p = 0.17$, $p = 0.33$ for Basins 1 and 2, respectively). Although not significant, $SO_4^{2-}$ concentrations were higher during the dry period (389 ± 293) versus the wet period (376 ± 199 mg $L^{-1}$).

Unlike for $SO_4^{2-}$, $Cl^-$ concentrations ($n = 144$) varied more significantly between the two hydrological periods (Fig. 3b; *Tukey's* HSD test, $p < 0.001$, $F = 56.69$) than among the sites (*Tukey's* HSD test, $p < 0.05$, $F = 2.717$) with higher concentrations in the dry than the wet period. In general, the concentration of $Cl^-$ in the inflowing creeks was lower than that of the effluent (15 ± 10 mg $L^{-1}$ versus 11 ± 6 mg $L^{-1}$, respectively for Blackie and Mazeppa). During the wet period, there was little change in concentrations from effluent input to the outflow of Basin 3 (272 ± 138 mg $L^{-1}$ versus 211 ± 74 mg $L^{-1}$, respectively; *Tukey's* HSD test, $p = 0.41$). This pattern in the wet period differed considerably from that of $SO_4^{2-}$. During the dry period, mean $Cl^-$ concentrations increased from the site of effluent input (183 ± 69 mg $L^{-1}$) to the Basin 3 outflow (1271 ± 827 mg $L^{-1}$; *Tukey's* HSD test, $p < 0.001$) (Fig. 3b). The average $Cl^-$ content of effluent decreased by ~33% from wet to dry periods, because of interventions at the beef processing facility between these two sampling periods that reduced the output of $Cl^-$ (*Tukey's* HSD test, $p = 0.08$) (Sean Murray, personal communication).

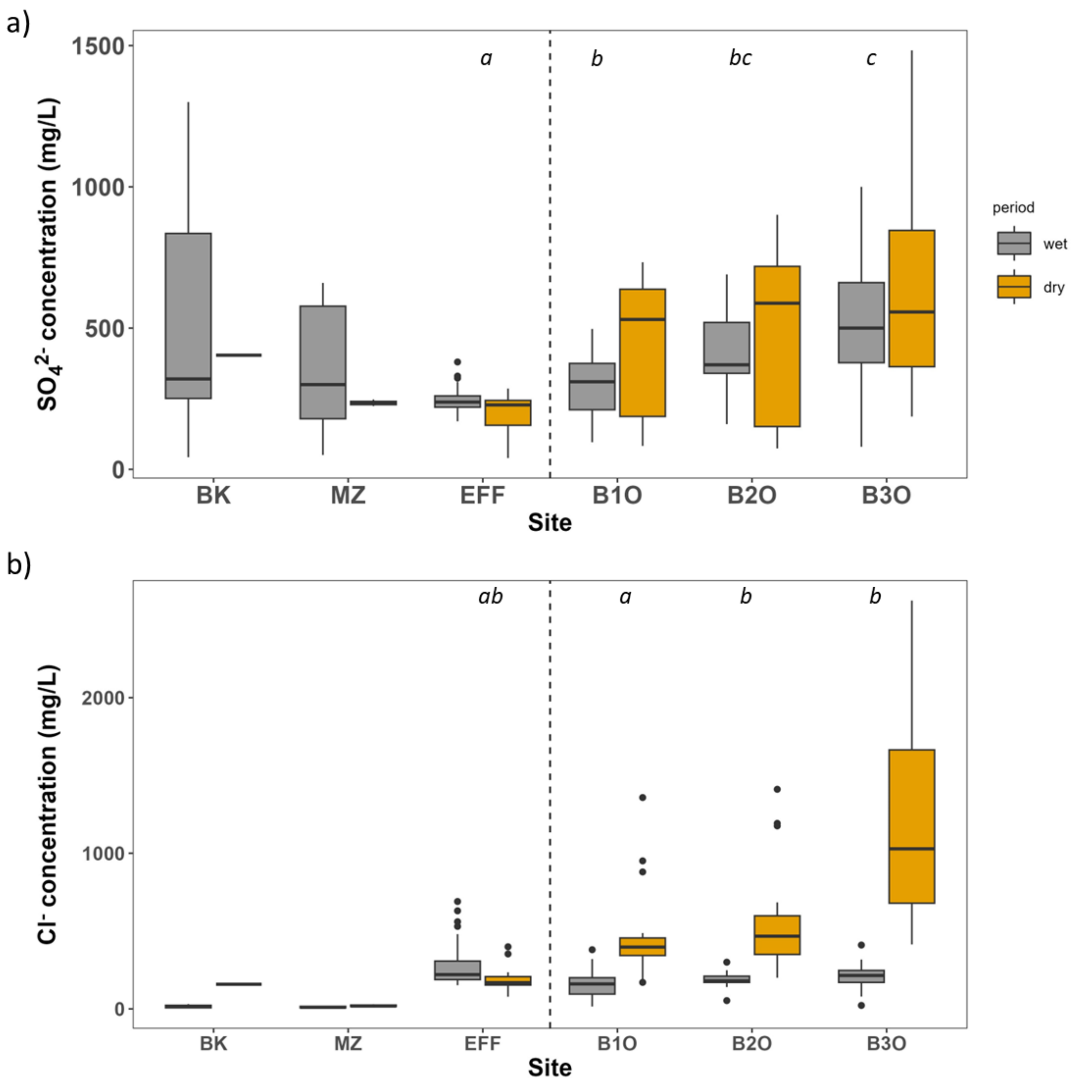


Figure 3. Concentrations of (a) $SO_4^{2-}$ and (b) $Cl^-$ across sites within the Frank Lake wetland. Significant differences from the two-way ANOVA (interaction effects included) are shown among sites, as denoted by lower case, italicized letters ($p < 0.05$). The concentrations at Blackie (BK) and Mazeppa (MZ) Creeks are shown, but not included in statistical analysis of differences due to limited data (during the dry period).

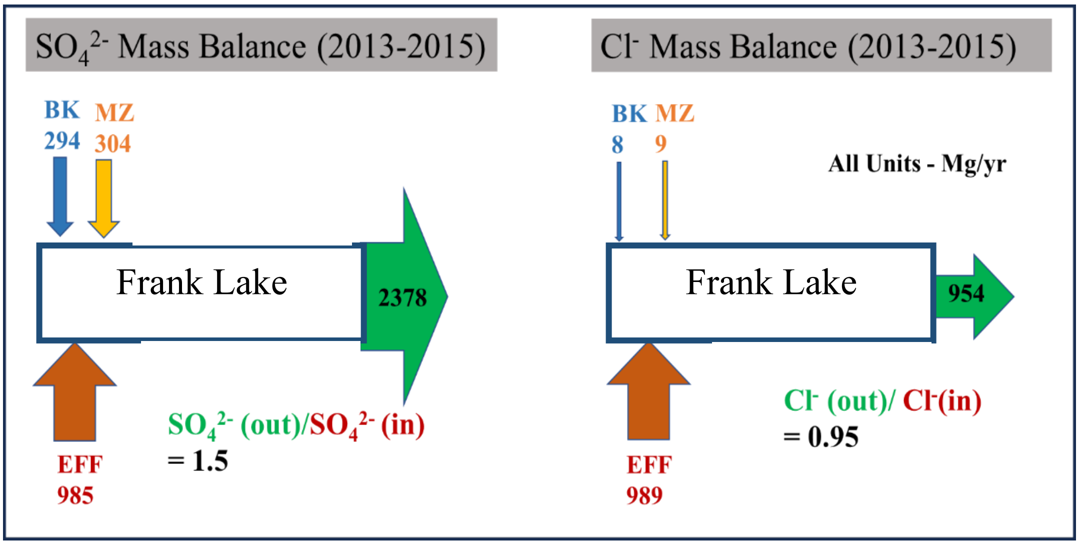
### *2.3.4 Mass balance of $SO_4^{2-}$ and $Cl^-$ in wet and drought periods*

The influx of $SO_4^{2-}$ into the wetland varied greatly between hydrologic periods, whereas $Cl^-$ exhibited little variation (Fig. 4). During the wet period (Fig. 4a), effluent input was 985 ± 55 Mg $SO_4^{2-}$ $yr^{-1}$ (mean ± 1.S.D., representing ~65% of total inputs), while Blackie and Mazeppa imported 294 ± 349 (17%) and 304 ± 438 Mg $SO_4^{2-}$ $yr^{-1}$ (18%) into the wetland, respectively. In the dry period (Fig. 4b), $SO_4^{2-}$ influx from effluent declined by ~25% from the wet period to 742 ± 55 Mg $SO_4^{2-}$ $yr^{-1}$ and was the single source of $SO_4^{2-}$ to Frank Lake. The patterns of $Cl^-$ flux into Frank Lake differed from $SO_4^{2-}$ as the creeks had a negligible role in $Cl^-$ inputs. During the wet period (Fig. 4a), effluent represented 98% of total $Cl^-$ influx (989 ± 53 Mg $Cl^-$ $yr^{-1}$), with only 8 ± 11 and 9 ± 11 Mg $Cl^-$ $yr^{-1}$ from Blackie and Mazeppa creeks, respectively. In the dry period (Fig. 4b), effluent was the only source of $Cl^-$ (632 ± 53 Mg $Cl^-$ $yr^{-1}$), which was ~36% less than effluent inputs during the wet period.

The export of $SO_4^{2-}$ and $Cl^-$ from the Basin 3 outflow during the wet period (Fig. 4a) was 2378 ± 385 Mg $SO_4^{2-}$ $yr^{-1}$ and 954 ± 174 Mg $Cl^-$ $yr^{-1}$ respectively, which further declined to 265 ± 43 Mg $SO_4^{2-}$ $yr^{-1}$ and 518 ± 95 Mg $Cl^-$ $yr^{-1}$ during dry years (Fig. 4b) due to limited outflow. The ratio of $SO_4^{2-}$ output to $SO_4^{2-}$ input ($SO_4^{2-}{}_{(out)}$/ $SO_4^{2-}{}_{(in)}$) was 1.5 and that of ($Cl^-{}_{(out)}$/ $Cl^-{}_{(in)}$) was 0.9 for the wet period, designating the wetland as a source of $SO_4^{2-}$ downstream compared to $Cl^-$ (no net retention or production) during the wet period. During the dry period, however, the output to input ratios decreased to 0.36 and 0.82 for $SO_4^{2-}$ and $Cl^-$, respectively, acting as a sink for $SO_4^{2-}$, with little retention of $Cl^-$.

**Wet period**

a)



**Drought period**

b)

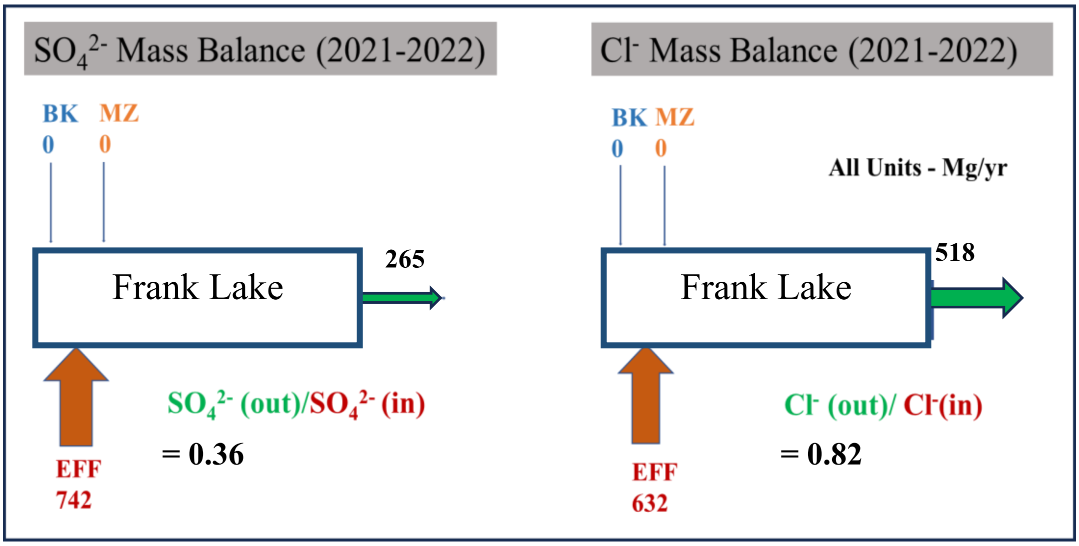


Figure 4. Mass balance of $SO_4^{2-}$ and $Cl^-$ in (a) wet and (b) dry periods in Frank Lake. All units are reported in Mg $yr^{-1}$.

### *2.3.5 Isotopic composition of $SO_4^{2-}$ and $H_2O$ during the drought period*

During the dry period, $\delta^{34}S$-$SO_4^{2-}$ varied little from the effluent inflow site (mean ± 1S.D. = 0.78 ± 1.35‰) to the Basin 1 (1.27 ± 0.92‰) and Basin 2 outflows (1.60 ± 0.99‰) (Kruskal-Wallis: post hoc=Dunn test, $p > 0.1$). However, from the Basin 2 outflow to the Basin 3 outflow, $SO_4^{2-}$ was enriched in $^{34}S$ ($\delta^{34}S$-$SO_4^{2-}$ = 5.2 ± 0.65‰) (Fig. 5a) (Kruskal-Wallis: post hoc = Dunn test, $p = 0.0011$). Conversely, $\delta^{18}O$-$SO_4^{2-}$ (Fig. 5b) and $\delta^{18}O$-$H_2O$ (Fig. 5c) increased considerably from the effluent site (-3.15 ± 1.07 ‰ and -17.75 ± 0.61 ‰ respectively) to Basin 1 outflow (6.19 ± 1.59 ‰ and -9.27 ± 1.75 ‰ respectively) but varied little from Basin 1 to Basin 2 outflow (7.28 ± 1.38 ‰ and -7.5 ± 1.56 ‰, respectively). In transit through Basin 3, both $SO_4^{2-}$ and water became further enriched in $^{18}O$, with a mean $\delta^{18}O$-$SO_4^{2-}$ value of 10.75 ± 0.77 ‰ and $\delta^{18}O$-$H_2O$ value of -6.5 ± 2.86 ‰.

The enrichment of $\delta^{18}O$ in $SO_4^{2-}$ and in $H_2O$ was strongly correlated in the outflows of Basin 1, 2, and 3 ($R^2 = 0.81$, $p < 0.001$; Fig. 6), with a regression slope of nearly 1 (Fig. 6). The observed enrichment of $\delta^{34}S$-$SO_4^{2-}$ and $\delta^{18}O$-$SO_4^{2-}$ of the residual pool was compared to an expected hypothetical enrichment pattern based on the Rayleigh distillation model, using an average fractionation of 25‰ each for $\delta^{34}S$ (Clark and Fritz, 1997; Kendall and Doctor, 2003) and $\delta^{18}O$ (Fritz et al., 1989) which are average values of the range of fractionation observed in various systems (Fig. 7).

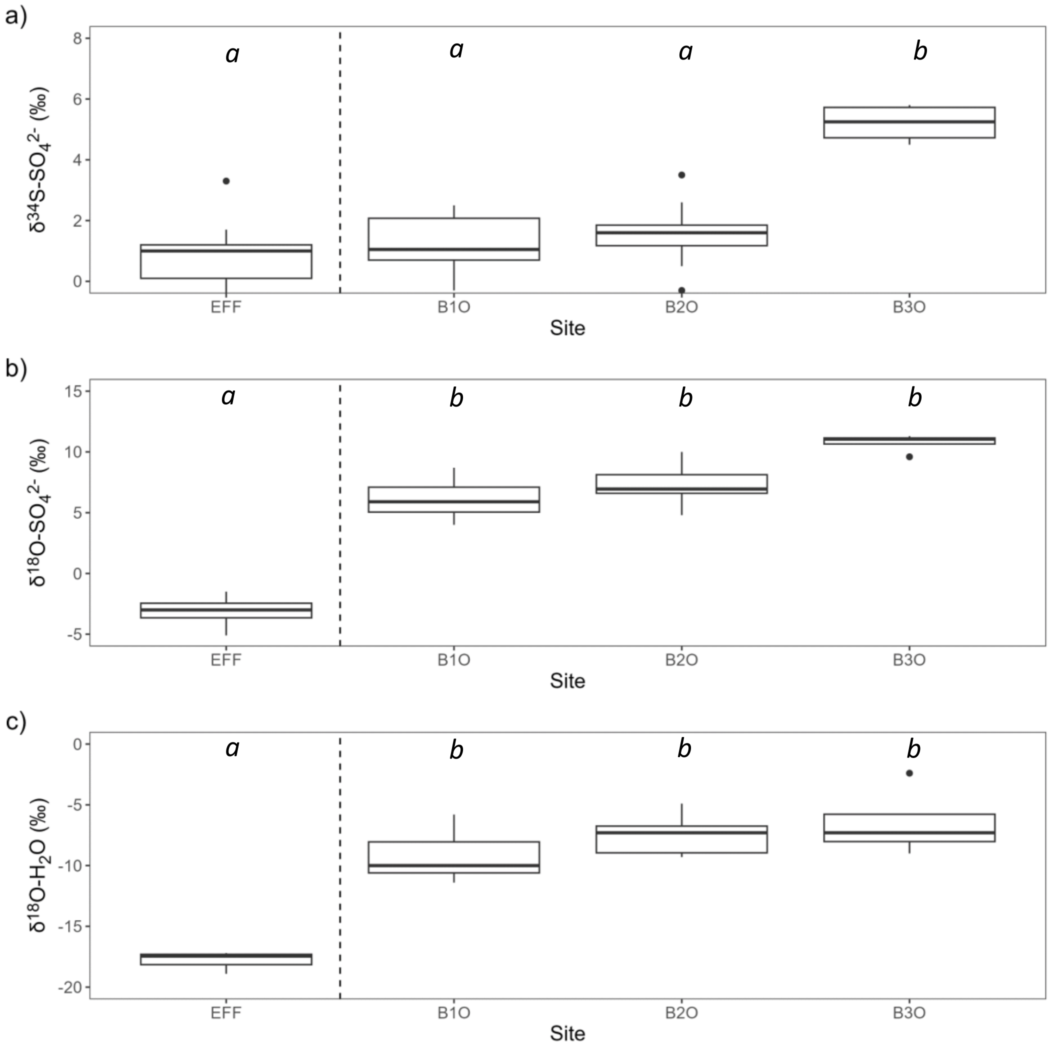


Figure 5. Stable isotopic composition of $SO_4^{2-}$ and $H_2O$, including measurements of (a) $\delta^{34}S\text{-}SO_4^{2-}$, (b) $\delta^{18}O\text{-}SO_4^{2-}$, and (c) $\delta^{18}O\text{-}H_2O$ at the effluent (EFF) and outflows of Basin1 (B1O), Basin 2 (B2O), and Basin 3 (B3O) in Frank Lake. Significant differences among the groups from the Kruskal Wallis test (post-hoc: Dunn test) are shown as different italicized, lower-case letters ($p < 0.05$).

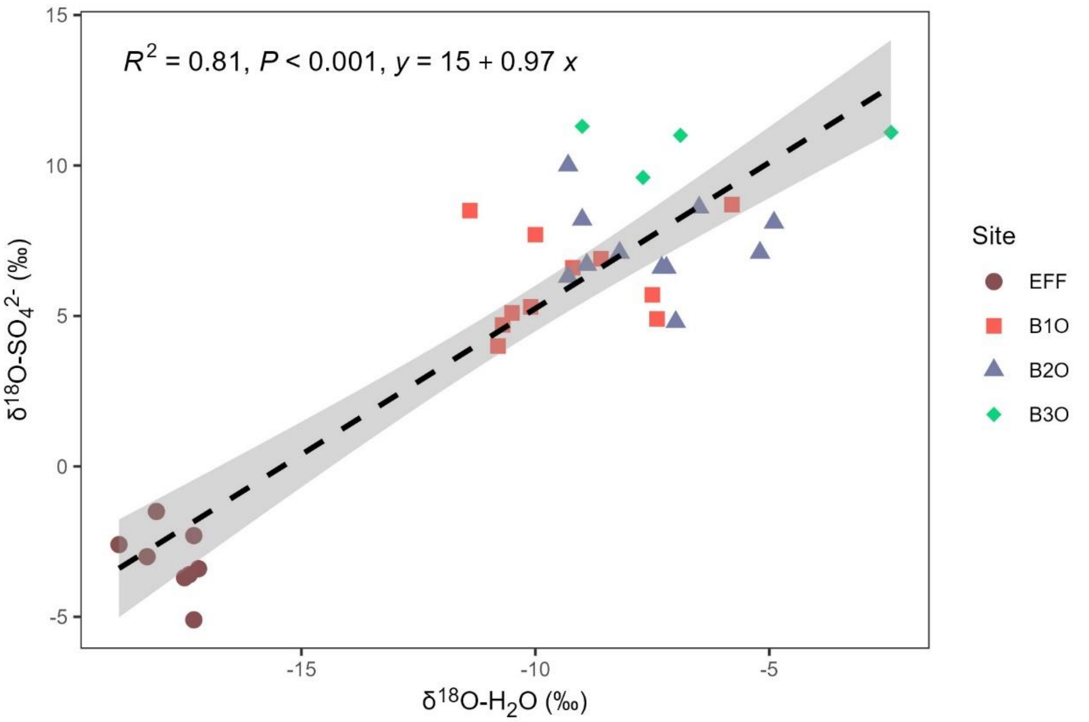


Figure 6. Plot of $\delta^{18}O\text{-}SO_4^{2-}$ versus $\delta^{18}O\text{-}H_2O$ across sites (effluent (EFF) and outflows of Basin 1 (B1O), Basin 2 (B2O), and Basin 3 (B3O)) within Frank Lake during the dry period.

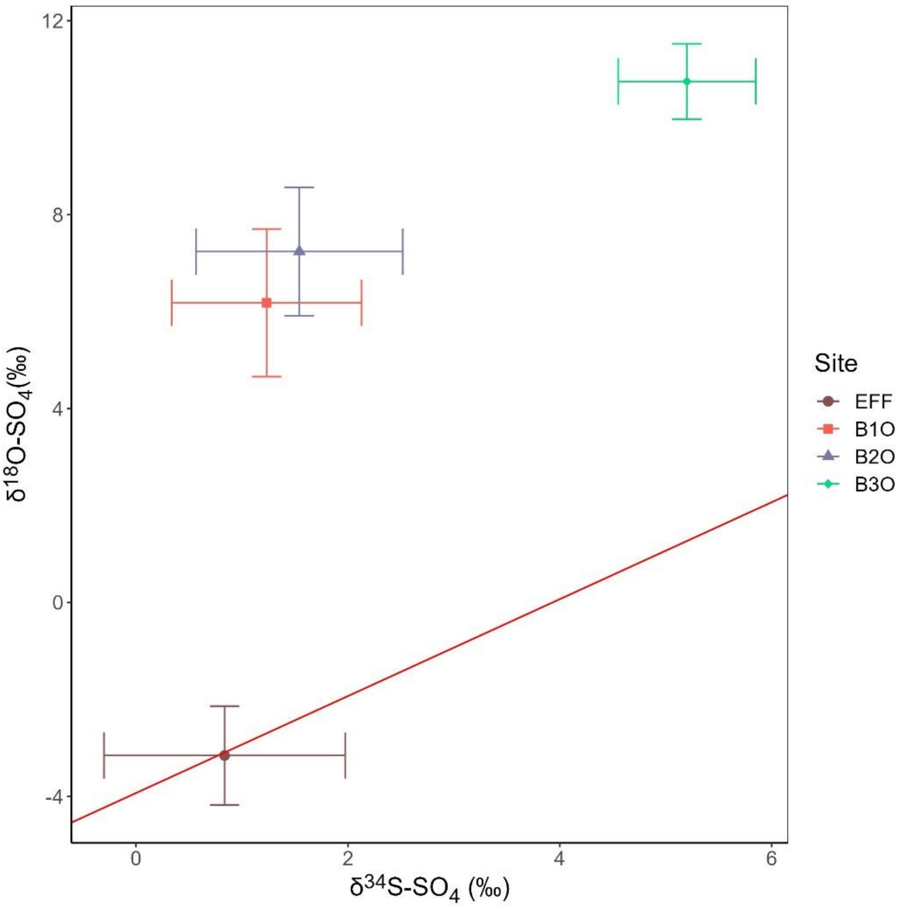


Figure 7. Plot of $\delta^{34}S\text{-}SO_4^{2-}$ versus $\delta^{18}O\text{-}SO_4^{2-}$ at the four sites (effluent (EFF) and outflows of Basin 1 (B1O), Basin 2 (B2O), and Basin 3 (B3O)) within Frank Lake. Points are the mean values for each site during the dry period, and error bars denote ± 1 S.D. The red line represents the hypothetical enrichment of dual isotopes in the residual $SO_4^{2-}$ pool according to the Rayleigh distillation model (the fractionation value used was 25‰ (Clark and Fritz, 1997; Kendall and Doctor, 2003) each for $^{34}S$ and $^{18}O$ (Fritz et al., 1989) which are average values of the range of fractionation observed in various ecosystems).

### *2.3.6 Sediment incubation experiment*

The incubations showed a range in net $SO_4^{2-}$ cycling patterns, with both increases and decreases in $SO_4^{2-}$ concentrations for different locations within the wetland complex. Incubations with sediment and water from Basin 1 underwent a net increase in dissolved $SO_4^{2-}$ concentration of water from the inflow and mid-basin locations (+9.73 and +3.91 mg $SO_4^{2-}$ $L^{-1}$ $d^{-1}$ respectively), while there was a net decrease of -21.77 mg $SO_4^{2-}$ $L^{-1}$ $d^{-1}$ in the outflow location (Fig. 8a). Incubations from all three locations in Basin 2 experienced net $SO_4^{2-}$ removal with rates of -6.17, -5.67 and -48.9 mg $SO_4^{2-}$ $L^{-1}$ $d^{-1}$ respectively at inflow, mid-basin, and outflow locations (Fig. 8b).

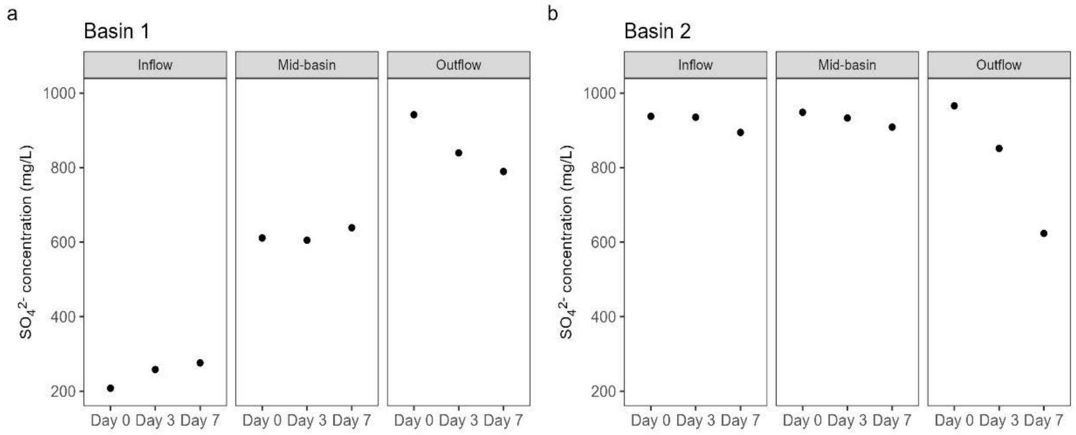


Figure 8. Changes through time in $SO_4^{2-}$ concentrations in sediment incubations, for different locations within Basin 1 and 2 of Frank Lake.

## 2.4 Discussion

### *2.4.1 Frank Lake $SO_4^{2-}$ content and safe consumption guidelines*

The mobilization of S through mining, agricultural additions and effluent release has led to the widespread increase in $SO_4^{2-}$ content of ground and surface waters (Zak et al., 2021). Globally, $SO_4^{2-}$ concentrations in fresh water often vary from 0 to 230 mg $L^{-1}$ in groundwater, up to 250 mg $L^{-1}$ in lakes, with concentrations as high as 630 mg $L^{-1}$ in rivers (Zak et al., 2021). The $SO_4^{2-}$ concentration in treated effluent entering Frank Lake was low compared to the $SO_4^{2-}$ content in effluent in other areas (Fernando et al., 2018; Wu et al., 2013), and importantly, below 250 mg $L^{-1}$, which is the safe limit for human consumption set by the World Health Organization (WHO) (Fernando et al., 2018). The $SO_4^{2-}$ concentration in Frank Lake effluent was also below the safe limit set by the surface water quality guidelines for livestock and irrigation water in Alberta (1000 mg $L^{-1}$) (Government of Alberta, 2018). However, the average $SO_4^{2-}$ content of water leaving the Frank Lake exceeds this limit by ~2 times or more (Fig. 3). Unlike other constructed treatment wetlands receiving S rich effluents (Chen et al., 2016; Wu et al., 2013) and mine drainage (Nyquist and Greger, 2009), Frank Lake is not a $SO_4^{2-}$ sink, and concentrations stay elevated due to multiple factors (as discussed below).

First, emergent macrophyte (Bulrush and Cattail) vegetation in Frank Lake was largely restricted to the outer edges of Basin 1 and 2 (Flanagan et al., 2022), and the more extensive bulrush vegetation in Basin 3 interacted little with water during the drought period. Further, microbial reduction of $SO_4^{2-}$ was limited in the wetland complex (Fig. 7, 8), which, in combination with limited extent of plant uptake, restricted the bioremediation capacity of the wetland. Accordingly, I saw little shift in the relative contribution of $SO_4^{2-}$ to the total S pool, relative to other forms of S, namely organic S in dissolved or particulate form (Fig. 2). Second, hydrologic conditions in this semi-arid region further enhanced $SO_4^{2-}$ content through evapo-concentration of solutes across the basins (Fig. 3; Zhu et al., 2019). The limited water inputs from creeks (Table 1) and little or no groundwater recharge (Zhou et al., 2023) also contributed to the high concentration of $SO_4^{2-}$ (Fig. 3; Zhu et al., 2019). Taken together, elevated $SO_4^{2-}$ concentration within the wetland limits the immediate useability of the water within Frank Lake, while export of water into the Little Bow River downstream leads to a dilution of $SO_4^{2-}$ concentrations to values well within safe consumption levels.

### *2.4.2 Hydrologic controls on wetland source and sink behaviour*

The mass balances from both wet and dry periods showed that Frank Lake alternated between a net $SO_4^{2-}$ source and sink, respectively, unlike the conservative tracer $Cl^-$, for which the wetland remained a net sink in both periods (Fig. 4a and 4b). I observed extreme shifts from 100% retention of incoming $SO_4^{2-}$ in the dry period, to an export of 150% during the wet period. The shift from source to sink in Frank Lake was unique compared to wetlands in other regions. The $SO_4^{2-}$ retention capacity of wetlands in other regions have been shown to decrease with elevated precipitation and rapid hydrological flushing, yet these wetlands typically remain net $SO_4^{2-}$ sinks (Bayley et al., 1986). For example, similar work in northwestern Ontario showed that the $SO_4^{2-}$ retention capacity of a fen during wet years was 4% but increased to 23% during dry years (Bayley et al., 1986). Previous work at Frank Lake has shown there is also net retention of N and P during wet periods (White and Bayley, 2001; White et al., 1999; Zhu et al., 2019), while Zhou (2023) observed a net export of DOC during the wet period. Taken together, the extreme shifts between net retention and export of $SO_4^{2-}$ in Frank Lake are not generalisable to all wetlands, nor are they representative of all bioavailable nutrients or conservative tracers (including $Cl^-$) in Frank Lake.

Hydrologic connectivity (Cheng et al., 2023) and water residence time (WRT) are crucial factors determining wetland nutrient retention capacity (Fisher and Acreman, 2004; Ghosh and Gopal, 2010; Zhang et al., 2022). The above-average precipitation (450 mm) (Zhu et al., 2019) during wet years facilitated greater flow between the basins and downstream into the Little Bow River. Consequently, the WRT of the wetland decreased to 2.56 years (Zhou et al., 2023) which limited both the time for effluent water to interact with microbes and plants (Perez et al., 2011), and the extent of binding and storage in underlying sediments as metal sulfides. On the other hand, during the dry period, Frank Lake was mostly hydrologically isolated from rest of the watershed, lacking inflow from the creeks, and limited outflow from Basin 3 (Table 1). Prevailing dry conditions favoured lower water levels and a longer WRT of ~16.4 years (Zhou et al., 2023). While longer WRTs could potentially enhance microbial $SO_4^{2-}$ reduction due to longer processing times in situ, more anaerobic conditions, and lower redox potential (Stark et al., 1995), we did not see evidence for this effect in dry years. Here, the small enrichment of $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$ across the basins (Fig. 5, 7) suggested limited bacterial reduction in the dry period. Taken together, the isotopic and incubation results indicated little biogeochemical processing of $SO_4^{2}$, despite the longer WRT and limited outflow from Basin 3, and that the apparent $SO_4^{2-}$ sink in the dry period mass balance was due to shifts in hydrologic conditions alone (i.e., abiotic factors). This observation contrasts with my initial hypothesis, as abiotic, rather than biological processes appear to play a decisive role in the net source or sink behaviour of Frank Lake.

### *2.4.3 Limited and localized $SO_4^{2-}$ reduction in Frank Lake*

The results from the mass balances (Fig. 4), dual isotope analyses (Fig. 5, 7), and incubations (Fig. 8) collectively point to a limited role of $SO_4^{2-}$ reduction in Frank Lake. Pure cultures of SRB typically enrich the residual $SO_4^{2-}$ pool by ~45‰ for $\delta^{34}S$ and 22 to 30‰ for $\delta^{18}O$ (Antler et al., 2013; Thode, 1991). My field-based observations showed moderate isotopic enrichment linked to $SO_4^{2-}$ removal. Had $SO_4^{2-}$ reduction been more extensive in Frank Lake, we would expect greater isotopic enrichment in the residual $SO_4^{2-}$ pool across the basins (Fig. 7). A previous study in a constructed wetland treating $SO_4^{2-}$-contaminated groundwater (286 mg $L^{-1}$) had 21% removal efficiency and showed relatively high $\delta^{34}S$-$SO_4^{2-}$ and $\delta^{18}O$-$SO_4^{2-}$ (enriched by 10.1‰ and 6.1‰) in outflowing water (Wu et al., 2011). Another study by Flege (2001) used five different constructed wetlands across Indiana and Ohio to treat acid mine drainage. There, two of the wetlands showed $SO_4^{2-}$ removal (from 1740 ppm at inflow to 831 ppm at outflow) through microbial reduction, that enriched $\delta^{34}S$ values in remaining $SO_4^{2-}$ by nearly 5.5‰, while removal (640 ppm at inflow to 290 ppm at outflow) in another wetland led to nearly 9‰ shifts in $\delta^{34}S$ values. Unlike in those studies, isotopic patterns in Basins 1 and 2 shifted little from effluent values (Fig. 7), indicating little $SO_4^{2-}$ removal. Incubations of sediment in each basin also showed that net $SO_4^{2-}$ removal was limited (Fig. 8), consistent with the observed lack of isotopic enrichment in Basins 1 and 2. The observed increase in $\delta^{18}O$-$SO_4^{2-}$ values throughout the basins were closely related to the shifts in the $\delta^{18}O$-$H_2O$ values (Fig. 5c and 6) caused by isotopic enrichment associated with evaporation of surface water (Zhu et al., 2019). The impact of shifting water isotopic composition on $\delta^{18}O$-$SO_4^{2-}$ can occur through multiple biochemical mechanisms (e.g., partial reduction of $SO_4^{2-}$, reoxidation of sulfide) during which O atoms in $H_2O$ can be incorporated into $SO_4^{2-}$ by S cycling bacterial consortia (reviewed in detail by Antler et al. (2013). Overall, Basins 1 and 2 were not active sites of $SO_4^{2-}$

remediation during drought conditions. The net export observed in the 2013-15 wet period mass balance (Fig. 4) suggested similar wetland functioning for this period.

In contrast, isotopic patterns suggested that there may have been more prevalent $SO_4^{2-}$ reduction occurring from the outflow of Basin 2 to Basin 3, though data were only available for the brief period when water flowed through or was standing in the outflow channel (Zhou et al. 2023). From Basin 2 to 3 outflows, both $\delta^{34}S$ and $\delta^{18}O$ were enriched by 3.66‰ and 3.51‰, respectively (Fig. 5a, b, 7), and the change of slope in the dual isotope plots between Basins 2 and 3 was more in line with predictions based on fractionation values of BSR (Fig. 7). Hence, the pairing of my mass balance with both isotopic and incubation techniques indicated that while the entire wetland complex showed limited net processing of $SO_4^{2-}$, there was indeed spatial variability in S cycling, and localized habitats were capable of removing S, particularly in Basin 3 and some vegetated locations at the south end of Basin 2.

### *2.4.4 Mechanism underlying the limited $SO_4^{2-}$ reduction in Frank Lake*

The limited $SO_4^{2-}$ removal and associated low isotopic enrichment in Frank Lake was likely due to multiple factors, including shifts in OC quality and quantity among different sections of the wetland complex. Availability of labile OC is an important regulatory control on microbial $SO_4^{2-}$ reduction (Hao et al., 2014), and increased availability of OC enhances DSR (Wiessner et al., 2005). Yet elevated DOC content alone was not the main factor controlling DSR and net $SO_4^{2-}$ removal in Frank Lake. Concentrations of DOC were elevated across all aquatic habitats ($\geq$ ~30 mg $L^{-1}$; Zhou et al. 2023), and past work has shown that heterotrophic microbial activity was generally high throughout the wetland complex (Zhou et al. 2023; Bogard et al. 2023). Conversely, the removal of $SO_4^{2-}$ in incubations was most intense in the sample from the Basin 2 outflow (a vegetation-dominated habitat). Beyond DOC availability, the presence or absence of emergent vegetation appears to be a potentially important factor controlling these patterns. While wetland plants can have a negative influence on BSR via root $O_2$ release (Stein et al., 2007; Wiessner et al., 2010), the supply of cattail litter to wetland soils enhanced $SO_4^{2-}$ removal in microcosm experiments using tertiary wastewater (Chen et al., 2016). As wetland plants are decomposed, humic materials can build up the soil OC pool (Pinney et al., 2000; Quanrud et al., 2001). The microbial decomposition of plant litter supplies soluble carbohydrates, consumes $O_2$, and ultimately provides electrons to sustain microbial DSR metabolism (Chen et al., 2016). Basin 3 in Frank Lake stands out from the other two basins, due to the presence of extensive bulrush vegetation. For water moving through Basin 3, Zhou et al. (2023) reported a structural shift in the DOC pool from aliphatic to aromatic (i.e., toward more plant and soil-like sources of DOC). This signifies that water masses in Basin 3 likely interact more closely with areas dominated by emergent vegetation, where enhanced litter decomposition could favour microbial SRB communities and $SO_4^{2-}$ metabolism. This interpretation is consistent with the enrichment of $SO_4^{2-}$ dual isotopic signatures from the outflow of Basin 2 to 3 (Figs. 5 and 7). Hence, a greater presence of vegetation within Frank Lake Basin 1 and 2 could have a positive influence on $SO_4^{2-}$ reduction, and this may be a management strategy to consider in the future.

Beyond the presence of emergent vegetation, wide redox gradients throughout Frank Lake likely favour different pathways of anaerobic microbial respiration that structure patterns of $SO_4^{2-}$ removal. In terms of thermodynamic constraints, anaerobic respiratory processes including $NO_3^-$ reduction (denitrification), then reduction of manganese ($Mn^{4+}$) and iron ($Fe^{3+}$) are energetically more favourable than the reduction of $SO_4^{2-}$ (Berner, 1980; Froelich et al., 1979). The effluent released to Frank Lake contains a range of nutrients and is especially rich in $NO_3^-$ (the bulk of the N pool; Flanagan et al. 2022). The effluent $NO_3^-$ (~100 mg $L^{-1}$) entering Frank Lake is mostly retained in Basin 1 (75 – 96%; Flanagan et al. 2022; Zhu et al. 2019), indicating intense denitrification here. High $NO_3^-$ availability likely inhibits $SO_4^{2-}$ reduction through increased denitrification, while simultaneously facilitating sulfide oxidation using $NO_3^-$ as an oxidant (Whitmire and Hamilton, 2005). This pattern was observed in small wetlands (a fen and a marsh) in Michigan, where $NO_3^-$ was efficiently consumed in groundwater, while $SO_4^{2-}$ was removed only after $NO_3^-$ was depleted (Whitmire and Hamilton, 2005). A similar pattern was observed in a constructed wetland microcosm (Guo et al., 2020). However, N availability decreases dramatically below Basin 1 in Frank Lake, as denitrification and sedimentation limit the transfer of N further downstream (Flanagan et al., 2022). In these locations below Basin 1, DSR may become more energetically favourable. The pattern of $SO_4^{2-}$ removal in my incubation studies supported this interpretation, as the accumulation in $SO_4^{2-}$ in samples nearer to the effluent source may reflect sulfide oxidation linked to denitrification (Fig. 8), while the loss of $SO_4^{2-}$ in samples taken farther from the source (i.e., Basin 1 outflow and beyond) may be due to the depletion of $NO_3^-$ . While the incubations used here cannot confirm these patterns due to their simple design (i.e., I did not measure denitrification or cycling of other terminal electron acceptors like $Mn^{4+}$ and $Fe^{3+}$), the spatial gradients in redox conditions appear to be important in structuring the cycling of $SO_4^{2-}$. This phenomenon should be explored in greater detail in future studies to help explain the mechanisms underlying the wetland's capacity (or lack thereof) to process $SO_4^{2-}$.

### *2.4.5 Implications of limited $SO_4^{2-}$ retention on downstream water quality*

Considering the findings from my study and past work at Frank Lake (Zhu et al., 2019), the wetland can be considered as a site that is not actively processing or retaining $SO_4^2$. This observation is important, because $SO_4^{2-}$ pollution plays a crucial role in the freshwater salinization syndrome unfolding in aquatic ecosystems worldwide (Kaushal et al., 2021; Kerr, 2017). Within the Oldman watershed and broader South Saskatchewan River network, salt concentrations including $SO_4^{2-}$ and $Cl^-$ have been increasing in recent decades, linked to the intensification of land use (Kerr, 2017). By exploring headwater $SO_4^{2-}$ cycling in the Oldman watershed, my study helps to provide context for these observed changes. My work also highlights the limited capacity for wetlands like Frank Lake to play an active role in combatting downstream salinization.

# CHAPTER 3. CONCLUSIONS

Globally, wetlands are considered efficient, low-cost sites for wastewater treatment. However, the capacity for wetlands to remove S is poorly understood, particularly in natural wetlands. My study explored S cycling in Frank Lake, a restored mineral-soil wetland receiving complex industrial and municipal effluents that are high in $SO_4^{2-}$ concentrations. The first part of the study quantified the $SO_4^{2-}$ retention capacity of the wetland using a mass balance approach, comparing $SO_4^{2-}$ patterns to those of $Cl^-$, a conservative tracer. I considered two different hydrological periods, a wet and dry period, and while Frank Lake behaved as a sink for $Cl^-$ in each hydrologic period, the wetland shifted from a net source of $SO_4^{2-}$ in the wet period to a net sink during the dry period. The drastic change in behaviour was solely attributed hydrological differences, primarily the lack of flow during the dry period that disconnected Frank Lake from the Little Bow River. Next, I used a combination of incubations and dual isotope analysis ($\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$) of dissolved $SO_4^{2-}$ to identify the potential role of microbial $SO_4^{2-}$ reduction in the wetland. The limited isotopic enrichment of $SO_4^{2-}$ together with low $SO_4^{2-}$ removal rates in incubations from most locations confirmed that there was some $SO_4^{2-}$ reduction in the wetland, but that the rates must be low when considered at the ecosystem level. These techniques also indicated that $SO_4^{2-}$ removal was localized mostly to Basin 3 and the outflow of Basin 2. Redox competition, mainly the preferential occurrence of $NO_3^-$ reduction in Basin 1 (and possibly other terminal electron acceptors) and limited emergent macrophyte habitats in Frank Lake Basins 1 and 2 may be the factors that limit $SO_4^{2-}$ reduction in the wetland complex.

In the present scenario of rapid anthropogenic salinization of freshwater systems around the world and hydroclimatic changes (Kaushal et al., 2023), my study is important, as it evaluates the impacts of these factors and the potential role for wetlands to remediate $SO_4^{2-}$ pollution. This study revealed the limited processing of $SO_4^{2-}$ compared to other nutrients like N and P (White and Bayley, 2001; White et al., 1999; Zhu et al., 2019), which showed that outflow from Frank Lake exacerbates ongoing salinization in the South Saskatchewan River watershed (Kerr, 2017). The export of S from Frank Lake will have downstream consequences, as S transformations do not occur in isolation, but influence other nutrient cycles such as that of C, N, P, Fe and Mn (Sturman et al., 2008). Hence, when hydrologically connected, the ionic composition of the wetland outflow is an important influence on the downstream ecosystem health and functioning.

Finally, this study demonstrated that even if wetlands are thought of as nutrient buffering zones in a landscape, the efficiency of this property depends on climate, hydrology, the chemical composition of the water, sediment composition, OC availability, and most importantly, the nutrient in question. As shown here, these factors have inconsistent influences on the removal of different nutrients, with Frank Lake removing or retaining N (Flanagan et al., 2022; White and Bayley, 2001), P (White et al., 1999; Zhu et al., 2019), $Cl^-$ (Zhu et al., 2019), and at times, C (Zhou et al., 2023), but not S. Thus, Frank Lake provides important ecosystem services related to the processing of some effluent constituents, but not others such as $SO_4^{2-}$ removal.

Although my study provides insight into the extent of $SO_4^{2-}$ transformation in Frank Lake, uncertainties remain regarding the S cycle in this important wetland. For instance, samples available for some locations were extremely limited, introducing uncertainties into my mass balance and interpretation of spatio-temporal patterns. Thus, a detailed study including more water and sediment samples from different regions of the wetland (especially from Basin 3) and at different times (including winter sampling) should be done in the future. Such sampling would help to refine our understanding of the $SO_4^{2-}$ removal capacity of Frank Lake. Moreover, it would be important for future work to explore interaction effects between $SO_4^{2-}$ and other elements (such as N, P, Fe and Mn) with that of S. Considering the isotope analysis ($^{34}S$) of sulfide in sediments will also be useful to differentiate their source to be underlying pyrite minerals or the microbial reduction of effluent derived $SO_4^{2-}$. These potential approaches would help to build on the observations that I make in my thesis.

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## APPENDIX - A

Figure A1. Effluent water inflow into the Basin 1 of Frank Lake

Figure A2. Basin 1 outflow of Frank Lake

Figure A3. Basin 2 outflow of Frank Lake

Figure A4. Basin 3 outflow of Frank Lake

# APPENDIX - B

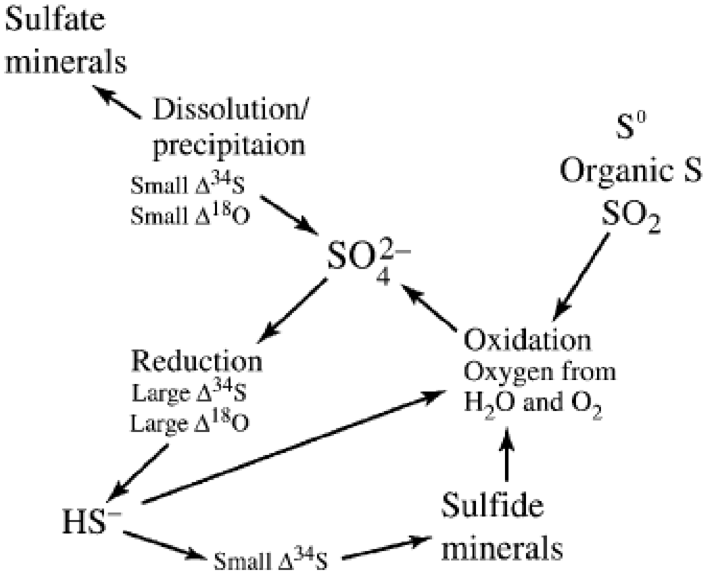

Figure B1. Relative isotopic shifts in $\delta^{34}S$ and $\delta^{18}O$ values of $SO_4^{2-}$ caused by different reactions in the S cycle. Taken from Kendall and Doctor (2003).

# APPENDIX - C

Table C1. Locations of sediment sample collection in the three basins of Frank Lake.

| Basin | Location | Latitude | Longitude |
|---|---|---|---|
| Basin 1 | Inflow | 50.56689 | -113.727 |
|  | Mid-basin | 50.55997 | -113.712 |
|  | Outflow | 50.55632 | -113.707 |
| Basin 2 | Inflow | 50.55522 | -113.708 |
|  | Mid-basin | 50.55096 | -113.704 |
|  | Outflow | 50.53973 | -113.706 |

Figure C1. Locations of sediment samples collected from Basin 1 and 2 of Frank Lake