# PATTERNS OF SULFUR AND CARBON BIOGEOCHEMISTRY IN ALPINE WETLANDS OF NIWOT RIDGE, COLORADO

by

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Patterns of sulfur and carbon biogeochemistry in three wetland types across an alpine landscape, Niwot Ridge, Colorado
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Wetlands serve as important locations of disproportionately high biogeochemical activity and plant productivity in many lowland regions. However, little is known about the function of alpine wetlands, or about how their biogeochemical cycling compares with the broader alpine landscape, which is usually composed of thin, rocky soils and tundra vegetation. In my thesis research, I compared the soil and water biogeochemistry among three types of alpine wetlands at Niwot Ridge, CO: alpine wet meadow (AWM), periglacial solifluction lobe (PSL), and subalpine wetland (SAW). Each wetland type exhibited unique biogeochemical characteristics. SAWs had high organic matter, high carbon concentrations, and low bulk density. Mean loss on ignition from SAW samples (70%) was significantly higher than AWM and PSL samples (17% and 13%, respectively), mean soil %C and DOC in surface water from SAW samples (37.2% and 12.8 mg $L^{-1}$, respectively) was higher than in AWMs (9.4% and 6.5 mg $L^{-1}$), and in PSLs (7.0% and 7.3 mg $L^{-1}$), and soil bulk density was lower in SAWs (0.16 g $cm^{-3}$) than in AWMs (0.48 g $cm^{-3}$) and PSLs (1.06 g $cm^{-3}$). PSLs had very high sulfur concentrations in soil and water as sulfate, at 0.603 mg S $g^{-1}$ dry soil and 16.4 mg S $L^{-1}$, compared to 0.016 mg S $g^{-1}$ dry soil and 0.5 mg S $L^{-1}$ in AWMs and 0.042 mg S $g^{-1}$ dry soil and 0.2 mg S $L^{-1}$ in SAWs. PSLs also had low soil pH, with a mean pH of 3.95 in interior soil samples from PSLs, compared to 4.56 and 4.23 in the AWMs and SAWs, respectively. Compared to surrounding alpine dry meadow, all wetlands had higher water content, higher organic matter, lower pH, and higher soil extractable sulfur as sulfate in interior pools, and AMWs and SAWs had higher total soil carbon. These findings suggest that wetlands may have a disproportionate effect on biogeochemical and ecological processes in alpine landscapes.

# DEDICATION

For my mom, who inspired my love of wetlands.

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| | |
|---|---|
| ANOVA | Analysis of variance |
| AVS | Acid-volatile sulfides, also known as monosulfides |
| AWM | Alpine wet meadow |
| BD | Bulk density of soil |
| BDSR | Bacterial dissimilatory sulfate reduction |
| C | Carbon |
| cm | Centimeter |
| C-S | Carbon-bonded sulfur, or shorthand for organic sulfur |
| DOC | Dissolved organic carbon |
| DS | Disulfides, also known as pyritic sulfides |
| $\delta^{34}S\text{-}SO_4^{2-}$ | Isotopic ratio of $^{34}S/^{32}S$ in a sulfate sample compared to the international standard, Vienna Canyon Diablo Troilite (VCDT). |
| $HS^-$ | Bisulfide, a form of reduced sulfur |
| $H_2S$ | Hydrogen sulfide, a form of reduced sulfur |
| L | Liter |
| LOI | Loss on ignition |
| LTER | Long-term ecological research site |
| MANOVA | Multivariate analysis of variance |
| MEM | Mixed effects model |
| mg | Milligram, equal to 1/1000 of a gram |
| OM | Organic matter |
| PSL | Periglacial solifluction lobe |
| S | Sulfur |
| SAW | Subalpine wetland |
| SE | Standard error of the mean, describing variability across multiple samples of a population |
| $SO_4^{2-}$ | Inorganic sulfate |
| spp. | Species |
| T | Time step |

# Chapter 1: Introduction

In lowland regions, high rates of biogeochemical processing in wetland ecosystems are known to support the ecological function of the greater landscape (Reddy & DeLaune, 2008; Schlesinger & Bernhardt, 2013). Organic matter (OM), dissolved ions, and contaminants are disproportionately retained and transformed in wetlands compared to surrounding drier areas, so wetlands ultimately regulate elemental cycling as ecosystem control points in lowland regions (Bernhardt et al., 2017; Reddy & DeLaune, 2008). This disproportionality is primarily driven by variations in oxygen content across spatiotemporal scales (Stolt et al., 2001; Sutula et al., 2003), which support oxidation and reduction reactions within a single wetland and ultimately affect water chemistry and nutrient cycling in draining streams and the rest of the watershed (Mandernack et al., 2000; Wieder & Lang, 1984).

The biogeochemical importance of lowland wetlands suggests that wetlands in high-elevation alpine ecosystems may play a similar role. Alpine areas are uniquely sensitive to the effects of climate change (Aber et al., 2012; Pepin et al., 2015), yet they provide water resources to surrounding catchments, and water quantity and quality could be affected by a changing climate. Therefore, alpine wetlands are important sites in studying how changing ecosystem function affects water resources, through processes such as oxidation-reduction reactions. While they tend to be less recognized among alpine or wetland science more broadly, alpine wetlands exist around the world and recent studies have described biogeochemical, hydrological, and ecological aspects of these saturated areas on almost every continent (Chignell et al., 2019; Ma et al., 2016; Nielson, 2008; Ritter et al., 2005). Most of this research has focused on responses to global change, particularly water table lowering and changes to carbon cycling (Carlson et al., 2020; Mercer, 2018; Wang et al., 2017; W. Zhang et al., 2016). However, more research is needed to understand the variability among different alpine wetland types and locations, on scales from hillslopes to continents.

At the Niwot Ridge Long-Term Ecological Research (LTER) site in the Rocky Mountains of the Colorado Front Range, researchers are trying to understand how alpine ecosystems are responding to climate change. Wet areas of Niwot Ridge have received some attention as locations with high rates of soil respiration, plant productivity, accumulation of soil organic matter, microbial biomass, and nitrogen cycling (Chen et al., 2020; Costello & Schmidt, 2006; Fisk et al., 1998; Garland, 2007; Knowles et al., 2015, 2016; Seastedt, 2020). However, the scope of previous work has either been limited to one wetland location, or classified all wetlands as a shared class. This ignores potential variability among alpine wetland types, which could serve different biogeochemical roles in the landscape.

In contrast with past studies, I identified three distinct wetland types with different morphological characteristics from the alpine to the subalpine. First, alpine wet meadows (AWMs, **Figure 1-1** panel A) are small ponded areas that remain saturated for much of the year due to pooling and lateral flow of snowmelt from the winter snow pack (Chimner et al., 2010; Taylor & Seastedt, 1994). Second, periglacial solifluction lobes (PSLs, **Figure 1-1** panel B) are slow-moving bodies of soil that creep across the landscape due to reduced internal friction from a seasonally-formed base ice lens. The ice lens serves as a source of water as it melts during the growing season, and prevents lateral subsurface flow, allowing snowmelt to accumulate in pools for long periods of time (Andersson, 1906; Benedict, 1970; Leopold et al., 2008). Both AWMs and PSLs are small in size and do not support the hydric vegetation characteristic of lowland wetlands (USACE, 1987), but meet the USDA definition of a wetland as "An ecosystem that depends on constant or recurrent, shallow inundation or saturation at or near the surface of the substrate" (USDA, 2013). Finally, subalpine wetlands (SAWs, **Figure 1-1** panel C), are larger wetlands below tree line that are dominated by *Sphagnum* spp., hummock-hollow topography, and thick organic soil (> 40cm). The differences in size, elevation, and morphology among wetland types could affect how well they retain and transform reactants of interest, particularly sulfur and carbon.

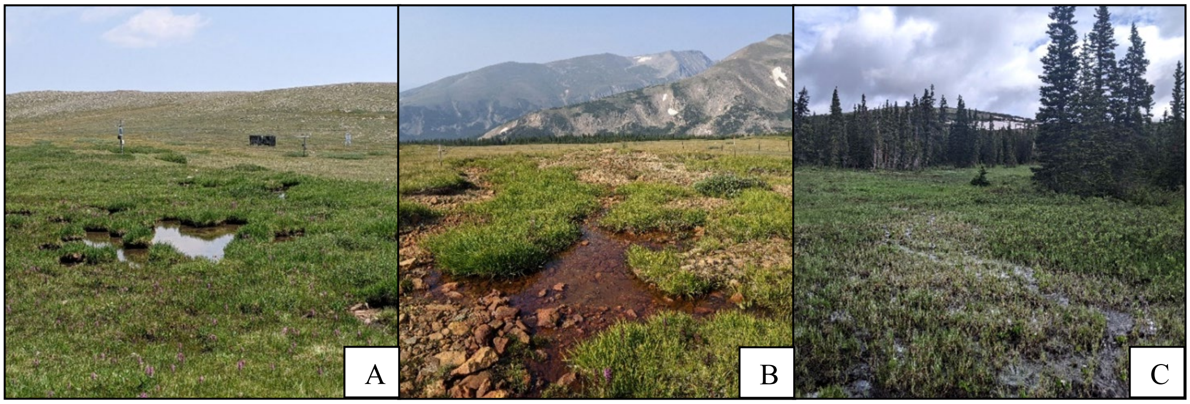

**Figure 1-1.** Wetland types studied: (A) alpine wet meadows (AWMs), (B) periglacial solifluction lobes (PSLs), and (C) subalpine wetlands (SAWs). Wetland types differed in dominant morphological features and elevation.

Recent research has demonstrated that sulfur (S) cycling is changing, and wetlands may be important locations supporting S transformations and transfers in the alpine. Although atmospheric deposition of S has decreased since implementation of the Clean Air Act, anthropogenic sources (primarily agriculture) are causing S loading in natural systems (E.-L. S. Hinckley et al., 2020; W. Orem et al., 2011). Additionally, effects of climate change are increasing weathering rates of minerals such as pyrite ($FeS_2$), and rising sulfate ($SO_4^{2-}$) concentrations in aquatic ecosystems have been linked to this source (Crawford et al., 2019; Todd et al., 2012). As S sources change and inputs rise, we can expect biogeochemical processing of S to be concentrated in wetter areas, where flooded anoxic soils can support communities of sulfur-reducing bacteria (Baldwin & Mitchell, 2011; W. Orem et al., 2011). Excess S in the environment can have significant consequences for ecosystem function, including stimulation of methylmercury production, acidification, eutrophication, disruption to coupled carbon, nitrogen, and phosphorus cycles, soil base cation depletion, and effects to downstream water quality (Baldwin & Mitchell, 2011; Bergman et al., 2012; Jeremiason et al., 2006; Kester et al., 2003; Lamers et al., 2001; van der Welle et al., 2008). Therefore, tracking concentrations and sources of S in alpine wetlands can help us understand how the changing S cycle could influence ecological function across alpine landscapes.

Wetlands could also play a significant role in carbon (C) cycling in the alpine. Wetlands are sites of high C storage that are prone to becoming C sources following anthropogenic wetland degradation or climatic change (Moomaw et al., 2018; Yarwood, 2018), and previous studies in alpine wetlands have tended to focus on quantifying C storage and projecting changes to C cycling (K. Bao et al., 2015; Daugherty et al., 2019; Drollinger et al., 2019; Ma et al., 2016; Reed et al., 2021; Wei & Wang, 2017; Wickland et al., 2001). Carbon is also coupled to other biogeochemical cycles as an important electron donor, so C cycling can be affected by changes to other cycles. For example, S loading can promote anaerobic oxidation of methane and organic matter decomposition (Baldwin & Mitchell, 2011; Feng & Hsieh, 1998; Pester et al., 2012). Variability in morphology, size, and elevation of alpine wetlands may influence whether these systems serve as C sources or sinks, and how C cycling is affected by global change.

Ecosystem control points are important areas at Niwot for examining how the sulfur and carbon cycles are changing. Nielson (2008) and Leopold et al. (2008) proposed that alpine wetlands across Niwot Ridge may act as biogeochemical "hotspots" with a disproportionate influence on biogeochemistry at aggregate scales. "Ecosystem control point" (*sensu* Bernhardt et al., 2017) has replaced "hotspot" to describe a particular place that has a disproportionately high effect, in space and time, on biogeochemical and ecological processes at larger scales. In order to diagnose ecosystem control points, biogeochemical properties and processes must be compared between wetlands and dry areas to determine whether this effect is present across the landscape. Additionally, at Niwot Ridge specifically, the differences among the three wetland types may affect how well they retain, transform, and export S and C, leaving some systems effectively isolated from their surroundings. In order to address these unknowns and to illuminate the role of wetlands in alpine ecosystems, I asked the following research questions:

1) Do alpine wetlands support higher concentrations of C and S compared to dry meadows, which comprise the majority of the landscape?
2) Do the morphological differences among the wetland types — potentially reflecting differences in hydrologic connectivity — drive distinct patterns in C and S biogeochemistry?

To address these questions, I measured a suite of soil physicochemical characteristics to establish wetland soil typologies, then investigated differences in S and C forms and concentrations among wetland types. Contrasting the patterns in C and S storage across wetlands and compared to the surrounding dry meadow is the first step toward understanding whether wetlands serve as ecosystem control points in alpine landscapes.

## Chapter 2: Methods

### *2.1: Study Site*

This research occurred within the Niwot Ridge LTER site in the Colorado Rocky Mountains, USA. Niwot Ridge is managed by the University of Colorado in cooperation with the US Forest Service and includes 1,200 ha of alpine and subalpine classified as Biosphere Reserve. Total wetland coverage studied was 0.15 $km^2$ across 8 sites that included three AWMs, three PSLs, and two SAWs (**Figure 2-1**). These wetlands span an elevation gradient from 3,105 to 3,541 m, from subalpine forest to alpine tundra, and each ranges in size from 165 – 75,000 $m^2$ in area. Previous work by Hermes et al. (2020) found that these persistently wet areas make up 0.3% of the alpine landscape. The majority of the landscape (58.3%) is dry alpine meadow, characterized by thin (< 2m in most areas) rocky soils with low soil moisture (≤ 0.2 v/v), classified as Cryochrepts, Cryumbrepts, and Cryorthents (Burns, 1980; Hermes et al., 2020). Dominant plant species in the alpine dry meadow are *Kobresia myosuroides, Carex rupestris, Selaginella densa,* and *Geum rossi* (Chen et al., 2020). The subalpine forest is characterized by coniferous trees, including *Picea engelmannii* and *Abies lasiocarpa* (Chen et al., 2020). Mean annual temperatures from the subalpine (3,048 m) to the peak of Niwot Ridge (3,750 m) range from -3.8 to 1.3°C, and mean annual precipitation is around 1,000 mm; long winters and short growing seasons characterize much of the region (Williams et al., 1996). Maximum soil temperature is 8-10°C in midsummer (Costello & Schmidt, 2006). Soils from AWMs at Niwot Ridge have previously been identified as Oxyaquic Cryepts and Gelepts as they are not extremely reducing even when flooded, but PSL soils in particular have the potential to be Cryaquepts (Garland, 2007).

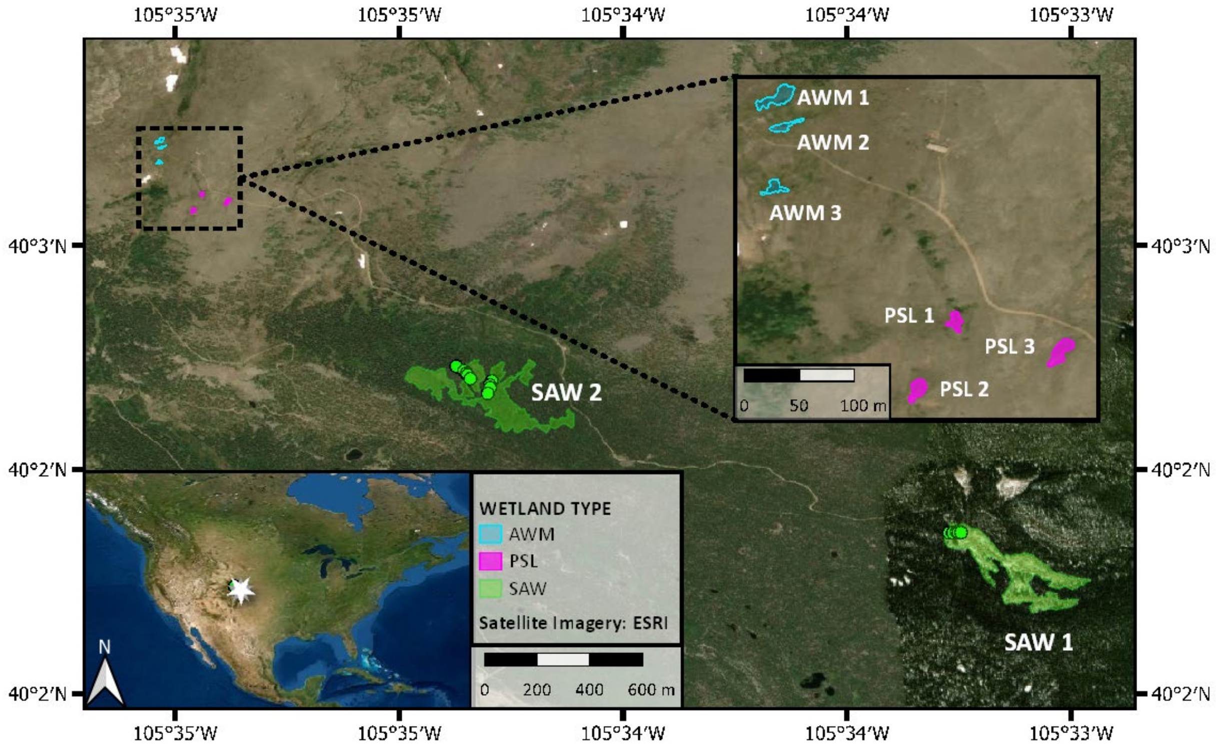

**Figure 2-1.** Map of sampling locations at the Niwot Ridge Long-Term Ecological Research (LTER) site. Colors indicate the wetland types studied. AWM = alpine wet meadow, PSL = periglacial solifluction lobe, SAW = subalpine wetland. Sampling nodes in SAWs are included as green dots to show the spatial extent of sampling.

### *2.2: Soil Sampling*

In July-September 2020, I collected soil samples from all wetland sites. I established transects from the dry edge into the center of each wetland, with sampling nodes in multiple pools of surface water (n = 4 interior nodes at each wetland) and the unsaturated edge for comparison (n = 1 edge node at each wetland). At SAW1, I used two paired parallel transects to sample soils from both flooded areas and raised hummocks at each node. I also set up two transects at SAW2 in central and eastern lobes of the wetland. At each sampling node within the transects, I collected soil samples at four depths (0-5, 5-10, 10-15, and 15-20 cm; n = 20 samples at each PSL and AWM, n = 36 samples at each SAW). In AWMs and PSLs, soil cores (20 cm in depth, 4-5 cm in diameter) were collected using a combination of pipes to avoid large rocks and sub-sectioned by depth interval. In SAWs, the organic soil was too dense to be cut into with a soil or peat corer, so a serrated knife was used to cut soil bricks 20 cm thick, which were then removed and cut into depth intervals. Sampling depths were limited to a 20 cm maximum because that was the deepest depth that could be sampled across all sites before refusal was hit. These soil samples were transported back to the laboratory in Ziploc bags and then air-dried prior to further processing.

In 2021, I collected one additional soil sample from each PSL site and maintained anaerobic conditions in air-tight storage containers using oxygen scrubbers while samples were transported to the laboratory. These samples were stored in Mylar bags flushed with $N_2$ gas and kept frozen until analysis.

### *2.3: Water Sampling*

From 16 June through 5 August 2021, I collected surface water and porewater samples from all wetland sites four times, each within week-long intervals: T1, 16-24 June; T2, 2-8 July; T3, 20-22 July; and T4, 4-5 August. During each sampling event, I collected 5 surface water samples at each PSL and AWM site and 10 surface water samples at each larger SAW site. Surface water collection sampling nodes were spread across the length (inflow to outflow) of each wetland, and I sampled from the same nodes at each sampling period. I used tension lysimeters (SoilMoisture Systems model 1909L-6") installed 15-20 cm below the soil or sediment surface to collect soil porewater. The location of each lysimeter was selected based on visible surface inflow and outflow points from each wetland. At PSL sites, the soil forms a "tongue" on the landscape from an accumulation of soil on the downslope side, so there was not a visible outflow; thus, I did not install lysimeters downslope of these features. I filtered all water samples in the field using Whatman glass microfiber syringe tip filters for future analysis (0.7 µm). Samples for DOC were stored in combusted glass amber bottles and acidified prior to analysis.

### *2.4: Laboratory Methods*

Following field collection, I analyzed all collected soil samples (n = 191) for a suite of soil physicochemical variables to establish patterns among the wetland types. I divided the soil samples into subsamples based on drying technique: one subsample was dried at 105°C to calculate gravimetric water content and loss on ignition, one subsample was dried at 60°C to determine total C, and the remaining soil was air-dried and used to measure pH and extractable $SO_4^{2-}$.

For loss on ignition (LOI) as a proxy for total soil organic matter, I combusted dried soil at 550°C for 4 hr following methods outlined by Heiri et al. (2001) and Burt (2011). For total C, dried soil was packed into tins and measured for %C by weight using a Thermo Finnigan FLASH EA 1112 CHN Analyzer at the Arikaree Environmental Laboratory at the University of Colorado, Boulder. To measure pH, I suspended 10 g of air-dried soil (5 g for SAW samples due to the absorptive properties of the peat) in 20 mL of 0.01M $CaCl_2$ for 1-hr and measured the pH of the solution with a benchtop pH probe. Additionally, I measured duplicates of each sample suspended in water to determine the difference between matrices for soil pH measurements. Finally, for extractable $SO_4^{2-}$, I ground 30 g of air-dried soil and shook the sample in 100mL of 0.006 M $Ca(H_2PO_4)_2{*}H_2O$ solution. I measured extractable inorganic $SO_4^{2-}$ in filtrate using ion chromatography, and converted to $SO_4^{2-}$-S using the ratio of atomic weights of S and $SO_4^{2-}$ (Metrohm 930 Compact IC Flex; detection limit 0.1 mg $L^{-1}$ $SO_4^{2-}$) (Tan et al., 1994).

In 2021, I analyzed the PSL soil samples collected under anaerobic conditions to estimate ratios of S species (acid-volatile sulfides, pyritic sulfides, sulfates, and organic S) in the soil, following methods outlined by Tuttle et al. (1986) and adapted to wetland soils by Orem & Bates (2013). Briefly, I treated soil samples with 6M HCl to evolve $H_2S$ from acid-volatile sulfides (monosulfides) in the sample, which were precipitated with 0.29 M aqueous $AgNO_3$ to produce $Ag_2S$. $Ag_2S$ was collected by filtration, dried, and weighed to determine acid-volatile sulfide content. I precipitated pyritic sulfides (disulfides) as $Ag_2S$ by the same protocol following reaction with a reduced $CrCl_2$ solution. I collected and washed the remaining sediment residue and precipitated sulfates in the sample as $BaSO_4$ to determine $SO_4^{2-}$ content. Finally, I oxidized organic S by reaction with Eschka's mixture at 800°C and precipitated resulting $SO_4^{2-}$ as $BaSO_4$ to determine organic S content.

For aqueous (surface and porewater) samples, dissolved organic carbon (DOC) was analyzed by a Total Organic Carbon Analyzer (Shimadzu Model TOC-L, detection limit 0.2 mg $L^{-1}$ DOC), and dissolved $SO_4^{2-}$ by ion chromatography (see model above). Dissolved $SO_4^{2-}$-S was calculated using the ratio of atomic weights of S and $SO_4^{2-}$. Additionally, I prepared one surface water sample from each wetland at each time point (n = 32 samples) for $\delta^{34}S$-$SO_4^{2-}$ stable isotope analysis by precipitation of $BaSO_4$ following methods from Carmody et al. (1998) and adaptations described in Hinckley et al. (2008). Briefly, acidified samples are boiled and reacted with 10% (w/v) $BaCl_2$ solution, and precipitated $BaSO_4$ is filtered onto non-sulfur 0.45 µm filters. $\delta^{34}S\text{-}SO_4^{2-}$ was measured on dried $BaSO_4$ precipitate via elemental analyzer/continuous flow isotope ratio mass spectrometry (EA-IRMS) using a Flash IRMS elemental analyzer (EA Isolink CN with ramped GC oven for triple NCS analysis) coupled with a Delta V Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). All stable isotope data are reported in delta (δ) notation in parts per thousand (‰), calculated as:

$$\delta^{34}S = \left( \frac{\left(\frac{^{34}S}{^{32}S}\right)_{sample}}{\left(\frac{^{34}S}{^{32}S}\right)_{standard}} - 1 \right) \times 1000$$

where the international standard for S isotope analysis is Vienna Canyon Diablo Troilite (VCDT), and normalization to the international stable isotope reference scale was based on the analysis of three laboratory reference materials with very different S delta values. Raw instrument data were corrected, if needed, for drift over time and sample size. These laboratory reference materials are calibrated annually against IAEA (International Atomic Energy Agency, Vienna, Austria) certified reference materials. An additional reference material with known sulfur stable isotope ratio was used as quality control. Long-term external precision for sulfur isotope analysis is ± 0.4‰. All isotope analyses were conducted at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley. Laboratory standard deviation for these stable isotope data was 0.43‰. No stable isotope readings were obtained from SAW samples, potentially due to interference of organics in the precipitation. Additionally, some early sample volumes were not high enough for sufficient precipitation, so 10 PSL and 7 AWM isotope values were measured.

### *2.5 Statistical Methods*

For each measured physicochemical variable, I ran a Kruskal-Wallis to test for significant ($p < 0.05$) differences among wetland types, including the differences between flooded interior and dry edge samples.

A nonparametric test was selected because these data violated the assumptions of normality using a Shapiro-Wilk normality test ($p < 0.05$ for each variable). Post-hoc pairwise Mann-Whitney U tests were run on the results to determine compact letter displays for each variable.

For each measured C and S variable (total %C in soil, DOC, extractable $SO_4^{2-}$-S in soil, and dissolved $SO_4^{2-}$-S), I tested the fit of different linear models with type, depth, and the interaction of type and depth as independent variables, as well as the significance of field replicate sampling location (i.e., PSL1 versus PSL3) as a random effect. As a result of these tests of model fit, I ran a linear model for total %C, with wetland type and depth as fixed effects; a linear model for DOC, with wetland type and time of sampling as fixed effects; a linear mixed effects model for extractable $SO_4^{2-}$, with type and depth as fixed effects and sampling location as a random effect; and a linear mixed model for dissolved $SO_4^{2-}$, with type and time of sampling as fixed effects and sampling location as a random effect. I assessed multicollinearity to determine the potential interaction effect of the measured S and C variables. I also calculated the model fit between linear models and multivariate models, concluding that linear models were a better fit for both soil and water samples. All models were run only on interior soil samples or surface water samples ($n = 159$ and 166, respectively).

I ran a Kruskal-Wallis to test for significant ($p < 0.05$) to determine statistical significance in DOC and $SO_4^{2-}$-S among samples from input lysimeters, output lysimeters, and surface water. These data violated the assumptions of normality using a Shapiro-Wilk normality test ($p < 0.05$), and data transformations did not raise the p-value above alpha. Post-hoc pairwise Mann-Whitney U tests were run on the results to determine compact letter displays for locations in each wetland type. Since there were no output lysimeters in the PSLs, I ran Mann-Whitney U tests to determine statistical significance between input porewater and surface water in the PSLs. A p-value of $<0.05$ was required for statistical significance in all tests.

For stable isotope data, the significance of the differences in $\delta^{34}S$-$SO_4^{2-}$ and $SO_4^{2-}$-S between PSLs and AWMs were assessed using a multivariate analysis of variance, or MANOVA. The variance of $\delta^{34}S$-$SO_4^{2-}$ was not equal between wetland types. Therefore, a lower alpha value of 0.001 was used for this

MANOVA. All data are reported as mean ± 1 SE, except where indicated. All statistical analyses were performed using R (Version 4.1.0), and R packages multcomp() and lme4() (Hothorn et al. 2008; Bates et al. 2015).

# Chapter 3: Results

## *3.1: Soil Physicochemical Characteristics*

Physicochemical characteristics in soil samples show distinct differences among wetland types. Results are shown in **Table 3-1** and are divided into sampling nodes in the dry edge (n = 12 AWM edge samples, 12 PSL edge samples, and 8 SAW edge samples) versus the flooded interior (n = 48 AWM interior samples, 47 PSL interior samples, and 64 SAW interior samples) of each wetland, grouped by wetland type. Soil water content was significantly higher in the SAW interior than in other sampling locations, and water content was lower in edge samples for all wetland types except AWMs. SAW samples (interior and edge) had significantly higher LOI than AWM and PSL sites (for all samples, 68 ± 2% versus 18 ± 1% and 12 ± 2%, respectively, $p < 0.05$). pH was lowest in PSL interior samples, with some measurements as low as 2.32. Significantly higher pH values were measured at the edges of the PSLs (5.14 ± 0.12, $p < 0.05$), but pH values in dry meadows were not statistically different from any wetland location. PSL samples had the highest bulk density (1.06 ± 0.37 $\text{g cm}^{-3}$), and SAW samples had the lowest bulk density (0.16 ± 0.01 $\text{g cm}^{-3}$), composed primarily of lightweight organic material.

**Table 3-1**
Soil physicochemical characteristics of wetland types studied (mean ± 1 SE).

| Wetland Type | Location | Water Content (%)[*] | LOI (%)[*] | pH[**] | BD ($\text{g cm}^{-3}$)[*] |
|---|---|---|---|---|---|
| AWM | Interior | $120 \pm 10^{b}$ | $17 \pm 1^{a}$ | $4.56 \pm 0.04^{a}$ | 0.48 ± 0.02[***] |
| | Edge | $124 \pm 21^{bc}$ | $21 \pm 4^{a}$ | $4.58 \pm 0.09^{a}$ | -- |
| PSL | Interior | $106 \pm 16^{c}$ | $13 \pm 2^{b}$ | $3.95 \pm 0.09^{c}$ | 1.06 ± 0.37 |
| | Edge | $24 \pm 4^{a}$ | $10 \pm 2^{b}$ | $5.14 \pm 0.12^{b}$ | -- |
| SAW | Interior | $584 \pm 21^{d}$ | $70 \pm 2^{c}$ | $4.23 \pm 0.05^{d}$ | 0.16 ± 0.01 |
| | Edge | $63 \pm 17^{c}$ | $51 \pm 7^{d}$ | $4.01 \pm 0.30^{acd}$ | -- |
| Dry Meadow[****] | | $20 \pm 4^{a}$ | $20 \pm 6^{ab}$ | $4.62 \pm 0.17^{ab}$ | 0.76 ± 0.07[***] |

Note: Different superscript letters in the same column indicate the values among wetland types are significantly different ($p < 0.05$).

[*] Water content, gravimetric water content as a percentage of dry soil weight; LOI, loss on ignition; BD, bulk density.

[**] pH measured in $CaCl_2$

[***] Bulk densities for the AWM and dry meadow from Chen et al., 2020.

[****] Dry meadow data courtesy of H.R. Miller.

### *3.2: Carbon Concentrations*

Total carbon concentrations measured in soil samples and DOC concentrations in water samples are shown in **Figure 3-1**. SAW sites had the highest overall C concentrations of any wetland type. Additionally, average %C in SAW soil was significantly higher than in dry meadow areas (37.2 ± 1.1% in SAW sites versus 8.8 ± 0.2% in dry meadows).

For total soil C, results of the linear model ($R^2 = 0.78$, $F(5,153) = 107.68$, $p < 0.001$) showed that total C from SAW sites and 0-5 cm depths were significantly higher than other wetland types and sampling depths. For the DOC in water samples, results of the linear model ($R^2 = 0.37$, $F(5,160) = 19.11$, $p < 0.001$) showed that DOC from SAW sites and from sampling time point 3 (7/20-7/22) were significantly higher than other wetland types and sampling times. Details of both models are shown in **Table 3-2**. For both variables, likelihood ratio tests comparing the linear model to a mixed effect model with field replicate sampling location as random effect (e.g., effect of PSL1 versus PSL3) showed the random effect was not significant ($p = 0.07$ and $0.16$ for %C and DOC, respectively).

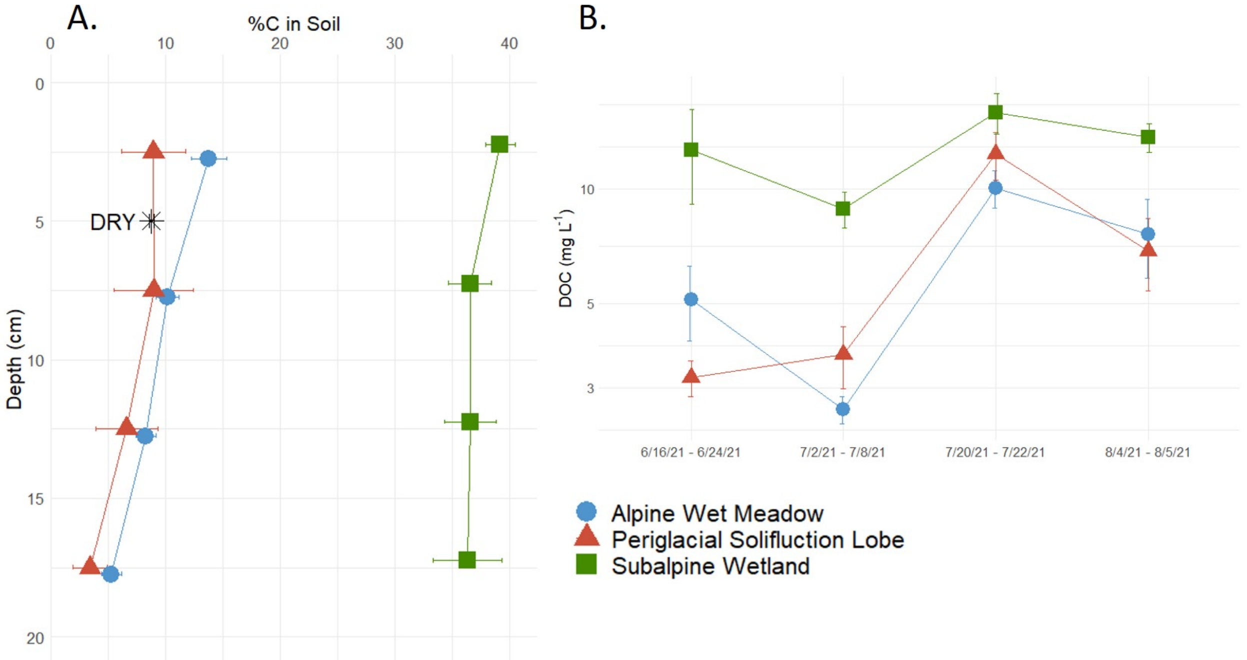

**Figure 3-1.** Mean total carbon (%) in soil (A) and dissolved organic carbon in surface water (B) by wetland types studied (mean ± 1 SE). Soil results are depicted along a depth profile and surface water results are depicted along a time series in 2021.

### *3.3 Sulfur Concentrations*

Extractable S as $SO_4^{2-}$ in soil samples and dissolved S as $SO_4^{2-}$ in water samples are shown in **Figure 3-2**. PSLs had the highest $SO_4^{2-}$-S concentrations of all wetland types in both the solid and aqueous samples (soil samples: 0.60 ± 0.21 mg S $g^{-1}$ dry soil versus 0.02 ± 0.00 and 0.04 ± 0.01 mg S $g^{-1}$ dry soil in AWMs and SAWs, respectively; water samples: 16.89 ± 0.49 mg S $L^{-1}$ versus 0.54 ± 0.08 and 0.22 ± 0.03 mg S $L^{-1}$ in AWMs and SAWs, respectively; $p < 0.05$). All wetland types had higher soil extractable $SO_4^{2-}$-S than the dry meadow areas.

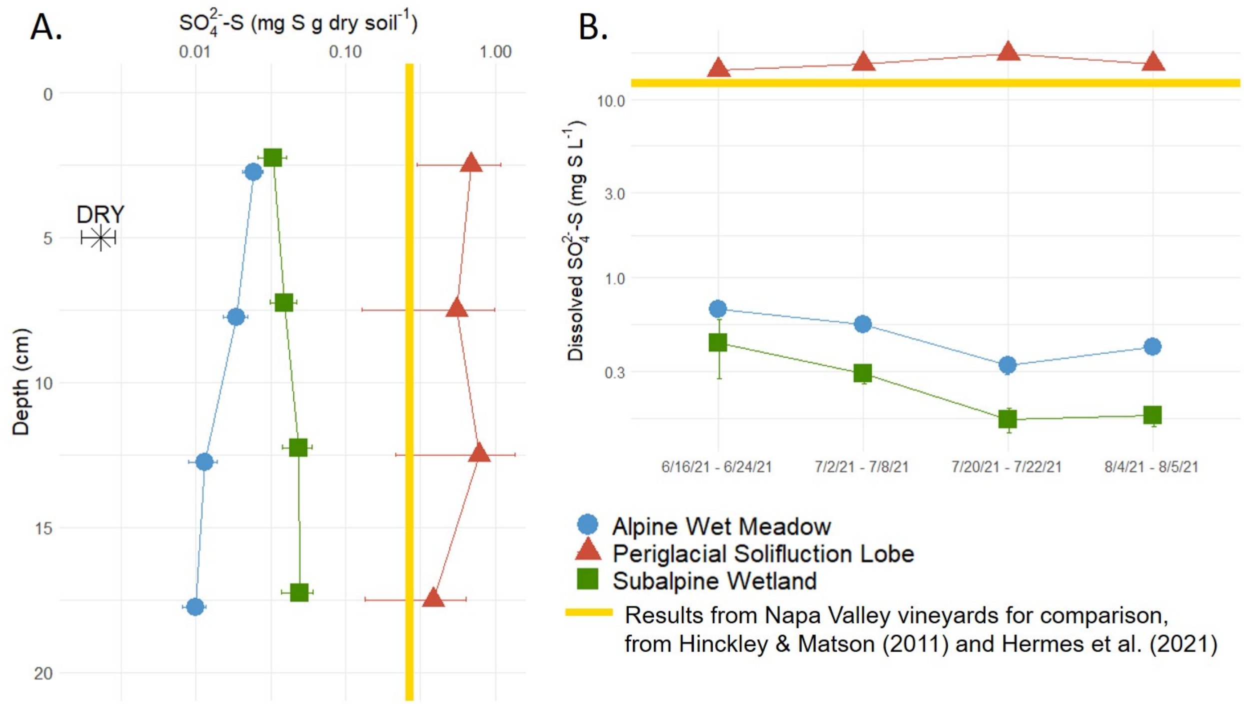

**Figure 3-2.** Mean extractable $SO_4^{2-}$-S in soil (A) and dissolved $SO_4^{2-}$-S in surface water (B) by wetland types studied (mean ± 1 SE). Soil results are depicted along a depth profile and surface water results are depicted along a time series in 2021. Results are compared to Napa Valley, CA samples with high sulfur loading, where comparison in A is extractable $SO_4^{2-}$-S measured in vineyard soils directly following sulfur application (E. L. S. Hinckley & Matson, 2011), and comparison in B is dissolved $SO_4^{2-}$-S from culvert water draining a vineyard (Hermes et al. 2021).

The linear mixed effects model showing the effects of type and depth (fixed) and sampling location (random) on soil extractable S as $SO_4^{2-}$ showed that all fixed effects were not statistically significant (conditional $R^2 = 0.34$, $R^2$ related to fixed effects alone = 0.09, **Table 3-2**). In fact, modeling the data using only sampling location as a random effect was a significantly better fit than modeling with fixed effects ($p < 0.05$). This significance indicates that one site (PSL1) was disproportionately affecting the average, as this site had much higher extractable $SO_4^{2-}$ than PSL2 and PSL3. Modeling dissolved $SO_4^{2-}$-S in water samples with wetland type and time as fixed effects and sampling location as a random effect (conditional $R^2 = 0.98$) showed that dissolved $SO_4^{2-}$-S was significantly higher in PSLs than other wetland types (**Table 3-2**).

**Table 3-2.** Coefficient values (Beta), standard deviation of beta (Std. beta), lower confidence level (LCL), upper confidence level (UCL), and p-value for all fixed effects in models based on model intercept. Shaded rows represent statistically significant variation from the model's intercept ($p < 0.05$). Intercepts for both models were AWM 15-20.

| Data Type | Model Name | Fixed Effect | Beta | Std. beta | LCL | UCL | p-value |
|---|---|---|---|---|---|---|---|
| Carbon | Linear: type + depth | Intercept | 6.77 | -- | 3.64 | 9.90 | <0.001 |
| | | Type: PSL | -2.42 | -0.15 | -5.58 | 0.75 | 0.134 |
| | | **Type: SAW** | **27.79** | **1.70** | **24.83** | **30.76** | **<0.001** |
| | | Depth: 10-15 | 1.93 | 0.12 | -1.55 | 5.40 | 0.275 |
| | | Depth: 5-10 | 3.17 | 0.19 | -0.30 | 6.65 | 0.073 |
| | | **Depth: 0-5** | **5.32** | **0.33** | **1.85** | **8.80** | **0.003** |
| DOC | Linear: type + depth | Intercept | 4.83 | -- | 2.06 | 7.60 | <0.001 |
| | | Type: PSL | 0.61 | 0.09 | -1.55 | 2.78 | 0.576 |
| | | **Type: SAW** | **6.01** | **0.88** | **3.95** | **8.08** | **<0.001** |
| | | Time: T2 | -1.99 | -0.29 | -5.20 | 1.21 | 0.221 |
| | | **Time: T3** | **5.63** | **0.83** | **2.44** | **8.82** | **<0.001** |
| | | Time: T4 | 2.37 | 0.35 | -0.82 | 5.55 | 0.144 |
| S as Sulfate (soil) | MEM: type + depth<br>Site as random effect | Intercept | -0.05 | -- | -0.63 | 0.53 | 0.863 |
| | | Type: PSL | 0.59 | 0.72 | -0.18 | 1.35 | 0.132 |
| | | Type: SAW | 0.03 | 0.03 | -0.81 | 0.87 | 0.948 |
| | | Depth: 10-15 | 0.12 | 0.15 | -0.20 | 0.44 | 0.455 |
| | | Depth: 5-10 | 0.05 | 0.06 | -0.27 | 0.37 | 0.756 |
| | | Depth: 0-5 | 0.09 | 0.11 | -0.23 | 0.41 | 0.578 |
| S as Sulfate (water) | MEM: type + depth<br>Site as random effect | Intercept | 0.26 | -- | -1.44 | 1.95 | 0.767 |
| | | **Type: PSL** | **15.95** | **2.12** | **13.67** | **18.22** | **<0.001** |
| | | Type: SAW | -0.29 | -0.04 | -2.82 | 2.24 | 0.822 |
| | | Time: T2 | 0.11 | 0.01 | -0.60 | 0.82 | 0.759 |
| | | Time: T3 | 0.63 | 0.08 | -0.08 | 1.34 | 0.083 |
| | | Time: T4 | 0.03 | 4.11e-03 | -0.68 | 0.74 | 0.932 |

Comparisons of the dissolved $SO_4^{2-}$-S and DOC in surface water with porewater from the input and output lysimeters in each wetland are shown in **Table 3-3**. Dissolved $SO_4^{2-}$-S in output water from the AWMs was significantly higher than surface and input water, and surface water was significantly lower in DOC. There was no statistical significance among water sampling locations in the PSLs and SAWs.

**Table 3-3**

Dissolved $SO_4^{2-}$-S and DOC in surface water compared to porewater for wetland types studied (mean ± 1 SE).

| Analyte | Wetland Type | Input Porewater[1] | N | Surface Water[2] | N | Output Porewater[1] | N |
|---|---|---|---|---|---|---|---|
| $SO_4^{2-}$ (mg $L^{-1}$) | AWM | $0.19 \pm 0.08^{a}$ | 6 | $0.47 \pm 0.03^{b}$ | 55 | $1.09 \pm 0.45^{ab}$ | 11 |
| | PSL | $18.86 \pm 1.85^{a}$ | 11 | $16.41 \pm 0.41^{a}$ | 49 | NA[3] | 0 |
| | SAW | $0.29 \pm 0.21^{a}$ | 8 | $0.21 \pm 0.02^{b}$ | 62 | $0.17 \pm 0.11^{ab}$ | 8 |
| DOC (mg $L^{-1}$) | AWM | $17.34 \pm 8.35^{ab}$ | 6 | $6.50 \pm 0.71^{a}$ | 55 | $13.13 \pm 3.25^{b}$ | 11 |
| | PSL | $7.78 \pm 2.32^{a}$ | 11 | $7.33 \pm 6.33^{a}$ | 49 | NA[3] | 0 |
| | SAW | $15.18 \pm 8.40^{a}$ | 8 | $12.78 \pm 0.87^{a}$ | 62 | $18.46 \pm 9.04^{a}$ | 8 |

Note: Different superscript letters in the same row indicate the values between locations are significantly different ($p < 0.05$).

[1] Porewater collected from lysimeters located along major surface water inflow and outflow paths.

[2] Surface water collected from aboveground pools.

[3] No output surface flow in PSLs.

### *3.4: Potential Variable Interaction*

The correlation between total %C and extractable $SO_4^{2-}$ in soil samples was r = -0.06, indicating low negative multicollinearity for a multivariate model. For water samples, the correlation between DOC and dissolved $SO_4^{2-}$ was r = 0.25, indicating low positive multicollinearity. Therefore, the linear models reported fit both datasets better than multivariate models.

### *3.5: Sulfur Stable Isotope Results*

Stable isotopic values of S-$SO_4^{2-}$ expressed in delta notation ($\delta^{34}S$) compared to concentrations of extractable $SO_4^{2-}$ in surface water samples are shown in **Figure 3-3**. Stable isotope data were not obtained from the SAW sites due to failed $BaSO_4$ precipitation. No visible clustering is observed between field replicate sampling locations (i.e., PSL1 versus PSL3), but clear clustering is observed between wetland types (PSL versus AWM). Results of the MANOVA showed a statistically significant difference in $\delta^{34}S$-$SO_4^{2-}$ and $SO_4^{2-}$-S between AWMs and PSLs ($p < 0.001$). Overall, surface water from PSLs is more depleted in $\delta^{34}S$-$SO_4^{2-}$ (mean = -6.7‰, relative to a mean of +1.8‰ in the AWMs). Comparison values shown in **Figure 3-3** are discussed in Chapter 4.3.

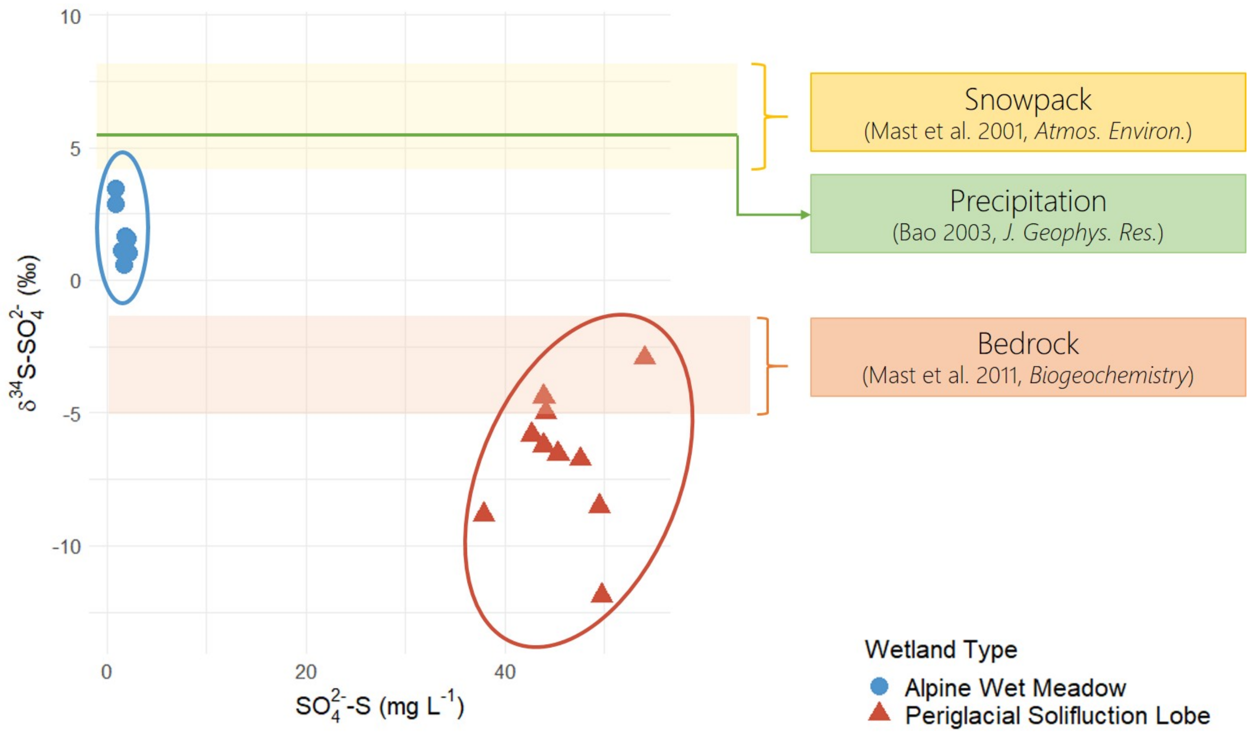

**Figure 3-3.** Stable isotope values of $SO_4^{2-}$-S versus dissolved $SO_4^{2-}$ in surface water from AWMs and PSLs. For reference, values are compared to the stable isotope value of precipitation in the southwest (H. Bao, 2003), the range of stable isotope values from 53 snowpack samples across the Rocky Mountains (Mast et al., 2001), and the stable isotope value of pyritic bedrock from a site south of Niwot Ridge (Mast et al., 2011).

### *3.6: Sulfur Speciation Results*

Only two samples of PSL soil were analyzed for S speciation due to time constraints. Results of those experiments are shown in **Table 3-4.** Due to the low sample number, these data should only be used as an indicator of other forms of S besides $SO_4^{2-}$ in these PSL soils.

**Table 3-4**

Results of S speciation experiment, where % of total extracted (non-elemental) S is expressed by S species for each sample. AVS = acid-volatile sulfides; DS = disulfides; $SO_4^{2-}$ = sulfate; C-S = organic S.

| Sample | % as AVS | % as DS | % as $SO_4^{2-}$ | % as C-S |
|---|---|---|---|---|
| PSL1 | 6% | 10% | 37% | 47% |
| PSL3 | 7% | 27% | 28% | 38% |

# Chapter 4: Discussion

Results of this study showed distinct patterns among wetland types that suggest key morphological differences in these ecosystems affect patterns of soil physicochemical characteristics and C and S biogeochemistry. Here I discuss patterns and potential drivers in these data to understand heterogeneity among wetland types. I also discuss the implications of this work for identifying ecosystem control points on the alpine landscape and prioritizing future research questions to understand the effects of global change on alpine biogeochemical cycling.

## *4.1: Physicochemical Characteristics*

Physicochemical characteristics reflected the patterns among wetland types, where SAWs are highly organic systems, PSLs are S-rich and acidic, and AWMs represent an intermediate type. The low pH values measured in all wetland interior samples are more acidic than other alpine wetlands described in the literature from across the globe. Previously reported average soil pH values from alpine wetlands in Tibet were all > 5.0 (E. Kang et al., 2021; Luan et al., 2014; Qiongyu Zhang et al., 2021), and Rocky Mountain subalpine wetland water ranged from 6.0-6.7 (Daugherty et al., 2019). The medium used to measure soil pH may be affecting these values, but when comparing my results measured in $CaCl_2$ to those measured in deionized water, water measurements were only 0.54 pH units higher in water than in $CaCl_2$ (4.86 versus 4.32, respectively). Garland (2007) also measured an average pH in wet alpine soils of Niwot Ridge of 4.6 in the epipedon (average 36 cm depth, measured in deionized water). Since pH is measured on a log scale, concentrations of acidity in alpine wetlands at Niwot Ridge were orders of magnitude higher than other measurements. However, Crawford et al. (2020) showed that deposition of strong acids has decreased at Niwot Ridge, and that overall, acidity across the watershed has stabilized. Despite decreasing acid deposition, acidity could be generated in alpine wetlands from ionization of organic acids, formation of sulfuric acid ($H_2SO_4$) as a byproduct of sulfate reduction, or the dissolution of $CO_2$ (Zhang et al., 2021). Soil pH should be monitored in future studies at Niwot Ridge to identify whether wetland acidity is increasing through time and potentially affecting ecosystem function.

Bulk density and water content results show distinct soil typologies among wetland types. Dense soils with visible rocks and gravel in the PSLs contrasted with lightweight organic soils with high water-holding capacity in the SAWs. High variability in characteristics like bulk density among the PSLs reflected the high variability observed in the PSL C and S pools. SAW soils were composed of sponge-like webs of interwoven organic material that needed to be cut with a serrated knife. This contrast in soil typologies shows how environmental conditions differ among wetland types, which set up different belowground environments for C and S biogeochemistry.

### *4.2 Patterns in Carbon*

Results from this study showed SAWs were highly organic C-rich wetlands compared to other types. Soils from SAWs had the highest total C and LOI values and water samples had the highest levels of DOC. The physical factors of SAW soils (low bulk density and high water content) were similar to peat soils (Inisheva, 2006). However, C concentrations in SAWs are lower than in wetlands classified as peatlands: measured LOI averaged 70% from 0-20 cm, compared to an average of 86-90% in 0-60 cm sediment cores from alpine peatlands of China (K. Bao et al., 2015); and DOC averaged 16.82 mg $L^{-1}$ (surface and porewater), on the lower end of DOC concentrations ranging from 5.6 to 44.4 mg $L^{-1}$ in other Rocky Mountain subalpine wetlands (Daugherty et al., 2019). Compared to AWMs and PSLs, SAWs are similar to lowland wetlands where high organic inputs and slow decomposition rates limited by oxygen lead to an accumulation of C. This pattern makes wetland ecosystems sites of high C storage and potentially valuable buffers against climate change. However, they are also potential C sources if disturbance to the system leads to faster turnover of organic matter or changes to belowground C cycling (Moomaw et al., 2018; Reed et al., 2021). For this reason, raising or lowering of the water table is a primary concern in alpine wetlands, as water table lowering (WTL) oxygenates surface soils or sediments and a rising water table can promote methane ($CH_4$) production (Gao et al., 2014; Wang et al., 2017; Wei & Wang, 2017; W. Zhang et al., 2016). Knowles et al., (2019) showed that Niwot Ridge is a net annual source of C to the atmosphere, and that flux could be altered by water table fluctuations in alpine wetlands. Future work should continue to monitor the hydrology in SAWs to determine how global change may affect the water table and C cycling at the landscape scale.

The other two wetland types supported significantly lower C pools. AWMs are not as C-rich as SAWs, but porewater still contained high concentrations of DOC (average 15.24 mg $L^{-1}$), and surface soil had higher total C than dry meadow soil. AWMs are therefore sites of disproportionately high C pools above tree line. Finally, PSLs are an extremely distinct wetland type. PSL soils had similar total C as alpine dry meadows, but surface water DOC concentrations were similar to those measured in AWMs. Knowles et al. (2019) suggested that the movement and disruption of solifluction lobes across the landscape could be redistributing soil organic C, so previously frozen occluded C could have been exposed and respired. With low C content, soil microorganism communities in the PSLs could be maintained through anaerobic respiration pathways linked to alternative sources of energy such as S.

### *4.3: Patterns in Sulfur*

The concentrations of $SO_4^{2-}$-S in the PSLs were significantly higher than other wetland types and dry meadow areas. For comparison, some PSL soil samples (from one node in PSL1 in particular) had over ten times the average concentration of $SO_4^{2-}$ measured in Napa Valley vineyard soils directly following an application of elemental S sprayed regularly as a fungicide (9.0 to 20.0 mg $SO_4^{2-}$ $g^{-1}$ dry soil versus 0.8 mg $g^{-1}$ dry soil) (Hinckley & Matson, 2011). Surface water $SO_4^{2-}$-S in PSLs was also higher than average concentrations in culvert water draining Napa vineyards, at 16.41 mg S $L^{-1}$ versus 12.23 mg S $L^{-1}$, respectively (Hermes et al., 2021, **Figure 3-2**). This comparison to crop systems with high rates of S inputs helps to put the magnitude of these measured S concentrations into perspective. Additionally, $\delta^{34}S$-$SO_4^{2-}$ values in the PSLs were highly depleted compared to AWMs, and were more depleted than almost all freshwater $\delta^{34}S$-$SO_4^{2-}$ values in global rivers inventoried by Burke et al., (2018). Here I explore potential biogeochemical processes that could be causing the differences in observed patterns of $SO_4^{2-}$-S concentrations and $\delta^{34}S$-$SO_4^{2-}$ isotopic fractionation between wetland types.

### 4.3a: Potential Sources of Sulfur

Historically, atmospheric deposition (both wet and dry) of S has been the primary source of S to remote ecosystems (Lovett, 1994). However, Crawford et al., (2019) showed that atmospheric deposition of $SO_4^{2-}$-S in the Green Lakes Valley has actually been decreasing since the early 2000s while river export of $SO_4^{2-}$-S has been increasing in areas that are not soil-mantled. Mast et al., (2001) showed that $\delta^{34}S$-$SO_4^{2-}$ values in snowpack across 52 high-elevation sites in the Colorado Rocky Mountains averaged +4.0 to +8.2‰, within the range of $\delta^{34}S$-$SO_4^{2-}$ stable isotope values measured in precipitation (average +5.8‰ in the southwestern US (H. Bao, 2003) and +1.1 to +12.7‰ in the eastern US (Y. Zhang et al., 1998)), but more enriched than all surface water $\delta^{34}S$-$SO_4^{2-}$ values in Niwot alpine wetlands (**Figure 3-3**). Atmospheric deposition is likely a source of S in AWMs, as $SO_4^{2-}$-S concentrations in AWMs are low enough to match low rates of atmospheric $SO_4^{2-}$ deposition (<5 kg $ha^{-1}$ $yr^{-1}$ in 2017, reported in Crawford et al. (2019)), and $\delta^{34}S$-$SO_4^{2-}$ values (mean: +1.8‰) were within the range of atmospheric deposition. This atmospheric source could be driven by the seasonally ponding snowmelt that forms the AWMs. Since $\delta^{34}S$-$SO_4^{2-}$ values in the AWMs are depleted relative to snowpack values, there could be a mix of S sources in these wetlands, including both atmospheric deposition and geologic weathering.

Geologic weathering of pyritic bedrock could be a significant source of S, particularly in the PSLs. Alpine landscapes often contain reactive sulfide minerals that can be prone to weathering as glaciers and snowpack retreat in a warming climate, and this process has been linked to increased $SO_4^{2-}$ concentrations in arctic and alpine ecosystems (Salerno et al., 2016; Szynkiewicz et al., 2013; Todd et al., 2012). The S stable isotopic composition of the bedrock has not yet been measured at Niwot Ridge, but pyrite values from a mining district in the alpine region to the south ranged from -1.7 to -5.0‰ (Mast et al., 2011, **Figure 3-3**). While these values are not as depleted as the mean value from the PSLs (-6.7‰), heterogeneity of stable isotopes in the bedrock could be substantial.

While there are many S biogeochemical transformations that could be occurring in these alpine wetlands, S stable isotope results do not show substantial evidence of these pathways. Preferential release of the light isotope during mineralization of OM could be releasing depleted $\delta^{34}S$-$SO_4^{2-}$ to surface water, but this isotope effect is small (~2‰) (P.G. Kang et al., 2014; Novák et al., 1994, 2001). Isotopic fractionation associated with plant uptake is also minor (Guo et al., 2020; Trust & Fry, 1992). Microbial sulfate reduction is associated with substantial fractionation, but if sulfate reduction is dominating in PSLs, the resulting $SO_4^{2-}$ pool would be enriched in $^{34}S$ relative to source values, not depleted (Bruchert, 2004). Reoxidation of $^{34}S$-depleted sulfide or mineralization of OM that has incorporated $^{34}S$-depleted sulfide could produce $^{34}S$-depleted $SO_4^{2-}$ or organic S (P.G. Kang et al., 2014; Mandernack et al., 2000; Mitchell et al., 2006), particularly if repeating cycling of reduction and oxidation cause further fractionation, but this would not explain the disappearance of $^{34}S$-enriched $SO_4^{2-}$ formed by initial sulfate reduction. Finally, bacterial disproportionation of intermediate S species ($S^0$, $S_2O_3^{2-}$, and $SO_3^{2-}$) can lead to preferential uptake of the light isotope (Canfield & Thamdrup, 1994; Habicht et al., 1998), and disproportionation can dominate over sulfate reduction and lead to significant fractionation (Pellerin et al., 2015). However, rates of the two processes must be compared directly because disproportionation can also be minor (Guo et al., 2020). Overall, stable isotope data from this study strongly indicates two different S sources between wetland types rather than distinct differences in S transformations.

### 4.3b: Sulfur Speciation

A final important consideration in interpreting these results is that S pools in both soil and water samples were measured only as $SO_4^{2-}$. This is an inorganic form of S that is highly soluble and can therefore be tracked through the landscape along hydrologic flow paths. However, S biogeochemistry is complicated by the fact that S can exist in 8 oxidation states and can therefore be incorporated into a wide variety of inorganic and organic molecules. While I was only able to measure the S speciation in two PSL soils, results of these experiments showed that only 28-37% of extracted non-elemental S in the soils was $SO_4^{2-}$. The highest fraction was extracted as organic S (38-47%), and these were in the PSL systems where OM was lowest among the wetland types. Therefore, S in AWMs and SAWs may primarily be in organic form, and all S species should be inventoried in future studies.

### *4.4: Implications: Alpine Wetlands as Ecosystem Control Points and Importance of Connectivity*

If these alpine wetlands serve as ecosystem control points, they would support high biogeochemical pools and/or process rates while disproportionately affecting the surrounding landscape. In this study, I measured higher concentrations of reactant C and S species in alpine wetlands compared to dry meadow areas, and found that these patterns differed by wetland type. Future studies can expand on these data to compare biogeochemical process rates and storage pools between dry and wet areas.

An additional priority for future work is determining the extent of hydrologic connectivity between each wetland type and the surrounding alpine landscape. Hydrologic pathways are the primary connection between ecosystem control points and their surroundings, and hydrologic connectivity can extend "across vertical, lateral, and longitudinal dimensions and span spatial and temporal scales" (Covino, 2017). If alpine wetlands serve as ecosystem control points, we might expect a regular transport of reactants into the surrounding landscape along hydrologic flow paths or a flush of reactants during a particular season or when a hydrological threshold is overcome (Bernhardt et al., 2017). Alternatively, they may be hydrologically isolated from the surrounding landscape, and support disproportionate biogeochemical pools and processes without disproportionately influencing their surroundings. Here I combine results of this study with a discussion of how the morphology of these three wetland types may affect their potential role in the landscape, summarized in **Figure 4-1**.

PSLs are a prime example of how morphology can influence hydrologic connectivity. The high concentrations of $SO_4^{2-}$-S measured in this study could affect the surrounding landscape through processes such as methylmercury production and heavy metal mobilization. But PSLs are relatively small, and the base ice lens prevents subsurface flow for much of the year. Therefore, hydrologic connectivity of PSLs is limited on both spatial and temporal scales. Additionally, global change may alter the role of PSLs: in the 1970s, discontinuous permafrost was reported as the base ice lens, but PSLs now support temperatures above freezing for multiple weeks each summer, and further warming could extend this period when the ice lens is melted (Knowles et al., 2019). If this period continues to increase, the biogeochemical dynamics both within and around each PSL site would be affected by subsurface flow and increase hydrologic connectivity of the wetland to the surrounding landscape.

AWMs are also small in size but have the potential to be more hydrologically connected than PSLs. Continuous surface flow paths can be observed in AWMs for much of the growing season, and while subsurface hydrology has not been modeled, there is no ice lens barrier preventing subsurface flow. The residence time of snowmelt and biogeochemical process rates in the AWM pools will affect how these wetlands change the chemistry of water draining from the top of the hillslope into the surrounding landscape.

SAWs have the greatest potential to support biogeochemical process rates that sustain productivity and affect water chemistry in the surrounding landscape. Their large spatial extent suggests that a significant proportion of water flowing out of the alpine could pass through a SAW before reaching the main stem of Boulder Creek, and areas just below tree line have been shown to contribute substantially to catchment streamflow (Carroll et al., 2019; Qinghuan Zhang et al., 2018). Multiple surface flow paths can be visually identified draining into and out of each SAW, and multiple pools of stagnant water are maintained throughout the summer months. While these areas are below tree line, they could affect the chemistry of water draining from the alpine into the greater Boulder Creek catchment, so future research should seek to investigate the biogeochemical heterogeneity and hydrologic connectivity of these wetlands.

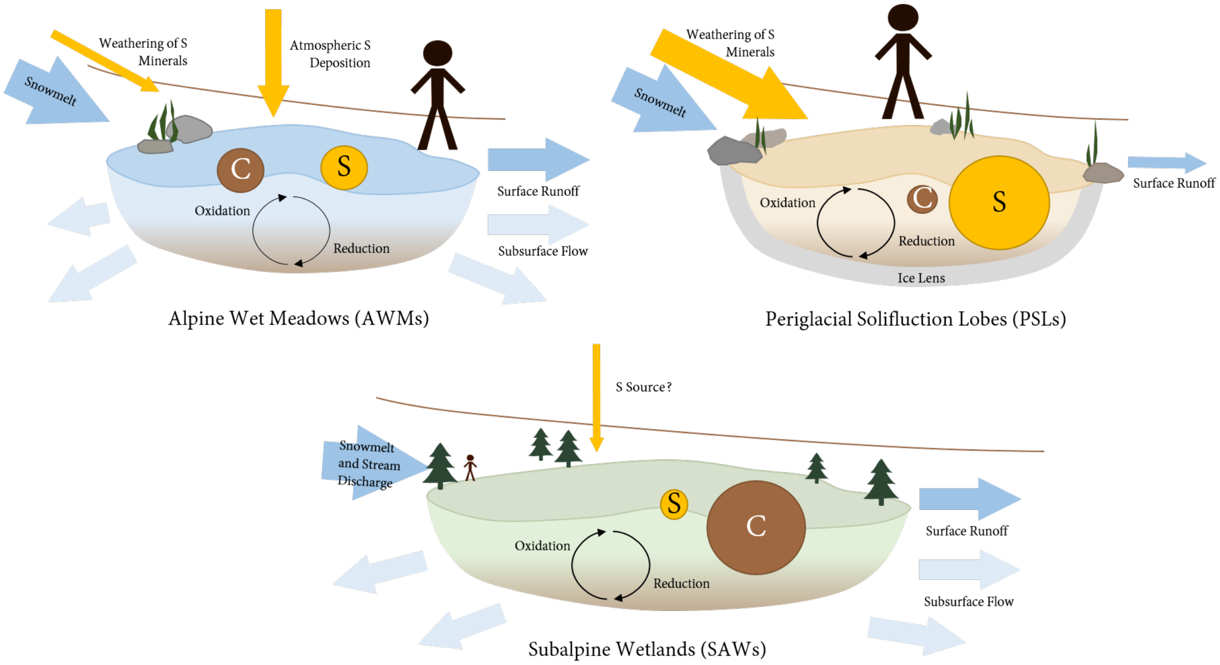

**Figure 4-1.** Conceptual model comparing results among wetland types studied. Wetland colors distinguish each type. Magnitudes of C and S concentrations are represented by the qualitative sizes of respective circles, and proposed S sources suggested from stable S isotope data are included. Suggested effects of morphology on hydrologic connectivity are represented by blue arrows, with darker blue showing surface flow and light blue showing subsurface flow. The relative size of each wetland type is depicted by the human figure, where the area of each SAW was over two orders of magnitude larger than AWMs and PSLs.

## Chapter 5: Conclusion

This study established the foundation for future biogeochemical studies in alpine and subalpine wetlands at Niwot Ridge, Colorado. I established three morphologically-distinct types of alpine wetlands, and used a combination of analytical methods to determine differences in biogeochemical patterns, including physicochemical characteristics and concentrations of reactive C and S. Alpine wetlands have been relatively understudied, and my research demonstrates the heterogeneity among the ecosystem types on a hillslope scale, and the importance in assessing the influence of morphology on biogeochemistry and hydrologic connectivity in alpine wetlands around the world.

I also showed that concentrations of S and C species were higher in alpine wetlands than surrounding dry meadow areas, suggesting that these wetlands are important reservoirs of reactive elements. I expanded on these findings by suggesting avenues for future work, particularly in the subalpine, to further explore the biogeochemical role of wetlands in the broader landscape. This is an important line of research to pursue because alpine ecosystems are especially sensitive to climate change, and the consequences of change are difficult to predict (Aber et al., 2012; Pepin et al., 2015). Future research must build on the patterns explored in this thesis to determine how warming and other changes in forcing will affect both wetland function *in situ* as well as their role in biogeochemical and ecological processes at broader scales.

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