

Research Title: Effects of Dissolved Oxygen & Iron II Concentration Fluxes in Beaver-Impacted Floodplains

Introduction:

Gravel-bed river floodplains are fundamental environmental contributors within glaciated mountain landscapes as they have disproportionately concentrated biogeochemical, hydrologic, and ecological processes within their converging terrestrial and aquatic systems.^{1,2} They perform critical groundwater recharge, promote riparian habitat, sustain heightened geodiversity, and support hydrologic, sediment, nutrient, and organic matter (OM) exchanges integral to ecosystem productivity and biodiversity.³ In terms of anthropogenic benefits, floodplains deposit fertile soils rich in minerals and OM to surrounding farms, while dampening peak flows, reducing flood risks, and decreasing flood depths downstream by slowing runoff and storing excess water.⁴⁻⁶ Floodplain systems are also especially adept to combat ongoing impacts of climate change.⁷ By increasing hydraulic gradients and biogeochemical cycles, floodplains not only eclipse climate impacts like aridification and hydrologic extremes, they also cool stream temperatures, store and remobilize heavy metals, and serve as crucial carbon sinks.^{8,9}

As one of Colorado's most sensitive living resources, floodplain ecosystems, their functions, and downstream capabilities are reliant on the hydrologic engineering of the North American beaver (*Castor canadensis*).¹⁰ Beavers promote hydraulic connectivity between floodplains and interdependent rivers and streams, generating key alluvial zones. The water spreading capabilities of beaver-built infrastructure make beavers central agents of climate change resilience.¹¹ Historically, beaver-assisted natural accumulation of OM cultivated varied aerobic respiration and redox conditions in riverine waters. These beaver-impacted, heterogeneous surface waters were once universally prevalent in North American streams.¹² With the introduction of the European fur trade in the 16th century, over-trapping, widespread wood removal from streams, and extensive land use alterations led to the virtual eradication of the Colorado (and much of North America's) beaver populations.^{13,14} As a result of the near extinction, the natural geomorphic, biogeochemical, hydrological, and ecological homeostasis of floodplain ecosystems were substantially compromised.

Beaver dam analogs (BDAs) are man-made structures often used in low-tech process-based restoration (LTPBR) to leverage crucial environmental processes and benefits of natural beaver dams.¹⁵ Re-instigating these natural processes enlivens endangered floodplains, returns channel stability, and increases historically-depleted hydraulic connectivity.¹⁶ BDAs are able to emulate natural beaver dam's maintenance of heightened flows later into the summer months, enhance surface and groundwater storage, level peak flows during flooding events, and amplify biogeochemical cycling of nutrients— a vital tool in mitigating stream-floodplain degradation.^{11,17} Both restorative LTPBR techniques and natural beaver dams are fundamental for conservation efforts in glaciated mountain ecosystems. However, these systems require an in-depth analysis of their biogeochemical significance.² Specifically, diurnal cycles and their fluctuations may drive a number of the capabilities of these systems.

Floodplains exhibit highly varied surface water flows which increases the diversity in the surface water temperatures. Those distinct environments cultivate niche and highly heterogeneous microbial communities. This enrichment expands the riverine waters' redox potential. The reducing or oxidizing capacity of a system determines a river or floodplain's ability to break down waste products or contaminants and to recycle available carbon and nutrients into soluble, mobile forms. Redox conditions and surrounding parameters are typically measured at the sediment-water interface.¹⁸ Quantifying redox conditions can be used to assess the redox role of floodplains which dictates the efficiency at which they cycle nutrients or store excess carbon dioxide.¹⁹ Beaver ponds are especially rich in dissolved organic carbon (DOC), which is an important electron source for redox reactions.²⁰ Microbes catalyze those redox reactions.^{21,19,22} Alkalinity quantifies water's capacity to neutralize acids. Changes in those measurements can be used as a signifier to infer the presence of microbial processes like respiration, nitrification, or reduction and the intensity of their impact on water chemistry. Alkalinity measurements are therefore indicative of the aggregate amount of microbial activity.²³

Dissolved oxygen (DO) can be used as a master variable in understanding redox dynamics of a system. It is insightful as to the types of microbial activity happening in a system. Increased water temperatures, elevated nutrient levels, or heightened plant oxygen demand reduces the solubility of oxygen in water, resulting in lower DO. Inversely, cooler water temperature, lessened nutrients, or decreased biotic activity increases the solubility of oxygen.²⁴ These indicators place DO as the master variable in controlling microbe operations, determining solubility and mobility of heavy metals, and mediating the redox dynamics of riverine waters. Elements and nutrients occurring in natural waters– like iron (Fe), manganese (Mn), carbon (C), oxygen, (O), and nitrogen (N)– have greater sorption capacity in plants when they are soluble and easily transported. This cycling is aided by the North American beaver’s hydrologic engineering. In the case of iron, the less frequently bioavailable ferrous iron, Fe(II), is more soluble, and therefore more easily absorbed into the system, than the more stable ferric iron, Fe(III).²⁵ Fe(III) is rarely found in aqueous form in a riverine system and is most abundant in solid form as ferrihydrite ($\text{Fe}_3\text{O}_4 \cdot 5\text{H}_2\text{O}$).²⁶ Fe(II), conversely, is always present and free in a riverine solution.^{27,28} If the nutrients are insoluble or a system is too dry, nutrients present are unable to be transported into or remediated by the system. Beaver-assisted environments ensure that those soluble nutrients are readily present, including Fe(II).²⁹ Given that iron is highly redox-sensitive, speciation is therefore highly indicative of the redox conditions within a system.

Beaver dam systems are critical in the face of changing climate. They mitigate hydrologic extremes like severe precipitation events, counteract warming riverine temperatures while maintaining water quality, and dictate indispensable biogeochemical cycles which keep essential elements available to plants and other organisms.^{7,29} However, growing climate extremes deplete summer baseflow and lessen floodplain capacities to store carbon, nutrients, and heavy metals in the floodplain. This lessened capacity is illustrated by decreased DO levels, signifying more insoluble nutrients that cannot be transported amongst the system. The role of floodplains is therefore reconditioned from serving as carbon sinks and architects of biogeochemical cycling to becoming carbon sources, emitters of ammonia gas, and generators of an overabundance of insoluble nutrients which contributes to eutrophication.³⁰ These impacts will be worsened by aridification and depleted baseflows.³¹

As such, the redox dynamics within a floodplain system’s surface waters– be it BDA, natural, or untreated– may serve as the foundation of riverine biogeochemical performance. Yet, much of this keystone redox fluctuation in BDA, natural, and untreated systems is predominantly documented during daylight hours, when DO is highest. The diurnal fluctuations in DO measurements demonstrate daytime super saturation of DO levels, while nighttime measurements show a swing to near anoxic conditions. Daytime measurements can only capture the redox conditions and species of those oxic states, meaning substantial portion of aerobic respiration and redox dynamics are underrepresented by missing nighttime observations.²⁹ Previous studies have examined redox dynamics within river-floodplain systems, but few have explored the complete 24-hour cycle of redox conditions across natural beaver dams, artificial beaver dams, and untreated floodplain waters.

This study investigated 1) how Fe(II) concentrations change with dissolved oxygen (DO) levels in beaver-impacted floodplains, 2) how the DO-Fe(II) relationship changes across surface water source types (I.e. ponds, seeps, and channels), 3) the diurnal relationship between Fe(II) concentrations and DO levels in beaver ponds, and 4) how Fe(II) and DO dynamics change across floodplain types (I.e. Natural beaver dam, degraded, and Beaver Dam Analog (BDA) floodplains).

The following hypotheses were tested to address our research questions:

- H1) Fe(II) and DO concentrations share a strong inverse relationship,
- H2) seeps have heightened Fe(II) concentrations than ponds or channels,
- H3) beaver ponds show a distinct inverse diurnal correlation between Fe(II) and DO measurements, and
- H4) BDAs and Natural Beaver ponds will have higher Fe(II) concentrations, while degraded floodplains will exhibit lower Fe(II) concentrations.

1. Specific Methodologies

1.1 Site Description & Sample Collection

We categorized sites into return seep, channel, and pond, focusing on beaver dam analogs (BDAs), natural, and untreated systems. Surface water samples at ponds and seeps were collected from the sediment-water interface. Surface water

samples from river channels were collected from the thalweg of the channel.. Sampling occurred at three locations: Crested Butte Land Trust (CBLT) Confluence Parcel (Crested Butte, CO; 38°52'41.64"N, 106°58'45.07"W), East River "Pumphouse" (Mount Crested Butte, CO; 38°55'39.49"N, 106°57'30.19"W), and Trail Creek Floodplains (Almont, CO; 38°54'3.55"N, 106°37'8.08"W) in July 2024 [Fig. 1]. Data from these sites provided a representative spread of hydrological and redox conditions across the study area. All discrete samples were extracted using 60 mL plastic syringes and filtered through single-use hydrophilic 0.45- μ m filters.



Figure 1: Sampling locations: Crested Butte Land Trust (CBLT) Confluence Parcel (Crested Butte, CO; 38°52'41.64"N, 106°58'45.07"W), East River "Pumphouse" (Mount Crested Butte, CO; 38°55'39.49"N, 106°57'30.19"W), and Trail Creek Floodplains (Almont, CO; 38°54'3.55"N, 106°37'8.08"W)

1.2 Sample Characterization & Electrochemistry

Measured parameters at each sampling location included dissolved oxygen, pH, specific conductivity, temperature, Fe(II) concentrations, and alkalinity. Sampling at CBLT Confluence Parcel included 3 pond locations, 3 return seep locations, and 2 in-stream locations. East River Pumphouse included 3 pond locations, 3 return seep locations, and 2 in-stream locations. Trail Creek focused on 3 BDA ponds, 2 degraded floodplain locations, and 3 natural beaver dam ponds.

In situ measurements of pH and specific conductivity were performed immediately after sample extraction using a Thermo Scientific Orion Star A pH/Conductivity Multiparameter Meter. Surface water samples were filtered, and Fe(II) measurements were preserved in ferrozine reagent, kept on ice, and shaded from sunlight.

1.3 Total Alkalinity Titration

Total Alkalinity was measured using the CHEMetrics Alkalinity Titret test kit. Surface water samples were filtered, mixed with activator solution, and titrated using pre-measured ampoules until a color change indicated the alkalinity level.

1.4 Dissolved Oxygen Monitoring

Dissolved oxygen was measured using a V-2000 Multi-Analyte Photometer for spot measurements and PME miniDOT Loggers for continuous monitoring. Covering specific conditions at the desired times, the spot DO measurements provided immediate data to gauge the system or further assessments needed while providing validation points for the continuous data. The continuous DO data identified the diurnal variations and supplied measurements for trend analysis of long term patterns or anomalies. The DO loggers and HOBO Pendant Temperature/Light loggers were utilized to include the influences of temperature changes, photosynthesis, and respiration cycles on the DO diurnal fluctuations. Atmospheric mixing may also impact DO concentrations. The V-2000 uses the Indigo Carmine method, with calibration prior to each measurement series. The miniDOT Logger recorded DO and temperature every 10 minutes continuously while in the field.

1.5 Fe(II) Concentration Measurements

Fe(II) concentrations were measured testing two colorimetric methods: the ferrozine technique and the Iron (Ferrous) Vacu-Vials Instrumental CHEMetrics kit technique. The ferrozine and CHEMetrics procedures were measured using a Microplate Reader and V-2000 Photometer, respectively, to measure a color change proportional to the concentration of our analyte. Ferrozine is an organic sodium salt that, when combined with 1 molar hydrochloric acid and ammonium acetate, can be used to detect and measure ferrous iron concentrations at a high sensitivity in aqueous samples. Ferrozine reacts with ferrous iron to form a stable, but light sensitive, fuschia-colored complex. We preserved our surface water samples in the field with ferrozine solution, measured the absorbance at the complex's absorption peak of 562 nanometers, and recorded Fe(II) concentrations according to the calibration curve of standard solutions of known Fe(II) concentrations (10 μM , 50 μM , 100 μM , 500 μM , and 1000 μM). Triplicate Fe(II) samples were preserved in the field under HCl and Ferrozine which chelated available Fe(II) into stable form until later analyzed in the lab on the plate reader.

Both colorimetric methods were employed for all spot sampling, including in-person and automated measurements.

1.6 24-Hour Sampling

Automated 24-hour samples were collected at the sediment-water interface by implementing an ISCO 6712 autosampler. Each collection time underwent a purge cycle to clear the intake tubing to avoid sample contamination. 1000 mL samples were distributed by the ISCO into acid washed bottles, collected after a complete sampling cycle, shaded from sun exposure to preserve redox conditions of Fe (II) concentrations until analyzed in the lab.

Time spent in the ISCO before ice preservation was also recorded for further statistical analysis of its contributions to total variance in the data.

Two 24-hr sampling campaigns were conducted. During initial ISCO deployment, paired in-person verification of Fe(II) concentrations occurred over a 24-hour period for the July 11-12th campaign. The following 24-hr campaign was fully automated. Surface water samples were collected hourly and in-field electrochemistry measurements were recorded. Subsequent ISCO sampling continued autonomously.

1.7 Statistical Analysis

Linear regression analysis was performed to determine the relationships and variations between DO and Fe(II) concentrations across floodplain systems. The ferrozine and CHEMetrics techniques' reliability was assessed using Bias analyses. Box-and-whisker plots provided a cleaner representation of the data trends across sample types while presenting

multiple analytes and sample site details, including the interquartile range, outliers, and medians of DO, Fe(II) concentrations, SpC, pH, and alkalinity across floodplain systems and sample types. Triplicate samples of each site reduced random error and ensured representative results. Comparison across surface water samples using all measured parameters was achieved by a KMeans clustering with three clusters, and a t-SNE nonlinear visualization on log-transformed data. Statistical significance and redox relationship strength were assessed using Python for Pearson Correlation Coefficients, Mutual Information tests, Kendall's Tau Correlation Coefficients, and p-values on log-transformed data.

2. Results & Discussion

2.1 DO and Fe(II) Dynamics of Beaver-Impacted Floodplains

A combination of in situ automated and in person surface water sampling across two floodplain locations and three sample source types indicated there is a relationship between DO levels and Fe(II) concentrations in floodplain systems. Fe(II) data collected at the Confluence and Pumphouse locations demonstrated an inverse relationship between the variability of Fe(II) as accounted for by the variability of DO measurements, but that degree of correlation varies between floodplain locations. At Confluence, DO and Fe(II) share a statistically significant inverse relationship where 62.68% of the variability in Fe(II) can be explained by the variation in DO measurements [Fig. 2].

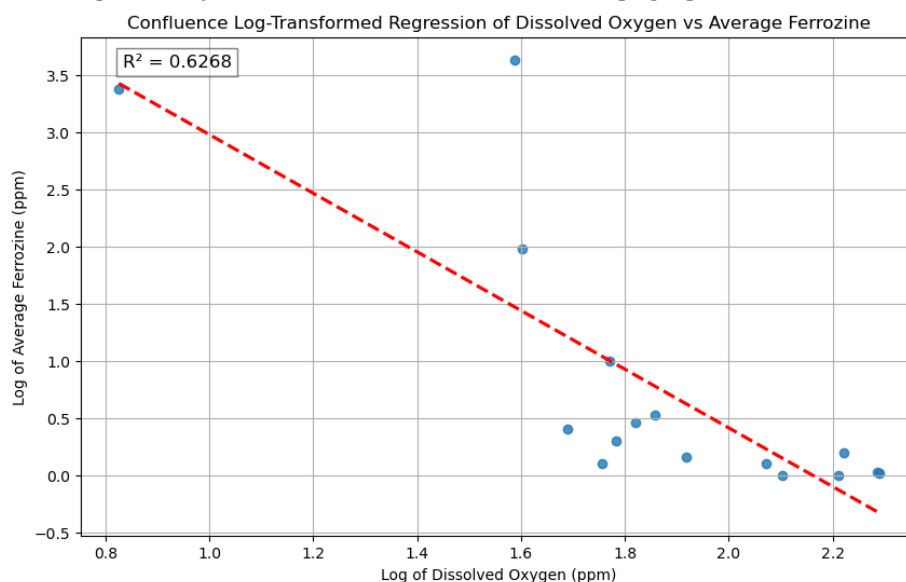


Figure 2: Confluence Regression of Dissolved Oxygen Concentrations (ppm) vs Average Ferrozine Concentrations (ppm). $R^2 = 0.6268$

However, at Pumphouse, while there is still an inverse relationship between DO and Fe(II), DO variations only account for 28.95% of the variability in Fe(II) measurements [Fig. 3]. This weaker inverse relationship indicates that other factors are likely influencing the Fe(II) fluxes at Pumphouse.

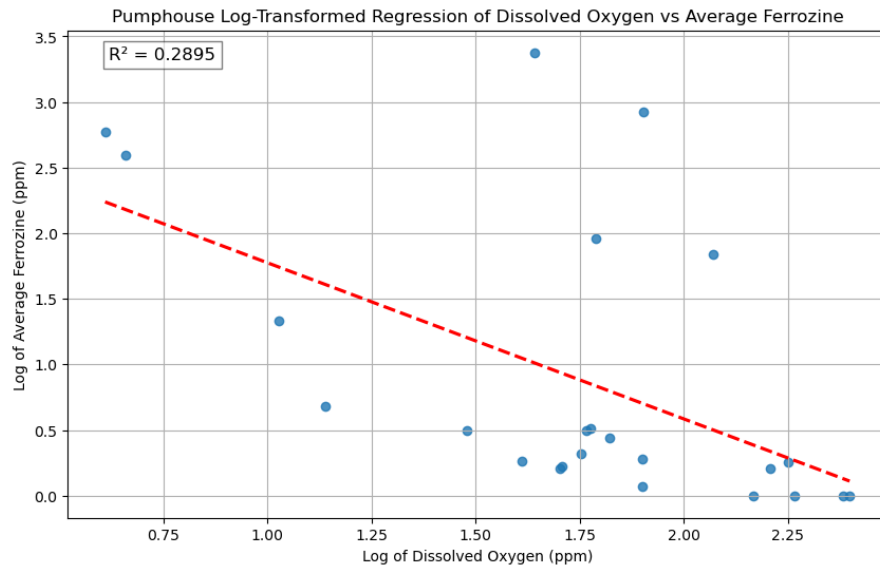


Figure 3: Pumphouse Regression of Dissolved Oxygen Concentrations (ppm) vs Average Ferrozine Concentrations (ppm). $R^2 = 0.2895$

Other possible determinants include residence times of water and iron, water-sediment interactions impacting sorption or desorption processes as a result of differing bedrock chemistry, microbial activity and diversity, presence of competing ions, available organic carbon, and diverse hydrodynamic conditions.^{32–34} Residence time of water, or the amount of time water remains in a particular area, impacts aerobic respiration rates and the amount of dissolved organic carbon present in that system.³⁵ Stagnant water sources, like ponds, experience high residence times where anoxic conditions persists and accumulate more dissolved organic carbon (DOC).³⁶ This creates favorable conditions for dissolution of iron oxides and releases Fe(II).³⁷ Residence time of Fe(II), or the average time Fe(II) remains in a dissolved, suspended or adsorbed state in a given system, determines both the degree of presence of Fe(II) in the floodplain and the ability to measure the soluble Fe(II).³⁸

Residence times of substances are determined by physical, biological, and chemical processes– part of which can be accounted for by the DO in the floodplain, but Pumphouse likely has additional dynamics at play. Holocene alluvial deposits along the East River increase and vary soil permeability, resulting in faster or complex groundwater flow paths which may reduce the residence time.^{39,40} Iron can also co-precipitate with or adsorb onto sediments, or compete with and form insoluble compounds with other ions like phosphate.⁴¹ Each of those aspects impact the residence time of Fe(II) and its mobility and soluble state measurability in floodplains. Additionally, interactions with other elements like sulfur can result in the formation of chemical compounds like iron sulfides in anoxic conditions, removing iron from the water column.^{42,43} These impacts on the solubility and mobility of iron in floodplains results in differing degrees of the inverse relationship between DO and Fe(II) at Pumphouse and Confluence.

2.2 Testing In-field Fe(II) Measurement Methodologies

In-field testing of methods for Fe(II) measurements yielded the CHEMetrics technique as being highly variable and having an increased bias in Fe(II) measurements as compared to the Ferrozine technique. Data from histograms of Fe(II) concentrations measured with Ferrozine versus CHEMetrics techniques provided differences in measurement ranges and frequencies to apply to Bias Analyses. Depicting the highlighted differences exemplifies any systematic biases or average differences between the two methods.

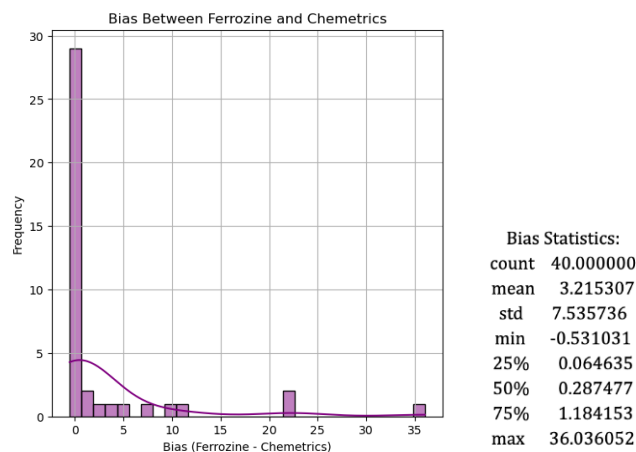
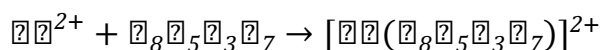


Figure 4: Bias analysis between Ferrozine and Chemetrics Fe(II) concentration measurements: Frequency vs Bias of Ferrozine Fe(II) measurements minus Chemetrics Fe(II) measurements.

CHEMetrics was on average biased to measure 3.215 mg/L less of Fe(II) concentrations than the Ferrozine technique. The standard deviation, however, was 7.536, indicating there was a high variability in the bias and the two methods varied widely. Bias was more likely to occur at higher concentrations, likely due to the 6.000 ppm limitation of the CHEMetrics technique. The maximum observed bias demonstrated that in some cases chemetrics measured 36.036 mg/L less than the Ferrozine technique [Fig. 4]. Chemetrics maintained a systematic error in its measurements, with a bias to measure lower Fe(II) measurements than were present in the system.

The bias measurements between Ferrozine and Chemetrics measurements can potentially be accounted for by examining the sensitivity, specificity, and ability of each method to avoid false positives. The rapid test Chemetrics kits utilize an adaptable iron determination analysis which uses 1,10-phenanthroline, a chelating agent, that targets Fe(II). This chemical compound binds to Fe(II), creating a stable metal complex measured by the photometer. That complex is dependent on reaction kinetics to provide enough of a duration for a complete binding of Fe(II) to 1,10-phenanthroline.^{44,45} If reaction time is not sufficient, the reaction will be incomplete and result in lower Fe(II) recordings, particularly at low Fe(II) concentration ranges. For the Chemetrics, Fe(II) was initially only measured at a set 1 minute after the sample pull. However, under or overshoot Fe(II) concentrations, especially at low Fe(II) ranges, prompted a re-evaluation of that reaction time. Measurements were then taken 1 and 5 minutes after the sampling, and again in the lab at the conclusion of the field day. These results yielded slightly closer values to what would be thermodynamically expected in the systems. Reaction time impacts measurements less at higher concentrations, as increasing the concentration of the reacting substances increases the likelihood that those particles will collide. Reaction rates are dependent on the frequency of those collisions, so higher Fe(II) concentrations resulted in faster reaction times and more reliable measurements. Overall, the Chemetrics technique is not as sensitive as the Ferrozine technique when looking at lower concentrations of Fe(II), or concentration values above 6.000 ppm. The Ferrozine technique is based on forming a highly sensitive stable complex with Fe(II) that produces a strong colorimetric signal.⁴⁶ So, the Ferrozine reagent's high sensitivity provides measurable signals across a wider range of concentrations, allowing for accurate readings even at low concentrations of Fe(II), or concentrations exceeding 6.000 ppm. The chemical reaction between Ferrozine and Fe(II) results in a chemical complex in which Fe(II) is coordinated to the Ferrozine reagent [Eq. 1].



Equation 1: The chemical reaction between Ferrozine and Fe(II).

There are no changes of species, leaving no loss of concentrations and making the Ferrozine complexation highly specific to Fe(II). Since Ferrozine does not react with Fe(III) or other ions present in the system, it can only measure Fe(II) concentrations and is incapable of producing false positives related to iron detection.⁴⁶

The Fe(II) and 1,10-phenanthroline reaction is also pH dependent. Too high a pH can result in incomplete formation of the Fe(II)-phenanthroline complex, leading to lower Fe(II) measurements.⁴⁴ Ferrozine concentrations, however, are preserved at a low pH, preventing the samples from precipitating out too quickly. pH can be impacted by temperature or

UV.⁴⁶ So, 1,10-phenanthroline’s stability is also highly dependent on the surrounding light and temperatures at the time of the reactions.⁴⁵ Most fieldwork was completed in open floodplains with exposure to direct sunlight and UV. Both the photometer and the Chemetrics kits were partially exposed to these UV and temperature increases, despite mitigation efforts. Higher light or temperature exposures could degrade the Fe(II)-phenanthroline complex, possibly accounting for results of lower Fe(II) measurements. In-situ observations showed that the CHEMetrics Vacu-viles also did not fill at consistent volumes. These differing sample volumes may have resulted in lower or higher samples compared to the chelating agent, likely creating variable reaction times for each of our samples. These properties indicate that the Chemetrics technique is not reliable for taking in situ measurements of Fe(II) concentrations due to its sensitivity, susceptibility to interference, and higher incidences of false negatives or positives.

2.3 DO and Fe(II) Clustering and Correlations of Beaver-impacted Floodplains by Source Type

In order to assess the relationships of DO and Fe(II) between the source types, we performed a KMeans clustering with three clusters, and a t-SNE nonlinear visualization to reduce dimensionality and clarify the distinct clusters [Fig. 5]. The data was log-transformed prior to all analyses to reduce impacts of outliers, stabilize any variance, and normalize the data. Running secondary analyses for the distributions of average Ferrozine Fe(II) measurements, average Chemetrics Fe(II) measurements, point DO, specific conductivity pH, and alkalinity by cluster demonstrated the Fe(II) measurements and DO as having the largest correlation by cluster assignment. A log-transformed ANOVA test of the KMeans clustering by sample type showed Ferrozine and Chemetrics as having statistically significant differences between sample types, while point DO, specific conductivity , pH did not. Alkalinity was close to being statistically significant, with a p-value of 0.055, but does not demonstrate a strong significance.

KMeans Clustering with t-SNE and Cluster Radii

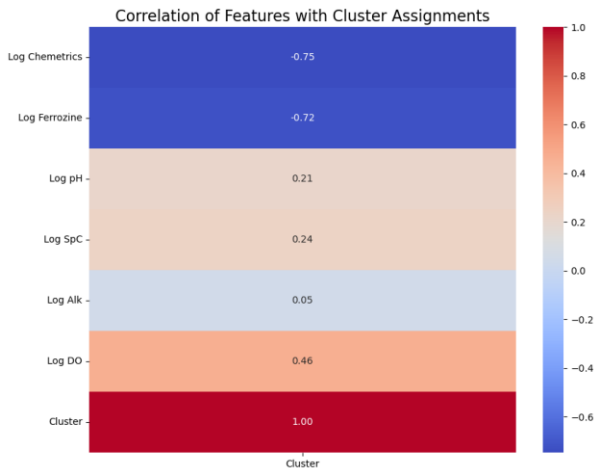
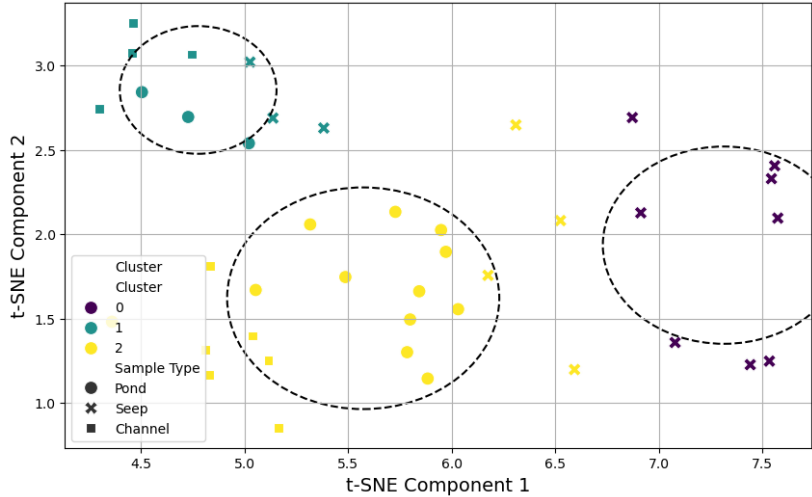


Figure 5: KMeans Clustering using t-distributed stochastic neighbor embedding (t-SNE) of surface water samples (left). Correlation matrix of measured parameters correlation to cluster assignments for KMeans Clustering (right).

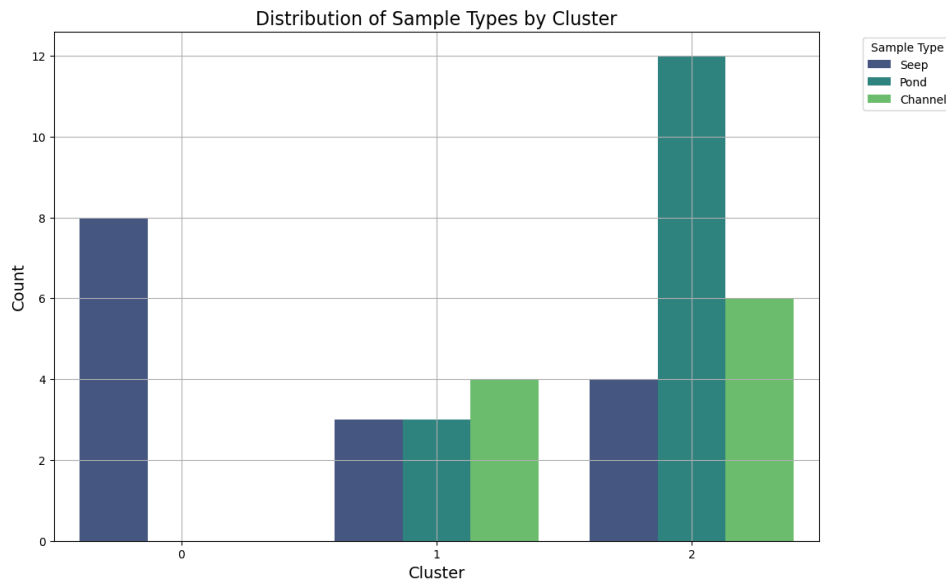


Figure 6: Distribution of Surface Water Sources Across KMeans Clusters.

Cluster 0 is exclusively made up of surface water samples from seeps [Fig. 6]. This cluster represents environments with high Fe(II) concentrations (2.80-36.76 ppm), moderate to high pH (6.56-7.93), moderate specific conductivity (198.53-829.37 $\mu\text{S}/\text{cm}$), high alkalinity (85-200 mg/L), and low DO measurements (0.84-5.72 ppm). Cluster 1 is characterized by low Fe(II) concentrations (0-1.72 ppm), moderate to lower pH (6.27-6.98), low specific conductivity (72.85-229.13 $\mu\text{S}/\text{cm}$), low alkalinity (36-80 mg/L), and high DO (4.42-8.87 ppm). This cluster is slightly dominated by channel (in-stream) surface water samples. Cluster 2 is represented by low Fe(II) measurements with multiple high outliers (0-6.24 ppm), moderate to high pH (6.58-7.88), high and more variable specific conductivity (192.33-1199.00 $\mu\text{S}/\text{cm}$), highly variable alkalinity (52-225 mg/L), and moderate but highly variable DO (2.13-10.01 ppm). Cluster 2 is predominantly surface water samples from ponds.

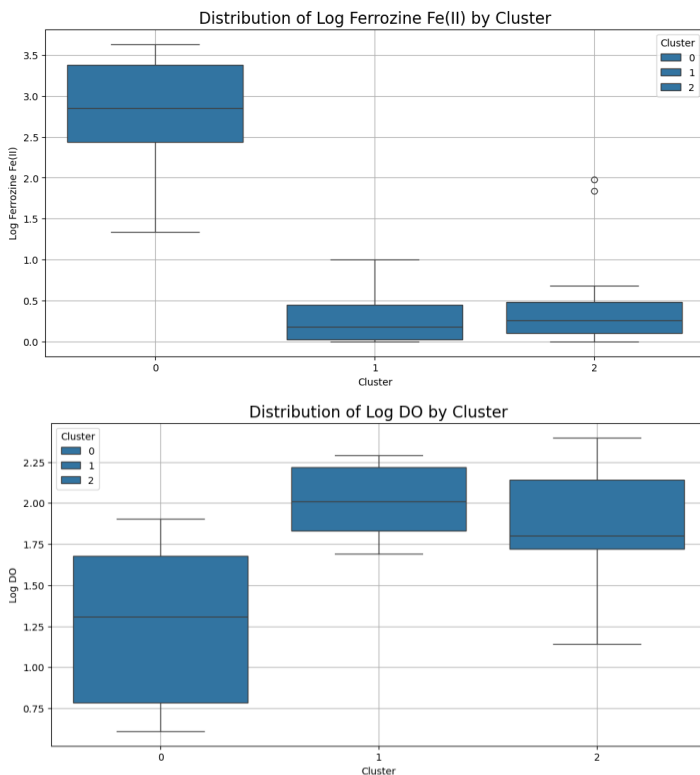


Figure 7: Surface Water Sample Clusters as compared by (left) distributions of Ferrozine Fe(II) concentrations, and (right) distributions of Dissolved Oxygen concentrations.

	Variable	Group 1	Group 2	Mean Difference	p-Value	Significant
0	Log Chemetrics Fe(II)	0	1	-1.4953	0.0000	Yes
1	Log Chemetrics Fe(II)	0	2	-1.4376	0.0000	Yes
2	Log Chemetrics Fe(II)	1	2	0.0577	0.8643	No
3	Log Ferrozine Fe(II)	0	1	-2.4651	0.0000	Yes
4	Log Ferrozine Fe(II)	0	2	-2.3463	0.0000	Yes
5	Log Ferrozine Fe(II)	1	2	0.1188	0.8355	No
6	Log pH	0	1	-0.0560	0.0189	Yes
7	Log pH	0	2	0.0089	0.8607	No
8	Log pH	1	2	0.0650	0.0006	Yes
9	Log SpC	0	1	-1.1438	0.0000	Yes
10	Log SpC	0	2	0.0753	0.9091	No
11	Log SpC	1	2	1.2192	0.0000	Yes
12	Log Alk	0	1	-0.9186	0.0000	Yes
13	Log Alk	0	2	-0.1695	0.5238	No
14	Log Alk	1	2	0.7492	0.0000	Yes
15	Log DO	0	1	0.7613	0.0001	Yes
16	Log DO	0	2	0.6318	0.0003	Yes
17	Log DO	1	2	-0.1295	0.5985	No

Figure 8: Multiple Comparison of Means for Surface Water Sample Clusters using Tukey. Compared across Chemetrics Fe(II) measurements, Ferrozine Fe(II) measurements, pH, specific conductivity, alkalinity, and Dissolved Oxygen.

Seeps were significantly different from ponds and channels with respect to the selected variables. Tukey's HSD tests across the clusters result in significant differences between clusters 0 and 1, and 0 and 2 for Ferrozine Fe(II) and DO measurements. 1 and 2 were not significantly different for DO and Ferrozine Fe(II) measurements. However, 1 and 2 were significantly different for pH, specific conductivity, and alkalinity [Fig. 8]. The distribution of sample types by clusters visualizes that seeps have significantly different Fe(II) and DO measurements when compared with the clusters dominated by ponds or channels. The significant differences between clusters dominated by ponds or channels is their pH, specific conductivity, and alkalinity measurements. Since the ponds demonstrate highly varied DO measurements, this accounts for the lack of significant distinction between clusters dominated by ponds and clusters dominated by channels. Additionally, throughout the flooding season, some downstream channel samples were cut off from the main channel and displayed altered redox conditions, potentially impacting the clustering, as well. Seeps, however, have a lower DO range with much less variance, making them statistically distinct from both ponds and channels [Fig. 7].

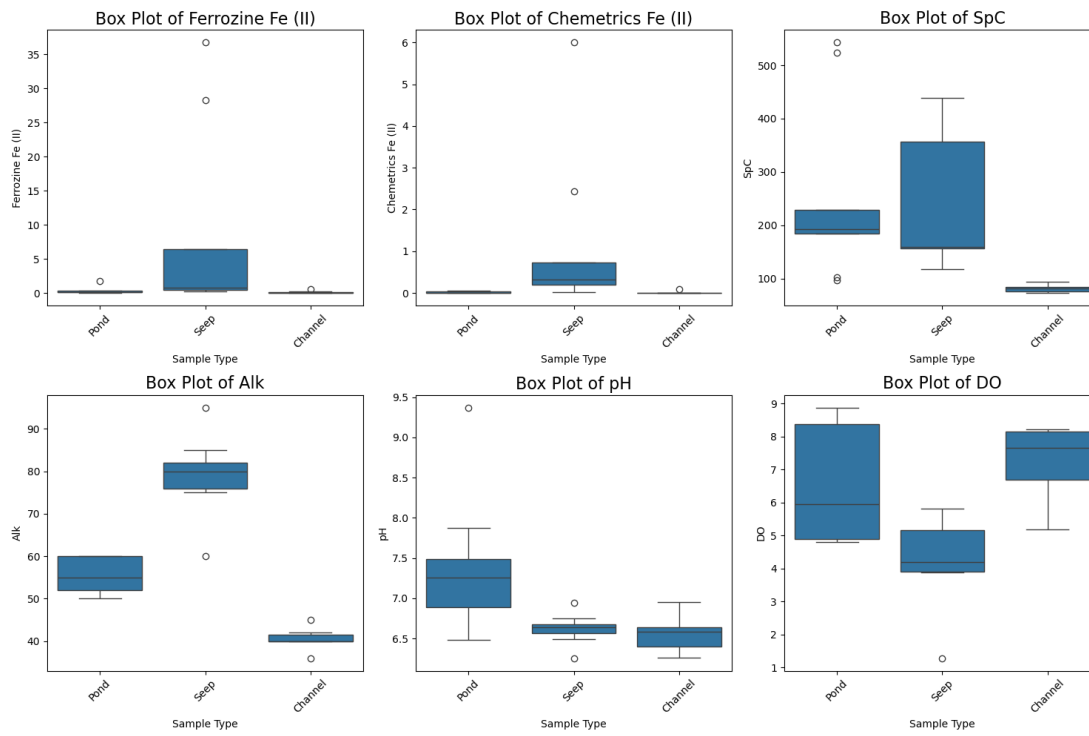


Figure 9: Confluence Box plots of Ferrozine Fe(II) measurements, Chemetrics Fe(II) measurements, specific conductivity, alkalinity, pH, and point Dissolved Oxygen across water sample source type

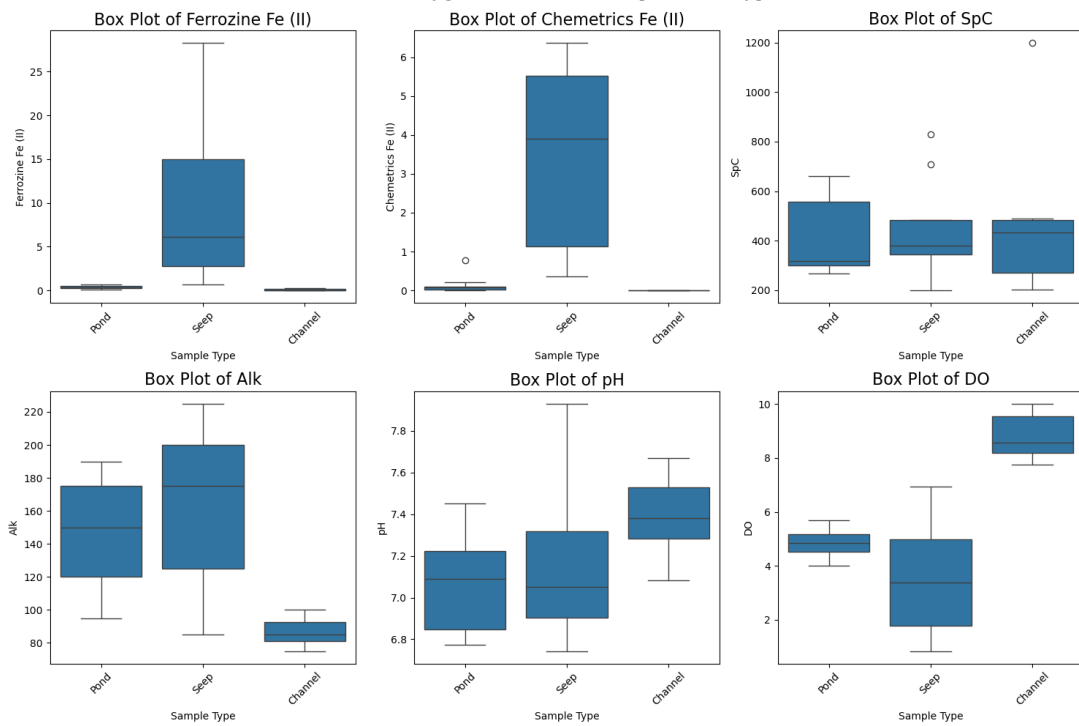


Figure 10: Confluence Box plots of Ferrozine Fe(II) measurements, Chemetrics Fe(II) measurements, specific conductivity, alkalinity, pH, and Point Dissolved Oxygen across water sample source type

	Variable	Sum of Squares (Between Groups)	Degrees of Freedom (Between Groups)	F-Statistic	P-Value	Sum of Squares (Residual)
0	Log Ferrozine Fe (II)	38.988402	2.0	20.643969	0.000011	19.830403
1	Log Chemetrics Fe (II)	43.303060	2.0	18.659333	0.000022	24.367545
2	Log SpC	0.024252	2.0	0.052248	0.949216	4.873799
3	Log Alk	1.403866	2.0	11.851225	0.000359	1.243803
4	Log pH	0.007688	2.0	2.290618	0.125929	0.035240
5	Log DO	4.980164	2.0	10.578868	0.000664	4.943036

Figure 11: Pumphouse log-transformed ANOVA for Surface Water Sample compared across Chemetrics Fe(II) measurements, Ferrozine Fe(II) measurements, pH, specific conductivity, alkalinity, and Dissolved Oxygen.

	Variable	Sum of Squares (Between Groups)	Degrees of Freedom (Between Groups)	F-Statistic	P-Value	Sum of Squares (Residual)
0	Log Ferrozine Fe (II)	27.474649	2.0	4.518706	0.032366	39.521319
1	Log Chemetrics Fe (II)	2.144533	2.0	0.422670	0.663985	32.979533
2	Log SpC	3.908636	2.0	6.938372	0.008906	3.661685
3	Log Alk	1.123266	2.0	43.319536	0.000002	0.168544
4	Log pH	0.012961	2.0	2.762594	0.100040	0.030495
5	Log DO	1.237226	2.0	3.851750	0.048568	2.087874

Figure 12: Confluence log-transformed ANOVA for Surface Water Sample compared across Chemetrics Fe(II) measurements, Ferrozine Fe(II) measurements, pH, specific conductivity, alkalinity, and Dissolved Oxygen.

At the Confluence site, seeps showed the largest median Ferrozine Fe(II), Chemetrics Fe(II), and alkalinity measurements, with high outliers, and the lowest median DO measurements [Fig. 9]. Ponds had the largest median specific conductivity, and the largest variance in pH and DO. Channels maintained low Fe(II), specific conductivity, alkalinity, and low but varied pH measurements with the largest median DO. Pumphouse demonstrated similar relationships, except with higher ranges of alkalinity, specific conductivity, and DO for the seep locations [Fig. 10]. A log-transformed ANOVA test demonstrated that the Ferrozine Fe(II), Chemetrics Fe(II), alkalinity, and DO had statistically significant differences across surface water source types, suggesting sample type plays a significant role in influencing these parameters at the Pumphouse site [Fig. 11]. Meanwhile, only Ferrozine Fe(II), specific conductivity, alkalinity, and dissolved oxygen had statistically significant differences between source types at Confluence [Fig. 12].

2.4 Diurnal Biogeochemical Cycling: DO and Fe(II) Concentration Correlations in Beaver Ponds

The diurnal inverse relationship between DO and Fe(II) concentrations was not consistently apparent throughout 24-hour sampling probably as a result of variability and complexity between the processes governing Fe(II) cycling and oxygen dynamics in beaver ponds, as well as sampling limitations. Over the course of two 24-hour sampling campaigns throughout July at the Confluence floodplain, DO and Fe(II) demonstrated a distinct diurnal inverse correlation at CP-BeavPond1-1 for the second campaign sampling. The July 11-12th sampling took both in-person and auto-sampled Fe(II) measurements to compare with both point and continuous DO. The July 23-24th sampling continued purely with auto-sampled Fe(II), point DO, and continuous DO measurements.

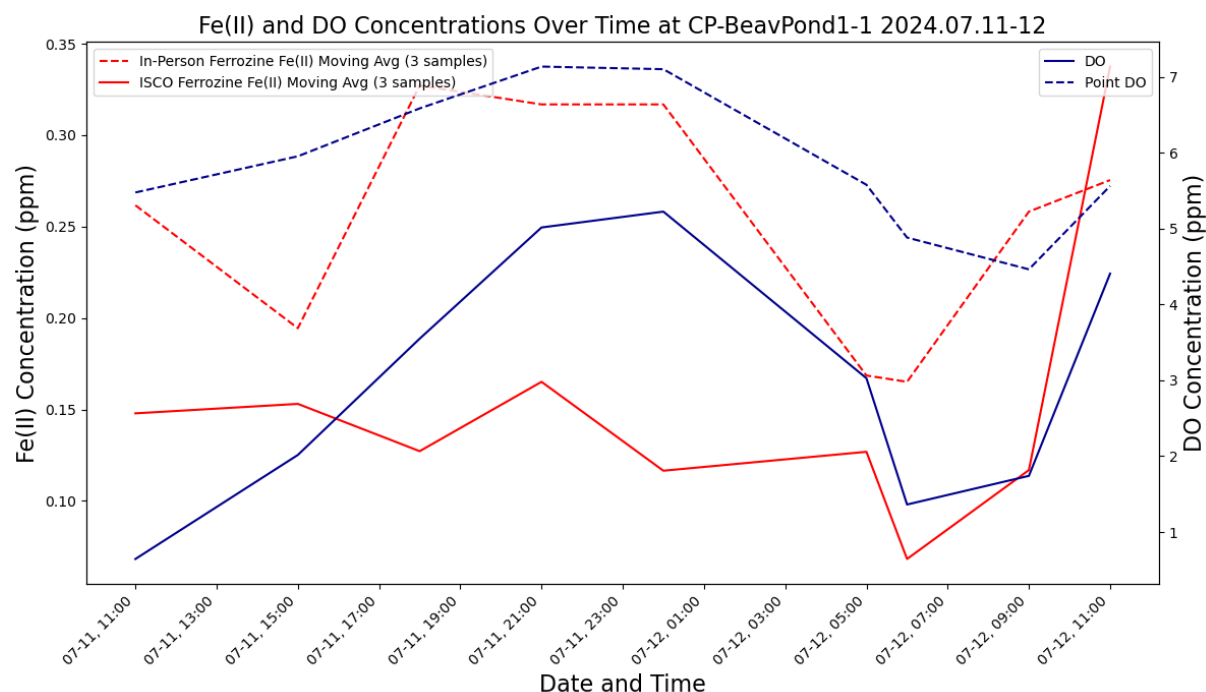


Figure 13: July 11-12th, 2024 24hr Sampling Campaign at Confluence: Fe(II) and DO concentrations over CP-BeavPond1-1's diurnal cycle with (i) In-person Fe(II) Ferrozine measurements, (ii) Auto-sampled Fe(II) Ferrozine measurements, (iii) Point Dissolved Oxygen, and (iv) continuous Dissolved Oxygen. Pearson correlation coefficient of 0.384 between continuous DO and ISCO Ferrozine Fe(II) measurements with a p-value of 0.308.

For July 11-12th, the Pearson correlation coefficient was 0.384 between continuous DO and ISCO Ferrozine Fe(II) concentrations with a p-value of 0.308 [Fig. 13]. At that time, the continuous DO and ISCO Ferrozine Fe(II) measurements held a weak positive correlation that was not statistically significant. Additionally, in-person Ferrozine Fe(II) measurements recorded higher concentrations than the auto-sampled Ferrozine Fe(II) measurements. Possible explanations include Fe(II) ISCO sample degradation due to exposure to oxidation or temperature changes over time, as well as different reaction kinetics between the times of in-person and auto-sampled measurements. As the beaver pond water samples must remain in the ISCO until retrieval, there is significant time in which the autosampler's lack of preservation conditions may have impacted the precipitation of Fe(II). As Fe(II) is highly sensitive to oxidation, any exposure to light, temperature, or oxygen changes could lead to Fe(II) oxidizing and cause a lower measured concentration of Fe(II).⁴⁷ In-person Fe(II) measurements capture the concentrations instantly and preserve the sample in a Ferrozine-HCl solution, maintaining pH and preventing any shifts in chemical equilibrium. As there was no 1 M HCl in the ISCO, delays in analysis may reduce measurable Fe(II).⁴⁶

Colorimetric point DO measurements were also notably higher than the continuous DO taken by the MiniDOT logger likely due to the inconsistent Vacu-vile filling, differing sensitivities and calibrations between the photometer and miniDOTs, any oxidation of the samples during extraction, or environmental interferences like temperature or light exposure to the photometer or samples.^{48,49}

DO levels persistently follow a diurnal cycle, with more oxic periods during the day driven by a number of factors. Photochemical reactions from UV and physical light, algal and microbial respiration, root oxygen release, dissolved organic matter generating reactive oxygen species, wind and water turbulence, or temperature-driven mixing can all lead to more aerobic conditions.^{50,51} Preliminary observations from miniDOT loggers showed the study sites all became anoxic at night while respiration exceeds oxygen production. So within a 24-hr period, CP-BeavPond1-1 would consistently become anoxic. However, the exact timing of that period can vary significantly. Preliminary observations saw ponds transition to anoxic conditions at several different times including anywhere from 6:00 pm, to midnight, to 6:00 am. The inconsistent timing of anoxia may therefore lead to unclear observable relationships between DO and Fe(II) concentrations without continuous sampling. Over the first campaign, feedback on the pond's oxygen dynamics could only be received instantaneously. In addition there was a limit to the number of times sampling could take place throughout the night as a result of in-person capacity and finite materials. These restrictions prevented continuous point sampling and possibly constrained the results to an incomplete oxygen cycle. The large gap in sampling from midnight, July 12th, until 5:00 am, July 12th is missing components of the DO variation.

Under the assumption that the anoxic period must have occurred during that gap in time, the full continuous DO data set was reviewed. All continuous DO points were plotted against the Continuous DO concentrations from the select three sampling times across that gap: 23:55 on July 11th, 04:55 on July 12th, and 05:55 on July 12th. The miniDOT loggers took continuous DO measurements 5 minutes prior to the ISCO sampling times of 24:00 on July 12th, 05:00 on July 12th, and 06:00 on July 12th to avoid any possible oxidation from turbulence during the auto-sampling pond water collection. When comparing the continuous DO data, much of the variability within the oxygen cycle is unaccounted for when only utilizing the continuous DO concentrations from the sampling times [Fig. 14]. Those partially recorded DO concentrations left missing variations in DO fluctuations, also indicating that only sampling Fe(II) concentrations at those times may also exclude portions of Fe(II) fluctuations, as well.

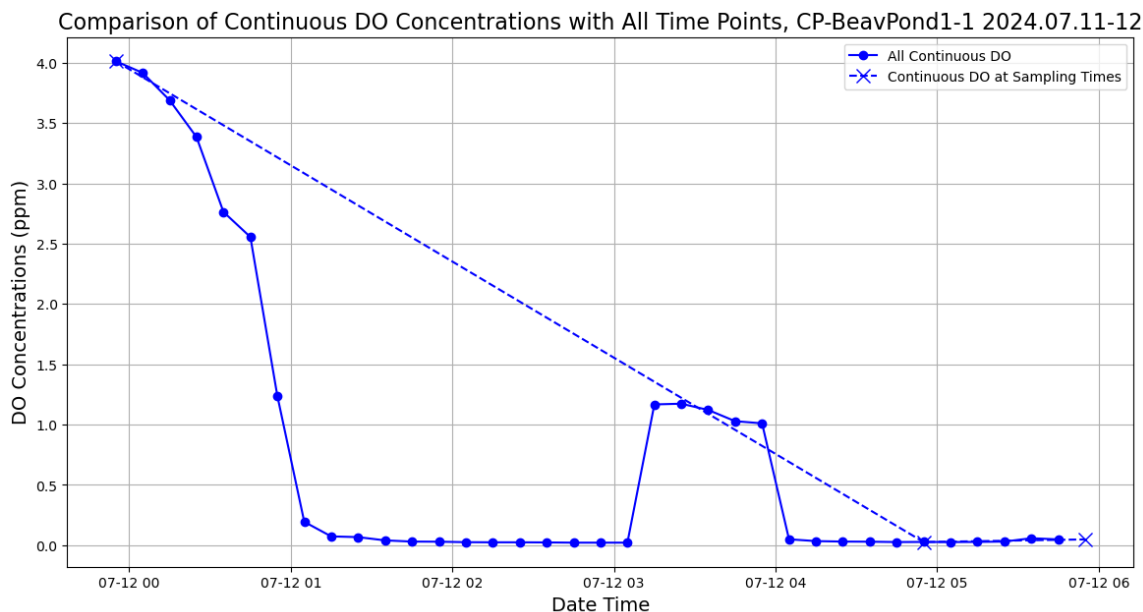


Figure 14: July 11-12th, 2024 24hr Sampling at Confluence: Continuous DO concentrations from 23:55, July 11th, to 5:55, July 12th over CP-BeavPond1-1's diurnal cycle.

However, when comparing Fe(II) concentrations with the full continuous DO data set over those select sampling times, there was a strong association between the variables with a Mutual Information value of 0.73. Using the Mutual Information captures both linear and non-linear dependencies, while the Pearson correlation can only account for the linear. The Kendall's Tau correlation coefficient was -0.44 with a p-value of 0.0002, suggesting a moderate inverse ordinal relationship between the continuous DO and ISCO Ferrozine Fe(II) concentrations [Fig. 15]. As this is a monotonic relationship, Kendall's Tau can assess categorical values that have a meaningful sequence but include values without uniform intervals. The results signify that DO and Fe(II) have a strong inverse relationship that is highly statistically significant over this segment of time. The lack of consistent apparentness of the diurnal inverse relationship between DO and Fe(II) concentrations throughout 24-hour sampling campaigns is therefore likely a result of inconstant sampling that is prone to excluding important fluctuations in the iron and oxygen cycle.

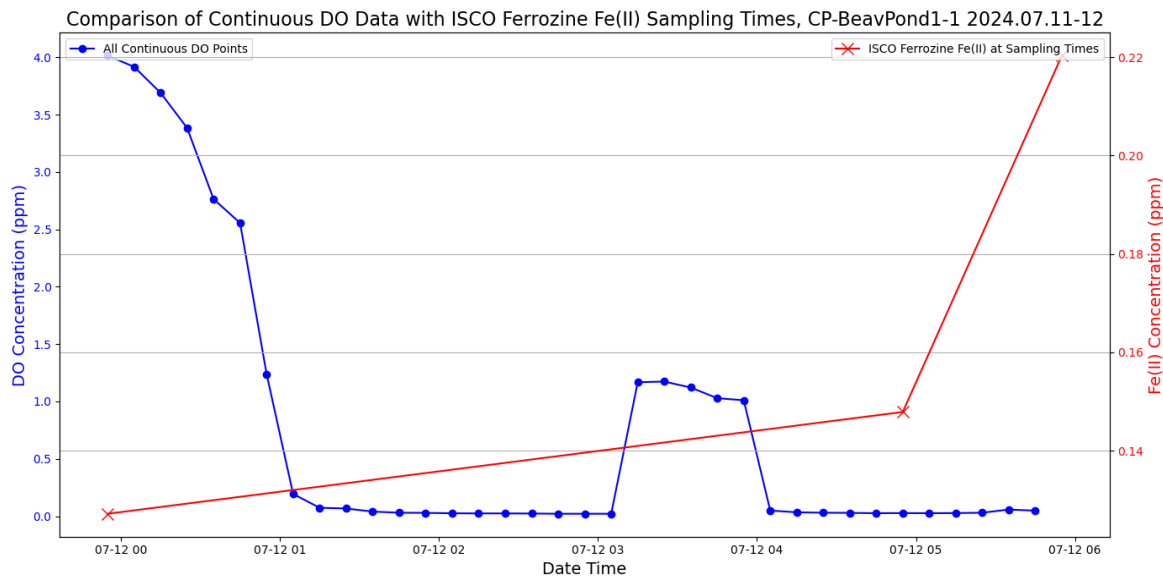


Figure 15: July 11-12th, 2024 24hr Sampling at Confluence: All continuous DO vs ISCO Ferrozine Fe(II) concentrations from 23:55, July 11th, to 5:55, July 12th over CP-BeavPond1-1's diurnal cycle. Mutual Information value of 0.73. Kendall's Tau Correlation Coefficient of -0.44 with a p-value of 0.0002.

For the second 24-hour sampling campaign, the Pearson correlation coefficient was -0.777 between continuous DO and ISCO Ferrozine Fe(II) concentrations with a p-value of 0.014 [Fig. 16]. At that time, the continuous DO and ISCO Ferrozine Fe(II) measurements had a strong inverse correlation that was statistically significant. For this campaign, only auto-sampling Fe(II) concentrations were taken. This allowed for in-situ review of the miniDOT loggers' data and appropriate selection of desired surface water samples for distinct oxic and anoxic periods. Therefore, there was a distinct diurnal inverse relationship between DO and Fe(II) concentrations in beaver ponds, however, the sampling required to properly document it necessitates either exclusively auto-sampled collection of surface water, or intensive in-person sampling in order to capture sufficient continuous data.

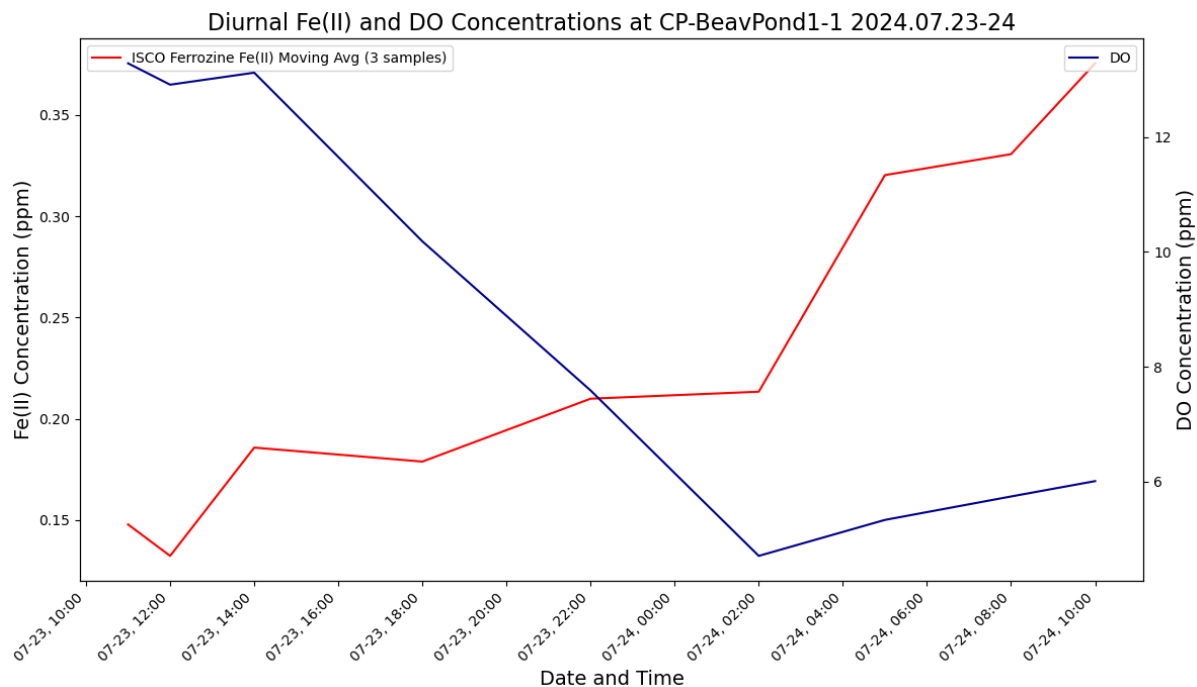


Figure 16: July 23-24th, 2024 24hr Sampling Campaign at Confluence: Fe(II) and DO concentrations over CP-BeavPond1-1's diurnal cycle with (i) In-person Fe(II) Ferrozine measurements, (ii) Auto-sampled Fe(II) Ferrozine measurements, (iii) Point Dissolved Oxygen, and (iv) continuous Dissolved Oxygen. Pearson Correlation coefficient of -0.777 between continuous DO and ISCO Ferrozine Fe(II) measurements with a p-value of 0.014.

2.5 Restoration: DO vs Fe(II) Fluctuations Across Floodplain Types

In examining three distinct sections across the Trail Creek floodplain (Natural, Degraded, and BDA), there appeared to be no distinct differences in the floodplain types 3 years post installation according to our parameters. A log-transformed ANOVA demonstrated that only the pH and specific conductivity had statistically significant differences across floodplain types, suggesting floodplain type does not play a significant role in influencing these parameters at this time [Fig. 17]. The lack of significant differences between Fe(II) and DO concentrations across floodplain type can possibly be attributed to the timeframe of the recovery and other ecological changes, any initial homogeneity in the landscape, hydrologic connectivity between sites, the effectiveness of the BDAs, or resilience to restoration alterations.^{15,52} The lack of time between BDA installation and this investigation may not have given the BDAs enough room to alter the surface water conditions and interactions. BDAs effectiveness is dependent on passing a certain threshold of hydraulic head.¹⁵ Insufficient time may have prevented the BDA's from achieving those size-dependent functions, limiting the hyporheic exchange and biogeochemical cycling around the BDAs from resembling the natural beaver dams. Additionally, BDAs are prone to being blown out by varied flow or circumnavigated, leaving them functionless. This was observed at a number of BDAs in the Trail Creek floodplain. Furthermore, the lack of mapping for flow or hydrologic connectivity prevents any elimination of its impact on Fe(II) availability and fluctuations across these floodplain types.

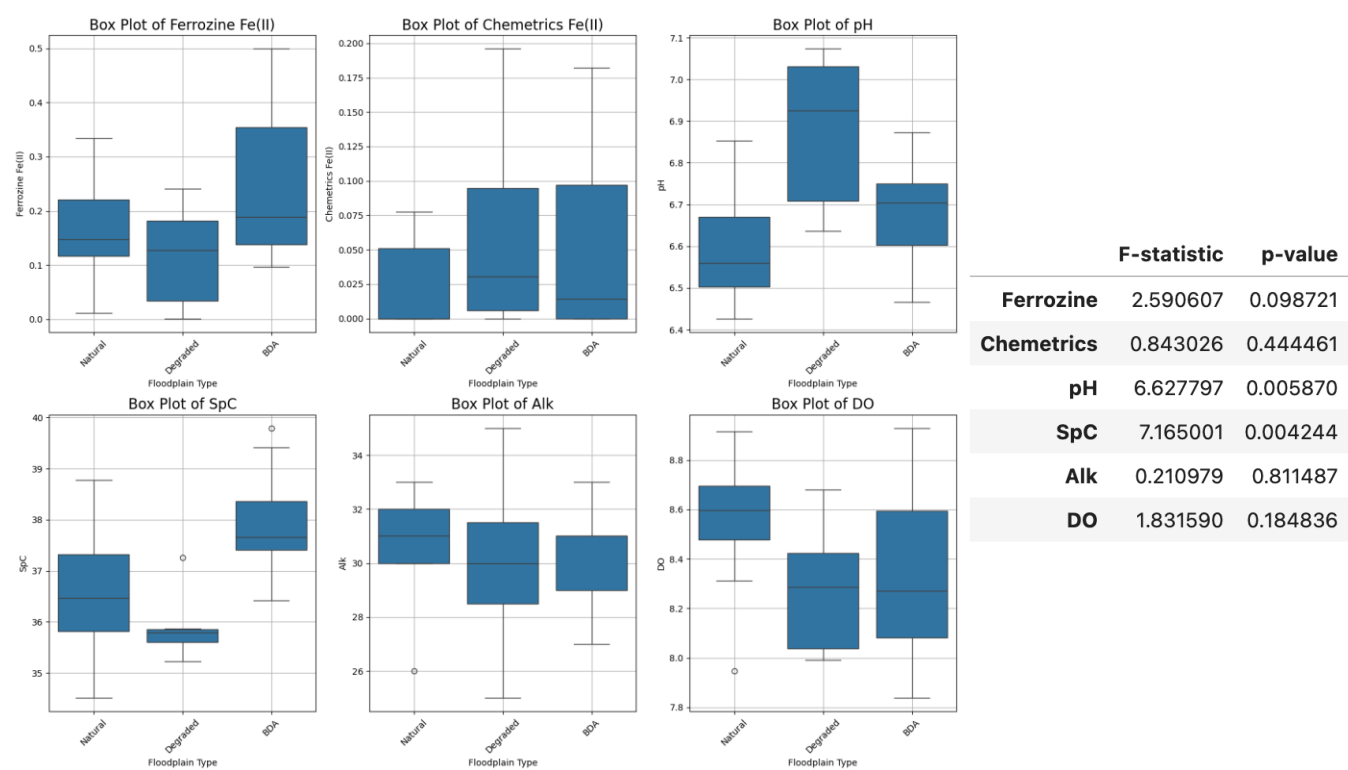


Figure 17: Trail Creek Box plots of Ferrozine Fe(II) measurements, Chemetrics Fe(II) measurements, specific conductivity, alkalinity, pH, and point Dissolved Oxygen across water sample source type (left), and log-transformed data ANOVA results (right).

However, graphing the Ferrozine Fe(II) concentrations as a progression along the stream corridor allows further investigation into Fe(II) dynamics across floodplain types [Fig. 18].

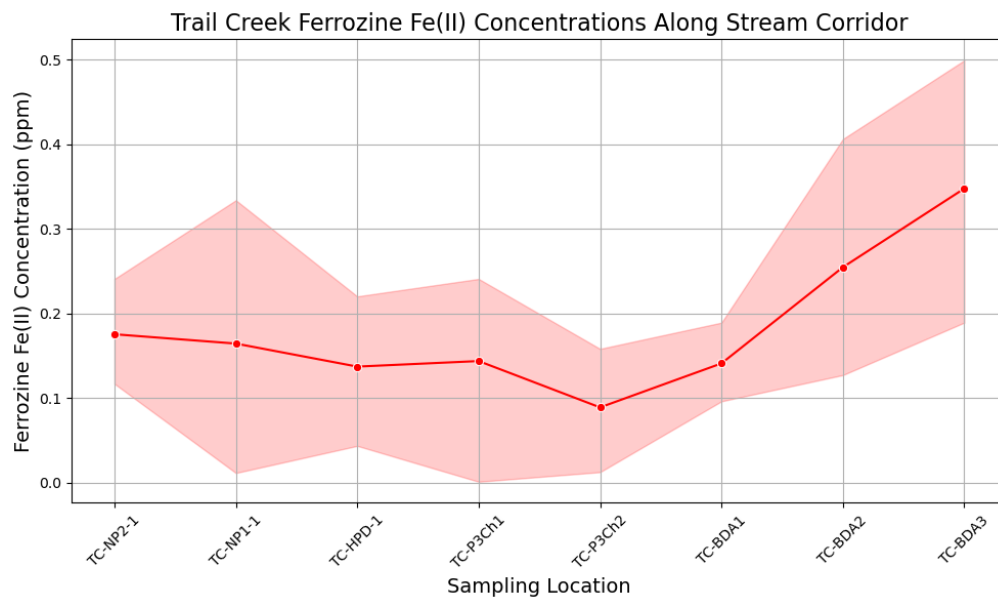


Figure 18: Ferrozine Fe(II) concentrations along the Trail Creek stream corridor.

The degraded sections showed a decline in Fe(II) concentrations followed by an increase in the BDA portion. This suggests possible oxidation or sedimentation of the Fe(II) in the degraded sections and a reintroduction of Fe(II) preservation throughout the BDAs or downstream iron accumulation.⁵³ The loss of Fe(II) in the degraded section of the floodplain points to either iron is being removed from the water column or converting into other forms like iron hydroxides, iron sulfides, mixed iron-organic complexes (like humic or fulvic complexes), or iron co-precipitation with phosphate or carbonate minerals.^{53,54} In the case of iron hydroxides, iron sulfides, or iron co-precipitation with phosphate or carbonate minerals, Fe(II) concentrations would be reduced in the degraded sections, and the BDA portion would have to reintroduce Fe(II).⁵⁴ However, mixed iron-organic complexes like the complexation of Fe(II) with organic molecules can form both soluble and insoluble complexes that can remain dissolved in the surface water, precipitate out, or be transported downstream.⁵⁵ Sedimentation and mixed iron-organic complexes could cause the transport of Fe(II) concentrations rather than the precipitation of them, resulting in Fe(II) accumulation downstream at the BDA portions.

Another possible explanation for the increase in concentrations along the BDAs could be that these structures either prevent iron oxidation or enhance Fe(II) release. BDAs can facilitate low flow conditions and low oxygen in their subsequent ponds, preventing the oxidation of Fe(II) and allowing it to accumulate.⁵⁶⁻⁵⁸ However, after graphing the point DO concentrations along the stream corridor and running a Pearson Correlation analysis between the point DO and Ferrozine Fe(II) concentrations there was a very weak inverse relationship that was not statistically significant between the two variables [Fig. 19-20]. Therefore, oxidation prevention is not likely. In order to determine further characteristics of this stream corridor and possible enhanced Fe(II) release, it would be prudent to track flow rates and water residence times. Comparing flow rates and Fe(II) and total iron concentrations into and out of each section of the floodplain could quantify any system loss or gain throughout the corridor. That quantification could indicate whether Fe(II) is being accumulated downstream or if its release is being enhanced.^{59,60}

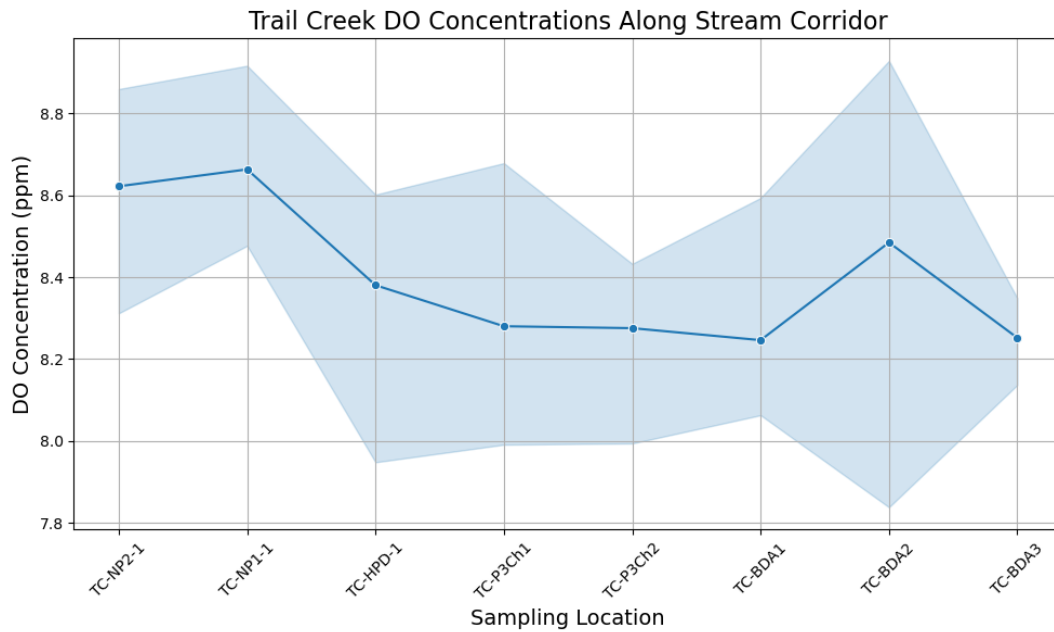


Figure 19: Point DO concentrations along the Trail Creek stream corridor.

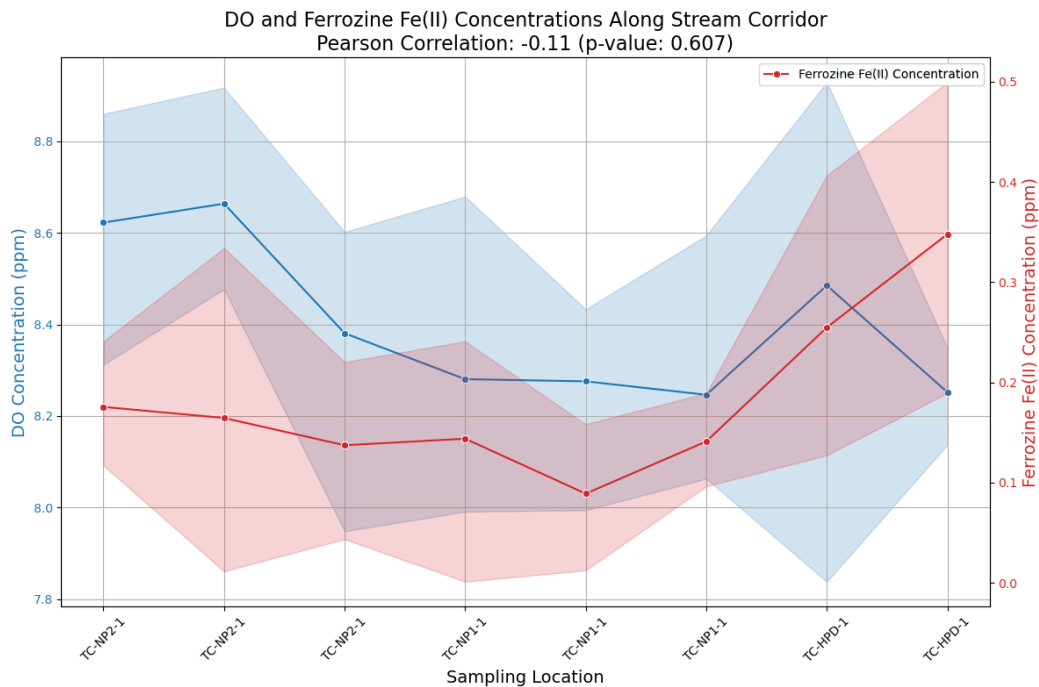
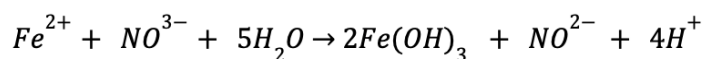


Figure 20: Point DO vs Ferrozine Fe(II) concentrations along the Trail Creek stream corridor.

Total Organic Carbon (TOC), inductively coupled plasma mass spectrometry (ICP-MS) samples, and light data from a HOBO light logger were also collected for future review. TOC and ICP-MS could provide insights into the trace elements (like Co, Cu, Zn, Pb) and organic matter influences on Fe(II) availability. Light data could assist in determining if the pond's oxid DO fluctuations are driven by photosynthesis.^{61,62}

Further considerations should examine differing metabolic pathways (like nitrogen) and microbial interactions, sequencing of iron microbes, quantifications of system loss or gain, and sampling a greater breadth of seep and pond locations. Fe(II) is able to precipitate into Fe(III) without the use of oxygen via microbial oxidation, coupled with denitrification [Eq. 2].⁶³ Heterotrophic nitrate-dependent Fe(II) oxidation is even more favorable in some environments, especially in anoxic zones, which assists in maintaining the availability of essential nutrients and energy in those environments.⁶⁴ This process both facilitates Fe(II) precipitation and has significant roles in the nitrogen and nutrient cycles of oxygen-limited ecosystems (like floodplains).^{65,66} As such, it is a highly important aspect to consider in future investigations of beaver-impacted systems.



Equation 2: The microbial oxidation of Fe(II) coupled to denitrification.

Sequencing iron-related microbes may contribute towards identification of key species involved in iron redox reactions across the floodplains, testing any similarities or differences between the Confluence and Pumphouse sites.⁶⁷⁻⁶⁹ Additionally, quantification of flow rates with Fe(II) concentrations assists in conducting mass and energy balances across redox-sensitive elements, especially iron. This enables quantification of system loss or gain, as mentioned above.^{59,60} Finally, expanded seep sampling breadth could look to include not only varied depths of seeps, but a greater number overall, with greater variation in microbial activity among them. This would provide a more comprehensive understanding of these floodplains, any changes, and the impacts of those changes.

In summary, beaver-impacted floodplains demonstrate an inverse correlation between Fe(II) and DO, however, the strength of that correlation varies by floodplain. Additionally, seeps are chemically different than ponds and channels, possibly indicating chemically distinct roles in the biogeochemical cycle. Furthermore, there is a distinct diurnal relationship between DO and Fe(II) in beaver ponds. Each of these aspects can be used to inform further studies into the biogeochemical capabilities of beaver-impacted floodplains.

Bibliography:

- (1) Morrison, R. R.; Jones, C. N.; Lininger, K.; Thoms, M. C.; Wohl, E. Chapter 3 - Resilient Floodplains in the Anthropocene. In *Resilience and Riverine Landscapes*; Thoms, M., Fuller, I., Eds.; Elsevier, 2024; pp 41–68. <https://doi.org/10.1016/B978-0-323-91716-2.00035-2>.
- (2) Hauer, F. R.; Locke, H.; Dreitz, V. J.; Hebblewhite, M.; Lowe, W. H.; Muhlfeld, C. C.; Nelson, C. R.; Proctor, M. F.; Rood, S. B. Gravel-Bed River Floodplains Are the Ecological Nexus of Glaciated Mountain Landscapes. *Sci. Adv.* **2016**, 2 (6), e1600026. <https://doi.org/10.1126/sciadv.1600026>.
- (3) DeBoer, J. A.; Thoms, M. C.; Delong, M. D. Chapter 10 - The Anthropocene: Rivers and Resilience. In *Resilience and Riverine Landscapes*; Thoms, M., Fuller, I., Eds.; Elsevier, 2024; pp 209–228. <https://doi.org/10.1016/B978-0-323-91716-2.00028-5>.
- (4) *Benefits of Natural Floodplains* | FEMA.gov. <https://www.fema.gov/floodplain-management/wildlife-conservation/benefits-natural> (accessed 2024-06-20).
- (5) *Agriculture is a Good Fit in Floodplains*. Extension | University of Nevada, Reno. <https://extension.unr.edu/publication.aspx?PubID=3014> (accessed 2024-06-20).
- (6) *Trail Creek: Low-tech restoration success*. US Forest Service. <https://www.fs.usda.gov/inside-fs/delivering-mission/sustain/trail-creek-low-tech-restoration-success> (accessed 2024-06-19).
- (7) Dewey, C.; Fox, P. M.; Bouskill, N. J.; Dwivedi, D.; Nico, P.; Fendorf, S. Beaver Dams Overshadow Climate Extremes in Controlling Riparian Hydrology and Water Quality. *Nat. Commun.* **2022**, 13 (1), 6509. <https://doi.org/10.1038/s41467-022-34022-0>.
- (8) Ciszewski, D.; Grygar, T. M. A Review of Flood-Related Storage and Remobilization of Heavy Metal Pollutants in River Systems. *Water. Air. Soil Pollut.* **2016**, 227 (7), 239. <https://doi.org/10.1007/s11270-016-2934-8>.
- (9) Dittbrenner, B. J.; Schilling, J. W.; Torgersen, C. E.; Lawler, J. J. Relocated Beaver Can Increase Water Storage and Decrease Stream Temperature in Headwater Streams. *Ecosphere* **2022**, 13 (7), e4168. <https://doi.org/10.1002/ecs2.4168>.
- (10) Larsen, A.; Larsen, J. R.; Lane, S. N. Dam Builders and Their Works: Beaver Influences on the Structure and Function of River Corridor Hydrology, Geomorphology, Biogeochemistry and Ecosystems. *Earth-Sci. Rev.* **2021**, 218, 103623. <https://doi.org/10.1016/j.earscirev.2021.103623>.
- (11) Brazier, R. E.; Puttock, A.; Graham, H. A.; Auster, R. E.; Davies, K. H.; Brown, C. M. L. Beaver: Nature's Ecosystem Engineers. *Wires Water* **2021**, 8 (1), e1494. <https://doi.org/10.1002/wat2.1494>.
- (12) Dahm, C. N.; Trotter, E. H.; Sedell, J. R.; Service, U.-F. ROLE OF ANAEROBIC ZONES AND PROCESSES IN STREAM ECOSYSTEM PRODUCTIVITY.
- (13) Polvi, L. E.; Wohl, E. The Beaver Meadow Complex Revisited – the Role of Beavers in Post-glacial Floodplain Development. *Earth Surf. Process. Landf.* **2012**, 37 (3), 332–346.

<https://doi.org/10.1002/esp.2261>.

- (14) Crego, R. D.; Rozzi, R.; Jiménez, J. E. Fur Trade and the Biotic Homogenization of Subpolar Ecosystems. In *From Biocultural Homogenization to Biocultural Conservation*; Rozzi, R., May Jr., R. H., Chapin III, F. S., Massardo, F., Gavin, M. C., Klaver, I. J., Pauchard, A., Nuñez, M. A., Simberloff, D., Eds.; Springer International Publishing: Cham, 2018; pp 233–243. https://doi.org/10.1007/978-3-319-99513-7_14.
- (15) Wade, J.; Lautz, L.; Kelleher, C.; Vidon, P.; Davis, J.; Beltran, J.; Pearce, C. Beaver Dam Analogues Drive Heterogeneous Groundwater–Surface Water Interactions. *Hydrol. Process.* **2020**, *34* (26), 5340–5353. <https://doi.org/10.1002/hyp.13947>.
- (16) Orr, M. R.; Weber, N. P.; Noone, W. N.; Mooney, M. G.; Oakes, T. M.; Broughton, H. M. Short-Term Stream and Riparian Responses to Beaver Dam Analogs on a Low-Gradient Channel Lacking Woody Riparian Vegetation. *Northwest Sci.* **2020**, *93* (3–4), 171–184. <https://doi.org/10.3955/046.093.0302>.
- (17) Pearce, C.; Vidon, P.; Lautz, L.; Kelleher, C.; Davis, J. Impact of Beaver Dam Analogues on Hydrology in a Semi-Arid Floodplain. *Hydrol. Process.* **2021**, *35* (7), e14275. <https://doi.org/10.1002/hyp.14275>.
- (18) Søndergaard, M. Redox Potential. In *Encyclopedia of Inland Waters*; Likens, G. E., Ed.; Academic Press: Oxford, 2009; pp 852–859. <https://doi.org/10.1016/B978-012370626-3.00115-0>.
- (19) Middelburg, J. J.; Soetaert, K.; Hagens, M. Ocean Alkalinity, Buffering and Biogeochemical Processes. *Rev. Geophys.* **2020**, *58* (3), e2019RG000681. <https://doi.org/10.1029/2019RG000681>.
- (20) Catalán, N.; Herrero Ortega, S.; Gröntoft, H.; Hilmarsson, T. G.; Bertilsson, S.; Wu, P.; Levanoni, O.; Bishop, K.; Bravo, A. G. Effects of Beaver Impoundments on Dissolved Organic Matter Quality and Biodegradability in Boreal Riverine Systems. *Hydrobiologia* **2017**, *793* (1), 135–148. <https://doi.org/10.1007/s10750-016-2766-y>.
- (21) Wang, X.-N.; Sun, G.-X.; Zhu, Y.-G. Thermodynamic Energy of Anaerobic Microbial Redox Reactions Couples Elemental Biogeochemical Cycles. *J. Soils Sediments* **2017**, *17* (12), 2831–2846. <https://doi.org/10.1007/s11368-017-1767-4>.
- (22) Islam, M. M. M.; Shafi, S.; Bandh, S. A.; Shameem, N. Chapter 3 - Impact of Environmental Changes and Human Activities on Bacterial Diversity of Lakes. In *Freshwater Microbiology*; Bandh, S. A., Shafi, S., Shameem, N., Eds.; Academic Press, 2019; pp 105–136. <https://doi.org/10.1016/B978-0-12-817495-1.00003-7>.
- (23) Fortin, D.; Praharaj, T. Role of Microbial Activity in Fe and S Cycling in Sub-Oxic to Anoxic Sulfide-Rich Mine Tailings. *J. Nucl. Radiochem. Sci.* **2005**, *6* (1), 39–42. <https://doi.org/10.14494/jnrs2000.6.39>.
- (24) Null, S. E.; Mouzon, N. R.; Elmore, L. R. Dissolved Oxygen, Stream Temperature, and Fish Habitat Response to Environmental Water Purchases. *J. Environ. Manage.* **2017**, *197*, 559–570. <https://doi.org/10.1016/j.jenvman.2017.04.016>.
- (25) Hopwood, M. J.; Statham, P. J.; Milani, A. Dissolved Fe(II) in a River-Estuary System Rich in Dissolved Organic Matter. *Estuar. Coast. Shelf Sci.* **2014**, *151*, 1–9. <https://doi.org/10.1016/j.ecss.2014.09.015>.
- (26) Balsamo Crespo, E.; Reichelt-Brushett, A.; Smith, R. E. W.; Rose, A. L.; Batley, G. E. Improving the Measurement of Iron(III) Bioavailability in Freshwater Samples: Methods and Performance. *Environ. Toxicol. Chem.* **2023**, *42* (2), 303–316. <https://doi.org/10.1002/etc.5530>.
- (27) *The flux of soluble organic-iron(III) complexes from sediments represents a source of stable iron(III) to estuarine waters and to the continental shelf - Jones - 2011 - Limnology and Oceanography - Wiley Online Library.* <https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.2011.56.5.1811> (accessed 2024-06-22).
- (28) Nagai, T.; Imai, A.; Matsushige, K.; Yokoi, K.; Fukushima, T. Dissolved Iron and Its Speciation in a Shallow Eutrophic Lake and Its Inflowing Rivers. *Water Res.* **2007**, *41* (4), 775–784. <https://doi.org/10.1016/j.watres.2006.10.038>.
- (29) Briggs, M. A.; Wang, C.; Day-Lewis, F. D.; Williams, K. H.; Dong, W.; Lane, J. W. Return Flows from Beaver Ponds Enhance Floodplain-to-River Metals Exchange in Alluvial Mountain Catchments. *Sci.*

- Total Environ.* **2019**, 685, 357–369. <https://doi.org/10.1016/j.scitotenv.2019.05.371>.
- (30) Sánchez-Carrillo, S.; Álvarez-Cobelas, M. Nutrient Dynamics and Eutrophication Patterns in a Semi-Arid Wetland: The Effects of Fluctuating Hydrology. *Water. Air. Soil Pollut.* **2001**, 131 (1), 97–118. <https://doi.org/10.1023/A:1011903300635>.
- (31) Salimi, S.; Almuktar, S. A. A. N.; Scholz, M. Impact of Climate Change on Wetland Ecosystems: A Critical Review of Experimental Wetlands. *J. Environ. Manage.* **2021**, 286, 112160. <https://doi.org/10.1016/j.jenvman.2021.112160>.
- (32) Smith, K. S. FACTORS INFLUENCING METAL SORPTION ONTO IRON-RICH SEDIMENT IN ACID-MINE DRAINAGE.
- (33) Hamid, A.; Bhat, S. U.; Jehangir, A. Local Determinants Influencing Stream Water Quality. *Appl. Water Sci.* **2019**, 10 (1), 24. <https://doi.org/10.1007/s13201-019-1043-4>.
- (34) Brannon, J. M.; Chen, R. L.; Gunnison, D. Sediment-Water Interactions and Mineral Cycling in Reservoirs. In *Microbial Processes in Reservoirs*; Gunnison, D., Ed.; Springer Netherlands: Dordrecht, 1985; pp 121–134. https://doi.org/10.1007/978-94-009-5514-1_7.
- (35) Helton, A. M.; Wright, M. S.; Bernhardt, E. S.; Poole, G. C.; Cory, R. M.; Stanford, J. A. Dissolved Organic Carbon Lability Increases with Water Residence Time in the Alluvial Aquifer of a River Floodplain Ecosystem. *J. Geophys. Res. Biogeosciences* **2015**, 120 (4), 693–706. <https://doi.org/10.1002/2014JG002832>.
- (36) Wang, L. L.; Song, C. C.; Yang, G. S. Dissolved Organic Carbon Characteristics in Surface Ponds from Contrasting Wetland Ecosystems: A Case Study in the Sanjiang Plain, Northeast China. *Hydrol. Earth Syst. Sci.* **2013**, 17 (1), 371–378. <https://doi.org/10.5194/hess-17-371-2013>.
- (37) Schwertmann, U. Solubility and Dissolution of Iron Oxides. *Plant Soil* **1991**, 130 (1), 1–25. <https://doi.org/10.1007/BF00011851>.
- (38) Meskhidze, N.; Völker, C.; Al-Abadleh, H. A.; Barbeau, K.; Bressac, M.; Buck, C.; Bundy, R. M.; Croot, P.; Feng, Y.; Ito, A.; Johansen, A. M.; Landing, W. M.; Mao, J.; Myriokefalitakis, S.; Ohnemus, D.; Pasquier, B.; Ye, Y. Perspective on Identifying and Characterizing the Processes Controlling Iron Speciation and Residence Time at the Atmosphere-Ocean Interface. *Mar. Chem.* **2019**, 217, 103704. <https://doi.org/10.1016/j.marchem.2019.103704>.
- (39) Hoffmann, T. Sediment Residence Time and Connectivity in Non-Equilibrium and Transient Geomorphic Systems. *Earth-Sci. Rev.* **2015**, 150, 609–627. <https://doi.org/10.1016/j.earscirev.2015.07.008>.
- (40) *Geological controls and effects of floodplain asymmetry on river-groundwater interactions in the southeastern Coastal Plain, USA*. CoLab. <https://colab.ws/articles/10.1007%2Fs10040-010-0595-z> (accessed 2024-08-09).
- (41) Du Laing, G.; Rinklebe, J.; Vandecasteele, B.; Meers, E.; Tack, F. M. G. Trace Metal Behaviour in Estuarine and Riverine Floodplain Soils and Sediments: A Review. *Sci. Total Environ.* **2009**, 407 (13), 3972–3985. <https://doi.org/10.1016/j.scitotenv.2008.07.025>.
- (42) Rickard, D.; Luther, G. W. Chemistry of Iron Sulfides. *Chem. Rev.* **2007**, 107 (2), 514–562. <https://doi.org/10.1021/cr0503658>.
- (43) Rickard, D.; Schoonen, M. A. A.; Luther, G. W. I. Chemistry of Iron Sulfides in Sedimentary Environments. In *Geochemical Transformations of Sedimentary Sulfur*; ACS Symposium Series; American Chemical Society, 1995; Vol. 612, pp 168–193. <https://doi.org/10.1021/bk-1995-0612.ch009>.
- (44) *1,10-phenanthroline - an overview | ScienceDirect Topics*. <https://www.sciencedirect.com/topics/chemistry/1-10-phenanthroline> (accessed 2024-08-11).
- (45) *Complexation kinetics of Fe²⁺ with 1,10-phenanthroline forming ferriox in acidic solutions - Adhikamsetty - 2008 - International Journal of Chemical Kinetics - Wiley Online Library*. <https://onlinelibrary.wiley.com/doi/abs/10.1002/kin.20336> (accessed 2024-08-11).
- (46) Viollier, E.; Inglett, P. W.; Hunter, K.; Roychoudhury, A. N.; Van Cappellen, P. The Ferrozine Method Revisited: Fe(II)/Fe(III) Determination in Natural Waters. *Appl. Geochem.* **2000**, 15 (6), 785–790. [https://doi.org/10.1016/S0883-2927\(99\)00097-9](https://doi.org/10.1016/S0883-2927(99)00097-9).
- (47) Morgan, B.; Lahav, O. The Effect of pH on the Kinetics of Spontaneous Fe(II) Oxidation by O₂ in Aqueous Solution – Basic Principles and a Simple Heuristic Description. *Chemosphere* **2007**, 68

- (11), 2080–2084. <https://doi.org/10.1016/j.chemosphere.2007.02.015>.
- (48) *V-2000 Photometer - Multi-Analyte Water Quality Machine*. CHEMetrics, LLC. <https://www.chemetrics.com/product/v-2000-multi-analyte-photometer-for-water-quality-analysis/> (accessed 2024-06-30).
- (49) *CHEMets Dissolved Oxygen Test Kit (K-7512) | CHEMetrics*. CHEMetrics, LLC. <https://www.chemetrics.com/product/oxygen-dissolved-chemets-visual-kit/> (accessed 2024-08-11).
- (50) Canfield, D. E.; Erik Kristensen; Bo Thamdrup. The Oxygen Cycle. In *Advances in Marine Biology*; Canfield, D. E., Kristensen, E., Thamdrup, B., Eds.; Aquatic Geomicrobiology; Academic Press, 2005; Vol. 48, pp 167–204. [https://doi.org/10.1016/S0065-2881\(05\)48006-2](https://doi.org/10.1016/S0065-2881(05)48006-2).
- (51) Das, K.; Roychoudhury, A. Reactive Oxygen Species (ROS) and Response of Antioxidants as ROS-Scavengers during Environmental Stress in Plants. *Front. Environ. Sci.* **2014**, *2*. <https://doi.org/10.3389/fenvs.2014.00053>.
- (52) Macfarlane, W. W.; Wheaton, J. M.; Bouwes, N.; Jensen, M. L.; Gilbert, J. T.; Hough-Snee, N.; Shivik, J. A. Modeling the Capacity of Riverscapes to Support Beaver Dams. *Geomorphology* **2017**, *277*, 72–99. <https://doi.org/10.1016/j.geomorph.2015.11.019>.
- (53) Vezina, A. F.; Cornett, R. J. Iron Transport and Distribution between Freshwater and Sediments over Different Time Scales. *Geochim. Cosmochim. Acta* **1990**, *54* (10), 2635–2644. [https://doi.org/10.1016/0016-7037\(90\)90001-2](https://doi.org/10.1016/0016-7037(90)90001-2).
- (54) Fernández-Remolar, D. C. Iron Oxides, Hydroxides and Oxy-Hydroxides. In *Encyclopedia of Astrobiology*; Amils, R., Gargaud, M., Cernicharo Quintanilla, J., Cleaves, H. J., Irvine, W. M., Pinti, D., Viso, M., Eds.; Springer: Berlin, Heidelberg, 2014; pp 1–4. https://doi.org/10.1007/978-3-642-27833-4_1714-3.
- (55) Kügler, S.; Cooper, R. E.; Wegner, C.-E.; Mohr, J. F.; Wichard, T.; Küsel, K. Iron-Organic Matter Complexes Accelerate Microbial Iron Cycling in an Iron-Rich Fen. *Sci. Total Environ.* **2019**, *646*, 972–988. <https://doi.org/10.1016/j.scitotenv.2018.07.258>.
- (56) *The Ecological Benefits of Beaver Dams and the need to study Beaver Dam Analogs | Stories | CSTEM | Eastern Washington University*. <https://www.ewu.edu/cstem/stories/the-ecological-benefits-of-beaver-dams-and-the-need-to-study-beaver-dam-analogs/> (accessed 2024-08-11).
- (57) Salt Lake Community College; Culbertson, A.; Wetterlin, L.; McIlwain, H.; Johnson, C. EFFECTIVENESS OF BEAVER DAM ANALOGS AS A RESTORATION STRATEGY TO MITIGATE INCREASED WATER TEMPERATURES AND DECREASED DISSOLVED OXYGEN; 2020; p 346621. <https://doi.org/10.1130/abs/2020RM-346621>.
- (58) Neace, H. *Water Quality and Soil Health Response to Beaver Dam Analogs in an Intermittent Stream in Southern Idaho*. Theses and Dissertations Collection. https://www.lib.uidaho.edu/digital/etd/items/neace_idaho_0089n_12622.html (accessed 2024-08-11).
- (59) Prescott, K. L.; Tsanis, I. K. Mass Balance Modelling and Wetland Restoration. *Ecol. Eng.* **1997**, *9* (1), 1–18. [https://doi.org/10.1016/S0925-8574\(97\)00015-3](https://doi.org/10.1016/S0925-8574(97)00015-3).
- (60) Yang, Z.-H.; Li, D.; Huai, W.; Liu, J. A New Method to Estimate Flow Conveyance in a Compound Channel with Vegetated Floodplains Based on Energy Balance. *J. Hydrol.* **2019**, *575*. <https://doi.org/10.1016/j.jhydrol.2019.05.078>.
- (61) Pezeshki, S. R.; DeLaune, R. D. Soil Oxidation-Reduction in Wetlands and Its Impact on Plant Functioning. *Biology* **2012**, *1* (2), 196–221. <https://doi.org/10.3390/biology1020196>.
- (62) Johnson, M. P. Photosynthesis. *Essays Biochem.* **2016**, *60* (3), 255–273. <https://doi.org/10.1042/EBC20160016>.
- (63) Madigan, M. T. *Brock Biology of Microorganisms*; Pearson/Benjamin Cummings, 2009.
- (64) Muehe, E. M.; Gerhardt, S.; Schink, B.; Kappler, A. Ecophysiology and the Energetic Benefit of Mixotrophic Fe(II) Oxidation by Various Strains of Nitratereducing Bacteria. *FEMS Microbiol. Ecol.* **2009**, *70* (3), 335–343. <https://doi.org/10.1111/j.1574-6941.2009.00755.x>.
- (65) Scholz, F.; Löscher, C. R.; Fiskal, A.; Sommer, S.; Hensen, C.; Lomnitz, U.; Wuttig, K.; Göttlicher, J.; Kossel, E.; Steininger, R.; Canfield, D. E. Nitrate-Dependent Iron Oxidation Limits Iron Transport in Anoxic Ocean Regions. *Earth Planet. Sci. Lett.* **2016**, *454*, 272–281.

<https://doi.org/10.1016/j.epsl.2016.09.025>.

- (66) *Elevated Fe(II) and Dissolved Fe in Hypoxic Shelf Waters off Oregon and Washington: An Enhanced Source of Iron to Coastal Upwelling Regimes* / *Environmental Science & Technology*. <https://pubs.acs.org/doi/abs/10.1021/es800144j> (accessed 2024-08-11).
- (67) Wang, J.; Krause, S.; Muyzer, G.; Meima-Franke, M.; Laanbroek, H. J.; Bodelier, P. L. E. Spatial Patterns of Iron- and Methane-Oxidizing Bacterial Communities in an Irregularly Flooded, Riparian Wetland. *Front. Microbiol.* **2012**, *3*, 64. <https://doi.org/10.3389/fmicb.2012.00064>.
- (68) Garber, A. I.; Cohen, A. B.; Nealson, K. H.; Ramírez, G. A.; Barco, R. A.; Enzingmüller-Bleyl, T. C.; Gehringer, M. M.; Merino, N. Metagenomic Insights Into the Microbial Iron Cycle of Subseafloor Habitats. *Front. Microbiol.* **2021**, *12*, 667944. <https://doi.org/10.3389/fmicb.2021.667944>.
- (69) Zhong, Y.-W.; Zhou, P.; Cheng, H.; Zhou, Y.-D.; Pan, J.; Xu, L.; Li, M.; Tao, C.-H.; Wu, Y.-H.; Xu, X.-W. Metagenomic Features Characterized with Microbial Iron Oxidoreduction and Mineral Interaction in Southwest Indian Ridge. *Microbiol. Spectr.* *10* (6), e00614-22. <https://doi.org/10.1128/spectrum.00614-22>.