

WATER QUALITY IN DYNAMIC REDOX ENVIRONMENTS: COUPLED
HYDROLOGIC-BIOGEOCHEMICAL CONTROLS ON METAL CONTAMINANT
MOBILITY

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Abstract

Rising population and changing climate threaten to increase the risks posed by anthropogenic and geogenic metal contaminants to our freshwater resources. Increased human demand for freshwater coupled with altered hydrologic cycles will shift (bio)geochemical conditions in soils and sediments, potentially driving transformations of metal contaminants from stationary (solid) phases to mobile forms subject to transport and human consumption. Understanding the fundamental controls on phase transformations of metal contaminants is thus essential for predicting the risk they impose. Often, redox processes control partitioning of metal contaminants in surface and subsurface environments, whether by changing the redox state of the metal itself, or by transforming the metal host(s). In the subsurface, redox processes are commonly driven by hydrologic conditions. My research seeks to understand how redox associated biogeochemical processes arising from and coupled to hydrologic conditions impact metal contaminant partitioning and mobilization. I employ a combination of experimental, spectroscopic, field and modeling approaches to study the partitioning of U, Pb and Cd to solid and dissolved phases in dynamic redox environments. In my first chapter, I find that the calcium-uranyl-carbonato species kinetically limit U(VI) reduction by Fe(II)(aq), thereby impeding transformation of U from a soluble form to an insoluble form. In my second chapter, I show that the affinity of Pb for a range of coordination environments limits dissolved Pb concentration in contaminated floodplain sediments, even during shifts in redox conditions and coupled dissolution of Pb hosts. In my third chapter, I reveal that soil redox environment influences the metal binding properties of DOM, leading to increased complexation of Cd by soft ligands in reduced environments. Finally, in my fourth chapter, I find that the impacts of beaver dams on hyporheic biogeochemical activity dwarfs that of seasonal hydrologic dynamics. Overall, my work both furthers our understanding of the biogeochemical cycles of U, Pb, and Cd and deepens our understanding of how changes in hydrology couple with biogeochemical redox processes to determine water quality.

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Table of Contents

Chapter 1: Introduction	1
1.1 Impact of redox processes on contaminant mobility.....	2
1.2 Relationship between hydrology and contaminant reactivity and transport	4
1.3 Dissertation overview.....	4
1.5 References	8
Chapter 2: Calcium uranyl carbonato species kinetically limit U(VI) reduction and lead to U(V)-bearing ferrihydrite	13
2.1 Abstract	13
2.2 Introduction	14
2.4 Materials and Methods	15
2.5 Results	20
2.6 Discussion	26
2.7 Conclusion.....	30
2.8 Acknowledgements	31
2.9 References	31
2.10 Figures.....	37
2.11 Supplementary Materials	41
Chapter 3: Collective partitioning limits porewater Pb concentrations during redox cycles in contaminated floodplain sediments	65
3.1 Abstract	65
3.2 Introduction	66
3.3 Materials and Methods	67
3.4 Results	71

3.5 Discussion	78
3.6 Conclusions	82
3.7 Acknowledgments.....	82
3.8 References	83
3.8 Tables	87
3.9 Figures.....	87
3.10 Supplementary Materials	92
Chapter 4: The impact of redox environment on metal-organic complexes: findings from East River floodplain soils.....	119
4.1 Abstract	119
4.2 Introduction	120
4.3 Materials and Methods	122
4.4 Results and Discussion.....	125
4.5 Conclusion.....	126
4.6 Acknowledgements	136
4.7 References	136
4.8 Figures.....	140
4.9 Supplemental Materials.....	145
Chapter 5: Assessing the impacts of meander-scale variability, seasonal snowmelt, and beaver dams on biogeochemical exports from floodplain sediments	148
5.1 Abstract	148
5.2 Introduction	149
5.3 Materials and Methods	150
5.4 Results	155
5.5 Discussion	162

5.6 Conclusion.....	165
5.7 Acknowledgements	166
5.7 References	166
5.8 Figures.....	169
5.9 Supplemental Materials.....	176

List of Tables

Table S2.1 Aqueous geochemical conditions for all reactors at all time points....	41
Table S2.2 Gibbs energy of formation for species used in thermodynamic cal ...	42
Table S2.3 Gibbs free energies of reduction of U(VI) species by FE(II)(aq)	49
Table S2.4 U EXAFS shell-by-shell fitting results.....	53
Table S2.5 U reduction rates at early and reaction stages	56
Table S3.1 Selected XRF results for OBJ-2 sediments	92
Table S3.2 Tabulated Pb, Fe, and S porewater concentrations	95
Table S3.3 Selected porewater pH measurements	96
Table S3.5 S XANES LCF result for samples collected on June 4, 2018	104
Table S3.6 S XANES LCF result for samples collected on June 20, 2018	105
Table S3.7 S XANES LCF result for samples collected on July 2, 2018	106
Table S3.8 S XANES LCF result for samples collected on August 8, 2018	107
Table S3.9 S XANES LCF result for samples collected on Sept. 11, 2018.....	108
Table S3.10 S XANES LCF result for samples collected on Oct. 26, 2018	109
Table S3.11 S K XANES LCF statistics for all samples	110
Table S3.12 Full LCF results for Pb L ₃ EXAFS for all June 20, 2018 samples..	114
Table S3.13 Full LCF results for Pb L ₃ EXAFS for all Aug. 8, 2018 samples....	115
Table S3.14 Full LCF results for Pb L ₃ EXAFS for all Oct. 26, 2018 samples...	116
Table S3.15 Results for shell-by-shell analysis of EXAFS.....	118
Table S5.1 XRD results from meander C and Z sediments	185

List of Figures

Figure 2.1 U concentrations and Gibbs energies for U(VI) reduction	37
Figure 2.2 Proportions of aqueous U	38
Figure 2.3 U L ₃ HERFD-XANES.....	39
Figure 2.4 TEM images and U EXAFS	40
Figure S2.1 Concentrations of dominant U species.....	45
Figure S2.2 Proportions of dominant U species	48
Figure S2.3 Gibbs free energies for mono-calcium uranyl carbonato sp.....	50
Figure S2.4 Fe(II)(aq) concentrations	51
Figure S2.5 Linear combinations of HERFD spectra	52
Figure S2.6 Fe K EXAFS spectrum.....	53
Figure S2.7 Energy dispersive x-ray spectroscopy results	55
Figure S2.8 Initial proportion of U as calcium-uranyl-carbonato.....	56
Figure S2.9 Natural Log of measured U concentrations over time	59
Figure 3.1 OBJ2 water table position May 1-Dec 31, 2018	87
Figure 3.2 Depth-resolved porewater concentrations of Pb, Fe, S	88
Figure 3.3 Linear combination fitting results of Pb L3 EXAFS spectra.....	89
Figure 3.4 Fraction of total Pb removed by sequential extractions	90
Figure 3.5 μ XRF Fe-Pb maps and correlation plots of sediment	91
Figure S3.1 Map showing the OBJ-2 sampling location.....	92

Figure S3.2 Image of beaver dam at OBJ-2.....	94
Figure S3.3 Summary of S K XANES linear combination fitting results	97
Figure S3.4 S K XANES spectra from samples collected on June 4, 2018.....	98
Figure S3.5 S K XANES spectra from samples collected on June 20, 2018.....	99
Figure S3.6 S K XANES spectra from samples collected on July 2, 2018	100
Figure S3.7 S K XANES spectra from samples collected on August 8, 2018.....	101
Figure S3.8 S K XANES spectra from samples collected on Sept. 11, 2018	102
Figure S3.9 S K XANES spectra from samples collected on Oct. 26, 2018.....	103
Figure S3.10 Pb L3 EXAFS spectra of samples collected on June 20, 2018	111
Figure S3.11 Pb L3 EXAFS spectra of samples collected on August 8, 2018	112
Figure S3.12 Pb L3 EXAFS spectra of samples collected on October 26, 2018 .	113
Figure S3.13 Pb L₃ EXAFS shell-by-shell fits.....	117
Figure 4.1 Distribution of C functional groups	140
Figure 4.2 Nominal oxidation state of carbon in oxic and anoxic sediments.....	141
Figure 4.3 Cd DOM complexes, Cd amendment.....	142
Figure 4.4 Cd DOM complexes, Ni & Cd amendment	143
Figure 4.5 ⁵⁶Fe traces for separations with Cd and Ni amendments.....	144
Figure S4.1 Map of field site.....	145
Figure S4.2 Ni DOM complexes, Ni amendment.....	146
Figure S4.3 Ni DOM complexes, Ni & Cd amendment	147
Figure 5.1 East River water surface elevations, 2018 and 2019.....	169

Figure 5.2 Geochemical composition of porewater from MCP (2018).....	170
Figure 5.3 Geochemical composition of porewater from MZT2 (2018).....	171
Figure 5.4 Dissolved oxygen concentrations, meander C	172
Figure 5.5 Dissolved oxygen concentrations, meander Z	173
Figure 5.6 Simulated redox zonation in 2018 and 2019	174
Figure 5.7 Simulated redox zonation in 2018 with contrasting permeabilities ..	175
Figure S5.1 Meander Z field instrumentation	176
Figure S5.2 Meander C field instrumentation.....	177
Figure S5.3 Model domain for idealized meander transect	178
Figure S5.4 Geochemical composition of porewater from MZT1 (2018)	179
Figure S5.5 Geochemical composition of porewater from MCT2 (2018)	180
Figure S5.6 Geochemical composition of porewater from MZT1 (2019)	181
Figure S5.7 Geochemical composition of porewater from MZT2 (2019)	182
Figure S5.8 Geochemical composition of porewater from MCP (2019)	183
Figure S5.9 Geochemical composition of porewater from MCT2 (2019)	184
Figure S5.1 Soil carbon, MZT1 and MCP transects.....	186
Figure S5.1 Soil Fe MZT1 and MCP transects.....	187

Chapter 1:

Introduction

Rising global population and accelerating climate change impose unprecedented challenges to the sourcing, treatment, and distribution of freshwater. The increase in global population drives increased demand for freshwater, while changing climate threatens to limit the freshwater supply. In the face of these challenges, providing freshwater for human consumption is often (deservedly) treated as an issue of water quantity (or scarcity); however, it is also an issue of water quality. Rising population coupled with increasing demand and a changing climate not only deplete existing sources of freshwater, but also threaten their quality, thereby further limiting the availability of water for human use.

Increased demand on existing freshwater sources impairs water quality when it increases concentrations of both anthropogenic and geogenic contaminants. For example, aquifer drawdown from increased pumping may introduce oxygen into anoxic aquifer solids, thereby initiating a suite of reactions capable of transforming contaminants such as U and Cr from a stationary, solid phase into a mobile phase subject to transport and human consumption.¹⁻⁴ Further, the development of new freshwater sources also may alter the partitioning of contaminants safely sequestered in the solid phase. For example, managed aquifer recharge of processed wastewater in Orange County, CA has resulted in the unanticipated release of naturally occurring arsenic into this novel freshwater supply.^{5,6}

Climate change, and the cascade of environmental impacts it creates, deepens the challenge of meeting increased demand for freshwater. Shifting precipitation patterns and warming temperatures alter the hydrologic cycle, thereby magnifying the strain on existing freshwater sources.^{7,8} Further, anticipated increases in extremes will result in precipitation coming as large, but less frequent pulses that will alter groundwater recharge.⁹⁻¹¹ Eventual warming around the equator may drive migration of human

populations toward the poles, further increasing freshwater demand in cooler mid-latitudes.^{12,13} Increased fire activity may lead to increased erosion and toxic runoff into freshwater sources.^{14,15}

Anticipating the impacts of rising population and changing climate on freshwater sources requires an understanding of the processes that control freshwater quality, as well as the ability to predict likely stressors on these processes. While the latter is essential for this goal, the work presented in this dissertation belongs in the former domain. Broadly, it seeks to further our understanding of the biogeochemical cycles of trace metal contaminants, of both anthropogenic and geogenic origin. Towards this aim, it focuses on the role of reduction-oxidation (redox) reactions in controlling the potential mobility of U, Pb, and Cd in surface and subsurface environments, and to resolve how changes in hydrology couple with biogeochemistry to dictate water chemistry.

1.1 Impact of redox processes on contaminant mobility

Redox processes drive the biogeochemical cycles of many geogenic and anthropogenic contaminants. The chemical speciation of redox-active contaminants, such as U, is controlled by redox transformations of the contaminant itself, while redox transformations of natural organic matter and mineral phases, particularly Fe- and Mn-(hydr)oxides and sulfide minerals, affect the speciation of non-redox active contaminants, such as Pb and Cd. In both cases, redox processes can transform geogenic contaminants from stationary, solid phases to mobile forms – and vice versa – thereby determining their potential for transport and influencing the risk they pose to human health and the environment. Understanding the myriad ways in which redox processes drive the biogeochemical cycles of contaminants is thus essential for predicting their impact on freshwater quality.

Uranium exemplifies the direct impacts of redox processes on contaminant fate. It is itself redox-active, occurring primarily in the +VI and +IV oxidation states in oxidized and reduced environments, respectively. Uranium(VI) forms the aqueous uranyl cation and, depending on solution chemistry, the series of hydroxyl, organic, carbonato, and calcium carbonato aqueous complexes.^{16,17} The latter series tends to dominate aqueous

U speciation when (bi)carbonate and Ca cooccur with U, due to the thermodynamic stability and limited reactivity of these complexes.¹⁸ In contrast, U(IV) tends to partition to the solid phase, forming the sparingly soluble mineral uraninite, and adsorbing on particulate organic matter (POM).^{17,19,20} Building evidence suggests that U(V) may also occur in environmental settings, likely incorporated into authigenic Fe mineral phases formed during redox transitions. However, while mixed U(V)-Fe phases have been formed in laboratory settings under environmentally relevant conditions, no such phase has yet been observed in field samples.^{21–25}

Reduction of U(VI) therefore transforms U from an aqueous and (potentially) mobile form to a solid and (generally) immobile one. U reduction proceeds by both biotic and abiotic pathways. In both cases, U reduction processes are closely linked to the redox cycles of other biogeochemical elements, notably C, S, and Fe. Abiotic reduction occurs via U(VI) reaction with aqueous and adsorbed Fe(II), aqueous S^{2-} and iron sulfide minerals.^{4,23,26–29} These abiotic pathways often occur alongside biotic pathways, in which U(VI) is used as a terminal electron acceptor by iron and sulfate reducing bacteria.^{30,31} The thermodynamics of U(VI) reduction are similar to Fe(III), though as with Fe(III), reduction energetics vary with U(VI) speciation.^{32,33} Reduction of the calcium uranyl carbonato species (by both abiotic and biotic pathways) is not facile, and thus the presence of these species tends to limit the transformation of U from the aqueous to the solid phase, even following the development of reducing conditions^{33–36}. The ternary complexes of uranyl also have a low affinity for soil and sediment solids, limiting U(VI) adsorption and further shifting U speciation towards non-aqueous phases.^{37,38}

The redox cycles of Fe and S, which have direct impacts on the redox cycling of U (among other elements), also drive the biogeochemical cycles of many non-redox active metals, such as Pb and Cd. The redox transformations of Fe result in (re)generation of Fe-(hydr)oxides, including ferrihydrite and goethite, with highly reactive surface sites and large surface areas, and thus a sizeable capacity for trace metal adsorption.³⁹ Both Pb and Cd form surface complexes with Fe-(hydr)oxides; the presence of Fe-

(hydr)oxides tends to limit the aqueous concentrations of these contaminants.^{40–47} Reductive dissolution of Fe(III)-(hydr)oxides, by both biotic and abiotic pathways, results in concomitant release of adsorbed metals (e.g., Pb, Cd) to the aqueous phase.⁴⁶ Whether the metals remain the aqueous phase depends on the availability of other sorbents and the favorability of mineral precipitation. Where the reductive dissolution of Fe(III)-(hydr)oxides occurs in tandem with sulfate reduction, precipitation of metal-sulfide phases with low solubilities may limit partitioning to the aqueous phase, alongside adsorption to Fe-sulfide phases. Both Pb and Cd form sparingly soluble sulfide minerals.⁴⁸ Oxidative dissolution of sulfide phases can release associated contaminants. While oxidative dissolution of large, low surface area crystalline sulfide minerals (e.g., galena, pyrite) tends to be slow, the dissolution kinetics of high surface area sulfide phases, often of poor crystallinity, such as those formed during rapid changes in solution redox conditions, are generally fast, leading to release of associated contaminants on the timescale of minutes to hours.⁴⁹

1.2 Relationship between chemical reactivity of contaminants, hydrologic conditions, and contaminant transport

The redox processes that drive contaminant transformations in soils and sediments are frequently coupled to hydrologic conditions. In saturated pores, the supply of dissolved oxygen is controlled by advective and diffusive fluxes, depending on the permeability of the soils or sediments and the hydraulic gradients across them. When the rate of oxygen demand exceeds that of supply, anoxic conditions develop, leading to the promotion of anaerobic metabolisms.^{50,51} Transient hydrologic conditions may drive transitions in redox conditions, thereby spurring transformations of redox-sensitive contaminants.^{52–54} Moreover, hydrologic conditions determine whether a mobile contaminant is transported. Hydraulic conductivity and gradient combine to determine the fluxes of mobile contaminants from their host sediments or soils.

1.3 Dissertation overview

The overall goal of this dissertation is to advance our understanding of the impacts of coupled hydrologic-redox processes on the biogeochemical cycles of trace-metal

contaminants (U, Pb, Cd), with an eye towards the chemical mechanisms that affect the mobility of these contaminants.

Chapter 2 develops an understanding of the impacts of the calcium uranyl carbonato ternary complexes on the transformation of U(VI) to reduced U species by reaction with Fe(II). The calcium uranyl carbonato complexes dominate aqueous uranium speciation in systems with dissolved calcium and (bi)carbonate. Their limited reactivity tends to restrict U(VI) adsorption and decrease the rate and extent of U(VI) reduction. In this study, I show that a kinetic constraint inhibits reduction of the calcium uranyl carbonato complexes by Fe(II)_(aq), while reduction by Fe(II) adsorbed on ferrihydrite proceeds. I also demonstrate that the homogeneous reaction produces an unexpected U(V)-bearing ferrihydrite phase, while the surface-catalyzed reaction yield uraninite. Through elucidation of the mechanisms that inhibit reduction of the calcium uranyl carbonato species and promote stabilization of U(V), this chapter advances our understanding of the controls on U solubility, thereby improving prediction of U transport in surface and subsurface systems.

This work has been published in *Environmental Science & Technology* with the title “Calcium-Uranyl-Carbonato species kinetically limit U(VI) reduction and lead to U(V)-bearing ferrihydrite”, and the authors listed as Christian Dewey, Dimosthenis Sokaras, Thomas Kroll, John R. Bargar, and Scott Fendorf. The author contributions are as follows: C.D. and S.F. conceived of the study and collaborated on study design. C.D. conducted laboratory experiments and reaction modeling, collected x-ray absorption measurements and transmission electron microscopy images, and analyzed x-ray absorption data. J.R.B. assisted with U L₃ EXAFS analysis. D.K. and T.K. developed the HERFD spectrometer and assisted with U L, HERFD-XANES measurements. C.D. wrote the manuscript; S.F. and J.R.B. provided editorial advice.

Chapter 3 assesses Pb partitioning in mining-impacted floodplain sediments under transient coupled hydrologic-redox conditions. While Pb, which occurs predominantly as Pb(II), is not itself redox-active in freshwater systems, common Pb hosts – Fe/Mn-(hydr)oxides, sulfide minerals – are sensitive to redox conditions. These hosts may be

dissolved during redox transitions, leading to spikes in porewater Pb concentrations. In this chapter, I show that the affinity of Pb for a range of ligands, its stability in a variety of coordination environments, and slow dissolution and desorption kinetics ensure that partitioning of Pb to the solid phase is more favorable than partitioning to the aqueous phase, even during redox transitions and shifts in phase hosting Pb. Furthermore, I show that if Pb is released during host-phase dissolution, Pb adsorption on particulate organic matter maintains low porewater Pb concentrations. This chapter shows that despite host-phase dissolution during redox transitions, partitioning of Pb to the aqueous phase is minimal and, therefore, transport of dissolved Pb is unlikely.

This work is currently being prepared for submission, with the authors listed as Christian Dewey, Sam Pierce, John R. Bargar, and Scott Fendorf. The author contributions are as follows: C.D. and S.F. conceived of the study and collaborated with J.R.B. on study design. C.D. installed field infrastructure and collected field samples. C.D. and J.R.B. collected x-ray absorption measurements. C.D. analyzed x-ray absorption data. C.D. and S.P. performed sequential extractions. C.D. wrote the manuscript, with editorial advice from J.R.B. and S.F.

Chapter 4 develops an understanding of the impact of redox conditions on the metal-binding properties of dissolved organic matter (DOM). The fundamental processes that regulate the metal-binding properties of DOM are largely unexplored. In particular, variation in DOM metal-binding properties arising from divergent microbial metabolisms has yet to be considered. In this chapter, I show that the metal-binding properties of DOM differ between soil redox environments and, further, that these differences are consistent with predicted changes in DOM functional composition arising from thermodynamic controls on microbial carbon mineralization. I use the contrasting chemical reactivities of Cd and Ni, with Cd preferring soft ligands and Ni hard ligands, to deduce the metal-binding properties of DOM from oxic and anoxic floodplain soils. Water extractable DOM was reacted with Ni and Cd, and the metal complexes, including Fe complexes native to the soil, were then characterized with tandem high-performance liquid chromatograph – inductively coupled plasma mass

spectrometry (HPLC–ICP-MS). With this approach, I reveal that DOM from the anoxic environment has a greater proportion of soft(er) ligands, and thus higher selectivity towards softer metals such as Cd, whereas DOM from the oxic environment has characteristically harder ligands with selectivity for harder metals such as Ni. This work has implications for transport of dissolved metals for which solubility is controlled by DOM. More dissolved soft metals are expected to be transported from reduced environments than from oxidized environments.

This work is being prepared for submission, with the authors listed as Christian Dewey, Rene Boiteau, Hannah Naughton, Peter Nico, and Scott Fendorf. C.D. and S.F. conceived of the study and collaborated with R.B. on study design. C.D. collected and prepared field samples for characterization. C.D. and R.B. performed HPLC–ICP-MS and FT-ICR-MS analyses. H.N. analyzed FT-ICR-MS results and collected and analyzed C NEXAFS spectra. P.N. identified the field site and assisted in design and implementation of field instrumentation. C.D. wrote the manuscript, with editorial input from S.F.

Chapter 5 investigates the impacts of meander-scale sediment variability, seasonal hydrologic conditions, and beaver dams on redox zonation in floodplain sediments in the East River watershed. The resurgence of the American beaver and its return to its historical range has led to increased construction of beaver dams. Beaver dams are known to influence the hydrologic and biogeochemical functioning of floodplains. In mountainous watersheds, seasonal snowmelt dynamics often dominate hydrologic conditions. In this chapter, I show that the transient hydrologic conditions imposed by a cross-channel beaver dam are distinct from the transient hydrologic conditions imposed by seasonal hydrologic dynamics, and further, that the beaver dam shifts redox zonation to a greater extent than seasonal hydrologic conditions. I use a combination of multi-year field observations of hydrologic and biogeochemical conditions, which captured the hydrologic and biogeochemical impacts of a channel-spanning beaver dam, and reactive transport modeling to assess and quantify the impacts of beaver dams on DO intrusion and the development of Fe(III) reducing conditions. This work reveals that

floodplain biogeochemical function varies on the meander scale, and that local hydrologic disturbances caused by beavers have a larger effect on exports from floodplain sediments than seasonal hydrologic conditions

This chapter is being further developed for eventual publication. Listed authors will include Christian Dewey, Patricia Fox, Dipankar Dwivedi, Carolyn Anderson, Marco Keiluweit, Shawn Benner, Peter Nico, and Scott Fendorf. C.D., S.B., P.N., and S.F. conceived of and designed the study. C.D., S.B., P.F., P.N., M.K., and S.F. installed field monitoring equipment. C.D. managed field operations. C.D. and C.P. collected field samples. P.F. performed lab analyses on field samples. C.D. developed the reactive transport model with input from D.D., S.B., P.N., and S.F. The manuscript will be written by C.D., with input from P.N., and S.F.

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Chapter 2:

Calcium-uranyl-carbonato species kinetically limit U(VI) reduction by Fe(II) and lead to U(V)-bearing ferrihydrite

2.1 Abstract

Reaction conditions and mechanisms promoting or inhibiting U reduction exert a central control on U solubility and, therefore, U transport and its associated risks. Here, we vary and track common aqueous uranium species to show that a kinetic restriction inhibits homogeneous reduction of the calcium-uranyl-carbonato species ($\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$) by $\text{Fe(II)}_{(\text{aq})}$, while ferrihydrite surface-catalyzed reduction of all aqueous uranyl by Fe(II) proceeds. Using U L_3 high energy resolution fluorescence detection (HERFD) x-ray absorption near edge structure (XANES) spectroscopy, U L_3 extended x-ray absorption fine structure (EXAFS) spectroscopy, and transmission electron microscopy (TEM), we also show that U(V) is generated and incorporated into ferrihydrite formed from homogeneous U(VI) reduction by $\text{Fe(II)}_{(\text{aq})}$. Through elucidation of the mechanisms that inhibit reduction of the calcium-uranyl-carbonato species and promote stabilization of U(V), we advance our understanding of the controls on U solubility, and thus improve prediction of U transport in surface and subsurface systems.

2.2 Introduction

Mining and refinement of uranium (U) ore has led to U contamination of surface and subsurface environments on all continents except Antarctica.¹ Natural U contamination of groundwater also occurs, often in U-bearing bedrock, jeopardizing aquifers that span these formations.² Given its chemical and radiological toxicity, U contamination poses risks to human health and the environment, especially when it occurs in dissolved (and potentially mobile) forms. Concentrations of dissolved U, and the propensity for U mobilization, vary extensively depending on U speciation.³ In surface and subsurface environments, U redox state is the primary determinant of U solubility, with the dominant oxidized state, U(VI), occurring as uranyl and the series of aqueous carbonate and calcium-carbonate complexes, of which the latter limit U adsorption and precipitation.⁴ The dominant reduced state, U(IV), forms sparingly soluble solids phases, such as uraninite (UO₂), and complexes with particulate organic matter (POM).^{3,5} Because reduction transforms U from a potentially mobile form to an immobile one, understanding U reduction pathways, and the mechanisms promoting or inhibiting these pathways, is essential for understanding U cycling and the risks it imposes.

Uraninite was long assumed to be the sole product of U reduction in surface and subsurface systems. However, this paradigm has recently been challenged, not only by mounting evidence for U(IV) adsorbed to POM, but also by emerging evidence for U(V) in systems with Fe and U. Indeed, U(IV)-POM has been shown to dominate U(IV) speciation in some organic-rich sediments, revealing unexpected variability in U(IV) speciation, while U(V) has been shown to incorporate into iron (hydr)oxides, including goethite, hematite, and possibly ferrihydrite, and to coprecipitate with magnetite and green rusts.⁵⁻¹⁶ The latter work establishes an evolving case for U(V) as an unexpected, but potentially relevant, U redox state in environmental systems, with unknown effects on U speciation and reactivity.

Uranyl speciation exerts a primary control on U(VI) reduction pathways. The formation of uranyl carbonate and calcium-uranyl-carbonate species limits microbial reduction of

U(VI), likely due to steric and thermodynamic factors.^{17–22} Similarly, abiotic reduction of U(VI) by aqueous sulfide, aqueous Fe(II), and surface-bound Fe(II) is fast for free uranyl and the uranyl hydroxides but slow for the uranyl carbonates, perhaps due to sluggish electron-transfer kinetics and more negative reduction half potentials for the uranyl carbonates than for free uranyl and its hydrolysis products.^{24–27} Although the kinetics of abiotic reduction of the calcium-uranyl-carbonato species have been considered obliquely, the reduction kinetics of these species by Fe(II)_(aq) are unresolved.^{17,19,28} Our objective, therefore, is to resolve the mechanisms and rates of U(VI) reduction by Fe(II), with a specific focus on the role these dominant uranyl species play in driving reaction rates, pathways and products.

2.3 Materials and Methods

Stock solutions

Stock solutions were prepared in an anaerobic chamber (95% N₂, 5% H₂ atmosphere; Coy Laboratory Products, Inc., Grass Lake, MI, USA) in acid-washed 25 mL serum bottles using boiled N₂-purged Milli-Q water and analytical-grade chemicals. The following stocks were prepared: UO₂Cl₂, 0.019 M; FeCl₂, 1.51 M; NaHCO₃, 1.04 M; CaCl₂, 2.99 M; piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer, 20 mM, adjusted to pH 7.10. Serum bottles were sealed with butyl rubber stoppers after preparation of stocks.

Analytical methods

Dissolved calcium and uranium were measured on a Thermo Scientific XSERIES 2 quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Fisher Scientific, Waltham, MA, USA). A modified ferrozine assay was used to measure Fe(II)_(aq).^{29,30} Total inorganic carbon (TIC) was measured on a Shimadzu TOC-L total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan).

Preparation of batch reactors

Five reactors were prepared by adding 140 mL of 20 mM PIPES buffer (pH 7.10) to 160 mL serum bottles, followed by aliquots of NaHCO_3 , UO_2Cl_2 , and CaCl_2 stock solutions. The volumes of the stock solutions were adjusted between reactors to achieve different initial proportions of calcium-uranyl-carbonato species ($\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ and $\text{CaUO}_2(\text{CO}_3)_3^{2-}$) (speciation of uranyl in the reactors is provided in Table S1). The reactors were filled with 20 mM PIPES buffer to within 1 mL of capacity, capped with butyl rubber stoppers and crimped, and wrapped in foil. The reactors were allowed to equilibrate for 24 h on a shaker table at 120 rpm, after which the stoppers were removed, 100 μL of FeCl_2 stock was added to each reactor, and the reactors were recapped and crimped. The reactors were sampled immediately after capping and at specified time points thereafter.

Reactors were sampled by syringe using a needle inserted through the butyl stopper into the reactor solution. pH was measured prior to addition of UO_2Cl_2 stock and at the conclusion of experiment. For measurements of dissolved metals, approximately 300 μL of solution was withdrawn from each reactor and filtered through a 0.2 μm regenerated cellulose membrane (Sartorius; Goettingen, Germany) into a 2 mL microcentrifuge tube. 200 μL of filtrate was dispensed into another 2 mL microcentrifuge tube, followed immediately by 200 μL of 0.5 M HCl. Acidified filtrate was stored in capped microcentrifuge tubes at room temperature until dilution and subsequent analysis by ICP-MS and ferrozine assay. For measurement of TIC, approximately 1.5 mL of solution was withdrawn from the reactors and filtered into 2 mL microcentrifuge tubes. One mL of filtrate was then dispensed into 8 mL serum bottles followed by 7 mL of N_2 -purged water. The bottles were capped with butyl rubber stoppers and crimped, after which there was no headspace in the bottles. The bottles were stored at 4 °C until analysis. Finally, two blank reactors were prepared and sampled alongside the reactors described above. One blank contained no iron, the other no uranium.

Solid phase reaction product was collected from duplicate reactors for characterization. After 12 h of reaction, the reactors were removed from the shaker table, allowing the solid phase to settle, after which the majority of the liquid phase was poured off, leaving a volume of approximately 50 mL of reaction solution and suspended solids. The resulting slurries were prepared for x-ray absorption spectroscopy and transmission electron microscopy as described below.

X-ray absorption spectroscopy

High energy resolution fluorescence detection (HERFD) uranium L₃ x-ray absorption near edge structure (XANES) spectra were collected at wiggler beamline 6-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) using an LN₂-cooled Si (311) double monochromator.³¹ The emission energy of the U L α 1 line was measured using a seven-crystal Ge (777) 1-meter radius Johann-type x-ray emission spectrometer at an energy of approximately 13615 eV. HERFD-XANES measurements were performed at room temperature with a spectral resolution of 1.6 eV. Uranium L₃ extended x-ray absorption fine structure (EXAFS) spectra were collected at wiggler beamline 11-2 at SSRL using an unfocused beam on a LN₂-cooled Si (220) double monochromator in the $\phi = 0^\circ$ orientation. The measurements were performed in transmission mode with N₂ ionization chambers. A LN₂ cryostat was used to maintain anoxic conditions and low temperature (77 K).

Samples for x-ray absorption measurements were prepared from slurries from duplicate reactors. For EXAFS measurements, the slurry was vacuum-filtered through 0.22 μ m filter paper, which was placed between two pieces of Kapton tape and mounted on a sample holder. The sample was stored under anoxic conditions until the measurements were performed. For HERFD-XANES measurements, the reactor slurries were pipetted into micro-centrifuge tubes and spun at 14000 rpm for 5 min, after which the supernatant was removed. The resulting pastes were washed three times with 1.5 mL N₂-purged Milli-Q water and spun at 14000 rpm for 1 min. After the third wash and spin cycle, the pastes were freeze-dried. The samples were prepared in an anaerobic chamber (95% N₂, 5% H₂ atmosphere) and stored there until analysis. The sealed micro-centrifuge tubes

were placed in the beamline sample holder, and x-ray absorption measurements were collected under ambient conditions over a period of approximately 2 h. No spectral changes were observed between consecutive scans that would indicate sample oxidation or incident x-ray beam damage during the measurements.

A uraninite slurry was prepared by addition of 200 μL N_2 -purged Milli-Q water to 0.2 g uraninite powder in a screw top micro-centrifuge with a rubber gasket. Uranyl adsorbed to ferrihydrite (Fh) was prepared by adding 0.046 g two-line ferrihydrite, prepared in accordance with Herbel and Fendorf,³² to 50 mL of 0.10 mM $\text{UO}_2\text{CO}_3^{4-}$ buffered at pH 7.05 with carbonate. The slurry was mixed for 4 h on a rotary shaker at 60 rpm, followed by centrifugation at 4000 rpm for 5 min. The supernatant was drained, and the resulting slurry was transferred into a micro-centrifuge tube with a rubber gasket. HERFD-XANES measurements were collected for the reaction products as well as the uraninite slurry and uranyl adsorbed to ferrihydrite. A monochromator crystal glitch at ~ 17274 eV was used as an internal standard to align consecutive energy scans within and across samples. Calibration and alignment of the EXAFS data were performed in SixPack.³³ The data were normalized, background-subtracted, and fit in the Athena and Artemis software packages, using FEFF 8.4.^{34,35} Calibration, alignment, and normalization of the HERFD-XANES data were performed in Athena.³⁴

Transmission electron microscopy

For examination with transmission electron microscopy (TEM), selected area diffraction (SAD), and energy-dispersive X-ray spectroscopy (EDS), solid phase reaction product was concentrated by centrifugation, similar to the protocol for HERFD-XANES analysis, with concentrated pastes suspended in 200 μL of degassed ethanol. The resulting slurry was applied to holey carbon films on copper support grids (Ted Pella, Inc., Redding, CA, USA). Samples were prepared and stored under an anoxic atmosphere (95% N_2 , 5% H_2 atmosphere) until transfer to the TEM vacuum chamber. Analysis was conducted at the Stanford Nano Shared Facilities using a FEI Tecnai G2 F20 X-TWIN transmission electron microscope (FEI, Hillsboro, OR, USA). The field-

emission gun was operated at an accelerating voltage of 200 kV. Images were collected with a CCD camera.

Reactor modeling and thermodynamic calculations

Equilibrium conditions were modeled with PFLOTRAN to determine aqueous chemical speciation.^{36,37} Model inputs were the measured concentrations of total uranium, total inorganic carbon, total ferrous iron, and total calcium; pH was set to the measured value of 7.10. Charge was balanced with Cl⁻. Activities generated by the model were used in calculations of the Gibbs free energy for reduction (ΔG_R) of the uranyl species by Fe(II)_(aq) for the following reactions:

- (1) $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3 + 2\text{Fe}^{2+} + 6\text{H}_2\text{O} \rightarrow 2\text{Ca}^{2+} + 3\text{HCO}_3^- + 3\text{H}^+ + 2\text{Fe}(\text{OH})_{3(s)} + \text{UO}_{2(s)}$
- (2) $\text{CaUO}_2(\text{CO}_3)_3^{2-} + 2\text{Fe}^{2+} + 6\text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + 3\text{HCO}_3^- + 3\text{H}^+ + 2\text{Fe}(\text{OH})_{3(s)} + \text{UO}_{2(s)}$
- (3) $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^- + 4\text{Fe}^{2+} + 9\text{H}_2\text{O} \rightarrow \text{HCO}_3^- + 8\text{H}^+ + 4\text{Fe}(\text{OH})_{3(s)} + 2\text{UO}_{2(s)}$

The Gibbs energies of formation used in the calculations were taken from Ginder-Vogel et al.³⁸ and Guillaumont et al.³⁹ (Table S1.2). To calculate the Gibbs free energies, we first determined U speciation in the reactors at each sampling point, employing equilibrium calculations (PFLOTRAN) with the measured pH and the concentrations of U(VI), Fe(II), Ca²⁺, and TIC. We then used the calculated activities of the three predominant U species – $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, $\text{CaUO}_2(\text{CO}_3)_3^{2-}$, $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ – and Fe(II)_(aq), Ca²⁺, H⁺ and HCO₃⁻ to calculate ΔG_R .

PFLOTRAN was used to construct a kinetic model of the reaction network in our reactors. Measured concentrations and pH were used as initial conditions. Only the three predominant aqueous U species – $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, $\text{CaUO}_2(\text{CO}_3)_3^{2-}$, $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ – were ultimately included in the model; inclusion of other aqueous U species did not affect model results, as their concentrations were extremely low. Change in aqueous U speciation was treated as an equilibrium process. Reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ by Fe(II)_(aq) was treated as a first-order reaction, and the rate constant was parameterized with experimental data. Surface-catalyzed reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ and the calcium-uranyl-carbonato complexes was represented as a second-order process

dependent on Fe(III) concentration. A complete description of model formulation appears in the SI.

2.5 Results

Reduction kinetics across U speciation conditions

To probe the pathways of reduction of the calcium-uranyl-carbonato species by $\text{Fe(II)}_{(\text{aq})}$, we performed a series of batch reactions in which reduction was thermodynamically viable. We varied the calcium and carbonate concentrations to yield different proportions of total calcium-uranyl-carbonato species. Assuming that U equilibration was much faster than U reduction, we used equilibrium modeling to determine U(VI) speciation and reactant activities at all time points (Table S1.1). We measured dissolved U(VI) and $\text{Fe(II)}_{(\text{aq})}$ to track the progress of the reaction, with the assumption that oxidized Fe and reduced U would form precipitates of limited solubilities.

As the reaction progressed, the rate of U removal decreased, concurrent with a shift in speciation away from $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ toward a greater proportion of total calcium-uranyl-carbonato species. For the reactor with U initially occurring as 96% calcium-uranyl-carbonato species, the concentration of U did not decline with time, and concentrations of dominant U species did not change appreciably over 98 h (Figure 1.1; Figure S1.1); the proportion of total calcium-uranyl-carbonato species was constant at 96.2% (of total U), and the proportion of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ was 3.8% (Figure 1.2; Figure S1.2). For the reactors with 68%, 51% and 22% of U initially occurring as calcium-uranyl-carbonato species, U concentrations decreased to 63.5, 16.3, and 6.98 μM , respectively, with the concentrations of all species declining over 98 h (Figure 1.1; Figure S1.1). In these reactors, the proportion of calcium-uranyl-carbonato species steadily increased and exceeded 90%, while the proportion of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ steadily decreased (Figure 2; Figure S2B-E). From 0 to 24 h, the decline in concentrations of mono and di-calcium-uranyl-carbonato species corresponded to an increase in the proportions of these species (Figures S1.1 and S1.2). After 36 h, however, the

proportions of the mono- and di-calcium uranyl species changed minimally, while their absolute concentrations decreased, albeit slowly ($\text{Rate} < 1 \mu\text{M} [\text{U}] \text{ h}^{-1}$) (Figures S1.1 and S1.2). In the absence of calcium, the concentration of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ decreased from 238 to 5.46 μM , and its proportion of total U fell from 96.0% to 71.5% (Figure 1.1; Figures S1.1A and S1.2A).

For all initial conditions, reduction of the three predominant uranyl species by $\text{Fe(II)}_{(\text{aq})}$ is thermodynamically favorable throughout the experiment. For the reactor with U initially occurring as 96% calcium-uranyl-carbonato species, the ΔG_R for reduction of the mono- and di-calcium-uranyl-carbonato species are -36 and -28 kJ/mol (respectively), and do not change over the course of the experiment (Figure 1.1; Table S1.3). For the reactors with 68%, 51% and 22% of U initially occurring as calcium-uranyl-carbonato species, the ΔG_R for reduction of the calcium-uranyl-carbonato species increased slightly over the course of the experiment, from a range of -40 to -42 kJ/mol to a range of -32 to -35 kJ/mol for the mono-calcium species, and from a range of -31 to -34 kJ/mol to a range of -24 to -27 kJ/mol for the di-calcium species, with greatest change occurring between 0 and 40 h of reaction (Figure 1.1; Table S1.3). The ΔG_R for reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ was also negative throughout the experiment in all reactors (Table S1.3).

From reactions (1-3), we expected removal of two mol Fe per mol U (through, respectively, oxidative and reductive precipitation). However, the calculated ratios of Fe:U removal, based on measured Fe and U concentrations, do not agree with our predictions (Table S1.1; Figure S1.4). At 24 h, the Fe:U removal ratios for the reactors with 68%, 51%, 22%, and 0% of U initially occurring as calcium uranyl carbonato species were 0.74, 0.73, 0.69, and 0.60, seemingly indicating a decrease in the extent of reduction with decreasing proportions of these species (the ratio from the reactor with U initially occurring as 96% calcium-uranyl-carbonato species is omitted, as there was no appreciable U removal in this reactor).

Oxidation State of Solid Phase Uranium

We collected U L₃ HERFD-XANES spectra of the solids, as well as uraninite and UO₂(CO₃)₃⁴⁻ adsorbed to ferrihydrite (U(VI)-Fh). The spectra of the solids contain a pre-edge feature at 17165.0 eV (feature A, Figure 1.3), an intense peak (white line) ranging from 17172.8 eV in the solid from the reactor without calcium to 17173.6 eV in the solids from the reactor with 68% U as calcium-uranyl-carbonate initially (feature B, Figure 3), and a broad resonance on the high-energy shoulder of the absorption edge at 17185.0 eV (feature C, Figure 1.3). The pre-edge feature also appears in the spectrum of U(VI)-Fh, as does the high-energy resonance, though the intensities of both features are more pronounced in the spectrum of U(VI)-Fh than in the spectra of the reaction product. Both the pre-edge feature and the high-energy resonance are absent in the spectrum of uraninite.

We first considered the possibility that the spectra of the reaction products were linear combinations of U(VI)-Fh and UO₂ spectra. Based on observed reaction stoichiometry, if UO₂ were the only reduced U phase in the solid, the solid would consist of 30-50% UO₂ and 70-50% U(VI)-Fh, depending on the reactor. Yet, linear combinations of 30% UO₂ and 70% U(VI)-Fh, and 50% UO₂ and 50% U(VI)-Fh did not fit the measured spectra. In both cases, the LCFs yielded spectra with a suppressed pre-edge feature and an intense resonance at 17185.0 eV (Figure S1.5A), and, further, a shift in the first EXAFS oscillation (~17210 eV) to lower energy relative to the first EXAFS oscillation in the measured spectra (Figure S1.5B). It was not possible to fit the measured spectra with any linear combination of UO₂ and U(VI)-Fh spectra, and only a combination of 10% UO₂ and 90% U(VI)-Fh produced a spectrum with the pre-edge feature (Figure S1.5B). Thus, the intensity of the pre-edge feature in the reaction product spectra, and its absence in the LCF results, indicate the reaction product contains <10% uraninite.

A second reduced U phase must therefore occur to satisfy observed reaction stoichiometry. Spectral features suggest the reaction product contains predominantly U(V) in a low-symmetry environment, and U(VI) as uranyl, but no detectable (by XAS) UO₂. The pre-edge feature, which has been previously documented in spectra of low-

symmetry U(V) and U(VI) compounds, arises from quadrupole $2p_{3/2} \rightarrow 5f$ electronic transitions.^{40,41} The resonance is masked in the spectra of U(IV)O₂ by splitting of the $6d$ band and a resultant broad, asymmetrical white line, which (in all U valence states) arises from dipole-allowed $2p_{3/2} \rightarrow 6d$ electronic transitions.^{41,42} Large field splitting of the $6d$ band, and subsequent masking of the pre-edge feature, is also theorized to occur in high-symmetry U(V) and U(VI) environments.^{43,44} Thus, the presence of the pre-edge feature in the reaction product spectra suggests that UO₂ is not a major component of the solid, and, further, that it is unlikely that the average local U environment is highly symmetrical. However, the broader white-lines in the reaction product spectra relative to the spectrum of U(VI)-Fh suggest an increase in symmetry in the reaction product relative to U(VI)-Fh. Incorporation of uranyl (V or VI) into ferrihydrite would drive such a change in symmetry, as would coordination of U(V) in a disordered local environment. Consistent with an increase in symmetry relative to adsorbed uranyl, the broad resonance occurring at 17185.0 eV in the reaction product spectra is less intense than the resonance at the same energy position in the spectrum of U(VI)-Fh (Figure 1.3). In the spectra of uranyl moieties, the resonance arises from multiple scattering of the $2p_{3/2}$ photoelectron along the axial O atoms.^{35,45,46} Its presence in the reaction product spectra indicates that uranyl is present in the solid, but its lower intensity (relative to the same feature in the spectra of U(VI)-Fh) points to either lengthened axial U–O bonds, which would occur in an incorporated uranyl (V or VI) phase, or a mixture of adsorbed uranyl and a disordered non-uranyl U phase. Finally, the white-lines of the reaction product fall between the white-lines of uraninite (17172.5 eV) and U(VI)-Fh (17173.6 eV), consistent with a U valence state between (IV) and (VI) (i.e., (V)). Their positions also increase with increasing initial proportions of calcium-uranyl-carbonato species, from 17172.8 eV in the reactor with no calcium to 17173.6 eV in the reactor with U initially occurring as 68% calcium-uranyl-carbonato species, suggesting that, in contrast to the Fe:U ratios, the proportion of reduced U decreases with increasing initial proportions of calcium-uranyl-carbonato complexes (Figure 1.3).

Local Structure and Phase Identification of U and Fe Solids

We performed TEM imaging of the produced solids, with simultaneous EDS and SAD analyses. The TEM images reveal the presence of nanocrystalline UO_2 , alongside an amorphous phase containing both U and Fe. The nanocrystalline domains (red arrow, Figure 4), which are assembled in elongated spindles joined at polycrystalline nodes, exhibit lattice spacings of 3.19 and 1.93 Å (Figure S1.7), consistent with the most intense diffraction lines for uraninite, and contain uranium but no iron (Figure S1.7). The amorphous phase appears as a haze in the TEM images (blue arrow, Figure 4), distinct from the holey carbon film. Lattice fringes cannot be discerned within this phase, indicating that it lacks crystalline structure. Given that it contains both Fe and U (Figure S1.7), the phase is likely ferrihydrite, with incorporated or adsorbed U. The observation of uraninite in the TEM images, alongside the absence of its features in the U L_3 HERFD-XANES spectra, indicates that a small U pool (<10%) occurs as uraninite.

We also collected U L_3 EXAFS measurements of the produced solids and performed a shell-by-shell fitting of the data. The U EXAFS spectrum and its Fourier transform (FT) are shown in Figure 4. We identified and fit multiple shells in the FT of the EXAFS: a first shell at 1.38 Å; a second shell at 1.90 Å; and three outer shells at 2.80, 3.30, and 3.80 Å. Peak distances in the FT are reported in units of Å and are uncorrected for phase shift. When referring to neighboring atoms, we report actual distances.

The fit results indicate that ~30% of solid U occurs as uranyl, and ~70% as a non-uranyl U phase. The amplitude of the peak in the FT at 1.38 Å indicates a shell of ~0.7 oxygens at ~1.78 Å (Table S1.4). Oxygens at this distance are diagnostic of the trans-dioxo structure of uranyl, in which two axial oxygens are triple-bonded to a central U atom.⁴⁵ Based on this feature, the coordination number (CN) estimated in the fit of 0.7 oxygens at ~1.78 Å indicates that ~30% of U in the produced solids occurs as uranyl.

Fitting the second shell suggests a mixture of U phases in the produced solid. The best fit for the second shell is one oxygen at ~2.33 Å (Table S1.4). While this U–O distance is consistent with that of the equatorial shell in uranyl phases and the first oxygen shell in uraninite, the CN is unrealistically low relative to expected values.^{45,47,48} In uranyl

carbonato phases, including uranyl carbonato complexes adsorbed on ferrihydrite, the equatorial shell contains four to six oxygens ranging from 2.33 Å to 2.41 Å, while in uraninite the U atom is coordinated with eight oxygens at 2.35 Å.^{47,48} We considered whether the low CN could be an artifact of data collection; the data quality appears sufficient to resolve the equatorial shell, and self-absorption would not affect our measurements, as we collected EXAFS in transmission mode. It is possible that the low CN reflects a disordered second shell with oxygens at a range of distances, producing frequencies that destructively interfere and reduce the second shell amplitude. U(V) coordination has been shown to exhibit such disorder, with U–O pairs ranging from 2.10 Å to 2.40 Å, resulting in suppressed second shell amplitude and unrealistically low CNs, though symmetrical U(V) coordination is also possible.^{14,49–51} We attempted to fit oxygens at a range of distances (between 2.10 Å and 2.40 Å) but were unable to improve the fit to the second shell CN. While a disordered U(V) coordination environment would result in destructive interference and subsequent amplitude reduction that cannot be captured by the Debye-Waller factor, the presence of uraninite and adsorbed uranyl alongside an incorporated U(V) phase would produce a similar effect.

Fitting the outer shells suggests that U occurs predominantly as a mixed U-Fe phase. We fit a U–Fe pair at ~3.49 Å, consistent with the U–Fe distances for U(V) incorporated in goethite and magnetite. Given that the analysis of the second shell is consistent with a U(V) phase, the fit results point to U(V) incorporated in an Fe(III) phase.^{12,47,48,51} Multiple scattering paths are known to produce low amplitude features between 3 and 4 Å in the FT of the EXAFS of uranyl carbonate moieties, but inclusion of these paths in the fit did not fully capture the outer FT structure.⁴⁵ Based on the TEM results, we also fit a U–U path at ~3.89 Å, a representative U–U distance in UO₂. Our interpretation of the third shell is consistent with that of the second shell, which points to multiple U phases in the produced solid.

Finally, we collected Fe K EXAFS measurements of the produced solids. The Fe EXAFS spectrum is shown in Figure S1.6. Linear combination fitting of the spectrum indicates that 99% of Fe occurs as ferrihydrite (Figure S1.6). This, alongside the

observation of an amorphous phase in the TEM images and the presence of Fe in the outer shell of the U EXAFS, indicates that U is incorporated in ferrihydrite formed after oxidation of $\text{Fe(II)}_{(\text{aq})}$ by U(VI). Incorporation in ferrihydrite would likely result in a disordered local U environment, consistent with our analyses of the U L_3 HERFD-XANES and EXAFS.

2.6 Discussion

A kinetic constraint appears to inhibit homogeneous reduction of the calcium-uranyl-carbonato species by $\text{Fe(II)}_{(\text{aq})}$. In the reactor with 96% of U occurring as calcium-uranyl-carbonato species, no visible solid is produced over the course of the experiment, and U concentrations decline by less than 3.3% (Figures 1 and 2), indicating that U reduction and coupled Fe oxidation are occurring at a negligible rate. Yet, reduction of the calcium-uranyl-carbonato species is thermodynamically favorable based on calculated ΔG_R (Figure 1.1, top panel; Figure S1.3). The apparent lack of reduction despite favorable thermodynamics (calculated using constants derived from conditions similar to those in our experiments, yielding confidence in their validity⁵²) thus indicates that a kinetic constraint inhibits the reaction.

We suggest that rigidity in the equatorial plane, coupled with shifts in electron density in the equatorial oxygens, both of which (we posit) arise from the bound calcium atom(s), render potential coordination sites unfavorable (sterically or energetically) for coordination of the smaller Fe^{2+} cation, thus inhibiting formation of (hypothetical) reactive intermediates, $\text{FeUO}_2(\text{CO}_3)_3^{2-}$ or $\text{FeCaUO}_2(\text{CO}_3)_3$. Given that reduction of both $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ is inhibited, a steric hindrance effect imposed by Ca does not seem likely; if steric hindrance were occurring, it would be reasonable to expect some reduction of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$, given it contains two potential coordination sites in the equatorial plane for a divalent cation (assuming that $\text{FeUO}_2(\text{CO}_3)_3^{2-}$ or $\text{FeCaUO}_2(\text{CO}_3)_3$ are necessary reactive intermediates).

In the reactors with lesser initial proportions of the calcium-uranyl-carbonato species, the reactive U component (toward $\text{Fe(II)}_{(\text{aq})}$) appears to be $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$. As the

initial proportion of the calcium-uranyl-carbonato species increases, the initial rate of overall U reduction, calculated as the change in U concentrations over the first 24 h of reaction, decreases (Table S1.5). This relationship likely results from the kinetic constraint noted above: as the initial proportion of calcium-uranyl-carbonato species increases (across initial reaction conditions), the initial proportion of unreactive U (toward $\text{Fe(II)}_{(\text{aq})}$; i.e., calcium-uranyl-carbonato species) also increases, resulting in a smaller concentration of labile U (i.e., $(\text{UO}_2)\text{CO}_3(\text{OH})_3^-$), and thus a smaller reduction rate. Further, assuming a pseudo-first order rate expression for overall U reduction, $R_{\text{overall}} = k * [U_{\text{total}}]$, the apparent rate constant, k , varies (non-linearly) with the initial percentage of calcium-uranyl-carbonato species, and therefore the initial percentage of the dominant non-calcium uranyl species, $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ (Figure S1.8). By definition, k is constant; thus, the apparent relationship between k and the concentration of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ in fact reflects a relationship between the overall reduction rate and the concentration of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ and, further, independence of the reduction rate and the total U concentration. Hence, the rate expression is reformulated as $R_{\text{overall}} = k * [(\text{UO}_2)_2(\text{CO}_3)(\text{OH})_3^-]$. This implies that initially $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ is the reactive U(VI) species in our experiment, and that the proportion (and therefore concentration) of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ determines the initial rate of overall homogeneous U reduction by $\text{Fe(II)}_{(\text{aq})}$.

As homogeneous reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ proceeds, U speciation shifts toward 100% calcium-uranyl-carbonato species, slowing the reduction rate. Following reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, there ensues a momentary disequilibrium between $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ and $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$. Uranium in the calcium-uranyl-carbonato species is converted to $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ in response to this disequilibrium, resulting in a decrease of the concentrations of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$. Yet, as the concentrations of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ decrease, the proportion of U occurring as these species increases; the decrease in total U concentrations amid fixed calcium and carbonate concentrations results in thermodynamic conditions that favor greater proportions of the calcium-uranyl-carbonato species. The reduction-equilibration process continues until the proportions

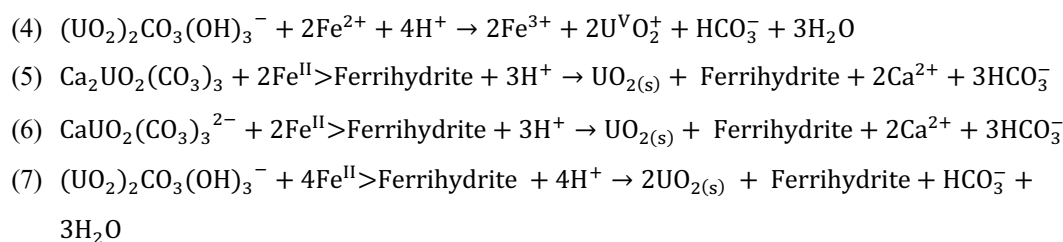
of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ are steady, and >92% of U occurs as these species. At this point, changes in aqueous speciation reach steady-state conditions.

After U speciation reaches steady-state and calcium-uranyl-carbonato species dominate U speciation, the reaction proceeds at a slower rate, and a heterogeneous U reduction pathway is promoted. Similar to the reactor with 96% of U as calcium-uranyl-carbonato, the shift in U speciation toward >92% calcium-uranyl-carbonato species results in negligible concentrations of reactive U(VI) species (toward $\text{Fe(II)}_{(\text{aq})}$). If homogeneous reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ were the sole reduction pathway in these reactors, we expect the reduction rate would be negligible. Ferrihydrite, produced by oxidation of $\text{Fe(II)}_{(\text{aq})}$ coupled to reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, allows for a heterogeneous (surface-catalyzed) reaction (no ferrihydrite was produced in the reactor with 96% of U initially as calcium-uranyl-carbonato). Sorption of the calcium-uranyl-carbonato species to ferrihydrite is possible, although its effect on U concentrations would be fast (occurring in seconds to minutes) and relatively small, given the propensity of the calcium-uranyl-carbonato species to remain in solution.⁴ Indeed, the sustained decrease of U concentrations suggests that sorption is not the dominant mechanism of U removal and instead reflects a heterogeneous U reduction pathway, activated by ferrihydrite, and capable of reducing the calcium-uranyl-carbonato species, such as the pathway described by Liger et al.²⁷ Heterogeneous reduction of the calcium-uranyl-carbonato species appears to be slow relative to homogeneous reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, but relatively fast compared to homogeneous reduction of the calcium-uranyl-carbonato species (Table S1.5). This suggests that the heterogeneous pathway overcomes the kinetic constraint inhibiting homogeneous reduction of the calcium-uranyl-carbonato species. Thus, as the U(VI)-Fe(II) reaction generates ferrihydrite, reduction shifts from occurring predominantly through the homogenous pathway and toward the heterogeneous one.

Reaction network modeling supports the conclusion of both a homogeneous and heterogeneous reaction pathway for uranyl reduction. Inclusion of heterogeneous pathways is necessary to reproduce the uranium concentrations observed in the reactors,

except in the reactor with 96% of U initially occurring as calcium-uranyl-carbonato (Figure 1.1). In this case, the improved fit with omission of the heterogeneous pathways illustrates the kinetic limitation arising from slow adsorption of the calcium-uranyl-carbonato species on trace amounts of ferrihydrite.

The activity of two reduction pathways indicates that proposed reactions 1–3 do not fully describe U(VI) reduction by Fe(II). The dominant presence of U(V) and the minor amount of UO_2 in the reaction product reveal that a two-electron transfer does not occur to the extent anticipated. We thus posit the following reactions:



Precipitation of U(V)-bearing ferrihydrite may prevent the second electron transfer (from either Fe(II) or U(V)) in the homogeneous reaction. Roberts et al.⁸ offers precedent for U(V) co-precipitating in various Fe phases, though not in ferrihydrite. Similarly, Massey et al.¹² show incorporation of U(V) into goethite, but find only a small proportion of U is incorporated as U(V), and do not observe incorporation of U(V) into ferrihydrite. Alternatively, it is possible that the second electron transfer cannot occur via a homogeneous pathway, by either U(V) disproportionation or transfer from $\text{Fe(II)}_{(\text{aq})}$ to U(V), or that a threshold $\text{U(V)}_{(\text{aq})}$ concentration is required for disproportionation at circumneutral pH. Density functional theory and Marcus theory calculations by Collins and Rosso⁵³ indicate that neither U(V) disproportionation nor U(V) reaction with $\text{Fe(II)}_{(\text{aq})}$ are feasible at circumneutral pH.

We propose that a second electron transfer by a homogeneous pathway is less favorable than U(V) precipitation with Fe(III). However, the production of ferrihydrite activates a heterogeneous reduction pathway with a lower activation energy for the second electron transfer. As the amount of ferrihydrite increases, the heterogeneous pathway is promoted, increasing uraninite yield. The shifts in position of maximum absorption in

the HERFD-XANES spectra thus likely reflect an increased proportion of UO_2 (Figure 1.3) and, therefore, increased activity of the heterogeneous pathway. The latter reconciles decreasing Fe:U removal ratios (indicating increased U adsorption) with decreasing maximum absorption positions (indicating increased U reduction). As the proportion of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ increases, ferrihydrite production also increases (via reduction of $\text{U(VI)}_{(\text{aq})}$ by $\text{Fe(II)}_{(\text{aq})}$). Simultaneously, U(VI) sorption becomes more favorable, due to a decrease in concentration of the calcium-uranyl-carbonato species. The result is an increase in U(VI) sorption, which decreases the Fe:U removal ratio, alongside promotion of a heterogeneous reduction pathway, which increases production of UO_2 . Because the solids were washed before the HERFD-XANES measurements, the maximum absorption positions largely reflect the speciation of non-adsorbed solid U.

Finally, the dual reaction network reconciles our findings with those of Massey et al.¹², in which uraninite was the dominant U product. In the Massey et al. experiments, ferrihydrite was initially present in all reactors, and thus the second electron transfer was viable and dominant.¹² The mechanism they propose for U(V) incorporation in goethite, and U(V) stabilization via a heterogeneous pathway, is consistent with the reaction network revealed in this work. Yet, the pathway they observe appears to yield a fraction of solid U(V) relative to the pathway we observe here due to the initial presence of ferrihydrite.

2.7 Conclusion

Here, we show that homogeneous reduction of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ or $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ by $\text{Fe(II)}_{(\text{aq})}$ is kinetically inhibited, while heterogeneous reduction of these species proceeds, albeit slowly (relative to homogeneous reduction of non-Ca uranyl species). By inhibiting homogeneous U(VI) reduction by $\text{Fe(II)}_{(\text{aq})}$ and slowing heterogeneous U(VI) reduction, Ca limits sequestration of U and promotes U solubility. Such an effect is necessary to consider particularly in Karst formations or arid environments, such as the western U.S., where calcium and carbonate are commonly abundant in surface and groundwater systems.

We also show that U(V)-ferrihydrite is a viable U reduction product under the conditions of our experiments. Our findings suggest that U(V)-ferrihydrite may occur in sediments that experience a sudden onset of iron-reducing conditions. Reduction of Fe(III)-(hydr)oxides would yield Fe(II)_(aq), which would react with U(VI)_(aq), yielding transient U(V)_(aq). Depending on the abundance of Fe(III)-(hydr)oxides and the rate of microbial U(VI) reduction, a portion of the U(V)_(aq) would precipitate with Fe(III) as ferrihydrite. Further work is necessary to evaluate the long-term stability of this phase, as it may serve as both a source and sink of aqueous U, depending on the timeframe considered.

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2.10 Figures

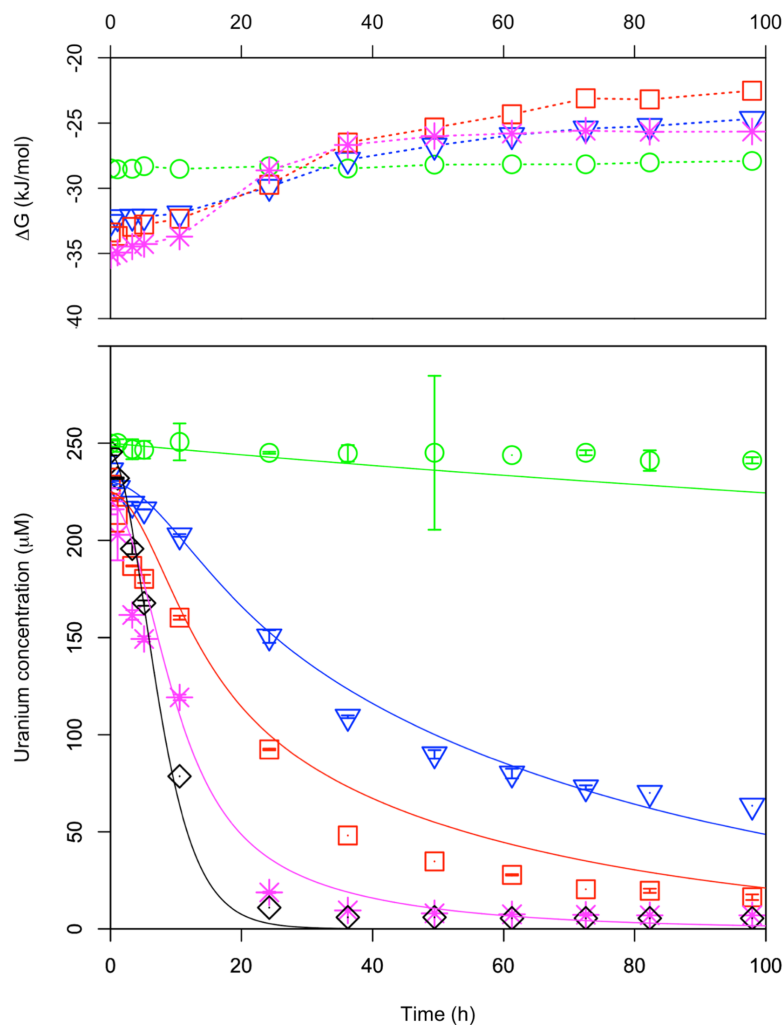


Figure 2.1. Bottom panel. U concentrations throughout the experiment (symbols) and output from the kinetic reaction model (lines) for the following initial proportions of U as calcium-uranyl-carbonato species: 96% (green circles/line), 68% (blue triangles/line), 51% (red squares/line), 22% (magenta stars/line), and 0% (black diamonds/line). Concentrations are averages of duplicates. Error bars do not appear for duplicates with equal concentrations. Top panel. Distributed Gibbs free energies of reduction for the calcium-uranyl-carbonato species. Plotted values are the weighted averages of the Gibbs free energies for the mono- and di-calcium species. At each time point, the Gibbs free energies were calculated for both mono- and di-calcium-uranyl-carbonato species. These values were multiplied by the fraction of total calcium-uranyl-carbonato occurring as each (mono- or di-calcium) species. The resulting values were summed and plotted.

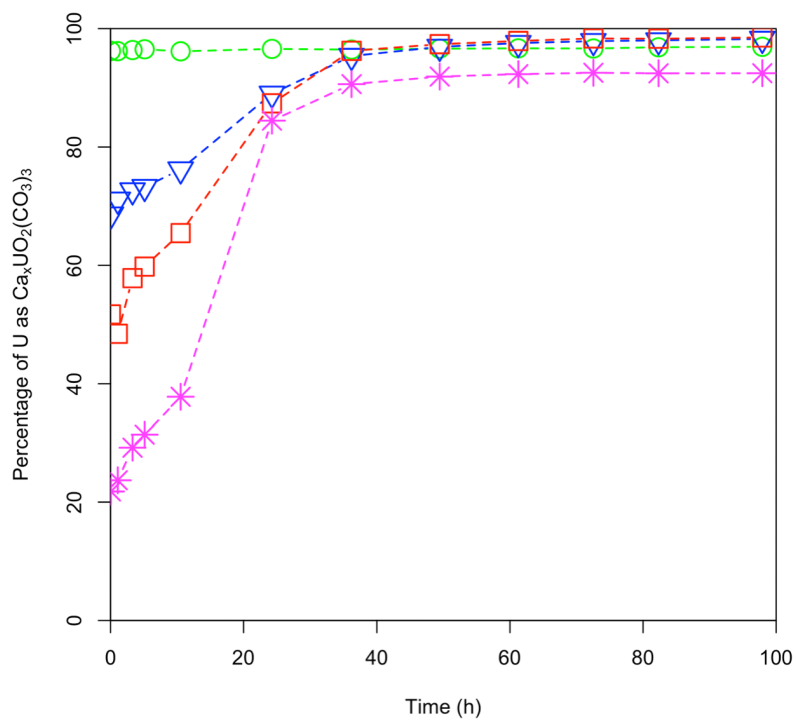


Figure 2.2. Proportions of aqueous U as total (mono- and di-) calcium-uranyl-carbonato species throughout the experiment, given the following initial proportions of these species: 96% (green), 68% (blue), 51% (red), and 22% (magenta).

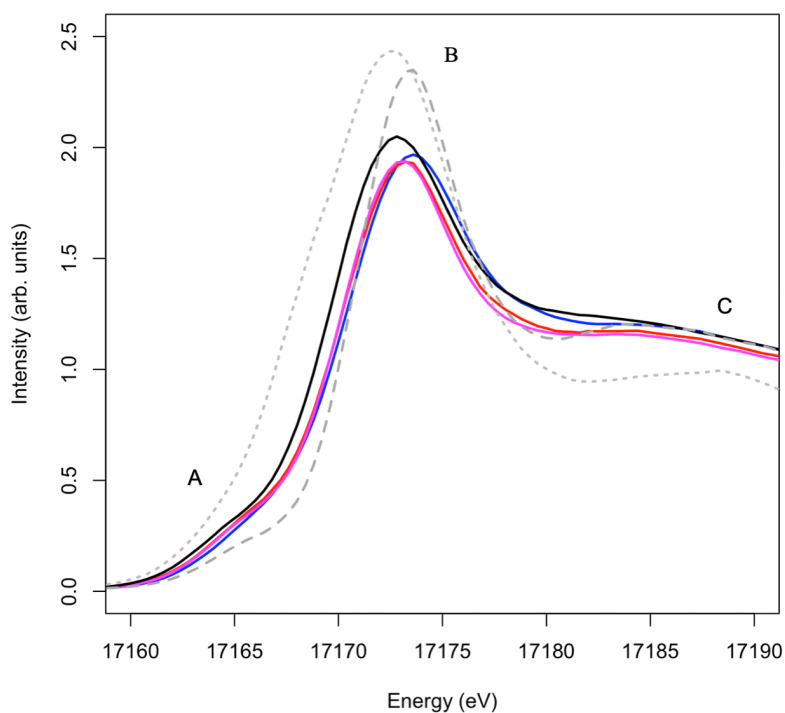


Figure 2.3. U L_3 HERFD-XANES spectra of reaction solids for the following initial proportions of U as calcium-uranyl-carbonate species: 68% (blue), 51% (red), 22% (magenta), and 0% (black). Spectra of uraninite (grey; small dashes) and uranyl adsorbed to ferrihydrite (grey; large dashes) are also shown. The pre-edge structure, white-line region, and post-edge resonance are marked with A, B, and C (respectively).

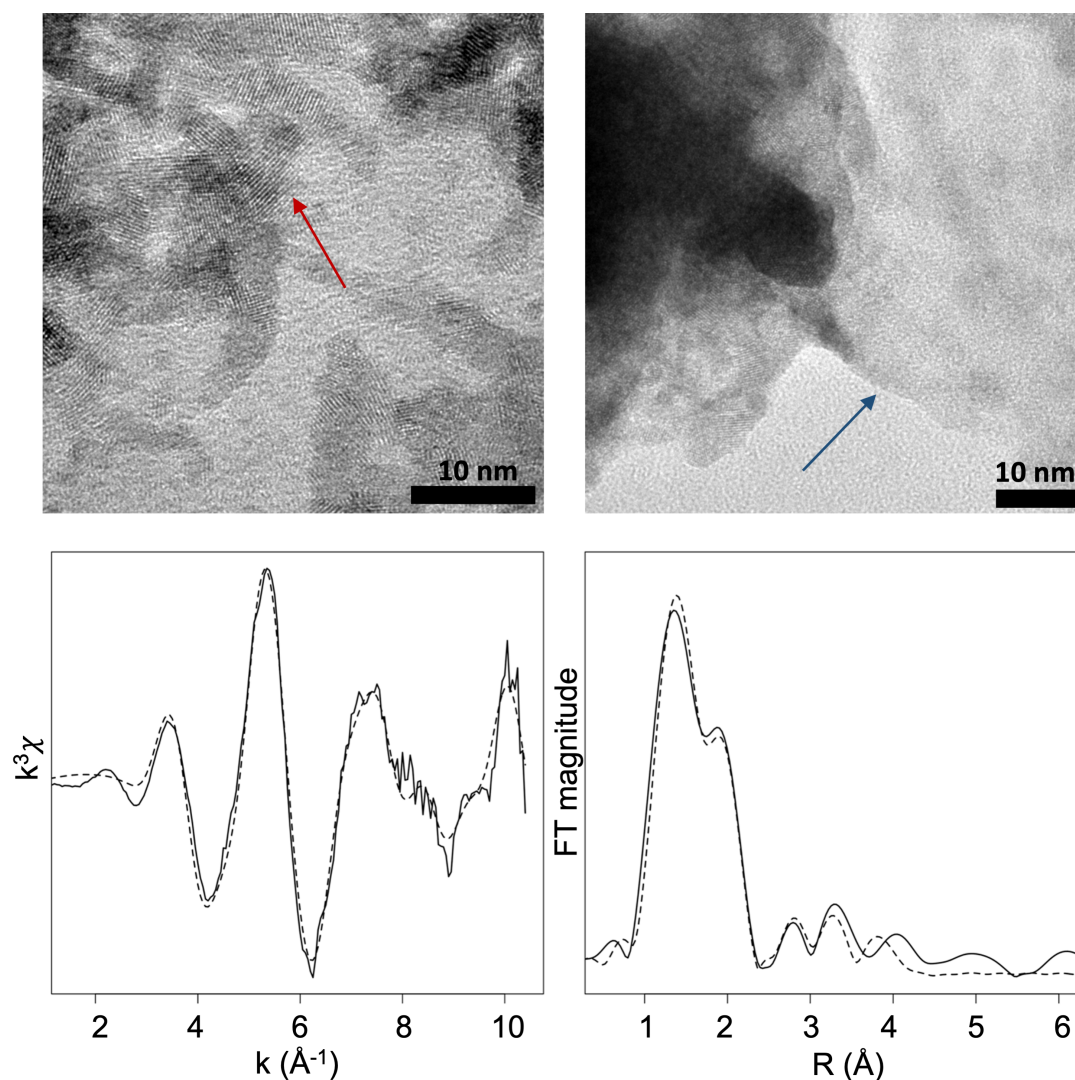


Figure 2.4. *Top.* TEM images of solid from the reactor with 68% of U initially occurring as calcium-uranyl-carbonato species. Nanocrystalline domains occur in the solid (upper left panel, red arrow), with lattice spacings of 3.19 and 1.93 Å, consistent with the dominant lattice spacings of uraninite (Figure S7). EDS analysis indicates the presence of uranium, but no iron, in these domains (Figure S7). An amorphous phase also occurs in the solid (upper right panel, blue arrow), and contains both uranium and iron (Figure S7). *Bottom.* U L₃ EXAFS spectrum (bottom left panel) and its Fourier transform (bottom right panel) of the solid from the reactor with 68% of U initially occurring as calcium-uranyl-carbonato species (solid line) and the shell-by-shell fit (dashed line).

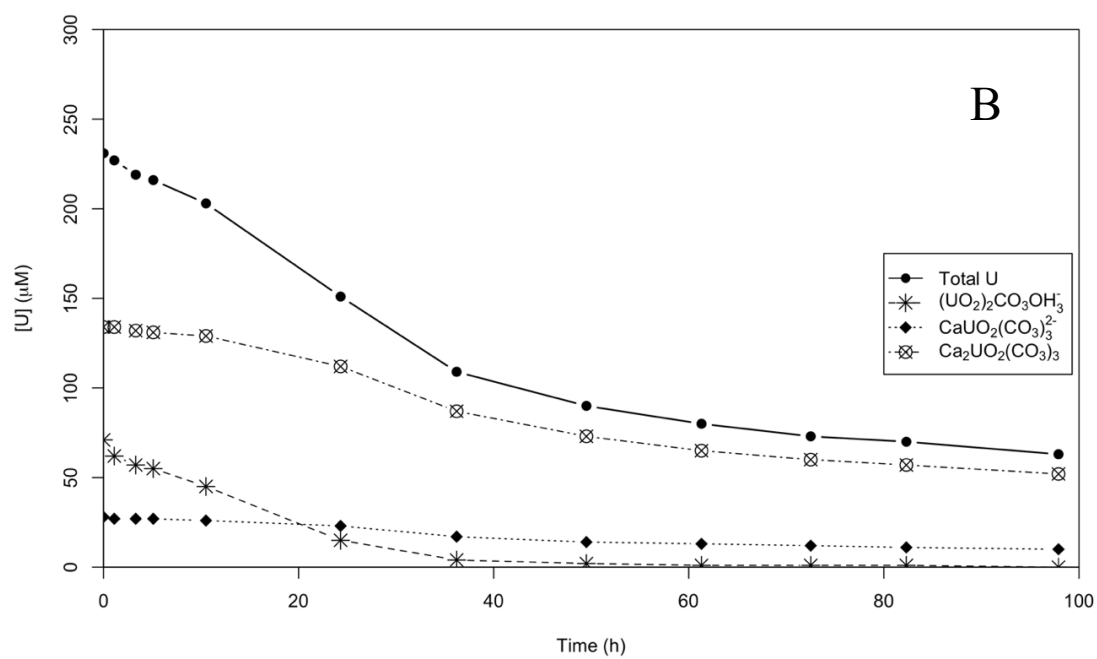
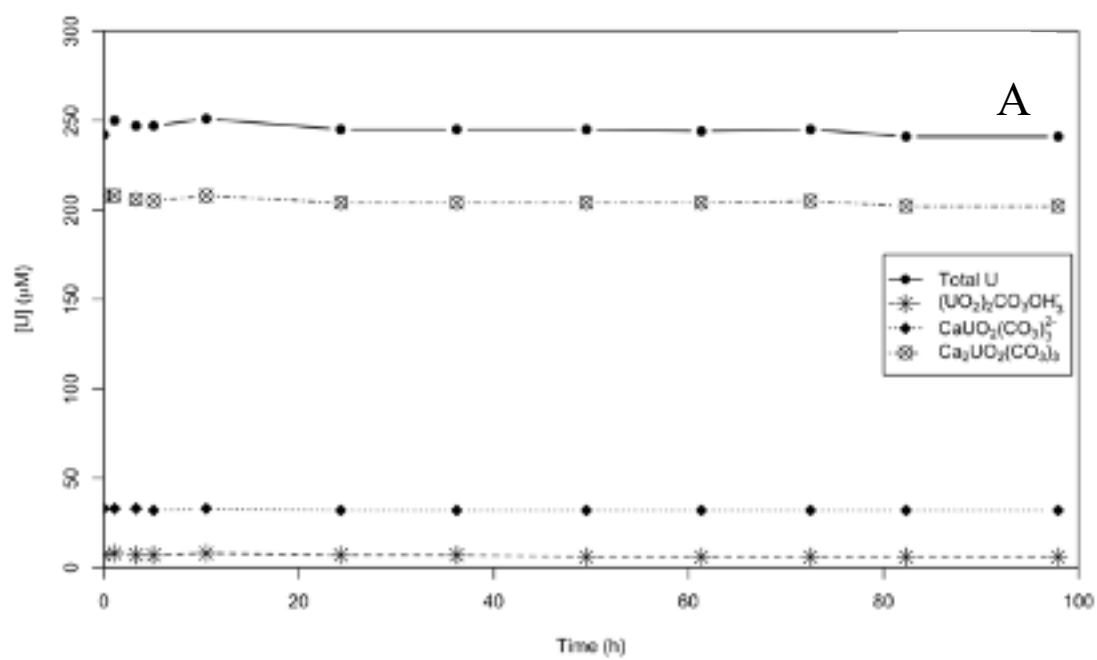
2.11. Supplemental Materials

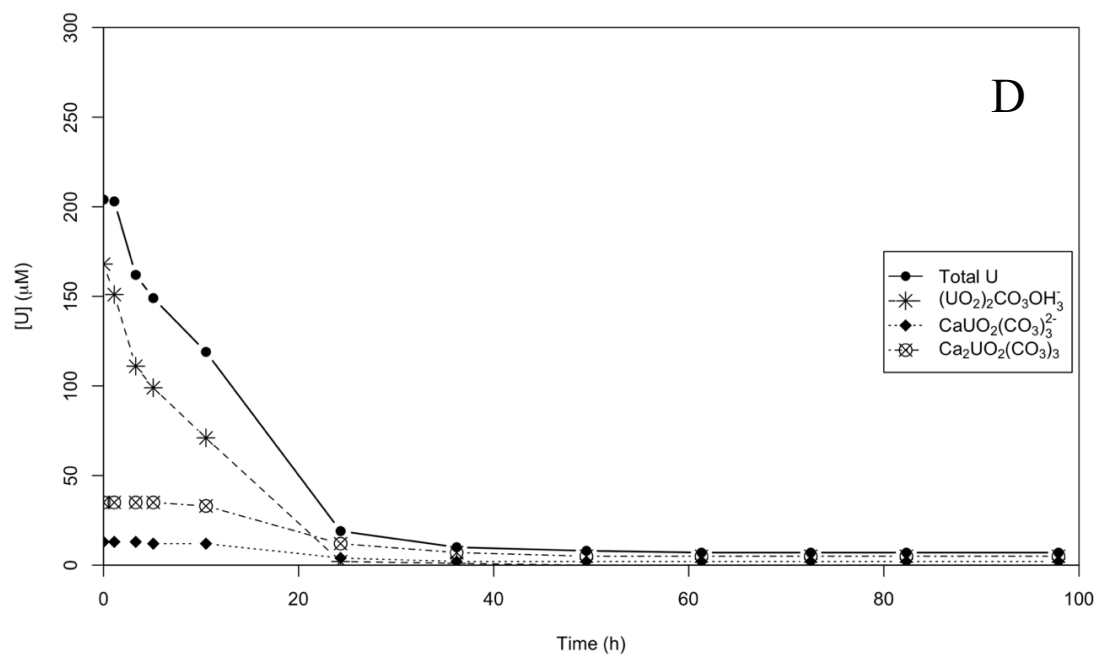
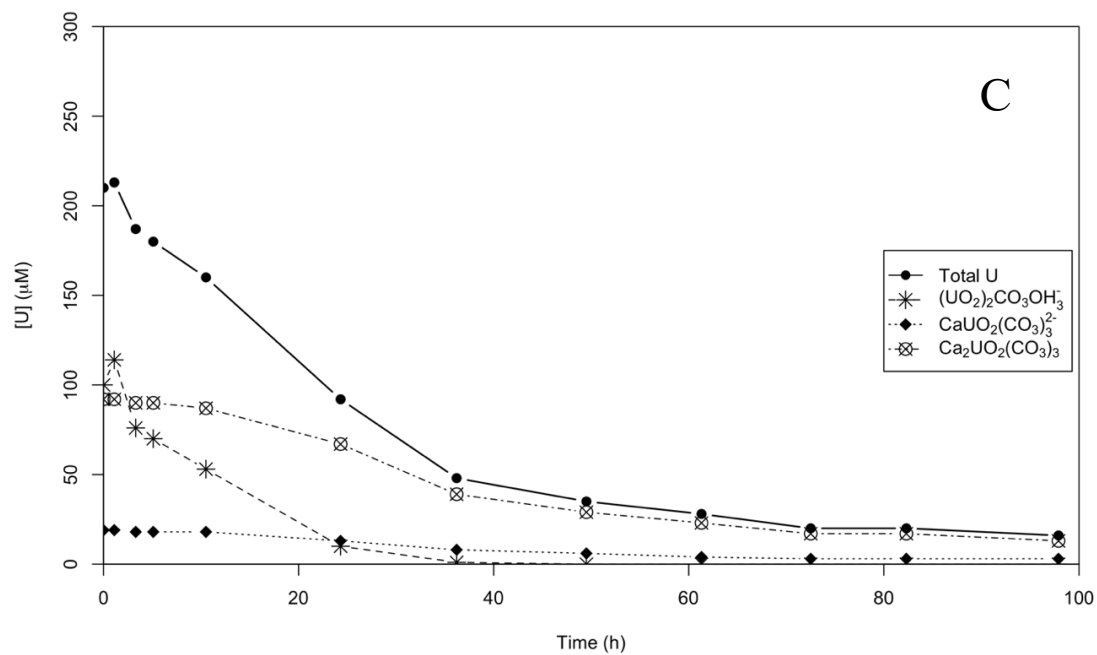
Initial % Ca-U-CO ₃ or blank conditions	Time (h)	U (M)	Fe (M)	Ca (M)	TIC (mol C/L)	pH	% U as mono Ca	% U as di Ca	% U as dimer U
96.3	0.0	2.42E-04	1.41E-03	6.60E-03	1.43E-03	7.10	13.2	83.1	3.0
	1.1	2.50E-04	1.43E-03	6.66E-03	NM	NM	13.2	83.0	3.0
	3.3	2.47E-04	1.44E-03	6.67E-03	NM	NM	13.2	83.2	2.9
	5.1	2.47E-04	1.41E-03	6.64E-03	NM	NM	13.2	83.3	2.7
	10.5	2.51E-04	1.41E-03	6.67E-03	NM	NM	13.1	83.0	3.1
	24.3	2.45E-04	1.42E-03	6.60E-03	NM	NM	13.2	83.4	2.7
	36.2	2.45E-04	1.45E-03	6.64E-03	NM	NM	13.2	83.3	2.8
	49.5	2.45E-04	1.39E-03	6.70E-03	NM	NM	13.2	83.4	2.6
	61.3	2.44E-04	1.40E-03	6.57E-03	NM	NM	13.2	83.5	2.6
	72.5	2.45E-04	1.39E-03	6.78E-03	NM	NM	13.2	83.5	2.6
68.6	82.3	2.41E-04	1.39E-03	6.73E-03	NM	NM	13.2	83.7	2.4
	97.9	2.41E-04	1.37E-03	6.80E-03	1.45E-03	7.05	13.2	83.7	2.4
	0.0	2.31E-04	1.43E-03	4.29E-03	1.02E-03	7.10	11.6	56.9	29.9
	1.1	2.27E-04	1.38E-03	4.33E-03	NM	NM	12.0	59.1	27.4
	3.3	2.19E-04	1.41E-03	4.27E-03	NM	NM	12.3	60.4	25.9
	5.1	2.16E-04	1.43E-03	4.29E-03	NM	NM	12.4	60.8	25.3
	10.5	2.03E-04	1.44E-03	4.33E-03	NM	NM	12.9	63.3	22.3
	24.3	1.51E-04	1.34E-03	4.29E-03	NM	NM	14.9	74.1	9.7
	36.2	1.09E-04	1.32E-03	4.30E-03	NM	NM	15.8	79.6	3.4
	49.5	8.99E-05	1.32E-03	4.36E-03	NM	NM	16.0	80.9	2.0
51.7	61.3	8.00E-05	1.29E-03	4.30E-03	NM	NM	16.0	81.6	1.4
	72.5	7.29E-05	1.28E-03	4.24E-03	NM	NM	16.0	81.8	1.1
	82.3	7.01E-05	NM	4.31E-03	NM	NM	16.0	82.0	1.0
	97.9	6.35E-05	1.27E-03	4.28E-03	1.09E-03	7.03	16.0	82.2	0.8
	0.0	2.10E-04	1.43E-03	4.38E-03	7.82E-04	7.10	8.7	43.0	46.8
	1.1	2.13E-04	1.45E-03	4.36E-03	NM	NM	8.2	40.2	50.1
	3.3	1.87E-04	1.43E-03	4.31E-03	NM	NM	9.8	48.1	40.6
	5.1	1.80E-04	1.41E-03	4.30E-03	NM	NM	10.1	49.7	38.6
	10.5	1.60E-04	1.41E-03	4.44E-03	NM	NM	11.0	54.4	32.9
	24.3	9.25E-05	1.35E-03	4.49E-03	NM	NM	14.6	72.8	11.0
21.8	36.2	4.81E-05	1.31E-03	4.48E-03	NM	NM	15.9	80.4	2.4
	49.5	3.48E-05	1.33E-03	4.39E-03	NM	NM	16.0	81.4	1.4
	61.3	2.78E-05	1.29E-03	4.46E-03	NM	NM	16.0	81.9	0.9
	72.5	2.04E-05	1.27E-03	4.41E-03	NM	NM	16.0	82.3	0.5
	82.3	1.96E-05	1.29E-03	4.54E-03	NM	NM	16.1	82.3	0.5
	97.9	1.63E-05	1.29E-03	4.36E-03	7.99E-04	7.06	16.1	82.4	0.4
	0.0	2.04E-04	1.40E-03	2.05E-03	6.46E-04	7.10	5.7	16.0	76.3
	1.1	2.03E-04	1.39E-03	2.05E-03	NM	NM	6.2	17.4	74.4
	3.3	1.62E-04	1.39E-03	2.07E-03	NM	NM	7.7	21.5	68.6
	5.1	1.49E-04	1.39E-03	2.04E-03	NM	NM	8.3	23.1	66.3
0	10.5	1.19E-04	1.37E-03	2.05E-03	NM	NM	9.9	27.9	59.6
	24.3	1.88E-05	1.29E-03	2.01E-03	NM	NM	21.7	62.8	11.7
	36.2	9.50E-06	1.26E-03	2.01E-03	NM	NM	23.1	67.5	5.5
	49.5	8.00E-06	1.25E-03	2.05E-03	NM	NM	23.4	68.5	4.2
	61.3	7.50E-06	1.28E-03	2.03E-03	NM	NM	23.5	68.8	3.8
	72.5	7.24E-06	1.24E-03	2.04E-03	NM	NM	23.5	69.0	3.6
	82.3	6.99E-06	1.25E-03	2.05E-03	NM	NM	23.5	68.9	3.7
	97.9	6.99E-06	1.25E-03	2.04E-03	6.74E-04	7.04	23.5	68.9	3.7
	0.0	2.48E-04	1.42E-03	0	6.91E-04	7.10	0.0	0.0	97.4
	1.1	2.32E-04	1.43E-03	0	NM	NM	0.0	0.0	97.3
Fe blank	3.3	1.96E-04	1.40E-03	0	NM	NM	0.0	0.0	96.9
	5.1	1.68E-04	1.34E-03	0	NM	NM	0.0	0.0	96.5
	10.5	7.86E-05	1.29E-03	0	NM	NM	0.0	0.0	94.5
	24.3	1.09E-05	1.29E-03	0	NM	NM	0.0	0.0	85.1
	36.2	5.97E-06	1.22E-03	0	NM	NM	0.0	0.0	79.9
	49.5	5.96E-06	1.25E-03	0	NM	NM	0.0	0.0	80.0
	61.3	5.46E-06	1.22E-03	0	NM	NM	0.0	0.0	78.2
	72.5	5.46E-06	1.24E-03	0	NM	NM	0.0	0.0	78.3
	82.3	5.46E-06	1.21E-03	0	NM	NM	0.0	0.0	78.2
	97.9	5.46E-06	1.21E-03	0	7.28E-04	7.05	0.0	0.0	78.2
U blank	0.0	2.33E-04	0	4.41E-03	1.09E-03	7.10	13.9	74.5	5.4
	24.3	2.16E-04	0	4.38E-03	NM	NM	14.4	77.0	7.9
	49.5	2.13E-04	0	4.38E-03	NM	NM	15.0	80.0	4.3
	72.5	2.11E-04	0	4.34E-03	NM	NM	15.1	80.0	4.2
	97.9	2.06E-04	0	4.29E-03	1.17E-03	7.05	15.2	80.2	3.8
	0.0	0	1.39E-03	4.42E-03	1.03E-03	7.10	NA	NA	NA
	24.3	0	1.37E-03	4.40E-03	NM	NM	NA	NA	NA
	49.5	0	1.39E-03	NM	NM	NM	NA	NA	NA
	72.5	0	1.37E-03	4.39E-03	NM	NM	NA	NA	NA
	97.9	0	1.38E-03	4.37E-03	1.03E-03	7.06	NA	NA	NA

Table S2.1. Aqueous geochemical conditions for all reactors at all time points.

Table S2.2. Gibbs energy of formation for all species used in thermodynamic calculations

Species	G _f (kJ/mol)	Reference
(UO ₂) ₂ CO ₃ (OH) ₃	-3139.53	(1)
CaUO ₂ (CO ₃) ₃ ²⁻	-3233.80	(2)
Ca ₂ UO ₂ (CO ₃) ₃	-3815.36	(2)
Fe ²⁺ (aq)	-78.90	(1)
H ₂ O(l)	-237.10	(3)
UO ₂ (cr)	-1031.833	(1)
2-line Fe(OH) ₃	-708.50	(4)
CO ₃ ²⁻	-527.90	(3)
HCO ₃ ⁻	-586.85	(1)
UO ₂ ²⁺	-952.551	(1)
OH ⁻	-157.00	(3)
Ca ²⁺	-552.806	(1)





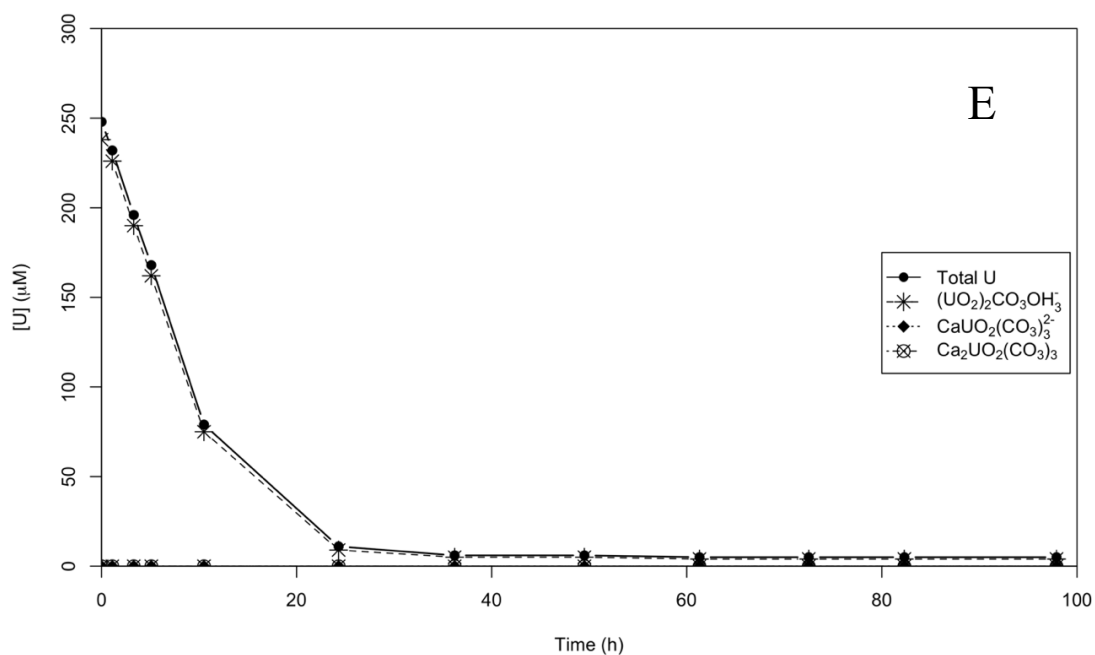
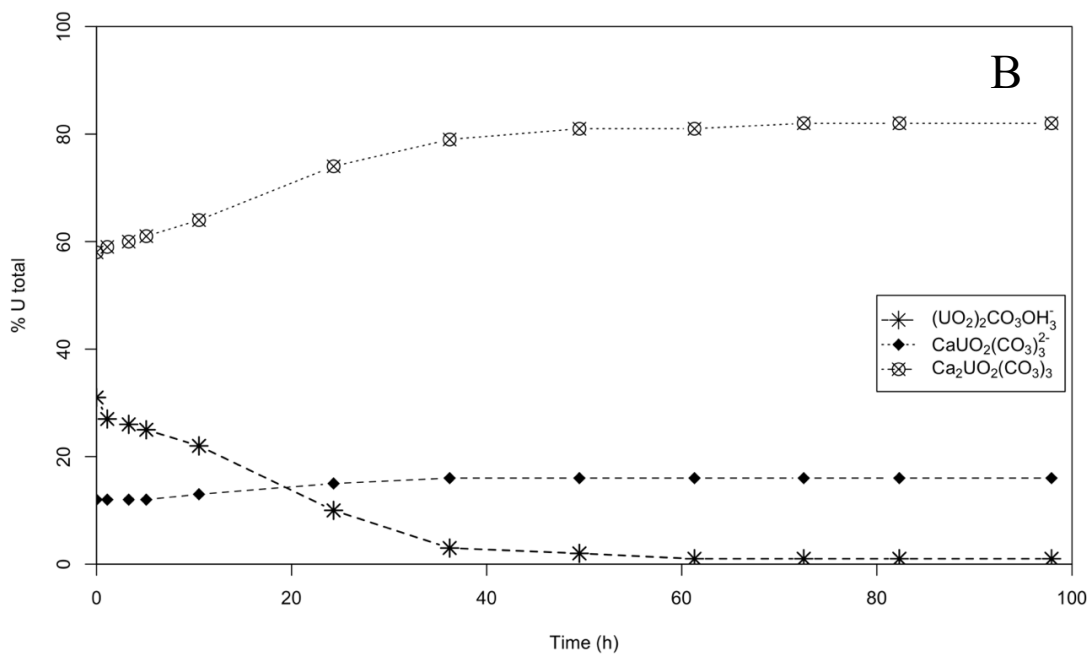
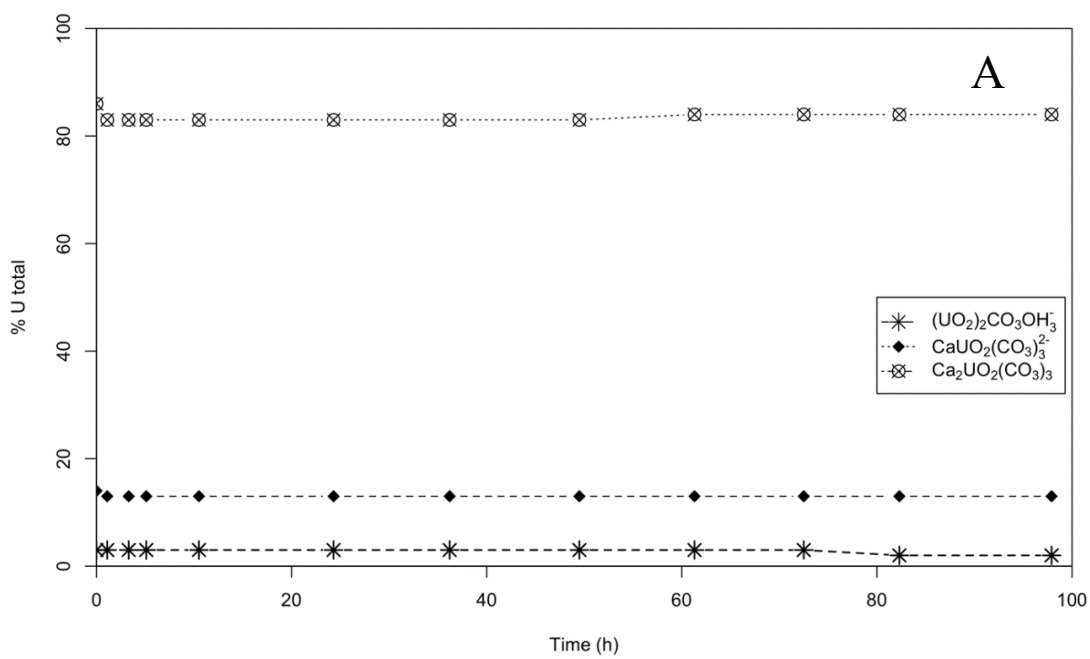
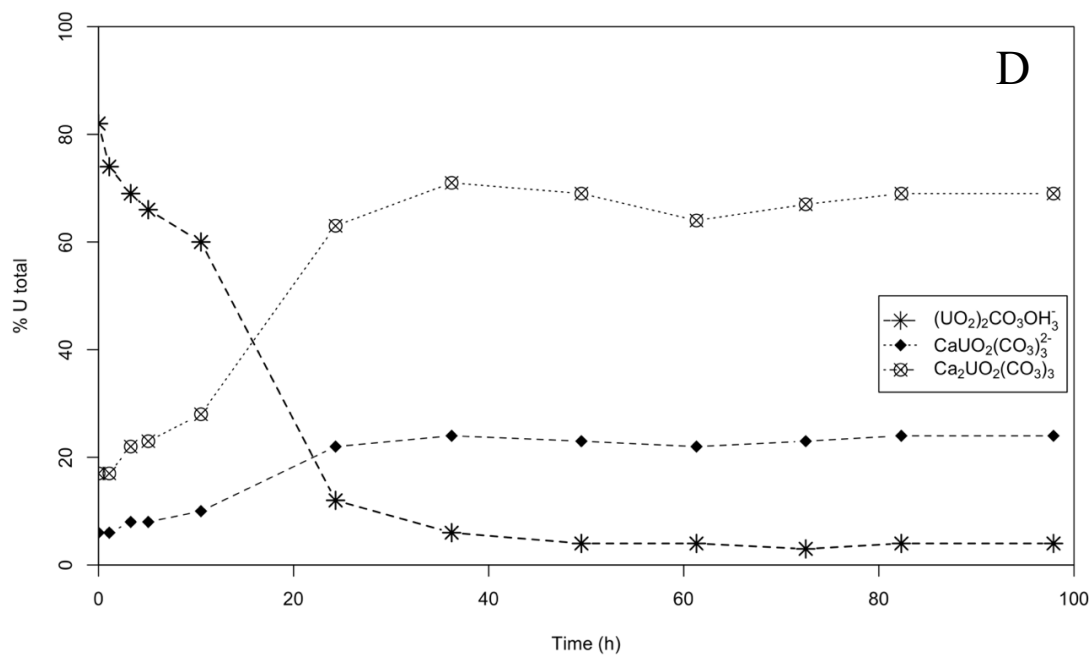
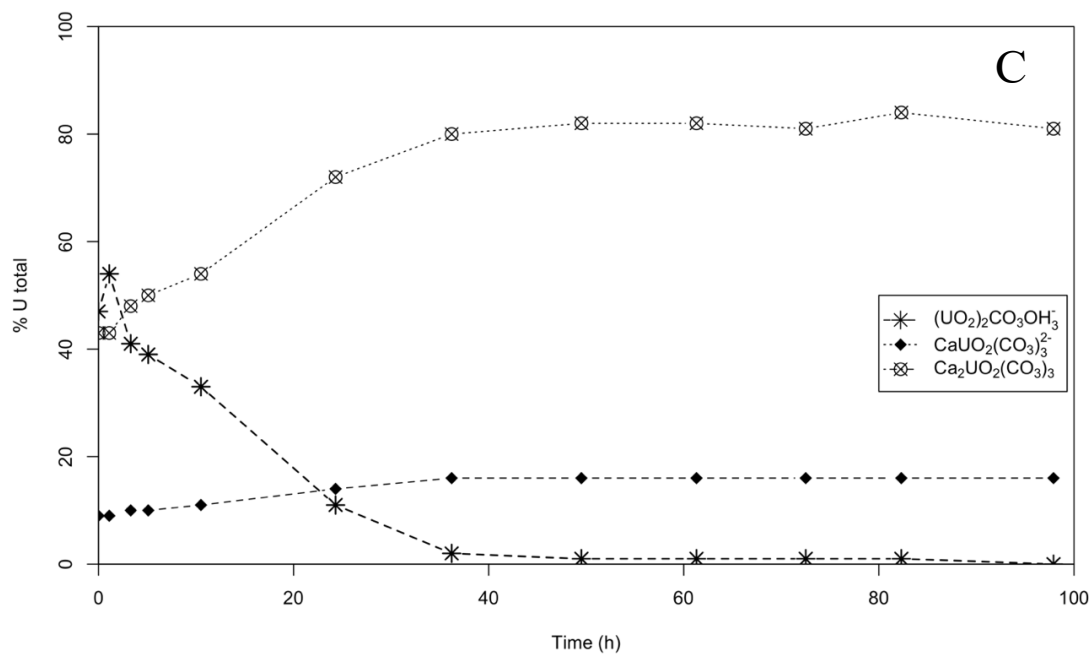


Figure S2.1. Concentrations for dominant U species through the experiment for the following initial proportions of U occurring as the calcium uranyl carbonate species: 96% (A), 68% (B), 51% (C), 22% (D), and 0% (E).





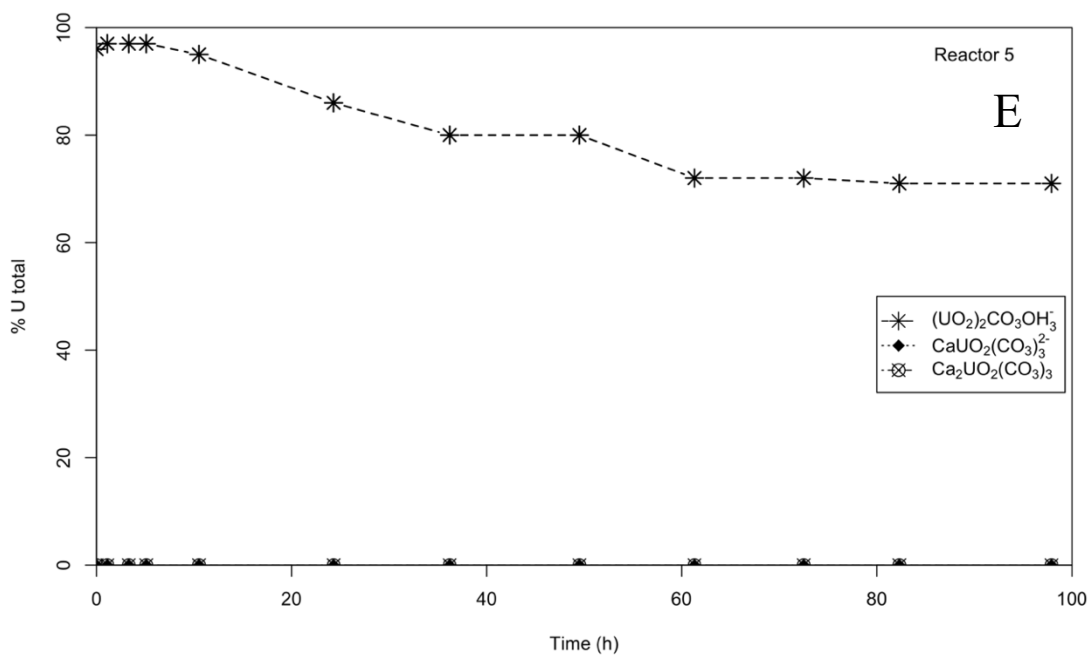


Figure S2.2. Proportions of dominant U species throughout the experiment for the following initial proportions of U occurring as the calcium uranyl carbonate species: 96% (A), 68% (B), 51% (C), 22% (D), and 0% (E).

Initial % Ca-U-CO ₃	Time (h)	dG mono (kJ / mol U)	dG di (kJ / mol U)	dG dimer (kJ / mol U)
96.3	0.0	-36.0	-27.3	-50.9
	1.1	-36.0	-27.4	-51.0
	3.3	-36.0	-27.3	-51.0
	5.1	-35.8	-27.1	-50.6
	10.5	-36.0	-27.3	-51.0
	24.3	-35.8	-27.1	-50.6
	36.2	-36.0	-27.3	-50.9
	49.5	-35.7	-27.0	-50.3
	61.3	-35.6	-27.0	-50.3
	72.5	-35.6	-27.0	-50.3
	82.3	-35.5	-26.8	-50.0
	97.9	-35.4	-26.7	-49.8
68.6	0.0	-39.8	-31.1	-58.5
	1.1	-39.4	-30.7	-57.8
	3.3	-39.4	-30.7	-57.8
	5.1	-39.4	-30.7	-57.7
	10.5	-39.1	-30.5	-57.2
	24.3	-37.1	-28.4	-53.2
	36.2	-35.0	-26.4	-49.1
	49.5	-34.0	-25.3	-46.9
	61.3	-33.2	-24.5	-45.3
	72.5	-32.7	-24.0	-44.3
	82.3	-32.5	-23.8	-43.9
	97.9	-31.9	-23.3	-42.9
51.7	0.0	-40.6	-31.9	-60.2
	1.1	-40.9	-32.2	-60.7
	3.3	-40.2	-31.5	-59.3
	5.1	-40.0	-31.3	-59.0
	10.5	-39.6	-30.9	-58.1
	24.3	-37.0	-28.3	-52.9
	36.2	-33.7	-25.1	-46.5
	49.5	-32.6	-23.9	-44.2
	61.3	-31.6	-22.9	-42.1
	72.5	-30.4	-21.7	-39.7
	82.3	-30.4	-21.8	-39.9
	97.9	-29.8	-21.1	-38.6
21.8	0.0	-41.5	-32.8	-62.0
	1.1	-41.3	-32.7	-61.6
	3.3	-40.9	-32.2	-60.7
	5.1	-40.7	-32.0	-60.3
	10.5	-40.1	-31.4	-59.2
	24.3	-35.1	-26.4	-49.1
	36.2	-33.1	-24.5	-45.3
	49.5	-32.5	-23.8	-43.9
	61.3	-32.3	-23.6	-43.5
	72.5	-32.1	-23.4	-43.1
	82.3	-32.1	-23.5	-43.2
	97.9	-32.1	-23.4	-43.2
0	0.0	NA	NA	-63.1
	1.1	NA	NA	-63.0
	3.3	NA	NA	-62.2
	5.1	NA	NA	-61.3
	10.5	NA	NA	-58.7
	24.3	NA	NA	-53.3
	36.2	NA	NA	-51.0
	49.5	NA	NA	-51.3
	61.3	NA	NA	-50.5
	72.5	NA	NA	-50.7
	82.3	NA	NA	-50.4
	97.9	NA	NA	-50.4

Table S2.3. Gibbs free energies of reduction of U(VI) species by Fe(II)(aq).

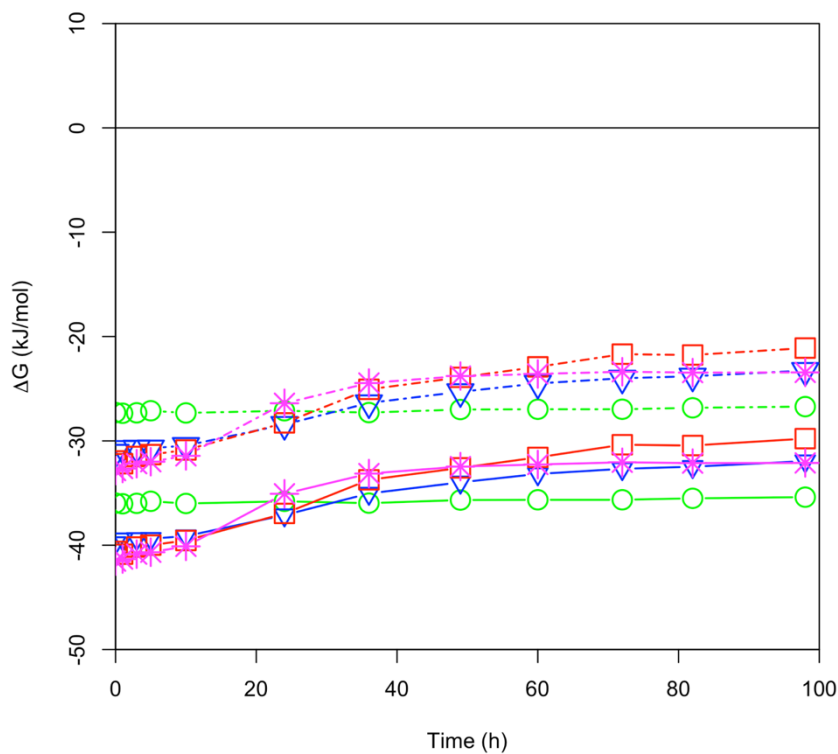


Figure S2.3. Gibbs free energies of mono-calcium uranyl carbonate (dashed lines) and di-calcium uranyl-carbonato-species (solid lines) reduction by $\text{Fe(II)}_{\text{(aq)}}$, for the following initial proportions of U as calcium-uranyl-carbonato species: 96% (green circles), 68% (blue triangles), 51% (red boxes), and 22% (magenta stars). Calculations are based on reactions 1-3.

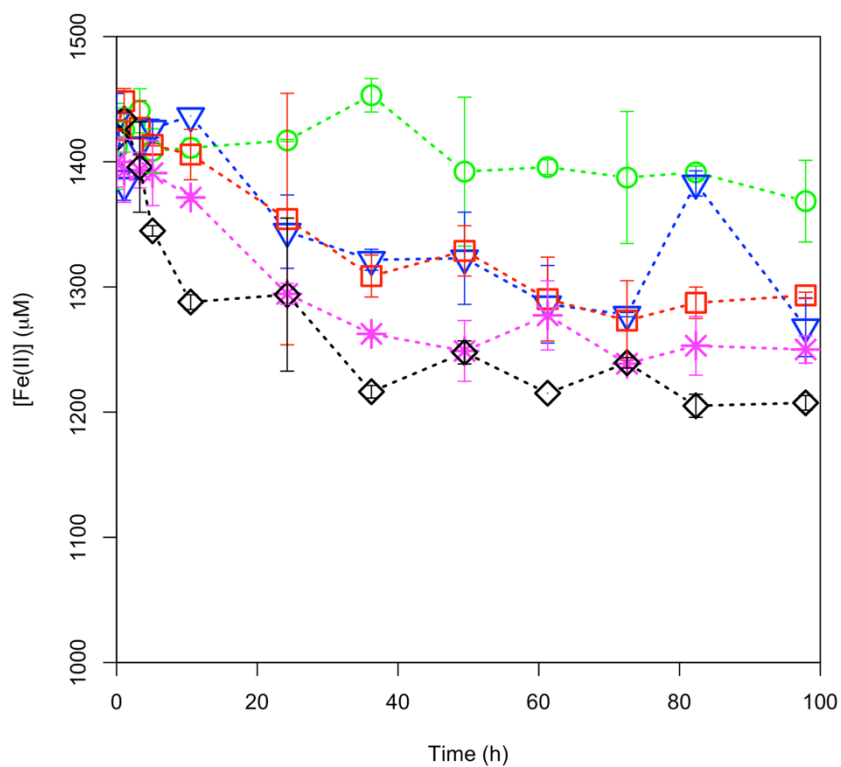


Figure S2.4. Fe(II)(aq) concentrations for the following initial proportions of U as calcium-uranyl-carbonato species: 96% (green circles), 69% (blue triangles), 51% (red boxes), 22% (magenta stars), and 0% (black diamonds).

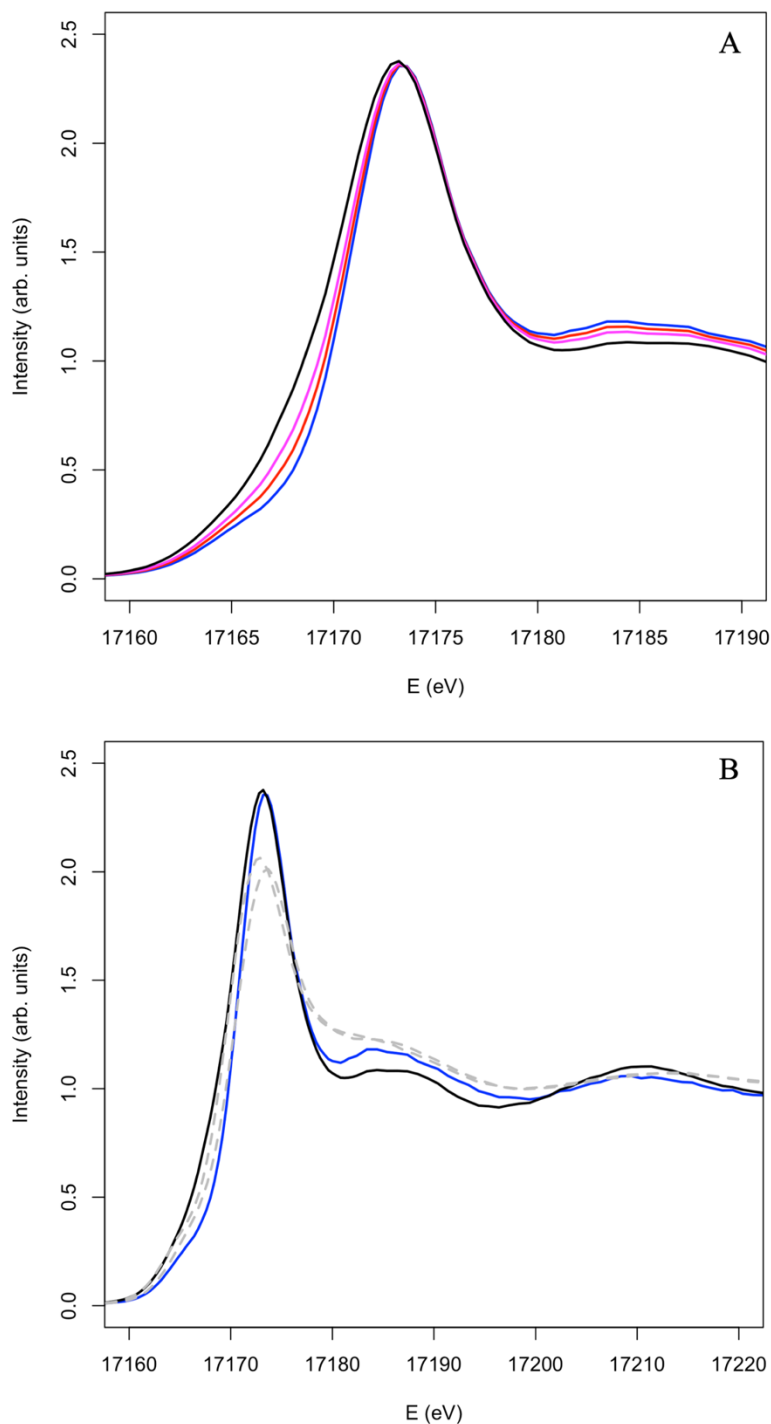


Figure S2.5. (A) Linear combinations of UO₂ and U(VI)-Fh HERFD-XANES spectra (blue: 10% UO₂, 90% U(VI)-Fh; red: 20% UO₂, 80% U(VI)-Fh; magenta: 30% UO₂, 70% U(VI)-Fh; black: 50% UO₂, 50% U(VI)-Fh). (B) Linear combinations of HERFD-XANES spectra of 50% UO₂ and 50% U(VI)-Fh (black line) and 10% UO₂ and 90% U(VI)-Fh (blue line) compared to spectra of the solid from the reactor with 68% U

initially as calcium-uranyl-carbonato (grey; small dashes) and the solid from the reactor with 0% U as calcium-uranyl-carbonato (grey; large dashes).

Table S2.4. U EXAFS shell-by-shell fitting results for the solid from the reactor with 68% of U initially occurring as calcium-uranyl-carbonato species.

	U–O _{ax}	U–O _{eq} ^a	U–Fe	U–U
S₀²	0.9 [*]			
E₀ (eV)	0.798 (2.22) ^b			
N	0.670 (0.053)	1.498 (0.39)	5.281 (6.68)	0.197 (0.11)
R (Å)	1.775 (0.010)	2.334 (0.017)	3.491 (0.047)	3.899 (0.038)
σ² (Å²)	0.0025 [*]	0.00788 (0.0028)	0.0401 (0.020)	0.003 [*]

^{*}parameter fixed during fitting process

^arefers to non-axial O atoms

^berror associated with parameter appears in parentheses

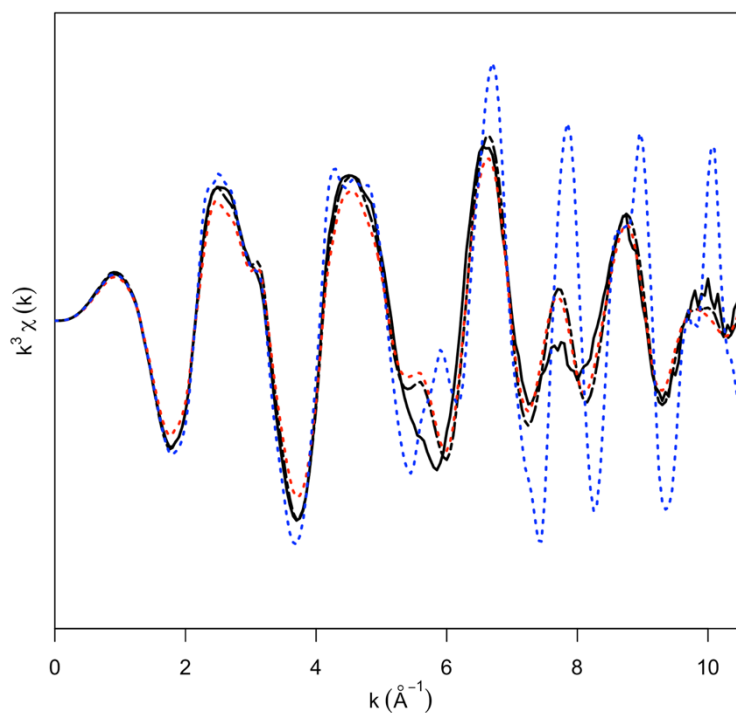
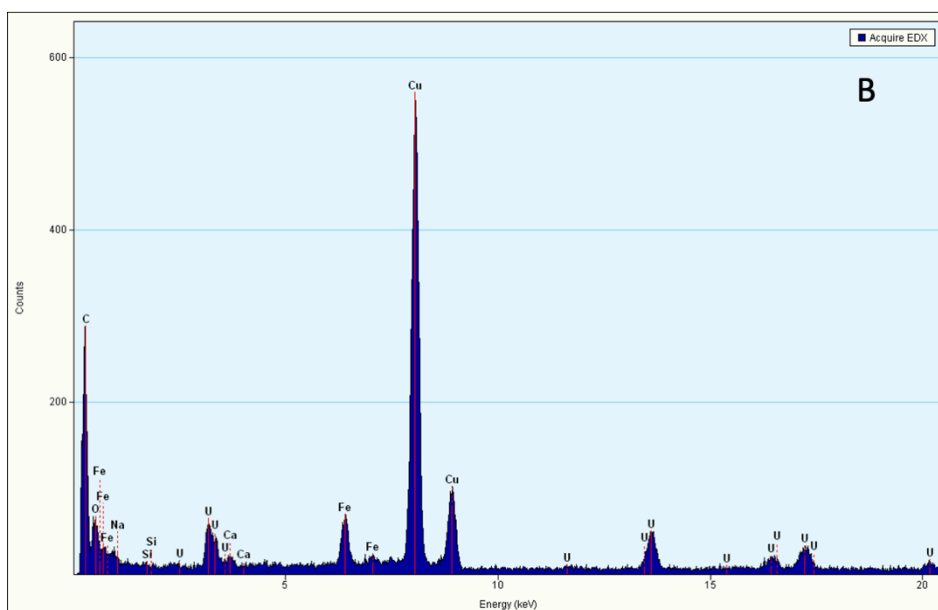
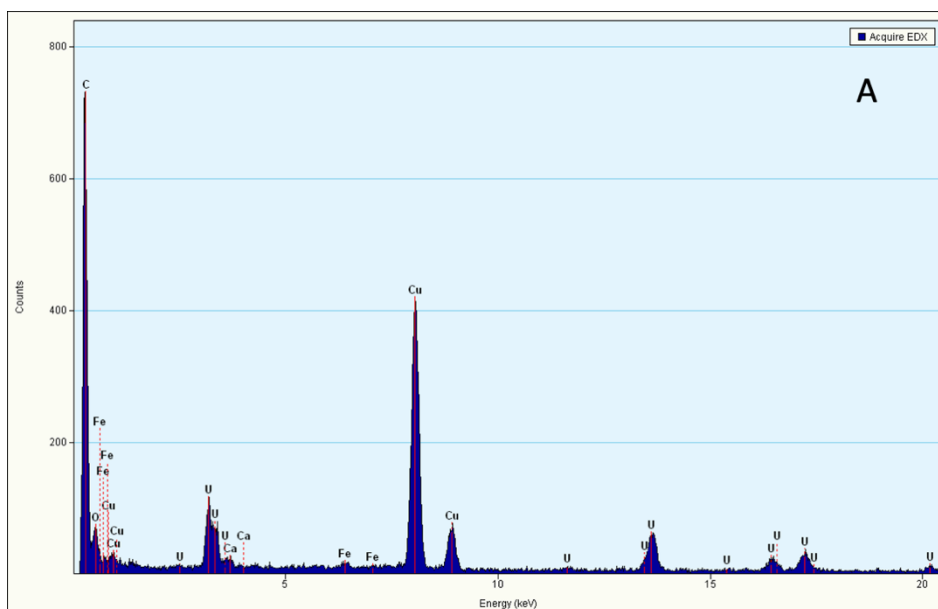


Figure S2.6. Fe K EXAFS spectrum of reaction solid from the reactor with 69% of U initially occurring as calcium-uranyl-carbonato species. A linear combination fit

(dashed black) of the spectrum from the reaction solid with 2-line ferrihydrite (dashed red) and goethite (dashed blue) standards indicates that Fe phase is 99% ferrihydrite.



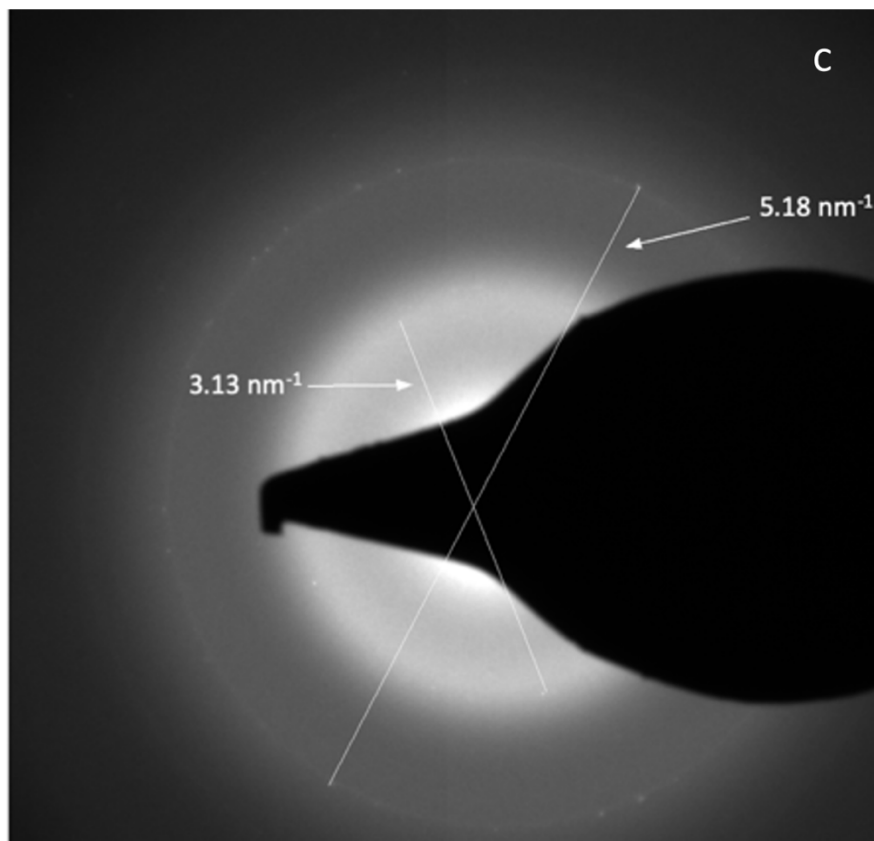


Figure S2.7. Energy dispersive x-ray spectroscopy results of the nanocrystalline phase (panel A), and the diffuse phase (panel B) in the solids produced in the reactor with 68% of U initially occurring as calcium-uranyl-carbonato species. To obtain these spectra, the electron beam was aimed at the locations identified with red and blue arrows (nanocrystalline and amorphous domains, respectively) in Figure 4. The Cu peaks in the spectra are likely from the Cu mesh supporting the holey carbon film. Their presence in the sample indicates that scattering of the electron beam and/or fluorescing photons causes florescence in atoms relatively far from the point of focus. Selected area diffraction pattern (panel C) of the solid in the reactor with 68% of U initially occurring as calcium-uranyl-carbonato species. The lattice spacings measured by selected area diffraction correspond to the most intense diffraction lines of uraninite (3.19 and 1.93 Å).

Table S2.5. U reduction rates at early and reaction stages, calculated as the change in U concentrations from 0 to 24 h and 24 to 98 h.

Initial % of calcium-uranyl-carbonato	Rate ($\mu\text{M/h}$) from 0 to 24 h	Rate ($\mu\text{M/h}$) from 24 to 98 h
96	-2.28	0.053
68	3.24	1.18
51	4.02	1.03
22	8.16	0.16
0	14.39	0.074

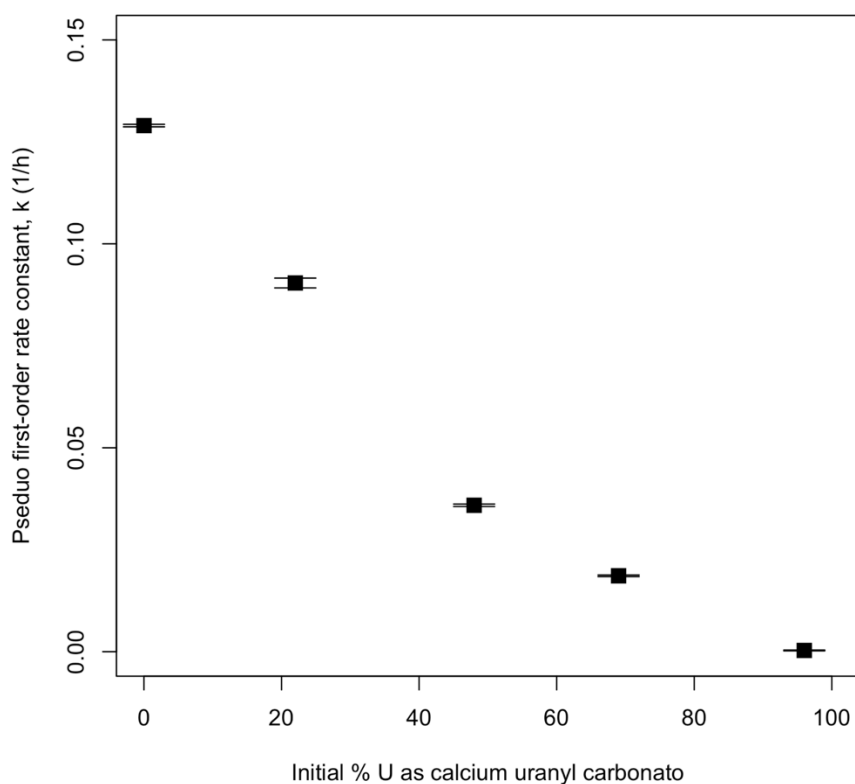
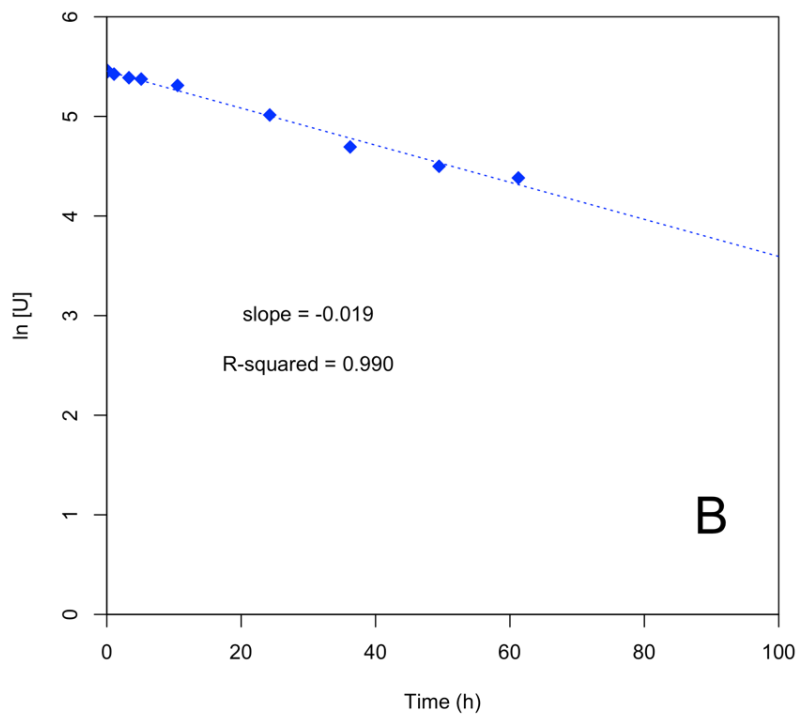
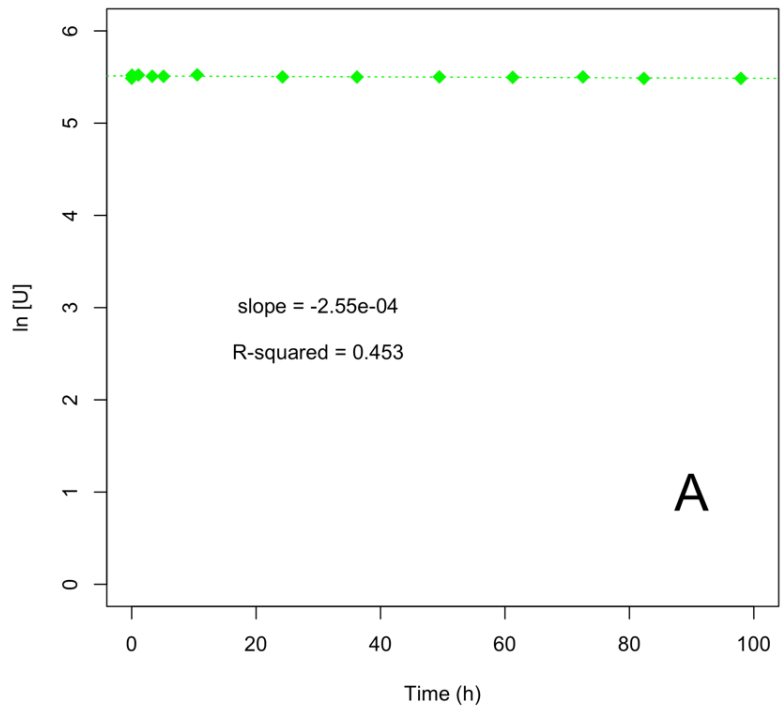
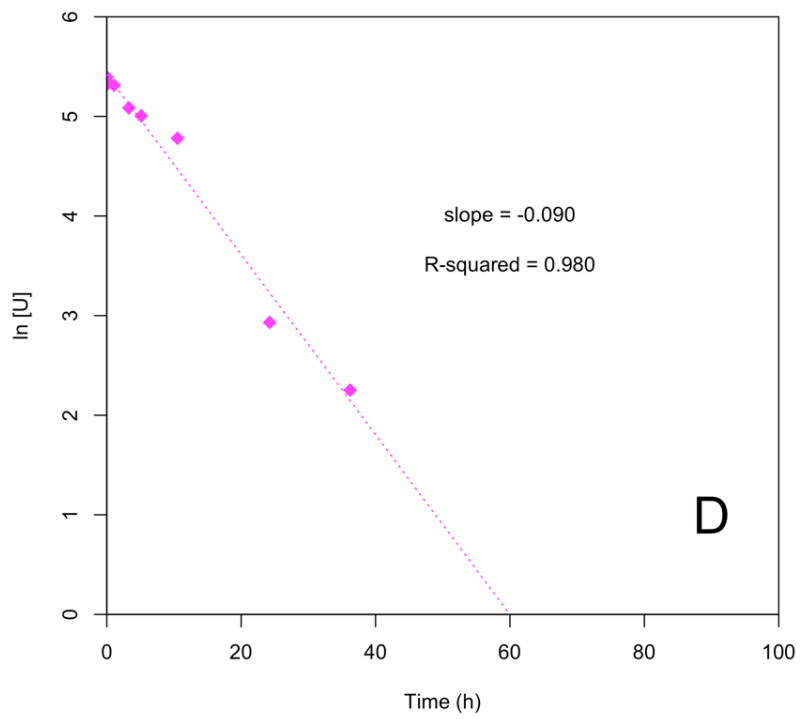
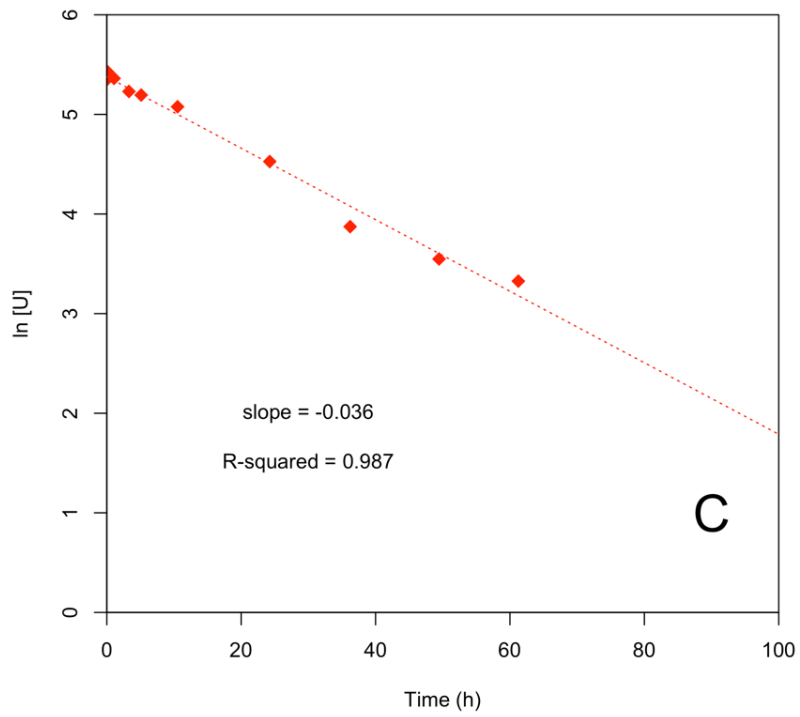


Figure S2.8. Initial proportion of U as calcium-uranyl-carbonato species plotted against the apparent (calculated) pseudo-first order rate constant for overall U reduction. The apparent relationship between the calculated constant and initial U speciation suggests that the rate is in fact a function of the concentration of the dominant non-calcium uranyl species, and therefore that this is the reactive species. Standard deviations for the rate constants were generally very low.





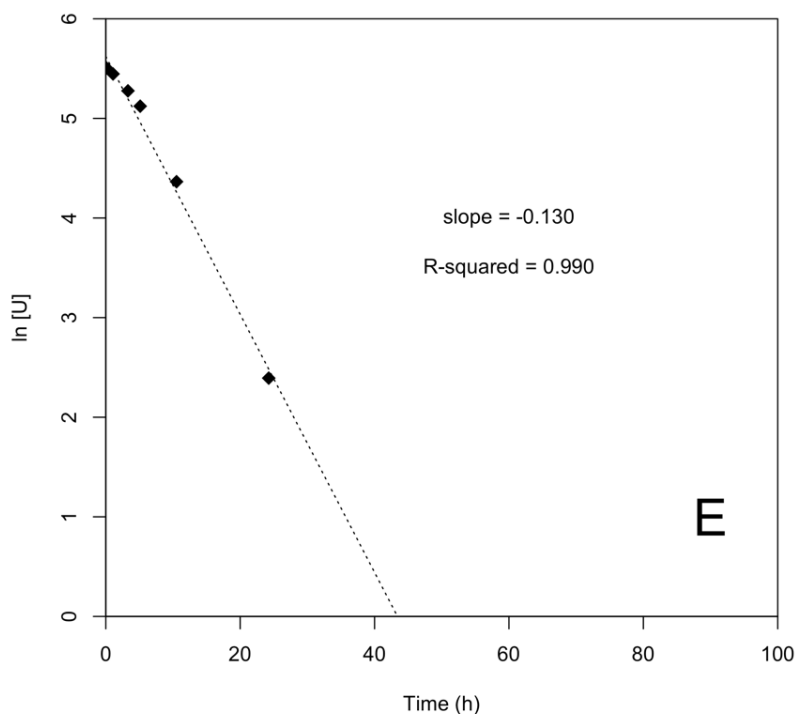


Figure S2.9. Natural log of measured U concentrations (solid symbols) over time for all initial proportions of U as calcium-uranyl-carbonato species (96%, panel A; 69% panel B; 49% panel C; 22% panel D; 0%, panel E). Linear regressions (dashed lines) were performed on the transformed concentration data to determine pseudo-first order rate constants. The slope and R-squared value of each regression appear alongside the plotted data. For the reactors with 69%, 49%, and 22% U initially calcium-uranyl-carbonato species, regressions were performed over the subset of concentration data for which aqueous U speciation was dynamic (i.e., approaching 100% calcium uranyl carbonato). For the reactor with 96% U initially as calcium-uranyl-carbonato, the regression was performed over the entire time series. For the reactor without calcium, the regression was performed until U concentrations were near zero and unchanging.

Description of U L₃ EXAFS shell-by-shell fitting process

We collected U L₃ EXAFS of the solid in the reactor with 69% of U initially occurring as calcium uranyl carbonato species. The measurements were performed at SSRL beamline 11-2 in transmission mode with a LN cryostat and N₂-filled ionization chambers. An Y foil was used for energy calibration. Vertical and horizontal slits were set to 1 and 9 mm, respectively. Seventeen scans were collected to 14.84 Å⁻¹. The scans were averaged in SIXPack, followed by background subtraction (pre- and post-edge) and normalization of the averaged scans (5). The chi function was extracted with the default background routine in SIXPack.

The EXAFS above ~10 Å⁻¹ contained large oscillations that appeared to be artefacts of data collection. We therefore tested three Fourier Transform (FT) window ranges to identify potential frequencies produced by artefacts. These ranges were 2.50 to 11.69 Å⁻¹, 2.50 to 10.40 Å⁻¹, and 2.50 to 9.70 Å⁻¹ (kmin to kmax). A Kaiser-Bessel window was used in all cases. All windows yielded a peak at 1.3 and 1.8 Å in the FT (FT distances reported here are uncorrected for phase shift; we report actual distances when referring to inter-atomic pairs). For the window to 11.69 Å⁻¹, several frequencies also appeared between 3 and 4 Å in the FT. Decreasing the upper window limit to 10.40 Å⁻¹ eliminated several of the low-amplitude frequencies in this range, resulting in an FT with two peaks between 3 and 4 Å, and a third peak with maximum amplitude at ~4 Å. Lowering the upper window limit to 9.70 Å⁻¹ yielded an FT identical to that of 10.40 Å⁻¹. We therefore concluded that the frequencies in the latter two FTs were not an artefact of data collection and instead reflected local chemical structure.

Shell-by-shell fitting was performed in SIXPack, using phase and amplitude scattering parameters generated by FEFF8.4 (6). We used a Kaiser-Bessel window from 2.50 to 10.40 Å⁻¹ with a *k*-weight of 3 and a *dk* of 1 to produce the FT for the fit. The fit was performed in R-space from 1 to 5 Å using a least-squares algorithm. S₀² was fixed at 0.9 throughout the fitting process. The frequencies were fit from low to high in the FT, with the exception of the first two (at ~1.3 and ~1.8 Å), which were fit simultaneously. Seed values for the fitting parameters (N, R, σ², ΔE₀, S₀²) were based on published

values from EXAFS of similar U phases. The complete fitting results are shown in SI Table 5. For the first peak, we began with a U–O path at 1.78 Å and fixed the variance of the mean free path length (σ^2) at 0.0025, a value representative of those published for the axial O atoms in uranyl (7–9). We fixed σ^2 because allowing the parameter to float yielded a negative value. For the second FT peak, we began with a U–O path at 2.33 Å, and we allowed σ^2 to float. Using the coordination number (CN) from the fit of the first shell, which represents the proportion of U as uranyl, we fixed the CNs for the known multiple scattering (MS) paths (U–O–C; U–C–O–C that yield frequencies between 3 and 4 Å in the FT. We allowed other parameters to float for these paths. Finally, since the known MS paths did not capture the full amplitude of the frequencies between 3 and 4 Å, we fit a U–Fe path at 3.49 Å, consistent the U(V)–Fe distance reported in Massey et al. (2014) (7). We also attempted a U–U path at 3.89 Å, given the presence of uraninite in the transmission electron microscopy results.

Description of Fe K EXAFS linear combination fitting process

We collected Fe K EXAFS of the solid in the reactor with 69% of U initially occurring as calcium uranyl carbonato species. The measurements were performed at SSRL beamline 11-2 in transmission mode with a LN cryostat and N₂-filled ionization chambers. A CAL foil was used for energy calibration. A harmonic rejection mirror set to an energy cutoff of 11 keV was used to minimize interference from higher order light. Vertical and horizontal slits were set to 1 and 7.5 mm, respectively. Two scans were collected to 15 Å⁻¹. The scans were averaged in SIXPack, followed by background subtraction and normalization of the averaged scans in Athena (5, 10). A spline from E = ~7150 eV to E = ~8009 eV was used to extract the EXAFS (chi) function from the normalized spectrum.

The normalized Fe K EXAFS were fit using the linear combination fitting routine in Athena (10). Goethite and 2-line ferrihydrite were used as standards in the fit. The fit was performed in *k*-space using a range of 2 to 15 Å⁻¹. The coefficients of the fit component were constrained to values between 0 and 1. The sum of the coefficients was allowed to exceed unity during the fit, after which the sum was normalized.

Description of reaction network model

We used the PFLOTTRAN Reaction Sandbox to develop a kinetic model of the batch reactors. The Reaction Sandbox permitted formulation of different reduction rates and mechanisms for each of the uranium species ($(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, $\text{CaUO}_2(\text{CO}_3)_3^{2-}$, and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$) with Fe(II), while allowing equilibrium between these species. The model domain consisted of a single cell. The cell was fully saturated, and fluxes of solutes and/or water were not permitted across any of the cell faces.

The reaction network was constructed by writing a custom reaction module which was compiled with the distributed PFLOTTRAN code. Constructing the custom module permitted reaction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ with $\text{Fe(II)}_{(\text{aq})}$ while simultaneously enforcing equilibrium conditions between $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, $\text{CaUO}_2(\text{CO}_3)_3^{2-}$, and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$. The reaction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ with $\text{Fe(II)}_{(\text{aq})}$ was modeling as a first order reaction, with a rate constant of 0.00001 (1/s). It was necessary to enforce equilibrium conditions between the aqueous U species, as a significant portion of U was transferred from the calcium-uranyl-carbonato species to $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$ and subsequently reduced during the experiment. In addition, the custom module allowed for inclusion of a pseudo-heterogeneous reaction pathway for reduction of the uranyl species. Two pseudo-heterogeneous reaction pathways were added: one for heterogenous reduction of $(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$, and a second for heterogeneous reduction of $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ (rate constants of 4.5 (mol/L-sec) and 1.25 (mol/L-sec)). The pseudo-heterogeneous reaction was formulated as a second-order reaction of a uranyl species and Fe(III), which was not allowed to precipitate in the model. This approach was necessary because it is not technically feasible to use a surface-complexed species in a chemical reaction without significant customization of the PFLOTTRAN code. In formulating our pseudo-heterogeneous model this way, we assume that the extent and rate of the surface reactions scale directly with the amount of Fe(III) produced, and further, that the surface reaction is not limited by the kinetics of surface complexation or nucleation and precipitation of ferrihydrite. For the reactors with visible solids at the conclusion of the experiment (those with U occurring initially as 69%, 49%, 22%, and

0% calcium-uranyl-carbonato), the model produced good fits to the observations, suggesting that our assumption is likely valid for these reactors. Yet, including the heterogeneous pathways in the model of the reactor with U occurring initially as 96% calcium-uranyl-carbonato did not yield results that fit the observations. Given that there was no visible Fe(III) in this reactor and only minor removal of U and Fe after 100 h of reaction, we concluded that the kinetics of surface complexation are in fact rate-limiting when trace amounts of Fe(III) are produced. In this case, U reduction is dominated by the homogeneous pathway throughout the experiment, and the heterogeneous reactions do not contribute to U removal. Thus, we used only the homogeneous model for the reactor with 96% of U occurring as the calcium-uranyl-carbonato species.

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Chapter 3:

Collective partitioning limits porewater Pb concentrations during redox cycles in contaminated floodplain sediments

3.1 Abstract

Lead contamination in soils and sediments presents acute and chronic risks to freshwater quality. While Pb, which occurs predominantly as Pb(II), is not itself redox active in freshwater systems, common Pb hosts – including Fe(III)-(hydr)oxides and galena (PbS) – are sensitive to redox transitions. Dissolution of Pb hosts during redox transitions may release Pb to porewater, leading to spikes in dissolved Pb concentrations. Here, we examine the impacts of hydrologically coupled redox transitions on Pb speciation and mobility in contaminated floodplain sediments. We find that the affinity of Pb for a range of ligands, and its stability in a variety of coordination environments, ensures that, across redox transitions, partitioning of Pb to solid phases is more favorable than partitioning to the aqueous phase. As porewater redox conditions shift with seasonal hydrologic conditions, dissolution of Fe(III)-(hydr)oxides and sulfidic phases occur, potentially releasing associated Pb to porewater. However, throughout these transitions, porewater Pb concentrations remain below 15 ug/L. Retention of Pb on particulate organic matter (POM), coupled with precipitation of galena and Pb adsorption on Fe(III)-(hydr)oxides, appears to limit dissolved Pb concentrations, even during shifts in redox conditions. Importantly, the predominance of Pb adsorbed on POM alongside low dissolved Pb concentrations suggests that if Pb is released from other hosts, it is retained by adsorption on POM. Thus, despite host-phase dissolution during redox transitions, partitioning of Pb to the aqueous phase is minimal and, therefore, transport of dissolved Pb is unlikely.

3.2 Introduction

Lead is a common contaminant in soils and aquifers, presenting acute and chronic risks to freshwater quality. Assessing these risks depends on understanding the processes that promote or inhibit the mobilization of Pb. Lead tends to partition strongly to solid phases under a range of biogeochemical conditions, which generally limits its mobility as a dissolved species. The low solubilities of common (and authigenic) Pb minerals potentially formed under anaerobic conditions such as PbS (galena) or PbCO₃ (cerussite) and the high affinity of Pb²⁺ for Fe/Mn-(hydr)oxides formed in oxygenated environments maintain low concentrations of dissolved Pb in sediment porewater.¹⁻⁹ Lead also adsorbs strongly to particulate organic matter (POM), further limiting dissolved concentrations.¹⁰

However, transitions in redox conditions may affect Pb partitioning, potentially releasing Pb to porewater. Although Pb is not redox-active in subsurface environments, shifts in redox conditions may alter the thermodynamic stability of Pb host phases, particularly Fe/Mn-(hydr)oxide and sulfide phases, as for other redox-inactive metals, such as Zn, Ni, and Cd.^{11,12} Indeed, the onset of anoxic conditions and subsequent reductive dissolution of Fe (hydr)oxides has been shown to release adsorbed Pb to the aqueous phase.¹³ Yet, the timescales of redox transitions and host-phase transformations may be incommensurate, potentially resulting in the formation of phases that do not conform with thermodynamic predictions, or in spikes in porewater Pb concentrations.^{13,14} Desorption of Pb from oxide surfaces tends to be slow, and dissolution of galena, even by oxidative mechanisms, even slower.¹⁵ Understanding the impacts of redox transitions on Pb partitioning is therefore needed to predict Pb behavior and assess the risks imposed by Pb-contaminated sediments to freshwater quality.

Here, we address this need by tracking redox conditions and solid Pb speciation in floodplain sediments in a mining-impacted watershed near Crested Butte, CO, USA. Our objectives were (1) to resolve Pb speciation during redox transitions coupled to hydrologic transients and (2) to determine the extent of kinetic control on Pb partitioning during these transitions.

3.3 Materials and Methods

Site Description

We examine sediments from a floodplain within the Slate River watershed, near Crested Butte, CO, USA. The watershed is a mountainous, high-elevation system (2700-4000 mASL), with hydrologic conditions dominated by seasonal snowmelt dynamics. Surficial Quarternary glacial sediments of variable thickness occur throughout the watershed, underlain by Cretaceous to Tertiary sedimentary rocks (Mancos shale, and the Mesa Verde, Ohio Creek, and Wasatch formations) intruded by porphyritic granitic stocks.^{16,17} In general, the floodplains and low-elevation slopes are overlain by Quarternary alluvium and colluvium. Our study site is located approximately 100 m downstream from the confluence of Slate River and Oh-Be-Joyful Creek at approximately 2726 mASL. Oh-Be-Joyful Creek drains the Upper Redwell Basin, host of the Daisy mine and other historic mining activities that targeted galena and sphalerite, among other minerals.¹⁶ Sediments in our study area host elevated concentrations of Pb and Zn (Table S3.1).

Field measurements and sample collection

Porewater and sediment samples were collected at a location 15 m south of the Slate River (13S 324138.46 m E 4308680.62 m N) (Figure S3.1). We refer to this location as OBJ-2. For collection of water samples, MacroRhizon pore water samplers (Rhizosphere Research Products, Wageningen, Netherlands) (hereafter referred to as rhizons) with 0.15 μm pore size were installed in October 2017 in duplicate at the following depths below ground surface: 50, 70, 90, 110, 130, and 150 cm. A 7.5-cm bucket auger was used to make a borehole for each porewater sampling depth. Two rhizons were inserted vertically into the bottom of each borehole. Tygon tubing was run from the rhizon port to the ground surface. After the rhizons were inserted, each borehole was packed with the sediment that was removed from it, with care to add sediment in the reverse order in which it was removed. Porewater was collected from the borehole depth to 9 cm deeper.

Water and sediment samples were collected from June 2 through October 26, 2018 at two- to four-week intervals. Porewater samples were collected by applying a vacuum with a 60 mL syringe to each rhizon for 30 to 60 min, after which the withdrawn porewater was dispensed into a 20 mL scintillation vial and a 40 mL amber glass vial for measurement of dissolved metals and organic carbon, respectively. Samples were acidified with 0.5 M HCl and stored at 4 °C until laboratory analyses. Porewater pH was measured in the field in approximately 30 mL of porewater dispensed into a 100 mL plastic cup. A 7.5-cm bucket auger was used to collect sediment samples at the depths of the rhizons. At each depth, approximately 20 g of sediment was removed from the tip of the auger with a stainless-steel spatula and packed into a glass serum vial, which was immediately capped with a butyl stopper and crimped. A hand pump was used to evacuate the vial headspace so that the sediments were in minimal contact with the atmosphere. All sampling locations at OBJ-2 were within a 4 m² area. The sealed vials were immediately placed on dry ice and stored at -18 °C until transfer to an anaerobic chamber (95% N₂, 5% H₂ atmosphere; Coy Laboratory Products, Inc., Grass Lake, MI, USA), where the vials were opened and recapped, providing an oxygen-free headspace. The recapped vials were stored at -18 °C until laboratory analyses.

Depth to groundwater at OBJ-2 was tracked hourly from December 1, 2017 through December 13, 2018. A pressure transducer (HOBO Water Level Data Logger U20-001-01, Onset Computer Corporation, Bourne, MA, USA) was installed for this purpose. The transducer was suspended on a galvanized steel cable to a depth of 130 cm in an open well of 2 m. Absolute pressure was logged at 1 h intervals. The measurements were converted to water pressure, and subsequently water depth, through use of barometric pressure measurements from the nearby East River floodplain (10.5 km distance; elevation = 2723 m ASL), which were logged at the same frequency with a transducer of the same make and model.

Sequential extractions

Sequential extractions were performed in triplicate on sediments collected on June 20, August 8, and October 26, 2018. The following extractions were performed, in the order

they are listed: anoxic Milli-Q water; 1 M MgCl_2 ; 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$; 1 M sodium acetate NaAc ; 0.1 M oxalic acid and 0.175 M ammonium oxalate; 30% H_2O_2 and 0.038 M HNO_3 ; and 1 M HCl . For each replicate, 2 g of dried and ground sediment was used. The sediments were prepared and stored in an anaerobic chamber (95% N_2 , 5% H_2 atmosphere) prior to the extraction procedure. Following each extraction step, sediments and extractants were centrifuged at 3000 rpm for 15 min, after which the supernatant was syringe-filtered through acid-washed polyethersulfone membranes with 0.45 μm pore size into acid-washed glass vials. For measurement of dissolved metals, 1 mL of filtered supernatant was transferred from the glass vials to 15 mL Falcon tubes and diluted 10- to 100-fold, depending on the extraction step, with 2% HNO_3 . The remainder of the filtered supernatant was reserved for measurement of TOC. The solutions were stored at 4 C in capped vials and tubes until analysis.

Elemental concentrations

Dissolved Pb was measured on a Thermo Scientific XSERIES 2 quadrupole inductively coupled plasma mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Dissolved Fe and S were measured on a Thermo Scientific ICAP 6300 Duo View inductively coupled plasma optical emission spectrometer with a solid-state CID detector (Thermo Fisher Scientific, Waltham, MA, USA). Samples were acidified with 2% HNO_3 prior to analyses. Total dissolved organic carbon was measured on a Shimadzu TOC-L total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Concentrations of solid-phase metals were measured by x-ray fluorescence (XRF) with a SPECTRO Xepos HE XRF Spectrometer (SPECTRO Analytical Instruments GmbH, Kleve, Germany). Sediments were dried and ground in an anaerobic chamber for XRF analysis.

X-ray absorption spectroscopy

Pb L_3 -edge extended x-ray absorption fine structure (EXAFS) spectra were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) at beamlines 7-3, 9-3 and 11-2. For collection of spectra, wet sediments were ground to a fine paste and pressed into

aluminum samples holders, which were sealed with Kapton tape. The samples were stored in an anaerobic chamber (95% N₂, 5% H₂ atmosphere) until transfer to a liquid helium cryostat at the beamline. Mineral specimens were obtained from Mineralogical Research Company (San Jose, CA, USA) for collection of x-ray absorption standards. Their purity was verified with x-ray diffraction prior to x-absorption measurements. Pb adsorbed on goethite was synthesized in the lab according to Schwertmann and Cornell¹⁸ and Bargar et al.,¹⁹ for use as an XAS standard.

For all measurements, incident x-ray energy was controlled with a LN₂-cooled Si(220) double monochromator in the $\phi = 90^\circ$ configuration. At beamline 7-3, the monochromator was detuned 30% at 13.4 keV to eliminate higher order harmonics. At beamlines 9-3 and 11-2, an in-hutch Rh harmonic rejection mirror was used to eliminate higher-order harmonics, with the cutoff set to 15 keV. Measurements were performed at 10 K. Florescence spectra of the sediments and the Pb-Gt standard were collected using a 30-element (BL 7-3) or 100-element (BLs 9-3 and 11-2) Ge detector array (Canberra, Meriden, CT, USA). Transmission spectra were collected for the mineral standards at BL 7-3 using an N₂-filled ionization chamber. An in-line Pb foil was used for energy calibration at all beamlines. Calibration and alignment of the EXAFS spectra were performed in SIXPack.²⁰ Spectra were normalized, background-subtracted, and fit in Athena, using FEFF 8.4.^{21,22} Linear combination fitting was performed in Athena on k^3 -weighted spectra between $k = 2 \text{ \AA}^{-1}$ and $k = 10.5 \text{ \AA}^{-1}$. The following standards were used in the LCF analysis: Pb adsorbed on goethite (Pb-Gt), Pb adsorbed on particulate organic matter (Pb-OM), galena, cerussite, anglesite, and pyromorphite. Shell-by-shell analyses were performed in SIXPack.²⁰

Elemental maps were collected by microscale x-ray fluorescence (μ XRF) mapping at SSRL beam line 2-3. Sediments were maintained frozen until subsampling for analysis. Sediments were opened and dried within an anaerobic chamber (5% H₂/95% N₂). Sediments were embedded in degassed epoxy (EpoTek 301) within the anaerobic chamber. Petrographic thin sections were prepared (Spectrum Petrographics, Vancouver, WA). XRM mapping measurements were performed in air, but thin sections

were otherwise stored anaerobically before and after the measurements. Incident x-ray energy was controlled with a water-cooled Si(111) double-crystal monochromator. A spot size of 2 x 2 μm was used. For XRM mapping, energy calibration was checked using an iron metal foil (K-edge taken to be 7112 eV). Incident energy on the sample was set to be 13,100 eV (*i.e.*, above the Pb L_{III} edge). XRM maps were measured at 10 μm step size (dwell time of 25 to 100 ms/point). X-ray fluorescence yield was measured using a Vortex Silicon Drift Detector (Hitachi, Schaumburg, IL, USA). The software package SMAK was used for processing and analysis of the elemental maps.²³

Sulfur K-edge x-ray absorption near edge structure (XANES) spectral measurements were collected at SSRL beam line 4-3. Prior to the measurements, sediments were dried, ground to a fine powder, and loaded into aluminum sample holders, which were sealed with polyethylene film and S-free Kapton tape. Samples were stored in an anaerobic chamber (95% N₂, 5% H₂ atmosphere) until transfer to the He atmosphere in the beam line sample box. Incident x-ray energy was controlled with a water-cooled Si(111) double monochromator in the $\phi = 90^\circ$ configuration. Florescence spectra were collected using a 4-element Vortex Silicon Drift Detector. Measurements were performed at room temperature. The thiosulfate pre-edge feature at 2472.0 eV was used for energy calibration. The spectra were calibrated, normalized, background-subtracted, and fit using Athena.^{21,22}

3.4 Results

Floodplain Hydrologic Conditions

Groundwater levels exhibit seasonal and diurnal variability. Water levels peak at 32 cm below ground surface (bgs) in late May, corresponding to maximum river discharge (Figure 3.1). High water at this time reflects maximum snowmelt across the watershed. Water levels decline to 105 cm bgs by early August, after which they rise until early October, reaching 55 cm bgs (Figure 3.1). The rise in water levels from August through early October is due to construction of a beaver dam on Slate River immediately

downstream from OBJ-2 (Figure S3.2). Increased precipitation and lower evapotranspiration may also contribute to the observed rise in water levels.

Porewater Chemistry

Porewater pH was measured in June and October at 50 cm and 130 cm bgs. Values ranged from 6.0 to 7.0 (Table S3.3). Throughout the sampling campaign, dissolved Pb concentrations are consistently below $15 \mu\text{g L}^{-1}$, which is the action level established by the US EPA Lead and Copper Rule.²⁴ They exhibit neither a depth trend nor a temporal trend. From June through September, Pb concentrations range from 0 to $7 \mu\text{g L}^{-1}$ and are consistent across depths (Figure 3.2; Table S3.2). On October 26, Pb concentrations range from 5 to $14 \mu\text{g L}^{-1}$ and decrease with depth (Figure 3.2; Table S3.2).

Dissolved Fe and S concentrations exhibit strong temporal and depth-dependent trends. In June and July, concentrations of dissolved S increased from 50 to 90 cm bgs, from 10 to 30 mg L^{-1} , and decreased from 110 to 150 cm bgs, from 10 to 2 mg L^{-1} (Figure 3.2; Table S3.2). In August, September and October, dissolved S concentrations decrease at all depths relative to their July levels, to approximately 4 mg L^{-1} from 50 to 90 cm bgs, and less than 1 mg L^{-1} from 110 to 150 cm bgs (Figure 3.2; Table S3.2). Dissolved Fe concentrations exhibit similar temporal trends, with maximum concentrations occurring in June and July, and reverse depth trends, with concentrations increasing from 90 to 130 cm bgs (Figure 3.2; Table S3.2). Maximum Fe concentrations occur at 130 cm bgs in June and July, reaching 195 mg L^{-1} . The 110 and 130 cm depths experience dissolved Fe concentrations between 115 and 185 mg L^{-1} in these months, while the 50 and 70 cm depths exhibit Fe concentrations below 10 mg L^{-1} , with the exception of the 50 cm depth on June 20 (54 ppm). Dissolved Fe concentrations decrease at all depths between July 7 and August 8. Maximum concentrations in August, September and October occur at 110 and 130 cm bgs, reaching 15 ppm. Fe concentrations are lower at 150 cm bgs, reaching approximately 10 mg L^{-1} . From 50 to 90 cm bgs, Fe concentrations are less than 5 ppm (Figure 3.2; Table S3.2).

Solid phase S speciation

S K-edge XANES spectra were measured on sediments collected from each depth on all sampling dates. Linear combination fitting of the spectra was performed with the following standards: dodecyl sulfate; sulfonic acid; sulfoxide; methionine; L-cystine; elemental sulfur; and mackinawite. The LCF reveals a clear transition in S speciation with depth, with more oxidized species occurring in the upper depths, and more reduced species occurring in the lower depths. Above 100 cm, 50-75% of solid S occurs as organic and inorganic sulfate species (fit with dodecyl sulfate and sulfonic acid; Figure S3.3); 25-30% of solid S occurs as reduced organic S species (fit with methionine and L-cystine; Figure S3.3); and 10-30% occurs as elemental sulfur (Figure S3.3). There is also a clear sulfoxide peak at 2476.0 eV in samples from the upper depths, accounting for up to 5% of total S signal (Figure S3.4-S3.9). The proportion of organic and inorganic sulfate species ranges from 30-50% at 110 cm, and 5-25% at 130 and 150 cm (Figure S3.3). Sediments from 150 cm bgs contain less than 10% sulfate on all sampling dates (Figure S3.3). The proportion of elemental sulfur below 100 cm depth increases as the proportion of sulfate decreases. Elemental S accounts for 10-65% of total S, with the greatest proportions generally occurring at 150 cm (Figure S3.3). Mackinawite is also present below 100 cm, occurring predominantly below 110 cm, and ranges from 2-25% of total S (Figure S3.3). Mackinawite is not present above 130 cm except for in sediments collected on June 4 (1% at 90 cm; Figure S3.3) and October 26 (2% at 110 cm; Figure S3.3). The proportion of organic reduced S (fit with L-cystine and methionine) below 90 cm was approximately equivalent to the proportion above 110 cm, with a greater proportion of methionine at depth (Figure S3.3). Sulfoxide was also detected below 90 cm, though less frequently and generally in smaller proportions than in the sediments above 110 cm (Figure S3.3). We did not observe a temporal trend in S speciation. The variation present in the S XANES fitting results likely reflects sediment heterogeneity across sampling points.

Concentration and Speciation of Solid Pb

Solid Pb concentrations ranged from 137 to 340 mg kg⁻¹, with an average of 209 mg kg⁻¹ and a standard deviation of 57 mg kg⁻¹ (Table S3.1). We attribute variability in solid Pb concentrations to sediment heterogeneity. There is neither a depth nor temporal trend in solid Pb concentrations.

We used sequential extractions, Pb L₃ EXAFS spectroscopy, and μ XRF mapping to characterize solid Pb speciation in the sediments. The sequential extractions and Pb EXAFS measurements were performed on sediments collected on June 20, August 8, and October 26 (Pb EXAFS spectra were not measured on the 50 cm and 150 cm samples collected on October 26). μ XRF maps were collected on sediments from 40 cm bgs and 130 cm bgs collected on October 16, 2017. The extractions, Pb EXAFS spectra, and μ XRF maps reveal that solid Pb occurs in three predominant phases: Pb adsorbed to particulate organic matter (Pb-OM); Pb adsorbed to Fe(III)-(hydr)oxides (Pb-HFO); and galena (PbS).

Linear combination fitting (LCF) of the Pb L₃ EXAFS spectra indicate that solid Pb occurs predominantly as Pb-OM, with lesser amounts of Pb-HFO and PbS. the proportions of Pb-HFO and PbS decrease and increase (respectively) with depth. A summary of the LCF results is shown in Figure 3.3. The LCF results reveal that Pb-OM comprises 39 to 75% of solid Pb, varying with season but not depth (Figure 3.3; Tables S3.11-S3.13). Pb-HFO accounts for 11 to 44% of solid Pb above 100 cm, and 0 to 20% below 100 cm, with the proportion of Pb-HFO decreasing with depth below 100 cm (Figure 3.3; Tables S3.11-S3.13). The proportions of PbS vary with depth, mirroring Pb-HFO, with PbS accounting for <5% of solid Pb above 100 cm, and 8 to 34% below 100 cm (Figure 3.3; Tables S3.11-S3.13). Finally, the LCF indicates that pyromorphite, anglesite and cerussite are below the detection limit of EXAFS, and if present, comprise less than 10% of total Pb (Figure 3.3; Tables S3.11-S3.13).

Shell-by-shell analysis of Pb EXAFS spectra above and below 100 cm supports the conclusion that the majority of Pb is adsorbed to organic matter. We analyzed spectra from the 50 cm and 130 cm samples collected on June 20 and October 26, respectively, treating these samples as representative of Pb speciation above and below 100 cm. In

both samples, a first-shell oxygen frequency characteristic of a Pb-O pair in Pb-OM is required to fit the Pb EXAFS spectra (Figure S3.13). Analysis of the EXAFS of the sample from 50 cm bgs yields an average first shell Pb-O distance of 2.33 Å (Table S3.14). This distance is 0.06 Å longer than Pb-O derived for Pb-Gt, and 0.04 Å longer than the maximum Pb-O distance expected for Pb adsorbed to iron(III)-(hydr)oxides (2.29 Å), which rules out Pb-HFO as the dominant phase (Table S3.14).^{5,25} Further, although the Pb-O distance is 0.04 Å shorter than the Pb-O distance derived for Pb-OM, it is well within the range of measured Pb-O distances for Pb adsorbed on organic matter (2.30-2.45 Å).²⁵ The Debye-Waller factor for the 50 cm bgs sample, which is twice that of Pb-Gt (but still within the expected range for first shell Pb-O), suggests that the Pb-O distance in the sample from 50 cm bgs reflects the average distance of either a disordered first oxygen shell or a mixture of Pb phases with Pb-O distances distributed around 2.33 Å.^{9,19} Consistent with this interpretation, the EXAFS-derived coordination number for the first oxygen shell in the sample ($N = 1.96$) is low relative to expected values of three or four for the most common coordination geometries (trigonal pyramidal and square pyramidal) for non-crystalline Pb.^{9,19,26} Such an effect is common in Pb EXAFS spectra, and points to a distribution of distances in the first oxygen shell, which produce destructive interference of backscattered photoelectrons and, therefore, suppressed amplitude in the EXAFS spectrum, yielding a low first-shell coordination number.⁹

The Fourier transform of the EXAFS spectrum of the 50 cm sample reveals an additional frequency arising from a second-shell iron backscatterer (Figure S3.13). The distance derived for this Pb-Fe pair is 3.27 Å (Table S3.14), consistent with Pb-Fe distances for Pb adsorbed in a bidentate edge-sharing configuration with FeO₆ octahedra in iron(III)-(hydr)oxides.^{5,19,25,27} However, the EXAFS-derived coordination number for the Fe atom ($N = 0.48$) is 25-50% lower than coordination numbers reported for adsorbed Pb.^{5,19,27} Given that the Pb-Fe distance is consistent with bidentate edge-sharing, it is unlikely that the low coordination number reflects a different surface configuration of adsorbed Pb.^{9,25} Instead, it likely indicates that Pb-HFO is not the predominant Pb phase in the sediment. This interpretation is consistent with the analysis of the first shell Pb-

O pair, which also suggest a mixture of Pb-OM and Pb-HFO. Altogether, analysis of the first and second shells indicate that Pb-OM is the dominant Pb phase above 100 cm, and that a smaller pool of Pb-HFO is also present. Although the LCF results suggests pyromorphite, anglesite, and cerussite are also present in low quantities, the shell-by-shell analysis neither substantiates nor disproves this conclusion—their contributions are likely too limited to make a substantial contribution to the local coordination environment. Nevertheless, their representation in the LCF might reflect first shell Pb-O distances not present in the Pb-OM standard that occur in Pb adsorbed to OM in the samples, given that the first-shell frequency dominates the EXAFS signal and, therefore, the LCF results.

The EXAFS spectrum of the sample from 130 cm is dominated by the crystal structure of galena. Fitting Pb-S and Pb-Pb pairs at 2.92 and 4.16 Å – distances characteristic of galena²⁸ –accounts for the majority of the EXAFS amplitude and structure (Figure S3.13; Table S3.14). However, the EXAFS-derived coordination numbers for the S and Pb shells (2.07 and 5.03, respectively), are less than half of the expected values for galena (6 and 12, for S and Pb, respectively) (Table S3.14). This indicates that PbS contributes less than half of the overall EXAFS amplitude in the spectrum of the sample and, further, points to the presence of a second predominant Pb phase. Given that the first Pb-S and Pb-Pb pairs from galena fit the majority of the EXAFS structure, it is unlikely that a phase with local order and heavy backscatterers accounts for the missing amplitude, as inclusion of such a phase would be necessary to capture the high k EXAFS structure. A phase with a weak second shell backscatterer likely dominates. Furthermore, the phase likely exhibits a highly disordered first shell: a narrow distribution of first-shell distances would produce a distinct frequency in the EXAFS and a prominent peak in the Fourier transform. This would rule out Pb-HFO as the predominant phase, as the distribution of first shell Pb-O distances for Pb adsorbed to iron(III)-(hydr)oxides is relatively narrow (2.27-2.30 Å). Pb-OM is a more likely candidate for the predominant phase. Not only can first shell O atoms in Pb-OM occur at a wide range of distances (2.30-2.45 Å), but given the abundance of reduced S below 100 cm (Figure S3.3) and the affinity of Pb for reduced S ligands, the first-shell structure

of Pb-OM structure might consist of a mixture of O and S atoms.²⁵ Such extensive first shell disorder would lessen the EXAFS amplitude arising from PbS without producing a defined peak in the Fourier transform.

The Pb EXAFS results do not suggest a temporal trend in Pb speciation. However, quantifying minor Pb phases is not possible with EXAFS, given its low sensitivity ($\pm 10\%$), and thus seasonal transformations of minor phases may not appear in the EXAFS results. We therefore performed sequential extractions to quantify Pb phases and determine their potential change in abundance over the season. Our goal with the sequential extractions was not to identify Pb phases, but to leverage the reactivities of the extractants to quantify minor Pb hosts. The sequential extractions do not indicate the presence of a minor, seasonally dynamic Pb pool. Five of the seven sequential extractions removed the majority of Pb in the solid phase. The efficiency of the extractions varied with depth but not season, suggesting that minor Pb phases in these sediments are stable over seasonal redox transitions. The Milli-Q water extraction and NaAc extraction steps removed $<5\%$ of solid Pb in all samples (Table S3.15). Between 15 and 20% of solid Pb was removed by the 0.1 M oxalate extraction in sediments collected above 100 cm (Figure 3.4). This treatment nominally targets Pb associated with reactive Fe(III)- (and other metal-) (hydr)oxide phases.²⁹ Less than 15% of Pb in the solid phase was extracted from sediments below 100 cm, with extraction efficiency decreasing with depth, from $\sim 15\%$ in sediments at 110 cm bgs to $\sim 5\%$ in sediments at 150 cm bgs (Figure 3.4). The peroxide extraction, which nominally targets sulfidic (or other reduced) phases,³⁰ removed between 10 and 25% of Pb in sediments collected below 100 cm bgs, and less than 5% in sediments collected above 100 cm (Figure 3.4). The 1 M MgCl_2 and 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ extractions, which nominally target (respectively) weakly adsorbed Pb and Pb associated with particulate organic matter,²⁹ respectively, each removed $\sim 10\%$ of total Pb in sediments from all depths and sampling dates (Figure 4). Finally, between 25 and 45% of solid Pb was removed by the 1 M HCl extraction, across sampling depths and dates (Figure 3.4). This treatment, which was performed last, is intended to extract less reactive Fe(III)-(hydr)oxides and other solids resistant to the preceding treatments. Importantly, the previous extractions may not completely

remove targeted phases and, further, Pb repartitioning during the sequential extraction procedure may occur.^{31,32} Indeed, given the affinity of Pb for a variety of surfaces, it is likely that, at all extraction steps, a portion of extracted Pb repartitioned to less reactive Fe(III)-(hydr)oxide phases.²⁵ In either case, the HCl extraction thus leads to a larger than expected extraction of Pb and reconciles the apparent differences between the sequential extraction results and the Pb EXAFS. The sequential extractions suggest that Pb-OM comprises <20% of total Pb, whereas the Pb EXAFS analysis indicates that up to 75% of total Pb is Pb-OM. This discrepancy likely reflects repartitioning of Pb to less-reactive Fe(III)-(hydr)oxides during the 0.1 M Na₄P₂O₇ extraction.

Micro-XRF maps were collected on sediments from 40 and 130 cm bgs. Maps showing Pb and Fe distributions are shown in Figure 3.5, alongside correlations plots for these elements. The maps and Pb-Fe correlations further suggest that, in the shallow sediments, Pb-OM and Pb-HFO are the predominant Pb phases, while in the deep sediments, PbS is a predominant Pb phase. In the sediment from 40 cm bgs, the majority of Pb is distributed throughout the particles and is correlated with the presence of Fe (Figure 5). The correlation of Pb and Fe points to Pb-HFO as a predominant Pb phase. Further, the Pb-Fe correlation is consistent with Pb-OM, as organic matter is often associated with Fe(III)-(hydr)oxides.^{33–36} A small pool of Pb occurs as 20 to 30 µm grains in which Fe is not present. These are likely small grains of galena undetected by in the EXAFS analysis. In the sediment from 130 cm bgs, the majority of Pb occurs as 20 to 60 µm grains in which Fe is not present. Based on the Pb EXAFS analysis, these grains are likely galena. There is also a sizeable amount of Pb that is distributed throughout the grains and associated with Fe, indicating the presence of Pb-HFO and Pb-OM at depth.

3.5 Discussion

Seasonal fluctuations of the water table drive dynamic porewater redox conditions along the sediment profile. In sediments above 100 cm, reducing conditions develop in June, concurrent with high groundwater levels, and are maintained through early July, after groundwater levels drop. In June, the elevated water table causes temporary saturation

of the upper sediments (Figure 3.1) and a subsequent diffusion limitation of dissolved oxygen supply. Iron(III) reducing conditions follow. Dissolved iron concentrations, presumed to be Fe(II), ranging from 5 to 50 mg L⁻¹, indicate reductive dissolution of Fe(III)-(hydr)oxides (Figure 3.2). Aqueous iron concentrations in late June and early July are nearly unchanged from early June levels, indicating ongoing iron reduction despite declining water levels (Figures 3.1 & 3.2). This perhaps reflects that, as the sediments drain, reduced species in partially saturated pores occur in concentrations sufficient to scrub entrained oxygen. There is no evidence for widespread sulfate reduction in the upper sediments. The low abundance of reduced sulfur phases alongside pronounced sulfate features in the S XANES for these depths (Figures S3.4-S3.9) suggest that elevated porewater sulfur (Figure 3.2) primarily occurs as sulfate. Sulfate reduction, if occurring in the upper sediments, perhaps only develops in occluded pores, aggregate centers, or other diffusion-limited features disconnected from preferential flow paths (which would be overrepresented in samples obtained from the rhizons). Alternatively, sulfide produced by sulfate reduction may immediately react with Fe(III), resulting in an abundance of solid S⁰ and minimal dissolved S²⁻.³⁷ The presence of S⁰ in the S XANES spectra is consistent with this interpretation. In either case, it is clear that reduced S is not retained in the solid phase in the upper sediments.

By the beginning of August, reducing conditions have diminished in the upper sediments, concurrent with a decrease in water table elevation and pore saturation (Figures 3.1 & 3.2). Continued draining of pores and entrainment of air further oxygenates the sediments and eliminates reduced species from porewater. Iron concentrations decline to detection limits by August and remain so through October (Figure 3.2). Reducing conditions that developed under high-water conditions in June and early July are thus reversed, and the sediments revert to an oxidized regime. The rise in the water table in early October, resulting from the construction of a beaver dam, does not drive reduction in the upper sediments before October 26, perhaps because the period between the sudden rise in the water table and the last sampling date is not long enough to drive the sediments into iron reducing conditions.

In contrast to the upper sediments, the deeper sediments (below 100 cm) are continuously saturated from late May through late October. These sediments do not experience entrainment of air or subsequent oxygenation, and reducing conditions are sustained throughout the season. In June and early July, dissolved iron concentrations below 100 cm range from 75 to 200 mg L⁻¹, indicating dissimilatory iron reduction, perhaps stimulated by sediment warming. Sulfate reducing conditions also develop below 100 cm. As dissolved Fe increases sharply from 90 to 110 cm, S concentrations decrease sharply, and mackinawite and other reduced sulfur phases appear in the S XANES spectra, while the sulfate peak is diminished. This reveals a transition in redox regime at approximately 100 cm, from early season Fe reducing conditions in the upper sediments to Fe and sulfate reducing conditions in the lower sediments (Figure 3.2). The transition is most pronounced in June and July, when concentrations of dissolved Fe and S are highest. Yet it is also well-defined August through October, when iron and sulfur concentrations are low relative to June and July levels. The presence of reduced sulfur phases at the lower depths throughout the season suggests that either these sediments do not experience seasonal oxidation events, or the concentration of reducing equivalents (solid and dissolved) is capable of protecting reduced sulfur phases and other reduced solids from oxidation.

Neither transient Fe reducing conditions nor sustained oxidizing conditions drive mobilization of dissolved Pb in the upper sediments, despite dissolution and reformation of host phases. Throughout the variable saturation states and redox conditions, dissolved Pb concentrations remain below 15 µg L⁻¹ in the upper sediments (Figure 3.2), and the proportions of Pb-OM and Pb-HFO, which are the predominant Pb species in the upper sediments, change minimally (Figures 3.3 & 3.4). Although reductive dissolution of Fe(III)-(hydr)oxides occurs in June and early July in the upper sediments, and oxidative dissolution of galena is favorable in August-October, dissolved Pb concentrations do not increase. Pb concentrations remain below 5 µg L⁻¹ in the upper sediments throughout the redox transitions (Figure 3.2). Lead released by reductive or oxidative processes appears to be re-partitioned sufficiently rapidly to avoid increases in dissolved concentrations, at least over the frequency of our sampling. Given the abundance of Pb-

OM and the documented affinity of Pb for organic matter,^{25,38} released Pb likely partitions to POM, rendering it immobile as a dissolved species.

Porewater Fe concentrations in the deeper sediments reveal large-scale dissolution of Fe(III)-(hydr)oxides in June and early July. Although Fe concentrations suggest a greater extent of host dissolution at depth than in the upper sediments, and therefore a greater extent of potential Pb desorption, dissolved Pb concentrations remain below 5 $\mu\text{g L}^{-1}$. This may reflect a lower proportion of Pb-HFO at depth than in the upper sediments (Figures 3.2 & 3.3). If Pb is released to porewater, it likely re-adsorbs to particulate organic matter and, to a lesser extent, the small remaining pool of undissolved Fe(III)-(hydr)oxides. With the onset of sulfate reducing conditions below 100 cm, Pb released during reductive dissolution of Fe(III)-(hydr)oxides may also precipitate as galena. Consistent with this interpretation, the proportion of Pb adsorbed to Fe(III)-(hydr)oxides decreases with depth, while the proportion of galena increases (Figures 3.2 & 3.3). Particulate organic matter may thus retain Pb released by reductive dissolution of host Fe(III)-(hydr)oxides, thereby limiting transport of dissolved Pb and facilitating partitioning of Pb from Pb-OM to PbS following the late-summer decline in Fe concentrations.

The affinity of Pb for a range of ligands, and its stability in a variety of coordination environments, ensures that partitioning to solid phases is more favorable than dissolution across redox transitions. The high affinity of Pb for the functional groups in POM (carboxylic acid and sulfhydryl), and the probable excess of these sites relative to dissolved Pb ligands, results in low dissolved Pb concentrations. The low solubility of PbS further ensures low dissolved Pb concentrations below the baseflow water table elevation.

However, seasonal changes in porewater redox conditions do not drive corresponding changes in solid Pb speciation. Neither the Pb EXAFS nor the sequential extraction results reveal a Pb pool that is temporally dynamic. Both analyses, however, show a clear transition in Pb speciation at the low water table position, from Pb-OM and Pb-HFO above to Pb-OM and PbS below. That this transition is mirrored in the S XANES

results suggests that the low water table elevation demarcates distinct redox regimes. Oxidized solid S phases dominate above the low water table elevation, despite occasional transient reducing conditions, while reduced S phases dominate below the low water table elevation. The absence of pyrite at depth in the S XANES spectra, alongside the presence of mackinawite and zero-valent sulfur, suggests that the sulfidic phases in the deeper sediments, including galena, are likely authigenic.

3.6 Conclusions

The affinity of Pb for a range of ligands and its stability in a variety of coordination environments limits porewater Pb concentrations during seasonal redox transitions. Although changes in porewater redox conditions dissolve Pb hosts, thereby releasing associated Pb, adsorption on POM and freshly formed mineral host phases maintain low porewater Pb concentrations. Particulate organic matter may be an important sink for released Pb if formation of a fresh mineral host phase occurs more slowly than Pb release. Our findings suggest that exports of dissolved Pb from floodplain sediments are minimal, even with active dissolution of Pb hosts. Nonetheless, redox transitions within the sediments may pose a risk to water quality if they drive formation of Pb-bearing colloids. The transport of suspended solids and colloids may redistribute Pb from upstream stores to downstream points of use, including diversions for irrigation and municipal water supply.

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3.9 Figures

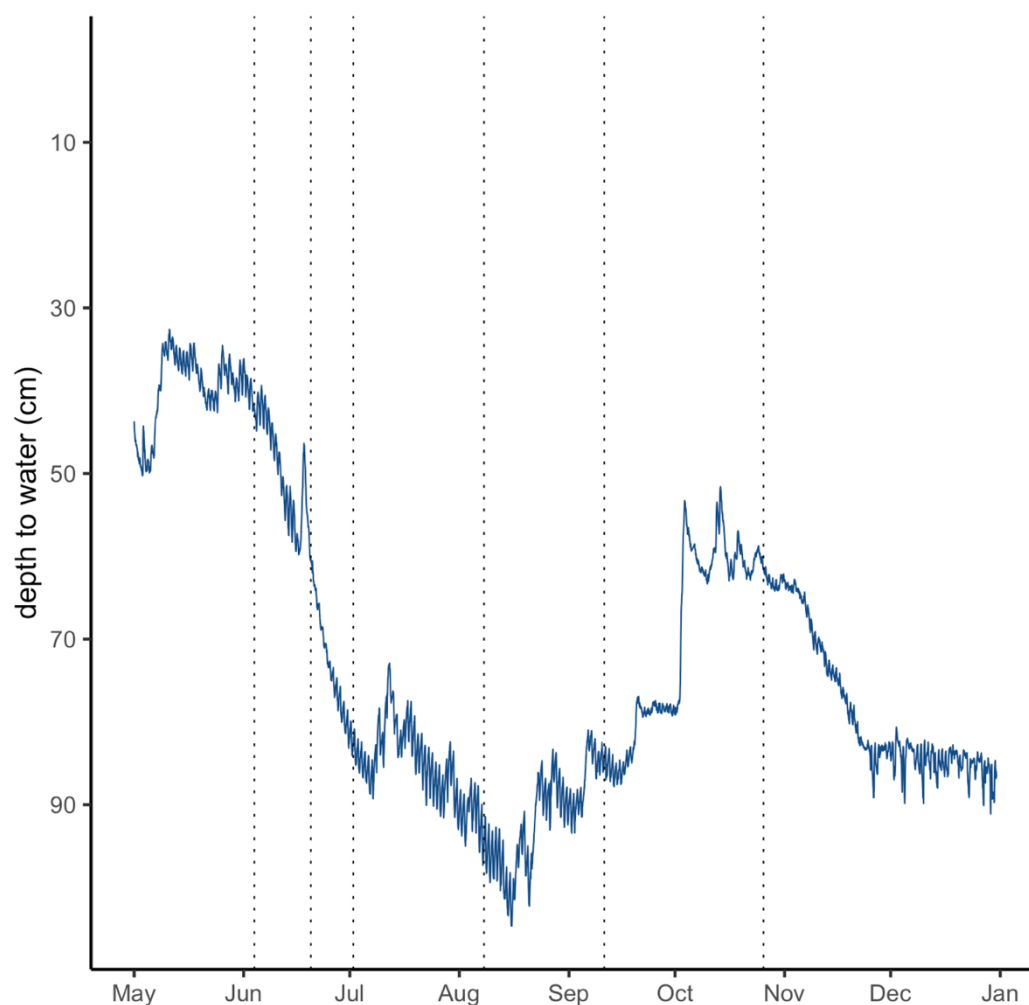


Figure 3.1. Water table position (cm below ground surface) from May 1 through December 31, 2018. Vertical dashed lines indicate porewater and solid-phase sampling dates (June 4, June 20, July 2, August 8, September 11, October 26). High water in May corresponds to peak in river stage due to snowmelt. The increase in water table position from mid-August to mid-October was driven by construction of a beaver dam in Slate River immediately downstream from the Oh-Be-Joyful floodplain.

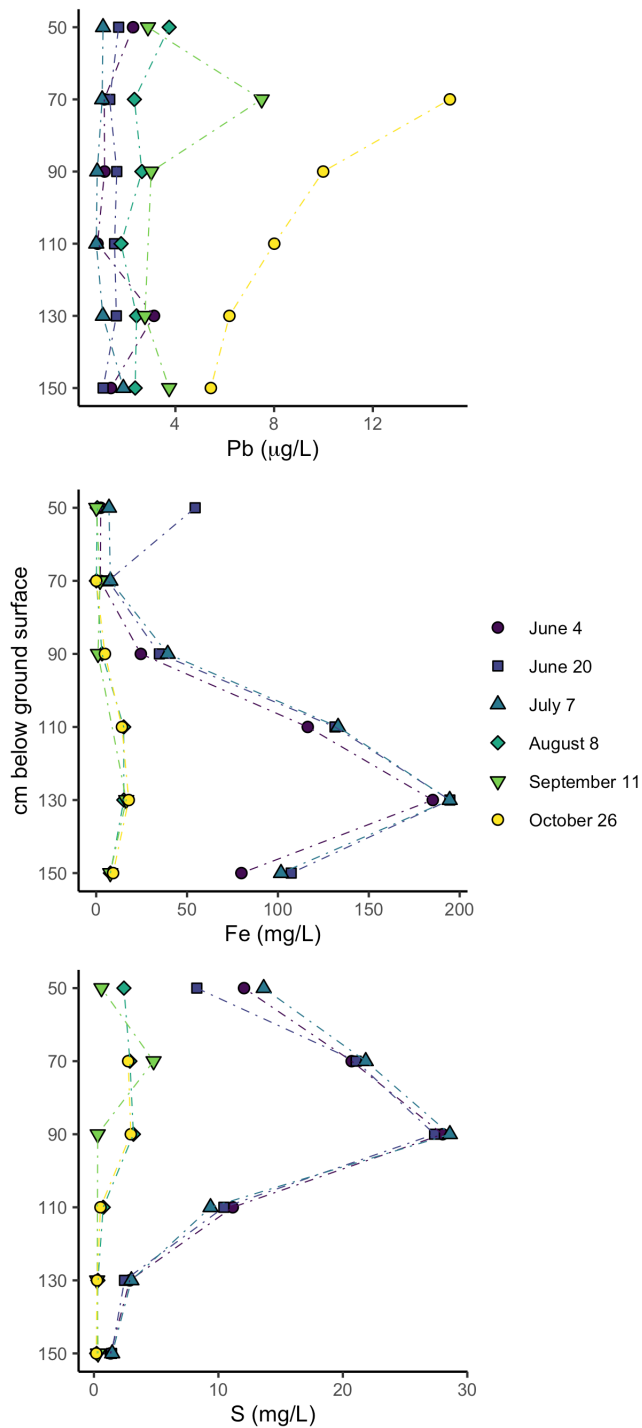


Figure 3.2. Depth-resolved porewater concentrations of Pb (top), Fe (middle), and S (bottom) from June 4 through October 26, 2018. The rhizons at 50 cm bgs did not yield porewater on October 26.

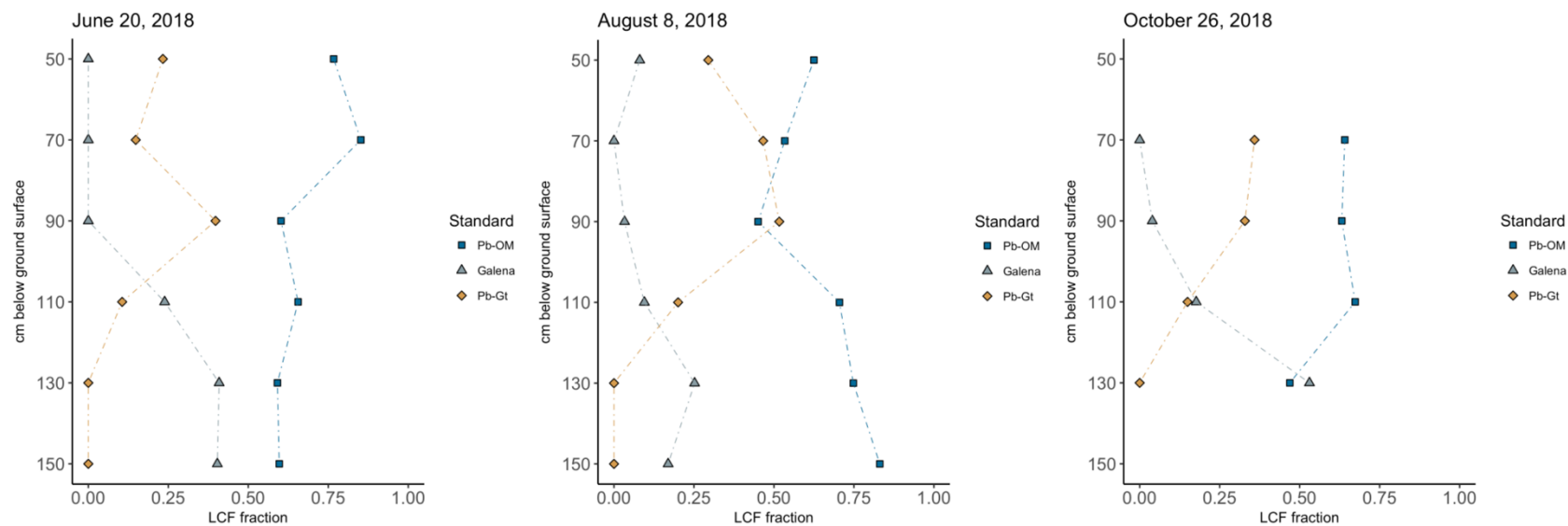


Figure 3.3. Linear combination fitting (LCF) results of Pb L3 EXAFS spectra of sediments collected on June 20, August 8, and October 26, 2018. The LCF was performed with the following standards: cerussite, galena, anglesite, pyromorphite, Pb-OM, and Pb-Gt. For clarity, only the Pb-OM, galena and Pb-Gt results are shown here, as the other standards comprised less than 10% of the overall fit. Full LCF results appear in the Supplemental Information (Figures S3.2-S3.4).

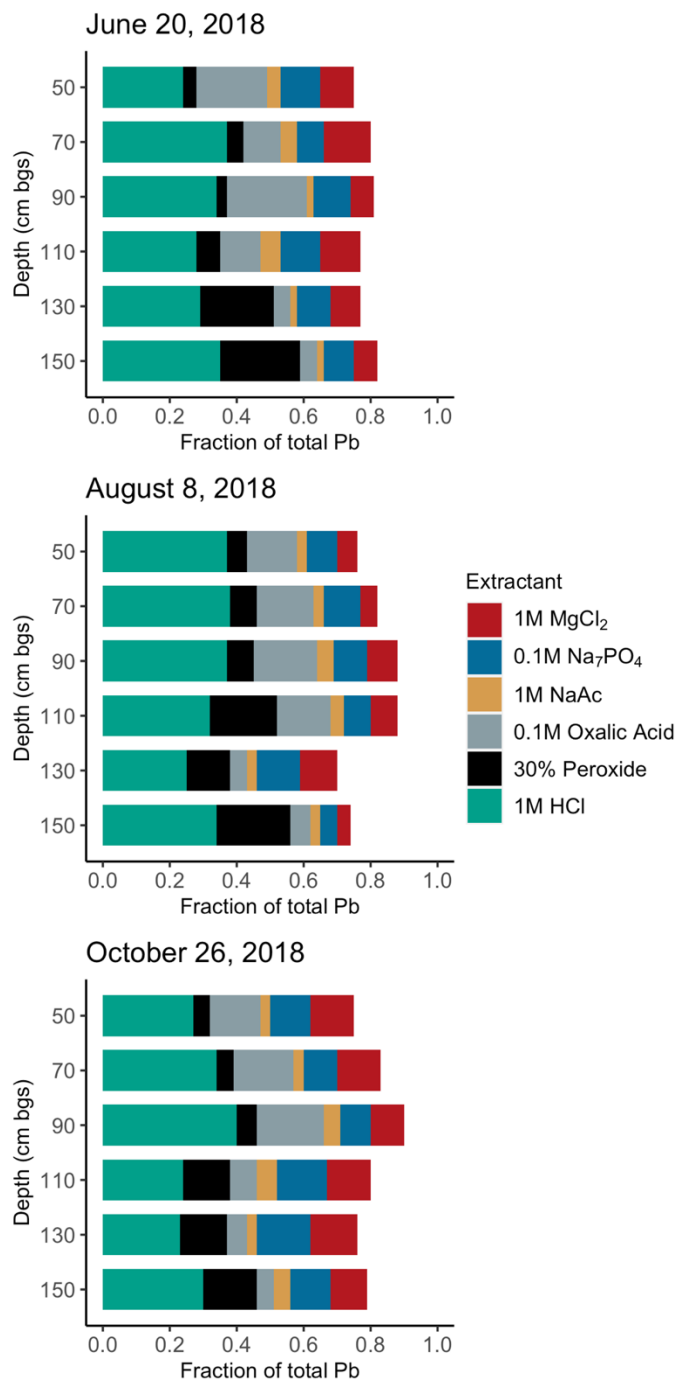


Figure 3.4. Fraction of total Pb removed by sequential extractions on sediments collected on June 20, August 8, and October 26, 2018 at OBJ-2. The extractions were performed in the following order (on the same the material): Milli-Q water; 1 M MgCl_2 ; 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$; 0.1 M oxalic acid; 30% H_2O_2 ; 1 M HCl. No Pb was extracted by the Milli-Q water step, and so the results from that step are not shown. The overall extraction efficiency was less than 100% for all samples.

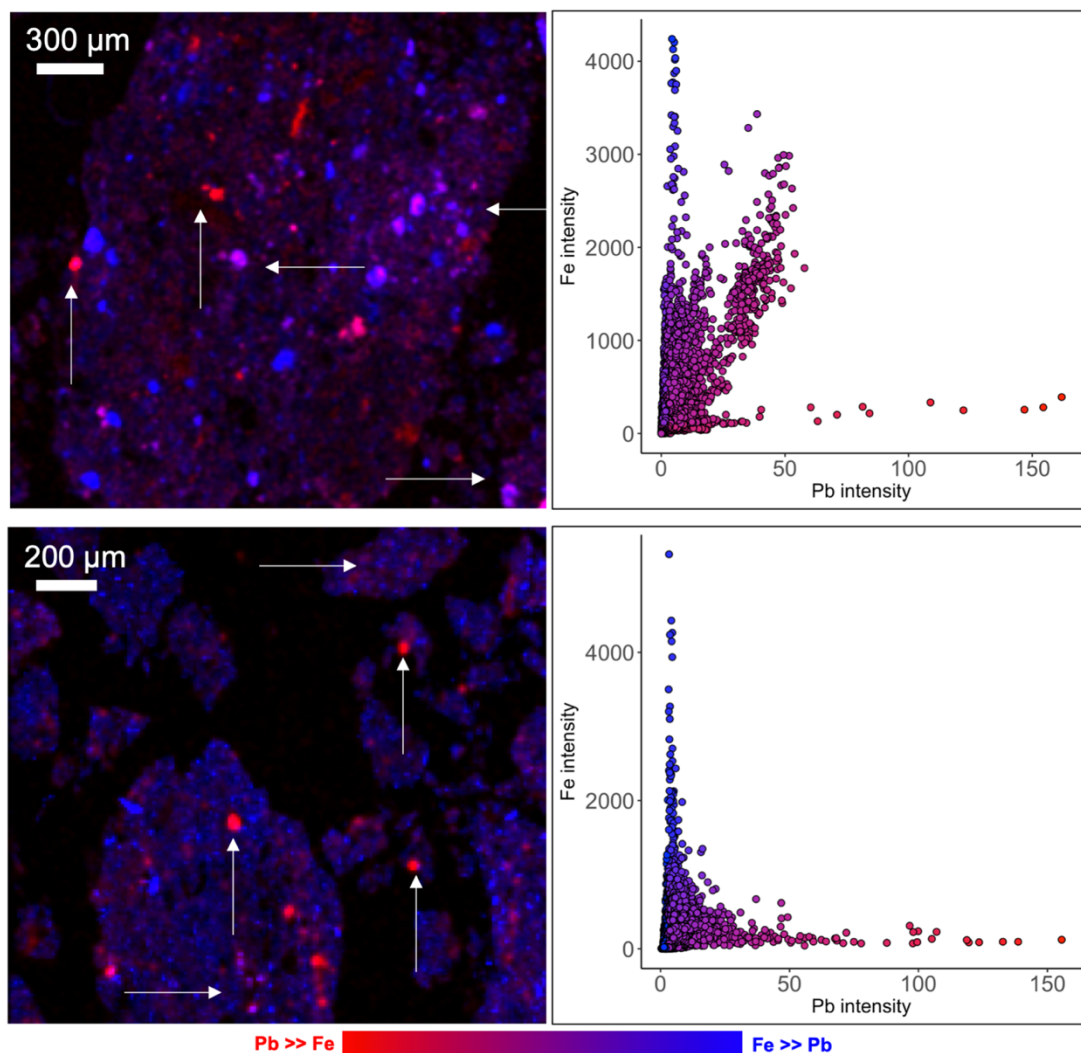


Figure 3.5. μ XRF Fe-Pb maps (left) and correlation plots (right) of sediment from 40 cm bgs (top row) and 130 cm bgs (bottom row). Maps of Fe (blue) and Pb (red) are overlain. In the sediment from 40 cm bgs (top row), the majority of Pb is distributed throughout the particles and is associated with Fe (purple features, horizontal arrows). A small pool of Pb occurs as 20 to 60 μ m grains in which Fe is not present (red features, vertical arrows). In the sediment from 130 cm bgs (bottom row), the majority of Pb occurs as 20 to 60 μ m grains in which Fe is not present (red features, vertical arrows). A smaller pool of Pb is distributed throughout the particles and is associated with Fe (purple features, horizontal arrows).

3.11 Supplementary Materials

Table S3.1. Selected XRF results for OBJ-2 sediments. Samples collected on July 2 and September 11, 2018 were not run on XRF.

Depth	Date	Na	Mg	Al	Si	P	S	Cl	K	Ca	Mn	Fe	Zn	Pb
		%	%	%	%	%	%	%	%	%	ug/g	ug/g	ug/g	ug/g
4-Jun-18	50	0.619	1.768	8.482	26.600	0.127	0.065	0.010	1.872	0.902	567.1	47390.0	789.1	302.6
	70	0.512	1.546	7.997	25.020	0.120	0.030	0.007	2.087	1.086	417.5	49260.0	371.1	340.2
	90	0.830	2.523	10.620	31.860	0.097	0.027	0.006	1.776	1.127	293.5	31920.0	468.2	179.9
	110	0.651	2.437	11.000	32.440	0.088	0.062	0.006	1.632	0.685	203.1	26860.0	434.6	181.4
	130	0.483	1.819	8.708	27.200	0.124	0.176	0.008	1.926	0.874	246.4	27830.0	639.9	167.2
	150	0.674	2.333	10.990	31.920	0.101	0.171	0.005	2.083	0.727	233.0	28130.0	466.4	142.1
20-Jun-18	50	0.769	1.879	9.582	30.240	0.124	0.070	0.004	1.939	0.995	688.5	40450.0	673.2	319.9
	70	0.803	2.426	10.050	31.920	0.132	0.054	0.004	1.820	0.946	310.9	40120.0	637.0	228.9
	90	0.717	2.317	10.240	31.680	0.115	0.043	0.004	1.764	0.819	294.6	45640.0	528.7	191.8
	110	0.926	2.374	10.530	31.820	0.105	0.062	0.008	1.904	1.198	215.9	31710.0	452.7	203.3
	130	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	150	0.895	2.457	10.250	31.960	0.111	0.159	0.007	2.032	1.364	244.5	28030.0	547.6	197.7
8-Aug-18	50	0.774	2.314	10.090	31.780	0.147	0.066	0.007	1.860	0.903	502.4	42780.0	539.4	210.2
	70	0.752	2.602	10.340	31.230	0.110	0.043	0.007	1.753	0.856	350.8	47150.0	605.8	231.2
	90	1.031	2.412	10.650	31.960	0.097	0.030	0.006	1.861	1.128	350.2	28560.0	398.7	164.2
	110	0.744	2.047	8.644	27.210	0.093	0.045	0.006	1.697	1.203	208.4	27830.0	411.1	183.5
	130	0.897	2.551	10.400	31.950	0.102	0.094	0.009	1.918	1.404	211.3	26900.0	517.1	196.8
	150	0.753	2.640	10.140	31.850	0.107	0.314	0.008	2.011	1.676	222.1	25070.0	463.6	167.4
26-Oct-18	50	0.573	1.868	8.986	28.455	0.134	0.066	0.007	1.799	0.836	575.1	43115.0	728.5	291.6
	70	0.660	2.499	10.750	30.920	0.105	0.055	0.004	1.774	0.718	359.3	49500.0	597.4	242.2
	90	0.866	2.343	10.650	32.030	0.090	0.032	0.005	1.837	1.085	267.6	30690.0	437.9	163.9
	110	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	130	0.838	2.582	10.670	32.050	0.097	0.104	0.008	1.815	1.269	205.4	24750.0	443.6	172.9
	150	0.882	2.581	10.340	32.000	0.101	0.187	0.008	2.024	1.498	227.9	24020.0	397.2	137.2
All depths	mean	0.757	2.287	10.005	30.641	0.110	0.089	0.007	1.872	1.059	327.1	34895.7	524.9	209.8
	std. deviation	0.138	0.317	0.875	2.194	0.016	0.071	0.002	0.122	0.267	139.7	9115.8	115.1	57.1
	max	1.031	2.640	11.000	32.440	0.147	0.314	0.010	2.087	1.676	688.5	49500.0	789.1	340.2
	min	0.483	1.546	7.997	25.020	0.088	0.027	0.004	1.632	0.685	203.1	24020.0	371.1	137.2

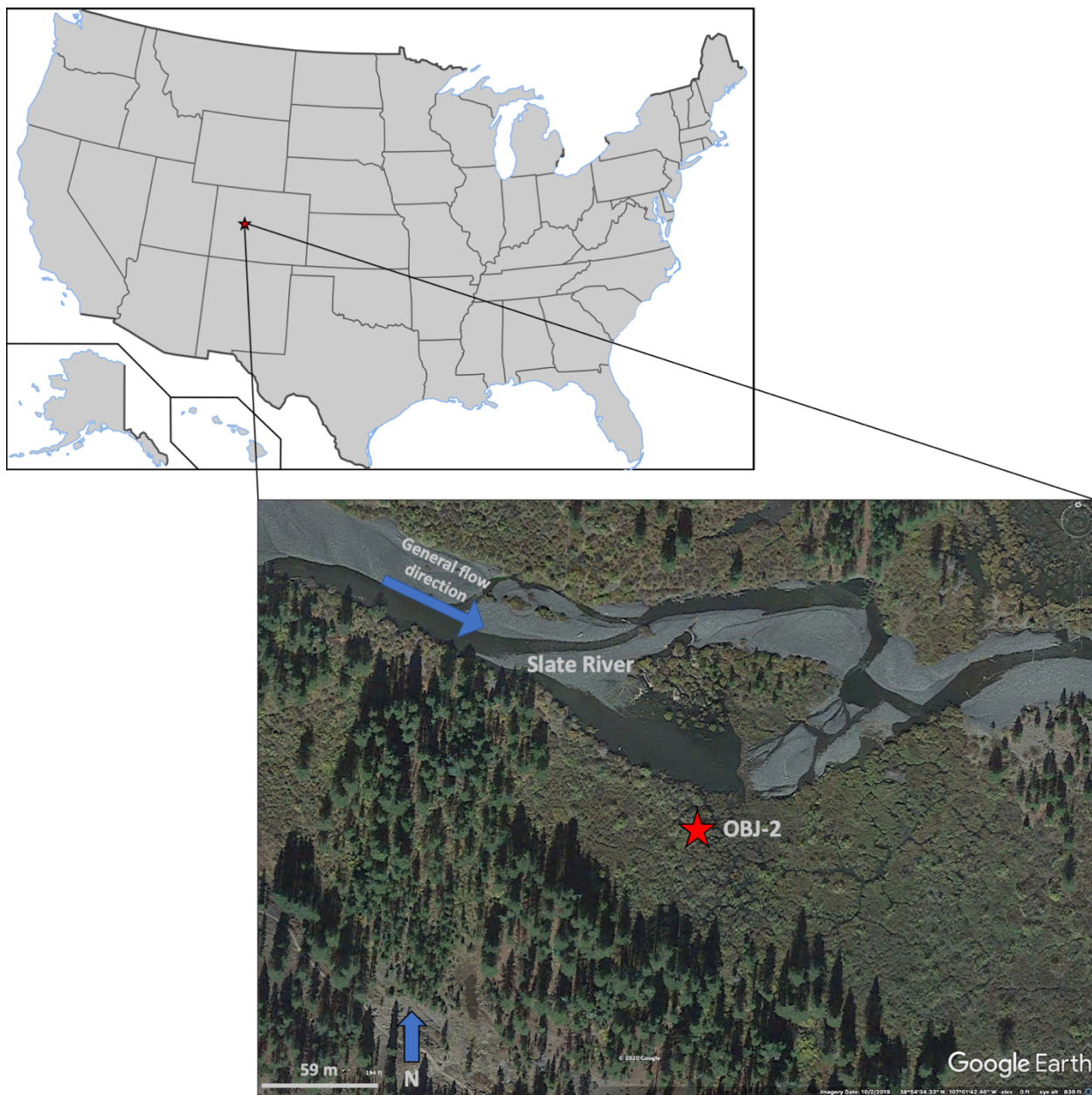


Figure S3.1. Map showing the OBJ-2 sampling location on the Slate River, near Crested Butte, CO, USA.



Figure S3.2. Image of beaver dam at OBJ-2. Construction began in late August, 2018.

Date	Depth	ICP-MS	ICP-OES	
		208Pb ppb	Fe2599-2 ppm	S_1820-2 ppm
4-Jun-18	50	2.3	2.48	12.06
	70	1.1	2.33	20.71
	90	1.1	24.56	28.02
	110	0.8	116.40	11.15
	130	3.1	185.20	2.88
	150	1.4	79.90	1.34
20-Jun-18	50	1.7	54.45	8.27
	70	1.3	6.68	21.07
	90	1.6	34.77	27.37
	110	1.5	131.40	10.44
	130	1.6	194.60	2.46
	150	1.1	107.30	1.42
2-Jul-18	50	1.1	7.05	13.64
	70	1.0	7.80	21.84
	90	0.8	39.37	28.60
	110	0.8	133.10	9.37
	130	1.1	194.50	3.01
	150	1.9	101.70	1.47
8-Aug-18	50	3.7	0.59	2.41
	70	2.3	0.09	2.89
	90	2.6	3.23	3.17
	110	1.8	15.14	0.74
	130	2.4	15.20	0.32
	150	2.4	7.85	0.22
11-Sep-18	50	2.9	0.10	0.61
	70	7.5	2.12	4.79
	90	3.0	1.00	0.30
	110	NA	NA	NA
	130	2.8	16.53	0.26
	150	3.7	7.75	0.33
26-Oct-18	50	NA	NA	NA
	70	15.1	0.05	2.75
	90	10.0	4.82	2.96
	110	8.0	14.18	0.53
	130	6.2	17.91	0.25
	150	5.4	9.31	0.19

Table S3.2. Tabulated Pb, Fe, and S porewater concentrations. Porewater samples from 50 and 110 cm collected on August 8 and October 26, respectively, did not surviving shipping.

Table S3.3. Selected porewater pH measurements. pH was only measured at the start and end of the season so as not to stress the rhizons.

Date	Depth	pH
4-Jun-18	50	7.1
4-Jun-18	130	6.8
26-Oct-18	50	6.9
26-Oct-18	130	6.5

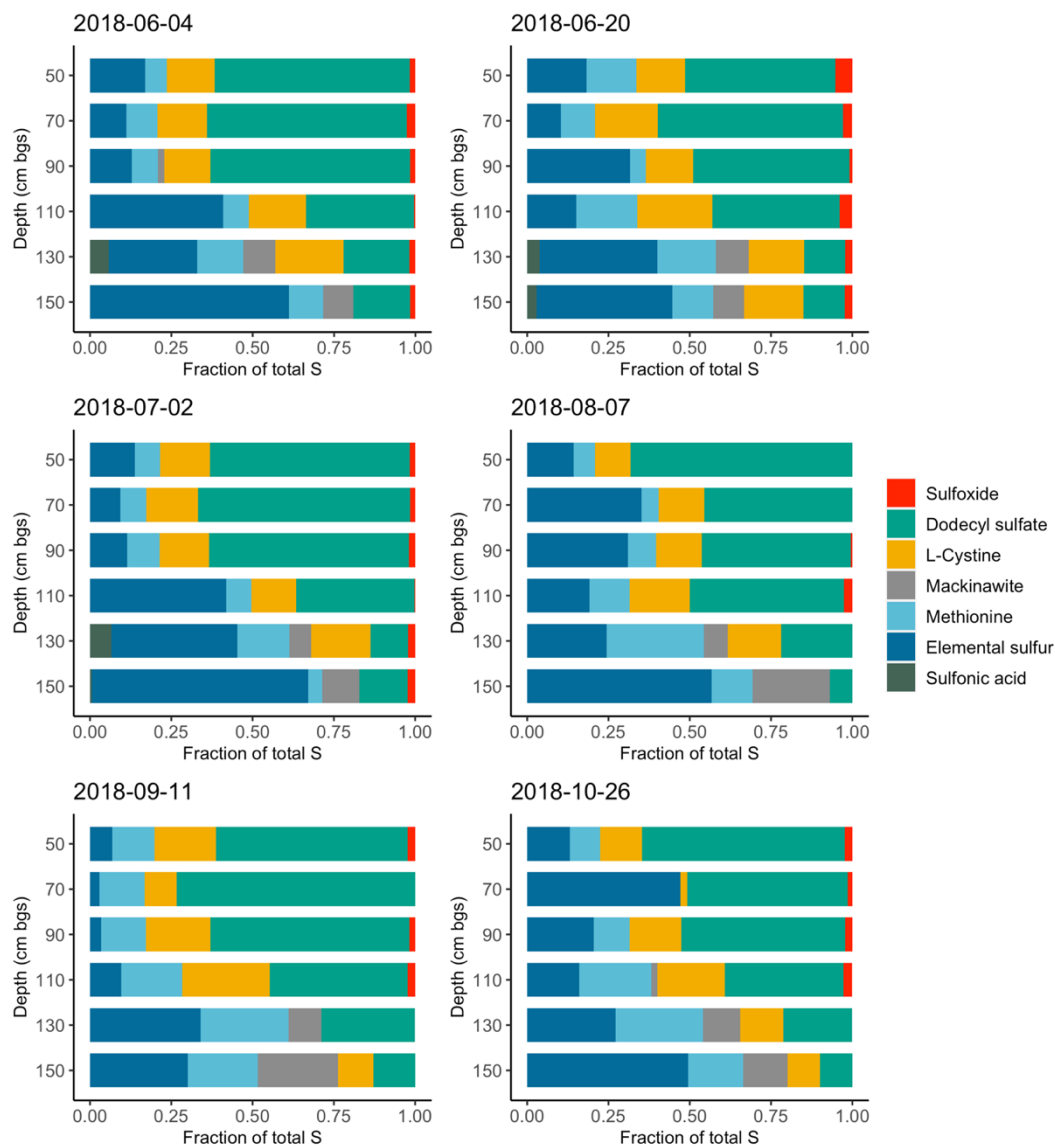


Figure S3.3. Summary of S K XANES linear combination fitting results.

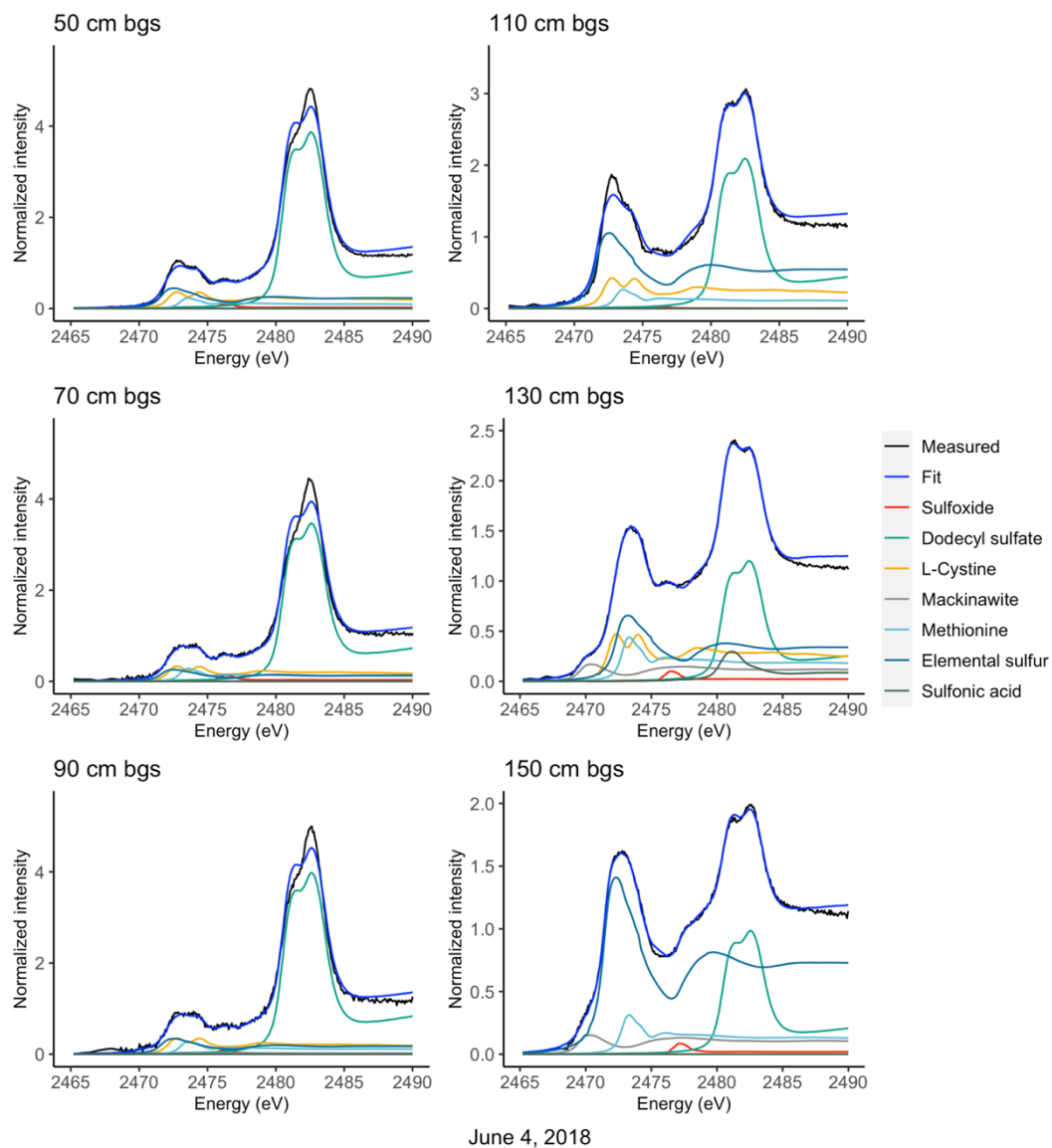


Figure S3.4. S K XANES spectra from samples collected on June 4, 2018

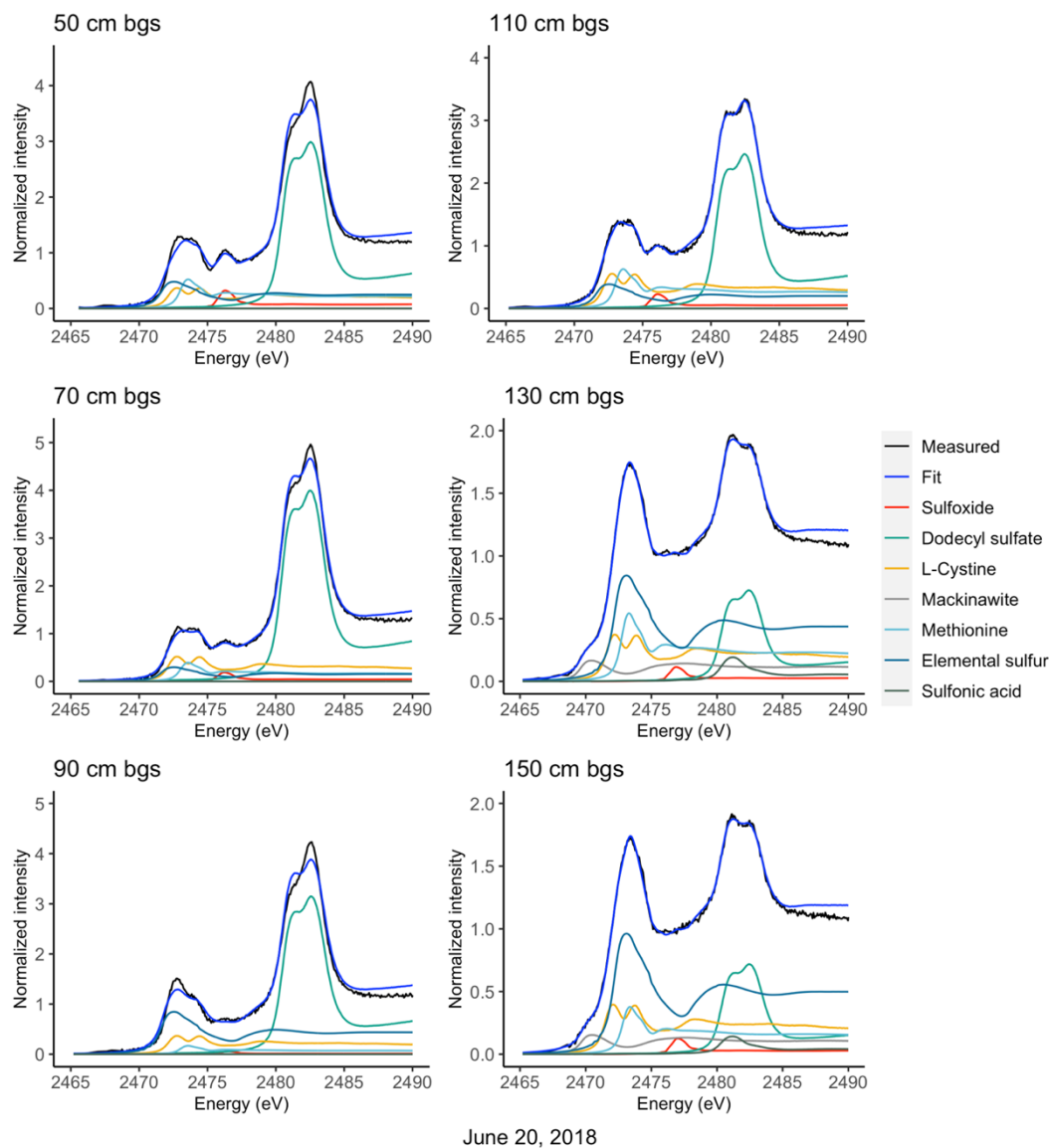


Figure S3.5. S K XANES spectra from samples collected on June 20, 2018

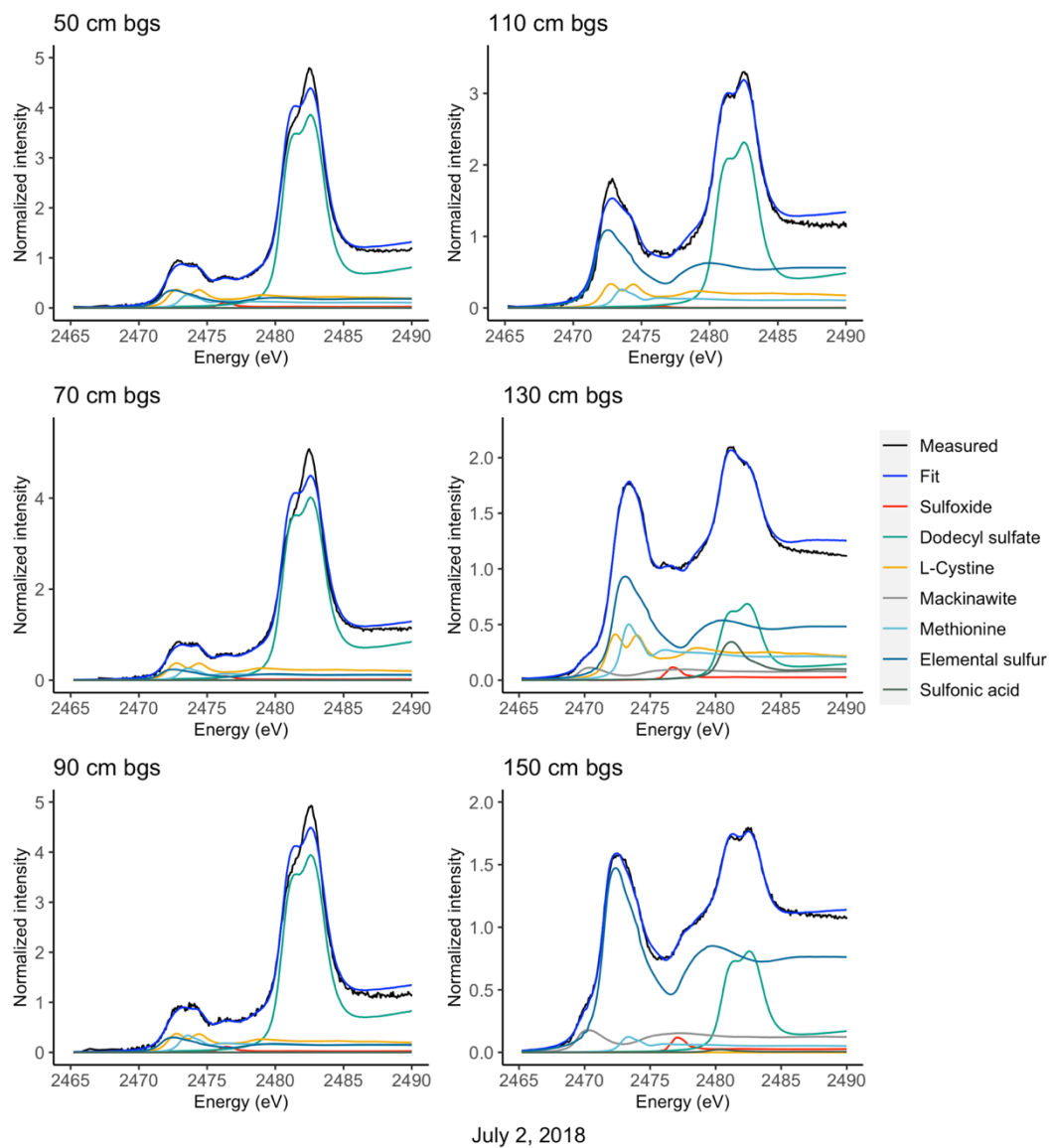


Figure S3.6. S K XANES spectra from samples collected on July 2, 2018.

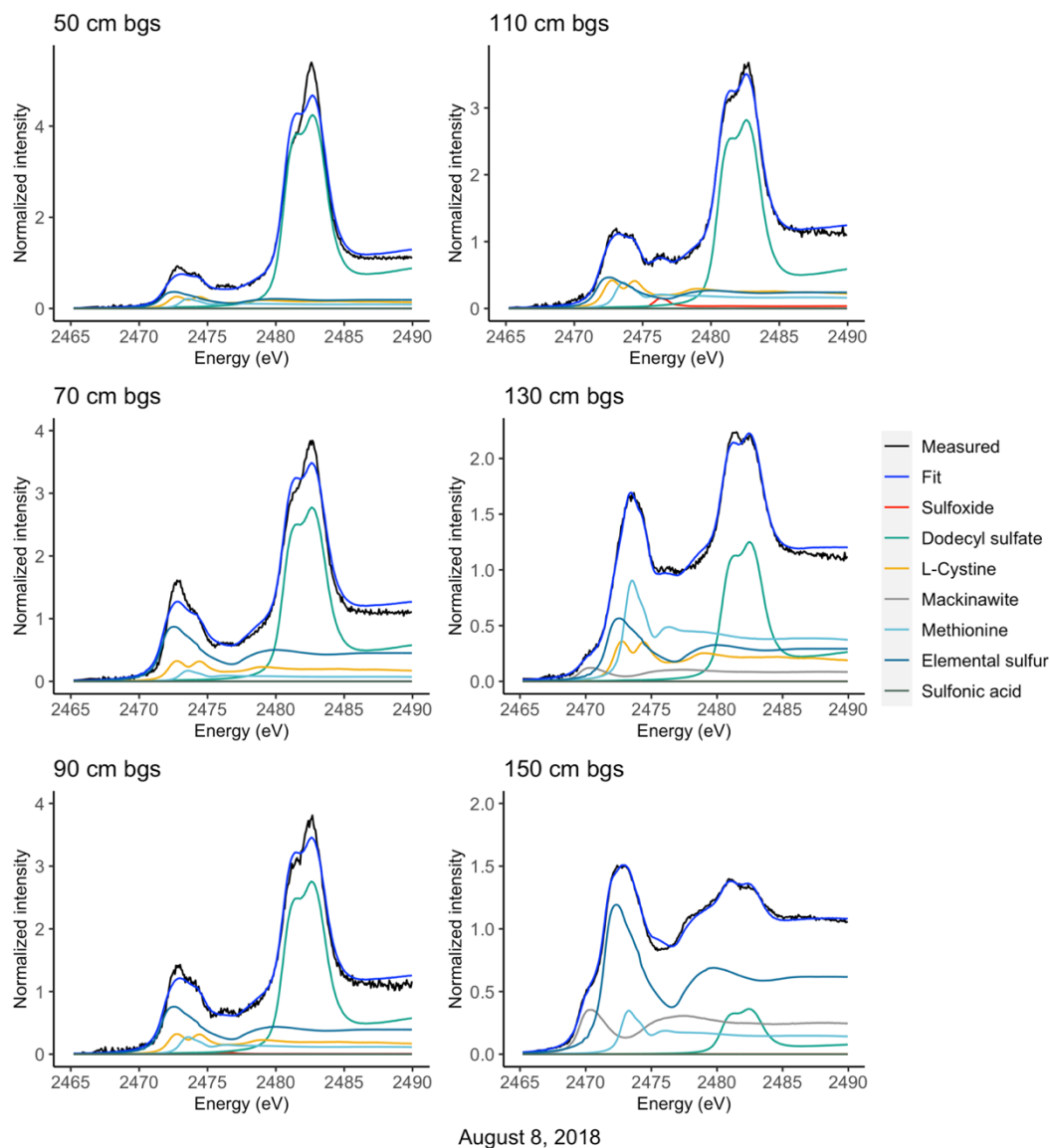


Figure S3.7. S K XANES spectra from samples collected on August 8, 2018.

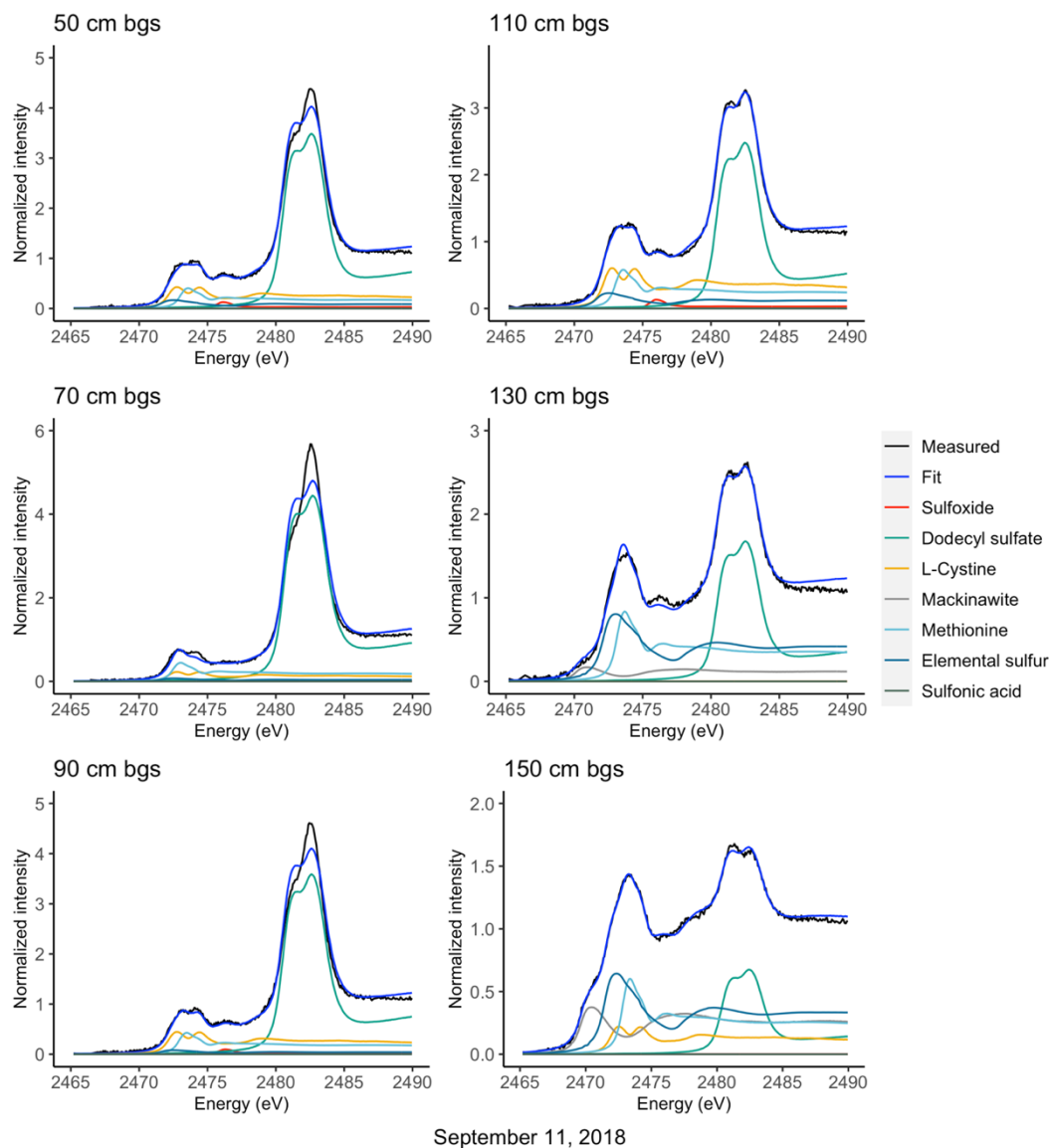


Figure S3.8. S K XANES spectra from samples collected on September 11, 2018.

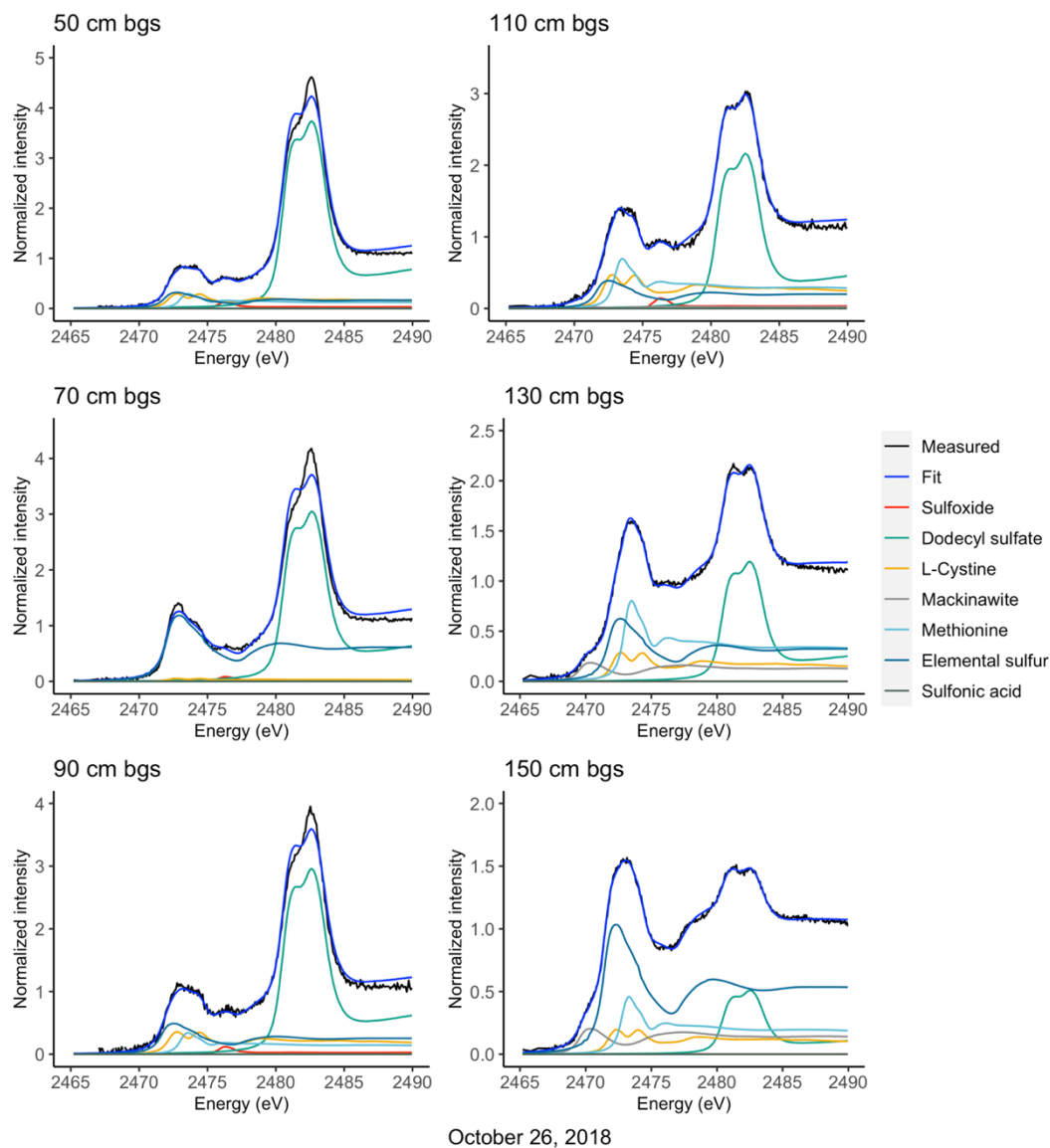


Figure S3.9. S K XANES spectra from samples collected on October 26, 2018.

Table S3.5. S XANES LCF results for samples collected on June 4, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-06-04	50	Dodecyl sulfate	0.762	0.005	-0.223	0.010
2018-06-04	50	Elemental sulfur	0.215	0.032	0.000	0.000
2018-06-04	50	L-Cystine	0.187	0.044	0.000	0.000
2018-06-04	50	Mackinawite	0.000	0.000	0.000	0.000
2018-06-04	50	Methionine	0.085	0.024	0.000	0.000
2018-06-04	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-04	50	Sulfoxide	0.021	0.008	-0.009	0.287
2018-06-04	70	Dodecyl sulfate	0.683	0.006	-0.194	0.014
2018-06-04	70	Elemental sulfur	0.124	0.040	0.000	0.000
2018-06-04	70	L-Cystine	0.169	0.055	0.000	0.000
2018-06-04	70	Mackinawite	0.000	0.000	0.000	0.000
2018-06-04	70	Methionine	0.106	0.030	0.000	0.000
2018-06-04	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-04	70	Sulfoxide	0.029	0.010	0.025	0.261
2018-06-04	90	Dodecyl sulfate	0.784	0.006	-0.181	0.011
2018-06-04	90	Elemental sulfur	0.165	0.038	0.000	0.000
2018-06-04	90	L-Cystine	0.182	0.050	0.000	0.000
2018-06-04	90	Mackinawite	0.024	0.020	0.000	0.000
2018-06-04	90	Methionine	0.102	0.027	0.000	0.000
2018-06-04	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-04	90	Sulfoxide	0.021	0.011	-0.009	0.336
2018-06-04	110	Dodecyl sulfate	0.413	0.003	-0.304	0.013
2018-06-04	110	Elemental sulfur	0.510	0.023	0.000	0.000
2018-06-04	110	L-Cystine	0.218	0.031	0.000	0.000
2018-06-04	110	Mackinawite	0.000	0.000	0.000	0.000
2018-06-04	110	Methionine	0.098	0.017	0.000	0.000
2018-06-04	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-04	110	Sulfoxide	0.006	0.006	0.078	0.843
2018-06-04	130	Dodecyl sulfate	0.237	0.121	-0.348	0.121
2018-06-04	130	Elemental sulfur	0.318	0.121	0.679	0.121
2018-06-04	130	L-Cystine	0.244	0.121	-0.430	0.121
2018-06-04	130	Mackinawite	0.116	0.121	0.043	0.121
2018-06-04	130	Methionine	0.164	0.121	-0.261	0.121
2018-06-04	130	Sulfonic acid	0.067	0.121	0.051	0.121
2018-06-04	130	Sulfoxide	0.021	0.121	0.204	0.121
2018-06-04	150	Dodecyl sulfate	0.194	0.123	-0.250	0.123
2018-06-04	150	Elemental sulfur	0.683	0.123	-0.230	0.123
2018-06-04	150	L-Cystine	0.000	0.000	0.000	0.000
2018-06-04	150	Mackinawite	0.104	0.123	-0.041	0.123
2018-06-04	150	Methionine	0.117	0.123	-0.268	0.123
2018-06-04	150	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-04	150	Sulfoxide	0.018	0.123	0.880	0.123

Table S3.6. S XANES LCF results for samples collected on June 20, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-06-20	50	Dodecyl sulfate	0.589	0.005	-0.252	0.013
2018-06-20	50	Elemental sulfur	0.233	0.032	0.000	0.000
2018-06-20	50	L-Cystine	0.190	0.044	0.000	0.000
2018-06-20	50	Mackinawite	0.000	0.000	0.000	0.000
2018-06-20	50	Methionine	0.195	0.024	0.000	0.000
2018-06-20	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-20	50	Sulfoxide	0.068	0.008	-0.003	0.090
2018-06-20	70	Dodecyl sulfate	0.788	0.004	-0.283	0.007
2018-06-20	70	Elemental sulfur	0.143	0.024	0.000	0.000
2018-06-20	70	L-Cystine	0.267	0.033	0.000	0.000
2018-06-20	70	Mackinawite	0.000	0.000	0.000	0.000
2018-06-20	70	Methionine	0.146	0.018	0.000	0.000
2018-06-20	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-20	70	Sulfoxide	0.039	0.006	-0.069	0.119
2018-06-20	90	Dodecyl sulfate	0.621	0.005	-0.217	0.012
2018-06-20	90	Elemental sulfur	0.409	0.031	0.000	0.000
2018-06-20	90	L-Cystine	0.189	0.042	0.000	0.000
2018-06-20	90	Mackinawite	0.000	0.000	0.000	0.000
2018-06-20	90	Methionine	0.063	0.023	0.000	0.000
2018-06-20	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-20	90	Sulfoxide	0.012	0.008	0.188	0.502
2018-06-20	110	Dodecyl sulfate	0.486	0.001	-0.333	0.005
2018-06-20	110	Elemental sulfur	0.187	0.010	0.000	0.000
2018-06-20	110	L-Cystine	0.286	0.013	0.000	0.000
2018-06-20	110	Mackinawite	0.000	0.000	0.000	0.000
2018-06-20	110	Methionine	0.234	0.007	0.000	0.000
2018-06-20	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-06-20	110	Sulfoxide	0.048	0.003	-0.201	0.039
2018-06-20	130	Dodecyl sulfate	0.143	0.124	-0.371	0.124
2018-06-20	130	Elemental sulfur	0.409	0.124	0.557	0.124
2018-06-20	130	L-Cystine	0.193	0.124	-0.548	0.124
2018-06-20	130	Mackinawite	0.113	0.124	0.073	0.124
2018-06-20	130	Methionine	0.203	0.124	-0.281	0.124
2018-06-20	130	Sulfonic acid	0.043	0.124	0.116	0.124
2018-06-20	130	Sulfoxide	0.024	0.124	0.595	0.124
2018-06-20	150	Dodecyl sulfate	0.142	0.126	-0.340	0.126
2018-06-20	150	Elemental sulfur	0.466	0.126	0.556	0.126
2018-06-20	150	L-Cystine	0.204	0.126	-0.677	0.126
2018-06-20	150	Mackinawite	0.105	0.126	0.127	0.126
2018-06-20	150	Methionine	0.141	0.126	-0.207	0.126
2018-06-20	150	Sulfonic acid	0.032	0.126	0.089	0.126
2018-06-20	150	Sulfoxide	0.027	0.126	0.722	0.126

Table S3.7. S XANES LCF results for samples collected on July 2, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-07-02	50	Dodecyl sulfate	0.761	0.005	-0.219	0.009
2018-07-02	50	Elemental sulfur	0.171	0.030	0.000	0.000
2018-07-02	50	L-Cystine	0.189	0.041	0.000	0.000
2018-07-02	50	Mackinawite	0.000	0.000	0.000	0.000
2018-07-02	50	Methionine	0.096	0.022	0.000	0.000
2018-07-02	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-07-02	50	Sulfoxide	0.022	0.008	0.004	0.262
2018-07-02	70	Dodecyl sulfate	0.792	0.007	-0.206	0.013
2018-07-02	70	Elemental sulfur	0.114	0.044	0.000	0.000
2018-07-02	70	L-Cystine	0.193	0.061	0.000	0.000
2018-07-02	70	Mackinawite	0.000	0.000	0.000	0.000
2018-07-02	70	Methionine	0.097	0.033	0.000	0.000
2018-07-02	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-07-02	70	Sulfoxide	0.019	0.011	0.024	0.457
2018-07-02	90	Dodecyl sulfate	0.777	0.005	-0.197	0.011
2018-07-02	90	Elemental sulfur	0.144	0.035	0.000	0.000
2018-07-02	90	L-Cystine	0.193	0.048	0.000	0.000
2018-07-02	90	Mackinawite	0.000	0.000	0.000	0.000
2018-07-02	90	Methionine	0.127	0.026	0.000	0.000
2018-07-02	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-07-02	90	Sulfoxide	0.024	0.009	0.108	0.282
2018-07-02	110	Dodecyl sulfate	0.457	0.003	-0.269	0.012
2018-07-02	110	Elemental sulfur	0.527	0.022	0.000	0.000
2018-07-02	110	L-Cystine	0.174	0.031	0.000	0.000
2018-07-02	110	Mackinawite	0.000	0.000	0.000	0.000
2018-07-02	110	Methionine	0.097	0.017	0.000	0.000
2018-07-02	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-07-02	110	Sulfoxide	0.004	0.006	0.109	0.958
2018-07-02	130	Dodecyl sulfate	0.135	0.118	-0.397	0.118
2018-07-02	130	Elemental sulfur	0.451	0.118	0.560	0.118
2018-07-02	130	L-Cystine	0.213	0.118	-0.414	0.118
2018-07-02	130	Mackinawite	0.077	0.118	-0.397	0.118
2018-07-02	130	Methionine	0.187	0.118	-0.204	0.118
2018-07-02	130	Sulfonic acid	0.077	0.118	0.109	0.118
2018-07-02	130	Sulfoxide	0.025	0.118	0.429	0.118
2018-07-02	150	Dodecyl sulfate	0.159	0.126	-0.225	0.126
2018-07-02	150	Elemental sulfur	0.713	0.126	-0.182	0.126
2018-07-02	150	L-Cystine	0.000	0.126	0.258	0.126
2018-07-02	150	Mackinawite	0.122	0.126	-0.094	0.126
2018-07-02	150	Methionine	0.047	0.126	-0.234	0.126
2018-07-02	150	Sulfonic acid	0.005	0.126	-0.724	0.126
2018-07-02	150	Sulfoxide	0.025	0.126	0.790	0.126

Table S3.8. S XANES LCF results for samples collected on August 8, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-08-07	50	Dodecyl sulfate	0.837	0.007	-0.111	0.014
2018-08-07	50	Elemental sulfur	0.176	0.049	0.000	0.000
2018-08-07	50	L-Cystine	0.134	0.067	0.000	0.000
2018-08-07	50	Mackinawite	0.000	0.000	0.000	0.000
2018-08-07	50	Methionine	0.079	0.036	0.000	0.000
2018-08-07	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	50	Sulfoxide	0.000	0.013	0.000	0.017
2018-08-07	70	Dodecyl sulfate	0.547	0.047	-0.154	0.047
2018-08-07	70	Elemental sulfur	0.422	0.047	0.000	0.000
2018-08-07	70	L-Cystine	0.168	0.047	0.000	0.000
2018-08-07	70	Mackinawite	0.000	0.000	0.000	0.000
2018-08-07	70	Methionine	0.063	0.047	0.000	0.047
2018-08-07	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	70	Sulfoxide	0.000	0.047	0.000	0.087
2018-08-07	90	Dodecyl sulfate	0.543	0.116	-0.170	0.116
2018-08-07	90	Elemental sulfur	0.367	0.116	0.000	0.000
2018-08-07	90	L-Cystine	0.166	0.116	0.000	0.000
2018-08-07	90	Mackinawite	0.000	0.000	0.000	0.000
2018-08-07	90	Methionine	0.102	0.116	0.000	0.116
2018-08-07	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	90	Sulfoxide	0.006	0.116	0.097	0.116
2018-08-07	110	Dodecyl sulfate	0.556	0.002	-0.199	0.006
2018-08-07	110	Elemental sulfur	0.225	0.014	0.000	0.000
2018-08-07	110	L-Cystine	0.217	0.019	0.000	0.000
2018-08-07	110	Mackinawite	0.000	0.000	0.000	0.000
2018-08-07	110	Methionine	0.144	0.010	0.000	0.000
2018-08-07	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	110	Sulfoxide	0.032	0.004	0.009	0.085
2018-08-07	130	Dodecyl sulfate	0.246	0.118	-0.324	0.118
2018-08-07	130	Elemental sulfur	0.274	0.118	0.000	0.118
2018-08-07	130	L-Cystine	0.185	0.118	0.000	0.118
2018-08-07	130	Mackinawite	0.083	0.118	0.000	0.000
2018-08-07	130	Methionine	0.337	0.118	-0.045	0.118
2018-08-07	130	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	130	Sulfoxide	0.000	0.000	0.000	0.000
2018-08-07	150	Dodecyl sulfate	0.071	0.118	-0.360	0.118
2018-08-07	150	Elemental sulfur	0.578	0.118	-0.212	0.118
2018-08-07	150	L-Cystine	0.000	0.000	0.000	0.000
2018-08-07	150	Mackinawite	0.242	0.118	0.000	0.000
2018-08-07	150	Methionine	0.129	0.118	-0.297	0.118
2018-08-07	150	Sulfonic acid	0.000	0.000	0.000	0.000
2018-08-07	150	Sulfoxide	0.000	0.000	0.000	0.000

Table S3.9. S XANES LCF results for samples collected on September 11, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-09-11	50	Dodecyl sulfate	0.687	0.117	-0.191	0.117
2018-09-11	50	Elemental sulfur	0.080	0.117	0.000	0.117
2018-09-11	50	L-Cystine	0.220	0.117	0.000	0.000
2018-09-11	50	Mackinawite	0.000	0.000	0.000	0.000
2018-09-11	50	Methionine	0.151	0.117	0.000	0.117
2018-09-11	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	50	Sulfoxide	0.027	0.117	-0.157	0.117
2018-09-11	70	Dodecyl sulfate	0.876	0.117	-0.094	0.117
2018-09-11	70	Elemental sulfur	0.035	0.117	0.000	0.117
2018-09-11	70	L-Cystine	0.116	0.117	0.000	0.117
2018-09-11	70	Mackinawite	0.000	0.000	0.000	0.000
2018-09-11	70	Methionine	0.166	0.117	-0.546	0.117
2018-09-11	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	70	Sulfoxide	0.000	0.117	0.000	117.000
2018-09-11	90	Dodecyl sulfate	0.707	0.118	-0.183	0.118
2018-09-11	90	Elemental sulfur	0.039	0.118	0.000	0.118
2018-09-11	90	L-Cystine	0.230	0.118	0.000	0.118
2018-09-11	90	Mackinawite	0.000	0.000	0.000	0.000
2018-09-11	90	Methionine	0.159	0.118	-0.099	0.118
2018-09-11	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	90	Sulfoxide	0.020	0.118	0.000	0.118
2018-09-11	110	Dodecyl sulfate	0.489	0.117	-0.302	0.117
2018-09-11	110	Elemental sulfur	0.111	0.117	0.000	0.117
2018-09-11	110	L-Cystine	0.312	0.177	0.000	0.000
2018-09-11	110	Mackinawite	0.000	0.000	0.000	0.000
2018-09-11	110	Methionine	0.216	0.177	0.000	0.117
2018-09-11	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	110	Sulfoxide	0.028	0.117	-0.294	0.117
2018-09-11	130	Dodecyl sulfate	0.330	0.118	-0.275	0.118
2018-09-11	130	Elemental sulfur	0.390	0.118	0.478	0.118
2018-09-11	130	L-Cystine	0.000	0.000	0.000	0.000
2018-09-11	130	Mackinawite	0.115	0.118	0.566	0.118
2018-09-11	130	Methionine	0.311	0.118	0.110	0.118
2018-09-11	130	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	130	Sulfoxide	0.000	0.000	0.000	0.000
2018-09-11	150	Dodecyl sulfate	0.133	0.119	-0.352	0.119
2018-09-11	150	Elemental sulfur	0.312	0.119	-0.204	0.119
2018-09-11	150	L-Cystine	0.114	0.119	-0.242	0.119
2018-09-11	150	Mackinawite	0.256	0.119	0.080	0.119
2018-09-11	150	Methionine	0.224	0.119	-0.208	0.119
2018-09-11	150	Sulfonic acid	0.000	0.000	0.000	0.000
2018-09-11	150	Sulfoxide	0.000	0.000	0.000	0.000

Table S3.10. S XANES LCF results for samples collected on October 26, 2018.

Date	Depth	Standard	Weight	Precision	e0	e0 precision
2018-10-26	50	Dodecyl sulfate	0.736	0.116	-0.182	0.116
2018-10-26	50	Elemental sulfur	0.155	0.116	0.227	0.116
2018-10-26	50	L-Cystine	0.151	0.116	0.000	0.000
2018-10-26	50	Mackinawite	0.000	0.000	0.000	0.000
2018-10-26	50	Methionine	0.110	0.116	0.000	0.116
2018-10-26	50	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	50	Sulfoxide	0.029	0.116	0.000	0.116
2018-10-26	70	Dodecyl sulfate	0.601	0.116	-0.157	0.116
2018-10-26	70	Elemental sulfur	0.573	0.116	0.378	0.116
2018-10-26	70	L-Cystine	0.026	0.116	0.000	0.000
2018-10-26	70	Mackinawite	0.000	0.000	0.000	0.000
2018-10-26	70	Methionine	0.000	0.116	0.000	0.116
2018-10-26	70	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	70	Sulfoxide	0.017	0.116	0.000	0.116
2018-10-26	90	Dodecyl sulfate	0.583	0.117	-0.188	0.117
2018-10-26	90	Elemental sulfur	0.237	0.117	0.000	0.117
2018-10-26	90	L-Cystine	0.184	0.117	0.000	0.000
2018-10-26	90	Mackinawite	0.000	0.000	0.000	0.000
2018-10-26	90	Methionine	0.127	0.117	0.000	0.117
2018-10-26	90	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	90	Sulfoxide	0.025	0.117	0.000	0.117
2018-10-26	110	Dodecyl sulfate	0.426	0.117	-0.266	0.117
2018-10-26	110	Elemental sulfur	0.187	0.117	0.000	0.117
2018-10-26	110	L-Cystine	0.242	0.117	0.000	0.117
2018-10-26	110	Mackinawite	0.021	0.117	0.000	0.000
2018-10-26	110	Methionine	0.258	0.117	-0.063	0.117
2018-10-26	110	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	110	Sulfoxide	0.030	0.117	0.000	0.117
2018-10-26	130	Dodecyl sulfate	0.235	0.118	-0.313	0.118
2018-10-26	130	Elemental sulfur	0.302	0.118	0.103	0.118
2018-10-26	130	L-Cystine	0.148	0.118	-0.102	0.118
2018-10-26	130	Mackinawite	0.127	0.118	0.000	0.118
2018-10-26	130	Methionine	0.299	0.118	-0.084	0.118
2018-10-26	130	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	130	Sulfoxide	0.000	0.000	0.000	0.000
2018-10-26	150	Dodecyl sulfate	0.100	0.118	-0.278	0.118
2018-10-26	150	Elemental sulfur	0.501	0.118	-0.235	0.118
2018-10-26	150	L-Cystine	0.101	0.118	-0.410	0.118
2018-10-26	150	Mackinawite	0.139	0.118	0.000	0.118
2018-10-26	150	Methionine	0.171	0.118	-0.276	0.118
2018-10-26	150	Sulfonic acid	0.000	0.000	0.000	0.000
2018-10-26	150	Sulfoxide	0.000	0.000	0.000	0.000

Table S3.11. S K XANES LCF statistics for all samples.

Date	Depth	Sum	Rfactor	Chi-sq.	Reduced Chi-sq.
2018-06-04	50	1.271	0.0075693	3.20075	0.0144178
	70	1.111	0.014937	5.00915	0.0222629
	90	1.278	0.009522	4.17327	0.0185479
	110	1.245	0.0092868	1.62365	0.0072162
	130	1.167	0.0006788	0.12105	0.1210451
	150	1.116	0.0016127	0.12251	0.122508
2018-06-20	50	1.274	0.0085528	1.80756	0.0106956
	70	1.384	0.003293	1.13048	0.0062114
	90	1.294	0.0097062	2.97472	0.133395
	110	1.242	0.001395	0.29363	0.0013167
	130	1.128	0.0012686	0.12415	0.124169
	150	1.116	0.0013971	0.12571	0.12571
2018-07-02	50	1.238	0.0068301	2.8397	0.0126209
	70	1.215	0.0141476	6.30513	0.0275333
	90	1.265	0.0088955	3.86953	0.0173521
	110	1.258	0.0079443	1.58	0.007057
	130	1.165	0.0011553	0.11811	0.1181054
	150	1.071	0.0023583	0.12571	0.12571
2018-08-07	50	1.226	0.015893	8.32099	0.0336822
	70	1.2	0.0147097	0.04732	0.0473189
	90	1.184	0.009417	0.11553	0.1155338
	110	1.173	0.0025221	0.067259	0.0028024
	130	1.125	0.002888	0.11754	0.1175432
	150	1.02	0.004262	0.11765	0.1176548
2018-09-11	50	1.165	0.0066738	0.11708	0.1170769
	70	1.192	0.0234876	0.1173	0.1172972
	90	1.155	0.0120278	0.11752	0.1175175
	110	1.155	0.0012717	0.11686	0.1168566
	130	1.147	0.0038939	0.11829	0.11829
	150	1.039	0.0016997	0.11862	0.118621
2018-10-26	50	1.18	0.0059489	0.1162	0.1161962
	70	1.217	0.0123799	0.11642	0.1164165
	90	1.156	0.0070345	0.11664	0.1166368
	110	1.165	0.0023301	0.11741	0.11741
	130	1.112	0.0020384	0.11799	0.11799
	150	1.012	0.0014901	0.11821	0.11821

June 20, 2018

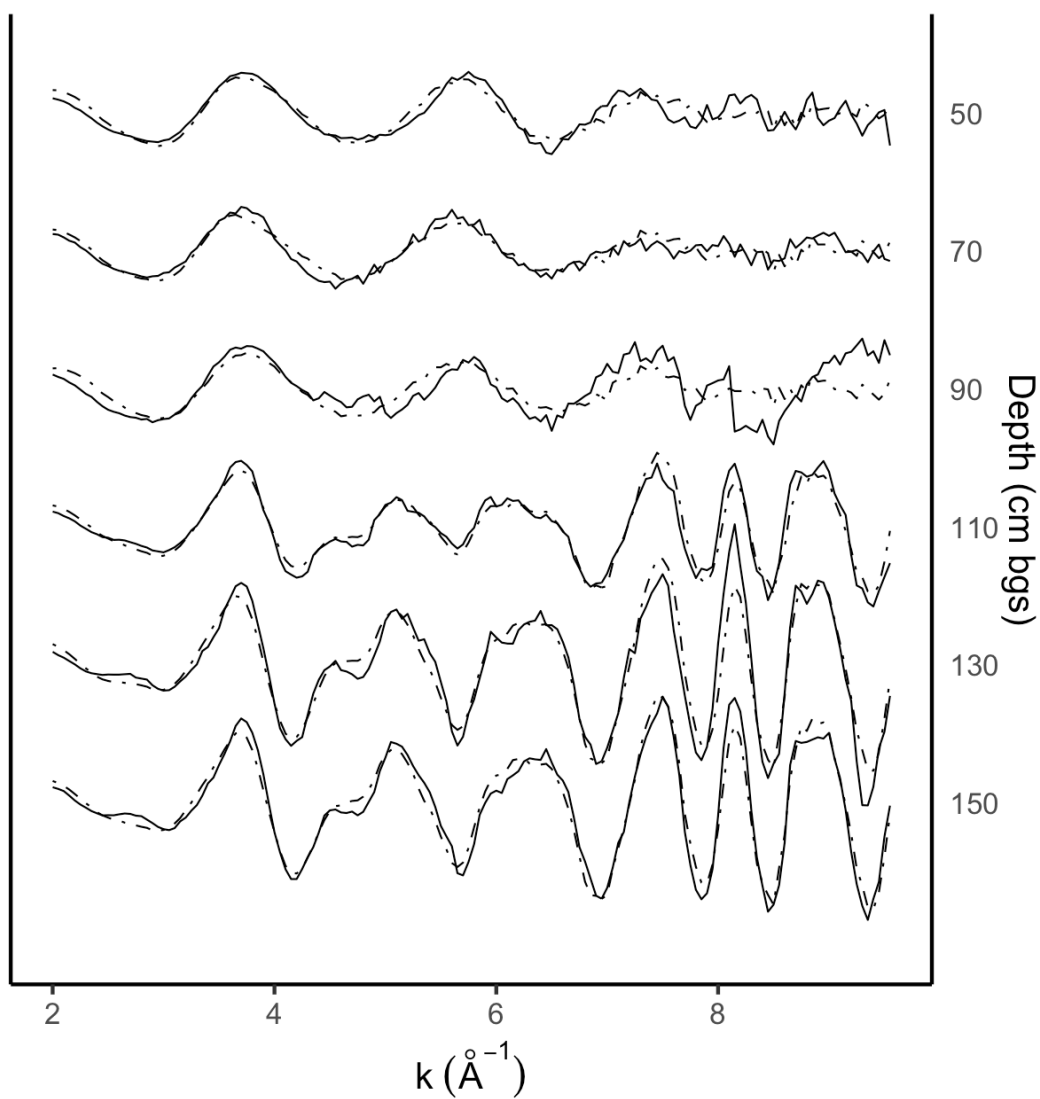


Figure S3.10. Pb L3 EXAFS spectra of samples collected on June 20, 2018.

August 8, 2018

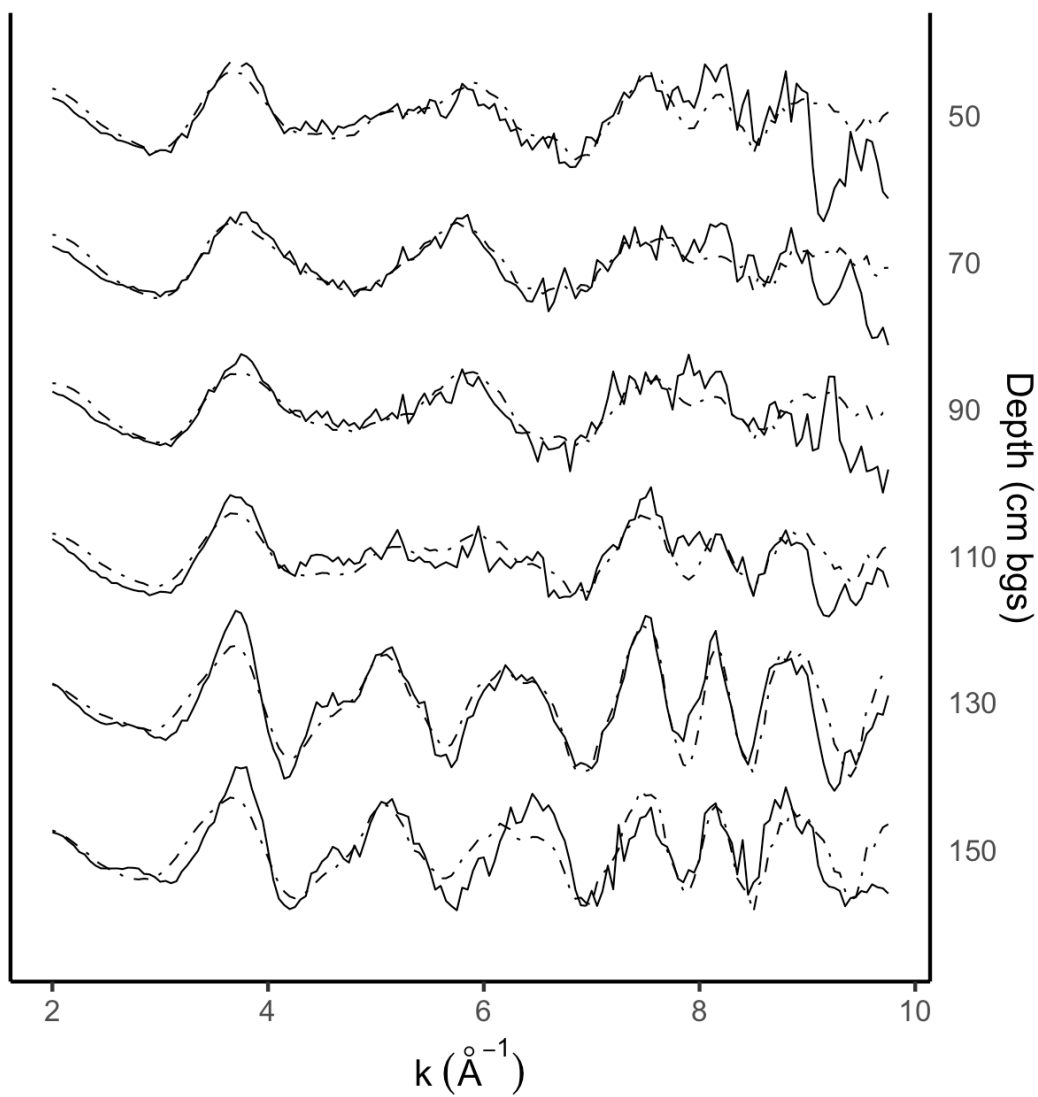


Figure S3.11. Pb L3 EXAFS spectra of samples collected on August 8, 2018.

October 26, 2018

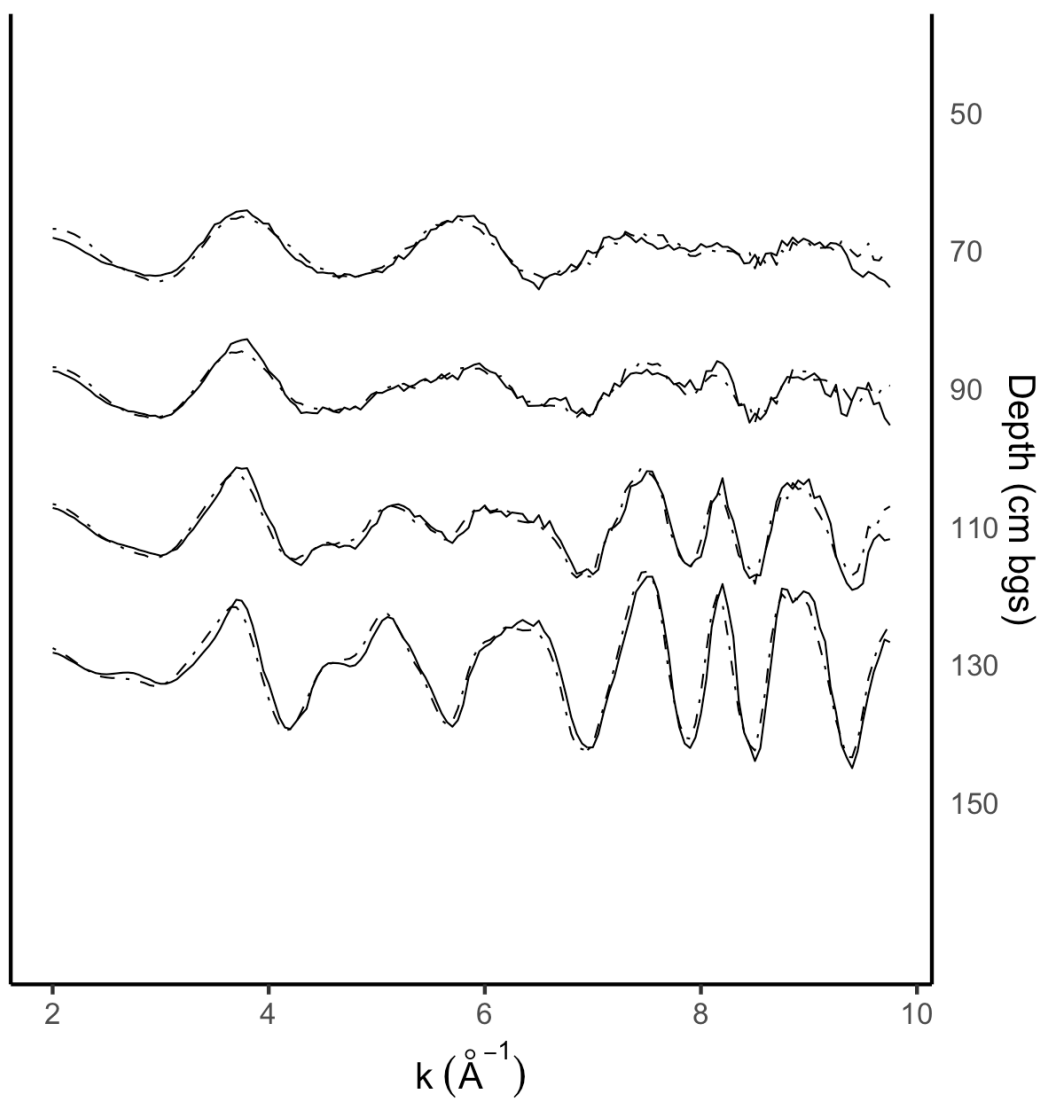


Figure S3.12. Pb L3 EXAFS spectra of samples collected on October 26, 2018.

Table S3.12. Full LCF results for Pb L₃ EXAFS for the samples collected on June 20, 2018.

date	depth	Rfactor	chisq	Red_chisq	std	weight
6/20/18	50	0.158	5.420	0.046	Pb-Gt	0.177
					Pyromorphite	0.100
					Pb-OM	0.705
					Galena	0.000
					Cerussite	0.000
					Anglesite	0.018
	70	0.169	5.205	0.039	Pb-Gt	0.113
					Pyromorphite	0.104
					Pb-OM	0.747
					Galena	0.000
					Cerussite	0.022
					Anglesite	0.014
	90	0.318	11.593	0.103	Pb-Gt	0.134
					Pyromorphite	0.048
					Pb-OM	0.808
					Galena	0.009
					Cerussite	0.000
					Anglesite	0.000
	110	0.074	6.792	0.051	Pb-Gt	0.058
					Pyromorphite	0.045
					Pb-OM	0.679
					Galena	0.195
					Cerussite	0.024
					Anglesite	0.000
	130	0.089	19.564	0.146	Pb-Gt	0.000
					Pyromorphite	0.085
					Pb-OM	0.510
					Galena	0.339
					Cerussite	0.066
					Anglesite	0.000
	150	0.054	14.738	0.098	Pb-Gt	0.000
					Pyromorphite	0.000
					Pb-OM	0.638
					Galena	0.297
					Cerussite	0.064
					Anglesite	0.000

Table S3.13. Full LCF results for Pb L₃ EXAFS for the sample collected on August 8, 2018.

date	depth	Rfactor	chisq	Red_chisq	std	weight
8/8/18	50	0.272	12.938	0.100	Pb-Gt	0.342
					Pyromorphite	0.115
					Pb-OM	0.390
					Galena	0.055
					Cerussite	0.021
					Anglesite	0.076
	70	0.206	8.386	0.064	Pb-Gt	0.328
					Pyromorphite	0.088
					Pb-OM	0.522
					Galena	0.005
					Cerussite	0.035
					Anglesite	0.023
	90	0.268	11.353	0.090	Pb-Gt	0.437
					Pyromorphite	0.037
					Pb-OM	0.425
					Galena	0.018
					Cerussite	0.045
					Anglesite	0.038
	110	0.344	17.597	0.131	Pb-Gt	0.204
					Pyromorphite	0.072
					Pb-OM	0.586
					Galena	0.078
					Cerussite	0.023
					Anglesite	0.038
	130	0.147	18.256	0.136	Pb-Gt	0.000
					Pyromorphite	0.031
					Pb-OM	0.640
					Galena	0.221
					Cerussite	0.026
					Anglesite	0.082
	150	0.351	29.491	0.223	Pb-Gt	0.000
					Pyromorphite	0.000
					Pb-OM	0.736
					Galena	0.117
					Cerussite	0.065
					Anglesite	0.082

Table S3.14. Full LCF results for Pb L₃ EXAFS for the sample collected on October 26, 2018.

date	depth	Rfactor	chisq	Red_chisq	std	weight
10/26/18	70	0.109	3.152	0.024	Pb-Gt	0.256
					Pyromorphite	0.067
					Pb-OM	0.648
					Galena	0.005
					Cerussite	0.006
					Anglesite	0.017
	90	0.140	4.218	0.030	Pb-Gt	0.223
					Pyromorphite	0.002
					Pb-OM	0.676
					Galena	0.026
					Cerussite	0.054
					Anglesite	0.020
	110	0.074	4.743	0.036	Pb-Gt	0.102
					Pyromorphite	0.043
					Pb-OM	0.677
					Galena	0.125
					Cerussite	0.034
					Anglesite	0.018
	130	0.054	22.446	0.159	Pb-Gt	0.000
					Pyromorphite	0.103
					Pb-OM	0.437
					Galena	0.375
					Cerussite	0.044
					Anglesite	0.042

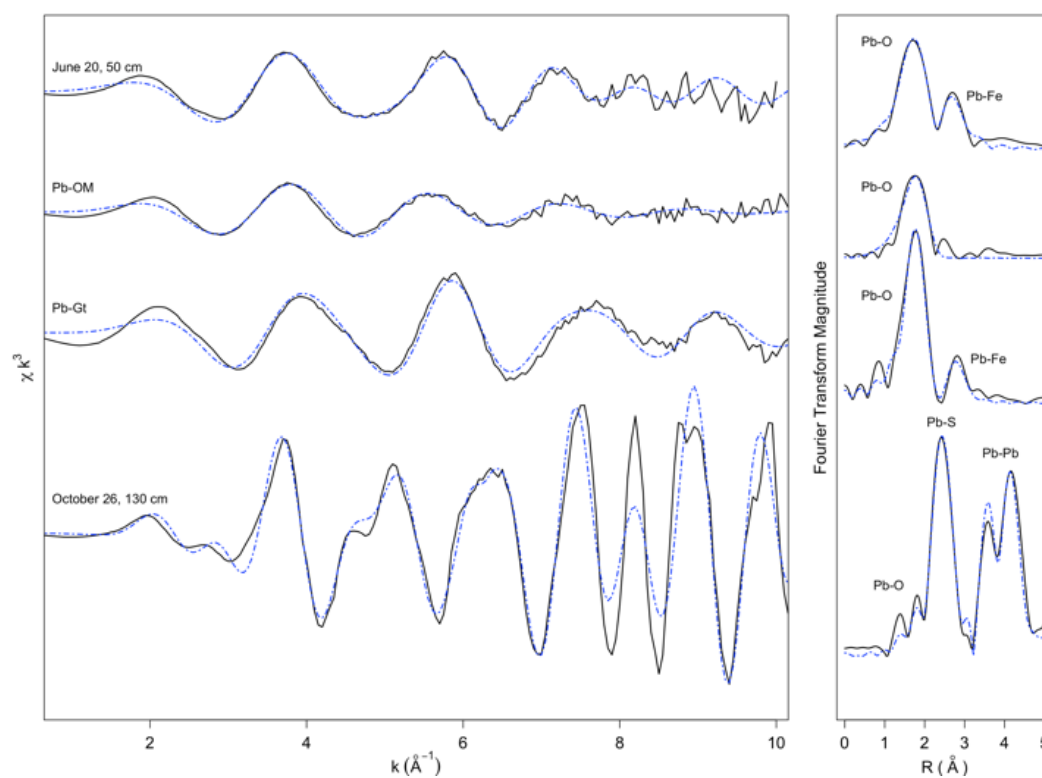


Figure S3.13. *Left.* Pb L_3 EXAFS spectra (solid black lines) of sample collected at 50 cm below ground surface on June 20, 2018; Pb adsorbed to particulate organic matter (Pb-OM); Pb adsorbed to goethite (Pb-Gt); and the sample collected at 130 cm bgs on October 26, 2018, plotted with shell-by-shell fits (dashed blue lines). *Right.* Fourier transforms of the EXAFS spectra. Fourier transform limits were from $k = 2.5 \text{ \AA}^{-1}$ to $k = 8.5$ to $10 \text{ \AA}^{-1}$. Distances in the Fourier transforms are uncorrected for phase shift.

Table S3.15. Results for shell-by-shell analysis of EXAFS for Pb-OM, Pb-Gt, and the sample collected at 50 cm below ground surface on June 20, 2018. CN = coordination number ($\pm 10\%$ for undistorted first shell neighbors), R = interatomic distance (± 0.03 Å for Pb-O bonds, and ± 0.04 Å for second shell distances), σ^2 = Debye-Waller factor, * = parameter fixed in fit. Least squares precisions are given in parentheses. $S0^2$ was set to 0.8 in all fits.

		50 cm	Pb-OM	Pb-Gt	130 cm
	E0 (eV)	-15.89	-12.64	-13.95	-6.57
Pb-O	R (Å)	2.33 (0.015)	2.37 (0.019)	2.27 (0.017)	2.46 (0.048)
	CN	1.96 (0.36)	1.54 (0.29)	1.52 (0.34)	0.70 (0.27)
	σ^2	0.016 (0.0029)	0.019 (0.0034)	0.0080 (0.0025)	0.015*
Pb-Fe	R (Å)	3.27 (0.017)		3.31 (0.039)	
	CN	0.48 (0.27)		1.17 (1.45)	
	σ^2	0.090 (0.0053)		0.023 (0.015)	
Pb-S	R (Å)				2.92 (0.0056)
	CN				2.07 (0.31)
	σ^2				0.0052 (0.0014)
Pb-Pb	R (Å)				4.16 (0.0056)
	CN				5.03 (0.80)
	σ^2				0.0047 (0.0011)

Chapter 4:

The impact of redox environment on metal-organic complexes: findings from East River floodplain soils

4.1 Abstract

Metal concentrations (e.g., Hg, Pb, Cu, Cd, U) in pore-waters of sediments and soils are often controlled by complexation with dissolved organic matter (DOM). However, the fundamental processes that regulate metal-binding properties of DOM are largely unexplored. In particular, variation in DOM arising from different microbial metabolisms across aerobic to anaerobic conditions have yet to be considered. Here, we show that soil/sediment redox environment influences the metal-binding properties of DOM. We use tandem high-performance liquid chromatograph (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) to characterize the metal binding properties of DOM from oxic and anoxic floodplain soils. We used the contrasting chemical reactivity of Ni and Cd, with Ni preferring hard ligands and Cd soft ligands, to deduce the metal binding properties of DOM. Water extractable DOM was with Ni and Cd; the evolution of metal complexes, inclusive of Fe native to the soils, were then tracked with HPLC–ICP-MS. We find that DOM from both redox environments leads to Ni binding predominantly by distinct classes of polar organic molecules and, to a lesser extent, by an unspecific pool of less polar organic matter. In contrast, Cd is predominantly bound by less polar DOM in the anoxic extract, and more polar DOM in the oxic extract. Furthermore, we find that the speciation of Fe displaced by Cd and Ni differ between redox environments. DOM from oxic and anoxic redox environments are fundamentally different. DOM from the anoxic environment has greater proportion of soft(er) ligands, and thus a selectivity for complexing with soft(er) metals such as Cd, while DOM from oxic environments has characteristically harder ligands that have a selectivity for harder metals such as Ni.

4.2 Introduction

Dissolved organic matter (DOM) often controls aqueous concentrations of trace metal in soils and soils. Metals such as Pb, Hg, Cd, Cu, U and Fe form sparingly soluble phases under the circumneutral pH conditions typically observed in soils, and dissolved concentrations of these metals are largely regulated by forming more soluble complexes with organic ligands.¹⁻⁹ The binding characteristics of organic ligands toward metals range in specificity and strength. Ligands can be produced by distinct metabolic pathways and for specific purposes, with strong and specific affinity for individual metals, such as mugineic acids,^{10,11} nicotianamines,^{12,13} and siderophores,¹⁴⁻¹⁶. However, the majority of DOM consists of complex organic material, originating from plant and animal detritus and transformed by progressive microbial decomposition,¹⁷ with a host of functional groups, many of which bind metals. Understanding the fundamental controls on the functional composition of this complex organic material (complex DOM) is essential for predicting its metal binding properties and, moreover, the propensity for metal solubilization and mobilization.

An emerging paradigm reveals that metabolic trajectories constrained by carbon mineralization rates determine the average composition of complex DOM in soils and soils. In short, oxygen-limited conditions, the carbon mineralization rate, R_C , decreases for electron-rich substrates with less favorable terminal electron acceptors, which results in enrichment of reduced compounds. The mineralization rate of organic compounds is proportional to the net thermodynamic driving force of the oxidation reaction, parameterized as F_T in the rate law derived by Jin and Bethke.^{18,19} The net thermodynamic driving force is the difference between the Gibbs free energy of the mineralization reaction, ΔG_{rxn} , and the energy required to phosphorylate ADP (approximately 50 kJ mol⁻¹).¹⁸ The latter diminishes the overall driving force of the mineralization reaction. Thus, as ΔG_{rxn} decreases, the impact of the energy required for phosphorylation on the overall driving force becomes more severe, and when the energy required for phosphorylation exceeds ΔG_{rxn} , the reaction stops. A decrease in ΔG_{rxn} , which is determined by the energies of the half reactions for reduction of the terminal

electron acceptor and oxidation of the carbon substrate, is driven predominantly by utilization of lower-yield electron acceptors. However, the energetics of substrate oxidation and, further, the activation energy required for electron removal must also be considered. LaRowe and van Cappellen (2011) showed that, on average, the removal of an electron from an organic compound becomes more favorable with increasing nominal carbon oxidation state (NOSC) of the compound. The formalism they propose, which relates the NOSC of an organic substrate to the Gibbs free energy for oxidation of that substrate, provides a means for calculating ΔG_{rxn} for different carbon substrates and electron acceptors, thereby facilitating calculation of F_T for an overall mineralization reaction.²⁰ Divergent mineralization rates and pathways of reduced (low-NOSC) substrates in oxic and anoxic environments. Keiluweit et al. (2017) and Boye et al. (2017) demonstrate that this thermodynamic constraint on substrate mineralization rates results in preservation of more reduced DOM in anoxic soils. Under anaerobic conditions, respiration of reduced DOM proceeds slowly, or not at all, relative to that of more oxidized DOM, resulting in depletion of the oxidized DOM pool and subsequent enrichment of the reduced pool.^{21,22}

The thermodynamic constraint on DOM composition may impart an (as yet) unrecognized control on the metal-binding characteristics of complex DOM. Enrichment of reduced compounds in anoxic soils reflects an increase in the proportion of lipids, proteins, and unsaturated hydrocarbons, and a depletion of carbohydrates, condensed hydrocarbons, and tannins.²² Such an effect has direct consequences for DOM functionalization and, therefore, metal-binding characteristics. In oxic environments, rapid depolymerization yields DOM enriched in oxidized functional groups, and particularly carboxylic acids.²³ In anoxic environments, enrichment of reduced DOM implies a relative abundance of less-oxidized and reduced functional groups, including phenol, amine, and thiol groups. Indeed, the relative abundance of these groups has been documented in anoxic environments.^{24–30}

Currently lacking is a systematic understanding of the controls on the composition of metal-binding functional groups in complex DOM. The goal of this work is to address

this gap. We posit that thermodynamic preservation of reduced carbon compounds results in the enrichment of reduced functional groups in complex DOM, and therefore increased selectivity of complex DOM (relative to complex DOM from oxic environments) for metals with affinity for these groups. Our specific objectives are (1) to compare complexes of Cd and Ni – representative soft and hard Lewis acids – with DOM from oxic and anoxic soils, and (2) to assess the selectivity of DOM from these soils for Cd and Ni.

4.3 Materials and Methods

Field site description and collection of soils

We examined soils from a floodplain within the East River watershed, near Crested Butte, CO, USA. The watershed is a mountainous, high-elevation system (2700-4123 mASL; average elevation of 3350 m), with hydrologic conditions dominated by seasonal snowmelt dynamics.³¹ Surficial Quarternary glacial soils of variable thickness were deposited throughout the watershed, underlain by Cretaceous to Paleozoic sedimentary rocks (Mancos shale and the Morrison, Maroon, and Gothic formations) with intrusions of Tertiary granodiorite.^{32,33} In general, the floodplains and low-elevation slopes are overlain by Quarternary alluvium and colluvium.

Soils were collected from an area of floodplain east of Crested Butte, at an elevation of 2723 mASL (Figure S4.1). The study site contains an array of instruments for characterization of subsurface hydrologic and geochemical conditions. A detailed description of the instrumentation appears in Chapter 5. Soils were collected from a known oxidized zone and a known reduced zone. The oxidized zone spans the N-S length of floodplain circumscribed by the river. The reduced zone is an oxbow feature northwest of the oxidized zone. Exact sampling locations are shown in Figure S4.1.

A 7.5-cm bucket auger was used to collect sediment samples at 20 cm below ground surface (bgs). Approximately 20 g of sediment was removed from the tip of the auger with a stainless-steel spatula and packed into a glass serum vial, which was immediately capped with a butyl stopper and crimped. A hand pump was used to evacuate the gas

from the vial so that soils were in minimal contact with the atmosphere. The sealed vials were immediately placed on ice and stored at 4 °C until transfer to an anaerobic chamber (95% N₂, 5% H₂ atmosphere; Coy Laboratory Products, Inc.), where the vials were opened and recapped, so that soils were stored in contact with an anaerobic atmosphere instead of vacuum. The recapped vials were kept at 4 °C until laboratory analyses.

Soil carbon characterization

Carbon K-edge near edge x-ray absorption fine structure (NEXAFS) fluorescence spectra were collected for the oxbow and MZT soils at the SGM 11ID-1 beamline at the Canadian Lightsource. Samples were prepared for analysis by mixing 1 g of ultrahigh purity water with 1 g of soil, and then placing a drop of the resulting mixture on clean gold foil. The liquid phase was allowed to evaporate before placement of the foil in the sample chamber. The dominant absorption feature in citrate, at 288.7 eV, was used for energy calibration. Sample spectra were background-subtracted using the baseline fluorescence collected on gold foil (>100 scans) and normalized in the Athena software package. Gaussian peak fitting was performed in Athena for the following functionalities: quinonic (284.05 eV), aromatic (285.1 eV), phenol (286.3 eV), aliphatic (287.5 eV), carboxyl/amide (288.6 eV), O-alkyl (289.5 eV), and carbonate (290.3 eV).

Chemical composition of DOM

The chemical composition of DOM was characterized using a 12 Tesla Bruker Solarix Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) at the Environmental Molecular Sciences Laboratory (EMSL) hosted at the Pacific Northwest National Laboratory. The FT-ICR-MS was run in negative mode. Samples were purified via solid phase extraction and suspended in 50/50 v/v MeOH prior to direct injection to the instrument. Ion accumulation time was optimized for each sample. Molecular formula assignments and nominal carbon oxidation state calculations were performed with scripts provided by EMSL. Details of the analysis, formula assignment, and NOSC calculations can be found in Boye et al., (2017).²²

Preparation of field samples for LC-MS analysis

Water extractions were performed on the soils. Degassed ultrahigh purity water at a ratio of 10:1 (mass water: mass soil) was used as the extractant organic matter. The extraction procedure was performed in an anaerobic chamber (95% N₂, 5% H₂ atmosphere). Briefly, 40 g degassed ultrapure water was added to 4 g sediment in trace metal-free polypropylene centrifuge tubes (VWR). The tubes were capped and rotated on a tube rotator at 60 rpm for 12 h. The resulting slurries were centrifuged at 4000 rpm for 15 min. Solid phase extractions were performed on the centrate using Superclean ENVI-Carb Plus SPE cartridges to retain dissolved organic compounds and 100% MeOH to elute the compounds. The resulting methanolic solutions were placed on a SpeedVac (Labconco) until the liquid phase was completely evaporated. The dried powders were then re-dissolved in 30 mL ultrapure water.

The resulting solutions were each divided into 10 clean polypropylene tubes (3 mL per tube). Aliquots of Ni and Cd solutions (10 ppm ICP standards in 2% HNO₃) were added to the tubes so that each set contained solutions of DOM extract with 1, 10, and 100 µM Ni and (separately) Cd, as well as solutions of DOM extract with 1, 10, and 100 µM of Ni and Cd together. This yielded two sets of solutions (one derived from extract of the oxidized soils, and one derived from extracts of the reduced sediment), with each set containing three subsets of solution with Ni and Cd additions (only Ni, only Cd, and Ni and Cd combined) at three metal concentrations (1, 10, 100 µM), and a subset of solution without addition of Ni or Cd. After metal additions, the solutions were stored at 20 °C for 24 h. We assumed 24 h was sufficient for the solution to reach equilibrium and that additional metal complexation by DOC would be minimal after this duration.

LC-MS analysis of field samples

The HPLC–ICPM-MS analysis was performed as described in Boiteau et al. (2013).³⁴ Chromatography was performed on a bioinert Thermo Ultimate 3000 HPLC system fitted with polyetheretherketone (PEEK) tubing and a C18 column (3 µm, 2.1 mm x 100 mm, Hamilton Corporation). Ultrahigh purity water (18.2 MΩ-cm), Optima-grade

methanol (MeOH), ammonium formate, and ammonium acetate (Fisher Scientific) were used. Samples were injected with 50 μ L injection volume and separated at a flowrate of 200 μ L/min. The chromatography method included an initial 2 min hold at 95% solvent A (5 mM aqueous ammonium formate) and 5% solvent B (5 mM methanolic ammonium formate), followed by a 20 min gradient from 95% solvent A (with 5% solvent B) to 95% solvent B (with 5% solvent A), and an isocratic elution at 95% solvent B (with 5% solvent A) for 10 min. A post-column PEEK flow splitter directed 50 μ L/min into a Thermo Element XR ICP-MS instrument fitted with platinum cones and an APEX desolvating nebulizer (Elemental Scientific) chilled to 0 °C with oxygen added at a flowrate of 50 mL/min. For all samples and additions, ^{56}Fe , ^{60}Ni , and ^{114}Cd were measured in the chromatographic separations.

4.4 Results and Discussion

We exploited fundamental differences in the complexation chemistry of Ni and Cd to probe the metal binding environment of DOM derived from oxidized and reduced soils. Ni was selected for its high affinity for hard donor ligands and low affinity for soft donor ligands, while Cd was selected for its opposed high affinity for soft donor ligands and low affinity for hard donor ligands. We assumed that complexation of added metals was fast relative to equilibration time (24 h), and therefore that differences in complexation kinetics between Ni and Cd (water loss rates for Ni and Cd: $k_{-w,\text{Ni}} = 2 \times 10^4 \text{ s}^{-1}$; $k_{-w,\text{Cd}} = 3 \times 10^8 \text{ s}^{-1}$) do not drive differences in metal profiles, and rather that the difference are from (thermodynamic) binding affinities. We compared the HPLC separations and tandem ICP-MS signals of these metals across redox environments to infer broad differences in binding characteristics of dissolved organic matter from oxidized and reduced soils.

Functional composition of organic compounds

The functional composition of NOM from the anoxic and oxic soils shows differences that are consistent with enrichment of low NOSC compounds in the anoxic soil. Compared to the oxic soil, the anoxic soil contains higher proportions of phenolic,

aliphatic and carboxamide functionalities, indicating relative enrichment of lipids and proteinaceous material (Figure 4.1). In contrast, the oxic soil NOM contains higher proportions of alcohols, aromatics and quinones. These differences in functionality suggest potential differences in the metal-binding characteristics of DOM between anoxic and oxic soils.

Nominal oxidation state of carbon in MZT and oxbow soils

The NOSC of organic compounds in the anoxic (oxbow) soils was, on average, lower than the NOSC of organic compounds in the oxic (MZT) soils (Figure 4.2). This indicates that thermodynamic constraints on carbon mineralization are a predominant control on DOM composition in these soils, consistent with other studies^{21,22} that link lower NOSC in anoxic soils with increased preservation of reduced organic compounds.

Distinct Cd & Ni species across redox environments

Polar compounds dominate Cd binding in DOM from the oxic environment. Three discrete polar Cd species are revealed by the well-resolved peaks at 1.8, 2.9, and 4.1 min (peaks 1, 2, and 3, respectively) in the HPLC–ICP-MS chromatogram of the MZT DOM extract spiked with 100 μM Cd (Figure 4.3). These peaks comprise the majority of ^{114}Cd signal, indicating that they dominate Cd speciation. The significant tailing on peak 3 likely arises from a distribution of low-concentration Cd species ranging in polarity, but less polar than the species represented in peak 3, as has been observed in other HPLC–ICP-MS studies of metal-DOM species.¹⁶ The small, broad peak at 20.0 min (peak 5) indicates a pool of non-polar, low concentration Cd species.

In contrast to the oxic environment, less-polar compounds dominate Cd binding in DOM from anoxic soils. The chromatogram of the anoxic DOM extract spiked with 100 μM Cd contains prominent peaks at 5.2 and 7.5 min (peaks 1 and 2) (Figure 4.3). The sharp leading edge of peak 1 suggests a discrete Cd species – likely similar to the species occurring at the 4.1 min in the chromatogram of the oxic extract. The peak at 7.5 min reflects a less-polar Cd species not present in the oxic derived DOM. These peaks are not well resolved and likely reflect a distribution of relatively abundant less-polar Cd-

DOM species. The tailing on peak 2 suggests additional less-polar Cd-DOM species, decreasing in concentration with decreasing polarity. The small broad peak at 20.0 min reveals the presence of low-polarity Cd-DOM species. These species are lower in concentration than the polar and less-polar species but are more abundant in the DOM from anoxic than oxic soils. Two small peaks also appear at 1.8 and 2.9 min, indicating that discrete, polar Cd species are a minor component of total Cd-OM species in the DOM of the anoxic soil.

In both the DOM derived from oxic and anoxic soils, Ni is predominantly complexed by a single polar compound. The chromatogram of the oxic soil NOM spiked with 100 μM Ni contains a large, well-resolved peak at 2.8 min, indicating a concentrated, discrete Ni species (Figure S4.2). Tailing is minor, which suggests low concentrations of less-polar Ni-binding ligands. The chromatogram also contains a small peak at 1.8 min, reflecting a second polar Ni species lower in concentration than the species represented in the peak at 2.8 min. The chromatogram of the anoxic soil NOM spiked with 100 μM Ni also contains a peak at 2.8 min (Figure S4.2). Tailing on this peak is more pronounced than in the oxic soil NOM, suggesting a relative increase in the concentration of less-polar Ni species.

Competition for binding sites

Concurrent additions of Ni and Cd reveals differences in ligand selectivity. A decrease in ^{114}Cd peak intensity following concurrent addition of Ni and Cd reveals competition for the ligand(s), either between Cd and Ni, or between Cd and a metal displaced by Ni. In either case, the competing metal would exhibit hard metal binding characteristics. Thus, the extent of relative decrease in ^{114}Cd peak intensity following concurrent Ni and Cd additions indicates the relative affinity of the ligand for soft metals over hard metals. A greater decrease in peak intensity suggest lower affinity for soft metals.

In anoxic soil, less-polar DOM exhibited higher selectivity for Cd than more-polar compounds. When the DOM from anoxic soil is spiked with both 100 μM Ni and 100 μM Cd, the ^{114}Cd signal intensity of the 20 min elution peak is unchanged relative to its

intensity in the chromatogram of the extract with only 100 μM Cd (Figure 4.4 & 4.3). This reveals that these ligands have a high affinity for Cd, and that Ni is not capable of displacing Cd these ligands. The peaks at 5.2 and 7.5 min are diminished relative to their intensities in the chromatogram of the DOM with only 100 μM Cd. This reveals that either Ni itself competes with Cd for these binding sites, or Ni displaces other OM-bound metals, which then compete with Cd for L_3 . The ^{60}Ni signal intensity in the chromatogram of the concurrent Cd/Ni additions does not contain a peak at 5.2 min, suggesting that L_3 does not bind Ni appreciably (Figure S4.2). It is therefore more likely that Ni displaces another naturally occurring metal with an affinity for L_3 sufficient to displace Cd. The decrease in the intensity of the peak at 7.5 min reveals a ^{114}Cd peak at 9.3 min, which is likely overlapped by the peak at 7.5 min in the chromatogram of the extract spiked with Cd alone (Figure 4.4). The appearance of this broad peak reveals the presence of relatively abundant less-polar ligands with higher selectivity for Cd than the ligands retained at 7.5 min. Cadmium is not displaced by Ni from ligands eluted at 9.3 min, unlike the Cd-ligand complexes eluted at 7.5 min. Finally, with addition of 100 μM Ni and 100 μM Cd, the ^{114}Cd signal intensity increased between 10 and 20 min relative to the same region in the chromatogram of the 100 μM Cd addition (Figure 4.4 & 4.3). This indicates increased Cd binding by a distribution of less/non-polar DOM species, which likely results from Cd being outcompeted by Ni, or hard metals displaced by Ni, for the more polar (and harder) ligands with shorter retention times. The fact that the ^{114}Cd signal increases in this region when Ni is present reveals higher selectivity for Cd than for Ni in less-polar DOM. If these ligands had higher affinity for hard metals than for soft metals, Ni (or metals displaced by Ni) would preferentially bind at these sites, and the ^{114}Cd intensity would not increase.

The ligands with the lowest affinity for Cd are revealed in the appearance of the peak at 2.8 min following concurrent addition of Ni and Cd. This peak is barely discernible in the chromatogram of the DOM from anoxic soil spiked with Cd alone (Figure 4.4 & 4.3). Competition between Ni and Cd for ligands eluted at 5.2 and 7.5 min results in Cd apparently binding to ligand eluting at 3.0 min. These ligands likely have the lowest affinity for Cd within the NOM from the anoxic soils.

In DOM from the oxic soil, the least polar species also exhibit higher selectivity for Cd than the more polar species. When the oxic soil NOM is spiked with both 100 μM Ni and 100 μM Cd, the ^{114}Cd intensity of the peak at 4.1 min decreases while the intensities of the peaks at 1.8 and 2.9 min increase, relative to their intensities of the chromatogram of the extract with Cd alone (Figure 4.4 & 4.3). This indicates that Ni, or a metal displaced Ni, competes with Cd for the ligands retained at 4.1 min. The portion of Cd that is outcompeted by Ni for the polar ligands is distributed over the ligands eluted at 1.8 and 2.9 min. A slight increase in the tailing following the peak at 4.1 min may indicate increased Cd binding in less-polar DOM, although to a much lesser extent than in the DOM from the anoxic soil.

Analysis of the ^{60}Ni signal supports the finding that the polar DOM species from both soils exhibit higher selectivity for Ni than Cd. The chromatograms of both NOM with concurrent Ni and Cd additions contain sharp peaks at 2.8 min, with higher intensities (4x) than in the chromatograms of the extract spiked with Ni alone (Figure S4.3, S4.2). The increase in intensity likely reflects binding of Ni that, in the NOM without Cd, was complexed at low concentrations to less polar compounds. Cd outcompetes Ni for these binding sites, and more Ni is therefore complexed with the discrete species at 3.0 min. This ligand is likely the same ligand that appears at 2.8 min the ^{114}Cd signal. The fact that the concentrations of Cd and Ni complexed with this ligand both increase with concurrent metal addition may reflect a binding mechanism that is only active at relatively high dissolved metal concentrations.

It is unlikely that complexation kinetics account for the observed selectivity of these ligands. The reactivity of a solvated ion is generally proportionally to its water loss rate. The water loss rate of Ni ($k_{-w,\text{Ni}} = 3 \times 10^4 \text{ s}^{-1}$) is four orders of magnitude lower than that of Cd ($k_{-w} = 3 \times 10^8 \text{ s}^{-1}$).^{35,36} Thus, Cd would have a kinetic advantage over Ni in competing for DOM binding sites, assuming that the majority of sites are unoccupied prior to metal addition. Increased binding of Ni and subsequent competition with Cd for binding sites must therefore reflect the affinity of the binding site for Ni, as opposed to faster Ni binding kinetics. The observed selectivity of the ligands thus likely reflects

thermodynamic properties of the ligands, and specifically their affinity for soft and hard metal ions.

Links to thermodynamic controls

Thermodynamic controls on DOM composition result in enrichment of reduced (low NOSC) carbon compounds in anoxic environments. Less-reduced carbon substrates, such as sugars, tannins, and condensed hydrocarbons are consumed faster than more-reduced substrates, such as lipids, proteins, and unsaturated hydrocarbons, leading to accumulation of these compounds in anoxic environments. This in turn implies enrichment of reduced functional groups, including phenolic, phenyl, amine, and thiol groups. As discussed above, increased relative abundance of these groups under reducing conditions has been demonstrated previously.^{24–30} In contrast, depolymerization and oxidation of DOM occurs rapidly in oxic environments, leading to abundant oxidized functional groups, and specifically carboxylic acid groups.²³ Consistent with this framework, DOM from the anoxic (oxbow) soils, which exhibits an average of NOSC of $-0.55 (\pm 0.35)$, contains higher relative abundance of phenolic, carboxamide, and aliphatic carbon, as well as a higher abundance of organic thiol species, than the oxic (MZT) soils, which exhibit an average NOSC of $-0.65 (\pm 0.28)$ (Figure 4.2). In contrast, the DOM from the oxic (MZT) soils contains higher relative abundances of alcohols and aromatic carbon.

These differences in DOM composition translate to differences in polarity. In oxygenated environments, the charge density of more oxidized functional groups will impart a dipole to the compounds, such that the compound as a whole is polarized. Remaining partially depolymerized compounds will have a relatively high density of O-containing functional groups, increasing with extent of depolymerization. Abundant O-containing functional groups will impart local dipoles throughout the structure, leading to abundant polarized regions of the compound, and therefore lower retention times.

In contrast, in reducing environments, a lack of oxidative transformation will lead to lower concentrations of polar DOM and an accumulation of nonpolar, reduced

compounds such as lipids, proteins and unsaturated hydrocarbons²². The relative abundance of less-/non-polar compounds will result in increased DOM retention times.

Lower NOSC and increased abundance of less-/non-polar carbon compounds points to a higher proportion of less-polar DOM in the anoxic (oxbow) than in the oxic (MZT) soil. This in turn results in greater abundance of less-polar metal-organic species in the anoxic environment than the oxic environment. The organic Cd species observed in the oxbow and MZT extracts are consistent with this prediction: in the oxic extract, Cd is predominantly bound by discrete, polar ligands; whereas in the anoxic extract, Cd is predominantly bound by a distribution of less-polar ligands (Figure 4.3). Furthermore, a greater proportion of Fe is bound to less-polar DOM in the in the anoxic than in the oxic soil, consistent with relative enrichment of reduced organic ligands in the anoxic environment (Figure 4.2).

In DOM from both environments, less-polar ligands demonstrate higher affinity for soft metals than for hard metals, consistent with a greater proportion of reduced ligands with higher affinity for soft metals in the reduced environment. A portion of OM-bound Cd is likely associated with reduced sulfur groups. Cd demonstrates a high affinity for thiol groups in DOM, and a lesser affinity for N- and O-containing groups.^{9,37,38} However, the sulfur content in DOM is likely low relative to the carbon and oxygen content (average C:S for soils is 359:1),³⁹ and thus the contribution of thiol groups to Cd binding may be small relative to O-containing groups – especially at amendment concentrations of 100 μ M. Instead, increased Cd binding likely occurs at the phenolic and amine/amide sites revealed by C NEXAFS (Figure 4.1). These sites are likely to be associated with less polar, proteinaceous material and partially degraded biopolymers.^{28–30} The apparent selectivity of these sites for Cd may reflect higher affinity of the less-/non-polar sites than the polar sites.

Fe-Cd and Fe-Ni exchange differ between redox environments

Alongside ^{60}Ni and ^{114}Cd , ^{56}Fe was measured in NOM from the oxic and anoxic soils before and after Ni/Cd additions. No Fe was added, and therefore the detected Fe species were formed in situ prior to the analysis. In both the oxic and anoxic soil, Ni and Cd displace NOM-bound Fe. However, the speciation of displaced Fe differs both between Ni and Cd additions, and between the oxic and anoxic soil NOM. Furthermore differences in the speciation of the displaced Fe, and variability in Fe displacement with progressively increased Ni and Cd concentrations, point to variable Fe-ligand dissociation mechanisms within and across redox environments, indicative of compositional differences in the DOM metal binding functional groups.

We assume that 24 h is sufficient for association of Cd and Ni with unoccupied functional groups to reach equilibrium. However, this duration may not be sufficient for equilibrium exchange of Cd and Ni for OM-bound Fe. In this case, the ligands that more quickly exchange Fe for Cd and Ni will exhibit a greater extent of Fe displacement over 24 h. Further, increasing the concentrations of Cd and Ni (the displacing ions) will increase the rate of exchange, leading to increased Fe displacement. If displacement is inhibited despite increasing the concentrations of Cd and Ni, it is likely inhibited by the stability of the Fe-OM complex.

In the oxic soil NOM, Fe is bound by two discrete polar ligands as well as by a distribution of less polar ligands. The ^{56}Fe chromatogram before metal addition contains two well-defined peaks at 1.7 and 2.9 min, which reflect the discrete polar ligands, and a broad peak between 6.0 to 12.0 min, reflecting a distribution of less-polar ligands (Figure 4.5). Progressive additions of Cd (1, 10, 100 μM) result in decreased intensity of the Fe peaks at 1.7 and 2.9 min, as well as in broad peak between 6.0 and 12.0 min (Figure 4.5). The decrease in ^{56}Fe intensity reflects displacement of Fe from the polar ligands eluted at 1.7 and 2.9 min, as well as from the less-polar ligands eluted between 6.0 and 12.0 min. However, Fe displacement is not uniform in the less polar ligands between 7.0 and 12.0 min. Comparing the chromatograms of the unamended extract and the extract spiked with 1 and 10 μM Cd, it is clear that the difference in ^{56}Fe intensity

decreases between 7.0 and 12.0 min (Figure 4.5). This reveals an Fe-ligand complex that is resistant to displacement by Cd either due to higher affinity of the ligand for Cd, or slow kinetics of metal exchange. Given the high water exchange rate for Cd, the Fe-Cd exchange reaction would likely be limited by slow dissociation of the Fe-OM complex, as opposed to the association of Cd with OM, in the case of a disjunctive exchange mechanism, or Fe-OM, in the case of adjunctive exchange.

The ligand complexes of Fe displaced by Ni differs from that of Fe displaced by Cd, with Cd displacing more Fe from less-polar Fe-OM species. Additions of 1 and 10 μM Ni result in nearly identical decreases in intensity of all peaks (Figure 4.5). This suggests that either the ligand-Ni affinity is not sufficiently high to drive complete Fe displacement, or that the metal exchange reaction is limited by slow dissociation of the Fe-DOM species. Compared to the 1 μM Cd addition, the 1 and 10 μM Ni additions displace a higher proportion of Fe from the polar and less-polar ligands, revealed in the lower intensity of the sharp ^{56}Fe peaks at 1.7 and 2.9 min, as well as the broad peak at 7.8 min, relative to the same peaks in the chromatogram of the 1 μM Cd addition (Figure 4.5). However, the 1 and 10 μM Ni additions displace less Fe from the polar ligands than does the addition of 10 μM Cd. In contrast, more Fe from the less-polar ligands is displaced by Ni than by Cd, particularly those retained after 10 min. This reveals a small group of less-polar ligands, retained between 10 and 12 min, with either higher selectivity for Fe and Ni than for Cd, or exchange mechanisms (and associated rates) that differ between Ni and Cd. However, the fact that intensity of the broad peak between 5 and 10 min decreases more with addition of 10 μM Cd than with 10 μM Ni points to the presence of a group of less polar ligands with higher affinity for Cd than for Ni.

The anoxic soil NOM contains more abundant less-polar Fe species than the oxic soil. A technical glitch during the analysis of the unamended anoxic extract prevented collection of this chromatogram. However, it is likely the chromatogram resembles that of the 1 μM Cd addition, albeit with higher ^{56}Fe intensities (Figure 4.5). As in the oxic soil, Fe in the anoxic extract is bound by two discrete, polar ligands, reflected in the

peaks at 1.4 and 3.0 min in the chromatogram, and a distribution of less polar ligands, retained between 5 and 15 min (Figure 4.5). The broad peak between 5 and 15 min exhibits approximately twice the intensity of the similar feature in the chromatogram of the oxic soil NOM, revealing a higher abundance of less-polar Fe-OM species. The peak intensity at 1.4 min decreases more with 1 μM Ni addition than with 1 μM Cd addition, suggesting that this ligand has a higher affinity for Ni than for Cd. Fe displacement from the ligands retained at 3.0 min and between 5 and 15 min is approximately equal for 1 μM Cd and Ni, based on changes in intensity of these peaks. Increasing the metal spikes to 10 μM results in displacement of Fe both from the ligand eluted at 3.0 min, with more Fe displaced in the Cd-amended NOM, and from the distribution of less polar ligands, with more Fe displaced by Ni in the ligands eluted between 7 and 15 min. Addition of 100 μM Ni resulted in no discernible peaks in the chromatogram, indicating that Ni displaced all bound Fe. In contrast, small peaks are apparent with the addition of 100 μM Cd. This reveals that Ni-Fe exchange in the NOM of anoxic soils is more favorable than Cd-Fe exchange. This likely reflects a higher affinity of Fe-binding sites for Ni than for Cd. It might also reflect more abundant binding sites with selectivity for Cd over Ni in the anoxic soil NOM. With more available favorable binding sites, Cd may effectively compete less for the ligands binding Fe.

We assume that 24 h is sufficient for association of Cd and Ni with unoccupied functional groups to reach equilibrium. However, this duration may not be sufficient for equilibrium exchange of Cd and Ni for OM-bound Fe. In this case, the ligands that more quickly exchange Fe for Cd and Ni will exhibit a greater extent of Fe displacement over 24 h. Further, increasing the concentrations of Cd and Ni (the displacing ions) will increase the rate of exchange, leading to increased Fe displacement. If displacement is inhibited despite increasing the concentrations of Cd and Ni, it is likely inhibited by the stability of the Fe-OM complex.

The rate of a metal exchange reaction depends on the rates of metal dissociation and association, as well as the concentrations of solvated metal ions being exchanged. For disjunctive exchange, in which the displaced metal first dissociates from the ligand, and

the displacing metal then associates with free ligand, the rate of association is predominantly determined by the water loss rates of the solvated ion.^{36,40} Therefore, if displacement of OM-bound Fe is limited by the association rate of Cd or Ni, Cd would uniformly displace more Fe than would Ni. The dissociation rate is strongly proportional to the stability of the dissociating metal-ligand complex. For adjunctive exchange, which requires the formation of a ternary complex with the displaced and displacing metal both bound to the ligand, the exchange mechanism and associated rates depend on the ligand structure, as either the formation or dissociation of the ternary complex can be limiting.³⁶

The observed variability in Fe displacement between Cd and Ni points to the presence of both fast- and slow-dissociating Fe-OM species across redox environments, occurring in different proportions. This suggests that the thermodynamic controls on the metal-binding preferences of DOM also affect metal exchange reactions on the DOM functional groups.

4.5 Conclusion

Developing a systematic understanding of the fundamental controls on the metal-binding properties of DOM is essential for predicting metal solubilization and transport. Here, we find that the metal-binding properties of DOM differ between oxic and anoxic environments and, further, that the differences are largely consistent with thermodynamic controls on DOM composition. DOM from the anoxic environment binds Cd in predominantly less-/non-polar ligands, while DOM from oxic environments bind Cd in predominantly more-polar ligands. In addition, we find that thermodynamic controls on DOM composition lead to variable DOM-metal exchange kinetics across redox environments. Altogether, these findings have significant implications for the migration of metals through soils and sediments. As redox transitions drive shifts in the functionality of DOM, metal partitioning will also shift. The onset of reducing conditions and subsequent transformations of DOM will lead to increased complexation of soft metals, such as Cd, by reduced DOM, which exhibits preference for softer metals.

More Cd and other soft metals will thus be transported from reduced sediments than from oxidized sediments.

4.6 Acknowledgements

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4.9 Figures

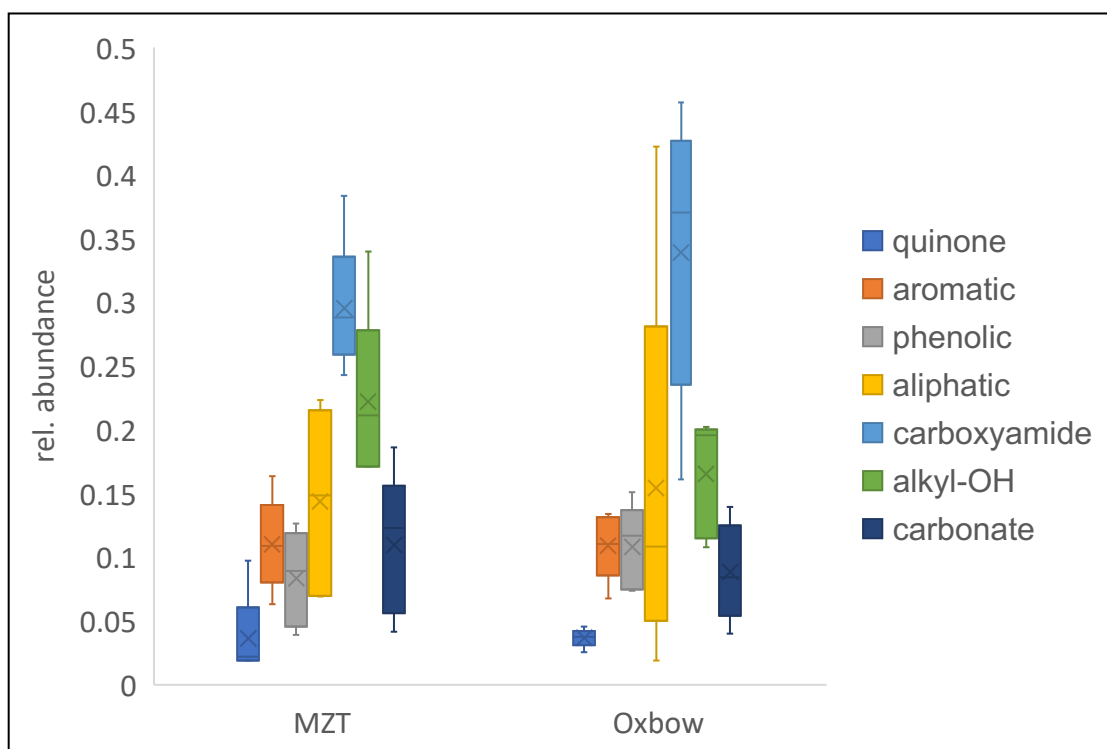


Figure 4.1. Distribution of C functional components in the oxic (MZT) and anoxic (oxbow) soils, as determined by C NEXAFS. The anoxic soils exhibit higher proportions of phenolic, aliphatic and carboxamide functional groups than the oxic environment.

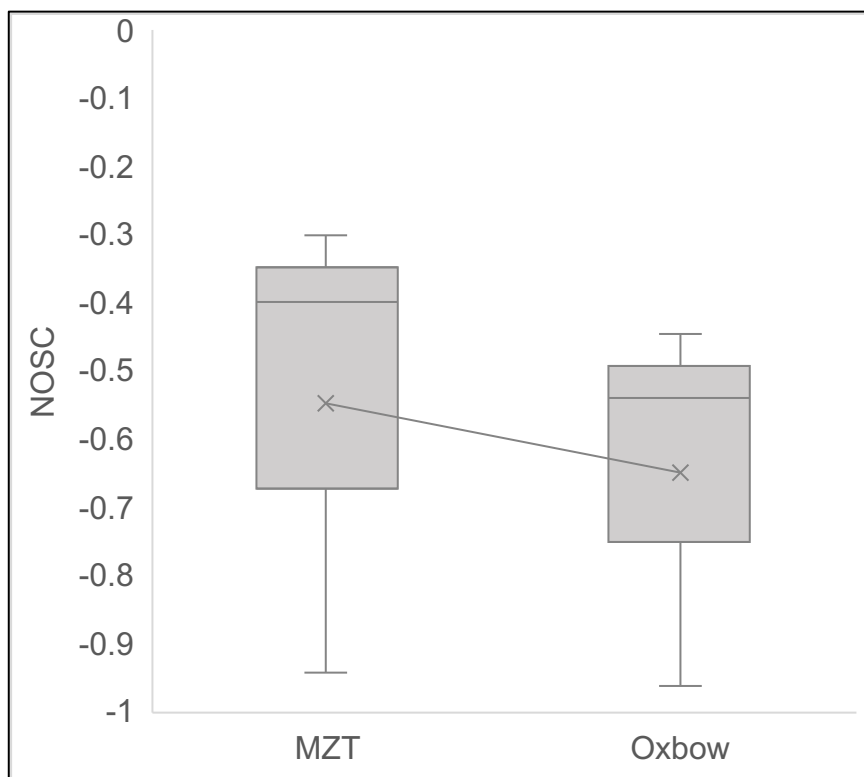


Figure 4.2. Nominal oxidation state of carbon (NOSC) values for NOM from oxic (MZT) and anoxic (oxbow) soils. The lower NOSC of NOM for anoxic versus oxic soils is inconsistent with the enrichment of reduced carbon compounds in anoxic environment via thermodynamic constraints on substrate mineralization rates.

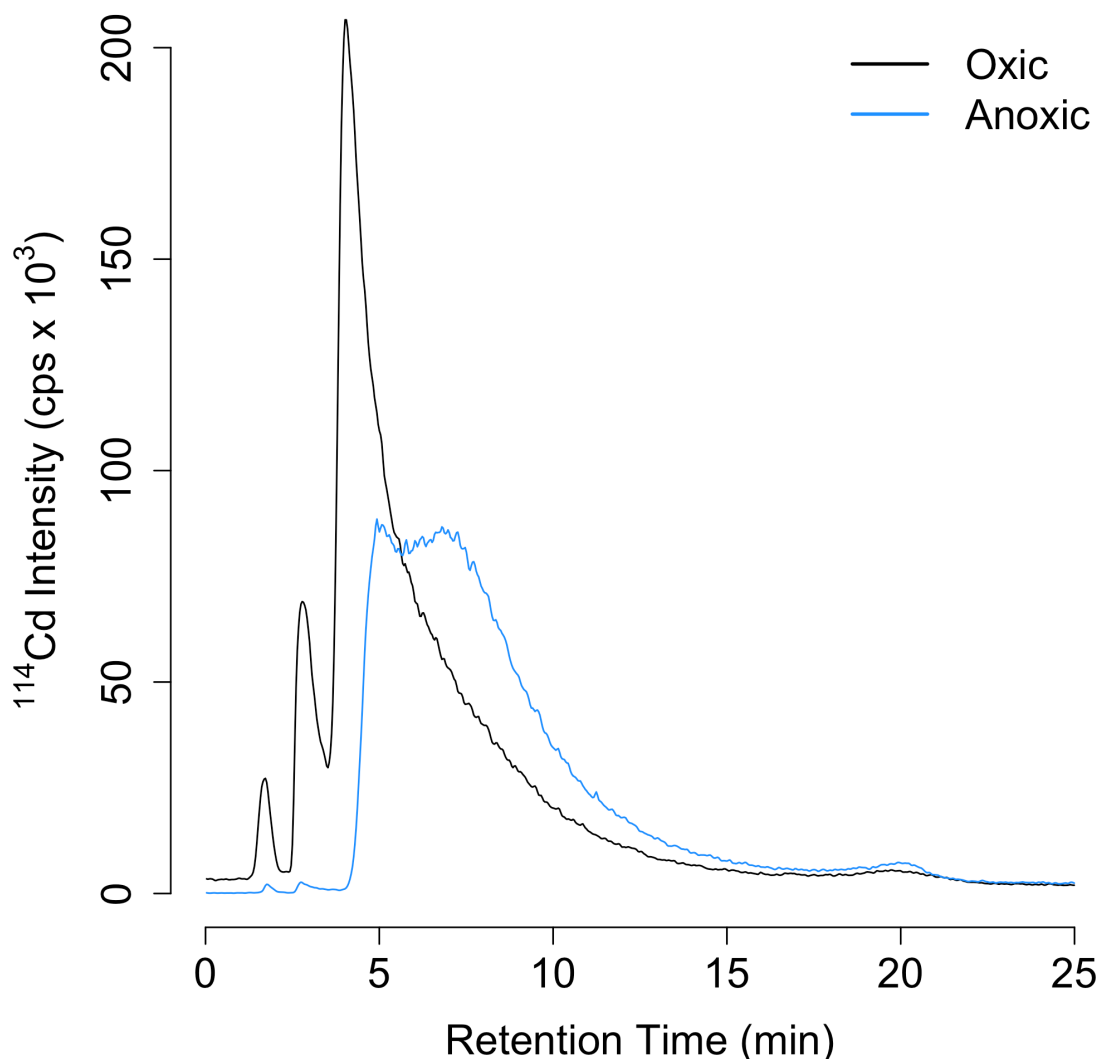


Figure 4.3. HPLC–ICP-MS profiles of complexes of Cd with DOM from oxic (MZT) and anoxic (oxbow) sediments amended with 100 μM Cd. Less-polar Cd ligands are more abundant in the anoxic environment than the oxic environment. Water extracts of soils from oxic (black) and anoxic (blue) environments (MZT and oxbow soils, respectively) were spiked with 100 μM Cd. The peaks at between 0 and 5 min indicate three distinct classes of polar Cd-binding organic ligands. In the extract from the anoxic sample, the majority of Cd is bound to ligands retained at 5 min and later. The peak at 7.9 min does not appear in the oxidized sample, and the peak at 20.0 min is more pronounced than in the oxidized sample.

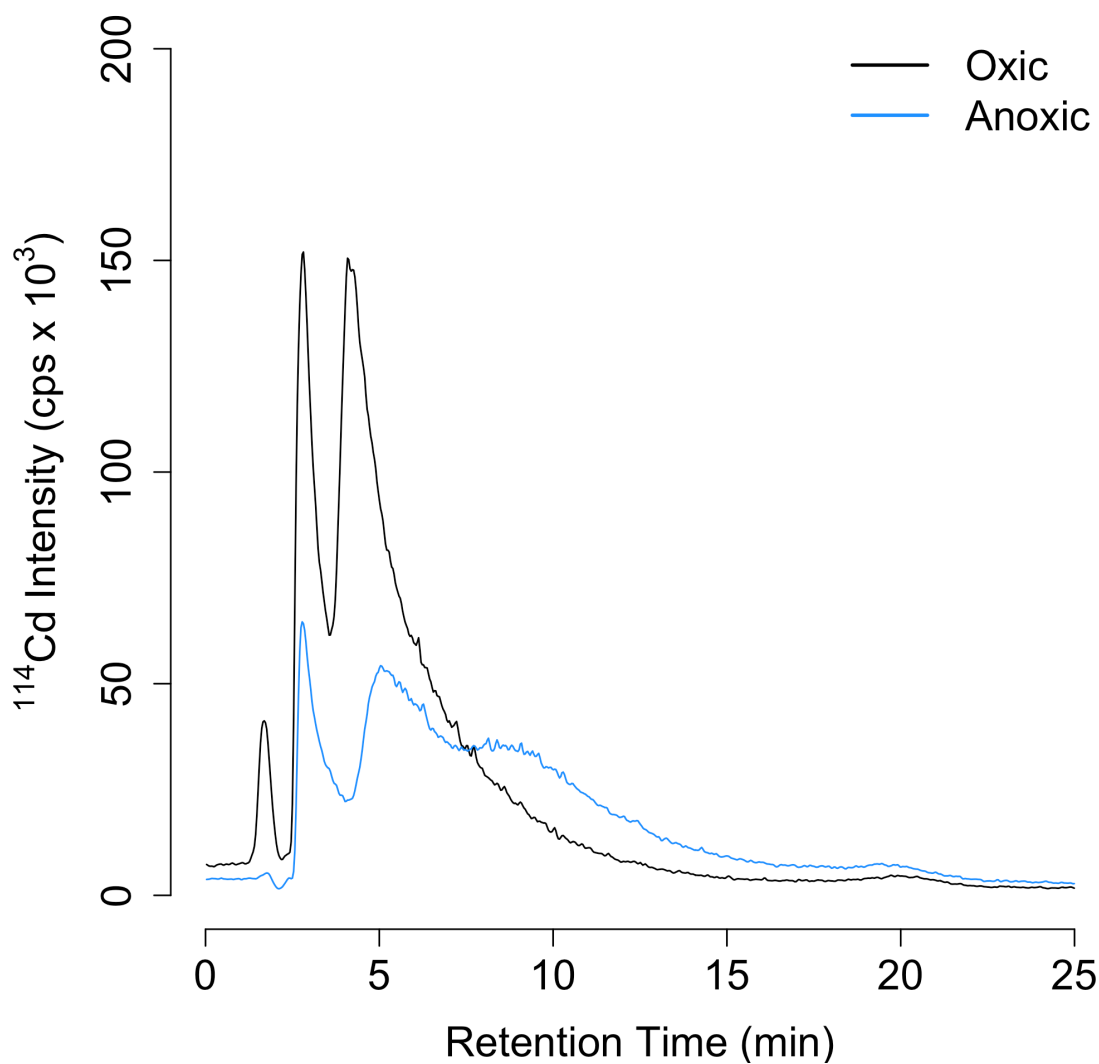


Figure 4.4. HPLC–ICP-MS profiles of complexes of Cd with DOM from oxic (MZT) and anoxic (oxbow) sediments amended with 100 μM Cd and 100 μM Ni. Concurrent additions of 100 μM Ni and Cd produce peaks at the same chromatographic time points as in separate metal additions, although relative intensities differ. Differences in intensity reflect competition between Ni and Cd for the ligand pools, or between Cd and a metal displaced by Ni.

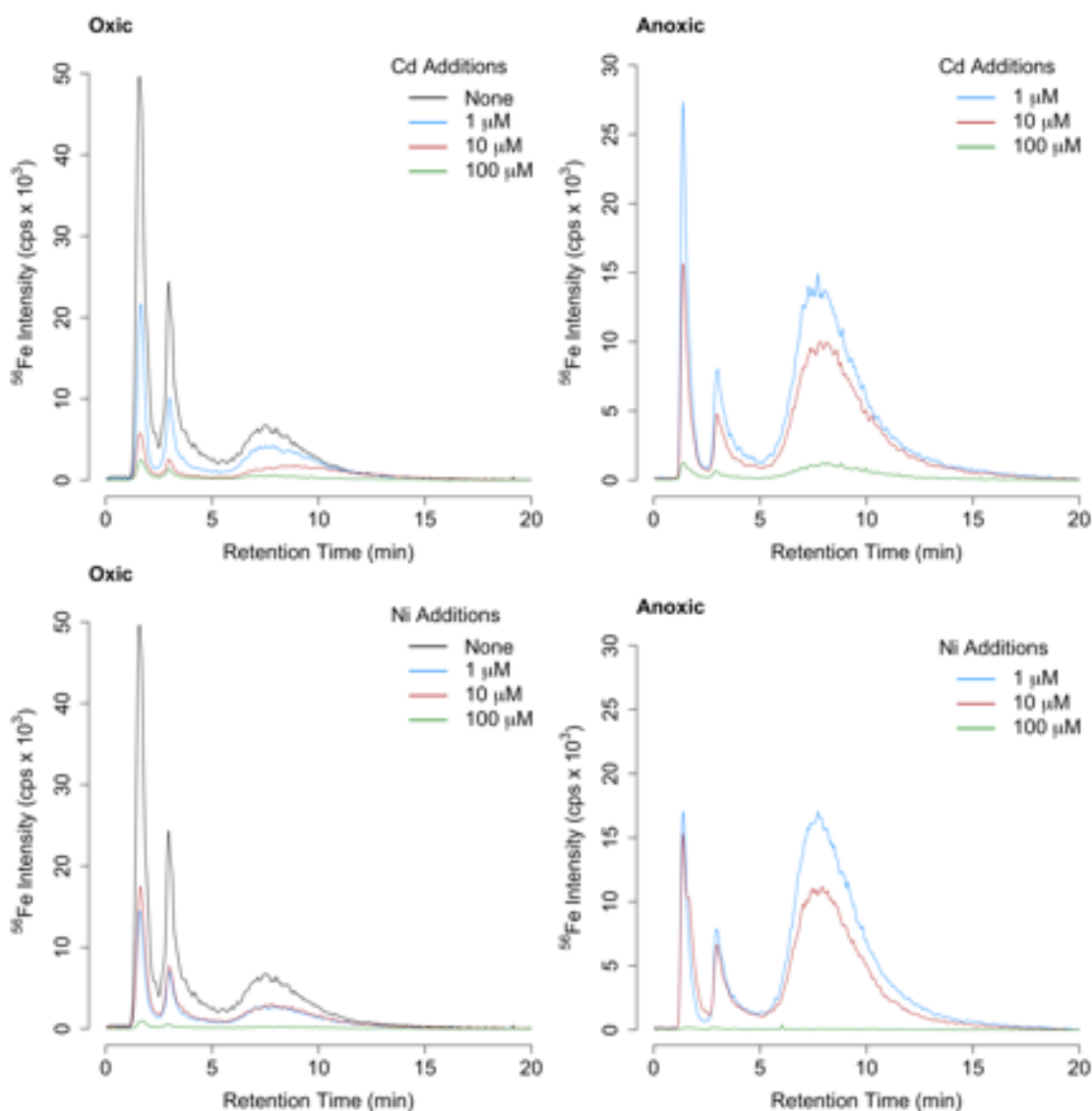


Figure 4.5. The ⁵⁶Fe traces for separations of oxic (left panels) and anoxic (right panels) soils with 1-100 μM Cd (top) and Ni (bottom). The majority of the ⁵⁶Fe signal in the oxic sample occurs in two distinct peaks between 0 and 5 min, likely corresponding to small polar organic ligands. The broad peak between 5 and 12 min likely reflects complexation of Fe by complex dissolved organic matter, as opposed to specific ligand pools. In the sample from the reduced environment, the majority of ⁵⁶Fe signal occurs in the broad peak between 5 and 15 min. The two distinct ligand pools between 0 and 5 min appear to comprise less of the overall organic-bound Fe pool. Ni and Cd displace Fe in all ligand pools. Increasing the concentration of added Ni and Cd results in increased Fe displacement.

4.11 Supplemental Materials



Figure S4.1. Map showing oxbow and MZT sampling locations at Meander Z on the East River floodplain.

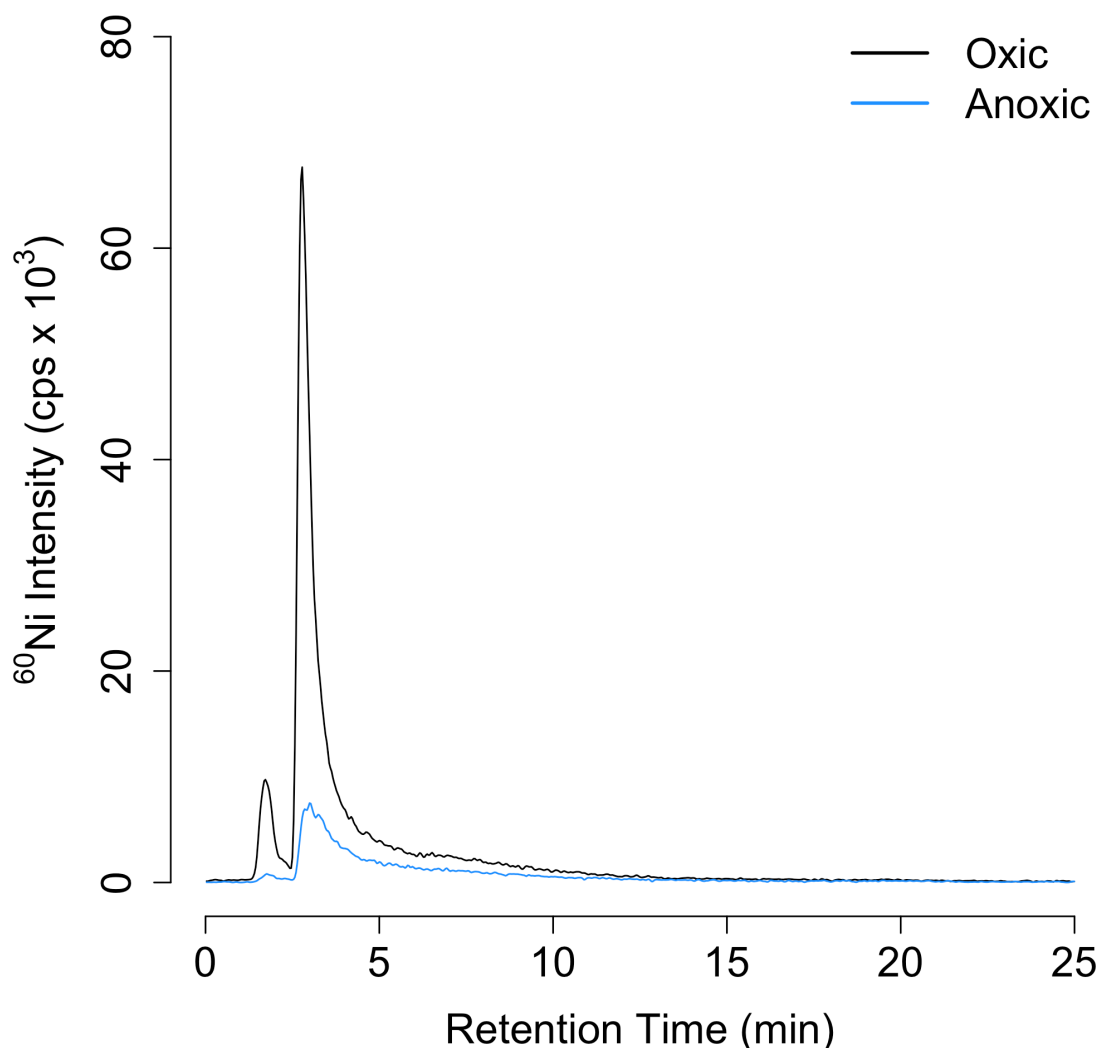


Figure S4.2. HPLC–ICP-MS profiles of complexes of Cd with DOM from oxic (MZT) and anoxic (oxbow) sediments amended with 100 μM Cd. Polar ligands predominantly bind Ni in both oxic and anoxic environments. The extracts of oxic (black) and anoxic (blue) soils were spiked with 100 μM Ni. In both samples, two peaks appear between 0 and 5 min and comprise the majority of the ^{60}Ni signal, indicating that similar Ni-binding ligand pools in both redox environments. The intensity of ^{60}Ni signal in the extract from the reduced environment is approximately 10 times less than the signal from the oxidized environment, suggesting less overall Ni binding capacity in the anoxic environment than in the oxic environment.

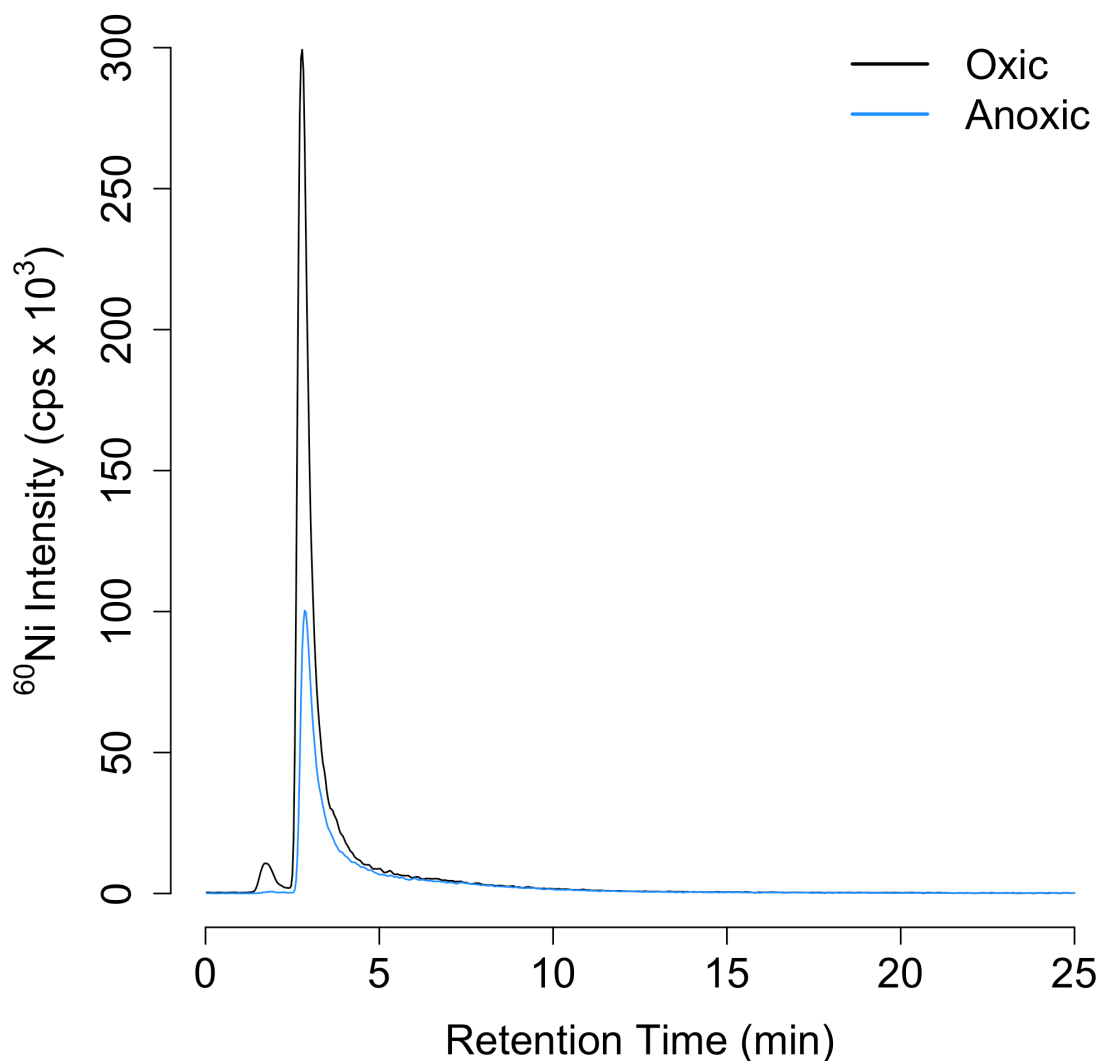


Figure S4.3. HPLC–ICP-MS profiles of complexes of Cd with DOM from oxic (MZT) and anoxic (oxbow) sediments amended with 100 μM Cd and 100 μM Ni. Concurrent additions of Ni and Cd reveal that Cd does not compete strongly for the ligands with high selectivity for Ni.

Chapter 5:

Assessing the impacts of meander-scale variability, seasonal snowmelt, and beaver dams on biogeochemical fluxes from floodplain sediments

5.1 Abstract

Floodplains buffer or enhance the exchange of contaminants and nutrients between surface and groundwater, depending on subsurface hydrologic and biogeochemical conditions. Currently, the extent to which hydrologic and biogeochemical conditions vary spatially and temporally within a single floodplain is unresolved. Further, the relative impacts of seasonal hydrologic conditions across the watershed and local hydrologic disturbances caused by beaver dams are unclear. Here, we compare the coupled hydrologic-biogeochemical conditions in two intra-meander regions within a single floodplain in a mountainous watershed, and assess the responses of sediments from these regions to (1) high water conditions imposed by a channel-spanning beaver dam and (2) seasonal high water conditions driven by snowmelt. We find that the two intra-meander regions exhibit contrasting redox conditions: one with predominantly oxidizing conditions, the other with predominantly reducing conditions. In addition, we find that beaver dams impose transient hydraulic gradients that are 360% greater than the maximum gradients imposed by seasonal snowmelt, and that beaver dams shift redox zonation much more significantly than seasonal hydrologic conditions. Our findings indicate that the biogeochemical functioning of intra-meander floodplain sediments is spatially variable at the meander scale. Moreover, we find that the transient hydrologic and biogeochemical conditions imposed by beaver dams drive large shifts in hydrologic and biogeochemical conditions in floodplains, whereas seasonal hydrologic conditions impose modest shifts.

5.2 Introduction

As interfaces between surface and groundwater, floodplains modulate the exchange of contaminants and nutrients between these freshwater stores. Floodplain sediments both attenuate and release contaminants and nutrients as they are transported from groundwater to surface water, and vice versa, thereby influencing water quality in rivers and shallow aquifers.¹⁻⁵ The extent to which floodplain sediments buffer or enhance contaminant/nutrient exchange between surface and groundwater depends on the hydrologic and coupled biogeochemical processes occurring in the sediments.^{4,6} Understanding the spatial variability of subsurface biogeochemical processes within a floodplain, and assessing the sensitivity of these processes to hydrologic conditions, is essential for predicting the impacts of changing climate on the exchange of contaminants and nutrients across floodplain sediments.

With the return of the American beaver to its historical range, beaver dams have become more common in watersheds throughout the western US. The variable hydrologic impacts of beaver dams include reduction in peak river discharge, modulation of the hydrograph at a catchment outlet, and increased water residence time along a river reach.^{1,2} These hydrologic impacts drive coupled biogeochemical responses in adjacent hyporheic sediments. Both increased oxygenation of sediments as well as increased transport of reduced metals along anoxic beaver-induced flow paths have been shown to occur following beaver dam construction.^{3,4} In addition, beaver dams have been shown to drive increases in shallow groundwater temperature.⁵ As beaver populations continue to grow, the hydrologic and biogeochemical conditions imposed by beaver dams will become more common across watersheds in the western US.

In high elevation watersheds, seasonal snowmelt dynamics drive transient hydrologic and associated biogeochemical redox conditions in floodplains. Two recent studies show that transient hydrologic conditions driven by seasonal snowmelt altered biogeochemical conditions in floodplain sediments, leading to increased exports of Fe and N.^{6,7} The importance of seasonal hydrologic processes coupled to biogeochemical

redox processes in floodplain sediments has been shown to impact a range of contaminant and nutrient cycles in moderate to high elevation watersheds.^{8–11}

In this study, we compare the hydrologic and biogeochemical conditions imposed by seasonal snowmelt dynamics and beaver dams in two intra-meander hyporheic regions within the East River floodplain, near Crested Butte, CO. Our objectives are (1) to assess the spatial variability in hydrologic and biogeochemical conditions across intra-meander floodplain regions; (2) to determine the impacts of seasonal hydrologic conditions on redox zonation and oxygen and iron fluxes from sediments in these regions; and (3) to compare the effects of beaver dams and seasonal snowmelt on hydrologic and biogeochemical conditions in these regions.

5.3 Materials and Methods

Description of field sites

We characterized hydrologic and biogeochemical conditions at two locations (meanders C and Z) in the East River floodplain near Crested Butte, CO. The East River watershed is a mountainous, high-elevation system (2700–4000 mASL), with hydrologic conditions dominated by seasonal snowmelt in late spring to early summer, and monsoonal rainfall in mid- to late-summer. Surficial Quaternary glacial sediments of variable thickness were deposited throughout the watershed. At our study sites, an unconfined alluvial aquifer is comprised of these sediments.¹² The glacial deposits are underlain by a suite of Cretaceous and Tertiary sedimentary rocks, including Mancos shale, a heterogeneous marine black shale with elevated metal and metalloid content.¹³

Field observations

At meander Z, we installed an array of pressure transducers (HOBO Water Level Data Logger U20-001-01, Onset Computer Corporation, Bourne, MA, USA) to characterize hydrologic conditions across intra-meander sediments and in the adjacent river reach. A transducer was also installed approximately 1 m above ground surface to record atmospheric pressure, which is needed to convert raw pressure measurements to water surface elevations. Transducers in the intra-meander sediments were suspended on

galvanized cable in 5-cm open wells approximately 1.5 m below ground surface. River transducers were suspended on galvanized cable in perforated 5-cm polyvinyl chloride (PVC) pipes, open at both ends and extending from 15 cm above riverbed to the elevation of the nearest bank. The PVC was secured to fence posts driven 50 cm into the riverbed. The transducer measuring atmospheric pressure was housed in a PVC sheath attached to a fence post. Measurements were automatically logged at 1 hr intervals from December 2017 through September 2019. A description of transducer calibration and conversion of raw pressure measurements to water surface elevations appears in the Supplemental Information. The transducers measured temperature in addition to pressure.

We also installed two transects of piezometers at both meanders (MZT-1, MZT-2; MCP1, MCT2) for collection of water samples across the intra-meander sediments. The transects were aligned with the general direction of hyporheic flow. Each transect consists of four to five piezometer locations, with each location containing piezometers at multiple depths (Figure S5.1). All locations in both transects contain piezometers at 100 cm and 25 cm bgs. The first, third and fifth locations on both transects also contain piezometers 200 cm and 50 cm bgs. Finally, the first, third and fifth locations along MZT-1 contain piezometers at 400 cm bgs. Stainless steel drive-point piezometers with a 6-inch screen length (Model 615, Solinst Canada, Ltd.) were used for the 200 and 400 cm bgs depths. The drive-points were fastened to galvanized pipe, with polyethylene tubing extending from the piezometer screen to the top of the galvanized casing. PVC piezometer tips with a 6-inch screen length (Model 601, Solinst Canada, Ltd.) attached to PVC pipe were used for depths of 100 cm bgs and shallower. The PVC piezometers were installed in 7.5-cm boreholes with washed silica sand and a bentonite seal.

At both study sites, water samples were collected approximately weekly over two consecutive summers (2018 and 2019, May through October). Approximately triple the volume of standing water was purged from the piezometers before sample collection. Samples were immediately filtered through in-line 0.45 μm polyvinylidene difluoride (PVDF) syringe filters and dispensed to multiple beakers and vials for specific

measurements and analyses. For quantification of dissolved carbon (organic, inorganic and total) samples were collected into 40 mL amber glass vials with rubber septa and no headspace. For quantification of dissolved ions, samples were collected into 20 mL scintillation vials. A second sample for quantification of ammonium was collected into a scintillation vial and acidified with 10 drops of 2 N HCl within two days of collection. The samples were stored at 4 C until laboratory analyses were performed.

The chemical composition of the water was characterized through field and laboratory measurements. Electrical conductivity and pH were measured in the field with a VWR sympHony MP8 Multisensor probe. Dissolved oxygen was measured in-field using a portable spectrophotometer (model # V-3000) and CHEMetrics vacu-vial kits (#K-7513: 1-15 ppm, ± 0.6 to 1.1 ppm; #K-7553: 0-1 ppm, ± 0.025 to 0.080 ppm) according to their protocols. Dissolved cations (with the exception of ammonium) were measured on a Thermo Scientific XSERIES 2 quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Fisher Scientific, Waltham, MA, USA). Dissolved anions were measured on a Dionex DX 500 Ion Chromatograph system. Dissolved ammonium was measured using a Lachat QuickChem 8500 Flow Analysis System (Hach, Loveland, CO, USA). Total inorganic carbon (TIC) and total organic carbon (TOC) was measured on a Shimadzu TOC-V total carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan).

Sediment samples were collected at the beginning and end of each field season. Hydraulic conductivity measurements were performed with a home-made permeameter. A description of permeameter construction and testing appears in the Supplemental Information. Soil carbon content was measured on a Shimadzu TOC-V total carbon analyzer equipped with a solids analyzer. Iron content was determined by a citrate-bicarbonate-dithionate extraction according to Loeppert and Inskeep (1996).¹⁴ Mineral phases were measured with a Rigaku MiniFlex 600 Benchtop X-ray Diffraction system (Rigaku, Tokyo, Japan).

Reactive Transport Modeling

The model was constructed as a two-dimensional y - z model with representations of physical and chemical properties alongside hydrological and biogeochemical processes. The modeling domain (Figure S5.3) consists of three stratigraphic layers: a topsoil unit with relatively low permeability $8.80 \times 10^{-14} \text{ m}^2$; a middle gravel layer with relatively high permeability $2.26 \times 10^{-11} \text{ m}^2$; and an underlying shale layer with low permeability $8.30 \times 10^{-15} \text{ m}^2$. The dimensions of the 2D domain were 54 m in length (y) and 6 m in depth (z). Domain surface topography was defined using a digital elevation model from a LiDAR dataset with 0.5 m resolution.¹⁵ The domain was discretized as a structured grid with uniform spacings of 0.5 m and 0.1 m in the horizontal (y) and vertical (z) dimensions and contains 7560 cells.

Model simulations were run in PFLOTRAN, an open-source, three-dimensional, groundwater flow and reactive transport code.^{16,17} Two groups of simulations were performed: (1) simulations of an idealized transect under beaver dam hydrologic conditions, and (2) simulations of the idealized transect under seasonal hydrologic conditions. Both sets included three simulations in the permeability of the topsoil layer was varied to assess the sensitivity of redox zonation to contrasting sediment permeabilities. A maximum timestep of 1 h was used in all simulations. The simulations were run in Richards mode for variably saturated flow. The relationship between capillary pressure and saturation was described with the van Genuchten model, and the Mualem formulation was used to relate relative permeability and the van Genuchten saturation function. Hydraulic conductivity values for the topsoil and gravel layers were measured with a permeameter (described above) and then converted to permeability values for model parameterization.

We use the reaction network described in Dwivedi et al., (2018) in our simulations.⁶ The reaction network contains 20 primary species (H^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Fe^{3+} , Al^{3+} , H_2O , acetate, $\text{O}_{2(\text{aq})}$, $\text{N}_{2(\text{aq})}$, $\text{NH}_{3(\text{aq})}$, NO_3^- , NO_2^- , Cl^- , HCO_3^- , S^{2-} , and SO_4^{2-}) and 72 secondary species. Aqueous speciation in the model is assumed to be at equilibrium. The minerals represented in the base model are ferrihydrite, pyrite, and calcite. Soil

organic matter is also treated as a mineral. Mineral precipitation and dissolution reactions are represented as being either at equilibrium or subjected to kinetic constraints, depending on the mineral. In the cases where precipitation and dissolution are represented as kinetically constrained, the reactions are modeled using transition state theory (TST) type rate laws. The reaction network also includes representations of microbial consumption of dissolved organic carbon (DOC) by aerobic respiration, denitrification, iron reduction, and sulfate reduction. Microbial DOC consumption pathways are represented by single Michaelis-Menten kinetic reactions using the following general rate law:

$$R_S = \mu_{max} \frac{C_S}{K_S + C_S} \frac{C_{TEA}}{K_{TEA} + C_{TEA}} \frac{K_I}{K_I + C_I} \quad (1)$$

where μ_{max} is the maximum rate of reaction, C_S is the substrate concentration, C_{TEA} is the electron acceptor (TEA) concentration, and C_I is the inhibitor concentration; K_S , K_{TEA} , and K_I are the half-saturation constants for the substrate, TEA, and inhibition species. Chemolithoautotrophic oxidation of Fe^{2+} and S^{2-} are also represented in the model. All reactions in the model are shown in the Supporting Information alongside associated rate laws and kinetic parameters.

The river water surface elevations measured immediately upstream and downstream of the MZT1 transect were used to impose hydrostatic boundary conditions at the upstream and downstream boundaries of the model domain. A no-flow boundary was imposed along the bottom of the domain, which represents the shale-saprolite interface. Imposing a no-flow boundary is justified given the low permeability of the underlying shale. To simulate recharge from rainfall, a constant specified flux was applied to the top boundary of the model domain. Given that Dwivedi et al. (2018) found that recharge had negligible impact on the biogeochemical functioning of the MCP transect, recharge in our model was not simulated as a time-varying process. The initial flow condition was a uniform hydrostatic pressure distribution set at 4.0 m above the model floor (approximately 2 m below ground surface).

Both river water and groundwater water were represented at the upstream and downstream boundary conditions. The average river water composition measured at the upstream transducer location was applied the upstream and downstream boundaries from 0 to 2 m below ground surface. Although the composition of the river water exhibits temporal variability, we found that the observed variability in river water composition had minor impact on model output. The groundwater composition used by Dwivedi et al. (2018) was applied from 2 m below ground surface to the model floor. The recharge composition used by Dwivedi et al. (2018) was applied to the top boundary.⁶ The average composition at MZT1-3 was used for the initial geochemical condition across the entire model domain. As in Dwivedi et al. (2018), the initial geochemical composition had little impact on model output after more than one pore volume was flushed through the model. The mineral and SOM composition of the model layers was initialized based on measured values. The model was run for 30 y with the hydraulic measured on the first day of ice-off in 2018 until near steady-state conditions developed. Simulations were begun following the 30-year spin up.

5.4 Results

Hydrologic conditions

Upstream and downstream river stage determine water table elevations and hydraulic gradients across the meander. Seasonal river stage is governed by snowmelt dynamics, which dominate hydrologic conditions throughout the catchment. The field observations for this study were collected in years with contrasting seasonal hydrologic conditions. In 2018, early warming and low snowpack led to early peaking of seasonal high water, followed by drought conditions across the watershed. At meander Z, peak river stage arrived in mid-May, reaching 2723.45 mASL upstream of the meander and 2723.00 mASL downstream (Figure 5.1; Figure S5.1). Thereafter river stage generally declined until early July, when water levels stabilized at 2722.90 mASL (upstream), and river stage reached baseflow conditions. In contrast to 2018, late warming and heavy snowpack resulted in late arrival of peak river stage in 2019, followed by high water sustained into August. Peak river stage arrived in mid-June at meander Z, with the

upstream water surface elevation reaching 2723.90 mASL, topping the bank (2723.70 mASL), and resulting in several weeks of overland flow (Figure 5.1). General decline of water levels followed, continuing steadily into September, and never dropping to the levels observed in 2018.

Construction of a beaver dam at the meander point (Figure S5.1) caused a rapid rise in upstream water levels in late July 2018. Upstream water levels steadily increased from extreme low water conditions in mid-July (2722.90 mASL) to near bank-full conditions in early October (Figure 5.1). Downstream water levels did not rise with upstream water levels due to the location of the dam. The dam broke on October 4, 2018, causing upstream water levels to fall 0.70 m in a matter of hours (Figure 5.1). Breach of the dam also caused a spike in downstream water levels (0.30 m).

Seasonal hydrologic conditions are characterized by coupled upstream and downstream changes in water level. The coupled nature of these changes results in relatively small changes in hydraulic gradient across the meander. The hydraulic gradient, i , defined as

$$i = \frac{\Delta h}{l}$$

where Δh is the difference between upstream and downstream water surface elevations and l is the distance across the meander, increases slightly with the arrival of peak seasonal discharge and decreases slightly thereafter. In 2018, the hydraulic gradient increases from 0.065 to 0.075 with the arrival of peak discharge, and then falls 0.05 in mid-July (Figure 5.1). Hydraulic gradients are lower in 2019, despite higher peak river stage, with values ranging between 0.055 and 0.065 between April and August, dropping steadily to 0.038 until measurements were ceased in early October (Figure 5.1). In both years, changes in hydraulic gradient never exceed 20% over any time period for which measurements were recorded.

In contrast, the beaver dam uncoupled upstream and downstream water levels, driving large changes in the hydraulic gradient within days. Three days after dam construction, the hydraulic gradient increased 90%, from 0.055 in late July to 0.010 in early August.

Two weeks after dam construction, the hydraulic gradient increased 255% from its value in late July, to 0.014 in mid-August (Figure 5.1). By early October, the hydraulic gradient increased 360% from its value in late July. Then, in just a few hours after the dam breach, the hydraulic gradient dropped 340%.

The changes in hydraulic gradient imposed by the beaver dam (and its breach) dwarf the changes imposed by seasonal peak discharge, both in rate and magnitude and magnitude of change. The beaver dam drives faster and larger shifts in gradient (both increasing and decreasing) than seasonal high water. The resulting transient hydrologic conditions are distinct from those arising from snowmelt dynamics.

Under seasonal hydrologic conditions and those imposed by the beaver dam, changes in river stage directly translate to changes in hydrologic conditions in the meander sediments. Water levels generally decrease across the meander, rising and falling with river stage during seasonal transitions (Figure S5.2). After construction of the beaver dam, water levels fall steeply across the meander. Subsurface flow generally occurs north-to-south in both cases.

Chemical composition of porewater

Only porewater from the deep (1-2 m bgs) piezometers are considered in this study. Porewater from the meander C sediments reveals active iron reduction across the meander. Along both MC transects, dissolved Fe concentrations generally increased with the direction of flow, from approximately 50 to 200 μM , until the approximate midpoint of the flow path (MCP-3 and MCT2-3; Figure S5.2), at which point Fe concentrations declined below 50 μM (Figures 5.2, S5.5, S5.8-10). The lowest Fe concentrations in both transects were consistently observed at the most downstream location along the flow path (MCP5 and MCT2-4). Dissolved organic carbon (DOC) concentrations mirrored dissolved Fe concentrations. Along both MC transects, DOC concentrations generally increased until the midpoint of the flow path, from approximately 0.25 to 0.60 mM, after which they declined to 0.25 mM (Figures 5.3, S5.5, S5.8, S5.9).

In contrast to meander C, iron reduction does not occur across meander Z sediments, except in local zones of reduction. Dissolved iron concentrations along MZT1 and MZT2 transects are generally below 5 μM throughout the 2018 and 2019 seasons, with the exception of the MZT2-1 location, which experiences transient increases in dissolved iron concentrations up to 65 μM (Figures 5.2 and S5.4, S5.6, S5.7). The increase in Fe concentrations at MZT2-1 occurs alongside declining water levels in both years (Figures 5.2 and S5.4, S5.6, S5.7). DOC is also low across both MZT transects, with concentrations generally below 0.10 and 0.15 mM in 2018 and 2019, respectively (Figures 5.2 and S5.4, S5.6, S5.7).

Temporal variability in Fe reduction differs within and between meanders and across seasons. Along the MCP transect, dissolved iron concentrations generally increase throughout both the 2018 and 2019 seasons at MCP-2 and MCP-3, but are relatively steady at MCP-1, MCP-4 and MCP-5 (Figures 5.3, S5.8). In contrast, along the MCT2 transect, dissolved Fe concentrations generally increase throughout 2018 at MCT2-2 and MCT2-3 and decrease throughout 2019 (Figures S5.5, S5.8, S5.9). In meander Z sediments, iron concentrations do not vary between years along the MZT1 transect, with concentrations consistently below 5 μM (Figures S5.4, S5.6). Along the MZT2 transect, dissolved iron concentrations rise at MZT2-1 in 2018, from below 5 μM in early June to 63 μM in late July, followed by a decline in concentrations to early June levels after construction of the beaver dam (Figure 5.3). In 2019, dissolved iron concentrations begin to rise in late July, reaching a maximum of 45 μM by late September (Figure 5.7).

In both meanders, porewater pH varied between 6.5 and 8.1, suggestive of calcite buffering. The ratios of dissolved bicarbonate to dissolved calcium point to calcite dissolution by respiration-derived carbonic acid as opposed to sulfuric acid produced by oxidation of sulfidic phases (Figures 5.2 & 5.3; Figures S5.4-8)).¹⁸ At both meanders in both years, pH is generally highest early in the season at the most upstream locations, with values near 8.0, and tends to decline as the season progresses. At meander Z, pH generally decreases along the flow path in both seasons, whereas along the MZT2 transect, pH generally increases along the flow path (Figures 5.3, S5.4, S5.6, S5.7). At

meander C, pH does not vary along the flow paths of either transect, nor does it vary significantly between transects across years (Figures 5.2, S5.8, S5.9).

Dissolved oxygen concentrations at the meanders further indicate anoxic conditions at meander C and (sub)oxic conditions at meander Z. Dissolved oxygen measurements along both transects in meander C were consistently below the limits of detection for the CHEMetric field kit throughout the 2017 field season, except at the most upstream and downstream locations, and at MCT2-4 (Figure 5.4). The elevated DO levels at MCT2-4 likely reflect oxygen contamination that occurred during the sampling procedure. Consistent bubbling during sampling in the polyethylene tubing at this location suggested a hole in the tubing that introduced oxygen during pumping of the piezometer. Given that DO measurements in the middle transect locations were consistently below detection limits for MCP and MCT2 transects, DO measurements were performed infrequently at meander C in 2018 and not at all in 2019 (Figure 5.4). In contrast, DO concentrations at meander Z varied from 5.5 mg/L and 4.2 mg/L at MZT1-1 and MZT2-1 (the upstream sampling locations), respectively, to below the limits of detection at downstream locations (Figure 5.5). The majority of measurements fell between 0 and 1 mg/L (Figure 5.5). The fact that we obtained DO measurements below detection limits at meander C gives us confidence that the concentrations measured at meander Z do not reflect contamination during the sampling procedure. Further, the presence of DO at low concentrations is consistent with the general absence of dissolved iron along the transects.

Meander Z DO concentrations also vary with hydrologic conditions, particularly at the upstream sampling locations. In 2018, the high water conditions resulting from the beaver dam construction cause increased DO concentrations at MZT1-1 and MZT2-1. At MZT2-1, the rise in DO is paralleled by a drop in dissolved Fe concentrations and occurs concurrently with the rise in water levels (Figures 5.5, 5.3, 5.1). At the MZT1-1, the rise in DO is occurs approximately one month after the rise in water levels (Figures 5.5, 5.1). In 2019, high water conditions in early summer result in DO concentrations between 1 and 6 mg/L at MZT1-1, and between 1 and 4.5 mg/L for most of the MZT2

transect (Figure 5.5). We note that most of the MZT2 transect was submerged when the high DO concentrations were observed (Figures 5.5, 5.1). Pumping may have drawn down overlying water through the borehole annulus, leading to contamination of the water at depth. As water levels dropped along the transects, DO concentrations also dropped below 1 mg/L, though rarely below the limits of detection for the method (Figure 5.5).

Physical and chemical composition of meander solids

Meanders C and Z both exhibit three distinct layers of unconsolidated material atop Mancos shale bedrock. The layers are a topsoil layer with relatively high organic material; a layer of well-graded sand and gravel, relatively low in organic material; and a weather-shale saprolite layer beneath the well-graded sand and gravel layer. However, the thicknesses of these layers vary between the sites. At meander C, the topsoil layer extends to approximately 1 m bgs across both the MCT and MCP transects. The sand/gravel layer extends from 1 m bgs to 1.5-2 m bgs, below which weathered shale is found. In contrast, at meander Z, the topsoil layer extends to 30-50 cm bgs. The sand/gravel layer is much thicker at Z than at C, extending beyond 2 m bgs. The weathered shale saprolite layer begins at depths between 2 and 4 m at meander C and Z, respectively.

The unconsolidated layers exhibit contrasting intrinsic permeability. This study relies on permeability values for meander C sediments estimated in Dwivedi et al. (2018) via textural analysis and a pedotransfer function.⁶ The intrinsic permeability of the saprolite, gravel, and soil layers were estimated at $8.30 \times 10^{-15} \text{ m}^2$, $2.26 \times 10^{-11} \text{ m}^2$, and $8.80 \times 10^{-14} \text{ m}^2$ respectively. The soil and saprolite layers at meander Z were assumed to have similar permeability values, given the relative proximity of the meanders. The hydraulic conductivity of the soil layer at meander Z was measured with a permeameter. Measurements on five cores collected within 3 m of each other yielded an average hydraulic conductivity of $1.17 \times 10^{-6} \text{ m/s}$, with a standard deviation of 4.98×10^{-7} . The average value was converted to an intrinsic permeability of $1.07 \times 10^{-12} \text{ m}^2$ assuming a water density of 997 kg/m^3 and a dynamic viscosity of water of $8.90 \times 10^{-3} \text{ Pa}\cdot\text{s}$. This

value is more than order of magnitude higher than the permeability value for the meander C soil layer.

Mineralogy at both meanders was dominated by quartz, phyllosilicate clays, and plagioclase. The distribution of these phases did not vary with depth (up to 100 cm bgs) (Table S5.1). Quartz was most abundant, comprising 45-63% of the mineral phase (Table S5.1). Plagioclase and clays comprised 8-30% and 12-19% of mineral phases, respectively. Within the clay component, muscovite and illite were most prevalent (5-16%), followed by chlorite and kaolinite (0-7%), and minor amounts of vermiculite (0-1%). Minor amounts of titanite (0-7%), calcite (0-6%), and dolomite (0-3%) were also detected at most locations and depths. A small amount of pyrite (1.5%) was detected at 90 cm bgs at MZT1-5.

Soil iron and organic carbon content were similar across both meanders. Soil organic carbon generally decreased with depth, from 1.8 to 7.4% in between 0 and 25 cm bgs, to less than 3.0% below 25 cm (Figure S5.10). Iron content was consistent along the depth profile, averaging 0.21 (± 0.06) mmol Fe per g sediment (Figure S5.11).

Reactive transport modeling

Based on the physical and chemical characteristics of meanders C and Z, we constructed a 2D reactive transport domain representing an idealized transect within the East River floodplain (Figure S5.3). The length and surface topography of the MZT2 transect were used to define the model domain. The observed hydrologic conditions at the meander Z river stations were used as upstream and downstream flow boundaries, and flow was validated with the observed water levels along the transect. Importantly, the model is not intended to capture the exact biogeochemical conditions at meander Z, but instead to represent an idealized system for assessment of the impacts of hydrologic conditions and sediment permeability on redox environment and hyporheic biogeochemical fluxes. To this end, parameterization for individual simulations was constrained by the physical and geochemical observations at the meanders. However, the reaction network was not

strictly calibrated to these observations and instead relied on the validity of the reaction network developed by Dwivedi et al. (2018) for meander C.⁶

As described above, two sets of simulations were run for the 2018 and 2019 hydrologic conditions (beaver dam and high seasonal water, respectively). For each hydrologic condition, three simulations were run in which the permeability of the topsoil layer was varied (low, mid, and high permeability conditions). The permeability conditions are representative of the conditions in meander C (low-k) and Z (mid-k), as well as hypothetical gravel-dominated meander (high-k).

A comparison of high water resulting from the beaver dam to seasonal high water conditions reveals greater DO penetration into mid-k topsoil sediments under the beaver dam conditions. Hydraulic gradient and thus flow velocities are significantly higher under the beaver dam conditions than under the seasonal high water conditions (Figures 5.1, 5.6). As a result, DO intrudes farther into the meander, limiting the onset of iron reduction, and restricting reducing conditions to the most downstream portion of the flow path (Figure 5.6). Further, the extent of DO intrusion into the conductivity gravel layer is also greater in the beaver dam condition than in the seasonal high water condition.

The permeability conditions in the topsoil layer modulate the effect of increased DO transport and subsequent redox zonation. In low-k meander C type sediments, reducing conditions dominate across the meander, even when the hydraulic gradient across the meander is at its maximum (Figure 5.7). In high-k gravel meander sediments, oxic to suboxic conditions dominate across the meander (Figure 5.7). In the mid-k soil layer, representative of meander Z, reduction is limited to the most downstream portion of the flow path, and the upstream portion of the flow is suboxic (Figure 5.6).

5.5 Discussion

A comparison of meanders C and Z reveals divergent biogeochemical functioning of the hyporheic zone within a single floodplain. Meander C exhibits reduction, evinced in sustained iron reducing conditions and anoxic conditions, while meander Z shows

minimal evidence of iron reduction, except in locally contained zones, and sustained oxic and suboxic conditions. Differing distributions of low-permeability soils and sediments across the meanders likely explains the differences in biogeochemical reactivity. At meander C, hyporheic flow primarily intersects low permeability soil and weathered shale, whereas at meander Z, flow occurs predominantly through more conductive topsoil and sand and gravel. Thus, flow velocity across meander Z sediments is higher than across meander C sediments, resulting in increased transport of DO from the river to the subsurface.

The beaver dam leads to increased fluxes of DO from the river to the meander Z-type sediments relative to the seasonal highwater conditions. The beaver dam imposes a much larger hydraulic gradient across the meander than the seasonal high water conditions (Figure 5.1). Our modeling results indicate that the increase in gradient leads to greater intrusion of DO under the conditions imposed by the beaver dam than under the seasonal high water conditions in an idealized meander Z system (Figure 5.6). The zone of reduction shrinks in response to increased DO intrusion. Thus, we interpret the measured rise in DO concentrations in 2018 in the MZT1-1 and MZT2-1 locations is driven by increased flux of DO into the sediments at these locations. The delay in DO rise at MZT1-1 may reflect that as the rate of DO supply increases in this location (driven by the increasing hydraulic gradient), the rate of microbial respiration (and therefore DO demand) increases proportionally, with the result the DO concentrations do not initially rise (Figure 5.5). As the hydraulic gradient, and therefore the rate of DO supply, continue to increase in September, the low concentration of DOC may limit microbial respiration rates, with the result that DO supply outpaces demand, leading to the observed rise in DO concentrations (Figure 5.5). At MZT2-1, the increased supply of DO leads to a reversal of the iron reducing conditions that developed at the location prior to the construction of the dam: the rise in DO concentrations at this location is mirrored by a decline in Fe concentrations (Figures 5.3, 5.5.).

In contrast, the idealized meander C system shows minimal disturbance as a result of the beaver dam. Despite high gradients, the topsoil layer is reduced across the meander

under the beaver dam conditions. DO is consumed quickly in the low-k sediments, leading to iron reduction within a meter of the river boundary (Figure 5.7). The lack of intrusion is in part the consequence of the large permeability contrast between the gravel and topsoil layers ($2.26 \times 10^{-11} \text{ m}^2$ in the gravel; $8.80 \times 10^{-14} \text{ m}^2$ in the (C-type) topsoil). The permeability contrast results in refraction of flowlines at gravel/soil interface. Flow from the soil layer is driven into the gravel layer as a result of the permeability contrast. Although the lateral (i.e., along the y-axis) gradients are equivalent in the soil and gravel layers, the impact of the increase in gradient is to increase flow from the soil layer into the gravel layer (i.e., downward). Lateral flow occurs overwhelmingly in the gravel layer. Thus, lateral intrusion of DO into the soil layer is minimal, and reducing conditions are preserved.

Nonetheless, because the refraction of flowlines is independent from the gradient across the meander, lateral fluxes of DO into the topsoil layer must increase for the low-permeability condition. The fact that increased lateral DO fluxes does not lead to oxidizing conditions can be explained through the Damkohler concept, represented by the dimensionless Damkohler number (Da). In brief, the Damkohler number relates the timescale of chemical reaction to the timescale of transport – in this case, advection.¹⁹ When $Da \gg 1$, chemical reaction processes control concentrations of a reactive species. When $Da \ll 1$, transport processes (advection) control concentrations of a reactive species. Thus, the lack of oxidation in the low-k topsoil layer indicates that the timescales of aerobic and anaerobic carbon mineralization exceed the timescales of transport by advection, despite a large hydraulic gradient across the meander. The low permeability of the topsoil layer ensures that advection is slow relative to DO consumption, even under the extreme hydrologic conditions imposed by the beaver dam.

The Damkohler concept also explains the lack of reduction in the soil layer in the high-permeability case. In this case, the gradients imposed by the beaver dam result in transport processes exerting control on redox zonation across the meander. The rate of DO transport exceeds that of DO consumption, and oxic to suboxic conditions occur across the idealized meander (Figure 5.7). The constraint on the rate of DO consumption

is likely the limited supply of DOC. Increasing the carbon content of the soils would lead to higher DO demand, increased biomass growth, and accelerated DO consumption.

In simulations of the idealized meander Z transect, in which the permeability of the topsoil layer is $1.055 \times 10^{-12} \text{ m}^2$, the beaver dam significantly changes the redox zonation across the meander relative to the seasonal high water condition. Put in terms of the Damkohler concept, the timescales of transport and mineralization reactions (aerobic and aerobic) appear to commensurate, and the concentrations of DO and Fe are affected by both transport (advection) and chemical reaction (mineralization reactions). The construction of the beaver dam imposes hydrologic conditions that increase the impact of advection on redox zonation, relative to the seasonal high water conditions (Figure 5.6). Nonetheless, the transport timescale is not increased to the point that reaction processes are irrelevant. DO consumption and subsequent Fe(III) reduction occur rapidly enough to yield reducing conditions in the downstream portion of the meander.

The exports from meander sediments to the East River are thus likely to depend on both the transport processes, which are affected by sediment permeability, and the kinetics of the biogeochemical reactions that transform potentially mobile species. The latter will vary depending on biogeochemical process. Beaver dams, and the large hydraulic gradients they impose, will have the most significant impact on exports from floodplain sediments in systems where the rates of biogeochemical transformations (and specifically transformations from immobile to mobile phases) are commensurate with transport.

5.6 Conclusion

The hydrologic conditions imposed by beaver dams are distinct from those imposed by seasonal high water conditions, leading to large increases in hydraulic gradient across meander sediments. These increases in gradient shift redox conditions where rates of transport are commensurate with rates of biogeochemical transformation. By modulating the rate of transport, sediment permeability is a fundamental constraint in determining where transport and reaction rates are likely to be commensurate.

Importantly, because the kinetics of biogeochemical reactions span orders of magnitude, transport rates may be commensurate with the rate of one biogeochemical process under certain permeability conditions, but incommensurate with the rates of other biogeochemical processes. Finally, in sediments where the timescale of biogeochemical reactions exceeds the rate of transport, such as in meander C, exports from the meander sediments to the river may be low relative to efflux from less reactive, but more permeable, sediments.

5.7 Acknowledgements

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5.9 Figures

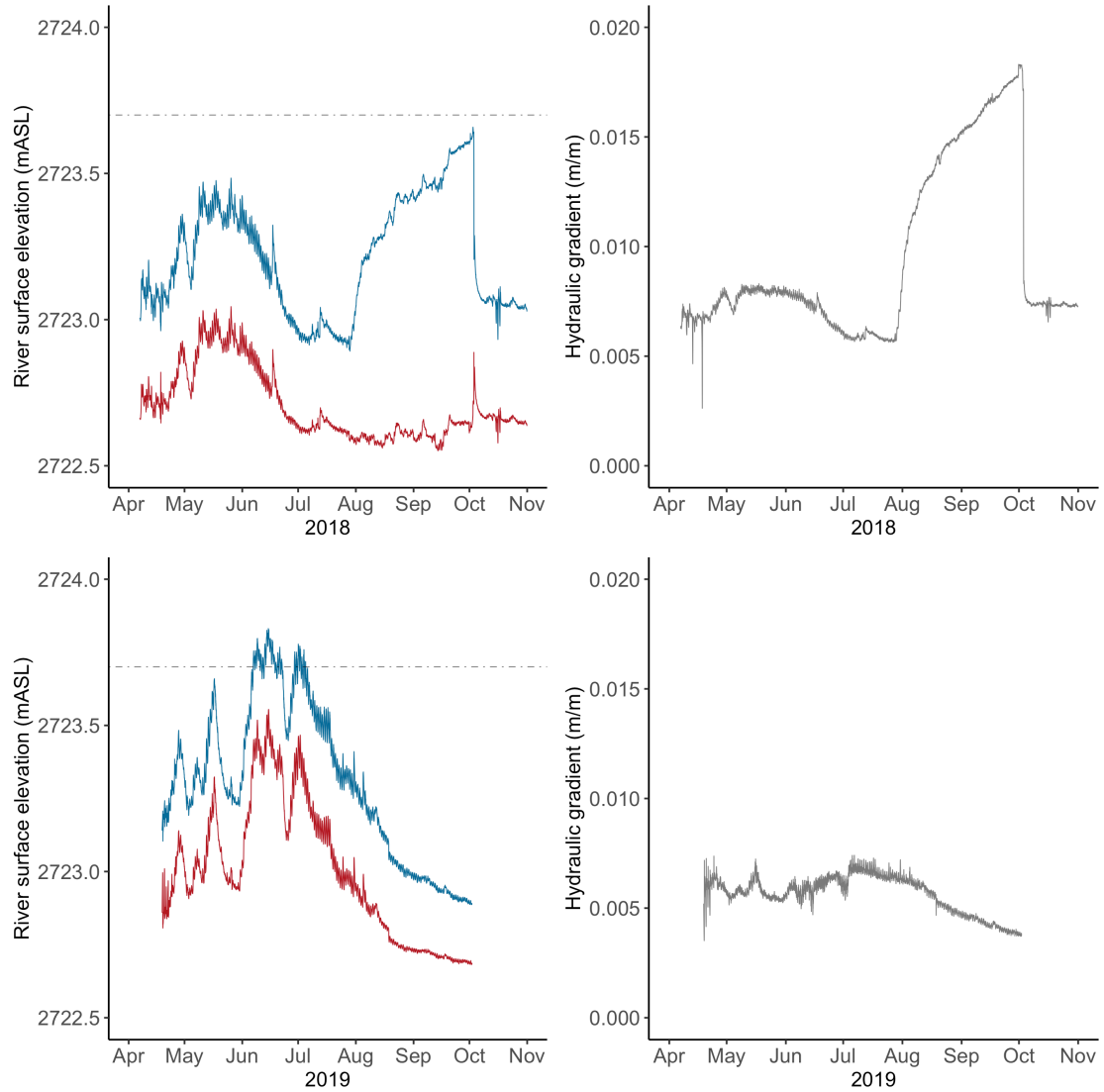


Figure 5.1. *Left.* River surface elevations at upstream (blue) and downstream (red) stations for 2018 (top) and 2019 (bottom). Dashed line at 2723.7 mASL indicates elevation of ground surface near the upstream station. The rise in upstream water level in late July 2018 was driven by construction of a beaver dam. *Right.* Hydraulic gradient across the meander in 2018 (top) and 2019 (bottom).

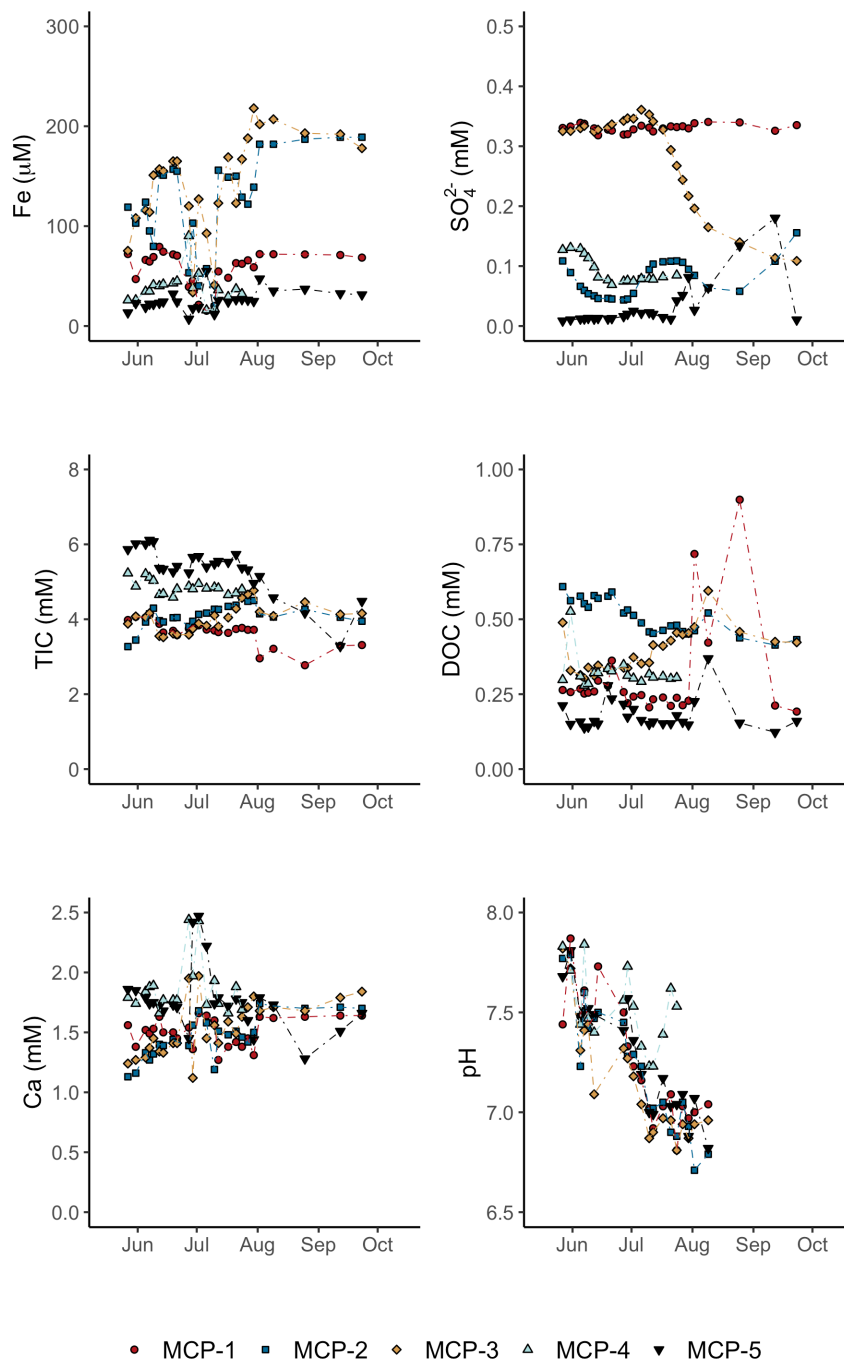


Figure 5.2. Geochemical composition of porewater from the MCP transect in 2018.

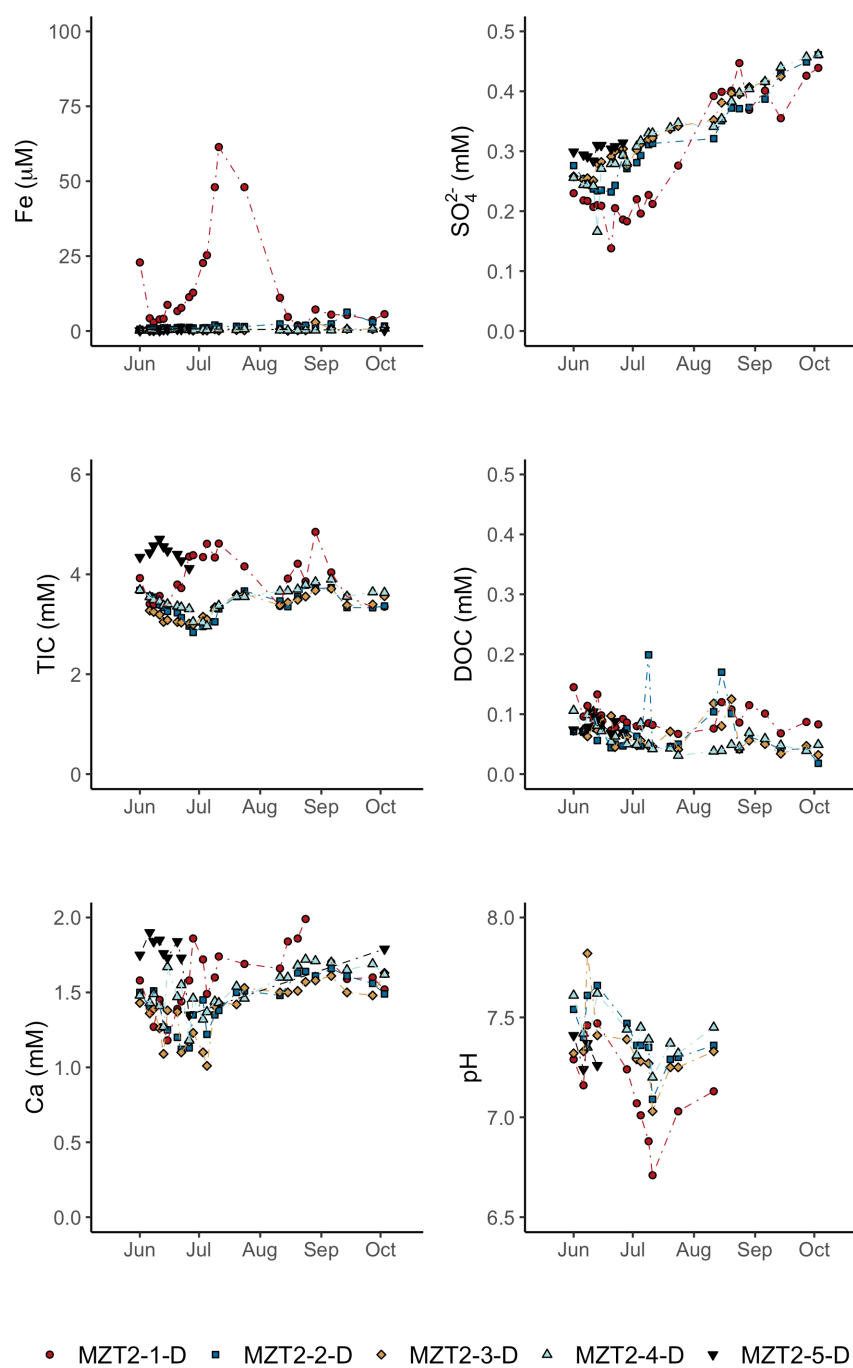


Figure 5.3. Geochemical composition of the MZT2-D transect in 2018.

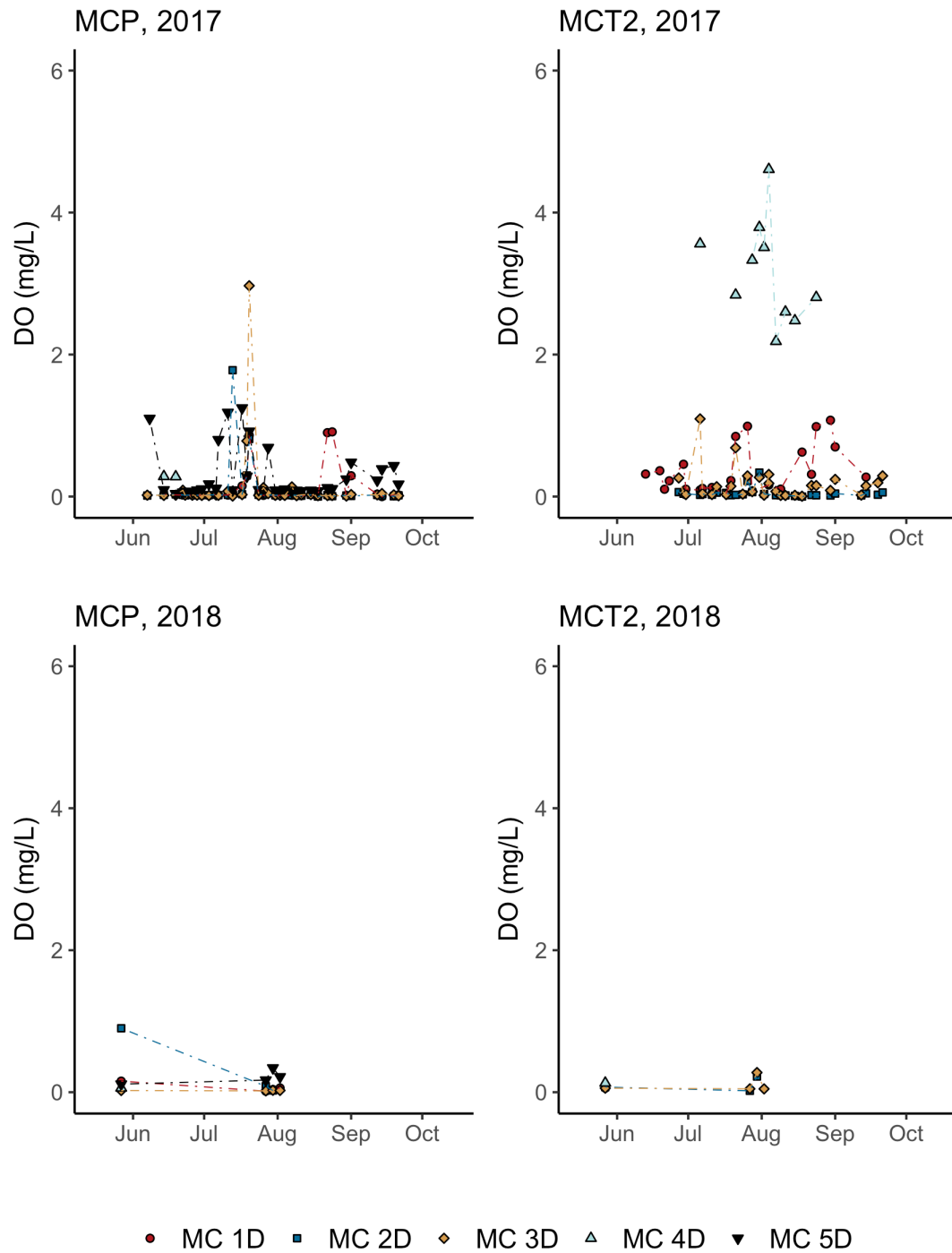


Figure 5.4. Dissolved oxygen concentrations along the MCT2 and MCP transects in 2017 and 2018. Measurements were performed in the field. Because most measurements were at or below the limits of detection in 2017, measurements were made infrequently in 2018 (and not at all in 2019).

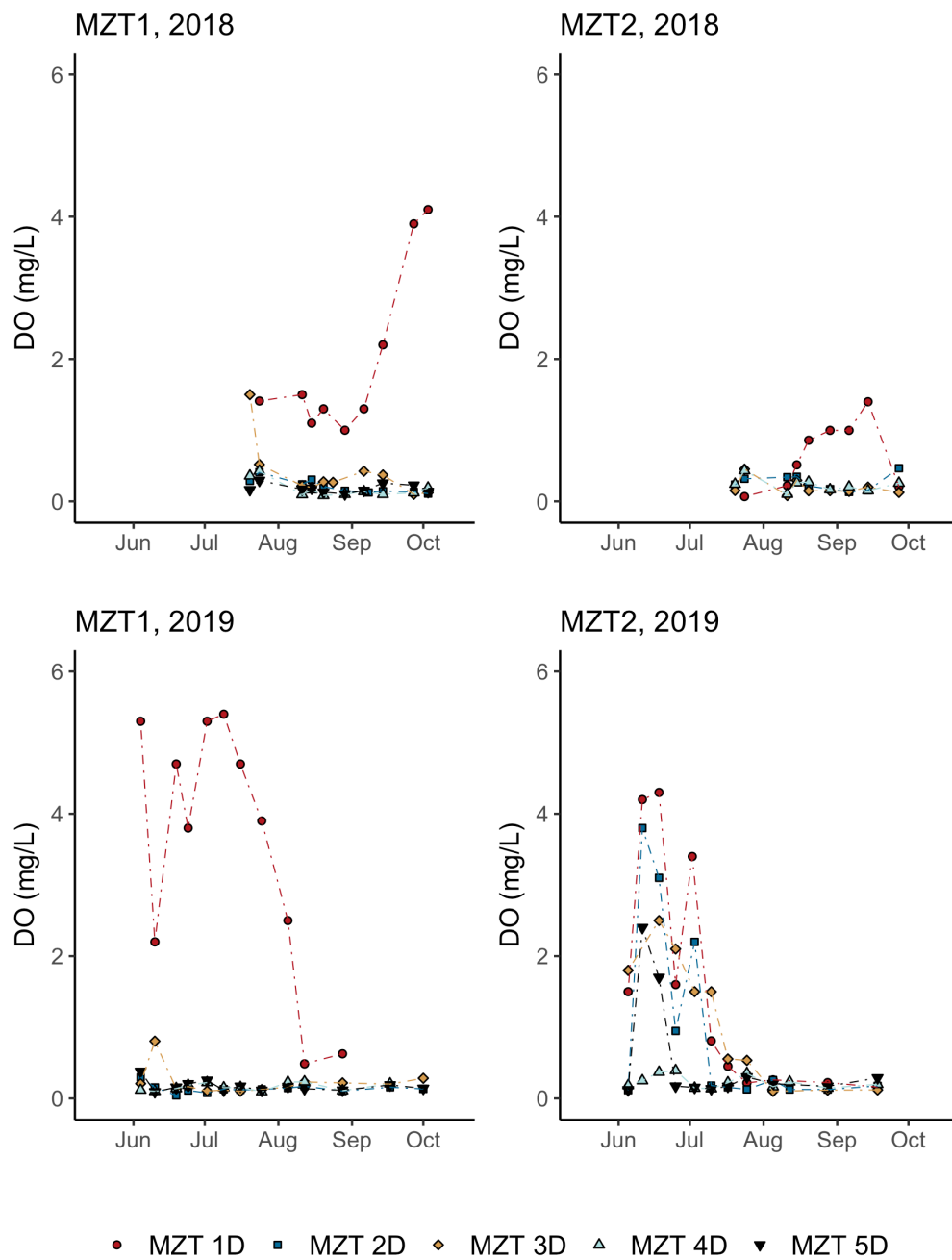


Figure 5.5. Dissolved oxygen measurements along the MZT transects in 2018 and 2019.

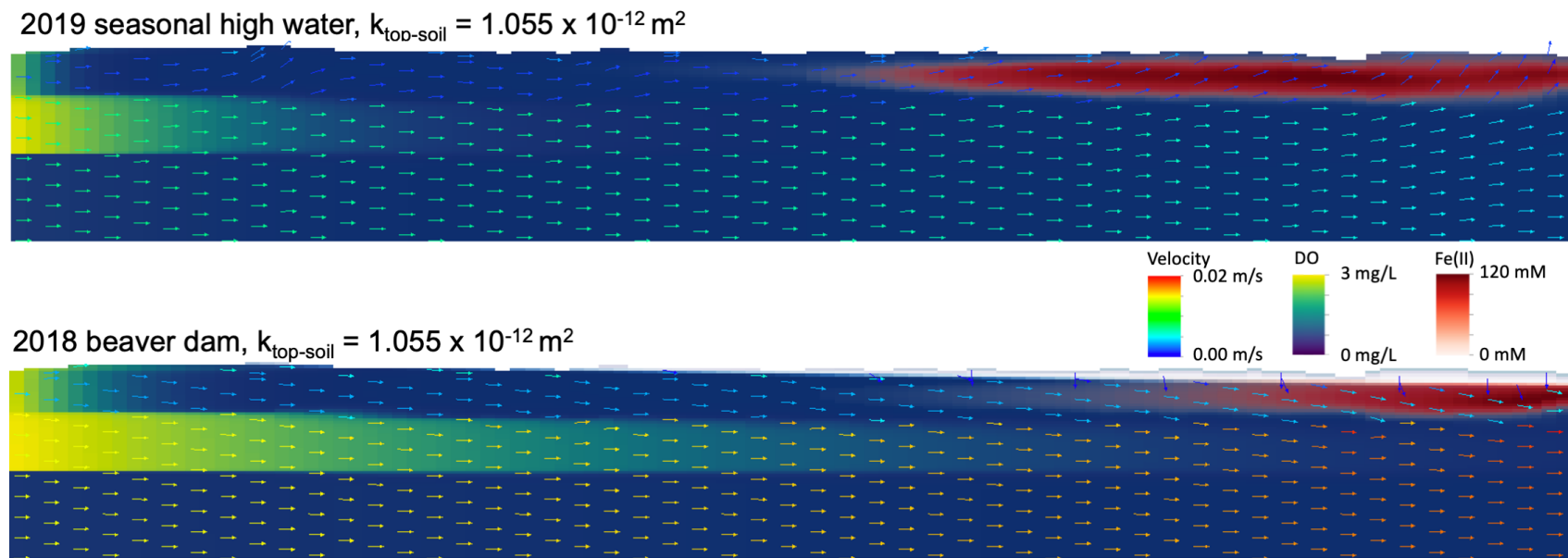
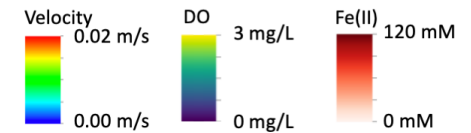
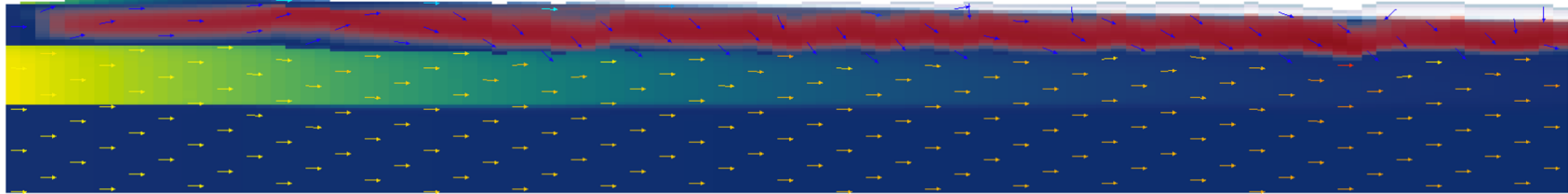


Figure 5.6. Comparison of redox zonation subjected to the largest gradients from the beaver dam (2018) and seasonal high water (2019) in an idealized meander transect based on the MZT2 transect. A schematic of the model domain is shown in Figure S5.3. The permeability of the topsoil layer for these simulations was set to value measured for MZT topsoil, $1.055 \times 10^{-12} \text{ m}^2$. The beaver dam leads to higher flow velocities across the meander, and therefore increased transport of DO, relative to the seasonal high water conditions. As a result, DO penetration into the topsoil is greater, limiting the extent of Fe reduction.

2018 beaver dam, $k_{\text{top-soil}} = 8.80 \times 10^{-14} \text{ m}^2$



2018 beaver dam, $k_{\text{top-soil}} = 2.26 \times 10^{-11} \text{ m}^2$

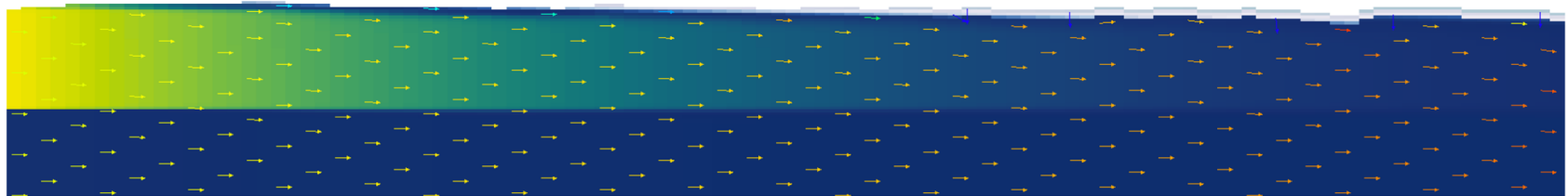


Figure 5.7. Comparison of redox zonation under low (top) and high (bottom) topsoil permeability conditions in an idealized meander transect at maximum hydraulic gradient resulting from the beaver dam. The low-k topsoil condition represents the C-type meander, while the high-k condition represents a meander consisting entirely of gravel (no topsoil layer). The mid-k topsoil condition that represents meander Z is shown in Figure 5.4

5.10 Supplemental Materials

Supplementary Figures and Tables

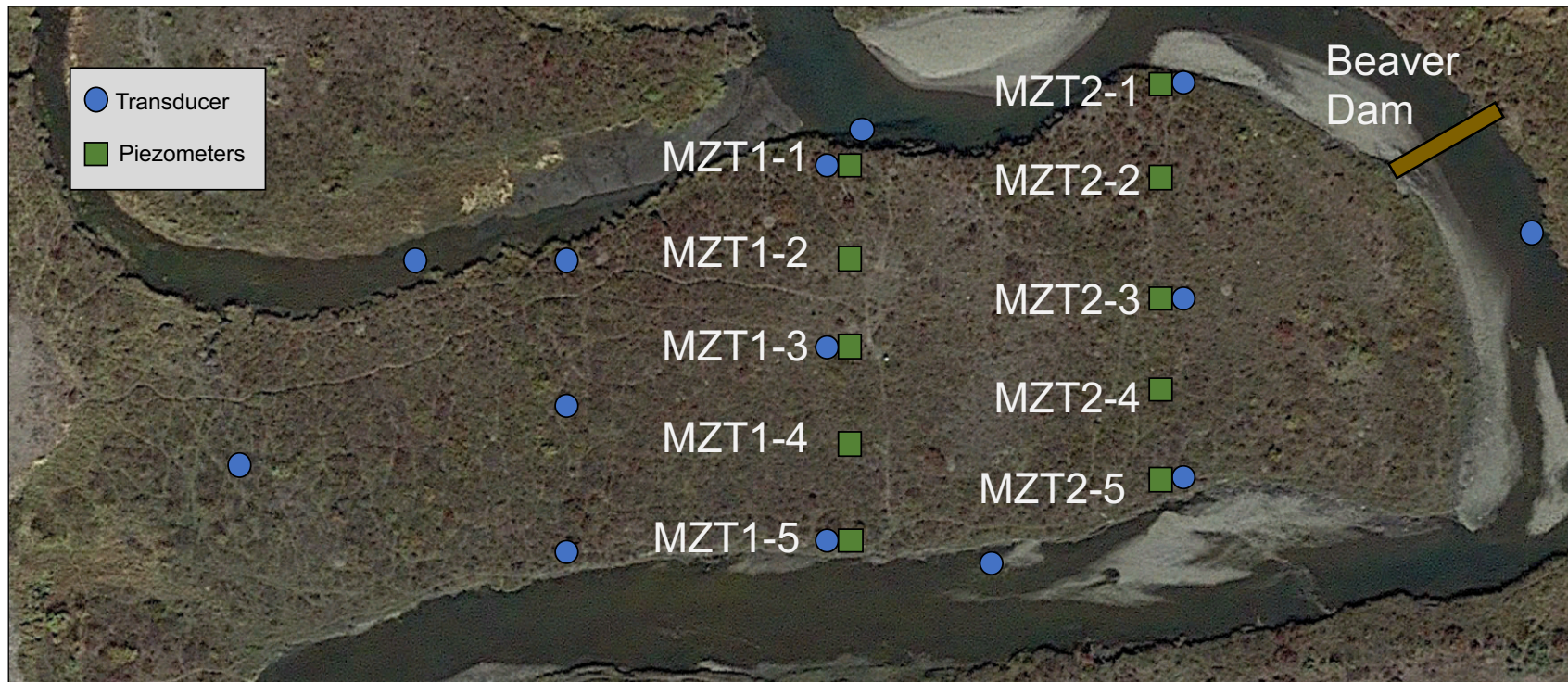


Figure S5.1. Meander Z field instrumentation. Blue circles represent locations of transducers. Green squares represent location of multi-level piezometers. The MZT1-1 and MZT2-1 locations are the most upstream locations on the transects. The location of the beaver dam, which was constructed starting in late July 2018, is marked on the northeast corner of the meander.



Figure S5.2. Meander C field instrumentation. Piezometers for water sampling.

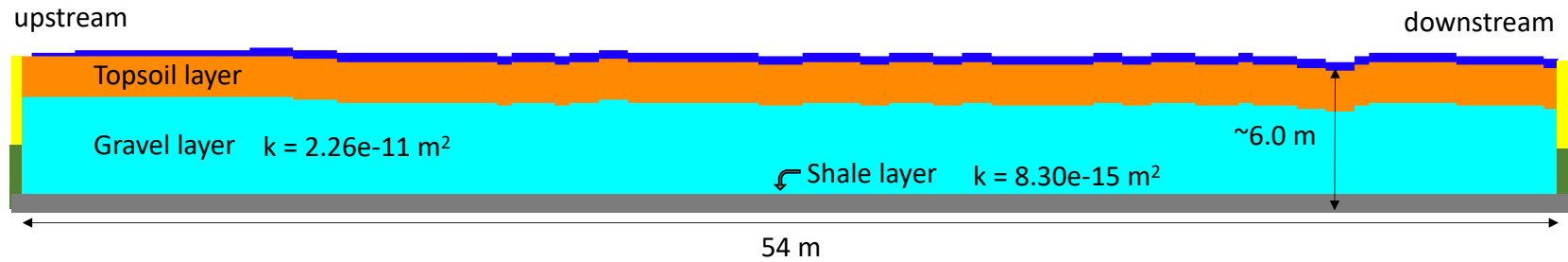


Figure S5.3. Modeling domain used in simulations. Permeability of the topsoil layer (orange) was varied between simulations. The permeabilities of the gravel (cyan) and shale (gray) layers were fixed at the values indicated. Upstream and downstream boundary conditions were dividing into river (yellow) and groundwater (green) inputs. The recharge boundary was applied at a constant rate uniformly across the top boundary (blue), for which the permeability was set to the topsoil value.

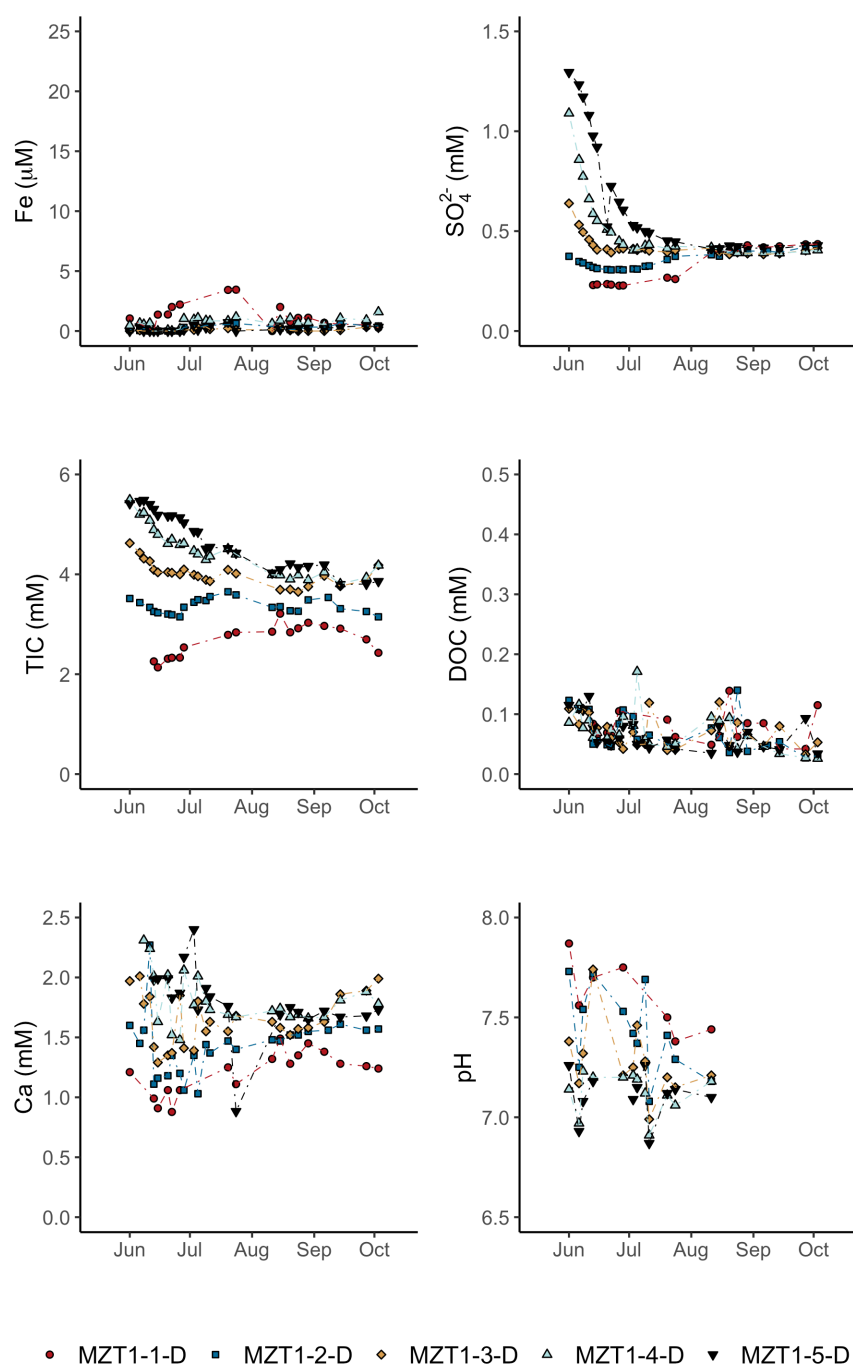


Figure S5.4. Geochemical composition of porewater from the MZT1 transect, 2018

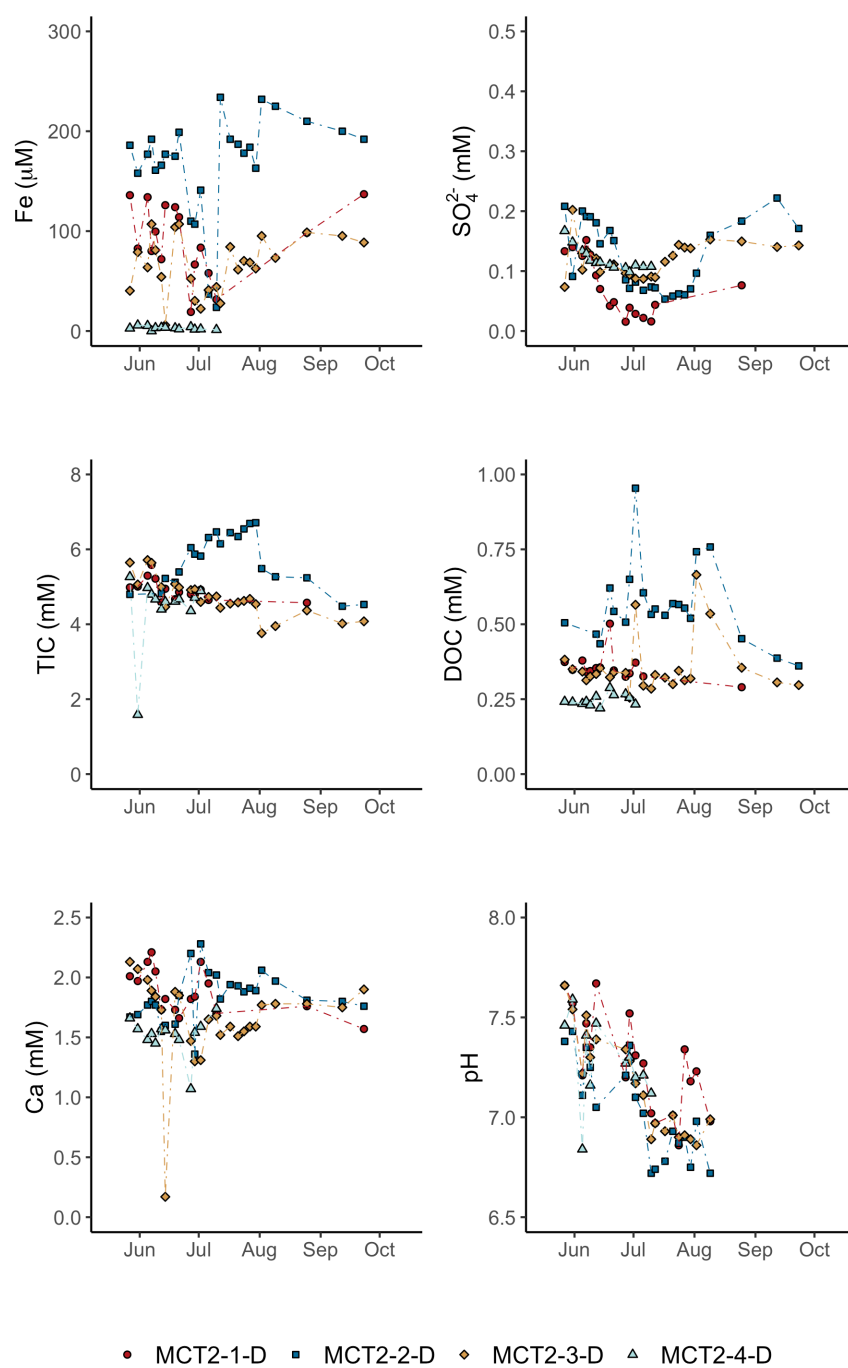


Figure S5.5. Geochemical composition of porewater from the MCT2 transect, 2018.

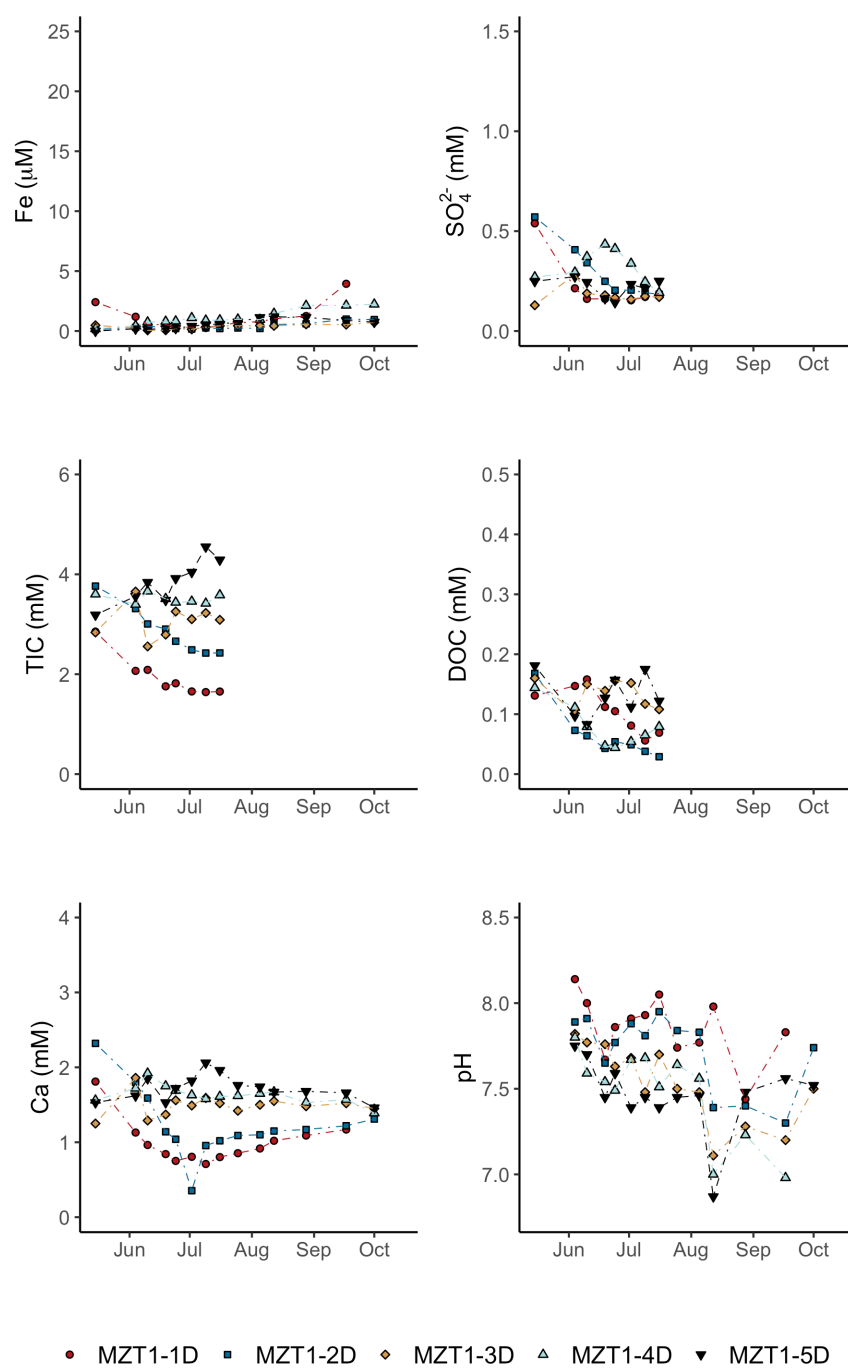


Figure S5.6. Geochemical composition of porewater from the MZT1 transect, 2019

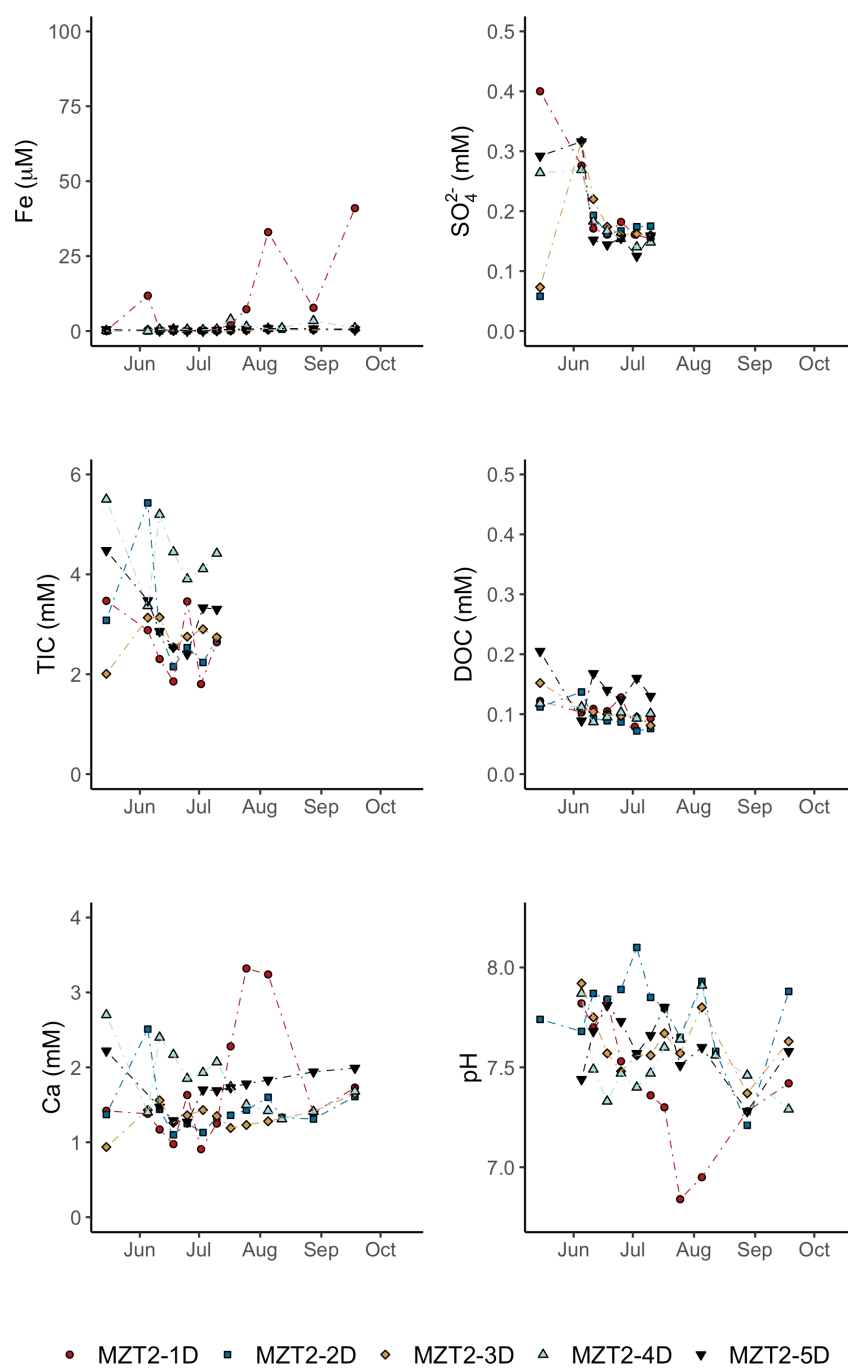


Figure S5.7. Geochemical composition of porewater from the MZT2 transect, 2019.

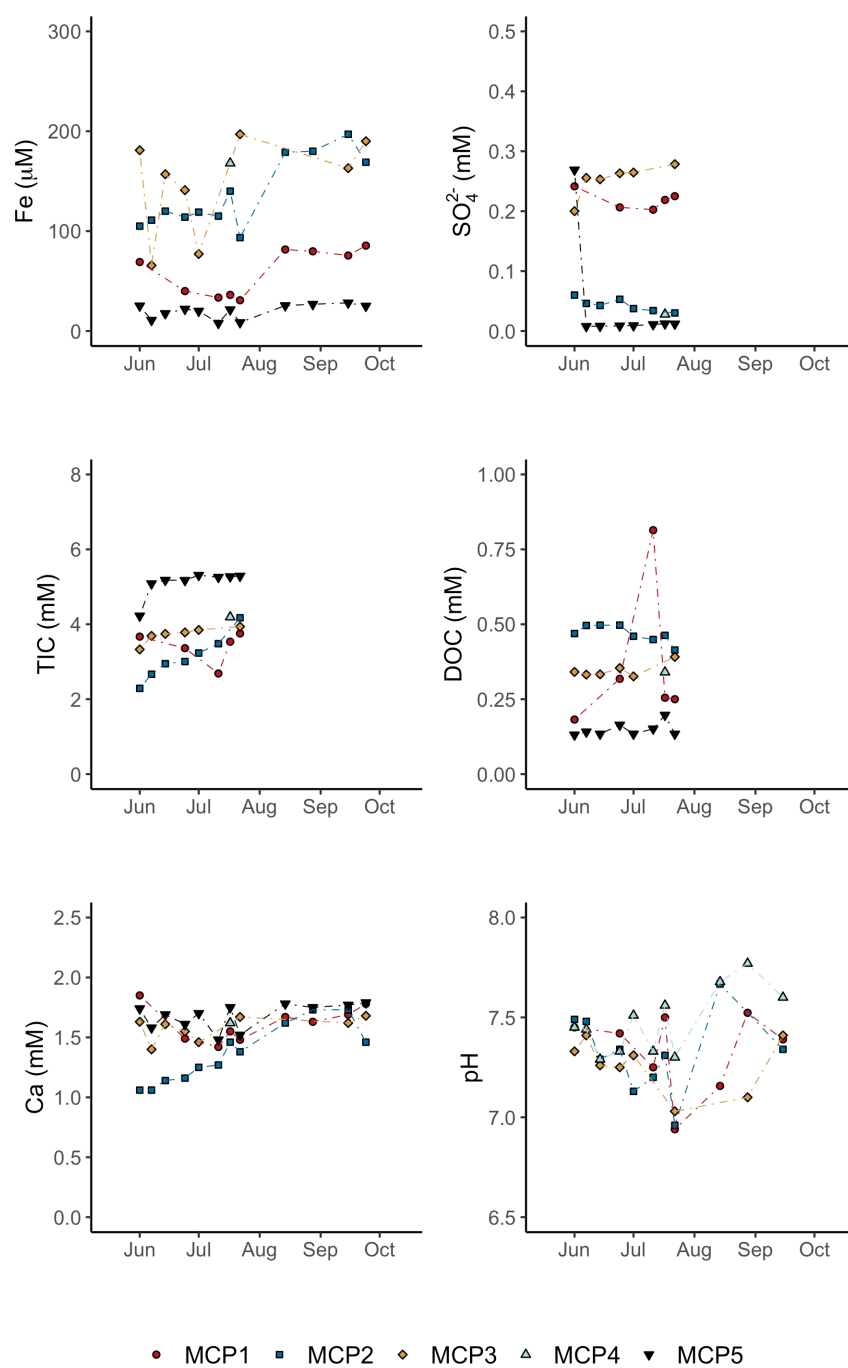


Figure S5.8 Geochemical composition of porewater from the MCP transect, 2019

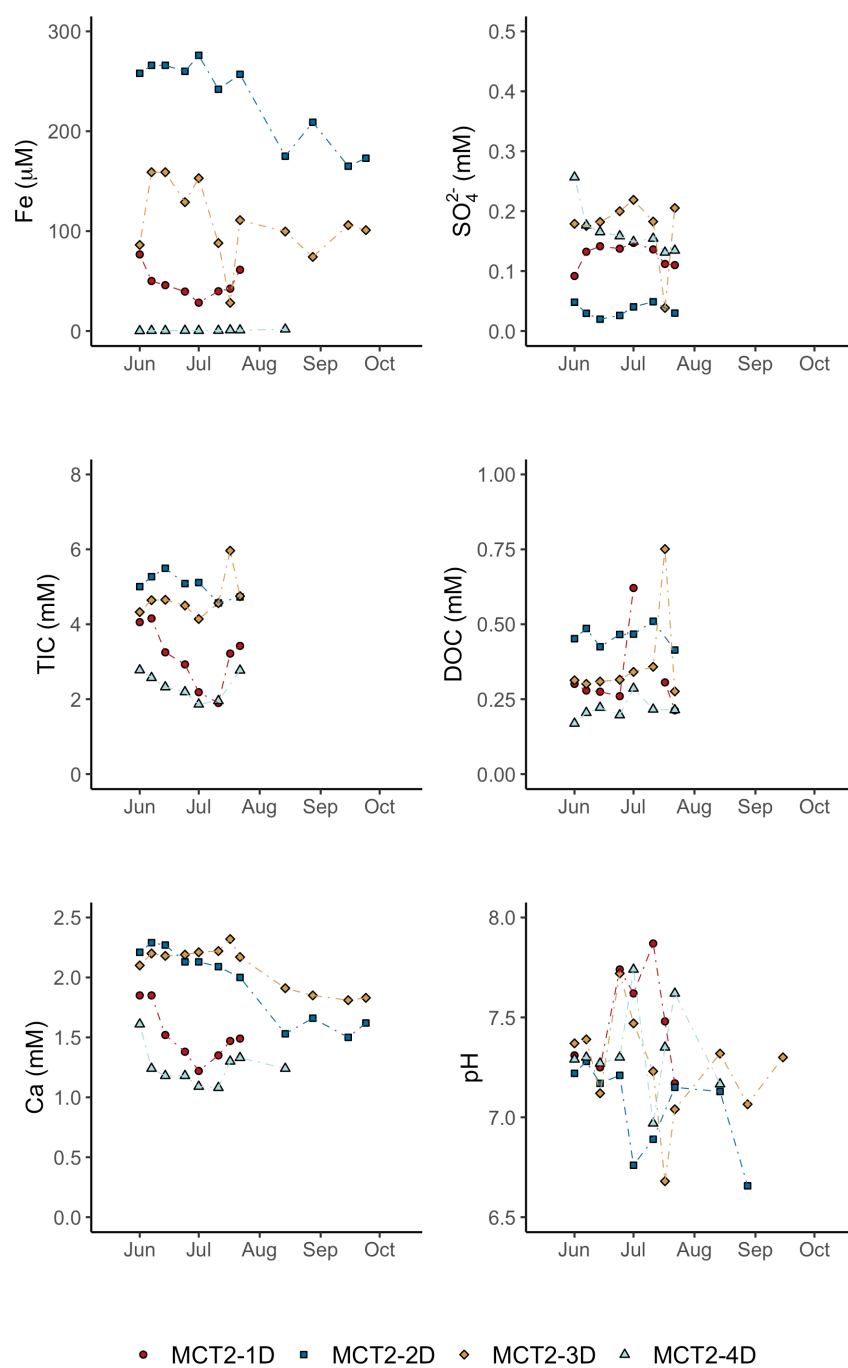


Figure S5.9. Geochemical composition of porewater from the MCT2 transect, 2019

Location	Depth, cm bgs	Avg Depth, cm bgs	Vermiculite	Kaolinite	Illite	Chlorite	Muscovite	Clay	Quartz	Palgioclase	Dolomite	Calcite	Pyrite	Titanite	Total
MCP-1	29-43	36.0	0.1	4.8	14.8	0.0	6.5	26.2	65.2	11.7	0.0	0.0	0.0	0.0	103.1
MCP-1	70-80	75.0	0.2	4.0	14.7	0.0	5.7	24.6	61.3	14.2	0.0	0.0	0.0	0.0	100.1
MCP-1	80-85	82.5	0.2	3.6	19.6	0.0	5.7	29.1	54.7	14.8	0.0	0.0	0.0	1.4	100.0
MCP-1	95-105	100.0	0.2	3.3	2.3	0.0	11.7	17.5	59.1	23.5	0.0	0.0	0.0	0.0	100.1
MCP-3	29-47	38.0	0.2	4.7	10.1	0.0	9.3	24.3	58.5	14.5	0.0	0.0	0.0	2.6	99.9
MCP-3	65-74	69.5	0.2	3.8	4.9	0.0	10.0	18.9	55.6	21.2	0.2	2.7	0.0	1.5	100.1
MCP-3	80-90	85.0	0.2	5.0	13.4	0.0	7.0	25.6	60.9	10.6	0.0	0.0	0.0	3.0	100.1
MCP-3	100-110	105.0	0.0	0.0	9.4	4.9	4.5	18.8	56.6	18.7	1.6	4.2	0.0	0.1	100.0
MCP-5	19-47	33.0	0.0	0.0	6.7	7.7	6.0	20.4	51.9	15.9	1.0	5.0	0.0	5.7	99.9
MCP-5	47-72	83.0	0.1	1.3	7.4	4.6	6.8	20.2	59.8	15.2	0.9	3.8	0.0	0.0	99.9
MCP-5	72-108	90.0	0.2	3.5	2.2	4.1	10.4	20.4	55.5	21.5	1.2	1.0	0.0	0.4	100.0
MZT1-1	0-20	10.0	0.0	1.3	10.5	3.8	5.1	20.7	53.7	14.6	2.0	1.9	0.0	7.1	100.0
MZT1-1	20-29	24.5	0.2	3.4	7.9	0.0	6.4	17.9	55.5	26.5	0.0	0.0	0.0	0.0	99.9
MZT1-1	45-60	52.5	0.2	0.3	5.3	5.2	9.2	20.2	56.6	20.8	0.0	0.0	0.0	2.5	100.1
MZT1-1	80-94	87.0	0.1	0.0	6.8	2.6	2.7	12.2	50.0	31.6	0.7	0.0	0.0	5.5	100.0
MZT1-5	0-18	9.0	0.0	1.5	8.9	4.2	5.7	20.3	60.0	12.5	1.1	4.2	0.0	1.8	99.9
MZT1-5	18-30	24.0	0.0	0.4	4.4	5.6	8.6	19.0	50.6	19.9	3.0	6.3	0.0	1.3	100.1
MZT1-5	50-70	60.0	0.2	4.1	12.3	0.0	4.7	21.3	57.9	18.3	0.4	0.0	0.0	2.1	100.0
MZT1-5	70-75	72.5	0.5	6.5	6.1	0.0	16.5	29.6	44.7	24.7	0.0	0.9	0.0	0.0	99.9
MZT1-5	75-87	81.0	0.1	2.1	5.9	2.4	7.9	18.4	57.5	21.3	0.8	0.3	0.0	1.7	100.0
MZT1-5	~90	90.0	0.0	1.2	0.0	4.6	13.0	18.8	43.7	28.7	0.8	0.0	1.5	6.4	99.9

Table S5.1. XRD results for MCP and MZT sediments. The clay fraction is the sum of the phyllosilicate clay components (vermiculite, kaolinite, illite, chlorite, and muscovite).

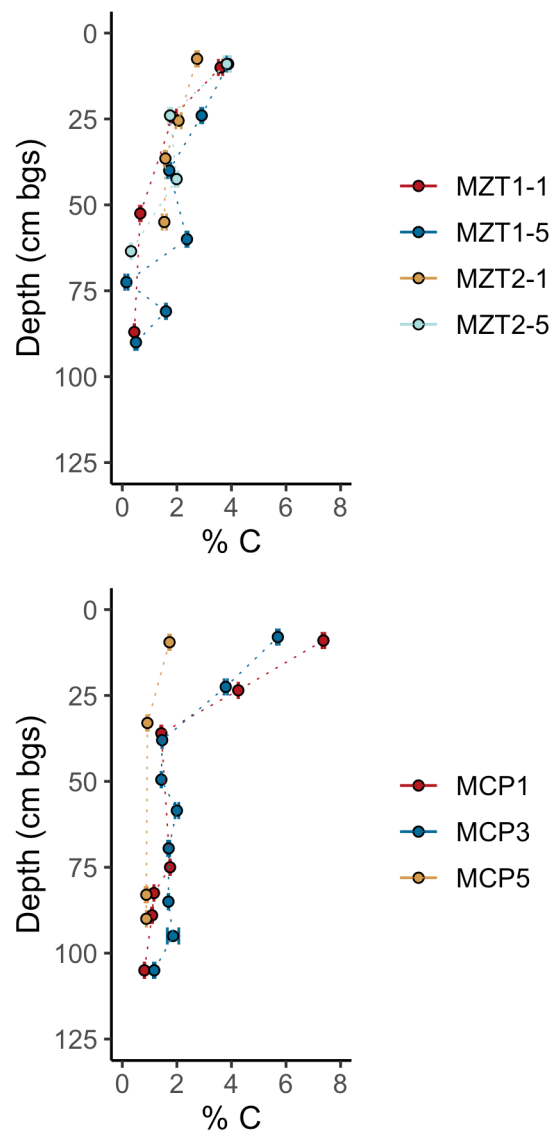


Figure S5.10. Soil carbon content along MZT1 and MCP transects.

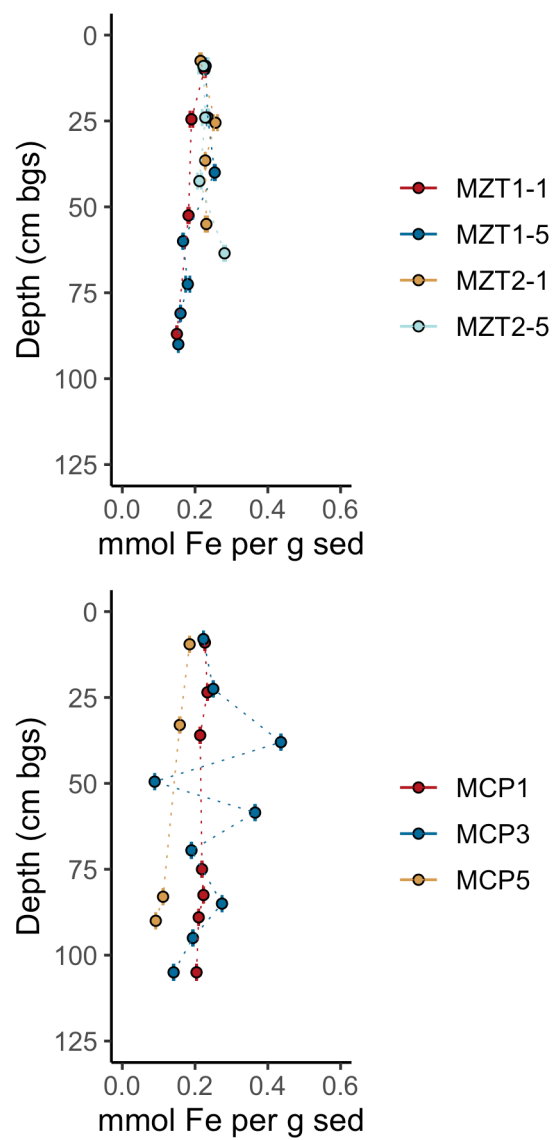


Figure S5.11. Soil Fe content along MZT1 and MCP transects.