**DOE Final Technical Report**
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# *Metabolic Constraints of Organic Matter Mineralization and Metal Cycling During Flood Plain Evolution*

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## EXECUTIVE SUMMARY

Floodplains are poorly understood and dynamic components of the global carbon cycle that not are well represented in Earth system models. Further, they have a dominant influence on the cycling of important metals within critical transport conduits between surface waters and groundwater. The physical characteristics of floodplains make the hydrology and associated coupled biology and geochemistry particularly responsive to ongoing and impending changes in climate, river management, and land development.

Using a combination of field-scale measurement with micro-scale laboratory experiments, we find that oxygen diffusion limitations lead to heterogeneous redox profiles, shifting microbial metabolism to less efficient anaerobic soil organic carbon oxidation pathways. During flooded periods, exhibiting strongly reducing conditions, dissolved organic carbon concentrations are elevated yet microbial C oxidation is limited by thermodynamic constraints on anaerobic respiration. Through laboratory incubations, we observed both kinetic and thermodynamic controls on carbon oxidation; anaerobic incubations were tested with nitrate and sulfate addition relative to an anoxic control and compared against aerobic conditions. Under anaerobic conditions, oxidation rates are consistent with thermodynamic succession in energy yields. Carbon oxidation rates are, however, highly sensitive to oxygenation, particularly in previously anaerobic soils, were oxidation rates are maximized. However, under field conditions, after a short-lived rise in carbon oxidation, rates decline again due to the formation of metal-organic complexes, which restrict the availability of dissolved organic carbon for microbial respiration. Our results suggest that these seasonally shifting controls on carbon oxidation rates across the floodplain are critical controls on carbon and metal export from the floodplain.

Export of metal-organic complexes varies depending on the hydrologic condition of the floodplain. We obverse different metal-organic complexes, and the preference for metals of differing chemistry, depending on the metabolic conditions of the soil. Combining our biogeochemical measurements with hydrological investigations of the East River flood plain, we developed a reactive transport modeling framework that examines inter-meander solute biogeochemistry and export to river water. We observe, for example, an accumulation of sulfate, calcium and bicarbonate in high-permeability meander sediments during the winter, followed by flushing of these constituents during spring melt. In the winter, the hydraulic gradient across the meander approaches zero, increasing the contribution of biogeochemical processes to porewater chemistry. As the hydraulic gradient increases with melting of snow and ice, advection of river water across the meander flushes the water from the meander sediments. Low carbon content and a steady supply of oxygen from river water prevents high-permeability sediments from reaching iron or sulfate reducing conditions. In contrast, low permeability sediments with higher organic carbon content are not flushed during spring melt, and these sediments experience iron reduction throughout the hydrologic cycle (i.e., annually). Increased water levels associated with spring melt increases the extent of sediment saturation and, therefore, anaerobic conditions. However, lower flow rates in low permeability sediments

offsets higher concentrations of dissolved constituents, resulting in slight differences in fluxes between high and low permeability sediments during seasonal hydrologic conditions (spring melt and summer baseflow).

A major factor altering the hydrologic conditions of the river and associated meanders results from the increasing beaver activity (a factor occurring through North America). We find that seasonal and semi-permanent beaver dams impose unique hydrologic conditions on the floodplain sediments: hydraulic gradients are at a maximum after beaver dam construction—even greater than during high water periods associated with spring melt. The increased hydrological gradients increases supply of oxygen to the meander sediments, oxygenating even the low permeability anaerobic zones, driving oxidation of these zones. The increase in gradient also increases biogeochemical fluxes across the floodplain sediments. Expansion of beaver population will continue to alter river hydrology and, as a result of meander biogeochemistry, water quality, while also having dramatic impacts on carbon and other elemental cycles within the river floodplains.

Collectively, our results illustrate the combined, and dynamic, impacts of mineral-associations and metabolic controls on carbon and metal fate and the overriding impact of hydrologic gradients imparted by the river and meander biogeochemistry and resulting impacts on river quality. The highly variable hydrology of floodplains leads to concomitant changes in biogeochemical processes within soils that ultimate control organic carbon, nutrients, and metal cycles.

## REPORT

Floodplains are poorly understood and dynamic components of the global carbon cycle that are not well represented in Earth system models. Further, they have a dominant influence on the cycling of important metals, such as uranium, within critical transport conduits between surface waters and groundwater. The physical characteristics of floodplains make the hydrology and associated coupled biology and geochemistry particularly responsive to ongoing and impending changes in climate, river management, and land development.

Carbon cycling within soils and sediments is largely controlled by microbial processes (inclusive of anabolism and catabolism) that are dependent on temperature and moisture levels. Further, there are principally two additional constraints on the rate and extent of microbial carbon oxidation: organic-mineral associations and oxygen limitations. Under oxygen limited conditions, microbial metabolisms are constrained by the respiratory pathway, and specifically the electron acceptor in respiration, which further serves to control metal fate and transport. Within floodplain soils and sediments, variations in hydrologic state (water saturation) coupled with structured porous media lead to extensive heterogeneity in redox environments, and thus metabolic trajectories controlling organic carbon oxidation, along with metal/mineral-organic associations. Moreover, the interconnection of floodplain soils/sediments with the river corridor

## Project Goals

Here we sought to decipher the coevolution of organic carbon and iron chemistry within floodplains, and its resulting determinant of carbon oxidation rates and metal contaminant fate and transport. Floodplains are dynamic environments that host large quantities of carbon and are critical conduits for surface and subsurface solute exchange. Our research will focus on the Colorado and associated river basins where a legacy of mining has left floodplain aquifers contaminated with uranium and other metals. Using a combination of field measurements and laboratory experiments, we are examining key processes in the associate carbon-metal cycles.

Our specific research goals are:

1. To define how organic matter, and specifically carbon chemistry, evolves over time within fine-grained flood deposits under different redox regimes.
2. To establish the roles of differing iron(III) minerals in serving as electron acceptors in anaerobic microbial respiration.
3. To determine the combined influences of organic matter—iron interactions on rates of carbon oxidation or methane production within the varying redox/hydrologic regimes of floodplains.

4. To determine how the combined evolution of organic carbon and iron mineralogy within redox regimes impacts uranium fate and transport within floodplains.

Our research focuses on anaerobic and cyclic aerobic-anaerobic regimes with four **hypotheses** that steer our research:

1. Within anaerobic regimes, deposited soil/sediment carbon will evolve toward lower oxidation states (e.g., the accumulation of lipids) and become thermodynamically limited for microbial metabolism with time and burial.
2. Oscillating redox regimes will not exhibit thermodynamic limitations to organic matter decomposition or goethite/hematite reduction during anaerobic periods, owing to the production of partially oxidized compounds aerobic cycles.
3. Iron-organic carbon complexes will dominate and resist decomposition within variable redox regimes.
4. Formation of metal-organic complexes will depend on the hydrologically-induced biochemical state of the soils/sediments.

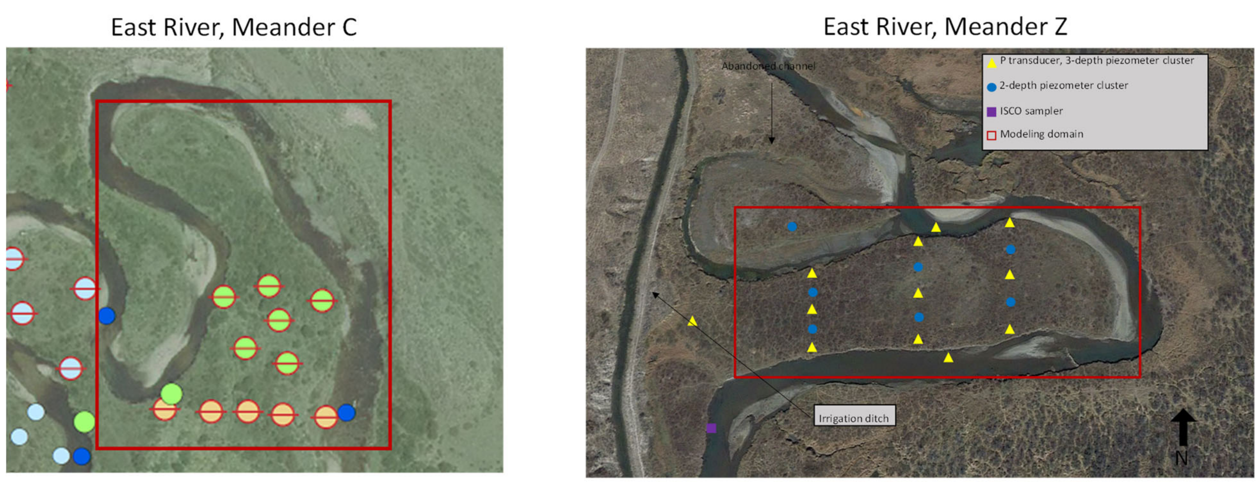


Figure 1. East River field sites illustrating the low permeability Meander C and high permeability Meander Z.

## Research Approach and Findings

Our research combines field measurement, field experimentation, and laboratory experiments, along with advanced spectroscopic techniques for product characterization, to meet the research goals. Further, we are utilizing a number of DOE user facilities, including the synchrotron light sources (SSRL and NSLS II), EMSL, and JGI. Our field research is centered largely along the floodplain of East River, CO (in concert with the LBNL-SFA research site) but also in the Wind River Floodplain (associated with the SLAC-SFA) and Hyslop field station in Oregon. Our findings are summarized in a series of publications (see References and Products);

here in we summarize our findings with an emphasis on work that is currently in the process of being published within peer reviewed literature.

Through both micro-scale laboratory experiments and field-scale observations, our findings show that oxygen diffusion limitations lead to heterogeneous redox profiles, shifting microbial metabolism to less efficient anaerobic organic carbon oxidation pathways. In both saturated and unsaturated systems, microsensor measurements in combination with gas flux measurements showed that particle size exerts a strong control on the extent of the anaerobic volume, thereby causing an overall decrease in organic carbon oxidation rates (Boye et al., 2017; Keiluweit et al., 2017; Wanzek et al., 2018 Lacroix et al., 2019, 2020). In model soils and sediments, we determined the distribution of operative microbial metabolisms and their cumulative impact on organic matter transformations and overall oxidation rates within anaerobic microsites (Keiluweit et al., 2018; Boye et al., 208; Masue-Slowey et al., 2020).

Using the varying hydrologic state of river meanders to determine central controls on microbial processes controlling carbon-metal fate, we instrumented two meanders along East River near Crested Butte, CO (Figure 1). The two meanders represent end-members for the river system. Meander C is a low permeability meander, having high clay content throughout the soil profile. By contrast, Meander Z is a high permeability meander that has hydrologically conductive and less conductive zones distributed within the soil profile. The two-end members allows us to scale our results across the entirety of the East River systems.

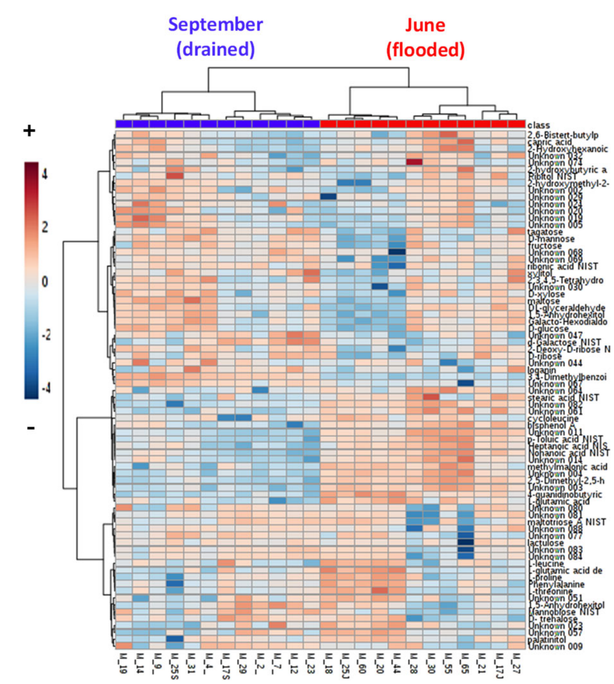


Figure 2. Heatmap visualization of low-molecular weight metabolites. Oxidized compounds (simple sugars and amino acids) are less common during flooding, especially in deep sediment, but become dominant upon drainage. By contract, Reduced compounds (fatty acids) are prominent during flooding, and significantly less abundant upon drainage.

Using pressure transducers along parallel transects across intra-meander soils/sediments and the river, hydrologic measurements were made continually throughout the various season. Correspondingly, solute chemistry was monitored in saturated soils/sediments using multi-depth piezometer clusters (~40, ~70, ~100 cm bgs) and in unsatured soils/sediments with rhizon samples (~10 and ~40 cm bgs) at transducer locations and with ISCO samplers on the river corridor. The biogeochemistry was tracked over the entire snow free season to elucidate the spatiotemporal coupling of hydrological, carbon and metal dynamics across the floodplain. Soil for incubations, metabolomics and solid phase analysis, samples were collected by augering 1 m – 2 m along transducer transects, kept in plastic core sleeves and $O_2$-scrubbed Mylar bag and stored 4°C until analyses/experimentation.

In collaboration with M. Tfaily, J. Kyle, and M. Lipton at EMSL (through an active FICUS proposal), examined changes in metabolome, lipidome, and proteome over a full flooding-drainage cycle across the meanders. Together, these analyses provided unprecedented insights into the metabolic factors controlling microbial respiration rates (Figure 2). Integrating these data using metabolic pathway analyses, we were able to show that flooding-induced shifts in the availability of low-molecular weight metabolites controls microbial respiration and, thus, both carbon oxidation rate and metal mobility across the floodplain (Figure 3). Using synchrotron based XAS (at SSRL, NSLS II and CLS) and high-resolution mass spectrometry (at EMSL), we were further able to demonstrate that amount, nature and stability of mineral bound C, N and P phases is a direct function of the redox conditions in the sediments.

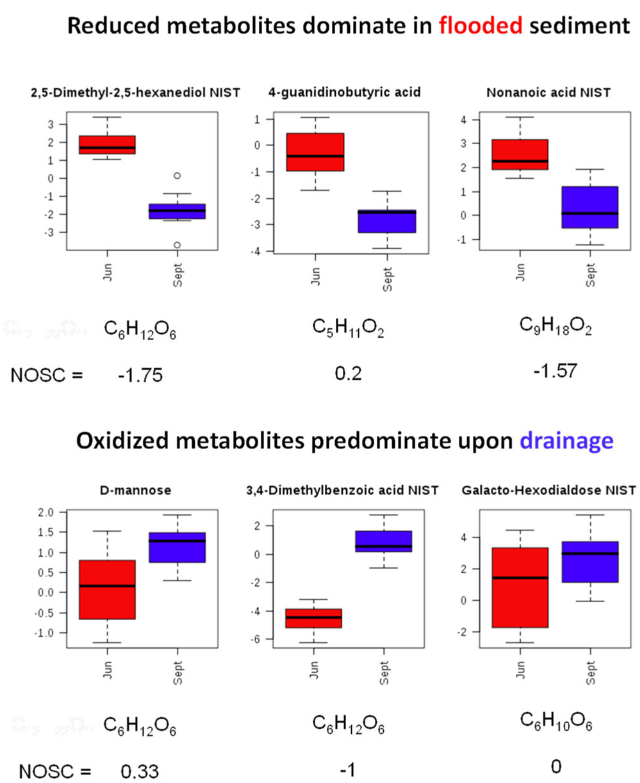


Figure 3. Nominal oxidation state of carbon (NOSC) values for floodplain metabolites during flooded and drained periods.

To compliment our field measurements, we also conducted laboratory experiments to determine hydrologically induced biogeochemical forcings of microbial metabolisms influencing carbon-metal cycles. Metabolic profiling showed that texture-induced anaerobic microsites reduced carbon oxidation rates by an order of magnitude relative to aerobic rates, with Fe(III) reduction dominating the overall metabolism. Consistent with our field measurements, particle size-induced anaerobic microsites resulted in the preferentially preservation of reduced (electron-rich) organic carbon compounds (both dissolved and particulate), a result corroborated by field measurements across multiple sites. Our results explain the selective preservation of aliphatic compounds such as lipids and waxes observed in soils globally. Further, shifting from anaerobic to aerobic conditions leads to a 10-fold increase in volume-specific mineralization rate, illustrating the sensitivity of anaerobically protected carbon to disturbance.

We additionally examined how operative microbial metabolisms, and associate rates, are impacting the mobility of metals ranging from iron to uranium. Solid-phase metal organic complexes were assessed using X-ray microscopy and near-edge X-ray absorption fine structure spectroscopy—along with more conventional density separations.

In order to test the relative importance of the thermodynamic and kinetic limitation, inclusive of depolymerization, on SOC degradation, we performed experiments using seasonally flooded soils with the addition of varying electron acceptors, pairing our field studies with laboratory experimentation. Key to our experiment was the separation of respiration due to SOC thermodynamic viability versus kinetic (EOE) facilitation, both of which are highest in oxygenated soils. Given the similar reduction potential of nitrate (to $N_2$) and oxygen (to water), and the ensuing high-efficiency carbon oxidation coupled with either oxic respiration or denitrification,[39] we assume that the bulk of additional respiration in soils incubated with equal amounts of oxygen versus nitrate must be attributed to the kinetic limitation on carbon degradation stemming from the absence of EOE activity in the absence of oxygen. The scale of the thermodynamic limitation can be assessed by comparing anoxic respiration across soils, which vary in redox potential, and with addition of different non-$O_2$ terminal electron acceptors (TEAs). We therefore performed incubations of variably saturated soils (surface, mid-core, and core bottom) and permanently flooded soils aerobically or anaerobically with nitrate, sulfate, and an anoxic electron acceptor control. We measured respiration, dissolved organic carbon (DOC), and redox indicators until $CO_2$ production saturated. Our results indicate a significant kinetic limitation on carbon degradation relative to thermodynamic and suggest the need to consider other controlling factors such as microbial community composition and total soil carbon.

Incubations were performed on four soil samples corresponding to three depths from Meander Z ("shallow", "deep anoxic", and "deep oxic") and a surface core from the neighboring oxbow channel ("flooded"), all of which constitute the "soil treatment". SOC ranged from 0.62 to 3.89% in the meander and 2.9% in the oxbow (Fig. 4). Soil nitrogen ranged from 0.052 to .35% in the meander and 0.221% in the oxbow, resulting in C:N ratios between 10.6 and 16.2 (Fig. 4). High SOC corresponded to high reduced iron with the shallow and flooded soils having Fe(II):Fe(total) ratios of 0.68 and 1.0 respectively, whereas the deep oxic soil had no measurable Fe(II) (Fig. 4). The anoxic middle and flooded soils showed higher tannin, lignin, condensed aromatic and unsaturated hydrocarbon relative abundance compared to the shallow and deep oxic soils, which had higher lipids and proteins, respectively (Fig. 4).

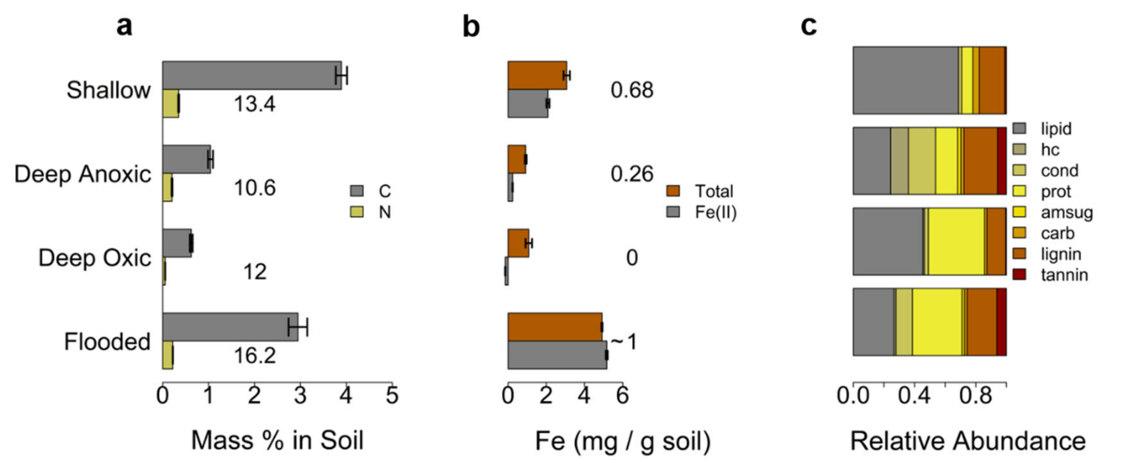


Figure 4. Field soil characteristics. (a) Mass percent organic carbon and nitrogen content per gram of dry soil with C:N indicated beside bars, (b) ferrous and total iron content taken from acid extracts, with Fe(II)/Fe(total) ratio indicated beside bars, and (c) relative concentration of soil carbon compound classes identified by van Krevelen diagrams of FT-ICR-MS data, where "hc" = unsaturated hydrocarbons, "cond" = condensed aromatics, "prot" = proteins, "amsug" = aminosugars, and "carb" = carbohydrates. All error bars indicate standard error of triplicate subsamples.

Cumulative respiration was 2 to 40 times greater with oxygen than with nitrate (Figure 5). In contrast, nitrate addition only increased respiration up to 1.6 times the anoxic control for carbon-poor deep soil, and less for other soils, highlighting the potential magnitude of enzymatic limitations on carbon cycling in variably saturated systems. Under anaerobic conditions, respiration rates scaled with thermodynamic yield, but the differential magnitude was small in comparison to aerobic conditions. Kinetic limitations appear to depend on carbon content and hydrologic state of the meander, the latter of which has yet to be incorporated into soil carbon modules utilized in earth system models.

Additionally, in association with Rene Boiteau at EMSL/Oregon State University, we have examined dissolved and colloidal metal-organic complexes using a novel, and state-of-the-art, ion chromatography with split, tandem ICP-MS and FT-ICR-MS, allowing unprecedented resolution of controls on metal solubility and mobility. Within drainage and pore-water of the East River meander soils/sediments, we find that Fe-organic complexes dominate. Moreover, specific binding sites compete for Fe, Ni, and Cd, indicating a defining functional group that dominates metal complexes, with limited discretion for the specific transition metal.

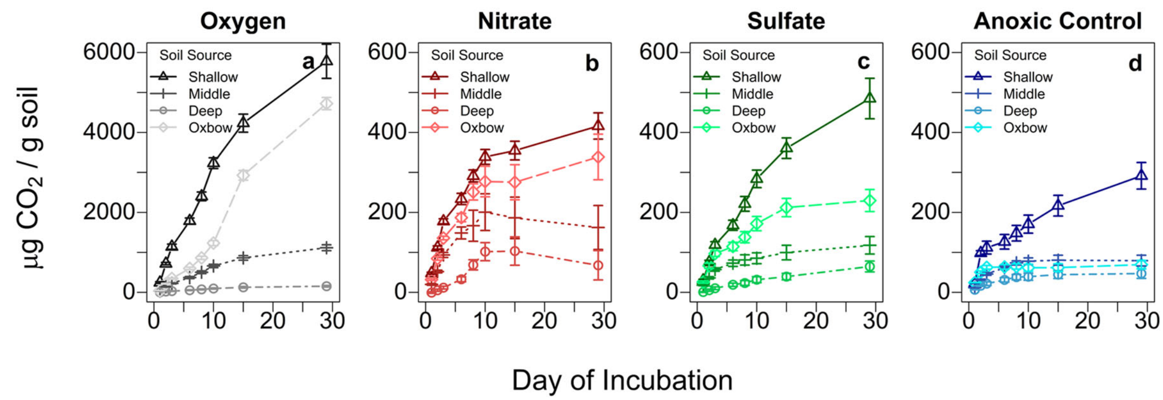


Figure 5. Batch reactors with equimolar added terminal electron acceptors. Soils from MZT12 and adjacent oxbow.

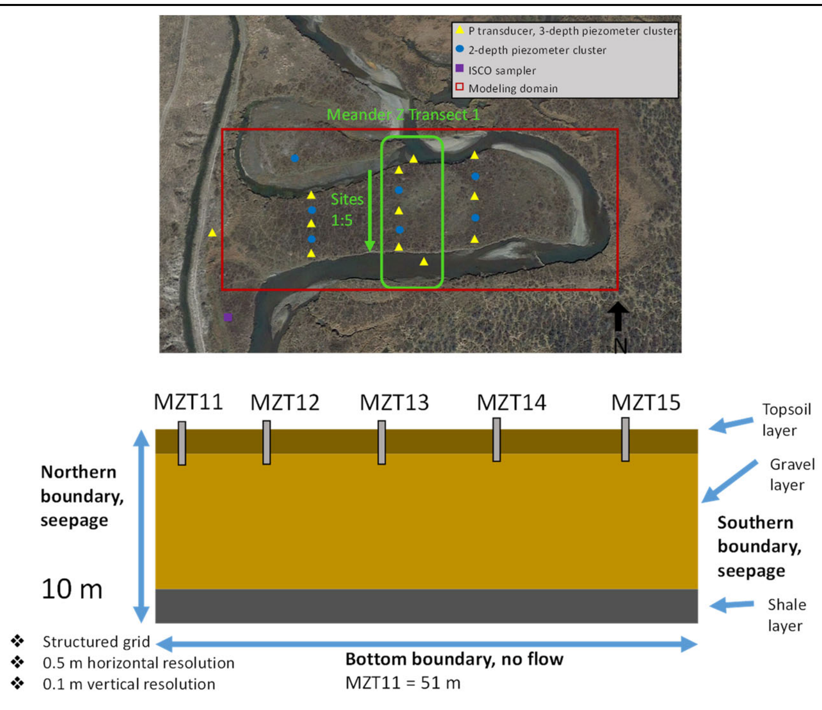


Figure 5. Meander Z transects used for reactive transport modeling.

Complimenting and integrating our field and laboratory experiments, we conducted reactive transport modeling to capture the integration of hydrological and biogeochemical processes controlling both elemental cycles in the meanders and their impact on river water quality. We employ reactive transport modeling of representative meanders on the East River (meanders C and Z) to determine the impacts of transient hydrologic conditions on redox zonation and biogeochemical fluxes across floodplain sediments. Model simulations are run in PFLOTRAN, an open-source, three-dimensional, groundwater flow and reactive transport code.. Simulations were performed in 1D, 2D and 3D. For 2D and 3D models, a structured grid of the domain was created from a digital elevation model derived from a 0.5 m resolution LiDAR dataset. The 2D and 3D domains were discretized with a uniform grid spacing of 0.5 m and 0.1 m in the horizontal and vertical dimensions. For meander Z (Figure 6), the modeling domains consist of three stratigraphic layers: a topsoil unit (0 to 1 m below ground surface) with relatively low permeability (8.80 x $10^{-13}$ $m^2$); a middle gravel layer (1 to 3 m below ground surface) with relatively high permeability (2.26 x $10^{-11}$ $m^2$); and an underlying shale layer (>3 m below ground surface) with low permeability (8.30 x $10^{-15}$ $m^2$). The East River serves as a hydrologic and geochemical boundary condition along the northern, eastern, and southern boundaries of the 2D and 3D model domains. The western edge and the bottom of the domains are treated as no-flow boundaries. Geochemical and hydrologic observations are used to define the boundary conditions.

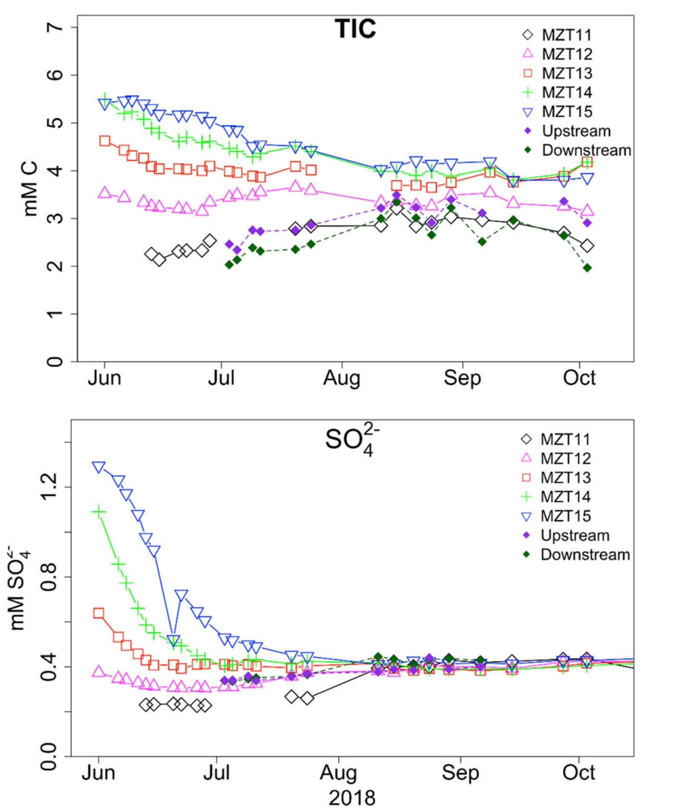


Figure 6. Pore-water total inorganic carbon (TIC) and sulfate across meander Z during the 2018 season.

Our modeling reveals an accumulation of sulfate, calcium and bicarbonate in high-permeability meander sediments during the winter, followed by flushing of these constituents during spring melt. In the winter, the hydraulic gradient across the meander approaches zero, increasing the contribution of biogeochemical processes to porewater chemistry (Figure 6). As the hydraulic gradient increases with melting of snow and ice, advection of river water across the meander flushes pores. Low carbon content and a steady supply of oxygen prevents high-k sediments from reaching iron or sulfate reducing conditions.

In contrast, low permeability sediments with higher organic carbon content are not flushed during spring melt, and these sediments experience iron reduction throughout the hydrologic cycle. Increased water levels associated with spring melt increases the extent of sediment saturation and, therefore, reduction. With respect to fluxes, lower flow rates in low permeability sediments offsets higher concentrations of dissolved constituents, resulting in slight differences in fluxes between high and low permeability sediments during seasonal hydrologic conditions (spring melt and summer baseflow).

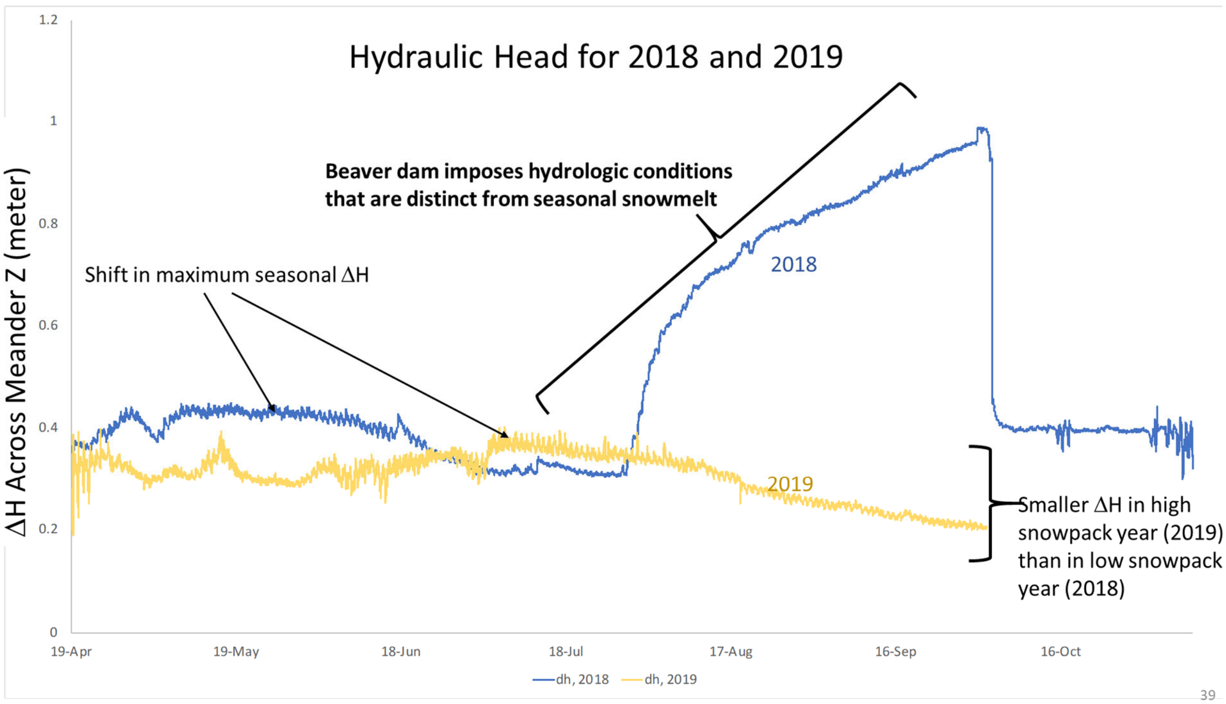


Figure 7. Hydraulic head for East River between the upstream and downstream zones of Meander Z for the years 2018 and 2019. During the 2018 year, a beaver dam develop mid-meander, altering the hydraulic head across the meander.

The construction of a beaver at the meander point in 2018 provided a unique opportunity to determine the effects of increased flow velocity on redox zonation and biogeochemical fluxes (Figure 7). We find that the beaver dam imposes a unique hydrologic condition on the floodplain sediments: the hydraulic gradient is substantially greater after beaver dam construction than during high water periods associated with spring melt. Simulations show that the increase in gradient increases supply of oxygen to low permeability reduced zones, driving oxidation of these zones. The increase in gradient also increases biogeochemical fluxes across the floodplain sediments. Moreover, the gradient resulting from the beaver dam overwhelms the seasonal gradients.

Collectively, our results show that hydrologic variability leads to distinct biogeochemical processes which exert dominant controls on carbon-metal cycling. Within anaerobic zones, kinetically and thermodynamically stranded reduced carbon compounds dominate while more oxidized compounds prevail within aerobic conditions. As a consequence, carbon is oxidized slowly and incompletely within anaerobic zones and limit metal-organic complexes. By contrast, oxygenated environments result rapid oxidation and greater metal-organic complexes. We observe these contrasting hydrological-biogeochemical environments manifested within the two end-member meanders. Meander C is dominated by low permeability material that maintain a high moisture content, leading to a dominance of anaerobic metabolic processes and products. By contrast, Meander Z is composed predominantly of highly permeable material and is generally well oxygenated by influxing river water or aeration. Moreover, the impacts on river water quality imposed by the meanders are also vastly different. Meander C develops pore-water chemistry consistent with reducing conditions that differs substantially from the river. However, low discharge rates lead to limited alterations in downstream river chemistry. The highly permeable soils/sediments of Meander Z lead, by contrast, to large discharge rates, but the pore-water chemistry in the inter-meander is not greatly altered from the river water. The most pronounced change in meander conditions, and concomitant river impacts, results from beaver dam emplacement. Sharp hydrologic gradients develop that overwhelm season changes and leads to radical changes in meander chemistry and discharge. In sum, our finding provide essential information on conditions that control metal concentrations in river and groundwater, allowing for both prediction and control of water quality. Summing our results from end-member meanders, combined with hillslope inputs, now provides the ability to forecast river water quality on East River and other similar mountainous watersheds.

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