

Mineral and Metabolic Constraints on Carbon Cycling Within and Export From Mountainous Floodplain Soils

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Mineral and metabolic constraints on carbon cycling within and export from mountainous
floodplain soils

A Dissertation Presented

by

CAM G. ANDERSON

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2023

Environmental Conservation

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ABSTRACT

MINERAL AND METABOLIC CONSTRAINTS ON CARBON CYCLING WITHIN AND EXPORT FROM MOUNTAINOUS FLOODPLAIN SOILS

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Floodplains contain soils rich in organic matter and are important ecosystems for agricultural production worldwide. Importantly, seasonal flooding results in drastic redox fluctuations, which have profound implications for transformations of soil carbon (C). However, we do not know the underlying biogeochemical controls on and spatiotemporal variation of soil C cycling in redox-dynamic floodplains. Using an active meander of the subalpine East River watershed (Gothic, CO), this dissertation first reviews the current understanding of floodplain C import, storage, and export, and further discusses how hydrology and corresponding redox patterns impact mineral and metabolic controls of floodplain C transformations. Using a combination of sequential extractions, physical fractionation, and high-resolution mass spectrometry, Chapter 2 finds that mineral-associated C constitutes a meaningful fraction of total C, suggesting that mineral-organic associations are quantitatively important for floodplain soil C protection. Chapter 3 investigates mechanisms of anaerobic protection that drive floodplain C cycling. Studying the same meander, metabolite data show evidence for kinetic constraints during flooding, leading to an accumulation of relatively higher molecular weight compounds.

Upon drainage, thermodynamic constraints are relieved in the surface soils, but deep soils remain reducing year-round. Chapter 4 aims to resolve the balance between mineral and metabolic constraints on floodplain C loss, across extreme low (2018) and high (2019) river discharge years. Using similar techniques as above, in combination with environmental monitoring, we found that reducing conditions (1) decreased the stability of iron-carbon associations, causing concomitant mobilization of iron and DOC, and (2) resulted in the selective preservation of reduced DOC compounds; both findings were stronger during extreme flooding. Connecting these mechanisms to riverine exports of floodplain C showed shifts in the balance of inorganic versus organic C export during extreme drought or flooding. Climate change is shifting the extent of flooding in mountainous floodplains, with prolonged megadrought expected to drive more low water years. Thus, understanding the mechanisms that control floodplain C cycling will be crucial to maintaining healthy floodplains that can sustain food productivity and clean water.

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CHAPTER 1

INTRODUCTION: MINERAL AND METABOLIC CONSTRAINTS ON FLOODPLAIN SOIL CARBON CYCLING

1.1 Abstract

Floodplain soils are large and dynamic reservoirs of carbon (C), where fluctuating redox conditions occur over relatively short temporal and spatial scales due to seasonal flooding and soil texture gradients. Importantly, climate change is shifting the extent of flooding, with implications on the cycling of floodplain C. In this introduction, we review the dynamics of floodplain C import, storage, and export. Specifically, we discuss how the dynamic hydrology and corresponding seasonal redox patterns drive mineral and metabolic controls on floodplain C transformations.

1.2 Introduction

Floodplain soils are large and dynamic reservoirs of carbon (C) (D'Elia et al., 2017; Sutfin and Wohl, 2017), where fluctuating redox conditions occur over relatively short temporal and spatial scales due to seasonal flooding and soil texture and organic matter gradients (Chen et al., 2017). Importantly, climate change is impacting snowpack levels in the western United States (Fyfe et al., 2017), resulting in drastic inter-annual differences in river discharge (Hubbard et al., 2018) and making these mountainous floodplains particularly vulnerable to increased episodes of flooding and droughts. This dynamic hydrology has profound implications for soil C retention and loss, as stored soil C could be released as the greenhouse gases carbon dioxide (CO₂) or methane (CH₄), or

exported as dissolved organic C (DOC) to riverine systems (Huang et al., 2020; Huang and Hall, 2017; Knorr, 2013), affecting both soil fertility and downstream water quality. However, we do not know the underlying biogeochemical controls on and spatiotemporal variation of soil C retention and export in redox-dynamic floodplain environments. To accurately predict if floodplains act as sinks or sources of C in a changing climate, we need to understand the mechanisms that stabilize or mobilize soil C. This introductory chapter details the mechanisms of floodplain C cycling (import, storage, and release), and reviews the implications of floodplain C loss in the face of climate change.

1.3 Import, storage, and export of floodplain soil C

The heterogeneous nature of floodplains has implications for the import, storage, and ultimately export of soil C. Organic C can enter floodplain soils through two primary mechanisms: (1) vegetation and litter inputs, such as root biomass and exudation, and (2) overbank deposition of dissolved and particulate organic C (Scott and Wohl, 2018). The balance between these inputs is controlled by features of the floodplain including flow regime, lateral connectivity during flooding, and sediment transport dynamics (Scott and Wohl, 2018).

Once C has entered floodplain soils, its storage is temporally and spatially heterogeneous, depending on factors including grain size distribution, soil moisture dynamics, and biogeochemical processes (Wohl, 2021; Wohl and Pfeiffer, 2018). For example, lower energy reaches, or reaches in wider valleys, are dominated by the deposition of finer materials, such as clays and silts (Scott and Wohl, 2018), which have been shown to be a factor controlling C stocks in floodplains (Heger et al., 2021). Burial

upon deposition is also considered a dominant mechanism of C storage: soil C in depositional environments has been shown to be less transformed and also more preserved than C in erosional environments (Berhe et al., 2012), due to increased moisture and associated oxygen limitations, for example (Berhe, 2012). Thus, textural and depositional dynamics impart physical controls on soil C storage that may impact floodplain C cycling.

The movement and transformation of C within floodplain soils are dynamic processes also impacted by seasonal shifts in hydrology due to flooding. Indeed, water table dynamics, and associated soil moisture fluctuations, can also control C storage: Sutfin et al. (2016) found that shallow water tables in floodplains can lead to soil organic C accrual due to decreased microbial metabolism. Importantly, floodplain soils and sediments are characterized by dramatic redox fluctuations over space and time, due to sediment characteristics (e.g., texture and mineral composition) and seasonal flooding dynamics (e.g., flooded and drained periods) (Chen et al., 2017). In these redox-dynamic floodplain environments, loss of soil C protected within mineral-organic associations is a two-step process impacting mineral-organic associations and the C decomposition cascade. First, soil C is made accessible from mineral-organic associations through both reductive dissolution and desorption through exchange reactions (Buettner et al., 2014; Kleber et al., 2015; Kramer et al., 2012; Mikutta et al., 2006). This process can increase the mobility of soil C in the environment leading to leaching of dissolved organic C (DOC) (Adhikari et al., 2017; Barcellos et al., 2018b; Buettner et al., 2014; Huang and Hall, 2017). Second, once soil C is mobilized and made bioavailable through enzymatic depolymerization, microbial metabolic constraints control whether the C is oxidized

(Boye et al., 2017; Keiluweit et al., 2016; LaRowe and Van Cappellen, 2011).

Importantly, oxygen limitations influence depolymerization by suppressing oxidative enzymes (Freeman et al., 2001). Mineral and metabolic constraints thus control the balance between soil C storage versus loss.

Spatial and temporal redox gradients affect both mineral and metabolic constraints on floodplain C cycling, and thus represent a critical control on soil C mobilization, availability, and oxidation (Fig. 1.1). The destabilization and subsequent loss of C from mineral-organic associations is a critical knowledge gap, especially in floodplain systems with drastic spatial and temporal redox gradients. Bailey et al. (2019) outlined dominant mechanisms of soil C destabilization, including release from physical occlusion, desorption processes, and microbial metabolic shifts. To understand C loss in redox-active environments, however, we must consider both mineral and metabolic constraints.

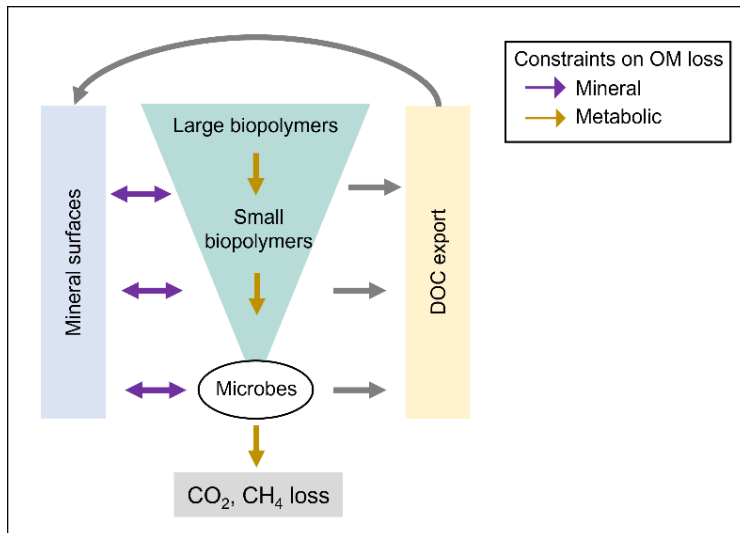


Figure 1.1. Conceptual diagram detailing mineral (purple arrows) and metabolic (yellow arrows) constraints on floodplain organic matter (OM) transformations and loss. Export includes gaseous loss as carbon dioxide (CO₂) and methane (CH₄), and loss in the dissolved phase as dissolved organic carbon (DOC). Modified from the soil continuum model (Lehmann and Kleber, 2015).

1.4 Mineral constraints on soil carbon cycling in floodplain soils

Mineral-organic associations represent an important and dynamic pool of OC (e.g., Kleber et al., 2021), and these associations are important controls on OC cycling across a diversity of ecosystems and soil types (Coward et al., 2017; Kirsten et al., 2021; LaCroix et al., 2019; Porras et al., 2017; Possinger et al., 2020a; Torn et al., 1997) (Fig. 1.1). A study of soils across humid to arid ecosystems in the United States showed that in wetter climates, such as those in floodplain ecosystems, mineral-organic associations drive the persistence of OC (Heckman et al., 2023). Indeed, stabilization in mineral-organic associations increases C turnover time (Lehmann and Kleber, 2015). Thus, associations between soil minerals and C play an important role in soil C transformations, but their specific importance within floodplain soils is less understood.

Iron (Fe) is a particularly important and dynamic metal that can form associations with soil C. It has been shown that Fe can form associations with organic matter, leading to soil C stabilization across both soils (Coward et al., 2017; Kleber et al., 2015) and deep sediments (Lalonde et al., 2012). Importantly, Fe is the most abundant redox-active metal in soils, meaning that it plays a dynamic role in soils that experience redox fluctuations, including floodplains.

Redox cycles impact Fe-C associations in several ways. First, they can impact Fe crystallinity and therefore the amount and type of C stored (Herndon et al., 2017). For example, more crystalline Fe phases associate with slower-cycling, more microbially-derived OM (Hall et al., 2018). Reducing conditions can also mobilize OM from Fe phases via reductive dissolution of Fe-bearing minerals (Adhikari et al., 2017; Han et al.,

2019; Knorr, 2013; Wordofa et al., 2019; Zhao et al., 2016). These dynamics lead to a heterogeneous composition of Fe-bearing minerals across redox-active soils.

In depositional environments, Fe-bearing minerals exhibit spatial patterns in composition. For example, along vertical floodplain redox gradients, Chen et al. (2017) found that relative to oxic layers, anoxic zones contained more crystalline Fe oxide phases, the most reducing zones did not contain any poorly crystalline oxides. In the Danube River floodplain, Mayer et al. (2018) found spatial patterns in redox and resulting Fe mineral composition. Specifically, they found dominant oxic conditions in the uppermost meter of Danube River floodplain soils, and spatial patterns in pedogenic Fe oxide distribution, which they attributed to artificial drainage (Mayer et al., 2018).

In addition to spatial variability, redox-dynamic soils exhibit temporal variation in Fe mineral composition. For example, in humid tropical soils, which experience redox fluctuations with precipitation dynamics, Fe mineral composition has been shown to shift with redox fluctuations, imparting different mineral stabilities. For example, lower crystallinity Fe oxides were protected against reductive dissolution, and higher crystallinity Fe oxides were not (Coward et al., 2018b).

The spatial and temporal variation in Fe mineral composition impacts the composition of mineral-associated organic matter (MAOM). While short-range ordered minerals and metals have similar capacities to bind unaltered organic matter, the longer-term stabilities of MAOM varies by mineralogy and thus in situ composition of MAOM can differ (Sanderman et al., 2014). For example, microbial metabolites have been shown to competitively sorb to Fe oxide mineral surfaces (Swenson et al., 2015). Furthermore,

at the nanometer scale MAOM has been shown to be enriched in nitrogen and oxidized OC, suggesting the importance of both microbial-derived residues and aromatic and carboxylic acids in the stabilization of MAOM (Possinger et al., 2020b). Thus, redox fluctuations can impart distinct signatures on MAOM chemistry across mineral phases, providing a fingerprint of the mechanisms that drive MAOM sequestration.

While Fe-bearing minerals respond to redox shifts, Al-bearing minerals are not redox reactive. Thus, C protection within Al associations might infer protection even in reducing conditions experienced at depth or during increased moisture events (e.g., flooding or precipitation) (Inagaki et al., 2020; LaCroix et al., 2019; Nitzsche et al., 2022; Possinger et al., 2020a). However, the relative influence of Fe and Al minerals on floodplain C storage and export is not well understood.

Mineral controls on floodplain C transformations are temporally and spatially diverse, driven by hydrology as well as mineral composition. However, despite the importance of mineral-organic associations in floodplain C cycling, we do not know how minerals and their associated C will be affected by predicted shifts in hydrology due to climate change, and how this impacts export of floodplain C and associated metals.

1.5 Metabolic constraints on C decomposition and oxidation

If the mineral constraint on floodplain C loss is lifted by disrupting mineral-organic associations, the newly available C is then subject to metabolic constraints (Fig. 1.1). Metabolic constraints on floodplain C loss include both kinetic and thermodynamic components. During flooding, the oxidative depolymerization of soil organic matter into assimilable monomers is kinetically constrained by the suppression of oxidative

depolymerizing enzymes (e.g., phenol oxidases). Instead, under such anaerobic conditions depolymerization relies primarily on hydrolytic depolymerization enzymes, which can be inhibited by the build-up of phenolic compounds, a phenomenon termed the “enzymatic latch” (Freeman et al., 2001). For example, oxidative enzyme constraints have been shown at the oxic-anoxic transition zone in soils through the accumulation of phenolic compounds (Naughton et al., 2021). Inhibition of oxidative enzymes could also result in the accumulation of relatively oxygen-poor and non-hydrolysable organic compounds, such as lignin (Hedges and Keil, 1995). Indeed, under anaerobic conditions, the enzymatic depolymerization of OC, and the subsequent fermentation of its byproducts, are the rate-limiting steps of OC decomposition (Glissmann and Conrad, 2002; Keiluweit et al., 2016; Megonigal et al., 2004).

Thermodynamic constraints are invoked when considering microbial respiration of assimilable C. Microbial respiration is catabolic reaction in which soil C compounds are oxidized to produce energy, a process which occurs in both aerobic and anaerobic environments. Soil C oxidation rates during respiration are dependent on the presence of electron acceptors (e.g., oxygen or Fe oxy(hydr)oxide minerals) and donors (i.e., soil organic matter). The thermodynamic favorability of the C oxidation reaction is dependent on the presence of both suitable electron acceptors and donors (LaRowe and Van Cappellen, 2011), both of which change seasonally in floodplain systems. The reduction half-reaction (i.e., the electron acceptor) is often assumed to be the main determinant of the thermodynamic favorability of soil C oxidation, with a cascade of electron acceptors yielding decreasing amounts of energy during reduction: O_2 , NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} , and

CO₂. In soils, when oxygen is depleted, Fe reduction often dominates due to the high abundance of Fe-bearing minerals.

However, it has been shown that the oxidation half-reaction (i.e., the electron donor) is equally important to consider in the process of C oxidation (LaRowe and Van Cappellen, 2011; Stuckey et al., 2015). To formalize this, we can use the combined kinetic-thermodynamic framework established by Jin and Bethke (2007), which incorporates the bioenergetics of microbial metabolism, to describe C oxidation rates:

$$Rate_{C\text{-oxidation}} = R_{\max} * X * F_k * F_T \quad [1]$$

where $R_{\max}$ and X are the maximum reaction rate and microbial biomass, respectively. F_k and F_T are non-dimensional factors ranging from 0 to 1. F_k represents a microorganism's ability to acquire and process OM substrates (often expressed within Michaelis-Menten or Monod equations), and it also incorporates OM protective mechanisms through minerals and physical occlusion. F_T is a measure of the catabolic energy yield of an OM oxidation reaction, linking the rate of reaction to the thermodynamic driving force (using Gibb's free energy of reaction).

The formulation in equation [1] thus accounts for both the kinetics of enzymatic reactions and the bioenergetics of microbial reactions. Within this framework, the energy yield of the bioavailable soil C determines its thermodynamic favorability and thus its reactivity: a more reduced C compound requires microbes to overcome more thermodynamic barriers to oxidize it. As a result, in reducing environments such as flooded soils, thermodynamic constraints on microbial metabolism selectively preserve otherwise bioavailable, reduced C compounds (Boye et al., 2017; Keiluweit et al., 2016).

LaRowe and Van Cappellen (2011) define a term called the nominal oxidation state of carbon (NOSC), which can be used to approximate OM reactivity through its relationship to the Gibb's free energy for the oxidation of C. NOSC can be determined for any mixture of organic substrates through their composition, using:

$$\text{NOSC} = -\left(\frac{-Z+4C+H-3N-2O+5P-2S}{C}\right) + 4 \quad [2]$$

where C, H, N, O, P, and S correspond to elemental stoichiometry values (measured using high resolution mass spectrometry, for example), and Z is equal to the net charge of the organic compound (assumed to be zero).

Kinetic and thermodynamic controls on C transformations are impacted by the redox fluctuations experienced in floodplain ecosystems. However, we have poor understanding of how the relative balance of these mechanisms shifts over seasonal flooding.

1.6 Conclusions and Implications

Mountainous floodplains represent a large and dynamic store of soil C, where seasonal flooding from snowmelt creates dynamic redox gradients which can strongly affect whether these soils retain or export C. To understand patterns of C export from floodplain soils, it is important to understand the mechanisms driving them. Redox dynamics can drive the balance between mineral and metabolic constraints on C loss. Specifically, reducing conditions during flooding can mobilize previously protected C, but kinetic and thermodynamic constraints can prevent its subsequent depolymerization and oxidation, respectively.

Therefore, a closer look at spatial and temporal controls on floodplain C transformations will yield mechanistic understanding of the mineral and metabolic constraints on C storage and export. This dissertation research aims to determine the biogeochemical controls on C cycling in redox-dynamic floodplains, knowledge which will improve our ability to predict C loss in redox-dynamic floodplain environments. This research is guided by the central hypothesis that the relative importance of mineral and metabolic constraints on floodplain C export shifts with redox conditions, which are dictated by seasonal flooding dynamics and spatial variations in soil characteristics, both laterally with distance to the river and vertically with soil depth. Hypothesized patterns of C loss through leaching (DOC production) and microbial respiration (CO_2 production), and corresponding Fe species and moisture variables, are shown in Fig. 1.2 over a seasonal redox gradient experienced in typical mountainous floodplains.

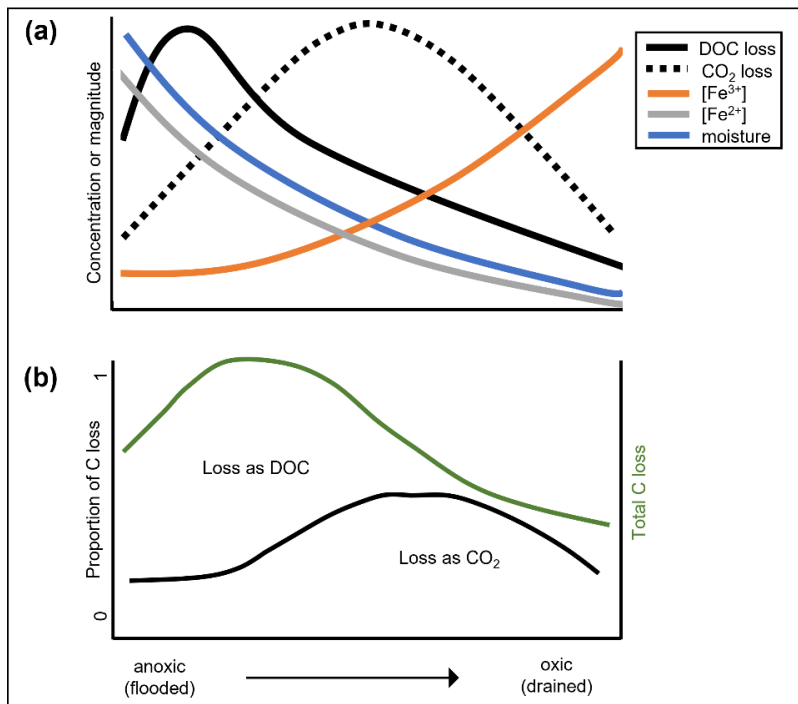


Figure 1.2. Hypothesized effects of redox on floodplain C losses, concentration of Fe species, and soil moisture over the season, from anoxic (flooded) to oxic (drained) conditions.

Importantly, we recognize that floodplains are heterogeneous systems that exist across climate zones with diverse underlying geology. Across floodplains—and even within a single floodplain—variation in water flow and sediment regimes drive differences in attributes such as erosion, deposition, and vegetation dynamics (Iskin and Wohl, 2023). While site-specific characteristics and measurements, and the variation thereof, are critical to informing specific management approaches, the mineral and metabolic mechanisms of C cycling outlined here are likely applicable to many floodplains across climates and geology.

Critically, changing snowmelt patterns in high-elevation floodplains will make these systems more vulnerable to increased floods and droughts (Diffenbaugh et al., 2013), potentially shifting floodplain soils from C sinks to sources and affecting soil fertility and downstream water quality. Because mountainous floodplains provide the majority of worldwide water resources (Viviroli and Weingartner, 2008), the potential mobility of C and metals in water with shifting climate patterns has implications for all who rely on this water. Thus, understanding the mechanisms driving C loss is important to predict the vulnerability of floodplain soils to climate change-related environmental perturbations. This knowledge has the potential to inform land management approaches by providing information about how dynamic flooding impacts the storage or export of carbon, nutrients, and metals, and to improve representation of redox-dynamic ecosystems in hydrologic and Earth System Models.

1.7 References

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CHAPTER 2

MOLECULAR NATURE OF MINERAL-ORGANIC ASSOCIATIONS WITHIN REDOX-ACTIVE MOUNTAINOUS FLOODPLAIN SEDIMENTS

2.1 Abstract

Floodplains are important terrestrial-aquatic interfaces that act as hotspots of organic carbon (OC) cycling, regulating ecosystem carbon storage as well as export to riverine systems. Within floodplain sediments, regular flooding and textural gradients interact to create dynamic redox conditions. While anaerobic protection of OC upon burial is a well-recognized carbon storage mechanism in redox-active floodplain sediments, the impact of protective mineral-organic associations is relatively unknown. Here, we determined the quantitative importance and chemical composition of mineral-organic associations along well-defined redox gradients emerging from textural variations and depth within meander sediments of the subalpine East River watershed (Gothic, CO). We characterized mineral-organic associations using a combination of sequential extractions, physical fractionation, and high-resolution mass spectrometry. Across the meander, we found that mineral-associated OC constitutes a meaningful fraction of total OC, and that extractable iron (Fe) and aluminum (Al) phases and high-density isolates were strongly correlated with total OC content, suggesting that mineral-organic associations are quantitatively important for floodplain sediment OC protection. Our mass spectrometry results showed OC associated with increasingly ordered Fe and Al phases are relatively enriched in low-molecular weight, oxidized, aromatic compounds. To our surprise, however, total OC content showed weak or no correlation with indicators of anaerobic protection, such as relatively

bioavailable OC pools (water-extractable and particulate OC) or the molecular weight and oxidation state of OC. Overall, this work highlights that mineral protection of OC bound to reactive mineral phases – in addition to anaerobic protection – can play a quantitatively important role in controlling soil carbon storage in redox-active floodplain sediments.

2.2 Introduction

Floodplains are important terrestrial-aquatic interfaces that can act as carbon sinks or sources, regulating ecosystem carbon storage within and export to riverine systems (D’Elia et al., 2017; Sutfin and Wohl, 2017). Globally, floodplain ecosystems are estimated to account for up to 8% of global soil organic carbon (OC) storage, despite covering only 1% of the land area surface (Sutfin et al., 2016). Each year, 0.25 Pg OC are transferred from floodplain to riverine systems, and eventually exported to oceans (Battin et al., 2008). OC stored within floodplains is increasingly precarious in the Anthropocene, as climate change and human alteration affect the timing and magnitude of flooding cycles (Syvitski et al., 2022). These changes are particularly pronounced in headwater systems of the mountainous western United States, with a current megadrought (Williams et al., 2022) foreshadowing further predicted declines in snow water equivalent (Siirila-Woodburn et al., 2021). To predict the impact of these drastic hydrological changes on OC retention and loss within floodplain sediments, specifically those in mountainous systems, a mechanistic understanding of the rate-controlling mechanisms is critical.

Seasonal flooding and the resulting depositional environment create a mosaic of floodplain sediments with varying characteristics and propensities to sequester OC. The

storage of sediment OC is temporally and spatially heterogeneous, depending on factors including grain size distribution, soil moisture dynamics, and biogeochemical processes (Wohl, 2021; Wohl and Pfeiffer, 2018). For example, seasonal flooding events result in particle sorting and accumulation of fine sediment, resulting in distinct textural patterns across floodplain meanders and with sediment depth (Sutfin et al., 2016). While sediment burial is a major factor governing OC storage in floodplains (Chaopricha and Marín-Spiotta, 2014; D'Elia et al., 2017; Lininger et al., 2019; Mayer et al., 2018), the mechanisms governing floodplain OC storage, and how burial interacts with texture, moisture, and biogeochemical gradients, are poorly understood.

In depositional floodplain sediments, textural gradients give rise to spatially dynamic redox conditions. Reducing conditions dominate both when sediments are saturated during flooding and in fine-textured sediments. It is commonly assumed that oxygen limitations experienced under reducing conditions are primarily responsible for OC preservation in the floodplain (Batson et al., 2015; Gurwick et al., 2008; Kroes and Hupp, 2010). The absence of oxygen imposes both kinetic and thermodynamic constraints on microbial activity and can limit depolymerization and respiration of organic compounds (Keiluweit et al., 2017, 2016), resulting in anaerobic protection of OC in the floodplain. Under anaerobic conditions, the activity of oxidative enzymes capable of catalyzing depolymerization reactions is restricted (Naughton et al., 2021), causing the preservation of complex particulate organic carbon (POC) (Keiluweit et al., 2017). Additionally, the degree of microbial respiration in anaerobic environments is not just dependent on the energetics of available terminal electron acceptors (e.g., Fe(III) or sulfate), but also on the oxidation state of the electron donor, i.e., the organic substrate

utilized by microbes (Boye et al., 2017). Boye et al. (Boye et al., 2017) found that in floodplain sediments, reduced organic compounds with nominal oxidation state of carbon values (NOSC) below a thermodynamic threshold that makes microbial respiration unfavorable are selectively preserved. The influence of such kinetic and thermodynamic constraints, here termed anaerobic protection, will likely vary along spatial redox gradients within floodplain environments, which can form within microsites, along textural transitions, or along the capillary fringe (Keiluweit et al., 2016; Lacroix et al., 2021; Naughton et al., 2021).

While anaerobic protection of OC within sediments has received widespread attention (Boye et al., 2017; Keiluweit et al., 2016; LaCroix et al., 2019; LaRowe and Van Cappellen, 2011), it is less clear how reactive metals influence OC preservation within floodplain environments. Associations with reactive minerals effectively protect organic compounds from microbial oxidation (Mikutta et al., 2007) and represent an important C storage mechanism in upland soil systems (LaCroix et al., 2019; Porras et al., 2017; Torn et al., 1997). Iron (Fe) and aluminum (Al) (oxy)(hydr)oxides (in the following abbreviated as “oxides”) are particularly important for the formation of mineral-organic associations (Kleber et al., 2021, 2015), especially in humid and/or redox-active systems (Coward et al., 2017; Kirsten et al., 2021; Rasmussen et al., 2018). This contrasts with relatively arid and alkaline systems, where organic complexes with calcium (Ca) can be an important OC stabilization mechanism (Rasmussen et al., 2018). Although the abundance of reactive Fe/Al/Ca phases can constitute a significant component of the mineral matrix in floodplain sediments (Chen et al., 2017; Darke and Walbridge, 2000; Wohl, 2021), their quantitative importance for OC retention has not been considered.

The importance of reactive metals in driving OC cycling likely shifts depending on the redox regime, because Fe, unlike Al and Ca, is redox-active (Anthony and Silver, 2020; Nitzsche et al., 2022; Possinger et al., 2020a). The stability of Fe-OC associations is impacted by redox oscillations: reducing conditions can lead to their disruption through reductive dissolution of Fe oxides and subsequent leaching (LaCroix et al., 2019); conversely, oxic conditions promote the formation of protective Fe-OC associations through precipitation reactions (Adhikari et al., 2017; Bhattacharyya et al., 2018, 2017; Tishchenko et al., 2015). In floodplains, it is expected that spatiotemporal variations in oxygen availability (and thus redox) as imposed by water table depth or textural gradients cause local variation in Fe reduction and oxidation, likely regulating the relative importance of Fe-OC associations (Chen et al., 2017; Mayer et al., 2018) as compared to protective associations between OC and Al or Ca (Das et al., 2019; Possinger et al., 2020a; Rasmussen et al., 2018). This is in contrast to systems dominated by organic soils, where pH plays an important role in OC storage: low pH values in acidic peatland soils have been shown to inhibit phenol oxidase activity (Kang et al., 2018) and methanogenesis pathways (Ye et al., 2012), for example.

In mineral-rich sediments, redox fluctuations alter both the degree of microbial OC transformation and mineral reactivity. Consequently, such fluctuations dictate the chemical nature of mineral-associated OC (MAOC) that might form. For example, plant-derived aromatic OC preferentially binds to increasing short-range order mineral content (Coward et al., 2018a; Hall et al., 2018; Kramer et al., 2012) in tropical and volcanic soils, which may be stabilized on Fe oxide surfaces during redox fluctuations (Bhattacharyya et al., 2018). However, because redox fluctuations affect the crystallinity

of Fe oxides (Bhattacharyya et al., 2022, 2018; Thompson et al., 2006), we expect that MAOC composition varies in redox-active soils. For example, in humid tropical soils which undergo frequent redox oscillations, Coward *et al.* found that varying Fe mineral phases, as determined by sequential extraction, associated with unique OC compounds (Coward et al., 2018a). Together, these observations suggest that the chemical composition of mineral-organic associations in floodplains will vary with redox regime.

In this study, we determined the quantitative importance and chemical composition of mineral-organic associations within redox-active subalpine floodplain sediments. To assess the impact of redox conditions on the characteristics of mineral-organic associations, we sampled along well-defined redox gradients emerging from textural variations and depth within an active floodplain meander of the East River (Gothic, CO). This study area is representative of headwater catchments in the Upper Colorado Basin, an area that supplies water resources to a large portion of the Western United States (Hubbard et al., 2018). We quantified the amount of mineral-associated OC (MAOC) using physical density fractionation and chemical extractions. To characterize the chemical composition of MAOC, we used a combination of selective dissolution with subsequent analysis by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Finally, we compared abundance and chemical composition of bioavailable OC and MAOC to infer the relative importance of mineral protection versus anaerobic protection on floodplain C cycling. We hypothesized that the chemical composition of mineral-organic associations is dictated by changes in mineral reactivity across redox gradients, and that the quantitative importance of MAOC will be lowest within more reducing environments.

2.3 Materials and Methods

2.3.1 Field site description

We established a sampling transect across an active meander of the East River floodplain (Gothic, CO; Fig. 2.1). This location was chosen as it is a representative meander of the subalpine region of the East River watershed, and has been studied extensively in terms of geochemical dynamics (Dong et al., 2020; Dwivedi et al., 2018) and carbon chemistry (Fox et al., 2020). Sediment profiles across the transect consisted of fine-grained material (< 2 mm) overlaying more coarse-grained alluvium, all underlain by Mancos shale (Fox et al., 2020). Floodplain vegetation included dwarf shrubs (e.g., American dwarf birch and mountain willow), potentilla, and grasses (Falco et al., 2019). Water table depth was calculated using pressure transducers (Hobo U20L-04) installed along the sampling transect (Dafflon and Dwivedi, 2020): over the course of seasonal flooding, the water table declined by 0.4 m at the near river position and 0.9 m at the mid-meander position (Table A.1). Measurements of soil moisture (Acclima TDR-315L) and redox potential (in-house platinum-tipped electrodes) were taken at the same sampling locations and depths in 2019, a year that experienced similar levels of river discharge and subsequent flooding as 2017 (Fig. A.1).

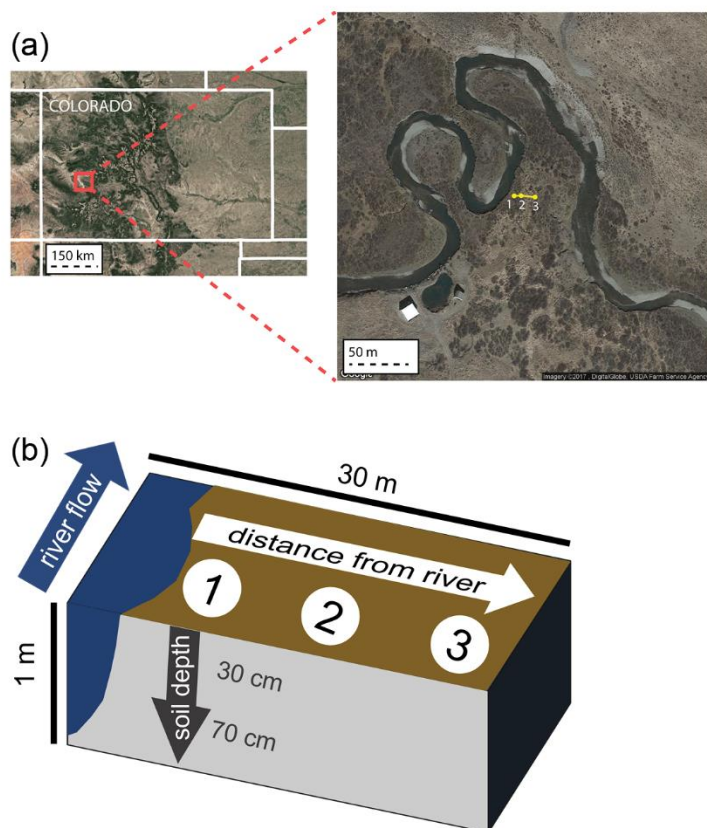


Figure 2.1. (a) Location of East River sampling transect in Colorado, and (b) schematic of the transect across the meander with distance from the river (positions 1, 2, and 3 representing near river, intermediate, and mid-meander positions, respectively) and sampled depths (30 cm and 70 cm). East River discharge values and corresponding sediment moisture and redox values are provided in Supporting Information (Fig. A.1, Table A.1).

2.3.2 Sediment sampling

We collected sediment samples in triplicate in June 2017 at three positions along the sampling transect, at shallow (30 cm) and deep depths (70 cm) (Fig. 2.1, Table A.2); these depths were chosen based on visual assessment of sediment redoximorphic features during field sampling. Sediments were air-dried and sieved to 2 mm. Sediments across the transect dry out to 27-59% volumetric water content upon drainage in September (Table A.1); thus precipitation of redox-active metal phases may have occurred during

sample processes and the data reported here likely represent the state of mineral-organic associations present within the sediments during fully drained, oxic periods spanning from mid-summer to the following spring. No unexpected or unusually high safety hazards were encountered in the sampling and subsequent analysis of sediment samples.

2.3.3. Bulk sediment characterization

We determined total sediment carbon and nitrogen in triplicate using an ECS 4010 CHNS-O elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA). We measured total metals (Fe, Al, Ca, Si, etc.) on finely ground samples using X-ray fluorescence (Spectro Xepos HE XRF Spectrometer). We determined sediment texture after loss on ignition to remove organic matter (4 h at 550 C) by a laser diffraction particle size analyzer (Beckman Coulter LS 13 320, Indianapolis, IN). We estimated root biomass by picking out coarse roots from air dried sediments. Analytical error was orders of magnitude lower than the variance among replicates, and thus only standard error of the means of replicates are reported.

2.3.4 Density fractionation and carbon content

To isolate organic matter of decreasing bioavailability (e.g., particulate to mineral-associated organic matter), we used a density fractionation procedure modified from the literature (Hatton et al., 2012; Keiluweit et al., 2018). Briefly, two sodium polytungstate (SPT) solutions were prepared in DI water at the following densities: 1.6 g cm³ and 2.2 g cm³ (solutions #1 and #2, respectively). Particulate organic matter was determined by adding and gently vortexing 30 ml of solution #1 and 7.5 g of sediment, shaking for 2 h at

low speed, and centrifuging for 1 h at 1000 x g. The supernatant was filtered (0.8 μm , Millipore), rinsed thoroughly with DI water to remove excess SPT, and the filter was oven-dried at 60 C and weighed to determine the amount of particulate organic matter. To determine the intermediate density fraction (> 1.6 and $< 2.2 \text{ cm}^3$), 30 ml of solution #2 was added to the remaining sediment pellet, and the above steps were repeated. The final density fraction ($> 2.2 \text{ g cm}^3$) was determined by thoroughly cleaning the remaining sediment pellet with DI water. 30 ml of DI water was added and vortexed until the pellet was suspended, then centrifuged for 1 h at 2000 x g. The supernatant was removed, and this process was repeated three times. The dry mass of the remaining sediment pellet was recorded. The intermediate and heavy fractions together comprised the MAOC pool. We determined carbon and nitrogen content of density fractions in triplicate as described above for bulk sediment.

2.3.5 Sequential extractions

To selectively dissolve mineral phases of varying degrees of order and their associated organic matter, sediments were extracted sequentially with four reagents (Fox et al., 2017; Heckman et al., 2018; Wagai and Mayer, 2007). After each extraction, the supernatant was filtered using 0.22 μm filters (Sigma-Aldrich). Briefly, 0.33 g sediment was extracted with 10 ml deionized (DI) water to extract bioavailable phases of minerals and organic matter (shaken 2 h at 200 rpm, centrifuged 20 min at 510 x g); this extraction represented water-extractable organic carbon (WEOC). The same pellet was then extracted with 10 ml of 0.1 M sodium pyrophosphate to target amorphous metal-organic complexes (Heckman et al., 2018; Kaiser et al., 2016) (shaken 16 h at 200 rpm,

centrifuged 1 h at 4000 x g) and combined with the filtered extract of a 6.7 ml DI water rinse (shaken 2 h at 200 rpm, centrifuged 1 h at 4000 x g). Then, 10 ml of 0.5 M hydrochloric acid (HCl) was added to the remaining pellet to target short-range order phases (Fox et al., 2017) (shaken 4 h at 200 rpm, centrifuged 20 min 4000 x g) and combined with the filtered extract of a 6.7 ml DI water rinse (shaken 2 h at 200 rpm, centrifuged 20 min at 510 x g). Lastly, 10 ml of 0.049 M sodium dithionite was added to the pellet to target total reducible oxide phases (Wagai and Mayer, 2007) (shaken 16 h at 200 rpm, centrifuged 20 min at 510 x g) and combined with the filtered extract of a 6.7 ml of 0.05 M HCl rinse (shaken 1 h at 200 rpm, centrifuged 20 min at 510 x g).

2.3.6 Metal and TOC analysis of sequential extractions

Extracts were analyzed for metals using Microwave Plasma-Atomic Emission Spectrometry (Agilent, Santa Clara, CA), diluting to a final concentration of 2% nitric acid. Extracts were analyzed for total organic carbon using a TOC-L Analyzer (Shimadzu, Kyoto, Japan), diluting in DI water.

2.3.7 Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) analysis of sequential extractions

Extracts were analyzed for organic matter composition using a 12 Tesla Bruker Solarix FTICR spectrometer located at the Environmental Molecular Science Laboratory in Richland, WA. Samples were first desalted and concentrated using solid phase extraction (Dittmar et al., 2008) with bond elute PPL cartridges. The analyte was eluted from the PPL cartridge in HPLC grade methanol and introduced directly to a standard

Bruker electrospray ionization source operating in negative mode. The instrument was externally calibrated weekly to a mass accuracy of < 0.1 ppm using a tuning solution from Agilent. The ion accumulation time varied to account for differences in C concentration between samples. A Suwannee River Fulvic Acid standard, obtained from the International Humic Substance Society, was used as a control to monitor potential carryover from one sample to another and ensure instrument stability in day-to-day operation. The instrument was flushed between samples using a mixture of Milli-Q water and HPLC grade methanol. One hundred and forty-four individual scans were averaged for each sample and internally calibrated using OM homologous series separated by 14 Da ($-\text{CH}_2$ groups). The mass measurement accuracy was < 1 ppm for singly charged ions across a broad mass-over-charge (m/z) range (i.e., $100 < m/z < 1100$). The mass resolution was 350,000 at 339.112 Da. BrukerDaltonik data analysis software (version 4.2) was used to convert raw spectra to a list of m/z values applying FT-MS peak picker module with a signal-to-noise ratio threshold of 7 and absolute intensity threshold of 100. Formularity software (Tolić et al., 2017) was used to assign chemical formulae based on the criteria previously described (Tfaily et al., 2018). Peaks were filtered to only include masses between $200 < m/z < 900$. Compounds were grouped into molecular classes (e.g., lipid-, protein-, carbohydrate-, lignin-, tannin-, condensed aromatic-like) based on C, H, and O for H/C and O/C ranges according to previous literature (Bailey et al., 2017). Each assigned molecular formula was used to calculate the nominal oxidation state of C (NOSC), double bond equivalence (DBE), and aromaticity index (AI) as in previous work (LaCroix et al., 2019).

2.3.8 Statistical analysis

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022). We used Shapiro-Wilk and Levene's tests to assess normality and equality of variances for all data. Parametric tests were used in cases in which data met assumptions for normality. To test our hypotheses, we grouped by sediment depth and assessed differences among meander positions or extractions using analysis of variance (ANOVA), combined with Tukey's honestly significant difference *post hoc* test ($\alpha = 0.05$ indicates significant differences), using the R package *agricolae* (Mendiburu and Yaseen, 2021). Specifically, we compared values within surface (30 cm) and deep (70 cm) sediments across the lateral meander transect from near river to mid-meander locations (Fig. 2.1) and among extractions. We performed linear regressions to determine relationships between sediment C and sediment properties ($\alpha = 0.05$ indicates significant differences).

2.4 Results and Discussion

2.4.1 Physical and chemical characteristics of meander sediments dictate redox regime

Along the meander transect and with depth, spatial variations in sediment texture, moisture, and root C inputs resulted in pronounced redox gradients. Clay and silt content was 53% greater in shallow (30 cm, 89.6%) than deep sediments (70 cm, 58.7%), and in deep sediments the clay and silt content was 105% greater at the meander center (79.6%) compared to the near river position (39.9%) (Table 2.1). Due to the finer texture in the meander center, the moisture content remained higher across both depths (65-67% volumetric water content, VWC) compared to the near river position (43%- 47% VWC)

during early season flooding (Table A.1). As a result of these texture and moisture patterns during flooding, the mid-meander position experienced lower redox potentials at both depths (-180 to 240 mV), while the coarse textured and well-drained near-river position remained relatively oxic (9 to 355 mV, Table A.1). Root biomass, used here as a proxy for root C inputs, decreased by 85% from shallow (1510.7 mg) to deep sediments (220.3 mg), with no significant differences among the meander positions (Table 2.1). Total metals varied significantly across the meander, but not with depth: total Fe in the mid-meander position (3.9%) was 22% greater than the near river position (3.2%), while total Ca in the near river position (2.3%) was 156% higher versus the mid-meander position (0.9%). There were no significant trends in total Al. Sediment pH was 11% greater in the near river position (7.9) than mid-meander position (7.1), and 13% greater in the deep (7.8) compared to shallow horizons (6.9) (Table 2.1).

Table 2.1. Physical and chemical characteristics of meander sediments (mean +/- standard error), across meander position and depth. Bulk metals included iron (Fe), aluminum (Al), and silicon (Si). Density isolates included particulate organic carbon (POC) and mineral-associated organic carbon (MAOC).

Position	Depth (cm)	Sand (%)	Silt + clay (%)	pH	Root biomass (mg)	Bulk Fe (%)	Bulk Al (%)	Bulk Ca (%)	Bulk Si (%)	POC (mmol C / g)	MAOC (mmol C / g)
Near river	30	13.9 ± 0.8	6.1 ± 0.8	7.3 ± 0.02	2011 ± 932	3.07 ± 0.08	4.97 ± 0.11	2.22 ± 0.08	23.91 ± 0.49	4.28 ± 1.22	3.88 ± 0.59
	70	61.1 ± 6.0	8.9 ± 6.0	8.3 ± 0.08	309 ± 183	3.34 ± 0.18	5.60 ± 0.04	2.42 ± 0.07	26.55 ± 0.09	2.44 ± 0.29	1.24 ± 0.21
Intermediate	30	8.7 ± 1.1	1.3 ± 1.1	6.5 ± 0.03	460 ± 84	3.25 ± 0.09	5.29 ± 0.08	1.25 ± 0.10	24.43 ± 0.57	3.41 ± 1.23	4.81 ± 0.53
	70	42.3 ± 9.1	7.7 ± 9.1	7.7 ± 0.10	128 ± 71	3.32 ± 0.12	5.72 ± 0.09	1.58 ± 0.12	26.89 ± 0.40	1.45 ± 0.02	1.18 ± 0.10
Mid-meander	30	8.6 ± 0.3	1.4 ± 0.3	6.7 ± 0.11	2062 ± 1705	3.75 ± 0.07	5.56 ± 0.11	1.01 ± 0.08	25.77 ± 0.55	3.40 ± 0.51	3.66 ± 0.73
	70	20.4 ± 2.0	9.6 ± 2.0	7.5 ± 0.02	223 ± 76	4.15 ± 0.27	5.30 ± 0.43	0.71 ± 0.01	25.55 ± 1.47	0.95 ± 0.12	0.93 ± 0.06

2.4.2 Mineral-associated carbon pools vary spatially across the meander

Total OC, as well as POC, MAOC, and root biomass, were significantly greater in the surface compared to deep sediments (Fig. 2.2a, Table 2.1, $p < 0.05$). We did not see a significant shift in root biomass across the floodplain meander (Table 2.1). However, contrary to our original hypothesis, total sediment C and POC significantly declined towards the finer textured, more reducing meander center, a trend particularly pronounced with depth (Fig. 2.2a, $p < 0.05$). Burial of POC and subsequent kinetic and thermodynamic protection due to limited oxidative depolymerization are common mechanisms of floodplain sediment C storage (Chaopricha and Marín-Spiotta, 2014; D'Elia et al., 2017; Lininger et al., 2019; Mayer et al., 2018; Naughton et al., 2021). If such protection mechanisms were dominant, we would expect the highest sediment OC and POC contributions in the more reducing meander center; however, we find both pools lowest at the meander center compared to the relatively oxic near-river position. This finding suggests that kinetic and thermodynamic constraints do not solely explain the patterns of C storage across the meander.

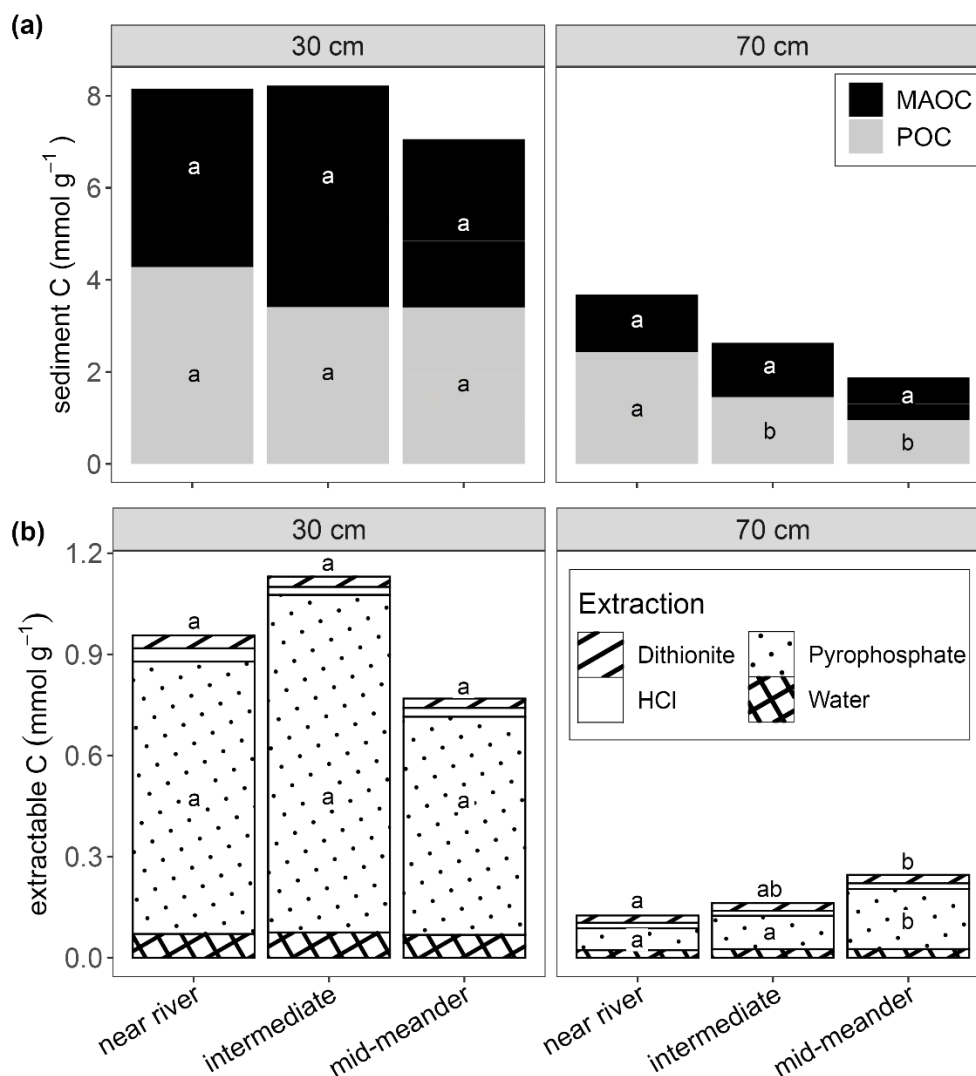


Figure 2.2. Sediment OC in shallow (30 cm) and deep (70 cm) sediment, across the floodplain meander in (a) bulk pools, separated by mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) (denoted by black and gray shading, respectively), and (b) extractable pools, separated by extraction (denoted by pattern fills). Units for both panels are mmol C per g sediment. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences along the meander in each depth: differences in MAOC and POC in panel (a), and differences in total extractable OC and pyrophosphate-extractable OC in panel (b). Mean and standard errors are reported in Table 2.1 for bulk pools, and in Table A.3 for extractable OC.

Our proxies for MAOC better predict the spatial distribution of total OC across the meander than POC. Notably, in deep sediments, the relative contribution of MAOC (isolated by density fractionation) compared to POC increased by 38% towards the center of the meander (Fig. 2.2a, $p < 0.05$). Similarly, the total amount of extractable OC (sum of water, pyrophosphate, HCl, and dithionite extractable OC) increased in the deep sediment at the mid-meander position (Fig. 2.2b, $p < 0.05$). Both independent lines of evidence indicate that mineral protection becomes relatively more important towards the finer-textured, more reducing meander center, and especially in deep sediments. This finding is counter to our expectation that mineral protection, particularly via bonds to Fe oxides, would decrease towards the more reduced deeper sediments of the meander center due to microbial Fe reduction (Chen et al., 2017). Rather, this suggests that mineral-organic associations play an important role in C storage, even in temporarily reduced locations of the floodplain meander.

Sequential extractions further highlight the importance of Fe and Al oxides in driving patterns of OC accumulation across the meander. Along the floodplain meander, total extractable Fe and Al concentrations as well as MAOC isolated by density fractionation strongly and significantly correlated with sediment C content (Fig. 2.3, $p < 0.05$). Extractable Ca, which can effectively protect OC in alkaline soils (Rasmussen et al. 2018), only significantly correlated with OC content in the more alkaline deep soils (Fig. A.2a, $p < 0.05$). In contrast, clay content did not significantly correlate with total sediment C content (Fig. A.2b). Our results therefore suggest that mineral protection of OC in our floodplain sediments is due to pedogenic (extractable) Fe and Al oxides, and Ca-organic complexes at depth, rather than phyllosilicates.

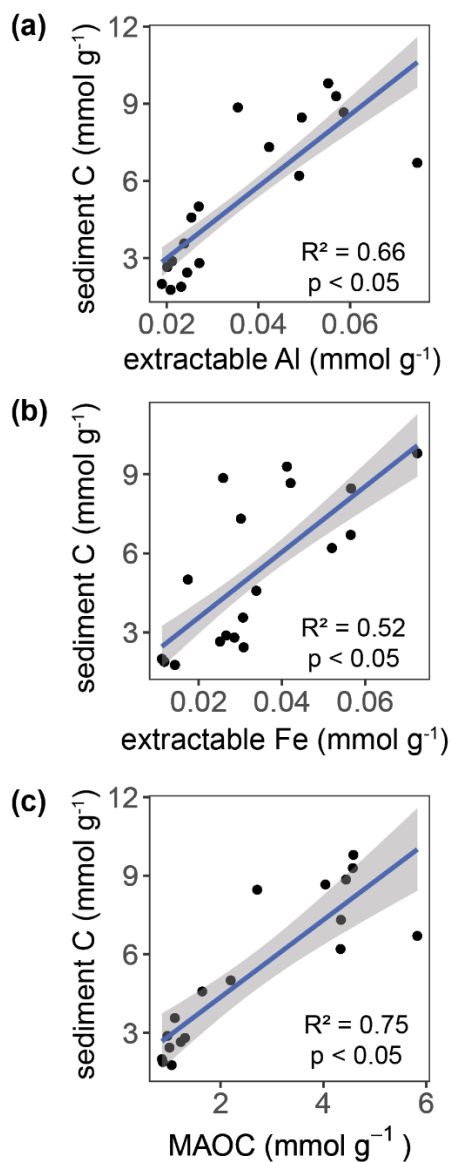


Figure 2.3. Correlations between sediment OC and (a) extractable aluminum (Al), (b) extractable iron (Fe), and (b) mineral-associated organic carbon (MAOC). Units for all panels are mmol C per g sediment. The blue line is the linear regression and statistics are provided, including the R^2 and p-value for model significance.

Total extractable OC was higher in surface compared to deep sediments and increased towards the meander center in the deep sediments (Fig. 2.2b, $p < 0.05$).

Pyrophosphate extracted significantly more OC than other extractants across both depths and all meander positions (Fig. 2.2b, $p < 0.05$). Comparison of the OC pools extracted

during selective dissolutions shows that in deep sediments, pyrophosphate-extractable OC is principally responsible for the significant increase in total extractable OC towards the meander center (Fig. 2.2b, $p < 0.05$). This increase in the contribution of pyrophosphate-extractable OC towards the more reducing meander center suggests protection of C within metal-organic complexes (Heckman et al., 2018; Wagai et al., 2013), although this pool might include nanoparticulate metal oxide phases (Regelink et al., 2014) and base-extractable organic matter (Heckman et al., 2018). Together, these results show that OC associated with extractable metals explains total sediment C distribution across the floodplain better than POC, signifying the quantitative importance of mineral-organic associations for C storage within floodplain sediments.

2.4.3 Changing composition of mineral-organic associations across redox gradients

Detailed examination of the extractable metal pools yielded further insights into the impact of mineralogical composition on MAOC pools across the floodplain meander. Shallow sediments contained greater amounts of extractable Fe and Al compared to deep sediments (Fig. 2.4, $p < 0.05$), which predominantly reside in less extractable (HCl and dithionite) pools. Similarly, the relative abundance of HCl- and dithionite-extractable Fe and Al pools was significantly greater near the river than in the meander center (Fig. 2.4, $p < 0.05$; except dithionite-extractable Al: $p = 0.052$). The dominance of these pools in sediments at the surface and close to the river are suggestive of the formation and preservation of more crystalline Fe oxides phases in the oxic meander domains. In contrast, although the total extractable Fe concentrations declined toward the meander center, and total extractable Al remained constant, pyrophosphate-extractable Fe and Al

concentrations increased towards the meander center, especially at depth (Fig. 2.4, $p < 0.05$). These results suggest that the more intense redox cycling in the meander center (Table A.1) may have caused the loss of more crystalline Fe and Al phases but maintained a larger pool of short-range order Fe and Al phases that may be subject to repeated dissolution and precipitation reactions. We suggest further research examine the impact of redox oscillations on the mobilization of reactive metal phases in floodplain sediments.

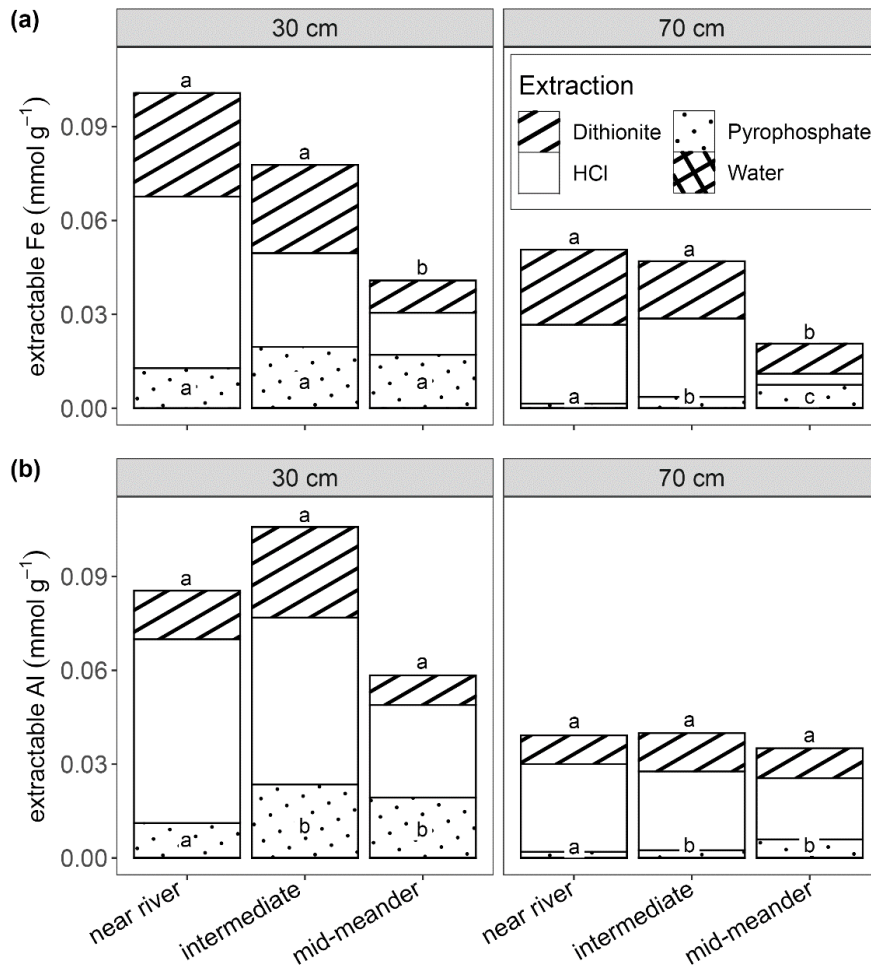


Figure 2.4. Composition of (a) extractable iron (Fe) and (b) extractable aluminum (Al) in shallow (30 cm) and deep (70 cm) sediment, across the floodplain meander. Extractable pools are denoted by pattern fills. Units for both panels are mmol metal per g sediment. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences along the meander in each depth: differences in total extractable Fe and pyrophosphate-extractable Fe in panel (a), and differences in total extractable Al and pyrophosphate-extractable Al in panel (b). Mean and standard errors are reported in Table A.4 for extractable Fe and Table A.5 for extractable Al.

In both surface and deep sediments, total extractable Fe decreased towards the meander center, whereas total extractable Al remained relatively constant across the meander (Fig. 2.4, $p < 0.05$). Consequently, the ratio of extractable Al/Fe increased by 93% from the near river position (0.82 ± 0.03) towards the meander center (1.58 ± 0.09 , Fig. A.3). These results suggest a relative enrichment of Al phases in the more reducing meander center, potentially due to loss of Fe oxides minerals via reductive dissolution (“gleying”). In deep sediments, while the pyrophosphate-extractable Al/Fe ratio decreased towards the meander center, the HCl-extractable Al/Fe ratio increased (Fig. A.3). This result suggests that, at least in deep sediments, short-range order phases are relatively enriched in Fe, while relatively ordered phases are enriched in Al. Amorphous Al oxides have been shown to strongly sorb and thus protect OC (Schneider et al., 2010), particularly in redox-active systems (Darke and Walbridge, 2000). Notably, similar shifts from Fe- in oxic domains to Al-protected OC in anoxic domains have been observed in soils experiencing seasonal flooding (LaCroix et al., 2019; Nitzsche et al., 2022; Possinger et al., 2020a) or contrasting precipitation (Inagaki et al., 2020).

Together, while the overall abundance of reactive Fe and Al phases declined towards the more redox-active meander center, we observed a relative increase in the abundance of water-extractable and pyrophosphate-extractable Fe and Al phases in comparison to the near river position. The greater abundance of such dissolved and short-range order Fe phases is likely a product of the more intense redox cycling that occurs here, which prevents formation of more crystalline phases due to repeated reduction cycles and export (Ginn et al., 2017; Winkler et al., 2018). The concurrent decline in extractable Fe relative to Al suggests a redox-induced preferential loss of Fe phases and

effective protection of remaining C within associations with Al phases. We acknowledge that the composition of mineral-organic associations in floodplain sediment is a function both of their original composition upon deposition, and of soil forming processes after deposition (e.g., redox cycling); however, in this study we are unable to tease these processes apart and we suggest that future research focus on these changes.

2.4.4 Imprints of mineral versus anaerobic protection on organic matter composition

To assess variations in OC composition along the floodplain meander, we used high-resolution mass spectrometry (FT-ICR-MS) on our sequential extractions. Our results confirm our hypothesis that variable mineral characteristics and contrasting redox environments impact the composition of relatively accessible WEOC and less accessible MAOC pools. Across both depths, WEOC had significantly lower NOSC values and significantly higher molecular weights compared to other extracts (Fig. 2.5a and b, $p < 0.05$), suggesting that this relatively accessible OC has undergone less depolymerization and oxidation than OC protected within associations to minerals, potentially due to kinetic and thermodynamic constraints on its decomposition (Boye et al., 2017; Naughton et al., 2021). With each extraction step, the NOSC of extracted OC consistently increased while molecular weight decreased (Fig. 2.5a and b, $p < 0.05$). This result indicates that MAOC becomes more oxidized and depolymerized the less extractable (and presumably more ordered) the Fe and Al phases become. WEOC contains relatively more lipid-, amino sugar-, and protein-like compounds compared to MAOC (Fig. 2.5c), suggesting that WEOC is more microbial in nature (i.e., more microbial metabolites and extracellular enzymes) (Guigue et al., 2016; Malik et al., 2016). With each subsequent extraction step,

the co-extracted OC becomes relatively more enriched in condensed hydrocarbon-, lignin-, and tannin-like compounds (Fig. 2.5c), suggesting that plant-derived aromatic compounds accumulate in association with increasingly stable Fe and Al oxides. These observations are consistent with evidence that low-molecular weight, oxidized, aromatic compounds preferentially bind to positively charged surfaces of Fe and Al oxides (Coward et al., 2018a; Kramer et al., 2012; Possinger et al., 2020b). While our results are supported in other studies, it is important to note that extraction conditions may alter SOM and thus impact downstream FT-ICR-MS analyses (Lv et al., 2020).

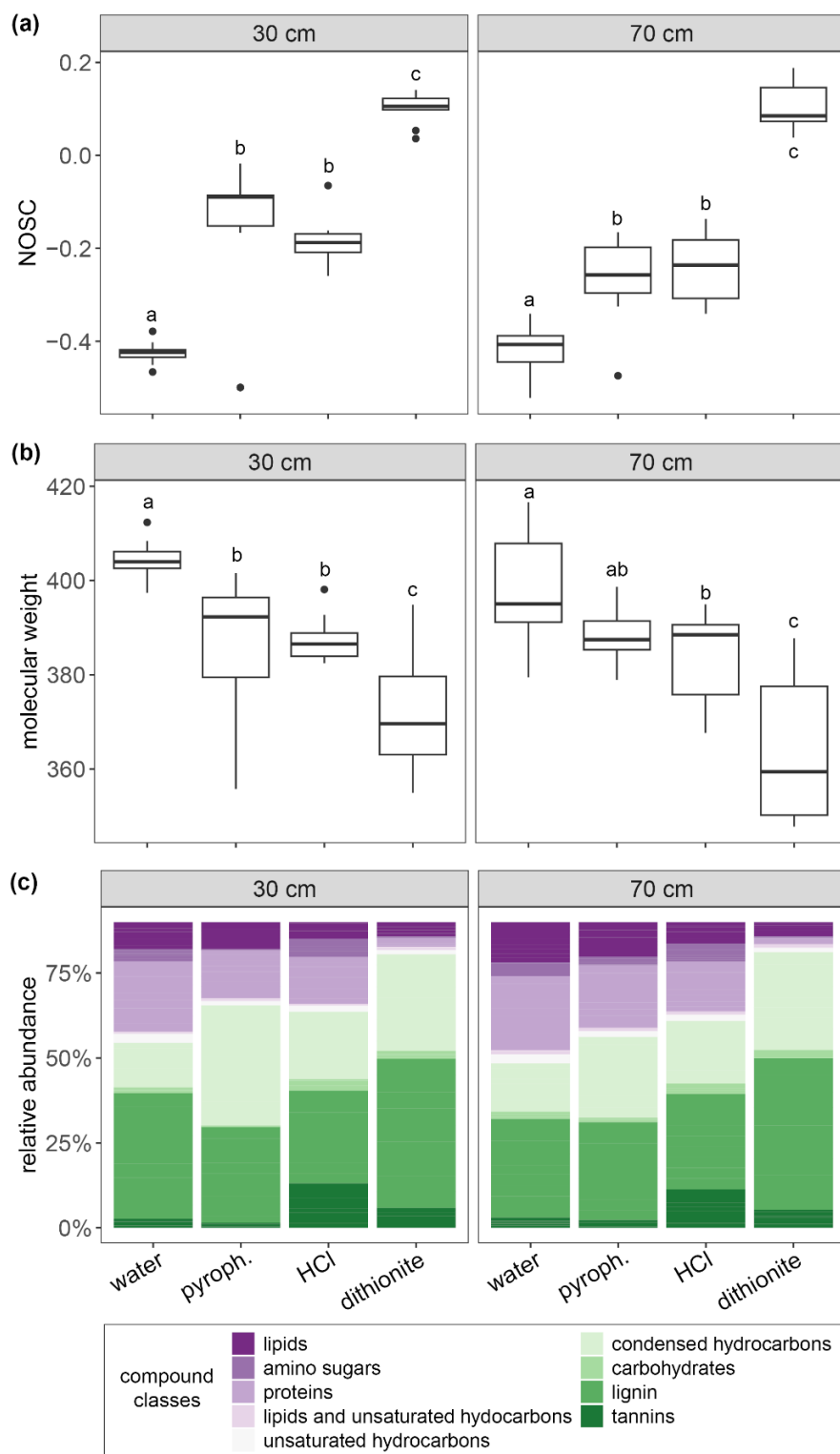


Figure 2.5. Composition of extracted organic matter in shallow (30 cm) and deep (70 cm) sediment: (a) nominal oxidation state of carbon (NOSC), (b) molecular weight, and (c) compound classes by extractant (i.e., water, pyrophosphate, HCl, and dithionite). Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across extractions in each depth.

We also found that the chemical composition of OC changed with sediment depth, specifically in the pyrophosphate-extractable pool that accounted for the largest portion of MAOC (Fig. 2.2b). As discussed above, OC co-extracted by pyrophosphate represents metal-organic complexes, although it possibly also includes a significant fraction of base-extractable OM (Heckman et al., 2018) or nanocrystalline phases (Regelink et al., 2014). Compared to shallow sediments, pyrophosphate-extractable OC in deep sediments consistently had lower NOSC (Fig. 2.5b) and aromaticity (double-bond equivalence, DBE; aromaticity index, AI, Fig. A.4), relatively more lipid-, amino sugar-, and protein-like compounds (Fig. 2.5c), and a higher proportion of N-, P-, and S-containing compounds (Fig. A.5). Furthermore, sediment C/N ratios also decreased with depth, especially towards the meander center (Fig. A.6), which can be indicative of greater microbial processing (Kramer et al., 2012; Wang et al., 2021). Together, these observations suggest that OC associated with amorphous metal complexes in deep sediments may have a stronger microbial signature compared to shallow sediments.

While lower NOSC values at depth possibly indicate that anaerobic protection (i.e., kinetic and thermodynamic constraints) is a stronger control on OC storage in deeper sediments than in shallow sediments, we did not find evidence that anaerobic protection becomes stronger in the more reducing meander center. Rather than decline towards the more reduced meander center, NOSC of pyrophosphate-extractable OC increased towards the center of the meander (Fig. A.7), albeit not significantly, suggesting a limited role for anaerobic protection in explaining the observed OC accumulation in this pool (Fig. 2.2b). Because, as discussed above, MAOC (Fig. 2.2) and pyrophosphate-extractable metals (Fig. 2.4) increased towards the meander center, these results indicate

that mineral protection via metal-organic complexes, rather than solely anaerobic protection, drives OC accumulation patterns along this floodplain meander transect. In sum, these results suggest that mineral protection of OC in metal-organic complexes imprints on its composition in redox-dynamic floodplain sediments.

2.5 Synthesis

2.5.1 Dynamic nature of mineral-organic associations along floodplain meander

Storage of OC within floodplain sediments is often attributed to constraints on microbial metabolism under reducing conditions, with oxygen limitations restricting both oxidative depolymerization and aerobic respiration, thus protecting OC anaerobically. However, redox transitions experienced by hydrodynamic floodplain environments also impact mineral transformations, with relatively unknown consequences on the importance of mineral-organic associations in OC storage. Our detailed investigation of defined redox gradients emerging from textural variations and depth offers important insights on the quantitative importance and chemical nature of mineral-organic associations within floodplain sediments.

Overall, we found a strong correlation between total sediment C and different proxies for MAOC (isolated by density fractionation and sequential extraction), suggesting that mineral-organic associations contribute to OC storage within the sediments of this mountainous floodplain. The fact that extractable Fe and Al, rather than clay content, predicted OC content across the meander points to a critical role of Fe and Al oxides in the formation of mineral-organic associations within these floodplain

sediments. While the importance of Fe and Al oxides for OC storage in upland soils (Wagai and Mayer, 2007) and even marine sediments (Lalonde et al., 2012) has long been established, our results support the notion that mineral protection, in addition to anaerobic protection, is also critical for OC storage in floodplain sediments with high mineral content. To this end, we acknowledge that our findings are limited to floodplains similar in nature to the East River: those with seasonal flooding, mineral-rich soils, and circumneutral pH. In floodplains and wetland systems dominated by organic soils and low pH values, such as peat-dominated wetlands, the mechanisms of OC storage may differ and include OC protection in anaerobic conditions (Wilmoth et al., 2021) or by leached phenolics from woody debris when the water table lowers (Fenner and Freeman, 2020), for example. We further note that since our study focused on a single sampling transect along a floodplain meander of the East River, our conclusions remain limited in scope to this spatial scope.

Interestingly, the chemical nature of MAOC showed no significant spatial variations across the meander but rather varied with crystallinity of the Fe and Al phases: with increasing crystallinity, we saw greater contributions of oxidized, low-molecular weight, aromatic compounds (Fig. 2.5). These results contrast with longer-term studies that examine the turnover of OC (Hall et al., 2018; Kramer et al., 2012), perhaps because of loss of more reactive short-range order phases in redox oscillating soils over longer timescales. In contrast, the absolute amount of MAOC showed significant spatial variations (Fig. 2.2). MAOC concentrations were greatest in the relatively oxic surface horizons, coinciding with greater contents of less extractable, and presumably more ordered, Fe and Al oxides (Fig. 2.4). In deeper sediments, despite the lower amounts of

MAOC, the relative contribution of MAOC to total sediment OC increased towards the more reducing meander center (Fig. 2.2a). This increase in the apparent importance of mineral protection (relative to anaerobic protection, as discussed above) in the meander center coincided with increases in the OC pool bound in amorphous metal-organic complexes. This result suggests that while the overall pool of extractable oxides declines (Fig. 2.4), a greater portion of the OC is sequestered in short-range order or amorphous metal-organic complexes (Fig. 2.2b). Given the concurrent increases in deep sediment clay content (Table 2.1) and pyrophosphate-extractable metals (Fig. 2.4), it is possible that nano-particulate Fe/Al oxide phases or phyllosilicates may also play a role in OC protection, although we did not directly measure phyllosilicate content.

Examination of the relative importance of mineral and anaerobic protection along redox gradients within the floodplain sediments yielded unexpected results. We initially hypothesized anaerobic protection to be most dominant in the more reduced meander sediments (Boye et al., 2017), resulting in greater OC concentrations. Instead, sediment OC concentrations were significantly lower in the fine-grained, reduced center of the meander and increased in the more oxic sediments near the river (Fig. 2.2a). If burial and subsequent anaerobic protection on floodplain C solely explained the C patterns along the floodplain meander, we further expected more relatively undegraded particulate or reduced OC to accumulate to be more prevalent in the reduced meander center. However, in the deep sediments, we found that POC declined towards the more reduced portion of the meander, while water-extractable OC and NOSC remained relatively constant. Conversely, we found that the relative contribution of MAOC increased towards the more reduced center of the meander (Fig. 2.2). Together, these results suggest that the relative

importance of mineral protection increased towards the reduced meander center, while that of anaerobic protection remained steady or diminished. While our study was qualitative in nature, future studies should consider quantifying the contribution of mineral versus anaerobic protection to floodplain sediment OC storage.

The prevalence of metal-organic complexes could be attributed to the fact that the meander center experiences more dramatic and persistent reducing conditions with seasonal flooding compared to sediments near the river (Table A.1). Reducing conditions prompt reductive Fe oxide dissolution and leaching of dissolved Fe from the system (i.e., gleying) (Bhattacharyya et al., 2018; Chen et al., 2017), consistent with the loss of extractable Fe (and Al) phases observed here. While the sediments become reoxygenated every season, the oxidation-reduction cycles may be dramatic enough to prevent formation of crystalline Fe oxides as has been observed in humid tropical soils that experience redox heterogeneity due to frequent rainfall (Bhattacharyya et al., 2018; Chen et al., 2018). Instead, repeated oxidation-reduction cycles in response to flooding may only allow for short-range order Fe (and Al) phases to form and persist (Ginn et al., 2017; Winkler et al., 2018). Because those short-range order Fe phases are likely dissolved and re-precipitated during each oxidation-reduction cycle, their presence may indicate that MAOC within such metal-organic complexes may not be as protected as the MAOC bound to crystalline Fe and Al oxides within the more oxic meander sediments. It is also possible that redox cycling increases the formation of reactive oxygen species, which can also stimulate organic matter decomposition (Hall and Silver, 2013), and we suggest that further studies of carbon cycling in dynamic floodplains should incorporate this mechanism.

2.5.2 Environmental Implications

Together, our results support the notion that mineral-organic associations are more important for OC storage in redox active floodplain environments than previously recognized and may act in combination with anaerobic protection to limit OC export through microbial decomposition (as CO₂) and leaching (as DIC/DOC). Our results demonstrate that, depending on the redox regime, the quantitative importance of mineral protection as well as the chemical nature of mineral-organic associations controlling their stability vary across floodplain sediments. High elevation floodplains like that of the East River, CO, experience dynamic redox transitions with seasonal flooding events. The magnitude and timing of these seasonal flooding cycles is expected to change due to greater variability in snowpack (Lute et al., 2015), declines in snowpack depth (Fyfe et al., 2017; Siirila-Woodburn et al., 2021), or slower snowmelt rates (Musselman et al., 2021), which will directly impact the redox regime in the floodplains. Combined, our results suggest that such changes will not only directly affect anaerobic protection but may also have immediate consequences for protection in redox-sensitive mineral-organic associations, warranting further exploration of potential impacts on OC storage within and export from floodplain sediments.

2.6 References

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CHAPTER 3

ANAEROBIC PROTECTION OF ORGANIC MATTER SHIFTS SEASONALLY IN MOUNTAINOUS FLOODPLAIN SOILS

3.1 Abstract

Floodplain soils are large reservoirs of organic carbon (OC), and they undergo seasonal flooding cycles that impact the retention and export of organic matter. During flooding, anaerobic conditions in saturated soils limit microbial organic matter decomposition, thereby protecting soil OC. What remains elusive is how such anaerobic protection mechanisms, including kinetic and thermodynamic constraints, respond to seasonal flooding cycles that cause oxic-anoxic transitions in the soils. Here, we show that seasonal flooding affects both kinetic and thermodynamic constraints on microbial organic matter degradation. Within a mountainous floodplain (East River, Colorado), targeted metabolite analysis shows that flooding leads to an accumulation of aromatic- and lipid-like, high molecular weight compounds in both shallow and deep soils, suggestive of kinetic and thermodynamic constraints on OC depolymerization and oxidation, respectively. Compared to shallow soils, deep soils had more reduced and lipid-like OC, suggesting that thermodynamic constraints are stronger in deep soils, which are less aerated and thus more reducing, and contain less OC. In deep soils, which remained relatively reducing year round compared to shallow soils, the accumulation of reduced OC compounds across both flooded and drained periods suggest that thermodynamic constraints on the oxidation of OC are active regardless of redox conditions. However, in deep soils, molecular weight of OC decreases upon drainage and

lignin content decreases, suggesting that drainage lifts kinetic constraints. As climate change continues to shift the timing and extent of seasonal flooding in mountainous floodplains, this work provides a mechanistic understanding of C cycling dynamics that will improve our predictions of the fate of floodplain C.

3.2 Introduction

Floodplain soils are large reservoirs of carbon (D'Elia et al., 2017; Lininger et al., 2019) which undergo seasonal flooding cycles that impact carbon, nutrient, and metal retention and export (Sutfin et al., 2016; Winnick et al., 2017). Snow-dependent floodplains, like those in the mountainous Western United States, are highly vulnerable to increased flooding and droughts predicted with climate change (Diffenbaugh et al., 2013). Understanding how these hydrologic shifts impact the mechanisms of floodplain C storage and loss can help us better predict changes to soil fertility and water quality with climate change.

Floodplain soils experience seasonal and spatial redox dynamics, across gradients of seasonal flooding and texture due to depositional processes, respectively. Reducing conditions, as measured by low redox potentials, are important controls on the fate of soil OC, as they affect both protective mineral-organic associations and microbial decomposition. During flooding, mineral-organic associations can be disrupted due to reductive dissolution of Fe-bearing minerals, increasing the mobility of soil OC in the environment (Adhikari et al., 2017; Barcellos et al., 2018b; Buettner et al., 2014; Huang and Hall, 2017). Once soil OC is mobilized, however, anaerobic protection mechanisms impact its subsequent decomposition, through both kinetic and thermodynamic

constraints (Keiluweit et al., 2016; Naughton et al., 2021). However, it is unclear how the seasonal flooding dynamics and soil textural gradients of floodplains impact the relative importance of kinetic and thermodynamic constraints on soil OC loss, making it difficult to predict how hydrological shifts due to climate change will impact the overall C budget of floodplains.

The soil continuum model posits that OC moves dynamically through the decomposer community, progressing from large plant biopolymers towards smaller, more oxidized and subsequently more soluble organic fragments that can then be utilized by microorganisms (Lehmann and Kleber, 2015). However, oxygen limitations, such as those experienced in reducing conditions, constrain the pathways of OC decomposition through both kinetic and thermodynamic mechanisms (Fig. 3.1). Together, these mechanisms lead to the anaerobic protection of OC in reducing conditions, such as those experienced in deep soils, anaerobic microsites, or during flooding.

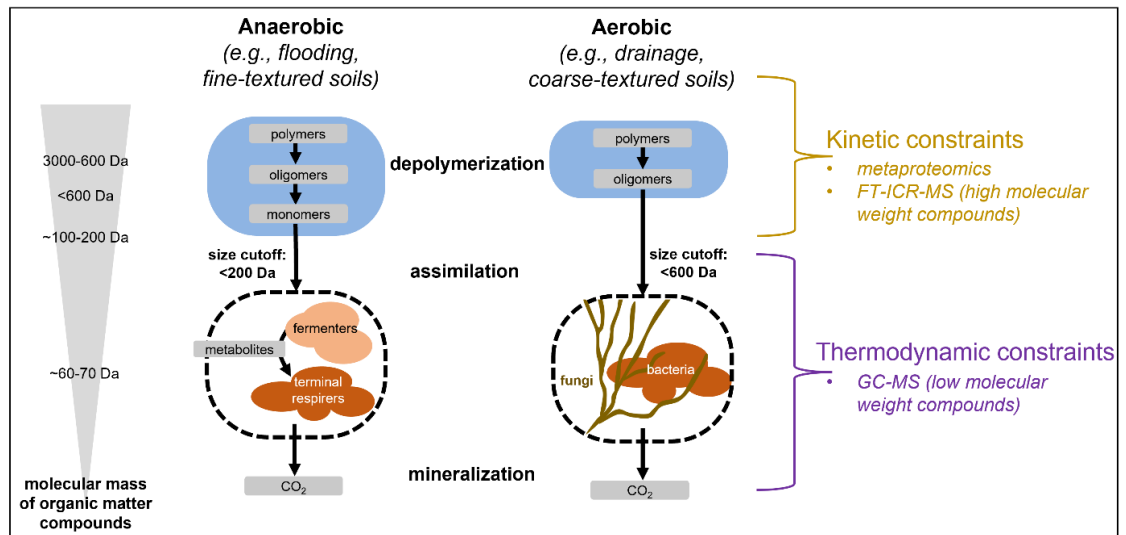


Figure 3.1. Conceptual diagram illustrating the process of organic matter depolymerization, assimilation, and oxidation across anaerobic and aerobic conditions. Kinetic constraints (measured using metaproteomics and FT-ICR-MS) can occur during the depolymerization step. Thermodynamic constraints (measured using GC-MS) can occur during mineralization of organic matter to carbon dioxide (CO₂). Figure based on Lehmann and Kleber (Lehmann and Kleber, 2015).

Extracellular enzymes directly regulate the transformation and turnover of soil OC, through activities of both hydrolytic (e.g., β -glucosidase and N-acetyl-glucosaminidase that hydrolyze polysaccharides, chitin, and amino sugars) and oxidative (e.g., phenol oxidases and peroxidases) enzymes. After enzymatic depolymerization, these smaller, soluble organic fragments can then be assimilated into and metabolized by soil microorganisms. However, under anaerobic conditions, the depolymerization of OM into assimilable monomers is kinetically constrained through the suppression of oxidative depolymerizing enzymes such as phenol oxidases. The subsequent build-up of phenolic compounds can inhibit hydrolytic enzymes (Freeman et al., 2001; Naughton et al., 2021) upon which depolymerization relies, although evidence of anoxic degradation of polyphenolic compounds has been shown in wetland soils (McGivern et al., 2021). If hydrolytic enzymes are inhibited, relatively reduced and non-hydrolysable compounds (e.g., lignin and lipids) can accumulate (Hedges and Keil, 1995; Keiluweit et al., 2016). Thus, if kinetic constraints are active, we would expect soil C to be higher in molecular weight, less oxidized, and relatively more enriched in non-hydrolysable organic compounds.

Redox dynamics also influence microbial respiration pathways (DeAngelis et al., 2010; Wilmoth et al., 2018) and thus impact greenhouse gas release from soils (Chen et al., 2018; Wang et al., 2018). Specifically, oxygen limitations constrain the microbial oxidation of soil OC: in the absence of oxygen, less favorable electron acceptors limit the thermodynamic favorability of OC compounds for microbial metabolism (LaRowe and Van Cappellen, 2011), resulting in the selective preservation of OC (Boye et al., 2017).

Indeed, flooding has been shown to impact metabolic pathways and C transformations, driven by the availability and oxidation state of dissolved OC (Boye et al., 2018).

Thermodynamic limitations on soil OC oxidation have been demonstrated across soil types and environments, including upland and seasonally flooded soils. In upland soils, anaerobic microsites have been shown to contribute to preservation of more reduced OC compounds, leading to the accumulation of OC with reduced functional groups (e.g., aromatic and aliphatic) (Keiluweit et al., 2017). In shallow tropical soils, which experience fluctuating oxic-reducing conditions due to high precipitation and fine-textured soils (Hall et al., 2013; Silver et al., 1999), the relative enrichment of reduced OC (e.g., aliphatic compounds), in combination with increasing abundance of anaerobic microorganisms, suggest that oxygen limitations and associated metabolic constraints limit the oxidation of OC (Lacroix et al., 2022a). In these upland soils, physical disruption of soil aggregates can remove thermodynamic constraints on OC oxidation, by increasing oxygen supply and disrupting anoxic microsites (Lacroix et al., 2022b).

In seasonally flooded soils, the selective preservation of thermodynamically unfavorable soil OC compounds in surface soils suggests that OC accumulation in these soils is driven by low redox potentials (LaCroix et al., 2019). In deep sulfidic floodplain sediments, thermodynamic constraints on OC oxidation led to the selective preservation and accumulation of reduced compounds such as proteins, lipids, and aliphatic compounds (Boye et al., 2017). In a reactor experiment, Naughton et al. found that the reaction favorability of metabolisms involving both sulfate and iron reduction depended heavily on the oxidation state of soil OC (Naughton et al., 2021). Thus, fluctuating redox

conditions across a diversity of soil types and ecosystems impact the thermodynamic favorability of OC oxidation, representing an important mechanism of C cycling.

While previous research has shown the importance of anaerobic protection of soil OC in upland and seasonally flooded soils, we have poor understanding of the spatial and temporal shifts in anaerobic protection mechanisms due to textural differences and seasonal flooding in depositional floodplain soils. For example, does aeration lift the episodic anaerobic protection experienced during flooding? Given the vast amount of OC stored in floodplains, understanding how seasonal flooding and depth dynamics impact the relative importance of kinetic and thermodynamic constraints on floodplain OC loss will provide critical information on the flow of carbon into and out of floodplains.

To determine how seasonal flooding and soil depth affect anaerobic protection mechanisms on the decomposition of floodplain OC, in this study we utilized a well-characterized floodplain meander of the East River in Colorado. To assess the impact of soil depth and flooding on anaerobic protection mechanisms, we sampled shallow and deep soils over the course of seasonal flooding. We hypothesized that both OC composition and protein abundances would show evidence of anaerobic protection mechanisms on floodplain OC oxidation, and that these would vary both across soil depth and over the course of seasonal flooding. We characterized seasonal changes in low- and high-molecular weight compounds using a combination of targeted and non-targeted metabolomics as well as seasonal dynamics of oxidative and hydrolytic enzyme classes using metaproteomics. We expected kinetic constraints to manifest themselves during flooding in higher concentrations of high-molecular weight compounds due to the lower abundance of oxidative enzymes. We further anticipated thermodynamic constraints

during flooding to cause a relative enrichment of energetically less viable, chemically reduced compounds.

3.3 Methods

3.3.1 Field site and soil sampling

In this study, we utilized a sampling transect across an active meander of the East River (Gothic, CO) (Dewey et al., 2022; Dwivedi et al., 2018; Fox et al., 2022, 2020) (see Chapter 2). This site is characterized by soil textural gradients as well as seasonal flooding dynamics: shallow soils and those closer to the mid-meander are finer in texture than their deeper and near-river counterparts, and particulate OC content is higher in shallow soils and at the near river location compared to deep and mid-meander soils (see Chapter 2).

To study the impacts of redox dynamics on anaerobic protection mechanisms, we sampled soils across both spatial and temporal gradients. We sampled soils at near river and mid-meander positions due to the distinct contrasts in texture, water and moisture dynamics, and carbon content. Specifically, we sampled soils in triplicate from near river and mid-meander positions, at shallow (17-39 cm) and deep (50-88 cm) depths; these depths were based on redoximorphic features observed during sampling. We performed this sampling in June and September 2017, corresponding to flooded and drained conditions, respectively (Fig. 3.2). Relative to historical levels, 2017 experienced relatively high snowpack and subsequent flooding conditions; we thus consider 2017 to be a high water year.

3.3.2 Organic matter characterization using FT-ICR-MS

To assess shifts in soil organic matter composition across seasonal flooding and with soil depth, we used Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) using a 12 Tesla Bruker Solarix FTICR spectrometer located at the Environmental Molecular Science Laboratory in Richland, WA. Briefly, soils water extracts were introduced directly to a standard Bruker electrospray ionization source operating in negative mode to generate negatively charged molecular ions. The instrument settings were optimized using a Suwannee River fulvic acid standard, obtained from the International Humic Substance Society, and blanks (HPLC grade methanol) were analyzed at the beginning and end of the day to monitor potential carryover from one sample to another. The instrument was flushed between samples using a mixture of water and methanol, and the ion accumulation time varied to account for differences in C concentration between samples. For each sample, 144 individual scans were averaged, and the spectra were internally calibrated using an OM homologous series separated by 14 Da (CH_2 groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (100-1,000 m/z), and $t \leq \text{H:C} < 0.8$) (Tfaily et al., 2017). BrukerDaltonik data analysis software (version 4.2) was used to convert raw spectra to a list of m/z values applying FT-MS peak picker module with a signal-to-noise ratio threshold of 7 and absolute intensity threshold of 100. Formularity software (Tolić et al., 2017) was used to assign chemical formulae based on the criteria previously described (Tfaily et al., 2018). Peaks were filtered to only include masses

between $200 < m/z < 900$; a total of 9534 peaks were retained for analysis, representing 10.8% of all peaks. Samples had a variable number of assigned peaks, ranging from 33 to 1351). Compounds were grouped into molecular classes (e.g., lipid-, protein-, carbohydrate-, lignin-, tannin-, condensed aromatic-like) based on C, H, and O for H/C and O/C ranges using van Krevelen plots according to previous literature (Bailey et al., 2017). Each assigned molecular formula was used to calculate the nominal oxidation state of C (NOSC), double bond equivalence (DBE), and aromaticity index (AI) as in previous work (LaCroix et al., 2019).

3.3.3 Low-molecular weight metabolite characterization using GC-MS

To assess shifts in soil metabolite profiles across seasonal flooding and with soil depth, we used gas chromatography-mass spectrometry (GC-MS). Water extracts were first freeze-dried (Labconco, Thomas Scientific, Swedesboro, NJ) and then derivatized as described previously (Kim et al., 2015). Briefly, 20 μ L of 30 mg/mL methoxyamine hydrochloride in pyridine (Sigma-Aldrich, Saint Louis, MO) was added to each freeze-dried sample for the protection of carbonyl groups. Samples were vortexed for 20 s, sonicated for 60 s, and were incubated at 37°C for 90 min with generous shaking (1000 rpm). After the incubation, 80 μ L of N-methyl-N-(tri-methylsilyl)trifluoroacetamide (Sigma-Aldrich) with 1% trimethylchlorosilane (Sigma-Aldrich) was added to each sample. The samples were vortexed for 20 s, sonicated for 60 s, and incubated for 30 min at 37°C with shaking (1000 rpm). After derivatization, the samples were allowed to cool to room temperature. Samples were transferred to autosampler vials and analyzed by GC-MS in random order. Blanks and fatty acid methyl ester (FAME) samples (C8–28) were

also included in the analysis for the background referencing and retention time calibration, respectively. Metabolites were separated using an HP-5MS column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m; Agilent Technologies) and analyzed in an Agilent GC 7890A coupled with a single quadrupole MSD 5975C (Agilent Technologies). For each analysis, 1 μ L of sample was injected in splitless mode. The injection port temperature was kept constant at 250°C for the duration of the analysis. The GC analysis started with an initial oven temperature fixed at 60°C for 1 min after injection, and then at a steady rate of 10°C/min, the temperature was increased to 325°C with the analyses finishing with a 5-min hold at 325°C. The GC-MS raw data files were processed with MetaboliteDetector as reported previously (Hiller et al., 2009). Briefly, retention indices (RIs) of the detected polar metabolites were calibrated based on the mixture of FAME internal standards, followed by deconvolution, and subsequent chromatographic alignment of the detected features across all analyses. Identification of the metabolites was carried out by first matching GC-MS spectra and RIs against a Pacific Northwest National Laboratory augmented version (> 900 metabolites) of FiehnLib (PMID: 19928838) library containing validated RIs and spectral information (Kind et al., 2009). Subsequently, unidentified metabolites were screened against the NIST14 GC–MS library by spectral matching alone. To eliminate false identification and reduce the deconvolution errors created during the automated data-processing, each metabolite identification was individually validated.

GC-MS data was processed using Metaboanalyst 5.0 (Pang et al., 2021). First, we removed features with signal-to-noise ratios lower than nine, and those overrepresented in blanks. We also removed features with > 50% missing values and estimated the

remaining missing values (405 or 8.4% in full dataset; 140 or 5.5% in dataset with unknown compounds removed) by replacing with the limit of detection (half of the minimum positive values in the original data). We then median-normalized and log transformed the data. NOSC values were calculated for each compound as described for the FT-ICR-MS data. Kyoto Encyclopedia of Genes and Genomes (KEGG) identifiers of the identified metabolites were mapped against bioinformatics databases in batch modes using KEGG Mapper to identify the biochemical pathways (Kanehisa and Sato, 2020); only pathways with more than one match are presented.

GC-MS compounds were assigned NOSC categories (reduced, intermediate, oxidized) based on thermodynamic limitations to Fe reduction, similar to other work (Boye et al., 2017; Naughton et al., 2021). Specifically, the NOSC cut-off of -1 was used, which represents a conservative measure of the NOSC value at which the thermodynamic driving force of Fe reduction becomes inhibited (Naughton et al., 2021).

3.3.4 Metaproteomic analysis

To test how seasonal flooding affects abundances of extra- and intracellular enzymes, we analyzed the proteome of soil extracts. Soil proteomics data was performed on methanol extracts from soil at the Environmental Molecular Science Laboratory in Richland, WA, as part of the MPLEEx extraction protocol (Nakayasu et al., 2016). MS analysis was performed using a Q-Exactive HF-X mass spectrometer (Thermo Scientific) outfitted with a nano-electrospray ionization interface. Electrospray emitters were homemade using 150 μm o.d. $\times$ 20 μm i.d. chemically etched fused silica (Kelly et al., 2006). The ion transfer tube temperature and spray voltage were 325°C and 2.2 kV,

respectively. Data were collected for 120 min following a 10 min delay after completion of sample trapping and start of gradient. FT-MS spectra were acquired from 300 to 1800 m/z at a resolution of 60 k (AGC target 3×10^6) and the top 16 FT-HCD-MS/MS spectra (Fourier-transform high energy collision tandem MS) were acquired in data-dependent mode with an isolation window of 0.7 m/z at a resolution of 45 k (AGC target 2×10^5) using a normalized collision energy of 30 and exclusion time of 45 s for charge states 2 to 6. Generated MS/MS spectra were searched using the mass spectral generating function (MSGF+) algorithm (Kim et al., 2008; Kim and Pevzner, 2014) against the FASTA file of protein sequences inferred from the genomes of matching samples sequenced at JGI (IMG Annotation Pipeline v.5.0.23) (see Supplemental Methods for DNA extraction). To optimize searches, samples and genomes were grouped in 4 groups with 12 samples in each, factored by meander location and soil depth. Duplicate sequences were removed from each FASTA file which varied from 16.2 to 19.2 million protein sequences, and 16 common contaminant sequences were appended to each FASTA file. MSGF+ was used in target/decoy mode with 20 ppm parent ion tolerance, tryptic rule, and no dynamic modification. Best matches from the MSGF+ searches were filtered at 1% FDR and only protein-specific peptides were used in consequent aggregation and quantitative analysis. Relative peptide abundances were determined by calculating the area under the curve of the peptide ion retention peak in the MS measurement, using MASIC software (Monroe and Gibbons, 2022; Monroe et al., 2008). Results were imported for aggregation and post-processing into the MS SQL (Microsoft) database. InfernoRDN software (Monroe and Gibbons, 2021) was used to transform peptides abundances ($\log_2$) and perform mean central tendency normalization. Protein

grouped normalized peptide abundances were de-logged, summed, transformed (log2) and normalized again in InfernoRDN to produce normalized abundances for the protein level roll-up.

To enable comparison across proteomics samples, we followed published methods (Diamond et al., 2019) to cluster proteins into their assigned functional orthology groups (e.g., Enzyme Commission number or EC) and summed spectral counts for proteins in the same sample with the same functional assignment. Before performing further statistical analysis, we removed various contaminants before analysis, including human and bovine proteins (Jouffret et al., 2021) and also removed functions that were present in fewer than five samples were removed from the analysis.

3.3.5 Statistical Analysis

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022). Because initial analyses of organic matter composition and proteomics showed no significant patterns across meander positions, we thus focused on soil depth and temporal dynamics. To assess differences in floodplain organic matter composition using FT-ICR-MS, we used Shapiro-Wilk and Levene's tests to assess normality and equality of variances for all data. Parametric tests were used in cases in which data met assumptions for normality. To test our hypotheses, we used analysis of variance (ANOVA) to assess the variation explained by soil depth and flooding condition. We combined this with Tukey's honestly significant difference *post hoc* test ($\alpha = 0.05$ indicates significant differences), using the R packages *agricolae* (Mendiburu and Yaseen, 2021) and *emmeans* (Lenth, 2023).

For our GC-MS data, we performed Partial Least Squares-Discriminant Analysis (PLS-DA) on all metabolites including unknowns, to see how samples clustered across variables (shallow vs. deep soils and flooded vs. drained). For all subsequent analyses of GC-MS, unknown metabolites were removed to allow us to characterize and link the compounds to metabolic pathways. To assess the significant metabolites that changed across flooding condition or soil depth, we used t-tests with equal group variance and p-value threshold of 0.05. We created a heatmap with dendrogram of significant metabolites using the R package *pheatmap* (Kolde, 2019), with the Pearson distance measure, Ward clustering algorithm, and scaling by metabolite.

To assess shifts in proteomics data, we used principal component analysis (PCA) to assess how proteins assigned EC numbers clustered by depth and flooding condition, using package *factoextra* (Kassambara, 2020). To assess specific changes across depth and flooding condition, we performed ANOVAs and post hoc tests as with FT-ICR-MS data, described above.

3.4.1 Spatiotemporal shifts in water table depth, soil texture, and soil OC at the floodplain meander

Both near river and mid-meander positions had higher water tables during flooding that decreased upon drainage (Fig. 3.2). The mid-meander position had a relatively higher water table during peak flooding (0 cm, ponded at soil surface) compared to the near river position (7.7 cm below ground surface, Fig. 3.2). Both positions had similar water table depths over the course of the season and remained at

similar levels upon late-season drainage (0.72 cm and 0.76 cm below ground surface at near river and mid-meander positions, respectively, Fig. 3.2).

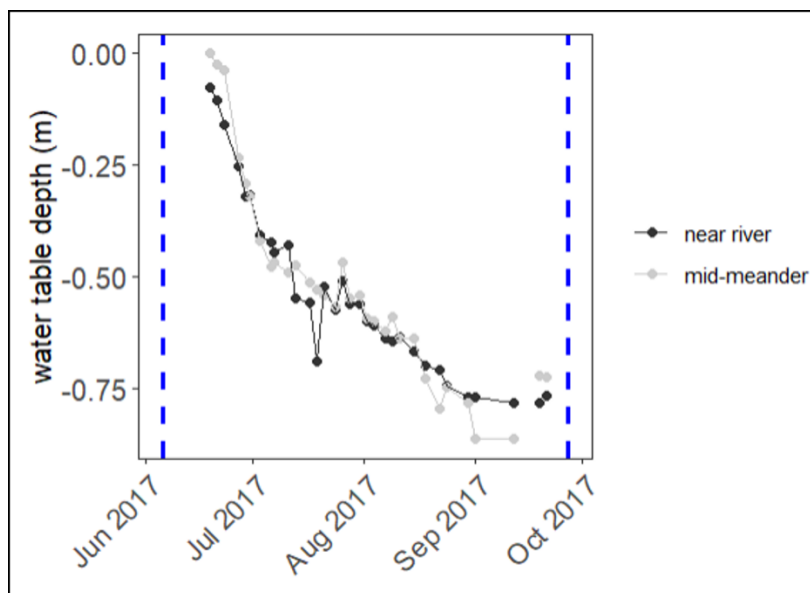


Figure 3.2. Water table depth of the East River, at near-river (black) and mid-meander (gray) positions. Dashed blue lines represent soil sampling dates.

3.4 Results

3.4.2 Soil metabolite composition using Fourier-transform ion cyclotron mass spectrometry (FT-ICR-MS)

Deep soil OC had significantly lower nominal oxidation state of C (NOSC) values (-0.64) relative to shallow soil (-0.46) (Fig. 3.3a). However, the mean NOSC did not significantly shift from flooded to drained conditions for either depth. Overall, the molecular weight of OC was significantly higher in shallow (393.3 Da) than in deep soils (346.5 Da) (Fig. 3.3b), but the molecular weight only decreased upon drainage in deep soils. Measures of aromaticity, including aromaticity index (AI) and double bond equivalence (DBE), were higher in shallow compared to deep soils, but this was only

significant for DBE. In shallow soils, aromaticity was significantly higher during flooding (128% higher DBE; 42.3% higher AI, Fig. 3.3c and d). However, in deep soils there was no significant change in aromaticity measures between flooding and drained periods (Fig. 3.3c and d).

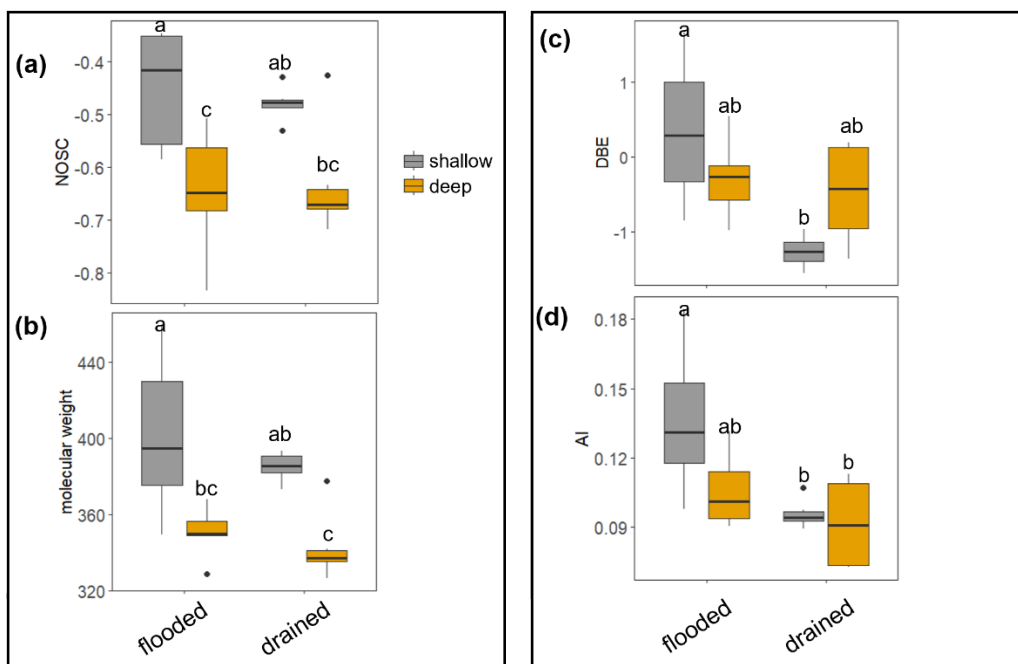


Figure 3.3. FT-ICR-MS data on soil water extracts: (a) nominal oxidation state of carbon (NOSC), (b) molecular weight, (c) double bond equivalence (DBE), and (d) aromaticity index. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of soil depth and flooding condition. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

Categorizing FT-ICR-MS data into compound classes using van Krevelen plots, we found that tannins were significantly enriched by 123% in shallow soils (Fig. 3.4). Similarly, lipids were enriched by 74% in deep soils. In shallow soils, lignin was significantly enriched by 32% upon drainage but lipids did not significantly shift upon drainage. In deep soils, lignin decreased by 20% upon drainage, but lipids did not significantly change over seasonal flooding.

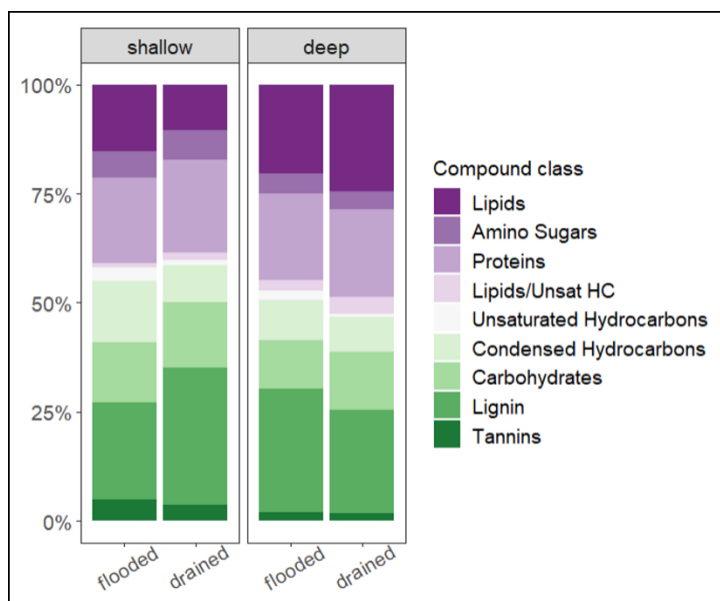


Figure 3.4. Relative abundances of compound classes identified in soil water extracts analyzed using FT-ICR-MS.

3.4.3 Soil metabolite composition using gas chromatography-mass spectrometry (GC-MS)

Metabolite data derived from GC-MS analysis showed significant shifts in metabolite composition both with depth and across seasonal flooding (Fig. 3.5).

Specifically, GC-MS metabolites significantly clustered by both flooding condition and soil depth using PLS-DA analysis (Fig. 3.5). Overall, 43.51% of variance was explained with the first two principal components (PC1 = 24.92%, PC2 = 18.59%).

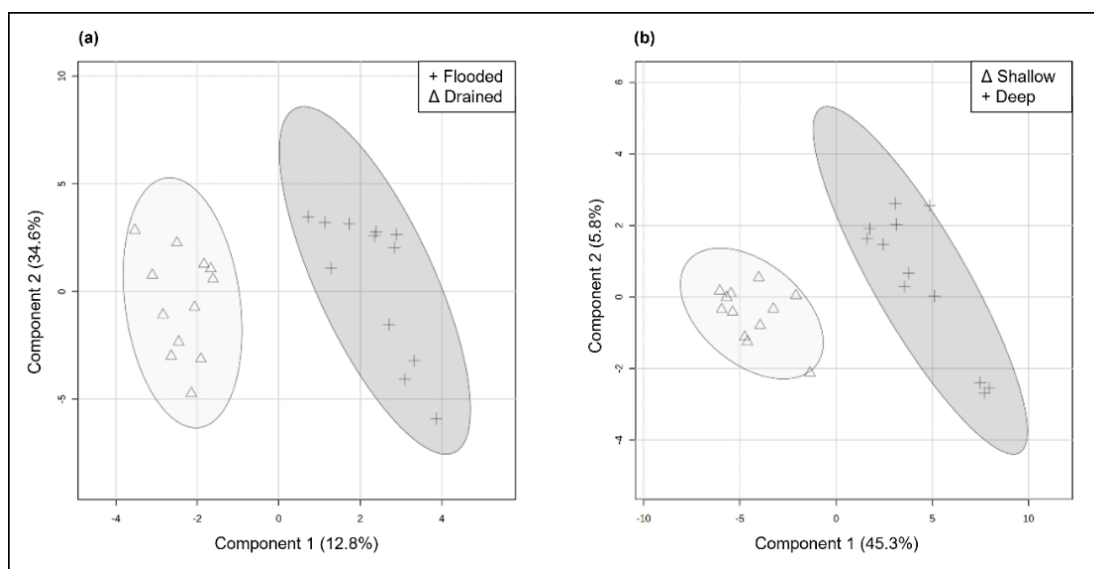


Figure 3.5. PLS-DA clustering of GC-MS metabolite data, by (a) soil depth and (b) flooding condition.

We assessed the metabolites significant both across soil depth and seasonal flooding gradients. After removing unknown metabolites (i.e., those not identified by the GC-MS library), we found 64 metabolites that significantly differed between shallow and deep soils (t-test, $p < 0.05$, Fig. 3.6, Table B.1). Specifically, deep soil metabolites had lower NOSC values (mean NOSC = -0.34) compared to shallow soils (mean NOSC = -0.06). Additionally, the difference in metabolite abundance between shallow and deep soils significantly correlated with NOSC and across NOSC classes (Fig. 3.7, $p < 0.05$). Notably, several compounds whose NOSC is below the thermodynamic threshold for Fe reduction were only present in deep soils including fatty acids and lipids (e.g., capric acid, nonanoic acid, caprylic acid, heptanoic acid, hydroxyhexanoic acid, and hydroxybutyric acid) or compounds involved in the biosynthesis of these compounds (e.g., glycerol 3-phosphate).

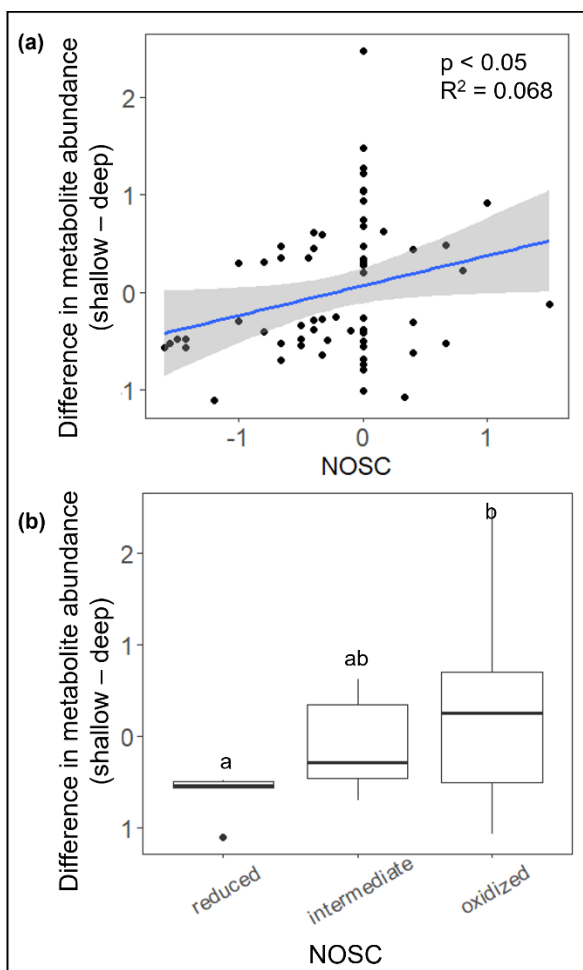


Figure 3.7. Differential abundance of GC-MS metabolites (shallow minus deep) (a) correlated with nominal oxidation state of carbon (NOSC), and (b) across different classes of nominal oxidation state of carbon (NOSC): highly reduced NOSC (< -1), intermediate NOSC (> -1 and < 0), and oxidized NOSC (> 0). In panel (a) the line indicates the results of linear regression analysis, and associated statistics are provided, including the R^2 and p-value for model significance. In panel (b) box plots in panel display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

To assess the potential metabolic pathways associated with the compounds significant at each depth, we mapped the metabolites to specific pathways using KEGG Mapper. While both shallow and deep soils both mapped to similar pathways, including

transport, biosynthesis, and metabolic pathways (Table B.2), there were distinct differences: only shallow soils contained pathway matches for amino acid metabolism, biosynthesis of secondary metabolites, and chemical structure transformation. Deep soils showed matches for lipid and energy metabolism pathways, including glycerolipid, propionate, and methane metabolisms, as well as fatty acid biosynthesis.

Analyzing differences in floodplain soil metabolites across seasonal flooding, we found 11 GC-MS identified metabolites that were significantly different between flooded and drained soils (t-test, $p < 0.05$, Fig. 3.8, Table B.2). During flooding, metabolites had lower NOSC (mean NOSC = -0.72), whereas during drainage they had higher NOSC (mean NOSC = -0.33). While the difference in metabolite abundance between drained and flooded periods showed a positive trend with NOSC, this was not significant (Fig. 3.9, $p > 0.05$). Similar to the results from deep soils, many of the relatively more reduced compounds that dominated during flooding are also involved in lipid metabolism and fatty acid biosynthesis (e.g., stearic acid, nonanoic acid, heptanoic acid, and methylmalonic acid) (Table B.3). However, there were no pathway matches with metabolites significant by flooding condition to the KEGG database, limiting our ability to infer active metabolisms from these metabolites; this was likely due to the smaller number of metabolites identified as significant across flooding condition.

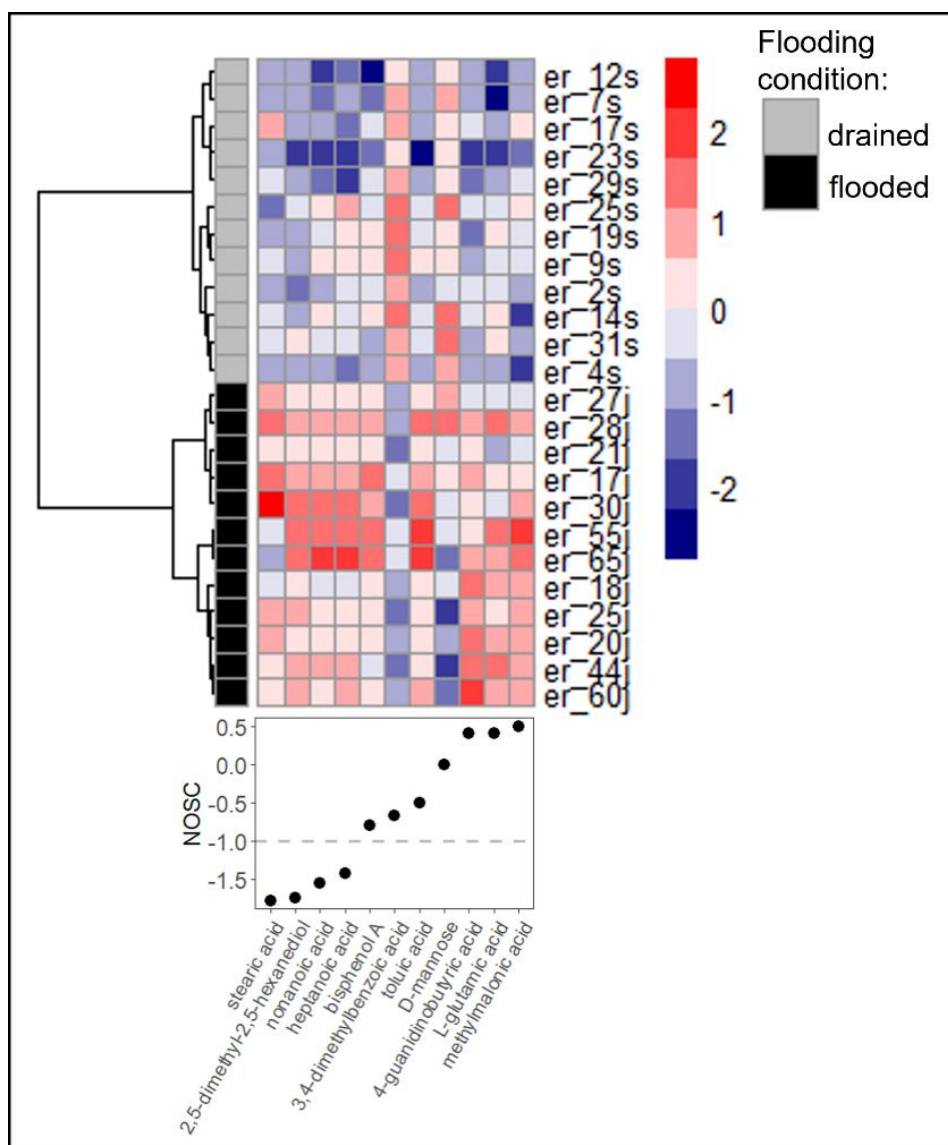


Figure 3.8. Heatmap of metabolite abundance by soil sample, clustered by flooding condition (drained = gray , flooded = black). Each row represents a sample, and each column represents metabolites that significantly differ between flooding condition. Metabolites are ordered by nominal oxidation state of carbon (increasing in NOSC moving right). Dashed gray line indicates thermodynamic threshold of NOSC = -1. The red and blue colors in each cell indicate relatively higher and lower concentrations of specific metabolites in each sample, respectively.

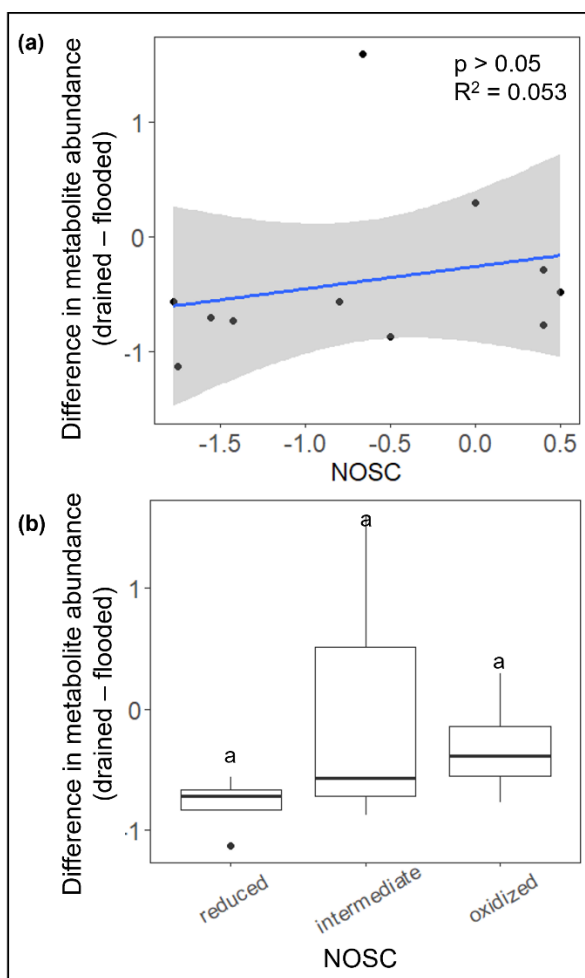


Figure 3.9. Differential abundance of GC-MS metabolites (drained minus flooded) (a) correlated with nominal oxidation state of carbon (NOSC), and (b) across different classes of nominal oxidation state of carbon (NOSC): highly reduced NOSC (< -1), moderate NOSC (> -1 and < 0), and oxidized NOSC (> 0). In panel (a) the line indicates the results of linear regression analysis, and associated statistics are provided, including the R^2 and p-value for model significance. In panel (b) box plots in panel display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

3.4.4 Shifts in soil protein profiles across soil depth and flooding status

We used metaproteomic data from all soil samples to provide insight into functions abundant across the floodplain meander soils with depth and over the course of seasonal flooding. Across all samples, we identified 4030 proteins with at least one uniquely mapped peptide. In total, 7.0% of proteins were assigned to one of six Enzyme

Commission (EC) numbers. Using PCA analysis, proteins assigned EC numbers significantly clustered by both depth and flooding condition (Fig. 3.10a and b). Overall, 77.6% of variance was explained with the first two principal components (PC1 = 56.4%, PC2 = 21.2%).

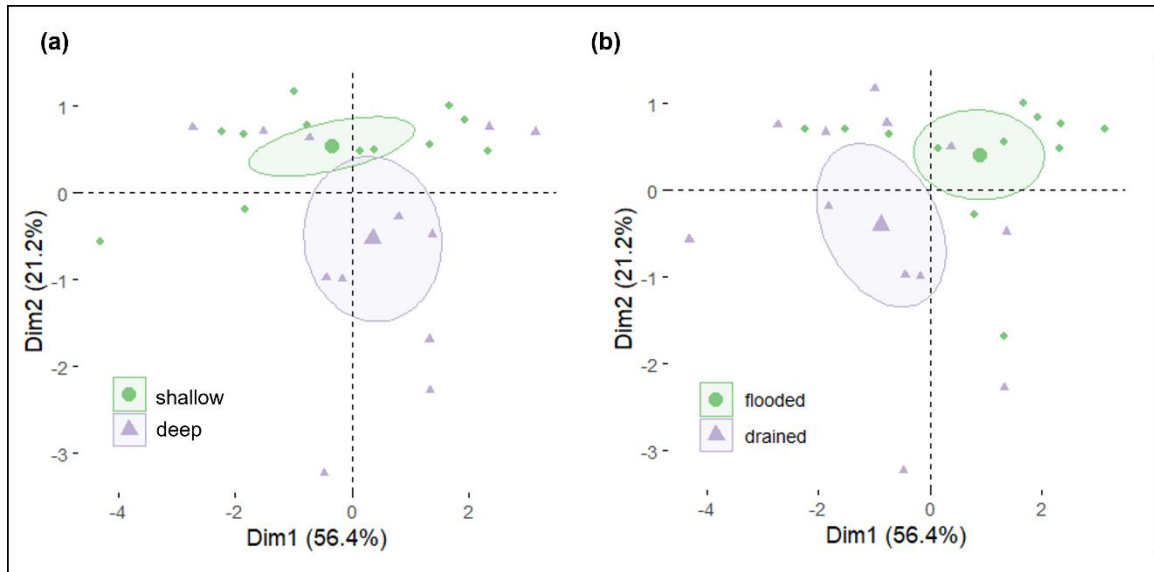


Figure 3.10. Principal component analysis (PCA) of proteins assigned EC numbers, colored by (a) depth (shallow vs. deep) and (b) flooding condition (flooded vs. drained).

Average numbers of proteins classified as oxidoreductases, transferases, and lyases increased upon drainage in shallow soils, although this was not significant (Fig. 3.11a, $p > 0.05$). The abundance of hydrolytic proteins in shallow soils increased almost two-fold upon drainage to levels that were significantly different than those found in deep soils ($p < 0.05$). Isomerase abundance did not significantly shift across either soil depth or flooding condition. Ligase proteins were significantly higher in deep soils ($p < 0.05$), but their abundance did not shift from flooded to drained timepoints.

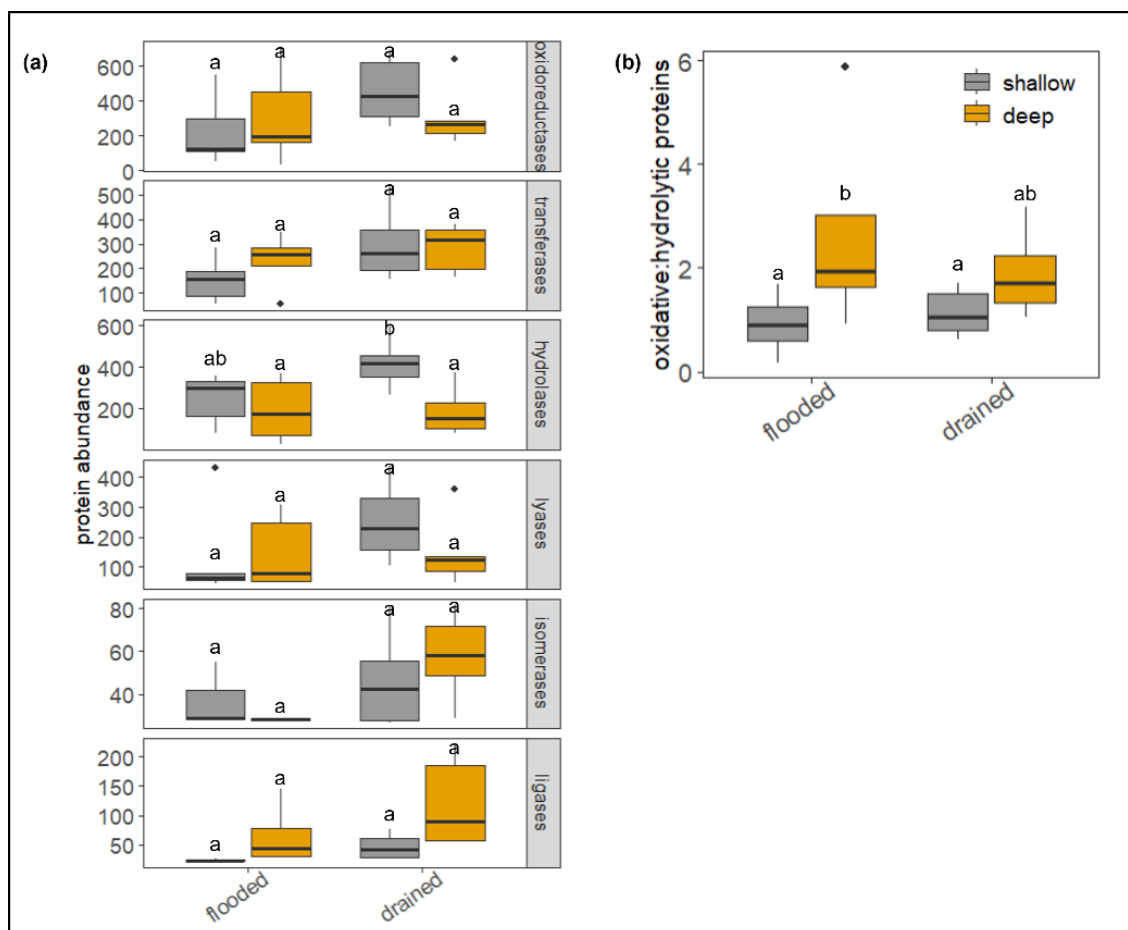


Figure 3.11. Protein data separated by depth (shallow and deep; colored gray and orange, respectively) and flooding conditions (flooded and drained): (a) Protein abundances of six EC classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases), and (b) ratio of oxidative:hydrolytic enzymes. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

To assess if oxygen limitations during flooding impose constraints on the relative abundance of oxidative compared to hydrolytic enzymes, we used the ratio of oxidative to hydrolytic enzymes. In shallow soils, the ratio of oxidative:hydrolytic enzymes only modestly increased upon drainage, and this was not significant (Fig. 3.11b). Deep soils showed a significantly (2-fold) greater oxidative:hydrolytic than shallow soils, especially during flooding.

3.5 Discussion

3.5.1 Evidence of kinetic constraints on OC depolymerization in floodplain soils

We hypothesized that kinetic constraints on OC depolymerization would manifest during flooding conditions and in deep soils, where we see higher moisture and lower redox potential (see Chapter 2). Specifically, we expected to see higher molecular weight OC compounds during flooding, especially in deep soils. In support of our hypothesis, the average molecular weight of OC in deep soils significantly decreased upon drainage, suggesting that its depolymerization was limited during flooding. In line with other studies, oxygen limitations associated with flooding and deeper sediments have been shown to inhibit oxidative depolymerization enzymes that process higher molecular weight OC compounds into smaller fragments (Freeman et al., 2001; LaCroix et al., 2019).

Additionally, the composition of soil OC across the floodplain meander was suggestive of shifting kinetic constraints. Following the “enzymatic latch” hypothesis (Freeman et al., 2001), we expected to see the accumulation of non-hydrolysable compounds such as lignin and long-chain aliphatic compounds in anaerobic portions of the floodplain, because oxidative depolymerizing enzymes would be inhibited. In support of this hypothesis, the relative abundance of lignin-like compounds in deep soils decreased upon drainage, a phenomenon observed in Mollisol soils subjected to sustained anaerobic conditions (Huang et al., 2020) and at the oxic-anoxic transition zone in incubation in different Mollisol soils (Naughton et al., 2021). This result suggests that in deep soils, drainage could relieve the kinetic inhibition of enzymes involved in the

oxidative decomposition of complex polymers such as lignin, effectively releasing the enzymatic latch (Freeman et al., 2001; Kleber, 2010; Naughton et al., 2021). We similarly found that lipid-like compounds were relatively enriched in deep compared to shallow soils; however, this did not change seasonally. Interestingly, compared to shallow soils, deep soils had significantly higher ratios of oxidative:hydrolytic enzymes. We posit that this oxidative potential in deep soils enables the lifting of kinetic constraints upon drainage, which we saw above. Together, these results suggest that even in deep soils with relatively low C content, seasonal oxygen limitations can impose kinetic constraints on the depolymerization of OC, which can be lifted upon drainage.

We also expected to see evidence of kinetic constraints in shallow soils, especially during flooding when oxygen limitations could prevent oxidative depolymerization. Overall, compared to deep soils, shallow soils contained relatively more tannin-like compounds, suggesting enrichment of plant inputs. Together with the higher average molecular weight of OC in shallow soils, this result fits with our previous work showing that shallow soils receive higher inputs of plant material compared to deep soils (see Chapter 2). During flooding, shallow soils contained relatively more aromatic and higher molecular weight compounds, in line with other studies in seasonally flooded wetlands found that periodic flooding imposed kinetic constraints on OC depolymerization, resulting in the accumulation of plant-derived, aromatic, and high molecular weight OC in surface soils (LaCroix et al., 2019). However, while the aromaticity of shallow soil OC decreased upon drainage, as we expected with the lifting of kinetic constraints, the molecular weight did not shift, possibly due to the continued accumulation of plant material in shallow soils throughout the growing season. We only found a modest (but not

significant) increase in oxidative enzyme abundance in shallow soils upon drainage. These results suggest that kinetic constraints may be less important in shallow compared to deep floodplain soils, and that continued plant inputs may confound the molecular signature expected with kinetic constraints. It is also possible that metaproteomics as a method did not resolve more subtle shifts in enzyme expression and activity over the course of seasonal flooding, similar to other studies (Graham et al., 2018), or that pools of extracellular enzymes do not change as rapidly as metabolite pools despite being the catalysts of metabolic reactions. It is also possible that enzymes may sorb onto mineral surfaces during oxic conditions, preventing their activity despite their presence (Kleber et al., 2007). Therefore, we suggest that future studies incorporate metatranscriptomic analysis to better resolve enzyme translation in dynamic floodplain systems. It is also possible that EC classification did not represent the full protein profile of the soils; we also recommend further studies examine other enzyme orthology systems, such as KEGG Orthology (Diamond et al., 2019), to better resolve protein shifts across dynamic hydrology.

3.5.2 Evidence of thermodynamic constraints on OC oxidation in floodplain soils

We hypothesized that thermodynamic constraints on the oxidation of floodplain OC would result in the selective preservation of thermodynamically unfavorable OC compounds in deep soils and during flooding, as seen in other reduced environments (Boye et al., 2017; LaCroix et al., 2019; Naughton et al., 2021). Consistent with our hypothesis, our FT-ICR-MS analysis of soil OC showed that deep soils were relatively more reducing compared to shallow soils, as seen in soils across lab (Naughton et al.,

2021) and field (Leewis et al., 2022) studies. Although deep soils are coarser compared to shallow soils, they still contain relatively high silt and clay contents, especially near the meander center. In combination with seasonal flooding dynamics, this results in relatively higher moisture content and lower redox values (see Chapter 2), especially during high water years, which suggests an environment conducive to the thermodynamic protection of soil C as our data show.

However, soil texture alone does not impart anaerobic protection: rather, texture couples with bioavailable OC content to regulate oxygen supply and demand (Keiluweit et al., 2018; Noël et al., 2017). Our previous work in these same soils showed lower total and water-extractable OC content in deep compared to shallow soils (see Chapter 2), which may represent an energetic constraint on its oxidation (Fontaine et al., 2007; Stegen et al., 2018), in addition to the thermodynamic constraint (i.e., oxygen limitations). An alternative hypothesis for the results we see is that in deep soils, substrate limitations in addition to anaerobic protection controls the metabolic activity of microorganisms. In this floodplain, deep sediments contain significantly lower OC compared to shallow soils. In a floodplain system, an increase in thermodynamically available OC upon drainage could lift bioenergetic constraints, a phenomenon observed in deep soils (Fontaine et al., 2007). Specifically, when this bioenergetic constraint is lifted (i.e., carbon substrate is made available to deeper soils), the decomposition of previously protected OC increases, known as the rhizosphere priming effect (Fontaine et al., 2007; Henneron et al., 2022). In this case, an increase in redox potential *or* carbon concentration may alleviate the thermodynamic protection mechanism in these soils. However, the significant correlation between metabolite abundance and NOSC values

suggests a relative enrichment of reduced metabolites at depth compared to shallow soils, providing more evidence of thermodynamic constraints at depth. We suggest that further research more mechanistically examine the tradeoffs between OC limitations and thermodynamic constraints across redox dynamics.

We also expected to see a lifting of thermodynamic constraints on OC oxidation upon floodplain drainage. Consistent with our hypothesis, our GC-MS data showed significant clustering of metabolites by flooding conditions, and specifically an increase in the average oxidation state of soil OC upon drainage. This shift in C oxidation state has been shown in comparisons between anaerobic and aerobic soil incubations (Huang et al., 2020); our study demonstrates that within a soil, C oxidation state can shift across dynamic hydrology. Interestingly, we did not see this seasonal signal in our FT-ICR-MS data, which may be because GC-MS can better identify smaller-molecular-weight compounds (e.g., < 400 Da), suggesting that this method may better indicate changes in critical low molecular weight metabolites such as sugars and amino acids (Bahureksa et al., 2021; Wilson et al., 2021).

During flooding we further expected to see an increase in the relative abundance of highly reduced aliphatics (e.g., lipids, waxes, and fatty acids), which have been shown to be enriched in reducing conditions (Boye et al., 2017; Keiluweit et al., 2016; Naughton et al., 2021). Our GC-MS data suggest that, while diverse metabolites occur across soil depths, relatively reduced fatty acid compounds accumulate at depth, regardless of flooding condition. This is corroborated by our FT-ICR-MS data, which showed the increased relative abundance of lipid-like compounds in deep soils. Previous research has explained the accumulation of lipids as a result of their non-hydrolysable nature under

anaerobic conditions (Hedges and Keil, 1995). While our results cannot definitively negate this hypothesis, we rather suggest an additional mechanism: in deep, relatively reducing floodplain soils, thermodynamic constraints associated with oxygen limitations result in the selective preservation of lipid-like compounds. Importantly, the relative composition of deep soil OC did not significantly change upon drainage, and neither did its aromaticity. Together, this suggests that these soils may stay relatively reducing year-round, especially in high water years, possibly maintaining thermodynamic constraints regardless of flooding stage.

Our metabolic pathway analysis suggests that lipid and energy metabolism pathways, including glycerolipid, propionate, and methane metabolisms, and fatty acid biosynthesis, dominate in deep soils. In deep soils, the dominance of lipid metabolism and fatty acid biosynthesis, in corroboration with low plant inputs (see Chapter 2), suggests that these are microbially derived metabolites. Propionate, for example, is an important intermediate in the degradation of OC in anaerobic environments such as wetlands (RoyChowdhury et al., 2018) and paddy systems (Gan et al., 2012), and is connected to methane (CH_4) oxidation. While CH_4 oxidation may be active in aerobic microsites within deep soils, and could contribute to overall CO_2 production, it is important to note that our data cannot differentiate whether these metabolisms are operative under reducing conditions, or whether we see an accumulation of their byproducts due to anaerobic protection under such conditions. We suggest that future research explicitly measure shifts in metabolite abundance, metagenomes, and associated gas (CO_2 and CH_4) over time, to better resolve the connection between metabolic activity and C loss pathways.

Additionally, while thermodynamic constraints may be selectively preserving reduced OC compounds in deeper soils, the high prevalence of more oxidized OC compounds across soil depths indicates that the abundance of more oxidized metabolites may be controlled by through mineral sorption mechanisms. Indeed, oxidized OC preferentially sorbs to mineral surfaces, which has been seen both in East River soils (see Chapter 2) and across diverse soil systems (LaCroix et al., 2019; Mikutta et al., 2007; Porras et al., 2017; Torn et al., 1997).

3.6 Synthesis

Floodplains experience fluctuating redox conditions due to seasonal flooding and variable soil texture with depth, but it is unclear how these dynamics impact floodplain carbon loss. Anaerobic protection of carbon, which includes both kinetic and thermodynamic constraints on depolymerization and oxidation, respectively, has been shown to control carbon loss in wetland soils (LaCroix et al., 2019) and deep floodplain sediments (Boye et al., 2017), yet we do not know how the relative importance of these constraints shifts in dynamic floodplain soils over space and time. We thus sought to understand the relative importance of kinetic and thermodynamic constraints on OC decomposition in a hydrodynamic floodplain of the East River, CO, across soil depth and flooding regime (Fig. 3.1).

Elevated moisture and associated anaerobic conditions have been shown to limit microbial metabolism in flooding-prone soils, leading to the accrual of soil OC in riparian and floodplain soils (Sutfin et al., 2016). Our study suggests that the composition of soil OC can provide clues to the mechanisms behind its accumulation. Importantly, our results

suggest that kinetic and thermodynamic constraints associated with flooded conditions and deeper sediment are mechanisms which act on OC to prevent its depolymerization and oxidation. Specifically, our data provide evidence for kinetic constraints during flooding, leading to an accumulation of relatively higher molecular weight compounds, and specifically more aromatic compounds and more lipid-like compounds in shallow and deep soils, respectively. Our data further suggest that energetic and thermodynamic constraints are relieved in the shallow soils, which are more aerated and thus more oxic, and contain more OC. Comparatively deep soils remain reducing year-round, and although there is some evidence of lifting of kinetic constraints upon drainage, thermodynamic constraints on the oxidation of OC (and possibly also substrate limitations) lead to the accumulation of reduced OC compounds regardless of flooding condition. Importantly, this work provides evidence for these mechanisms in a field setting, layering on to work in controlled environments (Naughton et al., 2021).

Our results indicate that the anaerobic protection mechanisms associated with seasonal flooding and soil depth control the utilization and preservation of bioavailable metabolites in floodplain soils. However, it remains unclear if anaerobic protection mechanisms are impacted by flooding extent, a crucial question as climate change is shifting hydrologic regimes in high altitude, snow-fed floodplains (Fyfe et al., 2017). Further, the connection between anaerobic protection mechanisms and C loss pathways in dynamic floodplains should be examined, specifically how the relative balance of protection mechanisms leads to a divergence in C loss dynamics.

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CHAPTER 4

SHIFTING MINERAL AND METABOLIC CONSTRAINTS REGULATE SOIL CARBON EXPORT FROM CLIMATE-SENSITIVE FLOODPLAINS

4.1 Abstract

Floodplains are critical terrestrial-aquatic interfaces which accumulate vast amounts of organic matter, including carbon, in their sediments. However, climate change is shifting snowpack levels in mountainous floodplains, making them vulnerable to more frequent extreme flood and drought years. The variable hydrology of floodplains drives spatial and temporal redox gradients which may impact soil carbon loss. Specifically, reducing conditions experienced during flooding can disrupt iron-organic matter associations and release dissolved organic carbon (DOC). However, microbial metabolic constraints due to oxygen limitations might selectively preserve this DOC. Here, we aimed to resolve the balance between mineral and metabolic constraints on floodplain carbon loss. We conducted a monitoring campaign across an active meander in the East River watershed (Gothic, CO) in 2018 and 2019, corresponding to extreme low and high river discharge years, respectively, and foreshadowing climate change predictions. Redox potentials and sequential extractions show that, especially in the extreme flooding year 2019, reducing conditions during flooding decrease the stability of iron-carbon associations, causing concomitant mobilization of iron and DOC. Metabolomics analysis (Fourier-transform ion cyclotron resonance mass spectrometry) shows the selective preservation of reduced DOC compounds during flooding, suggesting that oxygen limitations during flooding impose metabolic constraints on the oxidation of newly-available DOC; this pattern was especially pronounced in the high water year 2019.

Evidence for microbial metabolic constraints was corroborated by proteomics data, which, in deep soils, showed an increase in the ratio of oxidative to hydrolytic enzymes upon drainage in 2019. Together, these results provide evidence that spatiotemporal variations in redox in floodplains drive DOC loss pathways by controlling both mineral and metabolic constraints, and that these patterns shift across hydrological extremes. Our results suggest that hydrological shifts due to climate change may alter the magnitude and pathways of DOC export in watersheds, which has implications for the future carbon balance of alpine ecosystems.

4.2 Introduction

Mountainous floodplains contain vast amounts of soil carbon (C). Rich in organic matter, floodplains are critical zones of agricultural productivity and provide the majority of worldwide water resources (Viviroli and Weingartner, 2008). Seasonal flooding from snowmelt creates dynamic redox gradients, which can affect whether floodplain soils retain or export stored C. Critically, climate change is shifting snowpack levels in the western US (Fyfe et al., 2017). Decreased snowpacks are predicted for the rest of the 21st century, drastically impacting hydrology and water resources (Siirila-Woodburn et al., 2021), including in places like high elevation floodplains which are increasingly vulnerable to increased floods and droughts (Diffenbaugh et al., 2013; Hubbard et al., 2018). In particular, the mountainous western United States is already experiencing a megadrought and low-snow futures are predicted (Fyfe et al., 2017; Siirila-Woodburn et al., 2021; Williams et al., 2022). These dynamics can impact both C storage and the quantity and quality of downstream water (Dewey et al., 2022; Hubbard et al., 2018). However, we do not know the mechanisms of C storage and release in climate-sensitive,

redox-dynamic floodplains, limiting our ability to predict whether these ecosystems will act as a source or sink of C with climate change.

To improve our predictions of how floodplain C cycling is impacted by extreme shifts in flooding, as predicted with climate change, we need to understand how this flooding impacts the pathways and mechanisms of floodplain soil C export from meander soils as either dissolved organic C (DOC) or carbon dioxide (CO₂). In seasonally flooded soils, soil redox potential has been shown to impact pathways of C export (Boye et al., 2017; LaCroix et al., 2019). The dynamic hydrology and depositional nature of floodplains mean that their soils experience contrasting redox conditions over time and space. Within a season, floodplain soils undergo redox transitions from flooded and relatively reducing, to drained and relatively oxidic. Similarly, finer sediment deposited in lower energy areas can retain more soil moisture and limit oxygen movement through lower hydraulic conductivity, resulting in relatively reducing conditions compared to their coarser, more oxygenated counterparts.

It is unclear how extreme shifts in seasonal flooding patterns, and associated redox dynamics, affect C exports from meander soils, including CO₂ efflux and dissolved DOC loss. In seasonally flooded soils, surface soil CO₂ emissions have been shown to be influenced by soil temperature and moisture. While maximum soil CO₂ emissions have been shown to occur when temperatures are highest (Acosta et al., 2017; Pulliam, 1993), soil moisture also plays a strong role in soil C cycling, through its impacts on oxygen availability (Freeman et al., 2001; Keiluweit et al., 2016), root dynamics (Pulliam, 1993), and mineral composition (LaCroix et al., 2019). Specifically, soil CO₂ efflux is limited by oxygen limitations when soil moisture is too high, and by substrate diffusion limitations

when the soil is too dry, due to a lack of connecting water films (V. L. Bailey et al., 2019). Moisture controls on CO₂ efflux are especially important in systems with dynamic hydrology, such as floodplains, which experience wide ranges of soil moisture. For example, in an intermittently flooded paddy soil, microbial respiration rates were highest upon drainage (Miyata et al., 2000) and in a semi-arid floodplain respiration of flooded soils only matched that of non-flooded soils upon drainage (Sánchez-Andrés et al., 2010), likely owing to an increase in oxygen diffusion into the unsaturated soil and increased metabolic activity. In the lowland position of a seasonally flooded wetland, soil moisture and redox potential correlated more strongly with respiration than temperature, while soil temperature explained most variation in respiration in the upland position (LaCroix et al., 2019). In a semi-arid floodplain, the duration of flooding significantly impacted CO₂ emissions, but only after two consecutive flood-dry cycles (Sánchez-Andrés et al., 2010). We would thus expect that an increase in the extent of flooding should decrease microbial respiration, due to increased oxygen limitations, and soil temperature would impact respiration rates only upon moisture limitations. However, the mechanisms underlying microbial respiration in dynamic floodplain systems, which undergo seasonal flooding, are poorly understood.

Floodplain C exports from meander soils also occur as DOC fluxes out of the soil pore network. In general, DOC exports from riparian areas to streams has been largely attributed to hydrologic connectivity (Pacific et al., 2010). In contrast to the ‘teflon basin’ myth, which posits that snowmelt from high elevation watersheds simply flows over surrounding soils and sediments, surface-groundwater interactions are important control on the fate of watershed DOC (Williams et al., 2015). This DOC export pathway, termed

hydrologic nutrient flushing, occurs when large amounts of DOC is flushed from the system as a result of precipitation or snowmelt (Burns, 2005). In seasonally flooded ecosystems, such as floodplains, patterns of DOC export from floodplain meanders follow the hydrograph: low stream DOC concentrations during baseflow because of flow through low DOC mineral soils, followed by a rise in DOC concentrations due to the rising water table that moves through higher DOC organic soil (Pacifi et al., 2010).

While DOC export phenomena have been observed across a range of catchments and watersheds (Pacifi et al., 2010; Winnick et al., 2017), the mechanisms driving the flushing of DOC from floodplain soils are poorly understood, as is the fate of DOC upon floodplain drainage. An emerging area of uncertainty in winter biogeochemistry is the effect of variation in snow cover on DOC dynamics (Brooks et al., 2011). In redox-dynamic floodplain environments, loss of soil OC protected within mineral-organic associations is a two-step process involving both mineral and metabolic constraints. First, soil OC is made accessible through both reductive dissolution and desorption through exchange reactions (Buettner et al., 2014; Kleber et al., 2015; Kramer et al., 2012; Mikutta et al., 2006). The mobilization of OM from Fe phases via reductive dissolution of Fe-bearing minerals (Adhikari et al., 2017; Han et al., 2019; Knorr, 2013; Wordofa et al., 2019; Zhao et al., 2016), has been shown in dynamic watershed systems (Knorr, 2013). Second, once soil C is mobilized, oxygen limitations can kinetically constrain OC depolymerization reactions by suppressing oxidative enzymes (Freeman et al., 2001), and thermodynamic constraints control whether the C is oxidized (Boye et al., 2017; Keiluweit et al., 2016; LaRowe and Van Cappellen, 2011). Thus, mineral protection and metabolic constraints mechanistically control the balance between soil C storage versus

loss. Furthermore, this balance between mechanisms is driven by shifts in oxygen availability and associated redox dynamics, which we expect to change with flooding variability. However, it remains unclear how hydrological variability, both within a season and between contrasting water years, affects C export pathways (DOC versus CO₂), and the mineral and microbial mechanisms that control these C exports. Understanding these dynamics are especially important in mountainous floodplains, which in the Western United States are experiencing a prolonged megadrought corresponding to extremely dry years (Williams et al., 2022) interspersed with years of high flooding (Lute et al., 2015).

In this study, we assessed the impact of hydrological variability on controls on and pathways of C loss from mountainous floodplain soils. Over the course of an extreme drought and flooding year, we assessed the relative balance of mineral and metabolic constraints on CO₂ and DOC efflux from a well-characterized high-elevation mountainous meander within the Upper Colorado River Basin. Changes in mineral and metabolic constraints determined by spectroscopic and multi-omics approaches were linked to CO₂ and DOC efflux from the meander as determined by high-resolution monitoring. We hypothesized that seasonal flooding will shift the mechanisms of C loss from metabolic to mineral constraints during flooding and drainage, respectively, and specifically that, relative to low water years, extreme flooding will result in stronger increase in metabolic constraints and disruption of mineral constraints, resulting in an increase in DOC relative to CO₂ loss pathways, with subsequent impacts on DOC and DIC riverine export.

4.3 Methods

4.3.1 Field site description

To assess how seasonal flooding impacts mineral and metabolic constraints of soil carbon cycling, we established a sampling transect across an active meander of the East River floodplain (Gothic, CO; see Chapter 2). In the Rocky Mountains, winter 2017-2018 received record low snowpack, while the area received extremely high snowpack in winter 2018-2019; these contrasting snowpack levels correspond to low and high river discharge levels in 2018 and 2019, respectively (Fig. C.1). Importantly, these two seasons bookend climate change predictions and allow us to test the impacts of flooding both within and across contrasting seasons.

4.3.2 Environmental monitoring of water table depth and soil moisture, temperature, and redox potential

We used a combination of monitoring equipment to assess changes in hydrology and soil moisture, temperature, and redox regime over the course of seasonal flooding. We tracked water table depth using an electrical water level meter several times per week from June to September across both years. We measured soil moisture and temperature using Acclima sensors (model TDR-315L) set to measure every two hours, and installed along the sampling transect at depths 15, 30 and 70 cm. For the remainder of this study, we refer to 30 cm as “shallow” and 70 cm as “deep”. To measure soil redox potential, we installed platinum-tipped redox sensors and KCl salt bridges (built in-house) at the same locations and depths, which we measured manually using a multimeter over the course of seasonal flooding.

4.3.3 Sample collection: pore water, groundwater, and soils

We collected pore water and soil across seasonal flooding over years 2018 and 2019. For pore water sampling, we installed MacroRhizon pore water samplers (pore size 0.15 μm , Rhizosphere Research Products) in triplicate at the same positions and depths as the soil moisture, temperature, and redox sensors, and syringe sampled into evacuated amber vials over the course of seasonal flooding. Upon collection, these were refrigerated and analyzed for dissolved OC, metals, and FT-ICR-MS as described below.

We collected groundwater samples using a peristaltic pump from piezometers installed along the sampling transect (Fox et al., 2021). Briefly, we collected filtered water samples (0.45 μm PVDF syringe filter) for dissolved inorganic carbon (DIC) and organic carbon (DOC), which were refrigerated at 4 C for storage and shipment.

We collected soil samples in triplicate at the same positions and depths; these depths were chosen based on visual assessment of redoximorphic features during field sampling. Upon sampling, we flash froze soil samples using alcohol and dry ice, and stored them at -80 C for further laboratory analyses.

4.3.4 Pore water and groundwater analysis

4.3.4.1 Determination of DOC in pore water and groundwater

We analyzed pore water samples for DOC using a TOC-L Analyzer (Shimadzu, Kyoto, Japan), and groundwater samples for total DOC on a TOC-V Analyzer with a nondispersive infrared detector (Shimadzu, Kyoto, Japan). For both sets of samples, we

measured total dissolved C by catalytically aided combustion at 680°C, and analyzed DOC samples by non-purgeable organic C, for which we acidified samples with HCl and purged them with N₂ to remove inorganic C prior to analysis.

4.3.4.2 Determination of dissolved metals in pore water and groundwater

We measured elemental concentrations, including iron (Fe) and aluminum (Al), by inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer Elan DRC II) after acidification and dilution with ultrapure 0.16 M nitric acid and internal standard addition.

4.3.4.3 Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) analysis of pore water

FT-ICR-MS analysis was performed at the Environmental Molecular Science Laboratory in Richland, WA. Pore water samples were diluted to 5 mL, and then concentrated using solid phase extraction (SPE) following an established protocol (Dittmar et al., 2008). Briefly, the sample was acidified to pH < 2 with HCl (37%), the sorbent was washed with 50 mL of HCl (10 mm), and then eluted with 1.5 mL of MeOH. Spectra were acquired using a 12 Tesla FTICR mass spectrometer (Bruker Daltonics) equipped with Infinity Cell and Apollo II ESI source. An infusion system (Orton et al., 2018) automatically infused 20 µL of each sample at 3 µL/min with a backing solvent of MeOH/H₂O (70%). Negative mode ionization was used with a capillary voltage of 4.5kV, dry gas of 180°C at 4L/min. Ion accumulation was 100 ms. Spectra were recorded between *m/z* 98 and 900 with a

transient length of 1.1 s yielding a measured resolving power of 250k at m/z 400. Spectra were acquired in duplicate, with blanks and QCs.

Spectra were processed with CoreMS, and open-source python-based mass spectrometry framework (Corilo et al., 2021). Each spectrum was imported from the raw vendor format, processed, and peak-picked. For Thermo format files, peak picking was applied with noise threshold of 1 standard deviation as it was already noise-reduced data. For Bruker format files, a noise threshold of 3-standard deviations was used. Spectra were internally recalibrated against typical organic matter homologous series. Formulas were assigned with the following constraints: maximum mass error ± 0.5 ppm, C1-90, H4-200, O1-23, N0-2, S0-1, allowing for [M-H]⁻ ion types. Prior to statistical analysis, peaks were filtered to only include masses between $200 < m/z < 900$. Compounds were grouped into molecular classes (e.g., lipid-, protein-, carbohydrate-, lignin-, tannin-, condensed aromatic-like) based on C, H, and O for H/C and O/C ranges according to previous literature (Bailey et al., 2017). Each assigned molecular formula was used to calculate the nominal oxidation state of C (NOSC), double bond equivalence (DBE), and aromaticity index (AI) as in previous work (LaCroix et al., 2019).

4.3.4 Soil analysis

4.3.4.1 Sequential extractions of soils

To selectively dissolve mineral phases of varying degrees of order and their associated organic matter, we extracted soils sequentially with four reagents (Fox et al., 2017; Heckman et al., 2018; Wagai and Mayer, 2007). Specifically, we extracted soils

taken from two time points per year corresponding to early season flooding (May 28, 2018, and June 15, 2019) and late season drainage (September 22, 2018, and September 23, 2019). After each extraction, the supernatant was filtered using 0.22 μm filters (Sigma-Aldrich). Briefly, in an anaerobic chamber to maintain soil redox conditions, 1.0 g soil was extracted with 30 ml degassed and deionized (DI) water to extract bioavailable phases of minerals and organic matter (tubes lined with Teflon tape and shaken 2 h at 200 rpm, centrifuged 20 min at 510 x g); this extraction represented water-extractable organic carbon (WEOC). In the anaerobic chamber, the same soil pellet was then extracted with 30 ml of 0.1 M degassed sodium pyrophosphate with pH adjusted to 8.5 using concentrated HCl (Regelink et al., 2014); this extract targeted amorphous metal-organic complexes (Heckman et al., 2018; Kaiser et al., 2016) (tubes lined with Teflon tape and shaken 16 h at 200 rpm, centrifuged 1 h at 4000 x g). Then, 30 ml of 0.5 M hydrochloric acid (HCl) was added to the remaining soil pellet to target reducible short-range order phases (Fox et al., 2017) (shaken 4 h at 200 rpm, centrifuged 20 min 4000 x g). Lastly, 30 ml of 0.049 M sodium dithionite was added to the soil pellet to target total reducible phases (Wagai and Mayer, 2007) (shaken 16 h at 200 rpm, centrifuged 20 min at 510 x g) and combined with the filtered extract of a 10 ml of 0.05 M HCl rinse (shaken 1 h at 200 rpm, centrifuged 20 min at 510 x g).

4.3.4.2 Analysis of dissolved metals and DOC of sequential extractions

We analyzed soil extracts for dissolved metals using Microwave Plasma-Atomic Emission Spectrometry (Agilent, Santa Clara, CA), diluting to a final concentration of

2% nitric acid. We analyzed soil extracts for total non-purgeable DOC following the same method as the pore water analysis above.

4.3.4.3 Determination of Fe(II) in soil samples

We determined Fe(II) on the same soils as used in the sequential extractions. To measure Fe(II) production in soils, we used a modified ferrozine assay on acid extractions (Gibbs, 1979; Roden and Wetzel, 1996). Briefly, we extracted 1 g of each soil sample with 20 ml of 0.5 M HCl (shaken 1 h at 200 rpm, centrifuged 1 h at 4000 x g) and filtered the supernatant through 0.2 µm syringes. To measure Fe(II) content in the soil extracts, we combined 100 µl of the filtered supernatant with 2 ml of ferrozine HEPES buffer (0.406 µM ferrozine and 50 µM HEPES buffer, adjusted to pH 7 with HCl). After 15 minutes, we measured the absorbance of samples at 562 nm using UV-Vis spectroscopy (UV-1800, Shimadzu, Kyoto, Japan). To measure total Fe in the soil extracts, we combined 100 µl of the filtered supernatant with 2 ml of a 1% hydroxylamine HCl buffer, which acts as an Fe reducing agent. Samples were incubated for 4 h and then absorbance was read as above. For both Fe(II) and total Fe assays, we diluted samples as necessary and measured Fe using a standard curve from 1 mM ferrous ammonium sulfate in 0.05 M H₂SO₄.

4.3.4.4 Proteomics analysis of soil samples

Soil protein extraction and analysis was performed on methanol extracts of the same soils as used in the sequential extractions. The method followed that listed in Chapter 3. To enable comparison across proteomics samples, we followed published

methods (Diamond et al., 2019) to cluster proteins into their assigned functional orthology groups (e.g., EC number) and summed spectral counts for proteins in the same sample with the same functional assignment. Before performing further statistical analysis, we removed various contaminants before analysis, including human and bovine proteins (Jouffret et al., 2021) and also removed functions that were present in fewer than five samples were removed from the analysis.

4.3.5 Soil surface carbon dioxide (CO₂) efflux

To monitor soil CO₂ efflux, eight PVC collars (10 cm diameter) were installed to 5 cm depth along the sampling transect at near-river and mid-meander positions. We used a LI-COR (8100A, Biosciences) to monitor soil CO₂ efflux over the course of seasonal flooding. Briefly, CO₂ efflux was measured several times per week, and instrument parameters were as follows: CO₂ efflux was averaged across three readings per collar, with 15 s dead band and pre- and post-purge times. To limit the contribution of autotrophic respiration in our measurements, we periodically clipped vegetation inside the PVC collars.

4.3.6 Statistical analyses

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022). To assess differences in floodplain OC composition (e.g., FT-ICR-MS and proteomics) and composition of mineral-organic associations (e.g., sequential extractions and Fe(II) content), we used Shapiro-Wilk and Levene's tests to assess normality and equality of variances for all data. To test our hypotheses, we used analysis of variance

(ANOVA) to assess the variation explained by flooding condition and year, grouped by soil depth. We combined this with Tukey's honestly significant difference *post hoc* test, using the R packages *agricolae* (Mendiburu and Yaseen, 2021) and *emmeans* (Lenth, 2023). Additionally, we performed linear regressions to determine relationships between groundwater DOC and Fe. For all statistical analyses, $\alpha = 0.05$ indicates significant differences

4.4 Results

4.4.1 Seasonal patterns in river discharge, water table depth, and soil temperature, moisture, and redox potential

East River discharge levels varied drastically across the years 2018 and 2019 (Fig. 4.1a). In 2018, peak river discharge was $24 \text{ m}^3 \text{ s}^{-1}$ and occurred on May 26. In contrast, peak river discharge in 2019 was $77 \text{ m}^3 \text{ s}^{-1}$ and occurred on June 15. River discharge decreased towards base flow levels, which were reached in July in 2018 and in September in 2019.

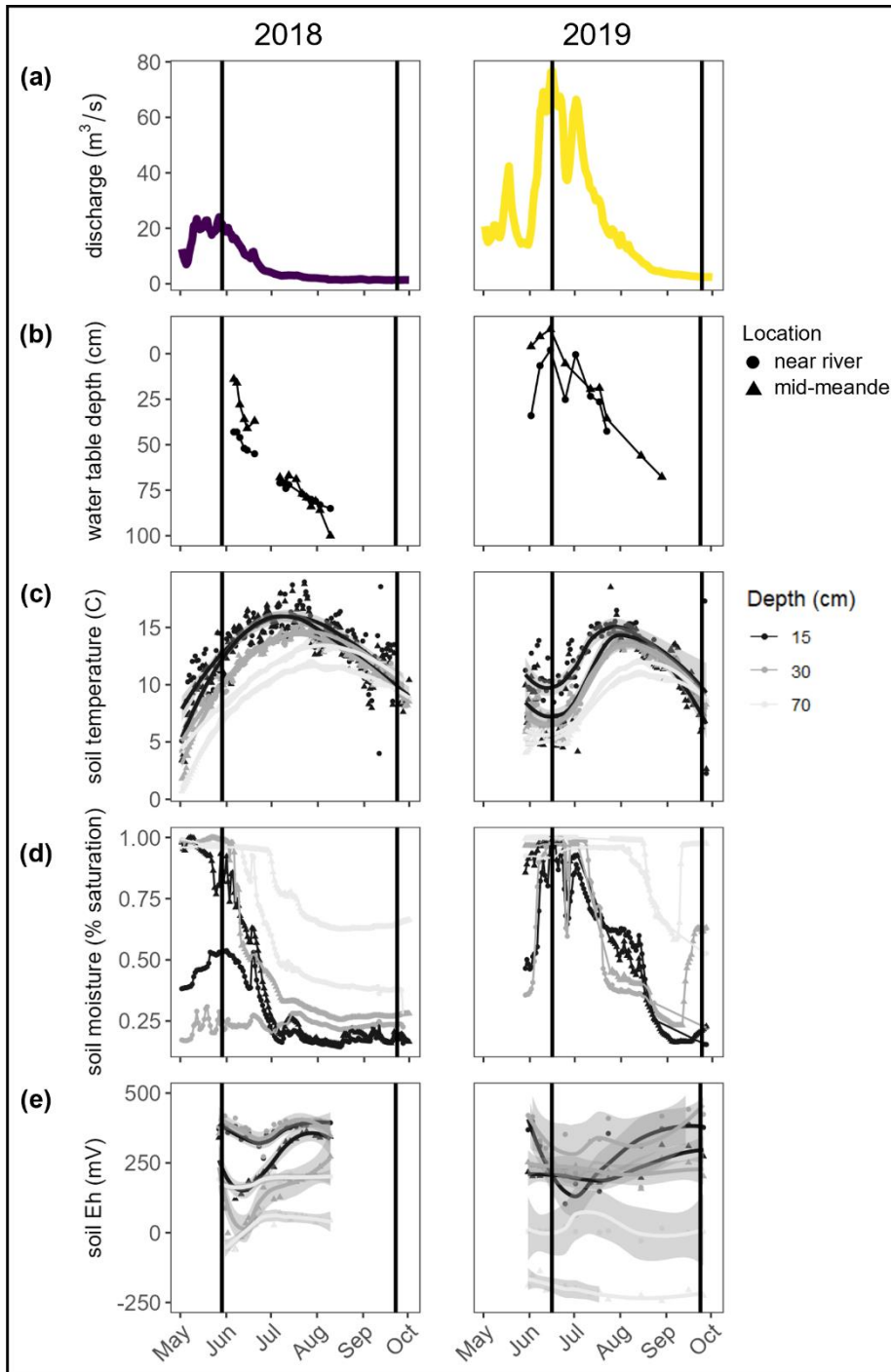


Figure 4.1. East River monitoring data across the years 2018 and 2019, including: (a) river discharge, (b) water table depth, (c) soil temperature, (d) soil moisture, and (e) redox potential. A water table depth of 0 cm indicates ponding at the soil surface. Vertical black lines indicate sampling dates. Data for panel (a) from USGS monitoring station 09112200 at East River Below Cement Creek (<https://waterdata.usgs.gov/monitoring-location/09112200/>).

Correspondingly, water table depth also differed across the years (Fig. 4.1b). Generally, water table depth decreased over the course of seasonal flooding. Specifically, in 2018 the water table at the sampling point nearest to peak flooding was 43 cm and 14 cm below ground surface at the near-river position and mid-meander positions, respectively. Upon drainage, the water table decreased to 85 cm and 100 cm below the ground surface at the near river and mid-meander positions, respectively. In 2019, during peak flooding the water table was 2 cm and 13 cm above the ground surface at the near river and mid-meander positions, respectively. Upon drainage, the water tables in 2019 reached 43 cm and 68 cm below the ground surface at the near river and mid-meander positions, respectively.

Soil temperature across both years 2018 and 2019 followed similar trends, regardless of soil depth: lower temperatures during early-season flooding, followed by an increase over the course of drainage, and a decrease at the end of the season (Fig. 4.1c). Overall, deeper soils stayed cooler than more shallow soils. However, there were distinct differences across the two years. Specifically, in 2018 temperatures during peak flooding were 10 C in surface soils (7-8 C in deep soils) and were similar across near river and mid-meander positions. In contrast, during peak flooding in 2019 temperatures were 8 C in surface soils at near river but 6 C at mid-meander; deep soils in both positions were at 5 C during peak flooding. Despite these early-season differences, soil temperature maxima for both years, across all depths and positions, were similar, reaching approximately 19 C towards the end of July.

Both near-river and mid-meander positions showed similar soil moisture patterns over the course of seasonal flooding: soils were more saturated during flooding, and less upon drainage (Fig. 4.1d). Specifically, in 2018 deep soils across both positions and all soils at the mid-meander position were nearly completely saturated during peak flooding, while surface and 30 cm soils at the near river position, however, remained at only 25-50% saturation during flooding, respectively. In 2018, deep soils drained past peak saturation in early June (near river soils) and late June (mid-meander soils). Soil moisture patterns in 2019 contrasted with those in 2018: during peak flooding in 2019 all soils across the floodplain meander were completely saturated. In 2019, surface and 30 cm soils began to drain late June and early July, regardless of meander position; in contrast, deep soils remained saturated for longer, not draining until August 2019.

Across both sampling years, soil redox potential generally was relatively more reducing (i.e., negative) during flooding and increased upon drainage (Fig. 4.1e). Overall, soils at the mid-meander position generally were more reducing than near river soils. Across all soil depths, soils were more reducing in 2019 than in 2018. Specifically, in 2018, soil redox potential in surface soils decreased after flooding occurred, and increased upon drainage, especially in the mid-meander soils which transitioned from 120 to 344 mV across the season. Soils from 30 cm followed a similar pattern as surface soils, but those at the mid-meander position were lower overall. Soil redox potential values in 70 cm soils followed a similar pattern across both sampling positions, but mid-meander soils were more reducing, reaching a low of -62 mV during peak flooding.

In contrast, redox potential in 2019 was generally more reducing than in 2018, across positions and depth. Surface soils followed similar patterns as in 2018, although

the near river surface soils become much more reducing (103 mV during peak flooding) and mid-meander surface soils generally stayed reducing, only increasing upon late season drainage. In 2019, soils at 15 cm and 30 cm followed similar patterns. Soil redox potential values in deep soils in 2019 were markedly different than in 2018: in 2019, redox potential values stayed reducing throughout the duration of seasonal flooding and did not increase upon late season drainage.

4.4.2 Spatiotemporal variation in biogeochemical factors

4.4.2.1 Extractable organic C

Total extractable OC significantly differed across sampling years, with soils in 2019 containing 26% more extractable OC than in 2018 (Fig. 4.2a, Table C.1, $p < 0.05$). While shallow soils did not differ across years or depths, in 2019 deep soils had significantly higher extractable OC content in than in 2018, and they also significantly decreased from flooded to drained conditions (Fig. 4.2a, Table C.1, $p < 0.05$).

Water-extractable OC was over four-fold higher in 2019 than 2018, regardless of flooding condition (Fig. 4.2a, Table C.1, $p < 0.05$). Moreover, while water-extractable OC content did not shift upon drainage in 2018, in 2019 it was significantly higher during flooding and decreased upon drainage; this was true across both soil depths (Fig. 4.2a, Table C.1, $p < 0.05$).

Pyrophosphate- and hydroxylamine extractable OC significantly differed by depth, with higher OC contents in shallow soils of both extracts ($p < 0.05$), but not by year or flooding condition (Fig. 4.2a, Table C.1). Dithionite-extractable OC similarly

differed significantly by depth ($p < 0.05$), but only shifted across seasonal flooding in deep soils: specifically, in 2018 flooded soils contained significantly more dithionite-extractable OC than during drainage, and overall deep flooded soils in 2018 contained more dithionite-extractable OC than in 2019.

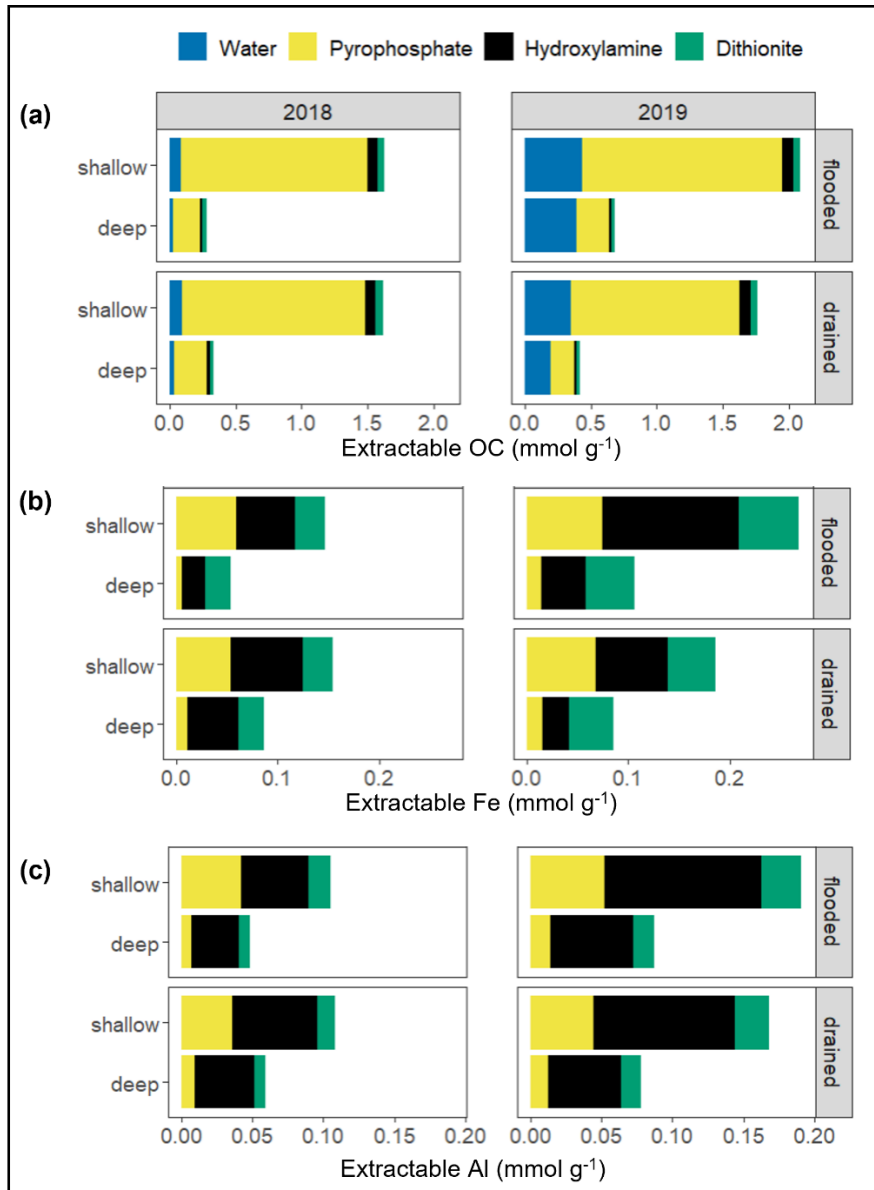


Figure 4.2. Extractable (a) organic carbon (OC), (b) iron (Fe), and (c) aluminum (Al) in different extracts (water in blue, pyrophosphate in yellow, hydroxylamine in black, and dithionite in green), across soil depth and from flooded to drained periods across years 2018 and 2019.

4.4.2.2 Spatiotemporal variation of extractable Fe and Al

Overall, total extractable Fe was nearly three-fold higher in shallow soils compared to deep soils, and 58% higher in 2019 than 2018, the latter driven by a significantly higher amount of extractable Fe during flooding in 2019 (Fig. 4.2b, Table C.2, $p < 0.05$). There was significantly more extractable Fe in shallow compared to deep soils ($p < 0.05$). Water-extractable Fe was two-fold higher in deep compared to shallow soils (Fig. 4.2b, Table C.2, $p < 0.05$), but there were no significant differences across years or seasonal flooding. Pyrophosphate-extractable Fe was significantly higher both in shallow soils and in 2019 (Fig. 4.2b, Table C.2, $p < 0.05$), but there were no significant differences across seasonal flooding. Specifically in deep soils, there was more pyrophosphate-extractable Fe in 2019 compared to 2018. Similarly, hydroxylamine-extractable Fe was significantly higher both in shallow soils and in 2019 (Fig. 4.2b, Table C.2, $p < 0.05$). Specifically, during flooding soils contained more hydroxylamine-extractable Fe in 2019 compared to 2018, and in 2019 the amount of hydroxylamine-extractable Fe decreased upon drainage. There was significantly more dithionite-extractable Fe in 2019 compared to 2018 (Fig. 4.2b, Table C.2, $p < 0.05$), and this was true across both flooded and drained sampling points.

Extractable Al followed similar patterns as extractable Fe, with some notable differences. There was significantly more total extractable Al in shallow compared to deep soils, and in 2019 compared to 2018 (Fig. 4.2c, Table C.3, $p < 0.05$). While there no difference in water-extractable Al by depth, it was higher in 2019 than in 2018, and this was driven largely by a significant increase in deep soils upon drainage. While pyrophosphate-extractable Al was higher in 2019 than 2018, within each depth category

there were no significant differences across years or flooding status. Similar to Fe patterns, hydroxylamine-extractable Al was higher during flooding in 2019 than 2018; however, unlike Fe, hydroxylamine-extractable Al remained high upon drainage. Dithionite-extractable Al followed similar patterns as Fe: it was significantly higher in 2019 compared to 2018, across both sampling points and depths.

Extractable Al:Fe ratios showed distinct spatial and temporal shifts with soil depth and over the season across 2018 and 2019 (Fig. C.2). Within each depth, Al:Fe ratios were significantly different across years ($p < 0.05$). In flooded soils, there were no differences in the extractable Al:Fe ratio across years 2018 and 2019 ($p > 0.05$). In contrast, upon drainage soils in year 2019 had significantly higher extractable Al:Fe ratios, regardless of soil depth ($p < 0.05$). During flooding, neither shallow nor deep soils significantly differed in extractable Al:Fe ratios between 2018 and 2019, but they did upon drainage ($p < 0.05$).

4.4.2.3 Fe(II) dynamics

There were no significant differences in extractable Fe(II) across years during flooding (Fig. 4.3). However, upon drainage Fe(II) levels were significantly lower in 2019 compared to 2018 ($p < 0.05$). In surface soils in both 2018 and 2019, Fe(II) levels decreased from flooding to drainage ($p < 0.05$).

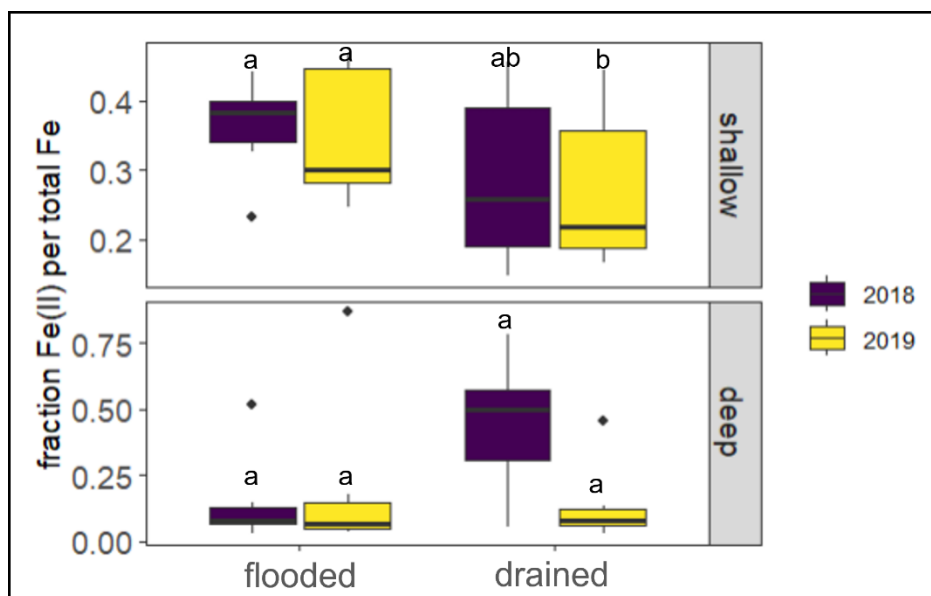


Figure 4.3. The fraction of HCl-extractable iron(II) (Fe(II)) relative to total Fe, by soil depth (shallow vs. deep) and year (2018 vs. 2019). Letters indicate Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of flooding condition and year, performed separately for each soil depth. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

4.4.2.4 FT-ICR-MS of pore water organic C

After filtering for mass (see Methods), 2018 pore water contained 6925 identified peaks, 2019 pore water contained 9798 peaks. During flooding, nominal oxidation state of carbon (NOSC) values were 40% higher in 2018 compared to 2019, a trend especially pronounced in shallow depths (Fig. 4.4a, $p < 0.05$). Across both years, NOSC values in both shallow and deep pore water were higher upon drainage than during flooding (Fig. 4.4a, $p < 0.05$). However, there were no significant differences in NOSC by depth.

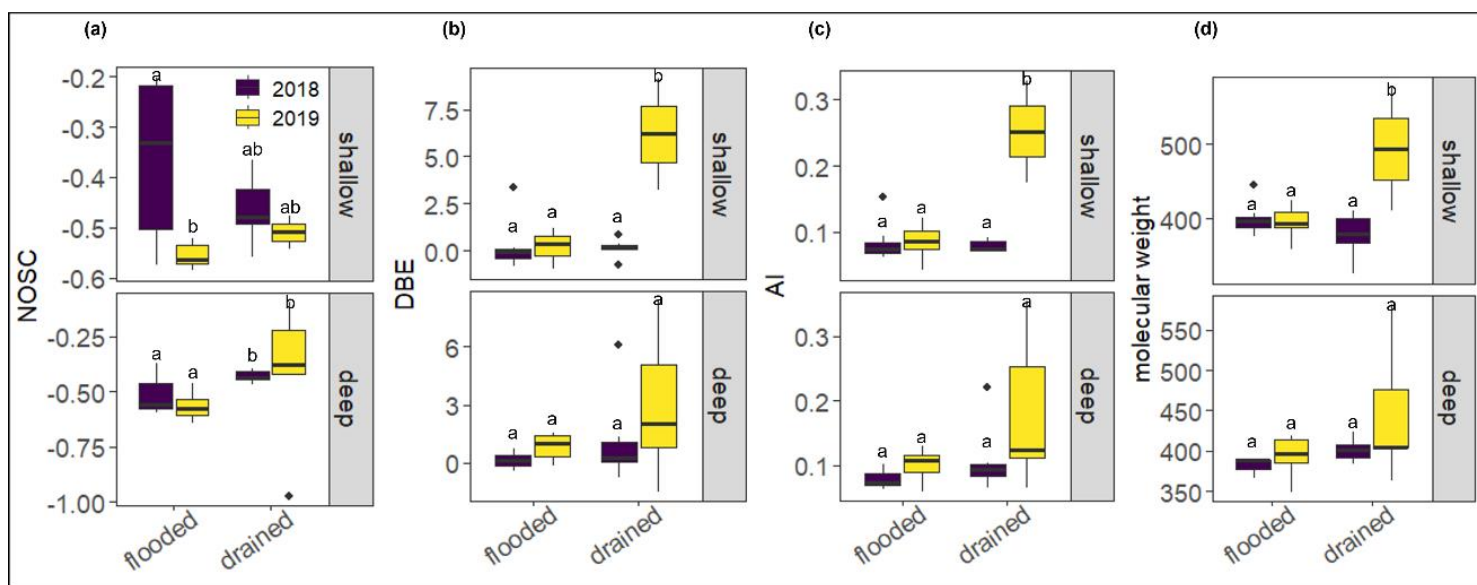


Figure 4.4. Pore water FT-ICR-MS data, including (a) nominal oxidation state of carbon (NOSC), (b) double bond equivalence (DBE), (c) aromaticity index (AI), and (d) molecular weight. Data is separated by depth (shallow vs. deep), flooding condition (flooded vs. drained), and years (2018 vs. 2019). Letters indicate Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of flooding condition and year, performed separately for each soil depth. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

During flooding, there were no significant differences in pore water double bond equivalency (DBE) across the sampling years. However, upon drainage, DBE values increased over four-fold in 2019 compared to 2018 (Fig. 4.4b, $p < 0.05$); taking soil depth into account, this trend was only significant in shallow soils. Another measure of aromaticity, aromaticity index (AI), showed similar patterns as DBE (Fig. 4.4c).

While the molecular weight of pore water OC during flooding did not significantly shift between years, upon drainage the average molecular weight increased by 18% in year 2019 compared to 2018 (Fig. 4.4d); this trend was especially pronounced in shallow depths.

4.4.2.5 Proteomics

Across years 2018 and 2019, a total of 47,548 proteins were identified, 10,785 of which were unique. Of these, 3935 were assigned one of six EC numbers. Across both years, shallow soils contained significantly higher abundances of oxidoreductase proteins compared to deep soils (Fig. 4.5a, $p < 0.05$), and individual year did not shift this relationship. Neither deep nor shallow soils showed significant differences across year or seasonal flooding; however, deep soils in both 2018 and 2019 showed moderate increases in oxidoreductase abundances upon drainage (Fig. 4.5a). Similar trends for transferase, hydrolase, and lyases proteins were seen (Fig. 4.5b, c, and d).

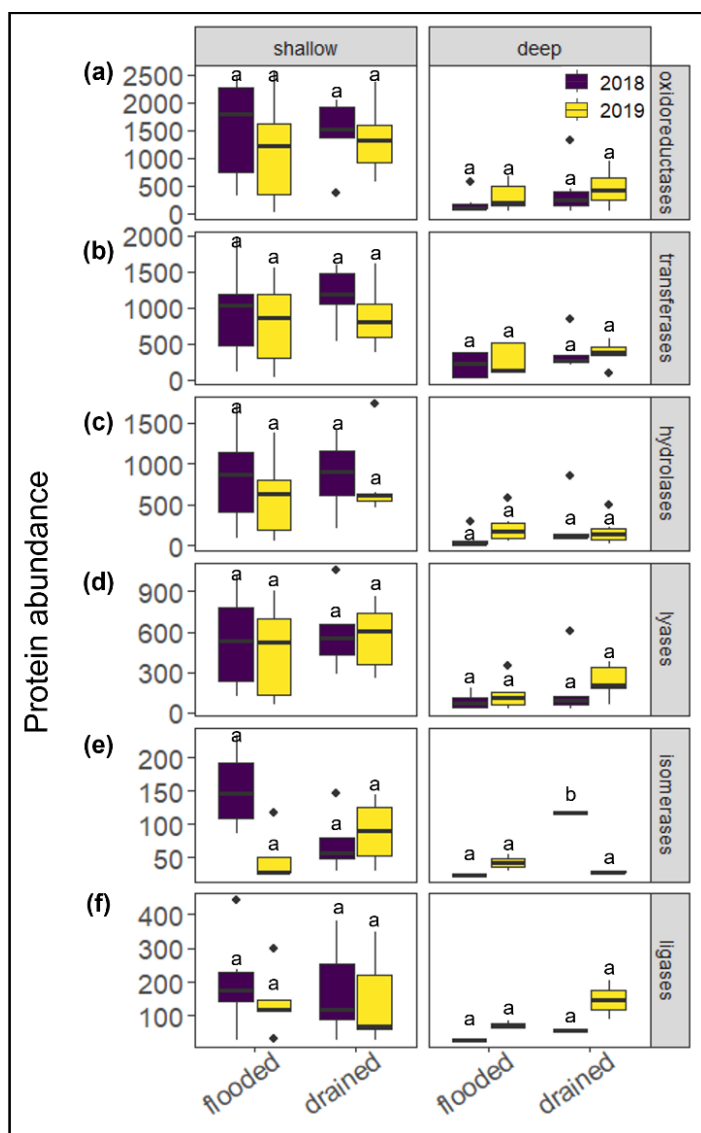


Figure 4.5. Protein abundances by Enzyme Commission (EC) class, in shallow and deep soils, across flooded and drained conditions, in years 2018 (purple) and 2019 (yellow). Letters indicate Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of flooding condition and year, performed separately for each soil depth. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

Overall, isomerase protein abundance was not significant across soil depth (Fig. 4.5e). However, in 2018, soils experiencing flooding contained more isomerase proteins than in 2019, although this was only marginally significant (Fig. 4.5e, $p = 0.056$). While

isomerase abundances in shallow soils did not significantly shift across flooding in either years 2018 or 2019, these abundances were significantly higher in deep soils in 2018 upon drainage (Fig. 4.5e, $p < 0.05$).

The abundance of ligase proteins did not significantly shift across soil depth, year, or flooding condition (Fig. 4.5f). However, in deep soils the abundance of ligases trended towards increasing upon drainage, although this was not significant.

In shallow soils, the ratio of oxidative to hydrolytic proteins did not significantly change from flooded to drained conditions in either year 2018 or 2019 (Fig. 4.6). During flooding, this ratio was 59% higher in 2018 than 2019 (Fig. 4.6, $p < 0.05$), although it did not significantly change across years when soils were drained. In deep soils, the oxidative/hydrolytic protein ratio was significantly higher during flooding in 2018 than in 2019, and upon drainage the ratio decreased in 2018 but increased in 2019 (Fig. 4.6, $p < 0.05$).

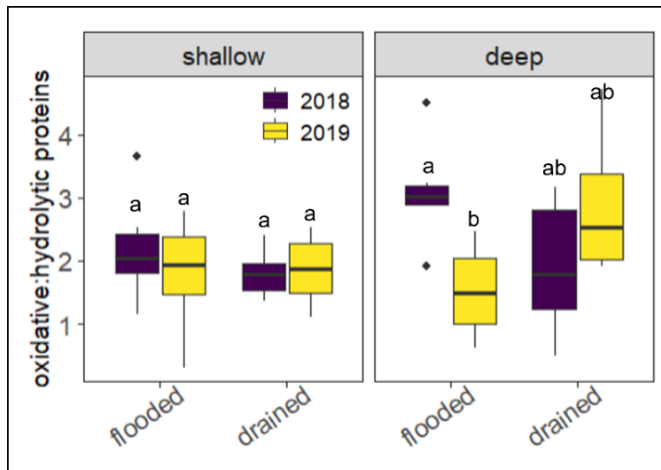


Figure 4.6. Ratio of oxidative to hydrolytic proteins, across shallow and deep soils, from flooded to drained conditions, in years 2018 (purple) and 2019 (yellow). Letters indicate Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of flooding condition and year, performed separately for each soil depth. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

4.4.3 Spatiotemporal dynamics of floodplain C loss pathways

4.4.3.1 Surface carbon dioxide (CO₂) efflux

In 2018, surface soil CO₂ efflux ranged from 1.140 to 4.13 $\mu\text{mol m}^2 \text{s}^{-1}$, and in 2019 ranged from 0.19 to 3.76 $\mu\text{mol m}^2 \text{s}^{-1}$ (Fig. 4.7b). Overall, CO₂ efflux was much higher in 2018 than 2019, although late season CO₂ efflux was similar across both years. Across both years, CO₂ efflux was low during peak flooding – and lower in 2019 than 2018 – and reached a maximum during peak soil temperatures and as deeper soils began to drain (Fig. 4.7b). Importantly, CO₂ efflux did not significantly shift with sampling location was thus averaged across position.

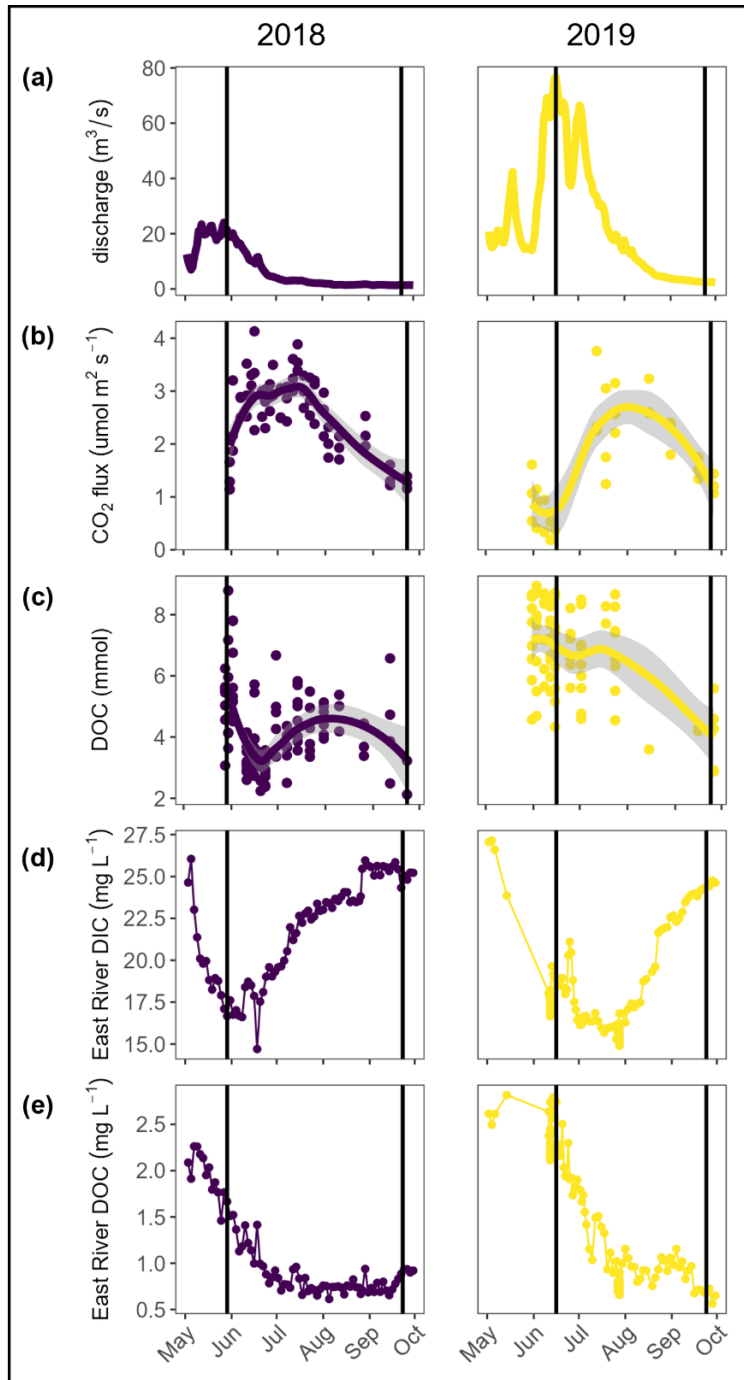


Figure 4.7. Soil carbon losses across years 2018 and 2019: (a) river water discharge, (b) soil CO_2 efflux, and (c) soil pore water dissolved organic carbon (DOC), (d) river water dissolved inorganic carbon (DIC), and (e) river water DOC. Data for panel (a) from USGS monitoring station 09112200 at East River Below Cement Creek (<https://waterdata.usgs.gov/monitoring-location/09112200/>). Data for panels (a)-(c) includes both sampling positions (near river and mid-meander). Data for panels (e) and (f) are from ESS-DIVE: doi:10.15485/1660459 (Dong et al., 2022). Vertical black lines indicate sampling dates.

4.4.3.2 Pore water DOC concentrations

Pore water DOC concentrations did not significantly differ by meander location and were thus averaged. In 2018, pore water dissolved OC (DOC) concentrations ranged from 0.61 to 2.26 mmol L⁻¹, and in 2019 ranged from 0.56 to 2.82 mmol L⁻¹ (Fig. 4.7c). Across both years, pore water DOC reached maximum levels during peak flooding, and decreased upon return to baseflow, which was nearly a month later in 2019 than 2018.

4.4.3.3 River water DIC and DOC concentrations

River water DIC concentrations ranged from 14.7-26.1 mg L⁻¹ in 2018, and from 14.9-27.2 mg L⁻¹ in 2019 (Dong et al., 2022) (Fig. 4.7d). In 2018, DIC levels fell to a minimum upon peak flooding (early June), and by mid-June, when the river reached base flow and soil moisture at 70 cm began to decrease (see Fig. 4.1a and d), were increasing at a steady rate (Fig. 4.7d). In contrast, in 2019 minimum DIC levels were reached several weeks after peak flooding (early July). DIC concentrations remained at their minimum for approximately two weeks longer in 2019 compared to 2018, after which river discharge approached baseflow and soil moisture at 70 cm decreased (see Fig. 4.1a and d), and DIC levels started to increase towards their maximum levels at a steady rate (Fig. 4.7d).

River water DOC concentrations ranged from 0.61 to 2.3 mg L⁻¹ in 2018, and from 0.60 to 2.8 mg L⁻¹ in 2019 (Dong et al., 2022) (Fig. 4.7e). Across both years, peak river DOC concentrations occurred during peak river discharge, and began to decrease as the river returned to base flow.

4.4.3.4 Groundwater dissolved organic C and Fe dynamics

In 2018, groundwater DOC ranged from 0.22 to 0.95 mmol L⁻¹, and in 2019 ranged from 0.16 to 1.24 mmol L⁻¹. Groundwater Fe ranged from 1.31 * 10⁻³ to 0.23 mmol L⁻¹ in 2018 and 1.49 * 10⁻⁴ to 0.28 mmol L⁻¹ in 2019 (Fig. 4.8a). Groundwater DOC positively correlated with groundwater Fe during both flooding and drainage in 2018 ($p < 0.001$, Fig. 4.8a) and only during flooding in 2019 ($p < 0.001$, Fig. 4.8b).

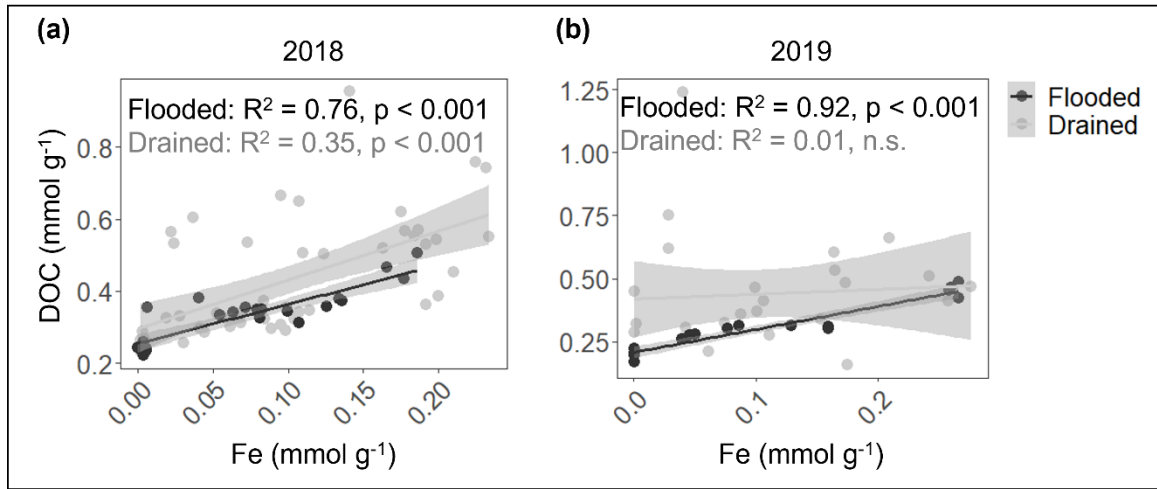


Figure 4.8. Groundwater DOC versus Fe during flooded (black) and drained (gray) timepoints, across years (a) 2018 and (b) 2019. Lines indicate results of linear regressions, and associated statistics are provided, including the R^2 and p-value for model significance.

4.5 Discussion

4.5.1 Seasonal flooding drives differences in water table depth and soil temperature, moisture, and redox potential

Contrasting flooding dynamics over the years 2018 and 2019 drove differences in water table depth, soil moisture, and redox conditions across the floodplain meander. Compared to the drier year 2018, higher river discharge levels in 2019 corresponded with higher water tables, and higher and sustained saturated and reducing conditions across the

meander and soil depths. Specifically, during peak flooding in 2019, the meander experienced ponding at the soil surface and sustained high soil moisture and low redox potential values, especially in deeper soils across the entire meander. In addition to receiving higher snowpack during the winter, 2019 experienced much later snow, corresponding to the higher and later peak flooding, and lower temperatures during peak flooding compared to 2018.

In contrast, the low-water year 2018 corresponded to relatively low soil moisture values near the soil surface, consistent with a relatively low water table and the lack of ponding. Despite this relative dryness in 2018, soils, especially deeper soils towards the meander center, still experienced relatively reducing conditions during early-season flooding. Similar coupling of river discharge, soil moisture, and redox potential has been observed in other floodplain systems (Lazareva et al., 2022) and seasonally flooded wetland soils (LaCroix et al., 2019). In years experiencing drastically different snowpack and subsequent snowmelt, the river discharge impacts environmental conditions along the floodplain meander; while these snowmelt-driven differences are large, they can still dwarf in comparison to hydraulic gradients imposed by beaver dams, for example, which are common in this region (Dewey et al., 2022).

4.5.2 Seasonal flooding disrupts mineral-organic associations

We expected that flooding would result in the disruption of mineral-organic associations and the subsequent loss of dissolved C and Fe. Consistent with our expectations, our sequential extraction data showed evidence for C mobilization during flooding, especially in extreme water years. Specifically, the increase in dissolved (water-

extractable) OC during the extreme flooding in 2019 compared to 2018 indicates initial flushing of pore water DOC, and/or reductive dissolution of Fe-bearing minerals and subsequent Fe and C loss during flooding. The concurrent increase in overall extractable Fe during flooding in 2019 suggests co-dissolution of Fe and C from mineral-organic associations, which is also reflected in the groundwater data. Indeed, the reductive dissolution of Fe oxides and Fe-bearing phyllosilicate clays has been shown to lead to the co-dissolution of Fe and C (Chen et al., 2017). Such a phenomenon has been seen in seasonally flooded soils, where low redox potential effectively removed reactive Fe phases during reductive dissolution reactions, leading to OC and metal loss (LaCroix et al., 2019), and also across other saturated mineral soils (Adhikari et al., 2017; Barcellos et al., 2018a; Buettner et al., 2014; Huang and Hall, 2017). Another indication of Fe reduction, shallow soils across both years contained higher HCl-extractable Fe(II) content relative to total Fe, which decreased upon drainage (significantly so in 2019). In a floodplain of the Piedmont region of Southeastern Pennsylvania and northern Delaware, which is dominated by anoxic buried wetland soils, a close relationship was found between high DOC and dissolved Fe in times of high discharge, which they attribute to reductive dissolution of Fe-bearing minerals (Lazareva et al., 2022).

Overall, we expected the relative importance of dissolved to mineral-associated OC to increase during flooding, especially in the high water year of 2019. The relatively higher contribution of mineral-associated OC in the drier year of 2018, coupled with relatively lower pore water DOC content that same year, suggests that mineral-organic associations are an important sink of floodplain soil OC, and that these are especially important in drought years. In 2019 water-extractable OC dominated at depth in the early

season, shifting to a more even contribution of water- and mineral-associated OC upon drainage. Together, this suggests that mineral-organic associations are important stores of soil C year-round in dry years, whereas they are relatively smaller pools of soil OC during high flooding years until drainage, a phenomenon we have seen across the spatial redox gradients of the meander (see Chapter 2).

Additionally, we expected flooding and associated reducing conditions to specifically disrupt Fe- but not Al-bearing minerals, as has been shown in other systems (LaCroix et al., 2019; Nitzsche et al., 2022). In line with our expectations, in 2019 hydroxylamine-extractable Fe was higher during flooding and decreased upon drainage, whereas the equivalent Al pools remained steady across seasonal flooding; this is suggestive of reductive dissolution of poorly crystalline Fe but not Al minerals. Additionally, in the high water year 2019, the Al:Fe ratios increased upon drainage in both shallow and deep soils, suggesting that Al-bearing minerals become relatively more important for OC storage than Fe-bearing minerals in high water years. This may be because soils in 2019 remained relatively reducing compared to 2018, even upon drainage. This result is in line with our previous study, where we found that the extractable Al:Fe ratio increased in more reducing meander locations (see Chapter 2), as well as other work in soils experiencing variable moisture (Anthony and Silver, 2020; Nitzsche et al., 2022). In contrast, during the relatively dry year of 2018, Al:Fe ratios decreased (albeit not significantly) upon drainage, suggesting that especially in low water years, the recrystallization of Fe-bearing minerals upon floodplain oxidation can once again impact C storage under oxic conditions; such a switch between Fe- and Al-

protection has been shown in other ecosystems with dynamic hydrology (LaCroix et al., 2019; Possinger et al., 2020a).

4.5.3 Oxygen limitations impose metabolic constraints on carbon loss during flooding

We expected that during flooding, reducing conditions would impose metabolic constraints that both prevent the depolymerization and oxidation of soil OC. Specifically, we expected that reducing conditions would impose these constraints on the depolymerization of OC, and that these constraints would be especially pronounced during the high water year 2019 due to sustained oxygen limitations and suppression of oxidative depolymerizing enzymes. Consistent with our expectation, in the high water year 2019, the ratio of oxidative to hydrolytic enzymes increased upon drainage in 2019, at least in deep soils. In contrast, this ratio remained relatively high across seasonal flooding in 2018, signifying little to no kinetic constraints in the drier year. Together, this indicates that kinetic constraints may be stronger during high water years and may be less relevant during dry years. The inhibition of oxidative enzymes in anaerobic conditions, known as the “enzymatic latch” (Freeman et al., 2001), has been shown in saturated peatland soils, where the ratio of oxidative to hydrolytic enzymes was lower under saturated conditions compared to soils held at a higher water holding capacity (Wen et al., 2019). Contrary to our expectations, in shallow soils we did not see evidence for kinetic constraints: there were no significant changes in the ratio of oxidative to hydrolytic enzymes across either year. Shallow soils, even in the high water year, have significantly higher redox potential values compared to deep soils, even during flooding; it is possible they remain oxidized enough to not manifest kinetic constraints.

We also expected thermodynamic constraints on the oxidation of OC to occur during reducing conditions associated with flooding. Consistent with our expectations, across both years pore water NOSC values were more reducing during flooding and increased upon drainage. In tandem with our connection between NOSC values and spectrometry-based identification of these highly reduced compounds (see Chapter 3), and existing literature showing the selective preservation of highly reduced OC compounds in studies in deep sulfidic sediments (Boye et al., 2017), seasonally flooded wetlands (LaCroix et al., 2019), and seasonally hypoxic waters (Chen et al., 2022), our results indicate that flooding, results in thermodynamic constraints on the oxidation of OC.

Further, we expected thermodynamic constraints to be especially pronounced during the high water year 2019, due to sustained saturated soils and associated reducing conditions. During flooding, surface pore water OC in 2019 was more reduced compared to that in 2018; this corresponds with the higher water table experienced in 2019 and associated higher soil moisture and lower redox potential values, and suggests that in higher water years, thermodynamic constraints on the oxidation of OC are relatively more important compared to during lower water years, imposing constraints too steep to lead to significant C loss through oxidation; we posit this shift leads to less CO₂ efflux from the soil during flooding in high water years. The selective preservation of reduced OC compounds (Boye et al., 2017) can prevent OC oxidation (Stuckey et al., 2015).

Importantly, because metabolic constraints may be less important during low water years such as 2018, when the soil redox conditions are relatively more oxic, it is possible that some DOC released from protective mineral-organic associations during

flooding could still be oxidized even during that flooding period, due to the relatively higher soil redox potential experienced in these years; we suggest this leads to the relatively higher CO₂ efflux we see in 2018 compared to 2019 during flooding. Indeed, it has been shown the mineralization of newly released OC during anaerobic conditions as CO₂ or CH₄ has the potential to offset metabolic constraints on SOM in anaerobic conditions, even as some SOM is still protected under these conditions (Huang and Hall, 2017).

4.5.4 Floodplain carbon loss pathways shift over seasonal flooding

Seasonal flooding results in spatial and temporal shifts in the pathways of floodplain carbon loss, with higher DOC production during flooding and higher CO₂ efflux in the later season upon drainage. Across both years 2018 and 2019, early season flooding suppressed CO₂ emissions. However, during peak flooding, CO₂ efflux was higher in 2018 compared to 2019, which we believe is due to both higher soil temperature and moisture at this time point. Comparatively, in 2019, relatively higher moisture and lower redox potential, concurrently with evidence for stronger metabolic constraints that year, likely also influenced the low CO₂ efflux in 2019 during peak flooding. Low CO₂ efflux has been attributed to high moisture and low redox conditions in other soil systems (Lacroix et al., 2021; LaCroix et al., 2019), signifying the importance of these variables.

Despite differences in water table depth and soil moisture and redox potential dynamics, years 2018 and 2019 both experienced peak soil CO₂ efflux and peak soil temperatures at the same period (late July). Maximum soil temperatures were slightly higher in 2018, which may explain why peak CO₂ efflux was also marginally higher that

year. However, the similarity between maximum CO₂ efflux and temperature across the two drastically different water years suggests that in this hydrodynamic floodplain system, temperature might not be the main driver of CO₂ efflux. Indeed, in a study of floodplain soil respiration in the Chesapeake Bay watershed, soil CO₂ efflux was correlated negatively with soil moisture (Batson et al., 2015). More specifically, high moisture and associated reducing conditions might drive low CO₂ efflux during the early season flooding, and low moisture might drive low CO₂ efflux during the late season, with intermediate moisture and maximum temperature driving peak CO₂ efflux mid-season upon floodplain drainage; this is in line with previous work in seasonal wetlands showing similar tradeoffs in temperature, moisture, and redox controls on CO₂ efflux (LaCroix et al., 2019). We acknowledge that our study only considers surface CO₂ efflux, but it is clear that whole soil profile respiration is important for overall C balance, as seen in a semiarid floodplain in Colorado (Tokunaga et al., 2016). Additionally, it is important to note that soil CO₂ efflux includes both respiration from plant roots (i.e., autotrophic) and microbial communities involved in the degradation of soil OC (i.e., heterotrophic). While live root respiration has been shown to account for over half of measured respiration rates (Pulliam, 1993), in our study, we clipped vegetation in the PVC collars to minimize autotrophic respiration. Thus, the CO₂ efflux measured here should more strongly reflect microbial respiration than total soil respiration, but we cannot definitively separate the contribution of root respiration.

Importantly, river DIC concentrations, which originate from dissolved CO₂, mirrored deep soil moisture patterns. Specifically, river DIC levels remained low during peak flooding across both years and did not begin to increase until deep soils began to

drain. This indicates that microbial respiration of OC – and subsequent CO₂ efflux – in these deeper soils was likely limited until deep soils became more ideal for microbial activity post-snowmelt flooding (i.e., increased temperatures, less saturated, and more oxidized conditions). This is similar to patterns observed in the East River, where river DIC concentrations are likely decoupled from initial pulses of river DOC with flooding (Winnick et al., 2017). While we did not explicitly measure deep soil CO₂ efflux, future research should include this measurement to better understand its connection and contribution to riverine DIC levels.

Pore water DOC production was highest across both years during peak flooding, a phenomenon largely tied to hydrologic nutrient flushing of shallow, organic-rich soils (Burns, 2005). During the high water year 2019, DOC levels were higher in pore water, groundwater, and the river, and in connection with the strong correlations between groundwater DOC and Fe as well as the loss of bioavailable OC from soils during flooding, this is indicative that the disruption of mineral-organic associations played a role in this increase in river DOC. The connection between reductive dissolution of Fe-C associations and floodplain C export has been observed in other floodplain soils (Lazareva et al., 2022) and simulated aquifers (Aeppli et al., 2022).

Another important consideration is the connection between upland soils and toeslope or floodplain soils. While we did not consider this source in this study, connectivity with DOC waters flushed from upland soils adds another potential source of DOC export that depends on extent of riparian zones and hydrologic connectivity between them and upland areas (Pacific et al., 2010). Further studies should examine such terrestrial connectivity to understand its influence on floodplain C cycling dynamics.

4.6 Synthesis

By examining hydrodynamic floodplain soils over the course of seasonal flooding, we found that flooding undermines mineral constraints and regulates the subsequent metabolism of newly available OC. Our results further suggest that there is a tradeoff between C loss pathways across extreme dry and wet years, a tradeoff which leads to relatively more DOC loss and less CO₂ loss in high water years (Fig. 4.9). Specifically, high water years can lift mineral constraints imposed by redox-active Fe-bearing minerals, leading to higher DOC loss from pore water to groundwater. Despite the relatively high DOC concentrations during the high flooding year, oxygen limitations associated with reducing conditions during flooding can limit microbial depolymerization and oxidation of these compounds, resulting in the selective preservation of relatively reduced OC compounds and thus limiting CO₂ efflux during extreme flooding years. In contrast, during extreme dry years such as 2018, relatively stronger mineral constraints lead to lower DOC losses, but more oxic conditions, even during flooding, lift the metabolic constraint and allow microbial depolymerization and respiration reactions to continue. Together, this results in relatively lower losses as DOC but higher losses as CO₂ during drier years. Previous work has seen similar tradeoffs in C loss: Huang et al. (2020) found that while anaerobic conditions reduced total C losses compared to the aerobic control, like previous findings (Huang & Hall 2017) they also found that sustained anaerobic conditions can still lead to the loss of bulk and mineral-associated OC. The results presented here show that these tradeoffs can be seen in field studies of floodplains.

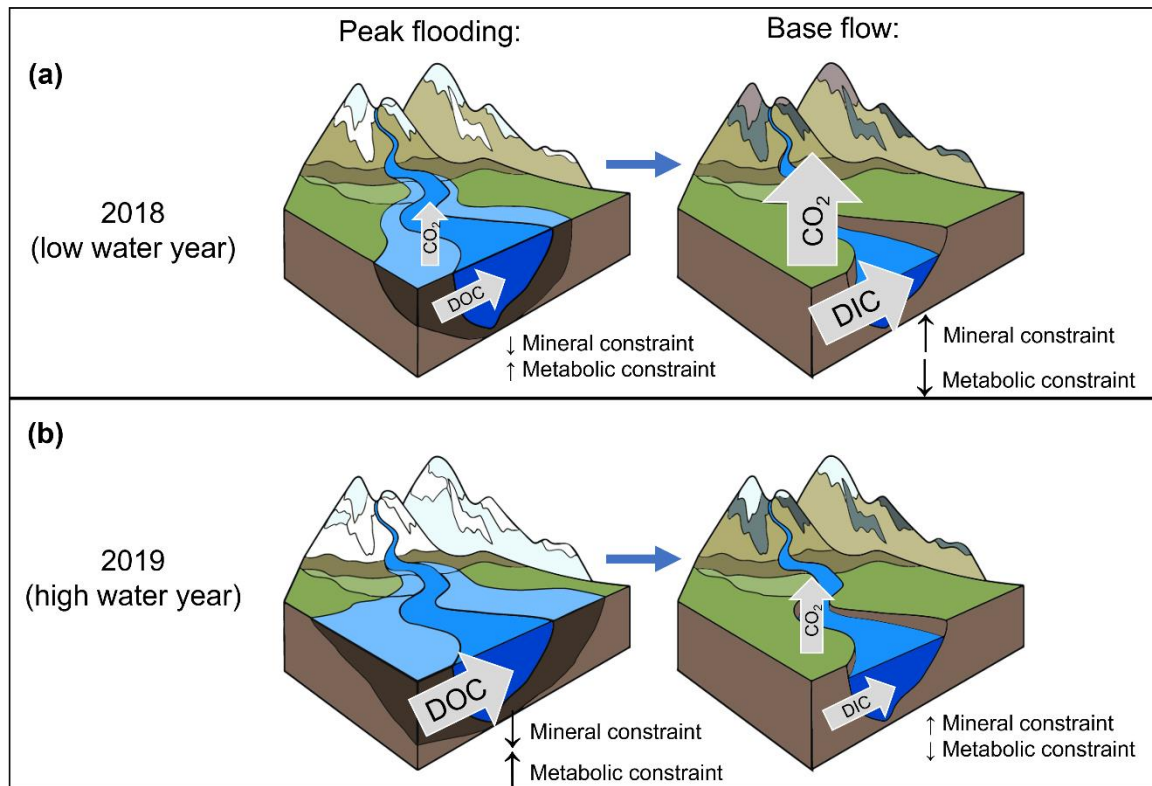


Figure 4.9. Conceptual model of floodplain soil C cycling mechanisms and loss pathways in an active meander of the East River (Gothic, CO), showing (a) low water years, and (b) high water years). Size of arrows indicates relative strength of constraint (mineral or metabolic) or loss pathway (DOC or CO₂).

We observed a strong link between pathways of C loss across variable flooding years and river transport patterns: in the high water year there was more riverine DOC export (due to decreased mineral constraints) but less riverine DIC loss (due to increased metabolic constraints). Indeed, the flux of C from terrestrial to riverine systems represents an important loss pathway from soils, and the balance of inorganic and organic C losses are shifting with climate change (Battin et al., 2023)

The shifting snowpack patterns predicted in the Western United States (Fyfe et al., 2017), and the ongoing megadrought experienced in this region (Williams et al., 2022),

make our understanding of shifting floodplain C dynamics crucial: a shift to drier conditions could lead to a net loss of floodplain C in inorganic forms as either soil CO₂ efflux or DIC export to river systems, with the occasional high water year resulting in DOC losses. Importantly, these mechanisms are likely applicable to other ecosystems, including those used for agriculture, connecting these C dynamics to critical questions of soil health and agricultural productivity in a changing climate.

4.7 References

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2: MOLECULAR NATURE OF MINERAL-ORGANIC ASSOCIATIONS WITHIN REDOX-ACTIVE MOUNTAINOUS FLOODPLAIN SEDIMENTS

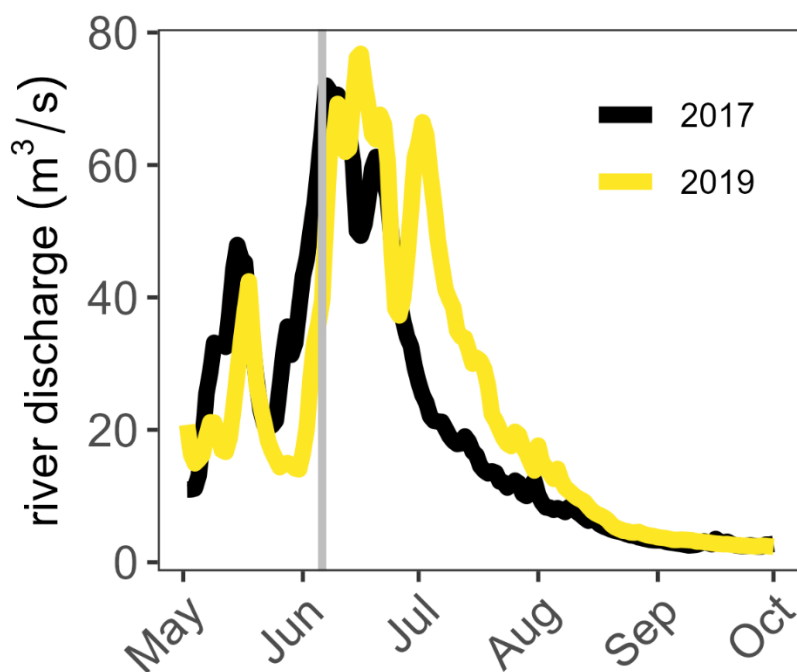


Figure A.1. East River discharge from May through September, across years 2017 (black) and 2019 (yellow). Data from USGS monitoring location at East River below Cement Creek, near Crested Butte, CO (USGS monitoring location 09112200). Solid gray line indicates sediment sampling date in 2017.

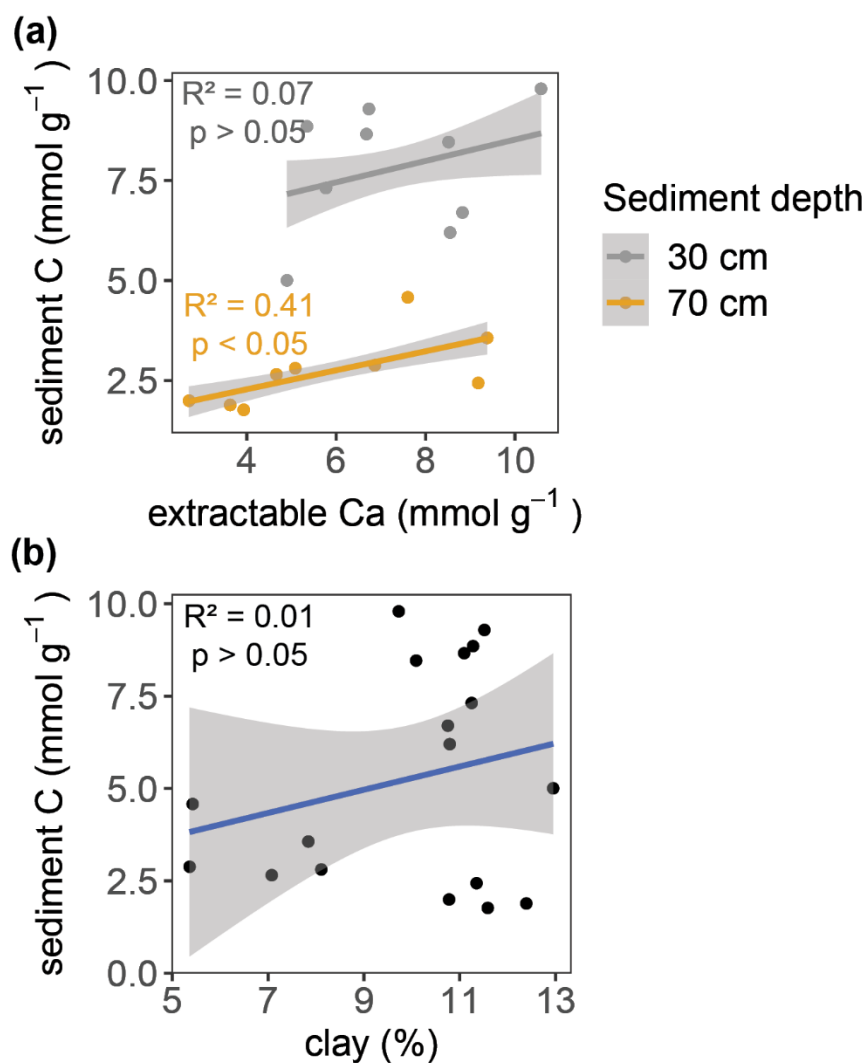


Figure A.2. Correlations between total sediment carbon (C) and (a) total extractable Ca and (b) clay content. Units for both panels are mmol C per g sediment. The linear regression and statistics are provided, including the R^2 and p-value for model significance.

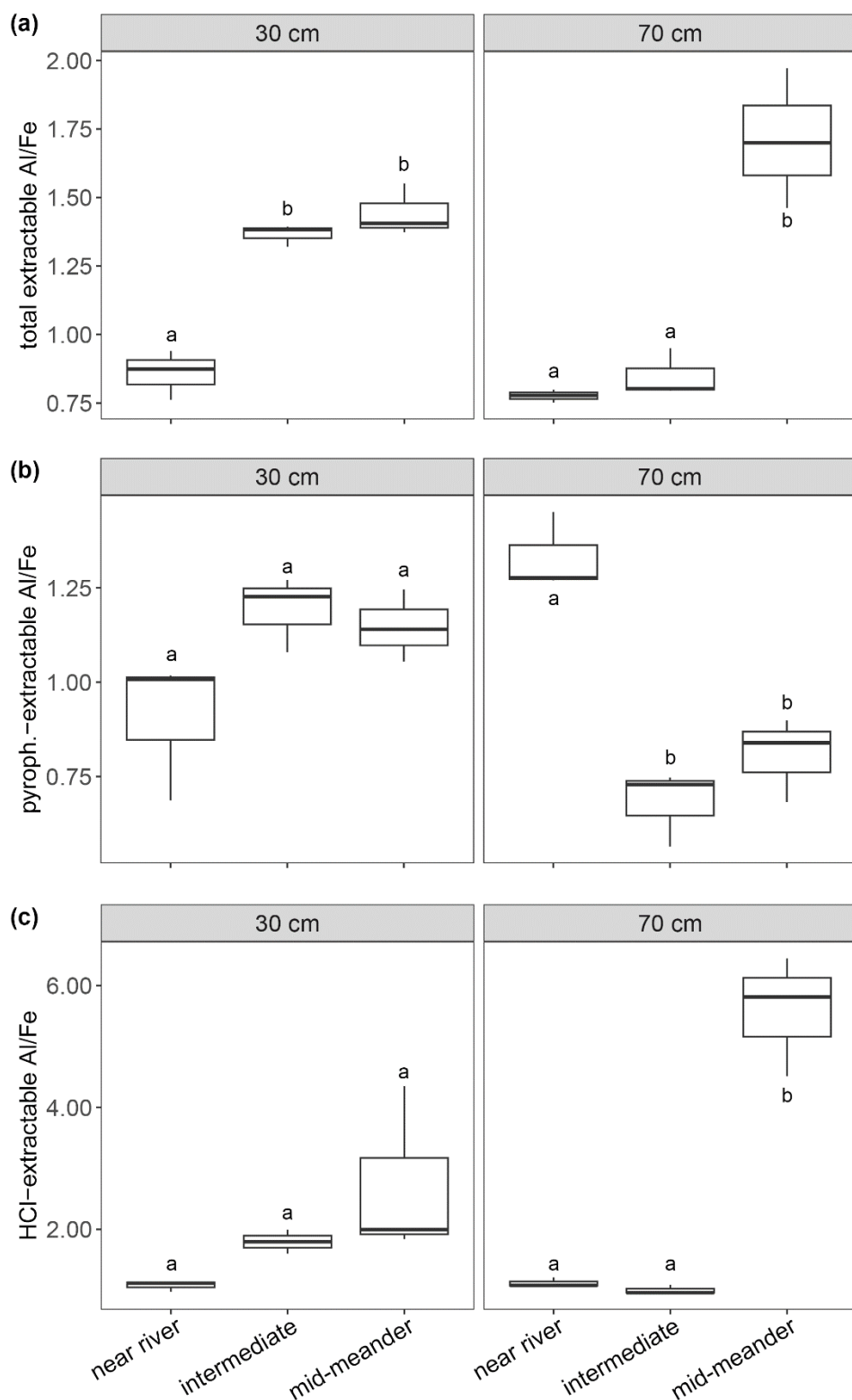


Figure A.3. Extractable Al:Fe ratios in shallow (30 cm) and deep (70 cm) sediment, across the floodplain meander: (a) total extractable Al/Fe, (b) pyrophosphate-extractable Al/Fe, and (c) HCl-extractable Al/Fe. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across the meander in each depth.

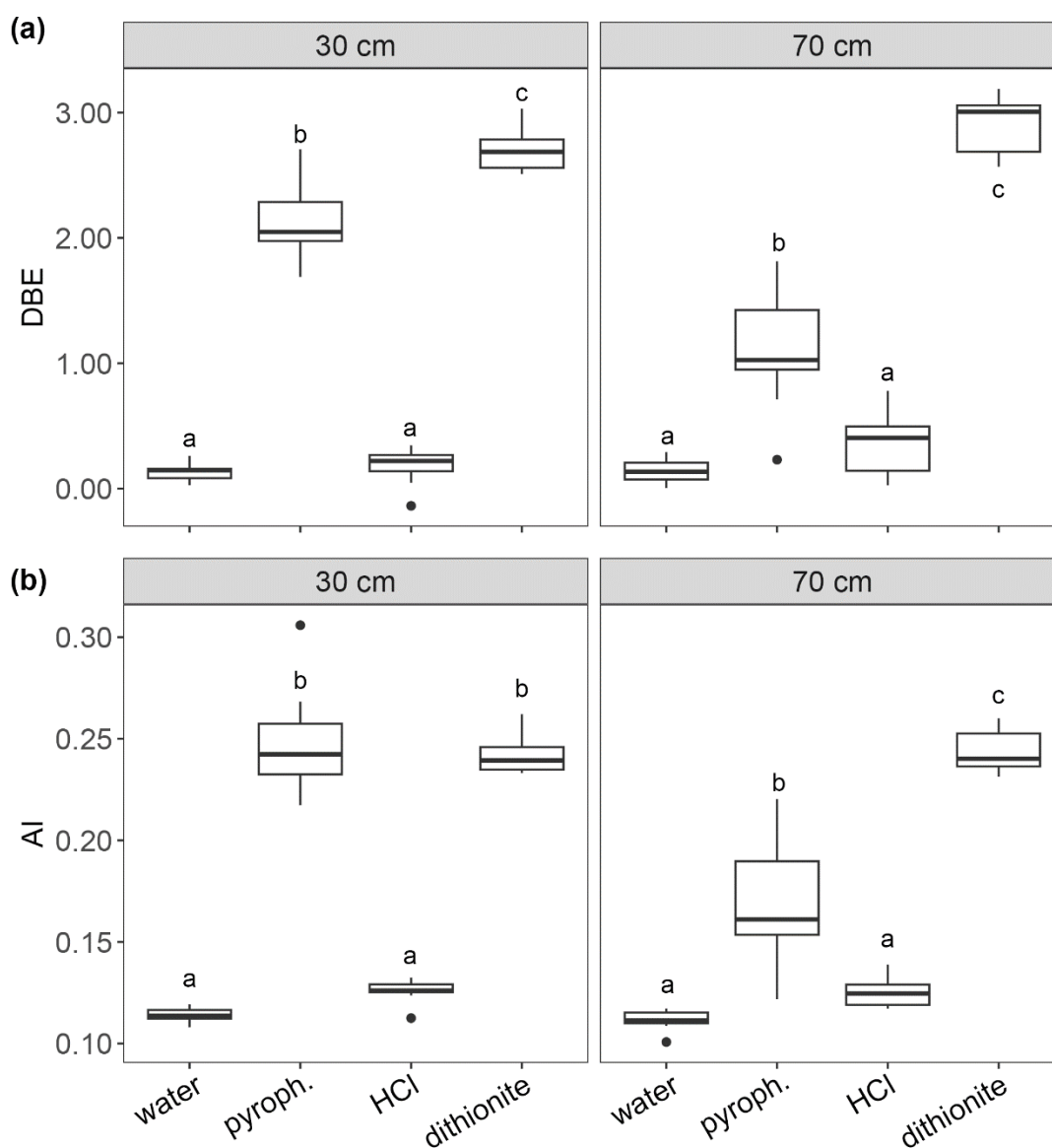


Figure A.4. Shifts in organic matter in shallow (30 cm) and deep (70 cm) sediment, across different extractants (water, pyrophosphate, HCl, and dithionite): (a) double-bond equivalence (DBE) and (b) aromaticity index (AI). Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across extractions in each depth.

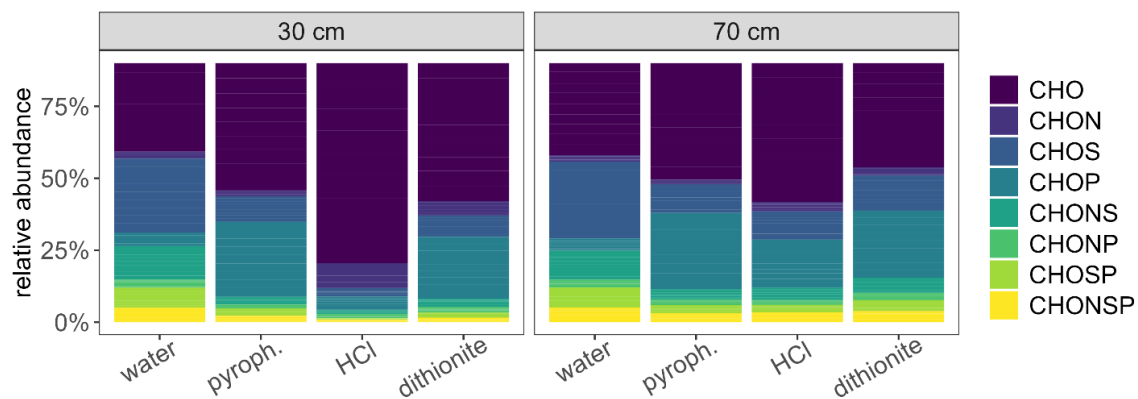


Figure A.5. Relative abundance of different elemental composition groups in sediment organic matter at 30 cm and 70 cm, extracted by water, pyrophosphate, HCl, and dithionite.

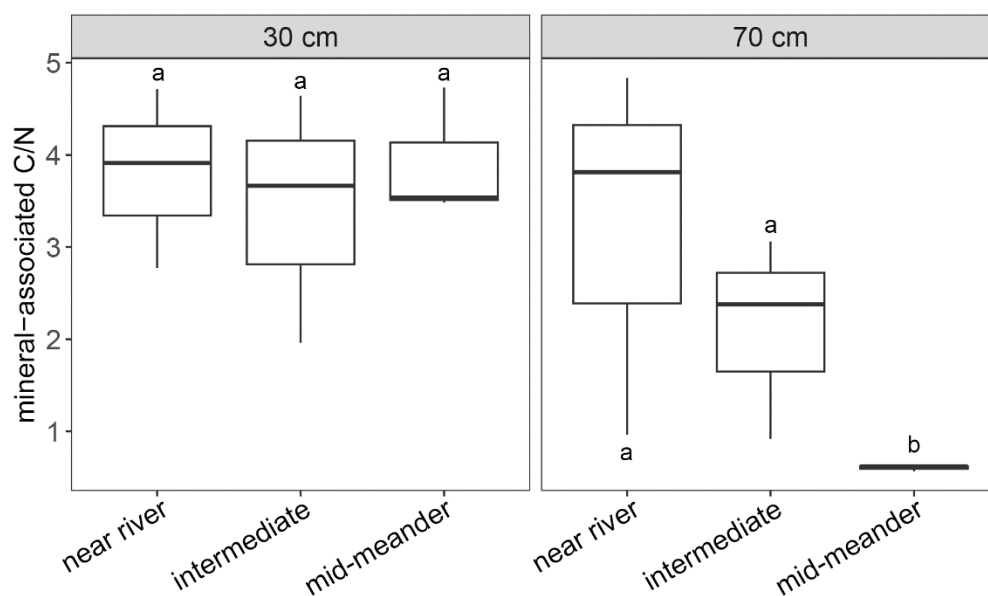


Figure A.6. Carbon to nitrogen ratios of mineral-associated organic matter in shallow (30 cm) and deep (70 cm) sediment, across the floodplain meander. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across the meander in each depth.

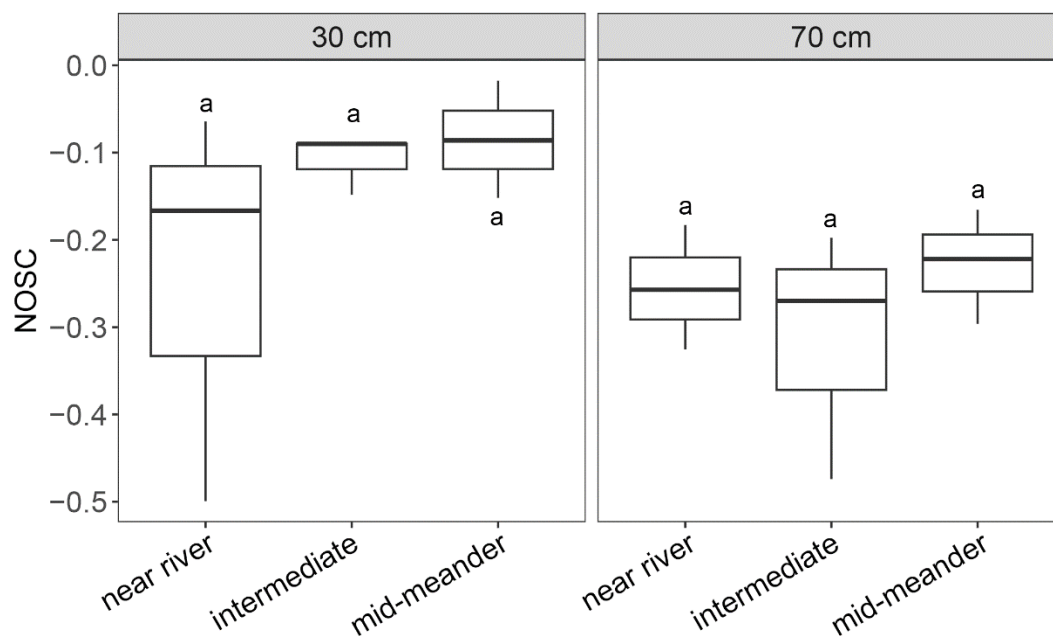


Figure A.7. Shifts in the nominal oxidation state of carbon (NOSC) of pyrophosphate extractable organic matter in shallow (30 cm) and deep (70 cm) sediment, across the floodplain meander. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across the meander in each depth.

Table A.1. Water table depth (m), moisture (percent volumetric water content, % VWC), and redox potential (mV) at the East River, across flooded (June) and drained (September) timepoints. See Fig. A.1 for comparison of 2017 and 2019 water years.

Position	Depth (cm)	Water table depth (2017, flooded) (m)	Water table depth (2017, drained) (m)	Soil moisture (2019, flooded) % VWC	Soil moisture (2019, drained) % VWC	Soil redox potential (2019, flooded) (mV)	Soil redox potential (2019, drained) (mV)
Near river	30			47.1 ± 0.7	30.7 ± 0.6	355.4 ± 51.0	341.2 ± 36.9
	70	-0.76 ± 0.0	-1.12 ± 0.1	43.5 ± 0.0	42.8 ± 0.2	8.6 ± 6.0	29.1 ± 29.2
Mid-meander	30			64.7 ± 2.4	27.3 ± 0.4	239.9 ± 6.8	221.2 ± 11.8
	70	-0.5 ± 0.1	-1.38 ± 0.0	67.3 ± 0.1	59.0 ± 0.4	-179.9 ± 11.7	-221.3 ± 5.4

Table A.2. Sampling location information including ESS-DIVE (<https://ess-dive.lbl.gov/>) nomenclature, elevation, latitude, and longitude. Latitude and longitude correspond to piezometers installed on the transect; sediment samples were taken within 1.5 m of these locations.

Position	ESS-DIVE designations	Elevation (m)	Latitude	Longitude
Near river	ER-MCB1	2759.56	38.92296836	-106.9501598
Intermediate	ER-MCB2	2759.24	38.92295536	-106.9500587
Mid-meander	ER-MCB3	2759.28	38.922948	-106.9499313

Table A.3. Sediment organic carbon (OC) extracted by water, pyrophosphate, HCl, and dithionite (mean +/- standard error), across meander position and depth. Units are mmol C per g sediment.

Position	Depth (cm)	Water-extractable OC	Pyrophosphate-extractable OC	HCl-extractable OC	Dithionite-extractable OC
		mmol / g	mmol / g	mmol / g	mmol / g
Near river	30	0.071 ± 0.01	0.81 ± 0.15	0.039 ± 2.8 e-3	0.038 ± 6.1 e-4
	70	0.064 ± 0.00	0.065 ± 0.00	0.015 ± 1.3 e-3	0.022 ± 1.2 e-3
Intermediate	30	0.075 ± 0.01	1.00 ± 0.17	0.024 ± 8.6 e-4	0.031 ± 3.8 e-4
	70	0.025 ± 0.00	0.10 ± 0.02	0.015 ± 1.3 e-3	0.023 ± 2.1 e-3
Mid-meander	30	0.068 ± 0.01	0.65 ± 0.10	0.026 ± 1.1 e-3	0.028 ± 1.3 e-3
	70	0.026 ± 0.00	0.18 ± 0.04	0.017 ± 3.3 e-3	0.025 ± 2.1 e-3

Table A.4. Sediment iron (Fe) extracted by water, pyrophosphate, HCl, and dithionite (mean +/- standard error), across meander position and depth. Units are mmol Fe per g sediment.

Position	Depth (cm)	Water-extractable Fe	Pyrophosphate-extractable Fe	HCl-extractable Fe	Dithionite-extractable Fe
		mmol / g	mmol / g	mmol / g	mmol / g
Near river	30	2.9 e-5 ± 3.6 e-6	7.6 e-3 ± 1.2 e-3	0.033 ± 2.7 e-3	0.020 ± 2.5 e-3
	70	5.1 e-5 ± 2.9 e-5	8.5 e-4 ± 9.9 e-5	0.015 ± 1.4 e-3	0.014 ± 6.6 e-4
Intermediate	30	6.1 e-5 ± 3.6 e-6	1.2 e-2 ± 7.8 e-4	0.018 ± 1.4 e-3	0.017 ± 3.5 e-3
	70	4.7 e-5 ± 3.6 e-6	1.0 e-2 ± 1.0 e-4	0.015 ± 9.5 e-4	0.011 ± 7.5 e-4
Mid-meander	30	4.7 e-5 ± 3.6 e-6	4.4 e-3 ± 1.2 e-3	0.0081 ± 2.8 e-3	0.0062 ± 8.0 e-4
	70	1.0 e-4 ± 3.6 e-6	3.1 e-3 ± 3.0 e-4	0.0021 ± 2.1 e-4	0.0058 ± 4.6 e-4

Table A.5. Sediment aluminum (Al) extracted by water, pyrophosphate, HCl, and dithionite (mean +/- standard error), across meander position and depth. Units are mmol Al per g sediment.

Position	Depth (cm)	Water-extractable Al mmol / g	Pyrophosphate-extractable Al mmol / g	HCl-extractable Al mmol / g	Dithionite-extractable Al mmol / g
Near river	30	3.7 e-5 ± 7.5 e-6	6.6 e-3 ± 1.7 e-4	0.035 ± 1.1 e-3	9.3 e-3 ± 8.4 e-4
	70	6.7 e-5 ± 0.00	1.1 e-3 ± 1.6 e-4	0.017 ± 9.7 e-4	5.5 e-3 ± 1.4 e-4
Intermediate	30	6.7 e-5 ± 0.00	1.4 e-2 ± 1.6 e-3	0.032 ± 1.1 e-3	1.7 e-2 ± 4.4 e-3
	70	5.2 e-5 ± 7.5 e-6	1.5 e-3 ± 1.4 e-4	0.015 ± 1.4 e-3	7.3 e-3 ± 5.8 e-4
Mid-meander	30	3.7 e-5 ± 1.5 e-5	3.5 e-3 ± 8.6 e-4	0.018 ± 2.9 e-3	5.7 e-3 ± 1.1 e-3
	70	5.5 e-5 ± 0.00	3.4 e-3 ± 1.6 e-4	0.012 ± 6.4 e-4	5.7 e-3 ± 4.6 e-4

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3: ANAEROBIC PROTECTION OF ORGANIC MATTER SHIFTS SEASONALLY IN MOUNTAINOUS FLOODPLAIN SOILS

Supplemental Methods

Soil DNA extraction

Soil DNA was extracted using Qiagen DNeasy PowerSoil kits (Qiagen, Hilden, Germany). The quick-start protocol was followed with several adjustments. First, 0.5 g of soil was weighed out for each extraction; this higher soil mass was used based on initial tests which showed equivalent or greater DNA yield without losing quality of DNA. Second, rather than using PowerBead tubes, soil was mixed with 750 μ L Lysing Matrix E solution in provided MP Bio tubes, which contained 1.4 mm ceramic spheres, 0.1 mm silica spheres, and one 4 mm glass bead (MP Bio, Santa Ana, California). Solution C1 was then added as instructed, vortexed, and then heated for 10 min at 65 C. The combination of Lysing Matrix E and heating has been shown to further increase DNA yields (G. Pold, personal correspondence). Additionally, instead of using a Vortex Adapter tube holder, a Fastprep (MP Bio, Santa Ana, California) set at 5.5 m s⁻¹ was used to mix the soil solution. As the final step, DNA was eluted using 70 μ L of Solution C6. DNA quality and quantity were assessed using a Nanodrop spectrophotometer (Thermo Fisher) and Qubit 1x dsDNA kit (Qiagen, Hilden, Germany), respectively. DNA extracts were then shipped to the Joint Genome Institute (JGI) for metagenomic sequencing, following IMG Annotation Pipeline v.5.0.23.

Table B.1. Metabolites measured by GC-MS, significant by depth (t test, $p < 0.05$). False discovery rates (FDR) are given.

Metabolite	t statistic	p value	FDR
DL-glyceraldehyde	-8.6422	1.60E-08	1.62E-06
3-hydroxybutyric acid	-7.8989	7.29E-08	3.17E-06
D-glucose	-7.7055	1.09E-07	3.17E-06
N-acetyl-D-galactosamine	7.6412	1.25E-07	3.17E-06
myo-inositol phosphate	7.2586	2.85E-07	4.97E-06
D-arabinose	-7.2424	2.95E-07	4.97E-06
melibiose	7.1388	3.70E-07	5.34E-06
maltotriose B	7.0736	4.27E-07	5.40E-06
lactic acid	-6.9738	5.33E-07	5.98E-06
1,3-dihydroxyacetone	-6.4671	1.66E-06	1.68E-05
glyceric acid	-6.3104	2.38E-06	1.99E-05
glycerol	-6.3023	2.42E-06	1.99E-05
benzoic acid	-6.2595	2.67E-06	1.99E-05
caprylic acid	-6.2457	2.76E-06	1.99E-05
1,5-anhydrohexitol B	-6.2125	2.98E-06	2.00E-05
maltose	-6.1195	3.69E-06	2.33E-05
1,5-anhydrohexitol A	5.6809	1.03E-05	6.13E-05
galacto-hexodialdose	-5.4772	1.67E-05	9.39E-05
capric acid	-5.4534	1.77E-05	9.41E-05
4-hydroxybenzoic acid	-5.3447	2.30E-05	0.00011043
D-(+)-melezitose	5.2967	2.58E-05	0.00011826
glycine	5.2705	2.74E-05	0.00012047
2,6-bis(tert-butyl)phenol	-5.2349	2.99E-05	0.00012578
threose	-5.1246	3.90E-05	0.00015749
lactose	5.0558	4.60E-05	0.00017883
D-xylose	-5.0312	4.89E-05	0.00018274
glycerol 3-phosphate	-4.8444	7.69E-05	0.00027721
2-keto-3-methylvaleric acid	4.0862	0.00048875	0.0016768
porphine	-4.0785	0.00049806	0.0016768
L-proline	4.0529	0.00053019	0.0017274
2-hydroxyhexanoic acid	-4.021	0.000573	0.0018085
2-hydroxymethyl-2-methylpropane-1,3-diol	-3.9968	0.0006078	0.0018602
2-hydroxybutyric acid	-3.8535	0.00086148	0.0025014
2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone	-3.8486	0.00087192	0.0025014
2-ketoisocaproic acid	3.8394	0.00089158	0.0025014
maltotriose A	3.7346	0.0011496	0.003138
2-O-glycerol-alpha-d-galactopyranoside	-3.7165	0.0012012	0.0031926
D-(+) trehalose	3.7055	0.0012337	0.0031949
6-hydroxynicotinic acid	3.6824	0.0013046	0.003294

raffinose	3.5746	0.0016921	0.0041683
D-galactose	3.5459	0.001813	0.0043599
L-methionine	3.5309	0.0018792	0.004414
L-valine	3.5102	0.001975	0.0045335
L-glutamic acid (dehydrated)	3.4901	0.0020725	0.0046516
ribitol	-3.4515	0.0022733	0.0049914
isomaltose	3.4264	0.0024144	0.0051883
lactobionic acid	3.3335	0.0030129	0.0063397
L-threonine	3.3073	0.003206	0.0065563
DL-isoleucine	3.2969	0.0032858	0.0065563
melibiose	3.2938	0.0033106	0.0065563
fructose	-3.277	0.003445	0.0066913
nonanoic acid	-3.0738	0.0055545	0.010585
D-mannitol	-2.9017	0.0082736	0.015475
thymine	2.763	0.011348	0.02084
D-mannose	-2.7295	0.01224	0.022076
mannobiose	2.5189	0.019543	0.034273
phenylalanine	2.5157	0.019681	0.034273
scyllo-inositol	2.4814	0.02121	0.036309
ribonic acid	-2.4563	0.0224	0.036682
bisphenol A	-2.4552	0.022455	0.036682
xylitol	-2.4472	0.022847	0.036682
heptanoic acid	-2.4465	0.022881	0.036682
uracil	-2.3412	0.028685	0.045269
toluic acid	-2.3093	0.030696	0.047697

Table B.2. Metabolic pathways mapped to each soil depth, using KEGG Mapper. Color coding of pathways is described in legend at the end of the table. "Other" includes pathways related to: translation, metabolism of cofactors and vitamins, xenobiotics biodegradation and metabolism, and signal transduction.

Depth	KEGG map	Pathway name	Hits
shallow	map01100	Metabolic pathways	20
shallow	map02010	ABC transporters	13
shallow	map01110	Biosynthesis of secondary metabolites	10
shallow	map01230	Biosynthesis of amino acids	9
shallow	map00970	Aminoacyl-tRNA biosynthesis	8
shallow	map01060	Biosynthesis of plant secondary metabolites	7
shallow	map01210	2-Oxocarboxylic acid metabolism	6
shallow	map00470	D-Amino acid metabolism	6
shallow	map01120	Microbial metabolism in diverse environments	5
shallow	map00966	Glucosinolate biosynthesis	5
shallow	map00052	Galactose metabolism	5
shallow	map02060	Phosphotransferase system (PTS)	4
shallow	map00460	Cyanoamino acid metabolism	4
shallow	map00290	Valine, leucine and isoleucine biosynthesis	4
shallow	map01240	Biosynthesis of cofactors	4
shallow	map00860	Porphyrin metabolism	3
shallow	map01064	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	3
shallow	map00280	Valine, leucine and isoleucine degradation	3
shallow	map00332	Carbapenem biosynthesis	2
shallow	map01070	Biosynthesis of plant hormones	2
shallow	map00260	Glycine, serine and threonine metabolism	2
shallow	map00997	Biosynthesis of various other secondary metabolites (e.g., Ditryptophenaline biosynthesis, Fumiquinazoline D biosynthesis, Paerucumarin biosynthesis, etc.)	2
shallow	map01200	Carbon metabolism	2
shallow	map00630	Glyoxylate and dicarboxylate metabolism	2
shallow	map00500	Starch and sucrose metabolism	2
shallow	map00960	Tropane, piperidine and pyridine alkaloid biosynthesis	2
shallow	map00480	Glutathione metabolism	2
shallow	map00330	Arginine and proline metabolism	2
shallow	map00999	Biosynthesis of various plant secondary metabolites (e.g., Crocin biosynthesis, Mugineic acid biosynthesis, Pentagalloylglucose biosynthesis, etc.)	2
shallow	map00562	Inositol phosphate metabolism	2
shallow	map01063	Biosynthesis of alkaloids derived from shikimate pathway	2
deep	map01100	Metabolic pathways	23
deep	map01120	Microbial metabolism in diverse environments	9
deep	map02010	ABC transporters	8

deep	map01110	Biosynthesis of secondary metabolites	7
deep	map00040	Pentose and glucuronate interconversions	5
deep	map02060	Phosphotransferase system (PTS)	5
deep	map00052	Galactose metabolism	5
deep	map00051	Fructose and mannose metabolism	5
deep	map00520	Amino sugar and nucleotide sugar metabolism	4
deep	map00561	Glycerolipid metabolism	4
deep	map00030	Pentose phosphate pathway	3
deep	map01220	Degradation of aromatic compounds	3
deep	map01200	Carbon metabolism	3
deep	map00500	Starch and sucrose metabolism	3
deep	map00680	Methane metabolism	2
deep	map00053	Ascorbate and aldarate metabolism	2
deep	map00362	Benzoate degradation	2
deep	map04024	cAMP signaling pathway	2
deep	map01240	Biosynthesis of cofactors	2
deep	map00627	Aminobenzoate degradation	2
deep	map00623	Toluene degradation	2
deep	map00640	Propanoate metabolism	2
deep	map00061	Fatty acid biosynthesis	2
deep	map04066	HIF-1 signaling pathway	2
deep	map01250	Biosynthesis of nucleotide sugars	2
deep	map00363	Bisphenol degradation	2
deep	map00010	Glycolysis / Gluconeogenesis	2

KEGG Pathway Maps:

Global and overview maps
Membrane transport
Chemical structure transformation
Amino acid metabolism
Biosynthesis of secondary metabolites
Carbohydrate metabolism
Lipid metabolism
Energy metabolism
Other

Table B.3. Metabolites measured by GC-MS, significant by flooding condition (t test, $p < 0.05$). False discovery rates (FDR) are given.

Metabolite	t statistic	p value	FDR
3,4-dimethylbenzoic acid	11.818	5.33E-11	5.38E-09
2,5-dimethyl-2,5-hexanediol	-9.3144	4.32E-09	2.18E-07
4-guanidinobutyric acid	-7.6601	1.20E-07	4.05E-06
toluic acid	-6.2666	2.63E-06	6.64E-05
nonanoic acid	-5.0408	4.77E-05	0.00096424
heptanoic acid	-4.6525	0.00012261	0.0018779
methylmalonic acid	-4.628	0.00013015	0.0018779
bisphenol A	-3.9484	0.00068402	0.0086358
L-glutamic acid (dehydrated)	-3.8535	0.00086155	0.0096685
stearic acid	-3.4588	0.0022343	0.022567
D-mannose	3.2573	0.003609	0.033137

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 4: SHIFTING MINERAL AND METABOLIC CONSTRAINTS REGULATE SOIL CARBON EXPORT FROM CLIMATE-SENSITIVE FLOODPLAINS

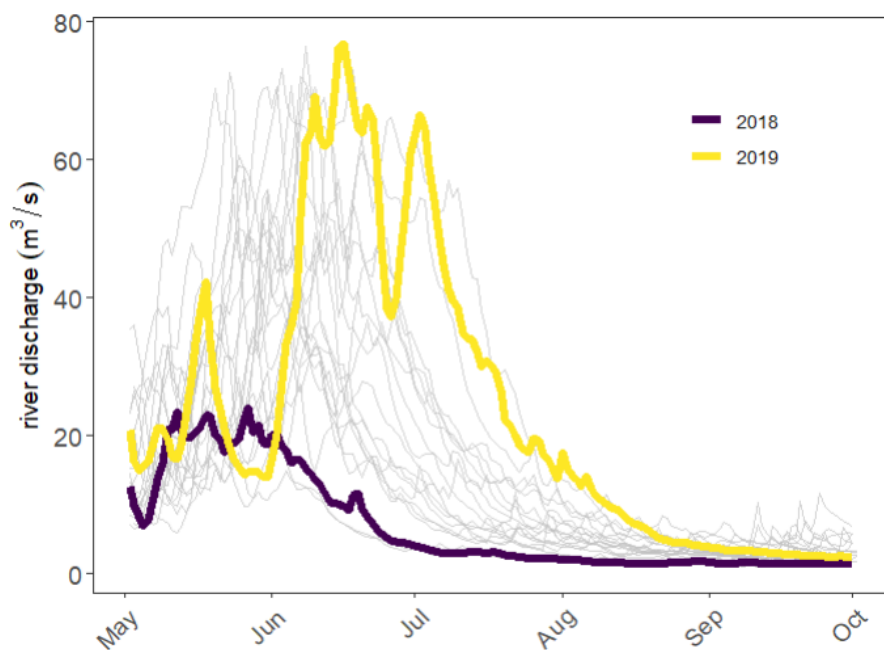


Figure C.1. East River discharge, with years 2018 and 2019 highlighted in purple and yellow, respectively. Historical river discharge from 1999-2017 is provided as light gray lines. Data from USGS monitoring station 09112200 at East River Below Cement Creek (<https://waterdata.usgs.gov/monitoring-location/09112200/>).

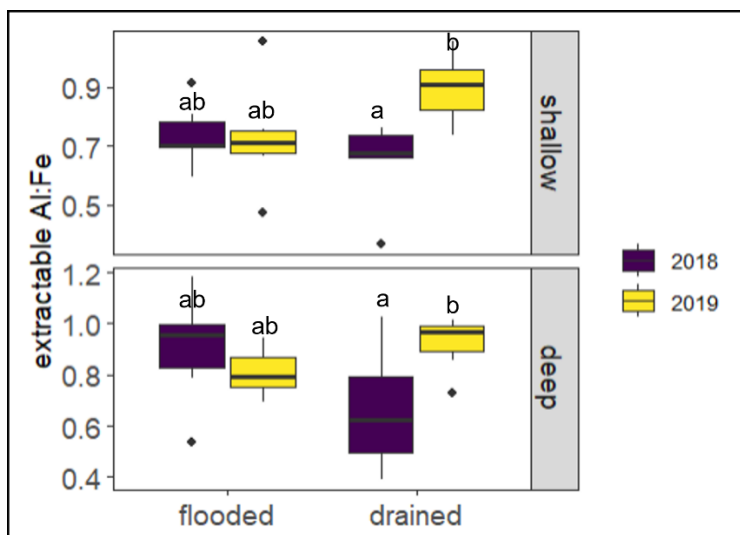


Figure C.2. Extractable Al/Fe ratio in shallow and deep soils from flooded to drained timepoints, across years 2018 (purple) and 2019 (yellow). Letters indicate Tukey's honestly significant difference test results ($p < 0.05$) based on differences across each combination of flooding condition and year, performed separately for each soil depth. Box plots display the median surrounded by the first and third quartiles, with whiskers extending from 1.5 times the inter-quartile range; outliers are displayed individually.

Table C.1. Extractable organic carbon (OC) in mmol g⁻¹ soil. Means and standard error are reported. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across flooding condition and year, within each soil depth.

Soil depth	Year	Flooding condition	Total extractable OC	Water extractable OC	Pyrophosphate extractable OC	Hydroxylamine extractable OC	Dithionite extractable OC
shallow	2018	flooded	1.48a ± 0.109	0.0898a ± 0.0102	1.27a ± 0.111	0.0695a ± 0.00357	0.0530a ± 0.00372
		drained	1.78a ± 0.123	0.0882a ± 0.0126	1.53a ± 0.117	0.0831a ± 0.00700	0.0593a ± 0.00441
	2019	flooded	2.08a ± 0.151	0.421b ± 0.0205	1.52a ± 0.146	0.0870a ± 0.00783	0.0583a ± 0.00279
		drained	1.76a ± 0.205	0.352c ± 0.0245	1.28a ± 0.182	0.0817a ± 0.00838	0.0504a ± 0.00726
deep	2018	flooded	0.279a ± 0.0225	0.0310a ± 0.00191	0.201a ± 0.0195	0.0209a ± 0.00225	0.0261a ± 0.00102
		drained	0.344ab ± 0.0381	0.0360a ± 0.00315	0.257a ± 0.0341	0.0251a ± 0.00226	0.0261ab ± 0.000404
	2019	flooded	0.708b ± 0.0378	0.401b ± 0.00970	0.265a ± 0.0366	0.0194a ± 0.000181	0.0222bc ± 0.00107
		drained	0.413c ± 0.0149	0.202c ± 0.00998	0.173a ± 0.0117	0.0162a ± 0.00220	0.0216c ± 0.000734

Table C.2. Extractable organic iron (Fe) in mmol g⁻¹ soil. Means and standard error are reported. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across flooding condition and year, within each soil depth.

Soil depth	Year	Flooding condition	Total extractable Fe	Water extractable Fe	Pyrophosphate extractable Fe	Hydroxylamine extractable Fe	Dithionite extractable Fe
shallow	2018	flooded	0.141a ± 0.00842	8.56 e-05a ± 1.11 e-05	0.0507a ± 0.00199	0.0565a ± 0.00945	0.0338a ± 0.00570
		drained	0.159a ± 0.0247	1.22 e-04a ± 2.72 e-05	0.0565a ± 0.0124	0.0723a ± 0.00929	0.0296a ± 0.00603
	2019	flooded	0.271b ± 0.0252	8.95 e-05a ± 1.36 e-05	0.0758a ± 0.0100	0.137b ± 0.0159	0.0581b ± 0.00936
		drained	0.186a ± 0.00877	8.56 e-05a ± 9.46 e-06	0.0681a ± 0.00482	0.0708a ± 0.00407	0.0472ab ± 0.00819
deep	2018	flooded	0.0536a ± 0.00481	3.03 e-04a ± 1.63 e-04	0.00517a ± 0.00159	0.0227a ± 0.00215	0.0254a ± 0.00335
		drained	0.0888ab ± 0.00934	3.99 e-04a ± 2.14 e-04	0.0120ab ± 0.00214	0.0498b ± 0.00752	0.0266a ± 0.00287
	2019	flooded	0.105b ± 0.00996	1.16 e-04a ± 4.93 e-05	0.0136b ± 0.00245	0.0494b ± 0.00963	0.0417b ± 0.00441
		drained	0.0813ab ± 0.00609	4.68 e-04a ± 1.74 e-04	0.0155b ± 0.00300	0.0248a ± 0.00211	0.0405b ± 0.00478

Table C.3. Extractable organic aluminum (Al) in mmol g⁻¹ soil. Means and standard error are reported. Letter designations are Tukey's honestly significant difference test results ($p < 0.05$) based on differences across flooding condition and year, within each soil depth.

Soil depth	Year	Flooding condition	Total extractable Al	Water extractable Al	Pyrophosphate extractable Al	Hydroxylamine extractable Al	Dithionite extractable Al
shallow	2018	flooded	0.107a ± 0.00806	1.94 e-04a ± 1.51 e-04	0.0431a ± 0.00940	0.0487a ± 0.00292	0.0153ab ± 0.00222
		drained	0.111a ± 0.0160	4.63 e-05a ± 1.54 e-05	0.0374a ± 0.00875	0.0603a ± 0.00740	0.0137a ± 0.000617
	2019	flooded	0.192b ± 0.0195	2.08 e-04a ± 4.69 e-05	0.0507a ± 0.00911	0.113b ± 0.0128	0.0287c ± 0.00167
		drained	0.168b ± 0.0146	1.91 e-04a ± 5.03 e-05	0.0444a ± 0.00539	0.0998b ± 0.00938	0.0241bc ± 0.00404
deep	2018	flooded	0.0486a ± 0.00689	9.68 e-05a ± 4.64 e-05	0.00693a ± 0.00231	0.0339a ± 0.00445	0.00766a ± 0.00241
		drained	0.0633a ± 0.0105	7.66 e-05a ± 3.11 e-05	0.00972a ± 0.00301	0.0449ab ± 0.00735	0.00858a ± 0.00113
	2019	flooded	0.0880b ± 0.0103	1.69 e-04ab ± 4.19 e-05	0.0133a ± 0.00210	0.0590b ± 0.00807	0.0155b ± 0.00126
		drained	0.0738ab ± 0.00563	3.03 e-04b ± 4.38 e-05	0.0112a ± 0.00113	0.0492ab ± 0.00496	0.0131ab ± 0.00216

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