TITLE: Effects of hypoxia on coupled carbon and iron cycling differ between weekly and multiannual timescales in two freshwater reservoirs

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# KEY POINTS

* Short-term (2–3 week) periods of hypoxia decreased iron-bound organic carbon and total organic carbon in reservoir sediments
* Multiannual periods of hypoxia increased total organic carbon in sediment, likely through decreased rates of respiration
* A substantial fraction of sediment organic carbon (~30%) was bound to iron in these two freshwater reservoirs

# ABSTRACT

Freshwater lakes and reservoirs play a disproportionate role in the global carbon budget, sequestering more organic carbon (OC) than ocean sediments each year. However, it remains unknown how global declines in bottom-water oxygen concentrations may impact OC sequestration in freshwater sediments. In particular, associations between OC and iron (Fe) are hypothesized to play a critical role in stabilizing OC in sediment, and these complexes can be sensitive to changes in oxygen. Under low-oxygen (hypoxic) conditions, Fe-bound OC (Fe-OC) complexes may dissociate, decreasing OC sequestration. However, rates of OC respiration are also lower under hypoxic conditions, which could increase OC sequestration. To determine the net effects of hypoxia on OC and Fe cycling over multiple timescales, we paired whole-ecosystem experiments with sediment incubations in two eutrophic reservoirs. Our experiments demonstrated that short (2–4 week) periods of hypoxia can increase Fe and OC concentrations in the water column while decreasing OC and Fe-OC in sediment. However, exposure to seasonal hypoxia over multiple years was associated with a substantial *increase* in sediment OC and no change in Fe-OC. These results indicate that the large sediment Fe-OC pool (~30% of sediment OC) contains both oxygen-sensitive and oxygen-insensitive fractions, and over multiannual timescales, OC respiration rates play a greater role than Fe-OC in determining the effect of hypoxia on sediment OC content. Consequently, we anticipate that global declines in oxygen concentrations in freshwater ecosystems are likely to alter OC and Fe cycling, with the direction and magnitude of effects depending upon the duration of hypoxia.

KEYWORDS: Carbon cycling, dissolved oxygen, iron-bound organic carbon, sediment, sediment-water interface, whole-ecosystem experiment

PLAIN LANGUAGE SUMMARY

Lakes play a remarkably important role in the global carbon cycle. Every year, more organic carbon (e.g., leaves, soil) is buried in lake sediments than in the sediments of all of the world's oceans. However, these organic carbon inputs can also be decomposed, releasing greenhouse gases. The extent to which lakes bury carbon vs. release greenhouse gases may be changing, as oxygen concentrations are decreasing in the bottom waters of many lakes around the world. Here, we added oxygen to the bottom waters of a whole reservoir to test how changes in oxygen concentration affect carbon cycling. We found that over short timescales (weeks), low oxygen conditions decreased the amount of carbon in sediment by breaking apart chemical complexes that can help retain carbon in sediment. However, over long timescales (years), low oxygen conditions appeared to *increase* carbon burial by decreasing the rate at which carbon inputs were decomposed. These results suggest that declining oxygen concentrations in lakes around the world may have important effects on global carbon cycling, with the direction and magnitude of the impact depending on the duration of low oxygen conditions.

INDEX TERMS:

* 0404 Anoxic and hypoxic environments (4802, 4834)
* 0408 Benthic processes (4804)
* 0428 Carbon cycling (4806)
* 0461 Metals
* 0471 Oxidation/reduction reactions (4851)

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# 1. INTRODUCTION

Freshwater lakes and reservoirs are increasingly recognized as hotspots in the global carbon cycle (David Bastviken et al., 2011; Battin et al., 2009; Carey, Hanson, et al., 2022; Raymond et al., 2013; Tranvik et al., 2018). Due to high organic carbon (OC) loading from the surrounding watershed, more OC is buried in lakes and reservoirs than in ocean sediments each year (Dean & Gorham, 1998; Knoll et al., 2013; Mendonça et al., 2017; Pacheco et al., 2014). Much of this OC remains sequestered in the sediments, especially in reservoirs, which alone account for 25% of the global carbon sink from all terrestrial and freshwater sources (Le Quéré et al., 2015). However, OC inputs can also be respired to carbon dioxide and methane, making lakes and reservoirs a source of greenhouse gas emissions equivalent to 20% of the global emissions from fossil fuels (Deemer et al., 2016; DelSontro et al., 2018). To refine global carbon budgets and manage water resources in a changing world, it is important to understand what factors control the balance between OC sequestration and carbon emissions in these ecosystems.

Recent research suggests that associations between OC and iron (Fe) may play a critical role in OC sequestration across soils and marine environments (e.g., Hemingway et al., 2019; Kramer & Chadwick, 2018; Lalonde et al., 2012), and these associations are hypothesized to also be important in freshwater sediments (Björnerås et al., 2017; Peter et al., 2016; von Wachenfeldt et al., 2008; Weyhenmeyer et al., 2014). Fe can promote OC stability through multiple mechanisms, including occlusion of OC in aggregates, which can result in physical inaccessibility to microbial degradation and subsequent burial of OC in deeper soil or sediment horizons (Markus Kleber et al., 2015 and references therein). Consequently, Fe complexation may facilitate OC sequestration over decades to millennia (Markus Kleber et al., 2015; Lalonde et al., 2012 and references therein)

Over shorter timescales (days to weeks), Fe-bound OC (Fe-OC) complexes are likely sensitive to the redox conditions of the surrounding environment (Figure 1). Fe-OC complexes form under oxic conditions (Riedel et al., 2013), as Fe(III) is more effective at complexing with organic matter than Fe(II) (Nierop et al., 2002). Under hypoxic (reducing) conditions, OC can be released from Fe-OC complexes through Fe(III) reduction and dissolution (e.g., Pan et al., 2016; Patzner et al., 2020; Peter & Sobek, 2018; Skoog & Arias-Esquivel, 2009), which can either result directly from hypoxia or through resultant increases in pH that promote OC release (Kirk, 2004; Thompson et al., 2006). Given these conflicting patterns—i.e., that Fe-OC complexes can be preserved over decades to millennia and yet may be unstable under the reducing conditions which commonly occur on day to month timescales in aquatic sediments—it remains unclear how changing oxygen dynamics will affect coupled OC and Fe cycling in freshwater ecosystems.

Currently, the duration of bottom-water hypoxia (low oxygen conditions) is increasing in many lakes and reservoirs around the world (Bartosiewicz et al., 2019; Jane et al., 2021; Jenny et al., 2016; Williamson et al., 2015), which could have varying consequences for OC sequestration (Figure 1). In many dimictic lakes and reservoirs, bottom-water hypoxia is interrupted by oxic conditions during spring mixing and fall turnover, resulting in dynamic oxygen conditions on the week to month scale. Combined, these short-term patterns sum to determine the net role of lakes and reservoirs in the global carbon cycle over multiannual timescales. Periods of hypoxia have the potential to decrease OC sequestration through reductive dissolution of Fe in Fe-OC complexes (Chen et al., 2020; Patzner et al., 2020). However, hypoxia also has the potential to increase OC sequestration by decreasing the rate of OC respiration (Carey et al., 2018; Carey, Hanson, et al., 2022; Hargrave, 1969; Peter et al., 2017; Sobek et al., 2009; Walker & Snodgrass, 1986), particularly if Fe-OC complexes are resistant to, or isolated from, changes in oxygen concentrations in overlying water. Decreased OC respiration rates under hypoxic conditions is thought to occur primarily because respiration is less thermodynamically favorable in the absence of oxygen (e.g., Arndt et al., 2013; LaRowe & Van Cappellen, 2011). Because reductive dissolution of Fe in Fe-OC complexes and decreased OC respiration under hypoxic conditions would have divergent effects on total OC sequestration, understanding the relative importance of these two processes across multiple timescales is critical for predicting the effect of hypoxia on OC sequestration in the bottom waters of lakes and reservoirs (Figure 1).

To date, few studies have explicitly examined Fe-OC in freshwater lakes and reservoirs, and those that have provide preliminary evidence that Fe-OC complexation may be lower in freshwater environments compared to better-characterized marine systems. Peter and Sobek (2018) analyzed Fe-OC in surficial sediment from five boreal lakes that spanned a gradient of oxygen conditions and found that less than 11% of sediment OC was bound to Fe, in comparison with ~20% across a range of primarily marine sediments (Lalonde et al., 2012). Further, Peter and Sobek (2018) found no association between Fe-OC content in sediment and oxygen in overlying water. However, it should be noted that the lakes in that study were particularly high in OC (9–42 mg/L DOC), and may not be representative of all freshwater ecosystems. Bai et al. (2021) studied Fe-OC along a salinity gradient in a subtropical tidal wetland and similarly found that freshwater areas had lower levels of Fe-OC (18% of sediment OC in freshwater and 29% in saltwater), but these results were attributed primarily to wetland plant characteristics, which may not be relevant in the bottom waters of lakes and reservoirs.

Despite limited research on Fe-OC in freshwater sediments, there are multiple reasons to expect that Fe may play an important role in OC sequestration in some freshwater ecosystems. Concentrations of Fe and dissolved OC (DOC) are strongly correlated in many freshwaters (Björnerås et al., 2017; von Wachenfeldt et al., 2008; Weyhenmeyer et al., 2014), and aqueous Fe concentrations are strongly correlated with sediment OC accumulation in boreal lakes (Einola et al., 2011). Moreover, hypoxic release of DOC from lake sediments has been well-documented, and is often attributed to reductive dissolution of Fe (Brothers et al., 2014; Kim & Kim, 2020; Lau & del Giorgio, 2020; Peter et al., 2017). Still, few studies have examined whether reactions involving Fe-OC complexes are the driving force for observed correlations between dissolved Fe and OC (but see Peter et al. 2018). Furthermore, it remains unknown how the Fe-OC cycling occurring on sub-annual time scales may affect OC sequestration on the multi-annual timescales relevant for global carbon budgets.

Analyzing the complex effects of oxygen on coupled OC and Fe cycling requires multiple experimental approaches. Field surveys have been effective at identifying correlations between OC and Fe (Björnerås et al., 2017; von Wachenfeldt et al., 2008; Weyhenmeyer et al., 2014). However, these observational approaches have limited capacity for identifying causal relationships. Whole-ecosystem experiments may be highly effective at identifying real-world impacts of freshwater oxygen on Fe and OC dynamics, while allowing for important ecosystem-scale processes such as turbulence and external loading (Carpenter, 1996; Dzialowski et al., 2014; Schindler, 1998). However, high levels of variability on a whole-ecosystem scale may limit the detection of subtle changes in OC and Fe processing. Small-scale incubations may be particularly useful for identifying changes that result from hypoxia (i.e., increased DOC and Fe release from sediment, decreased levels of Fe-OC, changes in sediment OC). However, small-scale incubations are limited by fouling and changes in microbial communities, among other microcosm effects, and do not reflect the full suite of processes that interact to control OC and Fe cycling in lakes and reservoirs. Consequently, integrating multiple approaches can provide complementary information on Fe-OC dynamics across spatial and temporal scales and overcome the limitations of single-approach studies.

To analyze how hypoxia impacts OC and Fe cycling over multiple scales, this study paired whole-ecosystem oxygen manipulations with laboratory incubations. We had two objectives: (1) characterize Fe-OC levels in sediment of two iron-rich reservoirs, and (2) analyze how hypoxia affects coupled OC and Fe cycling over both short-term (2–4 week) and multiannual timescales. Through this work, we aimed to provide insight on how increasing prevalence and duration of hypoxia in lakes and reservoirs may affect the critical role of these ecosystems in the global carbon cycle.

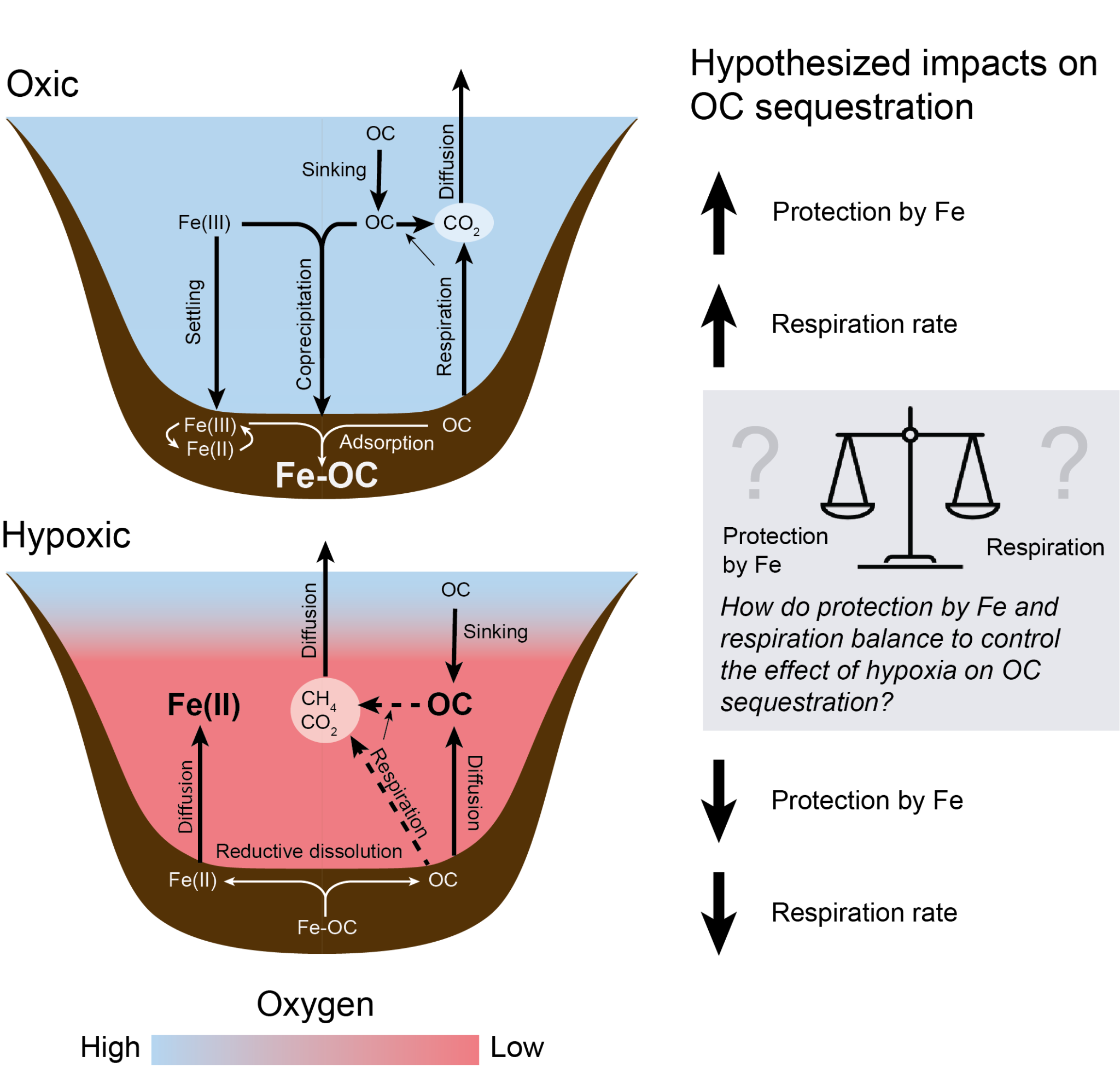


Figure 1: Conceptual diagram describing the hypothesized effects of changing oxygen conditions on coupled Fe-OC interactions. Under oxic conditions (top), complexation of Fe and OC (both through co-precipitation and adsorption) leads to increased concentrations of Fe-OC in sediments (increased Fe-OC protection), though oxic conditions may also lead to increased OC respiration rates. Under hypoxic conditions, reductive dissolution of Fe in Fe-OC complexes increases dissolved concentrations of Fe(II) and OC in the water column while decreasing the amount of Fe-OC in sediment (decreasing Fe-OC protection), though hypoxia may also decrease OC respiration rates. The net effect of these processes on OC sequestration remains unknown, motivating this study. This figure is a simplification of complex interactions happening on a whole-ecosystem scale, and focuses on hypothesized dominant processes operating on the time scale of days to years.

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# 2. METHODS

## **2.1 Study Sites: Falling Creek and Beaverdam Reservoirs**

Falling Creek Reservoir (FCR; 37.30ºN, 79.84ºW) and Beaverdam Reservoir (BVR; 37.31ºN, 79.81ºW) are small (FCR: 0.12 km2, 9.3 m deep; BVR: 0.39 km2, 11 m deep), eutrophic drinking water reservoirs located in southwestern Virginia, USA (Hounshell et al., 2021). Both reservoirs are located in forested catchments and both are dimictic, with summer stratified periods that typically last from May to October. BVR is located 3 km upstream of FCR and serves as the primary inflow source for FCR. Fe levels are high in sediment from these reservoirs as a result of weathering and erosion of Fe-rich metamorphic rocks (Chapman et al., 2013; Woodward, 1932); the bedrock underlying both reservoirs is layered pyroxene granulite (Virginia Division of Mineral Resources, 2003). Both reservoirs have been owned and operated for drinking water provision by the Western Virginia Water Authority (WVWA) since their construction (FCR: 1898, BVR: 1872; Gerling et al., 2016; Hamre et al., 2018).

A suite of variables are routinely sampled in FCR and BVR as part of a long-term monitoring program; all data analyzed in this manuscript are available in the Environmental Data Initiative (EDI) repository with detailed metadata (Carey et al., 2021; Carey, Lewis, et al., 2022; Carey, Wander, et al., 2022; Schreiber et al., 2022)

## **2.2 Whole-ecosystem oxygenation experiments**

In 2012, FCR was equipped with a side-stream supersaturation hypolimnetic oxygenation (HOx) system to improve water quality in the reservoir (Gerling et al., 2014). This type of HOx system functions by withdrawing water from the bottom of the reservoir, adding concentrated, pressurized oxygen gas to supersaturate the water with dissolved oxygen (DO), and then returning the oxygenated water at the same depth and temperature. Previous work in FCR has shown that the HOx system effectively increases DO concentrations throughout the hypolimnion without altering temperature or decreasing thermal stability (Carey, Hanson, et al., 2022; Gerling et al., 2014). From 2013–2019, the HOx system in FCR was operated at variable rates, maintaining an oxygenated hypolimnion for at least part of the summer (Carey, Thomas, et al., 2022). Conversely, oxygenation was reduced in 2020 and 2021, maintaining primarily hypoxic conditions throughout the summer stratified period. To assess the effect of multiannual changes in oxygen availability on OC and Fe-OC in sediment, we compared sediment core and sedimentation trap data from summer 2019 (which had a history of high-oxygen conditions during the preceding six years) to summer 2021 (which followed a summer of hypoxic conditions in 2020; Figure S2). Sediment data were not collected in 2020 due to the Covid-19 pandemic.

To assess how short-term changes in hypolimnetic DO concentrations impact Fe-OC on a whole-ecosystem scale, we operated the HOx in FCR on a variable schedule throughout the summer of 2019 (Carey et al. 2022 EDI). Oxygen was added in approximately two-week intervals at a rate of 25 kg O2 day-1 to the whole hypolimnion. Between oxygenation periods, we allowed the hypolimnion to become hypoxic over periods of at least two weeks without oxygenation. Because hypolimnetic volume varied throughout the summer (generally decreasing throughout the summer as the thermocline deepened), the mean concentration of oxygen added to the whole hypolimnion throughout an oxygenation period in 2019 ranged from 0.80 mg L-1 day-1 to 0.90 mg L-1 day-1.

BVR does not have a HOx system and experiences seasonal hypoxia from May through November (Hounshell et al., 2021). Consequently, BVR serves as a reference ecosystem to analyze the effects of oxygenation in FCR.

### *2.2.1 Oxygen*

We monitored DO concentrations throughout the full water column approximately two times per week in FCR and one time per week in BVR (Carey, Lewis, et al., 2022). High-resolution (~1 cm) depth profiles were taken using a conductivity, temperature, and depth profiler (CTD; Sea-Bird, Bellevue, Washington, USA) equipped with a DO sensor (SBE 43; Carey, Lewis, et al., 2022) from the reservoir’s surface to the sediments. We also measured dissolved oxygen using a YSI ProODO DO probe as a supplementary measurement method when the CTD was not available due to maintenance (YSI Inc. Yellow Springs, Ohio, USA; Carey, Wander, et al., 2022). YSI measurements were taken at discrete 1 m depth intervals. For a comparison of YSI and CTD measurements, see Carey et al. (2022).

### *2.2.2 Hypolimnetic Fe and DOC*

We collected water samples for DOC and Fe analysis at the deepest site in each reservoir with a 4-L Van Dorn sampler (Wildlife Supply Company, Yulee, FL, USA). Samples were collected once per week at seven depths in FCR (0.1, 1.6, 3.8, 5.0, 6.2, 8.0, and 9.0 m), which corresponded to the reservoir’s extraction depths, and five depths in BVR (0.1, 3.0, 6.0, 9.0, and 11.0 m). In 2019, we conducted a limited amount of additional sampling in FCR on a second day each week, and these measurements included DOC from 0.1, 1.6, 5.0, and 9.0 m depths.

We analyzed DOC by filtering water samples through a 0.7-µm glass fiber filter into an acid-washed bottle, which was rinsed with the filtered water three times before sample collection. The filtered samples were frozen for less than six months before analysis on an OC analyzer (Elementar Vario TOC cube, following Standard Method 5310B; APHA et al. 2017).

We collected both total and dissolved (filtered through 0.45-µm filters) samples for Fe. Samples were preserved in the field using trace metal grade nitric acid and analyzed using ICP-MS (Thermo Electron X-Series, Waltham, MA, USA) following APHA Standard Method 3125-B (American Public Health Association, 2018; Krueger et al., 2020; Munger et al., 2019; Schreiber et al., 2022).

### *2.2.3 Fe-OC in sediments*

We analyzed the concentration of Fe-OC in surficial sediments from both FCR and BVR on multiple dates throughout the summer stratified periods of 2019 and 2021. In 2019, sediment cores in FCR were collected immediately before the HOx system was turned on or off, resulting in the most oxic or hypoxic conditions during that SSS activation or deactivation interval, respectively. Sediment cores at BVR were taken once in the middle of summer and once approximately two weeks before fall turnover in 2019. In 2021, sediment core samples were taken from both reservoirs on the same dates, approximately once per month. Additional sediment core samples were collected in March 2021, when both reservoirs were unstratified and had oxic hypolimnia.

On each sampling date, we collected four replicate hypolimnetic sediment cores using a K-B gravity sediment corer (Wildlife Supply Company, Yulee, FL, USA). Cores were collected in the deepest part of each reservoir, approximately 20 m from where water samples were taken. In 2019, each core was capped and kept on ice while transported back to the lab, where the top 1 centimeter of sediment from each core was immediately extruded, collected, and frozen in scintillation vials for future analysis. In 2021, cores were extruded in the field, and the samples were kept on ice while being transported back to the lab.

### *2.2.4 Sediment traps*

To determine the amount of Fe-OC and total OC in samples of material settling from the water column (i.e., not deposition rates), we deployed 19-L buckets approximately 1 m above the sediments at the deepest point of each reservoir (8 m at FCR and 10 m at BVR). These sediment traps were deployed from June–December 2021 and sampled every two weeks by slowly bringing the bucket to the surface, decanting and discarding water from the bucket, collecting up to 5 L of the remaining water and particulate matter, and transporting this material back to the lab on ice. Upon arriving at the lab, we allowed the particulates to settle for approximately 5 minutes, before decanting and discarding as much water as possible and filling four 50-mL centrifuge tubes with the remaining material. The samples were centrifuged for 10 minutes at 3100 rpm, then combined into one vial and frozen for later analysis. No sediment traps were deployed for Fe-OC analysis in 2019.

## **2.3 Microcosm incubations**

To isolate the effects of oxygen from other interacting factors that affect Fe and OC on a whole-ecosystem scale, we conducted six-week microcosm incubations using hypolimnetic sediment and water from FCR. Incubations were conducted in 177-mL glass jars (Figure S1), after extensive pilot testing revealed that these jars were highly effective at maintaining hypoxic conditions when sealed and oxic conditions when uncapped. We started the experiment with 102 microcosms split evenly into oxic (uncapped) and hypoxic (capped) treatments. After two weeks (similar to the 2019 whole-ecosystem HOx manipulation), we switched the treatment of approximately half of the remaining microcosms, generating two additional oxygen regimes: hypoxic-to-oxic and oxic-to-hypoxic. Starting on week two, there were consequently a total of four oxygen regimes: hypoxic, oxic, hypoxic-to-oxic, and oxic-to-hypoxic.

To set up the experiment, we collected sediment and water from the deepest site in FCR on 30 June 2021, when the hypolimnion was hypoxic. Water was collected from 9 m depth using a Van Dorn sampler, and sediment was collected from the same location using an Ekman sampler. Samples were transported on ice back to the lab, then homogenized by stirring and shaking. We used a syringe to add the sediment slurry (20 mL) to each jar, then slowly added 150 mL of hypolimnetic water, making an effort to minimize sediment disturbance. We stored the microcosms in an unlit incubation chamber at 15 ºC for the duration of the experiment, which corresponded to warm, end-of-summer conditions in the hypolimnion of FCR (Carey, Lewis, et al., 2022).

### *2.3.1 Microcosm sampling*

Microcosms were sampled destructively for DO, total and dissolved Fe, total and dissolved OC, pH, sediment OC, and sediment Fe-OC. For the continuous oxic and hypoxic treatments, we sampled two times per week for four weeks (days 2, 6, 9, 13, 16, 20, 23). We added additional sampling for the hypoxic-to-oxic and oxic-to-hypoxic treatments: these treatments were sampled for the first three days after switching the oxygen regime (days 14, 15, 16), twice the following week (days 20, 23), and one more time a total of four weeks from when treatments were switched (day 34). Six replicate microcosms were sampled per treatment per day up until day 13, and three microcosms were sampled thereafter. All microcosms under a hypoxic treatment were sampled in an anaerobic chamber which maintained mean ambient oxygen conditions <200 ppm (Coy Laboratory, Grass Lake, MI, USA) to reduce oxygen exposure during sampling.

To begin sampling a microcosm, DO was measured using a YSI DO probe. While measuring DO, we used the probe to gently swirl the water in the microcosm, homogenizing the water sample while minimizing sediment disturbance. Next, we used an acid-washed syringe to withdraw 30 mL of water for total OC (TOC), 13 mL for total Fe, 30 mL of water for DOC, and 13 mL for dissolved Fe analyses. DOC samples were filtered through a 0.7-µm glass fiber filter, and dissolved Fe samples were filtered through 0.45-µm filters. After taking samples for Fe and DOC, we withdrew as much water as possible without disturbing the sediment and measured pH from this sample in a separate container using an Ohaus Starter 300 pH probe (Parsippany, NJ, USA). Finally, we swirled the sediment with remaining water (approximately 1–5 mL) and poured this mixture into a 20 mL glass EPA vial, which we then froze for Fe-OC analysis. Hypoxic microcosms were stored in the anaerobic chamber for approximately two hours before analysis to ensure oxygen concentrations in the chamber were sufficiently low before opening the jars. Oxic microcosms were sampled immediately after removal from the incubator.

All microcosm samples were analyzed following standard methods. We stored TOC and DOC samples in bottles that had been acid-washed and rinsed three times with the sample water. All DOC and TOC samples were frozen for < 6 months prior to analysis on an OC analyzer (Elementar Vario TOC cube, following Standard Method 5310B; American Public Health Association, 2018) Fe samples were preserved using trace metal grade nitric acid and analyzed using the ferrozine method (Gibbs, 1979). We also analyzed 22 of the Fe samples from day 23 using inductively coupled plasma mass spectrometry (ICP-MS).

## **2.4 Fe-OC analysis**

We analyzed the amount of Fe-OC in both the whole-ecosystem and microcosm sediment samples using the citrate bicarbonate dithionite (CBD) method. This method was first described for marine systems by Lalonde et al. (2012) and has since been adapted for freshwater lakes by Peter and Sobek (2018). It is important to note that our measurement of Fe-OC as the percentage of OC that is extractable using the CBD method is an operational definition (Fisher et al., 2021). We used this method to enable comparisons both between oxygen treatments and with other published work that used the same general approach (e.g., Lalonde et al., 2012; Peter & Sobek, 2018).

Following the CBD method and described by Lewis et al. (2022), each sediment sample was freeze-dried and divided into three treatments: initial, reduction, and control. “Initial” samples received no treatment and were used to measure the OC content of the sediment. “Reduction” samples were treated with a metal-complexing agent (trisodium citrate) and reducing agent (sodium dithionite) in a buffered solution (sodium bicarbonate) to measure how much Fe and OC were released as a result of Fe reduction. Control samples were used to account for the release of Fe and OC in the reduction treatment that resulted from processes other than Fe reduction. They were treated with the same buffer (sodium bicarbonate) and sodium chloride in the same ionic strength as the trisodium citrate and sodium dithionite of the reduction treatment.

For both the control and reduction treatments, we measured 100 mg of homogenized, freeze-dried sediment into 15-mL polypropylene centrifuge tubes (Falcon Blue, Corning Inc., Corning, NY, USA). We then added 6 mL of either control or reduction buffer solution (0.11 M sodium bicarbonate) to each tube. The reduction buffer contained 0.27 M trisodium citrate, while the control buffer contained 1.6 M sodium chloride. After heating samples to 80ºC in an oven, 0.1 g sodium dithionite was added to the reduction samples and 0.088 g sodium chloride was added to control samples, and samples were kept at 80ºC for an additional 15 min. Samples were centrifuged for 10 min at 3100 RPM, and the supernatant was collected in a 50-mL centrifuge tube. This extraction process was repeated two more times for both treatments (Peter and Sobek, 2018). Finally, samples were rinsed three times using artificial lake water, which was prepared by diluting Artificial Hard Water from Marking and Dawson (1973) to 12.5% with Type I reagent grade water.

After extraction, all sediment samples (including those in the initial treatment) were dried and acid-fumigated for 48 hours to remove remaining citrate and bicarbonate [(Harris et al. 2001)](https://www.zotero.org/google-docs/?TVAgXy). Samples were then run on a CN analyzer (Elementar VarioMax, Ronkonkoma, NY, USA) to determine the amount of OC per unit mass of sediment. We adjusted sediment mass to account for Fe loss during control and reduction treatments. The amount of OC removed with Fe reduction (CBD-extractable OC) was calculated as the difference between the OC content of the control and reduction samples and expressed as a percentage of the initial OC content of the sediment.

## **2.5 Data analysis**

All analyses were performed in R (version 4.0.3; R core team 2020), and all analysis code is available in the Zenodo repository (REFS).

### *2.5.1 Sediment Fe-OC characterization*

We calculated summary statistics to describe iron-bound organic carbon and total organic carbon in surficial sediment (2019 and 2021) and settling particulate material (2021 only) across both reservoirs. We then pooled data from both reservoirs to analyze the difference between settling material and surficial sediments, using Welch’s t-tests. Because data were unavailable for settling material in 2019, the comparison of settling material to surficial sediment was limited to 2021 data only.

### *2.5.2 Whole-ecosystem experiments: short-term responses*

We used Welch’s t-tests to assess whether sediment properties differed between the two-week periods of HOx activation compared to HOx deactivation during summer 2019 in FCR. Median values were not temporally autocorrelated between sampling events for any of the three sediment properties examined, justifying this approach.

To qualitatively assess whether oxygenation experiments led to differences in water column chemistry, we plotted DOC and Fe over depth and time throughout the summer stratified period of 2019 in both BVR and FCR.

### *2.5.3 Whole-ecosystem experiments: interannual differences*

We assessed whether there were significant differences in the three sediment properties among the four reservoir-years—BVR in 2019, BVR in 2021, FCR in 2019 (oxic) and FCR in 2021 (hypoxic). First, we used Levene tests to assess homogeneity of variance among reservoir-years (Table S1). While Fe-OC (both per unit sediment and as a percentage of sediment OC) met the ANOVA assumption of homogeneous variance, total sediment OC did not. Consequently, we used one-way ANOVAs and Tukey post hoc tests for Fe-OC metrics, but used Welch One-Way ANOVAs and Games-Howell post-hoc tests, both of which account for unequal variances, for sediment OC (Tables S2 and S3).

### *2.5.4 Microcosm incubations*

We used one-way ANOVAs and Tukey post-hoc tests to assess whether sediment properties differed between microcosm treatments, after testing for homogeneity of variance using Levene tests (Table S4). For this analysis, we only considered data from days 20 and 23 (pooled), as these were the final days where data were available for all treatments.

Speciation-solubility calculations were conducted for the 23rd day of the microcosm experiments using the Spece8 module of Geochemists’ Workbench (GWB; Aquatic Solutions LLC, Champaign, IL, USA) and the wateqf thermodynamic database (Ball & Nordstrom, 1991). The goal of the calculations was to assess the predicted speciation of Fe in the presence of OC under the environmental conditions of each microcosm treatment (following Oyewumi & Schreiber, 2017). Environmental conditions considered in this analysis included pH, DO, temperature, DOC, major cations (Ca, Na, K), Fe, and major anions (Cl, SO4; bicarbonate was not measured so we calculated that via charge balance). We assumed that DOC consisted primarily of humic acid for the calculations.

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# 3. RESULTS

## **3.1 Fe-OC levels in surficial hypolimnetic sediment are high and greater than in settling particulate matter**

A substantial proportion of sediment OC was associated with Fe in both FCR and BVR. In FCR (averaged across 2019 and 2021), one gram of surficial sediment contained a mean of 481 µmol Fe-OC (± 138, 1 SD), 31 percent (± 7.6) of the total sediment OC pool (n = 30). BVR had slightly lower Fe-OC than FCR on average, and one gram of surficial sediment contained a mean of 418 µmol Fe-OC (± 121), 24 percent (±6.8) of the total sediment OC pool (n = 20). Total OC comprised 8.8 (± 2.7) percent of sediment mass in FCR and 9.6 (± 1.0) percent of sediment mass in BVR.

Levels of Fe-OC, both as a fraction of sediment mass and as a fraction of total sediment OC, were significantly higher in sediment core samples than in settling material collected in hypolimnetic traps (Figure 2). In 2021, averaged across both reservoirs, one gram of the reservoir surficial sediments contained a mean of 443 (± 133) µmol of Fe-OC (n = 28), 69% higher than settling material collected in the traps, which contained a mean of 262 (± 143) µmol Fe-OC (n = 17; t(32) = -4.24, p < 0.001; Figure 2a). A mean of 24 ± 5.8 percent of the total sediment OC pool was bound to Fe in sediments (n = 28), while only 8.6 ± 3.9 percent of sediment OC was bound to Fe in settling material (n = 17; t(43) = -10.44, p < 0.001; Figure 2c). Total OC was higher in settling material (µ = 16.5, SD = 3.3) than in surficial sediments (µ = 10.3 ± 1.6; t(20) = 7.33, p < 0.001; Figure 2b).

Diagram

Description automatically generated

Figure 2: Summary of data from surficial sediment and settling material in 2021, including iron-bound organic carbon (Fe-OC), total sediment organic carbon, and Fe-OC as a percentage of sediment OC. Asterisks indicate statistical significance of the difference between surficial sediment and sediment traps: \*\*\* indicates p < 0.001 (Welch’s ANOVA). Note that only 2021 data are presented because settling particulate material was not collected in 2019.

## **3.2 Whole-ecosystem experiments: short-term responses**

Intermittent activation of the HOx in FCR in 2019 was associated with substantial changes in sediment characteristics. The amount of Fe-OC per g sediment was significantly higher during oxic (µ = 560 ± 70, n = 7) compared to hypoxic (µ = 394 ± 173, n = 9) periods in 2019 (t(11) = -2.6, p = 0.02; Figure 3b). Likewise, the total amount of OC (as a percentage of sediment mass) increased by 42% during oxic (µ = 8.5 ± 0.8, n = 7) compared to hypoxic periods (µ = 6.0 ± 1.55, n = 11; t(15) = -4.6, p < 0.001; Figure 3b). Fe-OC as a percentage of total sediment OC did not significantly change with variation in oxygen during these experiments (oxic: µ = 36.7 ± 3.8, n = 7; hypoxic: µ = 35.7 ± 7.9, n = 9; t(12) = -0.3, p = 0.747; Figure 3c).

In the hypolimnion of FCR, Fe concentrations tended to increase during hypoxic periods and increase following activation of the oxygenation system (Figure 4b). Consequently, Fe concentrations were generally lower in FCR compared to the unoxygenated reference reservoir (BVR) in 2019. Trends in DOC were more variable and less obviously related to oxygenation, though DOC concentrations were typically highest when the oxygenation system was not activated in FCR (Figure 4).

Calendar

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Figure 3: Intermittent operation of the hypolimnetic oxygenation system in Falling Creek Reservoir was associated with changes in sediment characteristics during the summer stratified period of 2019. Solid lines and dashed lines indicate activation and inactivation of the oxygenation system, respectively. Blue and black point colors indicate oxic and hypoxic conditions during the preceding two weeks, respectively. Left: time series data. Right: boxplots summarizing data based upon oxygenation status during the preceding two weeks. We note that on one occasion (mid July), the oxygenation system shut off prematurely and sediment samples were collected three days after the oxygenated period—these samples still experienced oxic conditions during the preceding two weeks and are therefore classified as “oxic.” Statistical significance of differences between oxic and hypoxic periods is indicated using asterisks: \* indicates p < 0.05, \*\*\* indicates p < 0.001.

Graphical user interface

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Figure 4: Whole-ecosystem oxygenation experiments during the summer stratified period of 2019 more strongly influenced Fe (b), compared to DOC (a) in Falling Creek Reservoir (FCR) and Beaverdam Reservoir (BVR).

## **3.3 Whole-ecosystem experiments: interannual differences**

Activation of the oxygenation system increased hypolimnetic DO concentrations in FCR from 2014 through 2019, and lower oxygen addition rates allowed for primarily hypoxic conditions in 2020 and 2021 (Figure S2). BVR exhibited hypolimnetic hypoxia from June through October throughout the duration of the study (Figure S2).

In FCR, the amount of OC in sediment increased by 57% as DO concentrations decreased from 2019 to 2021 (Figure 5b; Table S1, S2). Consequently, total OC was lower in FCR than BVR in 2019, but not in 2021 (Figure 5b; Table S1, S2). However, the amount of Fe-OC per gram of sediment did not change (Figure 5a; Table S1). As a result, the percentage of sediment OC that was bound to Fe decreased from 2019 to 2021 in FCR (Figure 5c; Table S1, S3). None of these three sediment characteristics differed between 2019 and 2021 in BVR (Figure 5d–f; Table S1, S2, S3).

Chart, scatter chart

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Figure 5: Differences in sediment organic carbon metrics between 2019 and 2021 in Falling Creek (FCR) and Beaverdam (BVR) Reservoirs. Metrics assessed include micromoles of iron-bound organic carbon (Fe-OC) per gram sediment (a), total sediment organic carbon (OC) as a percentage of sediment mass (b), and Fe-OC as a percentage of sediment organic carbon (c). Blue color indicates high-oxygen conditions (2019 in FCR) and letters delineate groups that are significantly different (p < 0.05; Table S1, S2, S3).

## **3.4 Microcosm incubations**

Experimental microcosm incubations successfully established four distinct oxygen regimes. DO concentrations increased rapidly when hypoxic microcosms were unsealed and decreased rapidly when microcosms were sealed (Figure 6). At the transition from hypoxic-to-oxic conditions, DO concentrations increased to approximately the same level as the continuous oxygen treatment (~7 mg/L) within one day. At the transition from oxic-to-hypoxic conditions, DO concentrations decreased below 1 mg/L within one day and declined to 0 mg/L by the end of the experiment.

Changes in oxygen conditions were associated with clear but asynchronous changes in aqueous OC and Fe. As microcosms switched from oxic-to-hypoxic conditions, TOC, DOC and total Fe decreased near synchronously, while dissolved Fe decreased below detection within one day of oxygen exposure. At the transition from hypoxic-to-oxic conditions, DOC and TOC rapidly increased to the same level as microcosms that had experienced continuous hypoxia (~10 mg/L; Figure 6). However, concentrations of both dissolved and total Fe only began to increase after three weeks of hypoxia (Figure 6). Measured DOC and TOC were strongly and linearly correlated, with DOC representing a mean of 96 ± 14 percent of TOC (Figure S3); thus, we focus our discussion on DOC hereafter, but the same trends apply to TOC.

At the end of the experiment, sediment OC differed significantly among treatments (one-way ANOVA: F(3, 20) = 9.09, p <0.001): sediment OC was significantly higher in microcosms that started under oxic conditions (oxic: µ = 4.6 ± 0.3, oxic-to-hypoxic: µ = 4.5 ± 0.3) than microcosms that started under hypoxic conditions (hypoxic: µ = 4.0 ± 0.0, hypoxic-to-oxic: µ = 4.1 ± 0.2; Figure 7). Fe-OC did not differ significantly between treatments as a proportion of sediment mass (F(3, 20) = 0.51, p = 0.683) or as a proportion of sediment OC (F(3, 20) = 2.40, p = 0.098).

Speciation calculations (Table S5) based upon ICP-MS results (Figure S4) suggest that oxygen conditions have primary control over Fe speciation, with a lesser impact on Fe-OC. The experiments that were maintained under hypoxic conditions had dominant Fe species of Fe2+, FeHCO3+, FeCO3, and FeSO4 (all of these species contain Fe in reduced state, Fe2+). For all of the microcosms that were exposed to oxygen at any time (hypoxic-to-oxic, oxic-to-hypoxic, oxic), the dominant Fe species were Fe(OH)3, Fe(OH)2+, Fe(OH)4-, FeOH2+ and FeHumate+ (all of these species contain Fe in the oxidized state, Fe3+). pH remained circumneutral across all treatments (Figure S5). These results indicate that 1) exposure to oxic conditions at any time in the experiment shifted the dominant oxidation state to Fe3+; 2) under oxic conditions, and to a lesser extent, hypoxic conditions, Fe complexed with DOC.

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Figure 6: Changes in water properties (dissolved oxygen, dissolved organic carbon, total iron, and dissolved iron) from experimental microcosms that were sampled destructively over the course of 34 days. Vertical lines indicate when oxygen conditions were switched, creating the oxic-to-hypoxic and hypoxic-to-oxic treatments.

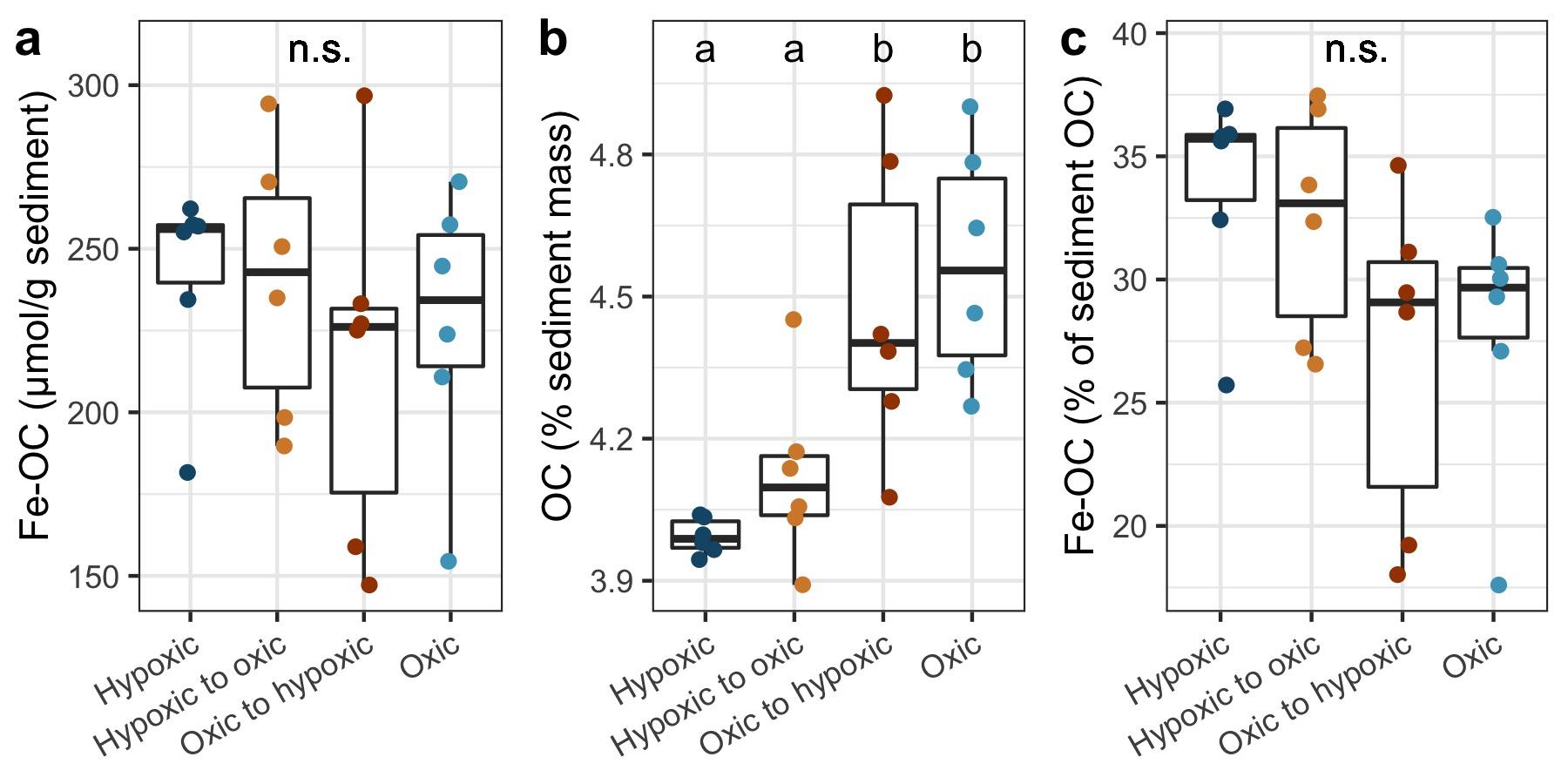


Figure 7: Differences in sediment organic carbon metrics at the end of experimental incubations (days 20 and 23). Metrics assessed include moles of iron-bound organic carbon (Fe-OC) per unit sediment mass (a), total sediment organic carbon (b), and Fe-OC as a percentage of sediment OC (c). Letters delineate groups that are significantly different (p < 0.05): no groups were significantly different for Fe-OC metrics (a, c). Days 20 and 23 were chosen for statistical comparisons as the last days in the experiment when data were collected from all treatments.

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# 4. DISCUSSION

Our results suggest that oxygen affects coupled OC and Fe cycling differently over short-term (several weeks) compared to long-term (multiannual) timescales (Figure 8). Short periods of hypoxia decreased total OC and Fe-OC in sediment and increased concentrations of DOC and Fe in overlying water, indicating that a portion of the sediment Fe-OC pool is sensitive to changes in oxygen. However, over longer timescales low oxygen conditions from 2019–2021 were associated with *increased* OC in sediments from FCR, indicating that the effects of hypoxia on Fe-OC may be outweighed by decreases in respiration rates under hypoxic conditions. Notably, Fe-OC comprised nearly one-third of surficial sediment OC in both FCR and BVR—regardless of oxygen status—which is substantially more than previously reported for freshwater lakes (Peter & Sobek, 2018). Below, we discuss short-term (section 4.1) and multiannual (section 4.2) results in the context of previous work, analyze net processing rates across the sediment-water interface (section 4.3), and discuss why Fe-OC levels may be higher in these reservoirs than other freshwater systems (section 4.4).

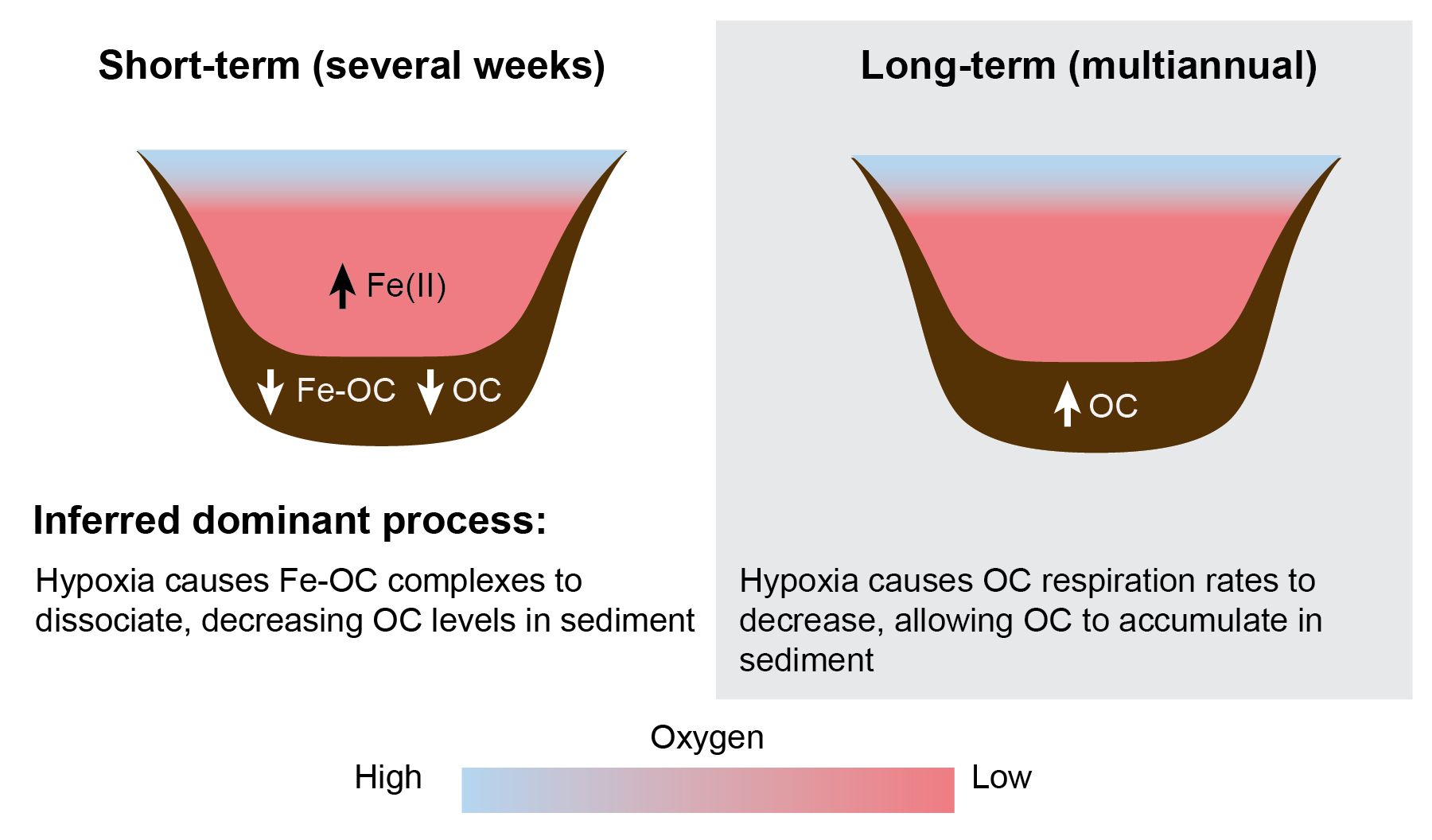


Figure 8: Experimental results suggest that the dominant means by which hypoxia affects sediment organic carbon differs between weekly and multiannual timescales. Left: in microcosm incubations, short-term (weeks) periods of hypoxia led to increased DOC and aqueous Fe, while decreasing sediment OC. On a whole-ecosystem scale, hypolimnetic Fe was closely correlated with oxygen concentrations, and short periods of hypoxia decreased both Fe-OC and OC in sediment. Consequently, Fe-OC protection appears to be a relatively more dominant control on sediment OC sequestration than respiration on short timescales. Right: two years of hypoxia in FCR led to increased OC in sediment on a whole-ecosystem scale, suggesting that respiration is a relatively more dominant control on OC sequestration than protection by Fe on this timescale.

## **4.1 Short-term periods of hypoxia lead to release of Fe-protected OC and decrease total sediment OC**

Both whole-ecosystem and microcosm experiments suggest that short-term (several weeks) periods of hypoxia can alter coupled OC and Fe cycling. Whole-ecosystem experiments revealed changes in hypolimnetic Fe, sediment OC, and sediment Fe-OC resulting from water-column oxygenation (Figures 4, 5, 7), while microcosm incubations showed clear changes in aqueous Fe, and DOC and sediment OC.

However, hypoxia did not clearly impact hypolimnetic DOC on a whole-ecosystem scale (Figure 4). A lack of relationship between hypoxia and DOC is counterintuitive and contrasts with previous experimental and observational studies which documented hypoxic DOC release (e.g., Björnerås et al., 2017; Brothers et al., 2014; Kim & Kim, 2020; Lau & del Giorgio, 2020; Peter et al., 2017; Weyhenmeyer et al., 2014). We anticipate that many interacting processes occurring on a whole ecosystem scale could have masked the effect of hypoxia on DOC. For example, DOC inputs from the epilimnion likely play a substantial role in determining hypolimnetic DOC concentrations in this eutrophic reservoir. Likewise, interacting effects of temperature and respiration rates may have altered water-column DOC concentrations, and increased oxidation of organic matter may increase its polarity and solubility, masking the effect of reductive dissolution of Fe in Fe-OC complexes on a whole-ecosystem scale.

In contrast to whole-ecosystem experiments, experimental incubations showed a clear signal of increased DOC following the onset of hypoxia, following previous studies (e.g., Skoog & Arias-Esquivel, 2009). However, this increase cannot be clearly attributed to the release of DOC from Fe-OC complexes, as Fe-OC did not differ significantly between experimental treatments and increases in Fe and DOC were asynchronous. Instead, we suspect that microbial mortality and lysis may be responsible for the rapid (<1 day) increase in DOC following the onset of hypoxic conditions in the experimental microcosms. Under oxic conditions, we observed the formation of orange (Fe-oxidizing) biofilms on top of the sediment layer (Figure S1), which may be partially responsible for the increase in sediment OC in these treatments. As oxygen concentrations were rapidly depleted, microbial mortality and lysis would have released organic matter into the water column, rapidly increasing DOC without affecting Fe.

The fact that Fe-OC appeared to vary with oxygenation on a whole-ecosystem scale but not among treatments in experimental microcosms was counterintuitive, as we expected microcosms to have decreased variability compared to whole-ecosystem experiments. We expect that this difference derives at least in part from sediment composition: whole-ecosystem samples were always composed of the top 1 cm of sediment from sediment cores, while sediment for the experimental incubations was sampled using an Ekman grab, and therefore included deeper layers of sediment. In soil, deeper horizons are thought to have more stable Fe-OC aggregates (Rumpel & Kögel-Knabner, 2011), and the same is likely to be true in sediments.

A number of alternative mechanisms could contribute to changes in Fe and OC in short-term experiments, in addition to reductive dissolution of Fe in Fe-OC complexes. Both Fe and OC can respond independently to changes in DO concentrations: Fe is transformed from Fe2+ (soluble) to Fe3+ minerals (insoluble) both biotically and abiotically under oxic conditions (Kappler et al., 2021). Furthermore, Fe reduction is often associated with an increase in pH, which may increase the solubility of OC (Tavakkoli et al., 2015). Increased microbial biomass may be partially responsible for the increase in sediment OC under oxic conditions on both whole-ecosystem and microcosm scales (as discussed above). While these mechanisms may play a role in Fe and OC release, the fact that Fe-OC decreased following inactivation of the oxygenation system in FCR suggest that, at least for some surficial sediments, short (~2 week) periods of hypoxia may cause reductive dissolution of a portion of the Fe-OC pool on a whole-ecosystem scale.

## **4.2 Over multiannual timescales, OC respiration rates play a greater role than Fe-OC in determining the net effect of hypoxia on sediment OC content**

Over multiannual timescales (2019–2021), exposure to seasonal hypoxia increased the amount of OC in sediments from FCR without changing the amount of Fe-OC. This clearly contrasts with short-term experimental results, which showed decreased OC content following short periods of hypoxia (section 4.3). While many factors could affect OC and Fe-OC over multiannual timescales, the fact that no comparable effects were seen in the unoxygenated reference reservoir (BVR) suggests that these changes can likely be attributed to changes in DO concentrations in overlying water. Over the course of two years of hypoxic conditions, OC levels in sediment from FCR increased to the extent that they were no longer significantly different from the hypoxic reference reservoir (Fig. 7).

Increases in sediment OC content with increased hypoxic duration are consistent with a reduction in sediment OC respiration rates under hypoxic conditions (Carey et al., 2018; Hargrave, 1969; Walker & Snodgrass, 1986). OC respiration rates are reduced under hypoxic conditions due to the fact that OC must be broken down using alternative electron acceptors which produce a lower energy yield (David Bastviken et al., 2003, 2004). As less OC is converted to CO2 and CH4 in both hypolimnetic water and surficial sediment, OC can accumulate more quickly in surficial sediments. Our results suggest that over multiannual timescales this process (decreased respiration under hypoxic conditions) outweighs the counteracting decrease in Fe protection of OC that we observed during short periods of hypoxia.

The fact that sediment Fe-OC content (per g sediment mass) did not change after two years of hypoxic conditions in FCR indicates that at least a fraction of these compounds are able to withstand fluctuations in environmental redox conditions. Long-term stability of Fe-OC complexes can be promoted by incorporation of OC into Fe oxides and other Fe minerals through co-precipitation and potential substitution (Markus Kleber et al., 2015 and references therein). Likewise, over time, weathering of Fe increases the porosity of mineral surfaces and allows for OC protection within pore spaces (REF). As Fe-OC persists over time, decreased accessibility to microbial decomposition (e.g., through burial) and increased chemical bonding may increase the stability of these compounds (Kaiser et al., 2007; Kaiser & Guggenberger, 2003). In FCR, the history of oxic conditions (2013–2019) may have contributed to the formation of particularly stable Fe-OC complexes in sediment, which were then able to withstand two summers of hypoxia.

Importantly, much of the OC that accumulates under hypoxic conditions does not end up being bound to Fe. This result may be a product of Fe oxidation state, as sorptive associations between DOC and Fe in sediment are much less likely to form if Fe is in a reduced state (Fe2+; Nierop et al., 2002). Because total OC increased following hypoxia and Fe-OC did not change, Fe-OC as a percentage of sediment OC was significantly lower after two years of hypoxia than before this hypoxic period. Declines in the percentage of OC that is bound to Fe may have important implications for ecosystem-scale carbon cycling, as OC that is associated with Fe is comparatively more protected from respiration. Increased stocks of OC that are not associated with Fe may increase rates of methane production and OC release from sediment (e.g., Hounshell et al., 2021), and could increase aerobic respiration rates under subsequent oxic periods.

## **4.3 Substantial OC and Fe cycling occurs at the sediment-water interface**

Notably, the OC content of the top 1 cm of sediment was significantly lower than that of settling particulate material in both FCR and BVR, and nearly three times as much of this OC was bound to Fe in sediments compared to settling material. These results imply a substantial level of OC and Fe processing at the sediment-water interface, which is a critical and understudied zone of freshwater lakes and reservoirs (e.g., Dadi et al., 2017; Hanson et al., 2015).

From a mass-balance perspective, the difference in Fe-OC between settling material and surficial sediments suggests that Fe-OC may be formed or preferentially preserved in sediments compared to OC that is not associated with Fe. Preferential preservation of Fe-OC is well-supported, as complexation with Fe has been shown to decrease OC turnover rates across multiple ecosystems (Eusterhues et al., 2014; Kaiser & Guggenberger, 2003; Kalbitz et al., 2005; M. Kleber et al., 2005; Lalonde et al., 2012; Mikutta & Kaiser, 2011). However, the difference in Fe-OC between settling material and surficial sediments likely also results in large part from Fe-OC associations formed in sediment (e.g., through adsorption of organic matter onto existing Fe minerals and Fe-OC complexes), as Fe(III) levels are much higher in sediments and the composition of OC in sediments may be more preferable for complexation with Fe. While we did not measure OC quality in this study, we anticipate that settling material may have higher autochthonous OC and be more rapidly respired, while sediment OC may be enriched in allochthonous aromatic OC, which preferentially associates with Fe (Kramer et al., 2012; Riedel et al., 2013; Shields et al., 2016). Documenting changes in Fe-OC throughout the process of sediment diagenesis enhances our understanding of OC sequestration, as few if any previous studies have quantified the difference between Fe-OC inputs and stocks in aquatic sediments. These results suggest that the sediment-water interface may be a hotspot for OC and Fe cycling in freshwater environments (following Hanson et al., 2015).

**4.4 High Fe-OC levels reflect site-specific characteristics**

## A mean of nearly one-third of sediment OC was bound to Fe (dithionite-extractable) across two years in FCR and BVR. This percentage is far greater than that documented by Peter et al. (2018), where Fe-OC comprised ≤ 11% of total sediment OC across five boreal lakes. Furthermore, the levels of Fe-OC recorded here are higher than the mean of ​​21.5% (SD = 8.6) reported for a broad range of marine sediments (Lalonde et al. 2012). With few other studies analyzing Fe-OC in freshwater lakes and reservoirs, our analysis provides new evidence that Fe-OC may play an important role in carbon sequestration in some freshwater sediments.

Differences in the percentage of organic matter that is bound to Fe may result from differences in overlying DO concentrations, as described throughout this study, as well as a number of other factors. For example, increasing ratios of Fe:OC and increasing absolute concentrations of Fe and OC can all increase the amount of Fe-OC coprecipitation (Markus Kleber et al., 2015 and references therein); these differences may derive from contrasting geology, catchment vegetation, and trophic status, among many other factors. Likewise, differing Fe forms and OC quality may also impact the formation of Fe-OC complexes: for example, aromatic OC tends to preferentially coprecipitate with Fe, and Fe weathering increases the availability of pore spaces for OC protection (Curti et al., 2021; Kaiser et al., 2007; Kaiser & Guggenberger, 2003; Markus Kleber et al., 2015). Variation in pH may increase the stability of Fe-OC in sediment, as inner-sphere associations between Fe and OC primarily form under low pH and outer-sphere associations prevail under high pH (Markus Kleber et al., 2015 and references therein). However, none of these factors fully explain differences in Fe-OC content between sites studied to date (e.g., Lalonde et al., 2012; Peter & Sobek, 2018).

Despite having higher Fe-OC levels (as a percentage of total sediment OC) than most aquatic sediments studied to date, FCR and BVR fall well within the range of other characteristics of these ecosystems. FCR and BVR have much lower sediment OC content than the boreal lakes analyzed by Peter and Sobek (2018; 14–38% of sediment mass), but higher sediment OC than the primarily marine sediments analyzed by Lalonde et al. (2012; 0–7% of sediment mass). Fe concentrations are high in sediment from FCR and BVR, with a mean of 53,466 mg/kg dry weight (Krueger et al., 2020). However, Peter and Sobek (2018) observed low Fe-OC as a percentage of sediment OC (µ = 6.7%) in one extremely high-Fe lake (Övre Skärsjön; 226,172 mg/kg reducible Fe in sediment). Likewise, pH in FCR and BVR is circumneutral (Figure S6), well within the range of 5.4–7.6 reported by Peter and Sobek (2018), and both Peter and Sobek (2018) and Lalonde et al. (2012) included a range of oxic and hypoxic sediments in their analyses. These observations from a range of aquatic sediments suggest that site-specific characteristics associated with catchment geology, water residence time, OC and Fe input rates, OC quality, and Fe mineral forms play an important role in determining the percentage of sediment OC that is bound to Fe. Understanding the controls on Fe-OC in freshwater sediment will require additional research, including Fe-OC characterization at a greater number and diversity of lakes and reservoirs. Such research will be essential to understanding how freshwater OC sequestration may be affected by global change in interacting factors such as Fe concentrations (Weyhenmeyer et al., 2014), temperatures (Dokulil et al., 2021; O’Reilly et al., 2015), and pH (Garmo et al., 2014; Gavin et al., 2018; Stoddard et al., 1999), among other factors.

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# 5. CONCLUSIONS

Results from this study help reconcile previous Fe-OC research and shed light on how declining oxygen concentrations may impact the role of lakes and reservoirs in the global carbon cycle. Previous work across terrestrial soils and marine sediments, among other environments, has provided contradictory evidence that Fe-OC complexes are (1) readily dissociated under hypoxic conditions and (2) capable of promoting sediment OC burial into deeper (hypoxic) layers over the course of decades to millennia. Here, we find that the timescale of analysis matters when discussing the stability of Fe-OC: a portion of the Fe-OC pool in surficial sediment is highly responsive to oxygen concentrations in overlying water, but over longer timescales the decrease in OC resulting from dissociation of Fe-OC complexes is outweighed by the increase in sediment OC that results from slower respiration rates under hypoxic conditions.

As the duration of hypoxia increases in lakes and reservoirs (Jane et al., 2021; Jenny et al., 2016), our results suggest that OC dynamics may respond non-linearly. While short periods of hypoxia may decrease OC burial, increasing prevalence and duration of hypoxia over multiannual timescales may increase OC burial in sediment. However, the increase in carbon burial is likely not sufficient to counter the increase in greenhouse gas potential that results from methane production under hypoxic conditions. While we found ~50% more OC in sediment under hypoxic conditions, previous research in these reservoirs has documented CH4 concentrations that were two orders of magnitude higher under hypoxic compared to oxic conditions (Hounshell et al., 2021). Furthermore, while total sediment OC levels increased under hypoxic conditions, the percentage of sediment OC that is bound to Fe decreased. Consequently, it is possible that hypoxic summers may lead to increased respiration rates during mixed conditions that follow fall turnover and spring mixing, though much remains unknown about how changing temperatures and mixing conditions would interact to affect these dynamics. Regardless, our work suggests that freshwater OC cycling involves multiple mechanisms interacting over different time scales. Our high sediment Fe-OC measurements indicate that Fe may play an important role in controlling OC cycling, at least at shorter time scales, in some freshwater ecosystems.

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# AUTHOR CONTRIBUTIONS

ASL conceptualized this study, with support from CCC and MES. Additionally, ASL performed chemical Fe-OC extractions, analyzed data, and wrote this manuscript. BRN made fundamental contributions to the analytical chemistry methods development and performed DOC analysis in 2019 and 2021. NWH and MES oversaw whole-ecosystem Fe sampling, data collation, and quality control, and helped with the conceptual framing for this manuscript. AD helped to design and run the in-lab experiment, process sediment samples, and discuss relevant literature with ASL. MEL helped interpret results and edit the manuscript. HLW helped collate and process 2019 and 2021 DOC and YSI data and review code for this manuscript. CCC helped to develop the conceptual basis for this manuscript, oversee oxygenation experiments, and interpret results. All coauthors edited and approved the final manuscript.

OPEN RESEARCH

All data used in this study have either already been published to the Environmental Data Initiative (Carey et al., 2021; Carey, Lewis, et al., 2022; Carey, Wander, et al., 2022; Schreiber et al., 2022) or are in the staging environment of the Environmental Data Initiative and will be published upon manuscript acceptance (REFS: incubations, sediment)

* [Whole-ecosystem OC/Fe-OC measurements](https://docs.google.com/document/d/19Q9S2e6sC9Cd0eD7Ei1EpAMvJT4MfImDxVnSp6cjHqE/edit)
* [Incubations](https://docs.google.com/document/d/1NNs5U2MFNKCAd3kbBq0o3P3JUnSZVWduWZdoKBRnG_I/edit) (DOC, Fe, Fe-OC, pH, DO, ICP)

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