Anoxia decreases the magnitude of the carbon, nitrogen, and phosphorus sink in freshwaters

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Abstract

Oxygen availability is decreasing in many lakes and reservoirs worldwide, raising the urgency for understanding how anoxia (low oxygen) affects coupled biogeochemical cycling, which has major implications for water quality, food webs, and ecosystem functioning. Although the increasing magnitude and prevalence of anoxia has been documented in freshwaters globally, the challenges of disentangling oxygen and temperature responses have hindered assessment of the effects of anoxia on carbon, nitrogen, and phosphorus concentrations, stoichiometry (chemical ratios), and retention in freshwaters. The consequences of anoxia are likely severe and may be irreversible, necessitating ecosystem-scale experimental investigation of decreasing freshwater oxygen availability. To address this gap, we devised and conducted REDOX (the Reservoir Ecosystem Dynamic Oxygenation eXperiment), an unprecedented, seven-year experiment in which we manipulated and modeled bottom-water (hypolimnetic) oxygen availability at the whole-ecosystem scale in a eutrophic reservoir. Seven years of data reveal that anoxia significantly increased hypolimnetic carbon, nitrogen, and phosphorus concentrations and altered elemental stoichiometry by factors of 2-5× relative to oxic periods. Importantly, prolonged summer anoxia increased nitrogen export from the reservoir by six-fold and changed the reservoir from a net sink to a net source of phosphorus and organic carbon downstream. While low oxygen in freshwaters is thought of as a response to land use and climate change, results from REDOX demonstrate that low oxygen can also be a *driver* of major changes to

freshwater biogeochemical cycling, which may serve as an intensifying feedback that increases anoxia in downstream waterbodies. Consequently, as climate and land use change continue to increase the prevalence of anoxia in lakes and reservoirs globally, it is likely that anoxia will have major effects on freshwater carbon, nitrogen, and phosphorus budgets as well as water quality and ecosystem functioning.

Keywords: Biogeochemistry, Ecosystem modeling, Hypoxia, Nutrient retention, Oxygen, REDOX, Reservoir, Stoichiometry, Water quality, Whole-ecosystem experiment

Introduction

Oxygen concentrations in lakes and reservoirs around the world are decreasing, which has the potential to substantially alter freshwater ecosystem functioning and water quality. As a result of climate and land use change, low oxygen availability (anoxia) is becoming more common in the hypolimnion, or bottom waters, of many lakes and reservoirs (Jenny et al. 2016a, Jane et al. 2021, Woolway et al. 2021). An increase in both the occurrence and duration of hypolimnetic anoxia in freshwaters is likely to substantially alter the cycles of carbon (C), nitrogen (N), and phosphorus (P), three fundamental elements that determine freshwater food web structure, water quality, and ecosystem functioning (Sterner and Elser 2002, Kortelainen et al. 2013). In particular, anoxia could disrupt the critical role of freshwater ecosystems as C, N, and P sinks in global biogeochemical cycles. Freshwaters retain 72% of the organic C, 56% of the total N, and 56% of the total P exported from land via sediment burial or release to the atmosphere, preventing these elements from being transported to downstream freshwater ecosystems or the oceans (Maranger et al. 2018). Altogether, the consequences of anoxia for C,

N, and P concentrations, stoichiometry (chemical ratios), and retention in freshwaters are likely severe and may be irreversible (Nürnberg 1988, Søndergaard et al. 2003, Brothers et al. 2014, North et al. 2014), necessitating ecosystem-scale investigation of how hypolimnetic anoxia affects freshwaters.

Biogeochemical cycles of dissolved and total C, N, and P will likely respond differently to hypolimnetic anoxia (Fig. 1). In the bottom waters of lakes and reservoirs, we expect dissolved organic C (DOC) concentrations to be higher in anoxic than oxic conditions, as DOC is mineralized much more efficiently by oxygen than by alternate terminal electron acceptors (Walker and Snodgrass 1986, Beutel 2003). Moreover, anoxia has been shown to stimulate the release of DOC from sediments to the water column (Fig. 1; Brothers et al. 2014, Peter et al. 2017), as well as increase hypolimnetic methane concentrations and subsequent greenhouse gas emissions (Vachon et al. 2019, Hounshell et al. 2021). DOC generally dominates the total OC (TOC) pool in lakes (Toming et al. 2020), thus we would expect TOC to exhibit similar responses as DOC to anoxia. For hypolimnetic dissolved inorganic nitrogen (DIN), ammonium (NH_4^+) concentrations would be expected to be higher in anoxic conditions due to ammonification and release from sediments (Fig. 1; Rysgaard et al. 1994, Beutel et al. 2006). In contrast, nitrate (NO_3^-) would be lower in anoxic than oxic conditions, as denitrification decreases NO_3^- in the absence of oxygen while nitrification increases NO_3^- in the presence of oxygen (Fig. 1; Sharma and Ahlert 1977, Downes 1987). Total nitrogen (TN) in the hypolimnion could either increase or decrease in anoxic conditions, depending on the balance of NH_4^+ vs. NO_3^- within the DIN pool, as the inorganic fraction of hypolimnetic dissolved N is generally greater than the organic fraction (Kim et al. 2006). For hypolimnetic phosphorus (P), we would expect that dissolved reactive phosphorus (DRP) concentrations would be higher in anoxic

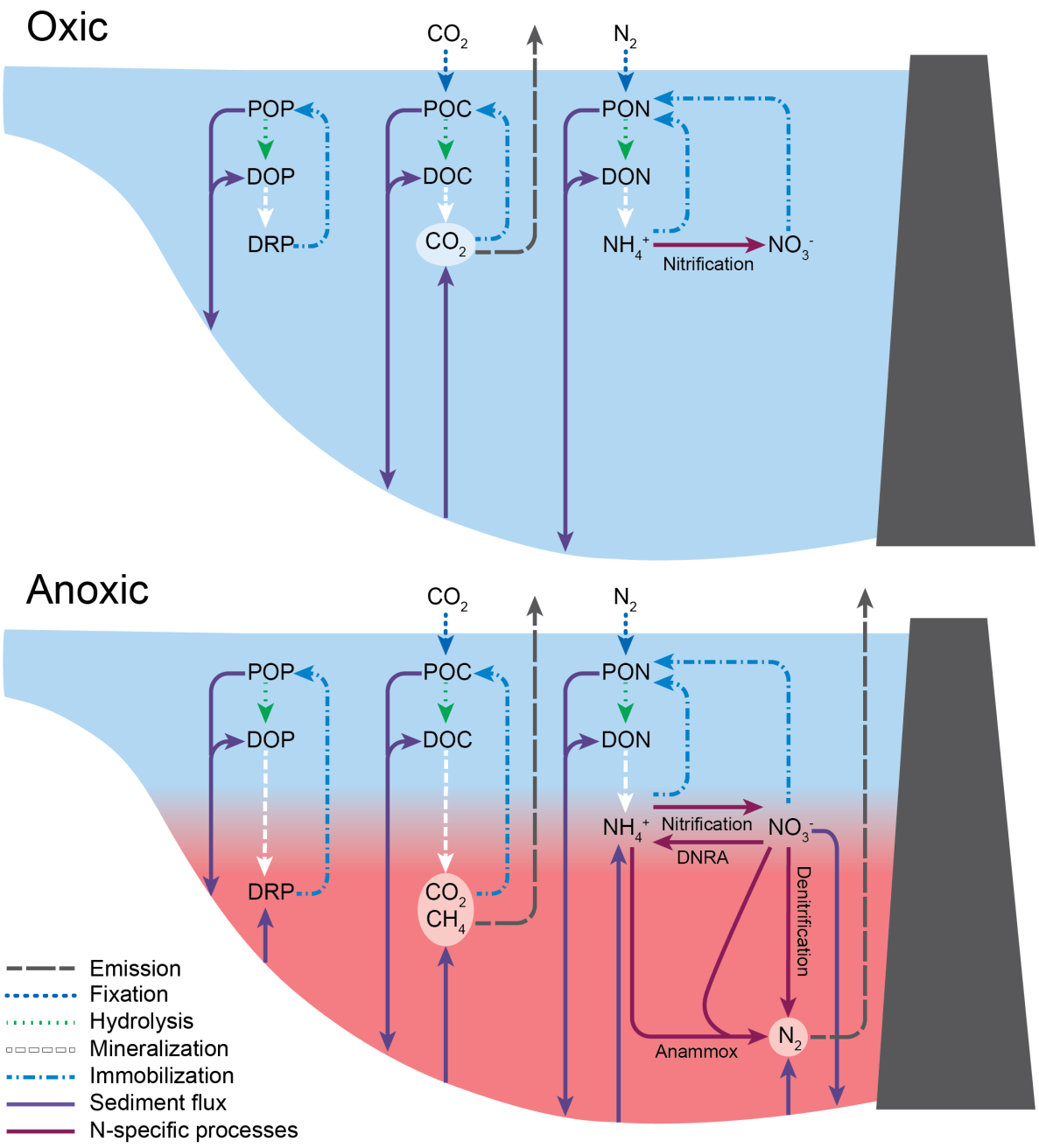


Fig. 1. Conceptual diagram of the dominant carbon, nitrogen, and phosphorus cycling processes (denoted by arrow lines) expected under oxic (top) and anoxic (bottom) conditions in the water column of a thermally stratified reservoir. Carbon processes include cycling of carbon dioxide (CO_2), dissolved organic carbon (DOC), methane (CH_4), and particulate organic carbon (POC). Nitrogen processes include cycling of dissolved organic nitrogen (DON), nitrogen gas (N_2), ammonium (NH_4^+), nitrate (NO_3^-), and particulate organic nitrogen (PON). Phosphorus processes include cycling of dissolved organic phosphorus (DOP), dissolved reactive phosphorus (DRP), and particulate organic phosphorus (POP).

conditions as DRP is released into the water column during iron reduction and particulate organic matter mineralization (Fig. 1; Mortimer 1971, Boström et al. 1988, Nürnberg 1988, Rydin 2000). Total P (TP) concentrations would likely exhibit a similar but more muted response to anoxia than DRP, as DRP is usually a small fraction of the TP pool (Wetzel 2001).

While these different C, N, and P processes have been well-studied individually, there have been no studies on the net effect of anoxia on all of these cycles operating concurrently at the ecosystem scale, likely due to the challenges of disentangling complex coupled biogeochemical cycling with observational field studies or laboratory experiments. Explicitly considering interconnected elemental cycles and their stoichiometry (following Sterner and Elser 2002) is essential to understanding the effects of anoxia on ecosystem functioning.

Increases in hypolimnetic anoxia have substantial implications for the fate of C, N, and P in freshwater ecosystems. There are two primary fates for C, N, and P entering into a waterbody: retention - by either remaining in the water column, burial in the sediments, or emission to the atmosphere (for C and N only) - or export downstream (following the ecosystem retention definition used by Dillon and Molot 1997, Harrison et al. 2009, Powers et al. 2015, Maranger et al. 2018, and many others). Anoxia may decrease the ability of lakes and reservoirs to retain NH_4^+ and DRP by reducing their burial in sediments (Rysgaard et al. 1994, North et al. 2014, Powers et al. 2015), thereby increasing their downstream export. Conversely, anoxia could increase the retention of NO_3^- by increasing its emission to the atmosphere via denitrification, thereby decreasing its downstream export (Fig. 1; Beaulieu et al. 2014). For C, the ecosystem-scale effects of anoxia are likely complex. The TOC pool includes dissolved and particulate fractions of OC that may respond to oxygen differently and are mediated by ambient environmental conditions, such as external loading, temperature, nutrients, and light (Hanson et

al. 2015). For example, anoxia could increase the retention of particulate OC (POC) by decreasing its mineralization, thereby potentially increasing its burial in sediments (Walker and Snodgrass 1986, Beutel 2003). Simultaneously, anoxia could decrease the retention of DOC by stimulating fluxes of DOC from the sediments into the water column (e.g., by reductive dissolution of iron-bound DOC complexes; Skoog and Arias-Esquivel 2009), thereby potentially decreasing burial in sediments (Brothers et al. 2014, Peter et al. 2017), and increasing DOC export downstream. Consequently, quantifying the effect of anoxia on C, N, and P retention vs. downstream export (and thus determining if a waterbody is a sink or source of C, N, and P downstream) is needed to improve our understanding of the changing role of lakes and reservoirs in global biogeochemical cycles.

In particular, human-made reservoirs, which retain substantially more inflowing C, N, and P per unit area than naturally formed lakes globally via either sediment burial or emissions to the atmosphere (Harrison et al. 2009, Powers et al. 2016, Maranger et al. 2018), may be very sensitive to the effects of hypolimnetic anoxia. Despite only covering 6-11% of the global lentic surface (Downing et al. 2006, Lehner et al. 2011, Verpoorter et al. 2014), reservoirs alone are estimated to account for ~40% of total annual global OC burial (Mendonça et al. 2017) and 26% of total annual global P burial (Maranger et al. 2018). Moreover, reservoirs globally emit 6.5 Tg N yr^{-1} to the atmosphere, primarily via denitrification (Harrison et al. 2009, Beusen et al. 2016). In an analysis of ~1000 lakes and reservoirs sampled once across the U.S., reservoirs were found to have lower organic C:P and N:P ratios than naturally formed lakes, which was attributed in part to a greater incidence of hypolimnetic anoxia in reservoirs than naturally formed lakes (Maranger et al. 2018). However, that study lacked accompanying oxygen data to examine how C, N, and P varied across a gradient of oxygen availability. Moreover, the amalgamation of data

148 from waterbodies with different climate and catchment land use makes it challenging to quantify
149 how changing oxygen alters water column C, N, and P concentrations, stoichiometry, and export.
150 We need new approaches that embrace the dynamic nature of reservoirs over time and allow us
151 to disentangle the effects of hypolimnetic anoxia on these waterbodies, especially as their
152 construction is increasing globally (Zarfl et al. 2015).

153 To mechanistically quantify the effects of anoxia on C, N, and P cycling, we devised and
154 conducted REDOX (the Reservoir Ecosystem Dynamic Oxygenation eXperiment), an
155 unprecedented, seven-year study that integrated a long-term hypolimnetic oxygenation
156 manipulation with ecosystem modeling in a eutrophic reservoir. Coupled whole-ecosystem
157 manipulations and ecosystem modeling provide a powerful approach for both quantifying the
158 effects of hypolimnetic anoxia on C, N, and P cycling and testing the mechanisms underlying
159 continental-scale patterns derived from thousands of waterbodies (e.g., Helton et al. 2015,
160 Maranger et al. 2018). Foundational work based on sediment core incubations in the laboratory
161 and small chambers placed *in situ* on the sediments of lakes and reservoirs (e.g., Frindte et al.
162 2015, Lau et al. 2016) have yet to be tested at the ecosystem scale, which is needed to overcome
163 the limitations of small volumes of water and mesocosm fouling. Studies that manipulate an
164 entire ecosystem are able to disentangle the effects of oxygen availability from other
165 environmental drivers, such as water temperature and biological activity, on C, N, and P cycling
166 (Cole 2013). However, it is logistically challenging to replicate these intensive experiments
167 under different meteorological and environmental conditions over time to assess robustness and
168 repeatability of ecosystem responses. Consequently, data from whole-ecosystem manipulations
169 can be used to calibrate ecosystem models (following Medlyn et al. 2015) that can simulate
170 complex ecosystem responses under a range of oxygen scenarios and weather conditions over

multiple years, thereby overcoming the constraints of separate empirical and model investigations.

The purpose of REDOX was to study ecosystem-scale functioning under contrasting oxygen conditions over multiple years in the same reservoir. First, we intensively monitored dissolved oxygen and total and dissolved C, N, and P chemistry, as well as a suite of accompanying water quality variables, in the reservoir during the seven-year field manipulation. Second, we used the empirical data to calibrate a coupled hydrodynamic-ecosystem model, which was used to quantify the effects of varying oxygen conditions over the seven years. To investigate further changes in reservoir C, N, and P cycling due to anoxia, we used the calibrated model to test hypolimnetic oxygen scenarios under a range of seasonal and meteorological conditions. We focused on two contrasting model scenarios: one in which there was oxygenation throughout the stratified summer periods in all seven years, resulting in continuous oxic conditions, and one in which there was no oxygenation, resulting in hypolimnetic anoxia every summer. We used the model output to address the following questions: 1) How does hypolimnetic oxygen availability affect total and dissolved C, N, and P concentrations and stoichiometry?, and 2) How does hypolimnetic anoxia affect reservoir retention and downstream export of C, N, and P?

Materials and Methods

Site description

We studied the effect of changing oxygen conditions on C, N, and P dynamics in Falling Creek Reservoir (FCR), a small eutrophic reservoir located in Vinton, Virginia, USA (37.303479,-79.837371; Fig. 2). FCR has a maximum depth of 9.3 m and surface area of 0.119

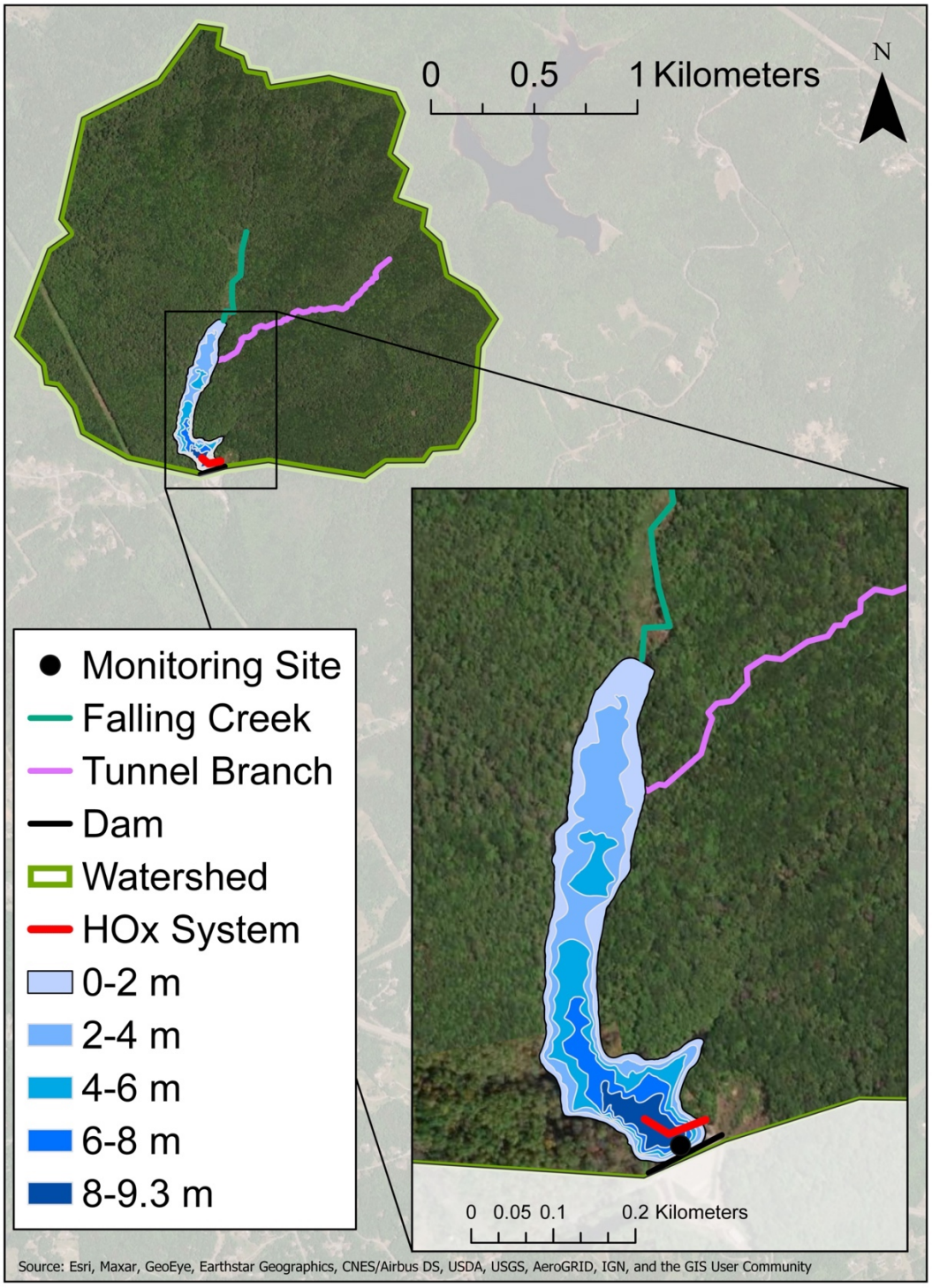


Fig. 2. Map of Falling Creek Reservoir, Vinton, VA, USA (37.303479, -79.837371). The map shows the reservoir watershed, locations of the two inflow streams (Falling Creek and Tunnel Branch), dam, hypolimnetic oxygenation (HOx) system, and monitoring site near the dam.

198 km^2 and is a drinking water source operated by the Western Virginia Water Authority (WVWA;
199 Gerling et al. 2014). FCR's watershed was farmland at the time of reservoir construction in 1898
200 and is almost completely deciduous forest today following agricultural abandonment in the
201 1930s (Gerling et al. 2016). The reservoir has never been dredged (Gerling et al. 2016), and had
202 a mean hydraulic residence time of 281 days (±12 days, 1 S.E.) during our study. FCR has
203 hypolimnetic outtake valves from which water can be withdrawn for treatment.
204
205 *Whole-ecosystem manipulations*
206 We manipulated hypolimnetic oxygen availability in FCR using an engineered
207 hypolimnetic oxygenation system (HOx) deployed by the WVWA in 2012, which allowed us to
208 generate contrasting summer oxic and anoxic conditions (Gerling et al. 2014). The HOx system
209 withdraws hypolimnetic water from 8 m depth, injects dissolved oxygen into the water at super-
210 saturated concentrations onshore, and returns the oxygenated water back to the hypolimnion at 8
211 m without altering thermal stratification or water temperature (Gerling et al. 2014).
212 During the summers of 2013-2019, the HOx system was operated at variable oxygen
213 addition levels and durations in collaboration with the WVWA (Carey et al. 2021d). Some
214 summers experienced intermittent 4-week periods of oxygenation (2013, 2014); some summers
215 had near-continuous oxygenation (2015, 2016, 2017), one summer had approximately half
216 oxygenation (2018), and one summer experienced intermittent 2-week periods of oxygenation
217 (2019; Carey et al. 2021d). These wide-ranging oxygenation conditions, which occurred because
218 the reservoir was an actively managed drinking water source, provided an ideal dataset for
219 calibrating the biogeochemical rates in the ecosystem model to variable hypolimnetic oxygen
220 conditions, as described below.

Monitoring data

FCR's physics, chemistry, and biology were intensively monitored throughout the REDOX manipulations (see Supplementary Text 1 for detailed sampling methods). On every sampling day, depth profiles of water temperature and dissolved oxygen were collected at the deepest site of the reservoir, near the dam (Carey et al. 2021b). We collected water samples for total and dissolved N, P, and organic C (hereafter, C) analyses from the reservoir's water treatment extraction depths (0.1, 1.6, 2.8, 3.8, 5.0, 6.2, 8.0, and 9.0 m) using a Van Dorn sampler. Water was filtered through glass-fiber 0.7-micron filters into acid-washed bottles and immediately frozen until analysis for dissolved C, N, and P samples (Carey et al. 2021e). Unfiltered water was frozen in separate acid-washed bottles for total samples (Carey et al. 2021e). We focused our sampling and analysis on organic C, rather than inorganic C, because of the important role of reservoirs in burying this pool in the global C cycle (Mendonça et al. 2017), and because previous work indicates that most terrestrial dissolved inorganic C loads are rapidly emitted to the atmosphere (McDonald et al. 2013).

We used standard methods for biogeochemical analyses (see Supplementary Text 2 for detailed laboratory methods). We used flow injection analysis to determine concentrations of N and P colorimetrically (APHA 2017), with an alkaline persulfate digestion for TN and TP fractions. DOC and TOC were determined by either heated persulfate digestion or high-temperature combustion followed by infrared absorbance (APHA 2017; see Table S1). All field and laboratory data are available with metadata in the Environmental Data Initiative (EDI) repository (Carey et al. 2019, Carey et al. 2020, Carey et al. 2021a, Carey et al. 2021b, Carey et al. 2021e, Carey et al. 2021f).

Model description and driver data

We used the empirical data to calibrate and validate the General Lake Model coupled to Aquatic EcoDynamics modules (GLM-AED, v.3.2.0a3) configured for FCR (see Supplementary Text 3 for detailed modeling methods). GLM-AED is an open-source, 1-D numerical simulation model that is widely used in the freshwater research community to model lakes and reservoirs (e.g., Bruce et al. 2018, Hipsey et al. 2019, Farrell et al. 2020, Ward et al. 2020). GLM-AED requires meteorological, inflow, and outflow driver data and simulates water balance and thermal layers using a Lagrangian strategy (Hipsey et al. 2019). GLM-AED has a flexible structure in which modules representing different ecosystem components can be turned on or off to recreate varying levels of ecosystem complexity; our configuration for FCR included modules for oxygen, C, silica (Si), N, P, organic matter, and phytoplankton (Carey et al. 2021c).

GLM-AED simulates the dominant processes controlling freshwater oxygen and C, N, and P cycling (see Supplementary Text 3; Farrell et al. 2020, Ward et al. 2020). Biogeochemical processes (e.g., sediment fluxes, mineralization) were modeled as a function of both oxygen following Michaelis-Menten dynamics and temperature following Arrhenius coefficients (Farrell et al. 2020). Consequently, processes that are favored in anoxic conditions (e.g., sediment fluxes of DOC, NH_4^+, and DRP into the hypolimnion) were still simulated in oxic conditions, but at much lower rates.

The ecosystem model provided important insight on the effects of anoxia that would have been impossible to obtain from the field manipulation alone. First, while we do report on the biogeochemical responses to the field manipulation to provide complementary data to the model output, ecosystems rarely experience such rapid shifting of redox conditions at sub-seasonal scales, as were created by abrupt additions of oxygen via the HOx system. Thus, to understand

how our FCR results applied to other waterbodies, we used the seven-year field manipulation as a proxy to contrast the consequences of seasonally oxic vs. anoxic hypolimnia for biogeochemical cycling in an ecosystem model. These highly contrasting scenarios were achieved in the model by manipulating hypolimnetic oxygen injection (described below). Second, to determine the cumulative fate of C, N, and P over an entire summer in response to oxygen dynamics, it is important to track these elements at a high temporal resolution. Because our field data were collected weekly to monthly, we used numerical modeling of hydrodynamics and ecosystem processes to capture daily dynamics. Third, the field manipulation included a variable oxygenation schedule which occurred against a backdrop of changing meteorology and hydrology. Consequently, the model enabled us to isolate the effects of oxygen availability on the reservoir's biogeochemistry and evaluate the robustness of ecosystem responses across varying environmental conditions.

Model configuration and calibration

All GLM-AED model configuration files, parameters, and driver data for FCR are available in the EDI repository (Carey et al. 2021c). GLM-AED driver data included hourly meteorological data from NASA's North American Land Data Assimilation System (NLDAS-2; Xia et al. 2012), stream inflow data, and outflow data. We developed stream inflow driver datasets – which consisted of daily discharge, water temperature, and chemistry – for the two primary streams entering FCR from observational data (Supplementary Text 3). To simulate the HOx system in the model, we added a submerged inflow that injected oxygenated water into the reservoir at 8 m, the same depth as in the reservoir (Supplementary Text 3). As the reservoir was managed to keep constant water level, outflow volume was set to equal inflow volume; the

physical and chemical properties of the outflow were determined by the state of the modeled reservoir (Supplementary Text 3).

We ran the model from 15 May 2013 to 31 December 2019, divided into calibration (15 May 2013-31 December 2018) and validation (1 January 2019-31 December 2019) periods for model verification. GLM-AED was run on an hourly time step throughout the total simulation period (Carey et al. 2021c).

We calibrated GLM-AED to observed conditions (Supplementary Text 3). First, we conducted a global sensitivity analysis to identify the most important parameters for simulating water temperature, dissolved oxygen, NH_4^+, NO_3^-, DRP, and DOC following Morris (1991). Second, we calibrated the identified sensitive parameters (Supplementary Text 3) using the covariance matrix adaptation evolution strategy for automated numerical optimization to minimize root mean square error (RMSE) between observations and model output (Hansen 2016) for all sampling depths in the water column.

We calculated multiple goodness-of-fit metrics to assess the model's performance during the calibration period, validation period, and total simulation period, including RMSE, the coefficient of determination (R^2), percent bias, and normalized mean absolute error (NMAE) (e.g., Kara et al. 2012, Ward et al. 2020, Ladwig et al. 2021). We calculated these goodness-of-fit metrics following the most common approaches used in 328 recent freshwater modeling studies (reviewed by Soares and Calijuri 2021; described in Supplementary Text 3).

Model scenarios

Following model calibration, we examined the effects of two different oxygen scenarios on the calibrated GLM-AED model: one in which the model was forced with a high level of

oxygenation to keep the hypolimnion oxic throughout summer thermal stratification (May 15-Oct 15) during 2013-2019 and one in which zero oxygen was added to the hypolimnion, so hypolimnetic anoxia quickly set up after the onset of thermal stratification each summer. All other driver data (meteorology, stream inflows, outflow) were held constant.

Statistical analysis

We used several approaches to answer the two research questions. For Q1, we first compared observed data from the oxygenated vs. non-oxygenated periods of our field manipulation to determine if oxygenation had an effect on empirical total and dissolved C, N, and P concentrations. We pooled all hypolimnetic C, N, and P samples from the two summers with the least oxygenation (July 15 - October 1 in 2018, 2019) when the HOx was deactivated and compared them with concentrations measured during the two summers with the most continuous oxygenation (July 15 - October 1 in 2016, 2017) when the HOx was activated. We also used the FCR field data to validate the model's ability to simulate the field manipulation. Second, because our goal was to compare completely oxic vs. completely anoxic summers and every summer had at least some oxygenation during the seven-year field manipulation, we focused our subsequent analyses on the anoxic vs. oxic model scenario output, which provided complementary data to the non-oxygenated vs. oxygenated empirical data. Focusing on the model output for this analysis also enabled us to overcome the limitations of comparing years with different numbers of sampling observations, as the model calculated daily C, N, and P concentrations and rates.

We compared hypolimnetic C, N, and P concentrations and rates between the oxic and anoxic model scenarios during 15 July - 1 October among years, the interval within the summer

thermally stratified period when the reservoir consistently exhibited hypolimnetic anoxia in non-oxygenated conditions. We calculated the median hypolimnetic dissolved and total concentrations of C, N, and P during this period for each of the seven years (2013-2019), and compared the median summer anoxic and oxic concentrations and their ratios using paired t-tests, as there was no temporal autocorrelation among median summer values. We also examined summer rates of all processes controlling increases and decreases in hypolimnetic C, N, and P to determine their relative importance and sensitivity to oxygen.

To examine how the uncertainty of our model outputs was affected by the model parameterization, we conducted an additional sensitivity analysis in which we doubled and halved the calibrated values of highly sensitive parameters for DOC, NH_4^+, NO_3^-, and DRP using a one-step-at-a-time (OAT) approach (following Brett et al. 2016). We then re-calculated the summer hypolimnetic concentrations of DOC, NH_4^+, NO_3^-, and DRP in the anoxic and oxic model scenarios for each variable and compared anoxic and oxic concentrations with paired t-tests, as described above.

For Q2, we estimated C, N, and P downstream export as a percent of inputs into the reservoir each summer (Powers et al. 2015, Farrell et al. 2020). Downstream export was calculated as:

$$Flux = 100\% \times ((\Sigma Outputs - \Sigma Inputs)/\Sigma Inputs) \quad \text{(eqn. 1)}$$

where Outputs and Inputs represent the daily mass of C, N, or P leaving and entering the reservoir, respectively, during 15 July - 1 October each year. Fluxes were calculated for both dissolved and total fractions of C, N, and P. Inputs were calculated by multiplying the individual stream daily inflow concentrations with their daily inflow volumes and then summing across the two streams. Outputs were calculated by multiplying the outflow water volume (leaving the

359 reservoir and going downstream) by hypolimnetic concentrations. A water budget calculated for
360 the reservoir in 2014-2015 (Munger et al. 2019) supplemented by monitoring data in this study
361 indicates that the two inflow streams represented approximately 97% of the reservoir's water
362 inputs (Supplementary Text 1), motivating our focus on those inputs.
363 Inputs and Outputs were summed across the 15 July - 1 October period to calculate C, N,
364 and P fluxes. Flux values of 0 indicated that the reservoir inputs balanced outputs; flux values <0
365 indicated that the reservoir was a net sink of C, N, or P; and flux values >0 indicated that the
366 reservoir was a net source of C, N, or P downstream. We compared summer retention (i.e., flux
367 values) in the anoxic and oxic scenarios with paired t-tests.
368 To ease comparison among C, N, and P concentrations and ratios, all analyses were
369 conducted in molar units. All analyses were conducted in R v.3.6.3 (R Core Team 2020).
370
371 **Results**
372 Our integrated whole-ecosystem REDOX field manipulation and modeling demonstrates
373 that hypolimnetic anoxia significantly alters water column C, N, and P concentrations and
374 stoichiometry. Importantly, our study also shows that prolonged hypolimnetic anoxia in the
375 summer decreases the ability of a reservoir to retain C, N, and P, substantially increasing its
376 downstream export.
377
378 *Observational data from whole-ecosystem manipulations*
379 Injection of oxygen into the bottom waters of Falling Creek Reservoir (FCR) over seven
380 years increased the reservoir's observed hypolimnetic oxygen, resulting in substantial changes in
381 total and dissolved C, N, and P concentrations (Fig. 3, Fig. S2). Due to the nature of our

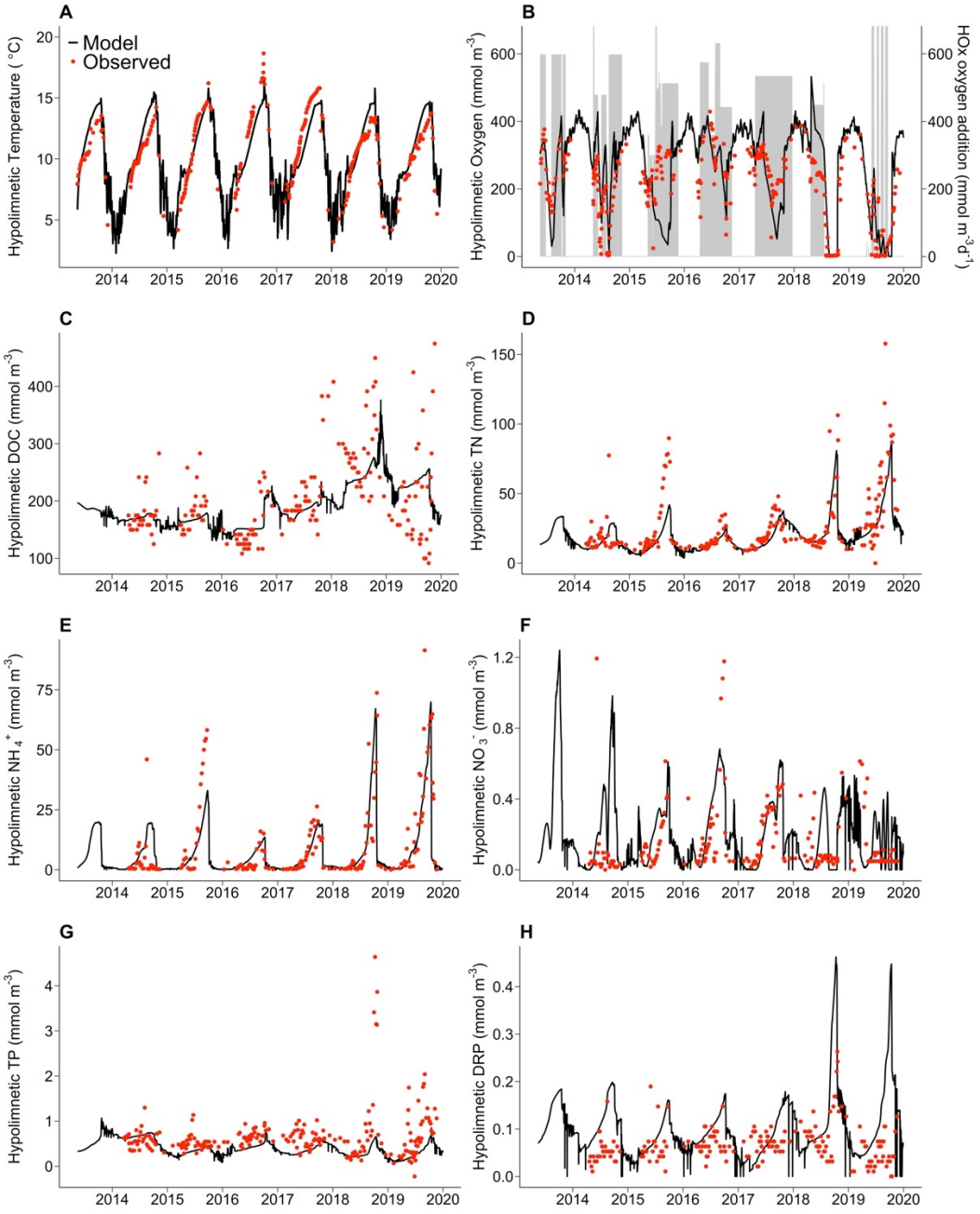


Fig. 3. The model was able to generally recreate observed reservoir dynamics. Modeled (black line) and observed (red points) hypolimnetic (9 m) water temperature (A), dissolved oxygen (B), dissolved organic carbon (DOC; C), total nitrogen (TN; D), ammonium (NH_4^+; E), nitrate (NO_3^-; F), total phosphorus (TP; G), and dissolved reactive phosphorus (DRP; H) in Falling Creek Reservoir (goodness-of-fit metrics presented for the full water column in Table 1). The grey shaded areas in panel B represent the periods and addition rates of oxygen injection into the hypolimnion from the hypolimnetic oxygenation system (HOx) during the seven-year field manipulation. Note varying y-axes among panels, and that many of the NO_3^- and DRP observations were below the limit of quantitation in laboratory analysis (0.11 and 0.08 mmol m^{-3}, respectively).

oxygenation manipulation, some years experienced low levels of oxygenation (i.e.., the HOx was off for prolonged periods throughout the summer), while others experienced high levels of oxygenation during the stratified period (Fig. 3B). Oxygenation resulted in substantially higher hypolimnetic oxygen concentrations without altering water temperature and thermal stratification in the reservoir (Fig. 3A,B). In 2019, oxygenation did not increase hypolimnetic oxygen concentrations to the same extent as preceding summers, likely because the HOx was only operated for intermittent 2-week periods (vs. 4-week or longer periods in all other years).

The median observed hypolimnetic DOC, NH_4^+, and DRP concentrations were 2.0, 6.9, and 1.3× higher in the summers with the least oxygenation (2018, 2019) than in the summers with the highest oxygenation (2016, 2017; Fig. 3C,E,H; Fig. S2), respectively. Following the patterns exhibited by the dissolved fractions, median observed hypolimnetic TN and TP concentrations were both 2.4× higher in the high vs. low oxygenation summers (Fig. 3D,G; Fig. S2). Conversely, median observed hypolimnetic NO_3^- concentrations were 5× lower in summers with low oxygenation than summers with high oxygenation (Fig. 3F, Fig. S2). Because our goal was to compare completely oxic vs. completely anoxic summer conditions and every summer had at least some oxygenation during the seven-year field manipulation at varying levels of oxygen injection, subsequent analyses focused on the anoxic vs. oxic model scenario output, described below.

Model performance

The field manipulation data were used to calibrate the ecosystem model, which generally reproduced observed water temperature, oxygen, dissolved and total C, N, and P concentrations, and stoichiometry (Table 1, Fig. 3, Supplementary Text 4). Similar to field observations, the

Table 1. Goodness-of-fit (GOF) metrics for comparing observations and modeled GLM-AED output for Falling Creek Reservoir, VA, USA. GOF metrics include root mean square error (RMSE), percent bias (PBIAS%), coefficient of determination (R^2), and normalized mean absolute error (NMAE); *n* is the number of observed measurements. Each GOF metric was calculated comparing model outputs and observational data for the water column for the Full simulation (2013-2019); Calibration (2013-2018); and Validation (2018-2019); see Supplementary Text 3 for details. Evaluated parameters include temperature (Temp, ºC), summer thermocline depth (TD, m), dissolved oxygen (mmol m^{-3}), dissolved organic carbon (DOC, mmol m^{-3}), total nitrogen (TN, mmol m^{-3}), ammonium (NH_4^+, mmol m^{-3}), nitrate (NO_3^-, mmol m^{-3}), total phosphorus (TP, mmol m^{-3}), and dissolved reactive phosphorus (DRP, mmol m^{-3}).

Time period	**Parameter**	**Temp**	**TD**	**Oxygen**	**DOC**	**TN**	**NH_4^+**	**NO_3^-**	**TP**	**DRP**
Full simulation	*n*	3639	324	3726	1277	1518	1277	1485	1724	1271
	RMSE	1.41	0.8	49.1	63.2	5.9	2.81	0.16	0.29	0.05
	PBIAS%	4.4	0.1	6.4	-14.5	-6.2	22.5	9.8	-31.7	26.5
	R^2	0.95	0.50	0.72	0.30	0.71	0.77	0.27	0.25	0.10
	NMAE	0.09	0.18	0.15	0.2	0.22	0.46	0.45	0.38	0.37
Calibration	*n*	3164	284	3251	1018	1250	1018	1014	1456	1012
	RMSE	1.39	0.9	45.9	61.5	5.0	2.81	0.16	0.25	0.04
	PBIAS%	3.3	-1.4	5.8	-16.8	-0.8	40.7	11.7	-29.6	21.1
	R^2	0.95	0.46	0.71	0.46	0.58	0.63	0.33	0.15	0.24
	NMAE	0.09	0.19	0.14	0.19	0.19	0.61	0.44	0.33	0.28
Validation	*n*	475	40	475	259	268	259	471	268	259
	RMSE	1.48	0.4	62.8	70.4	9.23	2.79	0.14	0.33	0.08
	PBIAS%	11.2	10.5	9.9	-4.2	-19.8	-7.2	3.3	-42.9	51.6
	R^2	0.97	0.95	0.74	0.52	0.94	0.94	0.12	0.85	0.03
	NMAE	0.12	0.1	0.2	0.24	0.36	0.22	0.49	0.38	0.57

simulation of oxygen injection in the model did not substantively alter modeled water temperature or thermocline depth (Table 1, Fig. 3A).

Model performance of most state variables in our study (Table 1) exceeded the median goodness-of-fit metrics for recent freshwater modeling studies reported by Soares and Calijuri (2021). For example, our water temperature R^2 was 0.95 for the full seven-year simulation period (vs. 0.94 in Soares and Calijuri 2021), our dissolved oxygen R^2 was 0.72 (vs. 0.61), our TN was 0.71 (vs. 0.61), and our NH_4^+ R^2 was 0.77 (vs. 0.35). No DOC data were reported by Soares and Calijuri (2021), but our R^2 values ranged from 0.30-0.52 for the full simulation, calibration, and validation periods. Phosphorus had less good fit, but was still reasonable: our TP R^2 was 0.25 for the full seven-year simulation period (slightly lower than the 0.30 reported by Soares and Calijuri 2021), but the validation period's R^2 for TP was much higher, at 0.85. Similarly, our DRP R^2 was 0.10 for the full seven-year period (vs. 0.32) but the six-year calibration period had better performance, at an R^2 of 0.24. NO_3^- had an R^2 of 0.27 for the full seven-year modeling period and R^2 of 0.33 for the six-year calibration period, which was lower than the 0.61 reported by Soares and Calijuri (2021).

Much of the variation in DRP and NO_3^- observations was within the analytical limits of quantitation. Consequently, while the model generally captured seasonal patterns of DRP and NO_3^-, it was simply not possible to reproduce short-term fluctuations in observations below method detection limits. NO_3^- concentrations in particular were extremely low in both field observations and model output (Fig. 3F). Throughout the study, NO_3^- was a very minor fraction of TN, representing a median of 0.5% (±0.9%, 1 S.D.) of TN at all depths in the field data and a median of 0.8% (±0.9%) of TN in the baseline simulation. Subsequently, a lower fit of NO_3^- did not affect the model performance of TN, as evident by its goodness-of-fit metrics (Table 1).

How does hypolimnetic oxygen availability affect total and dissolved C, N, and P concentrations and stoichiometry?

Model scenarios show that hypolimnetic anoxia significantly affected all three focal elemental cycles, but that N was the most sensitive (Figs. 4,5; see Table S3 for statistics). Summer TN molar concentrations in the reservoir were on average 3.0× higher in anoxic than oxic conditions, relative to a 1.1× increase of TOC and 1.6× increase of TP (Fig. 5A,C,G). The dissolved fractions accounted for most of the changes in total C, N, and P: following the field data, modeled summer hypolimnetic DOC, NH_4^+, and DRP concentrations in FCR were on average 1.1, 5.8, and 3.1× higher, respectively, during anoxic conditions than in oxic conditions (Fig. 5B,E,H). Conversely, hypolimnetic NO_3^- was much lower in anoxic conditions (usually at or just above 0 mmol m^{-3}) than oxic conditions, but DIN exhibited an overall increase because of the dominance of NH_4^+ over NO_3^- in the dissolved inorganic N pool (Fig. 5D,F). The statistical significance and overall magnitude of differences in concentrations between the anoxic and oxic scenarios were consistent even when focal parameters governing DOC, NH_4^+, NO_3^-, and DRP were doubled or halved in the parameter sensitivity analysis (Supplementary Text 3, Fig. S1).

The elemental stoichiometry in FCR exhibited rapid and large ecosystem-scale changes after the onset of anoxia each summer. While total and dissolved fractions of C, N, and P (except NO_3^-) significantly increased with anoxia (Figs. 4,5), the different fractions had varying sensitivities to changing oxygen, resulting in significant changes in C, N, and P ratios (Fig. 6, Supplementary Text 5). Hypolimnetic TN:TP and DIN:DRP were significantly higher (both by 1.9×, on average) in anoxic conditions than oxic conditions (Fig. 6G,H). Because modeled hypolimnetic NO_3^- concentrations were at or near zero during anoxic conditions (Fig. 5F), DOC:NO_3^- could not be consistently calculated (Fig. 6E). In contrast, TOC:TN, TOC:TP,

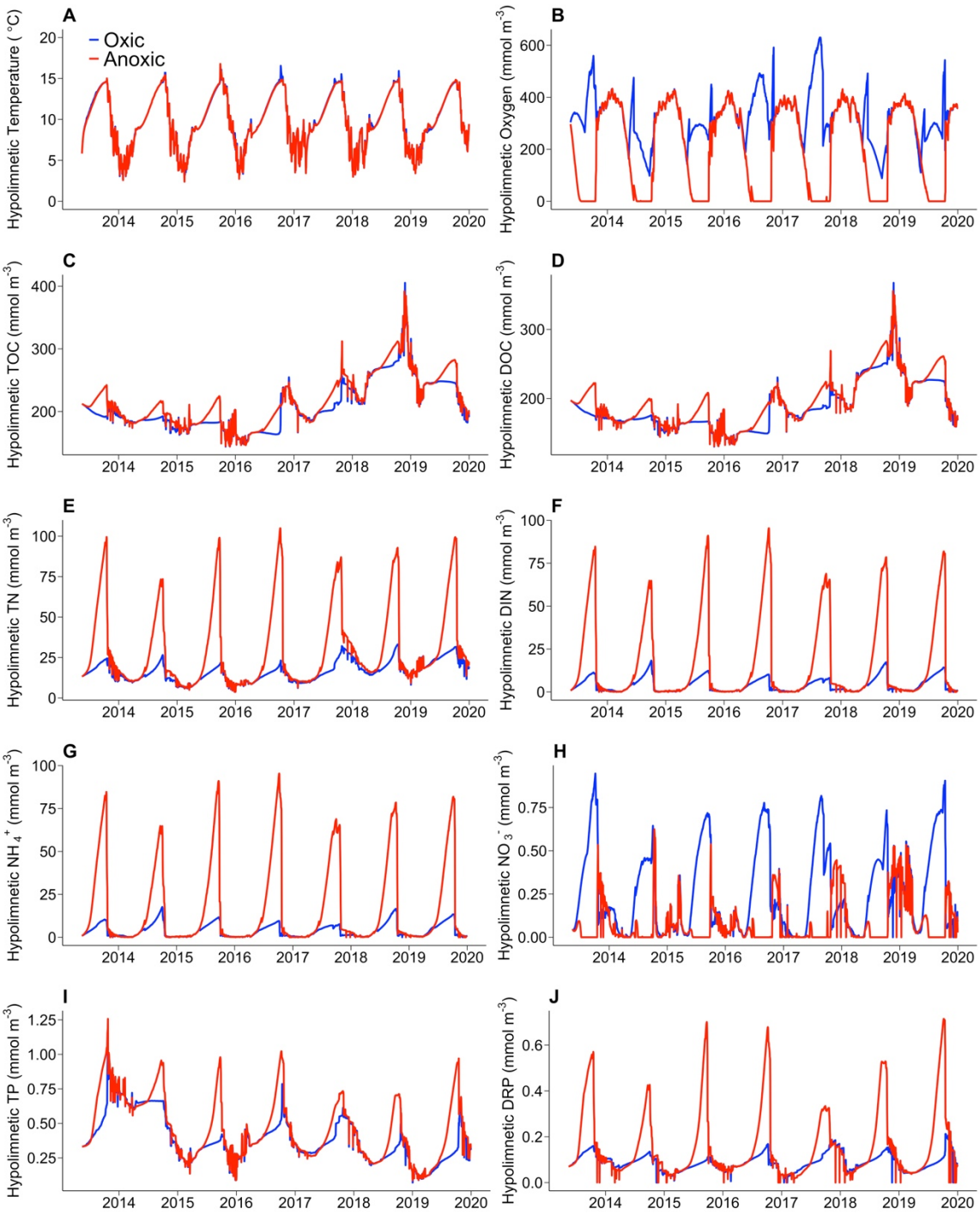


Fig. 4. Time series of oxic (blue) and anoxic (red) model scenarios in Falling Creek Reservoir. Model results are shown for hypolimnetic (9 m) water temperature (A), dissolved oxygen (B), total organic carbon (TOC; C), dissolved organic carbon (DOC; D), total nitrogen (TN; E), dissolved inorganic nitrogen (DIN, the sum of ammonium and nitrate; F), ammonium (NH_4^+; G), nitrate (NO_3^-; H), total phosphorus (TP; I), and dissolved reactive phosphorus (DRP; J). In the oxic scenario, oxygen was injected into the hypolimnion throughout the thermally stratified period each summer. In the anoxic scenario, no oxygen was added to the hypolimnion, resulting in prolonged hypolimnetic anoxia each summer. Note varying y-axes among panels.

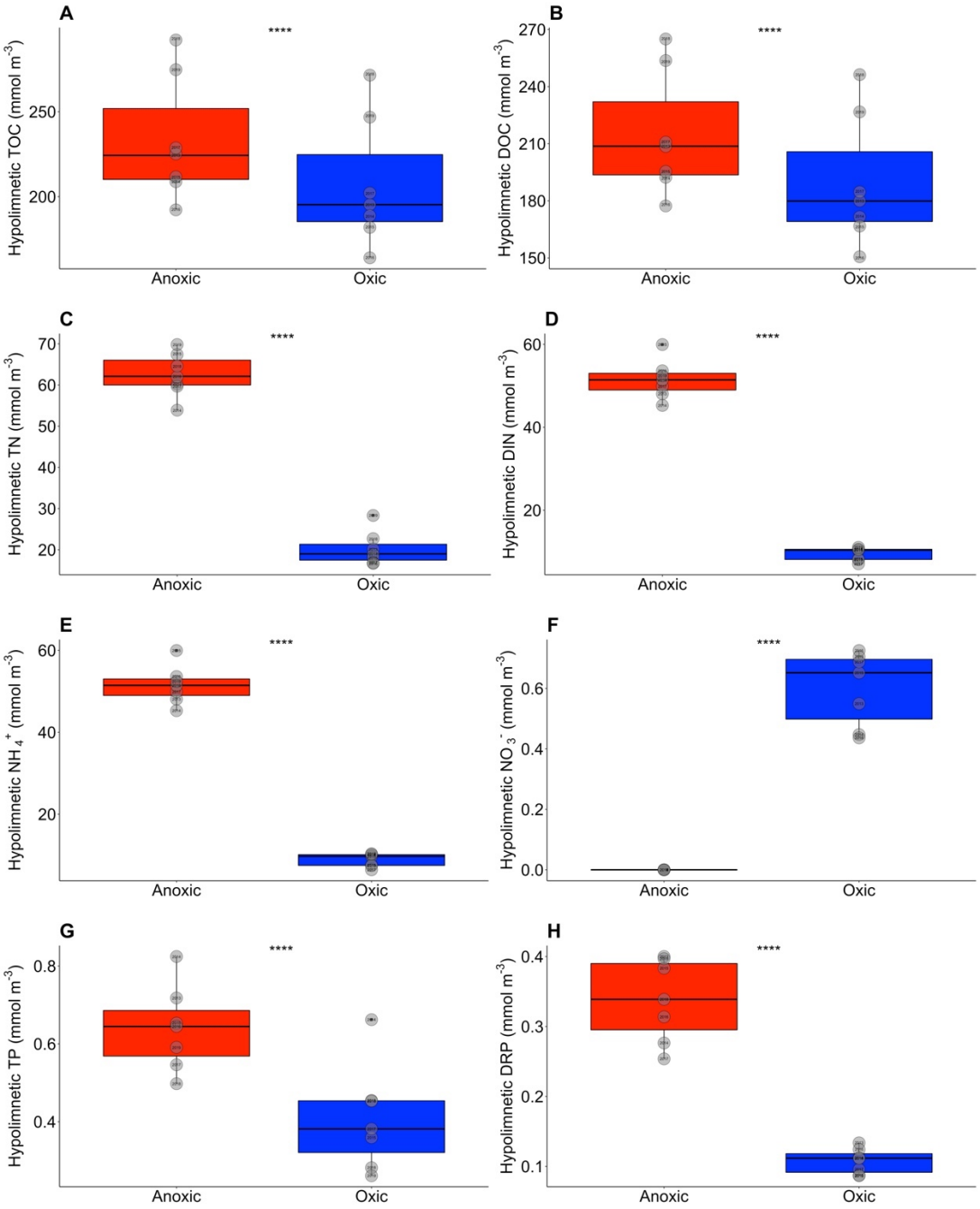


Fig. 5. Anoxia significantly altered bottom-water concentrations of carbon, nitrogen, and phosphorus. Median hypolimnetic (9 m) total organic carbon (TOC; A), dissolved organic carbon (DOC; B), total nitrogen (TN; C), dissolved inorganic nitrogen (DIN; D), ammonium (NH_4^+; E), nitrate (NO_3^-; F), total phosphorus (TP; G), and dissolved reactive phosphorus (DRP; H) concentrations between anoxic (red) and oxic (blue) scenarios during Falling Creek Reservoir's stratified period (July 15 - October 1) for all years of this study. The grey points are the median values from each of the seven years. The **** denotes that the difference between the median summer anoxic and oxic scenario concentrations was highly statistically significant (all paired t-tests $p \leq 0.0001$, see Supplementary Text 5 and Table S3 for statistics). Note varying y-axes among panels.

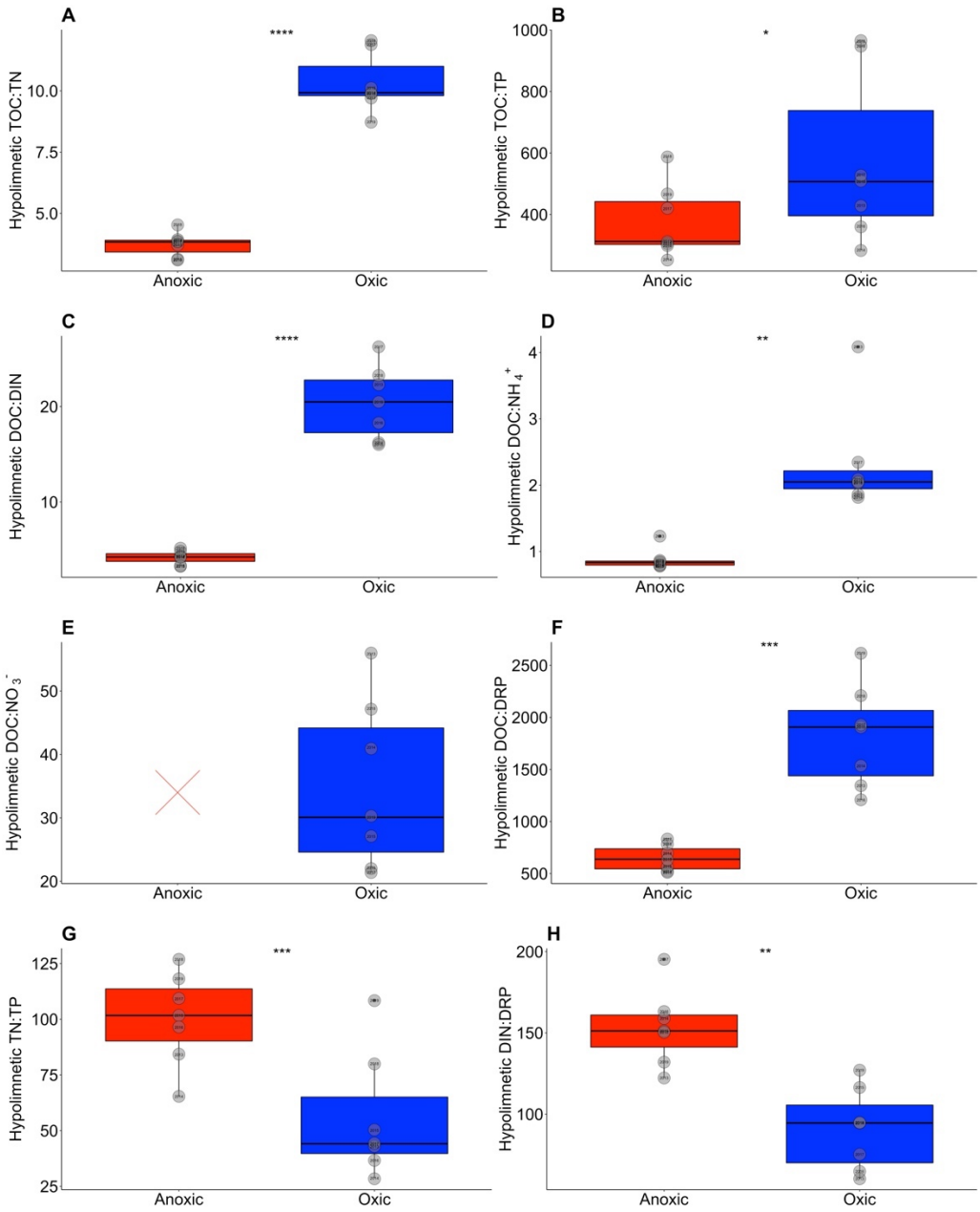


Fig. 6. Anoxia significantly affected water column stoichiometry. Total and dissolved molar ratios of hypolimnetic (9 m) total organic carbon:total nitrogen (TOC:TN; A), TOC:total phosphorus (TOC:TP; B), dissolved organic carbon:dissolved inorganic nitrogen (DOC:DIN; C), DOC:ammonium ($DOC:NH_4^+$; D), DOC:nitrate ($DOC:NO_3^-$; E), DOC:dissolved reactive phosphorus (DOC:DRP; F), TN:TP (G), and DIN:DRP (H) between anoxic (red) and oxic (blue) scenarios during Falling Creek Reservoir's stratified period (July 15 - October 1) for all years of this study. The grey points are the median values from each of the seven years. Because NO_3^- concentrations in the anoxic scenario were functionally zero, the ratio of $DOC:NO_3^-$ could not be calculated (hence the X in panel E). The asterisks denote the p-values from paired t-tests comparing the median summer ratios between anoxic and oxic scenarios: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ (see Supplementary Text 5 and Table S4 for statistics). Note varying y-axes among panels.

508 DOC:DIN, DOC:NH_4^+, and DOC:DRP were significantly higher (on average, by 2.7×, 0.7×,
509 4.7×, 5.0×, and 2.7×, respectively) in oxic conditions than anoxic conditions (Fig. 6A,B,C,D,F,
510 Supplementary Text 5).

511 The most important processes driving the biogeochemical responses to anoxia were much
512 higher fluxes of NH_4^+, DRP, and DOC into the hypolimnion from the sediments in anoxic
513 periods relative to oxic periods (Fig. 7). During anoxic summer conditions, the median release
514 rates of NH_4^+ and DRP from the sediments into the water column were 3.6× and 2.2× higher,
515 respectively, than in oxic conditions (Fig. 7). During oxic conditions, the sediment release rate of
516 NH_4^+ into the hypolimnion was 34× greater than the consumption of NH_4^+ by nitrification (Fig.
517 7, Fig. S5), thereby explaining the hypolimnetic accumulation of NH_4^+ that occurred during oxic
518 conditions (Fig. 4G). Although median labile dissolved organic N (DON) and P (DOP)
519 mineralization rates were both 4.0× times higher in oxic than anoxic conditions, their
520 contribution to hypolimnetic N and P budgets was much smaller than NH_4^+ and DRP sediment
521 fluxes. All biogeochemical rates involving the cycling of NO_3^- were much lower than for NH_4^+
522 overall, likely because of the much lower concentrations of NO_3^- within the DIN pool. For DOC,
523 the median sediment fluxes increasing DOC in the hypolimnion were 1.9× times higher in anoxic
524 than oxic conditions. Although labile DOC mineralization rates were 2.0× higher in oxic than
525 anoxic conditions, sediment flux rates were 19× higher than mineralization rates, resulting in
526 much greater hypolimnetic accumulation of DOC in anoxic relative to oxic periods (Fig. 7).

527 The time scales at which C, N, and P concentrations responded to shifts in hypolimnetic
528 oxygen availability differed as a result of multiple interacting biogeochemical processes (Figs.
529 4,7). For example, the onset of anoxia each summer triggered rapid decreases in NO_3^- (Fig. 4H),
530 due to sediment denitrification oxidizing NO_3^- to N_2 (Fig. 7). Similarly, the rapid increases in

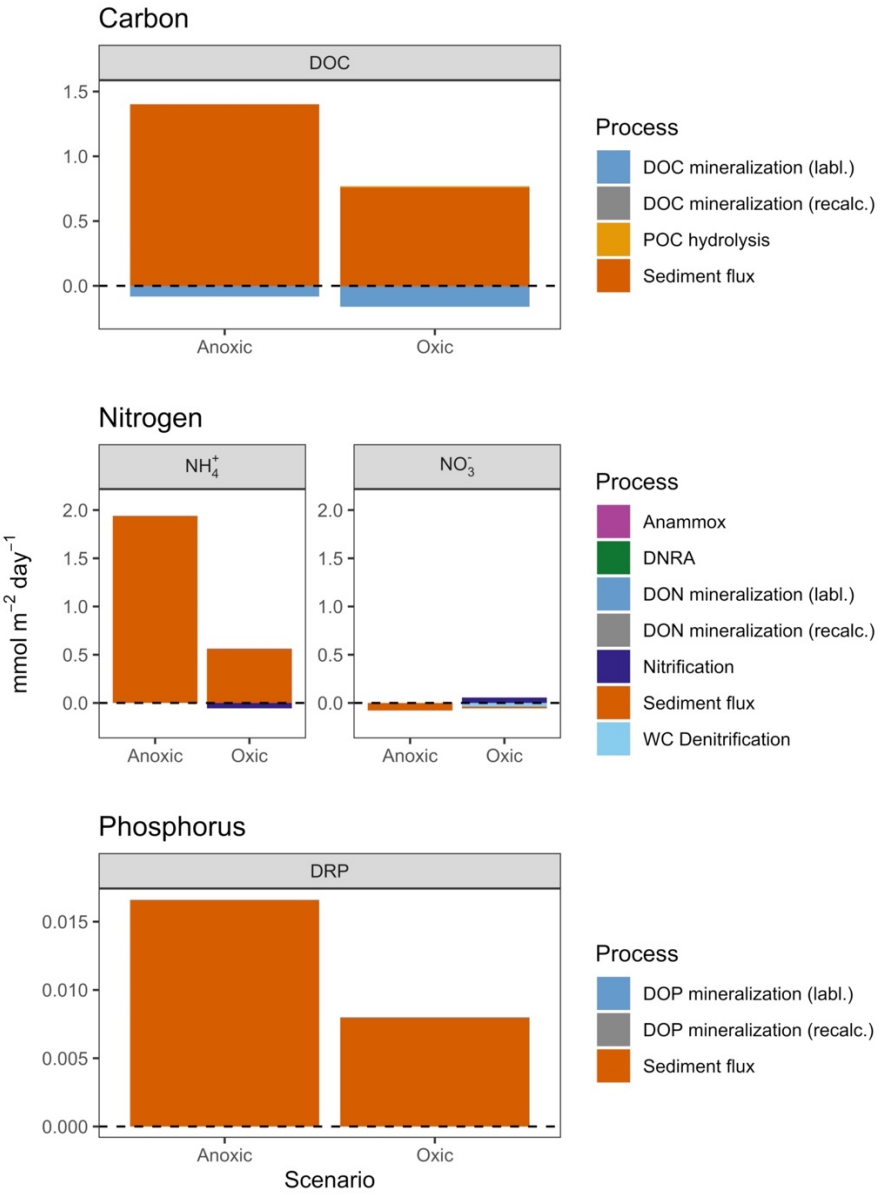


Fig. 7. Sediment fluxes dominated the biogeochemical cycling of dissolved carbon, nitrogen, and phosphorus and their responses to anoxia. Comparison of the dominant biogeochemical processes altering dissolved pools of carbon (dissolved organic carbon, DOC; top), nitrogen (ammonium, NH_4^+, and nitrate, NO_3^-; middle), and phosphorus (dissolved reactive phosphorus, DRP; bottom) in the hypolimnion of Falling Creek Reservoir under anoxic vs. oxic model scenarios. Rates shown represent the median contribution of each process to hypolimnetic concentrations of DOC, NH_4^+, NO_3^-, and DRP during Falling Creek Reservoir's summer stratified period (July 15 - October 1) for all years of this study. Positive rates indicate that the process increased hypolimnetic concentrations; negative rates indicate that the process decreased hypolimnetic concentrations. Mineralization is shown separately for both labile (labl.) and recalcitrant (recalc.) dissolved organic pools, and denitrification is partitioned for the water column (WC Denitrification) and sediment in the NO_3^- panel (Sediment flux). Note the varying y-axes among panels and that some rates are so small that they are not visible in the figure; Fig. S5 shows a modified version of this figure with the sediment fluxes excluded.

hypolimnetic NH_4^+ and DOC concentrations after the onset of anoxia (Fig. 4D,G) were attributable to the high rates of NH_4^+ and DOC sediment release (Fig. 7). In comparison, hypolimnetic DRP accumulation in response to anoxia occurred more slowly (Fig. 4J). This difference in time scale reflects the lower fitted value of the half-saturation constant of modeled DRP sediment fluxes (6.91 mmol m^{-3}) relative to the half-saturation constants of NH_4^+ sediment fluxes (41.25 mmol m^{-3}) and DOC sediment fluxes (93.13 mmol m^{-3}; Carey et al. 2021c). Consequently, oxygen concentrations in the hypolimnion had to decrease to near zero before anoxia stimulated an increase in DRP sediment fluxes, following Michaelis-Menten dynamics.

How does hypolimnetic anoxia affect reservoir downstream export of C, N, and P?

Overall, anoxia significantly increased downstream export of C, N, and P from FCR (Fig. 8). During the summer months, if the reservoir's hypolimnion was oxic, FCR served as a net sink for inflowing TOC and TP, decreasing the downstream export of those fractions (Fig. 8A,G). The reservoir served as a particularly important TP sink during summer oxic conditions, with 22% of inflowing TP buried in sediments, resulting in 78% of the inflowing TP exported downstream (Fig. 9). In comparison, while the reservoir was also a TOC sink in oxic conditions, only 8% of inflowing TOC was buried in sediments or removed via emission to the atmosphere, resulting in 92% export downstream (Fig. 9). However, in most anoxic summers, the reservoir became a net source of TOC and TP downstream, meaning that inflowing TOC and TP - as well as TOC and TP that were previously retained in the reservoir sediments - were released and transported out of the reservoir (Fig. 8A,G). Consequently, on average, the reservoir exported 105% of inflowing TOC and 123% of inflowing TP in anoxic conditions (Fig. 9). DOC and DRP fluxes largely mirrored the patterns of the total fractions, though DRP had much greater flux

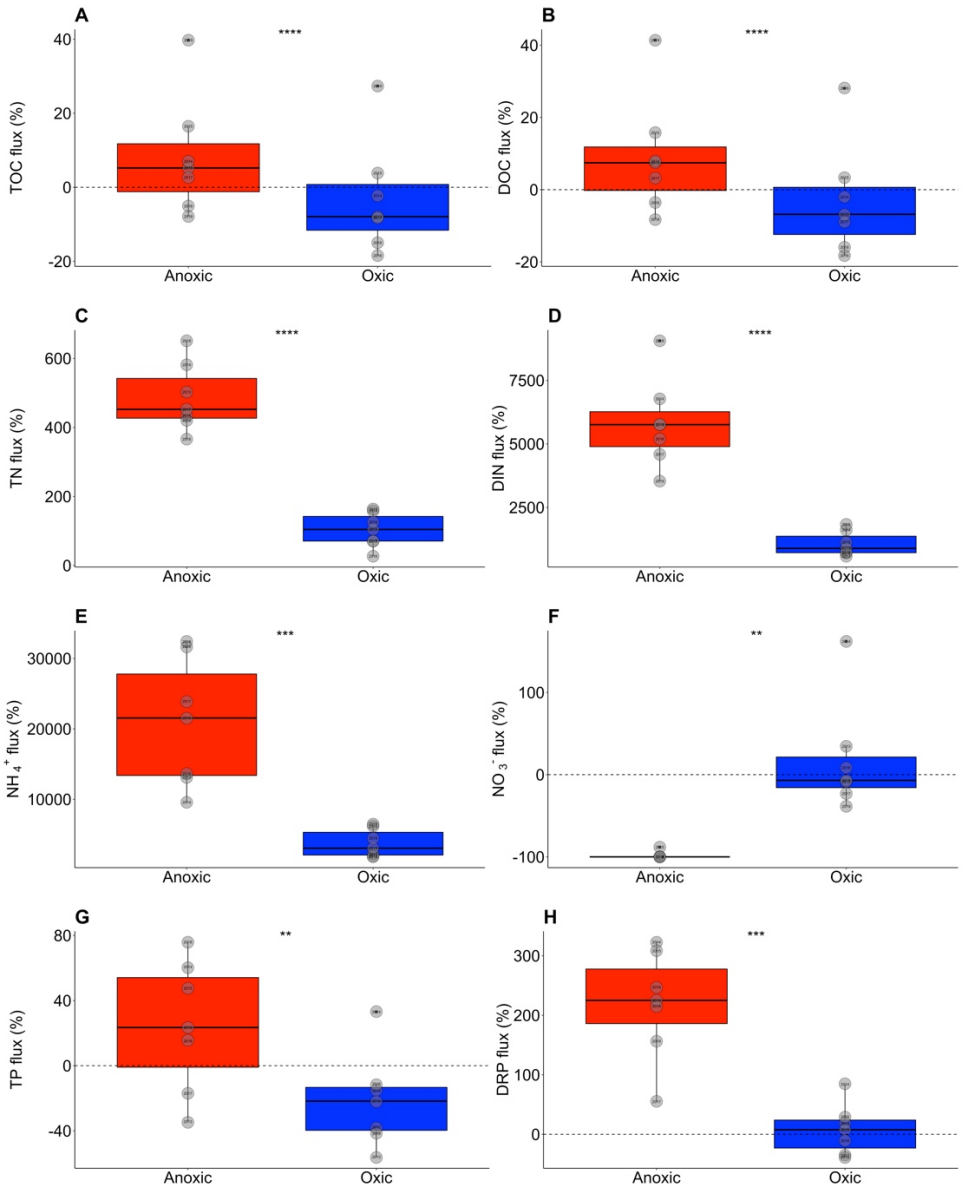
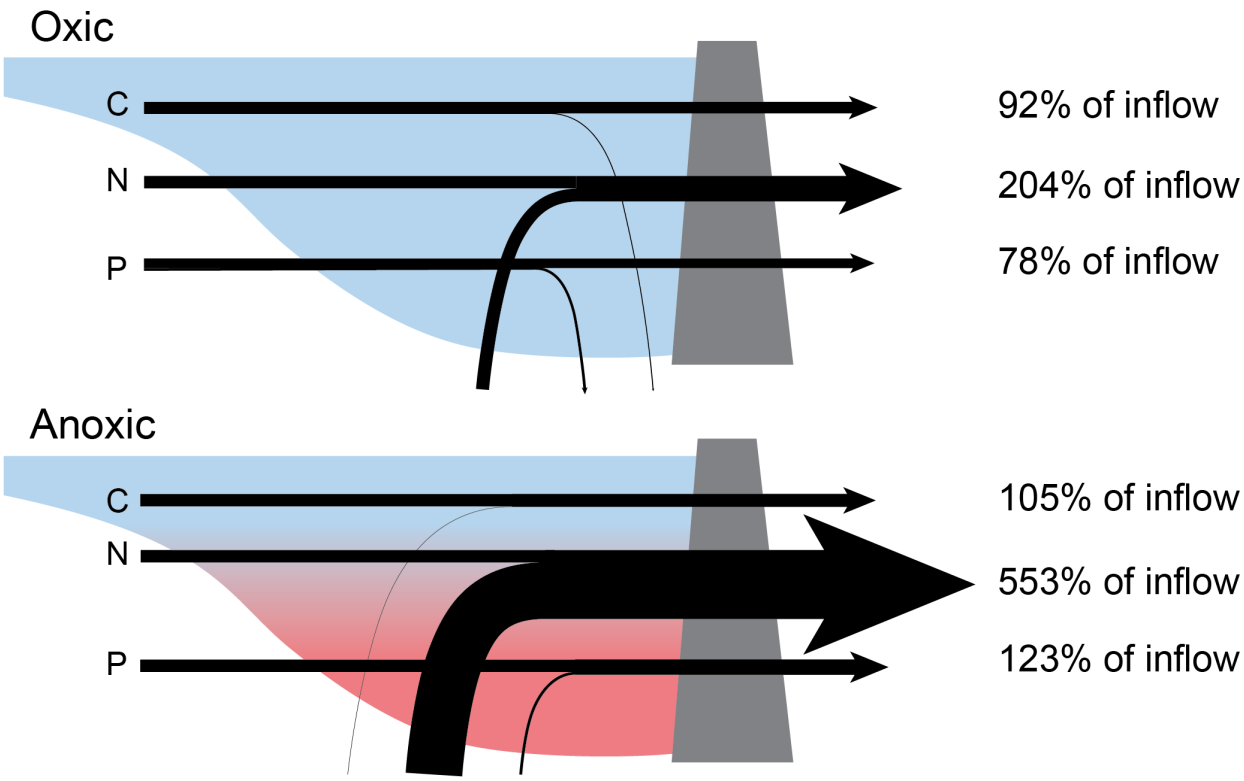


Fig. 8. Anoxia significantly increased the downstream export of total and dissolved fractions of organic carbon, nitrogen, and phosphorus. Percent downstream export (% flux) of total organic carbon (TOC; A), dissolved organic carbon (DOC; B), total nitrogen (TN; C), dissolved inorganic nitrogen (DIN; D), ammonium (NH_4^+; E), nitrate (NO_3^-; F), total phosphorus (TP; G), and dissolved reactive phosphorus (DRP; H) inputs into Falling Creek Reservoir for anoxic (red) and oxic (blue) model scenarios during the stratified period (July 15 - October 1) for all years of this study. Flux values of 0 (denoted by dashed horizontal lines) indicated that the reservoir inputs balanced exports; flux values <0 indicated that the reservoir was a net sink of C, N, or P; and flux values >0 indicated that the reservoir was a net source of C, N, or P downstream. The grey points are the median values from each of the seven years. The asterisks denote the p-values from paired t-tests comparing the median summer retention in anoxic and oxic scenarios: **** $p < 0.0001$, *** $p < 0.001$, and ** $p < 0.01$ (see Supplementary Text 5 and Table S5 for statistics). Note varying y-axes among panels.



583

584 **Fig. 9. Median summer downstream export of total organic carbon (C), total nitrogen (N),**
585 **and total phosphorus (P) inputs under oxic (top) and anoxic (bottom) conditions.** The "% of
586 inflow" value represents the percent of inflowing C, N, and P into the reservoir that is exported
587 downstream. A value of 100% indicates that reservoir inputs balanced exports; values <100%
588 indicated that the reservoir was a net sink of C, N, or P; and values >100% indicated that the
589 reservoir was a net source of C, N, or P downstream. Arrow widths are scaled to be proportional
590 to the median downstream export of each element.
591

592 downstream overall in anoxic summers than TP (Fig. 8B,H).

593 The reservoir was a net source of TN, DIN, and NH_4^+ downstream even in oxic
594 conditions, but this export significantly increased when the hypolimnion became anoxic in
595 summer (Fig. 8C,D,E). The only fraction of N that did not exhibit higher downstream export
596 during anoxic conditions was NO_3^- (Fig. 8F). During anoxic conditions, ~100% of inflowing
597 NO_3^- was removed due to sediment denitrification, whereas in oxic conditions, some of this NO_3^-
598 was exported downstream along with additional NO_3^- that originated from nitrified NH_4^+ in the
599 reservoir (Fig. 7). Overall, the reservoir exported 204% of inflowing TN in summer oxic
600 conditions and 553% in anoxic conditions (Fig. 9).

601 Aggregated across the seven years, FCR served as a small net sink of POC, PON, and
602 POP in its sediments in both oxic and anoxic model scenarios. All particulate organic fractions
603 exhibited significantly higher annual burial rates in anoxic scenarios than oxic scenarios, though
604 the differences were small, especially for POP (Supplementary Text 5, Table S6).

605

606 **Discussion**

607 Our study provides one of the first comprehensive analyses on the effects of oxygen on
608 multiple fractions of C, N, and P at the whole-ecosystem scale in a freshwater ecosystem. Our
609 unprecedented 7-year field manipulation coupled with ecosystem model simulations reveals that
610 anoxia may decrease the ability of reservoirs to serve as sinks of C, N, and P. Moreover, both the
611 empirical data and model output demonstrate that anoxia resulted in significantly higher summer
612 concentrations of hypolimnetic NH_4^+, DRP, and DOC and altered dissolved and total
613 stoichiometry by factors of 2-5×. Our integrated field manipulation and modeling study provides
614 important insight on the biogeochemical cycling of these three elements, which are already

615 changing in many freshwaters globally due to human activities (Powers et al. 2015, Maranger et
616 al. 2018), and likely will change substantially more in the future as the prevalence and duration
617 of anoxia in lakes and reservoirs increase (Tranvik et al. 2009, North et al. 2014, Jenny et al.
618 2016a, Jane et al. 2021). Below, we first examine the effects of anoxia on each elemental cycle
619 separately, then their combined stoichiometry, and ultimately whole-ecosystem biogeochemical
620 processing and fate.
621
622 *Hypolimnetic carbon and nutrient chemistry*
623 This study provides an answer to the critical question of how increased anoxia will affect
624 OC cycling at the whole-ecosystem scale (Sobek et al. 2009, Brothers et al. 2014, Peter et al.
625 2016, Mendonça et al. 2017, Carey et al. 2018). The shift in reservoir OC cycling in response to
626 anoxia is the consequence of changes in three linked processes: POC burial, DOC
627 mineralization, and DOC release from the sediments. Under anoxic conditions, POC burial
628 increased slightly, DOC mineralization rates were low, and DOC release from the sediments to
629 the water column was 2× higher than in oxic conditions (Fig. 7, Fig. S5). Under oxic conditions,
630 DOC mineralization rates, while higher than in anoxic conditions (Fig. 7, Fig. S5), were still an
631 order of magnitude lower than the rate of hydrologic flushing. The net outcome of these three
632 processes was a substantial difference in OC retention in the reservoir during oxic vs. anoxic
633 conditions. Under oxic conditions, the reservoir served as a net sink of DOC and TOC, with up
634 to 18% of inflowing DOC and TOC retained in a summer (Fig. 8A,B). Under anoxic conditions,
635 the decrease in net retention of inflowing DOC and TOC more than offset the slight increase in
636 POC burial, and nearly all of the inflowing DOC and TOC was exported downstream (as
637 indicated by 0% or positive flux in Fig. 8A,B). In five of the seven years, FCR even became a

net exporter of TOC and DOC in the anoxic scenario (Fig. 8A,B), meaning that both inflowing TOC and DOC and likely legacy TOC and DOC that were previously buried in sediments were released and transported out of the reservoir.

The finding that anoxia simultaneously decreased the reservoir's role as a DOC sink yet increased its role as a POC sink may explain some of the conflicting results that emerged from previous studies that focused on only one OC fraction. First, our study supports past work that observed increasing hypolimnetic DOC concentrations in anoxic conditions, suggesting that anoxia decreases the freshwater OC sink (Brothers et al. 2014, Peter et al. 2016, Mendonça et al. 2017). The increasing hypolimnetic DOC concentrations have been attributed to both reductive dissolution of iron-bound OC complexes in the sediments during anoxia (Skoog and Arias-Esquivel 2009, Peter et al. 2016, Peter et al. 2017) and decreased mineralization rates in anoxic conditions (Bastviken et al. 2004, Sobek et al. 2009). Our calibrated ecosystem model indicates that both processes are important, but that the much higher hypolimnetic DOC concentrations in anoxic conditions in FCR were primarily due to sediment release (Fig. 7). At the same time, our work also supports laboratory microcosm and sediment core studies that observed lower POC mineralization rates in anoxic than in oxic conditions (Bastviken et al. 2004, Sobek et al. 2009). In FCR, mean summer POC hydrolysis rates in the hypolimnion were five orders of magnitude lower in anoxic than oxic conditions (Fig. 7, Fig. S5), enabling slightly greater POC burial in anoxic than oxic conditions. Altogether, our work indicates that using an ecosystem model to simultaneously track both concentrations and rates of the major processes affecting dissolved, particulate, and total pools of OC is needed to understand the full effects of oxygen on OC cycling, as different fractions have different responses to anoxia.

660 Nitrogen was the most sensitive of the three focal elements to anoxia, with an NH_4^+-
661 dominated TN budget that increased dramatically during anoxic conditions. The dominant
662 mechanism driving the NH_4^+ increase in anoxic conditions were the approximately 4× higher
663 rates of ammonification and sediment release than in oxic conditions (Fig. 7). Anammox and
664 nitrification rates were very low in anoxic conditions (Fig. 7, Fig. S5), enabling NH_4^+ to
665 accumulate in the hypolimnion during anoxia. In oxic conditions, nitrification rates were unable
666 to balance sediment fluxes, resulting in much lower but still noticeable increases in summer
667 NH_4^+ concentrations (Fig. 4G). As a result, the reservoir functioned as an NH_4^+ source
668 downstream regardless of hypolimnetic oxygen availability, though anoxia increased
669 downstream fluxes by 5× relative to oxic conditions, on average. The high sediment NH_4^+ fluxes
670 - even in oxic conditions - indicate that FCR has a large sediment NH_4^+ pool, which is likely due
671 to historical agriculture in the catchment (Gerling et al. 2016). Until agricultural abandonment in
672 the 1930s, most of FCR's catchment was farmland (Gerling et al. 2016). Even though the
673 catchment did not experience industrial farming, agriculture can have century-long effects on
674 soil properties, erosion, and ecosystem functioning (Foster et al. 2003, Cusack et al. 2013),
675 resulting in a large pool of NH_4^+ that can be recycled between the hypolimnion and sediments for
676 many years before eventual export (Ahlgren et al. 1994, Gerling et al. 2016).
677 Following expectation, hypolimnetic NO_3^- concentrations were significantly higher in
678 oxic conditions than anoxic conditions. Despite an increase in NO_3^- during oxic conditions, the
679 dominance of NH_4^+ over NO_3^- in the DIN pool (due to high NH_4^+ sediment fluxes even in oxic
680 conditions; Fig. 7) resulted in overall similar patterns for TN and NH_4^+ (Fig. 5C,D,E). We
681 initially anticipated that an increase in NO_3^- in oxic conditions could balance an increase in NH_4^+
682 in anoxic conditions, thereby resulting in similar DIN concentrations regardless of oxygen level,

683 but low nitrification rates prevented increases in NO_3^- from occurring in oxic conditions (Fig. 7,
684 Fig. S5). Long-term water chemistry monitoring of FCR shows much lower summer NO_3^-
685 concentrations over time relative to NH_4^+ (Fig. 3E,F), and thus modeled results follow
686 observations. In the anoxic scenario, denitrification rates at the sediments were higher than in the
687 water column, whereas in the oxic scenario, denitrification rates in the water column were higher
688 than at the sediments (Fig. 7, Fig. S5), as also observed in Swiss lakes with varying oxygen
689 levels (Müller et al. 2021).

690 Altogether, anoxia significantly decreased FCR's role as a NH_4^+ sink and simultaneously
691 increased its role as an NO_3^- sink (Fig. 8E,F), to the extent that ~100% of inflowing NO_3^- was
692 removed via denitrification. A previous study reported an average TN retention rate of 26% (and
693 up to 78%) of inputs for agricultural reservoirs in the U.S. (Powers et al. 2015). It would be
694 expected that FCR, which is located in a forested catchment, would have much higher TN
695 retention than agricultural reservoirs because of its lower external TN loads, however, FCR's
696 high export of NH_4^+ resulted in the reservoir serving as a source of TN downstream regardless of
697 hypolimnetic oxygen availability (Fig. 9). We anticipate that a greater duration and prevalence of
698 hypolimnetic anoxia in lakes and reservoirs could increase freshwater NO_3^- retention, while
699 decreasing TN retention if a waterbody's DIN pool is dominated by NH_4^+, as in FCR.

700 Summer hypolimnetic DRP concentrations were approximately 3× higher in anoxic
701 conditions than oxic conditions (Fig. 5H). DRP cycling was primarily controlled by sediment
702 fluxes (Fig. 7), which encompassed both release from metal complexes and sediment organic
703 matter into the water column. Our observation of 2.2× higher sediment release rates of DRP in
704 anoxic than oxic conditions (Fig. 7) follows decades of work that have observed similar patterns

of increased P fluxes during anoxia (Mortimer 1971, Nürnberg 1987, Boström et al. 1988, Rydin 2000, Søndergaard et al. 2003).

A novel component of our study is that we simultaneously quantified both dissolved and total pools of P at the whole-ecosystem scale in our model simulations, allowing us to disentangle the responses of different P fractions to anoxia. While DRP concentrations tripled in response to anoxia, TP concentrations only increased by 1.6× (Fig. 5G,H), indicating a lower sensitivity of particulate P than DRP to hypolimnetic oxygen conditions in FCR. This result is supported by the negligible (albeit statistically significant) response of POP to changes in oxygen availability (Supplementary Text 5, Table S6). Consequently, we expect that the effects of anoxia on reservoir TP dynamics are dependent on the proportion of dissolved P vs. particulate P within the TP pool. If the hypolimnetic DRP pool comprises a sizeable proportion of TP, as observed in FCR (median of 11±1%), then TP retention will likely be sensitive to anoxia (e.g., Fig. 9), but if DRP is lower, then TP cycling may be more resilient to anoxia.

Shifts in stoichiometry in response to anoxia

The substantial difference in stoichiometric ratios between anoxic and oxic conditions has important implications for understanding how anoxia affects the ecosystem functioning of lakes and reservoirs. Because anoxia increased hypolimnetic NH_4^+ concentrations more than any other dissolved or total fraction in this study, and NH_4^+ dominated both the dissolved and total N pools, any stoichiometric ratios that included NH_4^+, DIN, or TN exhibited large shifts during anoxia (Fig. 6). The significantly higher TN:TP and DIN:DRP ratios observed during anoxia will likely affect water quality and food web structure (Fig. 6G,H). Higher N:P ratios favor non-N-fixing cyanobacteria and will shift the composition of other taxa in phytoplankton community

728 based on their N and P requirements (Reynolds 2006), as phytoplankton can access hypolimnetic
729 nutrients via multiple mechanisms (Cottingham et al. 2015). In contrast, the significantly lower
730 TOC:TN, TOC:TP, DOC:DIN, DOC:NH_4^+, and DOC:DRP ratios during anoxia (Fig.
731 6A,B,C,D,F) could increase organic matter mineralization rates (e.g., Coble et al. 2015).
732 Our results both support and contradict earlier studies that measured freshwater
733 stoichiometry across many waterbodies. Similar to an analysis of >27,000 freshwater samples
734 from U.S. waterbodies (Helton et al. 2015), we observed inverse relationships between NO_3^- vs.
735 NH_4^+ concentrations and DOC vs. DIN concentrations (Fig. 6). Our study provides experimental
736 evidence to support the hypothesis that redox gradients are a major driver of NO_3^-:NH_4^+ and
737 DOC:DIN ratios, which will increase in oxic conditions and decrease in anoxic conditions
738 (Helton et al. 2015). On the other hand, our work finds only partial support for earlier findings of
739 lower TOC:TP and TN:TP ratios in reservoirs than natural lakes in an analysis of ~1000 U.S.
740 waterbodies, which was attributed in part to a greater incidence of anoxia in reservoirs
741 (Maranger et al. 2018). Median TOC:TP and TN:TP ratios in the reservoirs of that study were
742 417 and 38, respectively, which are similar to the ratios observed in FCR (Fig. 6B,G). While
743 anoxia decreased TOC:TP ratios (Fig. 6B), it also increased TN:TP ratios (Fig. 6G), suggesting
744 that anoxia is not responsible for all differences in stoichiometry between reservoirs and natural
745 lakes. Our results indicate that individual waterbodies' responses to anoxia may be dependent on
746 the dominance of NO_3^- vs. NH_4^+ in their DIN pool prior to the onset of anoxia: if NO_3^-
747 dominates, then TN:TP ratios will likely decrease with anoxia, while if NH_4^+ dominates, then
748 TN:TP ratios will likely increase. In general, most lakes tend to have higher NO_3^- than NH_4^+
749 concentrations (Quirós 2003, Leoni et al. 2018), suggesting that anoxia may result in lower
750 TN:TP ratios in most waterbodies.

Opportunities and challenges of our whole-ecosystem approach

Our coupled field manipulation and modeling study provided a powerful approach for quantifying freshwater ecosystem responses to anoxia. By focusing on the same reservoir experiencing different oxygen conditions over multiple years, we were able to isolate the effects of oxygen on C, N, and P cycling without having to disentangle ecosystem-specific responses (e.g., if we were comparing across multiple waterbodies). Ideally, we would have run the REDOX field manipulation with multiple summers of continuous oxygenation and multiple summers of no oxygenation to contrast hypolimnetic conditions. However, we were constrained in our manipulation as the reservoir was an active drinking water source during the study, necessitating us to activate the oxygenation system every summer for the preservation of water quality. Consequently, we used the calibrated ecosystem model to simulate the biogeochemistry of continuously oxygenated and never-oxygenated scenarios, which uniquely enabled us to compare the effect of oxygenation while holding all other factors constant, such as temperature (Fig. 4A). The similar biogeochemical responses to anoxia between the non-oxygenated vs. oxygenated field data and the anoxic vs. oxic model scenarios support our integrated study approach and the robustness of our findings (e.g., Fig. 5, Fig. S2).

The simulation model provided insights to reservoir responses to anoxia that would have been challenging to glean from field observations alone. We used the model to calculate whole-ecosystem rates that are impossible to measure in the field (e.g., daily POC burial), determine the relative importance of different processes for biogeochemical budgets, and quantify how processes changed in anoxic vs. oxic conditions. While the model's biogeochemical rates were determined from automated optimization and calibration of numerical simulation parameters, they fall within reasonable ranges of biogeochemical rates observed in the field, supporting our

774 model results. For example, hypolimnetic sediment flux chamber measurements that were
775 collected in FCR in summer 2018 measured a mean sediment oxygen demand of ~20 mmol m^{-2}
776 d^{-1} (range 8-37.5 mmol m^{-2} d^{-1}), which compares well with our calibrated hypolimnetic flux of
777 29 mmol m^{-2} d^{-1} (Krueger et al. 2020). That study also measured NH_4^+, DRP, and DOC fluxes
778 from the sediment into the water column as the chambers became anoxic, with calculated release
779 rates up to 2.7 mmol NH_4^+ m^{-2} d^{-1}, 0.01 mmol DRP m^{-2} d^{-1}, and 14 mmol DOC m^{-2} d^{-1}
780 (Supplementary Text 1). These numbers are consistent with our maximum calibrated rates of 2.8
781 mmol NH_4^+ m^{-2} d^{-1} and 0.02 mmol DRP m^{-2} d^{-1} (Carey et al. 2021c). Our maximum calibrated
782 rate for DOC sediment flux, 1.4 mmol DOC m^{-2} d^{-1}, is an order of magnitude lower than the field
783 data, suggesting that our modeled sediment flux rate of DOC was likely conservative.
784 We note several limitations to our study that should be considered. First, we focused on
785 the hypolimnion of FCR as a reactor in which we could isolate coupled biogeochemical
786 processes occurring during summer stratification, when C, N, and P processing rates are usually
787 at their highest due to warm temperatures. This focus on the hypolimnion precluded the analysis
788 of other important processes that can have large effects on biogeochemical cycling in the
789 epilimnion (e.g., photodegradation). Second, FCR has a hypolimnetic withdrawal, which results
790 in increased downstream export of hypolimnetic water from the reservoir. While export of
791 hypolimnetic water is limited in many naturally formed lakes, hypolimnetic withdrawals are very
792 common in reservoirs that provide drinking water, hydropower, and flood risk protection (Hayes
793 et al. 2017), which represent a large proportion of the reservoirs in the U.S. (NID 2021). Third,
794 the ecosystem model is inherently limited in that it does not include all processes that can affect
795 C, N, and P cycling (e.g., microbial dynamics, bioturbation). Fourth, similar to many other lake
796 modeling studies (e.g., Kara et al. 2012, Farrell et al. 2020, Ward et al. 2020), it was challenging

to model NO_3^- and DRP. For these solutes in particular, most of the variation in observations was within the limit of quantitation (Supplementary Text 2), indicating that the model should not necessarily be penalized for the low performance in its evaluation metrics. Despite these challenges, the N and P parameters used for modeling FCR are consistent with other applications of the GLM-AED for other lakes (e.g., Kara et al. 2012, Farrell et al. 2020, Ward et al. 2020), and overall, we were generally able to recreate observed physical, chemical, and biological dynamics in both the epilimnion and hypolimnion (Fig. 3, Figs. S3,S4). Moreover, the similarity in results between the anoxic vs. oxic model scenarios and the field data from contrasting non-oxygenated vs. oxygenated summer days (Fig. 5, Fig. S2) supports our approach and overall results.

Conclusions

The duration, prevalence, and magnitude of anoxia in the bottom waters of lakes and reservoirs are increasing globally (Butcher et al. 2015, Jenny et al. 2016a, Jane et al. 2021). While low oxygen conditions are typically thought of as a response to land use and climate change (Jenny et al. 2016b, Jane et al. 2021), our analysis demonstrates that low oxygen can also be a *driver* of major changes to freshwater biogeochemical cycling.

Importantly, our work indicates that anoxia may alter the ability of freshwater ecosystems to serve as sinks of C, N, and P in the landscape. Consequently, while hypolimnetic anoxia is a result of increased C, N, and P loading into a waterbody, we also show that it may serve as an intensifying feedback that increases anoxia in downstream waterbodies. This is evident in our study, as we found significantly higher fluxes of C, N, and P downstream when FCR was exhibiting anoxic vs. oxic conditions during the summer. Anoxia thus has the potential to both

degrade the water quality of downstream waterbodies and necessitate greater treatment of water extracted from the reservoir for drinking. While more data are needed to evaluate the consequences of this feedback on downstream water quality, we hypothesize that it could be an important process affecting water quality in some freshwater ecosystems. Given the vital role that inland waters play in removing C, N, and P from downstream export (Harrison et al. 2009, Powers et al. 2016, Maranger et al. 2018), an increased prevalence and duration of anoxia in lakes and reservoirs will likely have major effects on global C, N, and P budgets as well as water quality and ecosystem functioning.

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