



Response of a River Channel-Hyporheic Zone System to Organic Matter Addition: Insights from Low-flow Experiments

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Abstract. River corridors are broadly important for nutrient and organic matter (OM) cycles because river channels and sediment with hyporheic zones (HZs) are hot spots for biogeochemical reactions. While many studies of OM dynamics and stream metabolism apportion a large part of river corridor metabolic functions to the HZ, effects of OM loading in the channel on HZ metabolism are not well understood. We conducted low-flow flume experiments to understand the relative contributions to aerobic respiration (AR) from the channel and from the HZ in response to OM loading. OM loading was promoted by addition of increasing masses of particulate organic matter (POM) inserted in the channel for 24 hours. AR was inferred from dissolved oxygen (DO) and CO₂ monitored in the channel and throughout the depth of the HZ. We measured dissolved organic carbon (DOC) concentrations, temperature, pH and other chemical parameters in channel water and porewater samples. Despite the common idea that HZs are biogeochemical hot spots, our low-flow experiments showed that OM addition to the channel magnified the channel's role as a biogeochemical reactor more, which in turn likely overwhelmed any potential contributions from the HZ. Channel DO increased and CO₂ decreased instantaneously in response to all POM additions, while in the HZ, there was a muted response limited to the shallow, downwelling portions of the HZ where DO decreased. The scenario where the HZ is a relative biogeochemical cold spot during a channel hot moment because of hydrologic conditions should also be considered when studying, assessing, and modeling OM cycles in river corridors.

1 Introduction

River corridors are central to cycling terrestrial and aquatic organic matter (OM) and nutrients, and play an important role in the global carbon (C) cycle. It is estimated that terrestrial ecosystems annually supply around 5.1 Pg-C (Drake et al., 2018) to rivers, and that a substantial amount of this C is delivered as leachate from leaf litter in the channel (McDowell and Fisher, 1976; Meyer and Tate, 1983; Meyer and Wallace, 1998). OM is transformed and C is mineralized to CO₂ in river corridors.



Therefore, river corridors are active participants in the C cycle (Harvey and Gooseff, 2015) and are major sources of CO₂ to the atmosphere, with contributions ranging from 0.7 Pg-C y⁻¹ (Lauerwald et al., 2015) to 3.9 Pg-C y⁻¹ (Drake et al., 2018).

35 Variabilities in and dependencies on parameters like flow velocity and turbulence (Raymond et al., 2012; Rocher-Ros et al., 2019), seasonal and anthropogenic influences (Pinardi et al., 2014), heterogeneity in reactivity of OM pools and respiration rates (Burrows et al., 2017; Ward et al., 2016), all contribute to uncertainties in estimating CO₂ efflux from rivers and in assessing the functions of rivers in OM cycling. Another complicating factor in evaluating rivers as OM biochemical reactors and as sources of CO₂ to the atmosphere is the unequal contributions from river channels and river sediments to OM

40 transformation (Garayburu-Caruso et al., 2026). Because these spatial domains are coupled through transport processes, partitioning the contributions from individual domains to OM cycling in whole river corridor is not trivial. Biogeochemical processes that occur in the channel affect those in the sediment and vice versa. Therefore, understanding mechanisms and feedbacks that influence metabolism in river channels and in river sediments is important for predicting how river corridors respond to stressors like increased OM loading.

45 Studies suggest that much of the OM in rivers is cycled within the zones of pronounced groundwater-surface water interaction known as hyporheic zones (HZs) (Boano et al., 2014; Gooseff, 2010; Harvey et al., 1996) and that the HZ can contribute up to 96% of river ecosystem respiration (Naegeli and Uehlinger, 1997). Therefore, HZs are typically considered the ‘hot spots’ for OM cycling, and due to their dynamic nature, HZs can also experience metabolic ‘hot moments’ (Kaufman et al., 2017; McClain et al., 2003). However, estimates for HZ contributions to river ecosystem respiration vary greatly (Garayburu-Caruso

50 et al., 2026; Battin et al., 2003; Fellows et al., 2001; Naegeli and Uehlinger, 1997) with some estimates reaching as low as 6% (Ingendahl et al., 2009). This suggests that under certain conditions HZs can be ‘cold spots’ for OM cycling and that the bulk of respiration occurs elsewhere within the river corridor. Across-site variations in hydrologic conditions, temperature, organic matter composition, and metabolic rates can impact the HZ’s role in OM transformations (Stegen et al., 2018; Findlay, 1995; Kaplan and Newbold, 2000). It remains unclear how an increase in OM loading would impact the HZ’s relative contributions

55 to river ecosystem respiration.

Studies of respiration dynamics in rivers use some common approaches to partition river respiration between the channel and the HZ domains. This is typically done by relating dissolved oxygen (DO) uptake, i.e., aerobic respiration (AR), to whole river metabolism (Ingendahl et al., 2009; Marzolf et al., 1994; Uzarski et al., 2001; Ward et al., 2018). Some common field methods take advantage of diurnal variations in DO and use respiration chambers or incubations (Fellows et al., 2001; Marzolf et al.,

60 1994; Uzarski et al., 2001). These methods can sometimes be uncertain because they often rely on: (1) approximations for water-atmosphere exchange fluxes, which can be highly variable and present an uncertainty in estimating AR and CO₂ evasion; (2) attributing the night-time DO depletion to respiration in sediments typically without considering impacts from interactions with low oxygen groundwater from the streambed and the floodplain; or (3) are time- and site-specific. Therefore, broadly understanding how respiration in the channel and in the HZ responds to environmental stressors such as OM loading requires

65 carefully accounting for atmosphere-channel-HZ interactions.



Gas exchange between rivers and the atmosphere, or reaeration when specifically referring to oxygen exchange, needs to be quantified robustly to produce reasonable estimates for AR (Aristegi et al., 2009). As the atmosphere is an unlimited source of O₂ fueling AR, and rivers are open to the atmosphere, atmosphere-river channel interactions are always taking place. Simultaneously, the exchange flux of material between the channel and the sediment (i.e., the HZ), mainly through advection of water and solutes, is equally important. Because the flux across the sediment-water interface is influenced by hydraulic properties of the riverbed material (Salehin et al., 2004), surface flow velocity (Thibodeaux and Boyle, 1987), and riverbed morphology (Buffington and Tonina, 2009), this flux can be best approximated through experimentally obtained parameters and numerical models.

Rivers are complex, heterogeneous environments that incorporate interactions with floodplains, riparian zones, and marshes – some major producers and sources of OM to rivers. To manage some of this complexity, we take advantage of a large flume. We conducted a series of controlled flume experiments designed to address the following questions: (1) How does a rapid increase in OM affect AR in a slow flowing river with a sandy bed; and (2), what are the contributions of the river channel and the HZ to AR? We hypothesized that the HZ will turn into a hot spot for AR due to delivery of OM from the channel, enhancing its role in OM cycling. We hypothesized that there would be strong feedback to the channel from HZ AR. Our experimental river is representative of shallow sandy rivers in coastal plains and not first-order channels in mountainous or steep terrain. To address the questions, we used high resolution DO monitoring via commercially available and custom sensors, CO₂ measurements, and other water chemistry parameters in both channel and the HZ.

2 Methods

A flume study that excludes complex interactions of natural open systems but allows for controlled input of OM offers an opportunity for a targeted evaluation of the atmosphere-channel and channel-HZ interactions and to observe processes that occur in the channel and in the HZ. To understand how periodic loading of OM supplied through the channel impacts relative contributions to AR from the channel and from the HZ, we added particulate organic matter (POM) to the flume channel and removed it after 24 hours. Specifically, the experiments used 200 grams, a repeat 200 grams, and 300 grams of crushed dry leaves as POM. These experiments are hereafter referred to as POM_{200A}, POM_{200B}, and POM₃₀₀.

2.1 Experimental Set-up

The study was conducted in a water recirculating flume measuring 7×0.3×1.2 m (length × width × height), with a 5×0.7 m (length × width) sediment bed (Fig. 1a). The sediment was manually shaped to include nine consecutive bedforms (0.45 m long × 0.07 m high) with a 3:1 stoss to lee side length ratio. The bedforms, which induce hyporheic flow, remained stationary during the experiments. Channel water moved into the HZ at the stoss or the downwelling side of the bedform and exited at the lee or the upwelling side of the bedform. Hereafter, the downwelling and the upwelling sides of the bedform are referred to as DW and UW, respectively. Sediment particle analysis showed that the flume sediment was composed of homogeneous,

coarse sand (Pioneer Sands, Industrial Sands Products, Brady, Texas, USA) with median grain size of $D_{50} = 1.8$ mm and a porosity of 0.27. The manufacturer's description of the sand is listed in Table C1. No POM was added to the sand to minimize the possibility of creating persistent biogeochemical hot spots inside the sediment bed. The channel water depth was kept at 30 cm above the bedform troughs and average flow velocity was set and maintained at 0.011 m s^{-1} . The open channel Reynolds number with the hydraulic radius as the characteristic length scale was 4,400, which is representative of low-gradient streams under baseflow conditions.

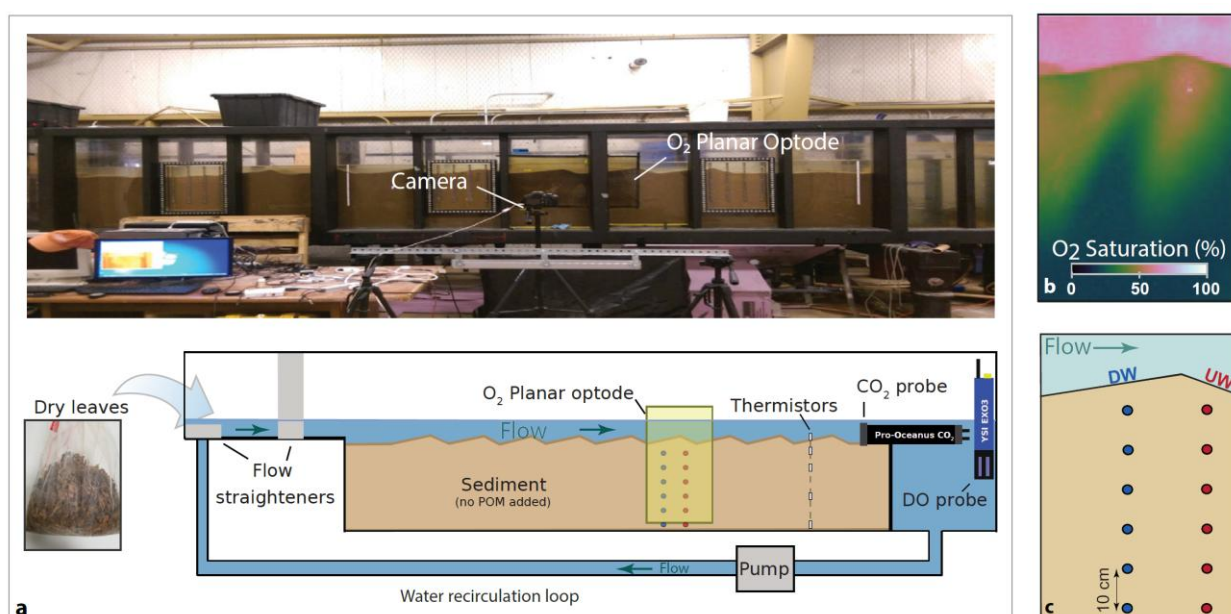


Figure 1: Experimental set-up for inducing and monitoring aerobic respiration in the flume. (a) Water recirculating flume with dissolved oxygen (DO) planar optode imaging system, a set of thermistors, a CO₂ probe (Pro-Oceanus), and a YSI-EXO3 sonde (modified from Kaufman et al. (2017)). A bag with dry leaves/particulate organic matter (POM) used during one of the experiments is shown in the lower left corner. (b) Example of planar optode image showing the DO field within the sediment (up to 53 cm depth) and in the channel. (c) Sampling scheme for porewater at the downwelling (DW) and upwelling (UW) sides of the bedform.

The water used in the flume was supplied from the Lower Colorado River (Austin, Texas, USA) to achieve biogeochemical and microbial conditions representative of a natural river ecosystem following previous studies (Kaufman et al., 2021). The flume was inoculated for approximately two months prior to the experiments to allow the establishment of microbial communities within the sand bed. During the inoculation period, dry leaves were periodically added to and removed from the flume channel to release OM and facilitate microbial metabolism and growth. The presence of OM during inoculation was confirmed through channel water and porewater analysis for dissolved organic carbon (DOC) (Table C2). Rigid insulating



foam and a black plastic sheet covered the top and the walls of the flume to minimize temperature and light variation, which are known to impact microbial metabolism, algal growth, and alter OM via photodegradation (Cory and Kling, 2018).

120 The water volume in the channel including the recirculatory system of the flume outside of the working section was 0.616 m³ and the volume of porewater within the HZ was 0.212 m³. To isolate the effects of OM loading from the effects of changing hydrologic conditions on AR, the relative channel-HZ volumes and fluid fluxes were kept constant during the incubation period and throughout the experiments.

The series of experiments began after the incubation period and following the development of a typical well-to-poorly oxygenated HZ profile. The experiments consisted of inserting POM, mostly dry *Morus alba* (white mulberry) leaves into the flume channel and removing the bag after 24 hours to avoid OM overloading which could lead to biofouling. The leaves were slightly crushed and loosely packed into polyester mesh bags (Fig. 1a) to allow leaching of OM while minimizing the input of particulates into the flume. Each experiment was conducted with a new set of dry leaves to keep the input rate and presumably the chemistry of the leachate consistent. The experiments were conducted back-to-back without replacing the flume water and porewater to go back to pre-experimental DOC concentrations. Between the experiments, the channel DO and CO₂ concentrations were allowed to return to initial conditions. The overall duration of each experiment from the time of addition of POM to the time of return of channel DO and dissolved CO₂ (CO_{2(aq)}) to the initial condition was approximately 8 days. Continuous and discrete measurements of DO, CO_{2(aq)}, pH, DOC, dissolved inorganic carbon (DIC), and temperature in the channel and in the HZ (at DW and UW; Fig. 1c) were collected throughout the experiments.

135 2.2 Continuous Measurements: Dissolved Oxygen, CO_{2(aq)}, pH, and Temperature

Continuous channel measurements of DO, pH, and temperature were collected at 30-minute interval using a YSI EXO3 sonde (Xylem Inc., NY, USA). The sonde's individual sensors were calibrated according to the manufacturer's instructions. The CO_{2(aq)} in the channel was measured in 30-minute intervals using a Pro-Oceanus CO₂-Pro CV probe (Pro-Oceanus Systems Inc., Nova Scotia, Canada), equipped with a gas-permeable membrane and a non-dispersive infrared gas analyzer. The CO₂ probe was pre-calibrated by the manufacturer with a reported accuracy of +/-20 ppm CO₂ over the range of our measurements. The EXO3 and the CO₂-Pro CV probes were placed side-by-side (Fig. 1a) at the downstream (outlet) end of the flume and away from the POM insertion point (upstream/inlet) to allow leachate to mix and to avoid readings impacted by the disturbances during placement and removal of the POM bag. Laboratory air temperature and the temperature in the flume (in the channel and HZ) were monitored with a set of thermistors (HOBO-Onset TMC and U12 Outdoor/Industrial loggers, Onset Computer Corporation, Bourne, MA, USA). Temperature was measured at 10-, 20-, 40-, and 60-cm depths within the HZ. DO in the HZ was monitored via a planar optode imaging system (Fig. 1a). Planar optodes have been used for two-dimensional DO field mapping in marine (Glud, 2008; Kessler et al., 2012) and riverine sediments (Kaufman et al., 2017). A planar optode, described in Kaufman et al. (2017) and in Larsen et al. (2011), was installed in a single bedform covering ~53 cm depth and 45 cm length of the HZ. The planar optode was calibrated using a PyroScience Firesting oxygen sensor with temperature module (Pyroscience, Aachen, Germany) prior to the experiments. Additionally, the measurements taken with the PyroScience



Firesting oxygen sensor were validated through gas chromatography. The calibration of the planar optode was conducted at ambient temperature ($\sim 22^\circ\text{C}$) with images taken at multiple DO saturation points between 0 and 100%. The DO optode images were taken at ~ 3 -hour intervals. However, due to camera trigger misfire, there are gaps in the optode image sampling series. The planar optode images were processed through the Python programming language (Van Rossum and Drake, 2009).

155 The DO map was spatially integrated and averaged into a single value, i.e., the average DO concentration within the bedform. Then, assuming that each bedform has the same DO distribution, the average DO concentration obtained from the images were applied to the entire volume of the nine bedforms in the flume which gives the total mass of DO (millimoles) in the HZ. The DO was converted from % saturation to mmol L^{-1} using temperature-adjusted Henry's constants which used the average temperature in the HZ measured with thermistors at the time when the DO images were taken.

160 2.3 Discrete Measurements: Sampling for Dissolved Organic Carbon, Dissolved Inorganic Carbon, Alkalinity, pH, major ions

Discrete channel water and porewater samples were collected throughout the experiments to capture chemical conditions in the flume and to calculate the HZ's $\text{CO}_{2(\text{aq})}$ before and after addition of OM. Porewater samples were collected with syringes attached to stainless steel tubes with screened holes at the lowermost two centimeters (Fig. A1). Channel water samples were collected directly using syringes. All samples were filtered ($0.2\mu\text{m}$ PES, Whatman Puradisc) into pre-combusted VOA vials. Porewater samples were collected from multiple depths (10-60 cm) at 10 cm intervals and at the DW and UW sides of the planar optode-hosting bedform (Fig. 1c). Channel samples were collected directly above the bedform crest (i.e., between DW and UW), approximately mid-depth of the channel (~ 6 cm). pH was measured using Accumet AP110 pH meter (Fisher Scientific) during sample collection. Ionic strength barely changed during each experimental run and across all experiments, making the activity coefficient for bicarbonate nominally constant. So, for our analysis and the discussion below, we assumed infinite dilution (i.e., activity $\cong$ concentration). Samples were analyzed for DOC and DIC (where: $\text{DIC} = \text{H}_2\text{CO}_3^* + \text{HCO}_3^- + \text{CO}_3^{2-}$) using a carbon analyzer (Apollo 9000, TOC Combustion Analyzer, Teledyne Tekmar Co.) and titrated for concentration of bicarbonate alkalinity (HCO_3^-). An additional check for bicarbonate concentration was performed through DIC speciation using pH measurements and dissociation constants for carbonic acid. Both pH and HCO_3^- were used to calculate the distribution of $\text{CO}_{2(\text{aq})}$ in the HZ using the first dissociation constant for carbonic acid and Henry's Law with temperature-adjusted constants following the equations below:

$$k_{a1} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3^*]} \quad (1)$$

where k_{a1} is the first constant for dissociation of carbonic acid equal to $10^{-6.38}$ at 20°C (Plummer and Busenberg, 1982); $[\text{H}^+]$ is the concentration of the proton in mol L^{-1} derived from $\text{pH} = -\log[\text{H}^+]$; $[\text{HCO}_3^-]$ is the concentration of bicarbonate ion in mol L^{-1} ; and $[\text{H}_2\text{CO}_3^*]$ is the concentration of $\text{CO}_{2(\text{aq})}$ in mol L^{-1} . We expressed the equilibrium relationship in Eq. 1 in terms of molarities rather than activities, because river water poses low ionic strength and ionic activities approach their molar concentrations. Gas concentration was calculated as follows:



$$gas_{(aq)} = K_H P_{gas} \quad (2)$$

where $gas_{(aq)}$ is the aqueous concentration of a gas in units of mol L⁻¹; K_H in mol L⁻¹ atm⁻¹ is the temperature-adjusted Henry's constant relating the partial pressure of a gas with the concentration of gas in the water; and P_{gas} is the partial pressure of gas in units of atm. Equilibrium concentrations of oxygen and CO₂ in water were calculated using atmospheric pressures of 0.2069 atm and 0.00041 atm, respectively. Channel and porewater pH, DOC, DIC, and (HCO₃⁻) measured across all POM experiments are reported in Tables C3-C5.

Temperature adjustment for Henry's constants for O₂ and CO₂, respectively, followed the van't Hoff equation:

$$\ln(K_H) = -60.2409 + 93.4517 \times \left(\frac{100}{T}\right) + 23.3585 \times \ln\left(\frac{T}{100}\right) \quad (2a)$$

$$\ln\left(\frac{K_H}{K_{H0}}\right) = 1202.79 \times \left(\frac{1}{T} - \frac{1}{T_0}\right) \quad (2b)$$

where T is the environmental temperature in units of K; T_0 is the standard temperature in units of K; K_{H0} is the Henry's constant in mol L⁻¹ atm⁻¹ measured at standard temperature. The K_{H0} for oxygen at 25 °C is 10^{-2.89} mol L⁻¹ atm⁻¹.

Channel water and porewater samples collected prior to the addition of POM_{200A} (Oct-02-2019) and immediately after the removal of POM_{200A} (Oct-03-2019) were analyzed for major anions and cations using a Dionex Integrion HPIC (Thermo Scientific) and a Dionex-ICS-2000 (Dionex), respectively (Table C6). The ionic composition of channel and porewater samples was speciated using the geochemical modeling software PHREEQC 3.6.2 (Parkhurst and Appelo, 2013). All water samples were kept in the dark at 4 °C prior to analysis.

2.4. Hyporheic Zone Flow Field Mapping and Modeling, and Sediment Hydraulic Characterization

Numerical models of flow were used to calculate the flow field in the sediment, which provides hydrologic context to the biogeochemical experiments, and to constrain sediment hydraulic properties. The modeling was executed through the multiphysics model *hyporheicFoam* (Li et al., 2020), a variant of OpenFOAM (Weller et al., 1998), which simultaneously modeled the coupled turbulent fluid flow in the channel and groundwater flow in the sediment. *hyporheicFoam* numerically solves the Reynolds-averaged Navier-Stokes equations with the $k-\omega$ closure scheme for the open channel and the groundwater flow equation for the sediment (HZ) using the finite volume method. We used the flume and sediment geometry, and the experimental flow and boundary conditions as inputs into *hyporheicFoam*. The model was used to estimate the permeability of the flume sand, visualize the flow paths throughout the extent of the HZ, and quantify the Darcy velocity distribution.

The flume sediment permeability was determined through combined experiments and modeling. A dye tracer test was performed on two bedforms. The dye traces were used to validate a particle tracking transport model executed through *hyporheicFoam*. The permeability was adjusted until the particle tracking model and the experimental dye tracer results were in reasonable agreement. Sand permeability obtained through this trial-and-error approach was 1.25×10⁻⁹ m². Additionally, column experiments and empirical approaches were used to ensure that the dye tracer test and the particle tracking model results for permeability were reasonable. Sand permeability obtained through column experiments was 1.32×10⁻¹⁰ m², and



through empirical calculations using the Kozeny-Carman equation (Carman, 1937; Kozeny, 1927) was $3.44 \times 10^{-10} \text{ m}^2$. All experimental and modeled permeabilities are typical for unconsolidated, well-sorted, coarse sand (Freeze and Cherry, 1979).

2.5. Reaeration Experiments for Water-Air Fluxes

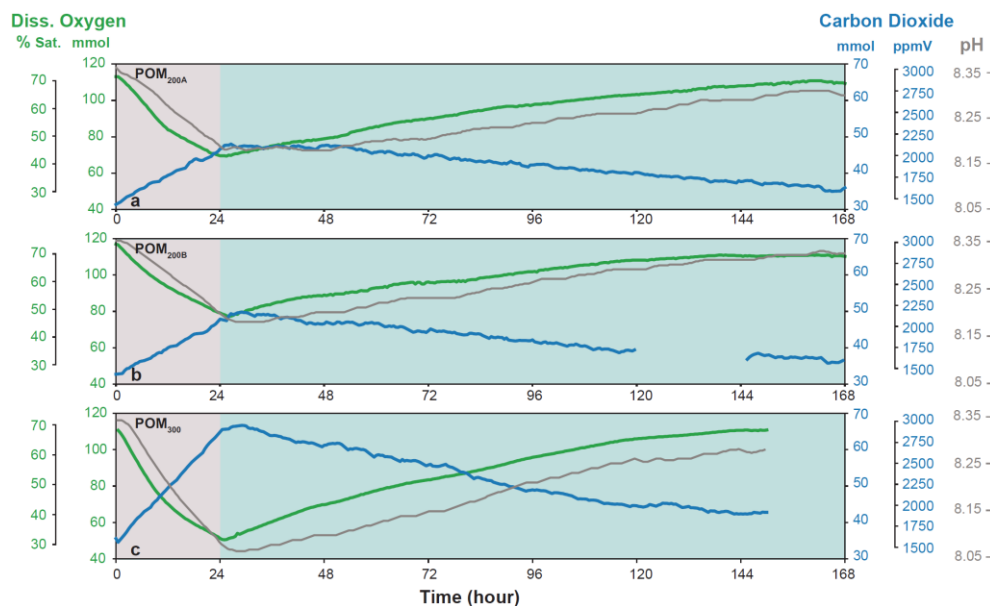
A sparging-reaeration experiment was conducted to get a sense of water-air fluxes. The channel was sparged with N_2 gas until DO concentrations reached low values of approximately 1 mg L^{-1} ($\sim 12\%$ saturation). Then the return curve of oxygen in the channel was monitored using three PyroScience FireSting fiber optic oxygen sensors. The conditions during the sparging-reaeration test were identical to the conditions during POM experiments, i.e., same channel flow rate, laboratory temperature range, and configuration of rigid and soft covers of the flume walls and the flume top.

2.6. Statistical Analysis of Geochemical Observations

In order to quantify any geochemical differences between different areas of the flume, including the channel and the upwelling and downwelling areas of the HZ, an unequal variances t-test (also known as Welch's t-test) was conducted on CO_2 and DOC. The Welch's t-test compares if the means of two sample groups (i.e., the channel vs HZ upwelling area vs HZ downwelling area) are different. The null hypothesis that sample means are equal is rejected if the p-value of the analysis is less than 0.05. Principal component analysis was conducted for the major ion concentrations and pH of water samples to determine if the channel and hyporheic zone samples will group together or separately upon linear dimensionality reduction.

3 Results

During the POM perturbation while the bag of leaves was present in the channel (hours 0 to 24 of the experiments), the channel DO, $\text{CO}_{2(\text{aq})}$, and pH showed an immediate and strong response (Fig. 2), while the DO throughout the HZ had a muted response (Figs. 3 and 4). A synchronous response in channel DO depletion and $\text{CO}_{2(\text{aq})}$ increase accompanied by a drop in pH was observed in the first 24 hours (Fig. 2). The overall return to initial conditions for channel DO, $\text{CO}_{2(\text{aq})}$, and pH was approximately 6 days (144 hours) after the removal of the bag. These in-channel trends were consistent across all three experiments for POM_{200A}, POM_{200B}, and POM₃₀₀ with larger DO deficit and $\text{CO}_{2(\text{aq})}$ levels during the POM₃₀₀ perturbation. A good agreement in DO, $\text{CO}_{2(\text{aq})}$, and pH values measured in the channel was observed during replicate POM_{200A} and POM_{200B} experiments, but not in the HZ.



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Figure 2: Dissolved oxygen, carbon dioxide, and pH measured in the channel for the three experimental particulate organic matter (POM) additions: POM200A (a) and POM200B (b) had 200 g of bagged crushed leaves placed in the channel for 24 hours denoted by the gray-shaded period, and POM300 (c) had 300 g of leaves placed.

Contrary to observations in the channel, the DO and $\text{CO}_{2(\text{aq})}$ dynamics in the HZ were less synchronous, with little to no trends (Figs. 4 and 5). The DO depletions in the HZ following the POM additions were not accompanied by notable increases in $\text{CO}_{2(\text{aq})}$ (Fig. 5). Only subtle increases in $\text{CO}_{2(\text{aq})}$ concentration were observed in the HZ at DW and UW at 10 cm depth (Fig. 5), whereas the DO dynamics occurred throughout the entire depth of the HZ. Most changes were noted in the shallow HZ (upper 25 cm; optode images in Fig. 3).

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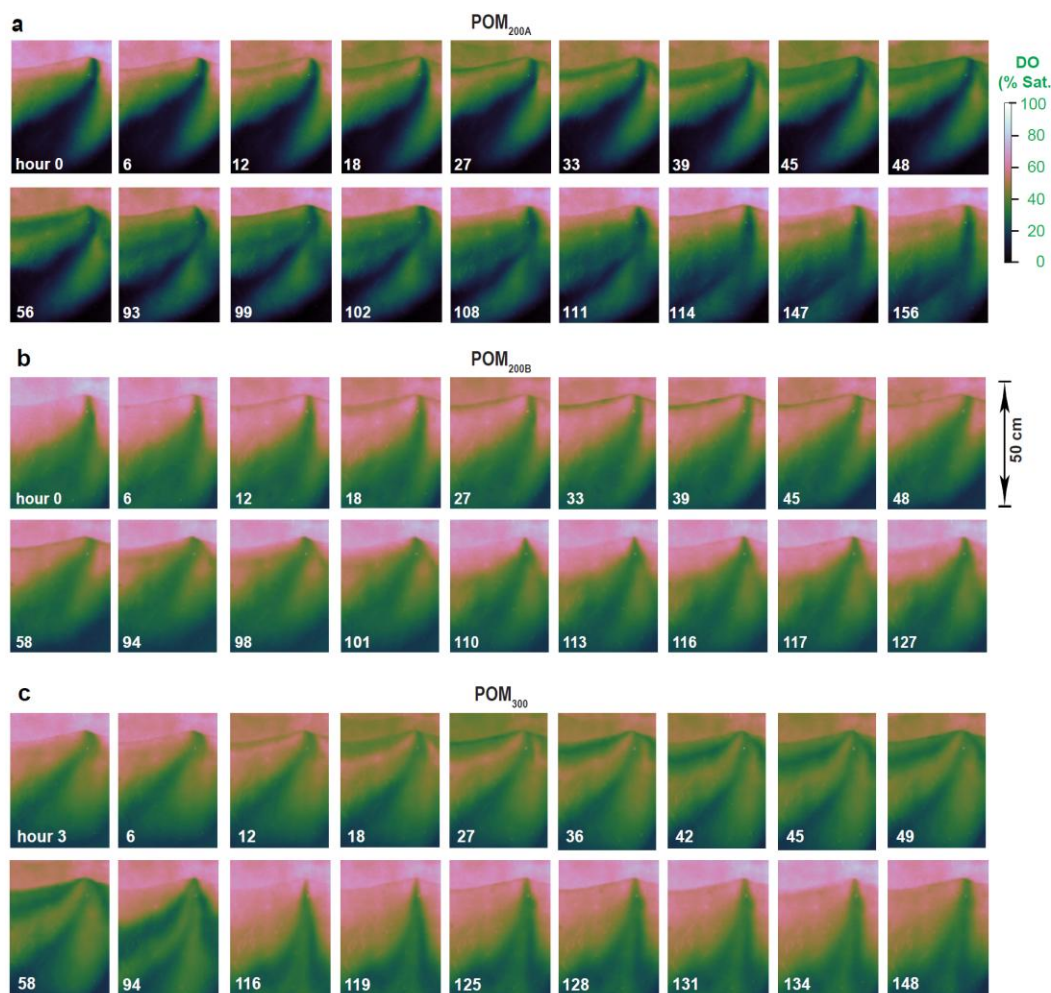


Figure 3: Images of dissolved oxygen (in % saturation) taken with a planar optode for the three experimental particulate organic matter (POM) additions: (a) POM_{200A}, (b) POM_{200B}, and (c) POM₃₀₀. Time marker 0 hour is the time when the bagged leaves were placed in the channel, which was then removed after 24 hours. Many additional images (nominally taken 3-hourly) were omitted to improve visualization, especially of changes.

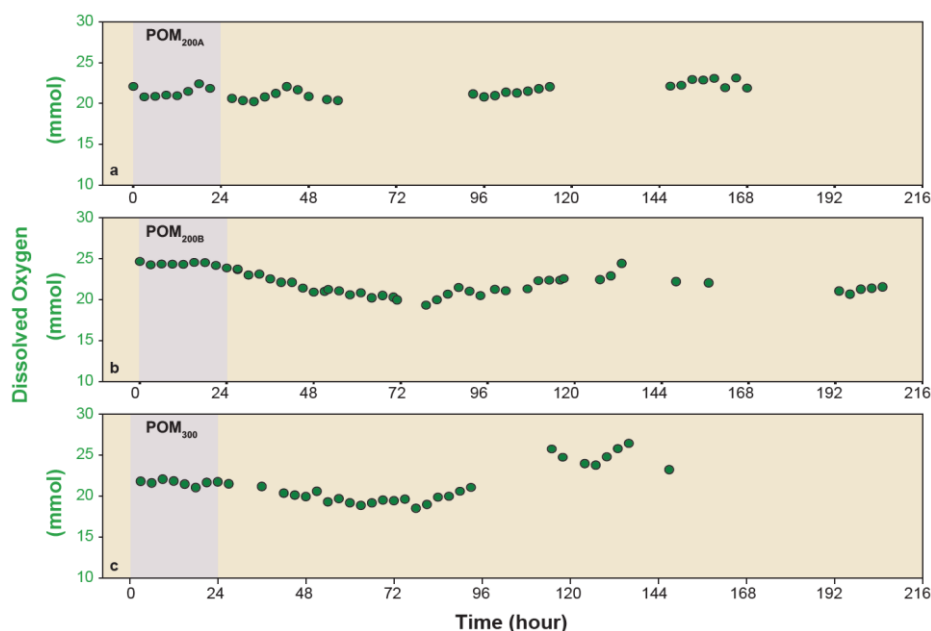


Figure 4: Calculated total mass of dissolved oxygen within the hyporheic zone (sediment) of the flume for the three experimental particulate organic matter (POM) additions: (a) POM200A, (b) POM200B, and POM300 (c). The mass was calculated by spatial integration of the images in Figure 3, corrected for porosity, and extrapolated for the entire area of the sediment section of the flume.

3.1 POM_{200A} Experiment

Observations in the Channel: Adding 200 g of bagged leaves resulted in a rapid response (within 30 minutes) in channel DO, CO_{2(aq)} concentration, and pH (Fig. 2). While the leaves were present in the channel, from hour-0 to hour-24, the DO in the channel (channel volume = 0.616 m³) decreased by 44 mmol (71.3% to 43.8% saturation), at an approximate rate of decrease of 1.66 mmol h⁻¹. Simultaneously, the total CO_{2(aq)} in the channel increased by 17 mmol (1417 ppmV to 2169 ppmV) at an approximate rate of 0.7 mmol h⁻¹. DO and CO_{2(aq)} returned to their initial conditions within ~144 hours (~6 days) post POM₂₀₀ perturbation.

The concentrations of DOC sampled before and immediately after the removal of POM_{200A}, increased from 9.7 mg L⁻¹ to 10.3 mg L⁻¹ (Fig. 5) – a 30.8 mmol-C increase in the flume channel (channel volume = 0.616 m³). The DOC trend showed an overall increase past the POM_{200A} removal, reaching a concentration of 11.6 mg L⁻¹ towards the end of this experiment.

Observations in the Hyporheic Zone: The response to the POM_{200A} perturbation in the HZ resulted in an overall 2 mmol fluctuation in DO (Fig. 4a), which was less pronounced when compared to the DO decrease in the channel. Between hours 0 and 3, the DO in the HZ (HZ volume = 0.212 m³) showed an initial decrease from 21.6 to 20.6 mmol. However, this decrease in DO was followed by an increase in DO, and by hour 18 of the POM_{200A} experiment the DO reached 22 mmol (Fig. 4a). This cyclical DO pattern repeated from hour 18 to 42 with an overall DO fluctuation of ~2 mmol. Optode images between hours 45

and 114 of POM_{200A} were not collected due to camera trigger misfire. Towards the end of the experiment, from hours 144 to 168, the DO in the HZ increased reaching the highest DO concentration observed through the POM-200A experiment (DO ~22.6 mmol).

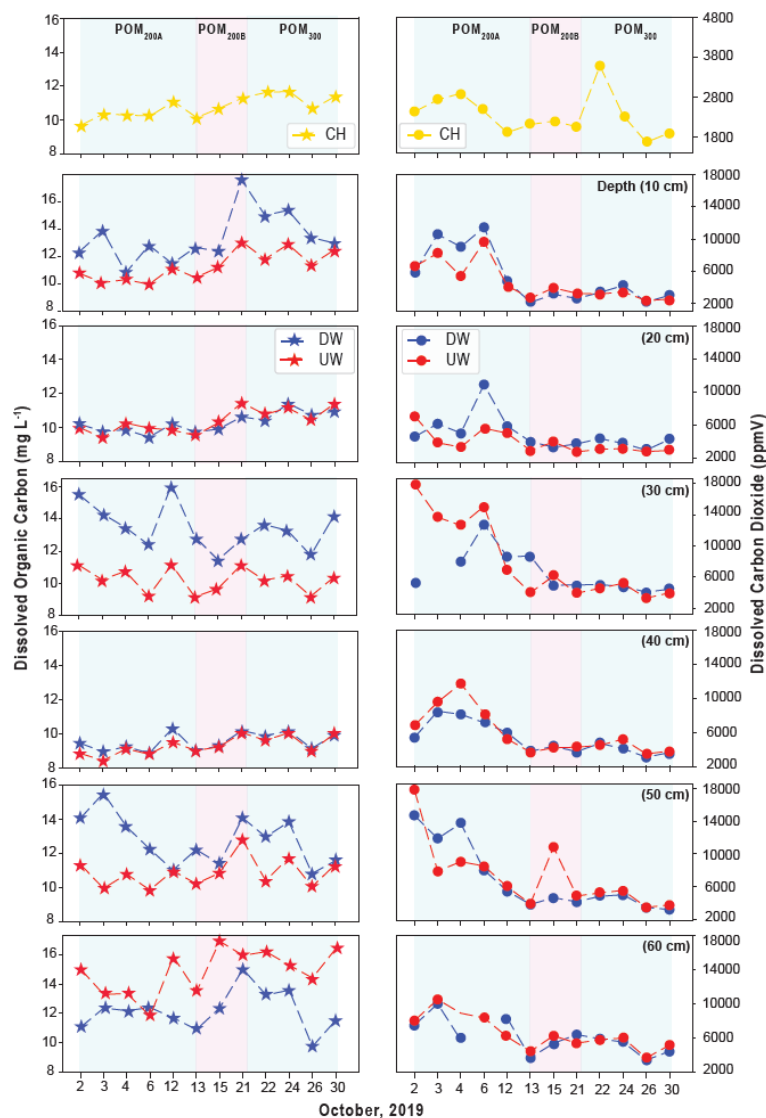


Figure 5: Dissolved organic carbon and dissolved carbon dioxide of porewater samples at different depths within the hyporheic zone taken through the entire time span of the three particulate organic matter additions. The blue and red stars denote downwelling (DW) and upwelling (UP) areas, respectively.



The DOC concentrations measured along the flow path through the HZ (i.e., at DW and UW) and with depth (i.e., 10-60 cm), showed a heterogeneous distribution of DOC concentrations across different depths and relatively static concentrations through time (Fig. 5, POM_{200A}; Table C2). At 10, 30, 50, and 60 cm depths, the DOC concentrations ranged between 8.4 mg L⁻¹ and 16.1 mg L⁻¹ during the POM_{200A} experiment. However, at 20 and 40 cm depths, the DOC concentration appeared more static, fluctuating around 10 mg L⁻¹ and 9 mg L⁻¹, respectively. No clear spatial or temporal patterns were found for the distribution of DOC within the bedform.

The CO_{2(aq)} in the bedform (Fig. 5, POM_{200A}) calculated using HCO₃⁻ and pH also showed no clear spatiotemporal pattern. However, the overall CO_{2(aq)} in the bedform was always higher than the CO_{2(aq)} observed in the channel, indicating the HZ is always an important source of CO₂. The highest CO_{2(aq)} of 18,000 ppmV was observed at 30 and 50 cm depths.

3.2 POM_{200B} Experiment

Observations in the Channel: The channel results from the repeat-200 g addition (i.e., POM_{200B} experiment) also showed an immediate response in DO, CO_{2(aq)}, and pH to the addition of leaves (Fig. 2). During the first 24 hours, the DO in the channel decreased by 39.75 mmol (from 71.2% to 48.0% saturation) at a rate of ~1.65 mmol h⁻¹. Simultaneously, the CO_{2(aq)} increased by 17.06 mmol (0.71 mmol h⁻¹), from 1403 ppmV to 2181 ppmV. Discrete water samples showed that channel DOC concentration increased from 10.8 mg L⁻¹ to 11.6 mg L⁻¹ throughout the POM_{200B} experiment (Fig. 5; POM_{200B}).

Observations in the Hyporheic Zone: During the POM_{200B} experiment, the DO in the HZ exhibited a cyclical pattern, where DO peaks coincided with the valleys in average temperature measurements (Fig. 4b). Between hours 0 and 18 of the POM_{200B} experiment the total DO content in the HZ was relatively static around 24 mmol. However, between hours 18 and 73, the DO trend showed a stepwise decrease by approximately 4.5 mmol and reached the lowest DO content in the HZ (19 mmol) during the POM_{200B} experiment. Past hour-73 the HZ DO began to recover, reaching 24 mmol at hour-132 of the experiment. This increase was then followed by another decrease.

The DOC concentration in the HZ was heterogenous (i.e., varying across 10-60 cm at DW and UW) ranging between 9 mg L⁻¹ and 17.6 mg L⁻¹ (Fig. 5, POM_{200B}). Throughout the experiment, the highest DOC concentration range was noted at 10, 30, 50, and 60 cm depths at both DW and UW. The narrowest range for DOC concentrations (9-10.8 mg L⁻¹) was noted at 20 and 40 cm depths. No clear spatiotemporal patterns in DOC concentrations were observed.

During the POM_{200B} experiment, the CO_{2(aq)} values in the HZ were higher than the CO_{2(aq)} observed in the channel (Fig. 5, POM_{200B}). Generally, the CO_{2(aq)} in the HZ fluctuated between 2,000 ppmV and 6,000 ppmV with no considerable differences across the flowpaths (i.e., DW/UW), but increasing with depth. Exceptionally high spikes of CO_{2(aq)} were observed at DW 30-cm and UW 50-cm depths (~ 9,000 ppmV and 11,000 ppmV respectively). Moreover, when compared to the POM_{200A} treatment, the repeat 200 g leaf addition resulted in lower CO_{2(aq)} in the HZ.



3.3 POM₃₀₀ Experiment

Observations in the Channel: Increasing the mass of leaves in the channel by a further 50%, from POM_{200A,B} to POM₃₀₀, caused a larger DO deficit in the channel, higher values of CO_{2(aq)}, and lower pH (Fig. 2) within the first 24 hours. The DO in the channel decreased by 60 mmol between hours 0 and 24 at a rate of 2.5 mmol h⁻¹ (from 69.4% to 30.4% saturation). Simultaneously, the CO_{2(aq)} increased by 32.1 mmol (1.33 mmol h⁻¹), from 1,546 ppmV to 2,945 ppmV. The time to return to the initial conditions past the removal of 300 g of leaves was approximately 6 days which is comparable with the POM_{200A} and POM_{200B} experiments.

Discrete samples showed that the DOC concentration continued to increase for the next two sampling events, even after the leaves were removed from the channel (Fig. 5, POM₃₀₀). The overall DOC concentrations in the channel during POM₃₀₀ experiment ranged between 10.8 mg L⁻¹ and 12.4 mg L⁻¹. No clear spatiotemporal DOC trends were observed.

Observations in the Hyporheic Zone: The results from POM₃₀₀ showed that the DO in the HZ did not respond quickly to the POM treatment (Fig. 4), which was similar to observations from the POM_{200A} and POM_{200B} experiments. The first DO response in the HZ was noted between hours 36 and 79, when DO content in the HZ began to decline from 20.1 mmol to 17.4 mmol. This 2.7 mmol reduction in DO was followed by a 7.6 mmol increase by hour 144 of the experiment, increasing the molar mass of DO in the HZ above its initial, pre-treatment condition.

The DOC concentration along the flow path in the HZ (i.e., DW and UW) and with depth ranged between 9.1 mg L⁻¹ and 16.4 mg L⁻¹ and was overall higher than DOC concentrations in the channel (Fig. 5, POM₃₀₀). At 20 and 30 cm depths, the DOC concentration was relatively static throughout the experiment, with little to no concentration differences between DW and UW. This observation was consistent with previous DOC concentrations observed at 20 and 30 cm depths during the POM_{200A} and POM_{200B} experiments (Fig. 5). No clear DOC trend indicative of concentration increase resulting from the addition of the source of leachate and DOC decrease following the removal of leaves was observed.

The CO_{2(aq)} profiles in the HZ showed subtle changes throughout the experiment, fluctuating between 2,100 and 5,950 ppmV across depths and had similar values at DW and UW (Fig. 5). The HZ CO_{2(aq)} was higher than in the channel which was consistent for all three POM experiments. Overall, the spatial profiles and temporal evolution of CO_{2(aq)} during POM₃₀₀ were more comparable to the results from the POM_{200B} experiment than to the results observed during POM_{200A}.

4 Discussion and Study Limitations

In this section we provide additional context to our observations, additional supporting analyses for the key insights, and present caveats and limitations of the study.

AR in the flume channel, inferred from decreasing DO and increasing CO_{2(aq)} concentrations, was promoted within 30-minutes from the addition of leaves across all POM experiments and increased with POM loading (Fig. 2). Relative to the HZ, the prompt response in decreasing DO and increasing CO_{2(aq)} in the channel following the addition of dry leaves could suggest that biochemical conditions in the channel and in the HZ were different and favored aerobic metabolism in the channel. For



example, the existence of C or nutrient limitations are known to impact microbial metabolism in rivers and can influence the
 350 reaction rates (Danczak et al., 2021; Garayburu-Caruso et al., 2020; Meyer and Johnson, 1983; Webster et al., 2016). When
 comparing DOC concentrations in the channel and in the HZ, the results showed that DOC concentrations in the channel were
 significantly lower than in the HZ across all POM experiments, except at 20 and 40 cm depths (Welch t-test $p < 0.01$; Fig. 6).
 The CO_2 in the channel and HZ are also have different means ($p < 0.0001$; Fig. 6), with much higher values in the HZ. However,
 the upwelling and downwelling areas of the HZ were not so statistically distinct from each other, especially for DOC. Flow
 355 modeling showed that the exceptions at 20 and 40 cm depths were likely flow stagnation points (see the blue-colored low
 velocity zone in Figs. 7f and streamlines in Fig. 7g in the other bedforms).

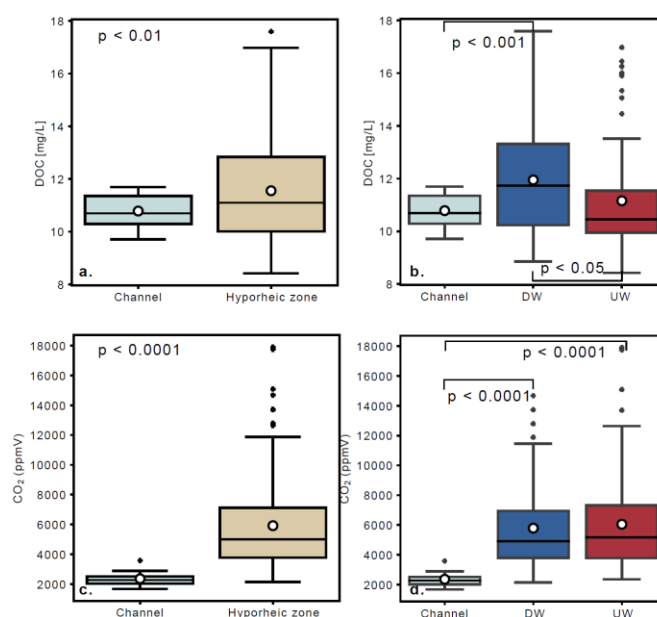


Figure 6: Boxplots of dissolved organic carbon (DOC) and carbon dioxide concentrations from the channel and hyporheic
 360 zone. The top row shows DOC concentrations measured in the channel (light blue) and in the HZ (tan) at the downwelling
 (dark blue) and upwelling (red) portions of the bedform measured across all three experiments (i.e., POM200A, POM200B,
 and POM300). The right columns show the DW and UW areas of the HZ separately. Sample means are shown in white circles.
 The boxes represent the quartiles (25th, 50th, and 75th percentiles), and whiskers represent the minimum and maximum
 excluding outliers. The bottom row shows the same information but for CO_2 . The p-values (e.g., < 0.05) denote the statistical
 365 significance of differences between two sample groups denoted by brackets analyzed with Welch's t-test. Only test results that
 are statistically significant are shown.

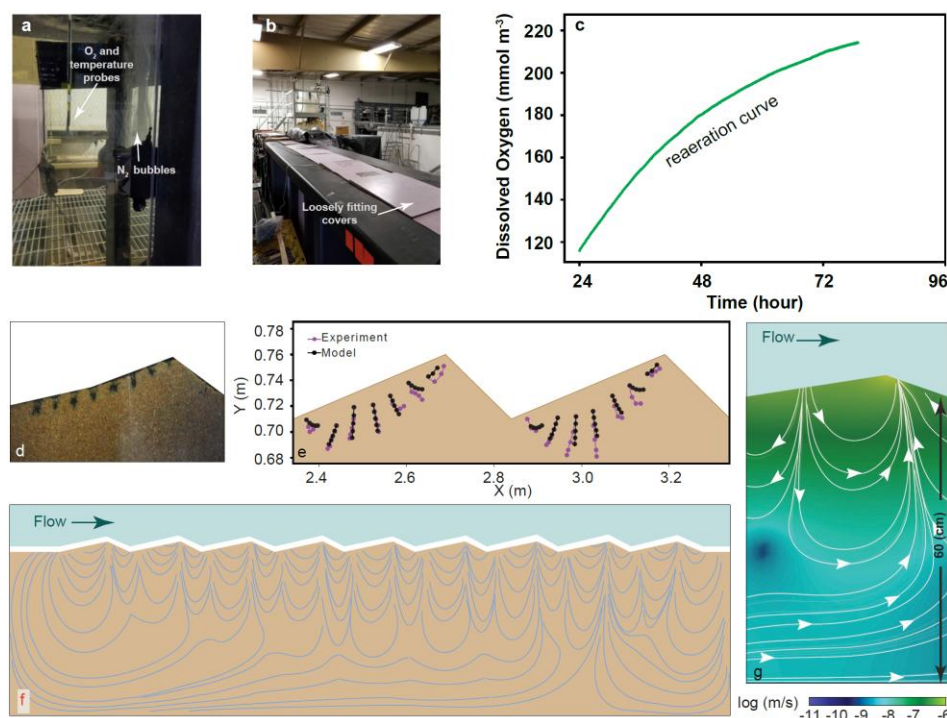


Figure 7: Results and experiments showing fluxes across the air-water interface (top) and sediment-water interface (bottom), and hyporheic exchange flow. The top row (c) shows results of oxygen re-aeration experiments in the channel. The middle row shows dye injection flow paths (d) compared with modeled flow paths (e). The bottom row and lower right image show modeled streamlines (f) and Darcy velocity field (g).

Under the assumption that the channel and HZ had different limitations where the channel was C-limited, and considering that leaf leachate is deficient in nutrients like nitrogen (N) and phosphorus (P) but rich in C-based organic compounds (Webster et al., 2016), introduction of a limiting compound such as C to the channel can explain rapid C uptake leading to prompt decrease of DO and increase in CO_{2(aq)}. Therefore, additional infusion of C into the already C-rich HZ may not result in noticeable increase in DO uptake. The OM addition also resulted in a drop in DO in the channel. This lower-DO water enters the HZ which in turn would result in lower AR. This is particularly important because DO might be the limiting reactant given that C is unlikely limiting in this HZ as previously shown by Turețcaia et al. (2023).

We additionally note that the repeat POM experiments (POM_{200A} and POM_{200B}) showed comparable results for DO uptake and CO_{2(aq)} increase observed in the channel but not in the HZ (Figs. 2, 4, and 5). This result also supports the hypothesis that the channel and the HZ likely had different biochemical conditions, where AR in the channel was limited by C supply, and that the additional POM loading did not overcome this limitation. Moreover, the comparable outcomes for replicate experiments for the channel but not for the HZ could have been influenced by the experimental set-up. The POM perturbations were conducted back-to-back, where the leaves were added to the flume channel without resetting the flume water and sand to pre-



experimental conditions, i.e., before POM insertion experiments began. Moreover, the addition of the next bag of leaves followed the return of channel DO and $\text{CO}_{2(\text{aq})}$ concentrations to conditions prior to the POM perturbation. Therefore, while the channel chemical conditions returned to the pre-perturbation state, the conditions in the HZ were still equilibrating with and responding to the OM perturbation, and thus the initial DO and $\text{CO}_{2(\text{aq})}$ concentrations for POM_{200B} and POM_{200A} were different (Fig. 2).

Despite the muted DO dynamics in the HZ as a result of channel OM loading, $\text{CO}_{2(\text{aq})}$ in the HZ was consistently and persistently higher than in the channel and increased with depth (Fig. 4). The highest $\text{CO}_{2(\text{aq})}$ (18,000 ppmV) was observed at 30 cm and 50 cm depths during the POM_{200A} experiment. Considering that POM additions induced relatively small fluctuations in DO in the HZ, and that DO was higher in the top 25 cm of the HZ (Figs. 4, 6, and 7), the elevated $\text{CO}_{2(\text{aq})}$ below 25 cm depth suggests other CO_2 sources, such as anaerobic respiration (e.g., denitrification).

If the HZ responded strongly to POM additions, one would expect pronounced geochemical differences going from downwelling to upwelling zones. This was clearly not the case. The statistical comparison showed that the DW and UW area DOC and CO_2 were statistically indistinguishable. This is also apparent when looking at all the data together in boxplots (Fig. B2). A broader analysis of the geochemical data (i.e., the data in Table C6) using principal component analysis further showed how similar the DW and UW areas were, and how both were distinct compared with the channel (Fig. B3).

The *hyporheicFoam* flow model results and dye tracing imply small fluxes across the sediment-water interface into and out of the HZ. This further illustrates and supports weak coupling and feedback between the HZ and the channel. Any changes in the channel barely propagated into the HZ as noted above, but it also means that any reaction products in the HZ such as CO_2 were not being released into the channel substantially. On the other hand, the channel was always connected to the atmosphere. On-/off-gassing was relatively still efficient despite the quiescent channel flow and atmospheric conditions, as shown by the results of the sparging-re-aeration experiments (Fig. 7c). Thus, while the HZ has weak coupling with the channel, the addition of POM in the channel should have led to increased DO fluxes from and CO_2 fluxes to the ambient air.

Other factors like absence of POM in the sediment, sorption, temperature differences between the channel and the HZ, and weak exchange between the channel and the HZ spatial domains could have contributed to variability in inferred AR across the experiments in particular for the HZ. The presence of POM in the HZ can focus biochemical reactions and serve as hot spots for microbial metabolism (Burrows et al., 2017). Considering that the flume sediment contained no buried POM and that the main source of OM into the HZ was from resident water and from leaf leachate supplied through the channel, the flume's HZ is likely different to most natural conditions. This suggests that natural processes like burial and collapse of riverbanks can contribute to the function of the HZs as 'hot spots' for biochemical reactions. However, when POM is introduced to the channel, it would lower or deplete DO in the channel, and when this water enters the HZ, oxygen would become the limiting reactant, resulting in low AR in the HZ.

Temperature affects reaction rates, and dynamic heat transport had been studied in the same flume before (Norman and Cardenas, 2014). For this experiment however, the temperature difference between the channel and the HZ was $\sim 1.5^\circ\text{C}$ throughout all the POM experiments, with lower temperatures always present in the HZ (channel temperature ranged from 19-



22 °C; HZ average temperature ranged 19-21 °C). The slightly lower and varying temperatures in the HZ could decrease biogeochemical rates but this effect would be small considering the ~2°C range. The muted HZ response to POM is more likely due to limited hydrologic exchange.

5 Conceptual Models for Hyporheic Zone Carbon Source-Sink Functions

425 We initially hypothesized the conceptual model that when geochemical conditions in the channel change, specifically when POM/DOC increases, the HZ would react quickly and turn into a hot spot for AR (Fig. 8a). CO₂ would increase throughout the HZ in this scenario as the CO₂ produced in downwelling zones flows across the HZ towards the upwelling zones. Thus, while the CO₂-rich portion of the HZ enlarges, fluxes into the channel also increase because of the elevated CO₂ and because of stronger advection. This would feedback with the channel and increase CO₂ in the channel as well as evasion. This is
430 potentially the case when hyporheic exchange is robust. Our experimental conditions revealed a different scenario and conceptual endmember. One that has muted hyporheic exchange and flow. Under such weakly coupled conditions, a dramatic geochemical change in the channel barely affected the HZ. There were changes in the HZ but they were delayed, muted, and did not feedback with the channel (Fig. 8b). In terms of C cycling, the HZ barely produced additional CO₂ and its capacity to store more CO₂ remained high. It is worth noting that the CO₂ remained high across the HZ through all experiments, typically
435 around 3,000-10,000 ppmV. Thus, there is clear potential for the HZ to be a CO₂ source for the channel and eventually the atmosphere. Yet, we found that under low-flow, high channel DOC conditions, this was not the case. Much of the CO₂ remained within the HZ and its production was not enhanced by the channel DOC loading.

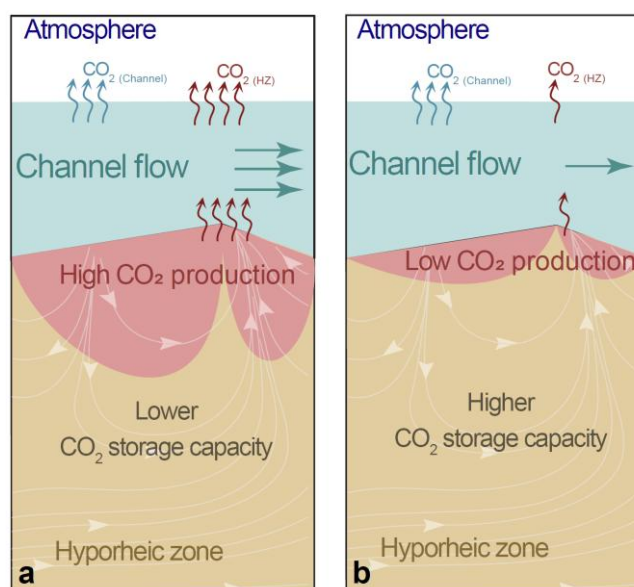


Figure 8: Conceptual model for the hyporheic zone sink-source functions for CO₂ under different river hydraulic conditions: (a) fast stream flow and (b) slow stream flow. (a) exhibits stronger coupling between the hyporheic zone and the channel due to faster flow in the channel. In (a), the hyporheic zone responds to changes in the channel, such as increases in dissolved organic carbon and oxygen content which enters the bed, leading to higher hyporheic zone CO₂ production. CO₂ efflux from the hyporheic zone to the channel is high in (a) and this CO₂ contributes significantly to evasion. (b) shows the hyporheic zone as mostly storing carbon dioxide (CO₂) with small efflux from the hyporheic zone to the channel, i.e., weak coupling between the channel and hyporheic zone. In (b), evasion is dominated by CO₂ produced in the channel.

6 Summary and Conclusions

Many studies have noted that the hyporheic zone is a hot spot for biochemical reactions in river corridors, yet the potential for a diminished role for the hyporheic zone in organic matter cycling has seldom been considered. Understanding the mechanisms that lead hyporheic zones to function as either a hot or cold spot for biochemical reactions can help improve predictive models for changes in water quality and CO₂ efflux from rivers under changing environmental conditions. In a set of flume experiments where organic matter was periodically added to a flume channel while employing relatively high spatiotemporal resolution mapping of dissolved oxygen, dissolved CO₂, and pH monitoring, our study found that the channel dominates aerobic respiration of organic matter. Under conditions of restricted exchange between the channel and the hyporheic zone and elevated aerobic respiration rates in the channel which depleted channel dissolved oxygen, the broad hyporheic zone functioned as a



460 relatively cold spot with only a narrow, very shallow zone showing significant response. Although when viewed internally and
 separately from the channel, reactions and changes are clearly happening in the hyporheic zone, they are likely too small
 relative to internal changes that happened in the channel to produce any feedback. Despite the limitations and controlled
 conditions, this experimental study resulted in the rare glimpse of the hyporheic zone as a relatively cold spot during organic
 matter addition to the river-hyporheic zone system. The channel, on the other hand, was the hot spot even under relatively low
 channel flow conditions. This perspective should also be considered in the hydro-biogeochemical analysis of river corridors.
 465 The source-sink (or storage) function of the hyporheic zone as part of the river corridor is strongly dependent on hydrologic
 conditions.

Appendix A: Porewater Sampling Method

Porewater within the hyporheic zone were extracted at target depths using a custom-fabricated mini piezometer shown in Fig.
 A1 below.

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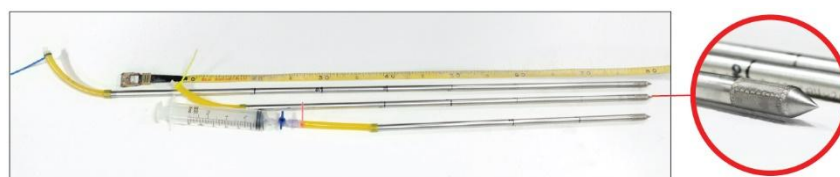


Figure A1: Mini piezometers with attached syringe for porewater sampling. The piezometers had a 2 cm porewater extraction
 screen covered with a fine steel mesh. The different length piezometers were used to access different sampling depths and
 minimize the sampling volume for a better representation of localized geochemical conditions.

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Appendix B: Results of Statistical Analysis of Geochemical Observations

The channel and porewater geochemistry were further analyzed to reveal differences, if any. These results are presented in
 Figs. B1 and B2.

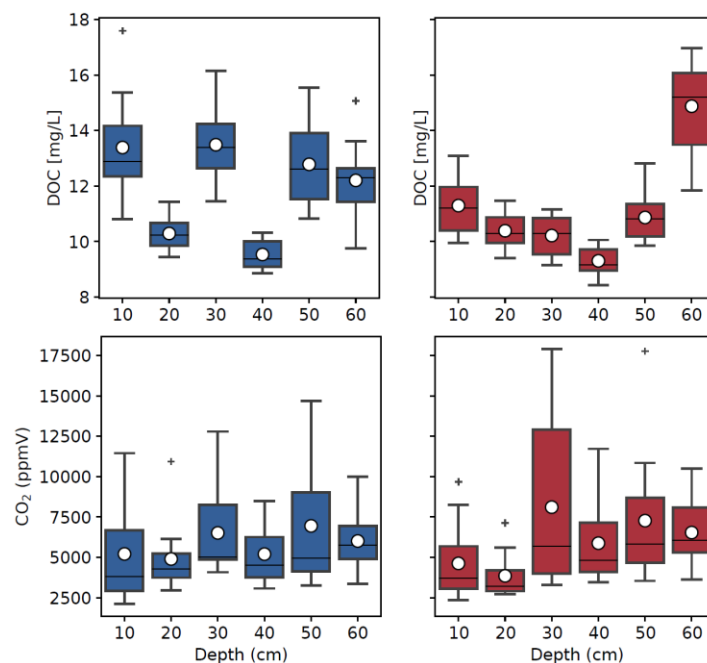


Figure B1: Dissolved organic carbon and carbon dioxide in porewaters from different depths in the hyporheic zone downwelling (left column, blue) and upwelling (right column, red) areas. The boxplots with quartiles and means (circles) include samples across all experiments, i.e., including all the data from Fig. 5.

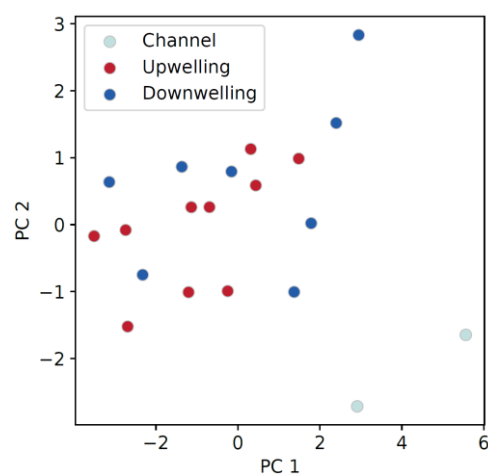


Figure B2: Results of principal component analysis (PCA) of geochemistry (cations, anions, pH) of the channel and the hyporheic zone, separated between upwelling and downwelling areas.



Appendix C: Geochemistry of the Sediment Used in the Flume and Water Samples and Results of Geochemical Modeling

Table C1: Manufacturer's specification of the sand (Pioneer Sands Industrial Sand Products, Brady, TX) used in the flume experiments.

Typical Physical Properties	
Mineral	Quartz
Roundness/Sphericity	0.6+
Acid Solubility	< 0.045%
Hardness	7.0
Specific Gravity	2.65
LOI (Loss on Ignition)	0.1%
Typical Geochemical Analysis (%)	
SiO ₂	99.48
Fe ₂ O ₃	0.06
Al ₂ O ₃	0.21
TiO ₂	< 0.01
CaO	< 0.01
MgO	< 0.01

Table C2: Dissolved organic carbon concentration in the flume channel and HZ during flume inoculation period.

Date	Channel DOC (mg- C/L)	DOC (mg-C/L) in DW						DOC (mg-C/L) in UW					
		10 cm	20 cm	30 cm	40 cm	50 cm	60 cm	10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
08/09/2019	10.09	NA	30.21	NA	9.25	NA	18.68	NA	NA	NA	NA	NA	NA
8/11/2019	10.13	NA	24.35	NA	17.98	NA	35.05	NA	NA	NA	NA	NA	NA
8/14/2019	10.99	NA	20.33	NA	12.25	NA	26.97	NA	NA	NA	NA	NA	NA
9/17/2019_bf4	8.95	13.14	9.34	13.93	9.43	14.21	14.28	11.95	8.25	11.21	8.23	10.46	11.96
9/17/2019_bf5	9.27	11.59	9.70	10.89	9.49	9.24	17.02	10.49	8.27	11.61	13.48	14.90	19.64
9/18/2019_bf6	9.77	14.53	10.69	15.89	9.52	14.25	12.51	12.15	10.25	11.15	12.79	13.34	18.50

*Note: bf4, bf5 and bf6 notation in 'Date' column indicates the flume bedform where the porewater samples were collected from. Bedform bf4 was the most upstream.

Table C3: Spatiotemporal flume water chemistry: POM_{200A} experiment. Concentrations of dissolved organic carbon (DOC) in mg-C L⁻¹, dissolved inorganic carbon (DIC) in mg-C L⁻¹, bicarbonate ion in mg L⁻¹ obtained through titration, and pH collected from the flume channel, the downwelling (DW), and the upwelling (UW) parts of the bedform.



Parameter	Channel	Depth at DW						Depth at UW					
		10 cm	20 cm	30 cm	40 cm	50 cm	60 cm	10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
Date 10/02/2019; Experimental hour 0													
<i>pH</i>	8.09	7.69	7.85	7.72	7.71	7.28	7.62	7.64	7.64	7.23	7.62	7.20	7.54
<i>DOC</i>	9.71	12.35	10.22	15.53	9.44	14.06	11.08	10.75	10.01	11.12	8.85	11.37	1.06
<i>DIC</i>	60.43	61.19	61.39	60.60	60.32	59.93	66.00	60.96	60.95	60.65	60.13	60.66	61.99
<i>HCO₃⁻</i>	341.8	333.4	374.6	327.0	322.8	327.0	365.0	331.2	360.8	344.7	330.9	329.1	324.9
Date 10/03/2019; Experimental hour 24													
<i>pH</i>	8.06	7.44	7.70	7.66	7.51	7.37	7.44	7.53	7.87	7.35	7.46	7.54	7.46
<i>DOC</i>	10.39	13.89	9.78	14.34	8.94	15.55	12.39	10.07	9.40	10.22	8.42	9.95	13.32
<i>DIC</i>	62.03	61.12	61.51	60.85	60.52	60.74	65.98	60.85	61.07	60.80	60.48	60.90	62.10
<i>HCO₃⁻</i>	344.8	335.5	356.6	NA	320.7	324.9	320.7	321.7	329.1	356.6	321.7	320.7	353.0
Date: 10/04/2019; Experimental hour 50													
<i>pH</i>	8.01	7.52	7.75	7.51	7.53	7.31	7.71	7.72	7.93	7.33	7.37	7.49	7.49
<i>DOC</i>	10.30	10.80	9.88	13.48	9.24	13.57	12.21	10.30	12.28	10.74	9.11	10.79	13.40
<i>DIC</i>	62.57	62.62	61.67	61.06	60.79	61.22	65.31	61.66	61.42	61.51	60.91	61.65	62.63
<i>HCO₃⁻</i>	343.2	344.7	327.0	305.0	320.7	327.0	343.9	325.9	325.9	322.8	320.7	327.0	320.7
Date: 10/06/2019; Experimental hour 89													
<i>pH</i>	8.12	7.41	7.42	7.34	7.58	7.55	7.15	7.52	7.72	7.27	7.54	7.52	7.54
<i>DOC</i>	10.26	12.81	9.45	12.41	8.85	12.26	12.43	9.95	9.98	9.19	8.79	9.86	11.84
<i>DIC</i>	58.53	59.03	57.88	56.82	57.11	57.63	61.45	57.95	58.33	56.87	56.87	57.26	58.47
<i>HCO₃⁻</i>	367.6	341.9	335.5	528.2	318.6	336.2	377.3	371.9	343.3	328.6	335.8	332.2	339.3

Table C4: Spatiotemporal flume water chemistry: POM_{200B} experiment. Concentrations of dissolved organic carbon (DOC) in mg-C L⁻¹, dissolved inorganic carbon (DIC) in mg-C L⁻¹, bicarbonate ion in mg L⁻¹ obtained through titration, and pH collected from the flume channel, the downwelling (DW), and the upwelling (UW) parts of the bedform.

Parameter	Channel	Depth at DW						Depth at UW					
		10 cm	20 cm	30 cm	40 cm	50 cm	60 cm	10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
Date: 10/12/2019; Experimental hour 0													
<i>pH</i>	8.14	7.73	7.64	7.46	7.63	7.67	7.51	7.79	7.71	7.56	7.69	7.63	7.63
<i>DOC</i>	11.10	11.46	10.23	16.15	10.32	11.02	11.72	11.20	9.86	11.15	9.51	10.98	15.91
<i>DIC</i>	62.56	62.96	62.50	61.94	62.19	62.75	66.50	62.61	62.13	61.47	61.97	62.68	63.63
<i>HCO₃⁻</i>	347.0	337.6	327.0	319.6	356.6	378.1	361.4	328.0	355.1	322.8	349.6	358.7	351.0
Date: 10/13/2019; Experimental hour 24													
<i>pH</i>	8.12	8.11	7.83	7.47	7.85	7.84	7.89	8.01	7.97	7.82	7.86	7.83	7.79
<i>DOC</i>	10.16	12.66	9.75	12.89	9.00	12.23	10.92	10.44	9.55	9.15	9.01	10.22	13.52
<i>DIC</i>	65.88	65.00	64.66	63.98	64.18	64.63	68.09	64.42	64.61	63.48	64.07	64.52	65.45



HCO_3^-	354.6	339.7	340.5	356.9	323.8	329.1	356.6	341.8	321.7	356.6	351.0	331.3	355.1
Date: 10/15/2019; Experimental hour 64													
<i>pH</i>	8.10	7.94	7.91	7.72	7.78	7.75	7.72	7.83	7.83	7.62	7.80	7.37	7.63
<i>DOC</i>	10.72	12.36	9.92	11.45	9.29	11.40	12.40	11.21	10.30	9.66	9.22	10.85	16.97
<i>DIC</i>	64.07	64.03	62.91	62.27	62.53	63.08	67.24	62.73	62.94	62.08	62.79	63.53	64.20
HCO_3^-	363.7	355.1	329.1	324.9	331.3	335.5	353.0	331.7	355.1	329.1	329.1	358.6	343.9
Date: 10/20/2019; Experimental hour 193													
<i>pH</i>	8.14	8.03	7.87	7.73	7.86	7.81	7.66	7.93	8.01	7.84	7.80	7.74	7.71
<i>DOC</i>	11.32	17.59	10.64	12.72	10.18	14.09	15.07	13.08	11.47	11.14	10.06	12.81	16.00
<i>DIC</i>	64.68	65.44	64.44	63.98	64.06	64.54	69.94	64.77	65.29	64.13	63.8	64.35	65.74
HCO_3^-	313.7	309.3	291.9	302.9	297.7	290.1	327.9	312.1	312.1	305.2	303.5	298.9	297.1

Table C5: Spatiotemporal flume water chemistry: POM₃₀₀ experiment. Concentrations of dissolved organic carbon (DOC) in 510 mg-C L⁻¹, dissolved inorganic carbon (DIC) in mg-C L⁻¹, bicarbonate ion in mg L⁻¹ obtained through titration, and pH collected from the flume channel, the downwelling (DW), and the upwelling (UW) parts of the bedform.

Parameter	Channel	Depth at DW						Depth at UW					
		10 cm	20 cm	30 cm	40 cm	50 cm	60 cm	10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
Date: 10/22/2019; Experimental hour 24													
<i>pH</i>	7.91	7.92	7.80	7.73	7.76	7.75	7.70	7.94	7.96	7.77	7.78	7.71	7.68
<i>DOC</i>	11.64	15.00	10.43	13.65	9.87	12.97	13.29	11.79	10.78	10.18	9.61	10.40	16.26
<i>DIC</i>	67.46	65.87	65.31	64.65	64.76	65.55	70.02	65.28	65.45	64.28	64.35	64.99	66.48
<i>HCO₃⁻</i>	314.6	307.6	298.3	297.6	296.5	303.5	327.9	305.3	306.4	297.7	299.4	307.0	308.7
Date: 10/24/2019; Experimental hour 70													
<i>pH</i>	8.06	7.80	7.84	7.82	7.79	7.70	7.69	7.88	7.92	7.68	7.68	7.65	7.63
<i>DOC</i>	11.69	15.38	11.42	13.29	10.18	13.86	13.62	12.88	11.17	10.45	10.04	11.76	15.33
<i>DIC</i>	62.49	63.32	61.73	60.27	60.25	61.00	64.81	61.70	61.14	59.90	60.14	60.39	61.61
<i>HCO₃⁻</i>	310.7	315.3	301.2	313.0	305.8	309.3	312.8	305.3	309.3	297.7	301.3	304.6	300.0
Date: 10/26/2019; Experimental hour 119													
<i>pH</i>	8.21	8.09	7.96	7.82	7.95	7.89	7.91	8.06	7.99	7.91	7.89	7.88	7.87
<i>DOC</i>	10.66	13.38	10.77	11.73	9.13	10.82	9.76	11.38	10.44	9.18	8.99	10.05	14.46
<i>DIC</i>	66.86	67.44	67.42	65.36	65.23	66.51	66.93	66.08	67.86	64.48	64.71	65.07	66.48
<i>HCO₃⁻</i>	310.3	317.1	331.5	319.2	304.6	286.6	295.9	302.6	302.2	296.6	300.4	292.5	305.8
Date: 10/30/2019; Experimental hour 188													
<i>pH</i>	8.16	7.94	7.79	7.76	7.85	7.90	7.79	8.01	7.96	7.81	7.83	7.84	7.71
<i>DOC</i>	11.42	12.96	10.97	14.21	9.95	11.57	11.55	12.48	11.42	10.38	10.01	11.35	16.45
<i>DIC</i>	64.90	65.38	65.39	64.01	63.90	64.31	68.08	64.95	65.74	63.99	64.05	65.45	66.12
<i>HCO₃⁻</i>	310.7	315.0	308.7	293.5	299.4	299.6	329.7	317.5	316.9	299.4	279.4	315.1	301.8



Table C6: Flume porewater and channel water chemistry and saturation indices (SI) with respect to aragonite, calcite, and gypsum calculated using the geochemical modeling software PHREEQC.

Parameter	Channel	Porewater depth (Downwelling side of the bedform)						Porewater depth (Upwelling side of the bedform)					
		10 cm	20 cm	30 cm	40 cm	50 cm	60 cm	10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
<i>pH</i>	8.34	7.69	7.85	7.72	7.71	7.28	7.62	7.64	7.64	7.23	7.62	7.2	7.54
	8.18	7.44	7.70	7.66	7.51	7.37	7.44	7.53	NA	7.35	7.46	7.54	7.46
<i>Temperature (C)</i>	21.74	21.24	21.07	20.09	20.83	20.82	20.81	21.24	21.07	20.9	20.83	20.82	20.81
	21.93	21.38	21.17	21.0	20.83	20.9	20.9	21.41	21.17	21.0	20.83	20.9	20.95
<i>Ca²⁺ (mg/L)</i>	101.4	100.1	99.8	97.6	97.4	97.5	100	98.7	99.2	97.9	97.3	97.7	97.9
	103.4	100.9	99.9	98.2	97.7	98.9	100.9	100.1	100.5	99.5	98.3	98.5	99.4
<i>Mg²⁺ (mg/L)</i>	30.8	30.3	30.2	29.5	29.4	29.4	30.4	29.9	30.0	29.6	29.4	29.5	29.5
	31.2	30.5	30.2	29.7	29.6	29.9	30.5	30.4	30.5	30.1	29.7	29.7	30.0
<i>Na⁺ (mg/L)</i>	41.1	40.7	40.5	39.5	39.6	39.8	40.7	39.9	40.4	39.8	39.6	39.7	39.6
	41.3	40.7	40.2	39.7	39.7	40.1	40.7	40.5	40.8	40.4	39.8	39.9	40.1
<i>K⁺ (mg/L)</i>	9.7	9.6	9.6	9.5	9.4	9.4	9.5	9.4	9.5	9.5	9.5	9.5	9.5
	9.8	9.6	9.6	9.5	9.4	9.5	9.6	9.5	9.6	9.5	9.5	9.5	9.6
<i>SO₄²⁻ (mg/L)</i>	67.1	70.1	68.8	67.0	69.9	66.6	66.8	65.7	67.0	65.6	64.9	64.9	65.9
	70.3	72.0	71.4	70.1	69.9	70.5	70.9	70.9	71.3	70.2	68.9	68.7	68.9
<i>NO₃⁻ (mg/L)</i>	19.1	18.1	18.9	16.7	18.71	16.1	3.2	16.2	17.9	16.6	18.7	16.5	15.2
	19.1	17.7	19.3	16.9	20.10	18.1	4.2	18.2	19.5	18.6	19.8	17.7	14.8
<i>Cl⁻ (mg/L)</i>	48.9	51.3	50.3	49.1	48.9	48.9	49.2	48.2	48.7	47.8	47.3	47.2	47.9
	51.5	53.1	52.6	51.7	51.6	51.9	52.5	52.4	52.3	51.6	50.3	50.3	50.2
<i>HCO₃⁻ (mg/L)</i> (speciation. in PHREEQC)	301.4	298.2	302.4	295.9	294.4	275.5	319.5	295.7	295.6	275.6	291.1	273.7	297.4
	308.5	289.3	299.9	295.7	289.3	284.2	312.3	291.6	301.2	283.3	287.1	292.2	294.8
<i>HCO₃⁻ (mg/L)</i> (titration)	280.2	273.3	307.1	268.1	264.6	268.1	299.2	271.5	295.7	290.5	271.2	269.8	266.3
	282.7	274.9	292.3	NA	262.9	266.3	262.9	263.7	269.8	292.8	263.7	262.9	289.4
<i>DIC (mg-C/L)</i>	60.2	61.2	61.4	60.6	60.3	59.93	66.00	60.9	60.9	60.6	60.1	60.7	61.9
	62.0	61.1	61.5	60.8	60.5	60.74	65.98	60.8	61.1	60.8	60.5	60.9	62.1
<i>Charge Balance (% error)</i>	-1.89	3.29	2.25	3.42	2.63	7.68	9.10	4.77	4.13	8.28	3.78	8.81	5.01
	-1.73	5.02	2.01	3.00	2.61	5.05	9.11	4.10	NA	5.43	3.75	3.97	5.78
<i>SI_{Calcite}</i>	1.22	0.56	0.73	0.57	0.57	0.09	0.52	0.52	0.51	0.04	0.47	-0.00	0.40
	1.09	0.30	0.58	0.52	0.35	0.20	0.33	0.39	NA	0.18	0.30	0.39	0.33
<i>SI_{Aragonite}</i>	1.08	0.42	0.58	0.42	0.42	-0.06	0.38	0.37	0.36	-0.11	0.32	-0.15	0.25
	0.94	0.15	0.43	0.37	0.20	0.05	0.18	0.25	NA	0.04	0.15	0.24	0.19
<i>SI_{Gypsum}</i>	-1.72	-1.69	-1.70	-1.71	-1.71	-1.71	-1.70	-1.72	-1.71	-1.72	-1.73	-1.72	-1.72
	-1.69	-1.67	-1.68	-1.69	-1.69	-1.69	-1.68	-1.68	NA	-1.69	-1.70	-1.70	-1.69



***Note:** Data collected on 10/02/2019 is shown in the top rows, and data collected on 10/03/2019 is shown in the bottom rows for each water chemistry parameter. Charge balances exceeding a 5% error are marked in red.

Data Availability

The dataset will be archived and made publicly available on Figshare once the manuscript is accepted for publication. The supporting data repository is currently set to private. We provide this secure access link (<https://figshare.com/s/a8610b60611cc47b7805>) exclusively for the editorial team to distribute to the reviewers. The data and link will be fully made public upon acceptance and publication.

Author contributions

Conceptualization: ABT, XC, XL, JCS, and MBC; Data curation: ABT; Formal analysis: ABT, BL; Funding acquisition: MBC, JCS, XC, and XL; Investigation: ABT with all authors; Methodology: MHK and AJK; Project administration: MBC, JCS, XC, and XL; Resources: PCB; Software: BL and XL; Supervision: MBC, JCS, XC, and XL; Visualization: ABT and BL; Writing (original draft and preparation): ABT; Writing (review and editing): All authors.

Competing interests

The contact author declares no competing interests on behalf of all co-authors.

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