JGR Biogeosciences

RESEARCH ARTICLE
10.1029/2021JG006610

Integrating Perspectives on Dissolved Organic Carbon Removal and Whole-Stream Metabolism

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Key Points:

- Water column biological assays underestimate organic carbon (OC) removal in streams by 10–100 times compared to whole-stream metabolism
- Simulation estimates of terrestrial OC inputs to streams differ depending on OC removal methods used
- Whole-stream metabolism and carbon spiraling metrics provide an integrated assessment of the role of streams in landscape carbon cycling

Supporting Information:

Supporting Information may be found in the online version of this article.

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Citation:

Plont, S., Riney, J., & Hotchkiss, E. R. (2022). Integrating perspectives on dissolved organic carbon removal and whole-stream metabolism. *Journal of Geophysical Research: Biogeosciences*, *127*, e2021JG006610. https://doi.org/10.1029/2021JG006610

Received 3 SEP 2021
Accepted 14 FEB 2022

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Abstract Quantifying organic carbon (OC) removal in streams is needed to integrate the functional role of inland waters into landscape carbon budgets. To illustrate how in-stream OC removal measurements can be used to characterize ecosystem and landscape carbon fluxes, we compared two common methods: (a) Bioassays measuring water column dissolved organic carbon (DOC) uptake and (b) daily rates of whole-stream metabolism and OC spiraling calculated from fluorescent dissolved organic matter, oxygen, and discharge measurements. We then assessed how OC removal rates from these two methods, measured in two low-productivity heterotrophic streams, affected estimates of terrestrial OC loading and export using a mass balance model. OC mineralization velocities calculated from whole-stream metabolism (0.06 ± 0.03 m d^{-1} (mean ± SD)) were greater than water column bioassay DOC uptake velocities (0.01 ± 0.01 m d^{-1}), which resulted in higher in-stream OC removal estimates (0.5%–15.2% and 0.02%–4.2% removal for whole-stream metabolism and bioassays, respectively). Furthermore, the terrestrial OC inputs needed to sustain in-stream OC concentrations differ among methods, with simulated inputs ranging from 79 to 1,300 or 3–350 g OC d^{-1} for whole-stream metabolism or bioassays, respectively. We show how in-stream OC removal can be used to quantify terrestrial-aquatic linkages by estimating OC inputs needed to fuel whole-stream metabolism in low-productivity streams, and offer future directions to better link OC removal with whole-ecosystem OC budgets. Without appropriate conversions to whole-stream processes, bioassays systematically underestimate whole-stream carbon cycling. By integrating whole-stream metabolism with OC transport, we can better elucidate the role of running waters in landscape carbon budgets and the global carbon cycle.

Plain Language Summary Streams play an important role in landscapes by moving carbon from land to downstream ecosystems and by removing carbon through in-stream biological activity. However, this connection between streams and the surrounding landscape is often left out of carbon budgets. To show how carbon removal in streams can be used to characterize carbon cycling across landscapes, we compared organic carbon (OC) removal estimated from two methods: (a) Bioassays measuring dissolved OC removal in the water column and (b) whole-stream metabolism (i.e., how much carbon is photosynthesized and respired daily by all organisms living in the stream). We then compared how different in-stream OC removal methods affected estimates of how much OC is loaded into the stream from the landscape. We show that bioassays underestimate rates of stream carbon cycling by up to two orders of magnitude (compared to whole-stream metabolism), resulting in lower estimates of in-stream OC removal and terrestrial OC loading. We show how in-stream measurements of carbon cycling can be used to estimate how much OC must enter streams from the terrestrial landscape. By pairing whole-stream metabolism with OC transport, we can understand how carbon moves into and cycles through running waters, highlighting the role of streams in landscape carbon cycling.

1. Introduction

Inland waters are active sites of carbon cycling; they process, store, and emit more than half of the carbon (C) they receive as terrestrial inputs (Battin et al., 2009; Butman et al., 2018; Cole et al., 2007). An estimated 5.1 Pg C is loaded into inland waters from terrestrial landscapes every year, which can comprise anywhere from 12% to 34% of terrestrial net ecosystem productivity when scaled to global surface area (Butman et al., 2015; Drake et al., 2018). The difference between estimates of terrestrial C loading into and export from inland waters to the ocean (0.9 Pg C yr^{-1} export) highlights the active role inland waters play in removing C (4.2 Pg C yr^{-1} stored or emitted) (Butman et al., 2015; Drake et al., 2018). Regional and global C budgets and earth system models, however, often exclude the loss of C from land to aquatic ecosystems. Including freshwater C fluxes and cycling in global C budgets requires estimates of freshwater ecosystem metabolism as well as metabolism-informed calculations of C fluxes to inland waters from the surrounding landscape (Hotchkiss et al., 2015; Vachon et al., 2021).

Streams and rivers are tightly linked to the terrestrial landscapes that drain into them. Most streams and rivers are heterotrophic, and rely on terrestrially-derived organic C (OC) inputs to support in-stream metabolism (Hall & Hotchkiss, 2017). Heterotrophic metabolism in streams removes and respires OC that might otherwise be transported downstream (Demars, 2018; Raymond et al., 2016) and metabolism of terrestrially-derived OC may account for 30% or more of CO_2 emissions from streams and rivers (Hotchkiss et al., 2015; Rocher-Ros et al., 2021). While our knowledge of the sources, transport, and transformation of OC across freshwater networks continues to expand (e.g., Casas-Ruiz et al., 2017; Creed et al., 2015; Hosen et al., 2021), few studies have quantitatively linked terrestrial OC inputs or transport with in-stream OC removal. Linking terrestrial OC inputs to streams with in-stream OC removal is paramount to elucidate the role of streams in landscape C budgets (Butman et al., 2018; Mineau et al., 2016).

Biotic OC removal in streams is most commonly estimated using one of two methods: biological assays ("bioassays," hereafter) and whole-stream metabolism. Bioassays (e.g., in vitro incubations of water samples) are often used to measure DOC biodegradability in the water column as a function of microbial DOC uptake using reproducible and standardized methods that allow for comparisons across different OC sources and ecosystem types (Guillemette & del Giorgio, 2011; Wologo et al., 2021). By measuring DOC uptake in a more controlled setting, bioassays can provide insight into how different environmental drivers such as nutrient concentrations, organic matter source, and temperature affect microbial DOC metabolism rates (Kelso et al., 2020; Mineau et al., 2016). While bioassay methods are limited in their scope and ability to represent in-stream conditions (e.g., they exclude benthic activity and fresh OC inputs of different forms and sources), DOC uptake rates derived from bioassays are still often extrapolated and used in whole-ecosystem assessments of C cycling, fate, and transport (e.g., Catalán et al., 2016; Kaplan et al., 2006; Raymond et al., 2016). By excluding bioreactive benthic areas and constraining the OC pool to ambient water column DOC, applications of bioassay-derived OC removal rates underestimate how much OC is delivered to and processed within streams. Comparisons between bioassay-derived and in situ rates of OC removal in streams are lacking, and understanding the magnitude of method-driven discrepancies in OC removal rates is needed to improve representation of the role of running waters in landscape OC budgets.

Compared to bioassay methods, whole-stream metabolism provides a more holistic, in situ measure of ecosystem C cycling. Whole-stream metabolism (i.e., gross primary production and ecosystem respiration (ER); GPP and ER) can also be used to assess OC removal via mineralization in streams (Hall et al., 2016). ER is an integrative daily measure of how much autochthonous and allochthonous OC is mineralized along a stream reach (Hall & Hotchkiss, 2017) and can be used to calculate OC mineralization relative to OC transport, which is the OC spiraling length (i.e., the distance traveled by OC before mineralization to CO_2; Hall et al., 2016; Newbold et al., 1982). Cross-site and seasonal comparisons of whole-stream metabolism and OC spiraling lengths found that in-stream OC removal is dynamic across regions, seasons, and land use (Griffiths et al., 2012; Hall et al., 2016; Plont et al., 2020). However, few studies directly compare both bioassay and whole-stream metabolism derived OC removal; this has potentially led to systematic differences in scientists' understanding of the role of streams and rivers in C cycling depending on the methods we use. Indeed, methodological biases in OC removal measurements can bias terrestrial OC loading estimates and landscape C budgets (Mineau et al., 2016). Assessing differences in OC removal rates derived from bioassays and whole-stream metabolism, and how those differences influence landscape C budgets, is required to gain a more holistic understanding of how streams receive, remove, and transport OC.

Understanding how much OC is metabolized in streams is needed to constrain terrestrial OC export estimates and integrate streams and rivers into landscape C budgets. Using OC removal measured with two common methods (bioassays and whole-stream metabolism), we sought to answer the following questions: (a) How different are in-stream OC removal rates calculated using water column bioassays versus whole-stream metabolism? (b) To what extent do different methods of inferring in-stream OC removal change estimates of external OC inputs to stream reaches? We predicted site-specific OC removal rates derived from whole-stream metabolism would be greater than those estimated using laboratory bioassays because ecosystem metabolism integrates highly reactive benthic areas, all sources and forms of OC (i.e., particulate organic carbon (POC) and DOC, allochthonous and autochthonous OC), and other environmental processes that bioassays exclude. Using a mass-balance modeling framework, we simulated how rates of OC removal derived from different methods influence estimates of terrestrial OC inputs to streams with low GPP and low autochthonous OC. Through applying measured OC

Table 1
Physical and Chemical Properties of Stroubles Creek and Walls Branch During the Study Period

Stream	Q ($m^3\ d^{-1}$)	$N{-}NO_3$ ($g\ m^{-3}$)	$N{-}NH_4^+$ ($g\ m^{-3}$)	SRP ($g\ m^{-3}$)	POC ($g\ m^{-3}$)	DOC ($g\ m^{-3}$)	DOC_{fdom} ($g\ m^{-3}$)
Stroubles Creek	10,187 (6,057, 12511)	1.47	0.031	0.023	0.28 (0.24, 0.32)	2.76 (2.39, 3.25)	2.70 (2.16, 3.23)
Walls Branch	2,419 (1,452, 2,635)	2.84	0.027	0.027	0.27 (0.21, 0.34)	1.70 (1.33, 2.02)	1.62 (1.26, 1.92)

Note. Discharge (Q, $m^3\ d^{-1}$) and dissolved organic carbon concentration (DOC, $g\ m^{-3}$, n = 12) are reported as mean values of all measurements over the study period in 2018. Particulate organic carbon (POC, $g\ m^{-3}$, n = 6) are reported as mean values of all measurements taken in November 2021. Nutrient concentrations ($N{-}NO_3^-$, $N{-}NH_4^+$, and soluble reactive phosphorus, SRP) are reported from samples collected at the beginning of the study period. All terms used in this table and throughout the manuscript are defined (with units) in Table 2. Values in parentheses are the minimum and maximum values observed (or DOC concentrations estimated from fluorescent dissolved organic matter sensor data, DOC_{fdom}) over the study period, excluding storm days on August 18 and August 22.

removal rates in simulated stream reaches, we highlight how whole-stream metabolism may be used to better understand in-stream OC cycling and landscape C budgets. We also offer future directions needed to use whole-stream metabolism to estimate terrestrial OC inputs and cycling in streams and link inland water C fluxes to their surrounding landscape.

2. Methods

2.1. Study Site

We conducted this study in two adjacent streams, Stroubles Creek and Walls Branch, upstream of their confluence in southwest Virginia, USA (37°11'45" N, 80°28' 58" W). Stroubles Creek is a headwater stream (Strahler order 3) draining a 25 km^2 watershed that is approximately 56% developed, 17% agricultural, and 27% forested land (Figure S1 in Supporting Information S1). Much of the developed and agricultural land in the Stroubles Creek catchment is located in the headwaters while the lower portion of the catchment leading up the Stroubles Creek-Walls Branch confluence flows through lower-density development and forested land. Walls Branch is a headwater stream (Strahler order 2) that drains a 4 km^2 watershed. Land use in the Walls Branch catchment is approximately 54% agricultural, 34% developed, and 12% forested. Both Stroubles Creek and Walls Branch have high NO_3^- concentrations (1.47 and 2.84 g N m^{-3} respectively, Table 1) and relatively low DOC concentrations (1.33–3.25 g C m^{-3}).

2.2. Sensor Deployment and Data Collection

We deployed dissolved oxygen (PME MiniDOT), water level (Onset HOBO U24 L), and fluorescent dissolved organic matter (fDOM, Turner Designs Cyclops-7F) sensors logging at 10-min intervals from 15–23 August 2018. In both Stroubles Creek and Walls Branch, we secured sensors approximately 25 m upstream of the Stroubles Creek-Walls Branch confluence. During the day on August 16 and August 22, we conducted solute pulse tracer additions of NaCl to measure velocity (u, m d^{-1}) and discharge (Q, $m^3\ d^{-1}$) along 200 m stream reaches immediately upstream of our sensor deployment sites. On these days, we also measured wetted channel width (w, m) at 15 transects along each study reach. We calculated mean stream depth (z, m) using the relationship between Q, u, and w (i.e., Q/(u*w)). We used these Q estimates, along with other Q estimates derived from tracer additions at these sites (n = 13 for Stroubles Creek and n = 10 for Walls Branch from June 2018 to June 2019) to develop site-specific Q-water level rating curves and estimate Q at 10-min intervals (Gore & Banning, 2017). Because storm events on August 18 and the night of August 22 (i.e., after Q was measured via dilution gauging earlier in the day) led to higher water levels outside the bounds of our Q-water level rating curve, we chose to exclude these 2 days from our OC spiraling calculations described below.

On four occasions throughout the sensor deployment, we collected grab samples and recorded other measurements to characterize physicochemistry (e.g., temperature, specific conductance, turbidity) at each deployment site. On August 16 and August 22, we also collected 4 grab samples at 50 m intervals for 200 m upstream of each deployment site to capture any longitudinal changes in DOC and nutrient concentrations over the study reaches. We filtered DOC samples through pre-ashed Whatman GF/F filters into acid-washed and ashed 40-mL amber borosilicate vials. After filtering, we acidified each DOC sample by adding a 2% (by volume) aliquot of 2N HCl and refrigerated them until analysis on a Elementar varioTOC Analyzer (as non-purgeable OC, NPOC).

We filtered nutrient samples through 0.22 µm PES filters into acid-washed 60-mL HDPE bottles, which we froze at −20°C until analysis. We analyzed nutrient samples for NH_4^+−N using the phenolhypochlorite method (Solorzano, 1969), NO_3^-N using the cadmium reduction method (APHA, 1998), and SRP using the ascorbic acid method (Murphy & Riley, 1962) on a Lachat Flow Injection Autoanalyzer (Quik-Chem methods 10-107-06-1-B, 10-107-04-1-A, and 10-115-01-1-B, respectively; Lachet Instruments). We used hand-held sensors to measure turbidity (NTU, Turner Designs AquaFluor) and specific conductance (µS/cm, YSI ProSolo). In November 2021, we measured both DOC and particulate OC (POC) in transport to account for POC in our OC spiraling estimates. While POC sampling occurred later than the bulk of our other analyses in 2018, DOC concentrations in 2021 were similar to those measured during 2018 (e.g., 2.42–2.56 g m^{-3} for Stroubles Creek and 1.49–1.65 g m^{-3} for Walls Branch in 2021) and turbidity was consistently low (e.g., 2–10 NTU) in Stroubles Creek and Walls Branch during both 2018 and 2021 sampling campaigns. Triplicate POC samples were collected at the upstream and downstream end of Stroubles Creek and Walls Branch reaches (n = 12 samples total). Each sample was filtered through pre-ashed and pre-weighed Whatman GF/F filters, which were then dried at 60°C for 48 hr and combusted at 550°C for two hours. Ash-free dry mass (AFDM, g) was calculated for each filter as the difference between combusted and dry mass, and we converted AFDM to OC using a 0.484 conversion factor (Griffiths et al., 2012; Thomas et al., 2005).

Over the course of the sensor deployment, we used discrete field measurements and laboratory tests of instrument-specific responses to correct our high-frequency sensor data. We corrected data from our dissolved oxygen sensors using three-point calibration tests. We deployed loggers in air-saturated calibration water in a 10 L bucket by vigorously bubbling with an aquarium pump and an airstone for the saturated calibration point. We added active dry yeast and beet sugar to the calibration bucket, which consumed oxygen for our low-oxygen calibration point. We checked the recorded oxygen concentrations of our sensors against oxygen concentrations measured by triplicate Winker titrations from saturated (bubbled), ambient stream water, and low-oxygen (yeast) calibration checks. We then corrected dissolved oxygen sensor data using sensor-specific, three-point calibration curves (low-oxygen, ambient oxygen prior to bubbling, air-saturated oxygen concentrations). We corrected the fDOM sensor data for temperature, turbidity, and light attenuation following Downing et al. (2012). Lastly, we developed site-specific DOC-fDOM rating curves using DOC concentrations from our grab samples and the recorded fDOM sensor signal at the time of sample collection. DOC-fDOM rating curves (R^2 = 0.99 and 0.74 for Stroubles Creek and Walls Branch, respectively) were then used to generate DOC concentration time series from the temperature- and turbidity-corrected fDOM data (DOC_{fdom}).

2.3. Whole-Stream Metabolism and Organic Carbon Spiraling

We used a single-station Bayesian inverse model to simultaneously estimate daily rates of gross primary production (GPP, g O_2 m^{-2} d^{-1}), ecosystem respiration (ER, g O_2 m^{-2} d^{-1}), and air-water gas exchange (K_O, d^{-1}) from sensor-derived diel O_2 in Walls Branch and Stroubles Creek using the *streamMetabolizer* R package (Appling et al., 2018). This model iteratively solves for the combination of GPP, ER, and K_O that provides the best match between measured (O, g m^{-3}) and modeled ($_mO$, g m^{-3}) dissolved oxygen data (Equation 1, Figures S2–S5 in Supporting Information S1). All parameters are defined, with units, in Table 2.

$$mO_i = mO_{i-\Delta t} + \frac{\mathrm{GPP} * \mathrm{PAR}_{i-\Delta t}}{z * \sum \mathrm{PAR}} + \frac{\mathrm{ER}}{z}\Delta t + K_O\left(O_{\mathrm{sat}(i-\Delta t)} - mO_{i-\Delta t}\right)\Delta t \quad (1)$$

Within *streamMetabolizer*, we selected the Bayesian model with both process error and observation error. Markov chain Monte Carlo (mcmc) simulations estimated unknown parameters using four mcmc chains, 500 burn-in steps, and 9500 saved steps. We modeled light (as photosynthetically active radiation, PAR, µmol m^{-2} s^{-1}) as a function of solar time, latitude, and longitude at our study sites based on methods in Yard et al. (2005). Oxygen saturation (O_{sat}, g m^{-3}) was calculated using temperature from dissolved oxygen loggers and barometric pressure from a nearby weather station (Garcia & Gordon, 1992). We visually checked model convergence using traceplot() in the *rstan* package and used the Rhat and N_eff values from fit() in the streamMetabolizer package to analyze model convergence. We established a quality-check threshold that would remove days from further analysis when Rhat values larger than 1.1, N_eff values larger than 38000 (i.e., the number of chains multiplied by the number of saved mcmc steps), or with unreasonable GPP or ER values (i.e., negative GPP or positive ER) (O'Donnell & Hotchkiss, 2019). After removing storm days that exceeded our rating curve ranges, there were no

Table 2
Definitions and Units of Terms Used in Manuscript

Term	Definition	Units
DOC	Measured dissolved organic carbon concentration	g m^{-3}
DOC_{fdom}	Dissolved organic carbon concentration estimated from fluorescent dissolved organic matter (fDOM) sensor data	g m^{-3}
Q	Discharge	m^3 d^{-1}
U	Velocity	m d^{-1}
W	Mean channel wetted width	m
Z	Modeled mean stream depth	m
O	Measured dissolved oxygen concentration	g m^{-3}
$_mO$	Modeled dissolved oxygen concentration	g m^{-3}
Δt	Measurement interval	day
GPP	Gross primary production	g O_2 m^{-2} d^{-1}
ER	Ecosystem respiration	g O_2 m^{-2} d^{-1}
K_O	Air-water gas exchange of oxygen	day^{-1}
O_{sat}	Dissolved oxygen at saturation	g m^{-3}
PAR	Photosynthetically active radiation	μmol m^{-2} d^{-1}
HR	Estimated heterotrophic respiration	g C m^{-2} d^{-1}
AR_f	Autotrophic respiration fraction	unitless
S_{OC}	OC spiraling length	m
$v_{f\text{-min}}$	OC mineralization velocity	m d^{-1}
k_{DOC}	Water column DOC uptake rate from bioassays	day^{-1}
$v_{f\text{-amb}}$	Water column DOC uptake velocity from ambient bioassays	m d^{-1}
$v_{f\text{-spk}}$	Water column DOC uptake velocity from bioassays with organic matter source added (i.e., "spiked")	m d^{-1}

additional days that needed to be removed from the data due to poor model fits (Rhat >1.1, N_eff > 38000) or unrealistic estimates of GPP, ER, and K_O (Table S2 in Supporting Information S1).

To better constrain our modeled parameter estimates, we used an informed prior for K_O. We used Equation 4 from Table 2 in Raymond et al. (2012) to calculate K_O from channel slope and u in each study reach. We then used the mean and standard deviation of K_O (from day-to-day stream-specific differences in u) to generate a normally-distributed prior for Stroubles Creek ($K_O = 3.09 \pm 1.10$ day^{-1}) and Walls Branch ($K_O = 5.01 \pm 1.10$ day^{-1}). We calculated Pearson correlation coefficients to test the degree of equifinality between modeled GPP, ER, and K_O. Posterior estimates of K_O were correlated with ER in Stroubles Creek ($r = -0.94$, $p < 0.001$, Figure S6 in Supporting Information S1) but were not correlated in Walls Branch ($r = -0.1$, $p = 0.83$, Figure S6 in Supporting Information S1). This correlation between ER and K_O outputs, as well as low rates of and variability in ER and K_O in both study sites, hinders our ability to discuss day-to-day changes in ER. However, we are confident that using constrained priors for K_O and low rates of K_O at our sites strengthened our metabolism modeling approach.

We calculated daily OC spiraling lengths (S_{OC}, m) in both of our study streams as the ratio of downstream OC transport relative to OC mineralization via heterotrophic respiration (as in Hall et al., 2016):

$$S_{OC} = \frac{Q * [\text{OC}]}{-\text{HR} * w} \quad (2)$$

We used cumulative daily Q (m^3 d^{-1}) and w (m) from stream-specific Q-w rating curves. Daily mean OC (±1 standard deviation) was calculated from the sensor DOC_{fdom} timeseries and the mean POC concentration for each stream. We estimated heterotrophic respiration (HR, g C m^{-2} d^{-1}) assuming an autotrophic respiration fraction (AR_f) of 0.44 ± 0.19 (i.e., the fraction of daily GPP respired by autotrophs) and calculated HR as in Hall and Beaulieu (2013):

$$\text{HR} = \text{ER} - (\text{AR}_f * \text{GPP}) \quad (3)$$

We also calculated OC mineralization velocity ($v_{f\text{-min}}$, m d^{-1}) as in Hall et al. (2016):

$$v_{f\text{-min}} = \frac{-\text{HR}}{[\text{OC}]} \quad (4)$$

For all metabolism and OC spiraling calculations and analysis, we converted GPP, ER, and HR from units of g O_2 m^{-2} d^{-1} to g C m^{-2} d^{-1} assuming a 1:1 M relationship (e.g., Guillemette & del Giorgio, 2011; McCallister & del Giorgio, 2008). We then compared $v_{f\text{-min}}$ from our study to previously published $v_{f\text{-min}}$ originally compiled in Hall et al. (2016) and Plont et al. (2020) (n = 126; Table S4 in Supporting Information S1).

2.4. OM Source Bioassays and Water Column DOC Uptake

We estimated rates of microbial DOC consumption in the water column using laboratory bioassays (methods as in Hotchkiss et al., 2014 based on Servais et al., 1989). When we started monitoring whole-stream metabolism (August 16), we collected stream water from Stroubles Creek and Walls Branch immediately downstream of the sensor deployment sites. On the same day of collection, we filtered stream water through pre-ashed Whatman GF/F filters into acid washed and pre-ashed 200 mL amber borosilicate bottles (four replicate bottles per treatment). We added a 1% (by volume; 2 mL) stream-specific microbial inoculum, which consisted of stream water filtered through a 2 µm pore size Whatman filter capsule to remove larger particulates. We chose to use water column microbial communities specific to each stream (e.g., 1% inoculum from the Stroubles Creek water column into Stroubles Creek bioassays and Walls Branch inoculums for Walls Branch bioassays) to reflect site-specific microbial metabolism.

To estimate microbial DOC uptake rates across a range of different dissolved organic matter compounds, we treated each bioassay with either no organic matter to represent microbial consumption of ambient stream DOC (hereafter, "ambient"), a sucrose solution (NOW Foods Beet Sugar), malted barley extract (Maillard Malts Amber Malt Extract Syrup), or roasted barley leachate (Simpson's Finest Roasted Barley). While water column DOC uptake from the ambient treatment bioassays provide a direct means of comparing OC reactivity across systems, these ambient uptake rates are low, often undetectable, and likely dominated by leftover, more persistent OC after rapid uptake of more reactive OC (Pollard, 2013). By using a combination of ambient and spiked treatments, we can better represent the diversity of OC reactivities present in streams and assess the potential uptake of new OC inputs to the water column that are likely missed by measuring ambient uptake rates alone. We added the organic matter source treatments as a 1% (by volume; 2 mL) spike filtered through a pre-ashed Whatman GF/F filter with a target enrichment of 2 g C m^{-3}. For the ambient bioassays, we added a 1% (by volume; 2 mL) aliquot of filtered stream water so that the total volume of all bioassays were equal.

We incubated bioassay bottles in the dark and submerged them in water baths to below their lids to maintain temperatures ~20°C for 4 days. Prior to incubation, we sacrificially sampled one bioassay in each treatment to confirm our target initial DOC concentrations. Samples were re-filtered, acidified, and analyzed for final DOC concentrations as described in water chemistry sampling methods above. To test for potential effects of contamination of our filtering equipment and bottles, we added a blank control consisting of only Milli-Q water. We note that there were no measurable changes in DOC concentration in the Milli-Q only bioassays over the course of the experiment.

We calculated bioassay DOC uptake rates assuming first-order reaction kinetics (Equation 5):

$$\text{DOC}_t = \text{DOC}_o e^{-k_{\text{DOC}} t} \quad (5)$$

DOC_o and DOC_t are DOC concentrations at the start and end of the bioassay incubation period t (t = 4 days), respectively. k_{DOC} is the bioassay-derived first-order DOC decay rate (day^{-1}) and represents the rate of microbial DOC uptake in the water column. To standardize k_{DOC} for in-stream conditions and allow for comparison between bioassay results and $v_{f\text{-min}}$ measured from whole-stream metabolism, We calculated DOC uptake velocities for ambient and spiked bioassay treatments ($v_{f\text{-amb}}$ and $v_{f\text{-spk}}$, respectively; m d^{-1}) by normalizing k_{DOC} by z (m; Equation 6; Baker & Webster, 2017):

$$v_{f-\text{amb}} = \frac{k_{\text{DOC-amb}}}{z} \text{ or } v_{f-\text{spk}} = \frac{k_{\text{DOC-spk}}}{z} \quad (6)$$

z is mean stream depth calculated from Q/(u*w) measured on August 16 (i.e., at the beginning of the bioassay incubation). We then compared $v_{f\text{-amb}}$ and $v_{f\text{-spk}}$ from our study to previously published bioassay DOC uptake velocities (n = 59; Amado et al., 2006; del Giorgio & Davis, 2003; Hood et al., 2009; Hotchkiss et al., 2014; Judd et al., 2006; Petrone et al., 2009; Wiegner et al., 2006; Wiegner et al., 2009; Table S5 in Supporting Information S1).

2.5. Simulating Whole Stream Carbon Loading and Fate

To demonstrate how in-stream OC metabolism can inform whole-ecosystem and landscape C budgets, we applied the ranges of OC removal rates and discharge from our study sites to a steady-state, mass balance model. Specifically, our goal was to test how differences in OC mass transfer coefficients ($v_{f\text{-}OC}$, m d^{-1}) influenced our estimates of terrestrial OC inputs to streams (OC_{lat}, g d^{-1}) given whole-stream OC removal (OC_R, g d^{-1}) and downstream export (OC_{out}, g d^{-1}; Equation 7).

$$OC_{out} = OC_{in} + OC_{lat} - OC_R \quad (7)$$

We assumed steady-state OC concentrations over our simulated reach and allowed the OC flux from upstream (OC_{in}) and stream width (w) to vary as a function of Q (Leopold & Maddock, 1953). Longitudinal sampling on August 16 and August 22 confirmed that DOC concentration did not vary significantly over our study reaches (<0.2 g m^{-3} variation over 200 m with no systematic increase or decrease in concentration). Based on the low rates of GPP in both study streams (see metabolism results below), we assumed in-stream autochthonous OC to be negligible in our simulations. We did not include OC in storage (e.g., burial in sediment) in our simulations. In-stream GPP, autochthonous OC sources, and OC in storage may be important to consider when applying this budget approach to other ecosystems (Kaplan et al., 2006; Webster & Meyer, 1997). We estimated OC_{lat} as the amount of OC inputs needed to maintain a constant OC concentration in our simulated reach (i.e., $OC_{lat} = OC_R$) after accounting for in-stream OC removal. We held the length (L) of our reach constant at 1,000 m and calculated the fraction of OC removed (R) over the simulated as (Equation 8; Bertuzzo et al., 2017; Helton et al., 2011):

$$R = 1 - e^{-(v_{f-OC}/H_L)} \quad (8)$$

We calculated the hydraulic load (H_L; m d^{-1}) as cumulative daily discharge (Q; m^3 d^{-1}) divided by the reach area (L*w; m^2). We calculated R for a given $v_{f\text{-}OC}$ across a range of Q values typical of the variation in Q on non-stormflow days at our study sites (i.e., between 0 and 25,000 m^3 d^{-1}). We used these R values for a given OC_{in} and Q to calculate OC_R, OC_{lat}, and OC_{out} fluxes, as well as the percentage of OC_{in} that was loaded in from the terrestrial ecosystem, removed, or exported over our simulated reach. We repeated this simulation across $v_{f\text{-DOC}}$ values ranging from 0.001 to 1 m d^{-1}. Lastly, we calculated R, OC_R, OC_{lat}, and OC_{out} for our observed bioassay and whole-stream metabolism $v_{f\text{-}OC}$ ($v_{f\text{-amb}}$ or $v_{f\text{-spk}}$ for bioassays and $v_{f\text{-min}}$ for whole-stream metabolism) and Q over our study period. As Q increases, metabolism may be enhanced (i.e., transport-limited) or suppressed (i.e., reaction-limited) (Covino, 2017; Demars, 2018; O'Donnell & Hotchkiss, 2019). We simulated OC fluxes for the minimum and maximum observed $v_{f\text{-}OC}$ and Q for both methods, but did not impose a positive or negative relationship between Q and OC metabolism in our simulations. Further details of our modeling approach can be found in the supplementary materials (Figure S7, Tables S7, and S8 in Supporting Information S1).

2.6. Statistical Analyses

We used a two-way analysis of variance with fixed effects to test for differences in bioassay k_{DOC} across organic matter sources and sites (Table S3 in Supporting Information S1). We used Shapiro-Wilk tests to check normality of residuals and a post-hoc Tukey's honest significant difference test to make pairwise comparisons between k_{DOC} from different organic matter sources. For all statistical analyses, we assigned a significance level of alpha = 0.05. We used a one-way analysis of variance to test for differences in whole-stream metabolism (i.e., GPP and ER) and OC spiraling (i.e., HR, S_{OC}, and $v_{f\text{-min}}$) between streams. All statistical analyses were conducted using R (R Core Team, 2021). We pooled $v_{f\text{-amb}}$, $v_{f\text{-spk}}$, and $v_{f\text{-min}}$ estimates across both sites for comparisons with $v_{f\text{-min}}$ and externally-sourced values of bioassay (i.e., water column DOC uptake velocity) and whole-stream metabolism-derived $v_{f\text{-}OC}$ (i.e., OC mineralization velocity). We used one-way analysis of variance to assess differences in $v_{f\text{-}OC}$ between bioassay (i.e., $v_{f\text{-amb}}$ and $v_{f\text{-spk}}$) and whole-stream metabolism (i.e., $v_{f\text{-min}}$) methods (Table S6 in Supporting

Table 3
Mean Whole-Stream Metabolism and Dissolved Organic Carbon Spiraling Estimates for Stroubles Creek and Walls Branch on Days Without Storm Events During August 2018

Stream	GPP (g O_2 m^{-2} d^{-1})	ER (g O_2 m^{-2} d^{-1})	K_O (day^{-1})	HR (g C m^{-2} d^{-1})	S_{OC} (km)	$v_{f\text{-}min}$ (m d^{-1})
Stroubles Creek	0.5 (0.3, 0.6)	−0.7 (−0.6, −0.8)	3.4 (3.4, 3.4)	0.2 (0.1, 0.3)	18.2 (11.2, 33.7)	0.07 (0.05, 0.09)
Walls Branch	0.02 (0.01, 0.03)	−0.3 (−0.2, −0.3)	5.5 (5.5, 5.6)	0.1 (0.1, 0.1)	5.7 (3.0, 8.9)	0.05 (0.04, 0.06)

Note. Values in parentheses represent the range (i.e., minimum and maximum) of mean daily parameter estimates over the study period. Daily estimates of uncertainty are in Table S1 in Supporting Information S1. Parameter abbreviations and their full names are gross primary production (GPP), ecosystem respiration (ER), air-water gas exchange (K_O), heterotrophic respiration (HR), organic carbon spiraling length (S_{OC}), and mineralization velocity ($v_{f\text{-}min}$).

Information S1). We conducted separate tests for $v_{f\text{-}OC}$ measured as part of our study and for $v_{f\text{-}OC}$ values from the literature to see if significant differences in $v_{f\text{-}OC}$ measured using bioassays or whole-stream metabolism were consistent across our study and published literature values (Tables S4 and S5 in Supporting Information S1).

3. Results

3.1. OC Removal From Metabolism and Bioassays

DOC was higher in Stroubles Creek (2.39–3.25 g m^{-3}, Table 1) than in Walls Branch (1.26–1.92 g m^{-3}, Table 1) over the study period. DOC concentrations estimated using fDOM sensor data (i.e., DOC_{fdom}) resulted in similar ranges in DOC in both streams (2.16–3.23 g m^{-3} for Stroubles Creek and 1.26–1.92 g m^{-3} for Walls Branch, Table 1). Seston POC was low with low variability across our study reaches (0.24–0.32 g m^{-3} for Stroubles Creek and 0.21–0.34 g m^{-3} for Walls Branch) and made up 10% and 15% of the total amount of OC in transport under base flow conditions in Stroubles Creek and Walls Branch, respectively. We therefore used the mean POC concentration in Stroubles Creek (0.28 g m^{-3}) and Walls Branch (0.27 g m^{-3}) as our daily estimates of seston POC in our OC spiraling calculations.

Both GPP and ER were higher in Stroubles Creek (mean GPP = 0.5 g O_2 m^{-2} d^{-1}, mean ER = −0.7 g O_2 m^{-2} d^{-1}) than in Walls Branch (mean GPP and ER = 0.02 and −0.3 g O_2 m^{-2} d^{-1}, Table 3). Both streams were heterotrophic (GPP < |ER|); ER was subsidized by external (i.e., upstream or terrestrial) C inputs. HR was also higher in Stroubles Creek than Walls Branch (0.2 vs. 0.1 g C m^{-2} d^{-1}, Table 3). However, S_{OC} was longer (i.e., OC traveled further before mineralization to CO_2) in Stroubles Creek than Walls Branch (11.2–33.7 vs. 3.0–8.9 km, Table 3). Despite S_{OC} being longer in Stroubles Creek compared to Walls Branch ($p = 0.017$), Stroubles Creek had higher $v_{f\text{-min}}$ than Walls Branch ($p = 0.019$), indicating that Stroubles Creek had a greater OC removal capacity than Walls Branch when normalized for stream size (Table 3).

Water column bioassay DOC uptake (k_{DOC}) differed among organic matter sources ($p < 0.001$), and between Walls Branch and Stroubles Creek ($p = 0.038$). The lowest k_{DOC} values were estimated in bioassays with ambient stream water from Stroubles Creek (0.01–0.08 day^{-1}) and Walls Branch (0.01–0.15 day^{-1}; Figure 1); all bioassays amended with organic matter had higher k_{DOC} values than the ambient bioassays ($p < 0.001$). Although the interaction term between organic matter source and stream in our bioassays was not significant ($p = 0.735$), we pooled k_{DOC} from Stroubles Creek and Walls Branch to compare how k_{DOC} changed as a function of organic matter source. k_{DOC} in bioassays amended with roasted barley leachate (0.08–0.18 day^{-1}) were the most similar to ambient bioassay k_{DOC} ($p = 0.079$). Beet sugar and malted barley extract amended bioassays had higher k_{DOC} than ambient k_{DOC} ($p = 0.004$ and $p < 0.001$, respectively). Despite the variability in DOC uptake among different organic matter sources, water column DOC uptake velocities for ambient ($v_{f\text{-amb}}$) and spiked ($v_{f\text{-spk}}$) bioassays were over an order of magnitude lower than $v_{f\text{-min}}$ ($p < 0.001$; Figure 2). This order of magnitude difference in $v_{f\text{-}OC}$ measured using bioassays versus whole-stream metabolism was also observed across previously published literature values ($p < 0.001$, Figure 2).

3.2. Simulated Ecosystem OC Fluxes and Fate

In the simulated stream reach, 15.2% of OC in transport was removed under the highest observed $v_{f\text{-}OC}$ scenario ($v_{f\text{-}OC} = 0.139$ m d^{-1}) when observed specific discharge was lowest (841 m^2 d^{-1}, Figure 3a). As specific discharge

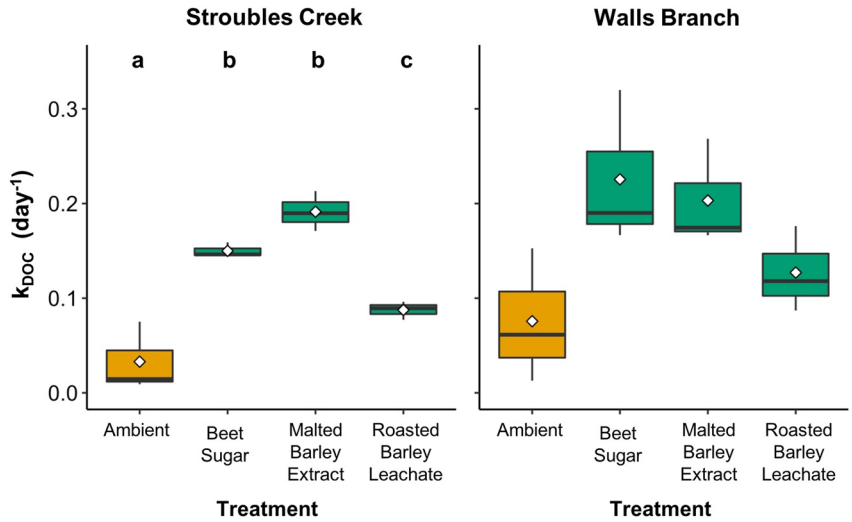

Figure 1. Water column dissolved organic carbon (DOC) uptake rate (k_{DOC}, in day^{-1}) across ambient bioassays (orange, no organic matter source added) and spiked bioassays (green, background DOC concentration elevated by 2 g C m^{-3} with either sucrose, malted barley extract, or roasted barley leachate). Letters above boxplots denote significant differences between organic matter source treatments based on a 2-way analysis of variance and post-hoc Tukey's honest significant difference comparison. There were no significant differences between treatments for Walls Branch bioassays. White diamonds represent the mean k_{DOC} for each organic matter source treatment.

increased, the fraction of OC removed decreased across all $v_{f\text{-}OC}$ simulations; the OC removal fraction differed 3-fold between the lowest and highest observed discharge conditions. Under all $v_{f\text{-}OC}$ scenarios in our simulations, the mass of OC removed, and therefore the mass of terrestrial OC inputs needed to sustain in-stream OC removal and downstream concentrations, increased at a diminishing rate with flow (Figure 3b). While there was overlap among the lower $v_{f\text{-}OC}$ for bioassays and whole-stream metabolism, R and OC_R were greater and more variable when applying whole-stream metabolism results compared to bioassay results (Figure 3). Simulated mass of OC removed using whole-stream metabolism results was similar to mass of OC removed calculated from observed

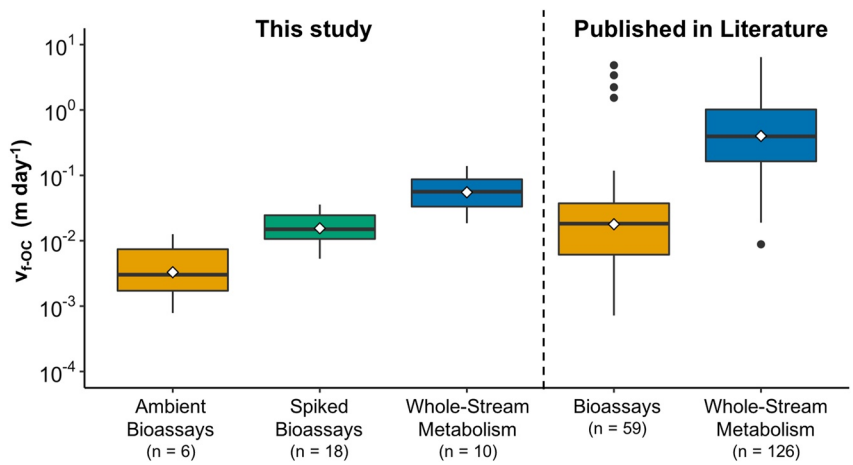

Figure 2. Organic carbon (OC) mass transfer coefficients ($v_{f\text{-}OC}$, m d^{-1}) for ambient bioassays (orange), spiked bioassays (green), and whole-stream metabolism (blue) from this study (left) and from other published studies (right; sources of $v_{f\text{-}OC}$ values in Tables S4 and S5 in Supporting Information S1). $v_{f\text{-}OC}$ for bioassays is water column dissolved organic carbon (DOC) uptake velocity calculated as the DOC uptake rate (k_{DOC}, day^{-1}) normalized for stream depth (z, m) at the time when bioassay incubations began. $v_{f\text{-}OC}$ for whole-stream metabolism (blue) is mineralization velocity ($v_{f\text{-min}}$), which represents the complete removal of OC (i.e., microbial assimilation of OC and mineralization into CO_2). White diamonds represent the mean $v_{f\text{-}OC}$.

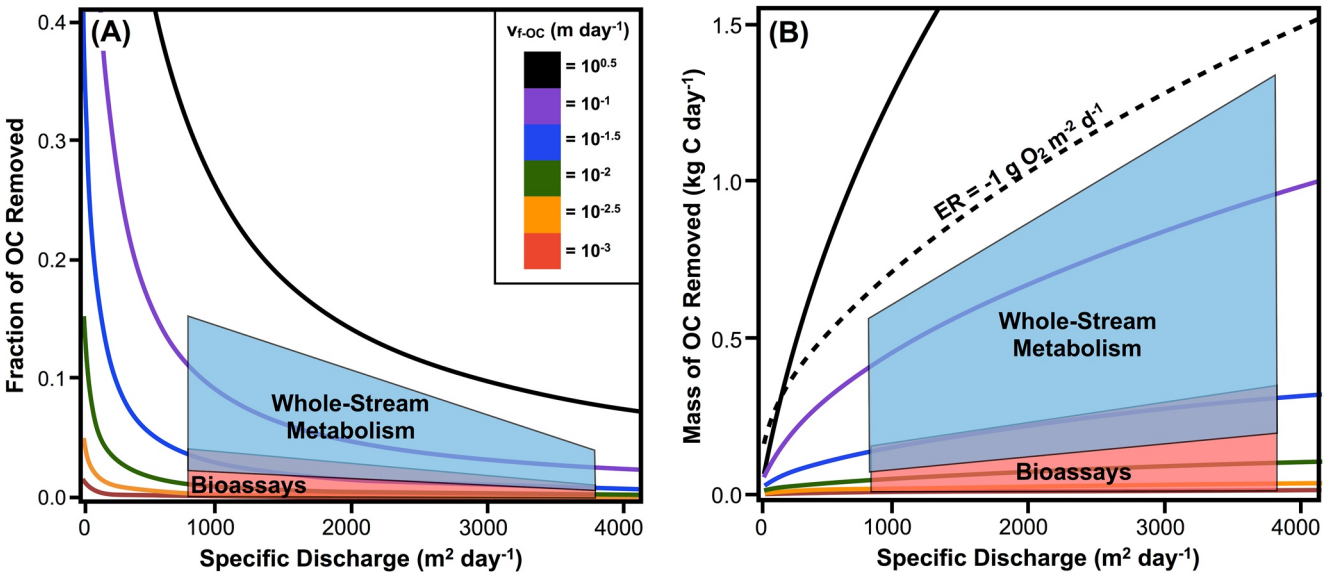

Figure 3. (a) Simulated fraction of organic carbon (OC) removed and (b) mass of OC removed across the range of specific discharge conditions typical of our study sites. Each solid colored line refers to a model run using a set OC mass transfer coefficient ($v_{f\text{-}OC}$, m d^{-1}). The dotted line is the mass of carbon removed across different specific discharge conditions for an ER rate of −1 g O_2 m^{-2} d^{-1}. The blue (whole-stream metabolism) and red (bioassays) transparent surfaces represent the range of (a) simulated fraction and (b) mass of carbon removed in our model, given the minimum and maximum measured $v_{f\text{-}OC}$ and specific discharge conditions across our two study sites.

ER in our study streams (−1 g O_2 m^{-2} d^{-1}, Figure 3). A greater percentage of the OC loaded into the simulated reach from upstream was removed for whole-stream metabolism $v_{f\text{-}OC}$ ranges (0.5%–15.2%) compared to bioassay $v_{f\text{-}OC}$ ranges (0.02%–4.2%, Figure 4). To sustain in-stream OC concentrations, simulated terrestrial OC loading ranged from 79 to 1,300 g C d^{-1} based on whole-stream metabolism and 3–350 g C d^{-1} when using bioassay estimates (Figure 4).

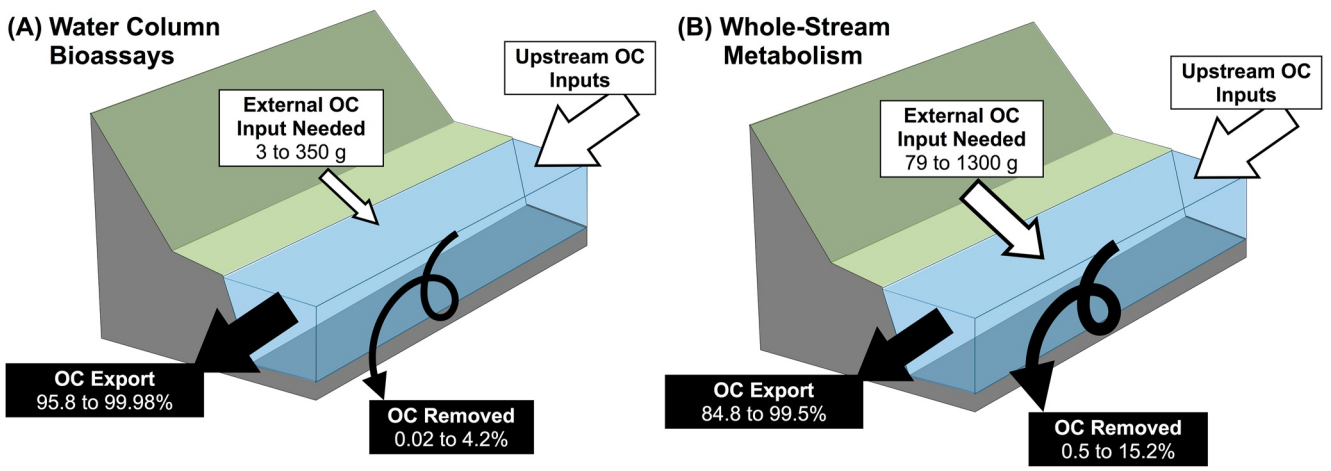

Figure 4. Percentages of organic carbon (OC) removed and exported, and daily terrestrial OC inputs needed to sustain in-stream OC concentrations in our model stream using (a) bioassay and (b) whole-stream metabolism results. Ranges in the OC fluxes are generated using observed minimum and maximum daily mean discharge in both streams and the minimum and maximum uptake velocity for bioassays (0.001 and 0.036 m d^{-1}, respectively) and mineralization velocity from whole-stream metabolism (0.020 and 0.139 m d^{-1}, respectively). Upstream OC input flux is loaded into our simulated stream reach assuming a constant OC concentration of 2.5 g C m^{-3} that scales proportionally with discharge.

4. Discussion

In this study, we estimated in-stream OC removal using both bioassays and whole-stream metabolism measurements in two heterotrophic streams, and applied OC removal estimates to stream C budgets to illustrate how we can better constrain terrestrial C inputs to freshwaters using whole-ecosystem process measurements. Based on our comparisons of OC metabolism methods and stream C budget simulations, we assert that (a) running waters are active sites of OC removal, (b) benthic metabolism makes up a large proportion of total stream OC removal, and (c) using bioassay-derived DOC removal rates underestimates terrestrial OC inputs to streams as well as the role of streams in watershed- and global-scale C budgets. Better integration of OC inputs, transport, and removal is needed to fully quantify the role of running waters metabolizing OC, as well as when, where, and how OC is supplied to streams and rivers.

4.1. In-Stream DOC Removal Estimated From Bioassays and Whole-Ecosystem Metabolism

Stroubles Creek and Walls Branch had low rates of GPP and ER, leading to high OC transport relative to removal (Table 3). In a previous study of multi-year patterns in ecosystem metabolism at a site on Stroubles Creek approximately 5 km upstream of our study sites, GPP and ER ranged from 0.01 to 17.3 g O_2 m^{-2} d^{-1} and −2.2 to −20.5 g O_2 m^{-2} d^{-1}, respectively (O'Donnell & Hotchkiss, 2019). GPP and ER at the lower Stroubles site analyzed here (mean GPP, ER = 0.5, −0.7 g O_2 m^{-2} d^{-1}) fall at the lower end of metabolism estimates from the upstream reach of Stroubles Creek. The upstream Stroubles Creek site has an open canopy throughout most of the reach and drains a higher percentage of developed and agricultural land compared to the downstream, more forested site on Stroubles Creek near Walls Branch in this study. GPP in our study streams was similar to that of nine other southern deciduous forested streams in North Carolina (0.1–3.0 g O_2 m^{-2} d^{-1} GPP; Bernot et al., 2010). However, ER and $v_{f\text{-min}}$ in our study streams fell below the observed ranges for North Carolina sites (1.6–17.9 g O_2 m^{-2} d^{-1} for ER, 0.41–4.13 m d^{-1} for $v_{f\text{-min}}$; Bernot et al., 2010; Plont et al., 2020) even though North Carolina sites were similar in Q to Stroubles Creek and Walls Branch (1,054–16,364 m^3 d^{-1}) and had a comparable range in S_{OC} (0.5–6.5 km; Plont et al., 2020). Storm events prior to and during the study period may have disturbed biofilms through scouring and increased turbidity, leading to lower rates of GPP, ER following periods of high stream discharge (O'Donnell & Hotchkiss, 2019, 2022). Higher flow events may suppress biological activity while also increasing aquatic-terrestrial connections and OC loading, leading to greater OC transport and thus longer S_{OC}, and should be part of future work linking OC metabolism and transport across different flow periods.

Whole-stream metabolism rates from this study and others suggest that streams and rivers may remove 10-100 times more OC inputs from the terrestrial landscape than estimated using bioassays alone. Regardless of whether the bioassays were enriched with a more biologically reactive organic matter source, OC removal measured using water column bioassays (i.e., $v_{f\text{-amb}}$ and $v_{f\text{-spk}}$) was up to 2 orders of magnitude lower than OC removal measured from whole-stream metabolism (i.e., $v_{f\text{-min}}$; Figure 2). Previous measurements of OC removal measured using bioassays and whole-stream metabolism in ten rivers support our results: $v_{f\text{-}OC}$ measured via whole-stream metabolism (0.05–0.68 m d^{-1}; Hall et al., 2016) was higher than bioassay $v_{f\text{-}OC}$ (0.001–0.03 m d^{-1}; Hotchkiss et al., 2014). When we compared previously published values of $v_{f\text{-}OC}$ measured using bioassays (n = 59) or whole-stream metabolism (n = 126), with the caveat that most bioassay and metabolism measurements were not made at overlapping sites, we observed a similar order of magnitude difference in $v_{f\text{-}OC}$ depending on the method used (Figure 2). We thus argue that whole-stream metabolism provides a more realistic estimate of in-stream OC removal because whole-stream metabolism integrates C processing in the water column as well as highly reactive benthic and hyporheic substrates (Griffiths et al., 2012; Kelso et al., 2020).

Given the notable discrepancy between OC removal estimated using bioassays or whole-stream metabolism, we suggest future investigators consider the context and tradeoffs of using bioassays to ecosystem OC fluxes in place of whole-stream metabolism. Although bioassays underestimate whole-stream OC cycling, they often allow us to isolate, control, and test how different physical (e.g., temperature, light) and biogeochemical controls (e.g., nutrient availability, stoichiometry) influence OC uptake (Kelso et al., 2020; Koehler et al., 2012) and assess the reactivity of different OC sources (Hotchkiss et al., 2014; Kelso et al., 2020) across different ecosystem types (Catalán et al., 2016). Bioassays can also provide standardized estimates of water column DOC uptake in aquatic ecosystems where sensor deployment or whole-stream metabolism modeling methods prove challenging (e.g., high air-water gas exchange, low GPP, or maintaining sensor deployments in remote regions; Ward et al., 2019;

Wologo et al., 2021). Previous comparisons of OC removal measured using whole-stream DOC additions versus DOC uptake estimated from bioassays found that neither bioassays nor whole-stream DOC uptake experiments produce representative estimates of ambient OC removal (Mineau et al., 2016). In contrast, OC removal estimated by whole-stream metabolism offers a daily, whole-stream representation of how streams cycle C, especially when additional measurements during higher flow periods support the future inclusion of metabolism estimates during both ambient and high flow days (e.g., Demars, 2018). Together with OC flux, whole-stream metabolism provides daily, integrative measures of C fixation (GPP) and mineralization (ER), as well as the balance between in-stream processes, external loading, and downstream transport (Hall et al., 2016). Whole-stream metabolism provides a more holistic measure of OC removal in streams than bioassays, and is a more appropriate method for quantifying the fluxes of OC to and through running waters.

4.2. Leveraging Whole-Stream Metabolism to Link OC Inputs and Fate Across Landscapes

Terrestrial C inputs into freshwaters are rarely measured directly and, consequently, the role of streams in the context of landscape C cycling remains poorly characterized. To date, most landscape- and global-scale C budgeting efforts have missed the opportunity to connect in-stream C cycling to the surrounding landscapes that fuel them (but see Butman et al., 2015; Vachon et al., 2021). We argue that whole-stream metabolism can be used to more directly estimate how much OC must enter from land to subsidize and sustain in-stream processes and C fluxes. Additionally, when paired with C emissions, storage, and export estimates (e.g., including CO_2 fluxes), whole-stream metabolism can better constrain the source and form of external terrestrial C inputs, either as organic or inorganic C, POC or DOC, and originating from terrestrial or internal production and respiration (e.g., Hotchkiss et al., 2015). One study that integrated whole-stream metabolism estimates into landscape C budgets suggested that 36 ± 18% of terrestrial OC inputs are removed and respired through in-stream biological activity during non-stormflow conditions (Demars, 2018). While in-stream OC removal was lower in our simulations (0.5%–15.2%), likely driven by the low metabolic activity in our study reaches ($v_{f\text{-}min}$ ranged from 0.04 to 0.09 m d^{-1} in our study compared to 0.21–2.67 m d^{-1} in Demars, 2018), we show that substantial amounts (up to 1,300 g OC d^{-1} in a 1 km reach) of externally-derived OC needs to be loaded into streams to subsidize observed rates of whole-stream OC removal and downstream OC fluxes. We recognize that assumptions in our budget simulations constrain the utility of the equations used here to low-GPP streams with low autochthonous OC inputs and low OC storage. Budget calculations for streams with higher GPP or GPP:ER relative to our study sites might require an estimate of autochthonous OC production in addition to allochthonous OC inputs, although we note that ER in excess of GPP over longer time periods can provide an appropriate estimate of OC inputs that were metabolized after accounting for GPP and resulting autochthonous OC stocks. With recent advances in low-cost, high-frequency environmental sensors, the research community is poised to not only elucidate temporal dynamics of stream C cycling and constrain uncertainty around autochthonous OC production and storage, but also link in-stream C fluxes back to the terrestrial landscapes that fuel them.

Our current understanding of how OC moves and cycles throughout landscapes and across freshwater networks fails to recognize substantial removal of OC in waterways by not integrating whole-stream metabolism into assessments of OC transport and fate. Large-scale sampling efforts, budget approaches, and syntheses have improved our understanding of how changes in flow, season, and location within the network influences the quantity, sources, and fate of OC (e.g., Casas-Ruiz et al., 2017; Creed et al., 2015; Raymond et al., 2016). However, as new hypotheses concerning the dynamics of OC loading, transformations, and downstream transport are developed, measurements of whole-ecosystem OC removal are still rarely integrated into our understanding of OC fluxes and fate within freshwater networks. By inferring OC removal solely from OC concentration, organic matter quality, and bioassay-derived OC uptake, we miss energetic losses of C from rapid mineralization of labile OC compounds that are metabolized before they can be measured in transport (Pollard, 2013). Furthermore, scaling findings around the balance between OC removal and export beyond a single stream reach and across dynamic flow conditions will lead to a more holistic understanding of how C not only moves, but cycles through freshwater networks. Pairing whole-stream metabolism with OC transport across both high and low flows can lead to a more holistic understanding of how, when, and where C is loaded into, cycled within, and transported through freshwater networks. Through our OC removal method comparison and mass balance simulations, we show how differences in OC removal, even in low-productivity streams, can have confounding effects on terrestrial OC loading estimates and the interpretation of the role of streams in removing and exporting OC. Integrating

whole-stream metabolism into landscape C budgets and network OC transport models sets a foundation for characterizing the inputs and fate of terrestrial-derived C in inland waters.

Data Availability Statement

Data are available through HydroShare and can be accessed at the following link: http://dx.doi.org/10.4211/hs.e3f7da13037c4b588040dd7c2f6a45a7

Acknowledgments
This work was supported by the National Science Foundation (DEB-1754237 to ERH and DGE-1840995 to SP) and a Society for Freshwater Science General Endowment Award to SP. We acknowledge Robert O. Hall Jr., Michelle A. Baker, and Melissa Castillo for helping develop ideas and methods used in this manuscript. We thank Benoit Demars and one anonymous reviewer for comments that greatly improved this paper. Many thanks to Bobbie Niederlehner for her assistance with analytical chemistry as well as members of the Hotchkiss Lab for their feedback on the ideas presented in this work.

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