Stream metabolism sources a large fraction of carbon dioxide to the atmosphere in two hydrologically contrasting headwater streams

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Abstract

Headwater streams are control points for carbon dioxide (CO2) emissions to the atmosphere, with relative contributions to CO2 emission fluxes from lateral groundwater inputs widely assumed to overwhelm those from in-stream metabolic processes. We analyzed continuous measurements of stream dissolved CO2 and oxygen (O2) concentrations during spring and early summer in two Mediterranean headwater streams from which we evaluated the contribution of in-stream net ecosystem production (NEP) to CO2 emission. The two streams exhibited contrasting hydrological regimes: one was non-perennial with relatively small groundwater inflows, while the other was perennial and received significant lateral groundwater inputs. The non-perennial stream exhibited strong inverse coupling between instantaneous and daily CO2 and O2 concentrations, and a strong correlation between aerobic ecosystem respiration (ER) and gross primary production (GPP) despite persistent negative NEP. At the perennial stream, the CO2–O2 relationship varied largely over time, ER and GPP were uncorrelated, and NEP, which was consistently negative, increased with increasing temperature. Mean NEP contribution to CO2 emission was 51% and 57% at the non-perennial and perennial stream, respectively. Although these proportions varied with assumptions about metabolic stoichiometry and groundwater CO2 concentration, in-stream CO2 production consistently and substantially contributed to total atmospheric CO2 flux in both streams. We conclude that in-stream metabolism can be more important for driving C cycling in some headwater streams than previously assumed.

Streams and rivers are control points in global carbon (C) cycling because of high carbon dioxide (CO2) emission rates (Caraco and Cole 2003; Cole et al. 2007; Drake et al. 2018). High emissions from small streams are attributed principally to groundwater inputs of dissolved CO2 from soil organic matter decomposition (Jones et al. 2003; Hotchkiss et al. 2015; Abril and Borges 2019). However, in-stream metabolic processes also influence CO2 emissions, with streams acting as net CO2 sources when ecosystem respiration (ER) from aerobic or anaerobic processes exceeds gross primary production (GPP) (Duarte and Prairie 2005; Hall and Hotchkiss 2017). Resolving stream CO2 sources and understanding their temporally and spatially varying contributions to CO2 emissions is a fundamental question in ecology.

Hydrology controls terrestrially derived sources of CO2 by influencing both its production in soils and posterior transport to streams (Liu et al. 2022). The precipitation regime strongly predicts stream CO2 emissions (Butman and Raymond 2011), suggesting conjoined effects on soil CO2 and runoff rates. Lateral inputs regulate stream CO2 emissions under high hydrological connectivity, suggesting transport limitation of terrestrial subsidies (Jones and Mulholland 1998). Moreover, terrestrial CO2 sources dominate under cold or high-flow conditions when in-stream respiration is typically low, but decline in importance under warm or low-flow conditions when in-stream respiration is enhanced (Jones and Mulholland 1998; Finlay 2003; Roberts and Mulholland 2007; Hotchkiss et al. 2015). Contrasting hydrological settings (e.g., gaining vs. losing reaches), seasons, and flow states

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(i.e., high vs. low flow) are thus expected to influence the relative importance of terrestrial vs. in-stream CO₂ sources (Hotchkiss et al. 2015; Leith et al. 2015; Horgby et al. 2019; Hutchins et al. 2019). However, the influence of hydrological setting on the temporal dynamics of CO₂ sources remains poorly understood, partially because in situ measurements of both riparian groundwater and stream CO₂ dynamics remain scarce (though see Duvert et al. 2018; Lupon et al. 2019).

Emission of CO₂ from stream sources is assessed using open-channel variation in dissolved oxygen (O₂), parsed into daytime production attributed to GPP and persistent O₂ consumption due to aerobic ER (Odum 1956; Hall and Hotchkiss 2017). Thus, ER is the dominant pathway for in-stream CO₂ generation, integrating organic matter consumption by both autotrophs and heterotrophs from both autochthonous (i.e., GPP) and allochthonous (i.e., terrestrial) sources. Net in-stream CO₂ emissions arise when ER exceeds GPP, yielding negative net ecosystem production (NEP). However, because ER is derived from O₂ concentrations, it quantifies only aerobic respiration such that equating in-stream O₂ and CO₂ dynamics tacitly assumes anaerobic processes contribute negligible CO₂. Although this assumption is plausible given reported stream denitrification rates (Mulholland et al. 2009), the impact of anaerobic pathways on stream CO₂ emission remains poorly tested. Divergence between observed and O₂-derived estimates of CO₂ production can also arise from values of respiratory quotient (RQ = molar ratio of CO₂ produced to O₂ consumed) different from unity. The RQ remains poorly constrained for streams, with substantial variation expected in response to varying composition of stream biota and organic matter (Beyers 1963; Berggren et al. 2012; Richardson et al. 2013). Simultaneously comparing stream O₂ concentrations, in-stream CO₂ production, and total CO₂ emission can help constrain these sources of variation (e.g., anaerobic respiration and RQ variation) to provide insights on the dynamic relative contributions of terrestrial vs. in-stream CO₂ sources in response to environmental drivers such as hydrology, light, temperature, redox conditions, and organic matter sources.

Our objectives were twofold. First, quantify the magnitude, temporal patterns, and sources of stream-atmosphere CO₂ exchange fluxes in two Mediterranean headwater streams. Second, compare these fluxes to NEP (NEP = GPP + ER) to parse contributions of terrestrial vs. in-stream sources to stream CO₂ emissions. We chose two streams permanently flowing during the study period but with contrasting hydrologic regime: one perennial gaining stream, and one non-perennial stream with seasonally and spatially varying losing reaches (Butturini et al. 2003; Bernal et al. 2015). We hypothesized that the relative contribution of NEP to CO₂ emission is generally low, but markedly different between streams based on contrasting contribution of lateral groundwater inputs to total discharge. Specifically, we expected lower groundwater inputs in the non-perennial stream would elevate the importance of in-stream metabolism on CO₂ emissions. As such, we predicted large and strongly coupled diel fluctuations of CO₂ and O₂ concentrations in this stream compared to the perennial stream. Moreover, we expected increased anaerobic respiration, and thus reduced contribution of NEP to CO₂ emission, as stream O₂ availability and flow decline during summer in the non-perennial stream. By contrast, we predicted stream CO₂ emissions dominated by groundwater inputs in the perennial stream, overwhelming NEP production. In both streams, predictable seasonal changes in light and discharge through spring and summer enabled empirical evaluations of the contributions of lateral and in-stream sources to stream CO₂ emission and their temporal dynamics under contrasting environmental conditions.

Materials and methods

Study sites

This study was conducted in two third-order streams of La Tordera, a granitic catchment in Catalonia (NE Spain). The climate is Mediterranean, with warm, dry summers and mild, humid winters. A pronounced altitudinal gradient (0–1700 m) results in microclimates that create strong zonation of vegetation and stream hydrologic regimes.

Both streams are spring fed and have similar drainage areas, with limited human impact (population density < 1 person km⁻²), and both have low alkalinity (400–700 μeq L⁻¹) with mean pH values ~ 6–7 (Piñol and Avila 1992). The non-perennial stream, Fuirosos, drains 9.9 km² at lower altitude in the Montnegre-Corredor Natural Park (41°41’N, 2°34’E; 80–760 m a.s.l.). The catchment is mostly forested, with riparian forests dominated by alder (Alnus glutinosa) and sycamore (Platanus hybrid). Mean annual precipitation is 658 ± 216 mm [mean ± standard deviation], and mean annual temperature is 13.9 ± 0.5°C (period 2009–2019). The stream has a well-preserved channel morphology, dominated by sand (29%), rocks (27%), and boulders (19%), with patches of cobbles and gravel (water width 2.4 ± 0.5 m). The stream seasonally loses water and surface flow can stop for several months in summer. The transition from wet to dry conditions starts with the stream losing water to the riparian groundwater, but preserving longitudinal surface flow connectivity. Further drying causes longitudinal disconnection resulting in isolated pools that can persist for several days until the stream dries completely (Butturini et al. 2002). Our study period encompassed both phases, though stream metabolism was calculated only during the phase with surface flow connectivity.

The perennial stream, Font del Regàs, drains 14.4 km² in the Montseny Natural Park (41°50’N, 2°30’E; 400–1600 m a.s.l.). The catchment is forested, with riparian forests of alder, black locust (Robinia pseudoacacia), sycamore, and ash (Fraxinus excelsior). Mean annual precipitation is 925 ± 151 mm, and mean annual temperature is 12.1 ± 2.5°C (period: 1940–2000). The stream has a well-preserved riffle–run structure, dominated by rocks (~ 30%), cobbles (~ 25%), and gravel (~ 15%) (wet
width 3.1 ± 0.4 m). This stream gains water along the mainstem, and thus precludes longitudinal disconnection of surface flow, though some reaches can lose water, especially in summer (Bernal et al. 2015).

Field sampling and sensor deployments

We deployed sensors in spring to early summer 2019 to measure dissolved CO2 and O2 concentrations continuously and estimate daily rates of stream metabolism in both streams. An optical dissolved O2 probe (HOBO U26; Onset Corporation) and an infrared CO2 gas analyzer contained in a watertight, gas-permeable sleeve (GMP251; Vaisala, Helsinki, Finland, range 0–5000 ppm) measured high-frequency (5 min) concentrations of CO2 and O2 from which atmospheric exchange fluxes were estimated. Stream water temperature was also recorded at 5-min intervals with the Vaisala sensor. The two probes were located < 10 m apart in areas with high advection and anchored to concrete blocks or submerged tree roots.

The deployments were conducted simultaneously at the two streams. The deployment at the non-perennial stream ranged from 15 May 2019 until the stream ceased flowing on 30 June 2019. We obtained 47 d of reliable CO2 and O2 data. On 07 May 2019, we verified the stream was losing water by measuring discharge with salt slug additions (Gordon et al. 2004) at three locations along a 160 m stream reach upstream of the deployment station. Specific discharge (i.e., discharge divided by drainage area) decreased through the reach by ca. 10%, indicating losing conditions already in early May. The deployment at the perennial stream ranged from 06 May 2019 to 09 July 2019. We observed abrupt declines in CO2 concentration in late May and June each time the probe was serviced (ca. every 10 d) (Supporting Information Fig. S1). These concentrations were removed from further analysis, yielding 16 and 65 d of reliable CO2 and O2 data, respectively.

Incident light (lux) was recorded at 10-min intervals with two HOBO UA-002-64 loggers (Onset Corporation) installed below the riparian canopy at each stream, one near the deployment station, and one ca. 100 m upstream. These measurements were averaged to represent stream surface light inputs. We converted lux to photosynthetic photon flux density (PPFD, μmol m−2 s−1), and summed to daily steps using PPFD > 4 mol m−2 d−1 as the minimum energy to support photautotrophs (Hill et al. 1995).

Stream discharge (Q, L s−1) was inferred from water level loggers (HOBO U20-001-04, Onset Corporation) measuring stage at 10-min intervals and using pre-established rating curves. For the non-perennial stream, the rating curve used data from 2 km upstream of the deployment station, with similar wetted width and stream water velocity (v) (Bernal and Sabater 2008). For the perennial stream, we used time series of groundwater head in the near-stream zone, which correlates well with measured Q (Ledesma et al. 2021). During 2018, we measured v and Q in several occasions, from which we estimated daily v (m s−1) using daily Q values. The relationship between v and Q was strong for the non-perennial (r2 = 0.9, df = 21, p<0.001) and the perennial (r2 = 0.6, df = 10, p<0.001) streams.

To estimate lateral CO2 fluxes, we measured CO2 concentrations in near-stream groundwater at the beginning and end of the deployment periods. Groundwater was sampled using a peristaltic pump (Masterflex 7533-40) from existing piezometers located 0.4 m from the non-perennial stream on 05 and 09 July, and from piezometers located 0.7, 1.0, and 1.7 m from the perennial stream at the perennial site on 06 May and 17 July. After purging each piezometer, we immediately submerged the CO2 probe in a clean bottle, sealed with parafilm, until readings stabilized (ca. 30–40 min). Concentrations were multiplied by lateral groundwater fluxes derived from drainage area increases along the reach. For each stream, reach lengths associated with CO2 turnover were estimated as 3 × v/KCO2 (Hall and Hotchkiss 2017). This reach length assumes that CO2 operated in isolation like O2, and thus can underestimate CO2 turnover lengths when dissolved inorganic carbon (DIC) is transported as bicarbonate, as in high-alkalinity systems. However, given the low alkalinity and pH of these streams (400–700 μeq L−1; 6<pH<7) and the absence of carbonate in riparian soils (unpublished data), we assumed this effect was small (Sets et al. 2017).

We used a regional digital elevation model to determine the incremental drainage area (ArcGIS 10.7, ESRI) per unit reach length, yielding 292 m2 and 961 m2 of stream length for the non-perennial and perennial stream, respectively. Lateral groundwater inputs were assumed zero at the non-perennial stream because the reach was losing water to the riparian zone. To estimate groundwater inputs at the perennial stream, we assumed stream flow increased proportionally with drainage area (Helton et al. 2018).

Stream metabolism and fCO2 calculations

We used time series of stream O2 concentrations, incident light, and stream water temperature to calculate daily metabolic rates for the whole deployment at the perennial stream, and for the period with longitudinal connectivity at the non-perennial stream (15 May to 16 June). The single-station method was applied because stream hydromorphology and longitudinal connectivity were similar along the spatial footprint of O2 measurements (ca. 400 m upstream) at both streams (Odum 1956). Groundwater inputs had minimal influence on stream O2 concentration at the non-perennial stream, which was net losing. At the perennial stream, dissolved O2 concentration was lower in riparian groundwater than the stream (4.7 ± 1.5 vs. 9.7 ± 0.6 mg L−1, Lupon et al. 2016). Considering a reach area of 1200 m2 (3 m wetted width × 400 m length) and groundwater inputs as low as Qw ~ 1.7 L s−1 (derived from mean Q = 58.3 L s−1 with a 3% increase in drainage area along a 400-m reach), we obtain a ratio between

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Qg and reach area <0.2 m d⁻¹. Thus, we concluded stream and groundwater mixing had minimal impact (<10%) on estimation of metabolic rates (fig. 2 in Hall and Tank 2005).

We estimated the normalized gas transfer coefficient (Kr600, d⁻¹), GPP and ER (g O₂ m⁻² d⁻¹) using the Bayesian inverse model from streamMetabolizer R package including both process and observation errors (Appling et al. 2018a). For the model, we assumed GPP is a linear function of light intensity (van de Bogert et al. 2007), while ER is constant throughout the day. The model does not account for factors that increase ER during the day such as photorespiration, which is presumably small in our forested headwater streams (Parkhill and Gulliver 1998). We fitted GPP and ER each day based on O₂ dynamics over a 28-h period starting at 23:00 the day before. Prior probability distributions for GPP and ER were equal for both streams based on previous reported values (0.5 ± 10 and –5 ± 10 g O₂ m⁻² d⁻¹ for GPP and ER, respectively) (Acuña et al. 2004; Lupon et al. 2016). To reduce equifinality, we pooled daily Kr600, estimated based on Q (Appling et al. 2018b) (Supporting Information Data S1).

Estimates of GPP and ER were used only when they passed a model output quality check. We removed all days with biologically impossible values (i.e., GPP <0 and ER >0), poor model convergence (i.e., R-hat >1.2 and number of effective samples >5000), or poor fit to O₂ data (i.e., R²<0.75, root mean square error (RMSE)>0.2, or mean absolute error (MAE)>0.2). Furthermore, we checked the feasibility of Bayesian-modeled Kr600 using independent Kr600 predictions from both the night-time regression method (Odum 1956) and hydraulic geometry (Raymond et al. 2012). We performed three model configurations (partial pooling with unconstrained Kr600, partial pooling with constrained Kr600, and pooling Kr600 by hand) and kept the configuration that best reproduced stream metabolism and Kr600 (see workflow and model implementation specifics in Supporting Information Data S1). The best option for the perennial stream was to set a constrained Kr600 for each Q class based on the distribution of Kr600 estimates from night-time and hydraulic approaches. For days that passed the quality test (60%), modeled Kr600 was used to estimate CO₂ and O₂ exchange fluxes, while the relationship between modeled Kr600 and Q (R² = 0.85, df = 36, p<0.001) was used to estimate Kr600 for the remaining days. For the non-perennial stream, GPP and ER were estimated by running the Bayesian model using a deterministic, hand-pooled Kr600 obtained from the linear relationship between binned Q and the combination of night-time and hydraulic estimates of Kr600. These Kr600 values were used to estimate CO₂ and O₂ exchange fluxes at this stream. Results showed significant ER vs. Kr600 covariance, and thus evidence of equifinality, at the non-perennial stream (Supporting Information Data S1).

NEP is defined as the sum of GPP and ER, the latter expressed by convention as a negative flux (Hall and Hotchkiss 2017). However, to better illustrate that ER is the main metabolic process contributing to in-stream CO₂ production, we expressed ER as a positive flux, and NEP as GPP minus ER. Negative values of NEP indicate ER is higher than GPP, and the stream contributes to net CO₂ emission. The units of NEP were converted from g O₂ m⁻² d⁻¹ to g CO₂-C m⁻² d⁻¹ assuming RQ = 1. Although widely adopted, this assumption can strongly influence CO₂ vs. O₂ coupling because actual values can vary between 0.7 (Beyers 1963; Kirk 2020) and more than 1.2 (Berggren et al. 2012; Alleson et al. 2016). We evaluated uncertainty from RQ variation by considering metabolic stoichiometry between 0.8 and 1.2.

The difference between daily CO₂ emission and NEP (both in g CO₂-C m⁻² d⁻¹) represented CO₂ outgassing not from in-stream aerobic metabolism but could thus attributed to lateral groundwater inputs or in-stream anaerobic processes. The relative contribution of stream metabolic activity to CO₂ emission was calculated as the ratio between the absolute value of NEP and daily CO₂ emission expressed as a percentage.

Data analysis

We computed instantaneous stream-atmosphere exchange fluxes of O₂ (mmol O₂ m⁻² min⁻¹) and CO₂ (mmol CO₂-C m⁻² min⁻¹) by multiplying the gas transfer coefficient (d⁻¹) by stream depth (m) and then by the difference between measured and atmospheric saturation concentrations (Raymond et al. 2012). Positive gas exchange indicates mass transfer from water to air (i.e., emission), while negative values indicate mass transfer into the water. We derived gas transfer coefficients for O₂ (Ko₂) and CO₂ (Kco₂) at 5-min intervals from Kr600 corrected for temperature and saturation based on Schmidt number scaling (Raymond et al. 2012). To align units of lateral and in-stream sources to daily CO₂ emission, we converted CO₂ exchange fluxes from mol CO₂-C m⁻² d⁻¹ to g CO₂-C m⁻² d⁻¹.

We used major axis regression models to explore the relationship between instantaneous CO₂ and O₂ exchange fluxes focused on the second half of each day (mid-day peak to late evening) to avoid hysteresis occasionally evident at the end of the deployment at the non-perennial stream. This analysis yields three types of information (Fig. 1): (i) the mean location of data points reveals departures from atmospheric equilibrium for both gases, (ii) the overall shape of the data cloud reveals the relevance of stream metabolism in structuring O₂ vs. CO₂ patterns, and (iii) the slopes for individual dates quantify metabolic coupling of gas concentrations at subdaily time scales. For this subdaily coupling, a slope of −1 indicates strong metabolic coupling given CO₂:O₂ molar stoichiometry is 1:1. We counted the number of days for which this slope was statistically significant and then the number of days for which the slope fell within ±20% of this theoretical range (i.e., from −1.2 to −0.8).

To further explore stream metabolism impacts on CO₂ dynamics, we analyzed the GPP vs. ER relationship, which we expected to be strong when GPP is large. We also analyzed the relation between O₂ vs. CO₂ gas exchange at daily time scales,
which better integrate hydrological and biogeochemical processes influencing CO₂ emissions (Vachon et al. 2020). Large deviation from slopes of −1 occur when (i) biofilm communities experience changes in composition or functioning, or (ii) there is shifting dominance of different CO₂ sources such as lateral groundwater inputs or anaerobic respiration processes.

To test how variability in environmental controls (Q, temperature, O₂ availability) impact CO₂ exchange fluxes, ER and NEP, we used linear regression (lm) and tested significance with analysis-of-variance. When time series exhibited strong autocorrelation in the residuals, we used generalized least squares (gls) models with a continuous autoregressive process as a correlation structure. Values of NEP, which were always < 0, were expressed in absolute terms to illustrate increases or decreases in response to varying environmental factors. We expected ER and NEP to increase with temperature, a fundamental driver of microbial activity, and daily CO₂ emissions to increase with increasing Q and rising influence of lateral groundwater inputs. Moreover, we expected the NEP contribution to daily CO₂ emission to increase as lateral groundwater inputs decrease over summer. Finally, we expected the contribution of ER to daily CO₂ emission to decline with reduced O₂ availability as conditions favor more in-stream anaerobic processes.

Differences in stream metabolic rates and the relative contributions of CO₂ sources to overall emission were assessed between the study streams with the Wilcoxon rank sum test, which is robust to violations of normality and equal variance assumptions. Statistical analyses were performed with R software (v.3.6.1) using a statistical significance threshold of p < 0.01.

**Results**

**Hydrological characterization and K₆⁰⁰**

Hydrology and K₆⁰⁰ differed substantially between the two streams. Water depth averaged 6.2 ± 1.6 and 9.7 ± 0.9 cm
Clear decline over time at both streams (Fig. 2A). At the non-perennial stream, connected and disconnected water pools were observed by 17 June, and water ceased flowing on 30 June. At the perennial stream, longitudinal connectivity persisted for the whole study period.

**Temporal patterns of light, temperature, and stream gas concentrations**

Daily light inputs averaged 7.7 ± 4.0 and 2.0 ± 1.5 mol m⁻² d⁻¹ at the non-perennial and perennial streams, respectively. Shading increased in both streams from May to July coinciding with riparian canopy leaf-out. At the non-perennial stream, light inputs exceeded 4 mol m⁻² d⁻¹ (minimum PPFD for photosynthesis; Hill et al. 1995) for almost the entire study period, but never exceeded that threshold at the perennial stream (Fig. 2B). Mean stream temperature rose in both streams over the study period (Fig. 2C), but was higher (16.1 ± 2.7°C) at the non-perennial than at the perennial stream (13.7 ± 2.2°C) with higher diel variations at the former (5.3 ± 1.6°C) than the latter (2.2 ± 0.6°C).

Mean dissolved O₂ concentrations were 7.3 ± 1.5 and 9.7 ± 0.6 mg O₂ L⁻¹ at the non-perennial and perennial stream, respectively. Diel cycles peaked in early afternoon (ca. 12:00–15:00 h) and were larger at the non-perennial stream. In late June, daily O₂ concentrations declined at the non-perennial stream (Fig. 2D), with suboxic conditions (O₂ < 5.0 mg O₂ L⁻¹) becoming increasingly common. At the perennial stream, no suboxic conditions were observed (Fig. 2D).

Mean dissolved CO₂ concentrations were 8.5 ± 5.7 and 2.9 ± 0.5 mg CO₂-C L⁻¹ at the non-perennial and perennial streams, respectively, with both exhibiting clear diel variation inversely related to O₂. The amplitude of diel CO₂ variation was higher at the non-perennial stream, especially during late June (Fig. 2E). At the non-perennial stream, daily CO₂ concentration averaged 5.5 mg CO₂-C L⁻¹ until early June, and then markedly increased, reaching 30 mg CO₂-C L⁻¹ in late June (Fig. 2E). At the perennial stream, this increasing pattern in CO₂ concentration was not observed (Fig. 2E).

**Exchange fluxes of CO₂ and O₂ with the atmosphere**

The relationship between instantaneous O₂ and CO₂ exchange fluxes varied in each stream. The data cloud was located above the −1 : 1 line for both streams, indicating substantial CO₂ sources other than aerobic metabolism. This was clearer at the perennial stream, which showed a more horizontal ellipse, suggesting greater variation in CO₂ than in O₂ concentrations (Fig. 3).

At the non-perennial stream, there was a strong and consistent negative relationship between instantaneous O₂ and CO₂ exchange fluxes (Fig. 3), with only 33% days exhibiting slopes within the expected range (−1.2 to −0.8). Subdaily slopes showed a gradual decline from mid-May to the end of June, ranging from −1.6 to −0.5 (slope β = 0.26 ± 0.002, R² = 0.78, F₁,₄₃ = 158, p < 0.001) (Fig. 4A). This decline was correlated

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**Fig. 2.** Temporal patterns of (A) mean daily stream flow (Q), (B) daily PPFD, (C) water temperature (Temp), (D) dissolved oxygen (O₂) concentration, and (E) dissolved carbon dioxide (CO₂) concentration, at the non-perennial (gray) and perennial (black) streams during the study period. The dashed line in (B) indicates minimum PPFD for photosynthesis threshold upon which photosynthesis is assumed to be no light limited (4 mol m⁻² d⁻¹; Hill et al. 1995). The dashed line in (D) indicates suboxic conditions (5 mg O₂ L⁻¹). Gaps at the end of the non-perennial stream deployment (C-E) are due to the stream going dry (open circles in A and B). Gaps in panel (E) for the perennial site correspond to anomalous stream water CO₂ concentrations recorded between 21 May and 30 June (Supporting Information Fig. S1).
Fig. 3. Relationship between instantaneous oxygen (O₂) and carbon dioxide (CO₂) exchange fluxes at the non-perennial and perennial streams. Positive values indicate stream concentrations above atmospheric equilibrium. The \(-1:1\) slope (dashed line) corresponds to the expected O₂:CO₂ relationship for photosynthesis (no other CO₂ sources). Subdaily regression fits are shown only for statistically significant major-axis slopes \((p<0.01)\). Purple and blue lines are fitted slopes within \(\pm 20\%\) of \(-1:1\) slope for the non-perennial and perennial streams, respectively; yellow and green lines indicate slopes outside that range.

With several environmental variables, including declining minimum daily O₂ concentration \((\beta = -0.21 \pm 0.02, R^2 = 0.73, F_{1,43} = 118, p<0.001)\) (Fig. 4B), declining stream flow \((\beta = -0.19 \pm 0.023, R^2 = 0.61, F_{1,43} = 68, p<0.001)\), and increasing water temperature \((\beta = 0.15 \pm 0.017, R^2 = 0.64, F_{1,43} = 77.1, p<0.0001)\).

At the perennial stream, instantaneous O₂ and CO₂ exchange fluxes exhibited no consistent relationship (Fig. 3). Although 76% of days showed a statistically significant negative relationship, only 14% of fitted slopes aligned with expected values. No relationship was observed between the temporal variation in fitted slopes and any measured environmental variables.

Both streams showed a negative relationship between daily O₂ and CO₂ exchange fluxes, though this was stronger at the non-perennial than at the perennial stream (Fig. 5). Moreover, the fitted slopes comparing daily exchange fluxes aligned more closely with theoretical expectations \((O₂:CO₂ \text{ slope} = -1)\) at the non-perennial stream.

Stream metabolism and contribution of NEP to daily CO₂ emission

Both streams were consistently heterotrophic, with GPP far lower than ER (Table 1), resulting in negative NEP. Mean GPP was \(0.42 \pm 0.05\) and \(0.24 \pm 0.02\) g O₂ m\(^{-2}\) d\(^{-1}\) at the non-perennial and perennial streams, respectively, corresponding to \(0.16 \pm 0.02\) and \(0.09 \pm 0.01\) g CO₂-C m\(^{-2}\) d\(^{-1}\) (Table 1). ER was higher at the perennial stream \((9.89 \pm 0.15\) g O₂ m\(^{-2}\) d\(^{-1}\) or \(3.71 \pm 0.06\) g CO₂-C m\(^{-2}\) d\(^{-1}\)) than the non-perennial stream \((3.8 \pm 0.39\) g O₂ m\(^{-2}\) d\(^{-1}\) or \(1.43 \pm 0.15\) g CO₂-C m\(^{-2}\) d\(^{-1}\)), leading to more negative NEP at the perennial stream (Table 1).

Both GPP and ER declined markedly from May to June in the non-perennial stream (Supporting Information Fig. S5). In contrast, ER slightly increased over time at the perennial stream, with no clear changes in GPP (Supporting Information Fig. S5). At the non-perennial stream, GPP and ER were positively related \((\beta = 5.9 \pm 0.8, R^2 = 0.65, F_{1,28} = 53.7, p<0.001)\), but this relationship was absent at the perennial stream \((F_{1,35} = 1.5, p>0.05)\) (Fig. 6A).

Daily metabolic rates and CO₂ emission fluxes were related to Q and water temperature, but differed between streams. Since Q and temperature are inversely related \((R^2 = 0.86\) and 0.92 at the non-perennial and perennial stream, respectively), their associations with metabolic rates and CO₂ emission fluxes were opposite. Moreover, strong correlation between \(K_{600}\), Q, and ER (Supporting Information Fig. S3, S4) preclude us from interpreting any cause of variation between ER (or NEP) and environmental variables at the non-perennial stream, such as the positive relationship between [NEP] and Q, or the negative relationship with temperature (Table 2; Supporting Information Table S4;
The magnitude of daily CO₂ emission fluxes exceeded NEP in both streams (Table 1; Fig. 7). However, mean daily CO₂ emission flux was threefold higher at the perennial stream (Table 1). Mean contribution of NEP to daily CO₂ emission fluxes was >50% at the two streams (Fig. 7). At the non-perennial stream, NEP accounted for half (50.8%) of daily CO₂ emission (%)NEP at the non-perennial and perennial streams. Different super-script letters indicate statistically significant differences between streams (Wilcoxon-rank sum test, p < 0.01). The number of cases is shown in parenthesis. Different super-}

**Table 1.** Mean (± SE) daily GPP, ER, NEP, carbon dioxide (CO₂) exchange flux, and relative contribution of NEP to daily CO₂ emission (%NEP) at the non-perennial and perennial streams. The number of cases is shown in parenthesis. Different super-script letters indicate statistically significant differences between streams (Wilcoxon-rank sum test, p < 0.01).

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<th></th>
<th>Non-perennial</th>
<th>Perennial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GPP</strong> (g CO₂-C m⁻² d⁻¹)</td>
<td>0.16 ± 0.02 (30)⁺</td>
<td>0.09 ± 0.01 (37)⁺</td>
</tr>
<tr>
<td><strong>ER</strong> (g CO₂-C m⁻² d⁻¹)</td>
<td>1.43 ± 0.15 (30)⁺</td>
<td>3.71 ± 0.06 (37)⁺</td>
</tr>
<tr>
<td><strong>NEP</strong> (g CO₂-C m⁻² d⁻¹)</td>
<td>-1.28 ± 0.13 (30)⁺</td>
<td>-3.61 ± 0.05 (37)⁺</td>
</tr>
<tr>
<td><strong>CO₂ exchange flux</strong> (g CO₂-C m⁻² d⁻¹)</td>
<td>2.57 ± 0.15 (47)⁺</td>
<td>6.7 ± 0.34 (24)⁺</td>
</tr>
<tr>
<td><strong>% NEP</strong></td>
<td>50.8 ± 1.5 (30)⁺</td>
<td>57.3 ± 3.0 (16)⁺</td>
</tr>
</tbody>
</table>

**Fig. 5.** Relationship between daily oxygen (O₂) and carbon dioxide (CO₂) exchange fluxes at the non-perennial and perennial streams. Positive values indicate concentrations above atmospheric equilibrium. Solid lines are the major axis regressions relating the gas fluxes (p < 0.001 for both). Coefficients ± SE for model regressions are shown (no R² calculated in major axis regression).

**Fig. 6.** Relationship between (A) GPP and ER, (B) stream discharge (Q) and NEP, and (C) stream water temperature (Temp) and NEP for the non-perennial (gray symbols) and perennial stream (black symbols). Error bars for ER and GPP are standard errors from the Bayesian model. Lines indicate statistically significant relationships (p < 0.01). For the non-perennial stream, relationships in (B) and (C) could be artifacts resulting from strong covariance between K₆₈₀ and ER. Statistical information in Table 2 and Supporting Information Table S4.

the daily CO₂ emission flux (Table 1) and was only moderately sensitive to variation in CO₂ : O₂ stoichiometry, with values from 42% for RQ = 0.8% to 63% for RQ = 1.2. At the perennial stream, NEP averaged 57.3% of daily CO₂ emission, and varied between 48% and 72% across the RQ range. The remaining daily CO₂ emission flux (49.2% and 42.7%) at the non-perennial and the perennial stream, respectively) was attributed to lateral groundwater inputs and anaerobic respiration. At the non-perennial stream, % NEP decreased with increasing stream water temperature, but this relationship was absent at the perennial stream (Table 2).
**Table 2.** Relationships between environmental drivers (discharge—Q, and temperature—Temp) and metabolic processes (net ecosystem production—NEP, carbon dioxide exchange flux—CO₂ emission and NEP’s contribution to CO₂ emission fluxes—%NEP). The generalized least squares (gls) models were used where ordinary least square (lm) model residuals showed autocorrelation. In each case, we report the slope (β ± SE) and degrees of freedom (df). Values of NEP were expressed in absolute terms (notice that in all cases NEP < 0). So a positive β indicates increases in the magnitude of NEP with increases in the associated driver. For the non-perennial stream, relationships between NEP and environmental variables may be artifacts resulting from the strong covariation between K₆₀₀ and ER.

<table>
<thead>
<tr>
<th>Environmental driver</th>
<th>Ecosystem variable</th>
<th>Final model</th>
<th>p-value</th>
<th>β</th>
<th>df</th>
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<tr>
<td>Q</td>
<td>NEP</td>
<td>lm</td>
<td>&lt;0.001</td>
<td>0.44 ± 0.03</td>
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<td></td>
<td>CO₂ emission</td>
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<td>0.47 ± 0.06</td>
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<tr>
<td></td>
<td>% NEP</td>
<td>gls</td>
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<td></td>
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<tr>
<td>Temp</td>
<td>NEP</td>
<td>lm</td>
<td>&lt;0.001</td>
<td>−0.37 ± 0.05</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>CO₂ emission</td>
<td>gls</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% NEP</td>
<td>gls</td>
<td>0.003</td>
<td>−3.18 ± 0.96</td>
<td>28</td>
</tr>
</tbody>
</table>

**Fig. 7.** Temporal pattern of daily carbon dioxide (CO₂) exchange flux (black circles), and NEP (orange circles) for the (A) non-perennial and (B) perennial streams. Bar charts indicate the relative contribution of NEP (orange) and other sources (i.e., lateral inputs and anaerobic respiration) (red) to daily CO₂ emission flux. We used |NEP| to facilitate comparison with daily CO₂ emission fluxes.

**Lateral groundwater CO₂ inputs**

We observed higher CO₂ concentration in riparian groundwater than in stream water at both sites, with an average of 41.5 ± 0.5 mg CO₂-C L⁻¹ (n = 2) at the non-perennial site and 16.9 ± 1.2 mg CO₂-C L⁻¹ (n = 4) at the perennial stream.

At the non-perennial stream, the reach length for CO₂ turnover averaged 303 ± 87 m. This reach accumulated a drainage area of 8.84 ha (292 m² increase in drainage area per m of stream length), yielding a mean specific Q = 0.020 mm d⁻¹. Assuming hydrologically gaining conditions and proportional increases in Q with drainage area, mean groundwater inputs along the entire reach equal 0.02 L s⁻¹ (< 1% of mean Q). This lateral water flux, in turn, contributes 0.11 ± 0.08 g C m⁻² d⁻¹ to stream CO₂ emission, representing 4.4% of measured mean daily CO₂ emission flux at the non-perennial stream. Since half of the measured CO₂ emission was attributed to NEP at this site, this lateral flux would explain just 8.8% of the additional CO₂ efflux.

At the perennial stream, the reach length for CO₂ turnover averaged 453 ± 30 m. This reach accumulated a drainage area of 435.3 ha (961 m² increase in drainage area per m of stream length), yielding a mean specific Q = 0.32 mm d⁻¹. Assuming proportional increases in Q with drainage area, mean groundwater inputs along the reach equaled 1.63 L s⁻¹ (or 3% of mean Q). This lateral water flux along with the measured CO₂ concentrations implies groundwater input contribution equals 1.6 ± 0.13 g C m⁻² d⁻¹ to stream CO₂ emission, or 25.2% of measured mean daily CO₂ emission flux at the perennial stream. This value represents 59% of the CO₂ efflux not attributed to NEP.

**Discussion**

Headwater streams are control points of CO₂ emission to the atmosphere, with most CO₂ attributed to terrestrial sources rather than to in-stream metabolic activity (Hotchkiss et al. 2015; Leith et al. 2015; Lupon et al. 2019). Our results suggest that the relative contribution of these sources varies with environmental conditions and magnitude of lateral groundwater inputs. We found that NEP was an important, sometimes dominant, source of CO₂ in the two headwater streams of this study. Although we expected this to be at the non-perennial stream because of low groundwater...
inputs, we expected lateral inputs to overwhelm NEP in the perennial stream. Potential mechanisms for observed patterns in CO₂ exchange fluxes and the varying contribution of in-stream sources to CO₂ emission are discussed given measured metabolism, flow, and temperature.

Stream metabolic rates and gas exchange fluxes

The non-perennial stream was heterotrophic, with ER dominating over GPP, and yet, these two rates were highly correlated. Although GPP was low (0.42 ± 0.05 g O₂ m⁻² d⁻¹) compared to similarly-sized streams (0.10–22 g O₂ m⁻² d⁻¹) (Roberts and Mulholland 2007; Hall et al. 2016; Savoy et al. 2019), it aligned with previously reported values for this stream (0.05–1.9 g O₂ m⁻² d⁻¹) (Acuña et al. 2004). Strong GPP vs. ER relationship is usually attributed to the primacy of GPP on ER (Demars et al. 2011; Beaulieu et al. 2013; Hall et al. 2016). Here, however, even the most generous estimates of photoautotrophic respiration support a small fraction of ER (5–7%) assuming photoautotrophic respiration consumes 44–69% of GPP (Beaulieu et al. 2013; Hall and Beaulieu 2013). Given the minimal lateral inputs, this result suggests that ER was fueled by stream organic matter sources. Large stocks of fine benthic organic matter typically accumulate over epithelial biofilms during low flows in this stream, which can amplify ER while limiting light inputs to photoautotrophs (Acuña et al. 2004). Moreover, limited nutrient supply could further suppress GPP (Myrstener et al. 2021), especially during late spring and early summer when dissolved inorganic N was <40 μg N L⁻¹ (unpublished data). We hypothesize that stream biota was highly sensitive to nutrient supply by remineralization, which may explain the strong non-stoichiometric coupling observed between GPP and ER.

Instantaneous gas fluxes highlight the major role stream metabolism plays on CO₂ emission, particularly in the non-perennial stream. A significant positive relationship between instantaneous CO₂ and O₂ exchange fluxes held throughout the study period, despite slopes falling outside the range of predicted metabolic stoichiometry in 33% of cases. Large daily variation in gas concentrations and the strong relationship between NEP and daily CO₂ emission fluxes imply O₂ and CO₂ were coupled by in-stream aerobic processes. Furthermore, this relationship persisted for daily exchange fluxes, though the slope (−0.61) was lower than expected (−1). Although these results illustrate how crucial summertime metabolism was for determining CO₂ emission at the non-perennial stream, they also suggest other in-stream sources, such as anaerobic respiration, must contribute to CO₂ emission. In our conceptual model (Fig. 1), the observed pattern in the non-perennial stream corresponds to scenario 3, where high O₂–CO₂ coupling leads to relatively elongated ellipses, and additional inputs lead to CO₂ oversaturated water (i.e., ellipses shifted rightwards).

At the perennial stream, GPP (0.24 ± 0.02 g O₂ m⁻² d⁻¹) was 40-fold lower than ER (9.89 ± 0.15 g O₂ m⁻² d⁻¹). These rates match those previously reported for this stream (Lupon et al. 2016), implying strongly negative NEP (−3.6 ± 0.05 g CO₂-C m⁻² d⁻¹) when compared to global scale values for headwater streams (e.g., −1.2 ± 0.15 g CO₂-C m⁻² d⁻¹ in Battin et al. 2008). This aligns with headwater forested streams as generally heterotrophic, driven by allochthonous dissolved organic matter mineralization (Roberts and Mulholland 2007). Together: (i) no relationship between GPP and ER, (ii) nor any consistent pattern between instantaneous CO₂ and O₂ exchange fluxes, and (iii) large, variable CO₂ emission fluxes, match our scenario 4 (Fig. 1). These patterns suggest limited influence of photoautotrophs on ER at the perennial stream, where terrestrial inputs likely dominate CO₂ temporal dynamics, especially compared to the non-perennial stream. Although limited reliable CO₂ data for this stream precluded more thorough comparisons between streams, anomalous increases in CO₂ concentrations observed between sensor maintenance (Supporting Information Fig. S1), suggest high local respiration induced by abundant coarse debris rapidly accumulated around the probe anchoring device. This observation is consistent with previous studies in this stream showing (i) high ER, and (ii) high proportion of protein-like dissolved organic matter providing easily degraded organic substrate (Bernal et al. 2018; Lupon et al. 2020). Other studies in subtropical areas have also reported abundant protein-like dissolved organic matter in stream water (Butturini et al. 2016; Larsen and Woelfle-Erkine 2018). It follows that in-stream dissolved organic matter degradation, and attendant ER and internal CO₂ production, is likely more important than previously thought in this type of streams.

Despite expecting ER to be highly temperature-dependent (Jones and Mulholland 1998; Demars et al. 2011; Song et al. 2018), we observed no consistent ER response to changes in temperature in either stream. At the non-perennial stream, ER and NEP actually declined despite dramatic increases in water temperature (up to 24°C in June) induced by high light inputs and low water depths. This pattern may be an artifact of the strong relationships between ER, Qₑ, and K₆₀₀ (Supporting Information Fig. S3, S4), or else, it could be related to decreasing lateral hydrologic connectivity and attendant declines in organic matter supply. At the perennial stream, NEP weakly increased with increasing temperature, indicating that NEP variation is mostly driven by other factors. This result implies challenges when imposing simple metabolic scaling laws to whole networks at regional or global scale (Demars et al. 2011) because factors beyond temperature can exert considerable control on ecosystem-level metabolic activity (Lynch et al. 2010; Beaulieu et al. 2013; Bernhardt et al. 2022).

Terrestrial vs. in-stream sources of CO₂ emission

At the non-perennial stream, NEP explained 51% of daily CO₂ emission (40–61% given uncertainty in metabolic stoichiometry). Furthermore, lateral CO₂ inputs could explain, at
most, less than 10% of the CO2 emission flux, supporting the expectation that lateral inputs of soil-respired CO2 minimally contributed to this stream’s CO2 emission. However, even considering the uncertainty in our calculations, a source other than aerobic respiration and lateral groundwater inputs is required to close the C mass balance of measured CO2 emission. Anaerobic respiration is a likely pathway for this unexplained source, especially at the end of the deployment period, when minimum daily O2 concentrations declined from 6 to 2 mg O2 L⁻¹. Increased anaerobic contributions could also explain the marked shift in subdaily CO2 : O2 slopes from close to stoichiometric expectations (−1) to values as low as −0.12. Shifting the association between gas fluxes, with increased CO2 respired per unit O2 produced, suggests coupling between aerobic and anaerobic processes as reported in other riverine systems (Harrison et al. 2005; Heffernan and Cohen 2010). Although in situ ¹⁵N addition experiments indicate anaerobic respiration rates in headwater streams are too small to substantially influence in-stream CO2 production (Mulholland et al. 2009; von Schiller et al. 2009), we argue that these processes are still not well quantified. Indeed, recent studies using ¹³C-DIC ratios suggest anaerobic processes (in soils or streams) represent important sources of CO2 in some boreal streams (Campeau et al. 2017) and are especially important during low flows (Gómez-Gener et al. 2020).

Lateral inputs were expected to dominate CO2 emission fluxes at the perennial stream because this net gaining reach more closely resembles the humid regions where lateral inputs convey significant DIC (Hotchkiss et al. 2015; Marx et al. 2017). This expectation was further supported by weak coupling observed between instantaneous CO2 and O2 exchange fluxes. Surprisingly, however, NEP accounted for 48–72% (given RQ uncertainty) of mean daily CO2 emission flux, while lateral inputs (1.6 ± 0.13 g C m⁻² d⁻¹) accounted for only ca. 25%. Because groundwater CO2 concentrations are notoriously difficult to constrain, we evaluated uncertainty (± 20%) in this variable, but this led to relatively small changes, with lateral inputs contributing 20–30% of measured CO2 emission. Although K_CO2 is also difficult to constrain, propagating ± 20% variation in this variable scarcely affect our estimates of relative contributions because it simultaneously influences CO2 emission and O2-derived metabolic rates, differing only by their respective Schmidt numbers. Finally, CO2 turnover length would need to increase fourfold (from 453 to 1797 m) for lateral groundwater inputs to explain 100% of mean daily CO2 emission. This expansion seems unrealistic given ambient alkalinity (400–700 μeq L⁻¹) and pH (6–7) in these streams (Piñol and Avila 1992). Yet, without actual measures of these two variables, we cannot completely rule out some time-lagged atmospheric equilibration arising from DIC transport as bicarbonate. Des our data set for the perennial stream was relatively small (n = 16), which increase our estimates of uncertainty, our results support the idea that NEP substantially contributed to CO2 emission. Although lateral CO2 fluxes may dominate in many settings, our results imply that this cannot be taken for granted because NEP contribution to total CO2 emission can be large under certain hydrological or environmental conditions as shown in this study.

Our results underscore the need to better constrain both terrestrial and in-stream CO2 sources in headwater streams to understand their time-varying relative contribution to stream CO2 emission. Combining direct measurements of dissolved CO2 and O2 concentrations helps unravel the different sources of CO2 emission from headwater streams (Pennington et al. 2018; Vachon et al. 2020). However, we found that divergences arise when confronting results obtained from CO2–O2 measurements and other approaches, such as in-stream metabolic rates directly inferred from stream O2 concentrations. The relative contribution of NEP to CO2 emission can vary substantially depending on metabolic stoichiometry, groundwater CO2 concentrations, or the presence of anaerobic respiration. Carbonate buffering can also complicate attribution of CO2 emission to different contributing sources, especially in high-alkalinity streams. Future studies should better constrain these uncertainties by carefully quantifying CO2 fluxes at the terrestrial-stream-atmosphere interfaces. High-frequency monitoring of gas concentrations in stream water and riparian groundwater combined with alkalinity (or pH) measurements and isotopic techniques constraining in-stream and terrestrial metabolic processes can help to reconcile results obtained from different empirical approaches and improve our mechanistic understanding of C cycling and CO2 emission from riverine ecosystems.

Recent studies suggest global CO2 emission from inland waters are 2.9 Pg C yr⁻¹, with low-order streams responsible for a disproportionate fraction of this efflux (Raymond et al. 2013; Marx et al. 2017; Liu et al. 2022). In boreal and temperate systems, lateral groundwater inputs dominate stream CO2 contributions (Hotchkiss et al. 2015; Leith et al. 2015; Lupon et al. 2019). Although dissolved CO2 concentrations in our streams were within the literature range (Gómez-Gener et al. 2016; Lupon et al. 2019), in-stream metabolic activity influenced both the magnitude and temporal dynamics of CO2 emission fluxes. NEP contributed 51% and 57% of daily CO2 emissions in both the non-perennial and perennial streams, respectively, despite differing in the relevance of lateral groundwater inputs. These values far exceed those previously reported for headwater streams (0–19%) (Hotchkiss et al. 2015; Gómez-Gener et al. 2016). Our results align with recent findings from tropical, boreal and Arctic streams suggesting that the influence of in-stream metabolism on CO2 emission may be larger than previously thought (Campeau et al. 2017; Rocher-Ros et al. 2020; Marzolf et al. 2022). Because losing streams and seasonal flow transitions are widespread in low relief and high water abstraction areas, even outside dry climates (Jasechko et al. 2021), our work may be broadly relevant to streams elsewhere.
findings suggest NEP could source substantial CO₂ in many headwater streams, particularly those with highly heterotrophic conditions and limited lateral inputs. In those cases, stream metabolic activity is less likely to be overwhelmed by transport and lateral groundwater inputs as occurs in lentic systems (Hotchkiss et al. 2018).

Data availability statement
Data set used for this study is public in Hydroshare (http://www.hydroshare.org/resource/5ca90b71b2774793b2d8a5793457e684).

References


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Conflict of Interest
None declared.