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LETTER

Groundwater-stream connections shape the spatial pattern and rates of aquatic metabolism

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Scientific Significance Statement

Freshwater systems rely strongly on surrounding terrestrial environments as sources of organic matter. To date, these land–water interactions have been conceptualized either aspatially or based on broad-scale transitions in resource availability along river systems, with limited understanding of how hydrological connections at watershed or reach scales shape aquatic processes. Here, we show that landscape structure can influence the spatial pattern and whole-reach rates of stream heterotrophic activity by determining where shallow groundwater flowpaths supply labile dissolved organic matter to streams. Recognizing these fine scale spatial patterns of metabolic activity is critical for understanding how stream ecosystems integrate diverse pathways of groundwater that extend laterally into terrestrial uplands, as well as for upscaling biogeochemical fluxes in river networks.

Abstract

A longstanding challenge in stream ecology is to understand how landscape configuration organizes spatial patterns of ecosystem function via lateral groundwater connections. We combined laboratory bioassays and field additions of a metabolic tracer (resazurin) to test how groundwater-stream confluences, or "discrete riparian inflow points" (DRIPs), regulate heterotrophic microbial activity along a boreal stream. We hypothesized that DRIPs shape spatial patterns and rates of aquatic heterotrophic microbial activity by supplying labile dissolved organic matter (DOM) to streams. Laboratory bioassays showed that the potential influence of DRIPs on heterotrophic activity varied spatially and temporally, and was related to their DOM content and composition. At the reach scale, DRIP-stream confluences elevated the spatial heterogeneity and whole-reach rates of heterotrophic

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Data Availability Statement: Data and metadata are available in the Zenodo data repository (Lupon et al. 2022; https://zenodo.org/record/4723597#.Y5i1Z3bMI2y). Additional supporting information may be found in the online version of this article.

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activity, especially during periods of high land–water hydrological connectivity. Collectively, our results show how the arrangement of lateral groundwater connections influence heterotrophic activity in streams with implications for watershed biogeochemical cycles.

Headwater streams are natural laboratories for exploring questions about how landscape connectivity and configuration influence ecosystem processes because they strongly rely on surrounding terrestrial environments as sources of particulate organic matter (POM) and dissolved organic matter (DOM) to aquatic heterotrophs (Marcarelli et al. 2011). Land–water interactions are thus central to theory in river science, but their consideration has often been either aspatial (Fisher and Likens 1973) or based on changes in terrestrial inputs or hydrogeomorphic structures along river continua (Vannote et al. 1980; Poole 2002). While important hydrological connections between soils and streams can operate at much finer scales (Krause et al. 2017), we still know very little about how such linkages organize stream processes.

Landscape topography, and particularly the size and arrangement of zero-order basins, may be an important source of spatial variation in land–water connections (Sidle et al. 2018). These unchannelized hollows create discrete conduits for groundwater transport, and are notably prominent in northern landscapes, where glacial till limits infiltration and forces lateral flowpaths through relatively shallow strata (Fig. 1A; Seibert et al. 2009; Hutchinson and Moore 2000). The confluences between these groundwater flowpaths and streams, referred to here as *discrete riparian inflow points* (DRIPs), can lead to abrupt longitudinal changes in streamflow, depending on the size of the upslope contributing area (Leach et al. 2017). DRIPs can also have elevated DOM concentrations relative to diffuse groundwater sources (Ploum et al. 2020) and thus create heterogeneity in water chemistry along stream reaches (Lupon et al. 2019, 2020). To the extent that DRIP-stream confluences supply limiting or bioreactive solutes, they may support locally elevated rates of stream ecosystem processes and thus serve as important metabolic "control points" (sensu Bernhardt et al. 2017). Yet, despite being prominent features of many landscapes (e.g., Briggs and Hare 2018), no studies have tested how such lateral connections give rise to heterogeneity in ecosystem functioning within drainage networks.

Here we ask how topographically-driven DRIPs influence the spatial configuration and whole-reach rates of heterotrophic microbial activity in boreal streams. Despite typically having high concentrations of DOM, microbial heterotrophs in northern streams are often limited by labile carbon (Burrows et al. 2017) and are responsive to flow events that deliver bioreactive DOM from adjacent soils (Demars et al. 2020). Thus, we hypothesized that relatively persistent supplies of terrestrial DOM from DRIPs support zones of elevated heterotrophic activity along streams. We tested this hypothesis using the resazurin–resorufin (Raz-Rru) system as a surrogate for heterotrophic microbial activity (Haggerty et al. 2009). We used Raz-Rru in bioassays to assess the reactivity of groundwater DOM from DRIPs and for in situ tracer additions to test how DRIP-stream confluences influence patterns of heterotrophic activity locally and at reach scales.

Materials and methods

Study site

The study was conducted in a first-order stream located in the Krycklan Catchment in northern Sweden (67.9 km^2, 114–405 m a.s.l; 64°12′N 19°52′E; Laudon et al. 2021; Fig. 1B). As with other boreal systems, Krycklan streams are generally DOM-rich, with POM accounting for only a small fraction (< 1%) of the total OM in transport (Laudon et al. 2011). We worked in a 1.4-km headwater section that has no tributaries and is bounded by two gauging stations (Fig. 1B). The upstream gauging station is located ~ 100 m downstream of a small humic lake that is partially surrounded by a mire complex. The rest of the catchment is dominated by coniferous forest composed of Scots pine (*Pinus sylvestris*) and Norway sprice (*Picea abies*), with some birch (*Betula pubescens*) flanking the stream. Several DRIPs enter the stream along this section (Fig. 1B), and their contribution to stream flow varies spatially and temporally (Leach et al. 2017). The stream, dominated by a riffle-run structure, has a mean channel width and water depth of 71 ± 24 and 15 ± 18 cm (mean ± SD), respectively. During summer and autumn (June–September), stream flow averages 6.6 ± 7.4 and 10.4 ± 10.9 L/s in the upstream and downstream gauging stations, respectively (period 2008–2016; Fig. S1). Yet, both stream flow and the contribution of different water sources (lake vs. groundwater) are highly variable in time (Fig. S1).

DOM characterization of DRIPs and associated heterotrophic activity

We first evaluated the potential of DRIP-stream confluences to fuel in-stream heterotrophic activity and how it relates to DOM content and composition using laboratory bioassays (appendix 1, Lupon et al. 2022). For the bioassays, we sampled the major water sources to the stream, including the lake outlet and five DRIPs that collectively account for ~ 70% of the groundwater inflows along the section (Leach et al. 2017; Fig. 1B). We identified DRIPs following Lidberg et al. (2017). Briefly, DRIPs were extracted with topographical modeling from a digital elevation model with a resolution of 2 × 2 m and preprocessed in a three-step breaching approach (*see* Lidberg et al. 2017). We then used the algorithms multiple flow direction infinity (MDInf) and stream index division equation (SIDE) to compute the side-separated contributions

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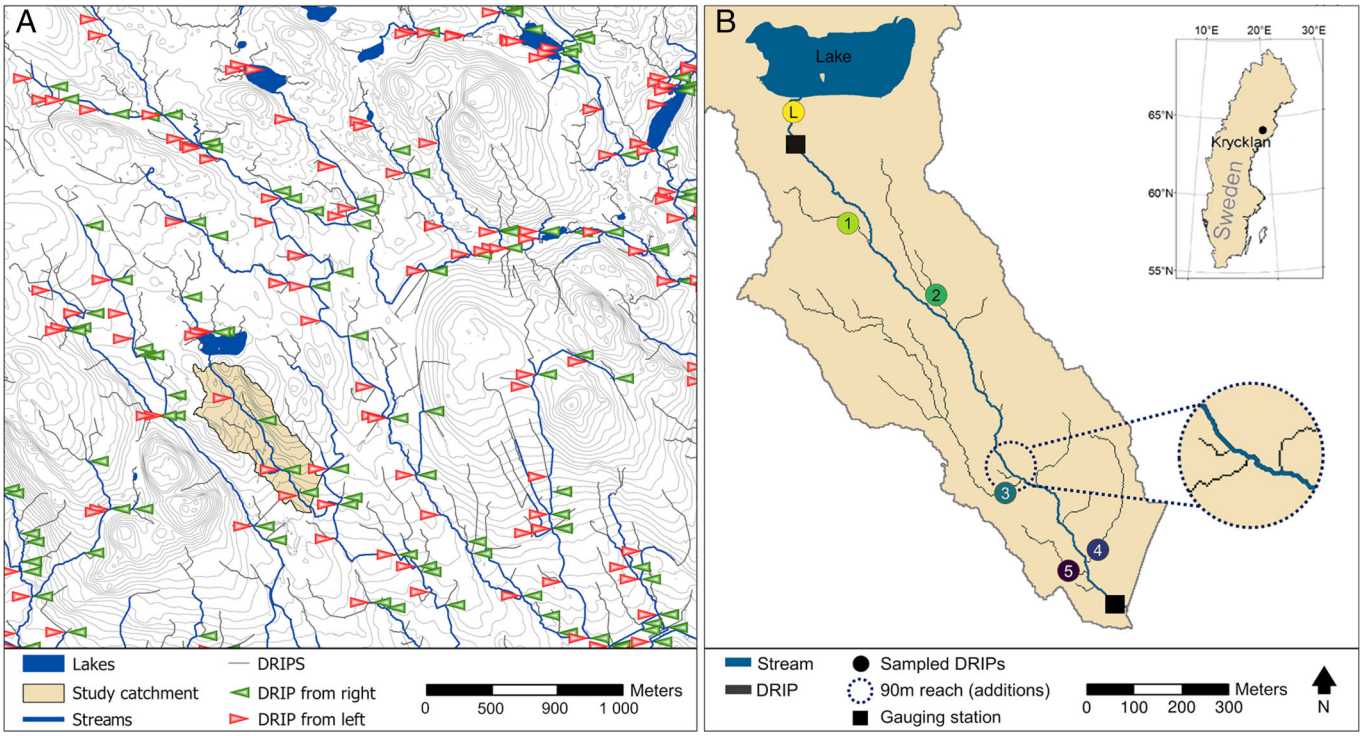

Fig. 1. Map of the study stream section and its catchment. (**A**) Section of the Krycklan catchment, its fluvial network and the main discrete riparian inflow points (DRIPs) discharging into headwater streams. (**B**) The 1.4 km long stream section (blue line), the upstream lake and the main DRIPs (gray lines). The map also shows the two gauging stations (black squares), the sampling locations for DRIPs (solid circles) and the 90-m stream reach selected for resazurin additions (dark blue open circle). A zoom into the reach selected for resazurin additions shows that there are three DRIPs entering to the stream. The inset shows the location of the Krycklan study catchment within Sweden (64°12′ N 19°52′ E; 67.9 km^2, 114–405 m a.s.l).

to the stream network (Seibert and McGlynn 2007). SIDE contributions between 2 and 10 ha were considered to be DRIPs.

We sampled all water sources (lake outlet and DRIPS) every 2–3 weeks during summer 2017. We collected samples from the thalweg of lake outlet, whereas water from DRIPs was sampled from fully screened, 1 m long PVC wells installed ca. 2 m from the stream edge. Water samples were filtered (Minisart Syringe Filter, 0.20 μm pore diameter) and kept cold and dark until laboratory analysis (< 12 h). To simulate what happens when these source waters reach the stream, we mixed 30 mL of filtered-water of each source with 100 μL of a slurry containing in situ stream microbial assemblages and incubated them for 24 h (Supporting Information Appendix S1). For each water source, we estimated the potential in-stream heterotrophic activity using the Raz-Rru tracer system (Supporting Information Appendix S1). Briefly, Raz transforms to Rru in the presence of aerobic respiration (Haggerty et al. 2009). Hence, we used Rru production over the incubation period (ΔRru, μg Rru L^{-1}) as a proxy of in-stream heterotrophic microbial activity. We also standardized the Rru production by the initial dissolved organic carbon (DOC) concentration in each incubation (ΔRru/DOC, μg Rru mg C^{-1}) to estimate the efficiency of heterotrophic activity per mass of DOC available in the water (Supporting Information Appendix S1). We measured DOC concentration by combustion on a Shimadzu TOC-VPCH analyzer after acidification and sparging to remove inorganic carbon. Finally, we also determined DOM composition by optical spectroscopy using an Aqualog spectrofluorometer (absorbance range: 220–800 nm; emission and excitation ranges: 220–800 nm; 1 nm steps). Samples were analyzed using a 1 cm path length quartz cuvette, and all excitation–emission matrices were corrected for blank absorption, instrument-specific biases, primary and secondary inner filter effects, and Rayleigh scatter, and were also normalized to Raman units (R.U.) using the built-in tools in the Aqualog software FluorEscence (Quatela et al. 2018). Furthermore, samples with high absorbance were manually diluted prior to analysis. We used absorbance and fluorescence data to calculate four indexes: the specific ultraviolet absorbance (SUVA), the fluorescence index (FI; McKnight et al. 2001), the biological index (BIX; Huguet et al. 2009), and the humification index (HIX; Zsolnay et al. 1999).

We used linear mixed-model ANOVAs followed by post hoc Tukey contrasts to test whether DOM characteristics and in-stream heterotrophic activity varied among water sources (package *lme4*, R 3.3.0 software, 2012; Bates et al. 2015). Water source was the independent variable and date was a random effect. Furthermore, we explored the relationship

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between chemistry and heterotrophic activity through linear regression models that considered either ΔRru or ΔRru/DOC as dependent variables, DOC concentration and DOM spectroscopic metrics as independent variables, and both water source and date as random effects. We selected the best-fit model by applying a step-wise analysis (package *lmerTest*; Kuznetsova et al. 2017). Model selection was based on backward elimination of random and fixed-effect terms using the Satterthwaite's degrees of freedom method (Fai and Cornelius 1996). We considered statistical tests significant when $p < 0.01$.

Spatial configuration and whole-reach rates of heterotrophic activity

We then evaluated how DRIP-stream confluences regulate the spatial configuration and whole-reach rates of heterotrophic activity by performing constant rate additions of Raz following Haggerty et al. (2009). We conducted additions in a 90-m reach located 850 m downstream of the head of the section (Fig. 1B). The reach, characterized by discrete up- and downwelling zones, was influenced by three DRIPs with potentially different inflow rate and chemical signatures (Fig. 1B). Additions were carried out on seven dates during summer 2017. To better understand the mechanisms driving spatial patterns of heterotrophic activity, we conducted the additions under both natural (four dates) and experimentally manipulated (three dates) flow conditions (Table S1). We experimentally manipulated stream flow by either damming the lake outlet (simulating drought) or pumping water from the lake (simulating floods). This manipulation modified the relative contributions of lake vs. groundwater to total streamflow, but did not directly alter the lateral DRIPs-stream connections. As a result, the contribution of groundwater to streamflow at the downstream gauging station ranged from 3% to 88%. This variability in groundwater contribution is similar to what can be observed at this site under natural conditions (Fig. S1).

Additions consisted of a solution containing Raz (target stream concentration = 200 μg Raz L^{-1}) and sodium chloride (conservative tracer) that was injected at constant rate at the top of the reach using a peristaltic pump (Model QGX, FMI Pump) over 2–3 h. We monitored electrical conductivity at 4 and 90 m downstream from the injection point to detect plateau conditions. At plateau, we collected samples from the surface and hyporheic water (two replicates) at 18 stations along the reach. Distances between sampling stations varied between 2 and 10 m and aimed to capture the spatial variability in heterotrophic activity near DRIP-stream confluences. We filtered all water samples and kept them dark and cold until analysis (< 4 h). We analyzed water samples for both Raz and Rru concentrations as described for lab experiments (Supporting Information Appendix S1). Furthermore, we measured channel wet width and depth at six transects along the reach and used conductivity breakthrough curves at 4 and 90 m from the injection point to estimate stream flow (L s^{-1}) at the top (Q_{TOP}) and the bottom (Q_{BOT}) of the reach. Groundwater inflow along the reach (Q_{GW}, L s^{-1}) was calculated as the difference between Q_{BOT} and Q_{TOP}. The relative contribution of groundwater inflows to stream flow was estimated as Q_{GW}/Q_{BOT}.

To assess the influence of DRIPs on the spatial configuration of in-stream heterotrophic activity, we analyzed the longitudinal profiles of Rru/Raz molar ratio in the surface and hyporheic water (Lupon et al. 2022). We used the molar ratio to account for potential dilution and adsorption effects (Haggerty et al. 2009). For the surface water, we calculated the relative increase in Rru/Raz molar ratios along the reach (ΔRru/Raz) and compared the relative increase in Rru/Raz molar ratios upstream vs. immediately downstream DRIPs using *t*-tests. For the hyporheic water, we used *t*-tests to compare Rru/Raz ratios between zones located upstream vs. immediately downstream DRIPs. We considered statistical tests significant when $p < 0.01$. We also estimated the whole-reach Raz to Rru transformation rate (k_T, /m) with an analytical model representing coupled parent–daughter steady state transport (Haggerty 2013; *see* appendix 2, Lupon et al. 2022). From k_T, we calculated three metrics to characterize heterotrophic activity at reach scale: the Raz transformation length (S_W, m), the Raz transformation velocity (V_f, mm min^{-1}), and the root mean squared error (RMSE) of the transport model. S_W and V_f are indicators of the efficiency of heterotrophic activity at reach scale, while RMSE describes the spatial heterogeneity in this activity based on longitudinal patterns (Supporting Information Appendix S2). Finally, we examined the role of hydrology on whole-reach heterotrophic activity by applying regression models (linear, potential, and logarithmic) between estimated metrics and hydrologic variables (*see* Table S1). Model selection was performed by ordinary least squares.

Results and discussion

Heterogeneity in microbial activity arising from DRIP-stream confluences reflected the amount and character of DOM supplied by these lateral flow paths. Laboratory bioassays showed that all water sources discharging into this stream had a strong terrestrial DOM signature, with relatively low FI (< 1.6) and BIX (< 0.6) values (Table 1). Yet, DOC concentration ($F = 31.31$, df = 5, $p < 0.001$), SUVA ($F = 9.03$, df = 5, $p < 0.001$) and HIX ($F = 10.43$, df = 5, $p < 0.001$) differed among sources, with lake water showing higher aromaticity and humic character than some DRIPs (Table 1). Likewise, the potential for DOM to support stream heterotrophic activity varied, on average, by 10-fold among sources ($F = 15.25$, df = 5, $p < 0.001$; Fig. 2A), even when correcting for differences in DOC concentration ($F = 5.09$, df = 5, $p = 0.006$; Fig. 2B). Specifically, ΔRru increased with DOC concentration ($R^2 = 0.77$, $n = 24$, $p < 0.001$; Fig. 2C), whereas ΔRru/DOC

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Table 1. Dissolved organic matter characterization of the main water sources draining along the stream section during summer 2017. Variables are: Dissolved organic carbon concentration (DOC), specific ultraviolet absorbance (SUVA), fluorescence index (FI), biological index (BIX), and humification index (HIX). For each variable, values are expressed as mean [minimum–maximum]. In all cases, different superscript letters indicate significant differences among water sources (robust Tukey test, $p < 0.01$).

Water source	DOC (mg C L^{-1})	SUVA (L mg C^{-1} m^{-1})	FI	BIX	HIX
Lake	23.5 [18.4–32.3]^{BC}	4.93 [4.40–5.24]^{A}	1.43 [1.40–1.45]^{A}	0.41 [0.38–0.43]^{A}	12.9 [9.9–18.2]^{C}
DRIP 1	39.6 [32.3–50.0]^{D}	3.16 [2.52–3.76]^{AB}	1.49 [1.46–1.52]^{A}	0.45 [0.40–0.50]^{A}	8.5 [6.9–10.7]^{ABC}
DRIP 2	36.2 [24.0–42.4]^{CD}	4.63 [4.04–5.34]^{A}	1.43 [1.39–1.44]^{A}	0.37 [0.34–0.43]^{A}	6.5 [3.7–9.7]^{AB}
DRIP 3	13.0 [9.4–15.2]^{AB}	4.32 [3.92–4.86]^{A}	1.60 [1.41–1.45]^{A}	0.38 [0.35–0.41]^{A}	13.9 [11.5–16.4]^{C}
DRIP 4	28.9 [24.7–33.6]^{CD}	2.99 [0.62–4.26]^{AB}	1.43 [1.51–1.81]^{A}	0.54 [0.48–0.70]^{A}	10.0 [8.4–12.1]^{BC}
DRIP 5	3.6 [2.2–6.8]^{A}	1.56 [0.77–1.95]^{B}	1.43 [0.63–1.99]^{A}	0.90 [0.71–1.20]^{B}	2.9 [2.3–3.5]^{A}

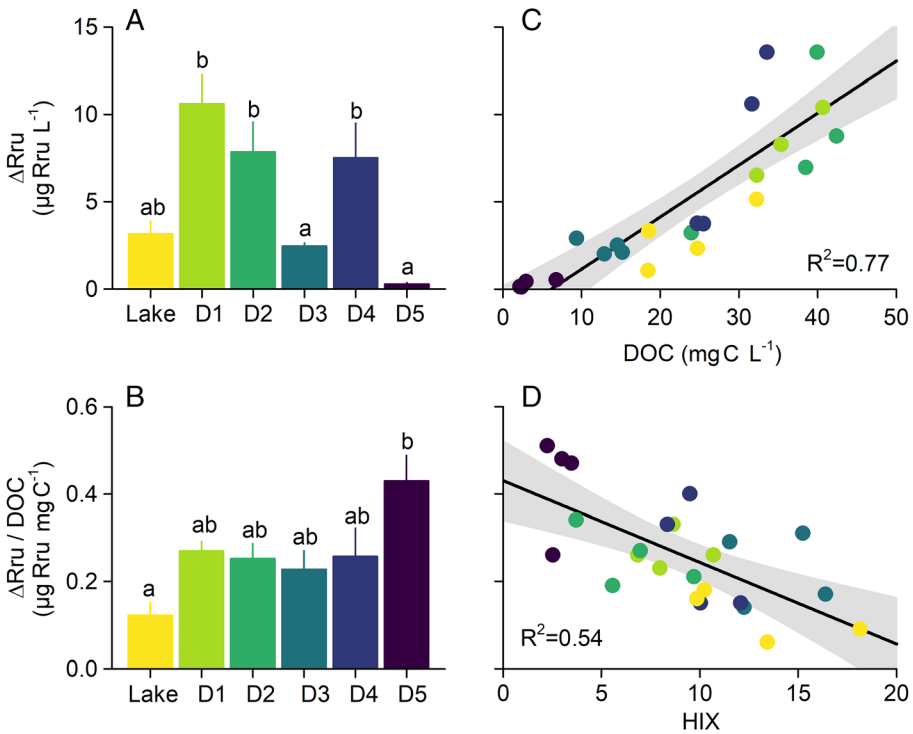

Fig. 2. Heterotrophic activity associated with the major water sources discharging into the stream section and its relationship to dissolved organic matter characteristics. Heterotrophic activity was measured as the Rru production over the incubation period (ΔRru, top panels) and as the Rru production standardized by the initial concentration of dissolved organic carbon (ΔRru/DOC). Colors of bars and points denote different water sources as labeled in panels (**A**) and (**B**). In panels (**A**) and (**B**), different letters indicate significant differences among water sources (Tukey test, $p < 0.01$). In panels (**C**) and (**D**), the black line shows the regression of the mixed-effect model, while the gray shadow shows the 95% confidence interval. The goodness of fit (R^2) of the model is also shown. Note that no relationship was observed between heterotrophic activity and SUVA, FI, BIX, or random terms.

was negatively related to the HIX ($R^2 = 0.54$, $n = 24$, $p < 0.001$; Fig. 2D). Collectively, these results show that the potential for a particular water source to fuel stream heterotrophic activity varied depending on the amount and the humic character of the DOM in transport. Relatively low rates of microbial activity for the lake outlet suggest that lake residence time was sufficiently long for bacterioplankton to process labile DOM forms before these reached the stream (Berggren et al. 2010). Furthermore, variation in DOM among DRIPs likely reflects the combination of local differences in groundwater table elevation relative to soil organic matter pools (Ledesma et al. 2018), shifts in riparian plant community composition (Kuglerová et al. 2015), and/or variable supplies of organic matter from root exudates and litter (Hensgens et al. 2020). Regardless of the mechanism, the implications of this variation are that DRIPs supplying low

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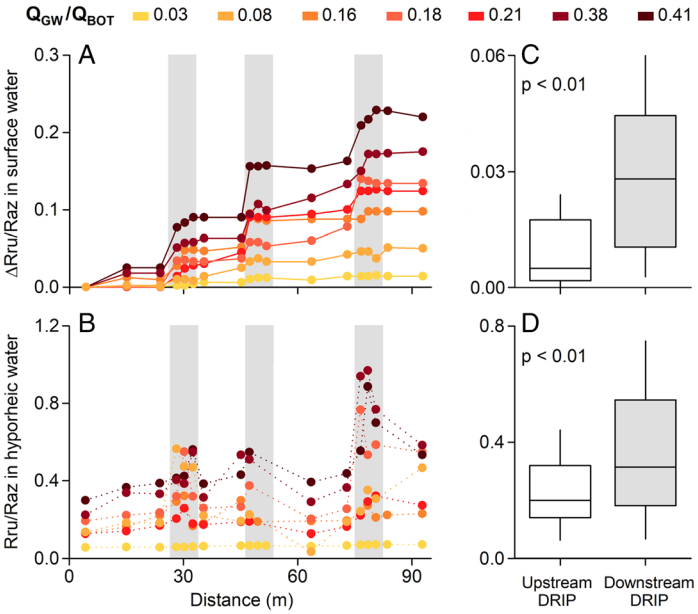

Fig. 3. Variation in the molar ratio between resorufin and resazurin concentrations (Rru/Raz) along the 90-m reach; and comparison of the Rru/Raz molar ratios between zones located upstream vs. immediately downstream discrete groundwater riparian input points (DRIPs) in either the surface (top panels) or the hypoheric (bottom panels) water. Different colors represent sampling dates with increasing groundwater contribution to streamflow (yellow: lowest contribution, dark red: highest contribution; *see* Table S1). In panels (**A**) and (**B**), vertical gray stripes indicate immediately downstream DRIPs zones. In panels (**C**) and (**D**), boxes indicate the 25th and 75th percentiles, and whiskers indicate the 10th and 90th percentiles. The *p* values of *t*-tests are also shown. For the surface water, values are expressed as the relative increase in Rru/Raz molar ratios along the reach (panel **A**) or upstream–downstream DRIPs (panel **C**). For the hyporheic water (panels **B** and **D**), values are expressed as bulk Rru/Raz ratios.

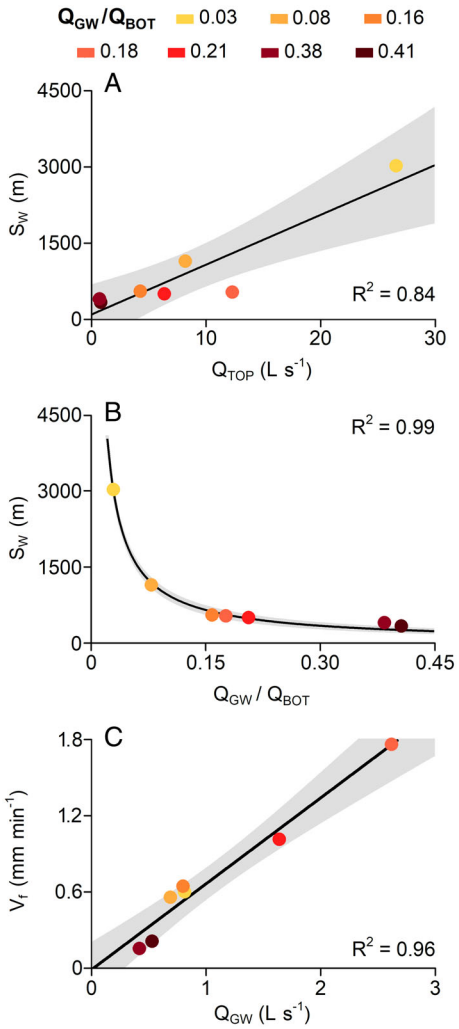

Fig. 4. Relationships between whole-reach resazurin (Raz) transfromation metrics and hydrological conditions. (**A**) Raz transformation length (S_W) vs. stream flow at the top of the reach (Q_{TOP}); (**B**) S_W vs. groundwater inflows contribution to downstream flow (Q_{GW}/Q_{BOT}); and (**C**) Raz tranformation velocity (V_f) vs. groundwater inflows along the reach (Q_{GW}). Different colors represent sampling dates with increasing groundwater contribution to streamflow (yellow: lowest contribution, dark red: highest contribution; *see* panel **B**, Table S1). The black line shows the model (either linear or potential), and the gray shadow indicates the 95% confidence interval. The goodness of fit (R^2) of each model is also shown.

DOC concentrations and/or humic-like DOM compounds likely lead to confluences with streams that passively transmit terrestrial DOM downstream. By contrast, DRIPs supplying DOM with more reactive constituents create confluences that are potentially key reactors for organic matter processing by microbes.

Tracer additions in the field supported the hypothesis that persistent supplies of DOM from DRIPs can generate zones of elevated microbial activity along streams. These patterns were generally consistent with our laboratory bioassays of DOM reactivity (Fig. 2), but we also recognize that organic carbon particle formation at the land–water interface could further promote microbial activity (Einarsdóttir et al. 2020). For all additions, Rru/Raz ratios increased and were more variable in DRIPs or immediately downstream of them, yet these longitudinal patterns were clearer in the surface than in the hyporheic water (Fig. 3). Most importantly, our results demonstrate that such confluences are disproportionately important for stream aerobic respiration at the reach scale, as 66–88% of Raz was transformed immediately downstream DRIPs during baseflow conditions (Table S1). Furthermore, the amount of Raz transformed immediately after DRIPs drove the overall spatial heterogeneity (i.e., RMSE) in microbial activity at the reach scale ($R^2 = 0.83$, $n = 7$, $p < 0.001$), as well as the whole-reach Raz transformation rate ($R^2 = 0.67$, $n = 7$, $p = 0.009$) (Fig. S2). Given this disproportionate influence on reach-scale metabolic activity occurring over relatively small spatial extents, DRIP-stream confluences clearly meet the criteria to be

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considered ecosystem control points (Bernhardt et al. 2017). Furthermore, such metabolic processes are also significant from a carbon cycling perspective since 17–51% of the carbon dioxide (CO_2) evaded from this same stream reach can be linked back to the in-stream, biological processes captured here (Lupon et al. 2019). Overall, we suggest that DRIPs are key elements of headwater streams that deserved further study as rates of several biogeochemical processes (e.g., DOM processing, nutrient uptake) are likely to vary widely among reaches with and without discrete land-stream confluences, especially in those ecosystems that rely strongly on terrestrial sources of DOM.

Our flow manipulation allowed us to further explore how increases in the contribution of groundwater to stream flow can amplify spatial heterogeneity and reach-scale rates of heterotrophic activity, thereby increasing the significance of these lateral confluences (Fig. 4; Table S1). Specifically, we found that S_W was positively related to Q_{TOP} ($R^2 = 0.84$, $n = 7$; Fig. 4A) and negatively related to Q_{GW}/Q_{BOT} ($R^2 = 0.99$, $n = 7$; Fig. 4B), whereas V_f was positively related to Q_{GW} ($R^2 = 0.96$, $n = 7$; Fig. 4C). Thus, the role of stream-DRIP confluences as regulators of aquatic heterotrophic activity is maximized during summer low flows, when DRIPs act as the major source of water to the stream (Fig. S1). Conversely, the influence of stream-DRIP confluences on whole-reach metabolism was the lowest during high flows, when upstream water sources overwhelm water flow through the system (e.g., Leach and Laudon 2019) and/or short water residence times limit the capacity of in-stream biota to act upon matter in transport (Fig. 4A; Raymond et al. 2016). We acknowledge that concentrations and potential sources of DOM might differ during natural droughts and floods and thus these results should be interpreted with caution. Indeed, the specific hydrologic thresholds at which DRIPs influence stream biogeochemical processes will likely change in time and space as a function of catchment geomorphology (regulating the contributing area of DRIPs), groundwater level (determining soil sources of DOM and nutrients), and water chemistry in a similar manner as observed in the laboratory bioassays.

To date, there is essentially no empirical research addressing how preferential groundwater inputs to streams shape metabolic activity at larger spatial scales. This limitation reflects, in part, the fact that most estimates of stream metabolism are based on measurements performed at reaches with low groundwater-stream exchange (Rocher-Ros et al. 2020), and the footprints of those assessments are far too coarse to resolve heterogeneity at this scale. Yet, a broader assessment of Krycklan landscape suggests that these lateral connections are ubiquitous (Fig. 1A). Indeed, considering the fraction of the landscape dominated by glacial till, and assuming a threshold for channel initiation of 2 ha (Leach et al. 2017), we estimate approximately 7.5 zero-order basins per km^2, which translates to about one DRIP every 250 m of stream length. While not all DRIPs are likely to amplify in-stream processes to the same degree, we suggest that these lateral connections represent a primary driver of heterogeneity in stream metabolic activity in headwater fluvial networks, at least during low flow conditions. Discrete lateral connections are common across headwater environments and may emanate from multiple geological (e.g., topography, bedrock fractures; Winter 1999; Briggs and Hare 2018) and anthropogenic (e.g., waste water treatment plants, ditches; Gücker et al. 2006) features; thus, the patterns observed here may arise in any systems where heterotrophic organisms are responsive to supplies of DOM. Recognizing these fine scale spatial patterns of metabolic activity is critical for understanding how stream ecosystems integrate flowpaths that extend laterally into terrestrial uplands (Fisher et al. 2004), as well as for upscaling biogeochemical fluxes in river networks (Saccardi and Winnick 2021).

Freshwater systems have long been at the forefront of testing the influences of cross-boundary energy flow on ecosystem functioning (Marcarelli et al. 2011). While much of this work in streams has emphasized aboveground organic matter inputs (e.g., litterfall, Wallace et al. 1997), recent studies highlight similarly important connections belowground (Demars et al. 2020). Yet, the general view of exchange between these "black boxes" remains simplistic, with little progress toward spatially resolving land–water interactions guided by lateral groundwater flowpaths. Similarly, while past studies have shown how lateral connections can influence spatial patterns of solutes (e.g., Dent et al. 2001) and gases (e.g., Duvert et al. 2018) in streams, these efforts have not extended to measures of ecosystem functioning. Our results show that, by acting as sources of reactive DOM, the often-ignored lateral connections arising from the confluence of streams and zero-order basins can create spatial heterogeneity in aquatic microbial processes that has important implications for ecosystem processes at larger scales.

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