Evaluating the Raz-Rru System for Use in Florida Springs

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"Smart tracer" methods such as the resazurin-resorufin (Raz-Rru) system have been introduced to quantify hyporheic exchange and microbial metabolism in hydrologic studies and provide insight as to the biogeochemical activity of stream systems in addition to hydraulic parameters describing advective transport and transient storage. However, preliminary reach scale tracer tests performed in small streams did not yield transformation of Raz likely due to insufficient residence times and inactive, homogeneous soils. This study aims to assess the effectiveness of Raz-Rru as an indicator of biogeochemical potential in areas where high rates of water-sediment exchange are expected. To compare activities in various Floridian aquatic environments, sediment was collected from the first magnitude spring-fed Silver River in Marion County as well as Jennings Creek, a small urban stream in Gainesville, and batch experiments were performed to determine rates of tracer transformation and adsorption. Results from microbially inactive vs live sediment samples indicate that the more organic Silver River sediments have greater capacities for adsorption and Raz transformation. Modeled kinetic parameters were utilized in an OTIS advection-dispersion model which showed that Raz to Rru conversion and retention is minimal in Jennings Creek sediment as compared to the Silver River. Overall, the kinetics of the Raz-Rru tracer system can be modeled effectively for a range of stream systems, however more extensive work is necessary to accurately separate fluorescence signals and determine the exact behavior of the tracers under the varying oxidation reduction potentials that drive biogeochemical activity.

INTRODUCTION

Due to more than 50 years of rapid population growth and increased agricultural activity, nitrate (NO_3^-) concentrations in Florida's groundwater, springs, and rivers have increased dramatically. Shifts from macrophytic to algal cover in many of these systems are thought to result from this change in nutrient loading; however the linkages between hydrology, microbial communities, and nutrient processing are not well understood. In-stream nitrate concentrations are typically determined by autotrophic uptake as well as denitrification in biofilms and sediments of the hyporheic zone (HZ). While the hyporheic zone has been identified as a biogeochemical processing "hot spot," its contribution to overall nitrate removal is debated (Chapman et al., 1995; Heffernan & Cohen, 2010).

Numerous studies have shown that respiration in the HZ accounts for 40-90% of total stream ecosystem respiration and Hinkle et al (2001) showed that on the 1-2 km reach scale, 1/3 of hyporheic zones showed almost complete nitrate removal either by plants or denitrification (Pusch, et al, 1998). As shown in Figure 1, these transient storage and removal zones occur as a result of water transferring from the water column to the sediment and reappearing further downstream (Mulholland et al., 2008). Overall, biogeochemical reactions are controlled by the flow of carbon and electron acceptors into the hyporheic zone where this exchange is increased by pressure changes induced near geomorphic structures (Briggs et al., 2013, Engelhardt et al., 2011). In terms of impacts on water chemistry, residence time dictates the extent to which oxidation- reduction (redox) reactions occur and is positively correlated with aerobic respiration (Briggs et al., 2013).

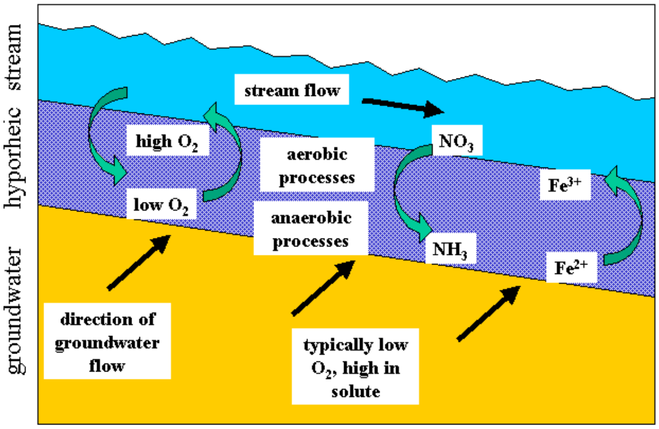

Figure 1. The hyporheic zone is critical in determining water quality (Gooseff, 2011).

Novel "smart tracer" methods such as the resazurin-resorufin system have recently been introduced to directly quantify the role of hyporheic microbial metabolism in streams (Haggerty et al., 2008; Lemke, et al., 2013). Resazurin (Raz) is a redox-sensitive compound that is reduced to resorufin (Rru) in the presence of metabolic activity by aerobes and facultative anaerobes; thus the Raz-Rru system can be used to determine the proportion of stream discharge subject to hyporheic exchange (Haggerty et al., 2008). Both compounds are weakly fluorescent and can be detected simultaneously at high temporal resolution using an in-stream fluorometer or with slightly improved signal separation using a benchtop instrument (Lemke et al., 2013). While the Raz conversion rate is found to be about 3 times higher in the hyporheic zone than in the water column, from the standpoint of tracer mass loss, the system may be ineffective in analyzing processes that occur over extremely long time scales (Haggerty et al., 2009, Zarnetske et al., 2009). The Raz-Rru method has been proven effective in several international studies but to the best of our knowledge has not been successfully applied in Florida.

Preliminary studies utilizing the Raz-Rru system in conjunction with a conservative tracer in small streams did not yield observed transformation of Raz to Rru likely due to short residence times and sandy soils that were not conducive to significant microbial activity. Furthermore, Raz and Rru loss to sediment adsorption was disregarded, which may have contributed to errors in reported concentrations. Lemke et al (2013) found that kinetic sorption is dominant over the equilibrium sorption mechanism for both Raz and Rru and in sandy, inorganic soils, sorption rates differ for the two tracers; Rru exhibits significantly higher rates especially at low pH.

The primary goals of this study are to determine the kinetic transformation and sorption rates for Raz and Rru in varying sediment types from Florida streams and rivers and to quantify the effectiveness of Raz as an indicator of microbial metabolism and biogeochemical potential in these systems through batch experiments. Results will aid in predicting Raz transformations under varying hydrologic conditions and identifying the presence of transient storage zones with high rates of biogeochemical activity.

EXPERIMENTAL METHODS

Raz & Rru Detection Wavelengths

Standard solutions of Raz and Rru were prepared in concentrations ranging from 0 to 200 μg/L (ppb) and for each, fluorescence was measured for a range of excitation and emission wavelengths on a bench-top fluorometer. As Rru exhibits greater fluorescence than Raz, there is a degree of error introduced in separating mixed signals and signal saturation can be an issue above 150 ppb. For both compounds, the strongest fluorescence signals were produced with excitation at 530 nm and emission at 645 nm (530/645 nm) and 480/590 nm, respectively. The best fit calibration equations for each compound are shown below

$$Raz_{590} = 3.8144Z + 136.31$$
$$Raz_{645} = 17.238Z + 88.833$$
$$Rru_{590} = 99.206U + 86.351$$
$$Rru_{645} = 134.41U + 36.564$$

where Z is the Raz concentration in ppb and U is the Rru concentration in ppb. The total signals for each wavelength were set equal to the sum of the Raz and Rru signals

$$S_{590} = Raz_{590} + Rru_{590}$$
$$S_{645} = Raz_{645} + Rru_{645}$$

and the resulting set of equations was solved for Z and U in all subsequent measurements.

Field Sites

Sediment was collected from two systems in Florida with significant differences in soil composition and hydrologic regime. The first was the Silver River near Ocala, FL, a well preserved state park area where the river is driven by first magnitude spring flows of approximately 650 cubic feet per second (cfs). The collection point was located on a vegetated slope where hyporheic exchange was likely forced by the direction of flow. The sediment was highly organic with a high water content and loamy texture.

For comparison purposes, sediment was also collected from Jennings Creek, a 1.41 cfs urban stream in Gainesville, FL that is impacted heavily by runoff from surrounding roadways. The sediment was characterized primarily by sand and gravel. The sample site was located on a similarly sloped area of the reach downstream of a small riffle-pool sequence where hyporheic exchange would be expected.

Batch Experiments

Batch culture experiments were performed according to methods adapted from González-Pinzón et al (2012). A total of 20 samples were prepared where 50 g of sediment from the Silver River were added to each of nine 200 mL sample bottles. Another nine were filled with sediment from Jennings Creek. The final two samples contained deionized water only. Sediment samples were filled to a final volume of 60 mL using collected stream water. The water samples and 6 sediment samples from each site were autoclaved at 121 °C for 20 minutes to eliminate the presence of microbial activity. Half of the autoclaved samples were then filled with the requisite volume of Raz for a final concentration of 100 ppb and the other half were treated with Rru to a concentration of 100 ppb. The 6 live samples were treated with Raz only. The samples were then placed on a shaker table and incubated at room temperature for a period of 6 hours. 250 μL samples were taken from each bottle at approximately 30 min intervals over the incubation period. Samples were buffered to a pH above 8 with 10 μL of 1 M NaOH to avoid the need for signal corrections and then centrifuged to remove residual sediment. 200 μL samples were pipetted to 96-well plates and total fluorescence was measured at 480/590 nm and

530/645 nm. Laboratory lights were kept off throughout the experiment to avoid photodegradation of the tracers.

Kinetics and Advection-Dispersion Modeling

The rates of Raz and Rru transformation and adsorption of to sediment particles were modeled by fitting measured concentrations from the batch experiments to the following equations

$$\partial Raz = -k_{fRaz} \cdot Raz \cdot Bac + k_{rRaz} \cdot Bac_{total} - k_{rRaz} \cdot Bac - k_{fsRaz} \cdot Raz \cdot S + k_{rsRaz} \cdot S_{total} - k_{rsRaz} \cdot S$$

$$\partial Rru = k_U \cdot Bac_{total} - k_u \cdot Bac - k_{fsRru} \cdot Rru \cdot S_u + k_{rsRru} \cdot S_{utotal} - k_{rsRru} \cdot S_u$$

$$\partial Bac = -k_{fRaz} \cdot Raz \cdot Bac + k_{rRaz} \cdot Bac_{total} - k_{rRaz} \cdot Bac + k_u \cdot Bac_{total} - k_u \cdot Bac$$

$$\partial S = -k_{fsRaz} \cdot Raz \cdot S + k_{rsRaz} \cdot S_{total} - k_{rsRaz} \cdot S$$

$$\partial S_u = k_{fsRru} \cdot Rru \cdot S_u + k_{rsRru} \cdot S_{utotal} - k_{rsRru} \cdot S_u$$

where k_f represents forward absorption by bacteria, k_{fs} represents forward sorption to sediment, and k_r represents reverse reactions. Bac_{total} is the total microbial concentration, Bac is number of bacteria occupied by Raz, k_u is the conversion of Raz to Rru, and S is the number of sorption sites available for Raz, while S_u represents sorption sites for Rru, and S_{total} is the total sorption sites. The conversion of Raz to Rru is assumed to be irreversible; however, Raz absorption by microbial cells does not necessarily indicate transformation. Sorption of both compounds to sediment is reversible.

Hydraulic transport parameters were estimated for two hypothetical reaches with sediments exhibiting the sorption and decay parameters fitted for Jennings Creek and the Silver River and applied to a modified OTIS solute transport model with transient storage and decay terms (Runkel, 1998; Hensley & Cohen, 2012).

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} + \frac{q_{LIN}}{A}(C_L - C) + \alpha(C_s - C) - kC$$

This model is based on the advection-dispersion equation which relates changes in solute concentration with respect to time and space to advection, dispersion, and transient storage in the stream system where Q is discharge (m^3/s), A is channel cross-sectional area (m^2), D is the dispersion coefficient (m^2/s), q_{LIN} is the sum of inflows to the system (m^3/s), C_L is solute concentration in inflows (ppb), α is the storage exchange coefficient (1/s), and C_s is solute concentration in transient storage (ppb). In this equation, α is mathematically equivalent to the arameter q_{he} developed by Lemke et al. (2013) which quantifies the discharge subject to hyporheic exchange per volume of stream water. The equation

$$\frac{\partial C_s}{\partial t} = \alpha \frac{A}{A_s}(C - C_s) - kC$$

describes the rate of concentration change in the transient storage zone as a function of stream of the effective stream and storage zone areas (Stream Solute Workshop, 1990) When reduction and adsorption of Raz (or Rru) is observed, a - kC term is included in the model to account for the combined predicted first order conversion of resazurin to resorufin in the hyporheic zone as well as adsorption to sediments (Lemke et al, 2013). Breakthrough curves of Raz and Rru in the two simulated systems were compared from the standpoints of adsorption capacity and microbial activity.

RESULTS

Raz and Rru Transformation vs Time

For samples with autoclaved soils (see Figure 2), the added tracer was the only compound assumed present throughout the experiment; concentrations over time were determined using a single calibration for the given tracer to avoid the introduction of error in solving the full set of equations. From the inactivated samples, the decrease of both Raz and Rru concentrations over the incubation period was more pronounced for the Silver River soils. As shown in Figure 3, this was also the case for live soils where the Raz concentration in Silver River soil decreased by more than 60% over the first 40 minutes of incubation *vs* the

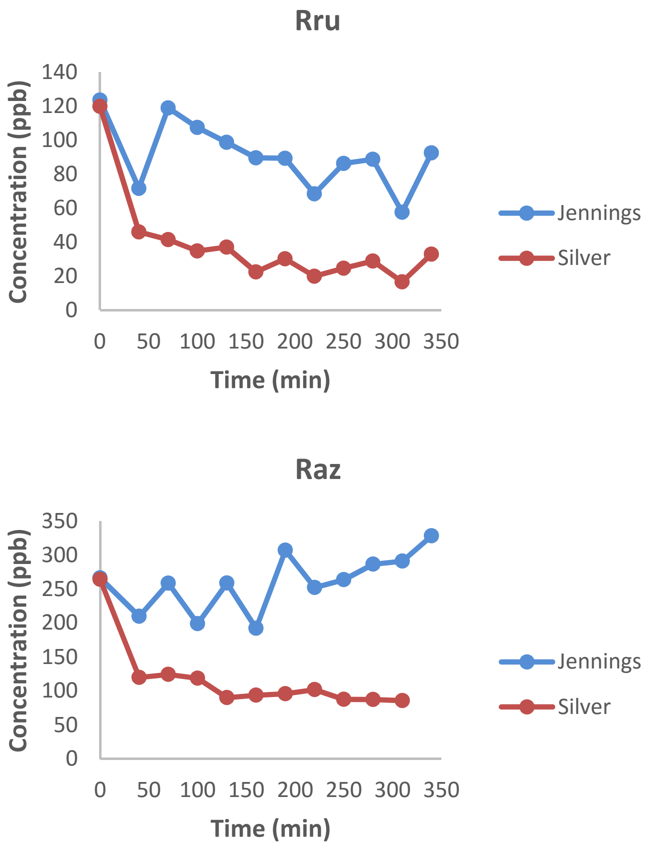

Figure 2. Adsorption to Inactive Sediment

intial 38% change observed in the Jennings Creek sediment. However, the reported increasing Raz trend for the inactive Jennings Creek sediment is not possible and likely the product of signal separation error.

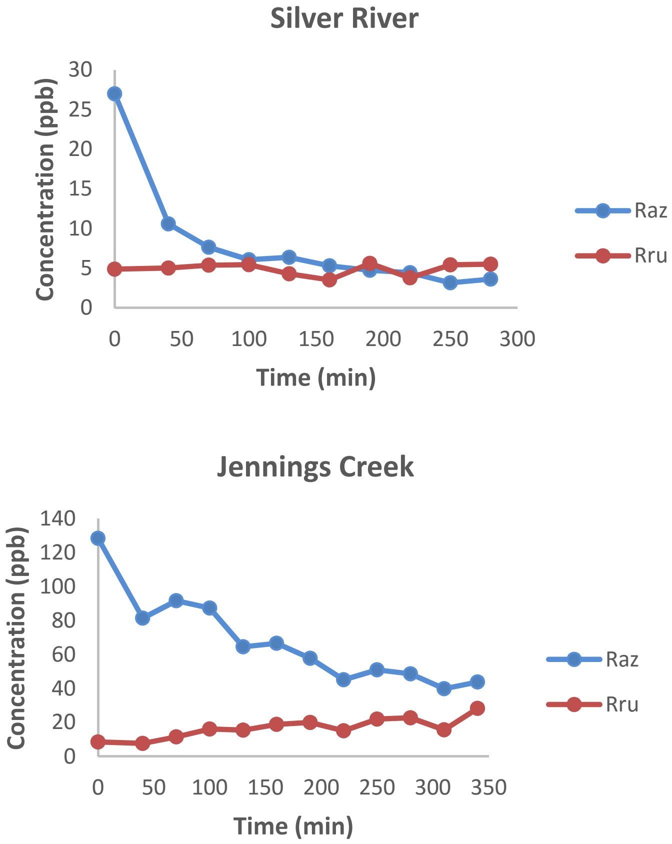

Figure 3. Combined Effects of Microbial Activity

Kinetic Parameters

Tables 1 and 2 summarize the sorption and kinetic transformation rates for Raz and Rru determined by fitting the batch experiment data to the set of equations relating bacterial concentration, soil sorption, and overall conversion of Raz to Rru. For both tracers, the Silver River sediment was found to have a higher total adsorption capacity and in both sites, the adsorption capacity for Rru was twice that of Raz. The Silver River sediment was also more biologically active with a relative microbial concentration five times that of the Jennings Creek sediment.

Table 1. Relative Sediment Sorption Capacities

System	Total Sorption Sites (M)	Total Microbes (M)
Jennings	1	2.2
Silver	5.8	11

In terms of transformation and sorption rates, values were consistently greater for the Silver River except for Raz conversion. In this organic sediment, adsorption may be the dominant removal mechanism for Raz. The results shown in Table 2 are also comparable to kinetic rates and sorption data reported for Raz and Rru in other studies (González-Pinzón et al., 2012, Lemke et al., 2013).

Table 2. Fitted Reaction and Sorption Rates

Tracer	Kinetic rate (1/M*s)		Adsorption (1/M*s)	
	Jennings	*Silver*	*Jennings*	*Silver*
Raz	$2*10^{-5}$	$8.33*10^{-6}$	$1.67*10^{-6}$	$3.46*10^{-5}$
Rru	$3.33*10^{-5}$	$6.67*10^{-5}$	$1.67*10^{-5}$	$2.97*10^{-5}$

Advection-Dispersion Modeling

Kinetic transformation and sorption rates were combined as a single decay coefficient for each tracer and utilized in OTIS modeling of Raz and Rru breakthrough curves (BTCs) for simulated stream reaches containing the two sediment types. The Raz and Rru BTCs are presented alongside that of a conservative tracer for both reaches. Hydraulic parameters of the systems are summarized in Table 3.

Table 3. OTIS Model Parameters for Tested Sediments

Parameter	Modeled Value
Flow (m^3/min)	0.283
Effective Area (m^2)	0.5
Storage Area (mm^2)	1
Dispersion Coefficient (m^2/min)	0.003
Exchange Coefficient (1/min)	0.12

As shown in Figure 4, a pulse injection of Raz and the conservative tracer fluorescein to a reach with Jennings Creek sediment would likely exhibit peak concentrations of 3.2 and 0.4 ppb Raz and Rru, respectively. This sums to the 3.6 ppb peak for fluorescein as expected. The Rru peak also occurs later due to retention in the transient storage zone.

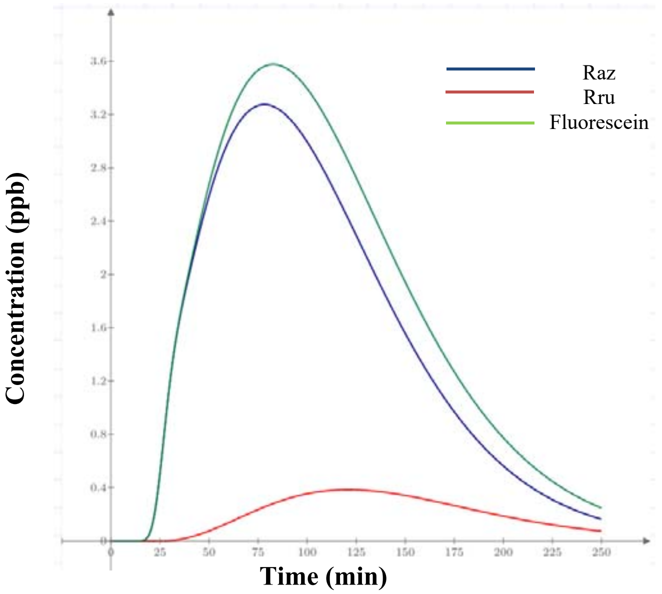

Figure 4. Raz and Rru breakthrough curves with Jennings Creek sediment

For the same reach with Silver River sediment, the same mean residence time of approximately 80 minutes is observed, however the Raz peak occurs sooner and the conversion of Raz to Rru is more pronounced as would be expected for more organic sediment. All three breakthrough curves also show longer tails than for the Jennings sediment which is likely a product of increased sorption and short-term tracer retention and release from hyporheic zones.

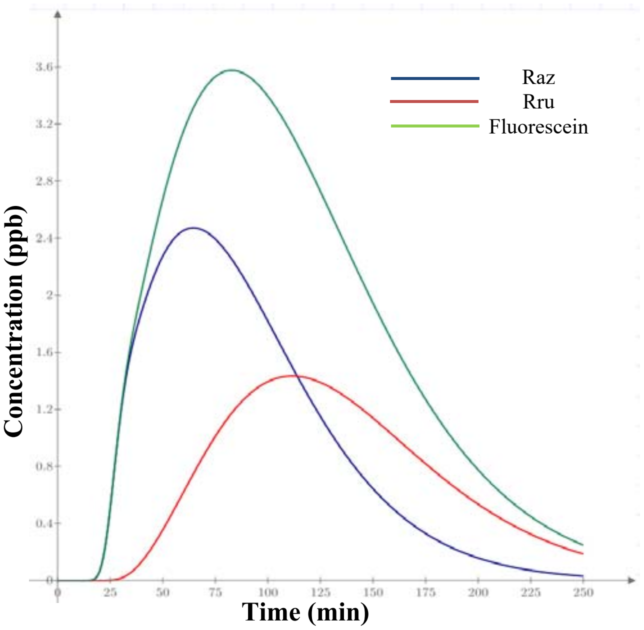

Figure 5. Predicted Breakthrough Curves for Silver River Sediment

DISCUSSION

As shown in Figures 2 and 3, measured Raz and Rru concentrations from the batch experiments contained a degree of uncertainty likely introduced by a combination of experimental and calibration error. Experimental issues could include pipetting and volume errors in sample preparation as well as slight photodegradation of the tracers. However, signal separation was likely dominant as preliminary tests of known Raz and Rru concentration mixes consistently produced overestimates of the Rru concentration. This is attributed to the fact that the fluorescence spectra for the two tracers overlap and that Rru is more fluorescent. Depending on the excitation and emission wavelengths employed, simultaneous measurements of both tracers below 1 ppb are not considered reliable (Lemke et al., 2013). In future laboratory work, calibrations will be performed with a set of wavelengths that will allow for more accurate Raz and Rru separation.

From the kinetic rate and sorption results summarized in Tables 1 and 2, the organic sediments of the Silver River were found to be orders of magnitude more active than those of Jennings Creek for some parameters. As Rru sorption was most significant in both systems, it is likely that this could be a major source of concentration detection error in reach scale studies and could make pulse injections infeasible even in small streams. The breakthrough curves shown in Figures 4 and 5 illustrate the predicted breakthrough curves for pulse injection tracer tests in reaches with Jennings Creek and Silver River sediment, respectively. As shown in Figure 4, Rru concentrations produced in Jennings Creek are below the 1 ppb detection limit for in-stream fluorometers which agrees with previous results in the actual stream (Lemke et al., 2013). For the same reach geometry with Silver River sediment, about 3 times more conversion of Raz to Rru can be expected with increased transient storage retention due to the higher sorption capacity and microbial activity of the system. Overall, fitted parameters from the batch experiments provide an accurate representation of the breakthrough curve trends that would be expected for sandy vs organic sediments.

In terms of the effectiveness of the system in estimating microbial activity for a specific site, the model utilized in this study did predict a microbial concentration for the Silver River that was five times that of Jennings Creek as expected for a more productive system. While full reach scale studies in large, highly organic spring systems may be impractical, this may indicate that for studies of isolated areas within a reach, the Raz-Rru system could provide an estimate of the overall biogeochemical activity given varying hydraulic parameters. However, further work is needed to determine whether this estimate could provide proportions of various reactions (e.g., aerobic respiration vs denitrification).

CONCLUSIONS

In this study, kinetic and adsorption rate parameters were determined for Raz and Rru in sediments from two Florida stream systems through batch culture experiments. Results support the hypotheses that inorganic sandy stream sediments have less capacity for adsorption and transformation of Raz than those originating in highly productive river systems. OTIS model results incorporating transformation and sorption parameters also support the lack of observed Rru production in previous work. As significant differences in microbial presence were also found between the two systems, it is likely that the Raz-Rru method would be effective in identifying the relative biogeochemical activities of sites throughout a given reach.

Future work will require refinement of fluorescence signal separation as well as analyses of Raz reduction efficiency over the gradient of oxidation-reduction and hyporheic exchange potentials present in stream sediments. A more complete understanding of the tracers' behavior over a range of biogeochemical conditions could aid in the development of a method for assessing the system's appropriateness for use in any stream of interest. While reach scale tracer studies may be cost-prohibitive in large systems such as the Silver River, in-stream controlled volume experiments may provide further information as to the correlations between Raz reduction, microbial respiration, and biogeochemical processes including denitrification.

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