Supporting Information for

Microbial transport, retention, and inactivation in streams – a combined experimental and stochastic modeling approach

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SUPPORTING INFORMATION: Experimental Methods

Field tracer injection experiment injectate preparation and in-stream sampling:

A stock culture of *E. coli* ($1.3x10^{10}$ MPN /100 mL) was prepared from Toenepi Stream water samples (n=8) by extracting medium from Colilert trays (IDEXX Laboratories, Inc., Westbrook, Maine, USA) from at least 5 positive wells with a hypodermic syringe for each water sample and incubating the *E. coli* suspension in 500 mL of sterile deionized water with added Colilert nutrients at 35˚C overnight. Clay (~15 kg) was sourced from the banks of the Toenepi Stream and disaggregated by vigorous mixing in stream water. The suspension of clay used in the injection had a mean diameter of 2.4 μm as measured by an EYE TECH laser particle analyzer (Ankersmid, Eindhoven, Netherlands). This clay suspension was added to a 200 L tank along with 100 L of stream water, 60 mL of rhodamine WT dye and 4 L of the *E. coli* culture. Seven liters of a slurry of fresh bovine feces (~5 kg) were added to provide an additional source of *E. coli* from a natural fecal source characteristic of the intensively dairy-farmed Toenepi catchment. The tank was filled to 150 L and mixed by a recirculating bilge pump for about 1 hour. Half (75 L) of the injectate was then transferred into a second 200 L tank using a second bilge pump. Both tanks were then diluted with stream water to 200 L to provide two reservoirs of injectate (total volume 400 L) with near-identical concentrations and quantities of tracers.

The injectate was pumped at a rate of 95 mL min^{-1} into the stream over much of the stream width using a diffuser manifold with seven ports. 170 L of the injectate was pumped into the stream from the first 200L tank over 30 minutes. Then the pump intake was transferred into the second injectate tank and the injectate pumping continued at the same rate for a further 30 minutes. The remaining 30 L of injectate in the first tank was added to the injectate in the second tank. In total, 342 L of injectate was delivered to the stream over a period of 1 hour.

The injectate tanks were stirred at intervals by hand throughout the injection. Injectate samples (1 L) were taken from both tanks at the start and end of the injection to confirm the homogeneity of the injectate suspension. The average injectate concentrations and coefficients of variation were $1.79 \times 10^4 \pm 11$ % ppb rhodamine WT, $8.4 \times 10^3 \pm 11\%$ NTU turbidity and $5.8 \times 10^7 \pm 14\%$ MPN / 100 mL *E. coli*.

The average of the Site 1 samples taken throughout the experiment was used to estimate background fluorescence, turbidity, and *E. coli* concentrations. These background concentrations were 0.281 ppb rhodamine, 0.7 NTU turbidity, and 700 MPN/100mL *E. coli*. As there was no rhodamine in the influent streamwater, the apparent rhodamine background represents naturally occurring autofluorescence that responds at the excitation/emission wavelengths used to detect rhodamine. The comparatively high *E. coli* concentration for the stream at baseflow reflects the multiple sources of fecal pollution from livestock in the intensively grazed Toenepi catchment. Auto-samplers (ISCO 3700) were used to collect stream water samples at 30 minute intervals at all sampling sites. An additional in-stream sample was taken at each sampling site the next day, 19 hours after the injection started. Fluorimeters and turbidity sensors were deployed at the three downstream sites to continuously measure rhodamine and clay concentrations, respectively.

Laboratory column filtration experiment injectate preparation:

A particle stock suspension was prepared with 500 mL of DI water, 0.42 g of the fluorescent fine particles, and dispersant (5 g L^{-1} of sodium hexametaphosphate) to assist with wetting and dispersion of the (slightly hydrophobic) particles. This dispersed suspension was diluted 10x in DI water to reach the desired concentration for injection (3.3×10^4 # / mL). The injectate consisted of 500 mL of the diluted fine particle suspension, 100 g of sodium

chloride, and 100 μL of *E. coli* concentrated from a Toenepi water sample following the same procedure as for the field experiment (3.3×10^9 MPN / 100 mL stock concentration).

Laboratory analysis of samples

E. coli concentrations were analyzed using the Colilert Quanti-Tray® /2000 (IDEXX) MPN method using x100 dilutions of water samples and sediment sub-core slurries. The precision of this method is about 30% (coefficient of variation of replicates) and is essentially independent of concentration (above a minimum) because the sample analyzed can be volumetrically diluted as required to give good enumeration. The limit of detection is necessarily greater than the limit of resolution (of 1 MPN in a 100 mL sample). Salt tracer concentrations in the column experiment effluent were measured using a YSI model 30 Conductivity meter, (Yellowsprings Inc., Ohio, USA). A flow cytometer (Becton Dickson FACS Calibur) was used to analyze fluorescent fine particle concentrations in the column effluent and a fluorescence microscope (Leica, Leitz DMRBE) was used to analyze distributions of fluorescent fine particles within the sediment column, following the methods previously described.[44]

SUPPORTING INFORMATION: Stochastic mobile-immobile model for microbial transport in rivers

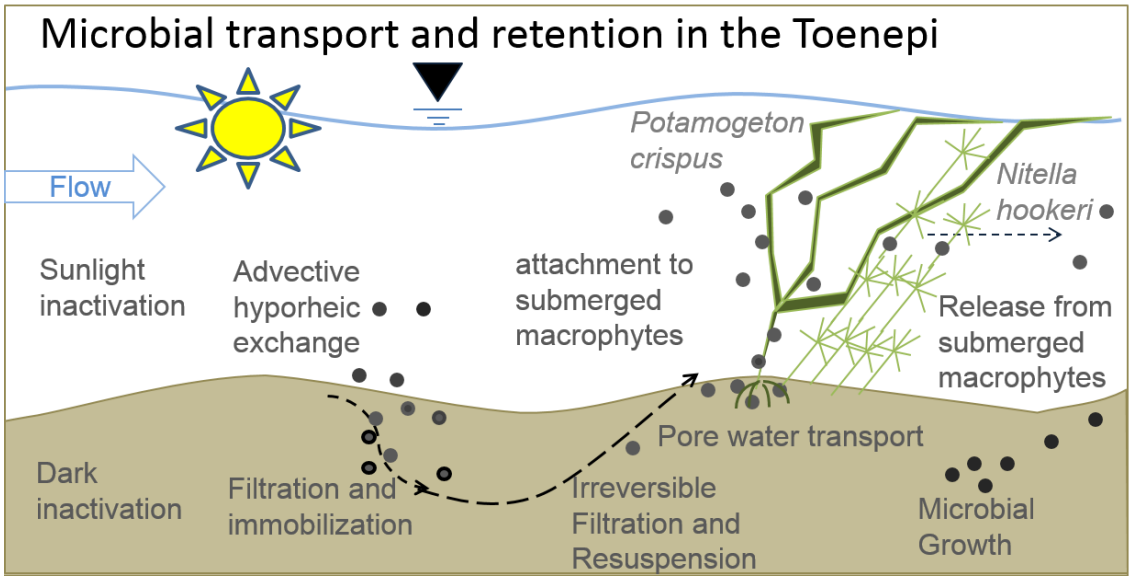

Figure S1: A conceptual model of the microbial transport, retention, and inactivation processes incorporated within the stochastic mobile-immobile model framework.

In-stream modeling

The mobile-immobile model framework is convenient for transport in rivers as the water column can be considered mobile and material retained in streambed sediments or in-stream structures, such as macrophytes, is effectively immobile.[1, 2] Advection and dispersion within the water column is convolved with a memory function that describes advective hyporheic exchange and immobilization (Equation 1)[3,4].

$$\frac{\partial C(x,t)}{\partial t} = \int_0^t M(t-t') \left[-U \frac{\partial C(x,t')}{\partial x} + K \frac{\partial^2 C(x,t')}{\partial x^2} \right] dt' \quad (1)$$

where C is in-stream concentration, t is the elapsed time, $M(t)$ is the memory function and U and K are respectively the velocity and dispersion coefficients that describe motion in the stream. The memory function represents the fraction of solute, fine particles, or microbes that are immobilized at time t and are still immobile at a later time ($t + dt$). The memory

function is normally written in Laplace space to simplify the expressions, where the Laplace transform, L{f}(u), of a function $f(t)$ is equal to $\int_0^\infty e^{-ut} f(t) dt$:[3,4]

$$\widetilde{M}(u) = u\bar{t}\frac{\tilde{\psi}_i(u)}{1-\tilde{\psi}_i(u)} \quad (2)$$

where $\widetilde{M}(u)$ is the memory function in Laplace space, u is the Laplace variable, $\bar{t}$ is the average travel time in the reach, defined as the stream reach length divided by the mean stream velocity, and $\tilde{\psi}_i(u)$ is the residence time probability distribution. This model can be used to represent transport of solutes, fine particles, or microbes with suitable residence time distributions, denoted by subscript i = S, P, or M, respectively. $\tilde{\psi}_i(u)$ depends on the probability of immobilization, Λ_i, and distribution of residence times in the immobile region, $\tilde{\varphi}_i$(u):

$$\tilde{\psi}_i(u) = \tilde{\psi}_0[u + \Lambda_i - \Lambda_i * \tilde{\varphi}_i(u)] \quad (3)$$

where $\tilde{\psi}_0$ is the residence time distribution in the mobile region (water column) and $\tilde{\varphi}_i(u)$ is the residence time distribution in the immobile region (e.g. hyporheic zone, biofilms or submerged macrophytes). $\tilde{\psi}_0$ is a much narrower distribution over time than $\tilde{\varphi}_i$(u) since it is controlled by in-stream transport. The brackets […] in Equation 3 indicate functionality, so the argument of the in-stream transport function is replaced with another function representing the effects of transport into and out of storage regions. Here, we assume that a single distribution $\tilde{\psi}_0$ characterizes the transport of solutes, fine particles, and microbes, since these materials should be transported very similarly in the water column. We take this as an exponential distribution $\psi_0(t) = e^{-t}$ or in Laplace space, $\tilde{\psi}_0$(u) = 1/(1+u). This

exponential distribution was chosen based on previous modeling work[3], and is only needed to be relatively narrow in comparison to the longer residence time distributions within the immobile region.

For solutes, the memory function represents both hyporheic exchange and incomplete mixing within the stream channel. Similarly, the residence time distribution for solutes, $\tilde{\varphi}_S(u)$, is based on the time solutes are retained within the streambed by hyporheic exchange or in the water column in dead zones, e.g., around in-stream structures. Solute residence time distributions have often been found to follow a heavy-tailed power law, where $\varphi_S(t) \sim t^{-(1+\beta_S)}$ or in Laplace space $\tilde{\varphi}_S(u) = 1/(1+u^{\beta_S})$, for $0 < \beta_S < 1$. "Heavy tailed" here means that the residence time distribution has an infinite mean or variance, which is true for power laws with slope $0 < \beta_S < 1$. Power-law exponents closer to 0 have less steep slopes and greater residence times.

For fine particles, the memory function is modified to incorporate the additional processes that cause immobilization. We assume that delivery of fine particles and microbes to the streambed is controlled purely by advective hyporheic exchange and that gravitational settling is negligible because the Stokes' settling velocity of fine particles is very low, especially organic particles and microbial cells that have low specific gravity. In this case, hyporheic exchange of solute and fine particles is similar, and $\Lambda_P \approx \Lambda_S$. Then, for fine particles, Equation 3 becomes:

$$\tilde{\psi}_P(u) = \tilde{\psi}_0[u + \Lambda_S - \Lambda_S * \tilde{\varphi}_P(u)] \quad (4)$$

Immobilization of fine particles is described by the particle residence time distribution in the immobile region, $\tilde{\varphi}_P(u)$:

$$\tilde{\varphi}_P(u) = \tilde{\varphi}_S[u + \Lambda_{HP} - \Lambda_{HP} * \tilde{\varphi}_{HP}(u)] \quad (5)$$

where $\tilde{\varphi}_S$ is the residence time distribution for solutes, Λ_{HP} is the probability that fine particles immobilize (i.e. filter, attach, or deposit) within the immobile region [T^{-1}], and $\tilde{\varphi}_{HP}(u)$ is the residence time distribution of fine particles that were immobilized within the immobile region. Thus, Equation 4 and 5 represent both fine particle transport into and out of regions of storage such as the hyporheic zone, and also uptake and resuspension within these regions. The residence time distribution of fine particles is also represented here as a heavy-tailed power-law distribution $\varphi_{HP}(t) \sim t^{-(1+\beta_{HP})}$ or in Laplace space $\tilde{\varphi}_{HP}(u) = 1/(1+u^{\beta_{HP}})$, for $0 < \beta_{HP} < 1$.

A conceptual model of the transport, retention, and inactivation processes included in the stochastic model are shown in Figure 1. We assume that microbes are transported similarly to fine particles, but are also subject to inactivation. Therefore advective delivery of microbes is controlled by hyporheic exchange and $\Lambda_M \approx \Lambda_S$. Equation 3 for microbes becomes:

$$\tilde{\psi}_M(u) = \tilde{\psi}_{0M}[u + \Lambda_S - \Lambda_S * \tilde{\varphi}_M(u)] \quad (6)$$

To represent inactivation in the water column, the residence time distribution of microbes within the mobile region is the solute residence time distribution subject to the first-order inactivation rate constant for sunlight, k_0, yielding $\psi_{0M}(t) \sim e^{-t}e^{-k_0 t}$ or in Laplace space

$\tilde{\psi}_{0M}(u) = 1/[1 + u + k_0]$. The residence time distribution of microbes in the immobile region, $\tilde{\varphi}_M(u)$, is written similarly to particles:

$$\tilde{\varphi}_M(u) = \tilde{\varphi}_S[u + \Lambda_{HM} - \Lambda_{HM} * \tilde{\varphi}_{HM}(u)] \quad (7)$$

where $\tilde{\varphi}_S$ is the residence time distribution for hydrodynamic transport in the immobile region, Λ_{HM} is the probability that microbes immobilize within the immobile region [T^{-1}], and $\tilde{\varphi}_{HM}(u)$ is the residence time distribution of microbes that were immobilized within the immobile region. We also take the residence time distribution of microbes as a heavy-tailed power-law residence time distribution subject to the first order rate for dark inactivation, k_H, yielding $\varphi_{HM}(t) \sim t^{-(1+\beta_{HM})}e^{-k_H t}$ or in Laplace space $\tilde{\varphi}_{HM}(u) = 1/[1 + (u + k_H)^{\beta_{HM}}]$, with $0 < \beta_{HM} < 1$.

Streambed sediment modeling

Equations 1 - 3 can also be used to model transport within streambed sediments. However, instead of exchange between a mobile water column and immobile zone representing hyporheic exchange and incomplete in-stream mixing, transport occurs through sediment porewater. In this case, porewater flow represents the mobile domain, and storage occurs by either retention in stagnant porewater, e.g., in dead-end pores or regions with very low permeability, or immobilization of fine particles and microbes due to filtration. These smaller-scale immobilization/remobilization processes are represented by another set of residence time distribution functions within sediments:

$$\tilde{\psi}_{Cj}(u) = \tilde{\varphi}_{CS}[u + \Lambda_{Cj} - \Lambda_{Cj} * \tilde{\varphi}_{Cj}(u)] \quad (8)$$

where *C* in the subscripts denotes column and *j*= *P* for particles and *M* for microbes, $\tilde{\varphi}_{CS}$ indicates the residence time distribution caused by heterogeneity along hyporheic flow paths, and Λ_{Cj} and $\tilde{\varphi}_{Cj}$ indicate the probability of immobilization and distribution of residence times in the immobile region of the sediments. $\tilde{\varphi}_{CS}$ can be exponential or power-law depending on sediment properties.

Fine particles and microbes are also subject to heterogeneous transport in porewaters, and can additionally be immobilized by filtration and subsequently resuspend from the surfaces of bed sediment grains. The probability of particle immobilization, Λ_{CP}, reflects filtration within sediment porewaters. The residence time distribution of fine particles within sediments is often found to be power-law, $\varphi_{CP}(t) \sim t^{-(1+\beta_{CP})}$ or in Laplace space, $\tilde{\varphi}_{CP}(u) = 1/(1+u^{\beta_{CP}})$, where the exponent is $0 < \beta_{CP} < 1$.

The residence time distribution of microbes undergoing reversible filtration in granular porous media as a combination of power-law and uniform distributions was previously found.[5] Here we represent reversible filtration of *E. coli* as a mixture of power-law and uniform residence time distributions, with both subject to exponential inactivation:

$$\varphi_{CM}(t) \sim c * \ t^{-\beta_{CM}} \mathrm{e}^{-k_H t} + (1-c) * \ T\mathrm{e}^{-k_H t} \quad (9)$$

where *c* is the fraction of power-law behavior, β_{CM} is the power-law exponent for microbes, k_H is the dark inactivation rate [T^{-1}], *T* represents the truncation time for the distribution, i.e., the maximum observation time for the breakthrough curve. In Laplace space $\tilde{\varphi}_{CM}(u) = \ c * 1/[1 + (u + k_H)^{\beta_{CM}}] + (1-c) * \ 1/(Tu + k_H)$. This multi-scale modeling framework allows us to compare the local- and reach-scale microbial immobilization parameters (i.e., Λ_{CM} vs. Λ_{HM}, and β_{CM} vs. β_{HM}) to assess if reach-scale retention of microbes primarily

reflects the timescale of remobilization within the sediment bed, or the timescale of other processes such as hyporheic exchange. Similarly, we can also compare the rates and timescales of local- and reach-scale solute and fine particle retention in order to evaluate the relative effects of hydrodynamic, particulate, and biological processes in microbial dynamics.

SUPPORTING INFORMATION: Multi-scale simulations of microbial transport

Observed in-stream breakthrough curves for the conservative solute (rhodamine), tracer particles, and *E. coli* at Sites 2, 3, and 4 are shown in linear space in Figure 1.

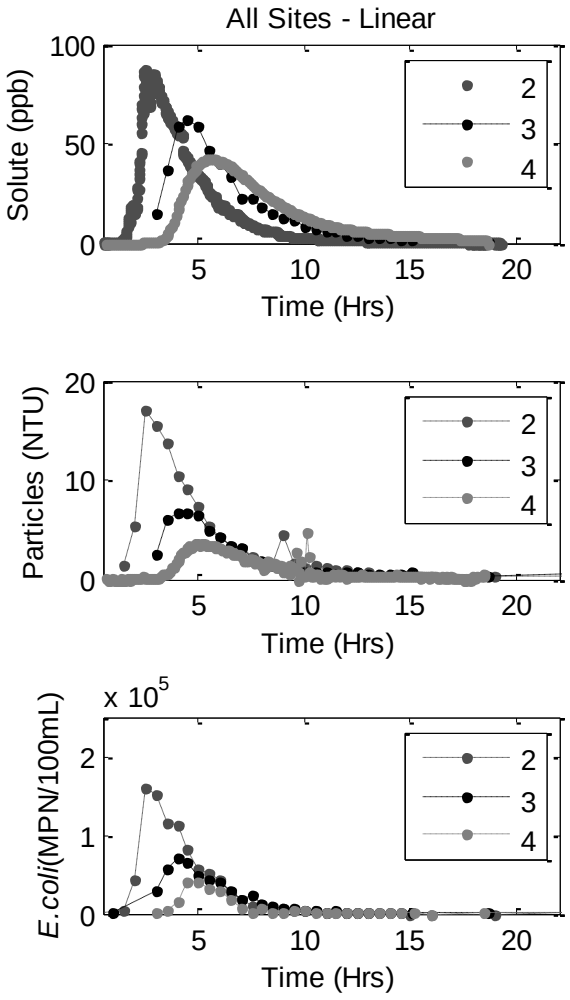

Figure S2: The breakthrough curves in the Toenepi stream for the conservative solute (rhodamine), tracer particles, and *E. coli* are shown in linear space.

The stochastic model best-fits for solute and fine particles at Site 4 are shown in Figure 2. All in-stream concentrations are normalized by the respective average concentrations at the injection site.

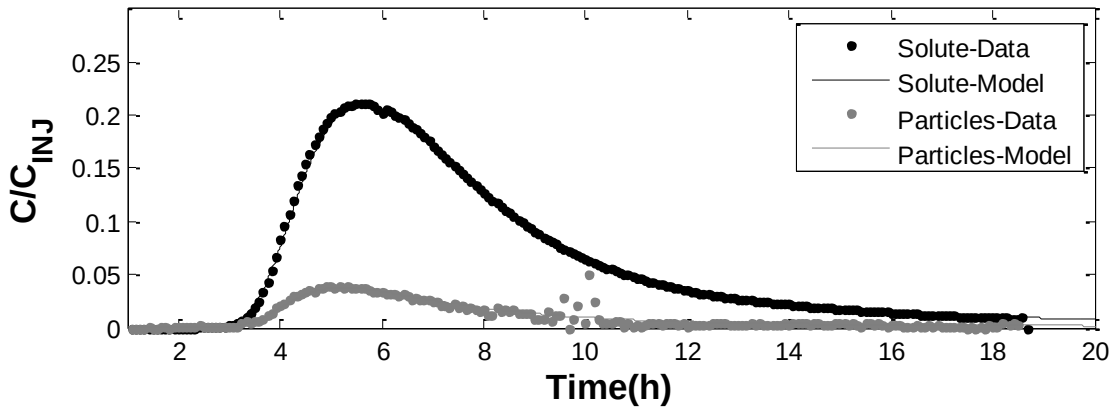

Figure S3: In-stream breakthrough curves and stochastic model first for the conservative solute and tracer particles at Site 4 in linear space. Concentrations are normalized by the respective average concentrations at the injection point.

Observations and simulations of *E. coli* breakthrough curves in the stream are presented in Figure 3.

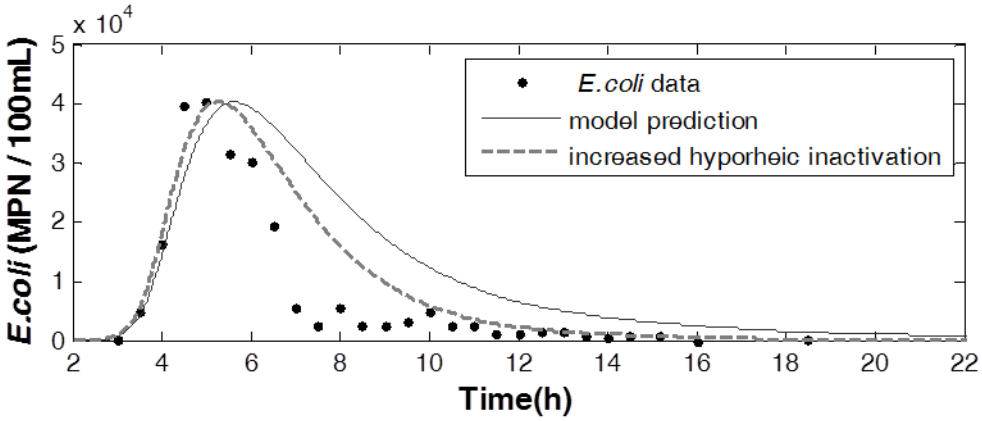

Figure S4: *E. coli b*reakthrough curves in the Toenepi stream at Site 4 in linear space. The dark line represents the model prediction and the dashed gray line is the model simulation with increased dark inactivation rate.

References

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