A Spectrum of Preferential Flow Alters Solute Mobility in the Critical Zone

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Abstract

Preferential flow reduces water residence times and allows rapid transport of pollutants such as organic contaminants. Conventional understanding of solute transport implies that preferential flow reduces the influence of soil matrix-solute interactions; however, this assumption lacks robust validation in the field. To better understand how physicochemical properties affect solute transport across a range of preferential flow conditions, we applied deuterium-labeled rainfall to field plots containing manure spiked with eight common antibiotics with a range of affinity for the soil. We then quantified preferential flow and solute transport using 48 soil pore water samplers spread along a hillslope. Based on >700 measurements, our data showed that solute transport to lysimeters was similar – regardless of antibiotic affinity for soil – when preferential flow represented less than 15% of the total water flow. When preferential flow exceeded 15%, however, concentrations were higher for compounds with relatively low affinity for soil. These results suggest that bypassing water flow can select for compounds that are more easily released from the soil matrix, thus providing fundamental insight into how flow heterogeneity affects pollutant mobility in soils. Moreover, because these data do not fully align with existing solute transport theory, they may be useful for building improved process-based transport models.

Main Text

A growing and increasingly affluent human population is releasing ever greater loads of organic contaminants into Earth's critical zone. Many of these compounds are susceptible to rapid movement via preferential flow, which can often lead to orders of magnitude greater solute leaching than predicted by equations specific to a homogenous soil matrix (e.g., the advection-dispersion equation). Preferential flow is ubiquitous in soils, representing as little as 1% to more than 70% of total water movement. The associated potential for rapid chemical transport threatens water quality in nearby aquifers and streams, making it important to understand the key physical and chemical factors controlling organic compound movement through unsaturated soils.

Preferential flow occurs as a disequilibrium between water flowing through the low-permeability bulk soil (i.e., the soil matrix) and the highly conductive fraction of the total soil volume such as macropores with hydraulic conductivities > 0.01 cm h⁻¹. Under such conditions, flow rates can sharply increase without uniform increases in soil pore water pressures as water bypasses the lower permeability matrix (i.e., bypass flow). This phenomenon is observed over a broad range of water contents. In dry soils, preferential flow may occur as partially water-repellant layers destabilize the wetting front forming fingered flow or as flow is concentrated through newly-formed cracks. As soils approach saturation, near-positive pore water pressures can force water from the matrix into highly conductive macropores, making total flow proportionally more preferential.

Preferential flow drastically reduces the residence time of water in the critical zone, thus limiting the opportunity for dissolved substances to sorb to soil particles. Therefore, when preferential flow is
minimal, more homogenous flow through the soil matrix dominates, favoring transport of compounds with low affinity to soil, such as those with a low sorption coefficient ($K_d$).\textsuperscript{30–32} The conventional assumption holds that the influence of solute-matrix affinity decreases as flow becomes proportionally more preferential\textsuperscript{33–36} such that the kinetics of rapid flow restrict sorption\textsuperscript{37} or enhance desorption.\textsuperscript{38} However, studies to date have mostly compared known preferential transport of solutes to more homogenous flow – either modeled based on advection-dispersion processes\textsuperscript{39,40} or focused on specific conditions such as frozen soils\textsuperscript{41} – but have never directly assessed how organic contaminants of varying chemical properties become mobilized along a spectrum of flow heterogeneity. As a result, the conditions necessary to dampen versus amplify the influence of compound physiochemical properties on solute transport are not well understood. The primary objectives of this study were to 1) quantify transport of eight veterinary antibiotics under different preferential flow conditions and 2) determine if preferential flow can eliminate the influence of solute-soil affinity on transport of these solutes. This analysis is necessary to provide a fundamental understanding of how preferential flow alters contaminant mobility and build process-based transport models needed to manage water quality and thwart water resource degradation.

Here we explore the influence of solute-matrix affinity across a range of preferential flow by applying simulated rainfall to field plots containing manure spiked with eight common veterinary antibiotics (listed by decreasing relative affinity to the soil matrix): erythromycin (ERY), tylosin (TYL), tetracycline (TC), pirlimycin (PLY), chlortetracycline (CTC), oxytetracycline (OCT), sulfadimethazine (SDM), and sulfamethazine (SMZ). Veterinary antibiotics were chosen for 1) their environmental ubiquity, as in the U.S. up to 52 million kg per year are applied to soils\textsuperscript{42,43} and for 2) their wide range of affinity to soils.\textsuperscript{44} Antibiotic-spiked manure was applied to field plots (200 x 150 cm) on the soil surface or injected to a depth of 10 cm ($n = 3$ plots per application method). After 7 days of rainfall suppression, we applied rainfall (7 cm h$^{-1}$) to these plots, plus additional 3 plots without treated manure to serve as controls for flow and transport. Rainfall simulations were labeled with deuterium to facilitate preferential flow quantification. Monitoring soil pore water isotope signatures and antibiotic concentrations in suction lysimeters across time (1 h before, 30 min into, and 1 h) and space (multiple locations and depths of 30 and 90 cm) allowed us to quantify solute transport with $> 700$ estimates of preferential flow. See Methods and Supporting information for more experimental details.

We defined antibiotic movement in terms of change in concentration, $\Delta C$, from samples collected 0.5 h into and 1 h after rainfall versus pre-event (background) values from the same lysimeter. We deemed $\Delta C$ to be zero whenever veterinary antibiotic concentrations decreased from background or were non-detectable. At the same time, we considered flow to be partitioned into two distinct hydrological domains assuming faster advection through preferential pathways (e.g., root channels and macropores) versus slower flow through the soil matrix via combined advection and dispersion mechanisms. Following the conceptual framework provided by Stumpp, et al.\textsuperscript{45}, the fractional contribution of preferential flow was
calculated by where sampled deuterium concentrations, $D_t(t)$, were used in a two-member mixing model that separated rainfall moving through preferential flow paths, $D_{PF}(t)$, from pre-event soil matrix water, $D_{MF}(t)$ (see Methods for full derivation). We estimate that the average of 7 cm of rainfall infiltrated in this experiment (Table S1) would have replaced ~ 20 cm of storage via pure advection. This calculation suggests that a homogenous wetting front would not have reached our most shallow pore-water samplers (30 cm) and that the sampled water was derived from some combination of rainwater bypassing the soil matrix and pre-event matrix storage. As a result, we consider the mixing model to be suitable for quantifying preferential flow during the simulated rainfall experiment.

A total of 153 of the 768 measurements (20%) resulted in zero or negative $f_{PF}$ values, which we considered to represent entirely matrix-derived water ($f_{PF} = 0$) in subsequent analyses. Though event water was applied at a constant rainfall intensity (7 cm h$^{-1}$) and infiltrated in similar rates between plots (Table S1), simulated rainfall produced nearly three orders of magnitude of variation in preferential flow ($f_{PF}$ from 0.0017 to 0.6; Figure S1). The range of positive $\Delta C$ values extended nearly four orders of magnitude, from 0.006 to 3.85 µg L$^{-1}$ (Figure S2), with probability of detection highest in the low range of preferential flow (Figure S3). These numerous point estimates of preferential flow in space (i.e., different lysimeter depths and random positions) and time (i.e., during and after rainfall) enabled analysis of solute mobility under a spectrum of flow heterogeneity.

A frequency analysis of samples with detectable changes in antibiotic concentration ($\Delta C > 0$) showed clustering in three distinct ranges of preferential flow: $0 < f_{PF} \leq 0.15$, $0.15 < f_{PF} \leq 0.35$, and $0.35 < f_{PF} \leq 0.61$ (Fig. 1a). In general, solutes with high relative affinity for the soil matrix (e.g., TYL, TC) were most frequently detected under low preferential flow conditions (i.e., $f_{PF} \leq 0.15$). The relatively low-affinity sulfonamides (SDM and SMZ) had a more uniform distribution across the range of preferential flow. While the high-affinity solutes ERY and PLY had similar distributions as the sulfonamides, it should be noted that they were detected less often (N = 11 for ERY and 19 for PLY versus N = 22 for SDM and 35 for SMZ). Further, the sulfonamides were always detected under high preferential flow conditions (e.g., $f_{PF} > 0.4$), whereas ERY and PLY continued to have non-detects (i.e., $\Delta C = 0$) in that range (Figure S4). Altogether, these results suggest that bypass flow preferentially mobilizes some solutes over others, with relative affinity to the matrix acting as an important factor in this process.

After binning data into the three preferential flow ranges ($0 < f_{PF} \leq 0.15$, $0.15 < f_{PF} \leq 0.35$, and $0.35 < f_{PF} \leq 0.61$), the compounds with the greatest contrast in relative affinity to soil (e.g., the high-affinity macrolides TYL and ERY versus the low-affinity sulfonamides SDM and SMZ) were similar in $\Delta C$ for the low range of preferential flow, but diverged with increasing bypass flow (Fig. 1b). For example, when $f_{PF}$ was $< 0.15$, TYL and SMZ had nearly identical $\Delta C$ values. However, when preferential flow exceeded 0.35, $\Delta C$ was more than an order of magnitude higher for SDM compared to ERY. This finding suggests that the influence of solute-matrix affinity on transport was weakest when bypass flow was minimal.
Here we note that the antibiotic PLY, which had a relatively moderate affinity for soil, produced the highest $\Delta C$ in drainage in the high range of $f_{PF}$ (Figs. 1b). However, a previous antibiotic transport study conducted in the same field site reported PLY as being highly mobile with 50x more PLY transported in runoff compared to the sulfonamide SMZ$^{46}$. This result suggests that PLY sorption to the $A_p$ soil sample used for $K_d$ determination may not have been representative of the entire field, or else that our ranking was accurate and the high apparent mobility of PLY seen in field-runoff studies$^{46,47}$ reflects the influence of other controls on transport (such as colloidal transport, as discussed in the Supporting Information). Some numerical simulations$^{33,48}$ and one recent column study$^{41}$ have also suggested that solutes with moderate affinity for soil may be most susceptible to preferential flow. Though the underlying mechanisms are not yet clear, we speculate that these compounds may have high enough $K_d$ to be sorbed throughout the soil medium, yet soluble enough to be partitioned or displaced into local bypass flow. Thus, when $f_{PF}$ approached $\sim 0.5$ (i.e., roughly equal matrix and preferential contributions to flow), compounds with moderate relative affinity for soil could be selected in higher proportions relative to other antibiotics.

Solute transport to our lysimeters also appears to have been most susceptible to preferential flow (i.e., $\Delta C/f_{PF}$ was highest) when $f_{PF}$ was $< 0.15$ (Fig. 2). The influence of preferential flow on the magnitude of $\Delta C$ was therefore dampened when solute-matrix affinity became more influential, indicating a fundamental shift in solute and flow partitioning. For example, in a situation where $\Delta C$ linearly increased across the range of $f_{PF}$ values (red dashed line fit to raw data in Fig. 2), the antibiotic detection in drainage would respond similarly (i.e., nearly constant $\Delta C/f_{PF}$) across the spectrum of preferential flow. Instead $\Delta C$ was an order of magnitude more susceptible to preferential flow at the lowest versus highest $f_{PF}$ values. These data thus further illustrate that solute responses to bypass flow differ along the spectrum of preferential flow, with low $f_{PF}$ conditions causing non-selective transport and high $f_{PF}$ conditions causing selective transport at the length scale of the lysimeters.

These differences in transport behavior can be explained by both the amount of preferential flow and the ability of this bypass flow to access antibiotics. For example, 7 days of rainfall suppression would likely have been sufficient time for compounds to diffuse into the soil matrix$^{13,50-53}$ and for sorption equilibrium to occur.$^{46,54}$ Consequently, solute transport in plots spiked with antibiotics was nearly identical to control plots (Figure S5), suggesting that these compounds may be stored in the soil matrix from previous applications. Therefore, when drainage water was less preferential (i.e., $f_{PF} < 0.15$), the likelihood of sampling all compounds was higher (Fig. 1a and S3) as most of the drainage water originated in the matrix. Infiltrating water may have mixed with a greater volume of pre-event storage before triggering preferential flow events with trace levels of antibiotics, allowing for compounds strongly sorbed to the soil matrix (e.g., high relative affinity) and compounds weakly bound to macropore walls (e.g., low relative affinity) to be transported in similar proportions. In contrast, higher proportions of preferential flow would have excluded flow through the matrix where much of the compounds resided,$^{52,55-58}$ causing the fast preferential flow domain to become more distinct from the slow matrix
flow domain\textsuperscript{59–61} and infiltrating water to select for compounds with a higher affinity for the aqueous phase.

We additionally note that initiation of macropore flow often requires contributions from the soil matrix,\textsuperscript{22} with the potential to dilute or displace the tracer signal in preferential flow paths.\textsuperscript{24,62} This process can lead to underestimations of event water contributions to preferential flow.\textsuperscript{24,25,62} Our method may not have distinguished these preferential flow scenarios from matrix water; rather, our analyses were intentionally focused on preferential flow paths that originated at or near the soil surface. Under the assumption that antibiotics were near the surface at the time of rainfall (max manure injection depth of 10 cm), our $f_{PF}$ calculations would have detected fast-flowing event water contributions with the greatest potential to rapidly transport these solutes to depth. Altogether, our $f_{PF}$ estimates should provide a useful representation of flow heterogeneity and identify source contribution of water and solutes in drainage.

Further, we encourage the use of alternative preferential flow detection methods,\textsuperscript{5} under similar experimental conditions, to determine the relevance of this range of detected preferential flow—and its bearing on relative solute transport—in other heterogeneous systems.

In this study we treated preferential flow as an explanatory variable, which revealed that conventional transport phenomena may hinge on the degree of flow heterogeneity. This distinction appears to be unprecedented in previous studies, in part, because none have considered how the magnitude of preferential flow alters the influence of solute-matrix affinity in soils. As a result, these findings contradict conventional understanding of solute transport, where the influence of compound properties was thought to be significantly reduced with bypass flow.\textsuperscript{33,63,64} To further explore this result, we used the conventional dual permeability model framework of Gerke and Van Genuchten\textsuperscript{65} with the HYDRUS 1D\textsuperscript{66} numerical platform to simulate analogous conditions to our experimental design (See Supplemental Information for details). Modeling results clearly predict that the difference between solutes of high and low relative affinity would decrease as the fraction of preferential flow increased (See Figure S6, and Tables S2, S3, and S4). Rather, our data indicated that when preferential flow intensified, $\Delta C$ in drainage became more influenced by the physiochemical interactions with the medium rather than just the medium itself (Fig. 3). Alternatively, if rainfall was applied much closer to the time of manure application one would expect the opposite trend: solute-matrix interactions might govern solute transport at low values of $f_{PF}$ but become less important as bypass flow mobilizes compounds with high and low relative affinity to soil equally. Using a similar experimental design, Le, et al.\textsuperscript{46} detected comparable losses to runoff for four antibiotics of varying mobility when rainfall occurred just 2 hours after manure application, yet losses differed by an order of magnitude when manure was undisturbed for 3 days. More directly, the timing of precipitation appears to be an important factor controlling compound behavior in the presence of preferential flow, due to sorption kinetics and physical partitioning of the compounds below-ground. We expect that this selective transport phenomenon will be observable in many other systems, as many farmers select periods without forecasted rain for manure application and organic contaminants in soil often reach sorption equilibrium within a few days.\textsuperscript{67,68}
Implications

It has long been known that solute diffusion and sorption equilibrium within the soil matrix may limit subsequent transport through preferential flow paths when rainfall timing is lagged relative to chemical application,\textsuperscript{52,53,56,58} with strongly sorbing substances most often affected.\textsuperscript{57} Similarly, preferential flow has been shown to non-selectively transport a range of compounds for decades.\textsuperscript{13,34,39,40,69} However, by simulating a range of preferential flow, we identified conditions necessary to dampen versus amplify the influence of compound physiochemical properties on solute transport, thus providing novel insight into subsurface water and solute partitioning. Our results suggest that under field-relevant scenarios the influence of solute-chemical properties appear damped below $\sim$15\% preferential flow, but amplified at higher contributions of event water. Mechanistically, this means that fast flow paths may preferentially select for solutes with low matrix affinity. Practically, this means that soil and solute physicochemical properties may become more, not less, influential as the magnitude of preferential flow increases. Given the ubiquity of preferential flow observations,\textsuperscript{8,9,19,70,71} it is important to develop better strategies for retaining mobile chemicals within the soil profile.

Here we recognize that this study only encompassed one particular set of field conditions. For example, by simulating just one storm with a constant rainfall intensity and timing we may have missed the opportunity to study precipitation-driven transport shortly after manure application, where the influence of compound properties is likely greatest.\textsuperscript{56,57} Simulating rainfall 7 days after manure application may have, rather, increased the likelihood of selective transport through macropores.\textsuperscript{57} Additionally, our suction lysimeters would not have necessarily intercepted all potential preferential flow paths. However, we posit that these results can be considered more broadly applicable to instances of non-equilibrium flow and transport because 1) our storm produced preferential flow estimates spanning 3 orders of magnitude, 2) the relative affinity of our eight antibiotics differed by up to two orders of magnitude, and 3) we collected $>700$ lysimeter measurements through time and space. In this way, we were able to detect both selective and non-selective transport. At the same time, our study quantified the response of antibiotics with wide-ranging chemical properties under varying levels of preferential flow, thereby emphasizing both the novelty and potential for broader transferability of these results.

Altogether, these results suggest that it may be necessary to re-evaluate common assumptions of solute transport under preferential flow conditions. Specifically, our findings indicate that: 1) the affinity between a solute and the soil matrix has little bearing on large-scale contaminant transport under conditions of low preferential flow, and 2) traditional reactive transport models (e.g., single domain flow and single sorption site) can better describe solute movement as the proportion of flow moving preferentially increases.

Methods
Field Study Site and Preparation

The field experiment was conducted in the spring of 2018 on a no-till agricultural field in Whitethorne, Virginia. The field had a 9 to 11% slope and was underlain by two loam-textured soil series: Braddock and Unison (Typic Hapludults) with moderate soil structure. Soil physiochemical and hydraulic properties methods are described in Table S3. A total of nine randomly placed rainfall simulation plots were installed in the field. Each plot consisted of a 200 x 150 cm steel frame inserted 10 cm into the soil surface, with adjacent 40 cm x 200 cm buffer strips maintained outside of the frame for installation of soil pore-water samplers. A steel pan was fitted to each frame, sealed for runoff collection, and piped down-gradient to a container for storage and quantification (Figure S7). Weed growth was then suppressed in all plots with glyphosate. Plots were differentiated into two treatments whereby manure was homogenously broadcasted on the soil surface (surface application; \( n = 3 \) plots) or injected below-ground into two 5 cm wide x 10 cm deep slits placed perpendicular to the slope and spanning the width of the plot frame and buffer strip (subsurface injection; \( n = 3 \) plots). The three remaining plots were used as controls by avoiding manure application and input of antibiotics. However, we detected some residual antibiotics in the control plots, which likely remained from manure applications in previous years (Figure S8). The presence of residue antibiotics in the control plots provided us with the opportunity to assess the mobility of compounds under short (up to 7 d) and long term (e.g., greater than 6 months) equilibration with the soil matrix.

Prior to manure application, we installed a series of suction lysimeters (200 kPa ceramic cups; Soil Moisture Equipment Corp., Santa Barbara, CA) in the plot buffer strips to sample veterinary antibiotic transport in the subsurface. These buffer strips received the same amount of rainfall and manure treatment yet were located outside of the metal plot frames. Soil pore water samples were withdrawn from two randomly positioned lysimeters in both the Bt1 (30 cm) and Bt2 horizons (90 cm) to detect vertical movement of antibiotics in surface application plots, with two probes per depth making four probes per plot (Figure S7 and S9). This same installation scheme was also adopted for control plots. In subsurface injection plots a series of nested (30 and 90 cm probes) lysimeters were installed both within and 25 cm down-gradient of the injection slit to detect vertical and lateral transport of antibiotics, with two probes per depth resulting in eight probes per plot.

A liquid slurry of dairy manure (5% solid content) was spiked with eight commonly used antibiotics: two macrolides, erythromycin and tylosin; two sulfonamides, sulfamethazine and sulfadimethoxine; three tetracyclines, oxytetracycline, chlortetracycline, and tetracycline; and one lincosamide, pirlimycin. A target concentration of 500 µg L\(^{-1}\) was used for all antibiotics. The spiked dairy manure slurry was then applied to each plot 7 days prior to rainfall simulations at a rate of 56 Mg wet mass ha\(^{-1}\). If natural rainfall occurred within the 7-day equilibration period, the plots were covered with plastic tarps to prevent unintentional water input to the plots.

Assessment of Antibiotic Relative Affinity to Soil
We performed a simple solute partitioning test in the laboratory to determine the linear sorption coefficient, $K_d$, for each of the eight antibiotics with soil from the field site (details in the Supplemental Information). Under this framework, each antibiotic was assigned a rank from 1 (highest affinity) to 8 (lowest affinity) based on measured $K_d$ values (Table S5). The antibiotics ERY and TYL were not detectable in the supernatant during the test, so were given respective rankings of 1 and 2. Additionally, we used the USEPA's BIOWIN model from the EPI (estimation program interface) Suite tool\textsuperscript{72} to estimate dissipation half-lives of each compound in soil following the methods described in Chen, et al.\textsuperscript{43}. Using these half-lives we projected that less than 10% of the originally applied antibiotic mass would have degraded during our 7-day experiments, so we therefore assumed that decay played a minor role during transport.

**Field Rainfall Simulations and Water Sampling**

After the 7-day equilibration period, we conducted rainfall simulations using deuterium-labeled well water to trace mobile infiltrating water and detect preferential flow contributions to pore water signature. The rainfall simulator (240 cm x 300 cm) followed the original design of Humphry, et al.\textsuperscript{73}, which has been adopted as standard protocol for the national research project for simulated rainfall-surface runoff studies\textsuperscript{74} because it provides constant droplet size and velocity between locations and studies. We conducted the rainfall simulations with the SERA-17 standard intensity of 7 cm h\textsuperscript{-1}, with rainfall continuing on each plot until the collection containers received 30 min of continuous runoff. Rainwater was isotopically labeled using a Dealglad venturi injector (9.0 x 5.5 x 5.5 cm; Shandong Jiujin Plastic Products Co., Shandong, China) fitted to the sprinkler inlet. This system dispensed an enriched deuterium solution into the well water at a desired ratio of ~ 4:100 (deuterium-spiked water: well water). Discrete pore water samples were taken from all lysimeters by applying 60 kPa of suction for 10 min, with samples collected 1 h before the simulation, 0.5 h into the simulation, and 1 hour after the simulation (Figure S9). All liquid samples were analyzed for $^2$H via cavity ring down spectroscopy (Model L1102-i, Picarro, Santa Clara, CA) and for all eight antibiotics via HPLC MS/MS, as detailed in the Supporting Information. To understand how these preferential flow estimates affected the transport of our eight veterinary antibiotics with a spectrum of relative affinity for soil, we quantified the change in concentration from lysimeter samples collected before versus during and after simulation ($\Delta C$) as a function of $f_{PF}$.

Here we note some potential constraints of using suction cup sampling to represent soil pore water. For example, suction lysimeters often have a smaller volume-of-influence compared to alternative pore water samplers,\textsuperscript{75-77} reducing the likelihood of intercepting every preferential flow path below the plots. Suction cups can also have biased representation of water in larger—more “mobile”—pores.\textsuperscript{78-80} We note that, though matrix and macropore waters can resist mixing during extreme rainfall,\textsuperscript{81} complete mixing between pores can occur within days.\textsuperscript{81-83} Thus, point measurements from our samplers (after 7 days of rainfall exclusion and equilibration) likely yielded representative samples of pre-event water from the
matrix and labeled event water from mobile water in macropores, while capturing a wide range of stable isotope signatures.

**Preferential Flow Analysis**

We considered flow to be partitioned into two distinct hydrological domains: faster advection through preferential pathways (e.g., root channels and macropores) and slower flow through the soil matrix via combined advection and dispersion mechanisms. Following the conceptual framework provided by Stumpp, et al.\(^{45}\) the isotope mass balance can be described as:

\[ Q_t(t) = Q_{PF}(t) + Q_{MF}(t) \]  \hspace{1cm} (1)

And:

\[ Q_t(t) \cdot C_t(t) = Q_{PF}(t) \cdot C_{PF}(t) + Q_{MF}(t) \cdot C_{MF}(t) \]  \hspace{1cm} (2)

where the preferential flow, \( Q_{PF}(t) \), and matrix flow, \( Q_{MF}(t) \), equal total discharge \( Q(t) [L^3 \cdot T^{-1}] \), and \( D_{MF}(t), D_{PF}(t), \) and \( D(t) [M \cdot L^{-3}] \) correspond to the isotope concentrations within each flow component. Assuming that preferential flow pathways translate to rainfall inputs during each sampling period, we consider the rainfall isotope signal to be equivalent to the preferential flow signal in the outlet:\(^{45,84}\)

\[ D_{PF}(t) = D_{rain}(t) \]  \hspace{1cm} (3)

Thus, the fractional contribution of preferential flow to the outlet signal is:

\[ f_{PF}(t) = \frac{D_t(t) - D_{MF}(t)}{D_{PF}(t) - D_{MF}(t)} \]  \hspace{1cm} (4)

and the preferential flow rate is:

\[ Q_{PF}(t) = f_{PF}(t) \cdot Q_t(t) \]  \hspace{1cm} (5)

We also note that mass transfer between the slow flowing matrix water is implicitly considered in the mixing model. For example, we can consider a scenario where event water infiltrates into the soil matrix and spills into a preferential flow path yielding an \( f_{PF} \) value of 0.50. Because event water reached the outlet before the wetting front it must have required preferential transport and thus 50% of the total water outflow is deemed preferential; with the remainder derived from pre-event matrix water.
Reactive Transport and Experimental Perspective

By applying labeled rainfall simulations to a heterogeneous no-till soil containing manure spiked with 8 antibiotics, we were able to quantify the amount of preferential flow in lysimeter drainage and assess the influence of compound properties on solute transport using the compounds’ wide range of relative affinity to the matrix. Additionally, because 1) the mass of antibiotics applied in manure was consistent between compounds and manure treatment (surface application versus subsurface injection), 2) estimated half-lives (described above) suggest that degradation was a minimal over the 7-day equilibration period, and 3) we were not concerned with metabolites of these antibiotics, our analysis did not require the explicit use of reactive transport models.

Statistical Analysis and Data Processing

We used a two-way analysis of covariance (ANCOVA) to statistically compare the slope of lines fitted to log-transformed $\Delta C$ as function of preferential flow ($f_{PF} > 0$ and $\Delta C > 0$) by manure application treatment (i.e., subsurface injection, surface application, and control plots with only levels of antibiotics) and lysimeter depth (30 cm vs 90 cm). Specifically, this allowed us to identify the statistical significance of manure treatment on $\Delta C$ across the observed range of the covariate (preferential estimates) while also testing for the interaction of depth on this relationship. The log-transformed data were found to meet ANOVA assumptions of normality (via normal-quantile plots) and homogeneity of variances (via Fligner’s test). We used R version 3.5.2 to conduct all statistical analyses with $\alpha = 0.05$. We found no significant difference between the slope of lines fitted to $\Delta C$ data across the range of $f_{PF}$ for all treatments, and no significant influence of lysimeter depth on this relationship (see Supplemental Information and Figure S10). Thus, we compiled all data together for subsequent analyses without separating treatment or depth.

Declarations

Supporting Information

This document contains information about soil characteristics, analytical procedures, field plot schematics, numerical simulations, and additional resources related background detection of antibiotics in soil.

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**Figures**
a) Smoothed density distributions of tested antibiotics in the detected range of estimated preferential flow (x-axis), indicating the total frequency of samples detected; N = the total number of samples with detectable changes in antibiotic concentrations ($\Delta C > 0$) and detectable preferential flow. b) Change in antibiotic concentration ($\Delta C$) as binned using three ranges of preferential flow with highest densities ($f_{PF} = 0 – 0.2$, $0.2 – 0.35$, and $0.35 – 0.61$). Error bars represent standard error of the mean (SE). Colors
indicate relative affinity to soil as ranked based on the sorption study (Kd values listed in Table S2): red indicates the compound with the lowest affinity (SMZ) and black indicates the compound with highest affinity (ERY) to soil. Lines track SMZ and ERY. R v3.5.2 was used to plot this figure.49

Figure 2

Solute susceptibility to preferential flow ($\Delta C / f_{PF}$) across the detected range of preferential flow. The red dashed line depicts a linear fit to raw (not log-transformed) data using all antibiotics ($\Delta C = 0$ and $f_{PF} = 0$ excluded). The linear fits indicate a possible condition where antibiotics have constant susceptibility to
leaching regardless of the amount preferential flow. Note that the y-axis in the inset figure has a logarithmic scale. R v3.5.2 was used to plot this figure.49

Figure 3

Different subsurface partitioning scenarios of solutes (dots) with high (black) and low (red) relative affinity to soil. Hypothetical solute concentration profiles (C vs x) are expressed at arbitrary locations spanning macropores surrounding a portion of the soil matrix. The top panel illustrates how both solutes would behave if rainfall simulations were conducted on the same day as antibiotic-spiked manure was applied. Compounds would have limited time to infiltrate into the soil matrix and come into sorption equilibrium, and high amounts of bypass flow through macropores could sample both compounds.
regardless of their relative affinity for soil. The bottom panel describes our experimental results, in which simulated rainfall occurred on the 7th day after antibiotic-spiked manure was applied to the plots. In this scenario, the elapsed time allowed solutes to diffuse into the soil matrix and sorption equilibrium to occur, so drainage with greatest macropore contributions (high preferentially flow) could select for compounds with low-affinity for the soil. In contrast, drainage with higher matrix contributions (low preferential flow) could sample all compounds in similar proportions.

**Supplementary Files**

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