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Emerging Contaminants

Culturable antibiotic-resistant fecal coliform bacteria in soil and surface runoff after liquid dairy manure surface application and subsurface injection

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Abstract

Land application of manure, while beneficial to soil health and plant growth, can lead to an overabundance of nutrients and introduction of emerging contaminants into agricultural fields. Compared with surface application of manure, subsurface injection has been shown to reduce nutrients and antibiotics in surface runoff. However, less is known about the influence of subsurface injection on the transport and persistence of antibiotic-resistant microorganisms. We simulated rainfall to field plots at two sites (one in Virginia and one in Pennsylvania) 1 or 7 d after liquid dairy manure surface and subsurface application (56 Mg ha^{-1}) and monitored the abundance of culturable antibiotic-resistant fecal coliform bacteria (ARFCB) in surface runoff and soils for 45 d. We performed these tests at both sites in spring 2018 and repeated the test at the Virginia site in fall 2019. Manure subsurface injection, compared with surface application, resulted in less ARFCB in surface runoff, and this reduction was greater at Day 1 after application compared with Day 7. The reductions of ARFCB in surface runoff because of manure subsurface injection were 2.5–593 times at the Virginia site in spring 2018 and fall 2019 and 4–5 times at the Pennsylvania site in spring 2018. The ARFCB were only detectable in the 0-to-5-cm soil depth within 14 d of manure surface application but remained detectable in the injection slits of manure subsurface-injected plots even at Day 45. This study demonstrated that subsurface injection can significantly reduce surface runoff of ARFCB from manure-applied fields.

Abbreviations: ARB, antibiotic-resistant bacteria; ARFCB, antibiotic-resistant fecal coliform bacteria; ARG, antibiotic resistance gene; CFU, colony-forming units; ERY, erythromycin; FCB, fecal coliform bacteria; SMZ, sulfamethazine; TET, tetracycline.

1 INTRODUCTION

Core Ideas

- Antibiotic-resistant fecal coliform bacteria (ARFCB) were detectable in dairy manure.
- ARFCB in runoff decreased 5–30 times 7 d after manure application.
- Less ARFCB in runoff from manure subsurface-injected plots than from surface-applied plots.
- ARFCB persisted up to 45 d in injection slits of manure subsurface-injected plots.
- Lateral movement of ARFCB away from manure injection slits was limited.

Antibiotic-resistant infections have been recognized as a global health care concern (WHO, 2014). An estimated 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 Americans die each year from these infections (CDC, 2019). Antibiotic overuse in health care and animal production is an important contributing factor to widespread antibiotic resistance (Martin et al., 2015; Shallcross, 2014). For example, up to 50–90% of the antibiotic mass administered to animals is excreted via urine and feces as parent compounds or their metabolites (Kumar et al., 2005). Previous experiments have shown that gut and fecal samples have elevated levels of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) within a week of antibiotic application (Aarestrup & Carstensen, 1998) and that concentrations of these compounds increase with subsequent treatments. Ruminant digestive tracts contain ARGs that can even be enhanced by antibiotic application (Cameron & McAllister, 2016). As a result, many researchers have linked animal production and manure use in agriculture with the spread of antibiotic resistance elements in the environment (Chang et al., 2015; Heuer et al., 2011; Marti et al., 2013), although the mechanisms of environmental transport are not entirely resolved.

In the United States alone, >10^6 t of animal manure (wet weight) are produced each year (Zhang & Schroder, 2014), and about 6.4 million ha of cropland receive livestock manure (Eghball & Power, 1999; MacDonald et al., 2010). Agricultural soils amended with manure generally have elevated concentrations of antibiotics, antibiotic resistance microorganisms (including pathogens), and antibiotic resistance genes (Cook et al., 2014; Hilaire et al., 2020; Liu et al., 2016; Marti et al., 2013; Yang et al., 2016). These conditions raise the potential for spread and dissemination of antibiotic residues, ARB, and ARGs. For example, storm-driven surface runoff to streams from livestock manure–applied areas is a source of fecal contamination in water (Crowther et al., 2002; Edwards et al., 2001; Tian et al., 2002). Field trials (Sistani et al., 2009; Soupir et al., 2006) and greenhouse studies (Brooks et al., 2009) have identified bacteria transport in runoff from manure-amended soils. Other studies have detected high concentrations of resistant fecal coliform bacteria (FCB) in amended soils, raising concerns of crop contamination and subsequent environmental transport (Geldreich & Bordner, 1971; Gerba & Smith, 2005). However, there has been limited research investigating the effect of these manure application methods on antibiotic-resistant FCB (ARFCB) in surface runoff and their spatial and temporal distribution in soil.

The traditional method of broadcast applying manure onto the surface of field soils is cost effective and time efficient but can leave the manure susceptible to being transported via surface runoff processes. Incorporation of manure via tillage promotes its mixing into the soil and reduces concentrations at the surface. However, tillage can result in undesired outcomes, including soil structure disturbance, compaction, and residue removal, which can increase erosion and carbon loss (López-Garrido et al., 2012; West & Marland, 2002). Therefore, manure injection techniques have been developed to apply manure below the soil surface without inducing the level of disturbance associated with typical tillage practices (Maguire, Kleinman, & Beegle, 2011; Maguire, Kleinman, Dell, Beegle, Brandt, et al., 2011). Previous studies have shown that manure injection can reduce nutrient leaching and hormone transport in surface runoff (Adeli et al., 2013; Kibet et al., 2011) and increase nutrient and water use efficiency (Maguire, Kleinman, & Beegle, 2011; Maguire, Kleinman, Dell, Beegle, Brandt, et al., 2011; Mina et al., 2016). When compared with surface application, manure injection has been shown to reduce pirlimycin concentrations by 21 times in transported sediment and by 32 times in runoff water (Kulesza et al., 2016). Another study showed that using manure injection compared with surface application reduced surface runoff losses of four antibiotics (sulfamerazine, chlortetracycline, pirlimycin, and tylosin) by 47–88% (Le et al., 2018). It is unclear, however, whether similar reductions of runoff-transported antibiotics could be translated to other cropping systems, to a wider range of antibiotics, or for culturable ARFCB.

To contribute to the development of best management practices to minimize output of ARFCB from manure-applied fields and their subsequent proliferation in the environment, we investigated (a) how manure application methods (surface application vs. subsurface injection) affect the total counts of culturable ARFCB in surface runoff, (b) how ARFCB move away from manure injection slits via lateral and vertical transport, (c) how the time gap between manure application and rainfall (1 vs. 7 d) affects ARFCB surface runoff and fate in soil, and (d) how differences in time of manure application (spring vs. fall) and antecedent moisture conditions affect ARFCB surface runoff and fate in soil.

2 | MATERIALS AND METHODS

2.1 | Study sites and experimental design

Two study site locations were used to contrast the roles of soils on ARFCB fate with alternative manure management practices (Supplemental Figure S1). The first set of field plots was located at the Rock Springs Agronomy Farm in Rock Springs, PA. The field had Hagerstown (fine, mixed, semiactive, mesic Typic Hapludalfs; silt loam) soil with an average slope of 3–5% and no history of manure applications.

The second set of plots was located in Whitehorne, VA, in a no-till field with Braddock loam (clayey, parasesquic, mesic Typic Hapludults; 46% sand, 44% silt, and 10% clay) and an average slope of 9–11% (Kulesza et al., 2016). The site had been previously harvested for corn (*Zea mays* L.), and manure had not been applied for at least 5 yr prior to the start of the study.

To better understand how the timing of manure application influences ARFCB transport, the Virginia site was used in spring 2018 and again in fall 2019. In the 2018 campaign, the site had 15 plots installed with three replicates of five treatments, including two manure application methods (surface application and subsurface injection), two manure–rainfall time gaps (1 and 7 d), and a no-manure control. For the fall 2019 study, a different side of the same field was used (Supplemental Figure S2). This part of the study used 18 plots installed with three replicates of the same six treatments that were used in the Pennsylvania campaign. During both campaigns, field plots were arranged in randomized complete block designs; additional details can be found in the supplemental information.

The Pennsylvania site was only used in spring 2018. There were 18 plots with six treatments, each with three replicates, including two manure application methods (surface application and subsurface injection), two manure–rainfall time gaps (1 and 7 d), and two sets of no-manure controls. Field plots were arranged in block design so that each treatment block had one representative of the rainfall time gaps for a total of nine blocks with 18 plots (Supplemental Figure S3).

For the Pennsylvania site, fresh liquid dairy manure was collected from a local dairy farm in Pennsylvania. For the Virginia studies, fresh liquid dairy manure was collected from a local dairy farm in Virginia 3 d prior to the first application day for each season. The liquid dairy manure was applied at the typical agronomic N application rate for Virginia (56 Mg ha^{-1} wet weight).

Rainfall was simulated rainfall on each experimental plot. Details on the rainfall simulator used in Pennsylvania and Virginia can be found in the supplemental information. The runoff generated was collected for 30 min after a continuous stream of surface runoff formed and reached the collection carboys. The time taken for continuous stream of surface runoff to form, the volume of runoff collected during the 30 min from each plot, and the total volume of water applied to each plot were recorded (Supplemental Table S1). Time to runoff was not measured in the Pennsylvania study. After 30 min of runoff generation, a 1-L subsample was taken from the collected water using a sterile 1-L amber glass bottle.

Soil samples were collected after the rainfall simulations had been completed at depths of 0–5, 5–15, and 15–25 cm to observe any movement of antibiotics throughout the soil profile. Soil moisture was calculated using the freeze-drying method. Samples were weighed, frozen, and then placed on a freeze drier at −40 ˚C for 2 d. After all moisture had been removed, samples were weighed again, and moisture content was calculated by subtracting the freeze-dried weight from the initial weight. Additional details on soil sample collection can be found in the supplemental files (Supplemental Information S2). All soil and water samples collected from the Pennsylvania site were shipped overnight on ice to Virginia Tech for analysis.

2.2 | ARFCB (*Escherichia coli*) sample analysis

The number of FCB resistant to sulfamethazine (SMZ), tetracycline (TET), and erythromycin (ERY) was determined using the colony-forming units (CFU) count method described in Marti et al. (2013) with minor modifications. Briefly, 0.1 ml taken from a collected runoff sample (Supplemental Information S2) was diluted stepwise by ten-fold (10^0, 10^{-1}, 10^{-2}, and 10^{-3}). Each solution was then spread onto a MacConkey agar (Difco Laboratories) on Gosselin Corning Gosselin 100 mm by 15 mm Polystyrene Petri Dishes (Fisher) containing selected concentrations of antibiotics (100 mg L^{-1} of SMZ, 8 mg L^{-1} of TET, and 40 mg L^{-1} of ERY) using sterile polypropylene cell spreaders (VWR). Antibiotic concentrations were added to the MaConkey agar once it had cooled to 45–50 ˚C to ensure antibiotic potency was not reduced by the temperature of the medium. The concentrations of antibiotics selected were adapted from a similar study (Wind et al., 2018). Their concentrations were selected based on comparisons to known European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum inhibitory concentrations where available (EUCAST, 2016) and trial enumerations of bacterial colonies on MacConkey agar supplemented with differing antibiotic concentrations when not available (Williams, 2016). For soil, 1.00 g of subsample was taken from a collected wet soil, mixed with 10 ml sterile saline solution (0.85% NaCl), and vortexed vigorously for 5 min to create a uniform suspension. Then, 0.1 ml of 10-fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) for each suspension was spread onto the MacConkey agar plates. All plates were incubated for 24 h at 37 ˚C, and colonies that appeared dark pink or red were counted.

2.3 | Statistical analysis

Data were processed using JMP Pro 15 (SAS Institute, 2019) with a significance level (α) of .1. All soil ARFCB concentrations, expressed as CFU g^{-1}, were $\log_{10}$ transformed before applying statistical analyses. For soil samples below detection limits, absolute abundance (CFU g^{-1} of wet soil) was assigned a value equal the method detection limit (10 CFU g^{-1} of wet soil, 1 $\log_{10}$ CFUg^{-1} of wet soil) for statistical analyses. The $\log_{10}$–transformed data were next tested for normality. If the data were normally distributed (tested using the Anderson–Darling Test Hypothesis), one-way ANOVA with Dunnett's test (all samples compared with background) was performed. If the data were non-normally distributed, the Wilcoxon/Kruskal–Wallis test (rank sums) with χ^2 approximation was conducted to compare data of different treatments.

For runoff samples, the percentage of manure-associated ARFCB initially applied to each plot that ended up in the surface runoff was calculated by

$$\text{Percent transported} = \frac{\text{ARFCB}_{\text{runoff}}\ (\text{CFU ml}^{-1}) \times V_{\text{runoff}}\ (\text{ml})}{\text{ARFCB}_{\text{manure}}\ (\text{CFU ml}^{-1}) \times V_{\text{manure}}\ (\text{ml})} \times 100\%$$

where $\text{ARFCB}_{\text{runoff}}$ and $\text{ARFCB}_{\text{manure}}$ are the respective ARFCB concentrations (CFU ml^{-1}) in the runoff and manure, and V_{runoff} and V_{manure} are the respective total volume of runoff collected during the rainfall simulation period and the volume of liquid manure applied to a plot. These percentages were tested for normality (using the same methods used with the soil samples). If normally distributed, a generalized regression fit to a beta distribution was performed; if non-normally distributed, treatments were compared using the same method used for the soil samples (i.e., Wilcoxon/Kruskal–Wallis test [rank sums]). The effect of individual treatment factors (e.g., manure application methods, soil depth, manure–rainfall time gaps, and the rain event) was analyzed using a generalized regression model with maximum likelihood estimation. These comparisons were between treatments within each of the three field sites (surface application vs. subsurface injection, surface application 1 d vs. surface application 7 d, and subsurface injection 1 d vs. subsurface injection 7 d).

Details from surface runoff (time to runoff [min], volume of water collected [L], and volume of water applied [L]) collected for all treatment plots at Virginia sites were compared with each other within seasons as well as with averages across seasons using ANOVA (Supplemental Table S1). Analysis of variance was also used to compare soil water content (%) based on treatments within and across seasons (Supplemental Table S5).

3 | RESULTS AND DISCUSSION

3.1 | Culturable ARFCB in surface runoff

3.1.1 | Injecting manure reduces transport of ARFCB in surface runoff

The concentrations of FCB resistant to SMZ, TET, and ERY in the liquid dairy manure used for the rainfall simulation studies are listed in Supplemental Table S2. The culturable ARFCB concentrations in the control runoff and well water used for rainfall simulation were below detection limits (1 CFU ml^{-1} runoff or well water).

For the Pennsylvania plots, 1.1 ± 1.2% of SMZ-resistant FCB, 0.7 ± 0.9% of TET-resistant FCB, and 0.9 ± 1.1% of ERY-resistant FCB initially applied to the plots via subsurface injection entered surface runoff when simulated rainfall was applied 1 d after manure application (Figure 1; Supplemental Table S3). In comparison, the surface-applied manure plots had relative transport percentages of 5.1 ± 3.8% (SMZ-resistant FCB), 2.7 ± 0.7% (TET-resistant FCB), and 4.5 ± 1.8% (ERY-resistant FCB). The surface-applied manure plots therefore had significantly higher amounts of transported FCB than the subsurface-injection plots ($p < .05$), with relative differences of 4.6 (SMZ-resistant FCB), 3.9 (TET-resistant FCB), and 5.0 times (ERY-resistant FCB). When the manure application–rainfall time gap was 7 d, these three ARFCBs were transported in statistically similar quantities in surface runoff from manure-injected plots and surface-applied plots ($p > .05$) (Supplemental Table S3).

In spring 2018, the Virginia plots had similar trends as the Pennsylvania plots. Specifically, when rainfall was simulated 1 d after manure application, surface runoff from subsurface-injection plots had 0.06 ± 0.07% (SMZ-resistant FCB), 0.07 ± 0.1% (TET-resistant FCB), and 0.08 ± 0.1% (ERY-resistant FCB) of the initially applied quantities (Figure 1; Supplemental Table S3). Runoff from surface application plots had significantly higher quantities of transported ARFCBs, with relative percentages that were 593 (SMZ-resistant FCB), 227 (TET-resistant FCB), and 279 times (ERY-resistant FCB) higher than the subsurface-injection samples (Supplemental Table S3). This result demonstrated that subsurface injection can be the preferred method of manure application for reducing target ARFCB leaving agricultural fields via surface runoff. As with the Pennsylvania

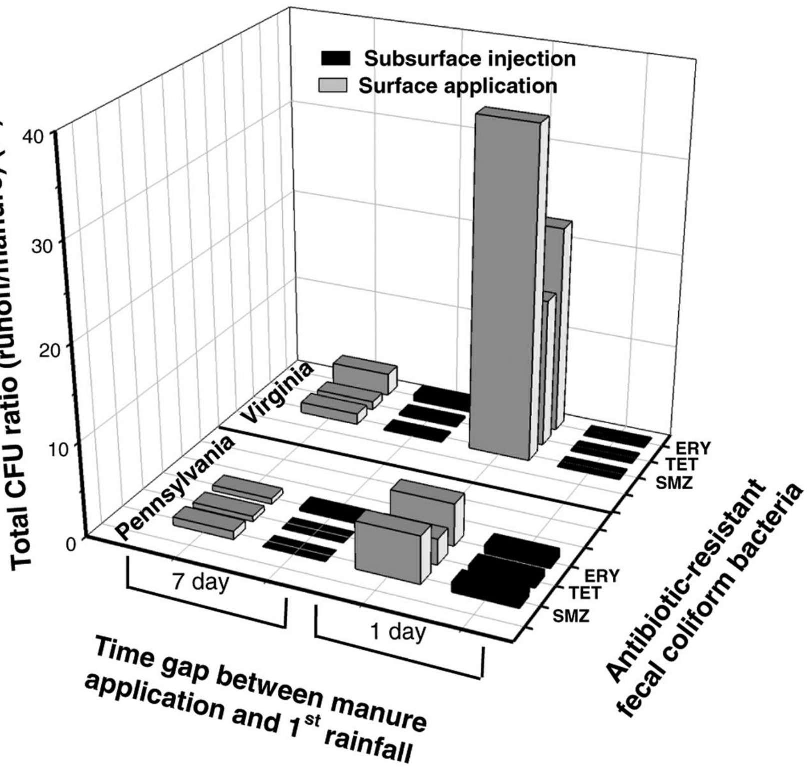

FIGURE 1 Culturable fecal coliform bacteria resistant to sulfamethazine (SMZ), tetracycline (TET) and erythromycin (ERY) detected in surface runoff. The manure was either surface applied or subsurface injected at two test locations (Pennsylvania and Virginia) in spring 2018. Simulated rainfall was applied at 1 and 7 d after manure application. Data are presented as mean colony-forming units (CFU) detected in surface runoff relative to the initial input with manure application. Standard deviations for each treatment are listed in Supplemental Table S3

plots, differences in runoff water quality were not significantly different ($p > .05$) between subsurface injection and surface application in the 7-d rainfall event (Supplemental Table S3).

Surface runoff in the Virginia site had substantially more ARCFB than in the Pennsylvania site, particularly in plots in which manure was surface applied and rainfall occurred 1 d after application. These differences likely result from several factors, including initial soil moisture, soil type, and topography, that encouraged more rapid and greater surface runoff in Virginia. For instance, the Virginia plots had slopes of 8–11%, compared with 3–5% in Pennsylvania. The Braddock soil at the Virginia site is capable of producing very high runoff when the slope is steep (USDA-NRCS, 2021). Therefore, even though there was no statistical difference for surface runoff volumes and time to runoff in the Pennsylvania site compared with the Virginia site (Supplemental Table S1), it is possible that more water infiltrated before runoff in Pennsylvania compared with Virginia. This process likely moved more ARFCB into the soil, making less available for loss due to runoff.

The manner of manure application can also influence infiltration and surface runoff processes. Previous work has shown that surface broadcast of manure can reduce the infiltration rate of soils having high clay content (Pote et al., 2001), and others reported reduced infiltration and increased runoff rates after manure application, which they attributed to pore clogging (Bottom et al., 1986; Khaleel et al., 1981). Researchers have also previously observed that when orchardgrass fields were not aerated before receiving broadcast liquid dairy or swine manures, the runoff volume generated was 47–81% higher compared with similar fields that were aerated (van Vliet et al., 2006). In this study, the subsurface injection of manure may have worked to similarly aerate the soils. The injection slits also likely created more preferential flow opportunities through the slits in the field plots. Practices such as incorporating compost into clay soil can enhance preferential flow pathways, leading to delayed and less total runoff generation (Maguire, Kleinman, Dell, Beegle, Brandt, et al., 2011; van Vliet et al., 2006).

Results from the current study are consistent with other studies pointing to the benefits of manure injection in curtailing runoff losses of manure constituents. For instance, researchers reported that using the surface application method caused 6.81% of fecal coliform–associated *Escherichia coli* from manure to enter runoff from simulated rainfall (Blaustein et al., 2015). Elsewhere, others monitored tile- and groundwater quality after land application of dewatered biosolids using both spreading and subsurface injection application methods but found no significant differences these methods of spreading biosolids (Gottschall et al., 2009). Another study performed in a tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] pastureland on a sandy loam soil in Crossville, AL, showed that subsurface injection of poultry litter resulted in >99% reduction of *E. coli* in surface runoff compared with manure surface application (Sistani et al., 2009). With our study, we show the benefit that injecting manure vs. surface application has on reducing ARFCB runoff from two distinct study sites. To the best of our knowledge, this is the first study examining this direct comparison of these manure application strategies on runoff of ARFCB.

3.1.2 | Greater time between manure application and rainfall reduces ARFCB losses in surface-applied plots

Comparing surface-applied manure in the Pennsylvania plots, waiting 7 d before simulating rainfall caused significant reduction in the ARFCB leaving via surface runoff vs. waiting 1 d ($p < .05$) (Figure 1). For these plots, 0.9 ± 1.5% of SMZ-resistant FCB, 0.6 ± 0.9% of TET-resistant FCB, and 0.6 ± 0.9% of ERY-resistant FCB that were initially applied to the plots left via surface runoff due to rainfall that was simulated 7 d later (Supplemental Table S3). Transported ARFCBs from 7-d plots had relative percentages that were, respectively, 5.7, 4.5, and 7.5 times lower than the 1-d plots. When manure was subsurface injected, waiting 7 d before simulating rainfall did not reduce total counts of ARFCB in surface runoff, in contrast to results from the surface-applied plots. In both cases (1- and 7-d gaps), reduced ARFCB left the subsurface injection plots via surface runoff compared with surface-applied plots. This finding showed that, if rainfall is expected within 1 d of manure application, subsurface injection can reduce ARFCB leaving plots via surface runoff. Although manure subsurface injection can be the preferred application method, when not feasible, our data suggest that surface applying manure somewhere between 1 and 7 d before a possible storm event can also help to reduce ARFCB entering surface runoff.

For the Virginia plots in spring, having a 7-d time gap before simulating rainfall caused a significant reduction in ARFCB leaving surface-applied manure plots via runoff compared with the 1-d gap before rainfall plots (Figure 1). Of the initial ARFCB applied to the surface-manured plots, only 1.2 ± 1.6% of SMZ-resistant, 0.9 ± 0.8% of TET-resistant, and 2.4 ± 3.2% of ERY-resistant FCB left via surface runoff when rainfall was simulated 7 d later (Supplemental Table S3). These ARFCB counts were, respectively, 29.7, 17.7, and 9.3 times lower than those associated with the 1-d gap rainfall simulations ($p < .001$). In contrast, ARFCB transport from injected-manure plots was statistically similar when rainfall was applied 1 vs. 7 d after manure application ($p > .1$). The Pennsylvania site had similar trends.

For the Virginia plots in fall, when manure was surface-applied with a 7-d time gap before simulated rainfall, the data showed ARFCB reduction entering runoff, just like the spring study. For the fall plots, 0.03 ± 0.02, 0, and 0.7 ± 0.2%, respectively, of SMZ-, TET-, and ERY-resistant FCB total counts initially applied to the plots via manure surface application were transported with surface runoff generated by rainfall 7 d later (Supplemental Table S4). These ARFCB percentages were, respectively, 23.3 times lower, 100%, and 3.7 times lower (Figure 2). When the manure was subsurface injected in the spring experiments, no benefit in applying 1 or 7 d before rainfall was observed. This was not the case for the fall experiment. In fall, total counts of SMZ- and TET-resistant FCB initially applied to the plots via manure subsurface injection were 2.3 times and 100% lower in runoff from the 7-d vs. 1-d treatments (Supplemental Table S4). In this season, increasing the time between manure application and rainfall was just as beneficial for both application methods.

Researchers found that, after conducting three consecutive simulated rainfall events separated by 24 h, fecal indicator *E. coli* loads were released in rainfall runoff from plots treated with fresh and aged cattle manure (Thurston-Enriquez et al., 2005). The treatment results ranged from 9.92×10^6 to 1.44×10^9 CFU per plot for fresh manure and from 2.68×10^7 to 1.10×10^8 CFU per plot for aged manure. Therefore, the aged manure caused a 10-fold reduction in *E. coli* entering runoff after the first event. Although our study was not exactly a replica of theirs, aging manure prior to application, if likened to ageing of manure after application to soil, shows a similar result. The outcome of both methods is reduction of FCB entering surface runoff after rainfall. Researchers found that increasing the lag time between manure slurry application reduced ARB concentrations in leachate and in the top 1 cm of soil in their columns (Bolster et al., 2018). Other studies showed that increasing time between manure application and runoff events decreases the amount of *E. coli* in surface runoff (Sistani et al., 2009; Wallace et al., 2013).

The general reduction in ARFCB transport via surface runoff with increased time between manure application and subsequent rainfall could have a few explanations. Increasing contact time between bacteria and soil could increase the sorption of bacteria to the soil surface. Most of the sorption would have occurred within the first day (most bacterial sorption studies make this assumption, e.g., Bolster & Abit, 2012; Mills et al., 1994; Zhao et al., 2012), so this may not be solely responsible. Another possibility is that as the ARFCB presence in soil increases, they become more actively involved in attaching to the soil surfaces by making biofilms and thus reducing their availability for transport (Bolster et al., 2018; Donlan, 2002). Lastly, some of the reduction in surface runoff could be due to die-off or inactivation of ARFCB in the soil. These results support another potential for reducing ARFCB runoff in situations where injecting manure might not be possible. Although it is not possible to predict rainfall events with 100% accuracy, surface application of manure can be scheduled with weather patterns in mind so that there is a time lapse before the next rainfall event, when possible. This will help reduce the potential for ARFCB when manure cannot be injected, as seen with our results.

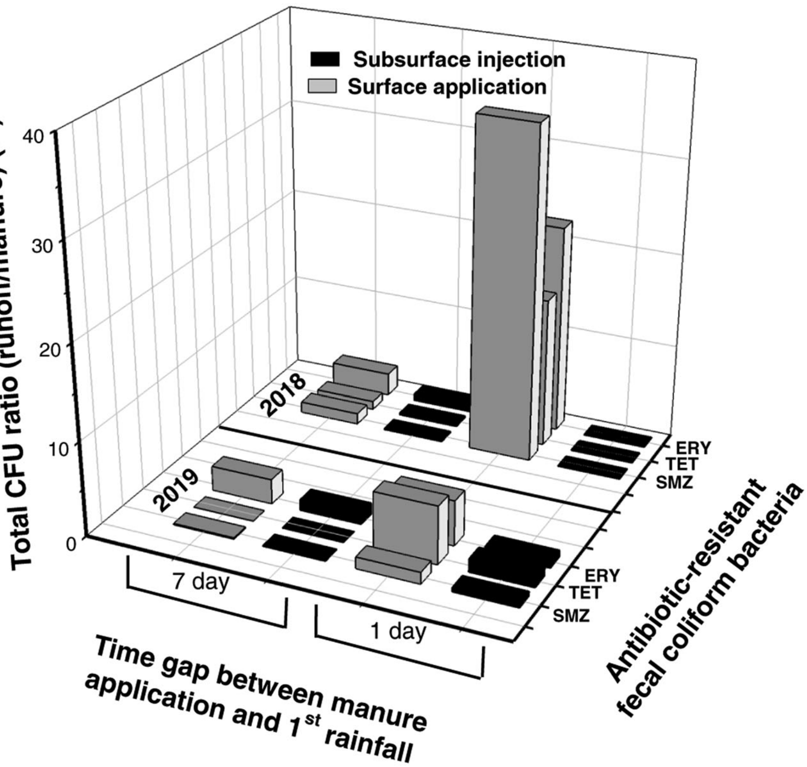

FIGURE 2 Culturable fecal coliform bacteria resistant to sulfamethazine (SMZ), tetracycline (TET), and erythromycin (ERY) detected in surface runoff. The manure was either surface applied or subsurface injected at one test location in spring 2018 and another in fall 2019, both in Virginia. Simulated rainfall was applied at 1 and 7 d after manure application. Data are presented as mean colony-forming units (CFU) detected in surface runoff relative to the initial input with manure application. Standard deviations for each treatment are listed in Supplemental Table S4

3.1.3 | Spring application of manure causes greater ARFCB loss in surface runoff

The fall season was expected to be drier than the spring season, and the runoff and soil moisture data collected supported this (Supplemental Tables S1 and S5). On average, the fall plots had 1,068.2 L of water applied (p = .02), took 43 min (p = .25) to generate runoff, and had lower soil moisture (17.2% during the fall; p = .004) (Supplemental Table S5). In the spring season, less water was applied to generate runoff.

When the experiment was repeated in the Virginia plots in the fall, the results differed in many ways from those collected in the spring (Figure 2). For example, when simulated rainfall was applied 1 d after manure application, relatively more TET-resistant FCB were transported, compared with SMZ-resistant and ERY-resistant FCB, even though TET-resistant FCB were the least transported in the spring. Furthermore, only the SMZ-resistant FCB were transported in significantly lower quantities from subsurface-injected vs. surface-applied plots (2.5 times reduction) (Supplemental Table S4), whereas ARFCB transport was significantly reduced for all three compounds in the spring (p < .001) (Supplemental Table S3). When the simulated rainfall occurred on Day 7 after manure application in the fall experiments, subsurface manure injection led to more SMZ-resistant FCB entering surface runoff (p < .05) than surface application (0.13 ± 0.05 vs. 0.03 ± 0.02%) (Supplemental Table S4). In contrast, the 7-d rainfall simulations in the spring did not produce any differences in ARFCB transport (Supplemental Table S3).

These contrasting results could be related to the difference in moisture concentrations in the soils during the different seasons: mean soil moisture was 17.2% during the fall vs. 24.5% during the spring (p = .004) (Supplemental Table S5). Lower moisture in the fall led to significantly less surface runoff being produced compared with the spring (Supplemental Table S1), which likely led to lower ARFCB transport. At the same time, the lower soil moisture in the fall may have encouraged greater ARFCB survival in the injection slits, where moisture content was greater (p = .004) (Supplemental Table S5). Altogether, manure injection was more effective for reducing ARFCB in spring 2018 compared with fall 2019.

There are many factors that can affect soil bacteria survival, such as pH, temperature, and soil moisture. Increased soil moisture, barring long-term saturation, can improve FCB survival rate (Bagdasaryan, 1964; Desmarais et al., 2002; Van Donsel et al., 1967). Additionally, drier soil could promote infiltration of liquid manure deeper into the soil profile after surface application, making less of the manure susceptible to surface runoff after heavy rainfall. Drier conditions, evidenced by differences in soil moisture, similar to the effect of rainfall timing, implied more potential migration into the soil matrix and could have reduced the amount of ARFCB available for transport so that less would move with surface runoff.

Manure treatment and rainfall timing combinations showed only one difference for ARFCB in runoff between spring and fall. Here we observed that the surface-application treatment had 4–50 times greater ARFCB counts in runoff with 1-d

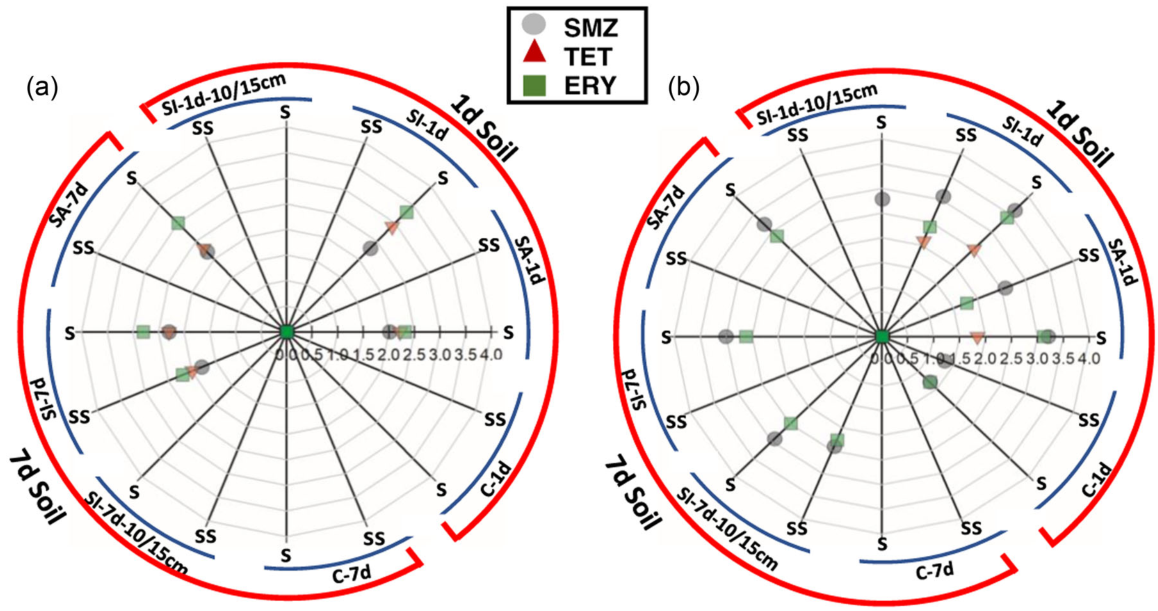

FIGURE 3 Average concentrations ($\log_{10}$ colony-forming units g^{-1} wet soil) of culturable fecal coliform bacteria resistant to sulfamethazine (SMZ), tetracycline (TET), and erythromycin (ERY) in soils where manure was surface applied or subsurface injected in (a) spring 2018 and (b) fall 2019 at the Virginia site. The soil samples were collected one hour before simulated rainfall on the first (1d) and seventh (7d) day after manure application. SI, SA, and C represent subsurface injection slit, surface application, and control without manure application, respectively; S and SS represent the 0-to-5- and 5-to-15-cm soil depths, respectively; 10 cm/15cm represents combined horizontal sampling distances of 10 and 15 cm from an injection slit

rainfall timing in spring compared with the fall experiment. Moreover, the surface-application plots had the highest ratio of ARFCB from applied manure leaving plots via surface runoff for both seasons (i.e., compared with subsurface injection). A soil column study to simulate fall and spring manure applications found that increased application rate of manure caused bacterial densities in leachate that were often significantly higher in the spring treatments compared with fall treatments (Warnemuende & Kanwar, 2002). Another study found that, generally, there was greater bacterial leaching from spring manure applications vs. fall manure applications (Stoddard et al., 1998). Fecal coliform mortality was significantly affected by the season. The greater percentage of ARFCB in runoff from spring compared with fall in our study is most likely due to the survival rate of the ARFCB being improved due to the higher soil moisture in spring. The increased time gap between manure application and subsequent rainfall was again seen as a good practice in both seasons but was more effective in the fall. The manure subsurface-injection method is beneficial in both seasons as well but is more effective in the spring.

3.2 | Spatial and temporal distribution of ARFCB in manure-applied soil in Virginia

For the spring 2018 experiment data (Figure 3a; Supplemental Tables S6 and S7), the background ARFCB concentrations were below the detection limit of the instrument. Soil samples collected from the 0-to-5-cm depth had detected ARFCB concentrations when taken from the surface-applied plots, as well as when taken from injection slits in subsurface-injected plots (Figure 3a). In contrast, samples taken at the same soil depth but at 10–15 cm from the injection slit before rainfall did not have detectable ARFCB concentrations, indicating negligible movement of ARFCB away from the injection slit prior to simulated rainfall. We also observed that the ARFCB remained above background concentrations in the 0-to-5-cm soil depth of surface-applied plots up to 7 d after manure application (Supplemental Table S6) but were not significantly different than background in the 5-to-15-cm depth samples (Supplemental Table S7). These results indicated that, even after 7 d, there was still potential for ARFCB transport via surface runoff in areas where manure was surface applied (Figure 3a).

The data showed no downward movement of ARFCB outside of the injection slit and no horizontal movement of these bacteria outside of the injection slit (Figure 4a,b). Elevated target ARFCB concentrations in manure soil surface–applied plots had returned to the background concentration by Day 14 after manure application and rainfall (Figure 4a; Supplemental Tables S6 and S7). On Day 14 after manure application and rainfall, target ARFCB in the 0-to-5-cm soil layer were above the background concentration at 5–10 cm away from the injection slit (Figure 4a), but by Day 45 they had returned to the

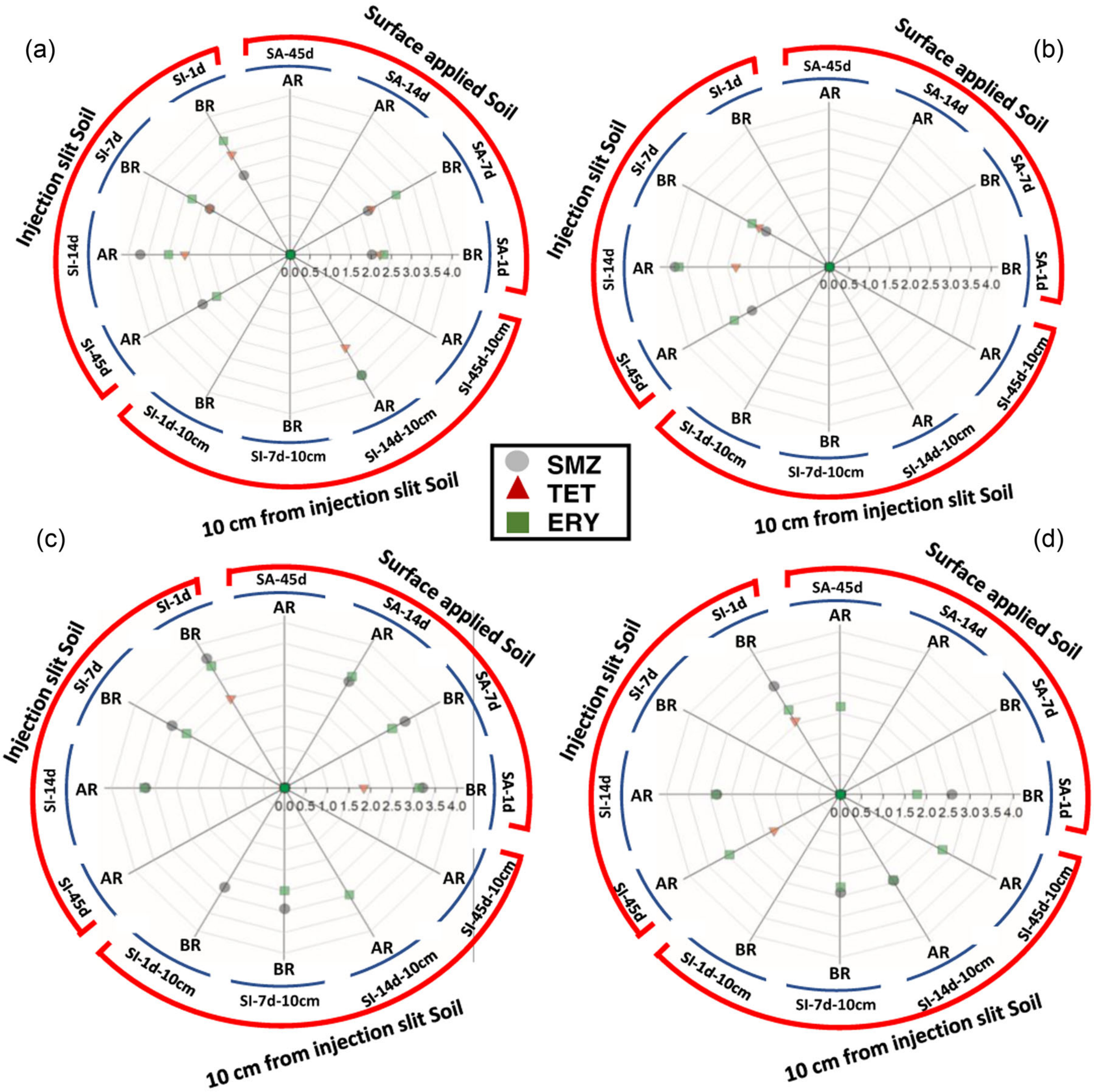

FIGURE 4 Average concentration ($\log_{10}$ colony-forming units g^{-1} wet soil) of culturable fecal coliform bacteria resistant to sulfamethazine (SMZ), tetracycline (TET), and erythromycin (ERY) where manure was surface applied or subsurface injected in the Virginia site. The soil samples were collected 1 h before simulated rainfall on the first (1d) and seventh (7d) day after manure application, as well as 14 d (14d) and 45 d (45d) after the simulated rainfall. (a) Spring 2018, 0-to-5-cm soil depth. (b) Spring 2018, 5-to-15-cm soil depth. (c) Fall 2019, 0-to-5-cm soil depth. (d) Fall 2019, 5-to-15-cm soil depth. SI, SA, and BG represent subsurface injection slit, surface application, and control without manure application, respectively; BR represents samples collected before simulated rainfall and AR represents samples collected after simulated rainfall; −10cm represents sampling locations that were 10 cm from an injection slit

background concentration. This indicates limited movement of ARFCB outside of the injection slit even after simulated rainfall; they were localized mostly to the injection slit (Supplemental Tables S6 and S7). This shows injected manure-associated ARFCB of concern are not transported as far over the soil surface as when manure is surface applied. The target ARFCB found within the injection slit persisted up to 45 d after manure application and simulated rainfall.

When the experiment was repeated in Virginia in fall 2019, ARFCB were more widely detected throughout the soil profile. First, the background concentration of ∼1.3 $\log_{10}$ CFU g^{-1} wet soil was not below detection. In the 0-to-5-cm soil depth, the soil samples from the manure injection slits and surface-applied plots also had ARFCB concentrations that were higher than the background (Figure 3b; Supplemental Tables S8 and S9) prior to rainfall. This time, there were slightly elevated concentrations of ARFCB away from the injection slit (Figure 3b). At 7 d after manure application on the surface-applied plots, there were still elevated ARFCB concentrations, which could be susceptible to surface runoff.

This trend was seen with the spring study also, revealing that 7 d may not be enough time for ARFCB in manure-applied soil to return to pre-application concentrations.

The injection slit and the "hotzone" observed in spring were not as clear in the fall experiment. In the fall, ARFCB persisted up to Day 14 in the injection slit in the 0-to-5-cm soil depth; however, by Day 45, ARFCB concentrations were below detection. This could be attributed to the overall lower soil moisture observed during this study, making less favorable conditions in the injection slit at that depth. However, ARFCB also persisted in the injection slit of the 5-to-15-cm soil depth up to 45 d (Figure 4c,d), as seen with the spring study. When comparing movement of the ARFCB outside of the injection slit, there was increased movement up to Day 14 in the 0-to-5-cm soil layer, but by Day 45 ARFCB had returned to the background concentration. As mentioned previously, for the 5-to-15-cm soil layer, elevated ARFCB concentrations were seen for the 5-to-10-cm distance outside of the injection slit tested. This is different from the spring experiment, when very limited horizontal and vertical movement of ARFCB outside of the injection slits was observed. For the surface-applied plots, in the VA spring experiment by Day 14, the ARFCB had returned to the background concentration. In the VA fall experiment, surface-applied plot ARFCB concentrations only returned to the background concentration in the 0-to-5-cm-soil layer by Day 45. For the 5-to-15-cm soil layer, elevated concentrations of ARFCB were mostly seen for ERY-resistant FCB (Supplemental Tables S8 and S9).

The environment created by the injection slit (moisture, nutrients, presence of antibiotics, potential transfer of antibiotic resistance genes, and presence of antibiotic resistant bacteria) induced a "hotzone" where these bacteria were able to persist. Although the injection method is beneficial for reducing the ARFCB entering runoff, it also appears to slow down degradation processes for ARFCB compared with surface applications. This phenomenon could encourage greater proliferation of antibiotic resistance in the environment (Berkner et al., 2014), which future work could consider in more detail. The injection method helps with protecting surface water from elevated ARFCB; however, the surface-applied method allows for quicker degradation of the ARFCB on the surface of soil. Stocker et al. (2015) found that concentrations of *E. coli* decreased with increasing soil depth in their rainfall study. In their study, *E. coli* was found to persist in soil layers up to 10 cm deep, and most of bacteria stayed in the top 1-cm soil depth after rainfall/runoff events (Stocker et al., 2015). In a study comparing the effect of manure fertilization and inorganic fertilization on ARB, soil populations of culturable ARB remained higher in the manure-treated soil for up to 94 d (Udikovic-Kolic et al., 2014). As seen with our study and others (Bolster et al., 2018; Ogden et al., 2001), after simulated rainfall, the majority of ARFCB remained in the uppermost layer of soil tested. These findings, combined with those of our current study, suggest that many ARFCB will only enter the subsurface when introduced via a process such as subsurface injection.

The spring data suggest limited transport; however, the fall data show that ARFCB can move if the conditions are right. This increased movement of ARFCB downward and laterally could be due to water movement (within the manure added) from high-potential to low-potential areas, carrying the ARFCB with it. It could also be attributed to the starting levels of ARFCB in manure applied. Fall manure had less TET-resistant FCB, which could explain the lower counts seen in soil. Conversely, the fall manure had higher concentrations of SMZ-resistant FCB, which could explain the increased transport. A drier soil profile would encourage more seepage of liquid manure into lower soil profiles after application as well, potentially explaining why the 5-to-15-cm layer had more instances of concentrations above the background in the fall study. Also of note is that in both seasons, even after a week of exposure, there were elevated concentrations of ARFCB in the 0-to-5-cm layer of the soil. Therefore, although increasing the time gap between surface applications and expected rainfall can be encouraged when manure injection is not possible, manure injection remains the best scenario whenever it is feasible.

4 | CONCLUSIONS

Results from this study show that subsurface injection of manure significantly reduced surface runoff of culturable ARFCB.

The resounding outcome was that subsurface injection is highly effective for reducing transport of ARFCB via surface runoff. Data collected showed that there was limited movement in spring applications; by Day 14, any ARFCB outside of the injection slit had returned to background concentrations. However, fall data show that the ARFCB can move if conditions are right. One unexpected finding was the ability of the ARFCB to persist in the injection slit for up to 45 d after simulated rainfall. This result was seen more in the spring application but was evident in the fall application as well, which could be a concern warranting more study.

Our data showed that increasing the time gap between manure application and subsequent rainfall was most beneficial when manure was surface applied in the spring. However, overall ARFCB counts in surface runoff were much lower when manure was applied in the fall. Altogether, these study results are particularly important for areas where water quality is affected by agricultural runoff and fecal pathogens are of concern. Ideally, manure injection should be encouraged along with no application before expected rainfall.

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Sheldon Shervon Hilaire: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Software; Visualization; Writing-original draft; Writing-review & editing. Chaoqi Chen: Data curation; Formal analysis; Investigation; Software; Visualization; Writing-review & editing. Jesse Radolinski: Investigation; Project administration; Writing-review & editing. Talia Leventhal: Conceptualization; Data curation; Investigation; Methodology; Project administration; Software; Visualization. Heather Preisendanz: Conceptualization; Data curation; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. Peter J. A. Kleinman: Conceptualization, Funding acquisition, Validation, Writing-review & editing. Rory Maguire: Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. Ryan D. Stewart: Project administration; Resources; Supervision; Validation; Writing-review & editing. Lou S. Saporito: Investigation; Methodology; Project administration; Writing-review & editing. Kang Xia: Conceptualization; Data curation; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest and the sponsors had no role in the design, execution, interpretation, or writing of the study.

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REFERENCES

Aarestrup, F. M., & Carstensen, B. (1998). Effect of tylosin used as a growth promoter on the occurrence of macrolide-resistant Enterococci and Staphylococci in pigs. *Microbial Drug Resistance*, *4*(4), 307–312. https://doi.org/10.1089/mdr.1998.4.307

Adeli, A., Tewolde, H., Shankle, M. W., Way, T. R., Brooks, J. P., & Mclaughlin, M. R. (2013). Runoff quality from no-till cotton fertilized with broiler litter in subsurface bands. *Journal of Environment Quality*, *42*, 284–291. https://doi.org/10.2134/jeq2012.0358

Bagdasaryan, G. A. (1964). Survival of viruses of the Enterovirus group (Poliomyelitid, Echo). *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, *8*, 497–505.

Berkner, S., Konradi, S., & Schönfeld, J. (2014). Antibiotic resistance and the enviornment: There and back again. *EMBO Reports*, *15*, 740–744. https://doi.org/10.15252/embr.201438978

Blaustein, R. A., Pachepsky, Y. A., Hill, R. L., & Shelton, D. R. (2015). Solid manure as a source of fecal indicator microorganisms: Release under simulated rainfall. *Environmental Science and Technology*, *49*, 7860–7869. https://doi.org/10.1021/acs.est.5b01095

Bolster, C. H., & Abit, S. M. (2012). Biochar pyrolyzed at two temperatures affects *Escherichia coli* transport through a sandy soil. *Journal of Environmental Quality*, *41*, 124–133. https://doi.org/10.2134/jeq2011.0207

Bolster, C. H., Brooks, J. P., & Cook, K. L. (2018). Effect of Manure application rate and rainfall timing on the leaching of antibiotic-resistant bacteria and their associated genes. *Water, Air, and Soil Pollution*, *229*, 130. https://doi.org/10.1007/s11270-018-3781-6

Bottom, J. D., Taraba, J. L., & Ross, I. J. (1986). Infiltration rate reduction on dairy manured plots. *American Society of Agricultural Engineers*, *86*, 4057.

Brooks, J. P., Adeli, A., Read, J. J., & Mclaughlin, M. R. (2009). Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. *Journal of Environment Quality*, *38*, 218–229. https://doi.org/10.2134/jeq2008.0029

Cameron, A., & Mcallister, T. A. (2016). Antimicrobial usage and resistance in beef production. *Journal of Animal Science and Biotechnology*, *7*, 68. https://doi.org/10.1186/s40104-016-0127-3

CDC. (2019). *Biggest threats and data: 2019 AR threats report*. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., & Hanage, W. P. (2015). Antibiotics in agriculture and the risk to human health: How worried should we be? *Evolutionary Applications*, *8*, 240–247. https://doi.org/10.1111/eva.12185

Cook, K. L., Netthisinghe, A. M. P., & Gilfillen, R. A. (2014). Detection of pathogens, indicators, and antibiotic resistance genes after land application of poultry litter. *Journal of Environmental Quality*, *43*, 1546–1558. https://doi.org/10.2134/jeq2013.10.0432

Crowther, J., Kay, D., & Wyer, M. D. (2002). Faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: Relationships with land use and farming practices. *Water Research*, *36*, 1725–1734. https://doi.org/10.1016/S0043-1354(01)00394-3

Desmarais, T. R., Solo-Gabriele, H. M., & Palmer, C. J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and Environmental Microbiology*, *68*, 1165–1172. https://doi.org/10.1128/AEM.68.3.1165-1172.2002

Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, *8*, 881–890. https://doi.org/10.3201/eid0809.020063

Edwards, D. R., Larson, B. T., & Lim, T. T. (2001). Runoff nutrient and fecal coliform content from cattle manure application to fescue plots. *Journal of the American Water Resources Association*, *36*, 711–721. https://doi.org/10.1111/j.1752-1688.2000.tb04300.x

Eghball, B., & Power, J. F. (1999). Phosphorus- and nitrogen-based manure and compost applications. *Soil Science Society of America Journal*, *63*, 895–901. https://doi.org/10.2136/sssaj1999.634895x

EUCAST. (2016). *MIC and inhibition zone diameter distributions of microorganisms without and with resistance mechanisms*. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf

Geldreich, E. E., & Bordner, R. H. (1971). Fecal contamination of fruits and vegetables during cultivation and processing for market: A review. *Journal of Milk and Food Technology*, *34*, 184–195. https://doi.org/10.4315/0022-2747-34.4.184

Grunwald, S., Corstanje, R., Weinrich, B. E., & Reddy, K. R. (2005). Sources of pathogenic microorganisms and their fate during land application of wastes. *Journal of Environmental Quality*, *35*, 378–389. https://doi.org/10.2134/jeq2005.0042

Gottschall, N., Edwards, M., Topp, E., Bolton, P., Payne, M., Curnoe, W. E., Coelho, B. B., & Lapen, D. R. (2009). Nitrogen, phosphorus, and bacteria tile and groundwater quality following direct injection of dewatered municipal biosolids into soil. *Journal of Environment Quality*, *38*, 1066–1075. https://doi.org/10.2134/jeq2008.0085

Heuer, H., Schmitt, H., & Smalla, K. (2011). Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, *14*(3), 236–243. https://doi.org/10.1016/j.mib.2011.04.009

Hilaire, S. S., Bellows, B., Brady, J. A., & Muir, J. P. (2020). Oxytetracycline and monensin uptake by Tifton 85 Bermudagrass from dairy manure-applied soil. *Agronomy*, *10*, 468. https://doi.org/10.3390/agronomy10040468

Khaleel, R., Reddy, K. R., & Overcash, M. R. (1981). Changes in soil physical properties due to organic waste applications: A review. *Journal of Environmental Quality*, *10*, 133–141. https://doi.org/10.2134/jeq1981.00472425001000020002x

Kibet, L. C., Allen, A. L., Kleinman, P. J. A., Feyereisen, G. W., Church, C., Saporito, L. S., & Way, T. R. (2011). Phosphorus runoff losses from subsurface-applied poultry litter on coastal plain soils. *Journal of Environment Quality*, *40*, 412–420. https://doi.org/10.2134/jeq2010.0161

Kulesza, S. B., Maguire, R. O., Xia, K., Cushman, J., Knowlton, K., & Ray, P. (2016). Manure injection affects the fate of pirlimycin in surface runoff and soil. *Journal of Environmental Quality*, *45*(2), 511–518. https://doi.org/10.2134/jeq2015.06.0266

Kumar, K. C., Gupta, S., Chander, Y., & Singh, A. K. (2005). Antibiotic use in agriculture and its impact on the terrestrial environment. *Advances in Agronomy*, *87*, 1–54. https://doi.org/10.1016/S0065-2113(05)87001-4

Le, H. T. V., Maguire, R. O., & Xia, K. (2018). Method of dairy manure application and time before rainfall affect antibiotics in surface runoff. *Journal of Environment Quality*, *47*(6), 1310–1317. https://doi.org/10.2134/jeq2018.02.0086

Liu, J., Zhao, Z., Orfe, L., Subbiah, M., & Call, D. R. (2016). Soil-borne reservoirs of antibiotic-resistant bacteria are established following therapeutic treatment of dairy calves. *Environmental Microbiology*, *18*, 557–564. https://doi.org/10.1111/1462-2920.13097

López-Garrido, R., Deurer, M., Madejón, E., Murillo, J. M., & Moreno, F. (2012). Tillage influence on biophysical soil properties: The example of a long-term tillage experiment under Mediterranean rainfed conditions in South Spain. *Soil and Tillage Research*, *118*, 52–60. https://doi.org/10.1016/j.still.2011.10.013

MacDonald, J., Ribaudo, M., Livingston, M., Beckman, J., & Huang, W. (2010). Manure use for fertilizer and for energy: Report to Congress. In *Manure use for fertilizer and energy* (p. 7). USDA Economic Research Service.

Maguire, R. O., Kleinman, P. J. A., & Beegle, D. B. (2011). Novel manure management technologies in no-till and forage systems: Introduction to the special series. *Journal of Environmental Quality*, *40*(2), 287–291. https://doi.org/10.2134/jeq2010.0396

Maguire, R. O., Kleinman, P. J. A., Dell, C. J., Beegle, D. B., Brandt, R. C., Mcgrath, J. M., & Ketterings, Q. M. (2011). Manure application technology in reduced tillage and forage systems: A review. *Journal of Environmental Quality*, *40*, 292–301. https://doi.org/10.2134/jeq2009.0228

Marti, R., Scott, A., Tien, Y.-C., Murray, R., Sabourin, L., Zhang, Y., & Topp, E. (2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at Harvest. *Applied and Environmental Microbiology*, *79*(18), 5701–5709. https://doi.org/10.1128/aem.01682-13

Martin, M. J., Thottathil, S. E., & Newman, T. B. (2015). Antibiotics overuse in animal agriculture: A call to action for health care providers. *American Journal of Public Health*, *105*(12), 2409–2410. https://doi.org/10.2105/AJPH.2015.302870

Mills, A. L., Herman, J. S., Hornberger, G. M., & Dejesús, T. H. (1994). Effect of solution ionic strength and iron coatings on mineral grains on the sorption of bacterial cells to quartz sand. *Applied and Environmental Microbiology*, *60*, 3300–3306. https://doi.org/10.1128/aem.60.9.3300-3306.1994

Mina, O., Gall, H. E., Saporito, L. S., & Kleinman, P. J. A. (2016). Estrogen transport in surface runoff from agricultural fields treated with two application methods of dairy manure. *Journal of Environmental Quality*, *45*, 2007–2015. https://doi.org/10.2134/jeq2016.05.0173

Ogden, I. (2001). The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. *International Journal of Food Microbiology*, *66*(1-2), 111–117. https://doi.org/10.1016/S0168-1605(00)00508-0

Pote, D. H., Reed, B. A., Daniel, T. C., Nichols, D. J., Moore, J., Edwards, D. R., & Formica, S. (2001). Water-quality effects of infiltration rate and manure application rate for soils receiving swine manure. *Journal of Soil and Water Conservation*, *56*, 32–37.

SAS Institute. (2019). *JMP® 15 basic analysis*. SAS Institute Inc.

Shallcross, L. J., & Davies, D. S. C. (2014). Editorials: Antibiotic overuse: A key driver of antimicrobial resistance. *British Journal of General Practice*, *64*, 604–605. https://doi.org/10.3399/bjgp14X682561

Sistani, K. R., Torbert, H. A., Way, T. R., Bolster, C. H., Pote, D. H., & Warren, J. G. (2009). Broiler litter application method and runoff timing effects on nutrient and *Escherichia coli* losses from tall fescue pasture. *Journal of Environmental Quality*, *38*, 1216–1223. https://doi.org/10.2134/jeq2008.0185

Soupir, M. L., Mostaghimi, S., Yagow, E. R., Hagedorn, C., & Vaughan, D. H. (2006). Transport of fecal bacteria from poultry litter and cattle manures applied to pastureland. *Water, Air, and Soil Pollution*, *169*, 125–136. https://doi.org/10.1007/s11270-006-1808-x

Stocker, M. D., Pachepsky, Y. A., Hill, R. L., & Shelton, D. R. (2015). Depth-dependent survival of *Escherichia coli* and Enterococci in soil after manure application and simulated rainfall. *Applied and Environmental Microbiology*, *81*, 4801–4808. https://doi.org/10.1128/AEM.00705-15

Stoddard, C. S., Coyne, M. S., & Grove, J. H. (1998). Fecal bacteria survival and infiltration through a shallow agricultural soil: Timing and tillage effects. *Journal of Environmental Quality*, *27*, 1516–1523. https://doi.org/10.2134/jeq1998.00472425002700060031x

Thurston-Enriquez, J. A., Gilley, J. E., & Eghball, B. (2005). Microbial quality of runoff following land application of cattle manure and swine slurry. *Journal of Water and Health*, *3*, 157–171. https://doi.org/10.2166/wh.2005.0015

Tian, Y. Q., Gong, P., Radke, J. D., & Scarborough, J. (2002). Spatial and temporal modeling of microbial contaminants on grazing farmlands. *Journal of Environment Quality*, *31*, 860–860. https://doi.org/10.2134/jeq2002.8600

Udikovic-Kolic, N., Wichmann, F., Broderick, N. A., & Handelsman, J. (2014). Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 15202–15207. https://doi.org/10.1073/pnas.1409836111

USDA-NRCS. (2021). *Web soil survey*. https://websoilsurvey.nrcs.usda.gov/

Van Donsel, D. J., Geldreich, E. E., & Clarke, N. A. (1967). Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. *Applied Microbiology*, *15*, 1362–1370. https://doi.org/10.1128/aem.15.6.1362-1370.1967

Van Vliet, L. J. P., Bittman, S., Derksen, G., & Kowalenko, C. G. (2006). aerating grassland before manure application reduces runoff nutrient loads in a high rainfall environment. *Journal of Environmental Quality*, *35*, 903–911. https://doi.org/10.2134/jeq2005.0266

Wallace, C. B., Burton, M. G., Hefner, S. G., & Dewitt, T. A. (2013). Effect of preceding rainfall on sediment, nutrients, and bacteria in runoff from biosolids and mineral fertilizer applied to a hayfield in a mountainous region. *Agricultural Water Management*, *130*, 113–118. https://doi.org/10.1016/j.agwat.2013.08.006

Warnemuende, E. A., & Kanwar, R. S. (2002). Effects of swine manure application on bacterial quality of leachate from intact soil columns. *Transactions of the American Society of Agricultural Engineers*, *45*, 1849–1857. https://doi.org/10.13031/2013.11436

West, T. O., & Marland, G. (2002). A synthesis of carbon sequestration, carbon emissions, and net carbon flux in agriculture: Comparing tillage practices in the United States. *Agriculture, Ecosystems and Environment*, *91*(1-3), 217–232. https://doi.org/10.1016/S0167-8809(01)00233-X

WHO. (2014). *The control of neglected zoonotic diseases*. https://www.who.int/neglected_diseases/ISBN9789241508568_ok.pdf

Williams, R. K. (2016). *Effect of composting on the prevalence of antibiotic resistant bacteria and resistance genes in cattle manure* [Master's thesis, Virginia Tech]. https://vtechworks.lib.vt.edu/handle/10919/74952

Wind, L., Krometis, L.-A., Hession, W. C., Chen, C., Du, P., Jacobs, K., Xia, K., & Pruden, A. (2018). Fate of pirlimycin and antibiotic-resistant fecal coliforms in field plots amended with dairy manure or compost during vegetable cultivation. *Journal of Environmental Quality*, *47*(3), 436–444. https://doi.org/10.2134/jeq2017.12.0491

Yang, Q., Wang, R., Ren, S., Szoboszlay, M., & Moe, L. A. (2016). Practical survey on antibiotic-resistant bacterial communities in livestock manure and manure-amended soil. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, *51*, 14–23. https://doi.org/10.1080/03601234.2015.1080481

Zhang, H., & Schroder, J. (2014). Animal manure production and utilization in the US. In *Applied manure and nutrient chemistry for sustainable agriculture and environment* (pp. 1–21). Springer. https://doi.org/10.1007/978-94-017-8807-6_1

Zhao, W., Liu, X., Huang, Q., Walker, S. L., & Cai, P. (2012). Interactions of pathogens *Escherichia coli* and *Streptococcus suis* with clay minerals. *Applied Clay Science*, *69*, 37–42. https://doi.org/10.1016/j.clay.2012.07.003

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