2013

# Fate and Transport of Antimicrobials and Antimicrobial Resistance Genes in Soil and Runoff Following Land Application of Swine Manure Slurry

Stacy R. Joy
*University of Nebraska-Lincoln*

Shannon L. Bartelt-Hunt
*University of Nebraska-Lincoln*, sbartelt2@unl.edu

Daniel D. Snow
*University of Nebraska-Lincoln*, dsnow1@unl.edu

John Gilley
*University of Nebraska - Lincoln*, john.gilley@ars.usda.gov

Brian L. Woodbury
*USDA MARC, Clay Center NE*, bryan.woodbury@ars.usda.gov

See next page for additional authors

Environ. Sci. Technol., 2013, 47 (21), pp 12081–12088
DOI: 10.1021/es4026358

Article

pubs.acs.org/est

# Fate and Transport of Antimicrobials and Antimicrobial Resistance Genes in Soil and Runoff Following Land Application of Swine Manure Slurry

Stacey R. Joy,[†] Shannon L. Bartelt-Hunt,[†] Daniel D. Snow,[‡] John E. Gilley,[§] Bryan L. Woodbury,[∥] David B. Parker,[∥,#] David B. Marx,[⊥] and Xu Li*[,†]

[†]Department of Civil Engineering, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States

[‡]School of Natural Resources, Water Sciences Laboratory, University of Nebraska-Lincoln, Lincoln, Nebraska 68503, United States

[§]USDA-ARS, Lincoln, Nebraska 68503, United States

[∥]USDA Meat Animal Research Center, Clay Center, Nebraska 68933, United States

[⊥]Department of Statistics, University of Nebraska-Lincoln, Lincoln, Nebraska 68503, United States

Supporting Information

**ABSTRACT:** Due to the use of antimicrobials in livestock production, residual antimicrobials and antimicrobial resistance genes (ARGs) could enter the environment following the land application of animal wastes and could further contaminate surface and groundwater. The objective of this study was to determine the effect of various manure land application methods on the fate and transport of antimicrobials and ARGs in soil and runoff following land application of swine manure slurry. Swine manure slurries were obtained from facilities housing pigs that were fed chlortetracyline, tylosin or bacitracin and were land applied via broadcast, incorporation, and injection methods. Three rainfall simulation tests were then performed on amended and control plots. Results show that land application methods had no statistically significant effect on the aqueous concentrations of antimicrobials in runoff. However, among the three application methods tested broadcast resulted in the highest total mass loading of antimicrobials in runoff from the three rainfall simulation tests. The aqueous concentrations of chlortetracyline and tylosin in runoff decreased in consecutive rainfall events, although the trend was only statistically significant for tylosin. For ARGs, broadcast resulted in significantly higher *erm* genes in runoff than did incorporation and injection methods. In soil, the effects of land application methods on the fate of antimicrobials in top soil were compound specific. No clear trend was observed in the ARG levels in soil, likely because different host cells may respond differently to the soil environments created by various land application methods.

## INTRODUCTION

Livestock wastes generated from confined animal feeding operations (CAFOs) represent a major source of antimicrobials and antimicrobial resistance genes (ARGs) in the environment.[1] Antimicrobials are often administered to livestock in CAFOs for growth promotion, prophylaxis, and disease treatment. However, a substantial portion of the antimicrobials administered to livestock are not absorbed by the animals.[2] Antimicrobial residues can cause the emergence of antimicrobial resistant bacteria in an animal's gastrointestinal tract and in the environment after land application of manure.[3,4]

There have been limited studies investigating the fate and transport of antimicrobials in soil and in runoff following land application of manure. One study detected no statistically significant differences in antimicrobial concentrations (chlortetracyline, monensin, and tylosin) in infiltration water and surface runoff when manure was applied using two land application methods (i.e., chisel plowing vs no-tillage).[5] In contrast, other studies suggest that soil tillage lead to reduced vertical transport of antimicrobials after broadcast application of liquid manure,[6] and incorporation could lead to reduced antimicrobial concentrations in runoff.[7] Once animal manure is land applied, the fate of manure-originated antimicrobials in soil and subsequent transport in runoff will also be affected by the compounds' sorption properties to soil particles[8−10] and susceptibility to biotic and abiotic degradation (e.g., photodegradation).[11−13] To date, there have been few studies that systematically evaluate multiple land application methods with respect to their effects on the fate and transport of different classes of antimicrobials in soil and runoff following land application of manure.

Received: June 13, 2013
Revised: September 8, 2013
Accepted: September 17, 2013
Published: September 17, 2013

Conflicting data exist in the literature on whether ARGs originating in livestock manure can persist in soil following land application of manure. Using sulfonamide resistance as an example, one study reported that the relative abundance of *sul* genes dropped by 1 order of magnitude over a 165 day period following manure incorporation into soil,[14] while another study reported increased antimicrobial resistance in soil 289 days after land application of manure.[15] In addition, antimicrobial resistant bacteria from manure may be transported to surface water via runoff. One study determined that heterotrophic plate count bacteria in runoff from soil amended with poultry litter decreased steadily with each of five successive rainfall events and some enterococci isolates demonstrated resistance to selected antimicrobials.[16] Unfortunately, this study only assessed culturable bacteria and included only one litter application method. Hence, there is a critical need to quantify ARGs, which allow for evaluation of the antimicrobial resistant populations in both culturable and nonculturable bacteria, in soil and runoff as a function of land application methods.

An understanding of the behavior of antimicrobials and ARGs in the environment is important in the development of manure management practices to control the proliferation of these contaminants from animal agriculture. The objective of this study was to determine the effect of various manure land application methods on the fate and transport of antimicrobials and ARGs in soil and runoff following land application of swine manure slurry. Two hypotheses were established for this study: (1) the extent of mixing of manure and soil during land application will determine the transport of antimicrobials and ARGs in runoff, and (2) the effect of land application methods on the fate of antimicrobials and ARGs in soil will be compound and gene specific. Three land application methods, broadcast, injection, and incorporation, were employed in this study, and rainfall events were simulated once a day for three consecutive days. Chlortetracycline (CTC), tylosin (TYL), and bacitracin (BAC), as well as their corresponding ARGs, were quantified in the soil and runoff from triplicate field plots for each experimental condition.

## MATERIALS AND METHODS

**Manure Generation and Collection.** Three groups of pigs housed in separate barns at the USDA Meat Animal Research Center (MARC) near Clay Center, NE were each fed a single antimicrobial. All hogs were fed a corn and soybean-based diet. Grower pigs received 66.2 mg CTC/kg ration, sows and gilts 75.0 mg TYL/kg ration and finisher pigs 39.7 mg BAC/kg ration. Manure from the finisher pigs was collected in pits under a slotted pen and was drained once a week using a pull-plug system. After draining, the plug was replaced and fresh well water was added to refill the pit to approximately a 0.5 m depth. Slurry was collected from this facility by removing a grate and dipping a plastic bucket to collect manure. Manure from grower pigs and manure from sows and gilts were pushed through slots in the pen floor and was collected in channels under the pen. Typically, 2000 L of well water was discharged every hour through the trough to flush the manure slurry to a treatment lagoon system. Slurries from each of these two facilities were collected at a point downstream where the two channels for each side of the building converged. To ensure sufficient solids content of the slurry, the flush system was turned off overnight to allow solids to accumulate in the trough systems. Slurry was collected in early morning using plastic buckets at the beginning of the first flush. Each week, swine slurries (defined as CTC-, TYL-, or BAC-manure) were collected at MARC and transported in 20 L plastic buckets to the land application site at the UNL Roger's Memorial Farm near Lincoln, NE. A subsample of the swine slurry was collected for solid and nutrient analyses at Ward Laboratories (Kearney, NE), and the results have been reported in a companion paper.[17] Another subsample was collected in 250 mL amber jars and transported in a cooler to UNL for antimicrobial and ARG quantification.

**Rainfall Simulation Experiments.** Experiments were designed to test three experimental treatments: land application methods (i.e., broadcast, incorporation, and injection), manure amendment (i.e., manure amended plots and control plots), and rainfall events (i.e., rainfall simulation test 1, 2, and 3). Test plots (0.75 × 2.0 m) were constructed using 20 cm-wide sheet metal frames driven approximately 10 cm into the soil. Swine slurry was weighed at the field site and applied on amended plots to meet the 1-yr N requirement for corn (i.e., 151 kg N $ha^{-1}$ $yr^{-1}$ for an expected yield of 9.4 Mg $ha^{-1}$, assuming that 70% of the total N in manure slurry is available to crops). For amended plots receiving manure (i.e., CTC-, TYL-, or BAC-manure) and control plots receiving no manure, each land application method was employed on three replicate plots. Over 5 weeks, a total of thirty six plots were established across the slope (5.8%) using a randomized block design (Supporting Information (SI) Figure S2). For broadcast plots, the slurry was slowly poured onto the soil surface and care was taken to ensure uniform distribution. For incorporation plots, a single pass with a tandem disk was used to mix the manure slurry into the soil to a depth of approximately 8 cm. For injection plots, four 13-cm deep trenches for slurry injection were established, 51-cm apart, across plots in a direction perpendicular to overland flow.

A portable rainfall simulator was designed according to a published study.[18] Rainfall simulation procedures were adopted from the National Phosphorus Research Project.[19] The first rainfall simulation event was conducted 24 h after land application of swine slurry, and two additional rainfall simulation runs were conducted at approximately 24 h intervals. In each rainfall event, the simulator applied rainfall for a 30 min duration at an intensity of 70 mm $hr^{-1}$.

Plot borders channeled runoff into a sheet metal lip that emptied into a collection trough located across the bottom of each plot. A galvanized steel trough diverted runoff into plastic buckets set below the soil surface. A sump pump was used to transfer runoff from the buckets into larger plastic storage containers, which were weighed at the completion of each simulated rainfall event to determine total runoff mass. Runoff samples were obtained from the storage containers immediately after agitation, and were collected in 1 L amber glass bottles with Teflon lined lids within minutes following the completion of the rainfall simulation tests. Soil cores were collected using acrylic tubes from the amended and control plots receiving broadcast and incorporation treatment before the first and after the third rainfall simulation. No soil samples were collected from injection plots because the methodology of the injection process did not yield a homogeneous soil surface. Runoff and soil samples were transported in a cooler promptly to UNL where they were stored at −20 °C until analysis.

**Antimicrobial Analysis of Runoff, Soil, and Manure Samples.** Sources and properties of the chemicals used in this study are provided in the text and Table S1 of the SI file. Within 24−48 h of collection, approximately 500 mL of runoff

Table 1. Antimicrobial and ARG Concentrations (Average ± Standard Error) In the Swine Manure Slurries Used in This Study[a]

| | antimicrobial[b] | | ARG | |
|---|---|---|---|---|
| manure slurry | (mg/kg ww) | (mg/kg dw) | (copy/mL) | |
| | CTC | | *tet*(Q) | *tet*(X) |
| CTC-manure | 3.3 ± 1.6 | 404 ± 138 | (2.5 ± 1.3) × $10^4$ | (1.3 ± 0.7) × $10^3$ |
| | TYL | | *erm*(B) | *erm*(F) |
| TYL-manure | 0.29 ± 0.12 | 32.5 ± 7.2 | (1.6 ± 1.1) × $10^4$ | (1.4 ± 0.5) × $10^2$ |
| | BAC | | *bcrA*, *bcrB*, *bcrC* | *bceA*, *bceR* |
| BAC-manure | 0.78 ± 0.75 | 320 ± 31.5 | ND[c] | ND |

[a]The averages and standard errors were calculated based on weekly fresh manure samples collected over the 5-week field experiment ($n$ = 5). [b]In addition to the primary antimicrobials, other antimicrobials were also detected in each manure type (SI Table S8). [c]ND, not detected.

water were vacuum filtered through a precombusted 0.5 μm Gellman A/E binderless glass fiber filter and then immediately through a preconditioned 200 mg Oasis HLB solid phase extaction (SPE) cartridge. SPE cartridges were stored at −20 °C prior to elution and analysis of extracts. SPE cartridges were eluted into borosilicate test tubes using 3 mL 0.1% formic acid in methanol, which also contained 16 ng oleandomycin as a surrogate to monitor analyte recovery. The solvent was reduced in volume to approximately 200 μL under a stream of dry nitrogen, and transferred to an autosampler vial with silane-treated insert and then mixed with 200 μL reagent water. Roxithromycin (internal standard for tylosin and bacitracin) and doxycycline (internal standard for chlortetracycline) were each added at 40 ng during the concentration step. Recovery of chlortetracycline, bacitracin (i.e., bacitracin A), and tylosin, was determined from extraction and analysis of fortified reagent water during the elution stage. Fortified blanks and method blanks were analyzed at a frequency of 1 in 20 samples (5%). Method detection limits were determined by extraction and analysis of eight replicates of reagent water samples fortified with antimicrobials at 0.005 μg/L. Recoveries determined using 0.004 μg/L fortified water were 137 ± 8% for chlortetracycline, 53 ± 7% for tylosin, and 28 ± 2% for bacitracin (i.e., bacitracin A). All samples were analyzed using electrospray ionization liquid chromatography-tandem mass spectrometry.[20−23]

Antimicrobials were extracted from solid samples (i.e., manure and soil) using solvent extraction followed by SPE cleanup. Well-mixed soil (10 g) or manure (0.2 g mixed with 5 g clean sand) samples were spiked with 16 ng surrogate, followed by addition of 14 mL of 5 mM ammonium citrate buffered to pH 6 using ammonium hydroxide and 6 mL methanol in 50 mL polypropylene centrifuge tubes. Mixtures were shaken briefly by hand and then on a Burrell Wrist-action shaker for 30 min. Solids and solvent were separated by centrifugation, with the supernatant decanted into a glass evaporation tube (RapidVap, Labconco Corporation). Solids were extracted a second time with 4 mL of ammonium citrate and 16 mL methanol, and a third time using 20 mL acetone. All extracts were combined and fortified with internal standards (doxycycline and roxithromycin, 40 ng each) and then concentrated on a Labconco RapidVap $N_2$ sample concentrator at 30 °C (90% rotation speed) until the volume was reduced in half. Purified reagent water was added to bring the volume to 100 mL and the resulting primarily aqueous solutions were extracted using 200 mg Oasis HLB SPE cartridges. Cartridges were then processed in a manner identical to the water samples using 130 mM ammonium citrate in methanol for the elution. Recoveries determined using 16 ng/g fortified soil (collected on site) were 57 ± 13% for chlortetracycline, 78 ± 6.5% for tylosin, and 12 ± 46% for bacitracin (i.e., bacitracin A).

All sample extracts were analyzed on a Waters 2695 high pressure liquid chromatograph (HPLC) interfaced with a Waters Quattro Micro triple quadrupole mass spectrometer.[21,24] Analytes were separated on a reverse phase (HyPurity C18, 250 × 2.1 mm, 5 μm particle size) column at 50 °C with a 50 μL injection volume. A gradient mobile phase (0.2 mL/min) was used consisting of A) 1 mM aqueous citric acid and methanol (97:3, v/v) and B) methanol and 1 mM aqueous citric acid (97:3, v/v). Initial gradient conditions (95% A) were held for 2 min, ramped to 5% A and held for 16 min, and then returned to 95% A for 5 min to equilibrate the column. Analytes were detected using multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI). The most intense MRM transitions were determined by infusion and monitored for each analyte (SI Table S2) and linear calibration curves were generated for all analytes and surrogates with $r^2$ values > 0.995. Antimicrobial mass loadings in runoff were calculated by multiplying the volume of runoff from each plot and the aqueous antimicrobial concentration in runoff.

**ARGs in Manure, Runoff, and Soil Samples.** For runoff samples, 500 mL of well-mixed sample was centrifuged for 5 min at 10 000$g$ at 4 °C in sterile 50 mL centrifuge tubes. Supernatants were decanted and pellets were stored at −20 °C until DNA extraction. Manure samples were handled in the same fashion, but only 30 mL of slurry was utilized. Soil cores (10−25 cm long) were extruded from plastic sleeves and separated into top, middle, and bottom sections. The top 5 cm and the bottom 5 cm of soil were separately homogenized and analyzed for ARGs.

DNA from manure, runoff solids, and soil was extracted using the MoBio UltraClean Soil DNA Isolation Kit (Solana Beach, CA) according to manual except that a 40-s bead beating was used to lyse the cells. DNA extracts were quantified using a NanoDrop spectrometer. qPCR conditions for tetracycline resistance genes *tet*(Q) and *tet*(X) and for tylosin resistance genes *erm*(B) *and erm*(F) were adopted from published studies.[25−27] Regular PCR or qPCR was also run on manure samples for bacitracin resistance genes *bceA* and *bceR*[28] as well as *bcrA*, *bcrB*, and *bcrC*.[29] The detection limit for each qPCR protocol was determined as the minimum concentration in the standard curve within the linear range. Key qPCR parameters and the linear range for each primer set can be found in SI Table S3. In addition to ARGs, the 16S rRNA gene in each sample was also quantified using qPCR.[30]

**Statistical Analysis.** Repeated measures analysis of variance (rANOVA) tests were conducted using SAS (Cary, NC) to determine the effects of three treatment methods, land application method (broadcast, injection, and incorporation), manure amendment (control and amended plots), and rainfall event (nos. 1, 2, and 3), on the concentrations of antimicrobials

**Table 2. rANOVA Tests on the Effects of Land Application Method, Manure Amendment, And Rainfall Event on the Concentrations of Antimicrobials and ARGs in Runoff**

| | CTC ($\mu$g/L) | TYL ($\mu$g/L) | *tet*(Q) (copy/mL runoff) | *tet*(X) (copy/mL runoff) | *erm*(B) (copy/mL runoff) | *erm*(F) (copy/mL runoff) |
|---|---|---|---|---|---|---|
| ***Application Method***[a,b] | | | | | | |
| broadcast | 0.009 | 0.125 | $4.5 \times 10^2$ | $1.3 \times 10^2$ | $4.2 \times 10^1$ a | $2.5 \times 10^1$ a |
| incorporation | 0.004 | 0.082 | $1.8 \times 10^2$ | $4.9 \times 10^1$ | $1.7 \times 10^1$ b | $9 \times 10^0$ b |
| injection | 0.005 | 0.034 | $1.3 \times 10^2$ | $3.2 \times 10^1$ | $8 \times 10^0$ b | $6 \times 10^0$ b |
| ***Manure Amendment*** | | | | | | |
| control plots | 0.003 a | 0.008 a | $5.8 \times 10^1$ a | $6 \times 10^0$ a | $5 \times 10^0$ a | $5 \times 10^0$ a |
| amended plots | 0.012 b | 0.650 b | $8.5 \times 10^2$ b | $5.6 \times 10^2$ b | $5.9 \times 10^1$ b | $2.2 \times 10^1$ b |
| ***Rainfall Event*** | | | | | | |
| 1 | 0.008 | 0.118 a | $3.7 \times 10^2$ a | $6.5 \times 10^1$ | $3.0 \times 10^1$ a | $1.5 \times 10^1$ |
| 2 | 0.006 | 0.080 ab | $2.1 \times 10^2$ ab | $6.8 \times 10^1$ | $1.6 \times 10^1$ b | $1.0 \times 10^1$ |
| 3 | 0.004 | 0.037 b | $1.4 \times 10^2$ b | $4.7 \times 10^1$ | $1.2 \times 10^1$ b | $8 \times 10^0$ |
| ***rANOVA values for***[c] | | | | | | |
| application method | 0.26 | 0.31 | 0.20 | 0.28 | 0.03 | 0.03 |
| manure amendment | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| rainfall event | 0.09 | 0.04 | 0.01 | 0.50 | 0.01 | 0.28 |
| application × amendment | 0.04 | 0.38 | 0.35 | 0.50 | 0.04 | 0.04 |
| application × rainfall | 0.39 | 0.95 | 0.45 | 0.74 | 0.77 | 0.90 |
| amendment × rainfall | 0.04 | 0.10 | 0.01 | 0.28 | 0.01 | 0.14 |
| application × amendment × rainfall | 0.09 | 0.14 | 0.03 | 0.09 | 0.19 | 0.50 |

[a]Values reported under "application method", "manure amendment", and "rainfall event" are treatment averages, which were calculated based on all the data for one particular treatment level. For example, 0.009 $\mu$g/L was calculated using CTC concentrations of all runoff samples from broadcasted plots, regardless whether they were from the control vs amended plots or from which runoff event. [b]Values followed by different letters are significantly different at the 0.05 probability level based on LSD tests. [c]rANOVA values are displayed as *p* values.

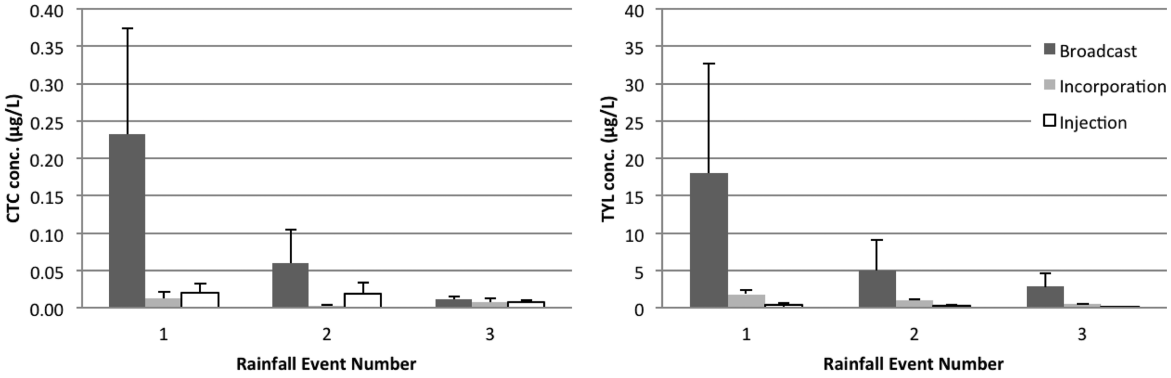



**Figure 1.** Aqueous concentrations of chlortetracycline (CTC) and tylosin (TYL) in runoff from manure-amended plots receiving broadcast, incorporation, and injection treatments over three rainfall events. Error bars show the standard errors over triplicate field experiments.

and ARGs in runoff. If a treatment method was determined as significant ($p < 0.05$), least significant difference (LSD) tests were conducted to determine the significance of the differences among the treatment levels.

## RESULTS

**Antimicrobials and ARGs in Manure.** The chlortetracycline level in the CTC-manure slurry was 3.3 mg/kg solids on a wet weight (ww) basis (Table 1). Similarly, tylosin and bacitracin concentrations were 0.29 and 0.78 mg/kg solids ww in the TYL- and the BAC-manure slurry, respectively (Table 1). ARGs were present in the manure slurry, and the absolute abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) was $2.5 \times 10^4$, $1.3 \times 10^3$, $1.6 \times 10^4$, and $1.4 \times 10^2$ copies/mL, respectively (Table 1). The relative abundance of these four ARGs in manure is reported in SI Table S4. Bacitracin resistance genes *bceA*, *bceR*, *bcrA*, *bcrB*, and *bcrC* were not detected in BAC-manure slurry samples using PCR-based methods.

**Antimicrobials in Runoff.** rANOVA tests on the three treatment factors (application method, manure amendment, and rainfall event) showed that manure amendment had significant impacts on the aqueous concentrations of both chlortetracyline and tylosin in runoff while rainfall event had significant impacts on tylosin only (Table 2). The chlortetracycline and tylosin in the runoff from most of the control plots were below method detection limits (SI Table S5), resulting in statistically significant difference between amended and control plots ($p = 0.01$ for both antimicrobials, Table 2). For tylosin, its aqueous concentration in runoff decreased across the three rainfall events (Figure 1, and $p = 0.04$ in Table 2). Because no bacitracin was detected in any runoff samples, this antimicrobial was not included in the rANOVA test.

The broadcast treatment of swine manure slurry resulted in higher total mass loading of antimicrobials in runoff than did the injection and incorporation treatments. After three rainfall events, a total of 5.8 $\mu$g/m$^2$ of chlortetracycline was transported

**Table 3. Mass Loadings of Chlortetracycline and Tylosin Exported in Runoff from the CTC- and TYL-Manure Amended Plots during Three Rainfall Events (Average ± Standard Error). Averages and Standard Errors Were Calculated Based on Triplicate Field Experiments**

| | chlortetracyline | | | tylosin | | |
|---|---|---|---|---|---|---|
| rainfall event no. | broadcast ($\mu g/m^2$) | incorporation ($\mu g/m^2$) | injection ($\mu g/m^2$) | broadcast ($\mu g/m^2$) | incorporation ($\mu g/m^2$) | injection ($\mu g/m^2$) |
| 1 | 4.54 ± 2.86 | 0.23 ± 0.20 | 0.30 ± 0.24 | 280.51 ± 213.66 | 33.56 ± 22.45 | 5.23 ± 2.28 |
| 2 | 1.11 ± 0.86 | 0.06 ± 0.04 | 0.27 ± 0.24 | 89.02 ± 70.28 | 11.55 ± 1.64 | 4.59 ± 1.84 |
| 3 | 0.15 ± 0.05 | 0.14 ± 0.07 | 0.09 ± 0.06 | 56.37 ± 34.12 | 4.50 ± 0.56 | 1.73 ± 1.25 |
| sum | 5.80 | 0.43 | 0.66 | 425.90 | 49.61 | 11.55 |
| fraction from #1 | 0.78 | 0.55 | 0.45 | 0.66 | 0.68 | 0.45 |

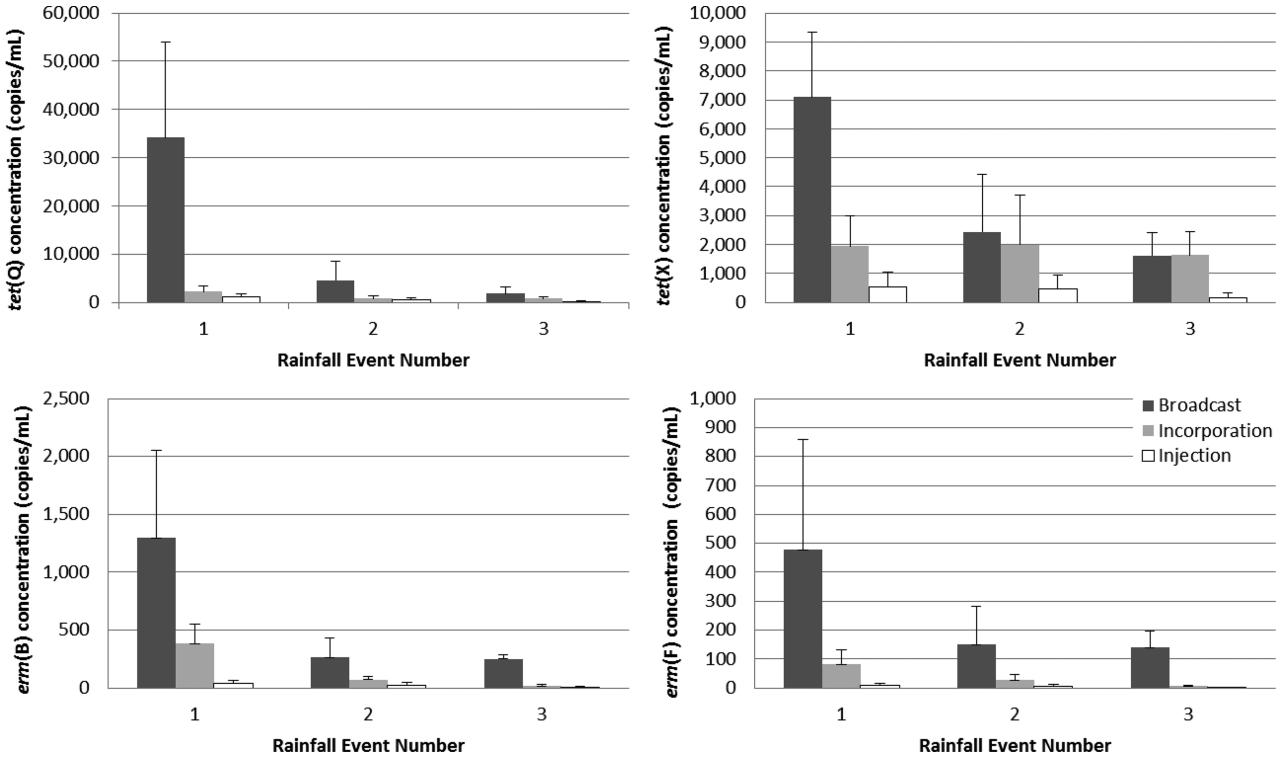



**Figure 2.** The absolute abundance (copy/mL runoff) of four ARGs (*tet*(Q), *tet*(X), *erm*(B), and *erm*(F)) in runoff from manure-amended plots receiving broadcast, incorporation, and injection treatments. Error bars represent standard errors from triplicate field experiments.

to runoff from the broadcasted plots (Table 3). As a comparison, a total of 0.43 and 0.66 $\mu g/m^2$ of chlortetracycline were transported from the incorporated and injected plots to runoff, respectively. Furthermore, 78%, 55%, and 45% of the total chlortetracycline load occurred during the first rainfall event for the broadcasted, incorporated, and injected plots, respectively. Similar trends were observed for tylosin (Table 3).

**ARGs in Runoff.** rANOVA tests showed that manure amendment had significant effects on the runoff concentrations of all the ARGs tested ($p = 0.01$, Table 2). No substantial levels of ARGs were detected in runoff from the control plots (Table S6). Land application method had significant impacts on the concentrations of *erm* genes in runoff ($p = 0.03$ for both *erm* genes): broadcast treatment caused significantly higher *erm* gene levels in runoff than did incorporation and injection treatments (Table 2). Rainfall events had significant impacts on the concentrations of *tet*(Q) and *erm*(B) in runoff (Figure 2, $p = 0.01$ for both ARGs in Table 2). Bacitracin resistance genes were not measured for runoff samples as they were not detected in manure samples.

**Antimicrobials in Soil.** Land applied slurry was the only source of antimicrobials in the soil of amended plots, as the soil samples collected from these plots prior to land application contained no detectable levels of antimicrobials (data not shown). After the rainfall tests, antimicrobial concentrations in the top soils of amended plots ranged between 3.7 and 63 ng/g soil dw (Table 4). Noticeably, broadcast led to higher tylosin concentrations in top soils in TYL-manure amended plots, while incorporation led to higher chlortetracycline concentrations in top soils in CTC-manure amended plots (Table 4).

**Table 4. Antimicrobial Concentrations (Average ± Standard Error) In Top Soils of Amended Plots after Three Rainfall Events[a]**

| | broadcast (ng/g soil dw) | incorporation (ng/g soil dw) |
|---|---|---|
| CTC in plots amended with CTC-manure | 3.7 ± 2.7 | 63 ± 8.7 |
| TYL in plots amended with TYL-manure | 32 ± 6.9 | 14 ± 13.2 |
| BAC in plots amended with BAC-manure | 0.0 ± 0.0 | 0.0 ± 0.0 |

[a]Standard errors were calculated based on triplicate field experiments.

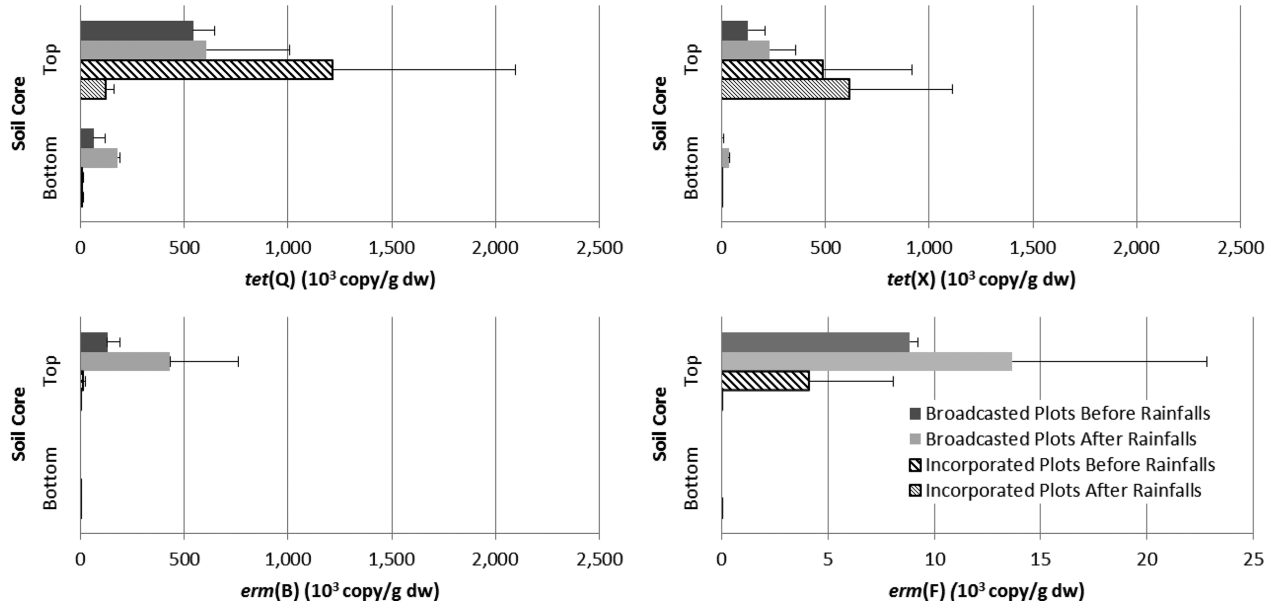



**Figure 3.** The absolute abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in top and bottom soil in manure-amended plots before and after three rainfall events. Error bars represent standard errors from triplicate field experiments.

Bacitracin was not detected in soil samples from BAC-manure amended plots.

**ARGs in Soil.** ARGs concentrations in soil were reported in absolute abundance (copies/g soil dw, Figure 3) and in relative abundance (ARG copies per copy of the 16S rRNA gene, SI Figure S4). Before the rainfall simulation tests, the average absolute abundances of *tet*(Q) and *tet*(X) in top soils were 1−2 orders of magnitude higher than those in bottom soils in broadcasted plots (Figure 3). After rainfall events, both *tet* genes increased in absolute abundance in the top soil of the broadcasted plots (Figure 3), while no common trend was observed in the top soil of the incorporated plots. The absolute abundance of *tet* genes increased in bottom soil in both broadcasted plots ($p$ = 0.17 for *tet*(Q), and $p$ = 0.03 for *tet*(X)) and in incorporated plots ($p$ = 0.83 for *tet*(Q), and $p$ = 0.97 for *tet*(X)) after the rainfall events, although most increases were not statistically significant. The *erm* genes increased in the top soil in broadcasted plots (Figure 3, $p$ = 0.42 for *erm*(B), and $p$ = 0.62 for *erm*(F)) and decreased in the top soils in incorporated plots (Figure 3, $p$ = 0.34 for *erm*(B), and $p$ = 0.35 for *erm*(F)) after the rainfall events. Finally, no appreciable amounts of ARGs were detected in control plots (SI Table S7), suggesting the occurrence of ARGs in the amended plot was due to land application of swine slurry.

## DISCUSSION

The antimicrobial concentrations in the swine manure slurry reported in this study are higher than those reported in similar studies. Chlortetracycline and bacitracin concentrations in swine manure are reported as high as tens of mg/kg dw[31−33] and 3.2−15 mg/kg dw,[34] respectively, whereas they were measured at 404 and 320 mg/kg dw in this study (Table 1). This is likely because the manure slurry was collected fresh in the current study. During manure aging, antimicrobials in manure can be sequestered[35] or degraded.[36] It is difficult to compare the ARG levels in our manure slurry samples with the literature, mainly because researchers often report ARG levels in the units of copies per gram of manure wet weight or fresh weight (fw), which could vary substantially in water content. In some studies, tetracycline resistance genes have been measured between $10^4$ and $10^9$ copies/g manure ww,[1,37] and tylosin resistance genes were detected between $10^4$ and $10^9$ copies/g of manure fw.[38,39] Although the target antimicrobial was the dominant compound measured in each manure type, other antimicrobials were also detected (SI Table S8). The presence of additional antimicrobials was likely due to contamination that occurred during the manure collection in the pits beneath the confinement facilities.

Broadcast generally resulted in higher antimicrobial concentrations in runoff than did incorporation and injection (Figure 1). Because swine slurry was spread on the soil surface, the antimicrobials were readily available for transport to runoff during rainfall events. In contrast, mixing manure slurry with surface soil to various extents (i.e., injection and incorporation) could reduce the loss of antimicrobials to runoff.[7,40] However, the main treatment factor, application method, was not considered statistically significant according to the rANOVA tests ($p$ = 0.26 for chlortetracycline and $p$ = 0.31 for tylosin, Table 2). This is partially due to the large variations among the triplicate plots, which are not uncommon in field-scale experiments. The differences in sorption partition coefficients between chlortetracyline (500−3715 L/kg[41]) and tylosin (1300 L/kg[42]) to silty clay loam, which is the soil type at the site of this study, might account for the differences in runoff concentrations. Due to the sorptive nature of chlortetracyline, it was not surprising that the aqueous chlortetracyline concentrations in the runoff were low (Figure 1). The range of tylosin concentrations in runoff measured in this study was 0.087−18 μg/L, which are similar to previously reported values of 0.01 and 6 μg/L.[5,8,9]

ARGs followed similar trends as the antimicrobials when being transported from plots to runoff. This is likely because indigenous soil microbes had not developed substantial levels of resistance during the field experiments and the resistant bacteria in the runoff were largely from the original manure slurry. This observation is supported by the largely similar relative abundances detected across the three rainfall events (SI Figure S3). Finally, high levels of ARGs in runoff from the

broadcasted plots suggest greater losses of manure to runoff from the broadcasted plots than the incorporated and injected plots.

The effect of land application methods on the fate of antimicrobials in top soil is compound specific. Broadcast application led to higher tylosin concentrations in the top soil, whereas incorporation resulted in higher chlortetracyline concentrations (Table 4). The trend for tylosin is expected: tylosin has a reported dissipation half-life of 7−8 days in soil,[11] hence during the field experiment (i.e., 4 days) the majority of the tylosin in manure would persist in top soil. On the other hand, chlortetracycline is photochemically labile with a photodegradation rate constant of 0.65 ± 0.30 $h^{-1}$ on clay surface.[12] A significant portion of the chlortetracyline could be photodegraded after being broadcasted on soil surface,[13] whereas mixing manure into soil (e.g., incorporation) would limit photodegradation. This hypothesis was supported by a previous study, which showed that when liquid manure was spread on soil surface, the concentration of tetracycline was lower in the top 0−10 cm than at a depth of 20−30 cm.[43] Bacitracin (i.e., bacitracin A) was detected in swine slurry samples (Table 1) but not in any soil or runoff samples, suggesting it was degraded soon after land application of swine manure slurry.[2,44] Bacitracin F, a common degradation product, was also detected negative in soil and runoff samples (data not shown).

In the amended plots, the absolute abundance of ARGs in top soils was orders of magnitude higher than that in bottom soils (Figure 3). Before the rainfall events, some level of ARGs was detected in the bottom soil of manure-amended plots. This was likely due to the infiltration of the diluted manure slurry used in the land application in this study. More pronounced vertical transport of ARGs was expected in incorporated plots than in broadcasted plots. However, neither the *tet* genes nor the *erm* genes exhibited this trend. The heterogeneity of the plot may account for the lack of the expected trend.

No clear trend was observed in the ARG levels in soil among land application methods. Before the rainfall events, *tet* levels in the broadcasted plots were lower than in the incorporated plots, whereas *erm* levels exhibited the opposite trend. This type of ARG-specific behavior was also observed in a previous long-term study.[45] The *tet* genes and *erm* genes may be carried by different host cells, and different host cells may respond differently to the soil environments created by varying land application methods.[46] Quick inactivation or growth of host cells may account for the ARG-specific behaviors in top soil. After rainfall, ARG levels in top soil increased in broadcasted plots but generally decreased in incorporated plots. In addition, ARGs were absent or at low levels in deep soil in most of the plots, suggesting that vertical transport of ARGs were not significant during the period of study.

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

*(X.L.) Phone: (402) 472-6042; fax: (402) 472-8934; e-mail: xuli@unl.edu.

### Present Address

[#]Palo Duro Research Center West Texas A&M University, Canyon, Texas


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

Funding for this project was provided in part by National Pork Board.

## ■ REFERENCES


(1) Wang, L. L.; Oda, Y.; Grewal, S.; Morrison, M.; Michel, F. C.; Yu, Z. T. Persistence of resistance to erythromycin and tetracycline in swine manure during simulated composting and lagoon treatments. *Microb. Ecol.* **2011**, *63* (1), 32−40.

(2) Sarmah, A. K.; Meyer, M. T.; Boxall, A. B. A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **2006**, *65* (5), 725−759.

(3) Chee-Sanford, J. C.; Mackie, R. I.; Koike, S.; Krapac, I. G.; Lin, Y. F.; Yannarell, A. C.; Maxwell, S.; Aminov, R. I. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J. Environ. Qual.* **2009**, *38* (3), 1086−1108.

(4) Heuer, H.; Schmitt, H.; Smalla, K. Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr. Opin. Microbiol.* **2011**, *14* (3), 236−243.

(5) Dolliver, H.; Gupta, S. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *J. Environ. Qual.* **2008**, *37* (3), 1227−1237.

(6) Kay, P.; Blackwell, P. A.; Boxall, A. B. A. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environ. Toxicol. Chem.* **2004**, *23* (5), 1136−1144.

(7) Kreuzig, R.; Holtge, S.; Brunotte, J.; Berenzen, N.; Wogram, J.; Schulz, R. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environ. Toxicol. Chem.* **2005**, *24* (4), 777−781.

(8) Davis, J. G.; Truman, C. C.; Kim, S. C.; Ascough, J. C.; Carlson, K. Antibiotic transport via runoff and soil loss. *J. Environ. Qual.* **2006**, *35* (6), 2250−2260.

(9) Kim, S.-C.; Davis, J. G.; Truman, C. C.; Ascough, J. C.; Carlson, I. I. K., Simulated rainfall study for transport of veterinary antibiotics—Mass balance analysis. *J. Hazard. Mater.* **2010**, *175* (1−3), 836−843.

(10) Sassman, S. A.; Lee, L. S. Sorption of three tetracyclines by several soils: Assessing the role of pH and cation exchange. *Environ. Sci. Technol.* **2005**, *39* (19), 7452−7459.

(11) Hu, D.; Coats, J. R. Aerobic degradation and photolysis of tylosin in water and soil. *Environ. Toxicol. Chem.* **2007**, *26* (5), 884−889.

(12) Werner, J. J.; McNeill, K.; Arnold, W. A. Photolysis of chlortetracycline on a clay surface. *J. Agric. Food Chem.* **2009**, *57* (15), 6932−6937.

(13) Eichhorn, P.; Aga, D. S. Identification of a photooxygenation product of chlortetracycline in hog lagoons using LC/ESI-ion trap-MS and LC/ESI-time-of-flight-MS. *Anal. Chem.* **2004**, *76* (20), 6002−6011.

(14) Heuer, H.; Focks, A.; Lamshoeft, M.; Smalla, K.; Matthies, M.; Spiteller, M. Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. *Soil Biol. Biochem.* **2008**, *40* (7), 1892−1900.

(15) Byrne-Bailey, K. G.; Gaze, W. H.; Kay, P.; Boxall, A. B. A.; Hawkey, P. M.; Wellington, E. M. H. Prevalence of sulfonamide resistance genes in bacterial isolates from manured agricultural soils and pig slurry in the United Kingdom. *Antimicrob. Agents Chemother.* **2009**, *53* (2), 696−702.

(16) Brooks, J. P.; Adeli, A.; Read, J. J.; McLaughlin, M. R. Rainfall simulation in greenhouse microcosms to assess bacterial-associated

runoff from land-applied poultry litter. *J. Environ. Qual.* **2009**, *38* (1), 218−229.
(17) Gilley, J. E.; Bartelt-Hunt, S. L.; Li, X.; Marx, D. B.; Snow, D. D.; Parker, D. B.; Woodbury, B. L., Runoff nutrient transport as affected by land application method, swine growth stage, and runoff rate. In *ASABE Annual International Meeting*; American Society of Agricultural and Biological Engineers: Kansas City, MO, 2013; Paper No. 131591897.
(18) Humphry, J. B.; Daniel, T. C.; Edwards, D. R.; Sharpley, A. N. A portable rainfall simulator for plot-scale runoff studies. *Appl. Eng. Agric.* **2002**, *18* (2), 199−204.
(19) Sharpley, A.; Kleinman, P. Effect of rainfall simulator and plot scale on overland flow and phosphorus transport. *J. Environ. Qual.* **2003**, *32* (6), 2172−2179.
(20) Zhu, J.; Snow, D. D.; Cassada, D. A.; Monson, S. J.; Spalding, R. F. Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr.* **2001**, *928* (2), 177−186.
(21) Snow, D. D.; Cassada, D. A.; Monson, S. J.; Zhu, J.; Spalding, R. F., Tetracycline and macrolide antibiotics - Trace analysis in water and wastewater using solid phase extraction and liquid chromatography-tandem mass spectrometry. In *Analysis of Emerging Contaminants using Liquid Chromatography/Mass Spectrometry (LC/MS/MS)*; Thurman, E. M., Ferrer, I. M., Eds.; American Chemical Society: Washington, DC, 2003; pp 161−174.
(22) Zhu, J.; Snow, D. D.; Cassada, D. A.; Monson, S. J.; Spalding, R. F. Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr.* **2001**, 177−186.
(23) Aga, D. S.; O'Connor, S.; Ensley, S.; Payero, J. O.; Snow, D.; Tarkalson, D. Determination of the persistence of tetracycline antibiotics and their degradates in manure-amended soil using enzyme-linked immunosorbent assay and liquid chromatography-mass spectrometry. *J. Agric. Food Chem.* **2005**, *53* (18), 7165−7171.
(24) Govaerts, C.; Li, C.; Orwa, J.; Van Schepdael, A.; Adams, E.; Roets, E.; Hoogmartens, J. Sequencing of bacitracin A and related minor components by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17* (12), 1366−1379.
(25) Koike, S.; Krapac, I. G.; Oliver, H. D.; Yannarell, A. C.; Chee-Sanford, J. C.; Aminov, R. I.; Mackie, R. I. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Appl. Environ. Microbiol.* **2007**, *73* (15), 4813−4823.
(26) Ghosh, S.; Ramsden, S. J.; LaPara, T. M. The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Appl. Microbiol. Biotechnol.* **2009**, *84* (4), 791−796.
(27) Koike, S.; Aminov, R. I.; Yannarell, A. C.; Gans, H. D.; Krapac, I. G.; Chee-Sanford, J. C.; Mackie, R. I. Molecular ecology of macrolide-lincosamide-streptogramin B methylases in waste lagoons and subsurface waters associated with swine production. *Microb. Ecol.* **2010**, *59* (3), 487−498.
(28) Yoshida, Y.; Matsuo, M.; Oogai, Y.; Kato, F.; Nakamura, N.; Sugai, M.; Komatsuzawa, H. Bacitracin sensing and resistance in Staphylococcus aureus. *FEMS Microbiol. Lett.* **2011**, *320* (1), 33−39.
(29) Murphy, T.; Roy, I.; Harrop, A.; Dixon, K.; Keshavarz, T. Elicitation effects of oligosaccharides on the transcriptional level of bacitracin ABC transporter genes in *Bacillus licheniformis*. *Biotechnol. Lett.* **2008**, *30* (9), 1665−1670.
(30) Suzuki, M. T.; Taylor, L. T.; DeLong, E. F. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. *Appl. Environ. Microbiol.* **2000**, *66* (11), 4605−4614.
(31) Ji, X.; Shen, Q.; Liu, F.; Ma, J.; Xu, G.; Wang, Y.; Wu, M. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. *J. Hazard. Mater.* **2012**, *235*, 178−185.
(32) Zhao, L.; Dong, Y. H.; Wang, H. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Sci. Total Environ.* **2010**, *408* (5), 1069−1075.
(33) Martinez-Carballo, E.; Gonzalez-Barreiro, C.; Scharf, S.; Gans, O. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environ. Pollut.* **2007**, *148* (2), 570−579.
(34) Zhou, L.-J.; Ying, G.-G.; Liu, S.; Zhang, R.-Q.; Lai, H.-J.; Chen, Z.-F.; Pan, C.-G. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. *The Science of the total environment* **2013**, *444*, 183−195.
(35) Wang, Q. Q.; Guo, M. X.; Yates, S. R. Degradation kinetics of manure-derived sulfadimethoxine in amended soil. *J. Agric. Food Chem.* **2006**, *54* (1), 157−163.
(36) Dolliver, H.; Gupta, S.; Noll, S. Antibiotic degradation during manure composting. *J. Environ. Qual.* **2008**, *37* (3), 1245−1253.
(37) Yu, Z. T.; Michel, F. C.; Hansen, G.; Wittum, T.; Morrison, M. Development and application of real-time PCR assays for quantification of genes encoding tetracycline resistance. *Appl. Environ. Microbiol.* **2005**, *71* (11), 6926−6933.
(38) Chen, J.; Yu, Z. T.; Michel, F. C.; Wittum, T.; Morrison, M. Development and application of real-time PCR assays for quantification of erm genes conferring resistance to macrolides-lincosamides-streptogramin B in livestock manure and manure management systems. *Appl. Environ. Microbiol.* **2007**, *73* (14), 4407−4416.
(39) Chen, J.; Michel, F. C., Jr.; Sreevatsan, S.; Morrison, M.; Yu, Z. Occurrence and persistence of erythromycin resistance genes (erm) and tetracycline resistance genes (tet) in waste treatment systems on swine farms. *Microb. Ecol.* **2010**, *60* (3), 479−486.
(40) Kay, P.; Blackwell, P. A.; Boxall, A. B. A. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere* **2005**, *59* (7), 951−959.
(41) Teixido, M.; Granados, M.; Prat, M. D.; Beltran, J. L. Sorption of tetracyclines onto natural soils: Data analysis and prediction. *Environ. Sci. Pollut. Res.* **2012**, *19* (8), 3087−3095.
(42) Clay, S. A.; Liu, Z.; Thaler, R.; Kennouche, H. Tylosin sorption to silty clay loam soils, swine manure, and sand. *J. Environ. Sci. Health, Part B* **2005**, *40* (6), 841−850.
(43) Hamscher, G.; Sczesny, S.; Hoper, H.; Nau, H. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Chem.* **2002**, *74* (7), 1509−1518.
(44) Pavli, V.; Kmetec, V. Pathways of chemical degradation of polypeptide antibiotic bacitracin. *Biol. Pharm. Bull.* **2006**, *29*, 2160−2167.
(45) Alexander, T. W.; Yanke, J. L.; Reuter, T.; Topp, E.; Read, R. R.; Selinger, B. L.; McAllister, T. A. Longitudinal characterization of antimicrobial resistance genes in feces shed from cattle fed different subtherapeutic antibiotics. *BMC Microbiol.* **2011**, 11.
(46) Boes, J.; Alban, L.; Bagger, J.; Mogelmose, V.; Baggesen, D. L.; Olsen, J. E. Survival of *Escherichia coli* and *Salmonella Typhimurium* in slurry applied to clay soil on a Danish swine farm. *Prev. Vet. Med.* **2005**, *69* (3−4), 213−228.

# Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry

*Stacey R. Joy*[a], *Shannon L. Bartelt-Hunt*[a], *Daniel D. Snow*[b], *John E. Gilley*[c], *Bryan L. Woodbury*[d], *David B. Parker*[d,#], *David B. Marx*[e], *and Xu Li*[a,*]

[a]Department of Civil Engineering, University of Nebraska-Lincoln, Lincoln, NE 68588; [b]School of Natural Resources, Water Sciences Laboratory, University of Nebraska-Lincoln, Lincoln, NE 68503; [c]USDA-ARS, Lincoln, NE 68503; [d]USDA Meat Animal Research Center, Clay Center, NE 68933; [e]Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68503

[*]Corresponding author:
844 N. 16th Street, N117 SLNK
Lincoln, NE 68588-6105
Phone: (402) 472-6042
Fax: (402) 472-8934
E-mail: xuli@unl.edu

[#] Current address:
Palo Duro Research Center
West Texas A&M University
Canyon, TX

This SI file includes:

17 pages, 8 tables, and 4 figures.

## MATERIALS AND METHODS

### Swine Manure Slurry

Manure slurry samples were collected from the sampling locations labeled in Figure S1. The wet weight and dry weight of the manure solids in the manure slurries from the finisher (BAC-manure), grower (CTC-manure), and sow and gilts (TYL-manure) were measured using gravimetric methods.

### Field Site

This field study was conducted in May and June 2011 at the University of Nebraska Rogers Memorial Farm located 18 km east of Lincoln, Nebraska. The study site had been cropped using a long-term no-till management system with controlled wheel traffic. Soybeans (*Glycine max*) were planted during the 2010 season and herbicide (glyphosate) was applied as needed to control weed growth. The soil at the site developed in loess under prairie vegetation, and is the Aksarben silty clay loam (fine, smectitic, mesic Typic Argiudoll) containing 15% sand, 57% silt, and 28% clay [1].

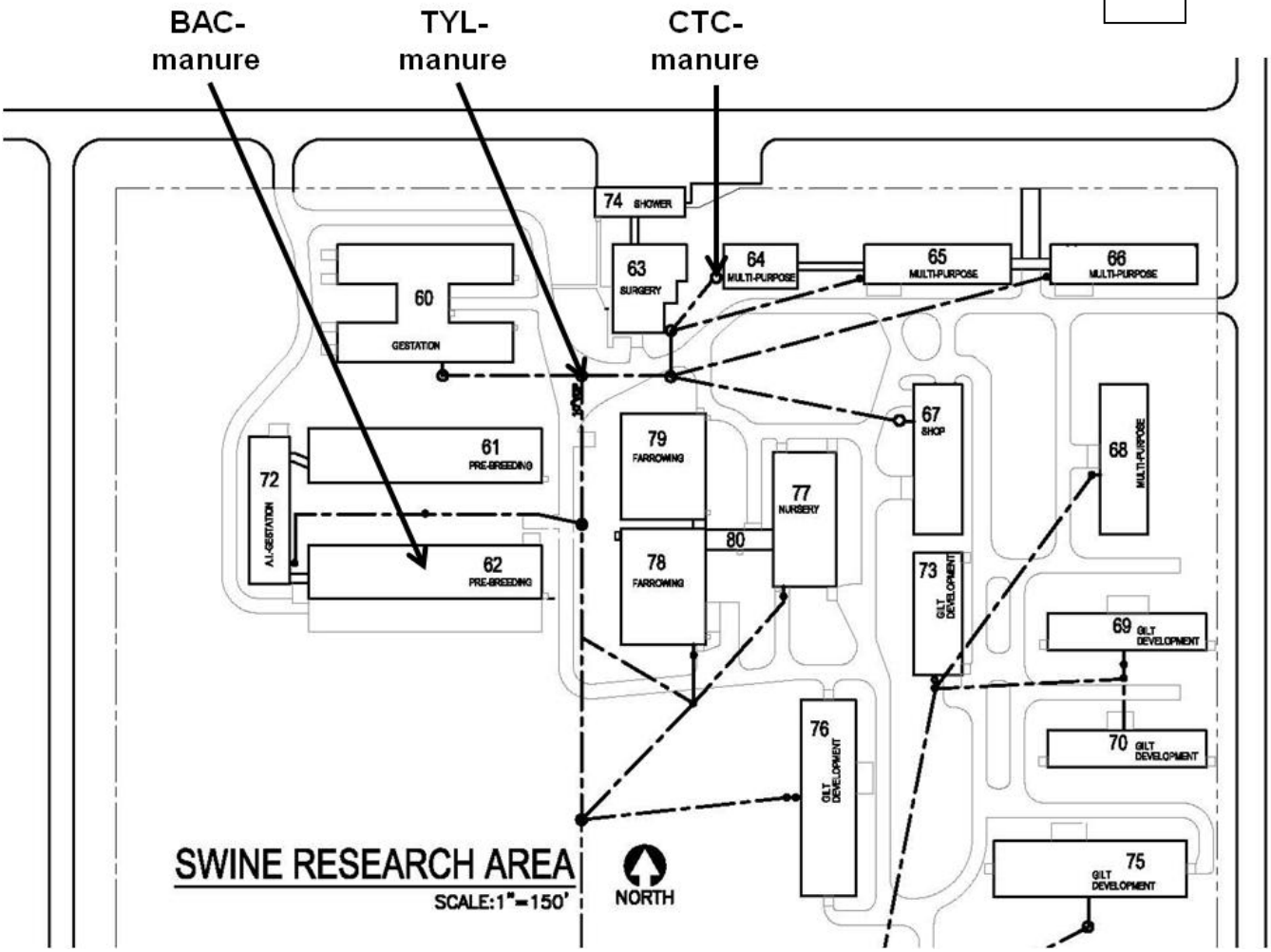
Soil samples for site characterization were obtained from the surface down to 2 cm just prior to manure application, and were air dried following collection. The study site had a mean slope gradient of 5.8%, an electrical conductivity (EC) of 0.38 dS $m^{-1}$ and a pH of 6.8. The organic matter and total carbon content of the soil was 4.7% and 2.62%, respectively. Mean measured concentrations of Bray and Kurtz No. 1 P, water-soluble P, $NO_3$-N, and $NH_4$-N were 43, 5.2, 8, and 4 mg $kg^{-1}$, respectively. The initial soil moisture condition prior to swine slurry application was not measured.

**Chemicals**

Standards for roxithromycin, doxycycline, bactracin A, and fenbendazole were purchased from Sigma-Aldrich (Fluka Chemicals). Oleandomycin, tylosin A and chlortetracycline were obtained from ThermoFisher Scientific (ICN Biomedicals and MP Biomedicals). Roxithromycin and doxycycline were used as internal standards and oleandomycin was used as a surrogate. Analytes were chlortetracycline, bactracin A, tylosin, and fenbendazole. Because bacitracin A is rapidly hydrolyzed in water at near neutral pH, a standard for bacitracin F (one of its degradation products) was synthesized and used to quantify this compound in the manure, soil, and runoff samples [2].

**FIGURES AND TABLES**

A



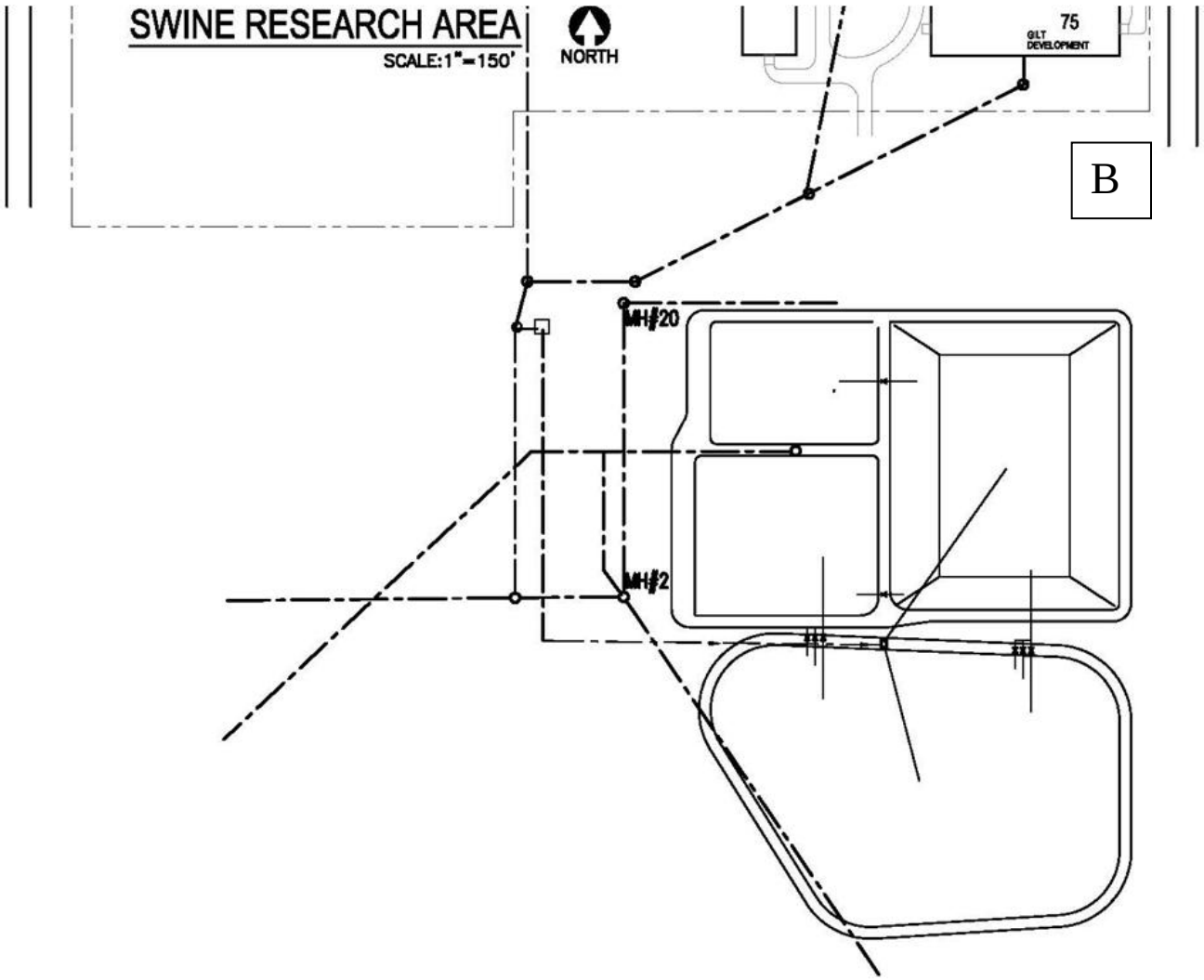



Figure S1. Map showing the locations of the barns and sampling points (A) and the lagoon systems (B) in the swine research area in the USDA Meat Animal Research Center.

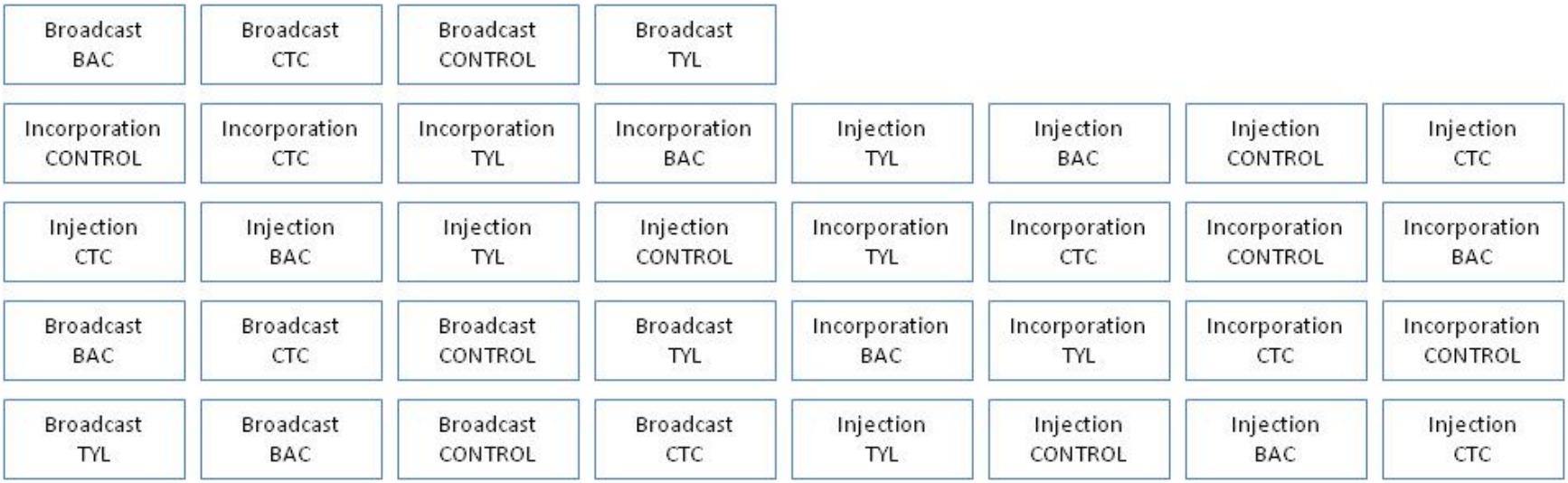



Figure S2. Randomized block design used in the field experiment. Plots in each row were constructed in the same week.

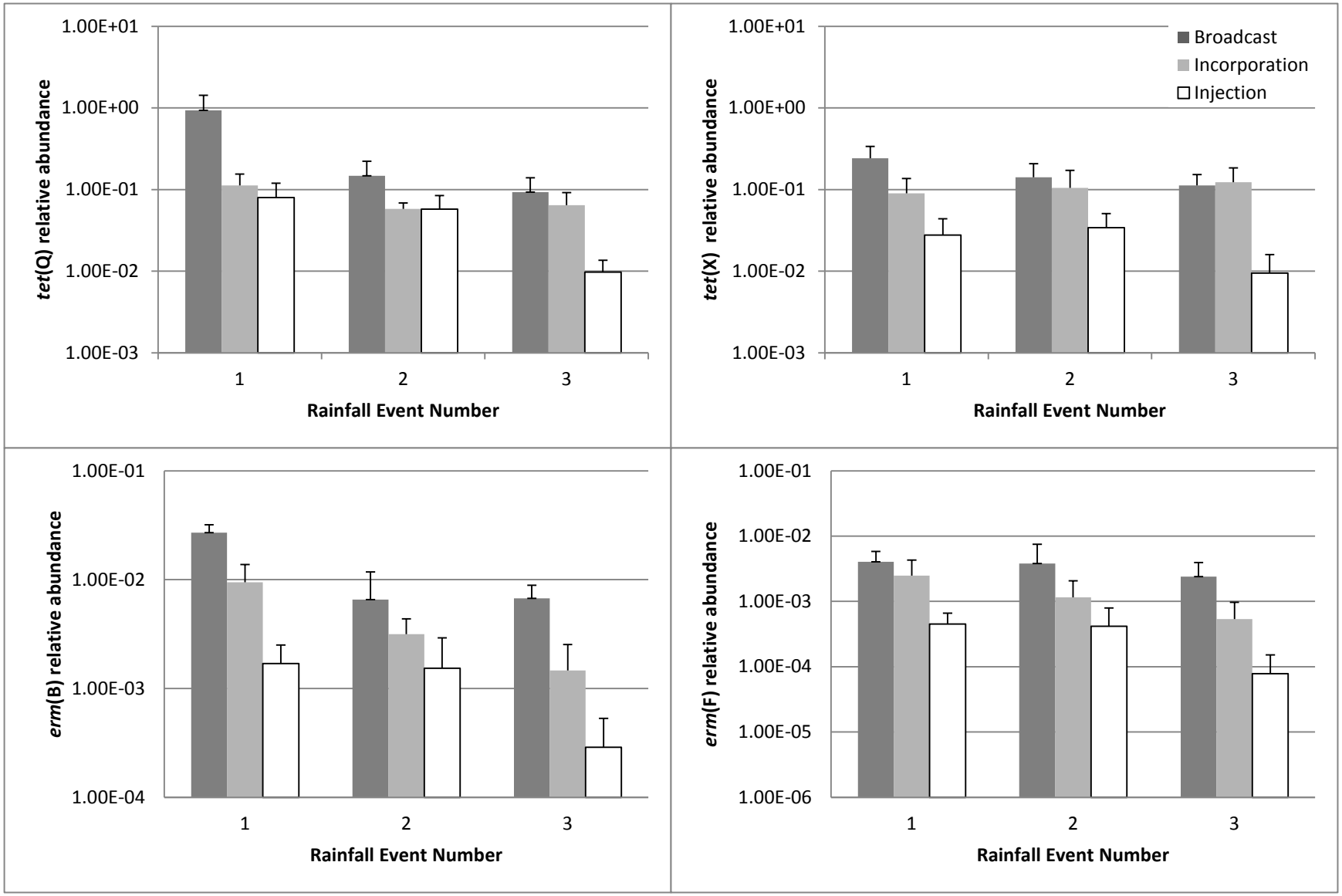



Figure S3. Relative abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in runoff from control and amended plots receiving broadcast, incorporation, or injection treatment. Error bars represent standard errors from triplicate field experiments.

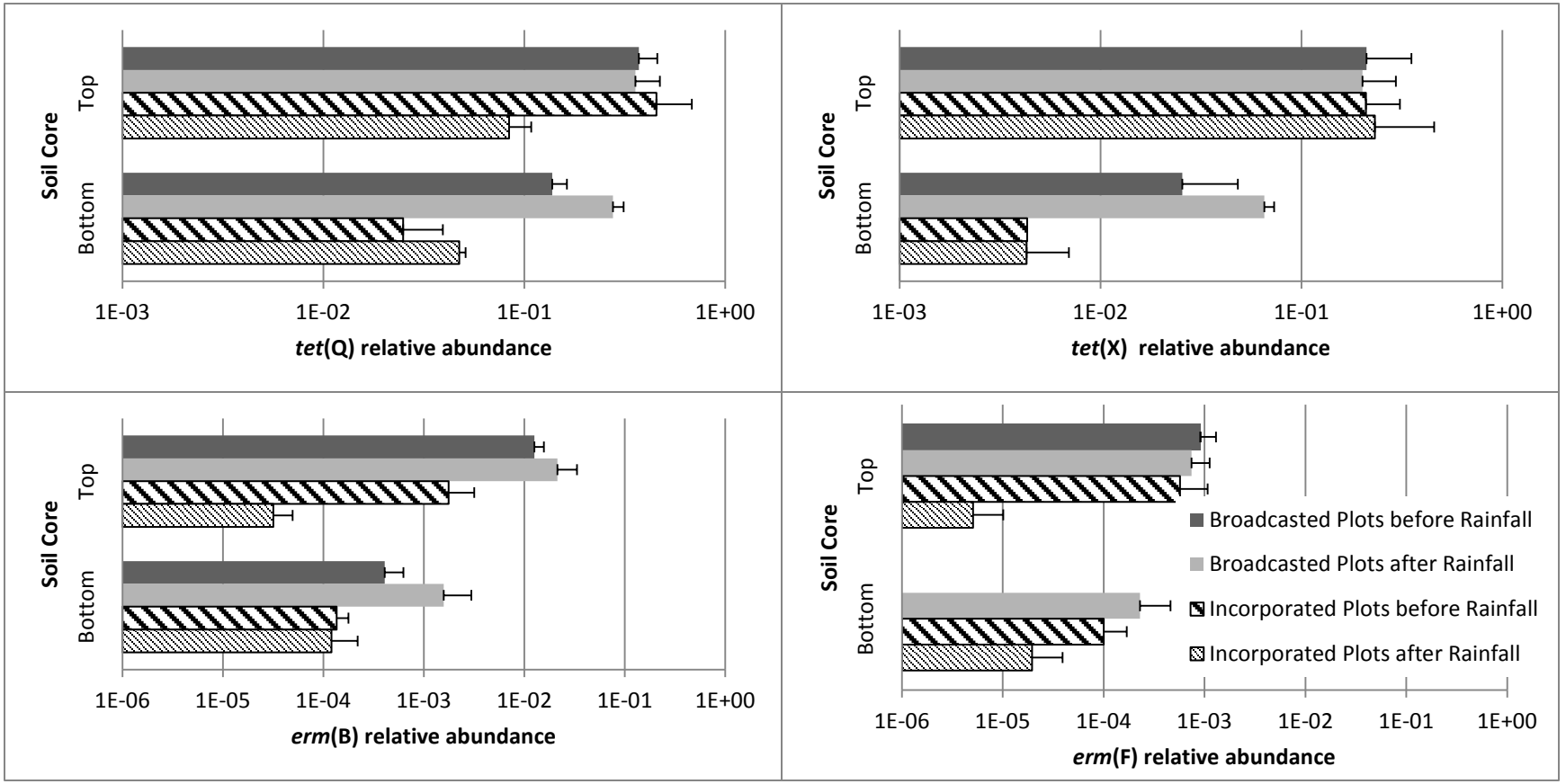



Figure S4. Relative abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in top and bottom soil in amended plots before and after three rainfall simulation tests. Error bars represent standard errors from triplicate field experiments.

Table S1. Properties of the antimicrobials used in this study.

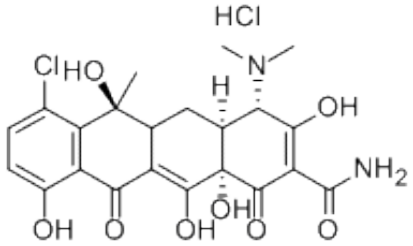
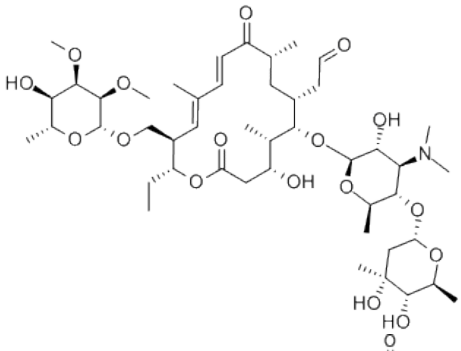
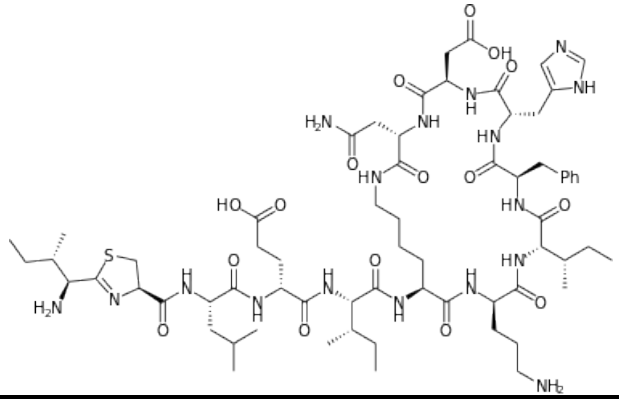
| Antimicrobial | Chemical Structure | Properties |
|---|---|---|
| Chlortetracycline |  | $K_d$ = 501-3715 L/kg [3]<br>Solubility = 500 mg/L<br>$t_{1/2}$ = 21 days [4] |
| Tylosin |  | $K_d$ = 1,300 L/kg [5]<br>Solubility = 6,000 mg/L<br>$t_{1/2}$ = 6-8 days [4, 6] |
| Bacitracin (Bacitracin A) |  | Environmental fate data for Bacitracin A are not available in the literature |

Table S2. Molecular weight, retention times, and MRM transition of antimicrobials, internal standards (IS), and surrogate (S) compound.

| Analyte | Molecular weight | Retention time (min) | MRM Transition (m/z) |
|---|---|---|---|
| Bacitracin A | 1422.7 | 9.82 | 712.10->86.20 |
| Bacitracin F | 1419.64 | 10.05 | 710.19->281.26 |
| Chlortetracycline | 478.88 | 8.71 | 478.90->444.00 |
| Fenbendazole | 299.35 | 10.63 | 300.20->268.20 |
| Tylosin | 916.10 | 10.40 | 916.9->174.2 |
| Doxycycline (IS) | 444.4 | 8.63 | 445.05->428.05 |
| Oleandomycin (S) | 687.86 | 10.51 | 688.35->544.10 |
| Roxythromycin (IS) | 837.05 | 11.58 | 837.55->679.50 |

Table S3. Relevant information of the qPCR and PCR reactions used in this study.

| Target ARG | Primer | Sequence (5'-3') | Annealing Temp ($^{o}$C) | Linear Range (copies/20µL) | $R^2$ | Efficiency (%) | Reference |
|---|---|---|---|---|---|---|---|
| *tet*(Q) | TetQ-FW | AGAATCTGCTGTTTGCCAGTG | | | | | |
| | TetQ-RV | CGGAGTGTCAATGATATTGCA | 63 | $10^2$-$10^9$ | 0.996 | 104.4 | [7] |
| *tet*(X) | TetX-FW | AGCCTTACCAATGGGTGTAAA | | | | | |
| | TetX-RV | TTCTTACCTTGGACATCCCG | 70 | $10^1$-$10^9$ | 0.997 | 81.8 | [8] |
| *erm*(B) | ErmB-FW | GGTTGCTCTTGCACACTCAAG | | | | | |
| | ErmB-RV | CAGTTGACGATATTCTCGATTG | 65 | $10^1$-$10^9$ | 0.978 | 111.1 | [9] |
| *erm*(F) | ErmF-FW | TCTGGGAGGTTCCATTGTCC | | | | | |
| | ErmF-RV | TTCAGGGACAACTTCCAGC | 65 | $10^1$-$10^9$ | 0.978 | 89.4 | [9] |
| *bceA** | BceA-FW | GCTACGACAGCACTTAATCA | | | | | |
| | BceA-RV | CACCTTCAGTTAGTCCATCA | 55 | | | | [10] |
| *bceR** | BceB-FW | TTAACCAACATCAACCTCAG | | | | | |
| | BceB-RV | CCCCATTTGTATTGCCAT | 55 | | | | [10] |
| *bcrA* | BcrA-FW | AAGTGGCAAGGCTTTTGAGA | | | | | |
| | BcrA-RV | CTCAGGATCAATCGGCAAAT | 60 | | | | [11] |
| *bcrB* | BcrB-FW | AAGTGGCAAGGCTTTTGAGA | | | | | |
| | BcrB-RV | AAATCACCGGGGGAATTAAG | 60 | | | | [11] |
| *bcrC* | BcrC-FW | AAGTGGCAAGGCTTTTGAGA | | | | | |
| | BcrC-RV | CTCAAGTTCCCCAGTTTCCA | 60 | | | | [11] |

* Regular PCR reactions.

Table S4. Relative abundance (average ± standard error) of ARGs *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in manure slurry.

| | ARG | Relative Abundance |
|---|---|---|
| CTC-Manure | *tet*(Q) | 1.33±0.26 |
| | *tet*(X) | 0.078±0.018 |
| TYL-Manure | *erm*(B) | 0.12±0.024 |
| | *erm*(F) | 0.0022±0.0004 |

Table S5. Aqueous antimicrobial concentrations in runoff from control plots (average ± standard error). MDL was 0.005 ng/µL.

| | Rainfall Event | CTC (ng/µL) | TYL (ng/µL) | BAC (ng/µL) |
|---|---|---|---|---|
| Broadcast | 1 | <MDL | 0.006* | <MDL |
| | 2 | <MDL | 0.011* | <MDL |
| | 3 | <MDL | <MDL | <MDL |
| Incorporation | 1 | <MDL | <MDL | <MDL |
| | 2 | <MDL | <MDL | <MDL |
| | 3 | <MDL | <MDL | <MDL |
| Injection | 1 | <MDL | 0.007[a] | <MDL |
| | 2 | <MDL | <MDL | <MDL |
| | 3 | <MDL | <MDL | <MDL |

*. Values are from one of the triplicate field experiments. No antimicrobials were detected in the other replicates.

Table S6. Concentrations of ARGs in runoff from control plots (average ± standard error). Standard errors were calculated based on triplicate field experiments. The MDL for each ARG is reported in Table S2.

| | | *tet*(Q) (copy/mL) | *tet*(X) (copy/mL) | *erm*(B) (copy/mL) | *erm*(F) (copy/mL) |
|---|---|---|---|---|---|
| Broadcast | Run 1 | < MDL | < MDL | < MDL | < MDL |
| | Run 2 | 423 ± 416 | 233 ± 231 | 25 ± 25 | 16 ± 16 |
| | Run 3 | < MDL | < MDL | < MDL | < MDL |
| Incorporation | Run 1 | < MDL | < MDL | < MDL | < MDL |
| | Run 2 | < MDL | < MDL | < MDL | < MDL |
| | Run 3 | < MDL | < MDL | < MDL | < MDL |
| Injection | Run 1 | < MDL | < MDL | < MDL | < MDL |
| | Run 2 | < MDL | < MDL | < MDL | < MDL |
| | Run 3 | < MDL | < MDL | < MDL | < MDL |

Table S7. No ARGs were detected in the top and bottom soil of control plots before and after the rainfall events in triplicate field experiments.

| | | Broadcast | | Incorporation | |
|---|---|---|---|---|---|
| | | Before Rainfalls | After Rainfalls | Before Rainfalls | After Rainfalls |
| *tet*(Q) | Top soil | <MDL | <MDL | <MDL | <MDL |
| | Bottom soil | <MDL | <MDL | <MDL | <MDL |
| *tet*(X) | Top soil | <MDL | <MDL | <MDL | <MDL |
| | Bottom soil | <MDL | <MDL | <MDL | <MDL |
| *erm*(B) | Top soil | <MDL | <MDL | <MDL | <MDL |
| | Bottom soil | <MDL | <MDL | <MDL | <MDL |
| *erm*(F) | Top soil | <MDL | <MDL | <MDL | <MDL |
| | Bottom soil | <MDL | <MDL | <MDL | <MDL |

Table S8. Antimicrobial concentrations in swine manure slurry (average ± standard error). Standard errors were calculated based on five weekly manure slurry samples.

| | Chlortetracyline | Tylosin (ng/g solid ww) | Bacitracin |
|---|---|---|---|
| CTC-manure | 3,324 ± 1,560 | 2 ± 1 | 172 ± 164 |
| TYL-manure | 102 ± 40 | 287 ± 124 | 7 ± 7 |
| BAC-manure | 16 ± 9 | 124 ± 68 | 777±753 |

1. Kettler, T. A.; Doran, J. W.; Gilbert, T. L., Simplifed method for soil particle-size determination to accompany soil-quality analyses. *Soil Sci. Soc. Am. J.* **2001,** *65*, (3), 849-852.
2. Pavli, V.; Kmetec, V., Pathways of chemical degradation of polypeptide antibiotic bacitracin. *Biol. Pharm. Bull.* **2006,** *29*, (11), 2160-2167.
3. Teixido, M.; Granados, M.; Prat, M. D.; Beltran, J. L., Sorption of tetracyclines onto natural soils: data analysis and prediction. *Environmental Science and Pollution Research* **2012,** *19*, (8), 3087-3095.
4. Carlson, J. C.; Mabury, S. A., Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. *Environ. Toxicol. Chem.* **2006,** *25*, (1), 1-10.
5. Clay, S. A.; Liu, Z.; Thaler, R.; Kennouche, H., Tylosin sorption to silty clay loam soils, swine manure, and sand. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes* **2005,** *40*, (6), 841-850.
6. Hu, D.; Coats, J. R., Aerobic degradation and photolysis of tylosin in water and soil. *Environ. Toxicol. Chem.* **2007,** *26*, (5), 884-889.
7. Koike, S.; Krapac, I. G.; Oliver, H. D.; Yannarell, A. C.; Chee-Sanford, J. C.; Aminov, R. I.; Mackie, R. I., Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Appl. Environ. Microbiol.* **2007,** *73*, (15), 4813-4823.
8. Ghosh, S.; Ramsden, S. J.; LaPara, T. M., The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Appl. Microbiol. Biotechnol.* **2009,** *84*, (4), 791-796.
9. Koike, S.; Aminov, R. I.; Yannarell, A. C.; Gans, H. D.; Krapac, I. G.; Chee-Sanford, J. C.; Mackie, R. I., Molecular ecology of Macrolide-Lincosamide-Streptogramin B methylases in waste lagoons and subsurface waters associated with swine production. *Microb. Ecol.* **2010,** *59*, (3), 487-498.
10. Yoshida, Y.; Matsuo, M.; Oogai, Y.; Kato, F.; Nakamura, N.; Sugai, M.; Komatsuzawa, H., Bacitracin sensing and resistance in Staphylococcus aureus. *FEMS Microbiol. Lett.* **2011,** *320*, (1), 33-39.
11. Murphy, T.; Roy, I.; Harrop, A.; Dixon, K.; Keshavarz, T., Elicitation effects of oligosaccharides on the transcriptional level of bacitracin ABC transporter genes in Bacillus licheniformis. *Biotechnol. Lett.* **2008,** *30*, (9), 1665-1670.