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Degradation of tetracycline, sulfamethazine, and tylosin in soil from prairie strips and row crops in Iowa

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Abstract

The livestock industry in the United States relies on antibiotics for disease prevention and treatment. As a result, antibiotic-laden manure is frequently applied to farmland to recycle nutrients. In the soil environment, antibiotics may accumulate and create selective pressure for antibiotic resistance in bacteria or travel to nearby water resources. This in vitro incubation study evaluated whether prairie buffer strips on farmland enhance degradation of three antibiotics—tetracycline, sulfamethazine, and tylosin—compared with degradation in soil from row crops adjacent to the strips. Soil from prairie strips of varying establishment ages and adjacent row crops were evaluated from three central Iowa sampling locations. Antibiotics mixed with swine manure slurry were added to soils at a starting concentration (10 μg kg^{-1}) that reflects common veterinary antibiotic concentrations in soil and runoff after manure application. Antibiotic concentrations were quantified at six time points throughout a 72-d incubation period and fit to a first-order model to calculate decay rate constants and half-lives. The mean half-life for tetracycline was 0.54 d longer in prairie strip soil than row crop soil, whereas sulfamethazine and tylosin demonstrated no significant difference in persistence in strip or crop soil. Time since the establishment of the prairie strip did not affect antibiotic persistence. Concentrations of each antibiotic decreased to near-background levels throughout the incubation period. This study suggests that prairie strips do not consistently enhance antibiotic degradation in farm fields, but that antibiotics are unlikely to persist throughout the growing season in soil under strip or crop management.

Abbreviations: HLB, hydrophilic lipophilic balance; INHF, Iowa Natural Heritage Foundation; LC–MS, liquid chromatography–mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; POM, particulate organic matter; SAX, strong anion exchange; SMZ, sulfamethazine; SOC, soil organic carbon; STRIPS, Science Based Trials of Row Crops Integrated with Prairie Strips; TET, tetracycline; TYL, tylosin; VA, veterinary antibiotic.

1 | INTRODUCTION

More than half of the 11.5 million kg of antibiotics sold in 2018 for livestock production in the United States were important to human medicine. Three drug classes accounted for 79% of total sales: tetracyclines, macrolides, and sulfonamides, which include the veterinary antibiotics (VAs) tetracycline (TET), tylosin (TYL), and sulfamethazine (SMZ), respectively (FDA-CVM, 2018). Each of these drug classes is listed as either highly important (tetracyclines and sulfonamides) or critically important (macrolides) by the World Health Organization (2018).

Because a large portion of antibiotic compounds administered to livestock remain unaltered in the gut prior to excretion, these compounds have been detected in swine manure slurry at concentrations ranging from <0.01 mg kg^{-1} up to >100 mg kg^{-1} (Van Epps & Blaney, 2016). Confined Animal Feeding Operations (CAFOs) in Iowa generate an estimated 50 Tg of manure annually, most of which is ultimately applied as fertilizer to farmland (Anderson, 2014), introducing antibiotics into the soil environment. Runoff from manure-amended sites thus allows the transfer of antibiotics and antibiotic-resistant organisms from soil to surface waters; numerous studies in recent decades have linked livestock operations to an increase in antibiotics and antibiotic resistant bacteria in surrounding soils as well as groundwater and surface waters (Campagnolo et al., 2002; MacKie et al., 2006; West et al., 2011). There is growing concern that these compounds can persist in soils and the surrounding environment, putting humans at risk for exposure and subsequent development of resistance to medically important antibiotics, although these exposure pathways are still poorly understood (Ben et al., 2019). Bacteria have been shown to acquire resistance at antibiotic concentrations up to orders of magnitude below minimum inhibitory concentrations (Gullberg et al., 2011). After the introduction of antibiotics to the soil, the transfer of resistant bacteria from soil to surface or groundwater supplies could result in chronic exposure to humans, potentially causing human pathogens to select for antibiotic resistance or acquire resistance through horizontal gene transfer (Chu et al., 2010; West et al., 2011).

Vegetated buffer systems are a common agricultural conservation practice, but they may also be a strategy for mitigating the spread of antibiotics from farmland. Vegetated buffers offer contaminant mitigation by improving microbial communities' capability to degrade agrichemicals, increasing infiltration and deposition of sediment-bound pollutants, and altering the ability of soil to sorb and degrade contaminants (Krutz et al., 2006). For these reasons, the use of vegetated buffer systems as in-field and edge-of-field agricultural conservation practices may provide opportunities to intercept and degrade antibiotic compounds in soil.

Core Ideas

- Prairie strip soil did not enhance the degradation of antibiotics.
- Antibiotics were degraded to nearly background levels in 72 d.
- Time since prairie strip establishment did not affect antibiotic degradation.
- Tetracycline, sulfamethazine, and tylosin degradation followed first-order kinetics.

One type of vegetated buffer system, prairie strips, is constructed using native prairie perennial grasses and placed along the contour of a hillslope in roughly 10% of a crop field (Schulte et al., 2017). An Iowa State University-based organization called STRIPS (Science Based Trials of Row Crops Integrated with Prairie Strips) has been investigating the functions and benefits of prairie strips on farm fields since 2003, with the objective of integrating prairie strips with other conservation practices to create a more sustainable agricultural system (STRIPS, 2020). Prairie strips have been proven effective at reducing dissolved and sediment-bound contaminants such as nutrients and pesticides (Hladik et al., 2017; Schulte et al., 2017), and a 2015 study by Soni et al. (2015) found that grass buffer systems were able to reduce TYL and its associated antibiotic resistance genes in agricultural runoff. Strips can also alter soil characteristics that influence sorption and dissipation of VAs, such as soil organic C (SOC) (Lertpaitoonpan et al., 2009), microbial activity (Cycoń et al., 2019), and pH (Chu et al., 2013; Sassman & Lee, 2005). The plant diversity of a prairie strip increases species richness and belowground biomass, resulting in positive effects on SOC content and soil microbial community activity (Chen et al., 2018; Lange et al., 2015). Soil pH can also be affected by the loss of organic matter, increased erosion, and fertilizer application on land used for row cropping when compared with land under prairie management (McCauley et al., 2017).

Several measurements have been made for degradation half-lives of SMZ, TET, and TYL, but results vary highly between studies, showing that half-lives range from 2.1 to 30 d for SMZ, <3 d and up to 31.5 d for TET, and 4.5 to 67 d for TYL (Carlson & Mabury, 2006; Halling-Sørensen et al., 2005; Lin et al., 2010; Pan & Chu, 2016). Higher half-lives indicate higher persistence, creating a higher risk for the accumulation of antibiotics in soil. Despite extensive existing research on dissipation rates of common VAs, how varying plant treatments and their effect on soil conditions might affect the persistence of VAs in the environment has been relatively unexplored. Similarly, there have been no studies comparing VA degradation in row crop soil treatment with that in a prairie strip system. Thus, the objectives of this laboratory study were primarily to compare the persistence of common VAs in row crop and prairie strip soils and, secondarily, to determine if prairie strip treatments improve soil health in a way that enhances dissipation of antibiotics. If prairie strip soil properties enhance VA degradation when compared with crop soil, this study would demonstrate enhanced degradation as an additional way in which prairie strips reduce the persistence of antibiotics in the environment (beyond mitigation of surface transport) and thus minimize their transfer from soil to surface and groundwater sources.

2 | MATERIALS AND METHODS

2.1 | Sampling sites and sample collection

Soil samples representing row crop production and adjacent prairie strips were collected from three STRIPs sites in central Iowa: the Neal Smith National Wildlife Refuge site, the Iowa Natural Heritage Foundation (INHF) Lanz Heritage Farm, and the Iowa State University Worle Research Farm. The three sites consist of varying proportions of reconstructed prairie vegetation (i.e., prairie strips) planted within row crops. These sites were selected due to the differing establishment ages of their prairie strips; Neal Smith was established in 2003, INHF in 2013, and Worle in 2015. Each of the sites is planted in corn (*Zea mays* L.) or soybeans [*Glycine max* (L.) Merr.] in alternating growing seasons. Worle was planted in corn in 2019, whereas Neal Smith and INHF were planted in soybeans. None of the three sites has a history of receiving manure amendment.

Soils were sampled at the Neal Smith National Wildlife Refuge farm, located at 41°33′14.0″ N, 93°15′10.8″ W, on 1 July 2019. Surface soil was collected on a side-slope with a 3-cm-diam. corer at a depth of 0–15 cm. Ten cores were collected on four transects approximately 10 m in length in the strip and the adjacent crop area at each site, resulting in 40 prairie strip soil cores and 40 row crop soil cores. Post-sampling, the soil cores were 1.27-cm (0.5-inch) sieved to remove roots and plant residue. The core samples from strips and crops from each site were then composited to form single bulk samples from each site for the cropped or prairie strip soil. Soils were sampled similarly at the Worle farm, located at 41°59′59″ N, 93°41′34″ W, on 9 July 2019. The INHF Lanz farm, located at 41°42′13″ N, 92°59′30″ W, was sampled in the same manner on 17 July 2019. Bulk density cores were also collected in duplicate at each of the three sites for soil bulk density analysis according to the method by the American Society for Testing and Materials (ASTM D6683-1 9; ASTM, 2019).

To determine the role of soil properties in VA degradation and to evaluate any differences between the different ages of prairie strips, physical and chemical soil properties were also measured on the composite samples. Two replicate soil moisture measurements were determined gravimetrically after drying at 105 °C for 24 h. A portion of crop and strip soil from each site was also set aside for air drying. The pH of each soil type was measured in a 2:1 solution of distilled water to the soil. Potentially mineralizable N was measured as the accumulation of mineral N during an aerobic 28-day incubation following the methods of Drinkwater et al. (1996), while respiration was measured simultaneously over the first 4 days of incubation by trapping CO_2-C in base followed by gas chromatography after acidification (Stott et al., 2011). Mehlich-3 extraction of major cations was quantified with OES-ICP (Stott et al., 2011). Organic C and N were measured by combustion and gas chromatography (EA1112 Flash NC Elemental Analyzer, Thermo Scientific) with correction for carbonate, and particulate organic matter (POM)-C was measured following the general methods of Cambardella and Elliot (1992).

Swine manure slurry was collected from a deep pit located northeast of Ellsworth, IA, and stored in the refrigerator until use. Manure from this pit had been previously shipped to the University at Buffalo for antibiotic analysis and was shown to contain 1,200 μg TET kg^{-1} dry manure and no detectable SMZ or TYL using the general methods of Wallace and Aga (2016) and Angeles and Aga (2018).

2.2 | Experimental design

Soils spiked with antibiotics and nonspiked control soils were incubated under aerobic conditions in dark, environment-controlled growth chambers set at 30 °C with triplicate replications of the six site and vegetation groups: (a) Worle prairie strip; (b) Worle row crop; (c) Neal Smith prairie strip; (d) Neal Smith row crop; (e) INHF prairie strip; and (f) INHF row crop. Soils were destructively sampled at six time points—0, 3, 7, 21, 36, and 72 d after manure application—and analyzed for SMZ, TET, and TYL to compare antibiotic degradation between prairie strip soil and row crop soil.

Soil from each of the six crop and strip composites was weighed in 36 aliquots of 100 g (dry weight) and placed into Mason jars. Soils were fortified with a mixture of antibiotic stock solution and swine manure slurry known to contain TET; therefore, a stock solution of 100 mg L^{-1} of SMZ and TYL and 64 mg L^{-1} TET was prepared in methanol. To simulate a concentration of manure transported in rainfall-runoff, 18 jars from each soil and vegetation combination received a pre-mixed solution of 3 ml of swine manure slurry, 0.1 ml of stock solution, and the amount of ultrapure water to bring soil moisture to 30%, resulting in a soil antibiotic concentration of 10 μg kg^{-1} (10 ppb). The concentrations of antibiotics and manure were chosen to represent concentrations delivered to the strips in runoff from manured croplands.

Each jar was thoroughly mixed by hand with a spatula. Jar weights were recorded on Day 0, and weights were remeasured every 7 d for the duration of the experiment. If water evaporation greater than 1 g occurred, ultrapure water was added in to maintain gravimetric soil moisture of 30%.

To get an initial (Day 0) concentration after spiking with antibiotics and a baseline background concentration, spiked and nonspiked jars from each soil group were destructively sampled in triplicate immediately after mixing. Jars from each soil group were then removed from the incubator and destructively sampled at the following five time points: 3, 7, 21, 36, and 72 d. After removal from the incubator, soils were freeze dried before extraction and analysis.

2.3 | Antibiotic extraction

Methods for extraction of antibiotics from soil and analysis were adapted from O'Connor et al. (2007) and Jacobsen et al. (2004). An automated Accelerated Solvent Extractor (ASE) 350 system (Dionex Company) was used for the antibiotic extractions. Five grams of freeze-dried soil was mixed with 2 g diatomaceous earth (DE) and packed into a 34-ml stainless steel ASE cell. The remainder of the cell was filled with approximately 23 g of Ottawa sand. The bottom of each vessel was covered with a circular 30-mm cellulose filter. After the cell was sealed, the sample was permeated by an extraction solvent of 50:50 0.2 M citric acid buffer (pH 4.6): methanol at 100 ˚C and 10.3 MPa (1,500 psi). Nitrogen gas was used to expel the extraction solvent into glass collection vials at the end of each extraction. The final volume of extract was roughly 52 ml. Extracts were transferred to 500-ml polytetrafluoroethylene (PTFE) bottles and diluted with Millipore Milli-Q water to 500 ml.

2.4 | Solid-phase extraction

The diluted extract solution was further concentrated using solid-phase extraction. Analytes were extracted using Oasis hydrophilic lipophilic balance (HLB) cartridges containing 500 mg sorbent from Waters connected in tandem to Isolute SAX (strong anion exchange) cartridges containing 200 mg sorbent from Biotage. Cartridges were attached to a Vac-Elute chamber and preconditioned with 5 ml of methanol, 5 ml of 0.04 M citric acid buffer (pH 4.7), and 5 ml of ultrapure water, each drawn through using vacuum at a rate of 1 ml min^{-1}. Samples were then drawn through the cartridges at 5 ml min^{-1}. The SAX cartridges were then removed from the HLB cartridges and thrown away. The HLB cartridges were washed with 3 ml of 0.04 citric acid buffer followed by a wash of 3 ml of 0.1 M ammonium acetate. The HLB cartridges were then transferred to a vacuum manifold and eluted in 2 ml of methanol into conical volumetric tubes. The extracts were concentrated under a flow of nitrogen gas to a volume of 0.3 ml and brought back up to a volume of 2 ml using 1.7 ml of 10 mM ammonium acetate. Using a 3-ml plastic syringe and stainless-steel cannula in conjunction with a 13-mm, 0.2-μm-pore nylon filter, the extract was transferred to a 2-ml amber silanized HPLC auto-injector compatible vial.

2.5 | Analysis of antibiotics (LC–MS)

Sample extracts were analyzed for SMZ, TET, and TYL using an ABSciex 5500 QTrap mass spectrometer (MS) with an Agilent 1260 Infinity liquid chromatographer (LC). Separation took place on an Agilent-Zorbax SB-C18 column (50 × 2.1 mm, 3.5 μm particle size) at a flow rate of 0.4 ml min^{-1}. Mobile Phase A was 0.1% formic plus 0.01 mM oxalic acid in water, and Mobile Phase B was 0.1% formic acid in methanol. The gradient begins at 97% A and holds for 3 min, then decreases to 85% A until 5.0 min, decreases again to 50% A by 8.0 min, and finally to 5% A at 10.5 min. The column re-equilibrates back to the initial conditions for a total of 20 min. The instrumental limit of detection (LOD) was determined to be 0.03, 0.06, and 0.02 ng L^{-1}, and the limit of quantification (LOQ) was determined to be 0.09, 0.21, and 0.08 ng L^{-1} for SMZ, TET, and TYL, respectively. Based on these instrumental values, LOD/LOQ values were back calculated for soil samples. Compounds were monitored using multiple reaction monitoring, with three product ions collected for each analyte. The most abundant transition was used for quantification, whereas the second and third product ions were used for ion ratio confirmation. Ratio acceptance criteria followed the European Standard, which uses a larger acceptance range for smaller ion ratios: the ratio is between 0 and 10% when the acceptable difference is 50%. If the ratio is 10–20%, the acceptable difference is 30%; a ratio range of 20–50% must agree with a difference of 25%, and a ratio above 50% has an acceptable difference of 20% (CEN, 2008). Calibration was conducted through single-point standard addition. The standard addition was made using 20 μl of approximately 100 pg $μl^{-1}$ standard added to 500 ul of extracted sample yielding a concentration near 4 pg $μl^{-1}$. Acquisition parameters were as follows: positive electrospray ionization with turbo spray source, curtain gas at 172 kPa, ion spray voltage of 4,000 V, and temperature of 600 ˚C. The precursor and product ion masses and optimized mass spectrometer conditions for the determinations of SMZ, TET, and TYL are shown in Supplemental Table S1. All concentrations reported for TYL are based on tylosin-A.

2.6 | Extraction recovery

Preliminary tests were conducted before the experiments to investigate the recoveries of the three antibiotics and the reproducibility of the method. Soils previously collected from the Northeast Iowa Research and Extension Farm (43.0° N, 92.5° W) near Nashua, IA were fortified with SMZ, TET, and TYL. The antibiotic fortification solution (0.025 mg ml^{-1}) was prepared in methanol. The extraction recoveries for a spiked concentration of 5 mg kg^{-1} in row crop soil were 48, 24, and 19% for SMZ, TYL, and TET, respectively. Antibiotics were detected in trace amounts in nonspiked samples (background), principally for TET, despite extensive efforts to eliminate these residues. The background concentration measured by the liquid chromatography–tandem mass spectrometry (LC–MS/MS) instrument was determined to be 0.0718 ± 0.070 μg kg^{-1}, calculated by taking the mean concentration of the unspiked control samples (Supplemental Table S2). This background concentration was subtracted from the concentrations of SMZ, TET, and TYL in the spiked soil sample extracts. Concentrations are reported without correction for extraction efficiency. The extraction efficiency for TET is somewhat lower than those reported previously (O'Connor & Aga, 2007) and reflects the inherent difficulties in optimizing extraction methods for these three antibiotics and the low antibiotic concentrations that were used in the experiment.

2.7 | Within-jar variability

To ensure that the variability of antibiotic distribution within jars was not greater than antibiotic concentration variability between jars, initial (Day 0), samples were subsampled in triplicate from each jar for treated and nontreated soils (Supplemental Table S3). A one-way ANOVA completely randomized design with subsampling in SAS was used to compare variability for three within-jar subsamples to the variability between the three true replicate jars. Within-jar variability was shown to be similar to between-jar variability. Therefore, a single subsample was taken from each jar for Days 3–72.

2.8 | Degradation kinetics

The decline in antibiotic concentration with time for SMZ, TYL, and TET was evaluated using the first-order linear regression kinetics (Equation 1) used to describe pesticide degradation in soil by Beulke and Brown (2001), as shown below. The integrated form of Equation 1 gives Equation 2. The logarithmic form of Equation 2 provides a linear relationship in which the slope is equivalent to the minus decay constant, k. Decay constants were used to calculate half-lives (Equation 4)—the time at which a concentration reaches half of the initial (Day 0) concentration—and were analyzed for significant differences between row crop treatments and prairie strip treatments.

$$\frac{dC}{dt} = -kC \tag{1}$$

$$C_t = C_0 \exp(-kt) \tag{2}$$

$$\ln C_t = \ln C_0 - kt \tag{3}$$

$$t_{1/2} = \frac{0.693}{k} \tag{4}$$

where C = concentration (μg kg^{-1} soil), t = time (days), k = degradation rate (days^{-1}), C_t = concentration at time t (μg kg^{-1} soil), C_0 = the concentration at time 0 (μg kg^{-1} soil), and $t_{1/2}$ = time for 50% degradation of the initial amount of antibiotic (days). A biexponential model was also evaluated, but R^2 values were not improved. Regression models were evaluated using JMP statistical software (SAS Institute).

2.9 | Statistical analysis

To test for any significant differences between degradation rates for crop soil and prairie strip soil, ANOVA with randomized block design followed by a t test was performed using JMP statistical software. The three sampling sites were treated as true replicate block groups to compare the effect of treatment (crop vs. strip soil) on k value. One-way ANOVA with randomized blocks followed by a t test was also used to evaluate the effect of antibiotic type (TET, SMZ, or TYL) on k value. A one-way ANOVA of k value vs. site followed by a t test was performed for each antibiotic to determine the influence of the sampling site on the decay constant.

3 | RESULTS AND DISCUSSION

3.1 | Degradation of SMZ, TET, and TYL in row crop and prairie strip soils

The quantity of SMZ, TET, and TYL recovered from row crop and prairie strip soils over the 72-d incubation period is shown for each sampling site in Figure 1. Initial antibiotic concentrations in soil closely reflected the concentrations in the mixture applied to the soil (~10 μg kg^{-1} soil) (Figure 1). Although each antibiotic decreased nearly to the detection limit during the incubation period, Day 72 concentrations of SMZ, TET,

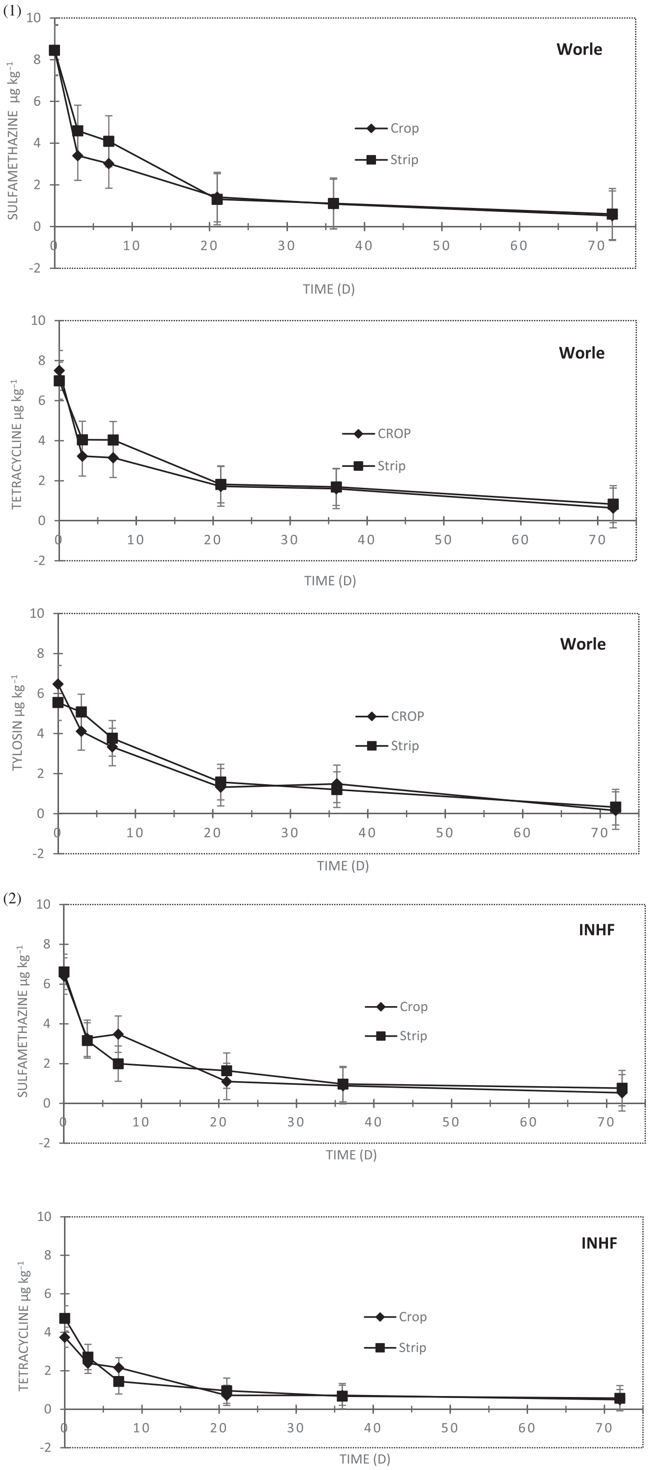

FIGURE 1 Degradation profiles of sulfamethazine, tetracycline, and tylosin in soil from prairie strips and adjacent cropped soil from three locations in Iowa: (1) Worle, (2) Iowa Natural Heritage Foundation (INHF), and (3) Neal Smith). Points indicate means ($n = 3$) and standard deviations

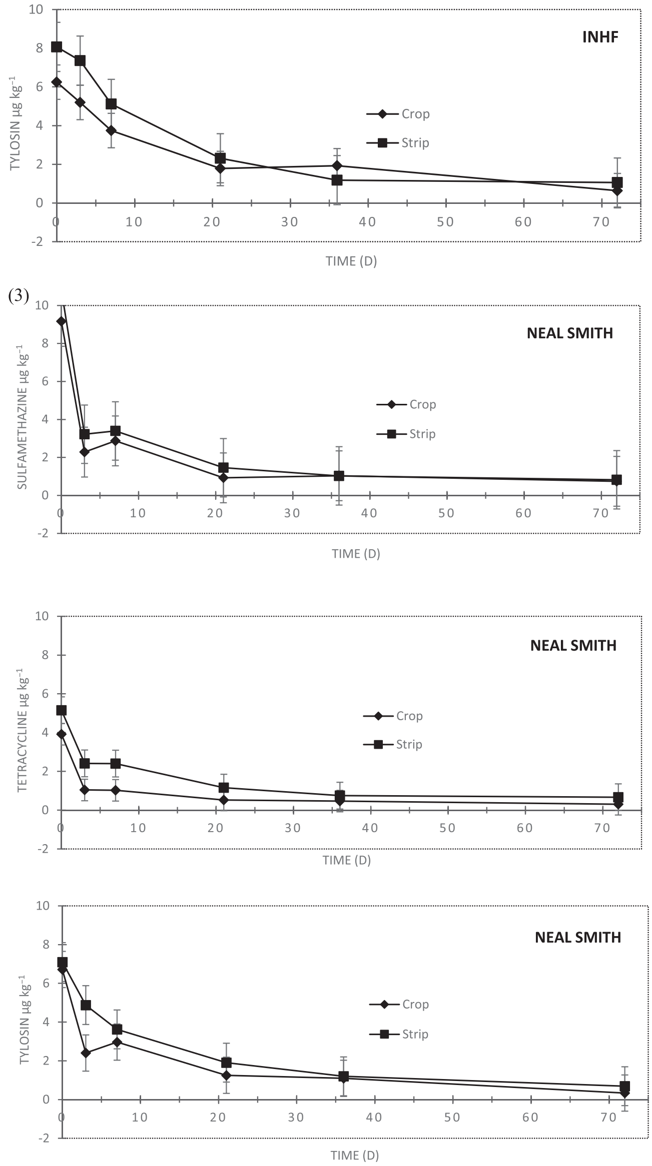

FIGURE 1 Continued

and TYL were significantly lower than concentrations on Day 36. In addition, the concentrations of SMZ, TET, and TYL were significantly different from background concentrations on the final sampling date (72 d), except for TYL in Worle crop soil. These results indicate that even at low concentrations, dissipation was still occurring throughout the last weeks of the study for the majority of treatments.

Half-lives and k values were the primary measure for persistence in this study, and these were determined from our linear regression model fit in JMP statistical software. Each regression fit was assessed by R^2 and the F statistic for

TABLE 1 Half-lives and rate constants (*k*) describing degradation of three antibiotics in cropped soils or in adjacent prairie strips soils at three sites in Iowa

Site	Antibiotic	Soil type	R^2	Half-life (days)	*k* value	*k* value confidence interval		*F* ratio[a]	*P* value[b]	*k* value SE
						Lower 95%	Upper 95%			
INHF	SMZ	Crop	.89	13.19	0.053	0.018	0.087	23.84	.016	0.011
		Strip	.79	16.12	0.043	0.003	0.083	11.58	.042	0.013
	TET	Crop	.86	15.00	0.046	0.012	0.081	17.70	.025	0.011
		Strip	.83	14.60	0.047	0.007	0.088	14.17	.033	0.013
	TYL	Crop	.82	20.09	0.035	0.005	0.064	13.89	.034	0.009
		Strip	.99	12.67	0.055	0.045	0.064	358.81	.000	0.003
Neal Smith	SMZ	Crop	.65	13.87	0.050	−0.017	0.117	5.68	.097	0.021
		Strip	.79	12.85	0.054	0.003	0.105	11.18	.044	0.016
	TET	Crop	.65	15.23	0.045	−0.016	0.107	5.62	.098	0.019
		Strip	.90	14.78	0.047	0.017	0.077	24.97	.015	0.009
	TYL	Crop	.75	16.44	0.042	−0.003	0.087	8.88	.059	0.014
		Strip	.96	14.78	0.047	0.028	0.065	64.63	.004	0.006
Worle	SMZ	Crop	.82	14.15	0.049	0.007	0.091	13.92	.034	0.013
		Strip	.89	12.67	0.055	0.018	0.09	23.68	.017	0.011
	TET	Crop	.73	19.53	0.036	0.004	0.075	7.99	.066	0.013
		Strip	.84	18.78	0.037	0.008	0.066	16.33	.027	0.009
	TYL	Crop	.80	5.81	0.119	0.003	0.078	11.88	.041	0.012
		Strip	.95	15.27	0.045	0.026	0.065	55.39	.005	0.006
Mean	SMZ[c]	Crop		13.73 (± 0.40)	0.051					
		Strip		13.88 (± 1.56)	0.051					
	TET	Crop		16.59 (± 2.08)	0.042					
		Strip		16.05 (± 1.93)	0.044					
	TYL	Crop		14.11 (± 6.06)	0.039					
		Strip		14.24 (± 1.13)	0.049					

Note. INHF, Iowa Natural Heritage Foundation; SMZ, sulfamethazine; TET, tetracycline; TYL, tylosin.
[a]*F* ratio, the ratio of mean square model to mean square error. *F* ratio close to 1 confirms the null hypothesis and indicates the regression model is inadequate.
[b]*P* value, the probability of a greater *F* statistic resulting from chance. *P* value < .05 indicates that the model *F* statistic is significant.
[c]Means (± SD) are obtained by averaging *k* values over the three sites.

the regression. Although the log-linear regressions produced significant regression models, improved models (based on increased R^2 and *F* statistics) resulted when the last sampling period was removed from the regression model. Half-lives and their corresponding *k* values for each antibiotic in row crop and prairie strip soil are presented in Table 1 along with R^2 values for each regression. Regression results including the final sampling period can be found in Supplemental Table S4. Half-lives calculated from Equation 4 decay rate constants are summarized in Table 1.

Using the mean rate constants for antibiotic degradation in prairie strip or crop soil from each site (Table 1), ANOVA showed that TET degraded significantly ($p < .05$) faster in prairie strip soil than it did in row crop soil (Supplemental Table S5); however, the magnitude of the difference was slight, with a mean half-life of 16.59 d in crop soil and 16.05 d in strip soil. Decay rate constants showed no significant difference between treatments for SMZ and TYL. Of the three antibiotics tested, TET persisted 23 and 16% longer than SMZ and TYL in crop soil, respectively, and persisted 14 and 12% longer than SMZ and TYL in strip soil, respectively, when comparing decay rate constants. However, a similar ANOVA (Supplemental Tables S6 and S7) that examined mean persistence of antibiotics in crop and strip soils with sites as replicates indicated that there was no significant difference in decay between the three types of antibiotics. Sulfamethazine, TET, and TYL demonstrated mean half-lives of 13.8 ± 1.2 d, 16.3 ± 2.0 d, and 14.2 ± 4.4 d, respectively.

3.2 | Soil properties

To inform degradation results, physical and chemical soil health properties were analyzed for the strip and crop soils

TABLE 2 Physical and chemical properties of soils collected from the root zone of row crops and prairie strips from three locations in central Iowa

	2015		2013		2003		Mean (± SD)	
	Worle		INHF		NEAL			
Property	Crop	Strip	Crop	Strip	Crop	Strip	Crop	Strip
Bulk density, μg g^{-1}	1.19	1.39	1.16	1.14	1.24	1.09	1.19 (± 0.03)	1.21 (± 0.13)
pH	5.4	6.0	6.4	6.5	6.5	6.5	6.1 (± 0.52)	6.3 (± 0.25)
Potentially mineralizable N, μg N g^{-1}	28	30	43	48	40	46	37 (± 6.14)	42 (± 7.93)
Total N, μg N g^{-1}	1,314	1,363	1,948	2,128	1,845	1,889	1,702 (± 277)	1,793 (± 319)
Soil organic C, μg g^{-1}	13,823	13,966	20,754	24,301	20,255	21,843	18,277 (± 3,156)	20,037 (± 4,408)
Respired CO_2–C, μg g^{-1}	132	168	223	340	160	286	171 (± 38.2)	265 (± 71.8)
K[a], μg g^{-1}	133	164	255	175	149	170	179 (± 54.2)	170 (± 4.60)
Mg[a], μg g^{-1}	233	283	461	473	436	617	376 (± 102)	458 (± 137)
Mn[a], μg g^{-1}	71	64	123	121	101	45	99 (± 21.2)	76 (± 32.3)
P[a], μg g^{-1}	27	31	21	20	10	8	19 (± 7.10)	20 (± 9.15)
POM N, μg g^{-1}	132	36	154	82	421	528	236 (± 131)	215 (± 182)
POM C, μg g^{-1}	2,117	1,079	4,310	8,239	5,980	6,026	4,027 (± 1,654)	5,000 (± 3,444)
Sand, %	53	51	8	24	9	12		
Silt, %	27	31	65	52	63	57		
Clay, %	20	18	27	24	28	31		

Note. INHF, Iowa Natural Heritage Foundation; POM, particulate organic matter.
[a]Mehlich extractable cations and P.

at each of the three sampling sites. Because of their extensive root system, prairie grasses are expected to increase soil organic matter, improving nutrient content and microbial activity as a result. Past studies analyzing VA degradation have suggested that microbial activity, pH, and SOC content tend to influence sorption and dissipation of VAs in a soil environment (Lertpaitoonpan et al., 2009; Lin et al., 2010; Pan & Chu, 2016). Increased VA sorption leads to reduced mobility and bioavailability and should increase persistence as a result.

The three prairie strip sampling sites were selected based on their differing establishment ages. The oldest prairie strip, established in 2003, was located at Neal Smith. The INHF prairie strip was established in 2013, and the Worle prairie strip was established in 2015. We hypothesized that as the age of the prairie strip increases, so does soil health and microbial activity, decreasing antibiotic half-lives as a result. Instead, differences in prairie strip age and corresponding soil properties resulted in no discernable difference in antibiotic decay across sampling sites. Soil properties for crop and strip soil at each sampling site are shown in Table 2 below.

Although some differences were observed in the physical and chemical properties of soils between sites, few differences were noted between cropped and STRIP soils within a site. The mean SOC in STRIPS soil was 9.6% greater than SOC of cropped soil, but the difference was not statistically significant (Table 2). Total N content was also numerically greater in STRIPS soil than in cropped soil, but the magnitude of the difference was slight. Potentially mineralizable N and respiration were uniformly higher in prairie strip soils than in row crop soils, indicating higher microbial activity in prairie strip soil. A similar trend was observed for POM-C (except at Worle), which is an indicator of recent additions to the organic matter pool. However, these three parameters were not statistically different between cropped and STRIP soil. Soil pH was similar for the two soils at each site, and no clear trend was observed in Mehlich extractable ions between soil types.

The most recently established prairie strip site, Worle, had lower soil respiration, pH, and POM-C, than INHF or Neal Smith soils, suggesting soil health may improve in the root zone of prairie strips over time. This may reflect greater soil erosion or tillage intensity at this site prior to STRIP establishment, the length of time since STRIP establishment, or some combination of these factors. Worle crop and strip soils also demonstrated lower SOC content than INHF or Neal Smith, possibly caused by farming practices prior to establishing the crop and strip management. Because SOC can affect nutrient retention and cycling, soil structure, and moisture retention, the decreased soil health at Worle may have also been influenced by reduced SOC (Blanco-Canqui et al., 2013). Soils at the three sites had similar clay content, but there was less silt and more sand at the Worle site compared with the other sites and little apparent difference in texture due to vegetation at each site.

3.3 | Degradation of SMZ, TET, and TYL in relation to soil properties

Sulfamethazine followed first-order degradation behavior, with R^2 values ranging from .65 to .89 (Table 1). Mean half-lives for SMZ were 13.73 ± 0.4 and 13.88 ± 1.6 d in crop and strip soil, respectively. Earlier laboratory-based studies have found SMZ half-lives to range between 1.2 and 63 d under varying soil treatments (Accinelli et al., 2007; Lertpaitoonpan et al, 2015; Pan & Chu, 2016). Previous studies have shown sulfonamide degradation to be impacted by starting concentrations of the compound, soil moisture, temperature, soil type, and extent of microbial activity (Accinelli et al., 2007; Lertpaitoonpan et al., 2015; Wang et al., 2006). Lower initial concentrations may have resulted in shorter half-lives due to the influence of the antibiotic concentration on the degrading microorganisms in the soil (Wang et al., 2006). The SMZ sorption to soil decreases with increasing soil pH (Chu et al., 2013; Lertpaitoonpan et al., 2009). However, Lertpaitoonpan et al. (2009) determined that SMZ sorption increases with increasing SOC. Whereas pH and SOC were higher in crop soils, the difference was not substantial in most cases, indicating that any soil health benefits provided by the presence of prairie grasses were not substantial enough to affect SMZ persistence.

Tetracycline followed first-order degradation behavior, with R^2 values ranging from .65 to .90 (Table 1). When treating sampling sites as true replicate blocks, TET decay rate constants were shown to be significantly higher in strip soil than in crop soil, though the mean half-lives of 16.59 ± 2.1 and 16.05 ± 1.9 d for crop and strip soil, respectively, were similar. Tetracycline may have been more susceptible to differences in microbial activity between the two soils, which was indicated by enhanced soil respiration in strip soils. However, sorption of tetracyclines is negatively correlated with pH and SOC, which may have lowered TET bioavailability in crop soils and accounted for the minimal difference in half-lives. Our result falls in the wide range of TET half-lives shown by prior laboratory-based studies. Pan and Chu (2016) determined TET to have a half-life of 31 d, whereas a similar incubation study by Lin et al. (2010) found that TET dissipated completely within 5 d in various soil treatments.

Tylosin followed first-order degradation behavior, with R^2 values ranging from .75 to .99 (Table 1). Mean half-lives for TYL were 14.11 ± 6.0 and 14.24 ± 1.1 d in crop soil and strip soil, respectively. A previous laboratory-based study determined TYL to have a half-life of 4.4 d (Carlson & Mabury, 2006), and field studies have found half-lives ranging from 4.5 to 67 d (Carlson & Mabury, 2006; Halling-Sørensen et al., 2005). Tylosin did not exhibit a significant difference between prairie strip and crop soil. Tylosin sorption is positively correlated with both clay content and SOC (Sassman et al., 2007). Differences in SOC between treatments were minimal, and the texture makeup from each sampling site (Table 2) indicates similar clay contents between crop soil and strip soil, potentially resulting in similar TYL sorption and thus similar bioavailability for degradation.

3.4 | Implications of antibiotic persistence in soils

This study addresses an environmentally relevant initial VA concentration of 10 μg kg^{-1} to model accurate environmental concentrations that would be found in agricultural soils or overland runoff after manure application. Some of the variance in VA persistence between this study and previous studies can likely be attributed to differing initial antibiotic concentrations of the fortified soils. Pan and Chu (2016) found that VA persistence tends to increase with increasing starting concentrations; however, the VAs used in this study showed similar half-lives compared with VA degradation studies using higher initial concentrations. This suggests that VAs may be more persistent than expected at low environmental concentrations. Earlier research on VA degradation has shown half-lives ranging from days to months; comparatively, the antibiotics in this study exhibited moderate persistence in each soil treatment, indicating that they would not be likely to accrue in soil between seasonal manure applications.

However, antibiotics at these concentrations may be more susceptible to accumulation in a field environment. The controlled conditions used in this study are not directly comparable with dissipation after field application of manure, where leaching, climate, and other variables will be influencing factors. Research has shown that degradation is also influenced by temperature, with half-lives throughout the nongrowing season up to eight times longer than half-lives during the growing season (Amarakoon et al., 2016). Thus, temperature differences may contribute to some of the discrepancies between studies. By the end of the 72-d study period, all three VAs returned to concentrations near the measured background level of 0.0718 μg kg^{-1}. However, soils in this study were incubated at 30 ˚C—if manure is applied during lower temperatures or in late fall, antibiotics may dissipate slowly, potentially persisting until spring rainfall. Further, if manure is applied in spring prior to planting, it is likely that some SMZ, TET, or TYL remains available during the growing season for transport to downstream waters. For example, Washington et al. (2018) detected the same VAs frequently (69–100% of the time over 3 yr) in a watershed with high intensity of animal production. Veterinary antibiotics have also been shown to dissipate more quickly in soils that have previously received manure (Topp et al., 2013); however, none of the sites used in this study have a history of manure application, so persistence may be longer in comparison.

Each of the three VAs in this study decayed similarly in crop and strip soils, with TET degrading at a significantly faster rate in prairie strips when accounting for differences in sampling sites. Previous studies found that VAs degraded significantly more quickly in nonsterilized soils compared with sterilized soils (Lertpaitoonpan et al., 2015; Pan & Chu, 2016), and that certain VAs such as TYL degrade more quickly in manured soils as compared with nonmanured soils (Carlson & Mabury, 2006), indicating that higher soil microbial activity can lower VA persistence. However, soil microorganisms do not quickly metabolize ^{14}C-SMZ to ^{14}C-CO_2 (Lertpaitoonpan et al., 2015), a limited number of microbial strains with TET degradation potential have been isolated (Ahmad et al., 2021), and microorganisms that grow on TYL have not been isolated. Instead, microbial degradation produces a variety of metabolites that may or may not have bioactivity (Aga et al., 2005; Garcia-Galán et al., 2008; Sassman et al., 2007). Higher soil respiration in prairie strip soils in this study suggests more microbial activity compared with crop soil—thus, our hypothesis that higher microbial activity would correlate to lower persistence was unproven for TYL and SMZ. In a similar incubation study, Lin et al. (2010) determined that SMZ was significantly more persistent in the root zone soil of a grass species when compared with a control soil containing no plants. This suggests that the root structure of grass species may have increased soil organic material, creating more adsorption surface area and decreasing VA bioavailability. Therefore, the effects of enhanced soil microbial activity in prairie strip soil could be offset by the decreased bioavailability of the antibiotics, resulting in higher persistence in strip soil, indicating that microbial activity may not be the strongest factor affecting VA degradation in a field setting.

4 | CONCLUSION

Tetracycline showed significantly higher persistence in row crop soil than prairie strip soil, but the magnitude of the difference was fairly small, with a difference in means of less than 0.6 d, suggesting that a prairie strip treatment is unlikely to enhance antibiotic dissipation in soil compared to a row crop treatment. Half-lives were generally in agreement with existing literature, with variability potentially explained by differences in initial concentrations, incubation temperature, and soil properties. These results suggest that these VAs applied at environmentally relevant concentrations in runoff are likely not persistent in crop or prairie strip soils to accumulate between seasonal manure applications. The most recently established prairie strip site did have lower nutrient content, soil respiration, pH, and POM than the other two sites, suggesting soil health may improve in the root zone of prairie strips over time. Despite differences in soil health parameters, however, the soil sampling site also did not have a significant influence on antibiotic decay rate constant.

While findings from this study identified VA degradation under controlled conditions, environmental sampling studies are consistently able to detect VAs in agricultural soils and nearby surface and groundwaters, indicating that despite relatively short measured half-lives, environmental movement is occurring and contributing to the broader development of antimicrobial resistance. Further work is recommended on biodegradation of TET as well as the development and transport potential of metabolites on the overall contribution to antimicrobial resistance.

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AUTHOR CONTRIBUTIONS

Alyssa N. Iverson: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing-original draft; Writing-review & editing. Thomas B. Moorman: Conceptualization; Funding acquisition; Methodology; Resources; Project administration; Supervision; Validation; Writing-review & editing. Michelle L. Soupir: Conceptualization; Funding acquisition; Methodology; Resources; Project administration; Supervision; Validation; Writing-review & editing. Amy J. Morrow: Investigation; Methodology; Resources; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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