Impact of Poultry Litter Application and Land Use on *E. coli* Runoff from Small Agricultural Watersheds

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ABSTRACT. *Fecal bacteria contamination of surface waters continues to be a critical water quality concern with serious human health implications, but relatively few land use specific data sets are available to guide management, restoration, policy, and regulatory decisions. In regions with substantial poultry production, litter application sites are often assumed to be major contributors to bacterial contamination, and grazing lands often receive a similar focus. Since most states use* Escherichia coli *as an indicator organism for fecal contamination, this study was designed to measure* E. coli *concentrations in runoff from small agricultural watersheds with various land uses. Specifically, three years of water quality data were collected from 13 watersheds and analyzed to evaluate the impacts of litter application and land use on* E. coli *concentrations in runoff. In this study, litter application did not impact* E. coli *concentrations in runoff, which can at least partially be attributed to the late summer target application date. Litter was produced and removed from poultry houses during hot, dry conditions unfavorable for* E. coli *survival. Thus, late summer application may be a recommended practice to minimize* E. coli *runoff from litter application sites. Cultivated watersheds with and without litter application produced the lowest* E. coli *concentrations in runoff, presumably due to limited wildlife presence and livestock exclusion. In contrast, the ungrazed native prairie reference site produced relatively high* E. coli *concentrations in runoff, presumably due to increased fecal deposition from abundant wildlife. The high concentrations of* E. coli *from grazed lands emphasize the need for livestock producers to follow best management practice recommendations to minimize bacteria contribution; however, it is important to note that high* E. coli *concentrations were measured in runoff from well-managed grazing lands as well as ungrazed native prairie, which indicates the difficulty of managing bacterial contamination.*

Keywords. *Fecal bacteria, Surface water, TMDL, Watershed planning, Water quality.*

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Fecal contamination, often assessed through measurement of the indicator organism *Escherichia coli* (Byappanahalli et al., 2003; Chin et al., 2009), is the leading cause of surface water impairment in the U.S. (USEPA, 2009a). Fecal contamination of streams results from a combination of human and animal fecal material from point and nonpoint sources. Major point sources typically include wastewater treatment plants (WWTPs), storm sewers, and other legal and illegal domestic point-source discharges (Teague et al., 2009). Nonpoint sources of *E. coli* include runoff from rural, agricultural, and urban landscapes, and subsurface flow from leaking on-site wastewater treatment systems (Paul et al., 2006; Teague et al., 2009). In addition, direct fecal deposition by wildlife and livestock often contribute *E. coli* (Zeckowski et al., 2005; Paul et al., 2006).

According to a 2008 report (TCEQ, 2008), 295 Texas streams were identified as impaired by bacteria and placed on the 2008 Texas 303(d) list. These impairments were based on the previous bacteria concentration criteria (geometric mean of 126 CFU per 100 mL and a single-sample maximum of 394 CFU per 100 mL for *E. coli*) designed to protect contact recreation use (Texas Administrative Code, 2004); however, recently revised criteria remove the single-sample criterion and establish additional recreational uses, such as secondary contact recreation.

In parts of Texas and in other states with concentrated large-scale poultry production, land-applied litter is often assumed to be a major contributor to bacterial contamination. However, field-scale data supporting this assumption are limited. On four field plots (1.3 to 2.7 ha) in Georgia with natural rainfall, Jenkins et al. (2006) reported an average concentration of 3,000 MPN per 100 mL for one runoff event that occurred within one month of fresh litter application. They also reported that concentrations in other runoff events were at background levels. In a three-year study of runoff from 0.6 ha cotton fields fertilized with raw poultry litter (7.2 to 9.2 Mg ha^{-1}), Vories et al. (2001) reported that *E. coli* concentrations in nine runoff events were quite variable, ranging from 13 to 9,800 CFU per 100 mL. Vories et al. (2001) also commented that the *E. coli* data set was much more limited than for traditionally sampled parameters (i.e., sediment, nitrogen, and phosphorus) in that study because highly restrictive quality-assurance protocols for bacteria eliminated numerous data points. On a small plot (4 m^2) study in Oklahoma, Guzman et al. (2010) reported average background *E. coli* concentrations of 6,800 MPN per 100 mL in runoff from pasture plots with simulated rainfall. In the Oklahoma study, plots with 4.9 Mg ha^{-1} of poultry litter applied (one-year-old litter applied the same day as clean out) produced event-mean *E. coli* concentrations that ranged from 7,100 to 22,000 MPN per 100 mL for simulated rainfall events within five days of application. Soupir et al. (2006) reported *E. coli* runoff concentrations between 9,270 and 16,500 CFU per 100 mL from 55 m^2 pasture plots treated with dried turkey litter stored under a shed for three weeks (2.8 Mg ha^{-1}) and simulated rainfall within 24 h. Sistani et al. (2010) reported much lower *E. coli* concentrations in runoff from plots with inorganic or no fertilizer applied (<100 CFU per 100 mL) and from plots with 9.0 Mg ha^{-1} of broiler litter applied (10 to 1,000 CFU per 100 mL); however, they also showed significant reductions in *E. coli* concentrations in simulated runoff occurring more than one week after application.

There is a critical need to address bacterial contamination of water and properly target contributing sources. Therefore, this study was designed to provide real-world data to support upland bacteria fate and transport modeling and science-based land management decisions related to poultry litter application and land use. Specifically, the objective was to collect runoff water quality data from 13 field-scale to farm-scale watersheds to evaluate litter application and land use impacts on *E. coli* runoff.

Materials and Methods

Site Description

All of the study watersheds were located at the USDA-ARS Grassland, Soil and Water Research Laboratory near Riesel, Texas (fig. 1). The research site is dominated by Houston Black clay soil (fine, smectitic, thermic, Udic Haplustert), which is recognized throughout the world as the classic Vertisol. These highly expansive clays, which shrink and swell with changes in moisture content, have a typical particle size distribution of 17% sand, 28% silt, and 55% clay. These soils are very slowly permeable when wet (saturated hydraulic conductivity of approx. 1.5 mm h^{-1}); however, preferential flow associated with soil cracks contributes to high infiltration rates when the soil is dry (Arnold et al., 2005; Allen et al., 2005; Harmel et al., 2006c).

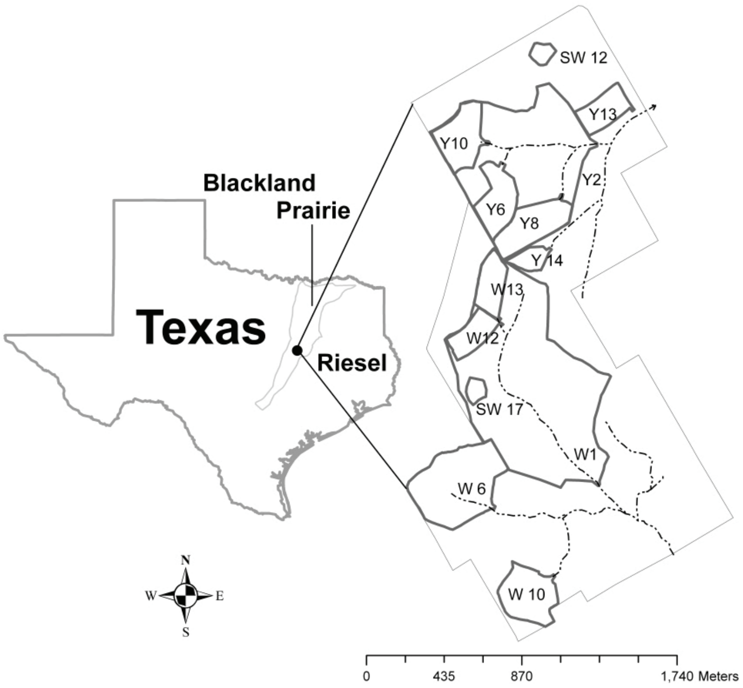

Figure 1. Field-scale and small watershed-scale sites (listed by name, e.g., SW12, Y2, W10) at the Riesel Watersheds.

Table 1. Land management and fertilizer data for cultivated and pasture watersheds.

	Cultivated Watersheds						Pasture Watersheds			
	Y6	Y13	Y10	W12	W13	Y8	SW12	SW17	W10	Y14
2008-2009										
Litter rate[a]	0.0	4.5	6.7	9.0	11.2	13.4	0.0	0.0	0.0	0.0
Crop/ land use	Corn	Corn	Corn	Corn	Corn	Corn	Native (hay)	Coastal (grazed, 1.5)[b]	Coastal (hay)	Klein (hay)
2009-2010										
Litter rate[a]	0.0	4.5	6.7	9.0	11.2	13.4	0.0	0.0	0.0	0.0
Crop/ land use	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Native (hay)	Coastal (grazed, 2.5)[b]	Coastal (hay)	Klein (hay)
2010-2011										
Litter rate[a]	0.0	4.5	6.7	9.0	11.2	13.4	0.0	0.0	0.0	0.0
Crop/ land use	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Hay grazer	Native (hay)	Klein (hay)	Coastal (grazed, 1.1)[b]	Klein (hay)

[a] Litter rate is expressed as Mg ha^{-1} year^{-1}.

[b] Grazing rate expressed as ha AU^{-1} year^{-1}.

Table 2. Descriptive data for the mixed land use watersheds.

	Mixed Land Use Watersheds		
	Y2	W1	W6
Area (ha)	53	71	17
Cultivated (%)	56	30	73
Improved pasture (%)	4	32	0
Rangeland (%)	39	38	27

Cultivated Watersheds

Thirteen fields (actually field-scale to farm-scale watersheds) were the experimental units for this study (tables 1 and 2). For the six cultivated watersheds, the within-year management was consistent to assess the litter rate treatment effect. Litter application was planned for the late summer each year, as soon as possible after 1 August, to increase the likelihood of dry field conditions for application; however, wet conditions delayed the 2008 application until October and the 2009 application until January 2010. The litter was obtained in the vicinity of the study site from the cleanout (either complete cleanout for multiple flocks or “cake out” from a single flock) of turkey or chicken (broiler) houses. The bedding material in the litter was either wood shavings or rice hulls.

In each year, each cultivated watershed received the same poultry litter application rate (0, 4.5, 6.7, 9.0, 11.2, or 13.4 Mg ha^{-1}), as determined *a priori* and randomly assigned in 2001 (table 1). Each field with applied litter also received supplemental inorganic N as recommended to meet agronomic requirements. The cultivated control site (Y6, 0.0 Mg ha^{-1} litter) received only inorganic N and P fertilizer. Each cultivated field had broad-base terraces on the contour and grassed waterways at the terrace outlets. Management consisted of typical practices including tillage, planting, harvest, and application of fertilizer, herbicide, and pesticide.

Pasture Watersheds

Four pasture watersheds were also included in this study (table 1). One of the pasture watersheds (SW12), a native (remnant) prairie that has never received litter or inorganic fertilizer, served as a control site. Litter application to pasture watersheds W10 and Y14 stopped in 2007 after seven annual applications. SW17 was rotationally grazed from

2007 to 2010, and W10 was rotationally grazed from 2010 to 2011. Overall, pasture management consisted of litter application (surface applied), hay harvest (or grazing), and herbicide application.

Mixed Land Use Watersheds

Three larger, mixed land use watersheds (table 2) were evaluated in addition to the ten homogeneous fields (field-scale watersheds). These mixed land use watersheds contained grazed pasture, hay pasture, and cultivated cropland. Two field-scale watersheds within Y2 received litter (Y8 and Y10). Similarly, two field-scale watersheds within W1 received litter (W12 and W13), but no litter was applied within watershed W6.

Water Quality Sample Collection

Data collection began in August 2008 and encompassed three study years from August through July (2008-2009, 2009-2010, and 2010-2011). For each site, runoff data and water quality samples were collected from a flow control structure (v-notch weir or a flume) located at the field outlet. For runoff events, water quality samples were collected with an ISCO 6700 automated sampler (ISCO, Inc., Lincoln, Neb.). Each sampler was programmed to collect frequent flow-interval (1.32 mm volumetric depth) samples and composite them into a single 16 L bottle, as discussed by Harmel et al. (2006a, 2006b). Prior to collection of each sample, each sampler executed a rinse of the sample tubing with ambient water. Because the samples were collected on equal flow intervals and composited into a single bottle, the resulting *E. coli* concentrations represent event mean concentrations (EMCs).

Storm event samples were retrieved from the field within 24 h of sample collection. For each sample bottle, a thoroughly mixed subsample was poured into a 0.71 L (24 oz.) sterile, polyethylene Whirl-Pak bag (NASCO, Inc., Fort Atkinson, Wisc.). In addition, a grab sample was collected in a Whirl-Pak bag for selected events by carefully opening the bag to avoid touching the interior and submerging it below the water surface pointing upstream. Once the sample bags were approximately 3/4 full, they were twirled, securely closed, and checked for leaks by gently squeezing. Samples were stored in a cooler on ice during transport to the laboratory.

E. coli Enumeration

Determination of the *E. coli* concentration in each water sample with EPA Method 1603 (USEPA, 2006) was initiated within 6 h of sample retrieval. Four dilutions (10, 1, 0.1, and 0.01 mL) were filtered using 0.45 µm membrane filters. The filters were then placed in petri dishes containing modified mTEC agar and incubated at 35°C ±0.5°C for 2 h ±0.5 h to resuscitate injured or stressed bacteria and then incubated at 44.5°C ±0.2°C for 22 h ±2 h. Finally, the red or magenta colonies were counted and recorded. Ideally, between 20 and 80 colonies appeared on the petri dishes. For quality control, 100 mL of phosphate-buffered saline was processed as a blank with each batch of samples, and a laboratory duplicate was evaluated with each batch. In addition, *E. coli* levels in selected litter samples (August 2010 and September 2011) were determined, using EPA Method 1603 as described above, immediately prior to litter application.

Data Analysis

Data analysis focused on determining the effects of poultry litter application and land use on *E. coli* runoff. The effect of litter application rate was evaluated with three years of data from the six cultivated watersheds (table 1). Box-and-whisker plots, regression analysis, and Mann-Whitney tests were used to examine the potential relationship between litter rate and *E. coli* concentrations. The effect of land use was evaluated with three years of data from the six cultivated, four pasture, and three mixed land use watersheds. Specifically, cultivated, native (remnant) prairie, hayed pasture, grazed pasture, and mixed land uses were compared graphically and with Mann-Whitney tests.

All statistical analyses used an *a priori* significance level of $\alpha = 0.05$ and were conducted with Minitab software (Minitab, 2000) according to procedures described by Helsel and Hirsch (1993) and Haan (2002). Since the majority of the data were not normally distributed based on Kolmogorov-Smirnov test results, the Mann-Whitney test was used to determine significant differences in median values between data sets.

Results and Discussion

E. coli in Runoff

In this three-year study, samples were collected from 262 runoff events and enumerated for *E. coli* concentrations. These data were then used to evaluate the impacts of litter application and land use. Descriptive statistics for each site and each year are listed in table 3. As with all measured data (Harmel et al., 2006a, 2009), the inherent uncertainty associated with determining *E. coli* concentrations contributed variability to the resulting data. Although detailed uncertainty analyses were not conducted specifically for these data, the uncertainty for individual *E. coli* concentrations can be estimated to average ±33% and range from ±15% to ±67% based on McCarthy et al. (2008).

Table 3. Annual summary statistics for event mean *E. coli* concentrations (CFU per 100 mL).

	Cultivated Watersheds						Pasture Watersheds				Mixed Land Use Watersheds		
	Y6	Y13	Y10	W12	W13	Y8	SW12	SW17	W10	Y14	Y2	W1	W6
2008-2009[a]													
Median	360	645	405	400	280	330	110	38,000	2,700	460	8,000	13,800	76,000
Mean	388	525	613	348	288	348	128	45,020	3,733	390	8,367	26,600	159,800
Max.	540	710	1,500	520	400	680	220	113,000	7,300	700	9,100	55,000	>400,000
Min.	290	100	140	70	190	50	50	7,100	1,200	10	8,000	11,000	3,400
n	4	4	4	4	4	4	5	5	3	3	3	3	3
2009-2010													
Median	690	575	390	240	380	675	2,750	3,550	1,080	800	4,200	2,450	2,150
Mean	1,326	905	895	607	1,078	1,456	5,854	63,703	966	1,134	6,923	7,679	4,177
Max.	3,700	2,800	3,800	3,500	8,300	7,600	21,000	>800,000	2,300	2,600	38,000	44,000	30,000
Min.	80	20	40	10	30	90	500	20	20	10	720	270	270
n	11	20	18	13	11	16	16	14	13	13	20	18	16
2010-2011[b]													
Median	-	-	-	-	-	-	-	-	-	-	-	-	4,350
Mean	-	-	-	-	-	-	-	-	-	-	-	-	4,350
Max.	160	1,100	150	150	3,100	3,300	1,200	200	6,900	410	2,500	2,000	7,800
Min.	-	-	-	-	-	-	-	-	-	-	-	-	900
n	1	1	1	1	1	1	1	1	1	1	1	1	2

[a] For the first storm (March 2009), *E. coli* concentration was determined by the membrane filter procedure using m-ColiBlue24 broth (Millipore, Billerica, Mass.) with an EPA-approved method developed by Hach (USEPA, 2009b).

[b] Due to the drought in 2010-2011, only one sample was collected from each site, except for W6, which produced two samples.

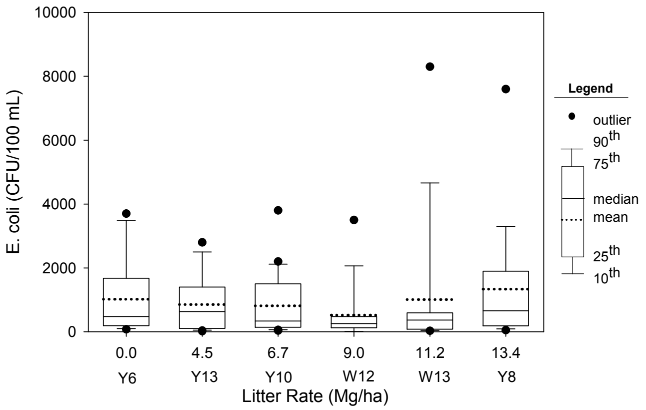

Figure 2. Runoff *E. coli* concentrations from cultivated watersheds with differing poultry litter application rates.

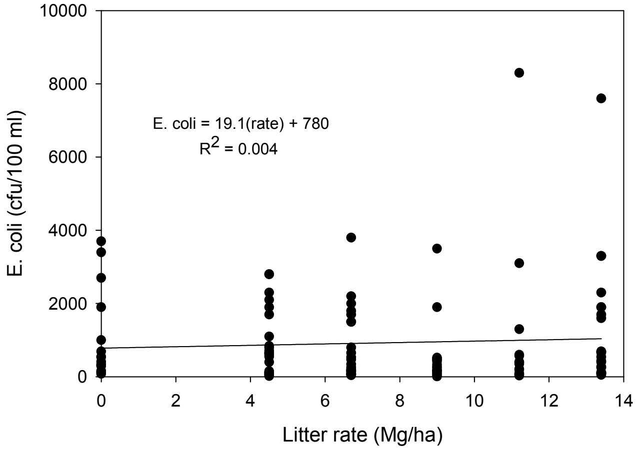

Figure 3. Regression relationship between litter rate and runoff *E. coli* concentrations.

Impact of Litter Application

Since the six cultivated watersheds received the same management within years (table 1), the treatment effect (litter application rate) was readily assessed. Litter application rate did not appear to affect the ranges or magnitudes of *E. coli* concentrations in runoff (fig. 2). The lack of treatment effect was confirmed by regression analysis (r^2 = 0.004, p = 0.51), as shown in figure 3. Furthermore, no significant differences in median concentrations were determined when data from the cultivated site with no applied litter (Y6) were compared with data grouped from all the cultivated watersheds that received litter. In fact, the overall median value for the cultivated watershed with no litter application was larger than that of watersheds with litter application. In terms of the extremely high *E. coli* concentrations (fig. 2), the 8,300 CFU per 100 mL value for the 11.2 Mg ha^{-1} litter rate (W13) occurred approximately one year after litter application, and the 7,600 CFU per 100 mL value for the 13.4 Mg ha^{-1} litter rate (Y8) occurred more than three months after application. Therefore, it was unlikely that poultry litter was the source of the high *E. coli* concentrations. Even if the initial *E. coli* levels in the applied litter were high, suboptimal temperature and moisture conditions in the environment typically result in rapid decline in introduced *E. coli* populations. Although specific die-off rates are difficult to predict, general estimates for first-order *E. coli* die-off are 0.51 d^{-1} for warm months and 0.36 d^{-1} for cold months (USEPA, 2000). While population decline is likely the typical occurrence, re-growth has been reported under some conditions (van Elsas et al., 2011).

Concentrations of *E. coli* in poultry litter can vary widely, with levels from below detection limits to 10^8 CFU (or MPN) g^{-1} being reported in various studies (Pope and Cherry, 2000; Terzich et al., 2000; Ashbolt et al., 2001; Brooks et al., 2009; Weidhaas et al., 2010). Therefore, *E. coli* in litter applied in August 2010 and September 2011 were enumerated using modified mTEC agar and EPA Method 1603 (USEPA, 2006) to further investigate the potential of litter to affect *E. coli* runoff (in hindsight, *E. coli* in litter should have been enumerated each year, but this realization was not made until 2010). *E. coli* levels in both samples were below the detection limit (100 CFU g^{-1} wet litter), likely due to the hot, dry conditions in Texas during the summer, which produced conditions unfavorable for *E. coli* survival at the time that litter was removed from the poultry houses and land applied (Wilkinson et al., 2011). Under these conditions, the lack of significant differences in runoff *E. coli* concentrations among watersheds with various litter rates was not surprising.

The substantial decrease in *E. coli* concentrations in drier litter relative to wetter litter or fresh feces is attributed mainly to litter desiccation. Once moisture levels fall below 70% to 75%, first-order die-off has been documented for *E. coli* in manure (Sinton et al., 2007). The typical hot summer conditions in Texas, which necessitate that poultry house cooling fans run almost full-time, produce relatively dry litter (average of 28% moisture, unpublished data from the Riesel application site) that favors more desiccation-tolerant microorganisms and is not conducive to *E. coli* survival (Dumas et al., 2011). A study by Brooks et al. (2009) in Mississippi using wetter litter (51.5%) containing a moderate level (~10^3 MPN g^{-1}) of thermotolerant coliforms (e.g., *E. coli*) found that litter application did not increase thermotolerant coliform release in rainfall simulations relative to the unamended control and that levels decreased to detection limits or below within 11 days. In contrast, Soupir et al. (2006) reported that application of turkey litter (49% water content; 3,000 CFU *E. coli* g^{-1}) significantly increased *E. coli* levels in runoff from rainfall simulations relative to the control. However, these rainfall simulations were conducted within 24 h of litter application. The combination of shorter time between application and runoff, higher moisture content of the litter, and lower temperatures explains these differing results, as studies have shown that microbial die-off is relatively rapid at elevated temperatures, especially when moisture content is reduced (Ferguson et al., 2003; Gallagher et al., 2012; Padia et al., 2012).

The median *E. coli* concentrations in runoff from the cultivated watershed with no litter (475 CFU per 100 mL) were similar to concentrations measured from other small cultivated watersheds in central Texas by Harmel et al. (2010) (965 and 300 CFU per 100 mL) but much higher than the concentrations reported by Jenkins et al. (2006) as background levels on cropped fields in Georgia (mean = 13 MPN per 100 mL). In the present study, the range of *E. coli* concentrations in individual storms for watersheds with litter applied (10 to 8,300 CFU per 100 mL) was similar to the range reported for cotton fields fertilized with 7.2 to 9.2 Mg ha^{-1} poultry litter (13 to 9,800 CFU per 100 mL) reported by Vories et al. (2001). Results from the present study, along with these previous field-scale studies under natural rainfall conditions, indicate that poultry litter application typically does not significantly increase *E. coli* concentrations in runoff. However, these field-scale studies, along with rainfall simulation studies such as Guzman et al. (2010), Soupir et al. (2006), and Sistani et al. (2010), generally conclude that application of litter with high initial *E. coli* levels may be a substantial contributor to bacterial impairment but only in runoff events occurring very soon after application. Those studies also pointed out that background populations of *E. coli* (e.g., *E. coli* from wildlife or naturalized populations of *E. coli*) are increasingly important in subsequent runoff events.

In the present study, only in 2009-2010 did runoff occur soon after litter application. In 2008-2009 and 2010-2011, the first runoff event occurred more than four months after litter application; however, in 2009-2010, litter was applied on 11-12 January 2010 and runoff occurred on 16 January. The *E. coli* concentrations in that first runoff event ranged from 50 to 200 CFU per 100 mL, which is lower than the annual means and medians shown in table 3. These results provide further support that litter application alone may not increase *E. coli* concentrations in runoff.

Impact of Land Use

The 13 study watersheds were categorized into five land uses: cultivated, native (remnant) prairie, grazed pasture, hayed pasture, and mixed land use. While neither litter presence/absence nor litter rate affected runoff *E. coli* concentrations, the impact of land use was readily apparent. Mean and median *E. coli* concentrations occurred in the following order: cultivated < hayed pasture < native prairie < mixed land use < grazed pasture (table 4, fig. 4). As the magnitudes increased, the standard deviations increased as well.

Further, wildlife habitat and presumably wildlife abundance and biodiversity also

Table 4. Summary statistics for event mean *E. coli* concentrations (CFU per 100 mL) for differing land uses.

Statistic	Cultivated	Hayed Pasture	Native Prairie	Mixed	Grazed Pasture
Median[a]	410 a	800 a	2,000 b	3,400 b	8,550 b
Mean	923	1,185	4,341	14,045	56,192
SD	1,330	1,406	5,790	49,679	176,990
Maximum	8,300	7,300	21,000	>400,000	>800,000
Minimum	10	10	50	270	20
n	119	34	22	67	20

[a] Medians followed by the same letter are not significantly different ($\alpha = 0.05$).

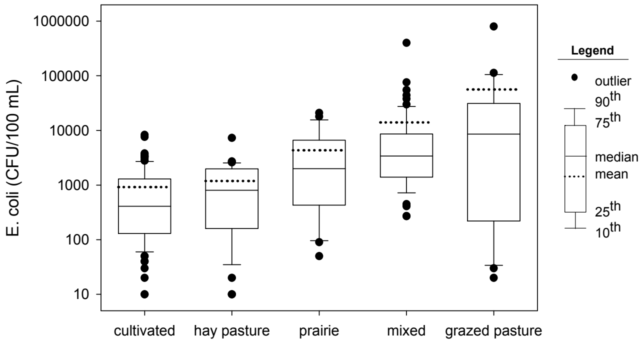

Figure 4. Runoff *E. coli* concentrations from various land uses.

improved along this same land use gradient, at least from cultivated fields to hay pasture to native prairie. As such, it was not surprising that the cultivated fields produced lower *E. coli* concentrations in runoff than did the hayed pasture or native prairie. In addition, the soil surface in cultivated fields was bare for prolonged periods during the year, which would have exposed the soil to more solar radiation and drying, thus likely reducing *E. coli* populations further (van Elsas et al., 2011). The further increase in *E. coli* concentrations in runoff from the native prairie relative to the hayed pasture likely resulted from a more abundant wildlife population resulting from the diverse vegetation and habitat on the native prairie (Aschwanden et al., 2007).

The effect of grazing cattle on *E. coli* concentrations was evident in this study (fig. 4, table 4), as the two land uses with grazing cattle (grazed pasture and mixed land use) produced the highest *E. coli* concentrations. The specific causes of the three *E. coli* concentrations greater than 100,000 CFU per 100 mL (table 3) are unknown. However, it is speculated that direct cattle access to the flume contributed to the >400,000 CFU per 100 mL value from W6 (mixed land use), and the small runoff volume (<2 mm) and lack of dilution contributed to the >800,000 CFU per 100 mL value from SW17 (grazed pasture). These two values are the only *E. coli* enumerations in the study that were too numerous to count (for statistical analyses, they were conservatively considered to represent 400,001 and 800,001 CFU per 100 mL, respectively). In any case, direct fecal deposition from cattle likely contributed substantial *E. coli* in these events, although cattle presence did not produce such extreme values in other events.

The concentrations of *E. coli* in runoff in the present study were generally higher than measured in other studies of grazed pastures. Wagner et al. (2012) reported median *E. coli* concentrations in runoff between 5,591 and 7,100 CFU per 100 mL for heavy and moderately grazed pastures near College Station, Texas, and 4,750 CFU per 100 mL for a grazed site near Sinton, Texas. Harmel et al. (2010) reported lower median *E. coli* runoff concentrations (2,465 CFU per 100 mL) for two heavily grazed horse and bull traps and concluded that moderate grazing would likely reduce *E. coli* concentrations. However, this was not the case in the present study, with higher *E. coli* concentrations resulting from a moderate stocking rate (1.1 to 2.5 ha AU^{-1} $year^{-1}$).

Harmel et al. (2010) concluded that higher *E. coli* concentrations in grazed pasture compared to cultivated land cannot be attributed solely to grazing cattle because of the likelihood of increased wildlife presence in pasture relative to cultivated land. Runoff data from the present study (from various land uses including ungrazed cultivated fields, grazed pastures, hayed pastures, and native prairie) made it possible to approximate the *E. coli* contribution of wildlife and cattle. Based on median *E. coli* concentrations for each land use (table 4), wildlife/background sources contributed 410 CFU per 100 mL on cultivated land and between 800 and 2,000 CFU per 100 mL on ungrazed pasture and native prairie. This represents an additional 390 to 1,590 CFU per 100 mL relative to cultivated land presumably due to wildlife. Similarly, grazing cattle and wildlife/background sources contributed 3,400 CFU per 100 mL on mixed land uses and 8,550 CFU per 100 mL on grazed pasture. Although these values are rough approximations, they agree with bacterial source tracking conducted on a limited number of surface waters in the region, which determined that wildlife and cattle (along with human sewage) were the leading sources of *E. coli* in the tested waters (Casarez et al., 2007). Together, these results indicate that wildlife/background should be considered in conjunction with livestock contributions when quantifying bacterial loads and when evaluating the contribution of agricultural practices to bacterial impairments.

Summary and Conclusions

This three-year study produced an extensive runoff *E. coli* data set (*n* = 262), which facilitated the examination of poultry litter application and land use impacts on *E. coli* runoff. Litter application did not appear to affect the range or magnitudes of *E. coli* concentrations in runoff from six cultivated sites differing only in applied litter rate. Regression analysis confirmed the lack of a significant relationship between *E. coli* concentrations and litter application rate. While not directly evaluated, the lack of litter rate effect on runoff *E. coli* concentrations is expected to also hold true for pasture land. The lack of impact can be at least partially attributed to the timing of litter application in this study. Litter was produced and typically removed from the poultry houses during the hot, dry summers, which likely produced conditions unfavorable for *E. coli* survival. It is possible that litter removed and land applied under cooler, higher moisture conditions would have higher *E. coli* levels that might impact *E. coli* runoff. Thus, late summer application may be a recommended practice to minimize *E. coli* runoff from litter application sites.

Although the present study indicated little impact of litter application on *E. coli* runoff, previous research indicated that runoff within a few days of litter application can be a significant source of *E. coli*. Therefore, application rates of organic soil amendments such as poultry litter should be carefully managed and be based on crop nutrient requirements. Similarly, application should be avoided when heavy rainfall is forecast within a few days to reduce environmental contamination and unnecessary loss of valuable crop nutrients.

In contrast to litter application rate, land use influences on runoff *E. coli* concentrations were readily apparent. Concentrations generally occurred in the following order: cultivated < hayed pasture < native prairie < mixed land use < grazed pasture. The magnitude of *E. coli* contribution by the native prairie (median = 2,000 CFU per 100 mL) was surprising, but clearly establishes the likelihood of considerable *E. coli* runoff from non-anthropogenic sources, especially in areas with abundant wildlife. The effect of grazing cattle on *E. coli* concentrations was also evident, with the highest concentrations coming from pasture and mixed land uses with cattle present. Because of regulatory attention on livestock producers and the need to protect the recreational use of U.S. waters, livestock producers should use recommended practices to properly manage grazing on upland and riparian areas to minimize the contribution of bacteria from their grazing operations. However, it must also be understood that high *E. coli* concentrations can be produced by well-managed grazing lands, as well as reference land uses such as native prairie, due to contributions from wildlife and other background sources.

While few would argue the importance of preventing direct sewage and confined animal feeding operation waste discharge into water bodies or the need to minimize the overall fecal contamination of water, defining and achieving acceptable bacterial concentrations in surface water is an extremely difficult task for many reasons. First, fecal deposition in water and throughout watersheds is a largely natural process. The need for and benefits of controlling wildlife, livestock, pets, and/or feral animals to reduce fecal contamination is a highly contentious issue. In addition, available data sets continue to indicate the presence of considerable background concentrations of bacteria (e.g., Ishii et al., 2006; Jensen et al., 2007; Guzman et al., 2010; Harmel et al., 2010; Wagner et al., 2012). Second, the scientific basis and understanding of *E. coli* fate, transport, and survival in terrestrial and aquatic environments is lacking (Ishii and Sadowsky, 2008; van Elsas et al., 2011), which makes it difficult to link bacterial sources and downstream concentrations, where standards attainment is commonly evaluated. Third, in most if not all areas, bacterial sources are numerous and are spatially and temporally variable. Thus, it is likely that controlling any one source will not alleviate all bacterial water quality concerns.

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