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Surface Water Quality

Escherichia coli efflux from rangeland ecosystems in the southcentral Great Plains of the United States

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Abstract
Bacterial contamination of surface water is a public health concern. To quantify the efflux of *Escherichia coli* into ephemeral and intermittent streams and assess its numbers in relation to secondary body contact standards, we monitored runoff and measured *E. coli* numbers from 10 experimental watersheds that differed in vegetation cover and cattle access in north-central Oklahoma. *Escherichia coli* numbers were not significantly different among the watersheds, with one exception; the grazed prairie watershed (GP1) had greater numbers compared to one ungrazed prairie watershed (UP2). Median *E. coli* numbers in runoff from ungrazed watersheds ranged from 260 to 1482 MPN/100 mL in comparison with grazed watersheds that ranged from 320 to 8878 MPN/100 mL. In the GP1 watershed, higher cattle stocking rates during pre- and post-calving (February–May) resulted in significantly greater bacterial numbers and event loading compared to periods with lower stocking rates. The lack of significance among watersheds is likely due to the grazed sites being rotationally (and lightly) grazed, data variability, and wildlife contributions. To address wildlife sources, we used camera trap data to assess the usage in the watersheds; however, the average number of animals in a 24-h period did not correlate with observed median *E. coli* numbers. Because of its impacts on *E. coli* numbers in water, grazing management (stocking rate, rotation, and timing) should be considered for improving water quality in streams and reservoirs.

1 INTRODUCTION

Bacterial contamination of water bodies is a public health risk that has been addressed jointly by the United States Environmental Protection Agency (EPA) and the Center for Disease Control (EPA, 2018). Because of the direct

Abbreviations: cfu, colony forming units; CTER, Cross Timbers Experimental Range; EPA, United States Environmental Protection Agency; MPN, most probable number; ODEQ, Oklahoma Department of Environmental Quality; WOTUS, Waters of the United States.

relationship between the water quality of surface runoff and the bodies of water that they flow into, it is critical to quantify the relationship between bacterial contamination of surface runoff and land use practices within watersheds (Harris et al., 2018). However, bacterial numbers in runoff water are highly variable due to the timing of rainfall in relation to sample collection and erratic bacteria sources. This has led to the labeled impairment of water bodies with only minimal anthropogenic land use impacts (Wagner et al., 2012).

To ensure water bodies are safe for secondary body contact, the Oklahoma Department of Environmental Quality

wildlife contributes to water bacterial contamination and the need to quantify "background" levels of *E. coli* has been

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(ODEQ), under EPA guidance, defined the acceptable levels of bacterial contamination to be anything below a consistent geometric mean of five times the EPA primary body contact standard of 126 colony forming units (cfu) of *Escherichia coli* per 100 mL of water, that is, 630 cfu/100 mL (EPA, 1986, 2012; ODEQ, 2021). *Escherichia coli* is often used to assess the safety of freshwater resources because it is an indicator of recent fecal contamination, which could contain pathogens known to cause illness in people (EPA, 1986; Jamieson et al., 2004; Jeng et al., 2005; Petersen et al., 2018). Using this benchmark to evaluate stream health results in the widescale labeling of streams as impaired. For instance, 4380 km of streams in Oklahoma were identified as impaired due to *E. coli* contamination (ODEQ, 2021). Grazing in riparian zones or shoreline zones, wildlife other than waterfowl, and upland rangeland grazing are three of the top five potential *E. coli* sources of stream impairment for the state (ODEQ, 2021). These findings are not unique to Oklahoma, as widescale impairment attributed to cattle grazing has also been reported both in nearby Texas and abroad in the United Kingdom and New Zealand (Crowther et al., 2002; McDowell et al., 2006; Wagner etal.,2012). Therefore, evaluating theimpactsof land use related to animal activities is important to understand the drivers of water quality impairment in rangelands.

There is a wealth of information on the overall increase in bacterial, nutrient, and sediment contamination of runoff from watersheds that have grazing cattle compared to those that are not grazed (e.g., Harmel et al., 2010, 2013; Jamieson et al., 2004; McDowell et al., 2006; O'Callaghan et al., 2019). The general observation is that as the number of grazers in a watershed increases, the additional fecal matter increasingly contaminates runoff. In perennial streams at base flow, results demonstrated that watersheds with land use dedicated to pastureland cover over 55% had a higher percentage of samples exceeding *E. coli* standards in comparison with watersheds with less percent (Scott et al., 2017). At the stream scale, the impact of cattle management within pastureland on water quality cannot be directly measured. Additionally, there is reason to believe that the contamination found in past studies was not solely due to the presence of cattle and that the current EPA water quality standards may not be appropriate for application to runoff in ephemeral streams (Wagner et al., 2012). The importance of understanding how

independently discussed in the literature (Harmel et al., 2010, 2013; Hong et al., 2018; Jamieson et al., 2004). Therefore, it is essential to quantify the variability in bacterial numbers and loadings in different environments at various scales, as they naturally fluctuate greatly across different land uses and ecoregions (Chen & Chang, 2014; Davies-Colley et al., 2008; Petersen et al., 2018; Rafi et al., 2018).

To understand the background sources of *E. coli*, the impact of vegetation types on wildlife abundance and

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Core Ideas

· Wildlife contributed to *E. coli* in runoff, but wildlife abundance measured using game cameras was unable to capture this relationship.

· Rotational grazing mitigated cattle effects on *E. coli* numbers in surface runoff.

· *Escherichia coli* loading totals were lower from forested watersheds due to lower runoff volumes.

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behavior should be considered. Vegetation is one of the primary factors influencing the habitat choice of animals. If animals are more abundant or tend to spend more time within a certain vegetation cover, then the frequency of fecal deposits is greater, leading to greater *E. coli* numbers in surface runoff. In the Cross Timbers ecoregion of the central Great Plains of the United States, small mammalian (e.g., rodent) communities tend to have the greatest diversity in tallgrass prairies compared to riparian woodlands (Horncastle et al., 2005). Additionally, eastern redcedar (*Juniperus virginiana*) encroachment into tallgrass prairie decreases small mammal density once eastern redcedar cover exceeds 42% (Matlack et al., 2008). Larger mammals, including feral hogs (*Sus scrofa*), raccoons (*Procyon lotor*), eastern cottontail (*Sylvilagus floridanus*), and nine-banded armadillos (*Dasypus novemcinctus*), have been identified as significant contributors to *E. coli* through fecal matter (Parker et al., 2013). Seasonalityplaysarolewhenitcomestohabitatchoice of wildlife for both mesocarnivores (Premathilake, 2018) and white-tailed deer (*Odocoileus virginianus*) (Dillard et al., 2006; Holtfreter, 2008).

In addition to influencing wildlife preference as a food source, vegetation type directly impacts runoff generation

and connection to the stream. Compared to bare ground, the existence of vegetation increases the quality of surface runoff (Bhandari et al., 2017; Butler et al., 2007; Mohammad & Adam, 2010). Additionally, native and non-native vegetation can serve as riparian buffers for mitigating the negative effects of land use on water quality (Chase et al., 2016; Schmitt et al., 1999; Udawatta et al., 2010). Compared to cropland, forests and well-managed grasslands are usually associated with greater canopy interception and higher infiltration, reducing surface runoff and its ability to carry sediments and other contaminants (Dosskey et al., 2010; Lyons et al., 2000).

Although previous research highlighted the impact of cattle grazing and vegetative cover on water quality, there is a lack of research that addresses these factors together. In addition, previous works on wildlife have either described habitat selection or utilized ungrazed sites to determine wildlife *E. coli* sources, but no studies have tied these ideas together to examine how habitat selection by wildlife influences *E. coli* contamination. Therefore, the three goals of this study were to (i) examine the influences of cattle grazing and vegetation type on *E. coli* numbers and loading; (ii) compare *E. coli* numbers in runoff from 10 small forest and grassland watersheds in Oklahoma to the secondary body contact standard; and (iii) correlate wildlife use and *E. coli* contamination of runoff.

2 MATERIALS AND METHODS

2.1 Site description

This study took place in the Cross Timbers region, which includes approximately 8 million ha in the southern Great Plains of the United States, extending from southern Kansas through central Oklahoma and into Texas. This region consists of a mosaic of tallgrass prairie and woodland/forest historically dominated by post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*). Recent land cover changes have included the densification of the forest and encroachment of eastern redcedar into both prairie and forested areas (Hoff et al., 2018; Joshi et al., 2019). Additionally, the majority of the Cross Timbers ecoregion is rural and used extensively for grazing and agriculture (Stallings, 2008; Thomas & Hoagland, 2011).

This study took place from April 2020 through October 2021 at the Oklahoma State University Cross Timbers Experimental Range (CTER), approximately 18 km southwest of Stillwater, Oklahoma. The climate of this region is highly variable with substantial seasonal variation. The nearest Oklahoma Mesonet weather station at Marena (2.5 km from CTER) recorded an annual average temperature of 15.6°C, an average minimum temperature in January of −3.3°C, and an average maximum temperature of 33.9°C in July. The annual rainfall for this area is ~890 mm with wet springs and falls and comparatively dry winters and summers (Qiao et al., 2017).

The 10 experimental watersheds at CTER were established to study water budget and sedimentation processes based on the dominant vegetation types (oak forest, eastern redcedar woodland, and tallgrass prairie) (Qiao et al., 2017; Zhong et al., 2022; Zou et al., 2014). For this study, the watersheds were re-named based on two factors: access to grazers (G for grazed and U for ungrazed) and the dominant vegetation types (O for oak forest, R for eastern redcedar woodland, S for switchgrass [*Panicum virgatum*] stand, and P for tallgrass prairie) (Figure S1). Numbers were also assigned to watersheds to differentiate the watersheds of the same grazer access and vegetation cover from one another.

The slopes of these watersheds are <5% with soils that are well drained, consisting predominately of the Stephenville–Darnell complex (StDD), Coyle soil series (Coy, CoyZ), and Grainola–Lucien complex (GrLE) (Table S1). Three hydrologic soil groups (B, C, and D) are included at different ratios between the watersheds with the greater portion of watershed areas consisting mostly of soils from hydrologic groups C and D (Table S2). The average depth of soil is approximately 1 m underlain by sandstone substrates. The understory or ground cover differed greatly in grass cover depending on the type of vegetation. For prairie and switchgrass watersheds (UP1, UP2, US1, US2, and GP1), grass cover ranged from 60% to 90%. In contrast, grass cover ranged from 3% to 33% for oak forest and eastern redcedar watersheds (GO1, GO2, GO3, GR1, and GR2). The cover of woody plants, forbs, and bare ground varied greatly among individual watersheds (Table S3).

2.2 | Vegetation type and herbaceous cover

Ground cover for the watersheds was evaluated using visual estimation with the Daubenmire method (Towne et al., 2005). For each watershed, the percentages of grasses, forbs, bare ground, and litter were visually estimated and placed into a coverage class corresponding to the estimated percentage for 20 (0.5 × 0.5 m) plots (Floyd & Anderson, 1987; Symstad et al., 2008). Additionally, we quantified differences in canopy coverage of the watersheds, using the classify tool in ArcMap 10.8 on a 1 m resolution National Agriculture Imagery Program imagery from 2019 (Ma et al., 2017; Table S4).

2.3 | Cattle grazing

Among all 10 watersheds, four (UP1, UP2, US1, and US2) were fenced and cattle were excluded. The remaining six (GO1, GO2, GO3, GR1, GR2, and GP1) were accessible to cattle (Figure S1). From June to January, cattle grazed at the relatively low stocking rate of 6.9 ha/head/year. In comparison, most ranches in the area stock at a rate of 3– 4 ha/head/year. During pre- and post-calving, 104 animals were concentrated in an area of 32.4 ha, which included watershed GP1 but did not include the other watersheds. As a result, GP1 had a stocking rate of 1.2 ha/head/year for February through May and a stocking rate of 6.9 ha/head/year for the rest of the study, resulting in an estimated effective stocking rate of approximately 4.9 ha/head/year, 40% higher than the other watersheds but still lower than typical commercial operations.

2.4 | Runoff measurement

To measure runoff volume and collect samples, H-flumes were equipped with Avalanche portable refrigerated samplers and 720 Submerged Probe Modules in all watersheds (Teledyne ISCO; Lincoln). Flow-weighted composite samples were collected in 18.9-L bottles, allowing the determination of event mean concentrations. Initially, we programmed all samplers based on a flow interval so that each took a sample for every 0.5 mm of runoff, as recommended by Harmel et al. (2006). Later, this was adjusted in forested watersheds to address the low runoff quantities observed. The threshold for sample collection at these forested watersheds was re-programmed so that samples were collected at the programmed enable depth (14mm)startingon May 16, 2021, and then for every 0.5 mm of runoff after that initial sample. This increased the number of samples collected from forested sites, such that 28 out of 38 (74%) of the samples for *E. coli* from these watersheds were collected using the adjusted program.

2.5 | *Escherichia coli* numbers and loading

During collection, each composite water sample was split for *E. coli* testing and other water quality analyses. Subsamples were stored and processed according to the Colilert-18 test manual (Crane et al., 2006). Within 5 h of collection, we used the IDEXX Quanti-Tray*/2000 most probable number (MPN) protocol to test each sample with two dilutions (1:10 and 1:100). Distilled/deionized water was mixed with sample water to ensure proper dilution. We quantified numbers using the IDEXX Quanti-Tray*/ 2000 MPN Calculator. This procedure is appropriate for making direct comparisons between MPN and cfu (Hulvey et al., 2021; Kinzelman et al., 2005). The *E. coli* loading of a given sampling event was calculated by multiplying the runoff volume by the *E. coli* number per 100 mL and then dividing it by the area of the watershed (Wagner et al., 2012). Uncertainty was estimated to be ±34% in *E. coli* numbers based on Harmel et al. (2006).

2.6 | Relative wildlife abundance as an index for use

Relative wildlife abundance in this study was assessed using pictures taken from 22 motion-activated infrared game cameras (Stealth Cam, Cabela, and Bushnell) distributed simultaneously across the 10 watersheds from the fall of 2020 through the fall of 2021 for 1 month per season. Sites for this studyweredefinedascameralocationsineachwatershed.The camera density was standardized by dividing the total area of all watersheds by the total number of cameras available and then overlaying a grid of that size over a map with the watersheds. Points were placed at the center of each grid that intersected the watershed. From these points, the surrounding area was assessed, and cameras were placed in locations that had a viewable area and were within 10 m from the predetermined point. With this design, sites were in close enough proximity that animals could move between sites. However, this is acceptable for the scope of this study because *E. coli* is likely influenced by the timing of defecation (i.e., usage) of the landscape by wildlife (Kendall et al., 2013). Therefore, we applied the term "use," rather than "abundance," to interpret the number of captures per 24-h period. After deployment, cameras collected images for 1 month per season, and the pictures captured on each camera per 24-h period were defined as each survey. Only animals of the size of rabbits and larger were included in our study, similar to the protocols from Parker et al. (2015).

2.7 | Data processing and statistical analysis

We organized the event-based *E. coli* numbers and loading data in R and examined the descriptive statistics for the watersheds. Consistent with many other water quality datasets, the data lacked normality, had many outliers, and contained inconsistent variances (Helsel & Hirsch, 1992). For these reasons, we used non-parametric approaches without transformation, but the data were log base 10 transformed for the figures. Each watershed was analyzed as a separate treatment. For each runoff event per watershed, the number and loading values were considered separate observations. We conducted Kruskal–Wallis rank sum tests to detect

differences between individual watersheds for numbers and loadings. If the Kruskal–Wallis rank sum test found that at least one watershed was significantly different from the others, we used a series of pairwise two-sample Wilcoxon rank sum tests between all watersheds with a Bonferroni correction to ensure we accounted for family-wise error (Hollander et al., 2013).

In addition, we compared the median bacterial numbers in runoff from each of the watersheds to the ODEQ *E. coli* standard for secondary body contact (630 cfu per 100 mL) using a series of one-sample Wilcoxon signed-rank tests. To maintain consistency with protocols for determining impairment, we also calculated the geometric means for the *E. coli* numbers for comparison with the secondary standard described above. Due to the variable stocking rates at GP1 that could impact *E. coli* numbers, we also conducted a two-sample Wilcoxon rank sum test to compare differences in *E. coli* numbers between runoff events with a stocking rate of 1.25 and 6.88 ha/head/year.

To assess the effectiveness of using game cameras to estimate the impact of wildlife on *E. coli* numbers in runoff, we estimated wildlife use and correlated this to median *E. coli* numbers for each watershed. We based wildlife use on the number of pictures of wildlife per trap night. For each condition available for the watersheds (vegetation cover, grazing, and season), the average number of animals captured per trap night was calculated, and then this number was paired with monthly median *E. coli* numbers for each watershed.

3 RESULTS

3.1 *Escherichia coli* numbers

Sample sizes for runoff events varied dramatically between watersheds, with the smallest sample size (n = 5) for watersheds GO2 and GR1. In comparison, the largest sample sizes (n = 32) were for watersheds UP2 and US2 (Table 1). Median *E. coli* numbers in surface runoff from the 10 watersheds ranged from 237 to 8878 MPN per 100 mL (Table 1). The individual runoff event numbers followed a similar trend of high variability, with minimum watershed numbers ranging from 3 to 580 MPN per 100 mL and maximum event numbers ranging from 4621 to 129,900 MPN per 100 mL (Table 1).

The only significant difference between watersheds was observed between GP1 and UP2, where *E. coli* numbers were significantly greater in the runoff from GP1 than those from UP2 (p = 0.024).

Half of the sites (GO1, GO2, GR1, GR2, and US2) had *E. coli* geometric means exceeding the secondary body contact standard (Table 1). Additionally, GP1, US1, and US2 had median *E. coli* numbers that were significantly greater than the secondary body contact threshold according to the Wilcoxon signed-rank tests (Figure 1).

Due to the differences in the stocking rate at GP1 depending on the time of year, we found significantly greater

TABLE 1   *Escherichia coli* numbers in runoff from the watersheds at Cross Timbers Experimental Range (CTER).

Watershed	Mean	SD	Min	Max	Median	Geometric mean	Sample size
GO1	1499	2030	18	4621	320	1432	9
GO2	2645	3855	580	9529	1046	670	5
GO3	2109	3121	60	8257	411	234	7
GP1	21,920	35,040	37	141,400	8878	340	20
GR1	33,780	55,640	150	129,900	2420	782	5
GR2	7596	20,710	3.0	72,700	374	4976	12
UP1	3063	5778	7.0	22,470	260	257	23
UP2	2479	4625	3.0	19,860	237	574	32
US1	1841	2664	6.0	9804	928	391	30
US2	4152	9597	9.0	51,720	1482	4482	32

Abbreviations: GO, grazed oak; GP, grazed prairie; GR, grazed redcedar; Max, maximum; Min, minimum; UP, ungrazed prairie; US, ungrazed switchgrass.

The relationship between the average number of animals per trap night and median *E. coli* numbers for each condition was assessed using Spearman's rank correlation method.

We also examined the percent forest cover, grassland cover, soil hydrologic group, and average slope for their correlation with median *E. coli* in each watershed using Spearman's rank correlation method.

E. coli numbers for runoff events during months when the stocking rate was 1.25 ha/head/year compared to runoff events during months when the stocking rate was 6.88 ha/head/year (p = 0.003). The *E. coli* numbers during the events with the greater stocking rate had a median of 28,900 MPN and ranged from 1733 to 141,360 MPN. In contrast, the *E. coli* numbers during the events with the lesser stocking rate

were over an orderofmagnitudelesswithamedianof1016MPNandrange from 37 to 79,150 MPN.

3.2 | *Escherichia coli* loading

Median *E. coli* event loadings in this study ranged from 7.81×10^6 to 5.19×10^9 MPN/ha (Table 2). Annual loadings for the watersheds during the 2021 water year ranged from 5.39×10^8 MPN/ha/year for GO1 to 2.37×10^{11} MPN/ha/year for GP1 (Table 2).

Individual runoff events exhibited a large range of *E. coli* loadings, differing six orders of magnitude in comparison to the two orders of magnitude range for annual loadings (Table 2). There were significant differences among individual watersheds for *E. coli* loading (p = 0.002) according to the Kruskal–Wallis rank sum test. There was one significant difference in median *E. coli* loading values between all the watersheds (Figure 2). The median event *E. coli* loading in GP1 (5.19×10^9 MPN/ha) was significantly greater than that in GO1 (7.81×10^6 MPN/ ha) (p = 0.048).

3.3 | Runoff volume impact on *Escherichia coli* numbers

The forested watersheds (GO1, GO2, GO3, GR1, and GR2) had less annual and monthly runoff in comparison with the grassland watersheds (GP1, UP1, UP2, US1, and US2) (Table S5). Additionally, there was a positive, statistically significant relationship between *E. coli* numbers and runoff volume, although the relationship was weak (ρ = 0.17, p = 0.03)
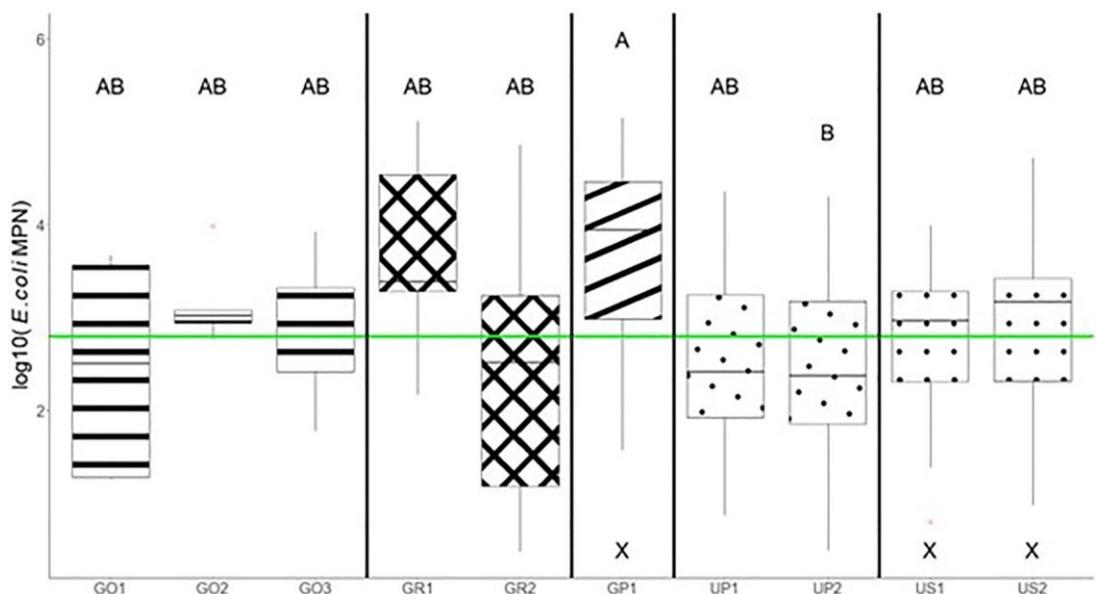
FIGURE 1 Log base 10 transformed boxplots of the *E. coli* numbers for the watersheds at Cross Timbers Experimental Range (CTER). Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The solid green line represents the secondary body contact standard. An "X" indicates that the median value for that watershed is significantly different from the secondary body contact standard. GO, grazed oak; GP, grazed prairie; GR, grazed redcedar; UP, ungrazed prairie; US, ungrazed switchgrass.

TABLE 2 *Escherichia coli* event and total annual loading from watersheds at Cross Timbers Experimental Range (CTER).

Watershed	Mean	Median	Min	Max	SD	Annual loading water year 2021
GO1	1.56×10^8	7.81×10^6	2.12×10^6	5.28×10^8	2.21×10^8	5.39×10^8
GO2	1.05×10^9	1.00×10^8	1.60×10^6	4.88×10^9	2.14×10^9	5.27×10^9
GO3	3.48×10^8	2.01×10^7	1.95×10^6	1.61×10^9	6.20×10^8	2.39×10^9
GP1	2.05×10^{10}	5.19×10^9	4.32×10^6	1.56×10^{11}	3.71×10^{10}	2.37×10^{11}
GR1	3.84×10^8	5.14×10^7	2.20×10^5	1.45×10^9	6.20×10^8	1.92×10^9
GR2	4.33×10^8	4.56×10^7	4.85×10^4	3.10×10^9	8.82×10^8	2.06×10^9
UP1	5.55×10^9	3.18×10^8	7.16×10^5	8.03×10^{10}	1.72×10^{10}	1.21×10^{11}
UP2	2.04×10^9	2.59×10^8	8.02×10^5	3.11×10^{10}	6.07×10^9	6.13×10^{10}
US1	1.42×10^9	6.61×10^8	5.47×10^5	1.23×10^{10}	2.46×10^9	3.25×10^{10}
US2	1.92×10^9	5.59×10^8	9.12×10^5	2.12×10^{10}	4.24×10^9	3.49×10^{10}

Abbreviations: GO, grazed oak; GP, grazed prairie; GR, grazed redcedar; Max, maximum; Min, minimum; UP, ungrazed prairie; US, ungrazed switchgrass.

(Figure S2). The log-transformed *E. coli* numbers also had a weak, positive relationship with volume ($\rho = 0.22$, $p = 0.004$) (Figure S2).

from 0 to 1.94. There was no correlation between these values and median *E. coli* numbers ($\rho = 0.14$; $p = 0.34$) (Figure S3).

3.4 | Correlation between *Escherichia coli* numbers and wildlife presence

The average number of animals captured on game cameras per 24-h period on the watersheds for each season ranged

3.5 | Correlation between *Escherichia coli* numbers and land cover/soil hydrologic groups

The percent forest cover, grassland cover, soil hydrologic group, and average slope were analyzed for their correlation with median *E. coli* in each watershed (Figure S4). No significant correlations were found between median *E. coli* numbers
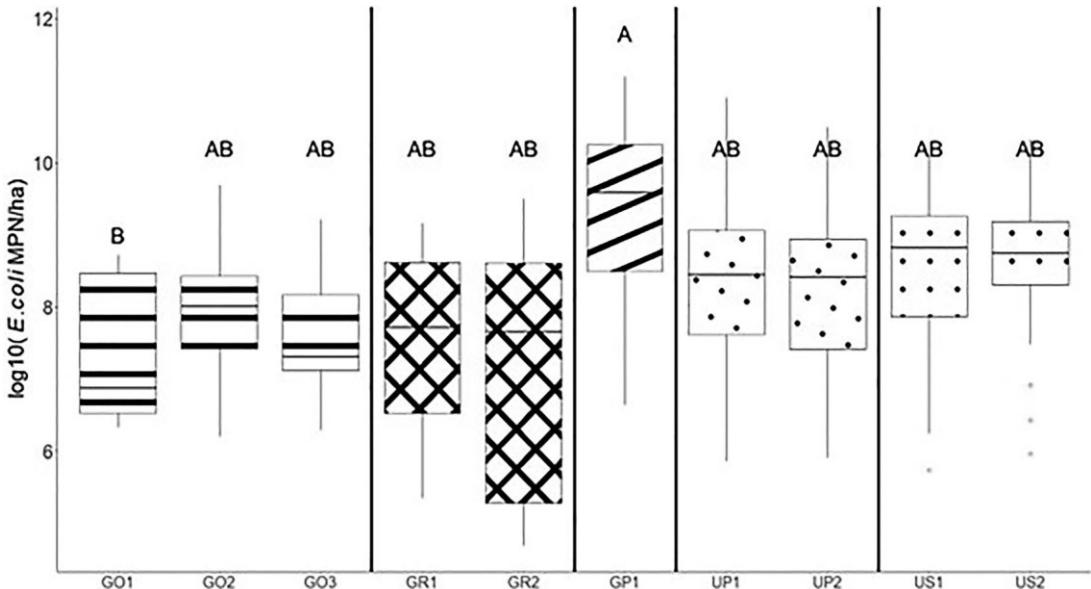
FIGURE 2 Log base 10 transformed boxplots of the event *E. coli* loading values (most probable number [MPN]/ha) for the watersheds at Cross Timbers Experimental Range (CTER). Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The red dots represent outliers with log-transformed data. Boxpl ots for individual watersheds are grouped according to the land use and vegetation cover of the watershed. GO, grazed oak; GP, grazed prairie; GR, grazed redcedar; UP, ungrazed prairie; US, ungrazed switchgrass.

and percent forest cover ($p = 0.49$), percent grassland cover ($p = 0.41$), slope ($p = 0.95$), percent soil hydrologic group B ($p = 0.88$), percent soil hydrologic group C ($p = 0.35$), or percent soil hydrologic group D ($p = 0.45$).

4 DISCUSSION

4.1 Vegetation cover, grazing, and wildlife impacts on *Escherichia coli*

We found no significant differences in median *E. coli* numbers among ungrazed watersheds (UP1, UP2, US1, and US2) and lightly grazed watersheds (GO1, GO2, GO3, GR1, and GR2), regardless of vegetation type. Significant differences were only found between one ungrazed watershed (UP2) and the most intensively grazed watershed (GP1). Results for bacterial numbers in the surface runoff collected

from the experimental watersheds at the CTER show the complexity of disentangling the impacts of cattle management, vegetation cover, and wildlife on surface water resources.

Consistent with previous studies, the *E. coli* numbers for all the watersheds at CTER were highly variable and righttailed skewed (Gregory et al., 2019; Wagner et al., 2012). Pastures stocked according to NRCS and/or Extension recommendations do not consistently cause significantly greater *E. coli* numbers in surface runoff. For example, no significant differences were detected between properly stocked rotationally grazed bermudagrass (*Cynodon dactylon*) and ungrazed pastures at sites near the Brazos River, Texas, but significant differences between properly stocked rotationally grazed bermudagrass and ungrazed native prairie were detected at sites in Riesel, TX (Wagner et al., 2012). The bacterial numbers in runoff from lightly grazed and ungrazed watersheds in central Oklahoma were generally not different, regardless of vegetation cover. However, we observed that heavier stocking rates during periods of increased rainfall and runoff led to significantly greater *E. coli* numbers and loadings. Median *E. coli* numbers for GP1 during events with higher stocking rates were significantly higher than the median *E. coli* numbers during events when this same watershed had lower stocking rates. This variability contributed to watershed GP1 having significantly greater median *E. coli* numbers than watershed UP2 but not UP1, despite watershed UP1 and UP2 both having the same land use and both excluding cattle.
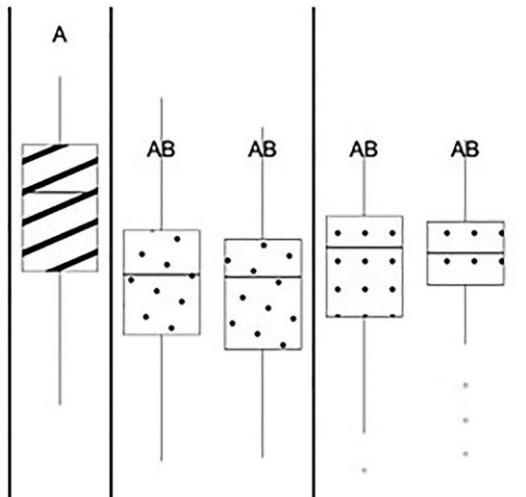
Therefore, *E. coli* numbers in surface runoff can be affected by cattle management, but other factors are also at play.

Other parameters associated with the watershed (soil type, slope, and cover) were not significantly correlated with median *E. coli* numbers. This is likely because *E. coli* deposition on the landscape is the primary driver of contamination. Previous study in the region found that greater pastureland percentage within the riparian zone buffer area of rivers was correlated with greater *E. coli* numbers in samples (Scott et al., 2017). This is consistent with the conclusion from our study that watershed physical characteristics cannot replace knowledge of grazing practices and wildlife usage of the landscape for predicting and mitigating *E. coli* contamination.

4.2 | *Escherichia coli* numbers and water quality concerns

There was some evidence that the quality of surface runoff from forested watersheds is less likely to exceed bacterial standards, although this was not always the case (Figure 2). Low bacterial numbers were reported in five experimental forested watersheds in Angelina National Forest near Lufkin, TX. The *E. coli* numbers for these sites had an average of 137 fecal coliform colonies per 100 mL, which was lower than the contact recreation standard at the time of the study of 200 fecal coliform colonies per 100 mL of at least five samples collected within a 30-day period (EPA, 1986; Hunter et al., 1983). One possible explanation could be the increased time between defecation and runoff events (Gregory et al., 2019). Due to reduced overall runoff and fewer runoff events in forested watersheds compared to grassland watersheds, there is a longer period between runoff events. This decreases the transport potential and allows bacteria to die off before potentially contaminating the runoff. However, the geometric mean *E. coli* numbers between the forested watersheds vary dramatically, suggesting that using a standardized metric for *E. coli* based on vegetation cover may not be suitable for modeling at larger scales.

Grassland watersheds (US1, US2, and GP1) had significantly greater median *E. coli* numbers compared to the secondary body contact threshold, with similar results reported for an ungrazed native prairie by Harmel et al. (2013). The sample sizes for these watersheds were much larger in comparison with the forested watersheds, which increased the possibility to capture runoff events sooner after defecation leading to a greater potential for high *E. coli* numbers (Gregory et al., 2019). Despite the lack of cattle grazing on watersheds managed for annual hay or forage harvest (e.g., US1 and US2), our study and others observed unexpectedly high *E. coli* numbers. One prior study found a watershed managed for coastal bermudagrass and harvested seasonally had a median of 5950 cfu per 100 mL (Gregory et al., 2019). Because wildlife were the only source of *E. coli* in these watersheds and these watersheds still significantly exceeded the secondary body contact standard, this adds support to exclude the application of this standard to edge-of-field runoff.

The significantly greater median *E. coli* numbers and loading from watershed GP1 compared to at least one watershed were expected due to the differences in cattle management. The significantly greater *E. coli* numbers for runoff events during periods with higher stocking rates at GP1 in comparison to numbers for that same watershed when stocking rates were lower is evidence that higher cattle stocking rates increase *E. coli* numbers in the runoff. However, this also suggests that the annual effects of higher stocking rates can be mitigated by rotational grazing. The lack of significant differences between GP1 and most of the watersheds provides further evidence that rotational grazing can be an effective management strategy for reducing *E. coli* numbers in runoff (Hulvey et al., 2021; Wagner et al., 2012).

4.3 | Vegetation cover and *Escherichia coli* loadings

Escherichia coli loading comparisons yielded similar results, as watersheds with higher *E. coli* counts under higher stocking rates resulted in greater loadings. Specifically, watershed GP1 had significantly greater median *E. coli* loading in comparison with GO1. Ideally, studies would consider *E. coli* loading on an annual scale. However, previous studies also examined differences among loadings on an event basis (e.g., Gregory et al., 2019). The loading differences in the individual watersheds appear to have been influenced by runoff volume. Watershed GP1 did not show significant differences in *E. coli* numbers with GO1 but had a significantly higher loading. This difference is likely due to the reduced runoff from forested watersheds in comparison with grassland watersheds. Greater bacteria loadings were observed from watersheds that had greater runoff, and runoff is heavily influenced by vegetation cover and soil hydrologic group (Bonan, 2002; Calder et al., 2007) (Table 2).

The *E. coli* loadings in this study were less than those observed in previous studies that observed median loading values of 8.1×10^{10} cfu/ha in runoff from grazed native rangeland and 4.2×10^{10} cfu/ha from ungrazed native rangeland (Wagner et al., 2012). The median values for the surface runoff from the CTER experimental watersheds had median loading an order of magnitude lower. The lower loading per event for this study could be due to differences in climatic variables such as temperature, solar radiation, and intensity of rainfall that influence the survival of *E. coli* in the secondary environment during a runoff event (Petersen &

Hubbart, 2020) or potentially lower wildlife numbers at CTER compared to previous studies. However, more field-based studies comparing values between regions are needed (Petersen & Hubbart, 2020).

4.4 | Wildlife and *Escherichia coli*

In addition to the impact of vegetation cover on *E. coli* numbers and loadings, the selection of these areas by wildlife was likely a contributor to the variation observed among the watersheds in this study. Past research has shown that wildlife species are a major contributor to bacterial contamination of surface runoff (Harmel et al., 2010; Parker et al., 2013; Wagner et al., 2012). The background levels, that is, the numbers of *E. coli* in runoff from sites where cattle grazing was excluded, were high enough such that the median *E. coli* numbers exceeded the secondary body contact standard for all but three of the watersheds. Despite this fact, the numbers observed in this study were lower than levels found in ungrazed sites of previous studies. The consistently lower background levels at our locations, in comparison to studies from Texas, provide preliminary evidence that runoff fromareasindifferentclimaticand/orgeographicregionsmay exhibit dissimilar bacteria numbers (Rafi et al., 2018).

The seasonality of wildlife preference for certain habitat types likely played a role in *E. coli* numbers, but the method of measurement was not sufficient to characterize this source. The game cameras at CTER captured variable numbers of animals per 24-h period for each treatment depending on the season. Premathilake (2018) found that most mesocarnivores in southcentral Oklahoma like striped skunks (*Mephitis mephitis*), Virginia opossum (*Didelphis virginiana*), bobcat (*Lynx rufus*), gray fox (*Urocyon cinereoargentus*), and northern raccoons (*Procyon lotor*), had higher site occupancy (captures on game camera) during the winter compared to the summer. The changes in animal behavior between season are considered a driver of fecal input to the landscape.

Despite our effort to correlate game camera data to *E. coli* numbers, the data showed no correlation between the number of animals captured in a 24-h period and the median *E. coli* across watersheds. There are a couple of explanations for this. First, there are other sources of *E. coli* outside of the meso-mammals measured. A 2006 bacterial source tracking study on the Trinity River in the Cross Timbers ecoregion in Texas found that the avian contribution to *E. coli* (23.2%) was greater than that of the mammalian wildlife source (13.4%) (TIAER, 2006). However, a study in the Ozark streams in Oklahoma found that the avian input was a much smaller percentage of identified gene copies in comparison with human and bovine copies (Browning et al., 2023). The inconsistency among studies in the region demonstrates the

variability that may be present among sites and the need for conducting bacterial source tracking on a case-by-case basis.

Second, this study had limitations in scope when it came to the data collection for the camera trap study itself. The closure assumption was violated due to the proximity of camera locations. Essentially, the sites at CTER are not close enough that animals can be detected at multiple sites per sampling event. This limitation causes the data to be skewed in cases where an individual tends to be captured on camera multiple times (Chandler & Royle, 2013). These limitations can be addressed in future studies by using wildlife-tailored models that either employ data from cameras based on a specific time interval (Moeller et al., 2018) or utilize paired cameras that face in opposing directions at the same location (Nakashima et al., 2022). Despite these limitations, this study is the first to employ game cameras to directly measure wildlife site use and assess the impact on water quality.

The watersheds used in this study were either absent of active management or had land use and land coverage associated with low-intensity ranching practices. For these reasons, we anticipated that the water quality of runoff is representative of these land uses under the best-case scenarios. These results suggest that wildlife inputs are variable and that even with best-case scenarios in this system, the ODEQ standards for regulated bodies of water discussed above for *E. coli* contamination are exceeded in runoff.

Previous studies have found that as the size of the watershed increases, *E. coli* numbers tend to decrease (Harmel et al., 2010; Rafi et al., 2018). Our study provides evidence that at the small watershed scale, *E. coli* numbers are consistently higher than water quality standards applied to recreational bodies of water, regardless of land management. There are currently no water quality standards for surface runoff. This study provides justification that (1) the existing criteria should be assessed during flow conditions reflective of use and (2) if new statutes (e.g., Waters of the United States [WOTUS]) require regulation of water quality in edge-of-field runoff, new runoff-specific thresholds for determining impairment must be developed.

5 | CONCLUSIONS

During this 1.5-year study, we observed *E. coli* numbers in surface runoff across a range of land uses in the Cross Timbers ecoregion. In most experimental watersheds where cattle were excluded, wildlife contributions resulted in *E. coli* numbers that exceeded regulatory standards currently applied to recreational bodies of water. The *E. coli* counts observed in this study (Oklahoma) were lower than those recorded in previous studies (Texas), contributing evidence that bacterial numbers are influenced by geographic and climatic

differences. For this reason, the data suggest that applying the definition of WOTUS to surface runoff could lead to the mislabeling of natural water resources as impaired. This is largely because of the variability contributed by wildlife sources. This study was unable to correlate *E. coli* numbers in runoff with game camera capture data. The direct impact that vegetation cover has on runoff volume resulted in variable *E. coli* event loadings among watersheds of different vegetation cover. Higher stocking rates during runoff events significantly impact bacterial contamination of surface runoff, but allowing a pasture to go ungrazed for months at a time can significantly reduce these impacts, even over a 1.5-year period.

AUTHOR CONTRIBUTIONS

Austin J. Phillippe: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Kevin L. Wagner**: Conceptualization; funding acquisition; project administration; supervision; writing—review and editing. **Chris B. Zou**: Supervision; writing—original draft; writing—review and editing. **Rodney E. Will**: Writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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