DETERMINING BACTERIAL AND NUTRIENT CONCENTRATIONS AND LOADINGS OF SURFACE RUNOFF FROM DIFFERING GRAZER ACCESS AND VEGETATIVE COVER IN NORTHCENTRAL OKLAHOMA

By

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Abstract: Water bodies in Oklahoma are primarily fed by runoff, making bacterial and nutrient contamination of surface runoff a significant water quality concern. The purpose of this thesis was to determine the impacts of grazing and vegetation cover on bacterial and nutrient concentrations and loadings in surface runoff at the field scale in northcentral Oklahoma. To address these concerns, I measured *Escherichia coli* (*E. coli*), total Kjeldahl nitrogen, total phosphorus, and ortho-phosphate concentrations and loadings from 10 experimental watersheds at the Cross Timbers Experimental Range, Stillwater, Oklahoma. Results showed that *E. coli* concentrations of runoff from the grazed prairie watershed with the higher stocking rate, were significantly greater than concentrations from ungrazed watersheds. Total Kjeldahl nitrogen concentrations were greatest from eastern redcedar (*Juniper virginiana)* woodland watersheds compared to all other land uses measured in the study, but small sample sizes created problems with detecting statistical significance. Total phosphorus concentrations and loading were lowest from switchgrass (*Panicum virgatum*) watersheds, but this was not the case with measurements for Ortho-P. Loading values were influenced by runoff volume because the more volume associated with an event, the more nutrients in total mass that are carried downstream. In cases where watershed covers of forest and grassland differ substantially, differences in runoff volume dictate loading differences rather than land use. Using concentration to compare water quality between watersheds in these instances should be implemented. These results indicate that cattle grazing and eastern redcedar impact the water quality of runoff, and land management practices such as biomass feedstock production systems have added benefits by reducing total phosphorus concentrations of runoff and loadings to streams.

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CHAPTER I

Impacts of Cattle Grazing, Vegetation Cover, and Wildlife on *E. coli* Contamination of Surface Runoff

Abstract

Bacterial contamination of surface runoff that flows into larger bodies of water is a public health concern. However, previous studies found that the primary contact standard is often exceeded due to natural sources of *Escherichia coli* (*E. coli*). This study aimed to determine the impacts of grazing, vegetation cover, and wildlife on *E. coli* concentrations and loadings in surface runoff, evaluate the correlation of wildlife abundance and *E. coli* contamination of runoff, and compare the *E coli* concentrations to the primary and secondary body contact standards set by the EPA.

I monitored runoff volume and measured *E. coli* concentrations from 10 experimental watersheds at CTER, which differed in vegetation cover and access by cattle. Data from camera traps were used to create and compare models based on factors that predict abundance. The grazed prairie watershed (8,878 MPN per 100 mL) had the highest *E. coli* concentrations, although this was not significantly different from any of the other watersheds except ungrazed prairie (237 MPN per 100 mL). Eight out of 10 watersheds at CTER had *E. coli* concentrations greater than the primary body contact standard. Watersheds with woody vegetation cover had lower *E. coli* loading in comparison to those with grassland cover likely due to the differences in runoff volume. Zero-inflated modeling of the game camera data with the Akaike information criterion (AIC) selection process revealed that the interaction between vegetation cover and season, and the presence of grazers were important variables to determine wildlife abundance. However, although the ungrazed watersheds exceeded the primary body contact standard, I did not find a significant correlation between *E. coli* concentration and the average number (rho = 0.19) and the modeled number of wildlife (rho = 0.24) in a 24-hour period. Based on these data, cattle management affected bacterial concentrations even at low stocking rates, and wildlife was a major source of contamination, but game cameras alone are not sufficient to quantify this source. This study indicates that attaining the primary body contact standard set by the EPA is not feasible for runoff associated with agricultural land use.

Keywords: *E. coli*, Runoff, Wildlife, Cattle Grazing

Introduction

Bacterial contamination of water bodies used for recreation is a public health risk that has been addressed jointly by the United States Environmental Protection Agency (EPA) and the Center for Disease Control (CDC) (EPA, 2018). With surface runoff as the primary source of water for Oklahoma's streams and reservoirs (Zou et al., 2010), it is critical to quantify the relationship between bacterial contamination of surface runoff and the land use practices of the watershed (Harris et al., 2018). However, this is a challenge because the inconsistency in water quality measurements due to the timing of rainfall events in relation to sample collection and wildlife sources of bacteria has led to the labeled impairment of water bodies that do not have a land use associated with anthropogenic impact (Wagner, 2011).

To ensure water bodies are safe for primary body contact recreation, the EPA defined the acceptable levels of bacterial contamination for recreational use to be anything below a geometric mean for at least 5 samples of 126 colony forming units (cfu) per 100 mL of water for *Escherichia coli (E. coli)* testing (EPA, 1986; EPA, 2012). *E. coli* is often used to assess the safety of freshwater resources because this bacterial species is an indicator of recent fecal contamination, which could contain pathogens known to cause illness in people (Petersen et al., 2018; Jeng et al., 2005; Jamieson et al. 2004; EPA, 1986). Using this benchmark to evaluate stream health has resulted in the labeled impairment of 2,722 stream miles in Oklahoma due to *E. coli* contamination (ODEQ, 2021). Additionally, this assessment determined that grazing in riparian zones or shoreline zones, wildlife other than waterfowl, and rangeland grazing are three of the top five potential sources of stream impairment for the state with each impacting approximately 6,000 river miles (ODEQ, 2021). A large portion of the contamination associated with the top potential sources is due to *E. coli* contamination, but these sources also impact sedimentation and nutrient impairment.

The majority of the cross timbers ecoregion is rural and used extensively for grazing and agriculture (Thomas and Hoagland, 2011; Stallings, 2008). 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 (O'Callaghan et al., 2019; Harmel et al., 2010; McDowell et al., 2006; Jamieson et al., 2004). The general theory is that as grazers are added to a watershed, the increase in fecal matter on the land area they inhabit contaminates rainwater during runoff events. However, there is reason to believe that the contamination found in these past studies is not solely due to the presence of cattle alone. Hong et al. (2018), Harmel et al. (2010), and Jamieson et al. (2004) independently discussed the importance of understanding how wildlife influences contamination and the need to quantify the "background" levels in various watersheds.

Prior studies directly and indirectly measured the background levels of *E. coli* and other contaminants. Control sites from previous studies provide insight into the levels of contamination with no cattle present. Harmel et al. (2010) found that there was no significant difference between bacterial contamination of runoff from a small watershed reported impaired by dairies and one that was deemed unimpacted by grazing. For the sites deemed "unimpacted," the mean *E. coli* concentrations were 1,446 colony forming units (cfu) per 100 mL and 941 cfu per 100 mL compared with the "impacted" sites that had mean *E. coli* concentrations of 1,817, 1,678, and 935 cfu per 100 mL. These similar concentrations of *E. coli* in both "impacted" and "unimpacted" watersheds far exceed EPA standards for primary body contact. In addition, Wagner (2011) found *E. coli* concentrations in runoff from ungrazed pastures in the east central Texas plains ecoregion ranged from 410 cfu per 100 mL to 261,000 cfu per 100 mL with a median of 7,600 cfu per 100 mL and in the Texas Blackland prairie ecoregion ranged from 110 cfu per 100 mL to 21,000 cfu per 100 mL with a median of 4,450 cfu per 100 mL. These high background concentrations indicate that the current EPA water quality standards are not appropriate for application to runoff in ephemeral streams but rather should only be applied to baseflows and reservoirs (Wagner, 2011). Also, quantifying the variability of these background concentrations and loadings in different environments is necessary as background values may vary greatly across different land uses and ecoregions (Rafi et al., 2018; Chen and Chang, 2014; Petersen et al., 2018; Davies-Colley et al. 2008).

In order to address background sources of *E. coli*, the impact of vegetation cover on the wildlife abundance and 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 in a certain vegetation cover, then the frequency of fecal deposit and, therefore, *E. coli* concentrations in surface runoff is likely to increase. In the cross timbers ecoregion, small mammalian (e.g., rodent) communities tend to have the greatest diversity in tallgrass prairies compared to riparian woodlands (Horncastle et al., 2005). Eastern redcedar encroachment into tallgrass prairie decreases animal species richness and diversity by causing one species, white-footed mice (*Peromyscus leucopus*) to dominate the rodent community in comparison with prairie vole (*Microtus ochrogaster*), fulvous harvest mouse (*Reithrodontomys fulevescens*), and cotton rat (*Sigmodon hispidus*) (Horncastle et al., 2005). Feral hogs (*Sus scrofa*), racoons (*Procyon lotor*), eastern cotton tail (*Sylvilagus floridanus)*, and nine-banded armadillos (*Dasypus novemcinctus*) depend on vegetation as food sources as well and have been described as significant contributors to *E. coli* through fecal matter (Parker et al., 2013). Seasonality and climate also play a role when it comes to habitat choice of wildlife. Premathilake (2018) found that the detection probabilities of mesocarnivores were altered due to seasonality. In addition, white-tailed deer (*Odocoileus virginianus*) movement radically changes in response to breeding seasons when bucks dramatically increase the area where they spend time (Holtfreter, 2008). Climate can impact forage availability and therefore species behavior. For instance, white-tailed deer rumens have a greater percentage of forbs in wet years and browse during dry years, most likely due to resource availability (Dillard et al., 2006). Therefore, seasonality should also be examined as an explanatory variable for analyzing wildlife habitat preference and *E. coli* in runoff.

In addition to influencing wildlife preference as a food source, vegetation type directly impacts runoff contamination by its effect on hydrologic processes and water budget. Healthy vegetation cover on a land surface compared with bare ground increases the quality of the soil and the water quality of surface runoff (Butler et al., 2007; Mohammad and Adam, 2010; Bhandari et al., 2017). Additionally, native and non-native vegetation have been successfully utilized as riparian buffers for mitigating the negative effects of land uses on water quality for grasslands and forests around the world (Chase et al., 2016; Udawatta, 2010; Schmitt et al., 1999). The key to improved water quality in runoff is a high and consistent ground cover. Compared to cropland, well-managed grasslands and forests increase the water quality of surface runoff by increasing infiltration and interception. When these two components in the water balance increase, the runoff generally decreases, diminishing its ability to carry sediments and other contaminants (Lyons et al., 2000; Dosskey et al., 2018).

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 as a combined treatment effect. In addition, previous works on wildlife have either described habitat selection or utilized control sites to determine wildlife *E. coli* sources, but no studies have tied these ideas together to examine how habitat selection by wildlife is reflected in *E. coli* contamination. Therefore, the three goals of this study are to: 1) examine the influences of cattle grazing and vegetation type on *E. coli* concentration and loading, 2) compare the concentrations in water quality from the watersheds at CTER to the primary and secondary body contact standards, and 3) evaluate the correlation of wildlife abundance and *E. coli* contamination of runoff.

Materials and Methods:

<u>Site Description</u>

This study took place from April 2020 through October 2021 at the Cross Timbers Experimental Range (CTER), a rangeland area dedicated for agricultural research purposes and managed by Oklahoma State University. CTER is located approximately 18 km southwest of Stillwater, Oklahoma (Zou et al., 2014). The climate of this region is highly variable with substantial seasonal variation. From 2005 to 2019, the nearest Oklahoma Mesonet weather station at Marena, located approximately 2.5 km from CTER, recorded an annual average temperature of 15.6˚C, 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 around 890 mm with wet spring and fall and comparatively dry winter and summer (Qiao et al., 2017).

Previous studies established ten experimental watersheds to study water budget and sedimentation processes based on the dominant vegetation types (oak forest, eastern redcedar woodland, and tallgrass prairie) in CTER (Zou et al., 2014; Qiao et al; 2017; Zhong et al., 2021). For the purposes of this study, the names of each of the watersheds corresponded with the access to grazers (G- Grazed and U-Ungrazed) and the vegetation cover type (O-oak forest, R- eastern redcedar, S-switchgrass, and P-prairie). The numbers associated with each of the watersheds are used to differentiate the watersheds of the same grazer access and vegetation cover from one another. The numbers associated with the replicates correspond as closely to those used by Schmidt (2021) as possible, but the names from her study did not include grazer access. Initially, each vegetation type had at least three watersheds as replicates – oak forest (GO1, GO2, GO3), eastern redcedar woodland (UP2, US2, GR1, GR2), and tallgrass prairie (US1, UP1, GP1) (Figure 1.1). In 2015, eastern redcedar in UP2 and US2 were removed to restore to native prairie (UP2) and plant switchgrass (US2) (Qiao et al., 2017). Vegetation in US1 was treated with herbicide in 2015 and planted to switchgrass in 2016. Since 2017, UP1, UP2, US1, and US2 were fenced to prevent cattle access (Figure 1.1). Switchgrass watersheds (US1, US2) were managed with annual harvest of aboveground biomass.

The slopes of these watersheds are less than 5%. The soils are well drained, consisting predominately of the Stephenville–Darnell complex (StDD), Coyle soil series (Coy, CoyZ), and Grainola–Lucien complex (GrLE) (Table 1.1). The average depth of soil is approximately 1 m underlain by sandstone substrates. The understory or ground cover differs greatly in grass cover depending on the type of vegetation. For prairie and switchgrass watersheds (UP1, US1, US2, UP2, GP1), grass cover ranges from 59.9 – 90.2% but grass cover ranges from 2.5 – 33.2% for oak forest and eastern redcedar watersheds (GO1, GO2, GO3, GR1, GR2). The cover of woody plant, forbs and bare ground vary greatly among individual watersheds (Table 1.2).

<u>Runoff measurement</u>

I installed ISCO Avalanche Portable Refrigerated Samplers equipped with a 720 Submerged Probe Module in the stilling well and a suction line attached to a strainer in the H-flume outflow in all watersheds (Grant and Dawson, 1991). All samplers were powered using solar panels wired to marine batteries. The 720 Submerged Probe Module measured the depth of the water in the stilling well every minute. This depth information was used by the ISCO Avalanche Portable Refrigerated Sampler to calculate the flow of water moving through the flume every 5 minutes. We programmed the samplers based on a flow interval so that each took a sample for every 0.5 mm of runoff that comes through the H-flume (Harmel, 2006). This sample interval was different depending on the size of the watershed, as watersheds with greater areas required larger sampling intervals in cubic feet. During a runoff event, the ISCO Avalanche Portable Refrigerated Sampler drew samples from the water moving through the H-flume based on the flow interval and the samples were composited into a five-gallon bottle.

During the study, it became apparent that the threshold for sample collection at grassland sites was not appropriate for collecting regular samples at forested sites. To account for this, these sites were programmed to sample when the water depth of the flume reached 14 mm on May 16th, 2021. This accounted for 28 out of 38 (74%) of the samples for *E. coli* from the forested watersheds.

E. coli Count

Once collected, the composite sample was split, providing 111 mL for *E. coli* testing and the rest for other water quality analysis. Subsamples were stored between 0 and 10 ^{o}C and away from any light sources according to the Colilert-18 test manual (Crane et al., 2006). Within 5 hours of collection, the bottles containing the samples were gently shaken to ensure the bacteria became suspended in the solution. The subsample was used for three tests: a 100 mL sample, a 10 mL with 90 mL dilution, and a 1 mL with 99 mL dilution. Next, one pack of the Colilert-18 reagent (IDEXX) was added to each dilution and shaken. After the reagent had dissolved, I poured the sample into the Quanti-Tray*/2000 and then sealed it with the IDEXX Quanti-Tray Sealer. Once sealed, the Quanti-Tray*/2000 was incubated at 35±0.5 degrees ^{o}C for 24 hours (IDEXX). After the incubation period, the concentrations for the samples were quantified using the IDEXX Quanti-Tray*/ 2000 MPN Calculator. Utilizing this method to make direct comparisons between MPN and cfu has been verified as valid and is common practice in current research (Hulvey et al., 2021; Kinzelman et al, 2005).

Vegetation Characterization

Ground cover for the watersheds was evaluated using visual estimation with the Daubenmire method. 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 twenty 0.5 m^2 plots (Floyd and Anderson, 1987; Symstad et al., 2008). Error for visual estimation was minimized across sampling in that all the observers had equal experience and training and consensus between two observers was utilized (Morrison, 2016). The plots used were the same as the ongoing clip plots that began with Schmidt et al. (2021) lending to comparison with previously defined vegetation covers for the watersheds in the study.

In order to evaluate canopy coverage of the watersheds, I used the classify tool in ArcMap 10.8 with 1-meter resolution National Agriculture Imagery Program (NIAP) imagery from 2019 from the Oklahoma GIS Clearinghouse to identify the differences in forest and grassland coverage between watersheds. This method has been found to be comparable to more costly and labor-intensive ground sampling methods (Ma et al., 2017). For the extent of this project, differences between oak and redcedar could not be determined because of a lack of imagery of the watersheds during winter months.

Access to Cattle Grazing

The cattle grazing operations at CTER caused variable stocking rates. The cattle were either located in a pasture with access to GO1, GO2, GO3, GR1, GR2, and GP1 or concentrated in a smaller area that included a large portion of GP1. During all months of the year, aside from pre- and post-calving, cattle were grazed at the stocking rate of 17 acres/head/year according to the cattle manager. During pre- and post-calving, 104 animals were concentrated in an area of 80 acres to allow for efficient care of the animals. This caused an increase in head over a smaller area which was incorporated in watershed GP1 but excluded cattle from the rest of the watersheds. This caused GP1 to have a stocking rate of 3.08 acres/head/year for February through May and a stocking rate of 17 acres/head/year for the rest of the study. In comparison, the other watersheds where cattle had access were not grazed during those months when the stocking rate was higher for GP1. Cattle were not equipped with tracking collars or forced to inhabit certain vegetation cover types allowing for cattle to range areas based on free choice.

Wildlife Abundance

Wildlife abundance on the watersheds was determined using pictures taken from 22 motion activated infrared game cameras (Stealth Cam G42NG, Cabela's Outfitter 14 MP Infrared HD Trail Camera, and Bushnell Color Viewer) for the fall season of 2020 through the winter season of 2022. Camera locations were chosen using a pre-determined grid point. In order to determine grid sizes, the sum of the total area for all watersheds was divided by the total number of cameras that could be used for the project. This allowed for me to standardize the area covered by the cameras on each watershed. Next, this camera density was applied to each watershed such that the densities were evenly distributed based on the areas for each watershed. I assigned cameras to each watershed such that the cameras per unit area were consistent. Thus, larger watersheds received more cameras. I then applied a customized grid to all the watersheds in ArcMap 10.8 and a point was placed at the center of each cell that encompassed the maximum area of 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 meters of the pre-determined point. After deployment, cameras collected images for one month per season and the pictures were used to determine both the number and species captured on camera per 24-hour period.

To examine the effect of wildlife abundance on bacterial contamination of runoff, the data collected from game cameras were analyzed and correlated to *E. coli* concentration. Analysis was limited to include only animals the size of rabbits and above including white-tailed deer, wild pigs, and meso-mammals consistent with the protocols from Parker et al. (2015). However, our study did not employ attractants and the cameras were placed randomly to avoid skewing results. I did this because the purpose of this study was to detect the animal abundance, rather than targeting certain species (Meek et al., 2014). Due to financial and personnel constraints, mark recapture activities typically associated with camera trap studies were not feasible.

Data Processing and Statistical Analysis

To quantify the vegetation cover associated with each of the watersheds, the Daubenmire frame data were analyzed by watershed and the descriptive statistics for each of the functional groups were calculated and arranged into a table using the dpylr package in R Studio. These statistics are found in Table 1.2 for each watershed. The GIS analysis that utilized the image classification tool for canopy cover for each watershed was used to calculate an approximate percentage of forest and grassland (Table 1.3). The percentages of canopy cover for grassland and forest were calculated by dividing the number of pixels associated with each canopy type by the total number of pixels in the watershed. The vegetation categories followed Schmidt et al. (2021).

To evaluate the impact of cattle grazing and vegetation cover on the contamination of *E. coli* in surface runoff, I examined the differences in concentrations and loadings between the ten watersheds at the CTER. Each watershed was treated as a separate experimental unit with the *E. coli* concentration and loading for each runoff event considered as the response variables. Concentration and loading values for multiple events within the same watershed were treated as replicates. Grouping by treatment was not possible for the statistical analysis because non-parametric statistics did not allow for me to generate a mixed effects model with watershed as a blocking factor/random effect. The *E. coli* loading of a given sampling event was calculated by multiplying the runoff volume by the *E. coli* concentration and then dividing by the area of the watershed (Wagner, 2011). I organized the data in R and examined the descriptive statistics for the watersheds. Due to the lack of normality, presence of many outliers, and inconsistent variances, I conducted Kruskal-Wallis rank sum tests to detect differences between individual watersheds for concentrations and loadings. If the Kruskal-Wallis rank sum test found that at least one watershed was significantly different from the others, I used a series of pairwise two-sample

Wilcoxon rank sum tests between all watersheds with a Bonferroni correction to ensure I accounted for family-wise error (Hollander et al., 2013). In addition, I compared the median concentrations of runoff from each of the watersheds to the EPA standard for primary body contact (126 cfu per 100 mL) and the ODEQ adjustment for secondary body contact (630 cfu per 100 mL) for *E. coli* concentration using series of one-sample Wilcoxon signed rank tests and created bootstrapped confidence intervals for reporting and comparison.

To examine the correlation between wildlife and *E. coli* contamination, I estimated wildlife abundance and correlated this to median *E. coli* concentrations for each watershed. I used two methods to estimate abundance, defined as the number of wild animals per 24-hour period (trap-night) occurring on each watershed for the purpose of this study. For the first method, we created zero-inflated generalized linear models GLMs with predictor variables with the package pscl in R studio often used in abundance modeling. From these models, I selected the best model using Akaike Information Criteria (AIC) from the bbml package (Risch et al., 2021; Shores et al., 2019). Due to the violations of the assumptions necessary for the Poisson distribution and the result of a Vuong test (p-value <0.001), I decided to employ a zero-inflated model. This model is a two-part model. The first part of the model addresses the factors that influence the binary component if any animals are captured on camera for a given trap-night. The second part of the model addresses the factors that influence the number of animals captured on camera for a given trap-night, assuming wildlife are present. This process allowed for us to formulate a series of models representing various hypotheses about which factors and interactions between factors (season, vegetation cover, and grazing) influenced the number of wildlife captured on camera per trap-night. Due to the highest weight observed by AIC for the H11 model compared to the others, I generated predicted values for each of the scenarios based on this model and then paired these results to *E. coli* data collected under those conditions. The predicted values from this model were then correlated to the *E. coli* concentrations from the watersheds of each corresponding treatment and season.

Using the same camera trap data, we based the wildlife presence directly 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 median *E. coli* concentrations for each watershed.

To compare the results of the correlation of the modeled number of animals per trap-night and the average number of animals per trap-night with median *E. coli* concentrations for each condition we used the Spearman's rank correlation method because, as mentioned above, the *E. coil* data violated normality.

Results

E. coli Concentration

Median *E. coli* concentrations in this study ranged from 237 MPN per 100 mL to 8878 MPN per 100 mL (Table 1.4). Concentrations had a large range of values that differed in an order of magnitude of 5 (Table 1.4). The Kruskal-Wallis rank sum tests showed significant differences among individual watersheds for *E. coli* concentration ($p = 0.009$). The median *E. coli* concentrations from GP1 were significantly greater than UP2 ($p = 0.024$) with no significant differences among the remaining watersheds (Figure 1.2). Only GO1 and GR2 had median *E. coli* concentrations that were not significantly greater than the primary body contact threshold (Table 1.5, Figure 1.2).

Watersheds GP1, US1, and US2 had median *E. coli* concentrations significantly greater than the secondary body contact values but the other watersheds did not (Table 1.5). It should be noted that sample sizes varied dramatically between watersheds with the smallest sample size for watersheds GO2 and GR1 of 5 in comparison with the largest sample sizes for watersheds UP2 and US2 of 32 (Table 1.4).

E. coli Loading

Median *E. coli* loadings in this study ranged from 7.81×10^6 MPN/ha to 5.19×10^9 MPN/ha (Table 1.6). *E. coli* loadings had a large range of values that differed in an order of magnitude of 6 (Table 1.6). The Kruskal-Wallis rank sum tests showed significant differences among individual watersheds for *E. coli* loading ($p = 0.002$). There was one significant difference of median *E. coli* loading values between all the watersheds (Table 1.6; Figure 1.3). The median *E. coli* loading in GP1 (5.19×10^9 MPN per ha) was significantly greater than that in GO1 (7.81×10^6 MPN/ ha) (p-value = 0.048).

Runoff Impact on *E. coli* Concentration and Loading

The forested watersheds had less annual runoff in comparison with the grassland watersheds. However, significant differences could not be detected due to the duration of this study being less than two full years (Table 1.6, Figure 1.4). Figure 1.4 shows the hydrologic influences of eastern redcedar and oak forests on runoff volume compared to the grassland watersheds, consistent with previous studies at the same sites (Schmidt et al., 2021; Zou et al., 2014).

There was a positive, statistically significant relationship between *E. coli* concentration and volume, although the relationship was weak (rho= 0.17, p-value = 0.03) (Figure 1.5). The log transformed *E. coli* concentration also had a weak, positive relationship with volume (rho = 0.22, p-value = 0.004) (Figure 1.5).

Wildlife Presence on the Watersheds

The zero-inflated H11 model performed the best with the highest weight out of the 17 zero-inflated models tested (Table 1.8). The interaction between season and vegetation cover type was included in the top models and in both the count and zero components of the top two models (Table 1.8). The grazing factor was also a key factor in the top model and the directionality of the coefficients is also important to note. The coefficients for the H11 model indicated the differences between the number of animals captured on camera per trap-night were influenced by grazer access and the interaction between season and vegetation coverage (Table 1.9).

Based on model simulation, wildlife will be more likely to be captured on game cameras under certain vegetation covers depending on the season. For example, the H11 model predicts a value of 1.18 (the number of animals anticipated to be caught in a trap night by a game camera) in a grazed oak watershed in the fall (Figure 1.6), contrasting a value of 0.53 in a grazed prairie watershed in the same season (Figure 1.6). Additionally, the forested watersheds, for example GO1, had consistently greater predicted and average number of animals per 24-hour period than the grassland watersheds (Figure 1.6). However, the interaction between season and vegetation cover is included in the selected model, and not vegetation cover alone (Table 1.8). This is reflected in that the spring values are similar among most of the watersheds, regardless of vegetation cover, but vary substantially during other seasons (Figure 1.6).

Correlation between *E. coli* concentrations and Wildlife Presence

There was no correlation between number of animals and median *E. coli* concentration. The number of animals modeled from the top model, H11 (Table 1.8) and the average number of animals per 24-hour period were highly correlated with a rho of 0.72 and a p-value of <0.001. The modeled number of animals per 24-hour period and median *E. coli* concentrations had a rho of 0.24 and a p-value of 0.099 and the average number of animals per 24-hour period had a rho of 0.19 and a p-value of 0.201 (Figure 1.7).

Discussion

Vegetation Cover, Grazing and Wildlife Impacts on *E. coli*

The cattle grazing management at CTER during calving season had a significant impact on *E. coli* contamination which was expected based on previous research. However, forested watersheds where cattle had access did not differ significantly from grasslands where cattle were excluded. The differences in stocking rates between the forested watersheds and GP1 create issues when drawing inferences to examine how vegetation cover can remediate the increased bacterial input from cattle grazing.

Similar to previous studies, I found that the standard deviations exceeded the means for *E. coli* concentration for all of the watersheds at CTER (Wagner et al., 2012; Gregory et al., 2019; Table 1.4). Even under average rainfall conditions for this area (average annual 890 mm compared with this study of 911 mm) limited sample sizes were received such that 4 out of 5 forested watersheds had sample sizes of less than 10. The only significant difference observed was between watersheds GP1 and UP2. Wagner (2011) found no significant differences between properly stocked pastures and ungrazed pastures at the sites near the Brazos River but found significant differences between properly stocked and ungrazed pastures at the sites in Riesel, Texas. The results from my study indicate that even certain watersheds with similar vegetation cover, soil type, cattle management, and geographic location, will differ in the detection of significant differences of *E. coli* concentrations in runoff from watersheds of different management. Specifically, watershed GP1 had significantly greater median *E. coli* concentrations than watershed UP2 but not UP1, despite watershed UP1 and UP2 both excluding cattle grazers. These variations in runoff water quality between individual watersheds observed in previous studies were evident in this study as well.

<u>*E. coli* Concentration and Water Quality Concern</u>

Each of the watersheds except GO1 and GR2 had median *E. coli* concentrations higher than the primary body contact standard. It is important to note that the forested watersheds (GO1 and GR2) that were not significantly different from the primary body contact standard had the highest number of samples for that treatment combination (Table 1.4, Table 1.5). This provides evidence that the forested watersheds could be potentially remediating *E. coli* concentrations in comparison with the grassland watersheds. The result of relatively low bacterial contamination was also found in 5 experimental forested watersheds in Angelina National Forest near Lufkin, Texas. Hunter et al. (1982) observed an average of 137 fecal coliform colonies per 100 mL which was lower than the standard at the time of the study of 200 fecal coliform colonies per 100 mL. One possible explanation could be the increased time between defecation and runoff events (Gregory et al., 2019). With a reduction in the overall runoff, there is a greater amount of time between runoff events, causing bacteria to die before contaminating the runoff.

Further, watershed US1, US2, and GP1 had significantly higher *E. coli* median concentrations compared to the secondary body contact threshold. The sample sizes for these watersheds are much larger in comparison with the forested watersheds, which increases the possibility to capture runoff events immediately after defecation leading to high *E. coli* concentrations (Gregory et al., 2019). Despite the lack of cattle grazing on watersheds US1 and US2, watersheds managed for annual harvest had been observed as having comparatively high *E. coli* concentrations. Gregory et al. (2019) observed a median of 5,950 cfu per 100 mL from a watershed managed for coastal bermudagrass and harvested seasonally. Because wildlife were the only source on these watersheds and these watersheds still significantly exceeded the secondary body contact, this standard should not be applied to runoff water quality.

The detection of a significantly higher median *E. coli* concentration and loading from watershed GP1 compared to at least one watershed was expected due to the differences in cattle management. The current stocking rate at CTER is 17 acres/head/year which is much lower in comparison to the stocking rate at GP1 of 3.93 acres/head/year during the calving season from February through March. With a higher stocking rate during and immediately before the wettest part of the year, it would have been reasonable to have observed the median from GP1 to be significantly higher than all of the watersheds at CTER. However, after calving season that pasture is no longer accessible to the cattle. Differences between GP1 and the rest of the watersheds were likely mitigated by the exclusion of cattle to a large portion of GP1 following the higher stocking rate (Hulvey et al., 2021; Wagner et al., 2012).

Vegetation Cover and *E. coli* Loadings

E. coli loading comparisons followed a similar trend to *E. coli* concentration. 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 have also examined differences between loading on an event basis (Gregory et al., 2019). The *E. coli* loading values in this study were in the general range of those observed in previous studies. Wagner et al. (2012) observed median loading values of runoff of 8.1×10^{10} cfu/ha from grazed native rangeland and 4.2×10^{10} cfu/ha from ungrazed native rangeland. The median value for watershed GP1 had a median loading of 5.19×10^{9} MPN/ha which is relatively low, but within the general range of the values previously observed. Consistent with Wagner et al. (2012), the ungrazed grassland watersheds (UP1 and UP2) had lower loading values in comparison to GP1 although these differences were not significant.

The loading differences in the individual watersheds appear to have been influenced by the impact of vegetation cover on runoff volume. Watershed GP1 did not show significant differences in *E. coli* concentration 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 (Table 1.7, Figure 1.4). Greater loading values were observed from watersheds that have higher amounts of runoff, a part of the hydrologic cycle that is known to be heavily influenced by vegetation cover (Bonan, 2002; Calder et al., 2007) (Table 1.6).

These results suggest one topic for a long-term study at CTER could evaluate the changes in loading and runoff following periods of woody encroachment as a result of fire exclusion. As woody species begin to encroach on these watersheds and influence the hydrology, the data from this study suggests that this will influence loading over time. Barger et al. (2011) claim that unmanaged/ungrazed rangelands in the central Great Plains change in woody cover at a rate of over 1.5% each year. Currently, there is a lack of information on the impacts of encroachment of eastern redcedar into the Great Plains on water quality with most of the previous work centered around documenting differences in sediment yield (Zou et al., 2018).

Wildlife and *E. coli*

In addition to the impact of vegetation cover on *E. coli* concentrations and loadings, the selection of these areas by wildlife was likely a contributor to variation between the watersheds in this study. Past research has indicated that wildlife is a major contributor to bacterial contamination of surface runoff (Wagner et al., 2011; Harmel et al., 2010; Parker et al., 2013). The background levels, the concentrations of runoff from sites where cattle grazing was not occurring, were high enough to exceed the EPA water quality standard for primary body contact for all the watersheds except GO1 and GR2. Despite the concentrations being significantly greater than the primary body contact standard, the concentrations observed in this study were also much lower than levels found from control sites of previous studies, possibly due to the method of cattle exclusion. This study used electric fencing at two of the sites and barbed wire at the other two ungrazed sites. Previous studies used barbed wire fencing (Harmel et al., 2010; Wagner et al., 2012; Gregory et al., 2019). The impact of the differences in exclusion between this study and previous studies on *E. coli* sources is an area of future study. Additionally, the consistently lower background levels at our locations provide evidence that runoff from areas in different climatic and/or geographic regions have dissimilar bacteria concentrations (Rafi et al., 2018).

The seasonality of wildlife preference for certain habitat types also played a role in *E. coli* concentrations. The result of the top model included the interaction between vegetation cover and season. This indicates that the season and the vegetation cover are important factors to consider for evaluating wildlife presence. In the case of this study, I found that both the averaged and predicted animals per 24-hour period tended to be greater during the spring and summer seasons in comparison with the other months (Figure 1.6). For instance, the ungrazed prairie watersheds (UP1 and UP2) had higher numbers of predicted and average number captured in a

24-hour period in the summer in comparison with the grazed oak watersheds (GO1, GO2, and GO3) that had higher numbers in the fall and spring compared to the summer months (figure 1.6). The influence of season on wildlife movement has been observed in other studies in this region. Premathilake (2018) found that most meso carnivores in southcentral Oklahoma like striped skunks (*Mephitis mephitis*), Virginia opossum (*Didelphis virginiana*), bobcat (*Lynx rufus*), grey fox (*Urocyon cinereoargentus*), and northern racoon (*Procyon lotor*), had higher site occupancy (captures on game camera) during the winter compared to the summer. I found the opposite of these results because the coefficients for interactions between vegetation cover and winter were typically lower or negative compared with those same vegetation covers for summer (Table 1.9). White-tailed deer also experience differences in behavior throughout the year. Holtfreter (2008) found that both juvenile and adult males in southeastern Oklahoma increased their daily movement patterns just before and during the breeding season. The increased numbers of wildlife observed in watersheds GO1 and GO2 could be explained, in part, due to greater numbers of bucks moving through these areas during the fall (Figure 1.6).

The access of grazers was important for determining wildlife presence both in the number of times animals were recorded during a trap night and, assuming animals were present, the number. This study found that the coefficient for ungrazed areas had a negative coefficient for the presence/absence part of the model but a positive coefficient for the count part of the model (Table 1.9). This suggests that there is a lower number of times an animal is caught on camera in the ungrazed areas compared to the grazed areas. However, assuming animals are captured on camera for that trap night, the number of animals that will be observed in ungrazed areas is greater than that of grazed areas. Brewer (2021) conducted a camera trap study on the impacts of cattle on mule deer behavior in Nebraska. This study found that mule deer generally avoid cattle, but the avoidance is temporal, rather than avoidance of areas where cattle are present altogether. The results from this study could have been influenced in a similar way. In this study, less presence/absence was modeled in ungrazed areas, but the number was higher in these areas once detected. Wildlife, therefore, were not avoiding areas with access to cattle grazers but they did not appear on camera when cattle were present or in large numbers. This difference is reflected in the predicted and average numbers between watersheds GO1, GO2, GO3, GP1, GR1, and GR2 in comparison with UP1, UP2, US1, and US2 (Figure 1.6). However, the impact of grazing for the model should be considered carefully because all the forested watersheds in the study had access to grazers, creating a potential for autocorrelation between forest cover and grazer access.

Despite our effort to correlate game camera data to *E. coli* concentrations, the data exhibited a weak, positive correlation between the number of animals captured in a 24-hour period and the median *E. coli* concentration. There are a couple of explanations for this. First, there are other sources of *E. coil* outside of the meso mammals used in the correlation for this study. A 2006 bacterial source tracking study on the Trinity River in the cross timbers ecoregion in Texas found that the avian (23.2%) contribution to *E. coli* was greater than that of the mammalian wildlife (13.4%) source (Miertschin and Water, 2006). Second, this study had limitations in scope when it came to the data collection for the camera trap study itself. Due to a lack of permitting, time, funding, expertise, and man-power, a mark-recapture study typically associated with camera trap data could not be conducted. This limitation causes the data to be skewed in cases where an individual tends to be captured on camera multiple times (Chandler and Royle, 2013). Also, the study employed various types of cameras in areas with different sizes of fields of view. This is a common issue for these types of studies and if models are used that do not take into consideration imperfect detection, like the one utilized in this study, the results can be misleading (Burton et al., 2015; Apps and McNutt, 2018). These things can be avoided 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, the study was effective at observing the general number of animals present in the different vegetation covers at CTER. I did not bait the traps, and I placed the cameras in even densities without selectively picking specific areas of traffic (Meek et al., 2014). The specific movements of a particular species could be considered in the future. For example, Holtfreter (2008) included data of GPS collared deer occurrences in different vegetation coverage types in southeastern Oklahoma. The publication of the data would have been useful for comparison with the findings from our camera trap study, but the analysis focused on habitat range size based on the heterogeneity of the land use rather than including results about how much time the deer spent in one habitat type compared to another. Despite the reasons mentioned above, this preliminary data indicated that mammalian wildlife could be impacting *E. coli* concentration on some level as indicated by the positive correlation.

The watersheds used in this study were either absent of active management or had land use and land coverage associated with responsible ranching practices. For these reasons, I anticipated that the water quality of runoff from the field scale, upland watershed with primary stormflow would pass both the EPA and ODEQ standards for the determination of impairment, but this was not the case for all watersheds evaluated. Previous studies found similar results and attribute this impairment largely to dilution differences. Analysis from Rafi et al. (2018) showed that there was a significant negative correlation between *E. coli* concentrations and increasing stream order size and watershed area. Essentially, edge of field runoff has no dilution associated with larger bodies of water used for recreation. This study provides further justification for finding an alternative method for determining impairment for *E. coli* concentrations of runoff water quality and continued monitoring of water quality from the experimental watersheds at CTER.

Conclusions

Determining the impairment of runoff by *E. coli* in agricultural areas is an important research topic because of the human health concern that bacterial contamination causes.

However, this study adds to the current body of work that demonstrates that attaining the primary body contact standard set by the EPA is not feasible for runoff associated with agricultural land use. This could improve through: 1) utilization of reference sites for ecoregion specific standards of *E. coli* at the edge of field scale, and 2) improving model predictions through comparison with direct observations, especially in eastern redcedar encroached watersheds. These suggestions provide policy makers and land managers with data driven suggestions about how much bacterial contamination at the field-scale is appropriate. The results and methods from the camera trap study demonstrate a need to conduct more thorough investigations on wildlife bacteria sources. The data from game cameras are not sufficient for correlating wildlife presence with *E. coli* contamination. This will require water quality scientists to collaborate with experts in wildlife ecology for assistance in collecting and analyzing movement and behavioral data. The loading data suggests that vegetation cover has influences on runoff volume and further assessment is needed regarding whether *E. coli* concentration or loading is a better measure of impairment of upland watersheds. Grazing in a prairie watershed had the highest concentration but this appears to be heavily influenced by the timing of cattle presence in relation to runoff and this should be accounted for in future study and analysis.

References

Apps, P., McNutt, J. (2018). Are camera traps fit for purpose? A rigorous, reproducible and realistic test of camera trap performance. *African Journal of Ecology*, *56*, 710-720.

Barger, N. N., Archer, S. R., Campbell, J. L., Huang, C., Morton, J. A., Knapp, A. K. (2011). Woody plant proliferation in North American drylands: a synthesis of impacts on ecosystem carbon balance. *Journal of Geophysical Research: Biogeosciences* *116*(G4).

Bonan, G. B. (2002). Hydrologic Cycle. *Ecological Climatology: Concepts and Applications, 1st edn. Cambridge University Press, New York,* 129-166.

Bhandari, S., Maruthi Sridhar, B. B., & Wilson, B. L. (2017). Effect of land cover changes on the sediment and water quality characteristics of Brays Bayou watershed. *Water, Air, & Soil Pollution*, *228*(9), 1-14.

Brewer, S. (2021). *Spatiotemporal Interactions Between Deer and Cattle* [Undergraduate Honors Thesis]. University of Nebraska-Lincoln, Lincoln, NE, USA.

Butler, D. M., Ranells, N. N., Franklin, D. H., Poore, M. H., & Green Jr, J. T. (2007). Ground cover impacts on nitrogen export from manured riparian pasture. *Journal of Environmental Quality*, *36*(1), 155-162.

Burton, A. C., Neilson, E., Moreira, D., Ladle, A., Steenweg, R., Fisher, J. T., Bayne, E., & Boutin, S. (2015). Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology*, *52*(3), 675-685.

Calder, I. R. (2007). Forests and water—ensuring forest benefits outweigh water costs. *Forest Ecology and Management*, *251*(1-2), 110-120.

Chandler, R. B., & Royle, J. A. (2013). Spatially explicit models for inference about density in unmarked or partially marked populations. *The Annals of Applied Statistics*, 936-954.

Chase, J. W., Benoy, G. A., Hann, S. W. R., & Culp, J. M. (2016). Small differences in riparian vegetation significantly reduce land use impacts on stream flow and water quality in small agricultural watersheds. *Journal of Soil and Water Conservation*, *71*(3), 194-205.

Chen, H. J., & Chang, H. (2014). Response of discharge, TSS, and E. coli to rainfall events in urban, suburban, and rural watersheds. *Environmental Science: Processes & Impacts*, *16*(10), 2313-2324.

Crane, R., Jackson, C. B., Sedlacek, R., & Walker, D. (2006). EPA approves new test procedures for the analysis of microbiological pollutants in wastewater and sludge. *Loveland, CO: Hach Company*.

Davies-Colley, R., Nagels, J., & Lydiard, E. (2008). Stormflow-dominated loads of faecal pollution from an intensively dairy-farmed catchment. *Water Science and Technology*, *57*(10), 1519-1523.

Dillard, J., Jester, S., Baccus, J., Simpson, R., & Poor, L. Texas Parks and Wildlife Department. (2006). *White-tailed deer food habits and preferences in the Cross Timbers and Prairies region of Texas*.

Dosskey, M. G., Vidon, P., Gurwick, N. P., Allan, C. J., Duval, T. P., & Lowrance, R. (2010). The role of riparian vegetation in protecting and improving chemical water quality in streams. *JAWRA Journal of the American Water Resources Association*, *46*(2), 261-277.

Floyd, D. A., & Anderson, J. E. (1987). A comparison of three methods for estimating plant cover. *The Journal of Ecology*, 221-228.

Grant, D. M., & Dawson, B. D. (1991). *ISCO Open Channel Flow Measurement Handbook*. Lincoln. Neb., ISCO Environmental Division.

Gregory, L. F., Harmel, R. D., Karthikeyan, R., Wagner, K. L., Gentry, T. J., & Aitkenhead-Peterson, J. A. (2019). Elucidating the Effects of Land Cover and Usage on Background Escherichia coli Sources in Edge-of-Field Runoff. *Journal of Environmental Quality*, *48*(6), 1800-1808.

Harmel, R. D., King, K. W., Haggard, B. E., Wren, D. G., & Sheridan, J. M. (2006). Practical guidance for discharge and water quality data collection on small watersheds. *Transactions of the ASABE*, *49*(4), 937-948.

Harmel, R. D., Karthikeyan, R., Gentry, T., & Srinivasan, R. (2010). Effects of agricultural management, land use, and watershed scale on E. coli concentrations in runoff and streamflow. *Transactions of the ASABE*, *53*(6), 1833-1841.

Harris, C. S., Tertuliano, M., Rajeev, S., Vellidis, G., & Levy, K. (2018). Impact of storm runoff on Salmonella and Escherichia coli prevalence in irrigation ponds of fresh produce farms in southern Georgia. *Journal of Applied Microbiology*, *124*(3), 910-921.

Havstad, K. M., Peters, D. P., Skaggs, R., Brown, J., Bestelmeyer, B., Fredrickson, E., Herrick, J., & Wright, J. (2007). Ecological services to and from rangelands of the United States. *Ecological Economics*, *64*(2), 261-268.

Helsel, D. R., & Hirsch, R. M. (1992). *Statistical methods in water resources* (Vol. 49). Elsevier.

Hollander, M., Wolfe, D. A., & Chicken, E. (2013). *Nonparametric statistical methods* (Vol. 751). John Wiley & Sons. NJ.

Holtfreter, R. (2008). *Spatial ecology of male white-tailed deer in the cross timbers and prairies ecoregion* [Master's thesis]. Auburn University

Hong, E. M., Park, Y., Muirhead, R., Jeong, J., & Pachepsky, Y. A. (2018). Development and evaluation of the bacterial fate and transport module for the Agricultural Policy/Environmental eXtender (APEX) model. *Science of the Total Environment*, *615*, 47-58.

Horncastle, V. J., Hellgren, E. C., Mayer, P. M., Ganguli, A. C., Engle, D. M., & Leslie, D. M. (2005). Implications of invasion by *Juniperus virginiana* on small mammals in the southern Great Plains. *Journal of Mammalogy*, *86*(6), 1144-1155.

Hulvey, K. B., Mellon, C. D., & Kleinhesselink, A. R. (2021). Rotational grazing can mitigate ecosystem service trade-offs between livestock production and water quality in semi-arid rangelands. *Journal of Applied Ecology*, *58*(10), 2113-2123.

Jamieson, R., Gordon, R., Joy, D., & Lee, H. (2004). Assessing microbial pollution of rural surface waters: A review of current watershed scale modeling approaches. *Agricultural Water Management*, *70*(1), 1-17.

Jeng 1, H. C., England 2, A. J., & Bradford 2, H. B. (2005). Indicator organisms associated with stormwater suspended particles and estuarine sediment. *Journal of Environmental Science and Health*, *40*(4), 779-791.

Kinzelman, J. L., Singh, A., Ng, C., Pond, K. R., Bagley, R. C., & Gradus, S. (2005). Use of IDEXX Colilert-18® and Quanti-Tray/2000 as a rapid and simple enumeration method for the implementation of recreational water monitoring and notification programs. *Lake and Reservoir Management*, *21*(1), 73-77.

Lyons, J., Thimble, S. W., & Paine, L. K. (2000). Grass versus trees: managing riparian areas to benefit streams of Central North America. *JAWRA Journal of the American Water Resources Association*, *36*(4), 919-930.

Ma, Q., Su, Y., & Guo, Q. (2017). Comparison of canopy cover estimations from airborne LiDAR, aerial imagery, and satellite imagery. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, *10*(9), 4225-4236.

McDowell, R. W., Muirhead, R. W., & Monaghan, R. M. (2006). Nutrient, sediment, and bacterial losses in overland flow from pasture and cropping soils following cattle dung deposition. *Communications in Soil Science and Plant Analysis*, *37*(1-2), 93-108.

Meek, P. D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'brien, T., O'Connell, A., Sanderson, J., Swann, D. E., Tobler, M., & Townsend, S. (2014). Recommended guiding principles for reporting on camera trapping research. *Biodiversity and Conservation*, *23*(9), 2321-2343.

Moeller, A. K., Lukacs, P. M., & Horne, J. S. (2018). Three novel methods to estimate abundance of unmarked animals using remote cameras. *Ecosphere*, *9*(8), e02331.

Mohammad, A. G., & Adam, M. A. (2010). The impact of vegetative cover type on runoff and soil erosion under different land uses. *Catena*, *81*(2), 97-103.

Miertschin, J., & Water, P. (2006). *Monitoring Report for Bacterial Source Tracking, Segments 0806, 0841, and 0805 of the Trinity River, Bacteria TMDL*. Texas Institute for Applied Environmental Research.

Morrison, L. W. (2016). Observer error in vegetation surveys: a review. *Journal of Plant Ecology*, *9*(4), 367-379.

Nakashima, Y., Hongo, S., Mizuno, K., Yajima, G., & Dzefck, Z. S. C. (2022). Double-observer approach with camera traps can correct imperfect detection and improve the accuracy of density estimation of unmarked animal populations. *Scientific Reports*, *12*(1), 1-10.

O'Callaghan, P., Kelly-Quinn, M., Jennings, E., Antunes, P., O'Sullivan, M., Fenton, O., & Huallachain, D. O. (2019). The environmental impact of cattle access to watercourses: a review. *Journal of Environmental Quality*, *48*(2), 340-351.

Oklahoma Department of Environmental Quality. (2021). *Water Quality in Oklahoma: Integrated Report, 2020*.

Parker, I. D., Lopez, R. R., Padia, R., Gallagher, M., Karthikeyan, R., Cathey, J. C., Silvy, N. J., & Davis, D. S. (2013). Role of free-ranging mammals in the deposition of Escherichia coli into a Texas floodplain. *Wildlife Research*, *40*(7), 570-577.

Parker, I. D., Lopez, R. R., Karthikeyan, R., Silvy, N. J., Davis, D. S., & Cathey, J. C. (2015). A model for assessing mammal contribution of Escherichia coli to a Texas floodplain. *Wildlife Research*, *42*(3), 217-222.

Petersen, F., Hubbart, J. A., Kellner, E., & Kutta, E. (2018). Land-use-mediated Escherichia coli concentrations in a contemporary Appalachian watershed. *Environmental Earth Sciences*, *77*(22), 1-13.

Premathilake, E. D. (2018). *Integrating camera trap data and occupancy modeling to estimate seasonal variations in occurrence, detection, and activity patterns of mesocarnivores in* *southcentral Oklahoma*. [Master's thesis, University of Central Oklahoma]. ProQuest Dissertations Publishing.

Qiao, L., Zou, C. B., Stebler, E., & Will, R. E. (2017). Woody plant encroachment reduces annual runoff and shifts runoff mechanisms in the tallgrass prairie, USA. *Water Resources Research*, *53*(6), 4838-4849.

Rafi, K., Wagner, K. L., Gentry, T., Karthikeyan, R., & Dube, A. (2018). Escherichia coli concentration as a function of stream order and watershed size. *Journal of Environmental Quality*, *47*(5), 949-957.

Risch, D. R., Ringma, J., Honarvar, S., & Price, M. R. (2020). A comparison of abundance and distribution model outputs using camera traps and sign surveys for feral pigs. *Pacific Conservation Biology, 27*(2), 186-194.

Schmitt, T., Dosskey, M. G., & Hoagland, K. D. (1999). Filter strip performance and processes for different vegetation, widths, and contaminants. *Journal of Environmental Quality*, *28*, 1479-1489

Schmidt, K. N., Zou, C. B., Kakani, V. G., Zhong, Y., & Will, R. E. (2021). Improved productivity, water yield, and water use efficiency by incorporating switchgrass cultivation and native ecosystems in an integrated biofuel feedstock system. *GCB Bioenergy, 13*(3), 369-381.

Shores, C. R., Dellinger, J. A., Newkirk, E. S., Kachel, S. M., & Wirsing, A. J. (2019). Mesopredators change temporal activity in response to a recolonizing apex predator. *Behavioral Ecology*, *30*(5), 1324-1335.

Stallings, C. V. (2008). *Analysis of land use, land cover change in the upland forests of Oklahoma: a comparison between the 1950s and 2000s*. [Master's thesis, Oklahoma State University]. ProQuest Dissertations Publishing.

Symstad, A. J., Wienk, C. L., & Thorstenson, A. D. (2008). Precision, repeatability, and efficiency of two canopy-cover estimate methods in northern Great Plains vegetation. *Rangeland Ecology & Management*, *61*(4), 419-429.

Thomas, R. E., & Hoagland, B. W. (2011). The changing forests of central Oklahoma: A look at the somposition of the cross timbers prior to Euro-American settlement, in the 1950's, and today. *Oklahoma Native Plant Record*, *11*(1).

Udawatta, R. P., Garrett, H. E., & Kallenbach, R. L. (2010). Agroforestry and grass buffer effects on water quality in grazed pastures. *Agroforestry Systems*, *79*(1), 81-87.

U.S. EPA. (1986). *EPA's ambient water quality criteria for bacteria – 1986*. EPA 440/5-84-002.

U.S. EPA (2012). *Recreational water quality criteria document: office of water.* EPA 820-F-12-058.

U.S. EPA (2018). *2017 Five-year review of the 2012 recreational water quality criteria.* EPA 823-R-18-001.

Wagner, K. L. (2011). *Evaluation of methods to assess and reduce bacterial contamination of surface water from grazing lands*. [Doctoral Dissertation, Texas A&M University]. ProQuest Dissertations Publishing.

Wagner, K. L., Redmon, L. A., Gentry, T. J., & Harmel, R. D. (2012). Assessment of cattle grazing effects on E. coli runoff. *Transactions of the ASABE*, *55*(6), 2111-2122.

Zou, C., Turton, D., & Engle, D. M. (2010). How eastern redcedar encroachment affects the water cycle of Oklahoma rangelands.

Zou, C. B., Turton, D. J., Will, R. E., Engle, D. M., & Fuhlendorf, S. D. (2014). Alteration of hydrological processes and streamflow with juniper (Juniperus virginiana) encroachment in a mesic grassland catchment. *Hydrological Processes*, *28*(26), 6173-6182.

Table 1.1 Slope and soil characteristics of each watershed (WS). The values in the slope column are in percent rise. The first value is the average slope and the second value is the standard deviation for the watersheds calculated with the slope tool in ArcMap 10.8. Values for each watershed are separated by a semi colon. Soil types are in percentage of area coverage of the watershed. Abbreviations are as follows: StDD: Stephenville-Darnell complex; Coy: Coyle loam; ReGr: Renfrow and Grainola soils; CoyZ: Coyle and Zaneis soils; GrLE: Grainola-Lucien complex; StSL: Stephenville fined sandy loam; CoLC: Coyle-Lucien complex; HaPE: Harrah-Pulaski complex; Zaneis-Huska complex; DooSL: Doolin silt loam.

WS	Area (m^2)	Slope	StDD	Coy	ReGr	CoyZ	GrLE	StSL	CoLC	HaPE	ZaHC	DooSL
GO1	23900	2.56; 1.04	100	0.00	0	0	0	0	0	0	0	0
GO2	28300	3.03; 1.69	0	47.2	1.55	12.7	0	34.9	0	0	0	3.83
GO3	46500	2.75; 1.66	0	22.6	7.77	0.989	0	68.8	0	0	0	0
GP1	40300	2.90; 1.40	55.2	44.8	0	0	0	0	0	0	0	0
GR1	29800	4.1; 2.6	91.9	0	0	0.320	7.92	0	0	0	0	0
GR2	13500	4.47; 2.67	21.9	0	0	56.7	21.5	0	0	0	0	0
UP1	22600	2.41; 1.65	63.5	19.9	0	0	0	0	0	15.4	1.12	0
UP2	25700	3.29; 1.66	77.4	0	11.3	0	2.88	8.66	0	0	0	0
US1	33300	2.77; 1.55	67.5	32.5	0	0	0	0	0	0	0	0
US2	37900	3.03; 1.62	29.3	0	28.8	0	13.0	8.55	20.3	0	0	0

Table 1.2 Understory/ground cover of each watershed excluding percentage of litter collected during the fall of 2020. The values for each vegetation column are the mean followed by the standard deviation of the percent cover estimated with Daubenmire frames. Values will not equal 100% because the ground cover by litter was not included.

Watershed	Treatment	Woody	Grass	Forb	Bare Ground
GO1	Grazed Oak	3.8 ± 3.9	2.5 ± 0	3.8 ± 3.9	23.6 ± 30.5
GO2	Grazed Oak	2.5 ± 0	19.1 ± 27.7	4.9 ± 8.2	5.50 ± 8.5
GO3	Grazed Oak	3.1 ± 2.8	28.6 ± 29.1	5.6 ± 5.6	3.1 ± 2.8
GP1	Grazed Prairie	6.0 ± 10.8	59.9 ± 21.3	8.8 ± 6.4	15.5 ± 15.6
GR1	Grazed Red Cedar	2.5 ± 0	5.5 ± 8.5	3.1 ± 2.8	11.5 ± 19.4
GR2	Grazed Red Cedar	2.5 ± 0	33.2 ± 38.1	4.5 ± 4.7	6.45 ± 5.9
UP2	Ungrazed Prairie	2.5 ± 0	72.5 ± 27.3	20.9 ± 18.1	3.1 ± 2.8
UP2	Ungrazed Prairie	3.2 ± 2.9	70.1 ± 30.1	20.8 ± 19.5	5.7 ± 8.7
US1	Ungrazed Switchgrass	2.5 ± 0	86.2 ± 21.0	6.12 ± 8.7	8.38 ± 19.7
US2	Ungrazed Switchgrass	2.5 ± 0	90.2 ± 11.1	2.5 ± 0	5.5 ± 8.5

Table 1.3 Cattle access and canopy cover characteristics for each of the watersheds at CTER. Percent forest and percent grassland canopy coverage were calculated using the image classification tool in ArcMap 10.8 on the NAIP imagery from 2019.

Watershed	Grazer Access	Dominant Vegetation	Percent Forest	Percent Grassland
GO1	Yes	Oak	57.4	42.7
GO2	Yes	Oak	66.1	33.9
GO3	Yes	Oak	57.1	42.9
GP1	Yes	Native Prairie	6.5	93.5
GR1	Yes	Redcedar	92.8	7.2
GR2	Yes	Redcedar	80.9	19.1
UP2	No	Native Prairie	0.1	99.9
UP2	No	Native Prairie	0.3	99.8
US1	No	Switchgrass	0.1	99.9
US2	No	Switchgrass	0.2	99.8

Table 1.4 Descriptive statistics for the *E. coli* concentrations in MPN/100 mL of runoff from the watersheds at CTER. P25 and P75 are the 25th and 75th percentiles of the distribution for each of the watersheds respectively.

Watershed	Mean	Std	Min	Max	P25	Median	P75	Sample Size
GO1	1,499	2,030	18	4,621	19	320	3,687	9
GO2	2,645	3,855	580	9,529	866	1,046	1,203	5
GO3	2,109	3,121	60.	8,257	288	411	2,730	7
GP1	21,920	35,040	37	141,400	966	8,878	29,100	20
GR1	33,780	55,640	150	129,900	1,890	2,420	34,480	5
GR2	7,596	20,710	3.0	72,700	16	374	2,695	12
UP1	3,063	5,778	7.0	22,470	84	260	1,789	23
UP2	2,479	4,625	3.0	19,860	71	237	1,493	32
US1	1,841	2,664	6.0	9,804	204	928	1,923	30
US2	4,152	9,597	9.0	51,720	206	1,482	2,668	32

Table 1.5 The results of the one-sample Wilcoxon signed rank tests between the median concentrations of runoff from each watershed and the EPA primary body contact standard (126 cfu per 100 mL) and Oklahoma secondary body contact standard (630 cfu per 100 mL).

Watershed	Wilcoxon Signed Rank Statistic	P Value (Primary)	Wilcoxon Signed Rank Statistic	P Value (Secondary)	Median	Bootstrapped CI
GO1	35	0.077	24	0.45	320	(19, 3,687)
GO2	15	**0.031**	14	0.063	1,046	(579, 1,203)
GO3	26	**0.026**	16	0.40	411	(60, 411)
GP1	207	**<0.001**	194	**<0.001**	8,878	(1,285, 24,382)
GR1	15	**0.031**	14	0.063	2,420	(148, 34,480)
GR2	35	0.25	15	0.90	124	(9, 921)
UP1	231	**0.002**	141	0.47	260	(87, 816)
UP2	412	0.003	302	0.24	237	(89, 1,159)
US1	432	**<0.001**	320	**0.037**	928	(281, 1,299)
US2	492	**<0.001**	429	**0.001**	1,482	(280, 2,213)

Table 1.6 Descriptive statistics for the *E. coli* loading values from individual runoff events for each of the watersheds at CTER. Loading values are in units of MPN/ha.

Watershed	Mean	Median	Min	Max	Std	Sample Size
GO1	1.56×10^{8}	7.81×10^{6}	2.12×10^{6}	5.28×10^{8}	2.21×10^{8}	8
GO2	1.05×10^{9}	1.00×10^{8}	1.60×10^{6}	4.88×10^{9}	2.14×10^{9}	5
GO3	3.48×10^{8}	2.01×10^{7}	1.95×10^{6}	1.61×10^{9}	6.20×10^{8}	7
GP1	2.05×10^{10}	5.19×10^{9}	4.32×10^{6}	1.56×10^{11}	3.71×10^{10}	20
GR1	3.84×10^{8}	5.14×10^{7}	2.20×10^{5}	1.45×10^{9}	6.20×10^{8}	5
GR2	4.33×10^{8}	4.56×10^{7}	4.85×10^{4}	3.10×10^{9}	8.82×10^{8}	12
UP1	5.55×10^{9}	3.18×10^{8}	7.16×10^{5}	8.03×10^{10}	1.72×10^{10}	22
UP2	2.04×10^{9}	2.59×10^{8}	8.02×10^{5}	3.11×10^{10}	6.07×10^{9}	31
US1	1.42×10^{9}	6.61×10^{8}	5.47×10^{5}	1.23×10^{10}	2.46×10^{9}	29
US2	1.92×10^{9}	5.59×10^{8}	9.12×10^{5}	2.12×10^{10}	4.24×10^{9}	26

Table 1.7 The annual runoff in millimeters for each watershed at CTER. Median, Max, and minimum values displayed are monthly values for the data for that year.

Watershed	Year	Runoff (mm)			
		Total	Max	Min	Median
GO1	2020	29.6	25.5	0	0
	2021	6.4	3.8	0	0
GO2	2020	2.8	2.6	0	0
	2021	13.7	6.4	0	0.1
GO3	2020	9.2	7.0	0	0.1
	2021	5.0	2.7	0	0
GP1	2020	68.2	56.3	0	0.9
	2021	108	49.8	0.1	8.5
GR1	2020	0.7	0.3	0	0.1
	2021	25.6	12.7	0	0.3
GR2	2020	27.8	7.3	0.6	1.9
	2021	26.8	16.6	0	0.9
UP1	2020	144.6	102.1	0.3	2.6
	2021	146.7	51.3	0	15.0
UP2	2020	205.2	179.4	0.4	2.8
	2021	176.3	46.6	1.1	17.0
US1	2020	100.9	64.2	0	1.6
	2021	172.3	61.3	0	18.0
US2	2020	78.7	42.2	0.01	3.1
	2021	106.5	22.8	0	12.0

Table 1.8 The AIC selection results for the zero-inflated model for predicting number of animals captured on game camera per 24-hour period. Δ AIC (Akaike Information Criteria) values represent the models' predictive performance with lower values suggesting better performance. Df (Degrees of freedom) are the number of combinations of the components included in the analysis.

Model	Δ AIC	Df	Weight	Zero Component	Count Component
H11	0	37	1	Vegetation*Season+Grazing	Vegetation*Season+Grazing
H12	40.6	33	<0.001	Vegetation*Season	Vegetation*Season
H13	69.3	23	<0.001	Season+Grazing	Vegetation*Season
H10	455	3.0	<0.001	Null Model	Null Model

Table 1.9 The coefficients for the best performing model (H11 model). Positive coefficients indicate a greater number of pictures predicted in a 24-hour period. The absolute value of the number indicates the magnitude of the change from the comparison group. Comparison groups are stated in the table.

	Zero Component	Count Component
	Ungrazed compared with Grazed	
	-0.71	0.33
	Season and Vegetation compared with Oak – Fall	
Prairie – Spring	0.10	23
Redcedar – Spring	-0.62	3.1
Switchgrass – Spring	3.30	-3.2
Prairie – Summer	1.90	20
Redcedar – Summer	0.90	1.1
Switchgrass – Summer	5.10	26
Prairie – Winter	-0.03	34
Redcedar – Winter	0.80	-15
Switchgrass – Winter	3.70	39

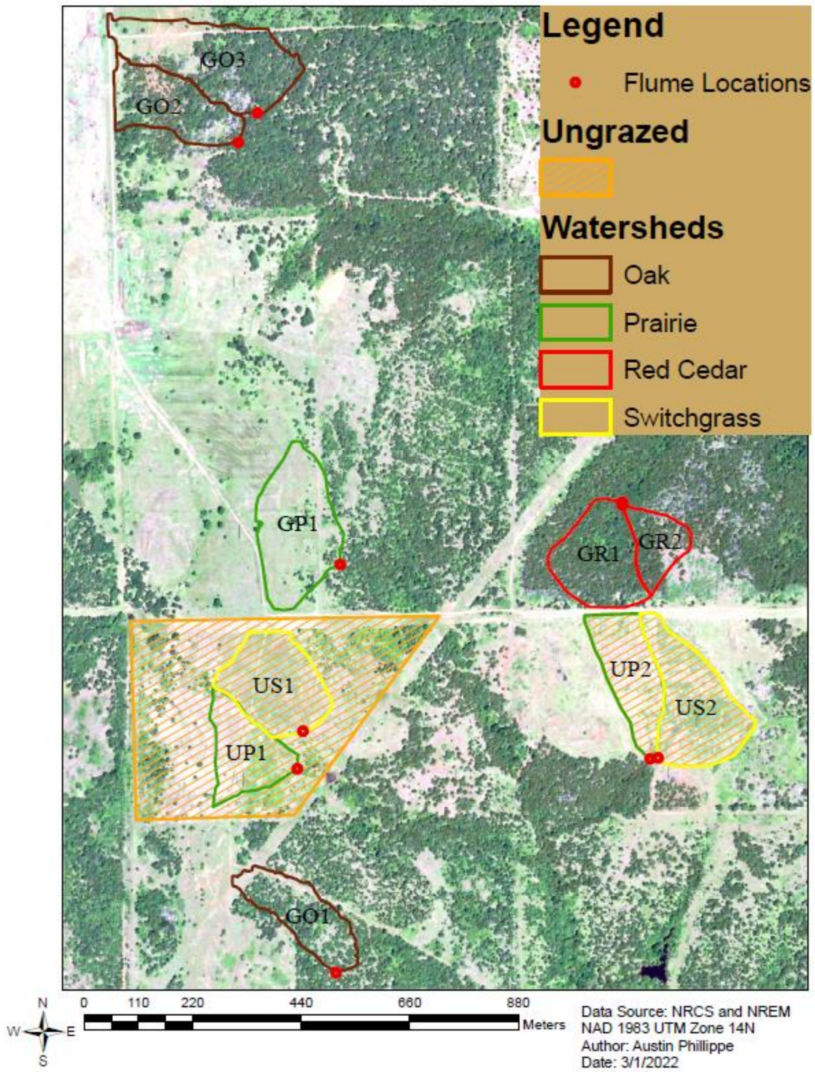


Figure 1.1 The size and locations of the watersheds monitored at CTER overlaid on a 1-meter resolution image from 2019 provided by the National Agriculture Imagery Program. The areas outlined in orange represent portions of the property that are excluded from cattle access. Cattle were not confined to any dominant vegetation cover for the purposes of this study.

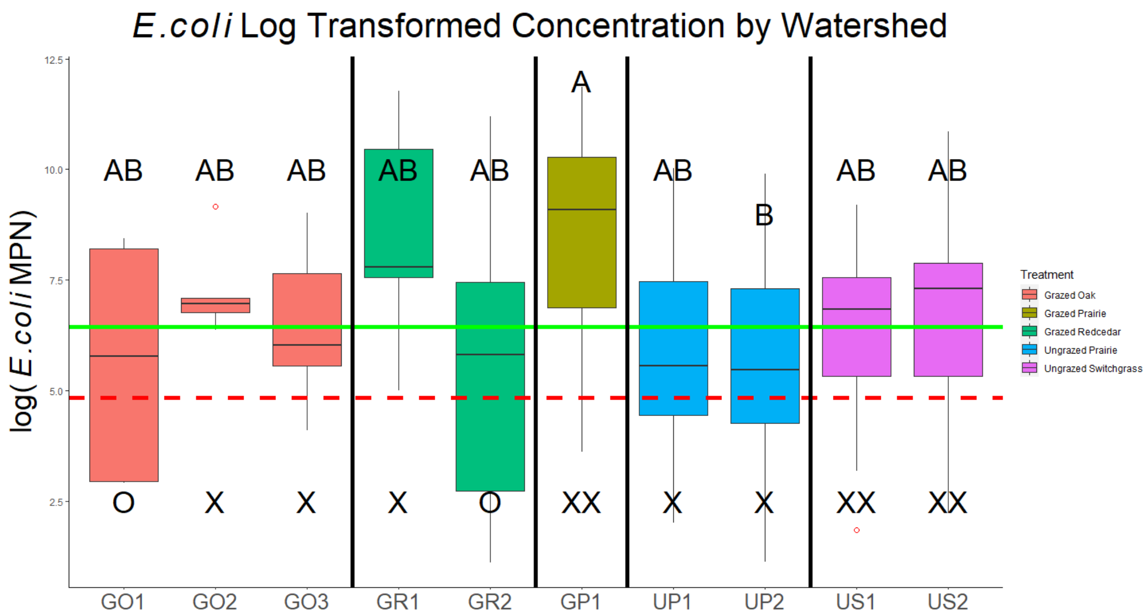


Figure 1.2 Boxplots of the *E. coli* concentrations for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The dashed red line represents the primary body contact standard and the solid green line represents the secondary body contact standard. "*XX*" indicates that the median value for that treatment is significantly different from both the primary and secondary body contact standard. "*X*" indicates that the median concentration of *E. coli* for that watershed is significantly higher than the primary body contact standard but not significantly higher than the secondary body contact standard. "*O*" indicates the median value for that watershed is not significantly higher than either the primary or secondary body contact standard.

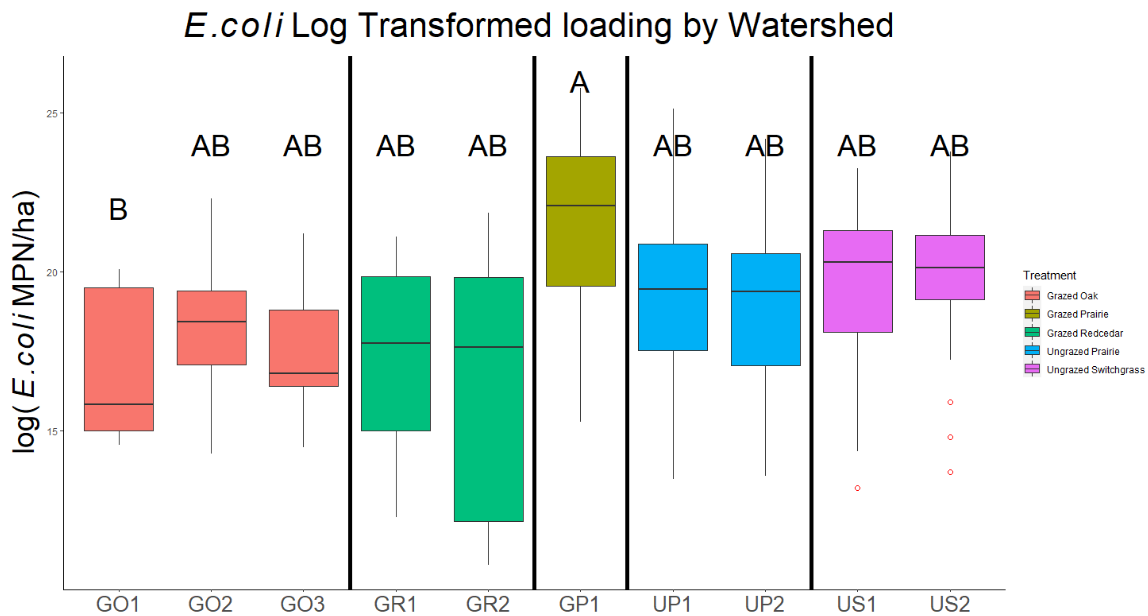


Figure 1.3 Boxplots of the *E. coli* loading values (MPN/ha) for the watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The red dots represent outliers with log transformed data. Boxplots for individual watersheds are grouped according to the land use and vegetation cover of the watershed.

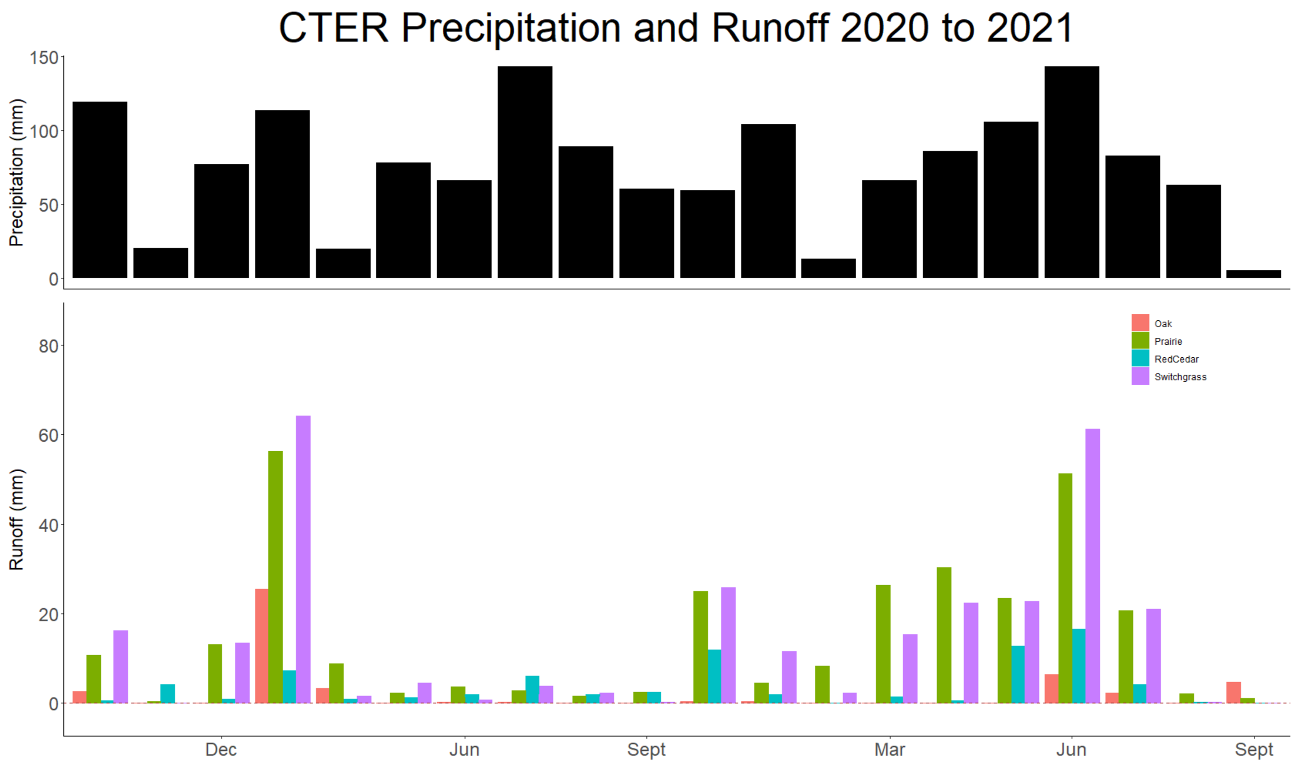


Figure 1.4 The monthly runoff from watersheds based on vegetation cover for visual purposes. On the bottom y-axis the runoff is presented in millimeters. The top y-axis is the precipitation in millimeters recorded from the nearest Mesonet station (Marena).

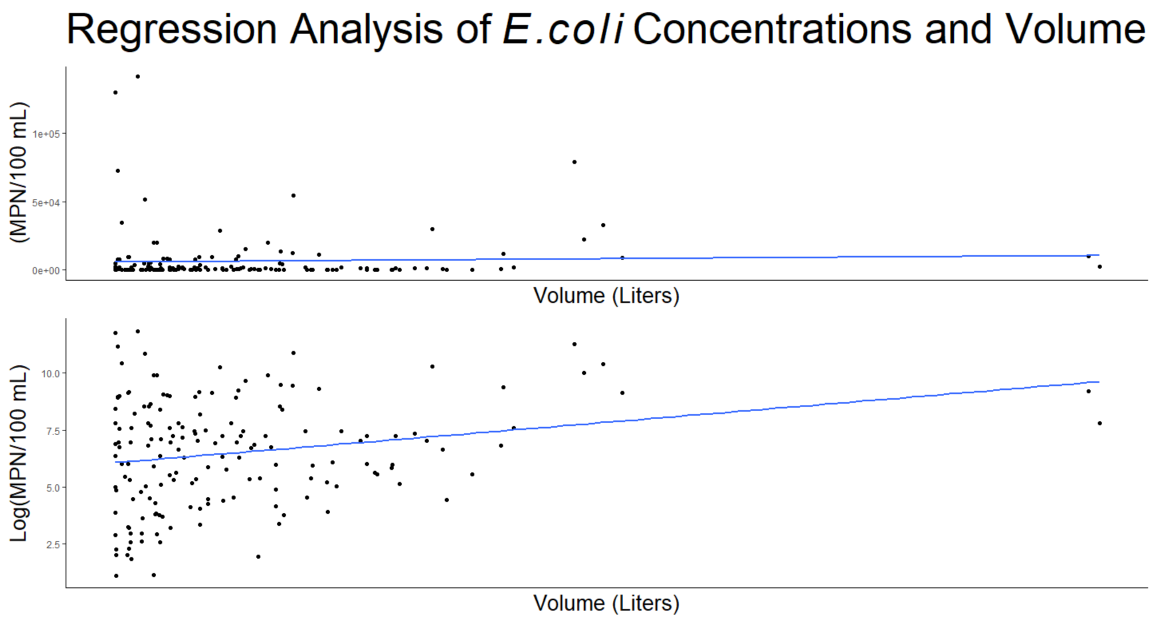


Figure 1.5 The linear regression of *E. coli* concentrations (MPN per 100 mL) and log transformed *E. coli* concentrations with volume (liters). The original data had a positive correlation that was significant but weak when using a Spearman's method (rho = 0.17, p-value = 0.03). The log transformed data using a Pearson's method also had a weak, significant, positive correlation (rho = 0.22, p-value = 0.004).

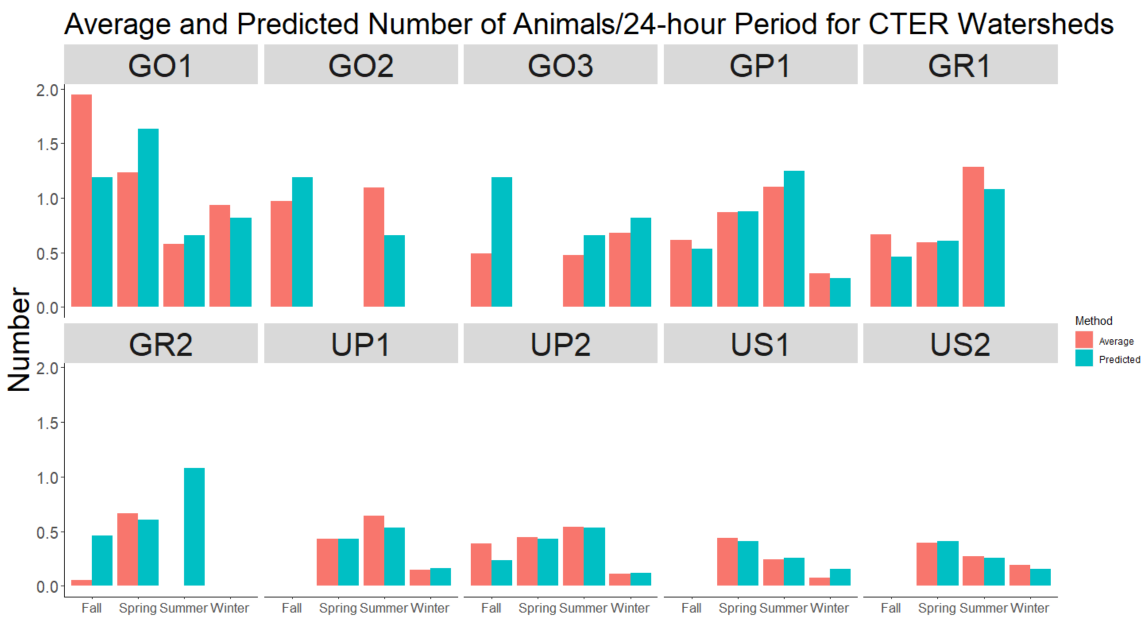


Figure 1.6 The average (*red*) and predicted (*blue*) number of animals per 24-hour period for each watershed for each season. Seasons of watersheds without bar graphs are seasons when at least one game camera failed in that watershed which did not allow for an average number of animals per 24-hour period to be calculated. That data was excluded from regression analysis.

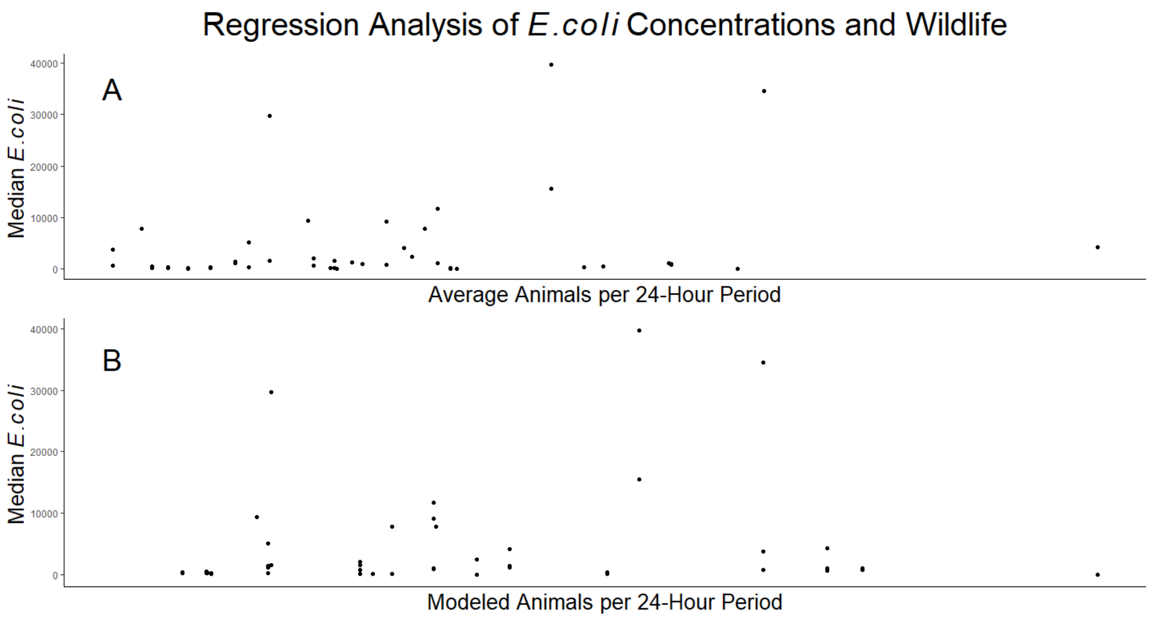


Figure 1.7 Correlations between wildlife and *E. coli* concentrations. A) Regression analysis with Spearman's rank correlation between average animals and median *E.coli* concentrations. B) Regression analysis with Spearman's rank correlation between modeled animals observed in a 24-hour period with median *E. coli* concentrations.

CHAPTER II

Impacts of Land Use and Vegetative Cover on Nutrient Concentrations and Loading in Runoff in the Cross-Timbers Ecoregion

Abstract

Runoff from agricultural areas is a significant source of nutrient contamination of water bodies in Oklahoma. The land use associated with a watershed is tied to the water quality of surface runoff, and quantifying the relationship between land use and runoff water quality requires direct measurements from experimental watersheds. To add to the information available for the edge of field water quality, I measured Total Kjeldahl Nitrogen (TNK), Total Phosphorus (TP), and Orthophosphate (Ortho-P) from 10 experimental watersheds that differed in vegetation cover and access to cattle grazing at the Cross Timbers Experimental Range. Data analyses were conducted using Kruskal-Wallis rank sum tests followed by pairwise two-sample Wilcoxon rank sum tests with a Bonferroni correction for post hoc analysis to evaluate the differences between each of the watersheds. I used one-sample Wilcoxon signed rank tests to compare the watershed concentrations with those from EPA reference sites (EPA, 2001).

The median TKN and TP concentrations in runoff from eastern redcedar watersheds were greater than concentrations from grassland and oak forest watersheds. However, small sample sizes from eastern redcedar watersheds and high variation between watersheds caused significant differences to be detected inconsistently. This was the case when comparing the EPA reference

values with the concentration data. As a result of greater runoff volumes, TKN loading were often greater from grassland watersheds (differences not significant). All median TP loading values were generally lower in the forested watersheds in comparison with the grassland watersheds but significant differences were only found between the grazed redcedar watershed and ungrazed prairie watershed. Ungrazed switchgrass watersheds had low TP concentrations and loading compared to other grassland watersheds. However, the Ortho-P loadings in the ungrazed switchgrass watersheds were significantly higher than the grazed redcedar watersheds indicating that biomass removal does not limit dissolved phosphorus loading. Vegetation cover affects nutrient efflux dynamics by altering the number and the volume of runoff events. The variability of the data and differences between the runoff concentrations and the EPA reference values suggest that numerical criteria with three levels (25^{th}, 75^{th} and 95^{th} percentiles) of nutrient concentrations of EPA reference streams would allow for more flexibility for managers to determine the status of runoff impairment based on the local information.

Keywords: Nutrients, Runoff, TKN, Ortho-P, TP, Loading, Concentration

Introduction

How landowners manage their properties has implications when it comes to both water quality and nutrient cycling. Previous studies found that poultry litter application, excessive grazing, and a lack of ground vegetation can cause eutrophication of water bodies downstream of agricultural runoff (Harmel et al., 2009; Butler et al., 2007). This creates problems because over-abundance of nitrogen and phosphorus in water sources leads to the increased risk of harmful algal blooms, reduction of aquatic species diversity, and increased potential for negative health impacts in humans (Smith et al., 1999; Camargo et al., 2005; Zhao et al., 2013). In Oklahoma, the fourth highest source of impairment of rivers is attributed to inadequate dissolved oxygen, likely caused from biological responses to nutrient contamination (ODEQ, 2021). To address this problem, it is important to identify sources of both nitrogen and phosphorus from prominent land uses and cover types in this region.

Land use and vegetation cover affect nutrient cycling through influencing water movement and nutrient uptake. Vegetation type influences runoff by altering important hydrologic processes including precipitation interception, infiltration, and evapotranspiration (Calder, 2007; Caterina et al., 2014). Well-managed grasslands and forests are associated with greater canopy interception and improved soil infiltration, reducing surface runoff and its ability to carry particulate forms of nutrients (Lyons et al., 2000; Dosskey et al., 2010). In addition, vegetation growth reduces total nitrogen and phosphorus loadings in runoff by taking up nutrients prior to runoff events (Hart and Cornish, 2012; Schmitt et al., 1999; Butler et al., 2007).

Grasslands and forests have different characteristics for remediating specific contaminants. For sediments, studies suggest that grassland vegetation is either not significantly different (Schmitt et al., 1999) or more effective than forests at reducing sediment loads due to the greater soil surface cover (Knight, 2007). Vegetation growth requires nutrient uptake to increase biomass (Kelly et al., 2007; Missaoui et al., 2005; Dosskey et al., 2018). Therefore, grasslands managed with grazing or mowed do a better job of removing nitrogen and phosphorous from soil prior to surface runoff in comparison with long-established forests where physical removal of vegetation is less frequent (Hefting et al., 2005; Kelly et al., 2007; Lyons et al., 2000). However, by this same logic, young, forested watersheds that are experiencing substantial plant growth are also highly effective at reducing nutrient loadings (Dosskey et al., 2010).

The lack of management can also be a problem for the water quality of surface runoff. Eastern redcedar (*Juniperus virginiana*) encroachment because of fire exclusion and passive management is suggested to degrade water quality (Thurow and Carlson, 1994; Engle et al., 1996). Eastern redcedar encroachment increased water loss to canopy interception and transpiration (Caterina et al., 2014; Zou et al., 2015), and the evapotranspiration accounted for a greater percentage of the water budget (Schmidt et al., 2021). In addition, the infiltration rates under the eastern redcedar canopy were greatly enhanced, thus, the amount of surface runoff potential was greatly reduced in the eastern redcedar encroached watersheds compared to grassland watersheds (Zou et al., 2014; Qiao et al., 2017). However, despite less runoff, modeling studies using the Water Erosion Prediction Project model predicted a similar sediment loading from an eastern redcedar heavily encroached watershed and an adjacent grassland watershed (Lisenbee, 2016). A recent study based on direct measurement using experimental watershed studies reported a similar sediment yield from both an eastern redcedar watershed and an adjacent grassland watershed restored from redcedar removal (Zhong et al., 2022). The herbaceous cover under the eastern redcedar canopy was generally low (Van Els et al., 2010). With less herbaceous vegetation to hold soil in place and filter runoff, the surface soil is more vulnerable to water kinetic force and, subsequently, increasing soil particles and nutrient particulates in runoff (Pierson et al., 2007). Therefore, the nutrient concentrations under eastern redcedar could be higher when runoff does occur, but the nutrient loadings could be lower overall due to few runoff events and smaller runoff volume per event.

On the other hand, management with high-intensity grazing can also negatively impact water quality. Cattle influence nutrient cycling by depositing nitrogen primarily in urine and phosphorus/nitrogen in feces both on the contribution watershed or directly into the stream (O’Callaghan et al., 2019). However, Butler et al. (2007) found that even with low ground cover (45% vegetative ground cover, 55% bare ground), the nutrient loading in runoff can be significantly reduced. Therefore, if the grazing intensity does not dramatically reduce the amount of vegetative ground cover and grazers are excluded from streams, water quality should be conserved from particulate contamination. Dissolved forms of both nitrogen and phosphorus in runoff would still be present, however, because these are often unaffected by the presence of dense vegetation (Heart and Cornish, 2012; Dorioz et al., 2006; Fiener and Auerswald, 2009; Gali et al., 2012). Because of the low cattle stocking rate and generally high vegetative ground cover at CTER, we do not expect large particulate contamination, and this study will focus on the nitrogen and phosphorus concentrations under different land use and vegetation cover.

The largest Oklahoma non-federal, rural land class is rangeland making up 33% of the total area of the state (USDA, 2020). In addition to being used extensively for grazing and agriculture, the cross timbers ecoregion has been described as a "mosaic" of different vegetation covers and associated land uses (Thomas and Hoagland, 2011; Stallings, 2008). For these reasons, this ecoregion is an excellent area to examine the impacts of different vegetation cover under light-intensity grazing on nitrogen and phosphorus efflux to stream. At the Cross Timbers Experimental Range (CTER), we measured 3 water quality metrics used to evaluate nutrient contamination in surface runoff; total Kjeldahl nitrogen (TKN), total phosphorus (TP), and Ortho-phosphorus (Ortho-P) from 10 experimental watersheds that differed in vegetation cover and grazing treatment. The objective of this study is to 1) determine how light-intensity cattle grazing under different vegetation cover impact nutrient concentrations and loadings in surface runoff and 2) evaluate the nutrient concentrations in runoff against the values from EPA reference sites in this ecoregion.

Materials and Methods

This study took place from April 2020 through October 2021 at the Cross Timbers Experimental Range (CTER), a rangeland area dedicated to agricultural research purposes and managed by Oklahoma State University. CTER is located approximately 18km southwest of Stillwater, Oklahoma. The climate of this region is highly variable, and the temperature and precipitation change substantially with season. From 2005 to 2019, the nearest Oklahoma Mesonet weather station at Marena, located approximately 2.5 km from CTER, recorded an annual average temperature of 15.6˚C, average minimum temperature in January of -3.3˚C, and average maximum temperature of 33.9˚C in July. The mean annual rainfall for CTER is around 890 mm with a wet spring and fall and comparatively drier winter and summer months (Qiao et al., 2017).

Previous studies established ten experimental watersheds to study water budget and sedimentation processes based on the dominant vegetation types (oak forest, eastern redcedar woodland, and tallgrass prairie) in CTER (Zou et al., 2014; Qiao et al., 2017; Zhong et al., 2022). For the purposes of this study, a unique identifier was given to each watershed based on its access to grazers ("G" for grazed and "U" for ungrazed) and the current vegetation cover type ("O" for oak forest, "R" for redcedar woodland, "S" for switchgrass, and "P" for prairie). Several watersheds had the same grazer access and vegetation cover, so a number was used to differentiate one from the other (Figure 2.1). UP2 and US2 were eastern redcedar woodland with cattle access until eastern redcedar was removed in 2015 and then fenced to prevent cattle access in June 2017. US1 was a prairie with cattle access until it was converted to switchgrass with herbicide application in 2015 and fenced along with UP1 in June 2017 (Qiao et al., 2017). US1 and US2 (switchgrass watersheds) were managed with an annual harvest of aboveground biomass.

The slopes of these watersheds are less than 5%. The soils are well drained, consisting predominately of the Stephenville–Darnell complex (StDD), Coyle soil series (Coy, CoyZ), and Grainola–Lucien complex (GrLE) (Table 2.1). The average depth of soil is approximately 1 m underlain by sandstone substrates. The understory or ground cover differs greatly in grass cover depending on the vegetation type. Grass cover ranges from 59.9 – 90.2% for prairie and switchgrass watersheds (UP1, US1, US2, UP2, GP1) but ranges from 2.5 – 33.2% for oak forest and eastern redcedar watersheds (GO1, GO2, GO3, GR1, GR2). The cover of woody plants, forbs, and bare grounds varies greatly among individual watersheds (Table 2.2).

<u>Runoff measurement</u>

Runoff was gauged by H-flumes and stilling wells located at each of the ten experimental watersheds' outlets. Next to each H-flume, an ISCO Avalanche Portable Refrigerated Sampler (Teledyne ISCO, 2013) was installed. The sampler was equipped with a 720 Submerged Probe

Module in the stilling well and a suction line attached to a strainer in the H-flume water flow. Each sampler was powered by a marine battery recharged by a solar panel. The 720 Submerged Probe Module measured the water depth in the stilling well every minute. The ISCO Avalanche Portable Refrigerated Sampler used this depth information to calculate the flow of water moving through the flume every 5 minutes (Grant and Dawson, 1991). The samplers were programmed based on a flow interval so that one sample was taken for every 0.5 mm of runoff (Harmel, 2006). During a runoff event, the sampler repeatedly drew water samples from the strainer attached to the H-flume based on the flow interval and the samples were composited into a five-gallon bottle. To account for the small and shorter runoff events, forested sites were programmed to sample at enable mode on May 16th, 2021. 22 out of 25 TKN/TP samples and all the Ortho-P samples from the forested watersheds were collected using sample at enable mode.

Nutrient Measurements

Three different chemical forms of nitrogen (total Kjeldahl nitrogen, TKN; nitrate/nitrite, NO_3−N; ammonium-N, NH_3−N) were measured. Total nitrogen is the sum of all nitrogen related to nitrates, nitrites, ammonia-bound nitrogen, and organically bound nitrogen (EPA, 2013). This differs from total Kjeldahl nitrogen in that total Kjeldahl nitrogen does not include nitrates and nitrites and is considered organic associated nitrogen (EPA, 2013). During sampling collection, samples were separated such that Oklahoma State University Soil and Forage Lab (SWFAL) analyzed the runoff samples for NO_3−N (Sechtig, 2001) and NH_3−N (EPA, 1993) and the Oklahoma Department of Agriculture, Food and Forestry (ODAFF) analyzed the samples for TKN (EPA, 1993). The values for TKN and NO_3−N were supposed to be added to TKN to get the TN measurement. However, 48.9% of the NH_3−N samples and 41.40% of NO_3−N samples were below the detection limit (< 2 ppm). Therefore, TKN was used to assess nitrogen contamination and loading.

The SWFAL processed the water samples for micronutrients (analysis not included in this study) and Ortho-P. ODAFF analyzed total phosphorus concentrations by semi-automated colorimetry (EPA, 1993). Ortho-P is the concentration of phosphate polyatomic ions in the solution and total phosphorus is the total amount of phosphorus in the water sample before filtration. SWFAL used inductively coupled plasma spectroscopy (ICP) to obtain the values for each measurement of water samples (EPA, 1993).

Data Analysis and Statistical Methods

Data analyses were conducted in R studio using the packages "MASS" for evaluating data structure, "boot" for calculating the bootstrapped confidence intervals for median, and standard base functions included in R for the non-parametric tests. Before conducting statistical analyses, the data for each watershed and water quality metric (TKN, TP, and Ortho-P) were tested for normality using a Shapiro-Wilk normality test and equal variances using a Levene's test. These tests were conducted to determine if parametric statistics were appropriate for data without transformation. The majority of the data sets met the normality assumptions, but the assumption of equal variances was only met for Ortho-P. Despite meeting those assumptions, the data appeared right-tailed skewed when graphed (Figure 2.4). The data for TKN (p-value <0.001) and TP (p-value <0.001) yielded p-values less than 0.05 for the Levene's test and, therefore, did not have equal variances. Because the assumptions necessary for parametric analysis were not met, we decided to run non-parametric tests (Kruskal-Wallis test, and Wilcoxon signed rank tests) for testing the median loadings and concentrations of TKN, TP, and Ortho-P. We conducted post hoc analysis by running a series of pairwise comparisons using the two-sample Wilcoxon rank sum test with a Bonferroni correction.

To evaluate the nutrient concentrations in the runoff against the values from EPA reference sites in this ecoregion, I used one-sample Wilcoxon signed rank tests. The document from the EPA provided reference values for TKN and TP for the central Great Plains ecoregion (EPA, 2001). The EPA provided no direct value for a reference for Ortho-P in this ecoregion. However, we did find data on various percentiles of the Ortho-P data from the reference sites

(EPA, 2001). To be consistent with the TKN and TP analysis, we used the 25th percentile because this was the same percentile provided as the EPA reference values for TKN and TP (EPA, 2001).

The nutrient loading for a given runoff event was calculated by multiplying the volume of runoff by the concentration and then dividing this value by the watershed area. Similar to the process for analyzing the concentration data, I used the Kruskal-Wallis rank sum test to test if any of the nutrient loading amounts per event for individual watersheds differed. I then used pairwise two-sample Wilcoxon rank sum test with Bonferroni corrections for post hoc analysis.

The runoff data were processed with Flowlink 5 software and summarized by month using the dplyr package in RStudio. To examine the differences in monthly runoff as depth between the vegetation cover types, the total monthly volume was divided by the area. We calculated the annual, monthly maximum, monthly minimum, and monthly median for each watershed (Table 2.9). To minimize the impact of season on runoff analysis, this data was summed to the annual values. Next, I ran a Friedman's test to see if there were detectable differences in annual runoff among individual watersheds. The experimental unit was the individual watershed and the response variable was the annual runoff in millimeters, with year as block. If significant differences were detected, a Nemenyi test for post hoc analysis was conducted.

Results

Total Kjeldahl Nitrogen

The Kruskal-Wallis rank sum tests indicated that there were significant differences of median TKN concentrations among the watersheds (p-value <0.001). With the exception of the grazed redcedar sites (GR1 and GR2), mean and median TKN concentrations were less than 2 mg/L. Post hoc analysis revealed the median TKN concentration of watershed GR1 was higher than watersheds US1 and UP1 (Table 2.3, Figure 2.2). Watershed US1 had the lowest median

TKN concentration, which was significantly lower than watersheds GR1 and GR2 (Table 2.3, Figure 2.2), but not significantly different from all others.

Based on one-sample Wilcoxon signed rank tests, the median TKN concentrations for all grassland watersheds (GP1, UP1, UP2, US1, and US2) and GR2 were significantly higher than the EPA reference value of 0.52 mg/L (EPA, 2001) (Figure 2.2, Table 2.4), but all forested watersheds other than GR2 (GO1, GO2, GO3, and GR1) did not significantly exceed the EPA reference value of 0.52 mg/L (Table 2.3). However, the 95% boot strapped confidence intervals indicate that all the watersheds have median TKN concentrations greater than 0.52 mg/L (Table 2.3).

Total Phosphorus

With the exception of the grazed redcedar sites, median TP concentrations were less than 0.2 mg/L, ranging from 0.06-0.18 mg/L. The Kruskal-Wallis rank sum tests showed there were significant differences of the median TP concentrations among watersheds (p-value <0.001). The post hoc analysis indicated that the median TP concentrations of GR2, GP1, and UP1 were significantly higher than UP2, US1, and US2 (Table 2.5, Figure 2.3). The median TP concentrations of GO1, GO2, GO3, and GR1 were not significantly different from any of the other watersheds.

The median TP concentrations in 7 of the 10 watersheds were not significantly higher than the 25th percentile (0.09 mg/L) of EPA reference streams (Table 2.6, Figure 2.3). The 95% bootstrapped confidence intervals for GO1, GO2, GO3, US1, US2, UP1 all included 0.09 mg/L or value below that. The 95% bootstrapped intervals for the grazed redcedar watersheds (GR1 and GR2) did not include 0.09 mg/L indicating that the median TP concentration was greater than 0.09 mg/L. Watersheds GR2 (p-value = 0.006), GP1 (p-value = 0.004), and UP1 (p-value = 0.008) had median TP concentrations that were significantly greater than 0.09 mg/L.

Orthophosphate

Median Ortho-P concentrations ranged from 0.03-0.08 mg/L, with most sites (7 of the 10) having medians between 0.04 and 0.05 mg/L. The Kruskal-Wallis rank sum tests showed there were significant differences of the median Ortho-P concentrations among watersheds (p-value <0.001). Post hoc analysis revealed one significant difference between watershed UP1 and GR2 (p-value= 0.049) (Table 2.7, Figure 2.4).

Only watershed GO1 and GR2 had median Ortho-p concentrations that were not significantly greater than the 25th percentile of the EPA reference streams, 0.0275 mg/L (EPA, 2001) (Figure 2.4, Table 2.8).

Runoff Quantity

The total annual runoff, median monthly runoff, maximum monthly runoff, and minimum monthly runoff in millimeters varied among watersheds (Table 2.9). Despite the large differences in total annual runoff volume between watersheds, the Friedman rank sum test found no significant differences (p = 0.06) (Table 2.9). Monthly runoff varied substantially and runoff volumes during months of little to no rainfall were similar between all vegetation covers (Figure 2.5, Table 2.9); however, in months where greater amounts of rainfall occurred, grassland watersheds (GP1, UP1, UP2, US1, and US2) tended to have more runoff than forested watersheds (GO1, GO2, GO3, GR1, and GR2) (Figure 2.5). This is also evident through the differences in maximum monthly runoff and total runoff observed for each year (Table 2.9).

Total Kjeldahl Nitrogen Loading

The annual loading of TKN ranged from 16.3 g/ha to 1,088.5 g/ha with median event loading ranging from 1.5 g/ha to 113.9 g/ha with no individual event loading greater than 425 g/ha (Table 2.10). The Kruskal-Wallis rank sum tests showed there were significant differences of the median TKN loadings among watersheds (p-value = 0.001). However, post hoc analysis did not identify any of the pairwise differences between watersheds as significant (Table 2.10, Figure 2.6). This can occur when the Bonferroni correction is large, which is likely the case when evaluating 10 experimental watersheds, many of which have relatively small sample sizes (Table 2.10). However, the annual TKN loading of each of the grassland watersheds (GP1, UP1, UP2, US1, and US2) are higher than the forested watersheds (GO1, GO2, GO3, GR1, and GR2) (Table 2.10).

Total Phosphorus Loading

The annual loading of TP ranged from 1.7 g/ha to 100.9 g/ha with median event loading ranging from 0.3 g/ha to 8.2 g/ha with no individual event loading greater than 60 g/ha (Table 2.11). The Kruskal-Wallis rank sum tests showed there were significant differences of the median TP loadings among watersheds (p-value < 0.001). Post hoc analysis revealed two significant differences between the median loading TP of watershed UP1 and GR2 (p-value = 0.03) and US2 (p-value = 0.03) (Table 2.11, Figure 2.7). Additionally, none of the annual TP loads for the forested watersheds (GO1, GO2, GO3, GR1, and GR2) exceeded any of the annual TP loads for the grassland watersheds (GP1, UP1, UP2, US1, and US2) (Table 2.11).

Orthophosphate Loading

The annual loading of Ortho-P ranged from 0.3 g/ha to 49.1 g/ha with median event loading ranging from 0.03 g/ha to 3.3 g/ha with no individual event loading greater than 35 g/ha (Table 2.11). The percentage of Ortho-P of the TP ranged from 6% in GR1 to 66% in UP2. The Kruskal-Wallis rank sum tests showed there were significant differences of the median Ortho-P loadings among watersheds (p-value < 0.001). The median Ortho-P loadings of GR1 and GR2 were significantly lower from those of the grassland watersheds except GP1 (UP1, UP2, US1, and US2). The median Ortho-P loading of the grazed oak watersheds (GO1 and GO2) were not significantly different from any of the other watersheds. GO3 had median Ortho-P loading significantly greater than GR2 (Figure 2.8; Table 2.12).

Discussion

Concentrations

Contamination of surface runoff by agricultural land use in the Great Plains region has been a focus of water quality research since the 1980s (Smith et al., 1983). The results from this study are consistent with what was expected but the EPA suggested concentration thresholds require more consideration if applying these standards to runoff water quality.

Although not statistically significant due to the limited sample size, the runoff from eastern redcedar encroached watersheds had concentrations that were consistently greater in TKN and TP in comparison with the other watersheds at CTER. This could be due to a couple of reasons. First, grasslands tend to be more effective at reducing erosion compared to woody dominated cover types, despite the potential for woody species to uptake nutrients in greater quantities (Lyons et al., 2000; Dosskey et al., 2010). Previous studies have found that eastern redcedar can alter the nitrogen composition of soils (Bekele and Hudnall, 2005). Therefore, this change, coupled with a reduction in understory found in a previous study (Van Els et al., 2010), could explain the higher nutrient output from the eastern redcedar encroached watersheds. However, the limited sample sizes in forested watersheds reduced the capacity of one-sample Wilcoxon signed rank test to detect significant differences. Specifically, watershed GO3 had a sample size of 4 and all those values were greater than 0.52 mg/L, but this still yielded a p-value of 0.06 because that is the lowest possible p-value for a sample size of 4 using this statistical approach. For this reason, it is important to pay attention to the sample size when one-sample Wilcoxon signed rank tests are used for detecting water quality impairment. Additionally, I changed the sampling protocol in the forested systems after one year of data collection to increase the number of samples. Samplers in the forested watersheds took a sample once the program enabled following a water depth of 14 mm in the strainer. This allowed sampling even at low thresholds for small runoff events and decreased the inaccuracy of runoff measurements when intervals are set too far apart (Harmel et al., 2003). However, the lower threshold depth in forested watersheds could result in over-estimating concentration due to the initial flux of nutrients.

The TKN values are in the general range reported for an ungrazed rangeland system (Nelson et al., 2020). Nelson et al. (2020) published results based on over 20 years of water quality and quantity measurement from experimental watersheds in El Reno, Oklahoma. The TKN concentrations in runoff from the native tallgrass prairie ranged from 0.1 to 575.0 mg/L with a median value of 1.8 mg/L. The median found in that study was higher than the 95% bootstrapped confidence intervals for all of the grassland sites at CTER (Table 2.3). The forested sites from this study had comparatively high TKN concentrations. Lowrance and Sheridan (2005) found that mature forested riparian buffers yielded a mean TKN concentration of 0.38 mg/L in Tifton, GA. Forested watersheds for both oak (GO1, GO2, and GO3) and eastern redcedar (GR1 and GR2) had confidence intervals that exceeded that value (Table 2.3). There was no TKN concentration data available for eastern redcedar for direct comparison.

The Grazed Prairie (GP1) had significantly higher median TP concentrations than the other grassland watersheds except for UP1 (Figure 2.3; Table 2.5). When the number of grazers on a watershed increases, the amount of fecal matter also increases, influencing higher nutrient contamination of surface runoff (McDowell et al, 2006). However, the connection between cattle grazing and nutrient contamination can be influenced by the timing of runoff event related to defecation, cattle management, and other processes (O'Callaghan et al., 2019). Due to the nature of the cattle management at CTER aside from GP1, it is unlikely that the grazing intensity is enough to significantly alter nutrient dynamics. When the concentrations for TP from this study were compared to the EPA reference value, only 3 out of 10 of the watersheds had significantly higher concentrations. The small percentage of watersheds identified as significantly higher than the EPA reference value and the grazed prairie watershed (GP1) identified as one of those suggests that using the EPA references values would identify watersheds with grazing practices as impaired.

The annual harvest of the switchgrass watersheds could have played a role in causing lower nutrient contamination from watersheds US1 and US2 (Dosskey et al., 2018). The TP concentrations were significantly lowest in the ungrazed switchgrass watersheds (US1 and US2) while ortho P concentration was not, providing evidence that annual removal of switchgrass could be an effective way to reduce TP, particularly for particulate P (Gali et al., 2012).

The TP concentrations observed in this study are lower than what has been observed from native rangeland in this region. Nelson et al. (2020), described earlier, found TP concentrations in runoff from native tallgrass prairie had a minimum value of 0.01 µg/L, a maximum value of 4.40 mg/L, and a median of 0.109 mg/L. In this study, only the median TP concentration at GR1 exceeded the native tallgrass prairie with intermittent grazing from Nelson et al. (2020) (Table 2.5). In comparison with the mean TP concentration of runoff of 0.11 mg/L from the mature forested area in Georgia (Lowrance and Sheridan, 2005), the oak forested watersheds had bootstrapped confidence intervals around that value but the eastern redcedar forested watersheds had higher values (Table 2.5). We observed generally lower median values of Ortho-P. The native tallgrass prairie from Nelson et al. (2020) had total reactive phosphate (another term for Ortho-P) concentrations with a median of 0.109 mg/L, a maximum of 3.801 mg/L, and a minimum of 0.1 µ/L. Although none of our median values were greater than 0.1 mg/L, I did observe maximum Ortho-P concentrations of 0.33 mg/L from watershed GP1 and 0.21 mg/L from GR1 (Table 2.7).

When comparing with the EPA reference values, the one-sample Wilcoxon signed rank test detected inconsistent significant differences in instances the watersheds had the same grazer access and vegetation cover. For example, GR1 and GR2 are both eastern redcedar forests where cattle have access but the median TKN concentration of runoff from GR1 was not significantly different from the EPA reference value whereas the median TKN concentration of runoff from GR2 is significantly higher (Figure 2.2, Table 2.3). Part of this can be explained by small sample sizes for the eastern redcedar watersheds. However, this same discrepancy occurred for grassland watersheds (UP1 and UP2) even though both have sufficient sample sizes (Figure 2.3, Table 2.5). Therefore, more watershed specific variables need to be taken into consideration and watershed level replications are essential to generate land use and cover impact on nutrient concentrations of runoff.

Currently, nutrient contamination in Oklahoma for non-wadable streams are evaluated using a Carlson's trophic state index with a value less than 62 determining impairment (ODEQ, 2020). This process requires a trophic state index (TSI) to be calculated from total phosphorus, chlorophyll a, or Secchi disk. TSI values from these various methods yield different results and require the consequences of impairment for the case of the Secchi disk and chlorophyll a methods (Osgood, 1982). Rather than waiting for an ecological response to eutrophication, the guidance of the EPA is to have a "numerical criteria modified to reflect site-specific conditions" (EPA, 2001). However, it is difficult to establish a numerical criteria when the data provided is the 25th percentile of all the rivers and streams in the region. One solution to this problem has been to use direct comparisons of reference sites to establish criteria based on a percentile above the 75th (Harmel, 2018). This idea has been displayed in Figure 2.4 for the Ortho-P analysis for which the EPA did not have a reference level but rather the 25th, 75th and 95th percentiles. Alternatively, when it comes to nutrient contamination determination for runoff, numerical criteria with three levels (25th, 75th and 95th percentiles) would allow more flexibility for managers to determine the status of impairment based on the local information. This is necessary if management is to be applied to runoff water quality because with such high variation between sites of the same land cover and land use, applying inappropriate standards could lead to impairment of sites by nutrients that are otherwise healthy.

Runoff

I measured lower runoff associated with forested watersheds in comparison with grassland watershed, which is consistent with previous studies at CTER (Schmidt et al., 2021; Qiao et al., 2017) (Figure 2.5). However, the annual runoff values during this study were generally higher in comparison to previous studies on the same watersheds largely due to the climate variability. The average annual rainfall during this study was 911 mm in comparison to the annual average of 890 mm.

In addition, the annual runoff from the grassland watersheds from this study are usually comparable or greater than previous studies in El Reno, OK and Bushland, Texas, both located in a drier climates. A study in El Reno, Oklahoma, observed runoff more than 75 mm during a year with annual rainfall equal to 828 mm. Another study that ran from 1978 to 1980 on clay loam soils in Bushland, Texas reported annual runoff of 12 mm at its driest grassland site that received 430 mm of rainfall (Smith et al., 1983).

Loadings

The loading results reflected the strong influence vegetation cover has on nutrient loading. For all three of the nutrients, the 95% confidence intervals for the median loading (g/ha) were greater in the grassland watersheds in comparison with the forested watersheds (Table 2.10, Table 2.11, and Table 2.12). This suggests that grassland watersheds tend to have higher nutrient loads compared with forested watersheds receiving the same amounts of precipitation and having similar soil characteristics (Figures 2.5, 2.6, and 2.7). Most of the studies on watersheds in this region have been centered on the impacts of grazing or cropping systems on nutrient loads (Harmel et al., 2009; Nelson et al., 2020). There are no data on nutrient loading of runoff from oak and redcedar woodland sites from this region for comparison.

The TKN loadings from redcedar watersheds were less compared with grassland watersheds although the eastern redcedar watersheds had higher TKN concentrations. The loading values are much lower because the amount of runoff is lower (Figure 2.5, Figure 2.6). The reason for this difference is the inclusion of volume in the calculation of TKN loading. Zhong et al. (2022) found that the same grassland watersheds used in this study had similar sediment loading in comparison to the eastern redcedar woodland watersheds despite having lower flows. This makes sense in comparison with my data because the grassland sites had lower TKN concentrations but higher TKN loading in comparison to eastern redcedar watersheds. The key impact of eastern redcedar encroachment on nutrient contamination is that it increased the concentration of nutrients in runoff but decreased the runoff volume.

The TKN loading quantities observed are high compared to previous studies. All the grassland watersheds, except US2 had greater TKN loading values in comparison with the median annual total nitrogen values for pasture/range land use (970 g/ha) found by Harmel et al. (2006) during a compilation study on measured nutrient load data for agricultural land uses (Table 2.10). Even when I examined data specific to this region of the country and under the same management conditions, the annual loading values for these grassland sites were higher than what has been observed on an idle grassland (500 g per hectare) (Table 2.10) (Smith et al., 1983). However, there were other watersheds from another study that yielded annual TKN values of 1,940 g/ha per year that were ungrazed and in fair condition (Smith et al., 1992). The forested sites from this study measured far below the rangeland values mentioned above.

TP followed the general trend with forested watersheds having lower median loading values per runoff event in comparison with grassland watersheds. However, watershed US2 had lower values, closer to what was observed for the forested sites, likely due to this watershed also having the lowest TP concentrations (Figure 2.7, Table 2.5, Table 2.11). The TP loading quantities are within the range of values observed in previous study. All the watersheds had lower loading values in comparison with the median annual TP values for pastures and rangelands (220 g/ha) found by Harmel et al. (2006) in the compilation studies mentioned before on agricultural land uses (Table 2.11). All watersheds have lower median values compared to previous data specific to this region under the same management conditions (780 g/ha) (Table 2.11) (Smith et al., 1983). In addition, other watersheds that were ungrazed and in fair condition yielded annual TP values of 490 g/ha (Smith et al., 1992). The lower TP loading values at CTER were expected because of the cattle management and high vegetative cover.

Ortho-P loading reflected how the variation of loading between individual watersheds can be influenced by differences in runoff volume. Table 2.7 showed that the Ortho-P concentrations of runoff from all the watersheds have 95% confidence intervals between 0.03 and 0.08 without much variation. However, the variation of loadings among the watersheds increased when the volume component was included in the calculation of loading. All watersheds had lower Ortho-P loadings when compared to dissolved phosphorus loading values previously observed at different location in the same ecoregion (Harmel, 2006). Relatively low nutrient losses from grazed pasture with well-established vegetation has been observed in other studies as well (Vadas et al., 2015; O'Callaghan et al., 2019). At a stocking rate of 17 acres/animal unit/year, observing higher nutrient loadings in comparison to other studies would have been unexpected.

Conclusions

The nutrient concentrations and loadings of runoff varied substantially between individual watersheds that differed in land use and vegetation covers. The concentrations of TKN and TP tended to be greater in the eastern redcedar dominated watersheds in comparison with the grassland watersheds. The ungrazed switchgrass watersheds where annual biomass removal occurs repeatedly appear to be reducing the concentrations and loading values of particulate nutrients but not dissolved phosphorus. Loading values were substantially influenced by the impact vegetation cover had on runoff volume. If the vegetation cover between areas varies dramatically, the data from this study suggest it could be best to compare differences in runoff concentration as well as the loading values. The purpose of this is to focus effort on the impacts of land use, rather than the impacts vegetation cover has on runoff volume. The results of this study provide baseline data and descriptive statistics for nutrient concentrations and loadings of runoff from multiple watersheds with different vegetation, recent land use history, and access of cattle. These baseline data provide opportunity to further study vegetation, land use changes, and management impact on nutrient efflux by incorporating paired watershed design to assist watershed management in improving water quantity and quality for the cross timbers ecoregion.

References

Bekele, A., & Hudnall, W. (2005). Response of soil δ15N and nutrients to eastern red cedar (*Juniperus virginiana*) encroachment into a relict calcareous prairie. *Plant and Soil*, *271*(1), 143-155.

Bidwell, T. G., Engle, D. M., Moseley, M. E., & Masters, R. E. (2009). *Invasion of Oklahoma rangelands and forests by eastern redcedar and Ashe juniper*. Oklahoma Cooperative Extension Service

Butler, D. M., Ranells, N. N., Franklin, D. H., Poore, M. H., & Green Jr, J. T. (2007). Ground cover impacts on nitrogen export from manured riparian pasture. *Journal of Environmental Quality*, *36*(1), 155-162.

Camargo, J. A., Alonso, A., & Salamanca, A. (2005). Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*, *58*(9), 1255-1267.

Calder, I. R. (2007). Forests and water—ensuring forest benefits outweigh water costs. *Forest Ecology and Management*, *251*(1-2), 110-120.

Caterina, G. L., Will, R. E., Turton, D. J., Wilson, D. S., & Zou, C. B. (2014). Water use of Juniperus virginiana trees encroached into mesic prairies in Oklahoma, USA. *Ecohydrology*, *7*(4), 1124-1134.

Dorioz, J. M., Wang, D., Poulenard, J., & Trevisan, D. (2006). The effect of grass buffer strips on phosphorus dynamics—a critical review and synthesis as a basis for application in agricultural landscapes in France. *Agriculture, Ecosystems & Environment*, *117*(1), 4-21.

Dosskey, M. G., Vidon, P., Gurwick, N. P., Allan, C. J., Duval, T. P., & Lowrance, R. (2010). The role of riparian vegetation in protecting and improving chemical water quality in streams 1. *JAWRA Journal of the American Water Resources Association*, *46*(2), 261-277.

Fiener, P., & Auerswald, K. (2009). Effects of hydrodynamically rough grassed waterways on dissolved reactive phosphorus loads coming from agricultural watersheds. *Journal of Environmental Quality*, (38), 548-559.

Gali, R. K., Soupir, M. L., & Mostaghimi, S. (2012). A comparison of nutrient losses from two simulated pastureland management scenarios. *Journal of Environmental Monitoring*, *14*(9), 2421-2429.

Grant, D. M., & Dawson, B. D. (1991). *ISCO Open Channel Flow Measurement Handbook*. Lincoln. Neb., ISCO Environmental Division.

Harmel, R. D., King, K. W., & Slade, R. M. (2003). Automated storm water sampling on small watersheds. *Applied Engineering in Agriculture*, *19*(6), 667.

Harmel, R. D., King, K. W., Haggard, B. E., Wren, D. G., & Sheridan, J. M. (2006). Practical guidance for discharge and water quality data collection on small watersheds. *Transactions of the ASABE*, *49*(4), 937-948.

Harmel, R. D., Smith, D. R., Haney, R. L., & Dozier, M. (2009). Nitrogen and phosphorus runoff from cropland and pasture fields fertilized with poultry litter. *Journal of Soil and Water Conservation*, *64*(6), 400-412.

Harmel, R. D., Pampell, R. A., Leytem, A. B., Smith, D. R., & Haney, R. L. (2018). Assessing edge-of-field nutrient runoff from agricultural lands in the United States: How clean is clean enough?. *Journal of Soil and Water Conservation*, *73*(1), 9-23.

Hart, M. R., & Cornish, P. S. (2012). Available soil phosphorus, phosphorus buffering and soil cover determine most variation in phosphorus concentration in runoff from pastoral sites. *Nutrient Cycling in Agroecosystems*, *93*(2), 227-244.

Hefting, M. M., Clement, J. C., Bienkowski, P., Dowrick, D., Guenat, C., Butturini, A., Topa, S., Pinay, G., & Verhoeven, J. T. (2005). The role of vegetation and litter in the nitrogen dynamics of riparian buffer zones in Europe. *Ecological Engineering*, *24*(5), 465-482.

Kelly, J. M., Kovar, J. L., Sokolowsky, R., & Moorman, T. B. (2007). Phosphorus uptake during four years by different vegetative cover types in a riparian buffer. *Nutrient Cycling in Agroecosystems*, *78*(3), 239-251.

Knight, K. W. (2007). *Effectiveness of naturally occurring riparian forest buffers and grass filter strips at buffering concentrated flow from row crop fields to streams in northeast Missouri*. [Master's thesis, Iowa State University]. ProQuest Dissertations Publishing.

Lisenbee, W. A. (2016). *Comparison of water quality and quantity in Eastern Redcedar-encroached woodland and native tallgrass prairie watersheds: A monitoring and modeling study* [Doctoral dissertation, Oklahoma State University]. ProQuest Dissertations Publishing.

Lyons, J., Thimble, S. W., & Paine, L. K. (2000). Grass versus trees: managing riparian areas to benefit streams of Central North America 1. *JAWRA Journal of the American Water Resources Association*, *36*(4), 919-930.

McDowell, R. W., Muirhead, R. W., & Monaghan, R. M. (2006). Nutrient, sediment, and bacterial losses in overland flow from pasture and cropping soils following cattle dung deposition. *Communications in Soil Science and Plant Analysis*, *37*(1-2), 93-108.

Missaoui, A. M., Boerma, H. R., & Bouton, J. H. (2005). Genetic variation and heritability of phosphorus uptake in Alamo switchgrass grown in high phosphorus soils. *Field Crops Research*, *93*(2-3), 186-198.

O'Callaghan, P., Kelly-Quinn, M., Jennings, E., Antunes, P., O'Sullivan, M., Fenton, O., & Huallachain, D. O. (2019). The environmental impact of cattle access to watercourses: A review. *Journal of Environmental Quality*, *48*(2), 340-351.

Oklahoma Department of Environmental Quality (2021). *Water quality in Oklahoma: integrated report, 2020*.

Osgood, R. A. (1982). Using differences among Carlson's trophic state index values in regional water quality assessment. *JAWRA Journal of the American Water Resources Association*, *18*(1), 67-74.

Pierson, F. B., Bates, J. D., Svejcar, T. J., & Hardegree, S. P. (2007). Runoff and erosion after cutting western juniper. *Rangeland Ecology & Management*, *60*(3), 285-292.

Qiao, L., Zou, C. B., Stebler, E., & Will, R. E. (2017). Woody plant encroachment reduces annual runoff and shifts runoff mechanisms in the tallgrass prairie, USA. *Water Resources Research*, *53*(6), 4838-4849.

Schmidt, K. N., Zou, C. B., Kakani, V. G., Zhong, Y., & Will, R. E. (2021). Improved productivity, water yield, and water use efficiency by incorporating switchgrass cultivation and native ecosystems in an integrated biofuel feedstock system. *GCB Bioenergy, 13*(3), 369-381.

Schmitt, T., Dosskey, M. G., & Hoagland, K. D. (1999). Filter strip performance and processes for different vegetation, widths, and contaminants. *Journal of Environmental Quality*. 28:1479-1489

Sechtig, A. (2001). QuickChem Method 12-107-04-1-A. *Determination of Nitrate/Nitrite by Flow Injection Analysis*.

Smith, S. J., Menzel, R. J., Rhoades, E. D., Williams, J. R., & Eck, H. V. (1983). Nutrient and sediment discharge from southern plains grasslands watersheds, Oklahoma, Texas, erosion, water quality hazards. *Rangeland Ecology & Management /Journal of Range Management Archives*, *36*(4), 435-439.

Smith, S. J., Sharpley, A. N., Berg, W. A., Naney, J. W., & Coleman, G. A. (1992). Water quality characteristics associated with southern plains grasslands. *Journal of Environmental Quality*, *21*(4), 595-601.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, *100*(1-3), 179-196.

Stallings, C. V. (2008). *Analysis of land use, land cover change in the upland forests of Oklahoma: a comparison between the 1950s and 2000s*. [Master's thesis, Oklahoma State University]. ProQuest Dissertations Publishing.

Thomas, R. E., & Hoagland, B. W. (2011). The changing forests of central Oklahoma: A look at the composition of the cross timbers prior to Euro-American settlement, in the 1950's, and today. *Oklahoma Native Plant Record*, *11*(1).

Thurow, T. L., & Carlson, D. H. (1994). Juniper effects on rangeland watersheds. *Texas A&M University-Texas Agricultural Experiment Station*.

U.S. EPA. (2001). *Ambient Water Quality Criteria Recommendations Rivers and Streams in Nutrient Ecoregion V.*

U. S. EPA. (1993). *Method 365.1: Determination of phosphorus by semi-automated colorimetry.*

U.S. EPA. (1993) *Method 351.2, revision 2.0: Determination of total Kjeldahl nitrogen by semi-automated colorimetry*.

U.S. Department of Agriculture. (2020). *Summary Report: 2017 National Resources Inventory*.

Van Els, P., Will, R. E., Palmer, M. W., & Hickman, K. R. (2010). Changes in forest understory associated with Juniperus encroachment in Oklahoma, USA. *Applied Vegetation Science*, *13*(3), 356-368.

Wilcox, B. P., Seyfried, M. S., Breshears, D. D., Stewart, B., & Howell, T. (2003). The water balance on rangelands. *Encyclopedia of water science*, *791*(4).

Zhao, X., Chen, L., & Zhang, H. (2013). Nitrate and ammonia contaminations in drinking water and the affecting factors in Hailun, northeast China. *Journal of Environmental Health*, *75*(7), 28.

Zou, C. B., Turton, D. J., Will, R. E., Engle, D. M., & Fuhlendorf, S. D. (2014). Alteration of hydrological processes and streamflow with juniper (Juniperus virginiana) encroachment in a mesic grassland catchment. *Hydrological Processes*, *28*(26), 6173-6182.

Table 2.1 Slope and soil characteristics of each watershed. The values in the slope column are in percent rise. The first value is the average slope and the second value is the standard deviation for the watersheds calculated with the slope tool in ArcMap 10.8. Values for each watershed are separated by a semi colon. Soil types are in percentage of area coverage of the watershed. Abbreviations are as follows: StDD: Stephenville-Darnell complex; Coy: Coyle loam; ReGr: Renfrow and Grainola soils; CoyZ: Coyle and Zaneis soils; GrLE: Grainola-Lucien complex; StSL: Stephenville fined sandy loam; CoLC: Coyle-Lucien complex; HaPE: Harrah-Pulaski complex; Zaneis-Huska complex; DooSL: Doolin silt loam.

Watershed	Area (m^2)	Slope	StDD	Coy	ReGr	CoyZ	GrLE	StSL	CoLC	HaPE	ZaHC	DooSL
GO1	23900	2.56; 1.04	100.0	0	0	0	0	0	0	0	0	0
GO2	28300	3.03; 1.69	0	47.2	1.6	12.7	0	34.9	0	0	0	3.8
GO3	46500	2.75; 1.66	0	22.6	7.8	0.9	0	68.8	0	0	0	0
GP1	40300	2.90; 1.40	55.2	44.8	0	0	0	0	0	0	0	0
GR1	29800	4.1; 2.6	91.9	0	0	0.3	7.9	0	0	0	0	0
GR2	13500	4.47; 2.67	21.9	0	0	56.7	21.5	0	0	0	0	0
UP1	22600	2.41; 1.65	63.5	19.9	0	0	0	0	0	15.4	1.1	0
UP2	25700	3.29; 1.66	77.4	0	11.3	0	2.9	8.7	0	0	0	0
US1	33300	2.77; 1.55	67.5	32.5	0	0	0	0	0	0	0	0
US2	37900	3.03; 1.62	29.3	0	28.8	0	13.0	8.6	20.3	0	0	0

Table 2.2 Understory/ground cover of each watershed excluding percentage of litter collected during the fall of 2020. The values for each vegetation column are the mean followed by the standard deviation of the percent cover estimated with Daubenmire frames.

Watershed	Treatment	Woody	Grass	Forb	Bare Ground
GO1	Grazed Oak	3.8 ± 3.9	2.5 ± 0	3.8 ± 3.9	23.6 ± 30.5
GO2	Grazed Oak	2.5 ± 0	19.1 ± 27.7	4.9 ± 8.2	5.5 ± 8.5
GO3	Grazed Oak	3.1 ± 2.8	28.6 ± 29.1	5.6 ± 5.6	3.1 ± 2.8
GP1	Grazed Prairie	6 ± 10.8	59.9 ± 21.3	8.8 ± 6.4	15.5 ± 15.6
GR1	Grazed Redcedar	2.5 ± 0	5.5 ± 8.5	3.1 ± 2.8	11.5 ± 19.4
GR2	Grazed Redcedar	2.5 ± 0	33.2 ± 38.1	4.5 ± 4.7	6.5 ± 5.9
UP2	Ungrazed Prairie	2.5 ± 0	72.5 ± 27.3	20.9 ± 18.1	3.1 ± 2.8
UP2	Ungrazed Prairie	3.2 ± 2.9	70.1 ± 30.1	20.8 ± 19.5	5.7 ± 8.7
US1	Ungrazed Switchgrass	2.5 ± 0	86.2 ± 21.0	6.1 ± 8.7	8.4 ± 19.7
US2	Ungrazed Switchgrass	2.5 ± 0	90.2 ± 11.1	2.5 ± 0	5.5 ± 8.5

Table 2.3 The descriptive statistics of the TKN concentrations in mg/L for the watersheds at CTER. Bootstrapped confidence intervals were calculated at the 95 percent confidence level with the bias-corrected and accelerated method.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals
GO1	1.7	2.0	1.1	1.9	0.5	3	(1.11, 1.86)
GO2	1.4	1.7	1.2	1.3	0.3	3	(1.20, 1.71)
GO3	1.4	2.1	1.1	1.2	0.5	4	(1.11, 2.11)
GP1	1.2	2.2	0.6	1.1	0.4	13	(0.82, 1.28)
GR1	5.6	7.5	1.7	6.7	2.7	4	(1.73, 7.33)
GR2	2.7	4.3	1.0	3.1	1.2	9	(1.48, 3.58)
UP1	1.2	1.9	0.1	1.2	0.4	15	(1.03, 1.39)
UP2	1.2	2.2	0.5	1.1	0.4	18	(1.06, 1.24)
US1	1.0	1.7	0.2	1.0	0.3	21	(0.85, 1.17)
US2	1.2	2.1	0.4	1.2	0.4	23	(1.06, 1.36)

Table 2.4 The results of the one-sample Wilcoxon signed rank tests of the median TKN concentration in mg/L for the watersheds at CTER to the EPA reference value of 0.52 mg/L from ecoregion 27 (EPA, 2001).

Watershed	Test Statistic	P Value
GO1	1.75	0.130
GO2	11.95	0.130
GO3	2.65	0.063
GP1	5.99	**0.001**
GR1	10.00	0.063
GR2	45.00	**0.002**
UP1	1.63	**<0.001**
UP2	7.88	**<0.001**
US1	2.58	**<0.001**
US2	5.18	**<0.001**

Table 2.5 The descriptive statistics of the TP concentrations in mg/L of runoff from the watersheds at CTER. Bootstrapped confidence intervals were calculated at the 95 percent confidence level with the bias-corrected and accelerated method.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals
GO1	0.15	0.19	0.09	0.18	0.06	3	(0.09, 0.19)
GO2	0.10	0.12	0.07	0.10	0.03	3	(0.07, 0.10)
GO3	0.10	0.12	0.09	0.10	0.01	4	(0.04, 0.18)
GP1	0.14	0.24	0.06	0.14	0.06	13	(0.07, 0.16)
GR1	1.27	1.89	0.22	1.48	0.79	4	(0.22, 1.88)
GR2	0.28	0.51	0.05	0.32	0.17	9	(0.13, 0.41)
UP1	0.16	0.88	0.07	0.10	0.20	15	(0.08, 0.10)
UP2	0.06	0.10	0.04	0.06	0.01	18	(0.05, 0.06)
US1	0.07	0.11	0.01	0.08	0.03	21	(0.06, 0.08)
US2	0.06	0.11	0.01	0.06	0.06	23	(0.05, 0.06)

Table 2.6 The results of the one-sample Wilcoxon signed rank tests of the median TP values for the watersheds at CTER to the EPA reference value of 0.09 mg/L from ecoregion 27 (EPA, 2001).

Watershed	Test Statistic	P Value
GO1	3	0.190
GO2	4	0.380
GO3	6	0.090
GR1	10	0.060
GR2	44	**0.006**
GP1	84	**0.004**
UP1	70	**0.008**
UP2	2	0.990
US1	27	0.990
US2	11	0.990

Table 2.7 The descriptive statistics of the Ortho-P concentrations in mg/L of runoff from the watersheds at CTER. Bootstrapped confidence intervals were calculated at the 95 percent confidence level with the bias-corrected and accelerated method.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals mg/L
GO1	0.04	0.05	0.03	0.04	0.01	3	(0.03, 0.05)
GO2	0.05	0.06	0.05	0.05	0.00	5	(0.05, 0.06)
GO3	0.05	0.05	0.04	0.05	0.00	5	(0.04, 0.05)
GP1	0.09	0.30	0.04	0.06	0.07	14	(0.05, 0.06)
GR1	0.09	0.20	0.04	0.08	0.07	5	(0.04, 0.08)
GR2	0.03	0.05	0.01	0.03	0.02	11	(0.01, 0.03)
UP1	0.07	0.20	0.03	0.05	0.04	14	(0.04, 0.05)
UP2	0.05	0.20	0.01	0.05	0.04	20	(0.03, 0.05)
US1	0.04	0.10	0.01	0.04	0.03	21	(0.02, 0.04)
US2	0.05	0.20	0.01	0.04	0.03	22	(0.03, 0.04)

Table 2.8 The results of the one-sample Wilcoxon signed rank test of the median Ortho-P values for individual watersheds to the 25th percentile of the Ortho-P concentration of EPA reference streams of 0.0275 mg/L from ecoregion 27 (EPA, 2001).

Watershed	Test Statistic	P Value
GO1	6	0.13
GO2	15	**0.02**
GO3	15	**0.02**
GP1	105	**>0.001**
GR1	15	**0.03**
GR2	46	0.13
UP1	105	**>0.001**
UP2	200	**>0.001**
US1	193	**>0.01**
US2	224	**>0.001**

Table 2.9 The annual runoff in millimeters for each watershed at CTER. Median, Max, and minimum values displayed are monthly values for the data for that year.

Watershed	Year	Runoff in Millimeters			
		Total	Max	Min	Median
GO1	2020	29.6	25.5	0.00	0.0
	2021	6.4	3.8	0.00	0.0
GO2	2020	2.8	2.6	0.00	0.0
	2021	13.7	6.4	0.00	0.1
GO3	2020	9.2	7.0	0.00	0.0
	2021	5.0	2.7	0.00	0.0
GP1	2020	68.2	56.3	0.00	0.9
	2021	108.0	49.8	0.08	8.5
GR1	2020	0.7	0.3	0.00	0.1
	2021	25.6	12.7	0.00	0.3
GR2	2020	27.8	7.3	0.61	1.9
	2021	26.8	16.6	0.01	0.9
UP1	2020	144.6	102.1	0.31	2.6
	2021	146.7	51.3	0.02	14.9
UP2	2020	205.2	179.4	0.35	2.8
	2021	176.3	46.6	1.07	16.8
US1	2020	100.9	64.2	0.00	1.6
	2021	172.3	61.3	0.00	18.2
US2	2020	78.7	42.1	0.01	3.1
	2021	106.5	22.8	0.02	12.4

Table 2.10 The descriptive statistics for the TKN loading of runoff in grams/ha from the watersheds at CTER. Annual loads were calculated by dividing the total load for the duration of the study by the number of years.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals	Annual Load
GO1	13.6	26.7	0.4	13.6	18.5	2	(0.4, 13.6)	16.3
GO2	37.2	61.4	12.5	37.8	24.5	3	(12.5, 37.8)	67.0
GO3	21.2	36.2	5.9	21.4	12.4	4	(5.9, 28.9)	50.9
GP1	96.7	274.8	1.4	74.5	83.8	12	(34.2, 142.1)	969
GR1	8.3	29.9	0.3	1.5	14.5	4	(0.3, 29.9)	19.9
GR2	33.4	160.7	3.1	13.1	51.2	9	(5.6, 21.8)	180.
UP1	116.8	403.9	0.6	113.9	102.4	15	(43.4, 148.0)	1,051
UP2	103.8	374.2	12.9	74.6	106.5	18	(29.4, 110.9)	1,021
US1	90.7	335.9	1.3	51.1	83.4	20	(34.7, 126.4)	1,089
US2	64.1	160.8	5.3	48.8	42.7	19	(31.6, 84.5)	731

Table 2.11 The descriptive statistics for the TP loading in grams/ha of runoff from the watersheds at CTER. Annual loads were calculated by dividing the total load for the duration of the study by the number of years.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals	Annual Load
GO1	1.4	2.7	0.04	1.4	1.9	2	(0.04, 1.40)	1.7
GO2	2.8	5.1	0.67	2.7	2.2	3	(0.67, 5.10)	5.1
GO3	1.4	1.8	0.59	1.7	0.6	4	(0.59, 1.70)	3.4
GP1	14.0	58.3	0.19	9.0	16.3	12	(3.50, 15.00)	101
GR1	2.1	7.9	0.03	0.3	3.9	4	(0.03, 7.90)	5.1
GR2	2.4	7.9	0.30	1.8	2.6	9	(0.53, 2.70)	13
UP1	9.7	32.2	1.40	8.2	7.5	15	(4.10, 9.60)	87
UP2	5.2	23.8	0.62	3.7	5.5	18	(1.80, 5.20)	56
US1	7.2	30.5	0.09	3.0	7.9	20	(2.34, 9.54)	86
US2	3.6	15.9	0.14	2.2	3.7	19	(1.24, 3.44)	41

Table 2.12 The descriptive statistics for the Ortho-P loading in g/ha of runoff from the watersheds at CTER. Annual loads were calculated by dividing the total load for the duration of the study by the number of years.

Watershed	Mean	Max	Min	Median	Std	Sample Size	Bootstrapped Confidence Intervals	Annual Load
GO1	0.36	0.7	0.01	0.36	0.5	2	(0.01, 0.36)	0.43
GO2	0.90	2.6	0.01	0.48	1.1	5	(0.01, 1.32)	2.7
GO3	0.40	0.9	0.01	0.24	0.4	5	(0.01, 0.67)	1.2
GP1	7.80	32.0	0.08	3.30	11.0	13	(0.39, 3.45)	61
GR1	0.11	0.3	0.01	0.03	0.1	5	(0.01, 0.22)	0.32
GR2	0.92	7.9	0.01	0.13	2.4	11	(0.02, 0.15)	6.1
UP1	5.20	19.0	0.20	3.32	6.2	14	(1.25, 4.85)	44
UP2	3.10	17.0	0.44	1.47	4.0	20	(1.16, 2.98)	37
US1	4.10	25.0	0.17	1.67	6.5	20	(1.07, 2.83)	49
US2	1.90	7.1	0.36	1.25	1.9	18	(0.89, 1.97)	21

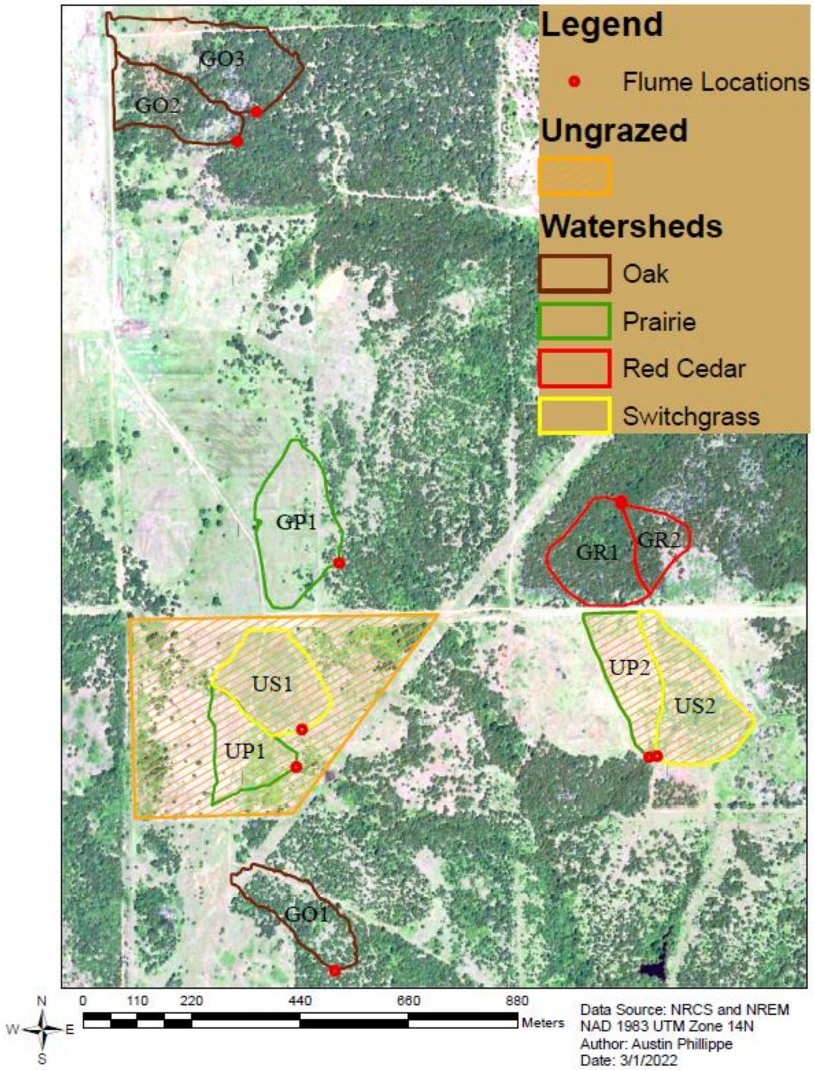
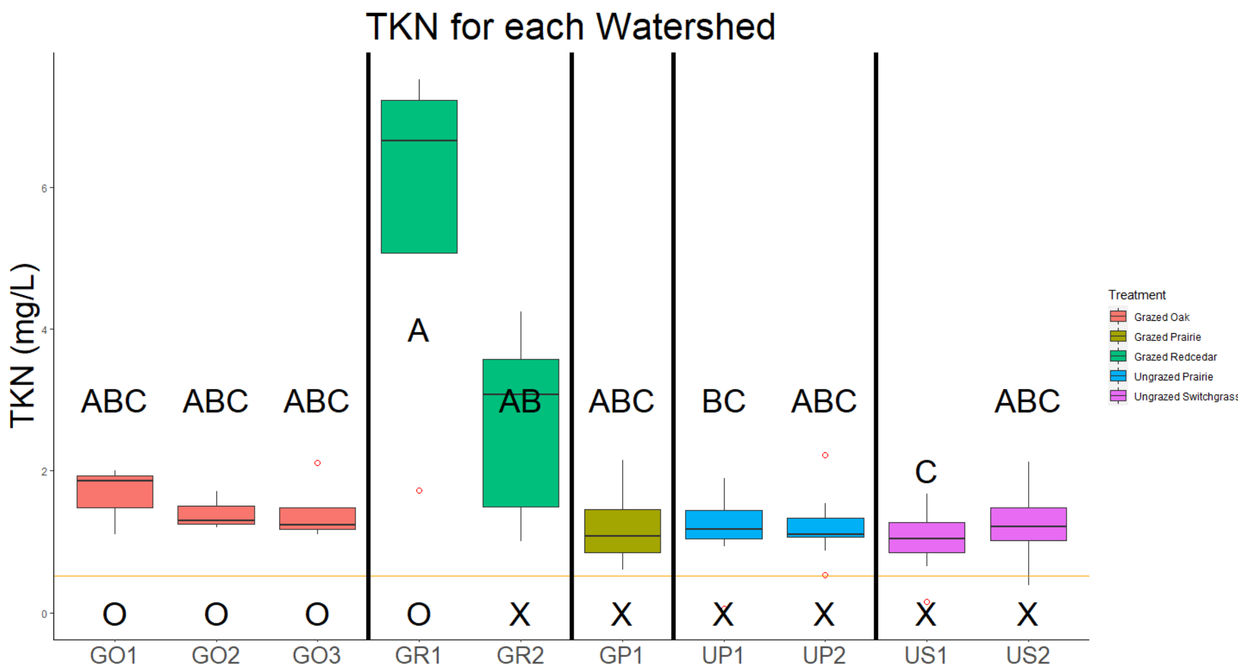
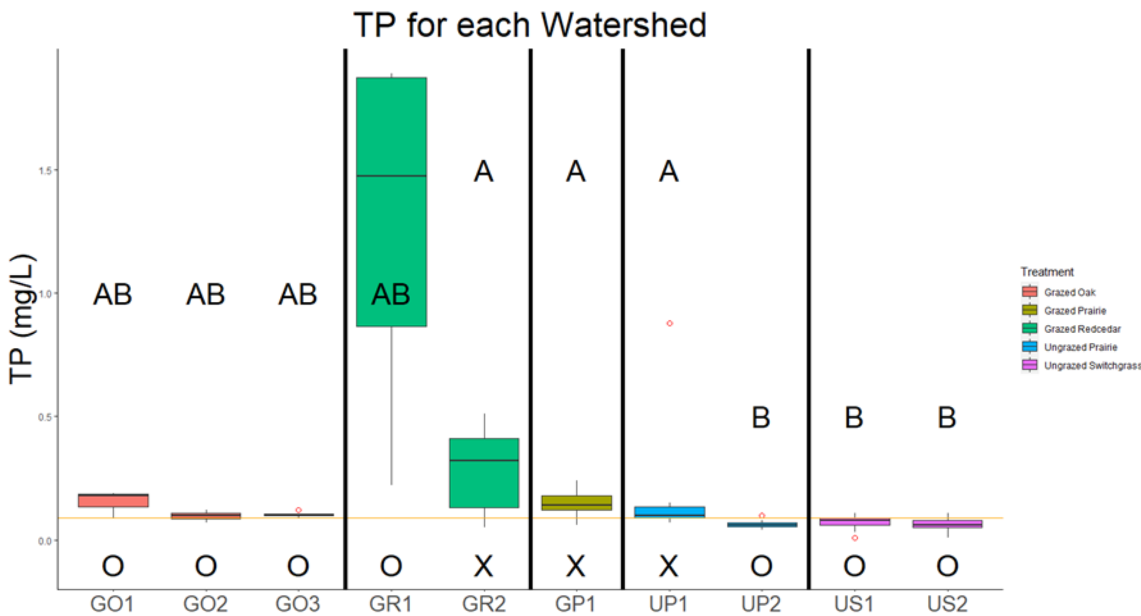


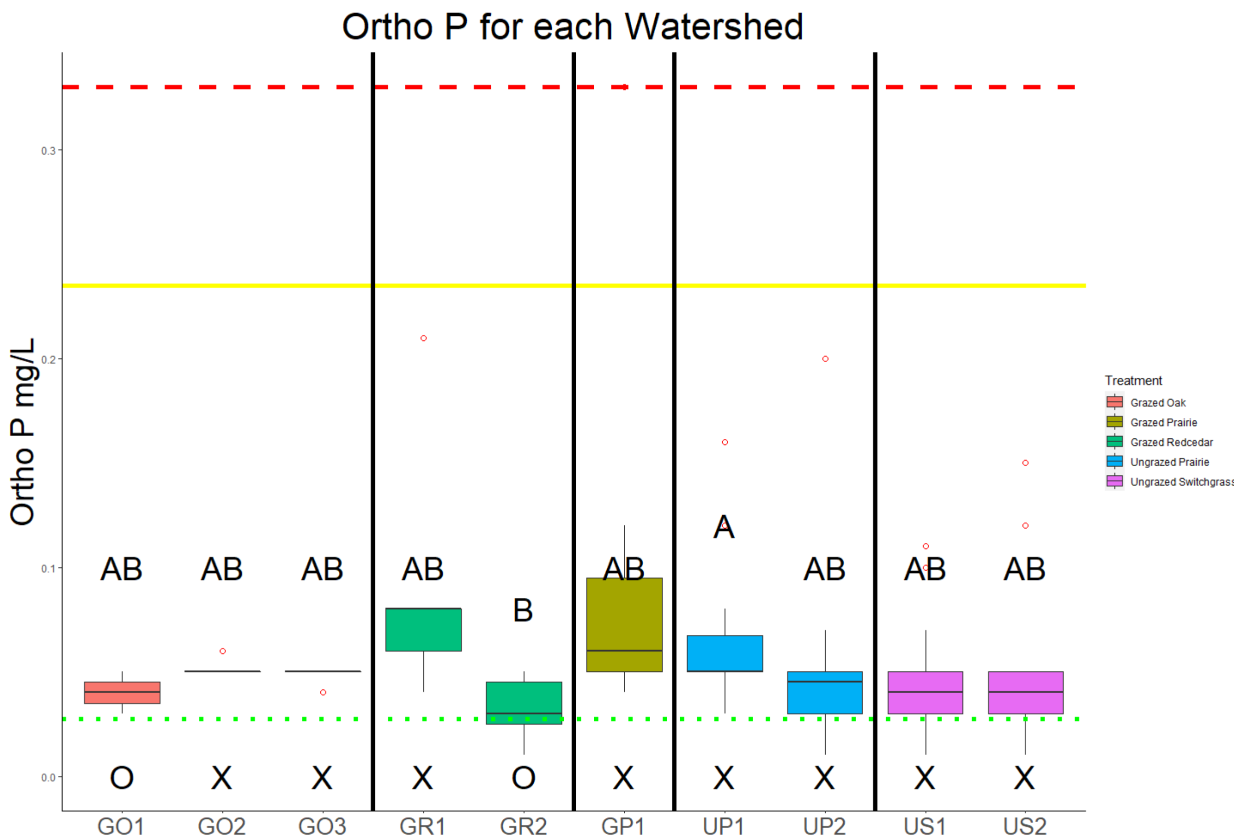
Figure 2.1 The size and locations of the watersheds monitored at CTER overlaid on a 1-meter resolution image from 2019 provided by the National Agriculture Imagery Program. The areas outlined in orange represent portions of the property that are excluded from cattle access. Cattle were not confined to any dominant vegetation cover for the purposes of this study.



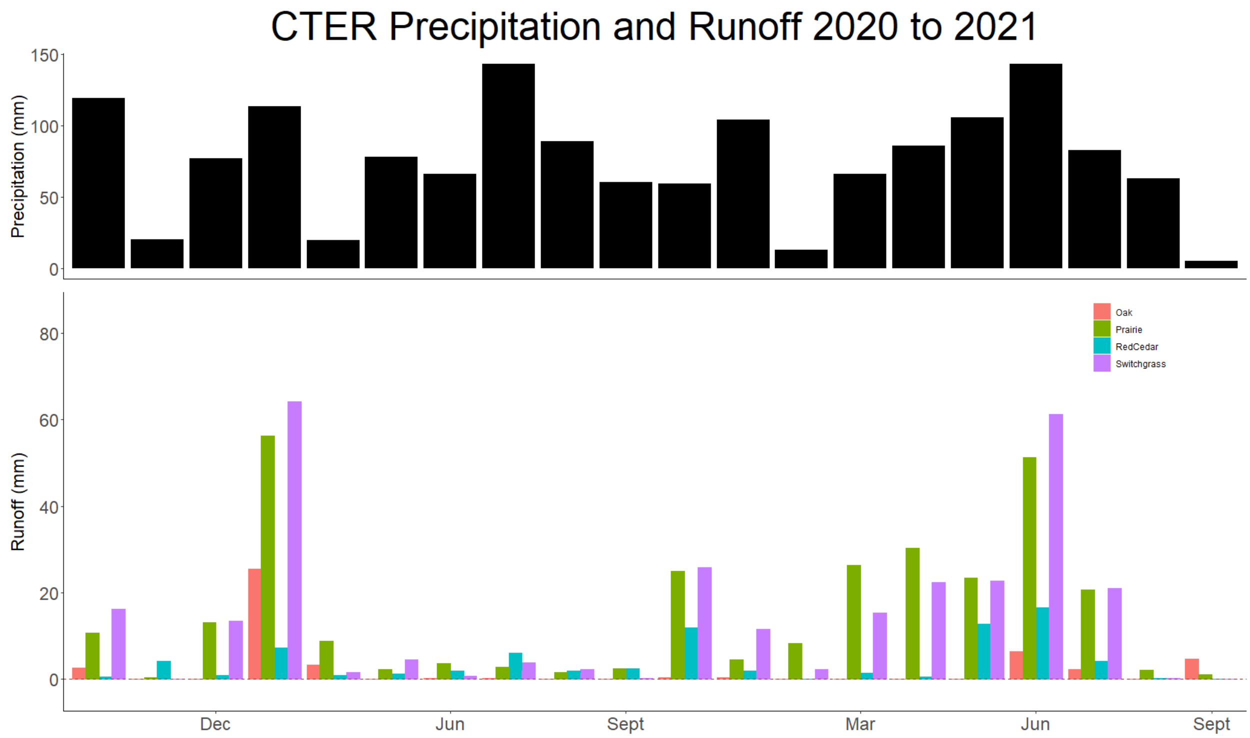
2.2 Boxplots of the TKN concentrations for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The solid orange line represents the EPA reference condition of 0.52 mg/L (EPA, 2001). "*X*" at bottom of the plot indicates that the median value for that watershed is significantly higher than the EPA reference condition. "*O*" indicates the median value for that watershed is not significantly higher than the EPA reference condition.



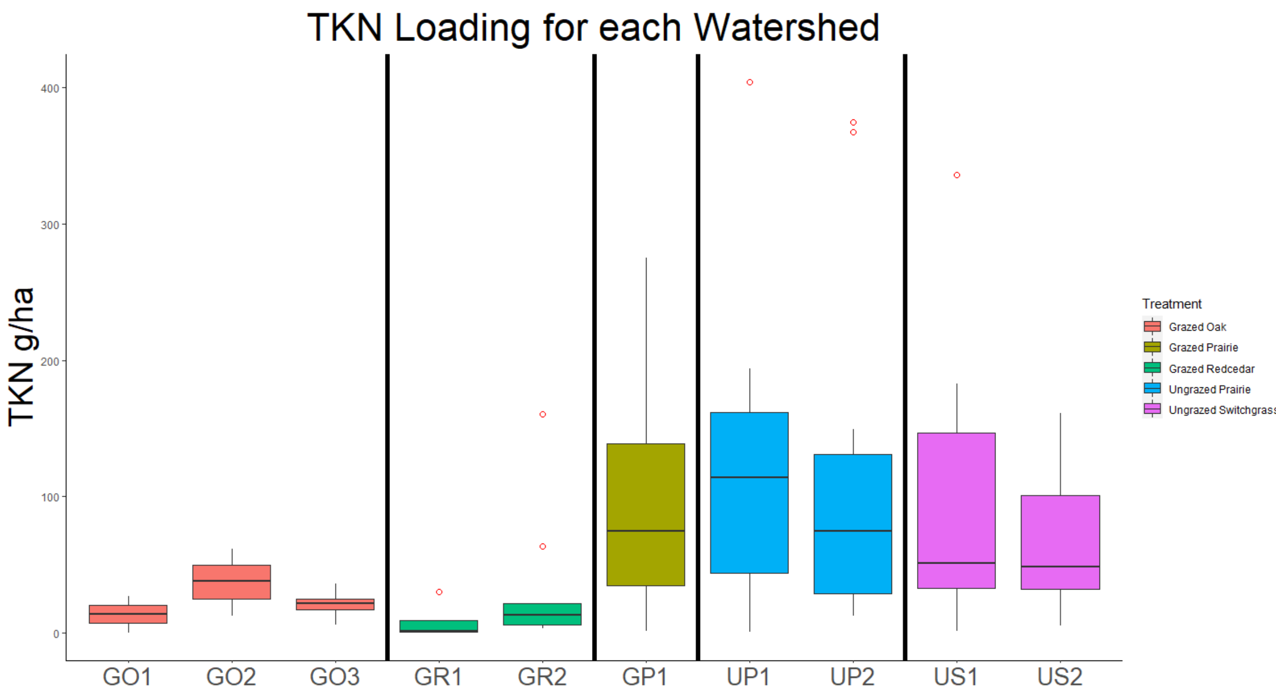
2.3 Boxplots of the TP concentrations for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The solid orange line represents the EPA reference condition of 0.09 mg/L (EPA, 2001). "*X*" at bottom of the plot indicates that the median value for that watershed is significantly higher than the EPA reference condition. "*O*" indicates the median value for that watershed is not significantly higher than the EPA reference condition.



2.4 Boxplots of the Ortho-P concentrations for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values. The dotted green line represents the 25th percentile for the EPA reference condition of 0.0275 mg/L (EPA, 2001); the solid yellow line represents the 95th percentile for the EPA reference condition of 0.235 mg/L; and the dashed red line represents the 95th percentile for the EPA reference condition of 0.330 mg/L. "*X*" at bottom of the plot indicates that the median value for that watershed is significantly higher than the 25th percentile for the EPA reference streams. "*O*" indicates the median value for that watershed is not significantly higher than the 25th percentile of EPA reference streams.



2.5 The monthly runoff from watersheds grouped based on vegetation cover for visual purposes. On the bottom y-axis the runoff is presented in millimeters. The top y-axis is the precipitation in millimeters recorded from the nearest Mesonet station (Marena).



2.6 Boxplots of the TKN loading per event for the individual watersheds at CTER.

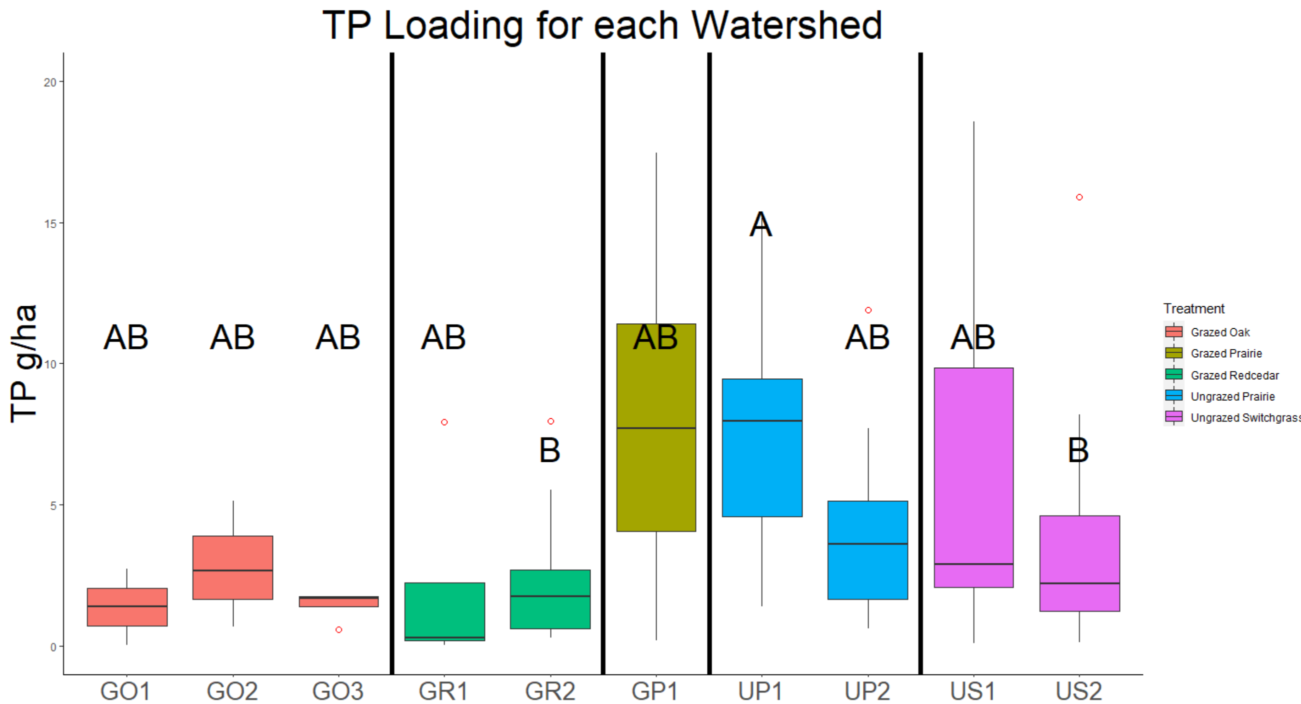


Figure 2.7 Boxplots of the TP loading per event for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values.

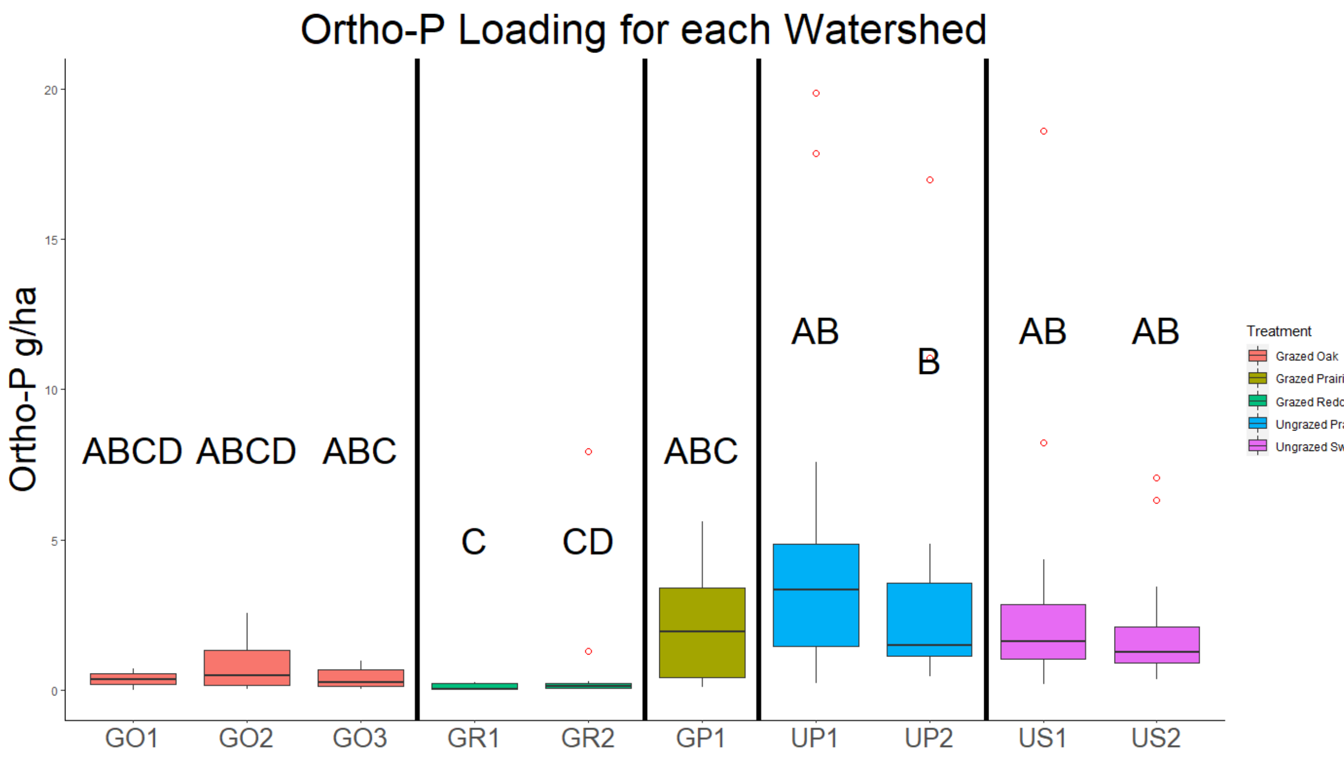


Figure 2.8 Boxplots of the Ortho-P loading per event for the individual watersheds at CTER. Boxplots with the same letter are not significantly different from one another. Letters earlier in the alphabet represent higher values.

VITA

Austin Phillippe

Candidate for the Degree of

Master of Science

Thesis: DETERMINING BACTERIAL AND NUTRIENT CONCENTRATIONS AND LOADINGS OF SURFACE RUNOFF FROM DIFFERING GRAZER ACCESS AND VEGETATIVE COVER IN NORTHCENTRAL OKALHOMA

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