Impacts of wild pigs (*Sus scrofa*) on nutrient cycling and water quality in riparian areas

by

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Abstract

Wild pigs (*Sus scrofa*) are a highly invasive species in the United States and millions of dollars are spent annually on removal efforts and damage reduction. Wild pigs may act as ecosystem engineers in areas where they are established, so it is important to fully understand their impacts to predict how environments they invade may change. Changes in riparian ecosystems should be of special concern as they provide important ecosystem services and are susceptible to disturbance. We examined changes in biogeochemical processes at the terrestrial-aquatic interface at a property that was densely populated by wild pigs. Nitrogen mineralization rates were estimated for floodplain soils disturbed by wild pig rooting, and erosion and accretion of stream bank sediment was recorded to estimate the effects of wild pigs on bank stability. Water quality parameters and fecal bacteria concentrations were measured to determine the impacts of wild pigs on water quality in small forested watersheds. Although the effects of wild pigs may vary depending on local environmental conditions and habitat types, our findings suggest that wild pigs impact nutrient cycling and water quality in riparian areas.

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List of Abbreviations

AICc	Akaike's Information Criterion corrected for small sample size
C	Carbon
CFU	Colony Forming Unit
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
FC	Fecal Coliforms
LME	Linear Mixed Effects
MBC	Microbial Biomass C
MBN	Microbial Biomass N
MNC	Monthly Net Change
N	Nitrogen
POTL	Privately-Owned Tract of Land
TN	Total Nitrogen
TSS	Total Suspended Solids
TUSK	Tuskegee National Forest

Chapter 1: Impacts of wild pig disturbance on nutrient cycling and sediment movement in riparian forests

INTRODUCTION

Riparian forests link terrestrial and aquatic ecosystems while fulfilling ecosystem essential roles of filtering, retaining, and transforming nutrients and sediment. Microbes found in riparian soil convert nitrogen into forms accessible to plants and other organisms, which is an essential function as nitrogen is a limiting factor of the primary production of most terrestrial ecosystems and can be rapidly lost in a system through root uptake, leaching, and denitrification (Fisher and Binkley 2000). Many commercial industries (i.e. agriculture, timber), and recreational hunting and fishing, are supported by riparian and wetland forest ecosystems. Despite the reliance of society on healthy and functioning riparian ecosystems, land use changes and degradation of water quality are issues of increasing concern and threaten the ability of these ecosystems to properly fulfill their ecological role. Riparian areas are some of the most degraded and altered landscapes around the world, with an estimated habitat loss of over 70% in the United States, and with riparian vegetation loss reaching 95% in some areas (Brinson et al. 1981, National Research Council 2002). While the main threats to riparian ecosystems have traditionally been seen as anthropogenic disturbances, other factors that are more difficult to control, such as invasive species and climate change, can have drastic impacts (Baldwin and Batzer 2012). Unfortunately, these impacts are usually poorly understood.

Riparian ecosystems are particularly sensitive to disturbance due to their status as an ecotone between terrestrial and aquatic environments (leading to a distinct microclimate), and the fact that changes occurring upstream have the ability to impact ecosystems far downstream of the initial source (National Research Council 2002, Alexander et al. 2007). Invasive species can be especially detrimental to riparian ecosystems due to their inherent disturbance regimes and abundant resources which make them easily accessible and attractive to invaders (King et al. 2012). Information is available regarding the impacts of invasive plant species on nutrient cycling and sediment transport in riparian habitats (Mineau et al. 2011, Mitchell et al. 2011, Greenwood and Kuhn 2014); however, we have little information regarding the impacts of invasive animals on these ecosystem-essential functions.

The wild pig (*Sus scrofa*) is an example of an invasive species that has the capacity to cause extreme disturbance to riparian ecosystems. In fact, wild pigs are considered ecosystem engineers as they are able to significantly influence and change the environment they inhabit (Crooks 2002, Sandom et al. 2012). Wetlands and riparian areas are preferred habitats of wild pigs due to the abundance of resources typically available in these areas, and their presence is common in riparian forests in the southeastern U.S. (Mayer and Brisbin 2009). Rooting and wallowing by wild pigs is a form of bioturbation, which is the movement of soil and sediment by organisms (Gabet et al. 2003, Platt et al. 2016), and results in drastic changes to both the physical landscape and community structure of the environment. Wild pigs root by using their snouts and hooves to dig and churn up soil in search of subsurface food, such as fungi, invertebrates, roots, small fossorial vertebrates, and tubers (Mayer and Brisbin 2009). Rooting may be shallow on the soil surface or extend deep into lower soil horizons, which can impact soil microfauna abundance, diversity, and community structure (Vtorov 1993, Mohr et al. 2005, Mitchell et al. 2007).

Current literature describing the effects of wild pigs on nutrient cycling and sediment transport in riparian areas is sparse and results are often inconclusive or conflicting (Beasley et al. 2018). Soil nitrogen (N) mineralization has been reported to increase due to wild pig rooting

(Singer et al. 1984, Cuevas et al. 2012), but other studies have indicated that N mineralization is unaffected by rooting (Groot Bruinderink and Hazebroek 1996, Moody and Jones 2000, Cushman et al. 2004, Barrios-Garcia et al. 2014). Soil microbial biomass has been reported to increase following rooting (Risch et al. 2010, Wirthner et al. 2012), but other studies have found that it remains unchanged (Taylor et al. 2011, Wirthner et al. 2011). Other studies have examined the impact of wild pig disturbance on soil erosion, but results were not significant (Dunkell et al. 2011, Strauch et al. 2016, Hancock et al. 2017). The discrepancy in results among studies may be due to different study environments, methods, varying densities of wild pigs, and the measurement of insensitive parameters. Therefore, a need clearly exists for a better understanding of the impacts of wild pig disturbance on nutrient cycling and sediment transport.

Our goal was to examine the impacts of wild pig activity on soil nutrient cycling and sediment transport in riparian zones. This information is critical to develop an improved understanding of invasive wild pigs on the environment, and specifically to guide wild pig management and control initiatives in riparian areas. Our specific research objectives were to:

1. Examine changes in soil nutrient cycling as a result of wild pig rooting by measuring nitrogen mineralization, microbial biomass, and soil physical parameters in riparian areas.
2. Examine the impacts of wild pigs on sediment transport by measuring stream bank erosion in forested watersheds with wild pig activity.

METHODS

Study Area

This research was conducted at a privately-owned tract of land (hereafter referred to as POTL) and Tuskegee National Forest (TUSK), which served as the reference area. POTL was a 4515-ha property located at 85°32'0.932"W 32°11'39.019"N in Bullock County, Alabama, USA. Management practices focused on maintaining habitat for white-tailed deer (*Odocoileus virginianus*) and eastern wild turkey (*Meleagris gallopavo silvestris*) populations. The most common habitat types were mixed pine (*Pinus* spp.)-hardwood forest and riparian hardwoods. The canopy was primarily composed of sweetgum (*Liquidambar styraciflua*), loblolly pine (*Pinus taeda*), and southern shagbark hickory (*Carya carolinae-septentrionalis*), and the understory was mainly herbaceous and semi-woody species such as blackberry (*Rubus* spp.), American beautyberry (*Callicarpa americana*), and eastern baccharis (*Baccharis halimifolia*). Wild pigs were present throughout the property and camera surveys estimated the density to be approximately 15.5 pigs/km^2, which is much greater than the average density of 6-8 pigs/km^2 in the southeastern U.S (Lewis et al. 2019).

The study area at TUSK was located approximately 25 km from the treatment area, and was closely aligned with POTL in terms of stream gradients, forest cover and habitat type, and stream size. Whereas wild pigs were present in some areas of TUSK, they were not yet established in the area selected for the study. This was confirmed with camera surveys conducted in March 2018. Trail cameras were placed 1-km^2 apart in a grid pattern at the sampling sites and baited with corn. After 1 week, remaining corn was collected and cameras removed. Analysis of camera data did not detect wild pig activity, nor was any sign (tracks, wallows, etc.) seen during field work.

Sampling sites (watersheds) at POTL and TUSK were restricted to certain criteria: low gradient, occupied by deciduous wetland forests, and streams 3rd order or lower in magnitude. Both sites were located in the Upper Coastal Plain physiographic region and in the Mantachie-luka-Bibb soil association. A total of 5 sites at POTL and 3 sites at TUSK were selected for sampling. The main tributaries were perennial, whereas most of the lower order streams were intermittent with flow only in winter and spring. At POTL, damage as a result of pig activity (rooting, digging, and wallowing) was observed at all sampling sites. This activity was observed on the floodplains and within the stream channels, even when the channels were dry.

Collection and Analysis of Soil Samples

Soil samples were collected at the POTL watersheds in June 2018 and October 2018, and at the TUSK watersheds in October 2018. The POTL watersheds were chosen for soil sampling if fresh rooting damage was present and there was a low density of herbaceous plants and grasses. At each POTL watershed, four “patches” of fresh rooting damage were chosen for soil collection. These sampling locations were referred to as plots. Fresh rooting damage was defined as disturbance that occurred less than a week prior to the sampling day, and was assessed by the condition of damaged vegetation and broken roots. Previous precipitation events also helped determine the age of rooted areas. An additional soil sample was taken from a randomly selected unrooted area within 3 m of each rooted plot, for a total of 8 plots at each POTL watershed. New freshly rooted areas at each watershed were sampled in October 2018 following the same method. Four individual locations were randomly chosen for soil collection at each TUSK watershed, for a total of 12 plots. Soil samples were extracted at a depth of 10 cm with a shovel and divided between two 207 mL Whirl-Pak® sterile sampling bags (Nasco Sampling, Madison, WI). A cylindrical container (volume 90.5 cm^3) was hammered into the ground next to the hole dug for soil collection and at the same depth to obtain a soil core sample for bulk density analysis. One of the bagged samples was put on ice for transport back to the Auburn University Biogeochemistry Laboratory and the other was immediately reburied in the hole and covered with the surrounding soil. HOBO TidbiT v2 Temperature Data Loggers (Onset Computer Corporation, Bourne, MA) were buried with 3 of the samples at each POTL watershed and removed after the incubation period. Plots were randomly chosen for temperature monitoring, but at least one rooted and one unrooted plot was monitored with the third plot being either rooted or unrooted. At TUSK, one randomly selected plot per watershed had a data logger. A 1x1-m wooden frame with metal wire was placed over the location of the buried bag to prevent wild pigs and other wildlife from tampering with it. In order for microbial processes to occur under natural conditions, the sample was left to incubate in the soil for approximately 30 days after which point it was retrieved and processed (Hart et al. 1994).

Soil samples were analyzed for bulk density, microbial biomass, and nitrogen mineralization at the Auburn University Biogeochemistry Laboratory. Samples to be analyzed for bulk density and soil moisture were dried in a Fisherbrand Isotemp 500 Series Economy Lab Oven (Fisher Scientific, Hampton, NH) for 48 hours at 105° C and then weighed. The mass of the oven-dried sample (g) was divided by the volume (cm^3) to calculate the bulk density of the soil. Bulk density values were used as a measure of soil compaction. Mass of the wet sample was subtracted from mass of the oven-dried sample and multiplied by 100 to obtain percent soil moisture.

In order to estimate N mineralization, 10 g of soil from pre-incubation and post-incubation samples was added to 100 mL 2 mol L^{-1} KCL, shaken for 1 hour, and filtered. Extracts were analyzed for NH_4-N and NO_3-N using a Bio-Rad Model 450 microplate reader

(Bio-Rad Laboratories, Hercules, CA). Negative values indicated that N was immobilized instead of mineralized. Pre-incubation total N (the sum of NH_4-N and NO_3-N) was subtracted from post-incubation total N and converted to a g $m^{2\ -1}$ day^{-1} rate.

Microbial biomass was estimated using the chloroform-fumigation technique described in Vance et al. (1987). Fumigated samples were analyzed by exposing 18.5 g of soil to $CHCl_3$ for 24 hours, after which the samples were extracted with 125 mL of 0.5 mol L^{-1} K_2SO_4, shaken for 30 minutes, and filtered. Extracts were analyzed using a Shimadzu TOC-VCPN combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD) for organic C and N. Microbial biomass was estimated by comparing organic C and N in the fumigated and unfumigated samples. Immobilization was accounted for by including constants for C and N (Shen et al. 1984, Brookes et al. 1985)

Erosion Sampling

Erosion sampling took place at the 5 POTL watersheds. TUSK watersheds were not included as the bank structure differed from POTL watersheds. Sediment erosion and accretion were measured by sediment pins, which were constructed of rebar approximately 1-meter in length with a metal washer welded to the middle. Between 16 and 24 pins were inserted in both sides of the stream bank in the approximate area where water samples were collected. Pins were placed approximately 5 meters apart with the washer flush with the soil level. Changes in soil level were measured approximately every 30 days by comparing the current soil level with the height of the washer (the soil surface at time 0). Change in soil level below the height of the washer was measured as erosion, and soil level above the washer was measured as accretion. Monthly net change (MNC) was calculated as the net change in soil level between sampling events, with negative values indicating erosion.

In addition to measuring changes in sediment transport associated with undisturbed banks, four bank slides utilized by wildlife in stream crossings were monitored at each watershed for sediment movement. Bank slides were worn paths that large mammals (such as wild pigs and deer) used to get from the floodplain to the stream channel. Five sediment pins were installed in each slide: one at the very top and two sets of parallel pins farther down. Changes in soil level were measured at each pin using the same methods as for bank pins. Presence or absence of wild pig activity (tracks and rooting) at each of the slides was recorded.

Statistical Analysis

Statistical analyses were conducted using R statistical platform version 3.5.3 (R Core Team 2019). Homoscedasticity and normality of the residuals were assessed visually with diagnostic graphs and statistically using Shapiro-Wilk and Levene's tests. Residuals of the data were not normally distributed, so non-parametric analyses were conducted. Wilcoxon Rank Sum and Kruskal-Wallis tests were used to compare parameters among treatments and sites. Dunn's Test of Multiple Comparisons with a Bonferroni adjustment was conducted post hoc for variables that differed significantly among treatments and/or locations. Bulk density, soil moisture, temperature, NH_4-N, NO_3-N, total N, microbial biomass N (MBN), microbial biomass C (MBC), and microbial C:N for paired (rooted and unrooted) soil plots were compared for June, October, and both sampling events combined. Reference (TUSK) samples were compared to October rooted and unrooted samples. Each set of paired plots was considered as an individual sample instead of a replicate due to high variability in the depth, age, and location of pig rooting within and among watersheds. Mean erosion, accretion, and MNC was calculated for bank and slide pins by watershed for each sampling event. Mean values were compared by pin position (bank/slide), seasons (wet/dry), and among watersheds. Seasons were delineated by precipitation and stream flow, which were greatest from November through April (wet season) and diminished from May through October (dry season). The percentage of slides with recent wild pig activity were calculated for each watershed by month. Slides were analyzed by wild pig activity (active/inactive) and season (wet/dry). A slide was considered active if wild pig tracks and/or new rooting damage were present in the immediate area.

RESULTS

The original intention was to collect soil samples from each plot at 3-month intervals; however, this was not possible due to lack of wild pig damage on the floodplains of the POTL watersheds. As a result, soil sampling occurred only twice at POTL (June/July and October/November 2018) and once at TUSK (October/November 2018). Sediment pins were analyzed approximately every 30 days from July 2018 through September 2019. Stream flow ceased at the end of July 2018 due to low rainfall and flowing water did not return until December 2018. A drought from mid-May 2019 to mid-October 2019 (Figure 1.1) meant that the majority of streams were dry or only contained stagnant pools of water.

Nitrogen Mineralization and Microbial Biomass

Paired soil plots did not differ in bulk density or temperature for either month, and there was no difference in temperature among all three plot types for October (Table 1.1). Bulk density values were significantly lower at reference plots ($\bar{x}$ = 0.84, SE = 0.09) than at rooted ($\bar{x}$ =1.14, SE = 0.03, $p < 0.01$) and unrooted plots ($\bar{x}$ = 1.18, SE = 0.03, $p < 0.01$) in October. Bulk density values did not differ between rooted and unrooted areas. Soil moisture was greater at rooted plots ($\bar{x}$ = 21.01%, SE = 1.61) than at unrooted plots ($\bar{x}$ = 18.52%, SE = 1.15, $p = 0.03$). Soil moisture ranged from 12.11 to 42.19% in June and 6.84 to 37.17% in October, and values between seasons were significantly different ($p < 0.01$). Mean daily temperature ranged from 23.6 to

25.0° C for rooted/unrooted plots during the summer and 13.08 to 14.6° C for all three plot types during the fall.

There were no differences in mean NH_4-N rates between paired plots, among treatments in October, or between seasons ($p > 0.05$). Mean NO_3-N rates were 0.018 g $m^{2\ -1}$ day^{-1} (SE = 0.003) at unrooted plots and 0.027 g $m^{2\ -1}$ day^{-1} (SE = 0.006) at rooted plots, and did not significantly differ between paired plots or among treatments ($p > 0.05$). There was a significant difference in NO_3-N rates between seasons, with values at paired plots ranging from 0.007 to 0.12 g $m^{2\ -1}$ day^{-1} in the summer and 0 to 0.032 g $m^{2\ -1}$ day^{-1} in the fall ($p < 0.01$). Overall mean total N was 0.026 g $m^{2\ -1}$ day^{-1} (SE = 0.005) at unrooted plots and 0.03 g $m^{2\ -1}$ day^{-1} (SE = 0.006) at rooted plots, and did not differ between paired plots or among treatments. Seasons differed significantly in total N, with values in the summer ranging from 0.002 to 0.115 g $m^{2\ -1}$ day^{-1} and from 0 to 0.052 g $m^{2\ -1}$ day^{-1} in the fall ($p < 0.01$).

Microbial biomass of N and C did not significantly differ between paired plots or among treatments ($p > 0.05$). Soil MBC and microbial C:N were greater in the fall than the summer ($p < 0.05$). Overall mean MBN was 29.88 μg g^{-1} soil (SE = 4.04) at unrooted plots and 34.71 μg g^{-1} soil (SE = 4.67) at rooted plots, and did not differ between seasons. Unrooted plots had an overall mean MBC of 348.88 μg g^{-1} soil (SE = 39.1) compared to 362.48 μg g^{-1} soil (SE = 33.85) at rooted plots. In June, microbial C:N was significantly greater in unrooted plots ($\bar{x}$ = 11.3 μg g^{-1}, SE = 1.12) than rooted plots ($\bar{x}$ = 9.34 μg g^{-1}, SE = 0.79, $p < 0.01$). In October, reference plots had greater microbial C:N ($\bar{x}$ = 8.98, SE = 0.75) than both rooted ($\bar{x}$ = 12.39, SE = 0.89) and unrooted ($\bar{x}$ = 13.28, SE = 1.01) plots ($p < 0.01$).

Bank Erosion and Accretion

Mean erosion and accretion differed by pin position and season, while monthly net change only differed by season (Table 1.2; Figure 1.2). Mean pin erosion throughout the year was significantly greater at slides ($\bar{x}$ = -1.81 cm, SE = 0.11) than banks ($\bar{x}$ = -1.35 cm, SE = 0.1, $p < 0.01$; Figure 1.3). Mean accretion was 1.34 cm (SE = 0.09) at bank pins and 1.19 (SE = 0.09) at slide pins, and was significantly greater at banks than slides ($p = 0.03$). Monthly net change throughout the year ranged from -34.6 to 21 cm at bank pins and from -61.3 to 58.4 cm at slide pins, and was not significantly different between pin positions ($p = 0.45$).

There were no significant differences in mean erosion at slide pins ($p = 0.40$), but mean accretion and MNC varied by wild pig activity and season (Table 1.3; Figure 1.4). Pin erosion ranged from -26 to 0 cm at slides with recent wild pig activity, and -34.2 to 0 cm at slides without recent activity. Pin accretion in the dry season significantly differed by activity with a mean accretion of 0.9 cm (SE = 0.19) and 1.35 cm (SE = 0.14) at active and inactive slides, respectfully ($p = 0.02$). Mean MNC in the dry season was significantly greater at active slides ($\bar{x}$ = -0.65 cm, SE = 0.24) than inactive slides ($\bar{x}$ = -0.11 cm, SE = 0.09) ($p < 0.01$). Slide activity varied by season with 57.5% of slides used in the wet season compared to 27.78% in the dry season ($p < 0.01$) (Figure 1.5). There were no differences in the percentage of slides used among watersheds.

DISCUSSION

Nitrogen Mineralization and Microbial Biomass

We did not observe differences in bulk density or temperature as a result of rooting activity. Differences in bulk density between rooted/unrooted and reference plots were likely due to microsite variability in soil properties. While rooted plots had greater soil moisture on average than unrooted plots, soil moisture varies due to microsite differences; therefore, mean values do not necessarily capture the amount of soil moisture on a larger scale. We expected that rooted plots would have lower bulk density than unrooted plots, indicating that the soil was less compacted, but we did not find a difference between the two treatments. This may have been due to pig traffic effects on bulk density in rooted areas which offset the disturbance effect of rooting. Cuevas et al. (2012) also found greater soil moisture in soils disturbed by wild pigs, but they observed that disturbed soils were less compacted than soil in undisturbed areas. The authors theorized that soil moisture may have been greater in rooted soils as rooting broke up the soil structure, thereby reducing capillarity and subsequent evaporation of moisture in the soil. Other studies have not observed differences in soil moisture in rooted areas (Moody and Jones 2000, Barrios-Garcia et al. 2014), or found that soil moisture was lower in areas disturbed by wild pigs (Risch et al. 2010, Bueno et al. 2013). Removal of vegetation and/or surface litter as a result of pig disturbance could expose the upper soil horizon to air and increase evaporation, leading to decreases in soil moisture. In alpine grasslands, bulk density was reported to have increased in rooted areas, which may have occurred as a result of vegetation loss and the structural support provided by roots (Bueno et al. 2013). However, similar to Cuevas et al. (2012), other studies have linked pig rooting to decreased bulk density in disturbed areas (Risch et al. 2010, Barrios-Garcia et al. 2014). Studies of other species have reported that bioturbation alters soil physical properties, changes nutrient content and microbial biomass, and influences soil production (Wilkinson et al. 2009, Platt et al. 2016). For example, moose (*Alces alces*) disturb benthic sediment when feeding on aquatic plants, which releases phosphorus and nitrogen into the water column (Bump et al. 2017). Impacts of wild pig rooting on soil moisture, compaction, and other physical properties likely vary as such parameters already differ greatly down to the microsite scale.

Plots did not significantly differ in NH_4-N, NO_3-N, or total N, although NO_3-N and total N were generally greater in rooted plots. Net N mineralization was significantly greater in the summer than in the fall. Nitrogen mineralization rates are influenced by soil moisture and temperature, with rates declining at the extreme ends of either range (Rosswall 1981). Soil moisture and temperature were greater in the summer than the fall, which suggests that environmental factors were the driving force behind overall higher rates of net N mineralization during the summer sampling event, or that the rooting activity in some of the rooted plots was sufficiently old enough that the initial, transient mineralization pulse already subsided. Other studies have also reported seasonal variations in N mineralization in riparian areas, with the greatest rates occurring during warm months (Jolley et al. 2010, Ricker and Lockaby 2014). On a treatment level, greater soil moisture at rooted plots potentially influenced the small increase in NO3-N and total N. We expected that N mineralization would be greater in rooted plots due to the mixing of organic material into the soil during rooting, but our small sample size likely precluded detection of difference in N transformation rates. It is possible there was a brief pulse in N mineralization immediately after rooting occurred, but that changes in N mineralization were brief. Nitrate-nitrogen is also rapidly lost in forests so elevated N may have leached, been taken up by plants, or rapidly immobilized between the time rooting occurred and the time we collected soil samples. While we attempted to sample rooted areas that had been disturbed no more than a week prior, it was sometimes difficult to determine the age of a rooted area. Similar studies have reported greater N mineralization in areas disturbed by wild pigs (Singer et al. 1984, Cuevas et al. 2012, Bueno et al. 2013), while others did not find a difference in soil N mineralization as a result of wild pig rooting (Groot Bruinderink and Hazebroek 1996, Moody and Jones 2000, Cushman et al. 2004, Barrios-Garcia et al. 2014). It is likely that the effects of rooting on N mineralization vary depending on climatic and ecosystem-specific factors, which has made it difficult to come to a definitive conclusion.

Microbial biomass of N and C varied by treatment and season, but rooted plots in the fall generally had the greatest MBN and MBC content. The increase in MBN and MBC during the October sampling event was likely a result of leaves senescing and becoming incorporated with the above-surface litter layer. Rooting by wild pigs potentially facilitated the mixing of dead leaves and other organic material into the upper soil horizons. The influx of organic material required a greater number of saprophytic bacteria for the decomposition process, which was represented by increased MBN and MBC. As MBN increases, the ratio of microbial C:N decreases due to more rapid decomposition of organic material. Lower microbial C:N is indicative of greater microbial activity and soil productivity (Li et al. 2016), which is generally inversely related with net N mineralization rates as microbes immobilize N to increase biomass (Fisher and Binkley 2000). This relationship was observed in our study with lower MBC and MBN in the summer when N mineralization rates were high. Siemann et al. (2009) reported lower C:N (measured as soil mineral content) in rooted areas and attributed the difference to mixing of litter and soil by wild pigs, which is what we theorize occurred in our study. Wild pig rooting has been observed to increase soil C and N concentrations and increase microbial biomass (Risch et al. 2010, Wirthner et al. 2012), while other studies did not observe changes in microbial activity spurred by rooting activity (Mohr et al. 2005, Taylor et al. 2011, Wirthner et al. 2011). Bueno et al. (2013) found that changes in soil C:N varied by plant community, with the lowest C:N and greatest soil organic matter content in rooted areas in the most productive plant community. Lastly, it is possible that wild pigs contributed organic nitrogen to the soil on a microsite scale in the form of fecal material and urine. Studies have shown that field application of bovine manure increases microbial biomass and soil N and C (Lovell and Jarvis 1996, Peacock et al. 2001), however these studies were conducted on a large scale and are not directly comparable to changes on a microscale level. The C and N content of domestic swine manure has been reported to be similar to that of bovine manure (Banwart and Bremner 1975), although there is currently no data on the nutrient content of wild pig feces. With a mean daily defecation rate of 3.8 – 4.3 dung/animal/day (Ferretti et al. 2015), high population densities of wild pigs could have the potential to influence soil microbial activity at a microsite level through the deposition of fecal waste material.

Bank Erosion and Accretion

We detected significant differences in sediment erosion and accretion between stream banks and slides, but monthly net change differed only by season. Mean erosion was greater at slides than banks overall and during the wet season. Seasonal effects are likely due to differences in precipitation and wild pig activity between the wet and dry season. Increased precipitation during the wet season could have weakened bank structure through subaerial processes, such as weathering, expansion, and contraction of soil due to fluctuations in soil moisture (Couper et al. 2002). Unstable banks are more susceptible to bank sloughing and scouring, which is the direct removal of sediment by flowing water, and bank collapse and slumping (mass failure). Accretion can occur when erosion processes affect the upper part of the bank and the displaced sediment is transported to the lower bank instead of being carried away by stream water. Trampling and sediment displacement by wild pigs moving up and down the bank face (slides) likely exacerbated natural erosion processes, especially if bank structure was already unstable.

Displaced sediment was either deposited on the lower part of the bank or transported into the stream channel where it mixed with streambed sediment or was carried downstream.

Active slides in the dry season had greater MNC and lower accretion than inactive slides, and a greater percentage of slides per watershed were active in the wet season compared to the dry season. Increased erosion due to seasonal effects may have made it difficult to detect a difference in sediment transport rates as a result of wild pig activity. Seasonal usage of slides by wild pigs likely varied in relation to the volume of water in the stream channel. Stream flow was greatly reduced during the dry season (May through October), so pigs were probably less likely to spend time (and subsequently utilize slides) in a particular watershed if there was little water available. The pattern of increased usage during the wet season is similar to results reported by Hancock et al. (2017), who also observed concentrated wild pig activity in a catchment during the wet season in Australia. The authors did not find evidence that wild pig disturbance increased erosion or influenced soil structure, and suggested that any changes may be slow and visible only over a longer period of time. Strauch et al. (2016) and Dunkell et al. (2011) studied wild pig disturbance on runoff and soil physiochemical parameters in Hawaiian wet forests, and came to the same conclusion, that changes in erosion processes likely take place over the long term. Neither study found an effect of wild pig disturbance on soil erosion nor total suspended solids (TSS) in runoff, which is a common method of measuring sediment transport. Concentration of TSS is influenced by environmental factors, such as sediment type and water flow, which may mask the true amount of erosion/accretion occurring in a watershed. At our watersheds, TSS concentrations in stream water were low (unpublished data) despite the volatility of the stream banks and the presence of wild pig disturbance. Low TSS concentrations were a function of the sandy bedloads that composed the local soil types as these sediment particles do not remain suspended in the water column for long. Our data suggest that sediment pins may be a viable method to measure changes in bank stability from large animal usage of watersheds. While previous studies did not find impacts of wild pig disturbance on erosion processes, other studies have reported increased erosion as a result of cattle grazing alongside streambanks (Kauffman et al. 1983, Trimble 1994). To our knowledge, this study is the first to examine fluctuations in bank sediment transport (erosion/accretion) as a result of large wild animals utilizing a stream bank and riparian interface, as well as providing evidence that wild pigs impact erosion rates in headwater riparian areas.

Conclusion

This study suggests that rooting by wild pigs may impact nutrient cycling in riparian areas by stimulating an increase in soil microbial biomass and influencing nitrogen mineralization rates. Wild pigs may also negatively affect stream bank stability by increasing bank erosion through trampling and disturbance of sediment, although impacts may vary by season. Further research on the interactions of wild pigs and environmental factors is necessary to fully comprehend the extent of their impacts in forested watersheds, but our results suggest that preventing wild pig encroachment into riparian areas may be important to ensure that ecosystem essential services, such as the cycling and retention of nutrients and sediment, are not negatively impacted.

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Table 1.1. Summary of soil samples collected at POTL in Bullock County, AL and TUSK in Macon County, AL in 2018 by month and treatment.

	UNROOTED				ROOTED					REFERENCE				
	Mean	**SE**	**Median**	**Range**	**Mean**	**SE**	**Median**	**Range**	**P**	**Mean**	**SE**	**Median**	**Range**	**P**
Bulk density (g/cm^3)														
June	1.15	0.04	1.19	0.82 - 1.39	1.14	0.04	1.13	0.70 - 1.42	0.83	-	-	-	-	-
October	1.18	0.03	1.21	0.91 - 1.41	1.14	0.03	1.12	0.83 - 1.36	0.39	0.84	0.09	0.79	0.42 – 1.53	**< 0.01**
Overall	1.17	0.02	1.2	0.82 - 1.41	1.14	0.03	1.13	.7 - 1.42	0.45	-	-	-	-	-
Soil moisture (%) *														
June	21.81	1.43	20.78	12.11 - 32.45	24.73	2.23	22.55	13.51 - 42.19	0.11	-	-	-	-	-
October	15.23	1.49	13.06	6.84 - 33.69	17.28	2.05	13.83	7.53 - 37.17	0.39	28.06	3.48	26.63	15.74 - 58.23	**0.02**
Overall	18.52	1.15	16.68	6.84 - 33.69	21.01	1.61	16.28	7.53 - 42.19	**0.03**	-	-	-	-	-
Temperature (°C) *														
June	24.64	0.15	24.65	24.16 - 25.02	24.53	0.12	24.62	23.6 - 24.98	0.83	-	-	-	-	-
October	13.62	0.15	13.65	13.08 - 14.01	13.91	0.17	13.77	13.5 - 14.6	0.31	14	0.22	13.79	13.76 - 14.44	0.48
Overall	18.63	1.74	14.01	13.08 - 25.02	20.55	1.33	24.39	13.5 - 24.98	0.29	-	-	-	-	-
NH_4-N (g m$^{2 -1}$ day^{-1})														
June	0.011	0.004	0.005	-0.005 - 0.049	0.006	0.005	-0.003	-0.006 - 0.049	0.43	-	-	-	-	-
October	0.004	0.002	0	0 - 0.020	0.001	0.001	0	-0.004 - 0.011	0.20	0.006	0.005	0	-0.006 - 0.062	0.70
Overall	0.008	0.002	0	-0.005 - 0.049	0.004	0.003	0	-0.006 - 0.049	0.16	-	-	-	-	-
NO_3-N * (g m$^{2 -1}$ day^{-1})														
June	0.027	0.004	0.022	0.01 - 0.06	0.041	0.008	0.033	0.007 - 0.12	0.21	-	-	-	-	-
October	0.007	0.003	0	0 - 0.032	0.009	0.003	0.005	0 - 0.036	0.61	0.015	0.004	0.016	-0.003 - 0.043	0.46
Overall	0.018	0.003	0.016	0 - 0.06	0.027	0.006	0.012	0 - 0.12	0.16	-	-	-	-	-
Total N * (g m$^{2 -1}$ day^{-1})														
June	0.038	0.006	0.038	0.005 - 0.091	0.047	0.008	0.046	0.002 - 0.115	0.38	-	-	-	-	-
October	0.011	0.005	0	0 - 0.052	0.01	0.003	0.005	0 - 0.038	0.92	0.021	0.009	0.015	-0.008 - 0.106	0.57
Overall	0.026	0.005	0.025	0 - 0.091	0.03	0.006	0.021	0 - 0.115	0.39	-	-	-	-	-
N biomass (µg g^{-1} soil)														
June	25.28	3.12	20.56	11.51 - 58.55	27.9	2.58	26.15	11.14 - 50.72	0.33	-	-	-	-	-
October	34.48	7.43	23.65	12.15 - 139.52	41.51	8.83	28.71	12.63 - 183.82	0.26	45.2	6.86	46.505	6.82 - 86.99	0.16
Overall	29.88	4.04	22.06	11.51 - 139.52	34.71	4.67	26.62	11.14 - 183.82	0.15	-	-	-	-	-
C biomass * (µg g^{-1} soil)														
June	280.36	31.36	278.65	66.15 - 650.96	270.64	28.41	232.88	43.29 - 495.54	0.70	-	-	-	-	-
October	417.41	69.28	312.71	128.51 - 1397.31	454.31	54.86	421.15	155.26 - 995.26	0.65	358.04	40.8	363.78	101.85 - 623.05	0.49
Overall	348.88	39.1	290.71	66.15 - 1397.31	362.48	33.85	316.35	43.29 - 995.26	0.89	-	-	-	-	-
C:N *														
June	11.3	1.12	11.54	5.43 - 26.03	9.34	0.79	9.64	3.67 - 16.03	**0.04**	-	-	-	-	-
October	13.28	1.01	11.79	8.63 - 25.17	12.39	0.89	11.63	4.67 - 18.34	0.57	8.98	0.75	7.86	6.28 - 14.93	**< 0.01**
Overall	12.29	0.76	11.79	5.34 - 26.03	10.87	0.63	10.1	3.67 - 18.34	0.06	-	-	-	-	-

Table 1.2. Summary of erosion, accretion, and monthly net change values at POTL in Bullock County, AL from July 2018 through September 2019 by season and pin position, with negative values indicating soil loss.

	BANK				**SLIDE**				
	Mean	**SE**	**Median**	**Range**	**Mean**	**SE**	**Median**	**Range**	**P**
Erosion *									
Dry	-1.55	0.13	0	-31.2 - 0	-1.93	0.15	0	-34.2 - 0	0.11
Wet	-0.9	0.14	0	-32.4 - 0	-1.56	0.17	-0.1	-31.0 - 0	< **0.01**
Overall	-1.35	0.1	0	-32.4 - 0	-1.81	0.11	-0.05	-34.2 - 0	< **0.01**
Accretion									
Dry	1.33	0.12	0	0 - 37.4	1.24	0.11	0	0 - 30.1	0.07
Wet	1.38	0.13	0	0 - 17.0	1.1	0.15	0	0 - 30.7	0.17
Overall	1.34	0.09	0	0 - 37.4	1.19	0.09	0	0 - 30.7	**0.03**
MNC *									
Dry	-0.09	0.08	0	-25.1 - 21.0	-0.25	0.09	0	-23.5 - 20.4	0.34
Wet	0.06	0.15	0	-34.6 - 10.0	0.36	0.24	0.1	-61.3 - 58.4	0.76
Overall	-0.05	0.07	0	-34.6 - 21.0	-0.05	0.1	0	-61.3 - 58.4	0.45

* Seasonal difference ($p < 0.05$)

Table 1.3. Summary of erosion, accretion, and monthly net change values at slides at POTL in Bullock County, AL from July 2018 through September 2019 by season and wild pig activity, with negative values indicating soil loss.

	INACTIVE				ACTIVE				
	Mean	**SE**	**Median**	**Range**	**Mean**	**SE**	**Median**	**Range**	**P**
Erosion									
Dry	-1.98	0.18	0	-34.2 - 0	-1.79	0.23	-0.6	-26.0 - 0	0.81
Wet	-1.89	0.33	-0.2	-31.0 - 0	-1.32	0.17	0	-19.0 - 0	0.06
Overall	-1.96	0.16	0	-34.2 - 0	-1.54	0.14	-0.3	-26.0 - 0	0.40
Accretion									
Dry	1.35	0.14	0	0 - 30.1	0.9	0.19	0	0 - 24.8	**0.02**
Wet	1.12	0.26	0	0 - 26.0	1.09	0.19	0	0 - 30.7	0.95
Overall	1.3	0.12	0	0 - 30.1	1	0.13	0	0 - 30.7	0.15
MNC *									
Dry	-0.11	0.09	0	-21.9 - 11.1	-0.65	0.24	-0.2	-23.5 - 20.4	< **0.01**
Wet	0.09	0.4	0	-61.3 - 14.9	0.56	0.29	0.1	-15.2 - 58.4	0.48
Overall	-0.07	0.11	0	-61.3 - 14.9	-0.02	0.19	0	-23.5 - 58.4	0.72

*Seasonal difference (p < 0.01)

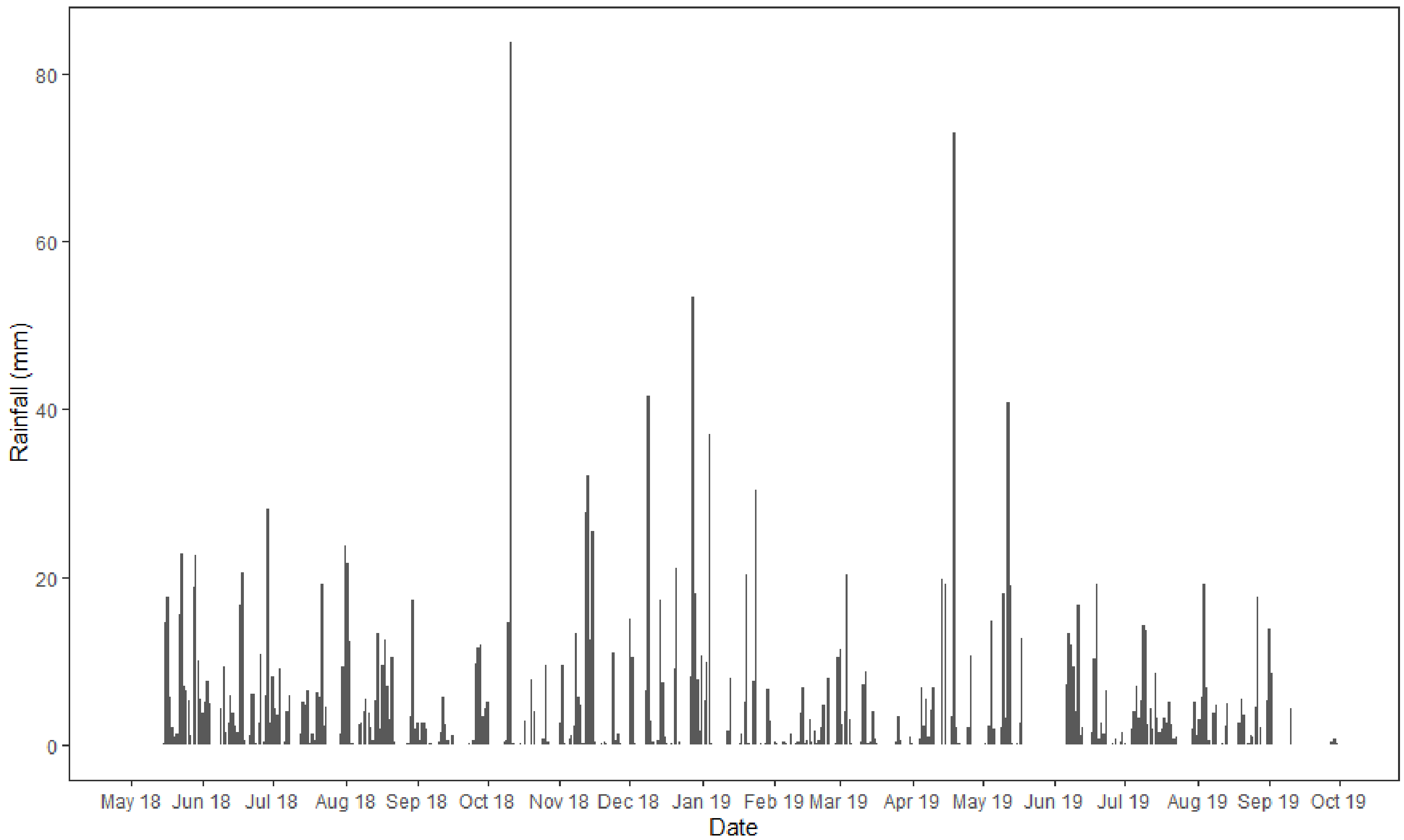

Figure 1.1. Mean rainfall (mm) at POTL in Bullock County, AL and TUSK in Macon County, AL from June 2018 through September 2019.

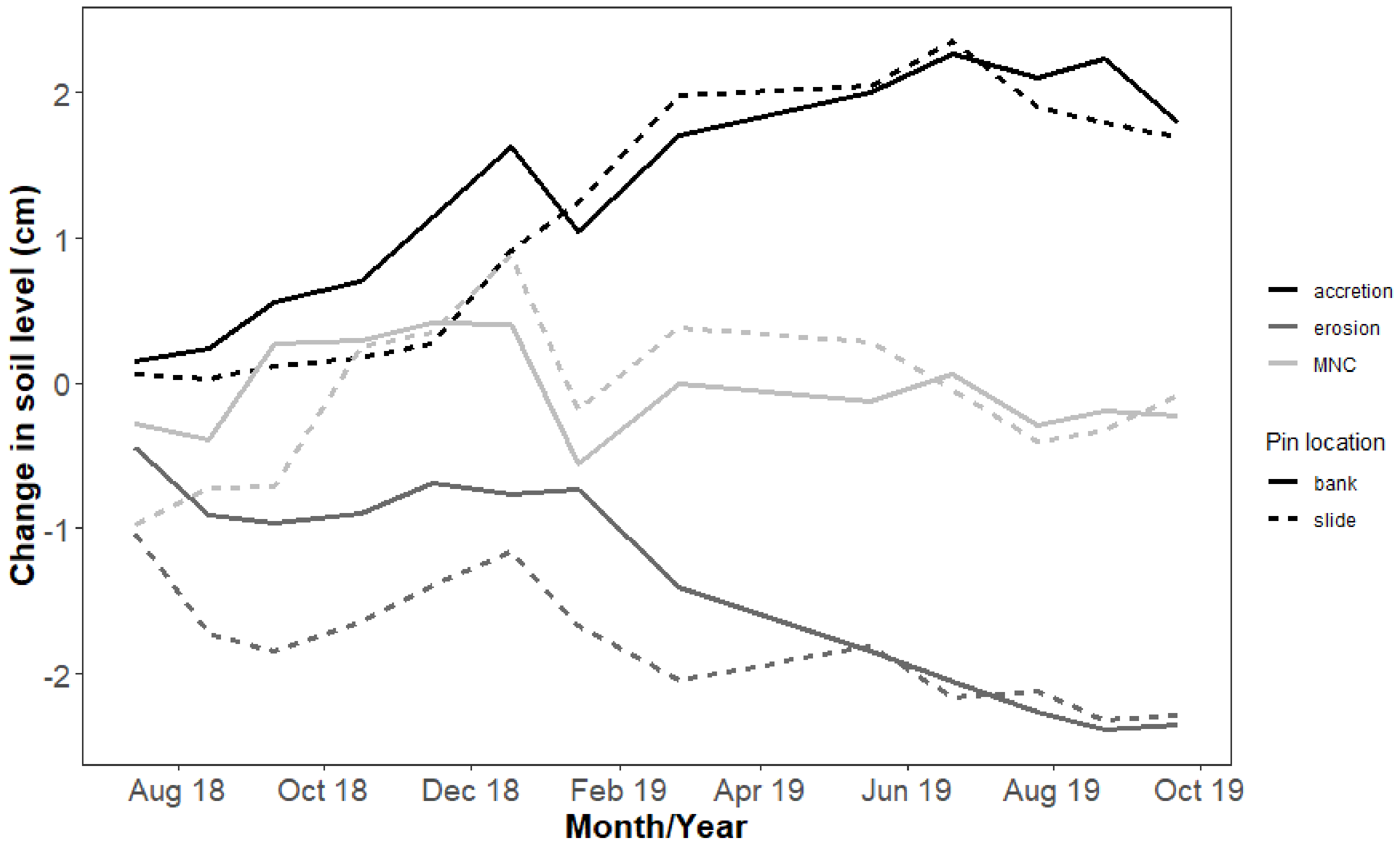

Figure 1.2. M Mean erosion, accretion, and monthly net change at watersheds at POTL in Bullock County, AL from June 2018 through September 2019 season by and pin position.

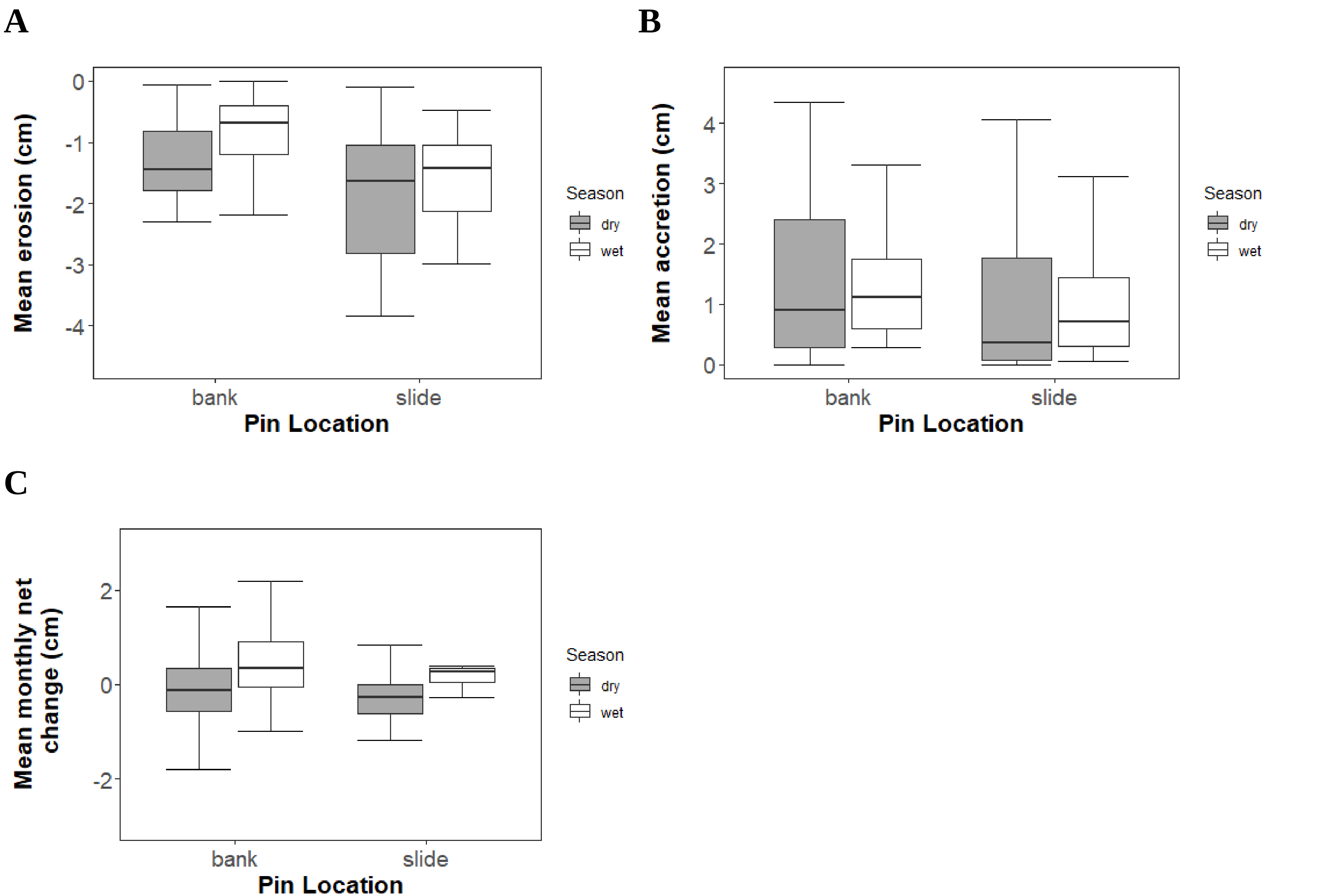

Figure 1.3. A) Mean erosion, B) mean accretion, and C) mean monthly net change at watersheds at POTL in Bullock County, AL from June 2018 through September 2019 by season and pin position.

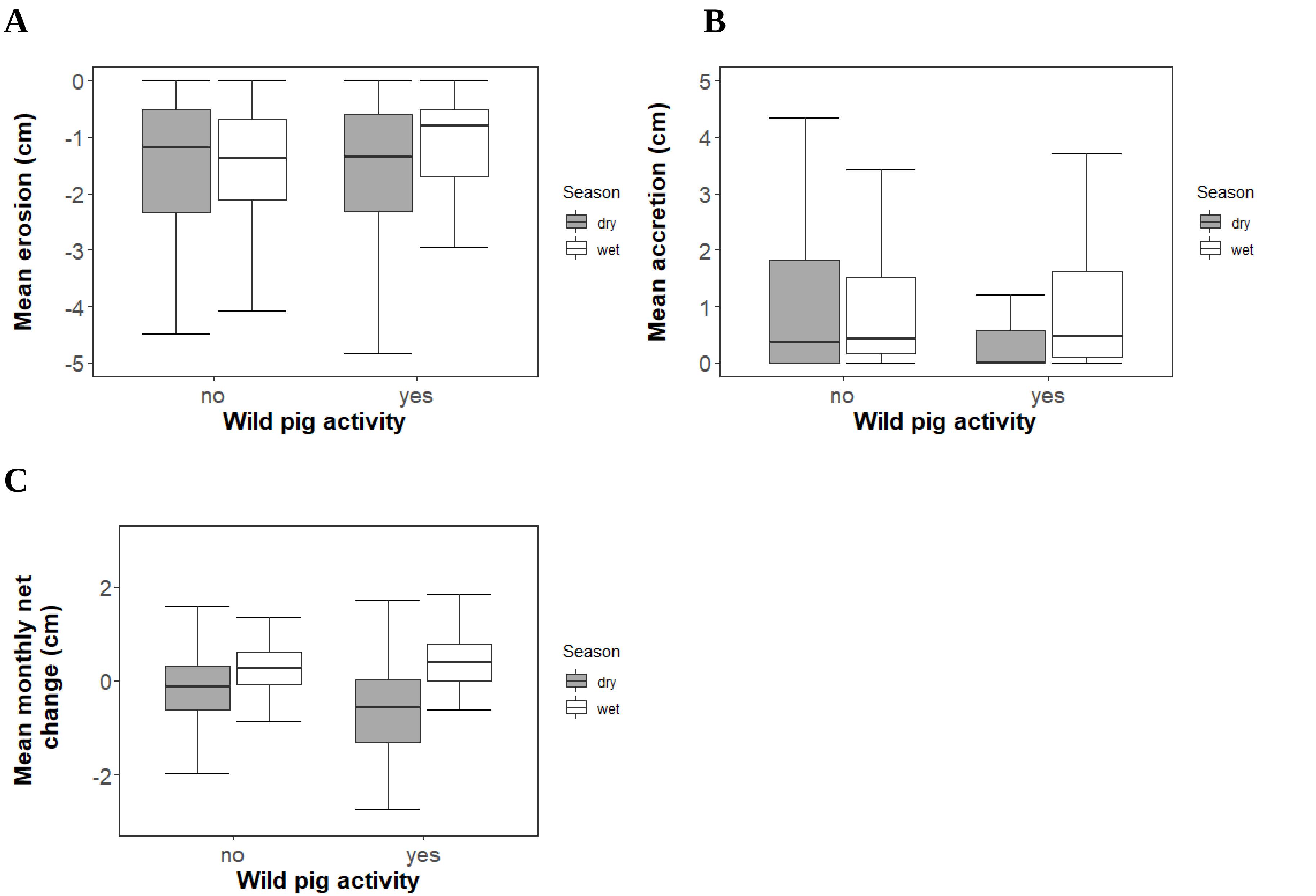

Figure 1.4. A) Mean erosion, B) mean accretion, and C) mean monthly net change at watersheds at POTL in Bullock County, AL from June 2018 through September 2019 by wild pig activity and season.

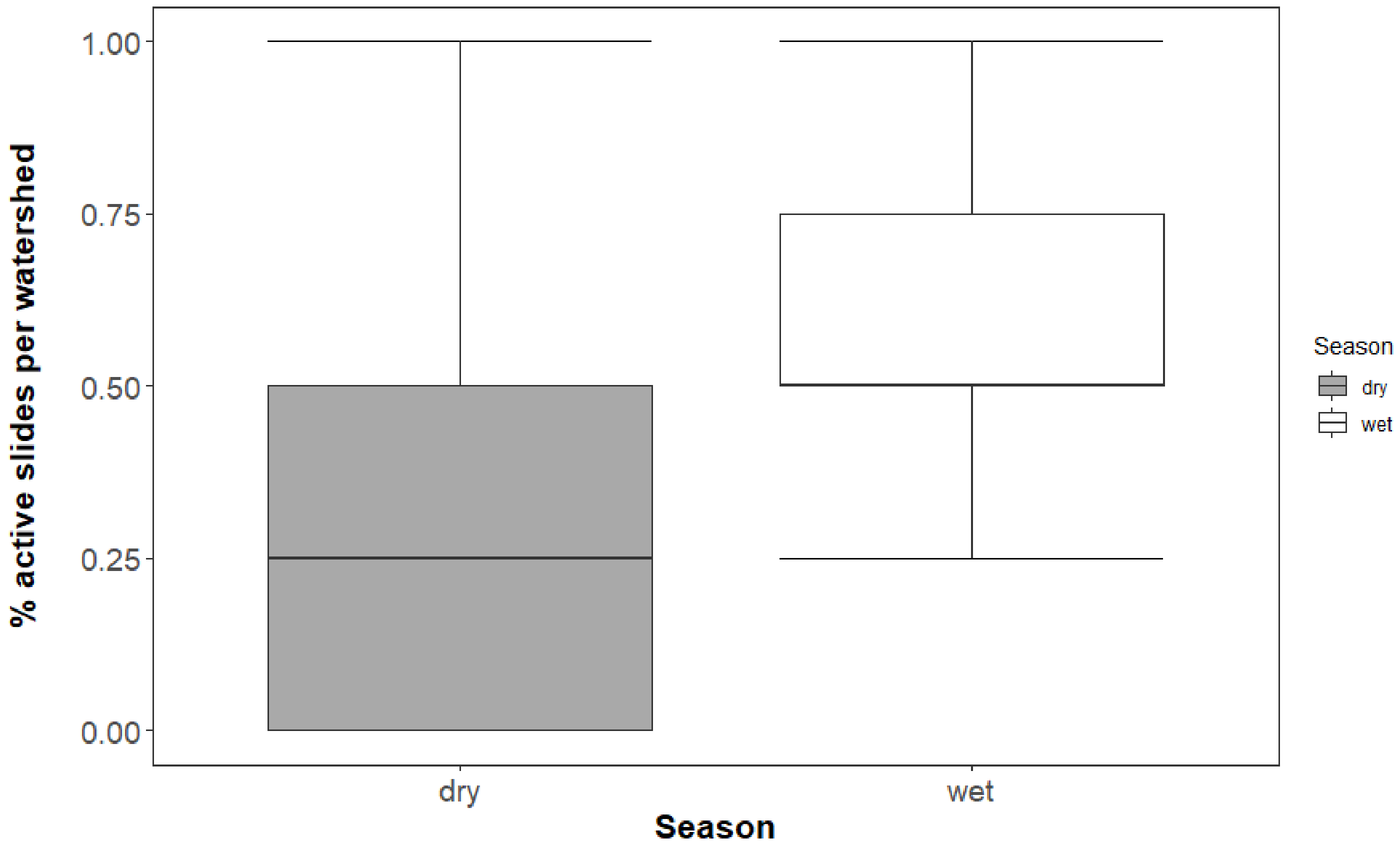

Figure 1.5. Percent active slides per watershed at POTL in Bullock County, AL from June 2018 through September 2019 by season.

Chapter 2: Impacts of wild pigs on water quality and fecal bacteria in headwater riparian systems

INTRODUCTION

As global development continues to bring urban and rural landscapes closer together, watershed health and security is increasingly threatened by land use changes and environmental conditions. Urban development, agricultural activities, and degradation and invasion of natural landscapes all influence local watershed health and can impact the quantity and quality of water available (Baldwin and Batzer 2012). Riparian and wetland forest ecosystems play a vital role as the “kidneys” of a watershed as they filter pollutants and sediment from the aquatic system through biological, chemical, and physical means (Jolley et al. 2010). Removal of dissolved chemicals and sediment improves water quality and nutrient uptake ensures adequate nutrient cycling through the terrestrial-aquatic interface. Riparian areas provide important ecological services, such as habitat and resources for plant and animal communities and surface water storage. Additionally, they support commercial industries, such as agriculture and livestock production, and recreational hunting and fishing sports.

There are many causes of degraded water quality in local watersheds, including improper sanitation management, storm water run-off from urban and residential areas, poor land-use practices, and agricultural runoff; however, the presence of livestock and wildlife in a watershed can reduce water quality as well. Livestock can contaminate stream water through direct contact or from runoff contaminated with feces and urine (Line et al. 2000, Davies-Colley et al. 2004), and subsequent use of contaminated water has been linked to disease outbreaks in humans (Ackers et al. 1998, Jay et al. 2007, Lindqvist et al. 2008). Other water quality indicators such as sediment particles, suspended solids, nutrients, and dissolved oxygen can be influenced by livestock presence in watersheds (Line et al. 2000, Line 2003, Ranganath et al. 2009). Wildlife species have also been documented as a source of waterborne fecal bacteria pollution. *Escherichia coli* and enterococci from gull (*Larus* sp.) feces has been detected at beaches in the Great Lakes (Fogarty et al. 2003), and *E. coli* from white-tailed deer (*Odocoileus virginianus*) and Canada geese (*Branta canadensis*) has been found in watersheds of the Finger lakes (Somarelli et al. 2007).

Direct impacts by wildlife on other water quality parameters are less documented, but indirect impacts of wildlife feeding or nesting behaviors can be substantial. Grazing by nonmigrating Canada geese can reduce the abundance of wetland vegetation, thereby limiting the ability of the watershed to filter and retain sediment and nutrients (Baldwin and Pendleton 2003). Fiddler crabs (*Uca pugnax*) oxygenate soils via burrowing and excrete waste which transports nutrients to subsurface plant roots (Montague 1982), while mussels (*Geukensia demissa*) deposit nutrient-rich feces and bind sediment particles on the surface (Kraeuter 1976). Filter-feeding by oysters (*Crassostrea virginica*) reduces suspended solids, nutrient loads, and seston biomass in the water column which improves water quality in estuaries (Grabowski and Peterson 2007). As ecosystem engineers, beavers (*Castor canadensis*) alter the vegetative structure, biogeochemistry, geomorphology, and hydrology of wetlands by felling trees and building dams (National Research Council 2002, Johnston 2012). For example, beaver impoundments can elevate the water table by controlling stormflow (Wigley and Lancia 1998), reduce water velocity and streambank erosion (Maret et al. 1987), increase ammonium concentrations in sediment (Naiman et al. 1994), and increase aquatic microbial activity (Songsteralpin and Klotz 1995). Beaver activity can result in dramatic changes to the ecosystem, but there are many benefits to their presence.

However, another mammalian vertebrate considered an ecosystem engineer is quickly gaining a reputation as a threat to wetland and riparian ecosystems: the wild pig (*Sus scrofa*). Invasive to North America, wild pigs frequently invade wetlands and riparian forests, and are densely populated in the southeastern United States (Mayer and Brisbin 2009, Lewis et al. 2019). Wild pigs dig and overturn soil in search of food, and create wallows for thermoregulation and ecto-parasite removal (Howe and Bratton 1976, Bracke 2011). This behavior is known as rooting, and can have serious consequences on the physical structure of ecosystems with increased erosion, destruction of vegetative communities, and introduction of pathogens. Therefore, the impacts of wild pigs are of great concern as riparian ecosystems provide essential ecological services and are sensitive to disturbance (National Research Council 2002, King et al. 2012).

Few studies have examined the impacts of wild pig disturbance on water quality and fecal bacteria in riparian areas, and those that have either did not find significant impacts or their results conflicted with other literature (Beasley et al. 2018). These inconsistencies have generally been due to differences in experimental design, environmental conditions and habitat type, various densities of wild pigs, and parameters selected for measurement. Doupé et al. (2010) conducted a study in northeastern Australia during the dry season with small ephemeral lagoons and concluded that the ability of wild pigs to access the lagoon affected certain water quality parameters. Lagoons with wild pigs had lower pH, greater turbidity, and lower dissolved oxygen concentrations than lagoons that were not accessible by wild pigs but nutrients and aquatic communities did not differ between treatments. A study in Louisiana, USA examined water quality in a single watershed and reported greater fecal coliforms and the presence of pathogenic bacteria at locations with evidence of wild pig activity (Kaller and Kelso 2003). However, in contrast to Doupé et al. (2010), they did not observe differences in dissolved oxygen and stream habitat due to wild pig disturbance. Singer et al. (1984) observed greater nitrate concentrations in stream water from a rooted hardwood stand in Great Smoky Mountains National Park compared to stream water from an unrooted stand, but did not find a difference in suspended solids. Two studies conducted in tropical forests in Hawaii analyzed runoff from fenced and unfenced plots with wild pig activity, and neither found differences in concentration of suspended solids or fecal bacteria due to fencing treatment (Dunkell et al. 2011*b*, *a*, Strauch et al. 2016). Brooks et al. (2020) compared runoff from a paddock containing wild pigs to a nearby stream, but runoff samples did not significantly differ from stream samples in concentrations of nitrate and nitrite, ammonium, fecal bacteria, or pathogenic bacteria. Wild pigs have been found to carry waterborne pathogens such as *Giardia* and *Cryptosporidium* (Atwill et al. 1997, Hampton et al. 2006) but to our knowledge, no study to date has been able to link waterborne pathogens in the environment to the presence of wild pigs.

The substantial variability in reported results, experimental designs, and environmental conditions and habitat types of previous studies examining impacts of wild pigs on water quality has created considerable confusion. As a result, our goal was to examine the impacts of wild pigs on water quality in headwater riparian systems using an experimental design that employed the conditions we felt must be met for a thorough assessment of wild pig impacts on water quality. These include (1) wild pigs must be present in and have access to the habitat/area being studied, (2) the pigs must be free to exhibit natural behavior, (3) the receiving body of water should represent natural flow, and (4) the water sampling technique must be sufficiently rigorous to detect subtle changes against a backdrop of high variability. Studies that employ these guidelines would have greater capability of assessing the impacts of wild pigs on water quality, and this information would be better suited for guiding wild pig management and control initiatives in riparian areas, and safeguarding water quality in local watersheds. As a result, our specific research objectives were to:

1. Identify multiple small watersheds with free-roaming wild pigs to observe changes in water quality parameters in a natural setting with minimal background variability.
2. Determine the impacts on water quality by measuring nutrient concentrations and physiochemical parameters in forested headwater stream systems.
3. Determine the impacts on fecal bacteria concentrations by analyzing stream water for the presence of swine fecal bacteria, and quantifying *E. coli* and fecal coliform concentrations.

METHODS

Study Area

This research was conducted at a privately-owned tract of land (hereafter referred to as POTL), which served as the treatment area, and Tuskegee National Forest (TUSK), which was the reference area. POTL was a 4515-ha property located at 85°32'0.932"W 32°11'39.019"N in Bullock County, Alabama, USA. Wildlife management practices focused on maintaining healthy white-tailed deer (*Odocoileus virginianus*) and eastern wild turkey (*Meleagris gallopavo silvestris*) populations. The most common habitat types were mixed pine (*Pinus* spp.)-hardwood forest and riparian hardwoods. The canopy was primarily composed of sweetgum (*Liquidambar styraciflua*), loblolly pine (*Pinus taeda*), and southern shagbark hickory (*Carya carolinae-septentrionalis*), and the understory was mainly herbaceous and semi-woody species such as blackberry (*Rubus* spp.), American beautyberry (*Callicarpa americana*), and eastern baccharis

(*Baccharis halimifolia*). Wild pigs were present throughout the property and camera surveys estimated the density to be 15.5 pigs/km^2, which is much greater than the average density of 6-8 pigs/km^2 in the southeastern U.S (Lewis et al. 2019).

The study area at TUSK was located approximately 25 km from the treatment area, and was closely aligned with POTL in terms of stream gradients, forest cover and habitat type, and stream size. While wild pigs were present in some areas of TUSK, they were not yet established in the area selected for the study. This was confirmed with camera surveys conducted in March 2018. Trail cameras were placed 1-km^2 apart in a grid pattern at the sampling sites and baited with corn. After 1 week, remaining corn was collected and cameras removed. Analysis of camera data did not show any signs of wild pig activity, nor was any sign (tracks, wallows, etc.) seen during field work. Ideally, the reference area would have been closer to the treatment area and be completely absent of wild pigs, however TUSK was the closest location to POTL that was known to be devoid of wild pigs and was still very similar to POTL.

Sampling sites (watersheds) at POTL and TUSK were restricted to certain criteria: low gradient, occupied by deciduous wetland forests, and streams 3rd order or lower in magnitude. Both sites were located in the Upper Coastal Plain physiographic region and in the Mantachie-luka-Bibb soil association. A total of 11 sites at POTL and 3 sites at TUSK were selected for sampling. The main tributaries were perennial, whereas most of the lower order streams were intermittent with flow only in winter and spring. At POTL, damage as a result of pig activity (rooting, digging, and wallowing) was observed at all sampling sites. This activity was observed on the floodplains and within the stream channels, even when the channels were dry.

Collection and Analysis of Water Samples

Sampling began in July 2018 at POTL and December 2018 at TUSK and continued through June 2019. Water samples were collected from each site (n = 14) every 2 weeks throughout the year as long as flow was present. Sampling at POTL and TUSK occurred within 24 hours of each other. Wide-mouth collection bottles were rinsed in stream water before collection of a 500-mL grab sample. All samples were collected at the outlet point where the main stream of the watershed (1^{st}-3^{rd} order) flowed into the connecting tributary thereby capturing the cumulative effect of wild pigs within the small watersheds.

Discharge was measured at the outlet point using the USGS mechanical current-meter method (Turnipseed and Sauer 2010). The outlet was divided into vertical subsections and the area of each subsection obtained by measuring width and depth. A FH950 Portable Velocity Meter (Hach Company, Loveland, CO) was used to measure the velocity of the water, which was then multiplied by the area of each subsection to determine discharge. Total discharge was calculated by summing the discharges of each subsection. Additionally, dissolved oxygen (DO), specific conductivity, temperatures, and pH were measured using a YSI Pro20 Dissolved Oxygen Instrument and a YSI Pro1030 Water Quality Meter (Xylem Inc., Yellow Springs, OH).

Water samples were kept on ice and transported to the Auburn University Biogeochemistry Laboratory where they were stored at 4° C. A Dionex ICS-1500 ion chromatography system (Thermo Fisher Scientific, Waltham, MA) was used to analyze anions (Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) and cations (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}). Dissolved organic carbon (DOC) and total nitrogen (TN) were analyzed with a Shimadzu TOC-VCPN combustion analyzer (Shimadzu Scientific Instruments, Columbia, MD). Concentrations of total suspended solids (TSS) were determined using filtration methods in accordance with EPA guidelines (USEPA 1999).

Analysis of Fecal Bacteria

Water samples for *Escherichia coli* analysis were taken from the 500-mL grab samples immediately after collection. Three 1-mL sub-samples were pipetted from each grab sample into three vials containing ColiScan Easygel. Upon return to the lab, the contents of each vial were transferred to petri dishes (n = 3), swirled, and left to sit undisturbed until the media gelled. The plates were then placed in an incubation chamber upside down to prevent condensation forming on the lid, and left to incubate at 29-37° C for 30 hours. After incubation, individual *E. coli* and fecal coliform (FC) colonies were counted using a microscope, pen, and click-counter. *E. coli* and FC concentrations were calculated by taking the mean of the three colony-forming unit (cfu) counts for each water sample and multiplying by 100-mL.

Additionally, water samples were sent to a private laboratory (Source Molecular, Miami Lakes, FL) to test for the presence of swine fecal bacteroidetes via microbial source tracking. During five sampling events (June 2018, July 2018, December 2018, April 2019, and August 2019) an additional 500-mL grab sample was collected at each sampling site and kept on ice. Within 24 hours, the samples were packed in a cooler and shipped to Source Molecular for analysis. Upon arrival, each water sample was filtered through a 0.45-micron membrane filter which was then placed in a 2-mL tube containing beads and a lysis buffer. The sample was homogenized for 1 minute and DNA extracted using a Generite DNA-EZ ST1 extraction kit (GeneRite, NJ). Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20-µL sample extract, forward primer, reverse primer, probe, and an optimized buffer. All assays were run in duplicate and quantification was achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers. A positive and negative control were run alongside the samples to identify any false negatives or positives (Source Molecular, personal communication, August 29, 2019).

Statistical Analysis

Statistical analyses were conducted using R statistical platform version 3.5.3 (R Core Team 2019). Homoscedasticity and normality of the residuals were assessed visually with diagnostic graphs and statistically using Shapiro-Wilk and Levene's tests. Data that did not meet assumptions of normality were natural log transformed, with a constant of 1 added to variables that contained one or more data points with a value of 0.

Linear mixed effects (LME) analysis was used to account for temporal autocorrelation and for the confounding relationship between treatment and study area. Models were developed using the lme4 (Bates et al. 2015)and lmerTest (Kuznetsova et al. 2017) packages to assess the importance of several variables in explaining water quality and fecal bacteria concentrations at the two study areas. A complete model was built with the fixed effects of Treatment (wild pig or reference), Season (wet or dry), and Discharge, as well as interaction terms for Treatment*Season and Season*Discharge. Seasons were delineated by flow, which was greatest from November through April (wet season) and diminished from May through October (dry season). Discharge was included as a fixed effect to account for changes in nutrient and fecal bacteria concentrations due to fluctuations in stream flow. A random effect of stream was included to account for inherent differences between watersheds and sampling areas.

Additionally, a random effect of time (day nested within month) was added to account for temporal autocorrelation as a result of repeated sampling (Chaves 2010).

A step-down model-building approach was used via the step function in the lmerTest package to eliminate non-significant ($\alpha > 0.05$) fixed effects and interaction terms, resulting in a final model for each water quality and fecal bacteria variable. Non-significant fixed effects were left in the model if interaction terms containing the effects were significant. Akaike's Information Criterion corrected for small sample size (AIC_c) was used to further evaluate models and confirm that the final model best fit the data.

RESULTS

Fourteen streams were sampled from May 2018 to June 2019 during 16 sampling events that occurred during flow periods at bimonthly intervals. Each stream was sampled at least four times. Flow ceased at the end of July 2018 due to low rainfall and flowing water did not return until December 2018. A drought from mid-May 2019 to mid-October 2019 (Figure 2.1) meant that the majority of streams were dry or only contained stagnant pools of water, and therefore were unable to be sampled. Streams were sampled only once in both March and April 2019 as access to the study sites was limited.

Model Selection

The fixed effect of Treatment was included in LME models for sulfate, sodium, calcium, TN, DOC, and specific conductivity (Table 2.1). An interaction term for Treatment*Season was included in models for magnesium, chloride, and nitrite. The effect of Treatment was not included in the models for ammonium, nitrate, potassium, TSS, temperature, or dissolved oxygen concentrations. None of the fixed effects or interaction terms were significant for pH or phosphate, and therefore were excluded from the models. The effect of Treatment was included in the models for *E. coli* and fecal coliforms, although it was significant only for *E. coli*. An interaction term for Treatment*Season was included in the FC model.

Water Quality

Concentrations of TN and DOC were greater at treatment watersheds than at reference watersheds ($p \leq 0.01$; Figure 2.2). DOC concentration in treatment watersheds was 10.62 mg/L (6.29, 14.95; 95% CL) greater than in reference watersheds ($p < 0.01$). Median DOC concentration was 17.83 mg/L in treatment watersheds and 4.51 mg/L in reference watersheds (Table 2.2). Treatment watersheds had 2.35 times (1.38, 4.00; 95% CL) the TN concentration of reference watersheds ($p = 0.01$), with median concentrations of 0.4 mg/L and 0.12 mg/L, respectively. Nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4^+) did not significantly differ between treatments ($p > 0.05$), although NO_3^- concentration in the dry season was 0.1 times (0.04, 0.16; 95% CL) concentration (mg/L) in the wet season ($p < 0.01$).

Specific conductivity was affected by treatment and discharge. At treatment watersheds, specific conductivity was 3.35 times (2.10, 5.33; 95% CL) the specific conductance (µS/cm) at reference watersheds ($p < 0.01$). Concentrations of sulfate (SO_4^{2-}) and sodium (Na^+) were affected by treatment, season, and discharge, while calcium (Ca^{2+}) was affected by treatment and discharge. Treatment watersheds had 10.25 times (5.67, 18.5; 95% CL) the SO_4^{2-} concentration (mg/L) in reference watersheds ($p < 0.01$), and 2.44 times (1.54, 3.89; 95% CL) the Na^+ concentration (mg/L) ($p < 0.01$). Calcium concentration in treatment watersheds was 4.84 times (2.5, 8.76; 95% CL) the concentration (mg/L) in reference watersheds ($p < 0.01$).

Dissolved oxygen differed by season and discharge ($p < 0.01$), but not by treatment. At treatment watersheds, median DO concentration was 6.29 mg/L in the dry season and 9.21 mg/L in the wet season (Figure 2.3). In comparison, median DO concentration was 8.05 mg/L in the dry season and 9.58 mg/L in the wet season at reference watersheds. For every 1% increase in discharge (L/s), DO increased by $4.74e^{-3}$ mg/L ($2.78e^{-3}$, $6.69e^{-3}$; 95% CL) ($p < 0.01$). Concentration of TSS was affected by discharge, but not by treatment or season. For every 1% increase in discharge (L/s), TSS concentration decreased by 0.11% (-0.21, -0.01; 95% CL) ($p = 0.03$).

Individual watersheds for both treatments differed in concentrations of Na^{+}, SO_4^{2-}, TN, Ca^{2+}, and DOC (Table 2.3). Reference watersheds significantly differed in specific conductivity ($p < 0.01$), but treatment watersheds did not ($p = 0.76$).

Fecal Bacteria

Treatment watersheds had 40.4 times (10.06, 153.95; 95% CL) the *E. coli* concentrations (cfu/100 mL) of reference watersheds ($p < 0.01$). Concentrations ranged from 0 – 70,767 cfu/100 mL among treatment watersheds and 0 – 967 cfu/100 mL at reference watersheds (Table 2.4).*E. coli* concentrations varied by treatment and idividual watershed (Figure 2.5). Median FC concentration was 8,067 cfu/100 mL at treatment watersheds compared to 3,000 cfu/100 mL at reference watersheds, but differences in concentration were not statistically significant ($p = 0.15$). Fecal coliform concentrations ranged from 1133 – 388,767 cfu/100 mL among treatment watersheds and 500 – 27,433 cfu/100 mL at reference watersheds, and were similar among individual watersheds (Figure 2.6). Analysis of Variance models showed that *E. coli* concentrations significantly differed between treatment watersheds, but FC concentrations did not ($p = 0.56$) (Table 2.6). Reference watersheds did not differ in either *E. coli* or FC concentrations ($p > 0.05$).

A total of 38 samples were sent for DNA analysis of swine fecal biomarkers from five different sampling events (Table 2.7). Overall, DNA from swine fecal bacteroidetes was detected in 23 of 33 (69.7%) samples from treatment watersheds and 0 of 5 (0.0%) samples from reference watersheds. Biomarker concentrations were quantified in 16 of 23 (69.6%) samples, while the remaining 7 samples had concentrations below the limit of quantification. Quantified concentrations ranged from $3.61 \times 10^2 – 1.92 \times 10^4$ copies/100 mL, with an overall mean of 4.07×10^3 copies/100 mL. While the biomarkers of interest were not detected in all watersheds each time samples were analyzed, each treatment watershed was positive for swine fecal bacteroidetes at least once during the five sampling events.

DISCUSSION

Water Quality

Nutrient concentrations and physiochemical parameters ranged widely and varied by treatment and watershed. Dissolved organic carbon and total nitrogen (organic and inorganic) were elevated at the treatment watershed compared to the reference watershed, however, concentrations of inorganic nitrogen such as NO_2^-, NO_3^-, and NH_4^+ were not elevated. Organic nitrogen content was likely elevated, which in turn increased TN concentrations. Inorganic nitrogen levels may not have been elevated due to low dissolved oxygen levels in the dry season. The transformation of NH_4^+ to NO_2^- is the rate limiting step of nitrification and is slowed when oxygen is not readily available. Wild pig feces and urine likely contributed to the increased levels of organic carbon and nitrogen in the treatment watersheds as reference watersheds were similar in geomorphology, hydrology, and habitat type. Singer et al. (1984) found increased annual nitrogen (measured as nitrate-nitrogen) concentrations in stream water from an area with rooting activity; however, statistical comparison between treatments in that study was not performed as drought conditions reduced the number of samples that were collected. Increased precipitation and storm flow during the wet season could have transported nutrients and organic material from the floodplain into the watershed, resulting in elevated nitrogen concentrations in stream water. The geomorphology and hydrology of the two sampling locations was not described but differences in slope, substrate, basin shape, watershed size, and stream flow could have contributed to different nitrogen concentrations in stream water. In comparison, Brooks et al. (2020) did not find a link between runoff from wild pigs and nitrogen (measured as nitrate-nitrite and ammonium) in stream water. However, the animals were in a pen and did not have access to the stream and riparian vegetation acted as a buffer for pen runoff. Other factors present in the study area likely influenced nitrogen and other nutrient levels in the stream, including livestock, agriculture, construction, and free-roaming wildlife species. Studies on livestock have shown that manure can increase nitrogen levels in water and is a threat to water quality in areas with livestock production (Hooda et al. 2000, Davies-Colley et al. 2004).

Dissolved oxygen did not significantly differ between treatments, although it differed by season and stream discharge. Across watersheds and treatments, DO was lower in the dry season and positively correlated with discharge. Dissolved oxygen content is affected by several factors including water temperature, flow, photosynthesis, and microbial decomposition of organic material. While disturbance (i.e. rooting) and introduction of animal waste in the aquatic-terrestrial interface can increase organic material in a stream, and therefore increase microbial consumption of DO, we did not find significantly lower levels of DO in watersheds with wild pigs. One explanation is that the watersheds included in this study had little to no riparian vegetation buffer between the floodplain and the stream channel, so there was very little plant matter that could be transported into the aquatic environment by rooting activity. We attribute the elevated DOC and TN in the treatment watersheds to wild pig feces, which suggests that microbial consumption of DO increased in order to decompose the increased amount of organic matter. The fact that we did not detect a decrease in DO levels could be due to spatial and temporal variability in the watersheds and wild pig usage of these habitats, especially if the change was minimal. A larger sample size and more intensive sampling may be needed to observe changes in DO levels resulting from wild pig activity. Doupé et al. (2010) reported lower DO in ephemeral lagoons accessible to wild pigs; however, lagoons significantly differed in plant, macroinvertebrate, and fish species composition, all of which impact DO levels. Additionally, sampling occurred during the dry season when water levels, and subsequently DO, continuously decrease because there is no replenishment from rainfall or runoff.

Specific conductivity, Ca^{2+}, SO_4^{2-}, and Na^+ were greater in the treatment watersheds, but also differed by season and/or discharge. Sulfur is released during the decomposition of organic material and oxidized to SO_4^{2-}, so wild pig feces could have increased SO_4^{2-} in the treatment watersheds. However, conductivity, Ca^{2+}, Na^+, and SO_4^{2-} are strongly influenced by soil type and subsurface geology and differences in concentrations are most likely due to geologic and soil morphologic variability. Greater Ca^{2+} concentrations at the treatment watersheds may be attributed to subsurface marine deposits referred to as the Selma Chalk region, which does not run under the reference watersheds. One explanation for the differences in SO_4^{2-} and Na^+ concentrations between the treatment and reference watersheds is the original water source. The treatment watersheds may be fed more by underground springs in comparison to the reference watersheds. Groundwater erodes and dissolves rock and minerals over time which introduces SO_4^{2-} and Na^+ ions to the aquifer (USEPA 2003). Conductivity is a measure of the ability of water to pass an electric current and is a reflection of the concentration of ions present (USEPA 2012*a*), so elevated specific conductivity at treatment watersheds is a function of greater Ca^+, Na^+, and SO_4^{2-} concentrations.

Although wild pig rooting was regularly observed within and adjacent to the stream channel, there was no difference between TSS concentrations in treatment and reference watersheds. The soils at both locations were mainly composed of sandy bedloads which means sediment particles drop quickly out of the water column and are not suspended for long. This is likely why phosphate was not elevated despite the input of fecal material and urine from wild pigs, as it binds quickly to sediment particles (Søndergaard et al. 2003). Also, there was little to no overland runoff crossing the terrestrial-aquatic interface as the floodplains at all watersheds were relatively flat. While we did not detect a difference in TSS between treatments in this study, other watersheds with different geomorphological and hydrological features (i.e. V-shaped catchment with clay substrate) could show more pronounced differences. Dunkell et al. (2011*b*, *a*) and Strauch et al. (2016) did not find a significant difference in TSS in runoff from fenced and unfenced plots with wild pig presence. However, plot size was small (10-m x 5-m) in comparison to the amount of soil that wild pigs can disturb and unfenced plots may not have experienced the intensity of rooting typical for the area. Water samples were only taken from stormflow runoff, which can highlight extreme values and does not reflect water quality under normal conditions.

Fecal Bacteria

There were stark differences in *E. coli* concentrations between the two watershed treatments. The United States Environmental Protection Agency (USEPA) recommends that recreational watersheds have a maximum geometric mean (GM) concentration of 126 cfu/100 mL in a 30-day sampling period, and that no more than 10% of samples taken have a concentration greater than the statistical threshold value (STV) of 410 cfu/100 mL (USEPA 2012*b*). All treatment watersheds had mean *E. coli* concentrations that exceeded 126 cfu/100, while the reference watersheds were below this threshold. Median *E. coli* concentrations at the treatment watersheds were similar to those measured in nearby urban watersheds (Crim et al. 2012), despite the lack of surface runoff from developed areas and other anthropogenic sources at our study sites. Treatment and reference watersheds had the same wildlife species (and sources of fecal matter) except for wild pigs, therefore, the elevated *E. coli* concentrations at the treatment watersheds are likely a result of wild pig activity.

Unlike *E.* coli, FC concentrations did not vary by treatment and were elevated in comparison to nearby urban watersheds (Crim et al. 2012). Fecal coliform concentrations historically were used to predict the presence of gastrointestinal illness-causing pathogens, but the USEPA no longer uses FC as an indicator of fecal contamination (USEPA 2012*b*). *E. coli* and enterococci are now the preferred method of identifying bodies of water potentially contaminated by fecal material, because their presence unequivocally indicates the occurrence of fecal contamination even if the detected strains are non-pathogenic (Edberg et al. 2000). Fecal coliform testing also detects thermotolerant non-fecal (“environmental”) coliform bacteria and can lead to an overestimation of fecal contamination and risk to public health (Francy et al. 1993). In our study, it is likely that environmental coliform bacteria was naturally present in runoff and streams and incubated along with FC, which made it difficult to detect an effect of wild pig presence on FC concentrations. Previous studies examining impacts of wild pigs on fecal contamination of watersheds have reported mixed results. Kaller and Kelso (2003) reported positive correlation of wild pig presence with fecal bacteria, but they measured FC and sampling occurred only three times in one watershed basin. Dunkell et al. (2011*b*, *a*) and Strauch et al. (2016) did not find a significant effect of wild pigs on *E. coli*, enterococci, or total coliforms in runoff from fenced and unfenced plots but, as mentioned previously, plot sizes were small and unfenced plots may not have experienced typical rooting intensity. Brooks et al. (2020) did not detect a difference in *E. coli* and enterococci concentrations between pen runoff and nearby stream water, which is likely because wild pigs did not have direct access to the stream and there were other sources of fecal matter in the area, such as livestock and free-roaming wildlife.

The positive detection of swine fecal bacteroidetes in treatment watersheds via microbial source tracking, and the absence of same in reference watersheds, further indicates that wild pigs can introduce fecal material and disease-causing pathogens to riparian areas. The effects of fecal contamination are not limited to the initial source area: downstream areas may be affected. Water-borne bacteria and pathogens accumulate as low-order streams flow into main tributaries of increasing magnitude. Stream sediments also serve as a reservoir for *E. coli* and potentially other pathogens (Garzio-Hadzick et al. 2010), and disturbance events that affect stream sediment (stormflow, anthropogenic activities, wild pig rooting) could re-suspend these microorganisms in the water column and cause them to travel further downstream or come into contact with a susceptible human or animal. Reducing wild pig presence in riparian areas during times of potential disturbance could reduce the amount of fecal contamination in the watershed, and therefore the *E. coli* deposited in stream sediment. To our knowledge, this study is the first that definitively links wild pig presence to the introduction of fecal material and waterborne pathogens in watersheds and meets the four conditions we believe are needed for an accurate assessment of wild pig impacts on water quality.

Conclusion

This study shows that wild pigs are a threat to water quality in forested watersheds by introducing fecal material and potentially disease-causing organisms. Further research on the downstream fate of pathogens and potential sources of contact with humans and animals is necessary for a clear understanding of the impacts wild pigs have on local water quality and ecosystem health. Our results suggest that microbial source tracking and *E. coli* monitoring may be effective ways to gauge wild pig activity in watersheds, as well as indicating that it may be important to reference wild pig populations upstream of major drinking water sources and recreational areas.

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Table 2.1. LME models for water quality and fecal bacteria concentrations for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019. Degrees of freedom is 1 for each fixed effect and dependent variable. Estimates and confidence limits have been back-transformed, if necessary. Concentration units: *mg/L*; specific conductivity: *µS/cm;* temperature: *°C*; fecal bacteria: *cfu/100 mL*.

* Data was natural log transformed prior to analysis

** Data was natural log +1 transformed prior to analysis

	TREATMENT			**SEASON**			**DISCHARGE ***			**TREATMENT*SEASON**			**SEASON*DISCHARGE**		
Y	Est	95% CL	P	Est	95% CL	P	Est	95% CL	P	Est	95% CL	P	Est	95% CL	P
NO_2^-**	0.08	$8.40e^{-4}$, 0.16	0.05	0.12	0.04, 0.21	< 0.01				0.20	0.14, 0.25	< 0.01			
DOC	10.62	6.29, 14.95	< 0.01				$-3.97e^{-3}$	$-7.30e^{-3}$, $-6.41e^{-4}$	0.02						
Ca^{2+}**	4.84	2.50, 8.76	< 0.01				-0.07	-0.09, -0.04	< 0.01						
NH_4^+ **							-0.02	-0.03, -0.01	< 0.01						
Na^+*	2.44	1.54, 3.89	< 0.01	1.22	1.08, 1.38	< 0.01	-0.08	-0.10, -0.06	< 0.01						
NO_3^- **				0.10	0.04, 0.16	< 0.01	$-5.65e^{-4}$	-1768.99, 0.01	0.93				0.2	$2.64e^{-3}$, 0.04	0.02
Cl^- *	2.12	0.94, 4.77	0.08	1.09	0.79, 1.50	0.60	-0.11	-0.15, -0.07	< 0.01	1.61	1.10, 2.38	0.02			
K^+ *							-0.04	-0.06, -0.01	< 0.01						
Mg^{2+} *	1.33	0.86, 2.06	0.20	1.04	0.86, 1.26	0.65	-0.07	-0.09, -0.05	< 0.01	1.31	1.05, 1.64	0.02			
TN *	2.35	1.38, 4.00	0.01												
SO_4^{2-} *	10.25	5.67, 18.50	< 0.01	1.29	1.04, 1.61	0.03	-0.11	-0.16, -0.06	< 0.01				1.12	1.04, 1.20	< 0.01
DO				2.57	1.55, 3.59	< 0.01	$4.74e^{-3}$	$2.68e^{-3}$, $6.69e^{-3}$	< 0.01						
TSS *							-0.11	-0.21, -0.01	0.03						
Sp Cond *	3.35	2.10,5.33	< 0.01				-0.11	-0.17, -0.06	< 0.01						
Temp				-8.72	-10.76,-6.69	< 0.01									
E. coli **	40.40	10.06, 153.95	< 0.01												
FC **	1.13	-0.25, 5.09	0.15	4.76	1.06, 15.12	< 0.01	0.09	0.02, 0.18	0.02	8.14	1.59,31.20	< 0.01			

Table 2.2. Summary of water quality values for water samples collected at POTL in Bullock County, AL and TUSK in Macon County, AL from May 2018 through June 2019. SE = Standard Error. Concentration units: *mg/L*; specific conductivity: *µS/cm*.

*Differences between treatments were significant (α = 0.05).

	TREATMENT				REFERENCE			
	Mean	**SE**	**Mdn**	**Range**	**Mean**	**SE**	**Mdn**	**Range**
SO_4^{2-}*	16.93	1.41	14.13	3.48 – 154.55	1.54	0.14	1.56	0.36 – 3.14
Cl^-	11.65	1.10	7.42	2.77 – 67.02	2.73	0.13	2.49	1.70 – 4.30
Na^+*	4.44	0.21	3.71	1.68 – 13.74	1.59	0.08	1.31	1.14 – 2.49
Ca^{2+}*	16.45	0.59	16.76	5.15 – 43.70	2.03	0.38	0.83	0.00 – 6.37
Mg^{2+}	1.56	0.05	1.50	0.69 – 4.02	1.77	0.20	1.13	0.85 – 4.17
NO_2^-	0.06	0.01	0.04	0.00 – 0.43	0.12	0.03	0.04	0.00 – 0.88
TN*	0.41	0.02	0.40	0.18 – 1.83	0.22	0.02	0.12	0.05 – 0.62
DOC*	17.61	0.40	17.83	7.16 – 28.11	5.94	0.40	4.51	2.90 – 19.11
K^+	1.47	0.04	1.42	0.63 – 2.55	1.26	0.09	1.12	0.88 – 3.62
NO_3^-	0.06	0.01	0.04	0.00 – 0.53	0.37	0.11	0.03	0.00 – 1.88
PO_4^{3-}	0.03	$3.98e^{-3}$	0.00	0.00 – 0.25	0.03	$8.14e^{-3}$	0.00	0.00 – 0.16
NH_4^+	0.08	0.01	0.00	0.00 – 0.73	0.02	0.01	0.00	0.00 – 0.10
TSS	0.06	0.02	0.03	0.00 – 1.58	0.03	0.01	0.01	0.00 – 0.24
Sp Cond*	0.14	0.01	0.13	0.00 – 1.54	0.03	0.01	0.02	0.01 – 0.10
pH	6.70	0.05	6.76	4.66 – 8.06	6.28	0.12	6.15	4.58 – 7.30

Table 2.3. Results of ANOVA models for water quality values for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.

* Data was natural log transformed prior to analysis

** Data was natural log +1 transformed prior to analysis

	Df	Sum sq	Mean sq	F-value	P-value
Treatment					
SO_4^{2-} *	10	19.94	1.94	9.10	< 0.01
Sp Cond *	10	3.66	0.37	0.66	0.76
TN *	10	3.94	0.39	4.90	< 0.01
Ca^{2+} **	10	8.82	0.88	9.63	< 0.01
DOC	10	1123	112.3	9.20	< 0.01
Na^{+} *	10	13.78	1.38	18.35	< 0.01
Reference					
SO_4^{2-} *	2	8.89	4.45	36.16	< 0.01
Sp Cond *	2	11.53	5.76	31.50	< 0.01
TN *	2	18.10	9.05	140.50	< 0.01
Ca^{2+} **	2	11.02	5.51	205.80	< 0.01
DOC	2	239.60	119.80	30.89	< 0.01
Na^{+} *	2	2.00	1.00	187.70	< 0.01

Table 2.4. Summary of fecal bacteria values for water samples collected at POTL in Bullock County, AL and TUSK in Macon County, AL from May 2018 through June 2019. SE = Standard Error. All concentration units are *cfu/100 mL*.

	E. COLI				FECAL COLIFORMS			
Watershed	**Mean**	**SE**	**Median**	**Range**	**Mean**	**SE**	**Median**	**Range**
Treatment								
1	6079.46	5393.91	300.0	133 – 70,767	16,813.92	3931.66	13,533.0	2867 – 48,167
2A	369.23	72.46	400.0	67 – 967	10,819.50	3143.11	7516.5	2267 – 42,400
2B	987.73	328.65	633.0	67 – 3300	15,610.10	3486.92	15,683.5	4267 – 38,933
3	1475.00	1047.60	550.0	100 – 4600	4600.00	991.16	4567.0	2900 – 6333
7	4991.63	3967.35	1033.0	0 – 32,667	30,081.00	16,834.82	11,300.0	1133 – 126,067
8	966.60	346.24	667.0	67 – 5500	15,835.71	4070.64	14,800.0	2867 – 59,633
9	1924.88	847.44	1066.5	100 – 7400	71,757.14	53,761.64	6267.0	2533 – 388,767
10	492.31	245.72	167.0	33 – 3267	14,827.75	5620.69	7116.5	1933 – 61,967
11	1674.31	908.15	600.0	100 – 12,066	11,813.92	2427.14	10,017.0	1600 – 26,233
12	628.64	330.20	150.0	33 – 4367	17,484.77	7446.73	7000.0	1900 – 96,767
14	399.92	298.12	116.5	0 – 3667	8605.67	3353.32	5350.0	1233 – 44,467
Overall	1711.25	632.91	333.0	0.00 – 70,767.00	18,301.22	3738.25	8067.00	1133 – 388,767
Reference								
T1	21.27	9.33	0.0	0 – 67	4818.18	1733.41	2267.0	500 – 20,000
T2	33.36	12.73	0.0	0 – 100	5148.55	1790.33	1967.0	600 – 16,300
T3	139.90	93.51	33.0	0 – 967	8773.20	2460.06	7400.0	833 – 27,433
Overall	62.50	30.16	0.0	0 – 967	6167.69	1156.64	3000.00	500 – 27,433

Table 2.5. Results of ANOVA models for *E. coli* and FC concentrations for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.

	Df	Sum sq	Mean sq	F-value	P-value
Treatment					
E. coli	10	80.21	8.02	3.04	<0.01
FC	10	10.19	1.02	0.88	0.56
Reference					
E. coli	2	9.99	5.00	0.95	0.40
FC	2	3.49	1.74	1.44	0.25

Table 2.6. Results from DNA analysis of swine fecal biomarkers in water samples collected at POTL in Bullock County, AL and TUSK in Macon County, AL during 2018-2019. Concentrations indicate Marker Quantified (copies/100 mL); ND: Not Detected; DNQ: Detected Not Quantified (concentration below limit of quantification). Bullet points indicate that samples were not analyzed from that watershed.

WATERSHED	JUNE 2018	JULY 2018	DECEMBER 2018	APRIL 2019	AUGUST 2019
Treatment					
1	ND	DNQ	5.69E+02	.	.
2A	DNQ	.	1.83E+04	6.92E+02	.
2B	ND	.	1.92E+04	ND	.
3	DNQ	ND	8.26E+02	.	.
7	ND	.	6.66E+02	.	.
8	3.61E+02	ND	5.21E+03	ND	.
9	3.54E+03	.	1.29E+03	.	.
10	ND	.	1.90E+03	DNQ	.
11	5.77E+02	.	1.05E+04	6.22E+02	.
12	ND	ND	6.19E+02	DNQ	.
14	DNQ	.	1.05E+03	DNQ	.
Reference					
T1	.	.	.	ND	ND
T2	.	.	.	ND	.
T3	.	.	.	ND	ND
Detections/Total	6/11	1/4	11/11	5/7; 0/3	0/2

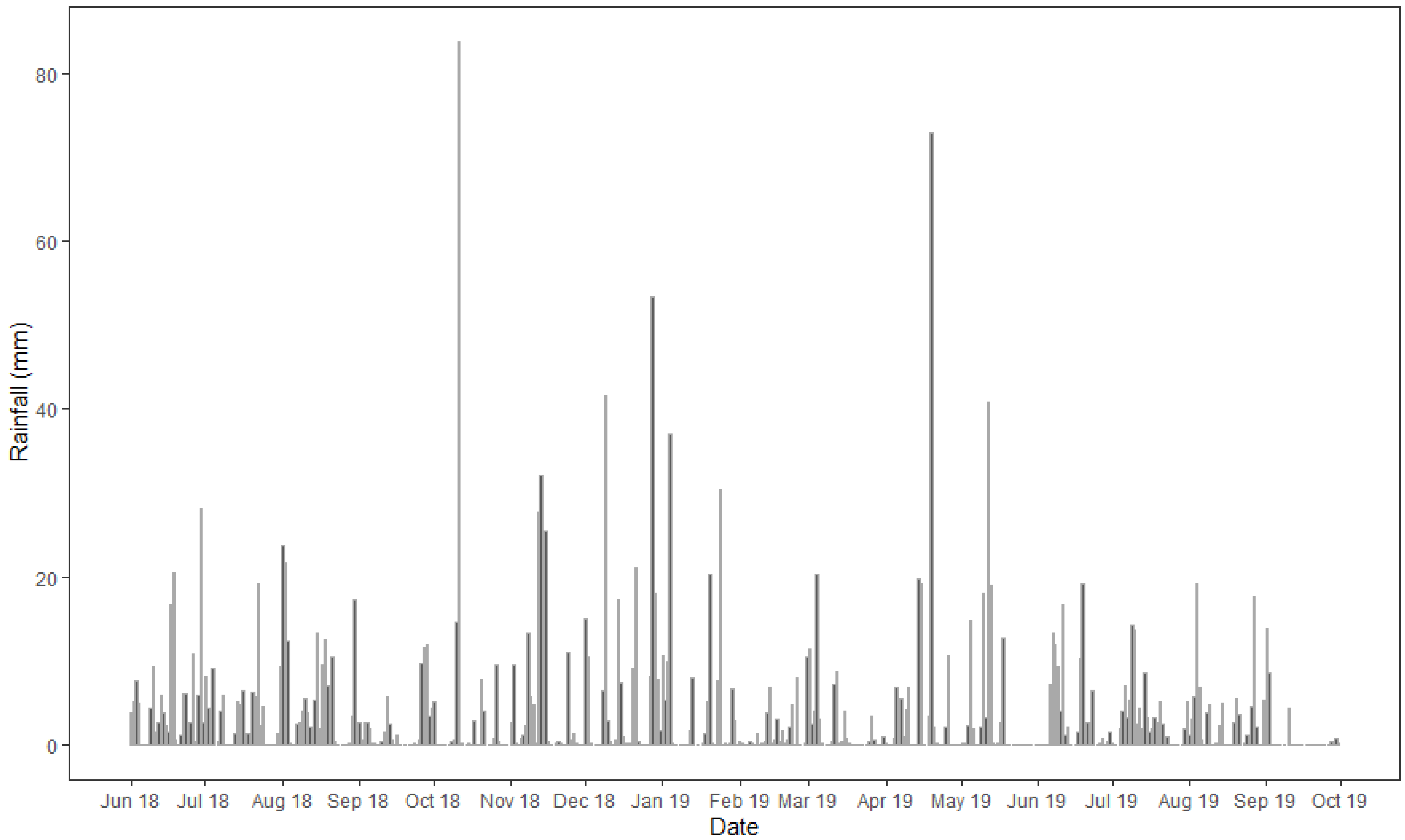

Figure 2.1. Mean rainfall (mm) at POTL in Bullock County, AL and TUSK in Macon County, AL from May 2018 through June 2019.

AB

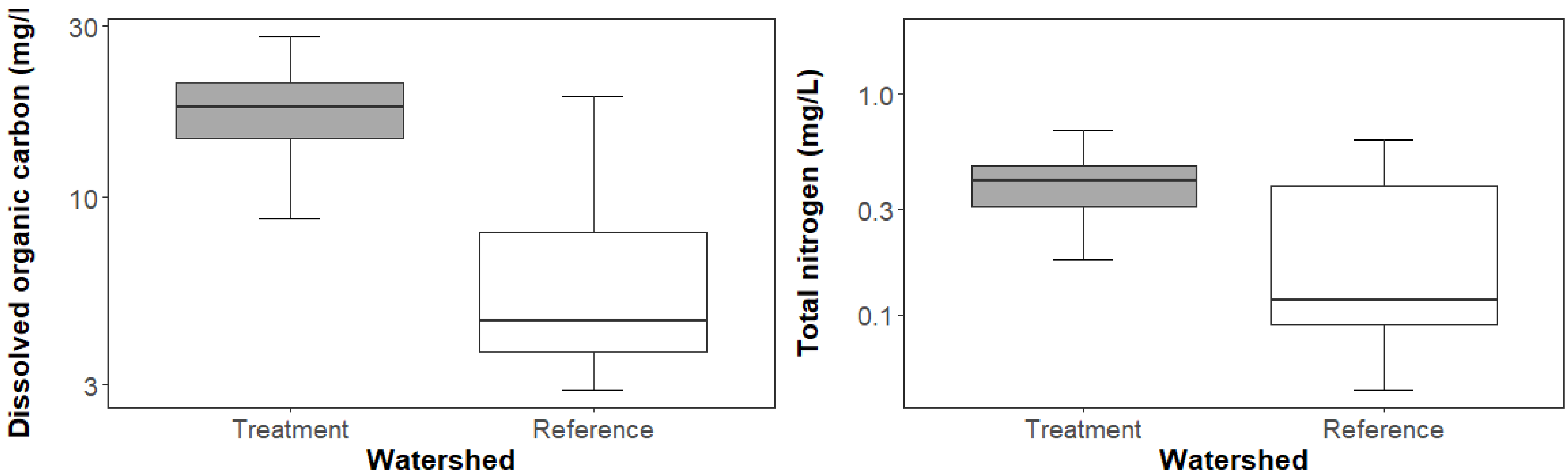

Figure 2.2. A) Dissolved organic carbon (mg/L) concentrations by treatment. B) Total nitrogen (mg/L) concentrations for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.

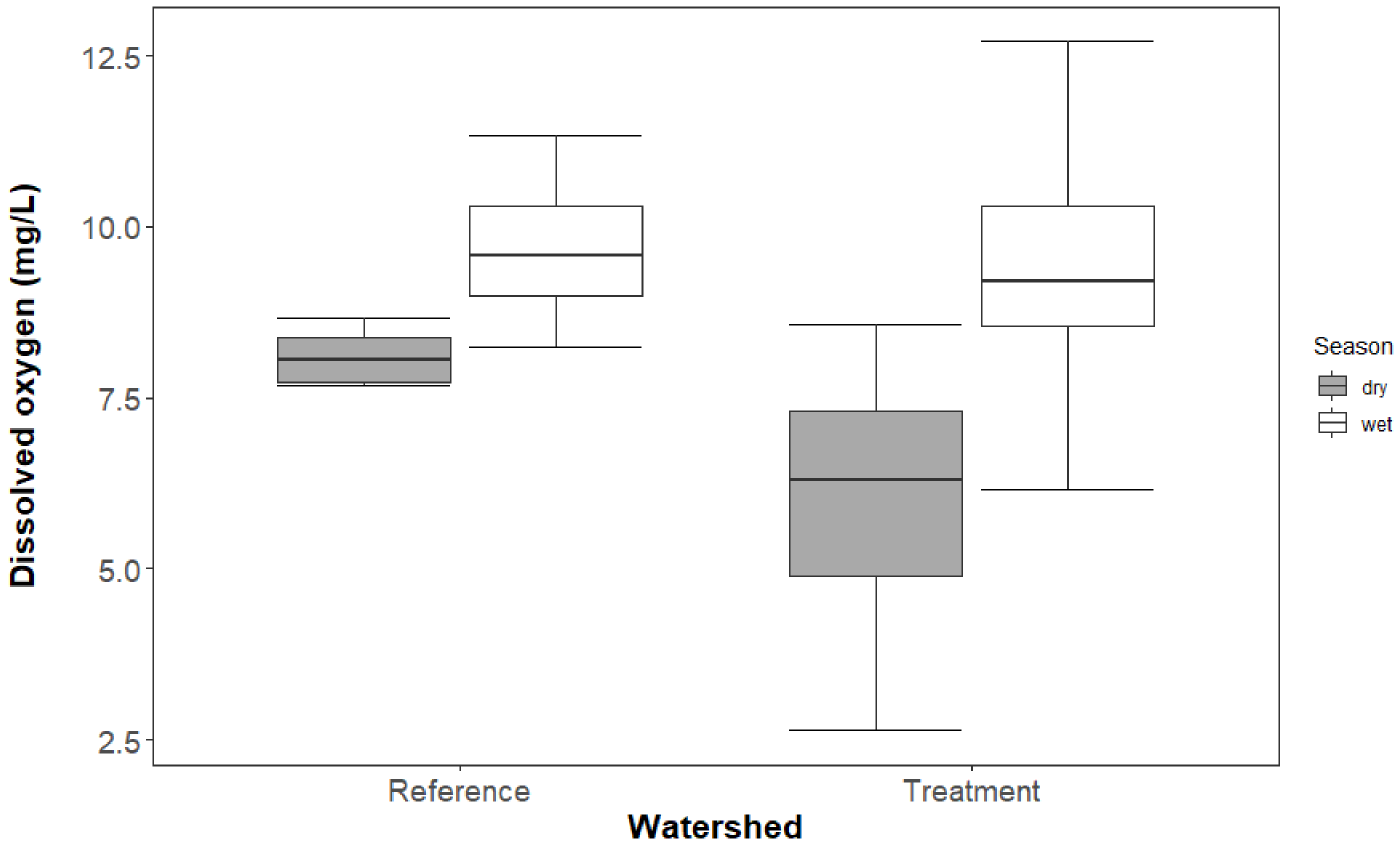

Figure 2.3. Dissolved oxygen (mg/L) concentrations for treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.

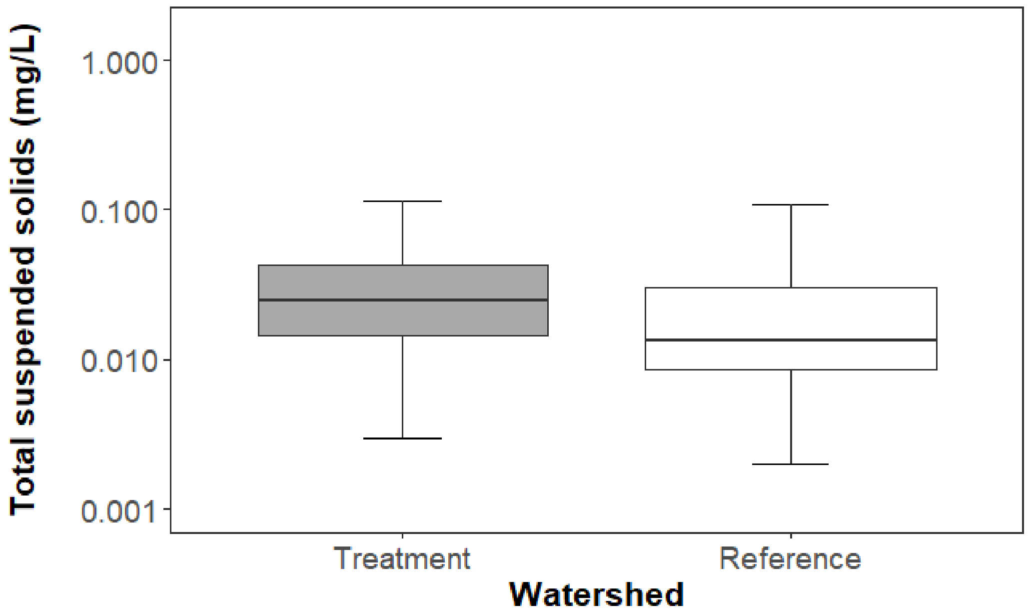

Figure 2.4. Concentrations of total suspended solids (mg/L) for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.

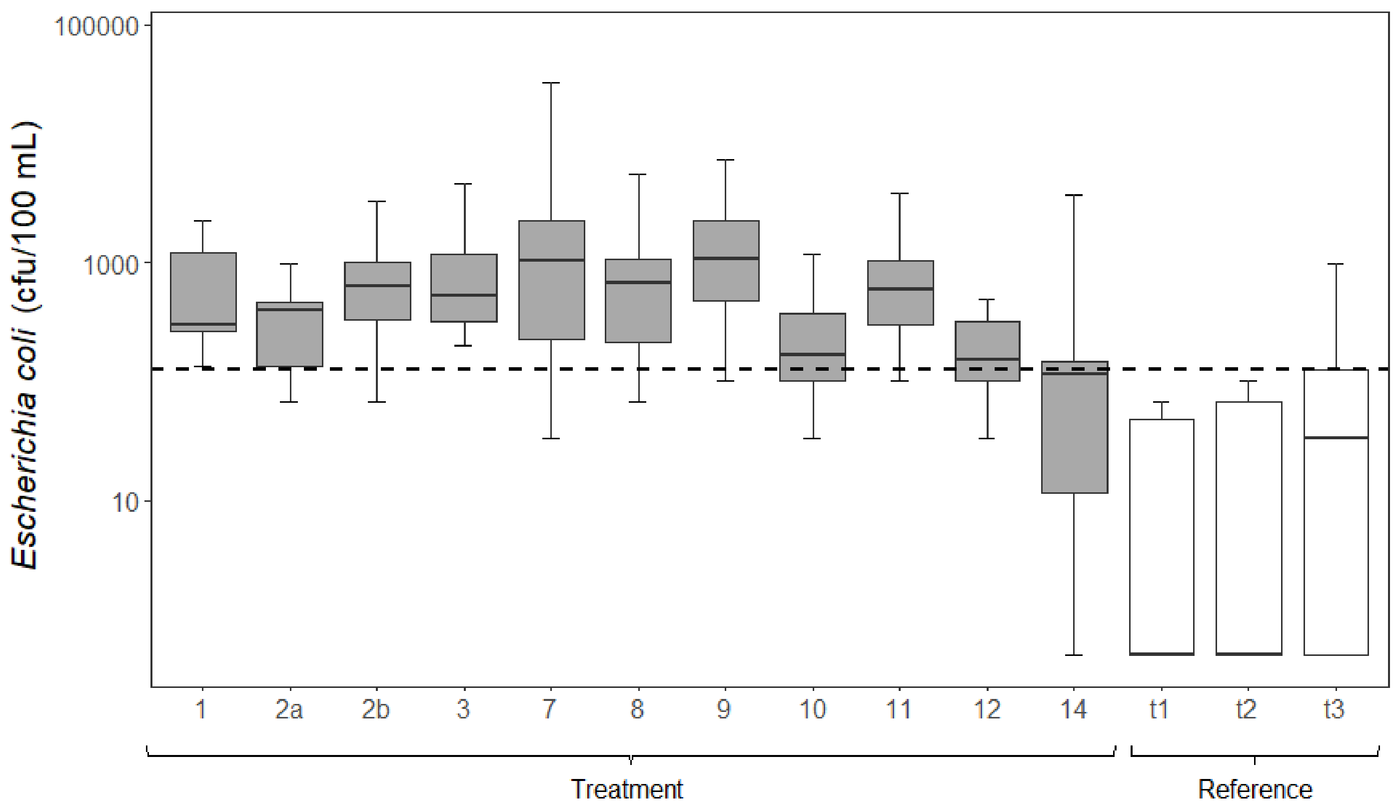

Figure 2.5. *Escherichia coli* concentrations (cfu/100 mL) for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019. The dashed line indicates the USEPA's recommended maximum GM for *E. coli* concentrations in recreational watersheds (126 cfu/100 mL).

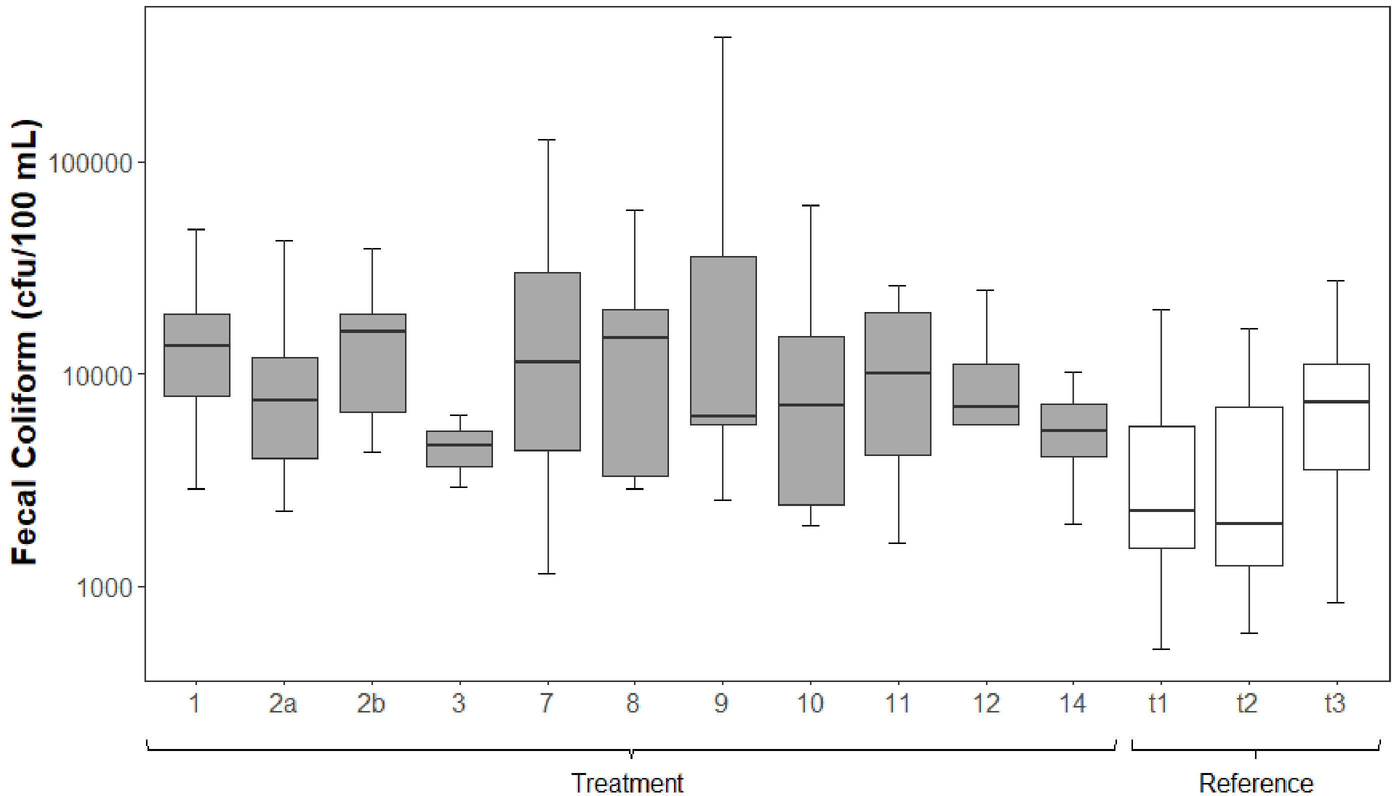

Figure 2.6. Fecal coliform concentrations (cfu/100 mL) for water samples collected at treatment watersheds (POTL in Bullock County, AL) and reference watersheds (TUSK in Macon County, AL) from May 2018 through June 2019.