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ORIGINAL PAPER

Wild pig removal reduces pathogenic bacteria in low-order streams

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Abstract Invasive wild pig populations have undergone enormous increases in the United States and particularly across the southern U.S. in recent years. High fecundity rates and abilities to adapt quickly to varied habitats have enabled pig populations to become entrenched and difficult to eliminate. The pigs cause many negative impacts on ecosystems including degradation of water quality through infusion of fecal contamination and other non-point source pollutants. Our goal was to determine the effects of pig removal on water quality in streams that were known to be significantly polluted by pig activity Bolds (J Environ Qual 50: 441–453, 2021). We compared *e. coli* and fecal coliform concentrations and loads in streams between a pre-removal period with those that occurred during the removal activities. Results suggest that *e. coli* and fecal coliform concentrations were reduced by 75 and 50% respectively through pig removal efforts. Questions remain concerning the longevity of the reduction especially once pig removal activities decrease in intensity.

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Introduction

Fecal contamination of streams and other bodies of water is an issue in many parts of the world and poses risks to human health. For example, Shiga toxin-producing *Escherichia coli* (STEC) infection in humans can cause severe stomach cramps, vomiting, fever, diarrhea, and even death (CDC 2017). Fecal bacteria can contaminate water and may arise from various sources including human waste, manure runoff, and feces from domestic animals, livestock, and wildlife. An increasing number of studies (Chase et al. 2012; Ahmed et al. 2010) show that animal waste is a contributing factor to impaired water quality in many areas, even those far from anthropogenic influences.

Invasive wild pigs have increased in number and distribution to the point that, due to their potential for destruction of natural resources, they are now recognized as the foremost large vertebrate problem in the US (Ditchkoff and Bodenchuk 2020). In addition, wild pigs are very problematic to control and considerable effort is being expended to develop effective control strategies (Ditchkoff and Bodenchuk 2020). One approach that may be promising is whole sounder removal which eliminates an entire breeding group of

pigs in a single trapping (Lewis 2019). However, results of wild pig removal strategies have been mixed in terms of effectiveness and an additional question is whether ecosystems, once subjected to high densities of wild pigs, may rebound when pig pressure is reduced. A key metric of wild pig damage within an ecosystem is water quality (Bolds et al. (2021) and, consequently, a critical question that we address in this paper is whether water quality, once diminished significantly by wild pigs, can respond positively to reduced pig densities.

Unlike livestock, fecal contamination of watersheds by wildlife is very difficult to control and to pinpoint sources is very challenging. In a study in the Finger Lakes, NY, *E. coli* found in stream water was originally blamed on human waste from leaking septic tanks and poor agricultural management practices, but analyses identified Canada geese (*Branta canadensis*) and white-tailed deer (*Odocoileus virginianus*) as two of the main sources (Somarelli et al. 2007). Several disease outbreaks have been linked to the consumption of fresh produce potentially contaminated by wildlife feces, including *E. coli* O157 from the feces of black-tailed deer (*Odocoileus hermionus*) (Laidler et al. 2013) and wild pigs (*Sus scrofa*)(Jay et al. 2007). Additionally, wildlife and livestock often exist in the same spaces and close contact can lead to indirect and direct disease transmission between individuals and populations. As examples, European starling (*Sturnus vulgaris*) abundance at feedlots in the United States was associated with increased shedding of *E. coli* in cattle feces (Carlson et al. 2020), and wild rodents trapped at swine farms showed a greater prevalence of *E. coli* than those trapped in developed areas and natural habitats (Allen et al. 2011).

Wild pigs in particular are widely-considered to be significant reservoirs for zoonotic diseases and can transmit pathogens to other animals and humans through direct contact, as well as indirect methods like fecal contamination of sediment, water, and food sources (Miller et al. 2017; Brown et al. 2018). Native to Europe and Asia, wild pigs are highly invasive and have established populations on every continent except Antarctica. While able to survive in a diverse range of conditions and habitats, wild pigs are often found in wetlands and riparian forests due to the availability of resources and their presence can result in immense damage to these sensitive ecosystems (Lewis et al. 2019; Mayer et al. 2020). Rooting and wallowing behaviors disturb soil macroinvertebrates, vegetative communities, and compromise soil structure and stability (Singer et al. 1984; Seward et al. 2004; Gray et al. 2020). The destructive capabilities of wild pigs combined with their role as a disease reservoir create a strong potential to drastically change stream and riparian habitats and animal populations.

Despite the potential for wild pigs to significantly impact water quality in riparian areas, only a handful of studies have sought to examine the relationship between fecal bacteria levels in stream water and the presence of wild pigs. Kaller and Kelso (2003) found greater fecal coliform (FC) concentrations in stream water near evidence of wild pig activity in Louisiana, while Brooks et al. (2020) found that fecal bacteria levels in runoff from a paddock containing wild pigs did not differ from levels in a nearby stream. Dunkell et al. (2011) and Strauch et al. (2016) studied wild pig impacts to Hawaiian watersheds by comparing runoff from fenced and unfenced plots, but did not detect a difference in fecal bacteria concentrations. In addition, the authors reported previously that *E. coli* concentrations in pig-inhabited watersheds were 40 times the concentrations in watersheds without pig activity, and microbial source tracking (MST) found swine fecal bacteria in 70% of water samples from pig watersheds (Bolds et al. 2021). Besides fecal bacteria, other studies have reported the presence of waterborne pathogens (i.e., *Giardia, Cryptosporidium,* and *Leptospira*) in wild pigs (Atwill et al. 1997; Hampton et al. 2006; Poudel et al. 2020).

The previous studies used a variety of experimental designs and have reported conflicting results which are most likely a function of variation in study design, habitat, climate, and land use practices. However, we are unaware of any studies that examined changes in fecal bacteria levels in riparian areas prior to and during wild pig removal. As fecal bacteria may remain in stream sediment for an extended period of time (Garzio-Hadzick et al. 2010), it is important to know whether removal of the main source (wild pigs) reduces fecal bacteria levels. This study sought to examine the impacts of wild pig removal efforts on *E. coli* and FC loads in headwater riparian systems, while addressing the four conditions for assessing wild pig impacts on water quality as described in Bolds et al. (2021). Specifically, our research objectives were to:

1. Determine wild pig impacts on fecal bacteria loads in headwater streams by comparing *E. coli* and fecal coliform concentrations before and after the initiation of targeted removal efforts.
2. Use microbial source tracking to link fecal bacteria in stream water to the presence of wild pigs in riparian areas by analyzing water samples for swine fecal Bacteroidetes.

Materials and methods

Study area

The study took place from May 2018 through September 2020 on a 4515 ha property in southeast Alabama, which served as the treatment area (Fig. 1). The property is managed for white-tailed deer and eastern wild turkey (*Meleagris gallopavo silvestris*), and was not used for agriculture or livestock farming. Dominant habitat types were mixed pine (*Pinus* spp.)-hardwood forest and riparian hardwoods, with a canopy mainly composed of loblolly pine (*Pinus taeda*), southern shagbark hickory (*Carya carolinae-septentrionalis*), and sweetgum (*Liquidambar styraciflua*). The understory was made up of herbaceous and semi-woody species, such as American beautyberry (*Callicarpa americana*), eastern baccharis (*Baccharis halimifolia*), and blackberry (*Rubus* spp.).

The treatment property is located in the Mantachie-Iuka-Bibb soil association and in the Upper Coastal Plain physiographic region. A handheld GPS was used to obtain coordinates for sampling points that occur at outlet points (locations just upstream of convergence points with higher order streams) of low-order streams on the property. Watersheds draining to the sampling sites were physically located and chosen for the study if they met certain criteria: low gradient, were third order or lower in magnitude, and were primarily occupied by deciduous wetland forests. Eleven watersheds met the criteria and had mostly intermittent flow in the winter and spring, although the main tributaries were perennial. During site selection, all 11 watersheds had damage from wild pig rooting and digging

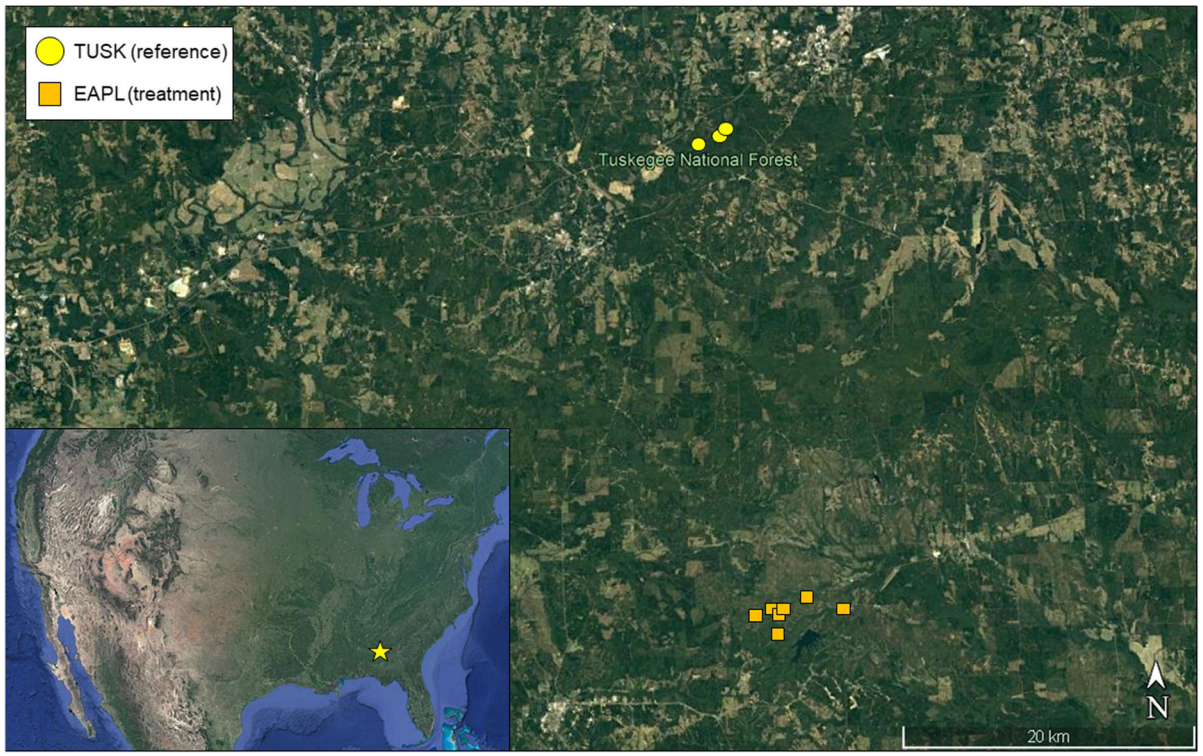

Fig. 1 Approximate locations of sampling sites for treatment streams 2A—14 (EAPL) and reference streams t1–t3 (TUSK) in southeast Alabama, USA

on the floodplains and/or within the stream channel. These are referred to as treatment streams (Fig. 1). The smallest watershed was 9.4 ha and the largest was 820 ha.

A reference area that showed little to no evidence of pig activity (determined from visual observation and camera surveys) was selected approximately 25 km away on the Tuskegee National Forest (Bolds et al. 2021; Fig. 1). The reference location was very similar to the treatment area in terms of topography, land cover, and stream characteristics. Three streams at the National Forest were selected for sampling.

At the start of the study, the density of wild pigs at the treatment property was estimated at 15.5 pigs/km^2 with camera surveys (Lewis et al. 2019). The average density of wild pigs in the southeastern U.S. has been estimated at 6–8 pigs/km^2 (Lewis et al. 2019) so density at the treatment property was quite high. Concentrated pig removal efforts by Auburn University and USDA-Animal and Plant Health Inspection Service personnel (conducted using whole sounder trapping) began in July 2019 and resulted in a reduced density of 10/km^2 by the conclusion of removal efforts in September 2020. The period during which pigs were removed (July 2019–September 2020) is referred to as Year 2 (Y2) while the pre-removal period (May 2018–July 2019) is referred to as Year 1 (Y1).

Collection and analysis of water samples

Water samples were collected from each stream (n = 11) every two weeks during Year 1 (Y1) and Year 2 (Y2) as long as flow was present. A 500 ml grab sample was collected at each site in the middle of the channel at the outlet point of each watershed where the stream flowed into the connecting tributary. Sampling small headwater and low-order streams enabled us to observe the cumulative effect of wild pigs within watersheds that were homogenous in terms of land cover / land use while eliminating other potential sources of fecal contamination. We measured discharge at the sampling points using the USGS mechanical current-meter method (Turnipseed and Sauer 2010).

Fecal bacteria (*E. coli* and fecal coliform) levels were measured for each water sample that was collected, with the exception of fecal coliforms for the first sampling event in May 2018. Immediately after collection, three 1 ml sub-samples were taken from each grab sample and transferred via pipette into vials containing Coliscan Easygel (Micrology Laboratories, Goshen, IN). The vials were kept on ice and transported to the Auburn University Biogeochemistry Laboratory, whereupon they were each transferred to a petri dish and incubated at 29–37 °C for 30 h. Colony types were distinguished by medium color: purple or blue for *E. coli* and pink or red for FC. After the incubation period, colony-forming unit (cfu) counts were performed using a microscope and handheld tally counter. Following cfu counts, the mean cfu of the three sub-samples was multiplied by 100 ml to calculate the concentrations of *E. coli* and FC (cfu/100 ml) for each stream. Concentrations were multiplied by corresponding discharge measurements to obtain loads.

In addition to examining fecal bacteria loads, we used MST to determine whether wild pig feces were contaminating stream water in the watersheds (Okabe et al. 2007). Using the previously described method, water samples intended for MST were collected in June, July, and December of 2018, April and August of 2019, and February and June of 2020. These months were chosen as they represented periods of low flow (May–October) and high flow (November–April). The samples were analyzed for the presence of swine fecal bacteroidetes using quantitative PCR (qPCR) at a private laboratory (Source Molecular, Miami Lakes, FL). Samples were filtered through 0.45 micron membrane filters, placed in 2 ml tubes containing beads and a lysis buffer, and homogenized for 1 min. DNA was extracted with a Generite DNA-EZ ST1 extraction kit (GeneRite, NJ). Using an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA), amplifications to detect the target gene biomarker were run in a final reaction volume of 20 µL sample extract, forward primer, reverse primer, probe, and an optimized buffer. All assays were analyzed in duplicate. To quantify the number of gene biomarker copies, a standard curve was generated from serial dilutions of known gene copy numbers from which target gene copy numbers were extrapolated. Positive and negative controls were run in tandem with the samples to aid in the identification of false negatives or positives (Source Molecular, personal communication, August 29, 2019) (Table 3).

Precipitation data were obtained from the Prism Climate Group at Oregon State University (PRISM

Climate Group 2021). A map depicting the locations of the study sites was created using Google Earth Pro (Google Earth 2021).

Statistical analysis

Statistical analyses were conducted using R statistical platform version 3.6.3 (R Core Team 2020). We calculated the cumulative *E. coli* and FC loads on a per site basis, and plotted cumulative loads by cumulative discharge to generate double mass regression curves (Searcy and Hardison 1960; Kara et al. 2015). Following clarification that the ANOVA assumptions were met for these data, Welch's t-tests were used to test for statistical differences between slopes for Y1 and Y2.

Results

Flow decreased between May–October due to increased evapotranspiration, while peak flow occurred during November–April when evapotranspiration was low as a result of leaf senescence and decreased temperatures. Precipitation patterns during Y1 and Y2 were generally similar (Fig. 2). In the treatment area, streams 1 and 7 did not have flow during Y2 and were subsequently omitted from analysis.

The breaks in slopes moving from Y1 to Y2 in the double mass curves developed from treatment area data clearly indicate changes in relationships between cumulative flow and cumulative loads of both *E. coli* and FC. At the treatment area, slopes in Y2 were significantly smaller than slopes in Y1 ($p < 0.01$; Figs. 3 and 4; Table 1). Median *E. coli* loads in Y1 were 3,522 cfu/s compared to 836 cfu/s in Y2, and ranged from 0 to 19,775,571 cfu/s (Table 2). Median FC loads were 109,533 cfu/s in Y1 and 45,916 cfu/s in Y2, and ranged from 38 – 3,810,226 cfu/s. Concentrations of *E. coli* and FC were generally lower in Y2 than Y1 and varied by watershed (Figs. 5 and 6). In Y1, median concentrations of *E. coli* and FC were 300 cfu/100 ml and 6,750 cfu/100 ml, respectively, compared to 67 cfu/100 ml and 3,100 cfu/100 ml in Y2. At reference streams (TUSK), *E. coli* and fecal coliform concentrations and loads did not differ between Y1 and Y2 (Figs. 5 and 6).

At the treatment area, 71.4% of the 28 MST samples collected in Y1, had a detectable number of swine fecal gene biomarker (Table 3). In Y2, 14 of the 19 samples (73.7%) were positive for the biomarker. The number of biomarker copies was too small to quantify in 6 samples in Y1 and 2 samples in Y2, while no copies of the biomarker were detected in 8 samples in Y1 and 5 samples in Y2. Of the samples with quantifiable numbers of the biomarker copy, values

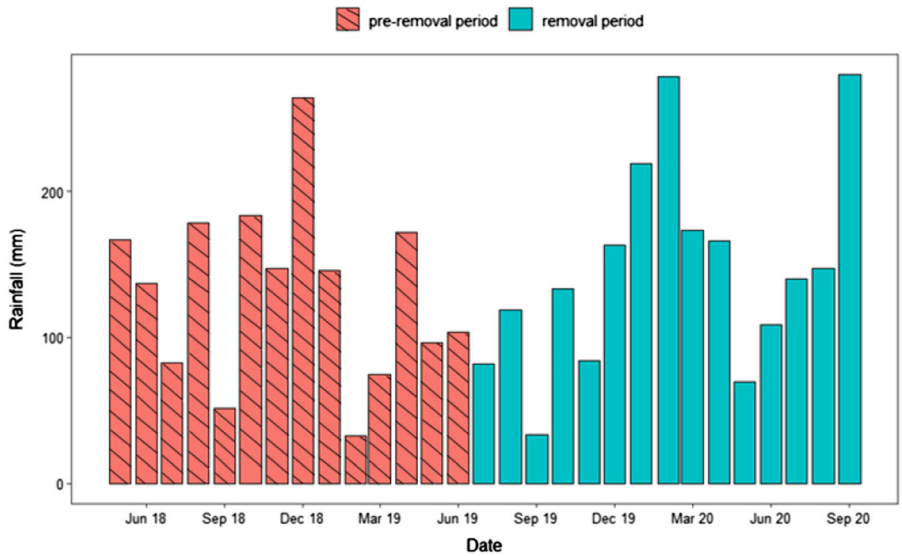

Fig. 2 Precipitation patterns at the study sites in southeast Alabama, USA for both the pre- removal and removal periods (May 2018–September 2020)

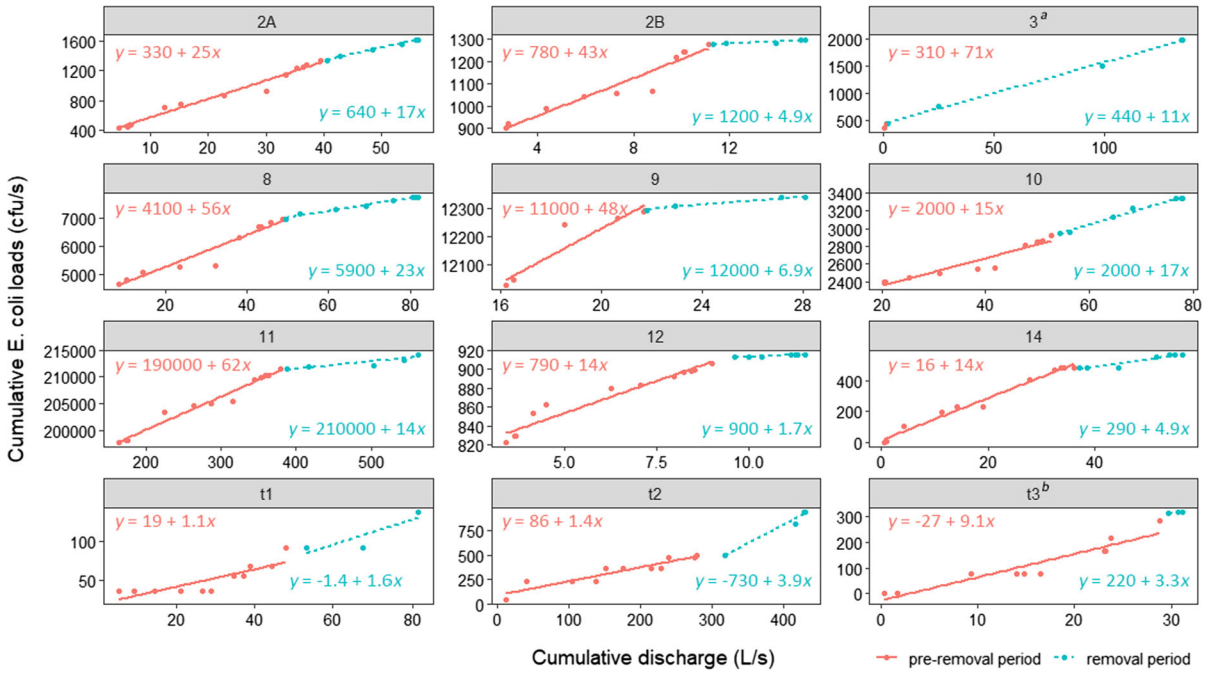

Fig. 3 Double mass curves of cumulative loads of *E. coli* for the 12 streams analyzed in the study during Year 1 "pre-removal period" (May 2018 to June 2019) and Year 2 "removal period" (July 2019 to September 2020). Streams 2A—14 were located at EAPL (treatment) and streams t1–t3 were located at TUSK (reference). Year 2 values are cumulative from Year 1. In each plot, the regression equations for Year 1 and Year 2 are in the upper left and lower right corner, respectively. Note that the scale of both axes differs by plot.[a] During Year 1 sampling events (n = 4), cumulative loads of *E. coli* ranged from 369 to 451 cfu/s and cumulative discharge ranged from 0.80 to 1.96 L/s. [b] During Year 2 sampling events (n = 3), cumulative loads of *E. coli* ranged from 315 to 320 cfu/s and cumulative discharge ranged from 29.70 to 31.22 L/s

ranged from 361 to 19,200 copies/100 ml with a median value of 1170 copies/100 ml in Y1. Values ranged from 762 to 12,700 copies/100 ml with a median value of 6050 copies/100 ml in Y2. At the reference area, 3 MST samples were collected in Y1 and none were positive for the biomarker. In Y2, the biomarker was detected in 1 of the 6 MST samples, but the number of biomarker copies was too small to quantify.

Discussion

The results suggest that reducing densities of wild pigs led to a reduction in *E. coli* and FC loads and concentrations in small forested watersheds in the treatment area. With one exception, all treatment watersheds in Y1 had mean *E. coli* concentrations that exceeded the USEPA recommendation of a maximum geometric mean of 126 cfu/100 ml for recreational watersheds (USEPA 2012; Fig. 5). In Y2, only three of the nine watersheds had mean concentrations that exceeded 126 cfu/100 ml. The watersheds included in this study had little surface runoff entering the stream channel, were not used for agriculture or livestock, and were free from potential sources of contamination by human waste (i.e., septic tanks), yet median concentrations in Y1 resembled concentrations found in urban watersheds in the Southeastern U.S. (Crim et al. 2012). Watershed characteristics remained the same and precipitation patterns were similar (Fig. 2) in Y1 and Y2. The only major change between the two periods was the reduction in the wild pig population. The change in slopes of the double mass curves occurred quickly suggesting that the initiation of pig removal had an immediate impact on *E. coli* and FC concentrations. Consequently, it is likely that pig removal efforts led to the decrease in fecal bacteria levels that was observed in Y2.

In reference watersheds where pig populations were low and no removal occurred, there were no statistical differences between Y1 and Y2 in terms of

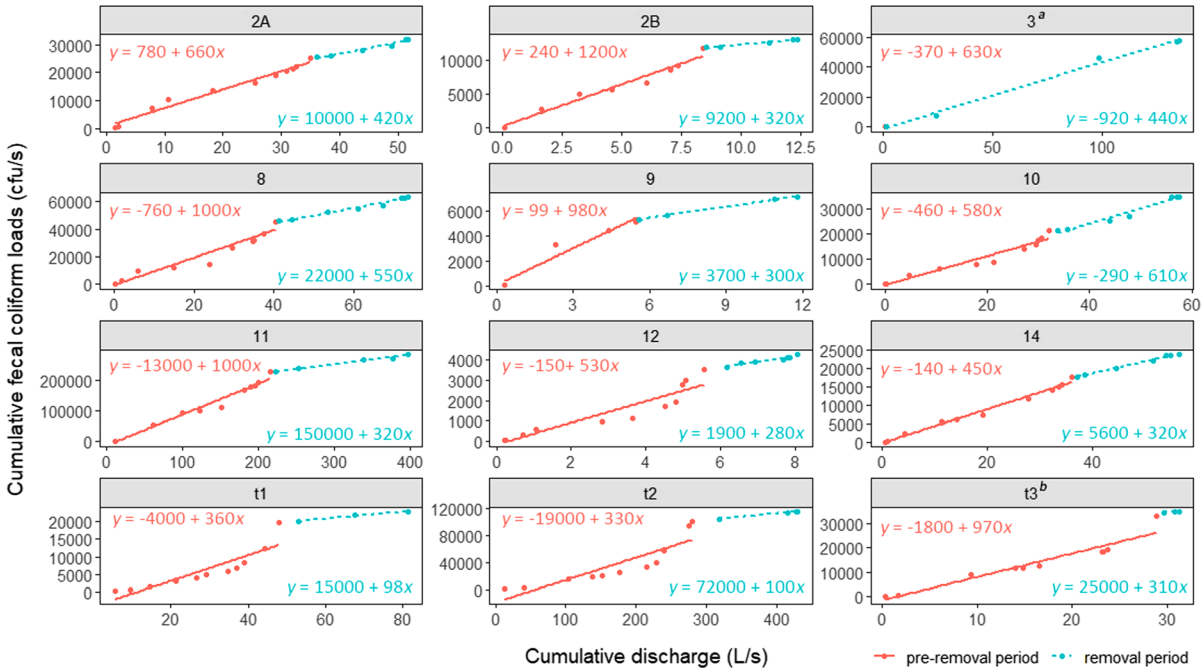

Fig. 4 Double mass curves of cumulative loads of fecal coliforms for the 12 streams analyzed in the study during Year 1 "pre-removal period" (May 2018 to June 2019) and Year 2 "removal period" (July 2019 to September 2020). Streams 2A—14 were located at EAPL (treatment) and streams t1–t3 were located at TUSK (reference). Year 2 values are cumulative from Year 1. In each plot, the regression equations for Year 1 and Year 2 are in the upper left and lower right corner, respectively. Note that the scale of both axes differs by plot.[a] During Year 1 sampling events (n = 3), cumulative loads of fecal coliforms ranged from 313 to 364 cfu/s and cumulative discharge ranged from 1.80 to 1.16 L/s.[b] During Year 2 sampling events (n = 3), cumulative loads of fecal coliforms ranged from 34,600 to 35,055 cfu/s and cumulative discharge ranged from 29.70 to 31.22 L/s

Table 1 Results from the Welch's t-tests comparing the volumetric flow-weighted slopes generated from the double mass regression curves for Years 1 and 2 at the overall location scale (treatment and reference)

	t	df	*p*-value	95% CI	
				Lower	Upper
Treatment					
E. coli	− 3.515	9.589	**0.006**	− 45.174	− 9.999
Fecal coliforms	− 3.880	10.930	**0.003**	− 603.019	− 166.253
Reference					
E. coli	− 0.360	2.266	0.75	− 11.356	9.416
Fecal coliforms	− 1.732	2.471	0.201	− 1176.045	412.742

p-values in bold represent statistical significance

concentrations or loads. This indicates that *E. coli* and fecal coliform levels were statistically stable within the reference area during those time periods.

Microbial source tracking results were similar in Y1 and Y2, even though the number of wild pigs decreased in Y2. It was apparent that wild pigs were still using the same riparian areas despite potential changes to home range size and location as sounders were being removed from the property. Our results indicate that while MST is useful to monitor sources of fecal contamination, it may not be the most accurate method to monitor levels of fecal contamination in

Table 2 Summary of concentrations and instantaneous loads of *E. coli* and fecal coliforms measured for treatment and reference streams during Year 1 (May 2018–June 2019) and Year 2 (July 2019–September 2020)

	YEAR 1				YEAR 2			
	Conc (cfu/100 ml)		Load (cfu/s)		Conc (cfu/100 ml)		Load (cfu/s)	
	Median	SE	Median	SE	Median	SE	Median	SE
E. coli								
Treatment	300	168.26	36.43	2082.63	67	16.55	9.43	30.69
Reference	0	30.16	0	8.20	33	35.46	2.51	32.60
Fecal coliforms								
Treatment	6500	1214.34	1024.25	1030.34	3133	393.77	466.25	918.77
Reference	3000	1156.64	2094.20	1287.46	1083.5	1822.13	1191.63	919.94

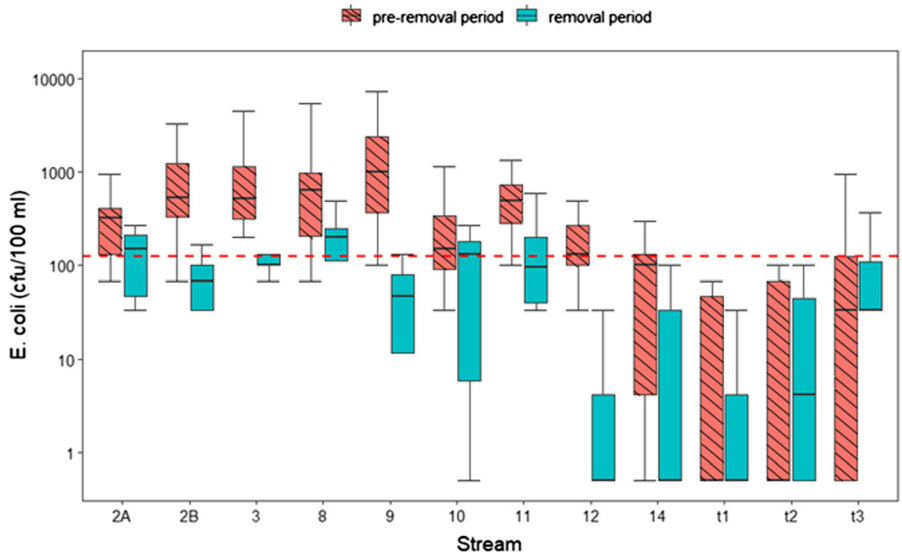

Fig. 5 *Escherichia coli* concentrations (cfu/100 ml) in water samples from the 12 streams analyzed in the study during Year 1 "pre-removal period" (May 2018 to June 2019) and Year 2 "removal period" (July 2019 to September 2020). Streams 2A—14 were located at EAPL (treatment) and streams t1–t3 were located at TUSK (reference). The dashed line indicates the USEPA's recommended maximum geometric mean for *E. coli* concentrations in recreational watersheds (126 cfu/100 ml). Note that the y-axis is on a log scale

streams. However, a major question remains—will water quality degrade again and, if so, at what rate, once intensive control efforts are discontinued?

While our study found that *E. coli* and FC levels decreased after pig removal began, future events that stir stream sediment could cause fluctuations in fecal bacteria levels. Stream sediment often acts as a sink for *E. coli* and other pathogens, and bacteria may persist longer in sediment than in the overlaying water column (Garzio-Hadzick et al. 2010). Additionally, fecal bacteria levels may differ downstream or in larger watersheds as smaller streams merge with larger tributaries. The reduction in fecal contamination of streams by removing wild pigs depends on several factors, such as stream order, the number of wild pigs, and stream hydrology and physical characteristics, so continued monitoring is necessary to observe changes in water quality. This study examined changes in fecal bacteria levels in streams in response to wild pig removal efforts and suggests that removal efforts can decrease fecal contamination of low-order streams. However, again, the longevity of the decrease is dependent on future fluctuations in wild pig populations combined with the intensity of future control efforts.

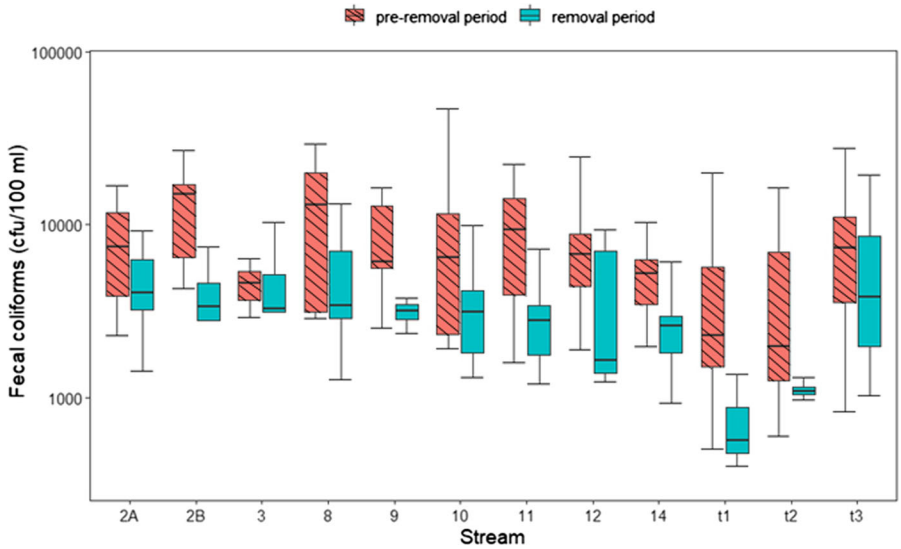

Fig. 6 Fecal coliform concentrations (cfu/100 ml) in water samples from the 12 streams analyzed in the study during Year 1 "pre-removal period" (May 2018 to June 2019) and Year 2 "removal period" (July 2019 to September 2020). Streams 2A—14 were located at EAPL (treatment) and streams t2–t3 were located at TUSK (reference). Note that the y-axis is on a log scale

Table 3 Results from DNA analysis of swine fecal biomarkers (copies/100 ml) in water samples collected from streams during Year 1 (May 2018–June 2019) and Year 2 (July 2019–September 2020) at treatment and reference locations

Stream	YEAR 1				YEAR 2		
	Jun 2018	Jul 2018	Dec 2018	Apr 2019	Aug 2019	Feb 2020	Jun 2020
Treatment							
2A	DNQ	–	18,300	692	–	1,440	3,230
2B	ND	–	19,200	ND	–	DNQ	–
3	DNQ	ND	826	–	ND[a]	10,800	–
8	361	ND	5,210	ND	ND[a]	10,200	12,700
9	3540	–	1,290	–	ND[a]	6,050	DNQ
10	ND	–	1,900	DNQ	–	1,070	171,000
11	577	–	10,500	622	–	7,790	12,300
12	ND	ND	619	DNQ	–	880	ND
14	DNQ	–	1,050	DNQ	–	762	ND
Reference							
t1	–	–	–	ND	ND	ND	–
t2	–	–	–	ND	–	ND	ND
t3	–	–	–	ND	ND	DNQ	–
Detections/Total	6/9	0/3	9/9	5/7; 0/3	0/3; 0/2	9/9; 1/3	5/7; 0/1

[a]Streams were stagnant on this collection date due to prolonged drought conditions

ND Not detected; *DNQ* Detected not quantified (concentration below limit of quantification). Dashes indicate that samples were not collected from that stream

Conclusion

Our results show that while wild pigs contribute to fecal contamination of streams, the contamination can be reduced by removing wild pigs from the area and decreasing population densities. Further research is needed to determine whether there are long-term effects from pig invasion regarding *E. coli* and fecal

coliforms persisting in stream sediment. Additionally, the downstream fate of *E. coli* and other pathogens introduced by wild pigs remains a critical question. These data indicate that targeted removal of wild pigs on a 4500 ha area can reduce fecal contamination of streams but does not indicate whether 'rebounding' of fecal contamination may occur if removal efforts are reduced in intensity and pig populations increase accordingly.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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