1-4-2022

Management-intensive grazing impacts on total *Escherichia coli*, *E. coli* O157: H7, and antibiotic resistance genes in a riparian stream

Laura M. Rubeck

James E. Wells

Kathy Hanford

Lisa M. Durso

Walter H. Schacht

See next page for additional authors

Authors

Laura M. Rubeck, James E. Wells, Kathy Hanford, Lisa M. Durso, Walter H. Schacht, and Elaine D. Berry

Science of the Total Environment 817 (2022) 152611

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Management-intensive grazing impacts on total *Escherichia coli*, *E. coli* O157:H7, and antibiotic resistance genes in a riparian stream

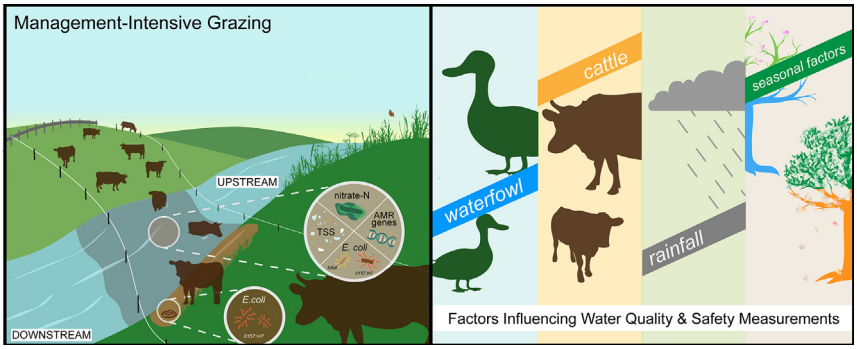
Laura M. Rubeck [a,1], James E. Wells [b], Kathryn J. Hanford [c], Lisa M. Durso [d], Walter H. Schacht [e], Elaine D. Berry [b,*]

[a] University of Nebraska-Lincoln, U.S. Meat Animal Research Center, 844 Road 313, Clay Center, NE 68933, USA
[b] USDA, Agricultural Research Service, U.S. Meat Animal Research Center, 844 Road 313, Clay Center, NE 68933, USA
[c] University of Nebraska-Lincoln, Department of Statistics, 343A Hardin Hall, Lincoln, NE 68583, USA
[d] USDA, Agricultural Research Service, Agroecosystem Management Research Unit, 251 Filley Hall, University of Nebraska-Lincoln East Campus, Lincoln, NE 68583, USA
[e] University of Nebraska-Lincoln, Department of Agronomy and Horticulture, 202 Keim Hall, Lincoln, NE 68583, USA

HIGHLIGHTS

- Grazing without reducing water quality benefits cattle producers and environment.
- Samples collected weekly, then monthly after grazing to determine long-term effects.
- Daily moves limited cattle access to any one section of the streambank.
- Preserving streambank and leaving 50% of forage reduced transport in runoff.
- Management-intensive grazing reduced extent of negative impacts on water quality.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 September 2021
Received in revised form 17 December 2021
Accepted 18 December 2021
Available online 4 January 2022

Editor: Jay Gan

Keywords:
Cattle
Water quality
Antimicrobial resistance
E. coli
Runoff
Sediment

ABSTRACT

The impacts of management-intensive grazing (MIG) of cattle on concentrations of total *Escherichia coli*, total suspended solids (TSS), and nitrate-nitrite nitrogen (NO_3 + NO_2-N), and occurrence of *E. coli* O157:H7 and selected antibiotic resistance genes (ARGs) in stream water and/or sediments were evaluated. Cattle were grazed for two-week periods in May in each of three years. Overall, grazing increased total *E. coli* in downstream water by 0.89 $\log_{10}$ MPN/100 mL ($p < 0.0001$), and downstream total *E. coli* concentrations were higher than upstream over all sampling intervals. Downstream TSS levels also increased ($p \leq 0.0294$) during grazing. In contrast, there was a main effect of treatment for downstream NO_3 + NO_2-N to be lower than upstream (3.59 versus 3.70 mg/L; $p = 0.0323$). Overwintering mallard ducks increased total *E. coli* and TSS concentrations in January and February ($p < 0.05$). For precipitation events during the 24 h before sampling, each increase of 1.00 cm of rainfall increased total *E. coli* by 0.49 $\log_{10}$ MPN/100 mL ($p = 0.0005$). In contrast, there was no association of previous 24 h precipitation volume on TSS ($p = 0.1540$), and there was a negative linear effect on NO_3 + NO_2-N ($p = 0.0002$). *E. coli* O157:H7 prevalence was low, but the pathogen was detected downstream up to 2½ months after grazing. Examination of ARGs *sul1*, *ermB*, $bla_{ctx\text{-}m\text{-}32}$, and *intI1* identified the need for additional research to understand the impact of grazing on the ecology of these resistance determinants in pasture-based cattle production. While *E. coli* remained higher in downstream water compared to upstream, MIG may reduce the magnitude of the downstream *E. coli* concentrations. Likewise, the MIG strategy may prevent large increases in TSS and NO_3 + NO_2-N concentrations during heavy rain events. Results indicate that MIG can limit the negative effects of cattle grazing on stream water quality.

* Corresponding author.
E-mail address: Elaine.Berry@usda.gov (E.D. Berry).
[1] Present address: USDA, Natural Resources Conservation Service, 120 W Fairfield, Suite 1, Clay Center, NE 68933, USA.

http://dx.doi.org/10.1016/j.scitotenv.2021.152611
0048-9697/Published by Elsevier B.V.

1. Introduction

Riparian ecosystems are transition zones between aquatic and terrestrial ecosystems, and serve as critical buffers that provide important ecological services relative to watershed function and water quality (Capon, 2020; Skinner, 1991). The presence of palatable forage, water, and/or shade make riparian locations inviting to grazing livestock. However, unmanaged grazing can degrade riparian ecosystems and negatively impact water quality. Potential ecological impacts of this unmanaged grazing include decreased productivity of streamside vegetation, deteriorated streambank stability, soil erosion, unrestricted runoff and the introduction of sediment and nutrients into surface waters, degraded habitat structure, and reduced water quality (Fitch and Adams, 1998; Schwarte et al., 2011). Fecal deposition directly in or beside surface water can result in the introduction of fecal bacteria and pathogens, potentially affecting the health of humans and other animals (Cooley et al., 2007; Sunohara et al., 2012). Cattle are an important reservoir of *E. coli* O157:H7, and can carry other pathogens and antimicrobial resistant bacteria (Wells and Berry, 2017). Manure contains numerous nutrients including nitrogen, which can affect microbial growth and eutrophication of water bodies, leading to increased algal growth and decreased oxygen levels (Belsky et al., 1999).

Despite the potential for negative impact, healthy riparian ecosystems can co-exist with livestock when grazing strategies are employed that vary grazing intensity, duration, timing, frequency, and length of recovery to balance with such things as the period of vegetation growth and production (Haan et al., 2006; Schwarte et al., 2011; Swanson et al., 2015). As an example, early season grazing can allow for sufficient time for new plant growth to protect the bank from erosion during the dormant season (Swanson et al., 2015). A simple reduction in stocking rate is often not an effective means of controlling grazing distribution and associated riparian function and recovery because grazing animals tend to concentrate in riparian areas regardless of stocking rate (Swanson et al., 2015). Instead, control within the grazing management unit can be increased through isolating the larger area into multiple smaller pastures and using rotational grazing to minimize the amount of time any one part of the riparian area is exposed to grazing. Management-intensive rotational grazing (MIG) is characterized by as many as seven or more pastures and high grazing pressure within each pasture during a short grazing period followed by a long recovery period (Gerrish and Ohlenbusch, 1998; Shawver et al., 2020). As with other rotational grazing systems, MIG matches available forage with livestock demand but greatly limits the amount of time each pasture is exposed to grazing (Shawver et al., 2020). The number of annual grazing cycles through the pastures is based on dominant vegetation type and annual precipitation. Compared to continuous grazing, riparian ecosystems and water quality can benefit from managed rotational grazing in regard to increased vegetation cover (Haan et al., 2006), reduced sediment and nutrient transport in runoff (Haan et al., 2006), reduced stream bank erosion (Lyons et al., 2000), reduction in concentration of fecal indicator bacteria (Sovell et al., 2000), and improved water clarity (Sovell et al., 2000). In particular, grazing strategies that limit the congregation of cattle and the concentration of their feces deposits near or in water bodies can limit the risk for introducing pathogens, other fecal bacteria, and nutrients (Hansen et al., 2020; Schwarte et al., 2011).

Fresh, safe water is a critical natural resource; hence, additional riparian grazing research is warranted for developing best management practices that reduce surface water pollution and improve the riparian habitat (Agouridis et al., 2005; Larsen et al., 1998). Grazing studies that have examined the introduction of fecal bacteria to riparian streams typically have determined the concentrations of fecal indicator microorganisms, rather than the occurrence of pathogens such as *E. coli* O157:H7. In addition to zoonotic pathogens, antibiotic resistant bacteria represent an emerging global health issue for humans, animals, and the environment, making a One Health approach necessary for more effectively addressing these problems. However, data are limited regarding the prevalence of naturally-occurring antibiotic resistant bacteria or genes in riparian or grazing environments, or the potential for their introduction into soils or surface waters by grazing cattle. Likewise, there is limited data on the persistence of these bacteria or their genes in riparian environments after removal of grazing cattle. A One Health panel of four antibiotic resistance genes (ARGs) was chosen to study antibiotic resistance in this production environment. Targets were chosen based on their relevance for human, animal, and environmental health. The *sul1* gene codes for sulfonamide resistance and is commonly targeted in environmental surveillance for ARGs. The *ermB* gene encodes macrolide resistance and the $bla_{ctx\text{-}m\text{-}32}$ gene encodes third-generation cephalosporin resistance. Sulfonamides, macrolides, and cephalosporins are categorized as "Highly Important" or "Critically Important" for human health by national and international public health agencies (WHO, 2019). Furthermore, select compounds within these three drug classes are used to treat infections in humans and food animals, making these gene determinants relevant for both human and animal health and for environmental surveillance of antibiotic resistance. The fourth PCR target was the *intI1* gene, which codes for the clinical class 1 integron-integrase that is associated with horizontal gene transfer of resistance to antibiotics, heavy metals, and other disinfectants, and has been proposed as a marker for human-impacted antibiotic resistance (Gillings et al., 2015). These four specific resistance determinants were selected by a group of scientists seeking to develop a core procedure for surveillance for agricultural antibiotic resistance, and have been used in previous studies of environmental resistance (Gurmessa et al., 2021; Yang et al., 2020; Yang et al., 2021).

The objectives of this study were to determine the immediate and long-term effects of MIG on microbial and physiochemical aspects of water quality and safety of a riparian stream, including the concentrations of total *E. coli*, total suspended solids (TSS), and nitrate-nitrite nitrogen (NO_3 + NO_2-N), and the prevalence of the pathogen *E. coli* O157:H7 and selected antibiotic resistance genes.

2. Materials and methods

2.1. Site description

The research site is along a stream at the US Meat Animal Research Center (USMARC) near Clay Center in south-central Nebraska. The USMARC is located on the site of the former Blaine Naval Ammunition Depot, used for the manufacturing and storage of munitions during World War II and the Korean Conflict. Groundwater contamination resulting from these activities was found on the USMARC property in the mid-1980s, and a groundwater remediation plan was developed and implemented by the US Army Corps of Engineers (USACE), with water treatment initiated in 2013 (USACE, 2010). Wells remove an estimated 14,000 L of groundwater per minute for treatment on a continuous basis. The treated groundwater is discharged into a 0.5 km-long rock-lined canal that was constructed by the USACE as part of the remediation project, to connect the discharged treated groundwater to an existing natural drainage. As previously described, the stream traverses approximately 11.3 km across USMARC and into a reservoir, with the grazing study site located at a distance of 0.5 km from the groundwater discharge point (Abimbola et al., 2020; Hansen et al., 2020).

The study site is located in a loamy plains ecological site in the Central Loess Plains Major Land Resource Area (MLRA) (ARS-NRCS-NMSU, 2021; NRCS, 2006). The physical landscape is comprised of nearly level to gently rolling plains altered by many narrow, shallow stream valleys. Additionally, the site is located in the Rainwater Basin wetland region, a broad ecological area encompassing 9700 ha that occupies portions of the 13 northernmost counties in the Central Loess Plains MLRA (ARS-NRCS-NMSU, 2021; NRCS, 2006). The dominant soil order in the Central Loess Plains is mesic, ustic Mollisols (NRCS, 2006), and the dominant soil series in the study site is Hastings silt loam (NRCS, n.d. Web Soil Survey). Most of the grassland at USMARC is a mixture of introduced and native grasses utilized for grazing and haying. Vegetation within the study site is dominated by introduced, cool-season perennial grasses such as Kentucky bluegrass (*Poa pratensis*) and smooth bromegrass (*Bromus inermis*).

The 30-year average annual precipitation at the study site was 73.1 cm of precipitation, with 75% falling during the growing season from April

through September (Table A.1). Rainfall during the growing season was recorded at a rain gauge located 0.6 km west of the study site. Precipitation was measured for 24 h periods starting at 0900 h daily. These data were supplemented with year-round rainfall and snowfall data collected by a weather station at the University of Nebraska-Lincoln South Central Agricultural Laboratory, located approximately 9.0 km east-northeast of the study site.

2.2. Management-intensive grazing of cattle

All animal use protocols were approved by the USMARC Animal Care and Use Committee.

A 1.03-km long stretch of grassland was selected along the stream. The fenced 12-ha area was divided into fifteen 0.8-ha pastures, each of which allowed cattle access to both sides of the bank along the stream (Fig. A.1). The individual pastures were fenced off using temporary polywire and step-in posts. The width of the stream averaged 5 m and the distance from stream bank to nearest boundary fence parallel to the stream ranged from 5 to 108 m. The stream was the sole water source.

The target for percentage forage utilization in each pasture was 45–50%. Forage availability for stocking rate calculations was estimated as described in the Supplementary Material. The pastures were rotationally grazed for two-week periods beginning in early May of each year. Fifty head of fall-calving heifers (ca. 500 kg each) were grazed in each of Years 1 and 2 (May 10–25, 2017 and May 9–24, 2018, respectively). In Year 3, the pastures were grazed by 39 head of mature cows (average 590 kg each) from May 8 to 23, 2019. Each pasture was grazed for 1 day with the cattle moved to a fresh pasture at 0900 h the next day. A blend of salt and mineral was moved each morning to the new pasture and its placement varied at distances between 50 and 109 m from the stream edge.

2.3. Sampling procedures: water, sediment, soil, and bovine feces

An upstream-downstream design was used to compare the microbial and physicochemical water quality of the stream before, during, and after grazing (NRCS, 2003). In each year, stream water and sediment samples were collected weekly during a six-week period starting two weeks before grazing, during the two weeks of grazing, and then ending two weeks after the grazing period (Weeks (Wk) −2, −1, 0, 1, 2, 3, and Month (Mo) 1; April into June) and then monthly during the remainder of the year (July to April).

During each sampling event, five water samples were collected at each of the immediate upstream and downstream locations of the stream as it entered and exited the grazing site (Fig. A.1). All downstream samples were collected first, followed by the upstream samples. In addition, five sediment samples were collected from the stream bottom at each of the upstream and downstream sites on each sample date. The water samples were collected before the sediment samples. In Year 1, two to three soil samples were also collected at each of the upstream and downstream sites on each sample date.

Water samples (approximately 1000 mL) were collected in sterile 1000-mL polypropylene bottles held on a telescoping sampler pole (Nasco, Fort Atkinson, WI). At each of the upstream and downstream sites, water was collected by dipping separate bottles at five locations spaced evenly across the width of the stream. Similarly, sediment samples (50 g) were collected from the stream bottom, by wading into the stream at five locations evenly spaced across the width of the stream, at each of the upstream and downstream locations. The sediment samples were collected using a hand trowel and placed in separate sample bags. The trowel was wiped clean, sanitized with 70% isopropanol, and wiped dry between each sample. The soil samples were collected using a JMC Backsaver soil sampler with 30.5-cm long, 1.9-cm diameter dry soil sampler tube (Forestry Suppliers, Jackson, MS). Each soil sample was placed in a separate sample bag, and the sampler tube was wiped clean and sanitized with 70% isopropanol before collecting the next sample.

To determine if the cattle were shedding *E. coli* O157:H7, freshly defecated feces (less than 24 h old) were collected twice weekly during the two-week grazing event in each year and analyzed for the presence of the pathogen. Fecal samples were randomly collected along both stream banks immediately after the cattle were moved to the next pasture, and placed in sterile sample bags. Twenty-five fresh fecal pats were sampled on each of the four sample days, totaling 100 samples annually.

For each sampling event, all sample types were collected about 0900 h, placed in coolers, and immediately transported to the laboratory for processing.

2.4. Analytical methods

2.4.1. Determination of total E. coli and E. coli O157:H7 in water, sediment, and/or feces

Total *E. coli* concentrations were determined in 100 mL of each water sample using IDEXX Colilert reagents with Quanti-Tray/2000 according to manufacturer's directions (IDEXX Laboratories, Inc., Westbrook, ME). Total *E. coli* concentrations are reported as the most probable number (MPN)/100 mL of water.

For determination of *E. coli* O157:H7 in water, 100 mL of each sample was measured into a sterile filtered bag and 100 mL of double-strength tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD) was added and mixed in by hand massage. For determination of *E. coli* O157:H7 in sediment and feces, 10 g of the sample were measured into tared sterile filtered bags and 90 mL of TSB containing 100 mM potassium phosphate buffer (TSB-PO_4; Barkocy-Gallagher et al., 2005) was added and massaged by hand to mix. The enrichments for all sample types were incubated at 25 °C for 2 h before being transitioned to 42 °C for 6 h, and then held at 4 °C overnight. Enriched water, sediment, and fecal samples were processed using immunomagnetic separation for *E. coli* O157:H7 isolation (Berry et al., 2010). The samples (1.0 mL) and 20 μL of anti-O157 Dynabeads (Invitrogen Corp., Carlsbad, CA) were placed into deep-well blocks. Blocks were shaken for 20 min and a Kingfisher 96 magnetic particle processor (Thermo Fisher Scientific Inc., Waltham, MA) was utilized for bead retrieval, washing, and elution. The concentrated beads (50 μL) were then spread onto CHROMagar O157 plates (DRG International, Inc., Mountainside, NJ) and incubated for 24 h at 37 °C. The plates were evaluated after incubation, and characteristic *E. coli* O157:H7 colonies were screened using DrySpot *E. coli* O157 latex agglutination tests (Oxoid Ltd., Basingstoke, UK). Suspect *E. coli* O157:H7 colonies were isolated and their identities were confirmed by multiplex PCR for genes for O157, H7 flagella, intimin, and Shiga toxin 1 and 2 (Hu et al., 1999). The $fliC_{H7}$ primer sequences used in the multiplex PCR were those of Gannon et al. (1997) and PCR conditions were those of Paton and Paton (1998). To be confirmed to be *E. coli* O157:H7, an isolate had the genes for O157, H7, intimin, and at least one of Shiga toxin genes.

2.4.2. Determination of total suspended solids and nitrate-nitrite nitrogen in water

The concentration of total suspended solids (TSS) in water was determined using US Geological Survey (USGS) Method I-3765-85 (USGS, 1989). Nitrate-nitrite nitrogen (NO_3 + NO_2-N) concentration in water was assessed using US Environmental Protection Agency (USEPA) Method 353.2 (USEPA, 1993).

2.4.3. Determination of antibiotic resistance genes

On each sampling day in Year 1, subsamples of bovine feces, water, sediment, and soil were frozen at −20 °C for later processing for PCR determination of four antibiotic resistance determinants: *sul1*, *ermB*, $bla_{ctx\text{-}m\text{-}32}$, and *intI1*. The DNA isolation and PCR reactions were performed as previously described (Meyers et al., 2020). The PCR primers and thermocycling conditions for each ARG are shown in Table A.2. Briefly, frozen samples were thawed and processed for DNA extraction using the Qiagen DNeasy Power Soil Kit (Germantown, MD) or PowerWater Kit, according to manufacturer's directions, with a bead beating step used for cell lysis. REDTaq®

ReadyMix™ Reaction Mix (Sigma Chemical, Darmstadt, Germany) was used with 2.0 μM primers to perform uniplex PCR reactions for each target (Table A.2), and agarose gel electrophoresis was used for amplicon visualization. Positive and negative controls were run with each reaction. Twelve of 170 water samples were not analyzed because there was not enough sample for DNA isolation or not enough DNA to measure.

2.5. Statistical analysis

Total *E. coli* concentrations in water were transformed to $\log_{10}$ MPN/100 mL for statistical analysis. Total *E. coli* concentrations, TSS (mg/L), and NO_3 + NO_2-N (mg/L) levels were analyzed using the PROC GLIMMIX procedure (SAS Institute Inc., Cary, NC). Approximate normal distributions were assumed for total *E. coli*, TSS, and NO_3 + NO_2-N. The initial model for the analysis included the fixed effects of year (1, 2 and 3), treatment (upstream or downstream), interval (17 sampling intervals from Week −2 to Month 11), year ∗ treatment, year ∗ interval, and treatment ∗ interval interactions and the random year ∗ treatment ∗ interval effect. All fixed effects were tested over the random year ∗ treatment ∗ interval effect. Any non-significant interaction terms were dropped from the initial model and the data were reanalyzed. Least squares means estimated from the final model are presented in the text and figures.

Linear regression was performed to determine if there was a linear relationship between the prior 24 h precipitation on the various response variables (interval ∗ year means), using InStat version 3.0 (GraphPad Software, Inc., San Diego, CA).

The prevalence of the antibiotic resistance genes in feces, water, sediment, and soils was determined in Year 1 only. Selected comparisons of gene frequencies were assessed using the two-tailed Fisher exact test (Uitenbroek, 1997).

Differences were considered significant at $p < 0.05$ and were considered tendencies at $0.05 < p < 0.10$.

3. Results and discussion

3.1. Total E. coli concentrations in water

The results from the initial model showed that the treatment, interval, and interval ∗ year terms had significant ($p < 0.05$) effects on *E. coli* concentrations. The treatment ∗ year and interval ∗ treatment terms were not significant ($p > 0.10$), indicating that differences in total *E. coli* concentrations in water collected at the upstream and downstream locations were consistent over the sampling intervals and over the three years, as demonstrated in Fig. 1. In the reduced model, the effect of treatment did not interact with either interval or year, and cattle grazing increased total *E. coli* concentrations in downstream water by 0.89 $\log_{10}$ MPN/100 mL ($p < 0.0001$). This result is consistent with previous reports of increased *E. coli* levels in water as a result of cattle grazing along or near riparian streams (Abimbola et al., 2020; Hansen et al., 2020; Sunohara et al., 2012; Vidon et al., 2008). In addition to a main effect of treatment, comparisons within treatment show that total *E. coli* concentrations in downstream water increased following the introduction of the cattle to the grazing site ($p < 0.05$) (Fig. 1). In downstream water, total *E. coli* concentrations increased from 0.88 $\log_{10}$ MPN/100 mL on Wk −1 to 2.65 $\log_{10}$ MPN/100 mL on Wk 2 ($p = 0.0085$) when the cattle were removed. The downstream total *E. coli* levels remained higher ($p < 0.05$) or tended to be higher ($p = 0.09$) through Mo 2 before dropping to lower levels.

Total *E. coli* in bovine feces may arrive in water via direct defecation by cattle, or more typically indirectly via runoff from bovine feces deposited in surrounding pastures. Among other factors, the persistence of viable *E. coli* after defecation is directly associated with the magnitude of the risk for its

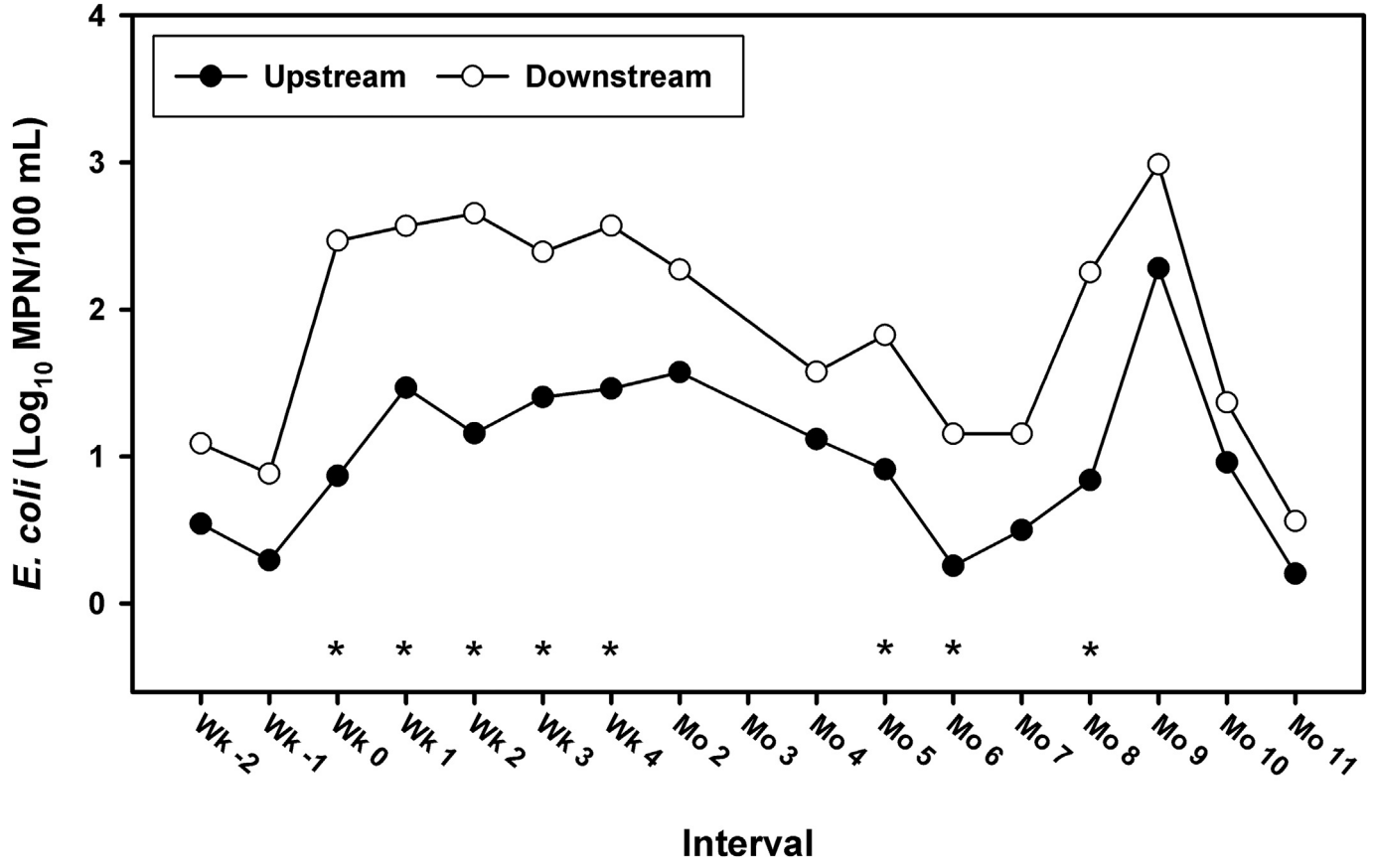


Fig. 1. Least squares means of total *E. coli* concentrations ($\log_{10}$ most probable number [MPN]/100 mL) in water collected at the upstream and downstream locations during the 3-year study. The standard error of the least squares means is 0.40. Asterisks denote intervals with significant treatment differences ($p < 0.05$).

detectable transport into water bodies. Once the cattle are removed and no longer replenishing the pasture with fresh feces, the numbers of *E. coli* generally will decrease, although this bacterium can persist and sometimes grow in feces or soils for weeks up to months (Avery et al., 2004; Berry et al., 2007; Oliver et al., 2005; Muirhead et al., 2005). The finding that total *E. coli* was consistently higher in downstream water samples compared to upstream further highlights the ability of this organism to persist in the environment outside of the gastrointestinal tract; however, other potential sources of *E. coli* in addition to cattle may have contributed to this outcome and are further discussed below.

Rainfall volume and intensity affected *E. coli* concentrations in stream water. There was a positive linear regression effect ($p = 0.0005$) of precipitation amount occurring in the 24 h prior to sampling on total *E. coli* concentrations in water. Each 1.00 cm increase in precipitation corresponded with an estimated 0.49 $\log_{10}$ MPN/100 mL increase in total *E. coli* (data not shown). Previous studies have shown that heavy rainfall events resulting in runoff can increase concentrations of *E. coli* and other fecal bacteria in surface waters in grazing areas or near crop land on which livestock wastes are applied (Hansen et al., 2020; Oliver et al., 2005; Tornevi et al., 2014). In summer storms, the presence and density of cattle in pastures within 50 m of a stream in the previous 30 days increased *E. coli* concentrations in stream water, with a stronger correlation when cattle were in pastures adjacent to the stream on the same day as the storm event (Hansen et al., 2020). These findings suggest that in addition to persistence, the higher downstream water concentrations of total *E. coli* observed from Wk −1 to Mo 2 (mid-May to July) may be due to increased rainfall and runoff during this period; the three months from May through July are normally the months with highest precipitation, due in part to spring and summer storms (Table A.1). It is notable that waterborne disease outbreaks are frequently associated with high rainfall and runoff events (Curriero et al., 2001).

In addition to runoff, stream bottom sediments are a significant source of *E. coli* and other bacteria for contamination of the overlying water. Outside of its primary habitat in the lower intestine of warm-blooded animals, *E. coli* may attach to the surfaces of soil, sand, or other particles; in water, these particles can settle out and *E. coli* can remain viable for long periods and even grow in the sediment environment (Ishii and Sadowsky, 2008; Savageau, 1983). Animals in the stream and/or heavy rainfall that increases stream volumes and flow can resuspend stream bottom sediments, increasing concentrations and transport of this microorganism in stream waters (Agouridis et al., 2005; Davies-Colley et al., 2008; Nagels et al., 2002). In the current study, increases in total *E. coli* concentrations coincided with increases in TSS at some intervals, and are further discussed below.

In the reduced model, there was a significant interaction of interval * year ($p = 0.0001$), indicating differences in water concentrations of total *E. coli* concentrations at a particular interval between different years. These differences were anticipated, given the likelihood for year-to-year differences in the timing and occurrence of storms and rainfall volumes. Fig. 2B shows the water concentrations of total *E. coli* averaged over the upstream and downstream locations in each of the 3 years. Total *E. coli* concentrations at Wk −2, Wk 0, and Mo 3 were different between Years 1 and 2 ($p < 0.05$). In addition, total *E. coli* concentrations at Wk 0, Wk 1, and Wk 3 differed between Years 1 and 3 ($p < 0.05$). With the exceptions of Wk −2 and Mo 3, these differences occurred in sampling intervals in May, the month in which the highest precipitation normally occurs at this location (Table A.1). Comparison of Fig. 2A showing the preceding 24 h rainfall with Fig. 2B highlights several coinciding instances of high rainfall and increased *E. coli* concentrations.

In Mo 3 of Year 3, two events combined to result in extremely high concentrations of total *E. coli* in the water at both the upstream and downstream sampling locations. Firstly, a herd of 283 cattle had been grazed for one week on a field of irrigated forage that was adjacent to the section of the stream that was immediately upstream from the upstream sampling location. Secondly, a storm occurring overnight before the Mo 3 sampling interval dropped 5.3 cm of rain (Fig. 2A). The storm runoff from the grazed forage entered the stream upstream from the study site, resulting in average water concentrations of 5.13 $\log_{10}$ MPN/100 mL for upstream samples and 6.15 $\log_{10}$ MPN/100 mL for downstream samples (data not shown). Because of the unusual circumstance of upstream contamination and also because these extreme outliers impacted the analysis by causing non-convergence, the Year 3 Mo 3 data were omitted from the reduced model.

Total *E. coli* concentrations increased in both upstream and downstream water samples from Mo 7 to Mo 9 (December to February; $p < 0.05$), over seven months after the cattle were removed from the grazing site (Fig. 1). At Mo 9, *E. coli* concentrations in upstream and downstream water were 2.28 and 2.99 $\log_{10}$ MPN/100 mL, respectively. These high *E. coli* levels are attributed to overwintering waterfowl. For portions of January and February of each study year, freezing temperatures persisted long enough to freeze much of the surface water in the region. Because of the continuous discharge of treated groundwater, the entire section of the stream in the study site and the approximately 0.5 km-long section immediately upstream remained open and was often some of the only unfrozen surface water available. On the January and February sampling events (Mos 8 and 9) in each study year, we observed flocks of mallard ducks (up to 200 birds) in the unfrozen water of this entire stretch. The mallard (*Anas platyrhynchos*) is the most common North American species of duck, and will winter as far north as conditions allow, so long as they can find food and open water (Drilling et al., 2020). Previous studies have documented the potential for negative water quality impacts due to waterfowl including geese and ducks, which may also transmit zoonotic pathogens or antibiotic resistant bacteria (Meays et al., 2006; Reed et al., 2003; Somarelli et al., 2007; Vogt et al., 2019). Indeed, in watersheds with multiple land uses that included grazing cattle, Meays et al. (2006) found wildlife was the major contributor of *E. coli* (>73%), with avian *E. coli* accounting for >20% of isolates from all sources.

Comparing the results of this MIG study with those reported for other grazing studies is difficult because of the many differences between studies, such as production and grazing systems, cattle numbers and density, experimental designs and sampling frequency, geography and climate, and season of study, etc. However, comparison of the total *E. coli* concentrations that we observed to those reported for grazing and storm events in other studies suggest that a similar MIG strategy may serve to limit the magnitude of the total *E. coli* concentrations in surface waters. With the exception of the Mo 9 levels ascribed to ducks, the peak water concentrations of *E. coli* recorded in each year were 3.08, 2.41, and 2.86 $\log_{10}$ MPN/100 ml in Mo 3 of Year 1, Wk 2 of Year 2, and Wk 0 Year 3, respectively (Fig. 2B). In Years 1 and 3, 1.50 and 2.54 cm of rainfall, respectively, had fallen in the 24 h before the water samples were collected. The Year 2 water samples were collected after the cattle had been grazed on the site for two weeks. In comparison, Vidon et al. (2008) reported total *E. coli* water concentrations up to 4.84 $\log_{10}$ MPN/100 mL when cattle had year-round unrestricted access to the stream. Similarly, Davies-Colley et al. (2008) observed *E. coli* water levels of 4.70 to 5.00 $\log_{10}$ MPN/100 mL during high stormflow in a stream located in a region dominated by dairy farming. *E. coli* stream water concentrations associated with animal access and storm runoff in cattle grazing areas between 4.00 and 5.00 $\log_{10}$ MPN/100 mL are typical (Davies-Colley et al., 2004; Nagels et al., 2002; Oliver et al., 2005; Stott et al., 2011). Hence, MIG when compared to other grazing systems may both limit the extent and duration of high *E. coli* concentrations in water.

3.2. Prevalence of E. coli O157:H7 in feces, water, and sediment

Freshly defecated bovine feces were collected during the two-week grazing periods in each year and analyzed for *E. coli* O157:H7 to determine if the cattle were shedding the pathogen. In Year 1, *E. coli* O157:H7 was identified in 10% of the 100 feces samples collected, and in 100% of downstream water samples that were collected one week after grazing was initiated (Fig. 3). Thereafter, the pathogen was detected sporadically in downstream water and sediment through Mo 3. In Year 2, *E. coli* O157:H7 was not detected in any sample of bovine feces, water, or sediment. In

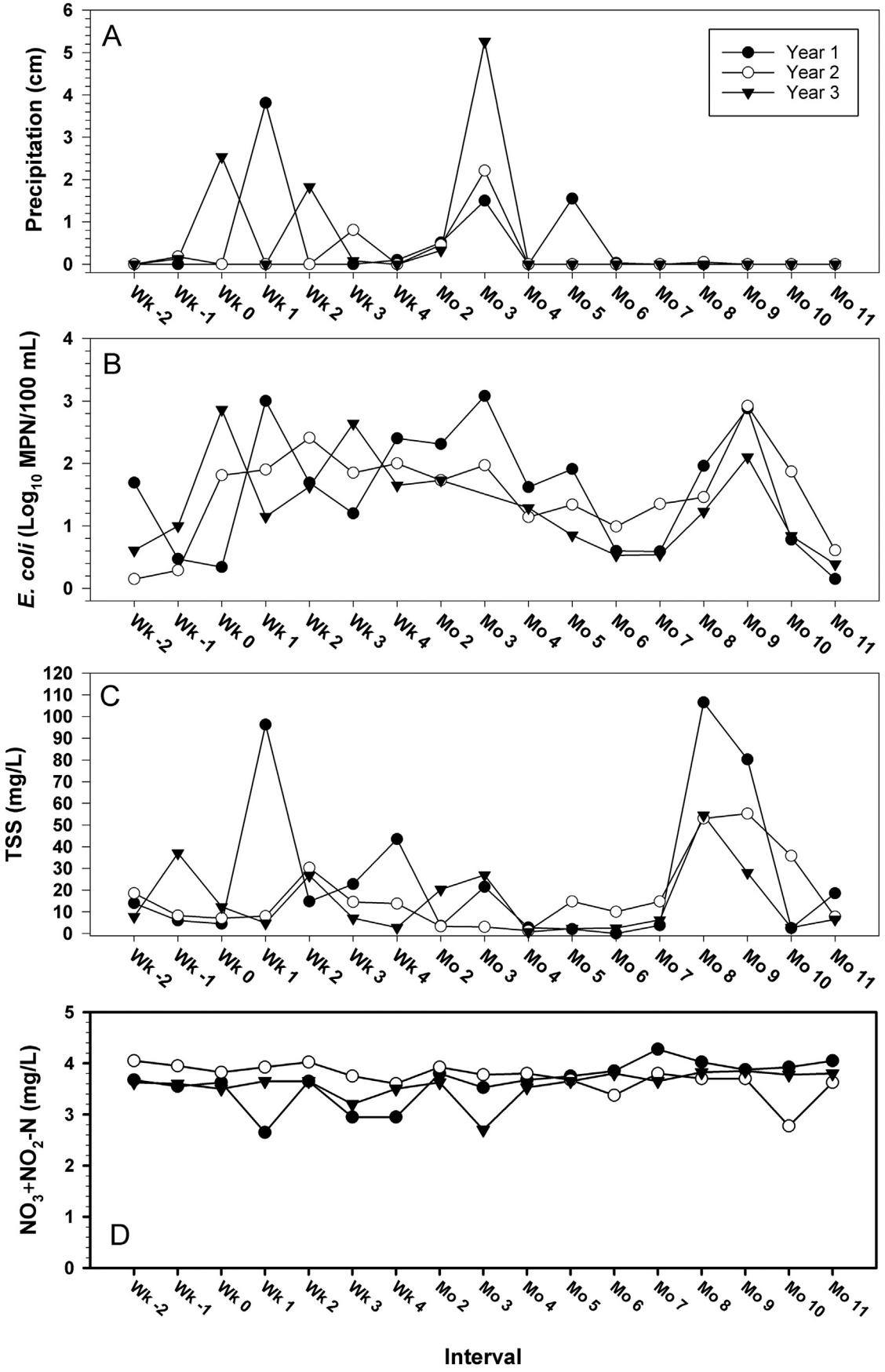


Fig. 2. (A) Precipitation that fell during the 24 h preceding the sampling interval, and concentrations of (B) total *E. coli* (log_{10} most probable number [MPN]/100 mL), (C) total suspended solids (TSS; mg/L), and (D) nitrate-nitrite nitrogen (NO_3 + NO_2-N; mg/L) in water at each interval averaged over treatment, in each year of the three-year study.

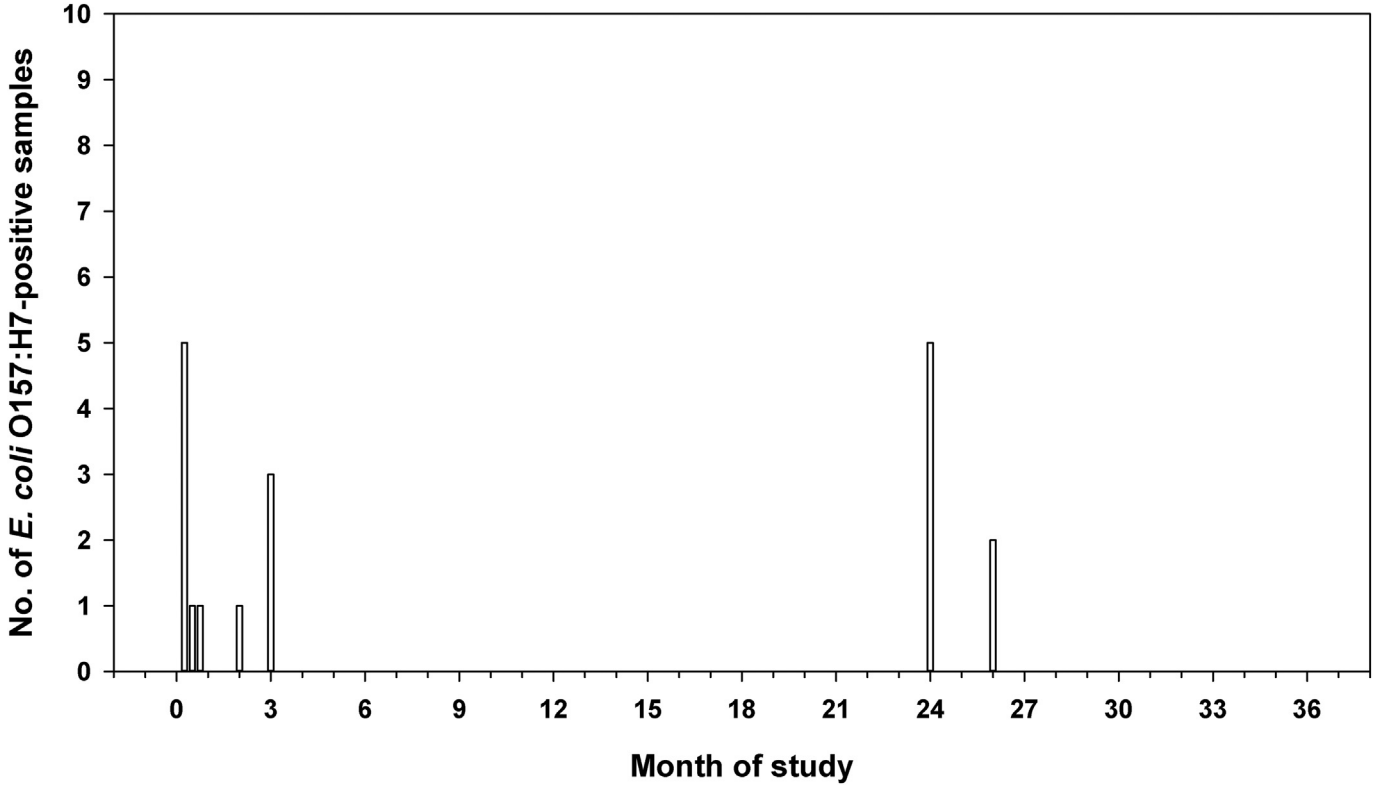


Fig. 3. Number of downstream water and sediment samples that were positive for *E. coli* O157:H7 among 10 samples collected at each sampling interval during the three-year study. Cattle were turned into the grazing site for two-week periods on Months 0, 12, and 24 (early May at Week 0 of each year).

Year 3, *E. coli* O157:H7 was not isolated from bovine feces, but was found in five downstream water samples collected at the initiation of grazing and in two downstream sediment samples at Mo 2.

This low prevalence of *E. coli* O157:H7 is consistent with our understanding of the seasonality of the shedding of this pathogen by cattle. The cattle were grazed on the site beginning in early May. The prevalence of *E. coli* O157:H7 in cattle feces typically is highest in the warmer months of summer and early fall (Barkocy-Gallagher et al., 2003; Van Donkersgoed et al., 1999). Furthermore, the shedding of higher concentrations of *E. coli* O157:H7 in bovine feces may also peak in summer and autumn (Ogden et al., 2004; McCabe et al., 2019). However, in combination with our fecal pat sampling plan, the occurrence of lower prevalence and/or concentrations of the pathogen may have caused us to miss its detection in feces of a shedding bovine. By randomly sampling 100 fecal pats over each annual two-week grazing period, we likely did not sample feces from each animal and/or did not sample feces from those cows that were actively shedding *E. coli* O157:H7 at detectable levels. This may account for the findings in Year 3 of *E. coli* O157:H7-positive samples of downstream water and sediment, without detection of the pathogen in bovine feces. Alternatively, other animal sources of *E. coli* O157:H7 may have provided the pathogen that was found in the downstream samples in Year 3. Deer, rabbits, raccoons, opossums, and starlings have been demonstrated to carry this pathogen, and are common at the USMARC (as reviewed by Berry and Wells, 2010). Wildlife is a likely source of *E. coli* O157:H7 isolated from one upstream water sample in the pre-grazing period (Wk −2) in Year 1 (data not shown).

Although the *E. coli* O157:H7 isolates were not subjected to molecular subtyping to confirm linkages between the cattle and environmental samples, the occurrence of the organism in downstream samples during and shortly after grazing suggests the cattle likely are the source. *E. coli* O157:H7 was detected up to Mo 3 in both stream water and sediment samples, from 84 to 99 days after defecation during the two-week grazing period (Fig. 3). Numerous studies have examined the in vitro survival of inoculated *E. coli* O157:H7 in different water types, including municipal, lake, river, and animal trough water. Reported survival ranges from several weeks up to several months, which support our observations of persistence for at least 84 to 99 days (Avery et al., 2008; Rice et al., 1992; Wang and Doyle, 1998). While generic total *E. coli* has been shown to adapt and become naturalized in soil and sediment environments, whether this occurs in *E. coli* O157:H7 is not clear (Ishii and Sadowsky, 2008; Jang et al., 2017). However, it is clear from this and other research that this pathogen can persist for long periods in environments outside the gastrointestinal tract. This presents risks where surface water is concerned, as high rains, runoff, and/or flooding can transport pathogens, potentially contaminating food crops or water sources for human or animal consumption (Cooley et al., 2007; Curriero et al., 2001).

3.3. Total suspended solids in water

The concentration of TSS was determined as a measure of water turbidity. In the initial model, the interval ∗ year term was not significant ($p = 0.5524$), indicating that the differences in TSS concentrations averaged over treatment between the three years were consistent over the intervals. In the reduced model, the significant interval ∗ treatment ($p = 0.0047$) indicated that the difference between the upstream and downstream TSS may differ among the intervals, so the simple effects of treatment were evaluated. The downstream concentrations of TSS were higher than the upstream TSS at Wk 1 ($p = 0.0016$) and Wk 2 ($p = 0.0294$) after the cattle were introduced to the grazing site (Fig. 4). Downstream TSS levels were also higher than the upstream TSS in Mo 8 and Mo 9 ($p < 0.0001$), when the mallard ducks were present. Comparison of Figs. 1 and 4 shows that concentrations of both TSS and total *E. coli* increased in response to the presence of cattle and ducks in the stream, with a notable exception. In Mo 8 and 9, TSS concentrations in the upstream water did not increase, likely because of the rock lining in the stream segment above the upstream sampling site. Hence, while the ducks contributed *E. coli* to the stream, the rocks prevented the resuspension of bottom sediments that is associated with their paddling and feeding activities.

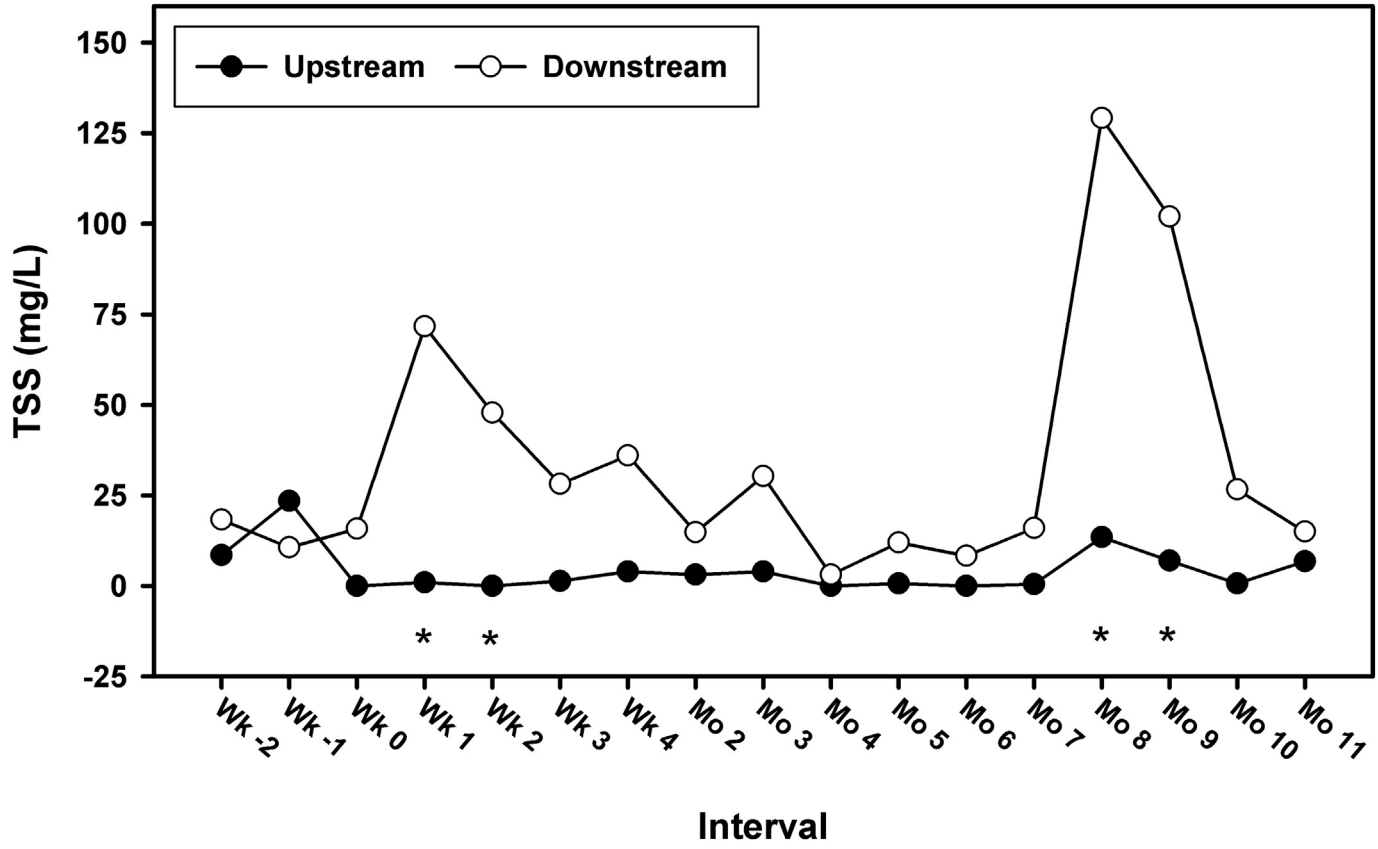


Fig. 4. Least squares means of total suspended solids (TSS; mg/L) in water collected at the upstream and downstream locations during the 3-year study. The standard error of the least squares means is 15.36. Asterisks denote intervals with significant treatment differences ($p < 0.05$).

In the reduced model, the treatment * year term approached significance ($p = 0.1040$), indicating that the differences between upstream and downstream TSS may differ between the years, so the simple effects of treatment at each year were evaluated. The nearing significance of treatment * year was due to the difference among the three years of the magnitude of difference between upstream and downstream TSS. These differences were significant in each year, with downstream TSS being significantly higher than upstream TSS. However, the difference was greater in Year 1 ($p < 0.0001$) than in Year 2 ($p = 0.0067$), than in Year 3 ($p = 0.0362$), as illustrated in Fig. 5.

Unmanaged grazing and unrestricted access to streams can result in decreased vegetation, soil erosion, reduced streambank stability, and unrestricted runoff, all of which exacerbate the introduction of sediments into streams, thereby increasing water turbidity (Belsky et al., 1999; Fitch and Adams, 1998; Schwarte et al., 2011; Vidon et al., 2008). Unrestricted cattle access resulted in an 11-fold increase in TSS in stream water in the summer when the animals spent more time near or in the stream (Vidon et al., 2008). In a long-term study examining the effects of grazing management on soil erosion from pastures, Pilon et al. (2017) found that TSS concentrations in runoff from continuously grazed watersheds averaged 126 mg/L compared to rotationally grazed watersheds that averaged 63 mg/L TSS. While the cumulative runoff volumes between these two grazing strategies did not differ, the cumulative TSS load (kg/ha) was higher for a continuously grazed compared to a rotationally grazed watershed (Pilon et al., 2017). In comparison, we did not observe a significant linear regression of the previous 24 h precipitation volume prior to interval measurement on TSS ($p = 0.1540$). This lack of association of TSS and rainfall maybe the result of the MIG strategy that we employed. While the cattle had access to the stream during grazing, with the daily moves, no one segment of the stream was continuously occupied by cattle. Additionally, aiming for forage removal of 45–50% left substantial vegetation cover on the pastures and streambank. Both approaches likely served to stabilize the streambank and surrounding ground, thereby limiting sediment transport during high rainfall events. Furthermore, the remaining vegetation may also have reduced the intensity of the influx of runoff into the stream, which in turn may have limited the resuspension of stream bottom sediments and subsequently TSS concentrations.

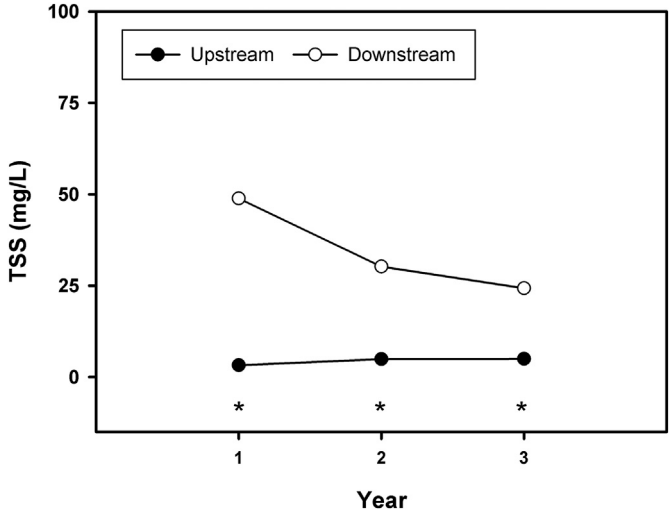


Fig. 5. Least squares means of total suspended solids (TSS; mg/L) in water collected at the upstream and downstream locations in each year during the 3-year study. The standard error of the least squares means is 6.38. Asterisks denote the years with significant treatment differences ($p < 0.05$).

3.4. Nitrate-nitrite nitrogen in water

Results from the initial model showed that treatment * year and interval * treatment terms were not significant ($p = 0.9966$ and $p = 0.6085$, respectively). The non-significant treatment * year term indicates that any differences in $NO_3 + NO_2$-N concentrations (averaged over interval) between upstream and downstream treatments were consistent over the three years. The non-significant interval * treatment term means that any differences in the $NO_3 + NO_2$-N concentrations (averaged over year) between upstream and downstream water were consistent over the intervals, as indicated in Fig. 6. In the reduced model, the effect of treatment did not interact with either interval or year, and there was an overall main effect of treatment for upstream $NO_3 + NO_2$-N concentrations to be significantly higher than downstream (3.70 versus 3.59 mg/L; $p = 0.0323$). In addition, there was a negative linear regression effect ($p = 0.0002$) of the previous 24 h precipitation volume on $NO_3 + NO_2$-N concentrations in water. Every 1.00 cm precipitation increase resulted in an estimated 0.160 mg/L decrease in $NO_3 + NO_2$-N concentration.

These results were not expected, given the many works reporting livestock grazing contributions of nitrogen to surface waters (Agouridis et al., 2005; Belsky et al., 1999; Hubbard et al., 2004). Cattle may deposit their feces containing nitrogen and other nutrients directly into water (Vidon et al., 2008) or nitrogen from fecal pats may be transported to surface water in runoff (Smith and Monaghan, 2003). However, in previous research conducted on this stream, Hansen et al. (2020) noted the decrease in nitrate-N as water flowed through from the groundwater discharge point to the reservoir, describing the stream as a nitrate-N "losing" stream system. Removal of nitrogen in stream systems occurs by denitrification and as plants, algae, and other aquatic biota utilize available nitrogen (Mazza and Johnson, 2009). Riparian vegetation influences nitrogen processing and uptake, and stream and/or grazing management efforts that develop healthy riparian plant communities may enhance nitrogen reduction from surface water. Furthermore, like TSS, the vegetation remaining after grazing may have limited $NO_3 + NO_2$-N transport in runoff. It is also possible that decreases in $NO_3 + NO_2$-N water concentrations associated with increases in the previous 24 h precipitation may be a result of dilution by rainfall (Hinckley et al., 2019).

There was a significant interaction between interval and year on $NO_3 + NO_2$-N ($p < 0.0001$), meaning that the differences in the $NO_3 + NO_2$-N concentration between the three years at a particular interval were different. These differences between the years occurred at sampling intervals Wk 1, Wk 3, Wk 4, Mo 3, Mo 7, and Mo 10 ($p < 0.05$), with no consistent pattern to the differences (Fig. 2D). Hansen et al. (2020) previously reported that nitrate-N concentrations of the groundwater at the discharge point of this stream ranged from 2.6 to 3.6 mg/L. Our interval * year measurements of $NO_3 + NO_2$-N concentrations ranged only from 2.65 to 4.28 mg/L, leading us to wonder if changes in the $NO_3 + NO_2$-N concentrations in the source groundwater were responsible for these differences.

3.5. Antibiotic resistance genes in bovine feces, water, sediment, and soil

Data regarding the types and prevalence of naturally-occurring antibiotic resistant bacteria and their genes in riparian grazing environments or the potential for introduction of antibiotic resistant bacteria into soils or surface waters by grazing cattle are limited. Hence, our examination was confined to Year 1 only, as a means to generate information to plan future work in these environments. Previous research has shown that this four gene panel of *sul1*, *ermB*, $bla_{ctx\text{-}m\text{-}32}$, and *intI1* is useful for studying the ecology of antimicrobial resistance in agricultural production systems (Durso et al., 2012; Gurmessa et al., 2021; Meyers et al., 2020; Yang et al., 2020; Yang et al., 2021).

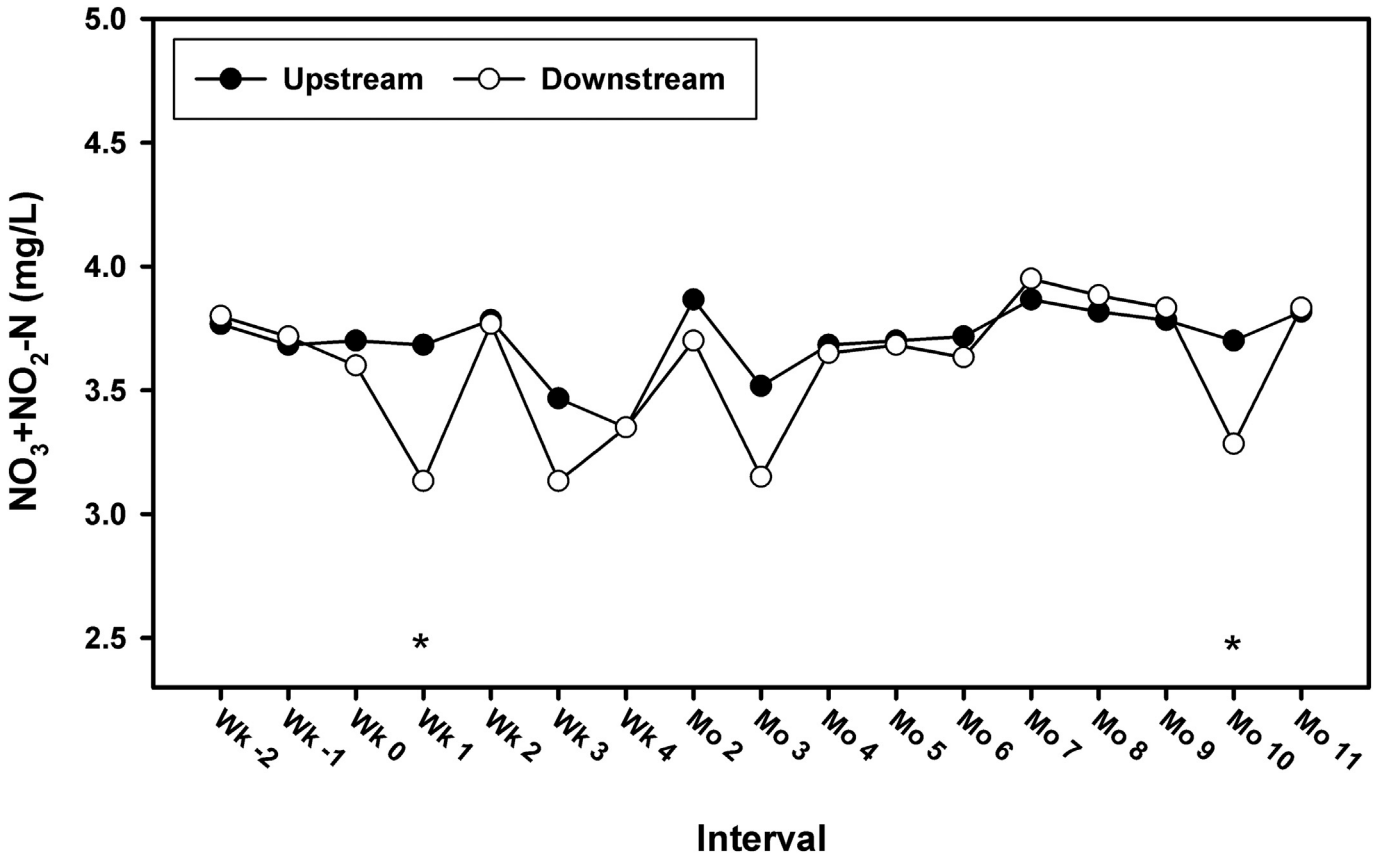


Fig. 6. Least squares means of nitrate-nitrite nitrogen ($NO_3 + NO_2$-N; mg/L) in water collected at the upstream and downstream locations during the 3-year study. The standard error of the least squares means is 0.15. Asterisks denote intervals with significant treatment differences ($p < 0.05$).

Of the four resistance determinants surveyed for this study, *sul1* was most frequently detected, and was detected in all sample types, including feces, sediment, water, and soil (Table 1). The presence of *sul1* gene in the pre-grazing soil, sediment, and water samples indicates a possible baseline level of *sul1*-containing bacteria in the watershed. In addition, the grazing cattle contributed *sul1* to the environment via their feces, which were 50% positive for the *sul1* gene. However, these facts alone cannot explain the results obtained from both upstream and downstream samples of water and sediment. Over the course of the entire study, 49 of the 79 downstream water samples were positive for *sul1*, which tended to be higher ($p = 0.08$) than the 37 *sul1*-positive of the 79 upstream water samples. In contrast, 82 of the 85 downstream sediment samples were positive for *sul1*, which was significantly higher ($p = 0$) than 6 of 85 upstream sediment samples (Table 1). The *sul1* gene is found in environmental bacteria, and the detection of sulfonamide-resistance genes is common in native soils and soils that have received limited or no inputs from livestock production (Durso et al., 2016; Yang et al., 2020). A localized population of *sul1*-containing bacteria may be well-adapted to the environment in the downstream sediment collection site. More likely, differences in the stream bottom sediments at the upstream and downstream locations may be influencing the differences in *sul1* prevalence. As noted above, the section of the stream immediately upstream from the study site was newly constructed for the groundwater remediation project. This short, new section (Fig. A.1) is rock-lined, receives limited runoff from the surrounding area, and is not next to a pasture, although cattle occasionally grazed on crop residue in an adjacent field. In addition, the water flowing through the section is principally the discharged groundwater. In comparing the stream bottoms, the stream bottom at the upstream collection site receives little sediment, and is firmer and clay-like, while the stream bottom at the downstream site has a thicker and softer layer of sediment. The looser

Table 1
Percentages of positive samples for each gene target in bovine feces collected in pastures, and in water, sediment, and soils collected at upstream and downstream locations, Year 1.

Sample type (total n)	Sampling interval	Month	No. of samples	*sul1*		*ermB*		$bla_{ctx\text{-}m\text{-}32}$		*intI1*	
Feces	Wk 0, 1, 2	May	100	**50**[a]		**92**		0		**1.0**	
(100)			(Up, down)	Up	Down	Up	Down	Up	Down	Up	Down
Water	Wk −2	April	5, 5	**60**	**60**	0	0	0	0	0	0
(158)	Wk −1	May	5, 5	**40**	0	0	0	0	0	0	0
	Wk 0	May	5, 5	0	0	0	0	0	0	0	0
	Wk 1	May	4, 4	**75**	**100**	**50**	**25**	0	0	0	0
	Wk 2	May	5, 5	**20**	**80**	0	0	0	0	0	0
	Wk 3	May	5, 5	0	**100**	0	0	**20**	0	0	0
	Wk 4	June	5, 5	**60**	**60**	0	0	0	**20**	0	0
	Mo 2	July	5, 5	**40**	**60**	0	0	0	0	0	0
	Mo 3	Aug	5, 5	**40**	**100**	0	**20**	0	0	0	0
	Mo 4	Sept	5, 5	**100**	**60**	0	0	0	**20**	0	0
	Mo 5	Oct	3, 5	**33**	**80**	0	0	**33**	0	0	0
	Mo 6	Nov	4, 3	**50**	0	0	0	0	0	0	0
	Mo 7	Dec	3, 4	**67**	**50**	0	0	**33**	**25**	0	0
	Mo 8	Jan	5, 4	**20**	**50**	0	0	**60**	**25**	0	0
	Mo 9	Feb	5, 5	**80**	**80**	0	0	**60**	**60**	0	0
	Mo 10	March	5, 4	**60**	**75**	0	0	**40**	**100**	0	0
	Mo 11	April	5, 5	**60**	**80**	0	0	**80**	**100**	0	0
Sediment	Wk −2	April	5, 5	0	**100**	0	0	0	0	0	0
(170)	Wk −1	May	5, 5	0	**100**	0	0	0	0	0	0
	Wk 0	May	5, 5	0	**100**	0	**40**	**20**	0	0	0
	Wk 1	May	5,5	**40**	**100**	**20**	0	0	0	0	0
	Wk 2	May	5, 5	0	**100**	0	0	**20**	0	0	0
	Wk 3	May	5, 5	0	**100**	0	0	0	0	0	0
	Wk 4	June	5, 5	0	**100**	0	0	0	0	0	0
	Mo 2	July	5, 5	**40**	**100**	0	0	0	0	0	0
	Mo 3	Aug	5, 5	0	**100**	0	0	0	0	0	0
	Mo 4	Sept	5, 5	0	**100**	0	0	0	0	0	0
	Mo 5	Oct	5, 5	0	**100**	0	0	0	0	0	**20**
	Mo 6	Nov	5, 5	0	**100**	0	0	0	0	0	0
	Mo 7	Dec	5, 5	**20**	**60**	0	0	0	0	0	**20**
	Mo 8	Jan	5, 5	0	**100**	0	0	0	0	0	0
	Mo 9	Feb	5, 5	0	**100**	0	0	0	0	0	0
	Mo 10	March	5, 5	**20**	**100**	0	0	0	0	0	**40**
	Mo 11	April	5, 5	0	**80**	0	0	**20**	**40**	0	0
Soil	Wk −2	April	3, 3	0	0	0	0	0	0	0	0
(85)	Wk −1	May	2, 3	0	**100**	0	0	0	0	0	0
	Wk 0	May	2, 3	**50**	**33**	0	0	0	0	0	0
	Wk 1	May	2, 3	0	**33**	0	0	0	0	0	0
	Wk 2	May	3, 2	0	0	0	0	0	0	0	0
	Wk 3	May	2, 3	0	0	0	0	0	0	0	0
	Wk 4	June	2, 3	0	0	0	0	**50**	0	0	0
	Mo 2	July	3, 2	0	0	0	0	0	0	0	0
	Mo 3	Aug	3, 2	0	0	0	0	**33**	0	0	0
	Mo 4	Sept	2, 3	0	0	0	0	0	0	0	0
	Mo 5	Oct	3, 2	0	**50**	0	0	0	0	0	0
	Mo 6	Nov	2, 3	0	**33**	0	0	0	0	0	0
	Mo 7	Dec	3, 2	**67**	0	0	0	0	0	0	0
	Mo 8	Jan	2, 3	**50**	0	0	0	0	0	0	0
	Mo 9	Feb	3, 2	0	0	0	0	0	0	0	0
	Mo 10	March	2, 3	**50**	0	0	0	0	0	0	0
	Mo 11	April	2, 3	0	0	0	0	0	0	0	0

[a] Percentages in boldface denote percentages greater than zero.

sediment in the downstream site may entrap more bacteria, including *sul1*-containing bacteria, making *sul1* presence in the sediment more highly correlated to *sul1* presence in the water flowing above the sediment, as compared to the upstream sediment.

The high frequency of *sul1* detection in both upstream and downstream water at most all timepoints, including before cattle were introduced into the system, makes it difficult to ascertain the impact of grazing on this target. In previous work, Naderi Beni et al. (2020) commonly detected sulfonamide antibiotics in the treated groundwater entering this stream, making it interesting to speculate on an association between the presence of these sulfa-containing drugs in the groundwater and the common occurrence of *sul1*-containing bacteria. Swine production facilities and other livestock operations near USMARC are potential sources of sulfonamide compounds (Naderi Beni et al., 2020).

The *ermB* gene was detected in 92 of the 100 bovine feces samples (Table 1). It was also detected in stream sediment and water, but not soil, during the time when cattle were grazing. The *ermB* contributions from cattle feces were not detected in soil, sediment, or water before or after the animals were removed with one exception, which was a single isolation in downstream water at Mo 3. Consistent with our observations, erythromycin-resistant enterococci with the *ermB* gene were commonly detected in bovine feces, regardless of whether or not the cattle had been treated with antibiotics (Agga et al., 2016). In addition, *ermB* is common in bovine manure, manure- and dairy wastewater-amended soils, and runoff from bovine manure-amended/impacted crop soils or pastured ground (Dungan et al., 2018; Gurmessa et al., 2021; Yang et al., 2020; Yang et al., 2021). Compared to pastures used for grazing, the soils of more concentrated cattle production in feedlots or dry lots can contain higher concentrations of ARGs. Agga et al. (2019) examined the persistence of several ARGs, including *ermB*, in soil following the removal of cattle after seven years of the continuous use of a beef cattle backgrounding facility that included both feeding and grazing areas. While there was a reduction in the concentrations of all ARGs two years after the cattle were removed, all targets were still present at detectable levels. At all of the time points, the concentrations of the ARGs were higher in the feeding area than the grazing area, likely due to greater fecal deposition. Similarly, Yang et al. (2020) found that the soil concentrations of *ermB*, *sul1*, and *intI1* genes were higher in pastures that were continuously grazed, compared to rotationally grazed pastures, suggesting that continuous deposition of cattle feces increases ARGs in the soil. In contrast, in the current work the *ermB* gene was not detected in any environmental samples before or four months after grazing, indicating that the use of MIG may limit the accumulation of ARGs shed by cattle.

The $bla_{ctx\text{-}m\text{-}32}$ gene was not detected in cattle feces samples and infrequently detected in soil and sediment (Table 1). Likewise, $bla_{ctx\text{-}m\text{-}32}$ was sporadically detected in both upstream and downstream water from Wk −2 (pre-grazing) through Mo 6 (post-grazing). Interestingly, the $bla_{ctx\text{-}m\text{-}32}$ gene was more consistently detected in water samples from Mo 7 through Mo 11 (December through April), with no difference in prevalence between upstream and downstream water ($p = 0.76$). Combined, these results support a conclusion that the grazing cattle were not the source of this target in these samples. While the source is uncertain, the timing of the more frequent detection of the $bla_{ctx\text{-}m\text{-}32}$ gene coincides in part with our January and February sightings of the overwintering mallard ducks in the section of the stream immediately upstream of the grazing site (discussed above). In addition, the earliest spring arrivals of migratory waterfowl in this region often can appear in late February or early March. Although speculative, it is important to note that migratory bird species can carry a variety of zoonotic pathogens and are considered high risk for spreading these disease agents along migration routes (Reed et al., 2003; Vogt et al., 2019). The carriage and shedding of antimicrobial resistant bacteria and ARGs by waterfowl have also been described, including genes for extended-spectrum beta-lactamases like $bla_{ctx\text{-}m\text{-}32}$ (Literak et al., 2010; Mathys et al., 2017; Vogt et al., 2019).

Alternatively, or in addition to waterfowl, other seasonal changes may impact the prevalence or concentration of $bla_{ctx\text{-}m\text{-}32}$ or other ARGs in rivers or surface waters. As an example, Herrig et al. (2020) found that concentrations of bla_{ctx} genes in river water varied with season. The bla_{ctx} gene concentration was higher in fall and winter, corresponding to greater runoff discharge following rainfall, accompanied by elevated turbidity and ammonium-nitrogen, and higher dissolved oxygen levels because of colder water temperatures. Other reported seasonal effectors of AMR genes in surface waters include UVA radiation and the presence of macrophytes (Reichert et al., 2021).

There was a single detection of the integrase *intI1* gene in a sample of cattle feces during grazing, and four *intI1*detections in sediment post-grazing, starting approximately four months after animals were removed (Table 1). This low detection rate was unanticipated, because the clinical *intI1* gene variant is commonly linked to *sul1* (Antunes et al., 2005: Gillings et al., 2015) and *sul1* was frequently detected. Environmental integron-integrase genes are more diverse, but also commonly co-located with *sul1* in the same isolate or community DNA sample (Chaturvedi et al., 2021; Gillings et al., 2008; Hardwick et al., 2008). As an example, Chaturvedi et al. (2021) found that detection of these two ARG targets was correlated as much as 85% of the time in rivers. In all five instances that the samples were positive for *intI1*, they were also positive for *sul1*. However, most *sul1*-positive samples in this study were negative for *intI1* (231 of 236 total *sul1*-positive samples, even after repeat testing for confirmation). While it is unexpected to have such a high percentage of *sul1*-positive samples negative for *intI1*, reports of *sul1*-positive isolates and samples that are negative for the class I integrase gene are not uncommon (Chaturvedi et al., 2021; Koczura et al., 2016).

Working on the premise that the *intI1* is indeed a marker for anthropogenic inputs, these data would suggest that the watershed used in this study is minimally impacted by human activities, even during times when the cattle are grazing. Given the remote location of this watershed, this is a possibility. However, additional work including sequencing-based studies would be needed before further consideration of this explanation. There have also been reports of *intI1* positive samples not showing a band during electrophoresis (Hardwick et al., 2008), and the possibility of false negative reactions here needs to be considered.

4. Summary

The management-intensive cattle grazing approach that we employed appeared to have limited the negative impacts on the water quality of the stream relative to continuous grazing, depending on the particular measurement of water quality.

Total *E. coli* concentrations in downstream water were consistently higher than in upstream water over the sampling intervals and over the three years of the study. We observed low prevalence of the pathogen *E. coli* O157:H7 but detected it sporadically in downstream water and sediment samples up to 2–1/2 months after the cattle were removed. These results corroborate previous data regarding the environmental persistence of total *E. coli* and *E. coli* O157:H7, and further demonstrate that grazing systems that protect water quality can also protect water safety. However, sampling and observation throughout the seasons also revealed waterfowl to be an important source of total *E. coli* to the stream and demonstrated the difficulty in ascribing any negative water quality effects to grazing cattle alone. Downstream water TSS concentrations were higher than upstream when the cattle or ducks were present. In contrast, there was an overall main effect of treatment for NO_3 + NO_2-N concentrations to be significantly higher in upstream water than in downstream water, which may be functions of nitrogen utilization and denitrification processes occurring in the stream.

Rainfall volume and intensity increased total *E. coli* water concentrations, due either to transport of *E. coli* from grazed pastures into the stream or by resuspension of stream bottom sediments containing the organism. However, when compared to *E. coli* levels reported after storm events in other cattle grazing systems, MIG may both limit the extent and duration of high *E. coli* concentrations in water. In contrast, there was no association of the previous 24 h precipitation volume on TSS water concentrations, and there was a negative linear effect of the previous 24 h precipitation volume

on NO_3 + NO_2-N water concentrations. These findings may also result from the MIG strategy that we employed, which both limited access to the streambank and controlled forage removal.

Understanding zoonotic pathogens like *E. coli* O157:H7 and antibiotic resistance in preharvest livestock environments is just one of the critical dimensions in developing One Health approaches to reducing these health risks for humans, agricultural systems, and natural ecosystems. Limited work has examined the prevalence and geospatial distribution of ARGs in pasture-based cattle production and their associated surface waters. Our examination was confined to Year 1 only, as a means to develop information and inform future antibiotic resistance work in the grazing environment. Among the four resistance determinants surveyed, *sul1* and *ermB* were commonly found in the feces of the grazing cattle. However, the high frequency of *sul1* detection in both upstream and downstream water at most all timepoints, including before cattle were introduced into the system, makes it difficult to determine the impact of grazing on this gene target. In contrast, *ermB* was detected in water and sediment primarily during the two-week grazing period when the cattle were present. These results point to knowledge gaps in our understanding of the ecology of individual antibiotic resistance genes, their persistence and distribution, and the relationships between the forces that drive microbial community structure and antibiotic resistance gene carriage in agricultural production systems and the natural environment.

Funding

This research was supported by the U.S. Department of Agriculture, Agricultural Research Service.

CRediT authorship contribution statement

Laura M. Rubeck: Investigation, Writing – original draft, Writing – review & editing. **James E. Wells:** Investigation, Writing – review & editing. **Kathryn J. Hanford:** Formal analysis, Writing – review & editing. **Lisa M. Durso:** Methodology, Investigation, Writing – review & editing. **Walter H. Schacht:** Conceptualization, Writing – review & editing. **Elaine D. Berry:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Shannon Ostdiek, Dee Kucera, Justine Condon, Brandon Nguyen, Morgan Meyers, Vanessa Whitmore, Angel Pham, and JoLynn Broweleit for outstanding technical support, and the USMARC Forage Department for assistance with forage evaluation and cattle. We also thank Glen Slater of University of Nebraska-Lincoln South Central Agricultural Laboratory for providing the weather data. We are particularly grateful to Ed Hebbert for assistance in data collection and consultation in drafting the manuscript, and to Grace Wong for her efforts on the artwork for the graphical abstract. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152611.

References

Abimbola, O.P., Mittelstet, A.R., Messer, T.L., Berry, E.D., Bartelt-Hunt, S.L., Hansen, S.P., 2020. Predicting *Escherichia coli* loads in cascading dams with machine learning: an integration of hydrometeorology, animal density, and grazing pattern. Sci. Total Environ. 722, 137894. https://doi.org/10.1016/j.scitotenv.2020.137894.

Agga, G.E., Cook, K.L., Netthisinghe, A.M.P., Gilfellin, R.A., Woosley, P.B., Sistani, K.R., 2019. Persistence of antibiotic resistance genes in beef cattle backgrounding environment over two years after cessation of operation. PLoS One 14, e0212510. https://doi.org/10.1371/journal.pone.0212510.

Agga, G.E., Schmidt, J.W., Arthur, T.M., 2016. Antimicrobial-resistant fecal bacteria from ceftiofur-treated and nonantimicrobial-treated comingled beef cows at a cow-calf operation. Microb. Drug Resist. 22, 598–608. https://doi.org/10.1089/mdr.2015.0259.

Agouridis, C.T., Workman, S.R., Warner, R.C., Jennings, G.D., 2005. Livestock grazing management impacts on stream water quality: a review. J. Am. Water Resour. Assoc. 41, 591–606. https://doi.org/10.1111/j.1752-1688.2005.tb03757.x.

Agricultural Research Service, and Natural Resources Conservation Service New Mexico State University (ARS-NRCS-NMSU), 2021. Ecosystem Dynamics Interpretive Tool. https://edit.jornada.nmsu.edu/catalogs/esd/075X/R075XY058NE. (Accessed 16 April 2021).

Antunes, P., Machado, J., Sousa, J.C., Peixe, L., 2005. Dissemination of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in Portuguese *Salmonella enterica* strains and relation with integrons. Antimicrob. Agents Chemother. 49, 836–839. https://doi.org/10.1128/aac.49.2.836-839.2005.

Avery, L.M., Williams, A.P., Killham, K., Jones, D.L., 2008. Survival of *Escherichia coli* O157:H7 in waters from lakes, rivers, puddles and animal-drinking troughs. Sci. Total Environ. 389, 378–385. https://doi.org/10.1016/j.scitotenv.2007.08.049.

Avery, S.M., Moore, A., Hutchison, M.L., 2004. Fate of *Escherichia coli* originating from livestock faeces deposited directly onto pasture. Lett. Appl. Microbiol. 38, 355–359. https://doi.org/10.1111/j.1472-765X.2004.01501.x.

Barkocy-Gallagher, G.A., Arthur, T.M., Rivera-Betacourt, M., Nou, X., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M., 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotype, and *Salmonella* in commercial beef processing plants. J. Food Prot. 66, 1978–1986. https://doi.org/10.4315/0362-028X-66.11.1978.

Barkocy-Gallagher, G.A., Edwards, K.K., Nou, X., Bosilevac, J.M., Arthur, T.M., Shackelford, S.D., Koohmaraie, M., 2005. Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. J. Food Prot. 68, 2264–2268. https://doi.org/10.4315/0362-028X-68.11.2264.

Belsky, A.J., Matzke, A., Uselman, S., 1999. Survey of livestock influences on stream and riparian ecosystems in the western United States. J. Soil Water Conserv. 54, 419–431.

Berry, E.D., Wells, J.E., 2010. *Escherichia coli* O157:H7: recent advances in research on occurrence, transmission, and control in cattle and the production environment. Adv. Food Nutr. Res. 60, 67–117. https://doi.org/10.1016/s1043-4526(10)60004-6.

Berry, E.D., Wells, J.E., Arthur, T.M., Woodbury, B.L., Nienaber, J.A., Brown-Brandl, T.M., Eigenberg, R.A., 2010. Soil versus pond ash surfacing of feedlot pens: occurrence of *Escherichia coli* 0157:H7 in cattle and persistence in manure. J. Food Prot. 73, 1269–1277. https://doi.org/10.4315/0362-028x-73.7.1269.

Berry, E.D., Woodbury, B.L., Nienaber, J.A., Eigenberg, R.A., Thurston, J.A., Wells, J.E., 2007. Incidence and persistence of zoonotic bacterial and protozoan pathogens in a beef cattle feedlot runoff control vegetative treatment system. J. Environ. Qual. 36, 1873–1882. https://doi.org/10.2134/jeq2007.0100.

Capon, S.J., 2020. In: Goldstein, M.I., DellaSalla, D. (Eds.), Riparian Ecosystems. Encyclopedia of the World's Biomes, 1st edition4, pp. 170–176. https://doi.org/10.1016/B978-0-12-409548-9.11884-6.

Chaturvedi, P., Singh, A., Chowdhary, P., Pandey, A., Gupta, P., 2021. Occurrence of emerging sulfonamide resistance (*sul1* and *sul2*) associated with mobile integrons-integrase (*intI1* and *intI2*) in riverine systems. Sci. Total Environ. 751, 142217. https://doi.org/10.1016/j.scitotenv.2020.142217.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J., Mandrell, R.E., 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS One 2, e1159. https://doi.org/10.1371/journal.pone.0001159.

Curriero, F.C., Patz, J.A., Rose, J.B., Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948-1994. Am. J. Public Health 91, 1194–1199. https://doi.org/10.2105/ajph.91.8.1194.

Davies-Colley, R., Nagels, J., Lydiard, E., 2008. Stormflow-dominated loads of faecal pollution from an intensively dairy-farmed catchment. Water Sci. Technol. 57, 1519–1523. https://doi.org/10.2166/wst.2008.257.

Davies-Colley, R.J., Nagels, J.W., Smith, R.A., Young, R.G., Phillips, C.J., 2004. Water quality impact of a dairy cow herd crossing a stream. N. Z. J. Mar. Freshw. Res. 38, 569–576. https://doi.org/10.1080/00288330.2004.9517262.

Drilling, N., Titman, R.D., McKinney, F., 2020. Mallard (*Anas platyrhynchos*), version 1.0. In: Billerman, S.M. (Ed.), Birds of the World. Cornell Lab of Ornithology, Ithaca, NY, USA https://doi.org/10.2173/bow.mallar3.01.

Dungan, R.S., McKinney, C.W., Leytem, A.B., 2018. Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. Sci. Total Environ. 635, 1477–1483. https://doi.org/10.1016/j.scitotenv.2018.04.020.

Durso, L.M., Miller, D.N., Wienhold, B.J., 2012. Distribution and quantification of antibiotic resistant genes and bacteria across agricultural and non-agricultural metagenomes. PLoS One 7, e48325. https://doi.org/10.1371/journal.pone.0048325.

Durso, L.M., Wedin, D.A., Gilley, J.E., Miller, D.N., Marx, D.B., 2016. Assessment of selected antibiotic resistances in ungrazed native Nebraska prairie soils. J. Environ. Qual. 45, 454–462. https://doi.org/10.2134/jeq2015.06.0280.

Fitch, L., Adams, B.W., 1998. Can cows and fish co-exist? Can. J. Plant Sci. 78, 191–198. https://doi.org/10.4141/P97-141.

Gannon, V.P.J., D'Souza, S., Graham, T., King, R.K., Rahn, K., Read, S., 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J. Clin. Microbiol. 35, 656–662. https://doi.org/10.1128/jcm.35.3.656-662.1997.

Gerrish, J., Ohlenbusch, P.D., 1998. Using terms: management-intensive grazing or management intensive grazing. Rangelands 20, 13–14.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J. 9, 1269–1279. https://doi.org/10.1038/ismej.2014.226.

Gillings, M.R., Krishnan, S., Worden, P.J., Hardwick, S.A., 2008. Recovery of diverse genes for class 1 integron-integrases from environmental DNA samples. FEMS Microbiol. Lett. 287, 56–62. https://doi.org/10.1111/j.1574-6968.2008.01291.x.

Gurmessa, B., Ashworth, A.J., Yang, Y., Savin, M., Moore Jr., P.A., Ricke, S.C., Corti, G., Pedretti, E.F., Cocco, S., 2021. Variations in bacterial community structure and antimicrobial resistance gene abundance in cattle manure and poultry. Environ. Res. 197, 111011. https://doi.org/10.1016/j.envres.2021.111011.

Haan, M.M., Russell, J.R., Powers, W.J., Kovar, J.L., Benning, J.L., 2006. Grazing management effects on sediment and phosphorus in surface runoff. Rangeland Ecol. Manag. 59, 607–615. https://doi.org/10.2111/05-152R2.1.

Hansen, S., Messer, T., Mittelstet, A., Berry, E.D., Bartelt-Hunt, S., Abimbola, O., 2020. *Escherichia coli* concentrations in waters of a reservoir system impacted by cattle and migratory waterfowl. Sci. Total Environ. 705, 135607. https://doi.org/10.1016/j.scitotenv.2019.135607.

Hardwick, S.A., Stokes, H.W., Findlay, S., Taylor, M., Gillings, M.R., 2008. Quantification of class 1 integron abundance in natural environments using read-time quantitative PCR. FEMS Microbiol. Lett. 278, 207–212. https://doi.org/10.1111/j.1574-6968.2007.00992.x.

Herrig, I., Fleischmann, S., Regnery, J., Wesp, J., Reifferscheid, G., Manz, W., 2020. Prevalence and seasonal dynamics of bla_{CTX-M} antibiotic resistance genes and fecal indicator organisms in the lower Lahn River, Germany. PloS One 15, e0232289. https://doi.org/10.1371/journal.pone.0232289.

Hinckley, B.R., Etheridge, J.R., Peralta, A.L., 2019. Storm event nitrogen dynamics in waterfowl impoundments. Water Air Soil Pollut. 230, 294. https://doi.org/10.1007/s11270-019-4332-5.

Hu, Y., Zhang, Q., Meitzler, J.C., 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. J. Appl. Microbiol. 87, 867–876. https://doi.org/10.1046/j.1365-2672.1999.00938.x.

Hubbard, R.K., Newton, G.L., Hill, G.M., 2004. Water quality and the grazing animal. J. Anim. Sci. 82, E255–E263.

Ishii, S., Sadowsky, M.J., 2008. *Escherichia coli* in the environment: implications for water quality and human health. Microbes Environ. 23, 101–108. https://doi.org/10.1264/jsme2.23.101.

Jang, J., Hur, H.G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T., Ishii, S., 2017. Environmental *Escherichia coli*: ecology and public health implications—a review. J. Appl. Microbiol. 123, 570–581. https://doi.org/10.1111/jam.13468.

Koczura, R., Mokracka, J., Taraszewska, A., Łopacinska, N., 2016. Abundance of class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors. Microb. Ecol. 72, 909–916. https://doi.org/10.1007/s00248-016-0843-4.

Larsen, R.E., Krueger, W.C., George, M.R., Barrington, M.R., Buckhouse, J.C., Johnson, D.E., 1998. Livestock influences on riparian zones and fish habitat: literature classification. J. Range Manag. 51, 661–664. https://doi.org/10.2307/4003609.

Literak, I., Dolejska, M., Janoszowska, D., Hrusakova, J., Meissner, W., Rzyska, H., Bzoma, S., Cizek, A., 2010. Antibiotic-resistant *Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and QnrS, in waterbirds on the Baltic Sea coast of Poland. Appl. Environ. Microbiol. 76, 8126–8134. https://doi.org/10.1128/AEM.01446-10.

Lyons, J., Weigel, B.M., Pain, L.K., Undersander, D.J., 2000. Influence of intensive rotational grazing on bank erosion, fish habitat quality, and fish communities in southwestern Wisconsin trout streams. J. Soil Water Conserv. 55, 271–276.

Mathys, D.A., Mollenkopf, D.F., Nolting, J., Bowman, A.S., Daniels, J.B., Wittum, T.E., 2017. Extended-spectrum cephalosporin-resistant *Enterobacteriaceae* in enteric microflora of wild ducks. J. Wildlife. Dis. 53, 690–694. https://doi.org/10.7589/2016-12-272.

Mazza, R., Johnson, S., 2009. Undercover isotopes: tracking the fate of nitrogen in streams. Science Findings 115. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR. https://www.fs.usda.gov/pnw/publications/undercover-isotopes-tracking-fate-nitrogen-streams. (Accessed 3 September 2021).

McCabe, E., Burgess, C.M., Lawal, D., Whyte, P., Duffy, G., 2019. An investigation of shedding and super-shedding of Shiga toxigenic *Escherichia coli* O157 and *E. coli* O26 in cattle presented for slaughter in the Republic of Ireland. Zoonoses Public Health 66, 83–91. https://doi.org/10.1111/zph.12531.

Meays, C.L., Broersma, K., Nordin, R., Mazumder, A., Samadpour, M., 2006. Spatial and annual variability in concentrations and sources of *Escherichia coli* in multiple watersheds. Environ. Sci. Technol. 40, 5289–5296. https://doi.org/10.1021/es060659q.

Meyers, M.A., Durso, L.M., Gilley, J.E., Waldrip, H.M., Castleberry, L., Millmier-Schmidt, A., 2020. Antibiotic resistance gene profile changes in cropland soil after manure application and rainfall. J. Environ. Qual. 49, 754–761. https://doi.org/10.1002/jeq2.20060.

Muirhead, R.W., Collins, R.P., Bremer, P.J., 2005. Erosion and subsequent transport state of *Escherichia coli* from cowpats. Appl. Environ. Microbiol. 71, 2875–2879. https://doi.org/10.1128/AEM.71.6.2875-2879.2005.

Naderi Beni, N., Snow, D.D., Berry, E.D., Mittelstet, A.R., Messer, T.L., Bartelt-Hunt, S., 2020. Measuring the occurrence of antibiotics in surface water adjacent to cattle grazing areas using passive samplers. Sci. Total Environ. 726, 138296. https://doi.org/10.1016/j.scitotenv.2020.138296.

Nagels, J.W., Davies-Colley, R.J., Donnison, A.M., Muirhead, R.W., 2002. Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. Water Sci. Technol. 45, 45–52. https://doi.org/10.2166/wst.2002.0408.

Natural Resources Conservation Service (NRCS), 2003. National water quality handbook. U.S. Department of Agriculture. https://directives.sc.egov.usda.gov/OpenNonWebContent.aspx?content=17843.wba. (Accessed 5 September 2021).

Natural Resources Conservation Service (NRCS), 2006. Land resource regions and major land resource areas of the United States, the Caribbean, and the Pacific Basin, U.S. Department of Agriculture Handbook 296. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051845.pdf. (Accessed 5 September 2021).

Natural Resources Conservation Service (NRCS), Web Soil Survey. U.S. Department of Agriculture. https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm. (Accessed 5 September 2021).

Ogden, I.D., MacRae, M., Strachan, N.J.C., 2004. Is the prevalence and shedding concentrations of *E. coli* O157 in beef cattle in Scotland seasonal? FEMS Microbiol. Lett. 233, 297–300. https://doi.org/10.1111/j.1574-6968.2004.tb09495.x.

Oliver, D.M., Heathwaite, L., Haygarth, P.M., Clegg, C.D., 2005. Transfer of *Escherichia coli* to water from drained and undrained grassland after grazing. J. Environ. Qual. 34, 918–925. https://doi.org/10.2134/jeq2004.0327.

Paton, A.W., Paton, J.C., 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. J. Clin. Microbiol. 36, 598–602. https://doi.org/10.1128/jcm.36.2.598-602.1998.

Pilon, C., Moore, P.A., Pote, D.H., Pennington, J.H., Martin, J.W., Brauer, D.K., Raper, R.L., Dabney, S.M., Lee, J., 2017. Long-term effects of grazing management and buffer strips on soil erosion from pastures. J. Environ. Qual. 46, 364–372. https://doi.org/10.2134/jeq2016.09.0378.

Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K., 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clin. Med. Res. 1, 5–12 doi.10.3121%2Fcmr.1.1.5.

Reichert, G., Hilgert, S., Alexander, J., Rodrigues de Azevedo, J.C., Morck, T., Fuchs, S., Schwartz, T., 2021. Determination of antibiotic resistance genes in a WWTP-impacted river in surface, sediment, and biofilm: influence of seasonality and water quality. Sci. Total Environ. 768, 144526. https://doi.org/10.1016/j.scitotenv.2020.144526.

Rice, E.W., Johnson, C.H., Wild, D.K., Reasoner, D.J., 1992. Survival of *Escherichia coli* O157:H7 in drinking water associated with a waterborne outbreak of hemorrhagic colitis. Lett. Appl. Microbiol. 15, 38–40. https://doi.org/10.1111/j.1472-765X.1992.tb00719.x.

Savageau, M.A., 1983. *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. Am. Nat. 122, 732–744 (accessed 7 September 2021). https://www.jstor.org/stable/2460914.

Schwarte, K.A., Russell, J.R., Kovar, J., Morrical, D.G., Ensley, S.M., Yoon, K.-J., Cornick, N.A., Cho, Y.I., 2011. Grazing management effects on sediment, phosphorous, and pathogen loading of streams in cool-season grass pastures. J. Environ. Qual. 40, 1303–1313. https://doi.org/10.2134/jeq2010.0524.

Shawver, C., Brummer, J., Ippolito, J., Ahola, J., Rhoades, R., 2020. Management-intensive grazing (MiG) on irrigated pasture. Colorado State University Extension, Livestock Series, Management, Fact Sheet 1. 635. https://extension.colostate.edu/docs/pubs/livestk/01635.pdf. (Accessed 2 August 2021).

Skinner, Q.D., 1991. Making riparian area protection a workable part of grazing management. December 3-5Range Beef Cow Symposium, Proceedings. Fort Collins, Colorado. https://digitalcommons.unl.edu/rangebeefcowsymp/245/. (Accessed 28 July 2021).

Smith, L.C., Monaghan, R.M., 2003. Nitrogen and phosphorus losses in overland flow from a cattle-grazed pasture in Southland. N. Z. J. Agric. Res. 46, 225–237. https://doi.org/10.1080/00288233.2003.9513549.

Somarelli, J.A., Makarewicz, J.C., Sia, R., Simon, R., 2007. Wildlife identified as major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints. J. Environ. Manag. 82, 60–65. https://doi.org/10.1016/j.jenvman.2005.12.013.

Sovell, L.A., Vondracek, B., Frost, J.A., Mumford, K.G., 2000. Impacts of rotational grazing and riparian buffers on physicochemical and biological characteristics of southeastern Minnesota, USA, streams. Environ. Manag. 26, 629–641. https://doi.org/10.1007/s002670010121.

Stott, R., Davies-Colley, R., Nagels, J., Donnison, A., Ross, C., Muirhead, R., 2011. Differential behavior of *Escherichia coli* and *Campylobacter* spp. in a stream draining dairy pasture. J. Water Health 9, 59–69. https://doi.org/10.2166/wh.2010.061.

Sunohara, M.D., Topp, E., Wilkes, G., Gottschall, N., Neumann, N., Ruecker, N., Jones, T.H., Edge, T.A., Marti, R., Lapen, D.R., 2012. Impact of riparian zone protection from cattle on nutrient, bacteria, F-coliphage, *Cryptosporidium*, and *Giardia* loading of an intermittent stream. J. Environ. Qual. 41, 1301–1314. https://doi.org/10.2134/jeq2011.0407.

Swanson, S., Wyman, S., Evans, C., 2015. Practical grazing management to maintain or restore riparian functions and values. J. Rangeland Appl. 2, 1–28 (accessed 26 July 2021) https://extension.unr.edu/publication.aspx?PubID=1400.

Tornevi, A., Bergstedt, O., Forsberg, B., 2014. Precipitation effects on microbial pollution in a river: lag structures and seasonal effect modification. PLos One 9, e98546. https://doi.org/10.1371/journal.pone.0098546.

Uitenbroek, D.G., 1997. SISA-Fisher exact test. http://www.quantitativeskills.com/sisa/statistics/fisher.htm. (Accessed 13 September 2021).

U.S. Army Corps of Engineers (USACE), 2010. Record of decision, sitewidegroundwater, former naval ammunition depot, Hastings, Nebraska. https://semspub.epa.gov/work/HQ/189068.pdf. (Accessed 13 September 2021).

U.S. Geological Survey (USGS), 1989. Techniques of Water-Resources Investigations of the United States Geological Survey. https://pubs.usgs.gov/twri/twri5-a1/pdf/twri_5-A1_n.pdf. (Accessed 11 May 2021).

U.S. Environmental Protection Agency (USEPA), 1993. Method 353.2, Revision 2.0: Determination of nitrate-nitrite nitrogen by Automated Colorimetry. https://www.epa.gov/sites/production/files/2015-08/documents/method_353-2_1993.pdf. (Accessed 13 September 2021).

Van Donkersgoed, J., Graham, T., Gannon, V., 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. Can. Vet. J. 40, 332.

Vidon, P., Campbell, M.A., Gray, M., 2008. Unrestricted cattle access to streams and water quality in till landscape of the Midwest. Agric. Water Manag. 95, 322–330. https://doi.org/10.1016/j.agwat.2007.10.017.

Vogt, N.A., Pearl, D.L., Taboada, E.N., Mutschall, S.K., Janecko, N., Reid-Smith, R.J., Jardine, C.M., 2019. Carriage of *Campylobacter*, *Salmonella*, and antimicrobial-resistant, nonspecific *Escherichia coli* by waterfowl species collected from three sources in southern Ontario, Canada. J. Wildlife Dis. 55, 917–922. https://doi.org/10.7589/2018-12-288.

Wang, G., Doyle, M.P., 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J. Food Prot. 61, 662–667. https://doi.org/10.4315/0362-028x-61.6.662.

Wells, J.E., Berry, E.D., 2017. Pathogens affecting beef, pp. 1–32. In: Acuff, G.R., Dickson, J.S. (Eds.), Ensuring Safety and Quality in the Production of BeefVol. 1. Burleigh Dodds Science Publishing, Cambridge, UK, p. 252.

World Health Organization (WHO), 2019. Critically important antimicrobials for human medicine, 6th revision. https://www.who.int/publications/i/item/9789241515528. (Accessed 13 September 2021).

Yang, Y., Ashworth, A.J., DeBruyn, J.M., Durso, L.M., Savin, M., Cook, K., Moore Jr., P.A., Owens, P.R., 2020. Antimicrobial resistant gene prevalence in soils due to animal manure deposition and long-term pasture management. PeerJ 8, e10258. https://doi.org/10.7717/peerj.10258.

Yang, Y., Ashworth, A.J., Durso, L.M., Savin, M., DeBruyn, J.M., Cook, K., Moore Jr., P.A., Owens, P.R., 2021. Do long-term conservation pasture management practices influence microbial diversity and antimicrobial resistant genes in runoff? Front. Microbiol. 12, 617066. https://doi.org/10.3389/fmicb.2021.617066.