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Escherichia coli and Enterococci Attachment to Particles in Runoff from Highly and Sparsely Vegetated Grassland

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Abstract Limited data on microbial partitioning between the freely suspended and particulate attached phases during transport along overland flow pathways have resulted in high uncertainty in bacterial fate and transport models and the application of these models to watershed management plans. The objectives of this study were to examine differences in attachment between *E. coli* and enterococci in runoff from plots with highly and sparsely vegetated grassland; investigate relations between flow regime, total suspended solids, and *E. coli* and enterococci attachment; and identify the particle size categories to which the attached cells were associated. Two rainfall simulations were conducted on large field plots 3 m wide by 18.3 m long with highly and both highly and sparsely vegetated covers and treated with standard cowpats. Results from the first experiment representing pasture with highly vegetated cover indicate that the majority of *E. coli* and enterococci are transported from the fresh manure source in the unattached state with only 4.8% of *E. coli* and 13% of enterococci associated with particles. The second experiment which compared partitioning in runoff from both highly and sparsely vegetated covers found lower bacterial attachment rates: the average *E. coli* percent attached was 0.06% from plots with highly vegetated cover and 2.8% from plots with sparsely vegetated cover while the corresponding values for enterococci were 0.98% and 1.23%, respectively. The findings from this study provide the first set of data on bacterial partitioning in overland flow from large field plots, and results may be helpful for parameterizing water quality models and designing conservation practices.

Keywords *Escherichia coli* · Enterococci · Microbial partitioning · Nonpoint source pollution · Water quality

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1 Introduction

Pathogens are a leading cause of water quality impairments in the USA and much of the world. Pathogens may originate from agricultural operations, leaking septic systems, combined sewer overflows, or wildlife; however, agricultural practices have been cited as the primary contributor to impairments of rivers and streams in the USA (US EPA 2003). The three most common pathogen indicators are fecal coliforms, *E. coli*, and enterococci (US EPA 1986). Although fecal coliform have been traditionally used

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as an indicator to detect the potential presence of pathogens in surface waters, *E. coli* and enterococci are thought to have a higher degree of association with outbreaks of gastrointestinal illnesses (US EPA 1986) and are currently the recommended indicator organisms (US EPA 1998, 2002). In an attempt to reduce pollutant loading to the water bodies, watershed assessments such as Total Maximum Daily Loads are being developed to identify pollution sources and determine pollutant reductions needed to restore and protect rivers, streams, and lakes.

Development and implementation of watershed management plans are costly, and therefore the ability to accurately predict the pollutant loadings from the potential sources within a watershed is essential. Currently, nonpoint source (NPS) pollution models are most frequently used to determine the maximum allowable loading rates of fecal indicators from diffuse sources that are necessary to meet water quality standards, and most NPS models simulate microorganism transport in the unattached or dissolved state (Paul et al. 2004). Previous studies have reported that fecal bacteria attach to particles in streams and estuaries (Characklis et al. 2005; Fries et al. 2006, Goulder 1977). Sediments in streams act as a reservoir of fecal indicators (Burton et al. 1987; Davies et al. 1995; Smith et al. 2008) that are resuspended during storms (Jamieson et al. 2005), and recent efforts have emphasized the importance of including the sediment-associated microbes when modeling in-stream microbial transport (Rehmann and Soupir 2009; Wu et al. 2009). Information is lacking regarding the mechanisms of microbial transport along overland flow pathways, contributing to the high uncertainty in efforts to model bacterial transport processes (Collins and Rutherford 2004).

In the presence of manure, indicator organisms may move freely with overland flow in an unattached state or be transported with particulates via association with soil, manure particles, or fecal particles attached to soil (Guber et al. 2007). Several researchers have found that the presence of manure reduces soil–bacteria attachment (Guber et al. 2005a, b) and that the release of fecal indicators to saturation excess flow from animal-waste-amended soils is in either the unattached state (Muirhead et al. 2005) or via association with fecal colloids (Soupir et al. 2010). Land management practices may further complicate understanding of microbe–particulate interactions because of differences in sediment concentrations in flow (availability of attachment sites) and contact between soil and microorganisms (i.e., surface-applied versus incorporation of animal waste).

Runoff from grazed pastures contributes significant bacterial loadings, resulting in downstream water quality impairments (Doran and Linn 1979; Doran et al. 1981; Soupir et al. 2006; Moore et al. 1982), but implementing conservation practices to reduce bacterial transport has been met with limited success. Stream bank fencing is often recommended when cattle have direct access to streams (Line 2003); however, other management practices, in addition to stream fencing, are likely necessary to reduce concentrations in runoff (Oliver et al. 2007b). Vegetated buffer strips are promoted as a practice to reduce pollution transport, but their effectiveness related to pathogen indicator reduction has produced mixed results (Coyne et al. 1995; Entry et al. 2000; Larsen et al. 1994; Lim et al. 1998; Roodsari et al. 2005). Meals (2001) reported that the combination of buffers, riparian fencing, and protected stream crossings is necessary for significant reduction of bacterial counts in an agricultural watershed in Vermont.

Grazed pastures are a significant source of pathogen indicators in agricultural areas, but tools are lacking to accurately predict movement from the land to surface waters; specifically, little is known about the transport mechanisms in overland flow pathways. This study seeks to expand upon the current knowledge of fecal indicator release from manure by examining partitioning between the attached and unattached phases during overland flow using large-scale field plots. The objectives of this study were to (1) examine differences in attachment between *E. coli* and enterococci in runoff from plots with highly and sparsely vegetated grassland; (2) investigate relations between flow regime, TSS, and *E. coli* and enterococci attachment; and (3) identify the particle size categories to which the attached cells were associated. The findings from this study provide the first data set on bacterial partitioning in overland flow from large field plots, and results may be used to parameterize water quality models and aid in the design and selection of conservation practices.

2 Materials and Methods

Two field experiments were conducted to examine the transport of *E. coli* and enterococci from highly and

sparsely vegetated cover grassland. Five field plots were constructed, four of the plots were treated with standard cowpats (one served as a control), and water was applied with a rainfall simulator (simulation 1 or S1). Runoff samples were collected at discrete intervals during the hydrograph and analyzed for attached and unattached *E. coli*, enterococci, and total suspended solids (TSS). Four months after the first experiment, 50% of the vegetation was removed from two of the four treatment plots; new cowpats were applied to all plots except the control, and a second rainfall simulation (simulation 2 or S2) was conducted.

Field Experiment plots were constructed on newly established vegetation on an area that had not received any manure applications during the previous 3 years. A seedbed of Kentucky 31 Tall Fescue was prepared during the fall prior to plot construction. To prepare the seedbed, the existing vegetation was sprayed twice with Roundup™, plowed twice, limed, fertilized, and broadcast with Kentucky 31 Tall Fescue. The area was irrigated weekly until vegetation emerged. The following spring five field plots, each 3 m (9.8 ft) wide by 18.3 m (60 ft) long, were constructed on a Groseclose silt loam (35% sand, 60% silt, and 5% clay) grassland on an approximately 9% slope dominated by a dense stand of Kentucky 31 Tall Fescue. A "V"-shaped outlet at the downslope end of each plot directed runoff into a 0.15-m (6-in.) H-flume equipped with a stilling well and a stage recorder for flow measurement. The stage recorder did not function properly on the control plot during S1, so flow data from the control plot during S2 were used for calculations. Four months later, vegetation was removed from two of the plots previously receiving cowpat applications to represent vegetation levels associated with overgrazed or poorly managed pasture. A dethatcher was used to remove approximately 50% of all established vegetation, and three equally sized bare areas were created in the top, middle, and lower third of the plots with a string trimmer. Areas void of any vegetation accounted for an average of 28.4% of the total plot area. Plots with vegetation removed are referred to as sparsely vegetated (SV) plots and the plots in which no vegetation was removed are referred to as highly vegetated (HV) plots.

Fresh dairy cattle fecal deposits were collected at the Virginia Tech dairy facility over a 24-h period. Standard cowpats (Thelin and Gifford 1983) were formed by mixing the manure in a cement mixer for 15 min. The homogenized manure was placed in molds with a diameter of 20.3 cm (8 in.) and a depth of 2.54 cm (1 in.) until a weight of 0.9 kg (2.0 lbs) was reached. Approximately 106 cowpats were applied to four of the five plots to represent grazed pastureland. The plot length was divided into 0.91 m (3 ft) segments, and five cowpats were randomly applied to each section. Six cowpats were applied to the "V"-shaped outlet at the downslope end of each plot. During the second field experiment, the same procedure was followed regarding manure collection and application. Standard cowpats were applied to the same plots that had received the previous applications: Previously applied cowpats had disintegrated due to warm temperatures, thick vegetation, and frequent mowing.

Due to the unreliability of natural precipitation for short-term field research, a rainfall simulator (Dillaha et al. 1988) generated a uniform rainfall event (2.8 cm/h) to all plots less than 24 h after application of manure to the plots. Discrete grab samples were collected at the outfall of the flumes at the onset of runoff, at 10-min intervals during the storm event, immediately following the end of the storm event, and 4 min after precipitation ceased. Three samples were collected during each sampling event, one for bacterial partitioning studies, one for total *E. coli* and enterococci concentration analysis, and one for TSS analysis. The rainfall event continued until runoff from all plots reached steady state: 3 h and 20 min for simulation one (S1) which was conducted in April and 4 h and 11 min for simulation two (S2) which was conducted in August. Steady state was defined as when flow at the outfall of the flumes remained constant (less than 10% change in stage) as determined by the stage recorder. Collected samples were transported to the laboratory immediately following the end of the rainfall simulation for analysis.

Sample Analysis Surface soil samples (0–8-cm depth) were collected with a soil probe from each transport plot prior to S1. The samples were sieved (2 mm) and stored prior to analysis. Soils were analyzed for Mehlich-1 P (11 mg kg^{-1}), organic matter by a modified Walkley–Black method (2.6%), and pH by 1:1 soil to distilled water ratio and solid-state pH meter (6.22) as described by Donohue and

Heckendorn (1994). Manure samples were collected prior to land application and analyzed by the Clemson Agricultural Service Laboratory. Water soluble P (2.02 g kg^{-1}) was determined by the method proposed by Sharpley and Moyer (2000). The pH (5.6) was measured potentiometrically in a 1:2 manure/water slurry (Peters et al. 2003). Average moisture content of fresh manure samples was 83.6%, determined gravimetrically. *E. coli* and enterococci concentrations in manure were enumerated on modified mTEC and mE agar (US EPA 2000) by membrane filtration (American Public Health Association 1998). Five samples of fecal material were diluted in phosphate buffer solution (Hach Co., Loveland, Colo.) at a 1:10 ratio. All samples were dispersed by treatment with a hand shaker for 10 min (Wrist Action shaker, Burrell Scientific, Pittsburgh, PA), and serial dilutions were performed in 1,000 mg L^{-1} dilutions of Tween 85 solution. *E. coli* concentrations in manure averaged 4.19×10^{6} colony-forming unit (CFU) g^{-1} dry wt (SD=3.16×10^{6}) before S1 and 2.62×10^{7} CFU g^{-1} dry wt (SD=1.42×10^{7}) before S2. Enterococci concentrations in manure averaged 2.54×10^{8} CFU g^{-1} dry wt (SD=1.06×10^{8}) before S1 and 1.07×10^{9} CFU g^{-1} dry wt (SD=5.33×10^{8}) before S2.

Water samples were analyzed for *E. coli* (attached and total concentrations), enterococci (attached and total concentrations), and TSS. Partitioning of pathogen indicators between attached and unattached phases was achieved by fractional filtration followed by centrifugation. Complete details regarding development and testing of the partitioning technique is described by Soupir et al. (2008). Briefly, samples were sequentially dispensed through four filters with average pore diameter of 500, 63, 8, and 3 μm to retain particles larger than coarse sand; medium, fine, and very fine sand; fine, medium, and coarse silt particles; and clay and very fine silt particles, respectively. Throughout the study, no measurable particulates (≥ 1.0 mg) passed through the 8-μm filter, and therefore cells associated with the 3-μm filter were classified as unattached. Following filtration, the retained solids were rinsed from all filter surfaces, resuspended in phosphate-buffered water (Hach Company, Loveland, CO), and centrifuged (Avanti J-25I, Beckman Coulter, Fullerton, CA) at 4,700 rpm (3,043×*g*) for 15 s (Huysman and Verstraete 1993; Lago 2005). A 1-mL aliquot of the supernatants (obtained from each of the four filter rinsates) and a 1-mL aliquot of the terminal filtrate (collected after passing through the 3-μm filter) were enumerated for *E. coli* and enterococci concentrations by membrane filtration (American Public Health Association 1998) using Modified mTEC and mE agar (US EPA 2000) to assess the unattached bacterial fraction. After centrifugation of each filter rinsate, each solution was treated with a hand shaker for 10 min to resuspend particulates and disperse attached and bioflocculated cells. The dispersed solution, representing the total concentration retained by each filter, was also enumerated for *E. coli* and enterococci concentrations by membrane filtration. TSS were analyzed by filtering samples through a 0.45-μm glass fiber filter (Pall Life Sciences, Ann Arbor, MI) and following the procedure recommended by the EPA method 160.2.

Calculations and Statistical Analysis All unattached and total concentrations (TC) were converted to number of culturable cells based on sample volume. The number of unattached cells was calculated by summing the number of cells in the supernatant of each of the four centrifuge tubes and the number of cells in the terminal filtrate. The total number of cells was determined by summing the number of cells in each of the four centrifuge tubes and the number of cells in the terminal filtrate. The attached portion was assumed to be the difference between the unattached and total *E. coli* and enterococci concentrations. The particulate associated fraction (PAF) was calculated by dividing the attached number of cells by the total number of cells. The attached number associated with each screen size was calculated by subtracting the total number of cells in the supernatant associated with each screen size from the total number of cells in each centrifuge tube. This value was divided by the TSS associated with each screen size to obtain the CFU per gram of particles. Flow-weighted concentrations were calculated by multiplying the sample concentrations by the subsequent runoff volume and then dividing by the total runoff volume from each plot. Loads were calculated by multiplying the sample concentrations by the subsequent runoff volume and converting the plot area to a per hectare basis. One of the two plots receiving the cowpat treatment with HV cover was excluded from bacterial load calculations because of missing data points during S2.

Statistical analysis of data was performed using the Statistical Analysis System (SAS Institute 2004).

During S1, the nonparametric Kruskal–Wallis rank test was used to test for significant differences between PAF and TC during the rising, peak (or steady state), and receding limbs of the runoff hydrograph. During S2, a 2-way analysis of variance (ANOVA) and Tukey's pairwise comparison were used to test for significant differences between partitioning coefficients and total concentrations during the rising, peak, and receding limbs of the runoff hydrograph and between highly and sparsely vegetated cover plots using the MIXED procedure. During S1, 19 samples were included in the rising limb analysis, ten samples in the peak limb analysis, and six samples in the falling limb analysis. During S2, 11 samples were included in the rising limb analysis, six samples in the peak limb analysis, and two samples in the falling limb analysis. Pearson correlation coefficients for PAF and TC and TSS were determined using PROC CORR, and a p test was performed to test for statistical significance (SAS Institute 2004). ANOVA and Tukey's pairwise comparison were used to test for differences in concentrations of bacteria associated with the different particle size categories. Data were normalized prior to analysis by natural log transformation, and statistical significance was determined when $p \leq 0.05$.

3 Results and Discussion

The rainfall event continued until runoff from all plots reached steady state. The longest runoff event lasted 90 min during S1 and 105 min during S2, both times occurring on one of the replicate treatment plots with HV cover. Few differences were noted between average runoff volumes among treatments during S1 or S2 (Fig. 1). The average runoff volume was 15% lower from the HV plots when compared to the control during S1 and the volume from the SV plots was 17% lower than the control during S2. The standard deviation of the runoff volume from highly vegetated plots during S1 and S2 was 173 and 310 L, respectively, and the standard deviation of the runoff volume from sparsely vegetated plots during S2 was 138 L. Differences are attributed to variation among plots and time for runoff to occur after the start of the rainfall event.

Flow-Weighted Concentrations in Runoff The average *E. coli*, enterococci, and TSS flow-weighted concentrations and average PAF values for S1 and S2 are presented in Table 1. Control plot bacteria samples were not partitioned between the attached and unattached phases because of the low cell counts. Background *E. coli* was detected in two of the ten samples collected during S1 and in five of the 12 samples collected during S2 from the control plots. Enterococci were not detected in samples collected during S1 but were present in all samples during S2. Background bacteria concentrations in runoff samples collected from the control plot is most likely attributed to wildlife (Doran et al. 1981; Patni et al. 1985) or to cross-contamination during manure application to treated plots.

Residual cowpats from the April application had disintegrated due to warm temperatures, thick vegetation, and frequent mowing. It is possible that a fraction of the total concentrations of fecal indicators detected during S2 was due to the previous manure applications (Kress and Gifford 1984); however, release of fecal indicators from aged cowpats has previously been found to be significantly lower than release from fresh cowpats (Soupir et al. 2010). If aged deposits acted as a source of fecal bacteria during S2, it is possible that the attached fractions would be higher: cells surviving for an extended time in the terrestrial environment are likely to be stressed and attach to sediments and organic particles to increase survival (Morita 1997). However, total bacteria concentrations were more than one order of magnitude lower in runoff during the second simulation, so even if bacteria from the aged fecal material

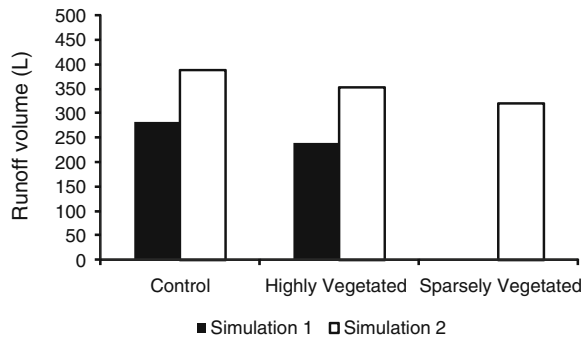

Fig. 1 Average runoff volumes measured during simulation 1 and simulation 2 from plots with highly and sparsely vegetated cover. The sparse vegetative cover treatment was only examined during the second simulation

Table 1 *E. coli*, enterococci, and total suspended solids average flow-weighted concentrations and average particulate attached fractions

	Treatment	FWC	Units	PAF	SD	Range
Simulation 1						
E. coli	HV	6.96×10^{5}	CFU 100 mL^{-1}	4.76×10^{-2}	7.12×10^{-2}	8.48×10^{-4}–0.343
	Control	176	CFU 100 mL^{-1}	–	–	–
Enterococci	HV	3.63×10^{5}	CFU 100 mL^{-1}	0.130	0.129	3.07×10^{-3}–0.460
	Control	0	CFU 100 mL^{-1}	–	–	–
TSS	HV	152	mgL^{-1}	–	–	–
	Control	19.8	mgL^{-1}	–	–	–
Simulation 2						
E. coli	HV	2.73×10^{4}	CFU 100 mL^{-1}	5.75×10^{-4}	5.67×10^{-4}	2.98×10^{-5}–1.99×10^{-3}
	SV	2.00×10^{4}	CFU 100 mL^{-1}	2.79×10^{-2}	6.24×10^{-2}	5.44×10^{-5}–0.230
	Control	2.52×10^{2}	CFU 100 mL^{-1}	–	–	–
Enterococci	HV	1.51×10^{4}	CFU 100 mL^{-1}	9.84×10^{-3}	2.00×10^{-2}	7.55×10^{-5}–7.89×10^{-2}
	SV	1.36×10^{4}	CFU 100 mL^{-1}	1.23×10^{-2}	4.79×10^{-3}	2.84×10^{-4}–0.114
	Control	4.02×10^{3}	CFU 100 mL^{-1}	–	–	–
TSS	HV	51.0	mgL^{-1}	–	–	–
	SV	670	mgL^{-1}	–	–	–
	Control	23.2	mgL^{-1}	–	–	–

were present in runoff, the high concentrations in fresh manure likely overwhelmed the aged contribution and might not be represented in the attached fractions. The concentration of both fecal indicators in manure was an order of magnitude higher in August when compared to April; yet, the FWC is an order of magnitude lower in runoff during S2. The reduced release and transport of bacteria during S2 could perhaps be attributed to warmer temperatures and higher solar radiation that would increase surface sealing of the fecal deposit and promote decay of bacteria in the outer crust (the bacteria readily available for release into overland flow).

During S2, the average *E. coli* PAF decreased to 0.06% from plots with highly vegetated cover and 2.8% from plots with sparse vegetative cover. The average PAF for enterococci decreased to 0.98% from well-managed plots and 1.2% from SV plots. In general, attachment from both highly and sparsely vegetated cover plots was low with less than 3% of all *E. coli* and enterococci classified as attached. Among the two fecal indicators, only *E. coli* PAF was significantly higher in runoff from plots with SV cover (p value=0.0002) when compared to the HV cover. *E. coli* and enterococci TC did not differ statistically between the HV and SV treatments.

Muirhead et al. (2005) found that *E. coli* in fresh and aged cowpats were primarily transported as single cells with an average attachment of 8%; however, Soupir et al. (2010) reported attachment rates between 28% and 49% in flow from small plots receiving high-intensity simulated rain. While it is likely that cells associate with soil when exposed to the external environment, evidence is building that the main mechanism of transport from simulated pasture receiving fresh manure application is via fecal particles (Soupir et al. 2010) or in the unattached state (Guber et al. 2007). The short time period (24 h) between manure application and runoff in our study also supports these findings since fresh, undisturbed cowpats have little opportunity to interact with soil particles. Further, bacteria present in runoff from lands treated with fresh manure are unlikely to be stressed and thus attach since nutrients and moisture are abundant. This study differs from previous reports because the impact of pasture management is evaluated, but removal of vegetation did not change total concentrations of indicators during S2 and only

resulted in a slight increase in *E. coli* attachment, while TSS concentrations increased significantly (p value<0.0001). Previous field studies examining association of microbes with particles have primarily focused on short intervals between manure application and saturation excess flow; therefore, future study is recommended to evaluate the impact of extended environmental exposure prior to rainfall on the attachment of fecal indicators to sediment and fecal particles.

Temporal Distribution of PAF and TC The temporal variation was evaluated to see if changes in flow regime affected indicator PAF, indicator TC, or TSS. Figure 2 presents the average PAF, TC, and TSS values versus time during S1 and S2. During S1, slightly higher attachment by both indicators was observed at the initial sampling event after the start of runoff (Fig. 2a); however, neither *E. coli* nor enterococci PAF or TC were significantly different between the rising, peak, and recession limbs of the runoff hydrograph.

During S2 (Fig. 2b, d), the enterococci TC in the rising limb was significantly lower than the TC at steady state from the plot with SV cover while *E. coli* TC was significantly lower from HV plots during steady state than during the rising limb of the hydrograph. The *E. coli* PAF was significantly higher during the receding limbs of the runoff hydrograph from the plots with SV cover when compared to the peak. No statistically significant differences were found between hydrograph stages for enterococci PAF. Relationships between bacterial attachment and flow have been noted previously (Guber et al. 2005b; Krometis et al. 2007); however, few statistically significant differences were detected in this study, possibly due to the low overall attachment in overland flow.

A correlation analysis found no significant linear relationships between bacterial TC or PAF and TSS concentrations during the S1 simulation. During the S2 simulation, only *E. coli* PAF was correlated with the TSS concentration (r=0.59). Previous attempts to correlate microbial attachment in saturation excess flow and environmental factors have also resulted in limited success (Soupir 2008); however, others have found linkages between in-stream fecal coliform partitioning and particle number concentrations (Characklis et al. 2005). In this study, a range of TSS concentrations were evaluated, and the amount of sediment appears to have little impact on bacterial partitioning, indicating that sediment is not a limiting attachment and that other factors are responsible for attachment.

The combined results from these rainfall simulations indicate that vegetation is very effective in filtering out particulates, but comparison of HV and SV cover did not result in differences in *E. coli* or enterococci concentrations. Manure was placed on the entire plots and a buffering zone was not present to allow for additional filtering of particles or indicators. Previously, Roodsari et al. (2005) found vegetation to decrease transportable fecal coliform by 23% to 67% when compared to bare soil plots. This indicates that a buffer zone at the edge of pastureland is necessary to reduce bacterial transport as HV cover used to represent well-managed pasture alone was not effective in preventing microbial movement.

Bacterial Loading Rates Loading curves describing the bacterial partitioning over the duration of the two rainfall events are presented in Fig. 3. Plots with the longest runoff event and therefore highest number of samples were used to create the curves. During S1, the unattached fraction consistently exceeded the attached fraction by more than an order of magnitude. During S2, the *E. coli*-unattached load was greatest from plots with HV cover while the *E. coli*-attached load was higher from plots with SV cover. The unattached enterococci load was similar between the two plot treatments, but the attached fraction was consistently higher in runoff from plots with sparse vegetative treatment. While the higher loading of the attached indicators from sparsely vegetated plots is to be expected, the overwhelming loading of both indicators in the unattached state suggests that management practices should focus on removal of cells transported in the free or unattached state to reduce bacterial transport from freshly grazed pasturelands, regardless of vegetative cover. The S1 control plot *E. coli* load was 8.47×10^{7} CFU ha^{-1}, and enterococci load was below the level of detection while the S2 control plot *E. coli* load was 1.67×10^{8} CFU ha^{-1} and enterococci load was 2.66×10^{9} CFU ha^{-1}.

Attachment to Particle Size Categories The distribution of TSS concentrations and attached bacteria

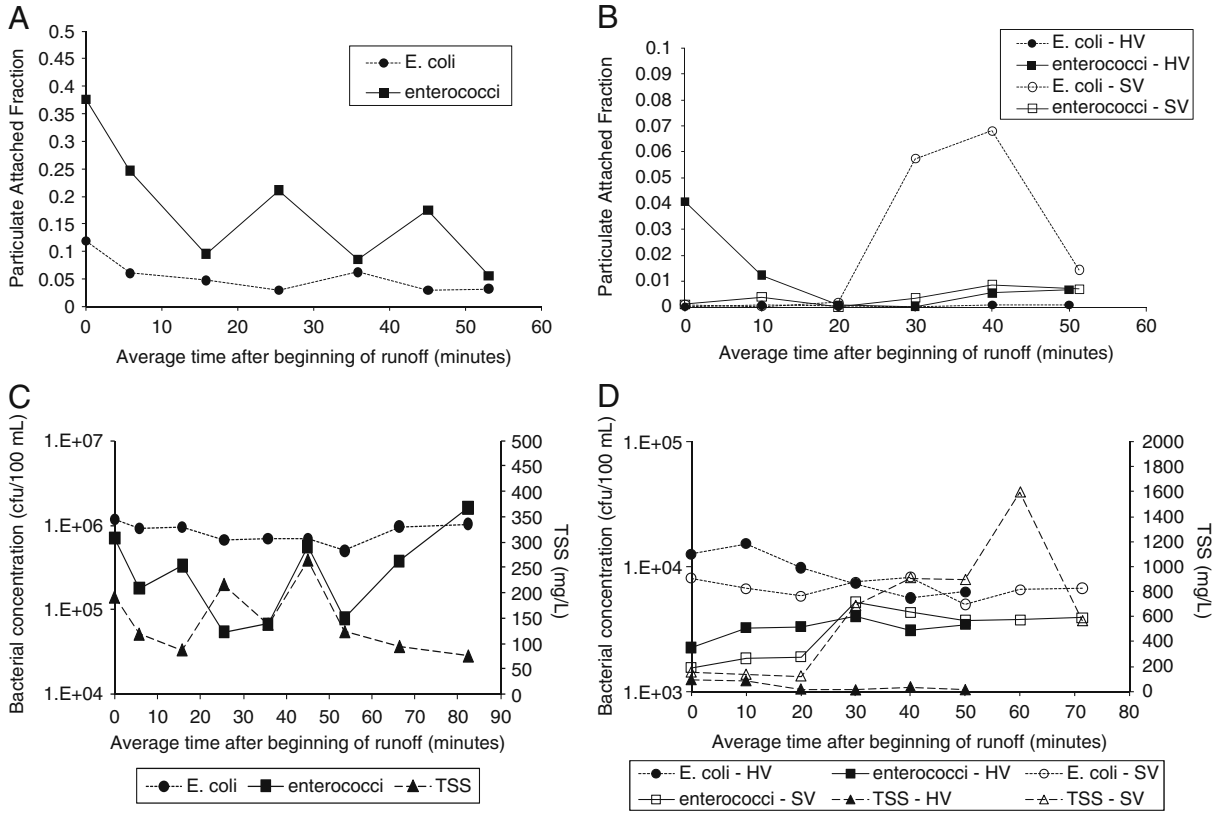

Fig. 2 Temporal distributions of *E. coli* and enterococci particulate attached fractions during simulation 1 (**a**) and simulation 2 (**b**) and *E. coli*, enterococci, and TSS concentrations during simulation 1 (**c**) and simulation 2 (**d**) in overland flow

among particle size categories is presented in Table 2. Total suspended solids associated with a screen size weighing less than 1 mg were considered to be negligible, and it was assumed that all cells retained by that screen size either remained in suspension or were bioflocculated but not attached to particulates. From plots with high vegetative cover during S1 and S2, the majority of particles were retained on the 8-μm screen. During S2, the majority of suspended solids from the plots with HV cover were retained on the 8-μm screen while the majority of particles from the plots with SV cover were retained by the 63-μm screen, indicating that the vegetation is effective in filtering and/or preventing detachment of larger particles.

The highest concentrations of *E. coli* and enterococci were both associated with particles retained by the 8-μm screen size during S1; however, during S2, *E. coli* and enterococci from HV plots both associated at a higher rate with particles >500 μm. From the SV cover plots, the attached cells were more frequently associated with the 8–63 μm particle size category, but only the *E. coli* associated with the 8-μm screen (92%) was statistically higher than the *E. coli* associated with particles retained by the 63-μm (6%) and 500-μm (2%) screens.

The distribution of attached cells was similar to the distribution of TSS among the three particle size categories for both indicator organisms during S1 and for enterococci in runoff from SV plots during S2. The larger total surface area associated with the smaller particle size fraction corresponds to a higher number of sites available for bacterial attachment. Recent studies suggest that the primary mechanism of cellular transport in saturation excess flow from small plots is manure particles which are typically between 0.6- and 17.8-μm diameter (Pachepsky et al. 2009). Previous studies have also identified predominant attachment of fecal indicators to smaller particle sizes (Auer and Niehaus 1993; Oliver, Clegg et al. 2007; Schillinger and Gannon 1985). The broad distribution of *E. coli*

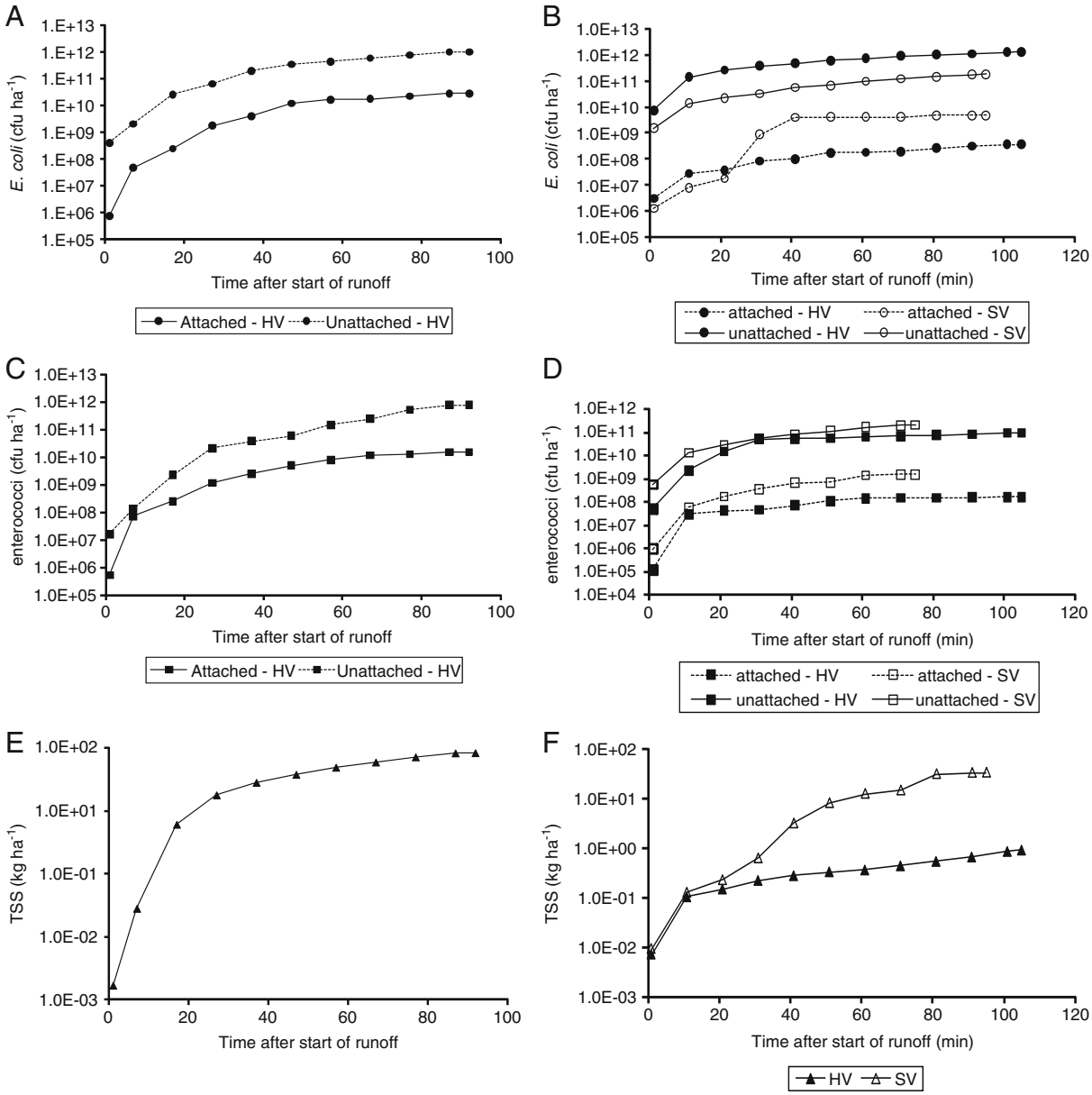

Fig. 3 *E. coli*, enterococci, and TSS loading rates from plots with highly and sparsely vegetated cover (HV and SV) treated with cowpats during an overland flow event during simulation 1 (**a**, **c**, **e**) and simulation 2 (**b**, **d**, **f**)

and enterococci across particles sizes during S2 might be due to the low attachment rates (PAFs). Only a very small fraction of the total indicator population was identified as attached, and therefore only a small sample of attached cells was available for classification. *E. coli* attachment to a broad range of suspended particle (as noted in S2–HV plots) diameters has been previously observed (Jeng et al. 2005) and is possibly due to the motility and rod shape of *E. coli* and its ability to attach to different angles or faces of the particles such as edge-to-edge or face-to-edge associations.

4 Conclusions

Experiments were conducted to collect the first data set on the partitioning of two fecal indicators, *E. coli*

Table 2 Particle sizes to which *E. coli* and enterococci attach in samples collected at the edge of the field in runoff from plots with highly and sparsely vegetated cover

Particle Size	Mean TSS (mg/L)	% TSS	*E. coli*		Enterococci	
			Mean CFU/mg solids	% attachment	Mean CFU/mg solids	% attachment
Simulation 1—highly vegetated cover						
>500 μm	43.9	15%	851ab	28%	240b	13%
63–499 μm	70.7	25%	433b	14%	549a	29%
8–62 μm	171.9	60%	1,766a	58%	1,095a	58%
Simulation 2—highly vegetated cover						
>500 μm	28.8	21%	76a	44%	120a	76%
63–499 μm	34.0	24%	25a	15%	23a	15%
8–62 μm	76.4	55%	70a	41%	15a	9%
Simulation 2—sparsely vegetated cover						
>500 μm	413.5	32%	18a	2%	34a	33%
63–499 μm	512.9	39%	49a	6%	32a	31%
8–62 μm	374.0	29%	726b	92%	37a	36%

Means followed by the same letter do not differ at the 5% level of significance according to Tukey's pairwise comparison

and enterococci, in overland flow from large-scale field plots. Standard cowpats were applied to vegetated plots planted with Kentucky 31 Tall Fescue to represent pasture. Two rainfall simulations were conducted: one on plots with highly vegetated cover and a second on plots with both highly and sparsely vegetated cover. Runoff samples were collected and analyzed for *E. coli* and enterococci total concentrations and particulate attached fractions and TSS.

Results from the first rainfall simulation representing pasture with highly vegetated cover found that the majority of *E. coli* and enterococci are transported from a fresh manure source in the unattached state. Average PAF value was 4.8% for *E. coli* and 13% for enterococci. Comparison of unattached and attached indicator loading rates found that the unattached fraction exceeded the attached fraction by at least two orders of magnitude. Fifty-eight percent of all attached cells were associated with particles smaller than 63 μm in size. Results from the second study comparing highly and sparsely vegetated cover lands also indicated that the majority of *E. coli* and enterococci are transported from the fresh manure source in the unattached state. The average *E. coli* PAF was 0.06% from plots with highly vegetated cover and 2.8% from plots with sparse vegetative cover. The average PAF for enterococci was 0.98% from plots with highly vegetated cover and 1.23% from plots with sparsely vegetated cover. Only the *E. coli* present in runoff from the poorly managed pastureland plots had statistically higher association with the 8-μm particle size range (92%) than the 63-μm (6%) and 500-μm (2%) particle size ranges.

Partitioning fractions presented here can be incorporated into water quality models that separate the transport of microorganisms into the attached and unattached phases. It appears that even slight changes in external environmental factors or cellular functions could potentially result in great variation in the percentage of fecal indicators associating with particles, and the conditions or associated responses driving these changes have not been identified. The majority of cells in this study were transported to the edge of the field in the unattached state from pasturelands receiving fresh fecal deposits regardless of vegetative cover, and therefore, conservation practices applied to grazed landscapes should focus on reduction of pathogen indicators moving in the unattached state. Future study is recommended to determine partitioning of indicators from different land uses with greater interaction between manure and soils and from aged fecal sources with potentially stressed microorganisms.

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