Journal of Environmental Management 110 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Management

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

Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry

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ARTICLE INFO

Article history:
Received 8 February 2011
Received in revised form
13 March 2012
Accepted 15 May 2012
Available online 15 June 2012

Keywords:
Vegetative filter strips (VFS)
Lysimeter
Escherichia Coli
Miniflumes
Salmonella
Runoff

ABSTRACT

Vegetated filter strips (VFS) are commonly recommended as a best management practice to prevent manure-borne microorganisms from reaching surface water resources. However, relatively little is known about the efficacy of VFS in mitigating bacterial runoff from land-applied swine manure. A field lysimeter study was designed to evaluate the effect of surface soil hydrologic conditions and vegetation on the retention of swine manure-borne *Escherichia coli* and *Salmonella* under simulated rainfall conditions. Experimental plots (6.5 m × 3.9 m) were set on a 5% slope lysimeter with loamy topsoil, clay loam or loam subsoil and a controllable groundwater level. Three small flow-intercepting miniflumes were installed 4.5 m from the plot's top, while all remaining runoff was collected in a gutter at the bottom. Plots were divided into bare soil and grass vegetation and upper surface soil moisture before rainfall events was controlled by the subsurface groundwater level. Swine manure slurry inoculated with *E. coli* and *Salmonella*, and with added bromide tracer, was applied on the top of the plots and simultaneously initiated the simulated rainfall. Runoff was collected and analyzed every 5 min. No substantial differences between retention of *E. coli* and *Salmonella* were found. In initially wet soil surface conditions, there was limited infiltration both in bare and in vegetated plots; almost all bromide and about 30% of bacteria were recovered in runoff water. In initially dry soil surface conditions, there were substantial discrepancies between bare and vegetated plots. In bare plots, recoveries of runoff water, bromide and bacteria under dry conditions were comparable to wet conditions. However, in dry vegetated plots, from 50% to 75% of water was lost to infiltration, while bromide recoveries ranged from 14 to 36% and bacteria recovery was only 5%. Substantial intraplot heterogeneity was revealed by the data from miniflumes. GIS analysis of the plot microtopography showed that miniflumes located in the zones of flow convergence collected the majority of bacteria. Overall, the efficiency of VFS, with respect to the retention of swine manure bacteria, varied dramatically depending upon the hydrologic soil surface condition. Consequently, VFS recommendations should account for expected amounts of surface soil water saturation as well as the relative soil water storage capacity of the VFS.

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1. Introduction

Contamination of drinking, irrigation and recreational water supplies with gastrointestinal pathogenic bacteria is a serious public health concern since exposure to these microorganisms can result in severe, and occasionally lethal, illnesses (Bitton and Harvey, 1992; Mead et al., 1999). A primary source of water-borne pathogens (e.g., *Salmonella,* pathogenic *Escherichia coli*) is animal manures, including poultry, cattle, and swine. A substantial threat to surface waters is runoff from agricultural fields receiving land applications of manure and/or direct fecal deposition (Patni et al., 1985; USEPA, 2000; Collins et al., 2005). In particular, the risk of surface water contamination is exacerbated where high rates of manure are applied to relatively small agricultural areas, such as occurs in the vicinity of confined animal feeding operations (CAFOs). Recent reports of water-borne *E. coli* 0157:H7 (0157) and *Salmonella* outbreaks (Haley et al., 2009; Nwachuku and Gerba, 2008) illustrate the need to develop mitigation strategies for microbial transport from fields to water resources.

Numerous studies have been conducted to investigate the efficacy of vegetated filter strips (VFS) as a best management practice

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0301-4797/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.jenvman.2012.05.012

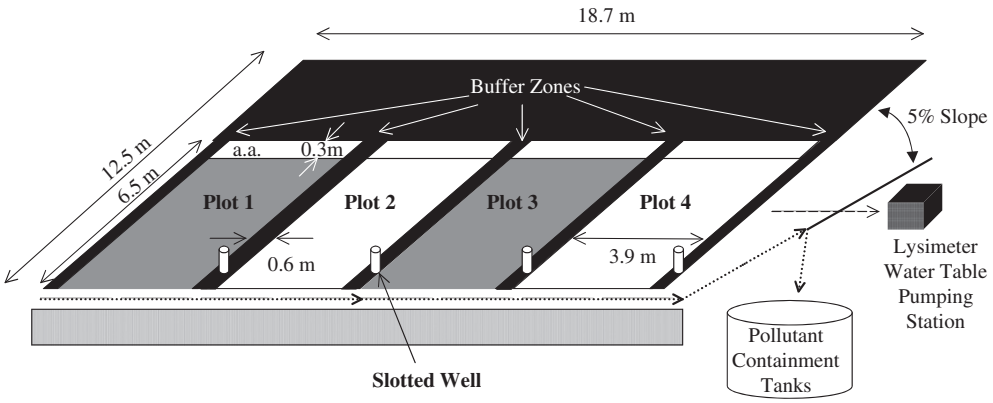

Fig. 1. Schematic diagram of lysimeter plots.

(BMP) for mitigating bacterial transport from land-applied bovine manure to surface waters (Dillaha et al., 1989; Edwards et al., 1996; Coyne et al., 1995; Fajardo et al., 2001; Mankin et al., 2006; Sullivan et al., 2007). By comparison, much less is known about VFS efficacy in retention of microorganisms originating from swine manure applications to agricultural fields (Koelsch et al., 2006). Given the differences in the consistency and organic matter composition of cattle and swine manures resulting from digestion and diet (Miller and Varel, 2003), it is unclear if the results from bovine manure are directly applicable to swine manure. In addition, there are inconsistencies in the available data for swine manure. Roodsari et al. (2005) reported virtually complete removal of *E. coli* and *Salmonella cholerasuis*, inoculated into a liquid swine slurry, from runoff after transport over a VFS (20% slope) with sandy loam soil texture. Conversely, Entry et al. (2000) reported that riparian filter strips, consisting of three different types of vegetation (grass, forest and maidencane), did not reduce fecal coliform numbers in runoff from areas treated with swine wastewater. In both studies, however, the impacts of detailed hydrological settings on bacteria transport were not fully evaluated.

The main objective of this study was to investigate the effects of initial soil moisture, caused by differences in subsurface water table fluctuations, on surface transport of the indicator microorganism, *E. coli* and *Salmonella enterica* Typhimurium, from land-applied liquid swine manure under simulated rainfall. Lysimeter experiments were conducted on bare and vegetated plots. In addition, the transport of bacteria was compared with transport characteristics of bromide-ion to help determine whether bacteria and inert tracer have different transport and retention properties.

2. Materials and methods

2.1. Site characteristics

The experimental site was located at the Patuxent Wildlife Research Refuge (US Department of Interior) at Beltsville, MD. A relatively large lysimeter (12.5 m wide by 18.7 m long) was instrumented to monitor surface water flow (Fig. 1). Specifically, the bottom and walls of the lysimeter were enclosed/lined with heavy-duty plastic. The average depth of the lysimeter was approximately 3 m and contained electric pumps which allowed for groundwater to be removed from the lysimeter. The lysimeter had a collection gutter around the bottom section to collect surface runoff. A V-notch weir was installed at the end of the gutter, prior to the water storage tank, to measure total surface runoff and to develop hydrographs.

One year prior to the initiation of these experiments, the original lysimeter soil was removed to a depth of approximately 20 cm, replaced with about 20 cm of loamy soil, and the surface graded to a slope of approximately 5%. The lower half portion of the lysimeter was partitioned into four adjacent and equal-sized 3.9 m (width) × 6.5 m (length) plots. Plots were delineated with thin metal sheets (10 cm × 85 cm) inserted into the soil, creating a 5 cm high wall around each plot. Plots were located approximately 60 cm apart from each other, creating buffer zones to facilitate plot access without disturbing the adjacent plots. Four slotted PVC wells (10 cm in diameter and 85 cm in length) were installed in the lower portion of the buffer zones next to the gutter to a depth of 50 cm, to monitor subsurface water level fluctuations. The first and third plots from the left side of the lysimeter were sowed with fescue grass seeds (vegetated plots) and designated as Plot 1 and Plot 3, respectively. The second and fourth plots from the left side of the lysimeter were kept devoid of vegetation (bare plots) and designated as Plot 2 and Plot 4, respectively. A 30 cm strip across the top of all plots was kept bare and designated as the manure application area. The soil textural analysis was performed for both the topsoil (top 20 cm) and the subsoil (20 cm depth) using the hydrometer method, on composite soil samples collected from five auger holes (Table 1). Results showed that the surface (top 20 cm) horizons of all four plots consisted of similar soil type, mostly loam soils.

Prior to rainfall experiments three portable miniflumes (more detail is provided by Roodsari et al., 2005) were installed along transversal transects located approximately 4.2 m from the bottom edge of the manure slurry application area (at about 2/3 of the plot length down the slope) in plots 1 and 3. Miniflumes were positioned (by visual inspection) at the lowest points (to grab most of the flow passing) along the horizontal transects and were connected to buried food-grade Tygon tubing that ran to the edge of plots and into sample containers.

A topographic map of each vegetated plot (Plots 1 and 3) was constructed by measuring the elevation at each pre-designated grids of 50 cm × 50 cm within each plot using a transit level.

Table 1
Surface (<20 cm) and sub-surface soil texture classification of the four plots at the lysimeter site.

Location	% Clay	% Silt	% Sand	Soil texture class
Plot 1 surface[a]	17.0	39.0	44.0	Loam
Plot 1 20 cm depth	23.0	33.0	44.0	Loam
Plot 2 surface[b]	24.0	42.0	34.0	Loam
Plot 2 20 cm depth	30.0	34.0	36.0	Clay loam
Plot 3 surface[a]	18.5	37.1	44.4	Loam
Plot 3 20 cm depth	29.0	35.0	36.0	Clay loam
Plot 4 surface[b]	22.0	38.0	40.0	Loam
Plot 4 20 cm depth	34.0	40.0	26.0	Clay loam

[a] Vegetated plots.
[b] Bare plots.

2.2. Rainfall simulations

A four-nozzle portable rainfall simulator (Roodsari et al., 2005) was utilized for rainfall simulations. The bars that the nozzles were mounted on were adjusted to be parallel with the plot surface (5% slopes) and the height of the nozzles were also adjusted to be 3 m above the ground so that raindrops would reach terminal velocity (Hirschi et al., 1990). The rain simulator was calibrated to deliver a rainfall intensity of approximately 80 mm h^{-1} (10-year average return period storm) with a uniformity coefficient of 90%.

Rainfall experiments were conducted under two different antecedent soil moisture conditions, wet and dry. For wet conditions, water from natural rainfall (between experiments) was allowed to accumulate inside the lysimeter. Additional water was added prior to experiments to ensure that the water table inside the lysimeter was near the level of the gutter (the lowest point in all plots). For dry conditions, water inside the lysimeter was regularly pumped out to maintain the water table 50 cm below the level of gutter. In addition, dry plots were covered with a tarp during natural rainfall events.

2.3. Manure slurry preparation

Swine manure was collected from a swine waste lagoon located on a farm in Germantown, MD. Due to settling in the lagoon, solids (at the bottom of the lagoon) and overlying water were collected independently and mixed just prior to experiments to achieve a solids content of ca. 4%. The slurry had no detectable *E. coli* or *Salmonella*.

The *E. coli* strain used in this study was isolated from fresh bovine manure collected at Beltsville Agricultural Research Center (BARC) Dairy Research, Beltsville, MD. The nonpathogenic *S. enterica* Typhimurium strain used in this study was purchased from the American Type Culture Collection® in Manassas, VA (ATCC® number 53648). Naladixic acid-resistant isolates were obtained by selection on naladixic acid amended growth medium. *E. coli* and *S. enterica* strains were grown to stationary phase overnight prior to experiments. Appropriate volumes of culture were added to the manure slurry to give 13 L of inoculated slurry containing approximately 10^6 cfu of each bacterium mL^{-1}.

Forty grams of potassium bromide, KBr, (i.e., 26 g of bromide − Br) were added to the 13 L of slurry, giving a bromide concentration of 2000 ppm.

2.4. Experimental procedures

Initial soil water content was determined gravimetrically by taking 6 soil core samples to a depth of 10 cm (3 samples from each side of each plot) and weighing the samples before and after drying at 105 °C for 24 h.

Thirteen liters of liquid swine manure (slurry) was applied uniformly throughout the application area above all four plots (approximating an application rate of 10.9 L m^{-2} or 10.9×10^4 L ha^{-1}). Just before slurry application, duplicate samples were taken to measure initial bacteria concentrations (C_0). Rainfall simulations were initiated immediately after slurry application.

Runoff volume collected in the gutter was measured and sampled (using 20 mL glass vials) at designated time intervals. Runoff volume collected by the three miniflumes was measured on-site and sampled at 5 min intervals after the onset of flow. All manure and runoff samples were stored on ice prior to being transferred to the lab for analysis.

2.5. Analytical procedures

E. coli and *S. enterica* in manure and runoff samples were diluted (as necessary to obtain individual colonies) and three 50 μL replicate sub-samples plated onto naladixic acid-amended MacConkey (for *E. coli*) and BG (for *S. enterica*) agar plates using a Spiral BioTech autoplater. MacConkey and BG plates were subsequently incubated at 44 °C and at 37 °C, respectively, for 18–20 h overnight. Bacterial colonies were counted using two methods. For samples with low background bacterial contamination (most *E. coli*), plates were counted using a Synoptic Limited Protocol Colony Counter. For samples with high background bacterial contamination (a few *E. coli* and all *S. enterica*), colonies were counted manually. To confirm correct identification of *S. enterica*, selected colonies of presumptive *S. enterica* were characterized using the BBL Enterotube II assay; the final count was adjusted based on the percentage of correct identifications. Bromide (Br) concentration (ppm) in runoff samples was measured using an ion-specific electrode Model-525 (Thermo Orion).

2.6. Microrelief analysis

Geostatistical interpolation techniques and GIS hydrologic tools were used to determine the location of surface flow pathways on plots 1 and 3. Elevation data from the experimental field sites were collected on a 50 cm × 50 cm grid in order to develop digital elevation models (DEMs) of their surface topography. The ArcGIS Geostatistical Analyst software package (ESRI Redlands, CA) was used to interpolate the elevation data in order to create a continuous map of surface elevations at a higher spatial resolution. As expected, the elevation data exhibited a high degree of trend which was removed by fitting the trend surface with a second-order polynomial that subsequently was subtracted from the original data values. Once trend removal assured the stationarity of the data, ordinary kriging was performed on the residuals. The residuals were best fit with a spherical model which was used to interpolate the surface elevations to a 10 cm × 10 cm raster grid.

Two ArcGIS (ESRI Redlands, CA) hydrologic tools were used to determine the location of the convergent surface flow pathways from the interpolated surface elevation raster grid map. The Flow Direction command was used to determine the direction of flow from each grid cell by identifying the neighboring cell with the steepest downslope value. The Flow Accumulation command was applied to the Flow Direction output to create a raster grid of the accumulated flow at each grid cell. This was accomplished by calculating the total accumulated flow at each grid cell from the surrounding upslope cells.

3. Results and discussion

3.1. Hydrographs

3.1.1. Bare plots

Runoff was observed within 2 min after initiation of rainfall under both wet and dry conditions in bare plots (Fig. 2). Under dry conditions, several additional minutes were required to reach maximum runoff, consistent with the volume of water required to saturate the soil surface. In all experiments >95% of water was recovered in runoff (Table 2), due to surface compaction and sealing resulting from the mechanical action of raindrops impacting exposed soil surface aggregates (Ward, 1995). Because of the small amount of infiltration (<5%), the distance to the water table was inconsequential.

3.1.2. Vegetated plots

There were substantial differences in hydrographs between wet and dry conditions in vegetated plots (Fig. 2). Under wet conditions, runoff was first observed within 3–4 min after initiation of rainfall while maximum runoff was reached after approximately 12–16 min. The volume of water recovered (>95%) was comparable

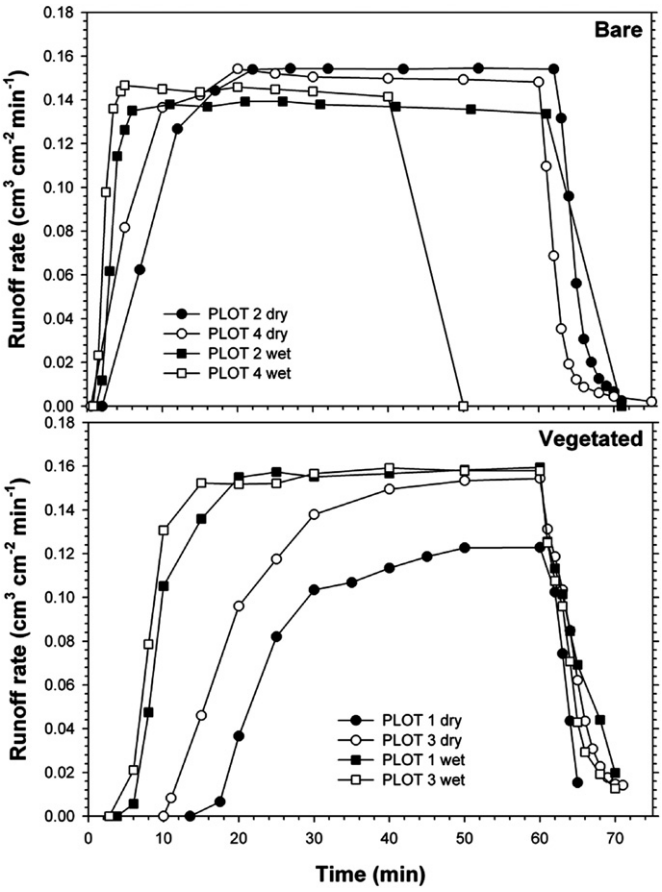

Fig. 2. Hydrographs of bare and vegetated plots under dry and wet conditions.

to bare plots (Table 2). However, initiation of runoff occurred substantially later under dry conditions; it took 13 min in Plot 1 and 10 min in Plot 3. These lag periods reflect the fact that, at the early stages of simulation, infiltration rates were equal to or exceeded rainfall rates. As rainfall simulations progressed and soils became wetter, infiltration rates decreased as rainfall either ponded in surface depressions or was discharged as runoff. Runoff rates eventually reached approximately 100% of maximum in Plot 3, but only approximately 75% of maximum in Plot 1; total water mass recoveries were approximately 52% and 75% for Plots 1 and 3, respectively.

It has long been recognized that vegetation attenuates surface runoff and favors infiltration processes. Mechanisms by which vegetation enhances infiltration include: (1) interception and dissipation of raindrops, thereby minimizing surface sealing; (2) resistance to overland flow, thus decreasing flow velocity; (3) increased soil hydraulic conductivity by plant root systems; (4) increased numbers of macropores from invertebrate activity; and (5) enhanced permeability of soil surfaces from accumulated organic matter residues (Shirmohammadi and Skaggs, 1984; Davies et al., 2004; Roodsari et al., 2005).

Table 2
Percent cumulative recoveries of rainfall and bromide from plots.

	Wet		Dry	
	Water	Br	Water	Br
Bare	98	114	98	96
Vegetated	98	96	52/75[a]	14/36[a]

[a] Individual results for plot 1/plot 2.

The discrepancies in runoff rates and percent recoveries between Plots 1 and 3 can be explained (at least partially) by differences in soils. Due to lysimeter anomalies, Plot 1 consisted of loam soil throughout the soil profile, while Plot 3 consisted of loam soil above 20 cm and clay loam soil below 20 cm (Table 1). Consequently, Plot 1 probably had a higher permeability throughout the soil column than Plot 3. This discrepancy was not manifested under wet conditions when the water table was only a few centimeters below the surface, but apparently was a major factor under dry conditions when the water table was approximately 50 cm below the surface.

3.2. Bromide and bacteria mass recovery

3.2.1. Bromide

Bromide recovery as a percentage of the applied mass is presented in Table 2. In bare plots, nearly all bromide was recovered under wet conditions while slightly less than 100% of Br was recovered under dry conditions. This is consistent with hydrographs shown in Fig. 2 where a small time interval was observed before runoff rate stabilized under dry conditions.

In vegetated plots, bromide was almost fully recovered under wet conditions (Table 2). This again is consistent with hydrographs shown in Fig. 2, where only a short period of time was available for infiltration. Much more interesting are data from the dry vegetated plots where bromide recovery was low and substantially less than the mass recovery of water. The most likely explanation for this discrepancy is the development of subsurface lateral flow within the soil and the seepage of this water to the runoff collector. In this case, a substantial portion of water coming to the runoff collector was the original soil water that did not contain bromide. This explains why percent bromide recovery was less than percent water recovery.

The development of subsurface lateral flow in runoff plots, and its effect on the mobilization of surface applied chemicals, has been previously noted experimentally (Nash et al., 2002) and in modeling (Kouznetsov et al., 2007). Sharpley and Kleinman (2003) described small plot studies in which overland flow tended to be produced at the lower end of the plots, where subsurface lateral flow accumulated. Given the low slope gradient, subsurface lateral flow might be intercepted by the lower plot boundary. As that water accumulates in the surface soil of the lower plot area, “infiltration” or “saturation excess” overland flow ensues. Under this process of overland flow generation, areas producing overland flow would be expected to expand upslope as rainfall continues. This description is consistent with the continuing growth of runoff rate observed in Fig. 2 for dry plots. The different recoveries of water and bromide in plots 1 and 3 are also consistent with this explanation. Soil texture in Plot 1 was uniformly loamy in the upper 40 cm soil profile, whereas plot 3 had a layer of clay loam as subsoil below the top 20 cm loamy topsoil. The presence of finer material below the coarser material should have resulted in formation of subsurface lateral flow, and subsequent overland flow in excess of saturation, much earlier at plot 3 than at plot 1. Gburek et al. (2006) indicated that saturation excess is a dominant mechanism of runoff in settings where vertical discontinuities in soil properties coincide with lower landscape positions where lateral flow can accumulate leading to saturated soil conditions. The textural discontinuity in plot 3 did not manifest itself when plot 1 and plot 3 were under wet conditions. The runoff in this case was generated almost exclusively by the infiltration excess; the subsoil had little effect on bromide transport which occurred almost exclusively in overland flow.

3.2.2. Bacteria

Cumulative recoveries of *E. coli* and *Samonella* in runoff are shown for all experimental conditions in Fig. 3. To facilitate

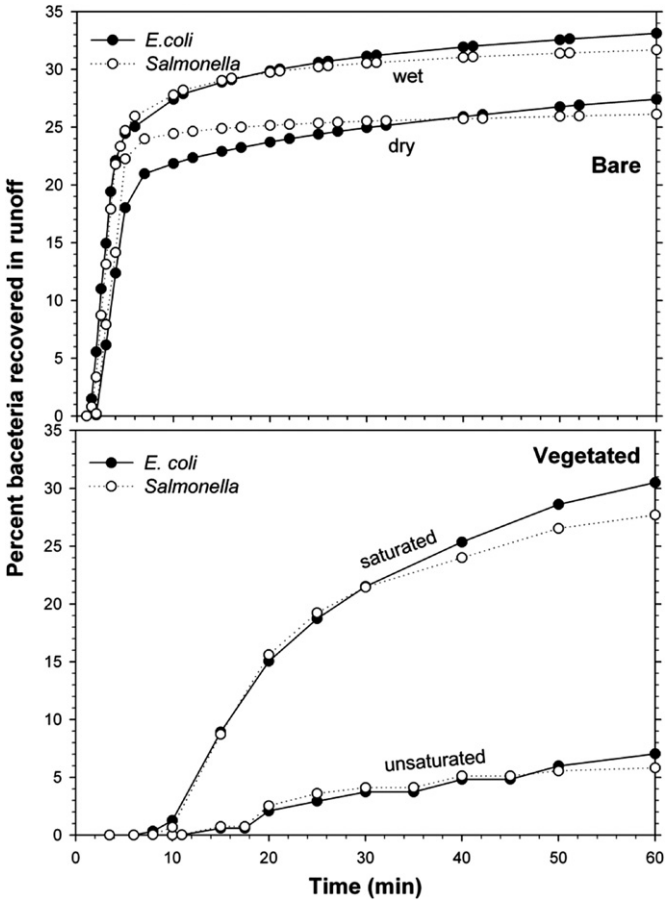

Fig. 3. Recovery of *E. coli* and *Salmonella* in runoff water from bare and vegetated plots under dry and wet conditions.

treatment comparisons, data for replicate plots have been averaged. In bare plots, *E. coli* and *Salmonella* were detected in the initial runoff from both wet and dry plots. Bacteria were rapidly transported across the bare plots; the majority of bacteria were recovered within the first 10 min (Fig. 3). Cumulative recoveries from bare wet and dry plots were approximately 32–33% and 26–27%, respectively. This discrepancy is presumably due to bacteria moving with infiltrating water into dry soil, and is consistent with hydrographs shown in Fig. 2 where a small time interval of infiltration can be observed before the runoff rate stabilizes under dry conditions. Note that the cumulative recoveries in runoff underestimate the total number of bacteria transported across the plots, since these values do not represent the bacteria attached to sediment particles in runoff. Our previous studies (unpublished data) indicated that $\geq$ 50% of bacteria were attached to the clay/silt sediments; consequently adjusted cumulative recoveries were $\geq$64% for wet and $\geq$52% for dry plots. We note that the attachment rate estimate we determined is slightly higher than the recent estimates of 28%–49% by Soupir et al. (2010) who experimented with bovine manure.

In vegetated plots, cumulative recoveries of *E. coli* and *Salmonella* were substantially reduced relative to bare plots (Fig. 3). Note that since no sediment was lost from vegetated plots, the values are indicative of actual bacterial transport across the plots. Cumulative recoveries of bacteria from wet plots were approximately 28–30% after 1 h (Fig. 3). Since bacteria were still being recovered in runoff after 1 h of rainfall, it is difficult to predict the total number of bacteria that would have eventually been transported across the plots. The hydrographs of wet vegetated plots (Fig. 3) indicate that most of the collected water came in overland flow. The relatively slow transport of bacteria across the vegetated plots, as compared to bare plots, suggests some type of adherence to either grass/root tissue or surface organic matter with subsequent release to runoff. Such a mechanism has been successfully used in a model developed to simulate transport of manure-borne microorganisms in vegetated filter strips (Guber et al., 2009). Recoveries of bacteria in dry vegetated plots were only 5–6%. It is likely that the majority of unaccounted bacteria were lost to infiltration. This is based on the observations that 1) initial runoff was not observed in plots until 10 min after rainfall initiation, 2) based on data from bare plots, 10 min was an adequate time for the majority of bacteria to be transported from the manure application area onto the plots, and 3) substantial infiltration occurred as judged from data on bromide recovery (Table 2) and hydrograph (Fig. 2).

The differences in cumulative recoveries between *E. coli* and *Salmonella* were relatively minor (Fig. 3). Although cell surface properties for these specific strains were not determined, the results suggest that differences were inconsequential.

3.3. Relative Br and bacteria concentrations in runoff

A comparison of bromide and bacteria concentrations in runoff provides additional insight into the mechanisms responsible for the observed transport. Relative concentrations, i.e. concentrations of bromide and *E. coli* normalized to their initial concentrations in manure, are shown in Fig. 4. In the bare plots, at the early stages of the simulation, when concentrations of both bromide and *E. coli* were highest, relative bromide concentrations were substantially larger that relative *E. coli* (data points below the line). This is an indication of *E. coli* transport not only in the free-floating form but also attached to sediment. At the later stages, when concentrations of both bromide and *E. coli* were low, there was not much difference between the two relative concentrations (data points near or on the line).

In vegetated plots, relative concentrations of bromide remained larger than those of *E. coli* at all times. This is an indication of retention of *E. coli* on soil or plant surfaces.

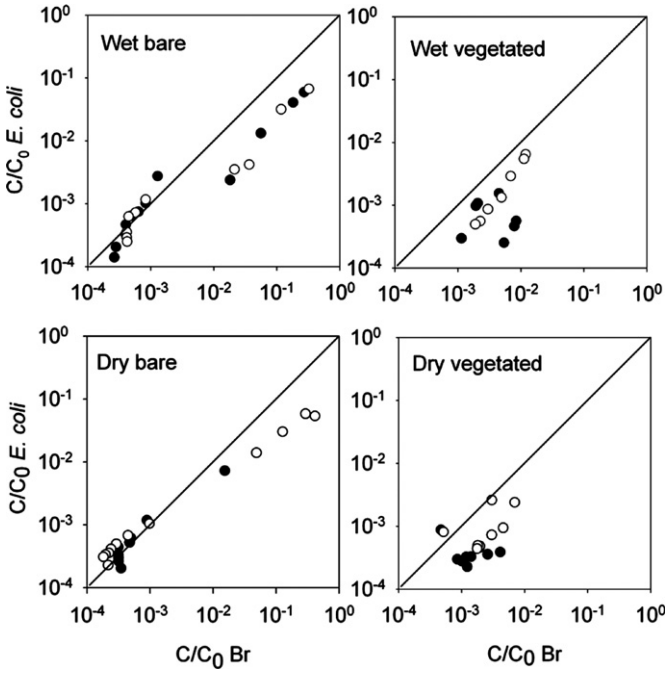

Fig. 4. Relative concentrations of *E. coli* as compared to relative bromide concentrations. Closed (dark) circles and white (open) circles indicate concentrations measured at 413 cm and 620 cm from the slurry application area, respectively.

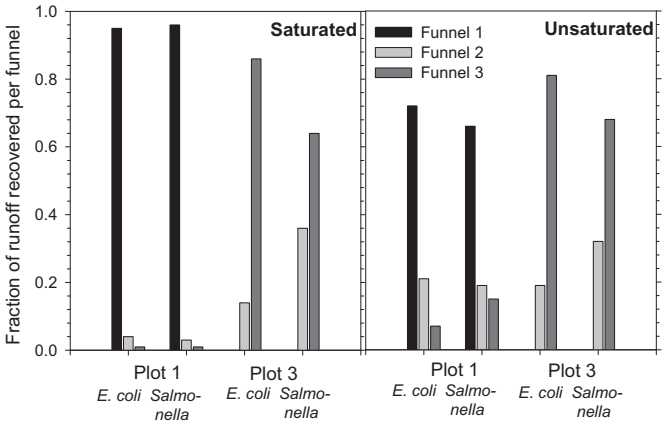

Fig. 5. Distribution of total number of bacteria collected in miniflumes in vegetated plots 1 and 3.

3.4. Small-scale heterogeneity of bacteria transport

Plot microtopography was responsible for causing substantial differences in the transport of bacteria within plots. In plot 1, miniflume 1 collected far more bacteria than miniflumes 2 or 3 (Fig. 5). Miniflume 1 intercepted 96% of the total intercepted bacteria during wet conditions, whereas during dry conditions, miniflume 1 intercepted about 70% of the total intercepted bacteria. The differences can be attributed to the intraplot heterogeneity of the flow and bacteria transport caused by the microtopography. As shown in Fig. 6, miniflume 1 was installed at the location where flow converged, whereas no flow convergence was expected to occur at the locations of miniflumes 2 and 3.

In plot 3, miniflume 1 collected no bacteria, while miniflume 3 collected approximately four times more than miniflume 2 (Fig. 5). An analysis of the plot 3 miniflume locations (Fig. 6) showed that the relative amounts of bacteria intercepted were in agreement with the locations of the miniflumes with respect to their proximity to the convergent flow pathways. Miniflume 3 was in the most active flow area of the plot, whereas miniflume 1 was in an area of flow divergence where intercepted rain was directed to other areas of the plot.

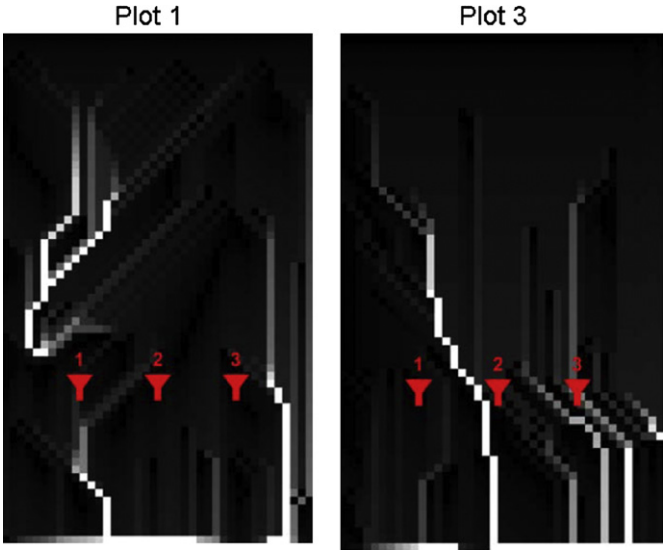

Fig. 6. Flow convergence zones and locations of miniflumes in vegetated plots 1 and 3; the lightest grid cells indicate areas where the accumulated flow was the highest.

Plot 1 miniflume collections during dry and wet conditions show that collection in miniflume 1 was less dominant in dry conditions than in wet conditions. This can be explained by the development of saturation excess runoff which affects all three miniflume locations in the same way – as the saturated zone expands uphill, the role of microtopography becomes inconsequential as the contribution from the subsurface flow becomes increasingly dominant under wet conditions.

In all simulations, the total number of bacteria cells intercepted by miniflumes was less than 1% of the total number of bacteria cells recovered in runoff that reached the gutter.

4. Conclusions

Consistent with previous studies, these results demonstrate the potential efficacy of VFS in mitigating bacterial runoff from swine organic waste sources. No substantial differences in retention of *E. coli* and *Salmonella* were observed. Very low bacterial retention was observed in bare plots whereas vegetated plots showed high retention capacity. Since the predominant factor minimizing bacterial runoff is infiltration into the soil column, the relative efficacy is dependent on the degree of soil saturation and distance to water table. Efficacy can vary dramatically depending on the amount of rainfall required to saturate the VFS, time required for runoff initiation from manured fields, and development of the subsurface flow in VFS. Consequently, VFS recommendations should account for expected amounts of water in runoff as well as the relative soil storage capacity of the VFS. For future research on testing the environmental fate of pathogens at a larger scale under natural conditions with VFS at the edge of fields, we recommend the following considerations:

1. Conduct similar investigations on environmental fate of pathogen at larger field scales under natural climatic conditions. This will permit accounting for different storms on the fate of pathogens through different pathways (e.g., surface runoff and infiltration).
2. Develop or modify existing field and watershed scale hydrologic and water quality models to account for the impact of VFS on attenuating pathogens under diverse land use and climatic conditions.

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