Effect of vegetated filter strips on infiltration and survival rates of *Escherichia coli* in soil matrix at Mau, Njoro River Watershed, Kenya

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Abstract Overland flows contaminated with manure borne pathogens pose risks to public health, because fecal pathogens may infiltrate into soil matrix from overland flows and contaminate soil water aquifers. The objective of this study was to evaluate the effect of vegetative filter strip (VFS) on infiltration rates (CFU 100 ml⁻¹ h⁻¹) of *Escherichia coli* (*E. coli*) in overland flow and their survival rates in soil matrix. Thirty samples of the specimen were collected from VFSs each sampling time. The samples were each filtered, followed by a series of ten dilutions; then analyses for *E. coli* using membrane filtration technique. Wet oxidation method and potassium persulfate technique were used to analyze particulate organic carbon (POC) and dissolved organic carbon (DOC) at ($p < 0.05$) level of significance, respectively. A strong relationship was obtained between *E. coli*, POC and DOC in the overland flows ($R^2 = 0.89$, $p \leq 0.05$; $df = 29$). This study confirms the hypothesis that DOC

released from Napier grass and Kikuyu grass exudates supported the initial survival, subsequent growth and adaptation of *E. coli* in its new secondary habitat outside its primary host. Thus, in the soil habitat, DOC and POC provided the initial energy for microbial cell multiplication from the VFS grasses. VFS influenced partitioning, infiltration and survival of *E. coli* in the overland flow into soil matrix. Thus, root zone retention data and information on *E. coli* in VFS systems are significant and could be used for scientific and management of soil erosion and the control of fecal pathogens entering surface water ecosystems both locally in Mau Ranges, Njoro River Watershed and internationally in other areas with similar environmental problems. VFS could be utilized under various designs of VFSs with different plants that have different setup of plants' root zone cover and penetrations systems that could help in infiltrating overland flow manure borne pathogens, a process that could be useful in the management of these pathogens in agro-pastoral systems locally and internationally.

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1 Introduction

Overland flows contaminated with manure borne pathogens pose risks to public health. Limited data on infiltration rates of manure borne pathogens in overland flows into soil

matrix and their survival rates in these microhabitats limit our understanding of the dynamics of microbial pathogens' fate and transport in overland flows (Tyrrel and Quinton 2003; Muirhead et al. 2006). Conservation of *Escherichia coli* mass balance in overland flow is controlled by fecal coliforms population dynamics, attenuation and diffusion (Tian et al. 2002). Soil and surface water fecal contamination is a matter of public health concern because zoonotic diseases emanating from bacterial, viral, protozoan and helminthic pathogens can be interchanged between livestock and humans (Pell 1997; Hill 2003). The need to understand manure borne microbial pathogens such as *E. coli* is important because they cause infections to humans either through zoonotic or pollution processes in livestock-based systems, therefore, this problem deserves new studies. Although *E. coli* are normal inhabitants of intestinal flora, some of the strains are highly versatile and serious pathogens that may cause diverse diseases in gastro-intestinal and extra-intestinal related organs. *E. coli* infect humans by means of virulence factors whereby they destabilize cellular processes. This implies that the dynamics of these pathogens need to be understood to generate information to help in their management and avert these infections in humans in agro-pastoral systems in future. It is unclear if cells sorbed to soil particles offer resistance to transport by overland flow or if they are transported along with eroding soil particles (Reddy et al. 1981; Tyrrel and Quinton 2003; Guber et al. 2005a, b; Oliver et al. 2005; Martinez et al. 2013; Allaire et al. 2015). Microorganisms such as *Clostridium parvum*, *Salmonella typhi* and *E. coli* O157 H7, which are harmful manure borne pathogens, occasionally contaminate lakes, rivers, streams and groundwater systems (Smith and Perdek 2004). However, pathogenic organisms are largely retained at or near the soil surface increasing the potential for pollution of surface waters (Tyrrel and Quinton 2003; Salisbury and Obropta 2015; Allaire et al. 2015). VFSs are some of the new low level technologies that could help trap nonpoint source pollutants from agricultural fields such as manure borne fecal coliforms, thus providing best management practices (BMP). VFS has been advanced as a practice to reduce pollutants transport and improve agricultural and livestock BMP, but their effectiveness concerning pathogenic indicator organisms shows varied results (Coyne et al. 1995; Lim et al. 1998; Entry et al. 2000; Roodsari et al. 2005; Lewis et al. 2009). VFS width influences fecal coliform trappings and performance of bacterial removal (Coyne et al. 1998; Lim et al. 1998; Mohanty et al. 2013). These include VFS drainage area ratio, which is a factor on overland flow volume (Mankin et al. 2006; Tate et al. 2006), the residence time of water (Fajardo et al. 2001), rainfall depth (Mankin et al. 2006; Guber et al. 2009a, b) and soil moisture content (Guber

et al. 2009a, b). Some evidence indicate that limited VFS removal of fecal coliforms occurs on soils that exhibit larger overland flow volumes and low infiltration including those of contaminants like pesticides and nutrients in soil (Sullivan et al. 2007; Fox et al. 2010). A wide range of contradicting opinions exists on the VFS efficiency and function with respect to pathogens and/or indicator organisms' removal (Munoz-Carpena and Parsons 1999; Collins and Rutherford 2004; Helmers et al. 2005; Pachepsky et al. 2006; Guber et al. 2007; Soupir et al. 2010). These pathogens also infiltrate into soil matrix through overland flows. Infiltration of pathogens from overland flows into soil matrix has hardly been managed by VFSMOD_W model unlike pesticides and nutrients (Poletika et al. 2009; Sabbagh et al. 2009; Fox et al. 2010; Muñoz-Carpena and Parsons 2011). Microbe infiltration might be governed by soil physical properties; vegetated cover; antecedent moisture content; rainfall intensity and inflow, and slope, while hydraulic resistance is a function of vegetation type and inflow volume (Munoz-Carpena and Parsons 1999; Soupir et al. 2010; Trowsdale and Simcock 2011; Davis et al. 2012; Martinez et al. 2013). It has been reported that the presence of manure reduces soil bacteria attachment (Soupir et al. 2006; Roodsari et al. 2005; Davis et al. 2009; Soupir et al. 2010; Gallagher et al. 2013; Martinez et al. 2013). Implementation of conservation practices to reduce bacterial transport has been met with limited success (Cardoso et al. 2012; Komlos et al. 2013). Little data exist on the transport mechanisms in overland flow pathways (Kasaraneni et al. 2014). In soil, *E. coli* could be recovered during its travel time into soil matrix by die-off, sedimentation, adsorption and filtration (Unc et al. 2015). *E. coli* transport mechanisms could be linked to organic carbon levels on those soils. However, the relationship between *E. coli* organic carbon has received theoretical and empirical attention in modeling energy and matter cycling in lakes, but limited attention in VFS systems (Riemann and Sondergaard 1986). Particulate organic carbon (POC) and dissolved organic carbon (DOC) have been reported as the likely energy and mass stock or reserve inducing delays in ecosystem recovery (Callieri et al. 1986; Bertoni et al. 1991a; de Bernadi 1991). Conversely, data based on bovine manure borne pathogenic infiltration processes into the soil matrix are still few in literature. This study relates to the previous work in the area, which evaluated the water quality of the Njoro River by examining the prevalence of diarrheagenic pathogens and the level of biological oxygen demand, and reported that enteropathogenic *E. coli*, necrotogenic *E. coli* and enteroaggregative *E. coli* levels were high in the river (Shivoga and Moturi 2009; Kiruki et al. 2011). The study concluded that Njoro River was highly contaminated as a result of diarrheagenic pathogens and organic material, and

stressed the significance and need to educate people on the good health practices; good waste disposal to help alleviate diarrheal diseases in the area. Reports on both Njoro Centre and Nakuru Municipality health facilities indicated the prevalence of diarrheagenic diseases often related to diarrheagenic pathogens ((Kiruki et al. 2011; Waithaka et al. 2015). Previous studies have also strongly supported the prevalence of these manure borne pathogens in the Njoro River Watershed (Shivoga and Moturi 2009; Olilo et al. 2016a, b, c). However, these studies did not show the pathogenic pollutants infiltration and survival rates in the soil matrix in the Njoro River Watershed. This study therefore encompasses environmental surface runoff and infiltration of overland flow into soil matrix under natural climatic rainfall conditions to account for different seasons at Mau, Njoro River Watershed. The objective of this study was to assess the effect of VFS on *E. coli* infiltration and survival rates in soil matrix at Mau, Njoro River Watershed, Kenya.

2 Materials and methods

2.1 Study site description

The study site was located at Tatton Agriculture Park (TAP), a livestock and crops research and demonstration unit and facility adjacent to and bordering Njoro River in eastern escapement of Njoro River Watershed, Mau Ranges, Kenya (Fig. 1). The site was located in the eastern escarpments of Mau Ranges that drains Lake Nakuru, Lake Baringo and Lake Victoria. The farm itself is located 22 km from Lake Nakuru and 172 km west of Nairobi in the East African Rift Valley. The topography at TAP comprises hilly land area with slopes ranging from 5 to 45%. The site was located down slope from Mau Forest and slightly above Njoro River accompanying underlying shrubby vegetation. The site location was at TAP, field 18; S 0022°319, E 03555°460; 2297.89 m above sea level (a.s.l); to S 0022°338; E 03555°456; 2288.13 m a. s. l. April–August was wet season, September–December was short rainy season, and January–March was dry season. Mean annual precipitation was 935.65 mm, with over 60% falling in April–August. The mean air temperatures ranged from a minimum of 17.6 °C to a maximum of 22.5 °C. The mean radiation ranged from a minimum value of 500 to a maximum value of 650-calorie cm⁻² day⁻¹. The mean evaporation ranged from 3.2 to 5.6 mm day⁻¹. The mean humidity ranged from 42 to 79%. The mean wind speed ranged from 3.5 to 7.4 km per hour. These weather characteristics justify this study because these slow showers of rainfall gather runoff, which eventually transport nutrients and fecal coliforms to the drainage systems of rivers that

reach and contaminate the receiving water bodies. The experimental field at TAP and the riparian forest area were Miocene age material and clay loamy soil type. Mixed indigenous African couch grass (*Cynodon dactylon*) (L.), Pers., Wire grass, *Eleusine indica* (L.) Gaertn; African bristle grass, *Setaria sphacelata*, (Schumach.) M. B. Moss var. *Sericea* (Sapf) W.D. Clayton; buffel grass *Cenchrus ciliaris* L.; and rescuegrass *Bromus catharticus* Vahl were the most predominant vegetation on the study site.

2.2 Study design

This experiment was designed to establish the transport form (unattached, attached or clumped) of *E. coli* in the overland flow in the erosion sedimentation system. Field experimental trials were performed from August, 2013–December, 2014 in randomized complete block design at different proportions of VFS I, VFS II and VFS III (Fig. 2). Fresh dairy manure samples were collected at the soil base of a dairy shed holding pen from cattle fecal material deposits of Tatton Agriculture Park (TAP) of Egerton University. Nine plots of 44 m length and 4 m wide each, made into three blocks, in replicate including VFS I, VFS II, and VFS III were established. The fields were constructed in an area that had clay-loam soil (34% sand, 32% silt and 34% clay) grassland on an approximately 15% slope dominated by indigenous grass (*Cynodon dactylon* L.). In each block, two of the fields were vegetated on clay loam with exotic Napier grass cuttings and Kikuyu grass rhizomes that were collected from TAP farm of Egerton University, while one was uniformly planted with a 30-m indigenous grass on clay loam. Field experimental plots were constructed on newly established vegetation on an area that had not received any manure before. In each of the blocks, each field was isolated with soil boulders (10 cm width) to prevent cross contamination (Guber et al. 2009a, b). Overland flow was generated from natural rainfall. The average natural rainfall rate was 3.8 ± 1.7 cm h⁻¹. Uniform grass heights of about 50 cm Napier grass and 50 cm Kikuyu grass were maintained throughout the duration of the experiment. Each of the nine plots was treated with standard cowpats. The experiment was run for four different consecutive seasons, namely short rains (August–December 2013) dry period (January–March, 2014), long rains (April–August, 2014) and short rains (August–December, 2014).

2.3 Micro topography estimation and *E. coli* infiltration rate modeling using VFSMOD-W in VFS

Best management practices (BMPs) are structural (water quality inlets, porous pavement, infiltration basins and wet

Fig. 1 Study site of Tatton Agriculture Park at Mau, Njoro River Watershed

pond/detention ponds), vegetative (swales, and vegetative filter strips) or managerial (preventing flooding, etc.) practices needed to treat, prevent or control surface water pollution. Structural and management BMPs can be too expensive or provides minimal flood protection, and for some of these practices, grained pollutants can clog infiltration basin. In this study, vegetative filter strips (VFSs) technique was chosen as an alternative method/technique over other existing management practices/techniques because it assists reduce peak overland flows downstream and can reduce overland flow velocity, through infiltration and storage. VFSs are also important in controlling emerging colloidal particles contaminants, including fecal pathogens particles, heavy metals and engineered nanoparticles (Wu et al. 2014).

In this study, microtopography survey was performed using total station (Sokkia SET4110 Co. Virginia, USA) as described by Moser et al. (2007) on transects of 0.5, 1.0, 2 and 5 m, diameter. *E. coli* infiltration rate modeling was performed using VFSMOD-W in VFS as described by Munoz-Carpena and Parsons (1999) and Wu et al. (2014). Infiltration rate of *E. coli* in the overland flow into soil matrix was simulated using the Green and Ampt (1911) relationship. This model assumes that a sharp wetting front

exists between the infiltration zone and soil at the initial water content. It also assumes that the length of the wetted zone increases as infiltration rate progresses. VFSMOD-W model that uses the Green and Ampt equation was used to approximate infiltration rates of overland flow into soil matrix (Munoz-Carpena and Parsons 1999). One-day-old cowpats were applied at the rate of 5.8 kg ha⁻¹ at the top of simulated pasture area 14 m above the start of grassed area of the fields. Natural rainfall was used to generate overland flow for the VFS of each experimental field that ranged from 1 to 3.8 cm h⁻¹.

2.4 Determining overland flow infiltration rates of *E. coli* into soil matrix

Prior to manure application into the VFS, soil water content was measured by taking gravimetric samples in triplicates from each site at 0–5, 5–10, 10–15, 15–20 cm depth. Rainfall rates were measured with rain gauge of the university's meteorological station situated 500 m away from the field site. Overland samples were collected in Coshocton bucket wheels at 0, 10, 20 and 30 m downslope within the VFS for the duration of rainfall at 10 min intervals. Overland flow samples were collected at four

LIVESTOCK PASTURE / MANURE APPLICATION SITE

14 metres
10 metres
20 metres

0 m
10 m
20 m
30 m

Block 1
Block 2
Block 3

KG
NG
CI
NG
KG
CI
NG
KG

Sampling Site

Collecting Troughs

Legend

- Vegetated Filter strips
- CI Control indigenous
- NG Napier Grass
- KG Kikuyu Grass

rainfall events. Upon collection from the VFS, 200-mL subsamples were prepared into three subsamples for laboratory bacterial partitioning studies, total coliforms, fecal coliforms and *E. coli* and TSS content analyses. The rainfall events overland flow reached steady state from 30 min to 2 h and 30 min of storm duration depending on the intensity. The hydrographs for each overland flow were computed at each sampling location in the field. Surface soil samples (0–20 cm depth) were collected next to Coshocton wheel bucket at 5 and 30 m from the top of the VFS. The samples were sieved (2 mm) and stored on a cool box prior to analysis. Manure samples were collected prior to land application for analysis of its constituents.

Water samples were analyzed for total suspended solids (TSS). Partitioning of pathogen indicators between

attached and unattached phases was achieved by fractional filtration followed by centrifugation as described by APHA (1998). 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. No measurable particulates (more than 1.0 mg) passed through the 8- μm filter, so cells associated with the 3- μm filter were classified as unattached. Following filtration, the retained solids were rinsed from all filter surfaces, suspended in phosphate-buffered water (Hach Company Loveland, Co) and centrifuged (Avanti J-251, Beckman Coulter, Fullerton, CA) at 4700 rpm ($3.043 \times g$) for 20 s (Huysman and Verstraete 1993). A 1 mL of aliquot of the supernatants (obtained from each of the four rinsates) and 1 mL aliquot of the terminal filtrate (collected after passing through the 3 μm filter) were enumerated for *E. coli* concentrations by membrane filtration (APHA 1998) using modified mTEC agar (Cerilliant Corp, Texas, USA) (USEPA 2000, 2008) 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 bio flocculated cells. The dispersed solution, representing the total concentration retained by each filter, was also enumerated for *E. coli* concentrations by membrane filtration. Suspended sediment concentration (SSC) was analyzed by filtering samples through a 0.45- μm glass fiber filter (Pall Life Sciences, Ann Arbor, MI) (USEPA 2000). Sediment collected from 0, 10, 20 and 30 m from the edge of the VFS was put in subsamples of 200 mL. The sediment concentration was determined by filtering 200 mL of the subsamples of overland flow water with vacuum pump at 150 mm Hg of pressure, through pre-weighed 0.45- μm pore-size filters. After the filtration, the filter papers were dried in an oven at approximately 105 °C for 24 h. The filter paper was then reweighed to determine sediment mass.

2.6 Interaction between *E. coli*, soil matrix, particulate organic carbon (POC) and dissolved organic carbon analyses (DOC) in VFS

A known weight of manure in sterile dilution water was suspended in sterile bottles (200 g). *E. coli* used in this study were fecal bacteria isolated from dairy cows' fresh cowpats from the Tatton Agriculture Park commercial farm of Egerton University, Kenya. The 200 g of manure that was weighed and applied on pasture area at the experimental site helped in understanding the amount of *E. coli* that entered each of the three sets of VFSs, through sampling and computation of *E. coli* concentrations and loads

along the transport within the VFSs, namely Couch grass–Buffel grass, Kikuyu grass and Napier grass. The procedure was as follows: First, the concentration of *E. coli* in the 200 g was determined prior to its application onto the farm; secondly, it was assumed that different amount (loads) of *E. coli* would enter different VFSs including Napier grass, Kikuyu grass and Couch grass–Buffel grass combinations; thirdly, the concentrations and loads of *E. coli* entering each of the VFS were sampled at the edge of each VFS at 0 m, then 10, 20 and 30 m at the exit; fourth, the amount of *E. coli* entering the VFS was assumed to be proportional to the amount of *E. coli* exiting the VFS and also proportional to the amount of *E. coli* in the initial manure (200 g) applied at the model pasture. Overland flow rates and peaks were measured using a flow meter Model (Hach FH950 Loveland Co. OH, USA).

The general protocol for *E. coli* identification was performed at the Microbiology Department Laboratory of Kenya Marine and Fisheries Research Institute, Kisumu, using DelAqua Water Testing Kit. Overland flow samples or their dilutions were analyzed for total coliform, fecal coliform and *E. coli* bacteria with the membrane filter technique (Greenberg et al. 1992). Approximately 500- μL subsamples were centrifuged at $100 \times g$ (Avanti J-251, Beckman Coulter, Fullerton CA, USA) to remove sediment. Thus, an appropriate volume of a water sample (100 μL) or dilutions of it were filtered through a 47-mm, 0.45- μm pore-size cellulose ester membrane filter (Sigma-Aldrich Co., Surrey, UK) that retains the bacteria present in the sample. From various ten dilution series, 0.1 ml was spread plated on 4-methylumbelliferyl β -D-galactopyranoside/Indoxyl β -D-glucuronide (MI) media (Cerilliant Corp, Texas, USA) in an incubator (Water testing Kit, DelAqua co, Surrey, UK) in replicates and incubated for 24 h at 35 °C for determination of total coliforms and *E. coli*, and 44.5 °C for determination of fecal coliforms. *E. coli* with β glucuronidase and β galactosidase activity on substrates in the growth medium appeared blue. Other coliforms with only β galactosidase activity appeared red. Results for manure samples were reported as number of colony forming units (CFUs) per gram-wet weight.

To ensure the purity of the isolates, well-isolated colonies from the membrane Thermo tolerant *E. coli* (mTEC) agar plates (at least two per plate) were streaked for primary isolation on MacConkey agar (Cerilliant Corp, Texas, USA) followed by a secondary isolation on the same medium. At least two colonies from each MacConkey plate were confirmed for their β -D-glucuronidase activity, which is a positive test for *E. coli*, by growing them on nutrient agar with 4-methylumbelliferyl β -D-glucuronide. Subsequently, confirmed populations were stored in tryptic soy broth for later use. Finally, *E. coli* populations were speciated by using the

BBL crystal identification scheme (Becton–Dickinson Microbiology Systems, Sparks, Md. USA). *E. coli* standard isolate (*K-12* ATCC 25922) (Cerilliant, Texas, USA) was included in the identification protocol for quality control and quality assurance. A series of biochemical tests were performed to determine if the isolates were *E. coli*, including Indole, methyl red, carbohydrate utilization, citrate utilization and Voges-Proskauer (Cerilliant, Texas, USA) by picking *E. coli* colony from the gel for the analysis. In order to determine the final confirmation of the purity of the *E. coli* isolates, Analytical profile index (API) 20 E test kit (Bio-Merieux, Marcy l'Etoile, France) was performed. Results were reported as colony forming units per 100 mL (CFU/100 mL). For soil sample, 1 g of soil was transferred to a test tube, diluted with 0.0425 g L⁻¹ sterile diluent Buffer field's Phosphate (Sigma-Aldrich Co. Surrey, UK) adjusted to pH 7.2 solution and stirred. For the overland flow sample, one mL of the sample was pipetted to a test tube and the dilution and plating proceeded as with the soil samples.

In order to examine if POC or DOC concentrations affect *E. coli* concentrations and growth, and also if DOC was absorbed by *E. coli* cells; samples of manure (20 g) were filtered through sterilized 50-mL bottles to obtain DOC filtrates, and the residues were used as sources of POC, while the filtrate was the sources of DOC. Three sets of isolated *E. coli* were plate spread onto three separate sterilized petri dishes inoculated with 5 g of locally prepared POC granules, 25 µL of locally prepared DOC medium and a control, which had pure water without any additional medium. The growths of *E. coli* in the two media were examined, and the procedure was performed as described by Greenberg et al. (1992).

The total solids were analyzed according to standard methods (APHA 1998) by drying an overland volume of 25 mL to constant weight. Soils were also analyzed for Mehlich-1 phosphorus (8 mg kg⁻¹), organic matter (OM) by a modified Walkley–Black method (2.5%) and pH by 1:1 soil to distilled water ratio and solid pH-form pH meter as described by Sharpley (1993). Water-soluble phosphorus (2.04 g kg⁻¹) was determined by the method described by Sharpley (1993). The pH (6.02) was measured potentiometrically in 1:2 manure/water slurry. Average moisture content of fresh manure samples was 84.8%, determined gravimetrically. *E. coli* concentrations in manure were enumerated on modified mTEC and MI agar (USEPA 2000) by membrane filtration (APHA 1998). Twelve samples of fecal material were diluted in phosphate buffer solution (Hach Co., Loveland, Col.) 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 1000 mg L⁻¹ dilutions of Tween 85 solutions. Wet oxidation technique of Maciolek (1962) was used to analyze particulate organic carbon, while dissolved organic

carbon was analyzed using potassium persulfate as described by Wetzel and Likens (1991) and APHA (1998).

2.7 Root zone retention and survival rates of *E. coli* in soil matrix in VFS (root zone, macropores and subsoil horizon)

E. coli samples were collected by scooping of soil attached to the roots of VFS grass (Napier, Kikuyu and Couch–Buffel) using a sterile corer rod with a table spoon shaped end measuring 1 m long. The samples were kept in a cool box at zero degrees Celsius and transported to the nearby Nakuru Municipal Laboratory for analysis within 6 h after collection. The samples were incubated at 44.5 °C (Degrees Celsius) and analyzed after 18–48 h using methods described by Greenberg et al. (1992). *E. coli* samples were collected by scooping of soil attached to the roots of VFS grass (Napier, Kikuyu and Couch–Buffel) using a sterile corer rod with a table spoon shaped end measuring 1 m long. The samples were kept in a cool box at zero degrees Celsius and transported to the nearby Nakuru Municipal Laboratory for analysis within 6 h after collection.

2.8 Data analysis

Analysis of variance (ANOVA) was used to test for differences in concentrations of bacteria associated with the different particle size categories. Statistical significance was determined at $\alpha \leq 0.05$. Differences if any were determined by the least squares means test (Kirk 1982; Snedecor and Cochran 1980; Zar 1996) for both independent and dependent variables. Statistical analyses of data were performed by PAST (Hammer et al. 2001; Helmers et al. 2005) and Systat (SYSTAT Institute Inc. 2007).

3 Results

3.1 Micro topography estimation and *E. coli* infiltration rate modeling using VFSSMOD-W in VFS

The mean hydro-environmental factors were measured in the VFSs and tabulated as shown below (Table 1). *E. coli* was significantly different ($p < 0.05$) in different VFSs. The hydraulic parameters obtained through VFSSMOD-W were tabulated in Table 2. The microtopography of the VFS was estimated using total station at a transect of 0.5, 1, 2 and 5 m. The *F* value shows significant differences between Napier grass, Kikuyu grass and Couch–Buffel grasses for tortuosity, limiting slope and limiting elevation differences at different scales of 0.5, 1, 2 and 5 m (Table 3).

Table 1 Mean (SEM $\pm$ $\sigma\bar{x}$) hydro-environmental factors measurements in VFS at Mau, Njoro River Watershed from August 2013 to Dec 2014

Variable Parameter	Unit	Applied manure/ Napier grass	Kikuyu grass	Couch–Buffel grasses	Degrees of freedom (<i>df</i>)	<i>F</i> value <i>F</i>	<i>p</i> value $\alpha = 0.05$
Farm area	m ²	1584 $\pm$ 15	1584 $\pm$ 15	1584 $\pm$ 15			
Farm slope	%	15.0 $\pm$ 1.5	15.0 $\pm$ 1.5	15.0 $\pm$ 1.5			
Annual rainfall	mm	935 $\pm$ 12	935 $\pm$ 12	935 $\pm$ 12	11		
Air temperature	°C	19.3 $\pm$ 7.4	19.3 $\pm$ 7.4	19.3 $\pm$ 7.4	(2, 9)	3.58	<0.05
Evaporation rate	mm h ⁻¹	20.5 $\pm$ 0.5	20.5 $\pm$ 0.5	20.5 $\pm$ 0.5	(2, 9)	3.62	<0.05
Sun-radiation	C m ⁻² d ⁻¹	580 $\pm$ 25	580 $\pm$ 25	580 $\pm$ 25	(2, 9)	3.42	<0.05
Humidity	%	70 $\pm$ 12	70 $\pm$ 12	70 $\pm$ 12	(2, 9)	3.30	<0.05
Wind speed	km-h ⁻¹	5.45 $\pm$ 0.05	5.45 $\pm$ 0.05	5.45 $\pm$ 0.05		3.43	<0.05
Rainfall	mm	54 $\pm$ 5.2	54 $\pm$ 4.5	54 $\pm$ 4.2	(2, 9)	3.58	<0.05
Overland flow rate	cm ³ s ⁻¹	2.5 $\pm$ 0.03	2.2 $\pm$ 0.02	3.5 $\pm$ 0.02	(2, 9)	3.6	<0.05
Soil moisture content	%	24.12 $\pm$ 0.5	24.10 $\pm$ 0.24	23.46 $\pm$ 0.34	(2, 9)	3.9	<0.05
Total dissolved solids	mgL ⁻¹	50.21 $\pm$ 0.3	50 $\pm$ 3.2	53 $\pm$ 3.2	(2, 9)	3.6	<0.05
pH	pH units	5.76 $\pm$ 0.24	5.76 $\pm$ 0.12	5.84 $\pm$ 0.12	(2, 9)	3.89	<0.05
Temperature	°C	21 $\pm$ 0.2	21 $\pm$ 1.05	23.2 $\pm$ 1.05	(2, 9)	2.31	<0.05
SS concentration	mgL ⁻¹	154 $\pm$ 6.8	78 $\pm$ 2.31	153 $\pm$ 2.4	(2, 9)	12.5	<0.05
DOC	µg/L ⁻¹	35 $\pm$ 5.3	123.3 $\pm$ 12	132.6 $\pm$ 12.3	(2, 9)	7.9	<0.05
POC	µg/mL ⁻¹	56 $\pm$ 6.7	132 $\pm$ 14	152 $\pm$ 21	(2, 9)	16.5	<0.05
<i>E. coli</i>	CFU 100 mL ⁻¹	6.18 $\times$ 10 ⁴	22. $\pm$ 3.2	50 $\pm$ 3.2	(2, 9)	4.28	<0.05

3.2 Determining overland flow infiltration rates of *E. coli* into soil matrix

The decrease in infiltration rate was gradual within 2.8 h of the initial events, but increased sharply during the first half hour and ceased 1 h after the start of the rainfall in the following week of natural rainfall. These differences were likely caused by the alteration of soil surface during intensive rainfall (18.8 mm h^{-1}). Commencement of overland flow that occurred 10 min after the start of natural rainfall event indicated a lag time. These lag periods reflected that at the start of rainfall event, infiltration rates were equivalent to or exceeded rainfall events. It rained progressively and the soils became wetter, accompanied with decreased infiltration rates as ponding was formed or overland flow was generated. In VFS I, the overland flow rate measured using flow meter was 10 cm s^{-1} , which, was the highest recorded rate in Couch grass–Buffel grass system. In VFS II, 73% of the maximum overland flow was recorded in Kikuyu grass. Least overland flow rate (58%) was recorded in VFS III in Napier grass. The recoveries of water mass in these VFS were, respectively, 49% (VFS II), 67% (III) and 76% (I). The *E. coli* concentrations entering the VFSs were higher than the *E. coli* concentrations exiting the VFSs (Fig. 3).

3.3 Selective partitioning of *E. coli* on manure wastes, root zone, soil macropores and subsoil particles in VFS

This study assessed the selective partitioning of microorganisms (*E. coli*) between suspended sediment-waste particles and water in VFS at Mau, Njoro River Watershed from August 2013 to December 2014. Cumulative recoveries in VFS I were significantly ($p < 0.05$, $df = 29$) higher than VFS II and VFS III. Cumulative recoveries of bacteria from VFS I ranged from 32 to 38%, while cumulative recoveries in VFS II and VFS II ranged from 2 to 8%. The other observation was significant ($p < 0.05$, $df = 29$) heterogeneity of bacterial transport between VFS

II, VFS III and the VFS I. There was slow transport of *E. coli* in the VFS II and III field plots as compared to VFS I. *E. coli* adhered to the grass leaves and roots tissue surface organic matter then later released into the overland flow. There was selective partitioning of microorganisms (*E. coli*) between suspended sediment-waste particles and water in VFS one meter from the edge during the study period (August 2013–December 2014) and shows that *E. coli* was attached to soil particles, epiphytes and clumps (Table 4). *E. coli* loading in the overland flow was linked to the concentration of *E. coli* in the overland flow, soil matrix. *E. coli* were epiphytic to the plants root systems, showing that the *E. coli* populations once released from the manure were infiltrated through the soil pores where they interacted with the plants fibrous root systems and soil matrix.

3.4 Interaction between *E. coli*, soil matrix, particulate organic carbon (POC) and dissolved organic carbon analyses (DOC) in VFS

There was a significant ($p < 0.01$; $df = 29.0$) spatial variation of DOC concentration in the sampling sites within the VFS in the overland flow during the investigation. The VFS II had the highest average DOC values in the overland flow. The DOC values decreased from the upper part to the exit of VFS. VFS III recorded the highest value due to the Napier grass leaves cover. VFS I (indigenous grass) had lower values of DOC. The mean temporal concentrations of DOC values were 160.05 ± 278.49 (SE) $\mu\text{g L}^{-1}$. DOC parameter was strongly related to POC parameter (Fig. 4) ($R^2 = 0.99$; $p \leq 0.01$, $df = 29$) in the overland flow in all VFSs.

The process of converting POC to a more absorbable DOC proceeded well in the VFSs. This is supported by the close relationship observed between POC and the *E. coli* cell abundance ($R^2 = 0.89$; $p \leq 0.05$; $df = 29$). POC impacted on *E. coli* cell abundance in VFS I, VFS III and VFS II in ascending order, respectively, during dry season (January 2014–March 2014). DOC was also strongly

Table 2 Soil hydraulic parameters in Couch–Buffel grass, Kikuyu grass and Napier grass in VFS at Mau, Njoro River Watershed from August 2013 to Dec 2014

Soil hydraulics	Unit	Couch grass–Buffel grass	Kikuyu grass	Napier grass	Degrees of freedom	F value	P value
Saturated hydraulic conductivity (K_s) cm h^{-1}	cm h^{-1}	0.00113	0.00133	0.00213	10	3.3	<0.05
Average suction at wet front (SAV)		0.379	0.399	0.469	10	3.4	<0.05
Saturated soil–water content (OS) $\text{cm}^3 \text{cm}^{-3}$	$\text{cm}^3 \text{cm}^{-3}$	0.301	0.321	0.351	10	3.1	<0.05
Initial soil–water content (OI) $\text{cm}^3 \text{cm}^{-3}$	$\text{cm}^3 \text{cm}^{-3}$	0.135	0.146	0.172	10	4.2	<0.05
Initial soil–water deficit (M)	$\text{cm}^3 \text{cm}^{-3}$	0.172	0.186	2.37E–07	10	4.8	<0.05
Green-Ampt parameters (A, B)		9.37E–07	9.38E–07	9.38E–07	10	3.2	<0.05

Table 3 Measurements of microtopography of Napier grass, Kikuyu grass and Couch–Buffel grass in the VFS at Mau, Njoro River Watershed from August 2015 to December 2014

Variable	Napier grass				Kikuyu grass				Couch grass–Buffel grass				Degrees of freedom ($df = 2, 8$)
	Turtuosity (T)	Limiting slope (LS)	Limiting elevation difference (LD)	Turtuosity (T)	Limiting slope (LS)	Limiting elevation difference (LD)	Turtuosity (T)	Limiting slope (LS)	Limiting elevation difference (LD)	Limiting slope (LS)	Limiting elevation difference (LD)	F value	p value
Scale (m)													
T	0.5	1.001	1.011	1.006	1.002	1.024	1.001	1.034	1.002	1.034	1.002	(6.81)	<0.01
	1	1.031	1.015	1.02	1.001	1.021	1.002	1.021	1.022	1.021	1.022		<0.01
	2	1.023	1.012	1.041	1.006	1.021	1.004	1.032	1.032	1.032	1.032		<0.01
	5	1.014	1.03	1.031	0.146	1.62	1.008	1.046	1.004	1.046	1.004		<0.01
LS	0.5	0.258	0.214	0.132	0.186	0.18	0.826	1.115	1.054	1.064	1.054	((6.83))	<0.01
	1	0.225	0.224	0.068	0.046	0.68	0.912	0.082	0.024	0.082	0.024		<0.01
	2	0.205	0.216	0.066	0.052	0.142	0.142	1.336	1.118	1.336	1.118		<0.01
	5	0.451	0.212	0.68	0.71	0.32	0.154	1.156	0.352	1.156	0.352	((6.83))	<0.01
LD	0.5	2.04	2.01	2.7	2.4	10.6	0.262	1.4	1.82	1.4	1.82		<0.01
	1	4.4	4.2	4.8	4.2	4.6	0.267	1.6	2.4	1.6	2.4		<0.01
	2	2.6	3.4	3.6	3.2	3.4	2.146	2.12	4.6	2.12	4.6		<0.01
	5	3.4	3.2	3.5	4.02	5.21	1.164	2.13	1.81	2.13	1.81		<0.01
Cover	%	99	97	95	99	97	95	99	95	97	95		<0.01

Fig. 3 Effect of various grass species on *E. coli* in overland flow in the VFSs at Mau, Njoro River Watershed from August 2013 to December 2014

related to *E. coli* cell abundance ($R^2 = 0.88$; $p \leq 0.005$) in ascending order of magnitude in the VFS I, VFS III and VFS II, respectively, during dry season (January 2014–March 2014). This was due to the absorption of DOC by *E. coli* cells. A very tightly coupled relationship was obtained between *E. coli* cell abundance and DOC of the overland flows ($R^2 = 0.89$, $F = 33.74$; $p \leq 0.05$; $df = 29$) (Fig. 5). Similarly, the *E. coli* cell abundance was tightly coupled to POC in the VFS II overland flow ($R^2 = 0.88$, $p \leq 0.005$; $df = 29$).

The VFS II and VFS III sampling sites had the highest POC (minimum $1010 \mu\text{gL}^{-1}$ and maximum $1200 \mu\text{gL}^{-1}$,

respectively). Similarly, the same sampling sites had the highest DOC values ($6011 \mu\text{gL}^{-1}$ and $7241 \mu\text{gL}^{-1}$), respectively. This was due to these sites having litters of plants leaves from Napier grass and Kikuyu grass. The high levels of particulate and dissolved organic matter characterized these areas. The spatial changes in POC and DOC concentrations in the VFS can be explained in terms of organic matter production and decomposition–mineralization set in the context of sedimentation gain and allochthonous input during the wet season (April–July, 2014) in the VFSs.

A significant regression observed suggested that the concentration of DOC increases with that of POC in direct proportions. DOC and POC released from cowpat into the soil environment continued to supply the *E. coli* with the rich source of carbon nutrients to utilize for metabolism during initial stages outside its primary host ($R^2 = 0.99$; $p \leq 0.05$; $df = 29$). The initial sugar sources and nutrients (nitrates and phosphates) were supplied from cowpat until *E. coli* adapted to the new soil habitat. The surface of *E. coli* got into direct contact with the nutrients supplied from DOC and POC in the soil. DOC and POC provided the initial energy for metabolic activity. This process provided the first energy for the division and growth of these bacterial cells. The possible and likely sources of DOC were recognized as DOC entering the vegetated filter strip field with the runoff from the catchment area; DOC from released from the cowpat; DOC released from the background soil; and finally DOC released from exudates continuously from Napier grass and Kikuyu grass. The

Table 4 Selective retention of partitioned microorganisms (*E. coli*) in overland flow onto VFS grass parts, soil particles, waste particles at Mau, Njoro River Watershed from August 2013 to December 2014

VFS type	Retention medium	Retention level by the medium, <i>E. coli</i> concentration (CFU 100 mL ⁻¹)	The proportion (%) of <i>E. coli</i> retained in relation to overland flow <i>E. coli</i> concentration on soil surface (CFU 100 mL ⁻¹)
Napier grass	Soil particle	4001	7.0
	Waste particle	28,105	50.9
	Roots epiphytic	18,001	32.72
	Clumps	800	14.55
	Overland flow free <i>E. coli</i>	55,000	100
Kikuyu grass	Soil particle	3000	7.1
	Waste particle	30,000	71
	Roots epiphytic	11,000	20.
	Clumps	9000	21.4
	Overland flow free <i>E. coli</i>	42,000	100
Couch grass–Buffel grass	Soil particle	4200	8.7
	Waste particle	27,000	56.13
	Roots epiphytic	9000	16.36
	Clumps	9100	18.91
	Overland flow free <i>E. coli</i>	48,100	100

Fig. 4 Relationship between $\ln$ (particulate organic carbon, $\mu\text{g L}^{-1}$) and $\ln$ (dissolved organic carbon, $\mu\text{g L}^{-1}$) in VFS at Mau, Njoro River Watershed from August 2013 to December 2014

Fig. 5 Growth rate (h^{-1}) $\pm$ SE σ of *E. coli* in the POC and DOC media isolated from cowpat in VFS at Mau, Njoro River Watershed from August 2013 to December 2014

DOC and POC released from the cowpat, and DOC released from Napier grass, Kikuyu grass and background soil regressed with *E. coli* survival and growth rates ranged from $r = 0.85$ to $r = 0.95$; ($p < 0.05$; $df = 29$).

3.5 Root zone retention and survival rates of *E. coli* in soil matrix in VFS (root zone, macropores and subsoil horizon)

Large quantities of infiltrated water leached with over 50% of the *E. coli* into the root zone system of Napier grass through soil macropores into the subsoil horizon. Two processes occurred in the root zone soil system of Napier grass, namely retention of *E. coli* cells onto the soil

macropores due to infiltration and filtration of overland flows and adsorption of *E. coli* cells onto the root zone soil particles (Table 4). The root zone retention by Napier grass was 32% of the *E. coli* in proportion to quantity of *E. coli* the overland flow, while waste particle had the highest amount of *E. coli* at 50.9. In the Kikuyu grass, the retention of *E. coli* relative to overland flow was 20% in the root zone and 7.1% in soil particles. In Couch grass–Buffel grass, the soil particle had 8.7% of the *E. coli* in the overland flow; its root zone had a proportion of 16%.

4 Discussion

4.1 Micro topography estimation and *E. coli* infiltration rate modeling using VFSSMOD-W in VFS

Microtopography and *E. coli* infiltration rate modeling using VFSSMOD-W in VFS are significant processes because they support differences in bacterial interactions. The following reasons support this statement: First, VFS had a significant effect on *E. coli* infiltration and survival rates in soil matrix at Mau; secondly, differences in micro topography of Couch grass, Kikuyu grass and Napier grass could be explained by significance differences in tortuosity, limiting slope, limiting elevation differences and percentage cover of VFS (Moser et al. 2007); thirdly, the reason why microtopography, development of saturation excess runoff, small-scale heterogeneity of bacterial transport and the lag periods of runoff flow, are important mechanisms is because these processes support differences in bacterial interceptions (Duchemin and Hogue 2008; Davis et al. 2009; Cardoso et al. 2012; Martinez et al. 2013; Liu and Davis 2014; Allaire et al. 2015; Miller et al. 2015); fourth, mechanisms that enhance attachment of *E. coli* to the grass leaves and roots tissue, surface organic matter then later getting released into the overland flow is important on the dynamics of infiltration process into the soil matrix; fifth, models developed to simulate fate and transport of manure borne microbes in vegetative filters strips have successfully exploited such mechanisms (Guber et al. 2009a, b); sixth, infiltration is supported by lag time phenomenon, which shows that bacteria in overland flow in VFS disappear through infiltration process (Cardoso et al. 2012).

Infiltration of *E. coli* in the overland flow into soil matrix was simulated using the Green and Ampt (1911) relationship. This relationship assumes that a sharp wetting front exists between the infiltration zone and soil at the initial water content and that the length of the wetted zone increases as infiltration progresses. The droplet impact on soil surface increased with the rainfall rate. This resulted in seal formation and macropore sealing that reduced

hydraulic conductivity and became the infiltration-limiting factor. Several explanations could be deduced from this study: First, the two order of magnitude difference between saturated hydraulic conductivity was in general agreement with equilibrium data on coliform partitioning between soil and bacteria suspensions (K_s) (Guber et al. 2011); secondly, the value of K_s equal to one indicates that the infiltration rate does not affect the bacterial losses, and kinetic attachment is the sufficient approximation of the bacteria exchange between the runoff and soil (Pachepsky et al. 2006); thirdly, soil hydraulic conductivity values between 20 and 2000 mL g⁻¹ from data on *E. coli* partitioning between runoff and sediment induces *E. coli* release from a bovine manure strip (Guber et al. 2007); fourth, when values of K_s ranged from 0.9 to 0.96, it could also be used to evaluate infiltration (Skaggs et al. 1969), infiltration equation (Tollner et al. (1976), and sediment filtration and fecal transport modeling (Guber et al. (2009b).

VFS enhances infiltration process through soil matrix ecosystem functions including: First, accumulated organic matter residues that enhance permeability of soil surfaces, plants roots that increase soil hydraulic conductivity, interception of dissipated raindrops that reduces surface sealing, resistance to runoff flow that decreases flow velocity, and increased number of soil macropores resulting from macroinvertebrates ecosystem balancing actions (Cardoso et al. 2012; Houdeshel et al. 2015); secondly, the efficiency of VFS in retaining cowpat manure bacteria (e.g., *E. coli*) varies depending on hydrologic soil surface condition (Cardoso et al. 2012); thirdly, there is a fast decline of fecal coliforms in cow part manures in the soil-runoff mixing zone that has a persistence of only 6–10 days after application date (Gessel et al. 2004); fourth, the decay process occurs because of the inability of the *E. coli* to reduce its metabolic rate to meet the low availability of usable dissolved organic carbon (Klein and Casida 1967); fifth, in the weakened state of nutrient shortage, *E. coli* is also stressed by other environmental factors, including high soil temperatures of up to 35 °C, strong solar radiation (550 cd in this study) and the acidic soil (pH 4.56) in the present study (Hill 2003); sixth, *E. coli* die-off rate in clay loam in dry, moist and wet conditions increases as temperature also increases from 25 to 35 °C (Ling et al. 2002b, 2005).

4.2 Determining overland flow infiltration rates of *E. coli* into soil matrix

During the mechanical filtration of *E. coli* in the soil column, the observed *E. coli* concentrations and loadings in the soil decreased gradually from top 1 to 5 cm after the natural rainfall, showing continuous downward movement and retention of *E. coli* through the soil depths. The

attenuation of overland flow by VFS was evidenced with the population density of *E. coli* and fecal coliform measured in the runoff flow and sediment not detectable after 7 days following natural rainfall in the VFS. The drop in the *E. coli* population density in the VFS could be attributed to losses resulting from rainfall overland flow, leaching and decay. Overland flow contributed to less than 50% of the total *E. coli* loss. The main loss of *E. coli* in soil surface resulted from die-off, accounting for over 50% because most of the microorganism concentrations were detected on the top 1 cm of the soil. Leaching process was observed to result into the loss of *E. coli* as not all the natural rainfall was accounted for in the overland flow. There was a substantial infiltration of rainfall runoff through the soil surface facilitating leaching of *E. coli*, which was indicated by 50% of the runoff flow being recorded at the outlet of VFS field plots.

4.3 Selective partitioning of *E. coli* on manure wastes, root zone, soil macropores and subsoil particles in VFS

There was a significant selective partitioning of microorganisms (*E. coli*) between suspended sediment-waste particles, and water in VFS. *E. coli* was attached to soil particles, epiphytic and clumped. There was a significant correlation between *E. coli* concentrations and total suspended solids, because of the Napier grass and Kikuyu grass filtering the cowpats, which are organic material. *E. coli* loading in the overland flow was linked to the concentration of *E. coli* in the overland flow, soil matrix and became epiphytic to the plants root systems. This shows that the *E. coli* populations from the manure infiltrated through the soil pores where they interacted with the plants fibrous root systems and soil matrix. Epiphytic *E. coli* concentration in VFS was negatively related to Epiphytic *E. coli* concentration in VFS. This shows that both VFSs interacted with *E. coli* from the manure in the overland flow. Increased interaction between *E. coli* in VFS was accompanied by a decreased interaction within the VFS II. *E. coli* loading in overland flow was directly related to the *E. coli* concentration in the soil matrix.

In this study, five reasons can be attributed to these observations: First, the concentration of *E. coli* in runoff was in the order of 4 log (CFU) mL⁻¹ compared to the concentration of *E. coli* in runoff in the order of 2 to 3 log (CFU) mL⁻¹ in simulated experiment for the short duration rainfall of 5 min (Ling et al. 2009); secondly, <25% of *E. coli* was isolated from feces mixed with soil, which was a low attachment level compared to >50% of *E. coli* attached to feces alone (Muirhead et al. 2005; Muirhead et al. (2006); thirdly, the physical filtration of bacteria at the soil surface also increased the chances of losses during

runoff (Crane et al. (1983); fourth, in overland flow, more than 50% of bacteria were not settled or filtered (Schilling and Gannon 1985); fifth, the use of soil trays (where the fecal material source contains mostly organic material with low density) is more erodible than grassed vegetated filters on soil surface rather (Khaleel et al. 1979). Thus, VFS on soil surface provided a better design for research and management of soils for the control of manure borne pathogens in rangelands and agro-pastoral systems.

4.4 Interaction between *E. coli*, soil matrix, particulate organic carbon (POC) and dissolved organic carbon (DOC) in VFS

This result indicates that both POC and DOC increase in the same proportion with increasing trophic in the VFSs. This did not imply a similar increase in extra cellular organic carbon release. Twelve reasons could explain this phenomena: First, DOC increases with increasing productivity (Baines and Pace (1991); secondly, in the regression model, the POC increase explains 99.9% of the variance in DOC increase; thirdly, two key factors contributed to the POC and DOC concentrations regime observed in the VFSs, namely allochthonous POC and DOC input from the neighboring upper part of the field strips and overland flows, and autochthonous organic matter leakage into the VFS's fluid system; fourth, these observations reassert the phenomenon of energy storage within the POC and DOC in the overland flows (de Bernadi 1991); fifth, since DOC is more accessible to *E. coli* than POC; therefore, DOC concentration was quickly reduced through mineralization (Bertoni et al. 1991b); sixth, POC and DOC are tightly coupled in the overland flows; seventh, in VFS overland flows, the concentration of POC in contrast is more stable in time since particle settling and particle microbial removal can be slow processes; eighth, organic carbon synthesis and mineralization processes in the soil help explain this assertion (Callieri et al. 1996); ninth, the large volume to surface area of *E. coli* made it get into direct contact with the nutrients supplied from DOC and POC in the soil; tenth, this relationship supports the hypothesis that it is DOC and POC released from the cowpat during *E. coli* release from the cowpat into soil environment that the *E. coli* utilizes initially to ensure their survival and subsequent growth in the new soil environment; eleventh, this ensures that the *E. coli* continues to possess its physiological vigor to survive and grow in the new soil environment; twelfth, the presence of DOC and POC helps *E. coli* survive despite the average monthly sunlight exposure of 500 cd in this area, which is in the tropical region. Thus, these organic carbons in the soil habitat provided the initial energy for *E. coli* microbial cell multiplication.

4.5 Root zone retention and survival rates of *E. coli* in soil matrix in VFS (root zone, macropores and subsoil horizon)

In the root zone of Napier grass, >50% of the *E. coli* in the overland flow was recovered. This implied that root zone was the most important interaction area of the *E. coli* and plant material in the Napier grass VFS. Two processes occurred in the root zone soil system of Napier grass, namely retention of *E. coli* cells onto the soil macropores due to infiltration and filtration of overland flows and adsorption of *E. coli* cells onto the root zone soil particles. This is a significant angle both scientifically and from a management standpoint. It shows that Napier grass and Kikuyu grass could be used for further investigations into the VFSs. This system could be tried to find other plants to mix in that mimic or complement with other plants. For management purposes these grass species could be used to control manure borne pathogens both in rangeland and in small-scale farms near water bodies locally and internationally in other areas with similar environmental problems.

The retention of *E. coli* in root zone could be attributed to six reasons, namely: First, the retention was because of the filtration of *E. coli* in the soil and adsorption of *E. coli* cells to soil particles (Ling et al. 2009); secondly, the size of bacteria ranged from 0.2 to 5 μm compared to the fine to medium pore size of 10 nm to 10 μm , mechanical filtration of bacteria could occur, though it would be incomplete (Matthess et al. 1988); thirdly, adsorption occurs between *E. coli* and soil particles and two strains of *E. coli* have shown that cells adhered rapidly to clay particles and formed cell-clay complexes, which adhere to each other or to other clay particles and form cell-clay aggregates at a much lower rate (Hattori 1970); fourth, *E. coli* established equilibrium in the soil water system, and the percentage of *E. coli* adsorbed depended on the clay and clay loam soil content in the VFSs; fifth there is equilibrium of *E. coli* in the soil water system in clay soil (Ling et al. 2002b); and sixth, the *E. coli* die-off, sedimentation, adsorption and filtration with biofilms attachment on soil macropores will enhance the decline of bacteria during the transport of active vegetative microbial organisms, in VFSs (Unc et al. 2015). This study had some limitations despite.

This study shows that individual *E. coli* cells could survive in the soil matrix. The survival could be explained in terms of the following: First, biotic factors including extra cellular organic carbon significantly influenced the survival of *E. coli* in the root zones of VFS; secondly, in the root zone of various plants, *E. coli* survival depends on soil geochemical conditions including ambient temperature; thirdly, the survival of these microorganisms also depends on the availability of extracellular materials such

as organic carbon in the root zone in VFSs; fourth, Napier grass root zone provided exudates in the form of organic carbon.

There was very little interaction observed in the root zone of both Kikuyu grass and Couch grass–Buffel grass. At the macropores stage there were attachments, which was similarly significant at subsoil horizon. Individual *E. coli* cells were adsorbed at both the micro pores and subsoil horizon. The following reasons could be attributed to this adsorption at both the soil macropores and subsoil horizon: First, <15% of bacteria movement through the soil matrix passed beyond 5 cm (Gannon et al. 1991); secondly, adsorption of *E. coli* could occur at all depths, while filtration of individual cells or adhering of cells to soil particles could occur during the filtration process (Ling et al. 2009); thirdly, evidence of attachment of *E. coli* to the soil particles; and fourth, the short duration rainfall produced a slower runoff velocity whereby some *E. coli* attached to soil particles could be deposited along the macropore flow pathway in the soil matrix, a mechanism that could improve the survival of *E. coli* along the soil matrix.

Several limitations in some aspects of this study could be pointed out: First, space and economic restrictions may make VFSs BMPs unsuitable for some sites; secondly, the ability of VFs to remove pathogenic pollutants depends on the design and maintenance of the VFSs BMPs; thirdly, when designing VFSs, the designs may be complex when many parameters and uncertainties need to be considered; the runoff flow in open fields is limited depending on soils found in the area of study, where infiltrates could readily leave little on the surface for runoffs; fourth, shallow water presence (inundations) could also reduce the infiltration time; fifth, the limitations of this study included key assumptions, which were made during the sampling and computations of the data. It was assumed that different amount (loads) of *E. coli* would enter different VFSs including Napier grass, Kikuyu grass and Couch grass–Buffel grass combinations. Sixth, it was also assumed that the amount of *E. coli*, entering the VFS was proportional to the amount of *E. coli* exiting the VFS and also proportional to the amount of *E. coli* in the initial manure (200 g) applied at the model pasture. However, these assumptions could not help much in enhancing the entire *E. coli* populations either to reach the VFS exit before either being adsorbed onto soil particles or plants parts; therefore, these could be the source of error in this study.

5 Conclusions and recommendations

Microtopography, development of saturation excess runoff, small-scale heterogeneity of bacterial transport, and the lag periods of runoff flow are important mechanisms that

support differences in bacterial interceptions. Particulate organic carbon (POC) and dissolved organic matter (DOC) waste influence variations in tortuosity and continuity of soil macropores and alters the soil's effective capacity to retain *E. coli*, which then influenced management decisions in VFSs. The VFS design, enhanced levels of POC–DOC levels and nitrogen levels in the cowpat helped improve the infiltration and survival rates of *E. coli* in the soil matrix, through increased levels of the survival, persistence and growth rates of *E. coli* in the soil column in the VFS, because of enhanced microhabitat and additional. These *E. coli* could continually move to the soil matrix as long as 3 months provided they could get into micro pores to move along with water films. The spread of undecomposed manure onto agricultural fields may pose serious potential on-farm pathogen management problems with crop, surface and groundwater contamination. Long rainfall hours with low volume generated runoff that produced higher *E. coli* concentration in the runoff flow because of greater detachment and entrainment associated with longer-duration rainfall event and higher volume runoff in the VFS. The dissolved organic carbon and particulate organic carbon released from the cowpat continue to support the survival and growth of *E. coli* released into soil environment as it adapts to its new habitat without losing its physiological strength from primary host. The extracellular DOC released from Napier grass and Kikuyu grass exudates supported the survival, subsequent growth and adaptation of *E. coli* in its new secondary habitat outside its primary host. Thus, in the soil habitat, DOC and POC provided the initial energy for microbial cell multiplication from the VFS grasses. The background soil DOC was too insignificant to support the initial survival and subsequent growth of *E. coli* released from manure from the primary host. The potential sources of DOC were recognized as DOC entering the vegetated filter strip field with the runoff from the catchment area; DOC released from the cowpat; DOC released from the background soil; and finally DOC released from exudates continuously from Napier grass and Kikuyu grass. Thus, root zone retention data and information on *E. coli* in VFS systems are significant and could be used for scientific and management of soil erosion and the control of fecal pathogens entering surface water ecosystems both locally in Mau Ranges, Njoro River Watershed and internationally. Various VFS could be utilized under various designs of indigenous grasses and with different setup of plant root zone cover and penetrations, that could help in various decision making processes including policy and the governance issues, legislation, management and evaluation of decay discrepancies across organisms locally and internationally.

The following recommendations could be considered in future studies: Initiate or modify the existing field and

watershed hydrologic and water quality models to explain the impact of VFS on reducing manure borne pathogens under different land use and climatic conditions and soil matrix. Studies are needed to investigate effect of background POC and DOC in enhancing the physiological vigor of *E. coli* released from cowpat into overland flows and into soil matrix.

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