ABSTRACT

Title of Document: RELEASE AND RUNOFF/INFILTRATION REMOVAL OF *ESCHERICHIA COLI*, ENTEROCOCCI, AND TOTAL COLIFORMS FROM LAND-APPLIED DAIRY CATTLE MANURE

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Simulating the rainfall-induced release of indicator bacteria from manure is essential to microbial fate and transport modeling with regard to water quality and food safety. Experiments were conducted to determine the effects of rainfall intensity, surface slope, and scale on the release of *Escherichia coli*, enterococci, and total coliforms from land-applied dairy manure. Rainfall intensity did not affect bacterial release dependencies on rainfall depth, but it did have a significant effect on the post-rainfall quantities of indicator bacteria in soil. While bacterial concentrations were evenly released into runoff and infiltration, the surface slope controlled the partitioning of total released bacterial loads. The proportion of *E. coli* released from manure exceeded enterococci, especially with infiltration flow. Scale had strong, inverse effects on the recovery of land-applied bacteria with runoff. These results will be used to improve microbial fate and transport models, critical for risk assessment of microbial contamination in the environment.

RELEASE AND RUNOFF/INFILTRATION REMOVAL OF *ESCHERICHIA COLI*, ENTEROCOCCI, AND TOTAL COLIFORMS FROM LAND-APPLIED DAIRY CATTLE MANURE

By

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Dedication

I dedicate this thesis to my parents, Sharon and Edward Blaustein who have loved and encouraged me since birth. It is also dedicated to my sister, Rachel, my brother, Robbie, and Dena Cohen, my partner, all of whom bring joy to my life. An additional dedication goes to my grandmother, Dr. Lee Joyce Richmond, for her guidance and inspiration throughout my academic career. From an early age, she encouraged me to follow my dream of pursuing higher education and a career path in academic science. The love of family along with the support of friends has sustained me throughout my graduate program.

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Chapter 1 – Introduction

During rainfall on lands where manure has been applied, microbial indicators and pathogens are released and transported with surface runoff and subsurface flow. Simulating the rainfall-induced release of fecal indicator bacteria is an essential component of microbial fate and transport modeling with regard to water quality and food safety. This thesis presents a critical literature review on microbial release (Chapter 2), and the results from three original experiments on the release of indicator bacteria – *Escherichia coli*, enterococci (*Enterococcus* spp.), and total coliforms – and a surrogate tracer – chloride ion – from dairy cattle manure and their removal with runoff/infiltration (Chapters 3-5). The final chapter (Chapter 6) summarizes significant results from the three experiments. Chapters 2-6 are briefly discussed in the following paragraphs of this introduction.

Chapter 2, the literature review, provides the comprehensive discussion of the release of indicator microorganisms and pathogens from manure as affected by biological, physical, and chemical factors. An annotated bibliography on the relevant work to-date is provided as well as a meta-analysis on the microbial release dependency in relationship to rainfall depth. Knowledge gaps are described and avenues for future research are suggested, which leads into the following chapters that describe three original research projects performed to advance this field of study.

Chapter 3 describes the first research experiment and discusses microbial release from a manure matrix into runoff and infiltration. The main objectives were to determine the impact of rainfall intensity and land surface slope on the release kinetics of *Escherichia coli*, enterococci, total coliforms, and chloride ion from dairy cattle manure in partitioning boxes during simulated rainfall. Particular attention was given to the relative concentration of manure constituents in the initial release (i.e., the content removed with initial runoff or infiltration divided by the starting

concentration in manure). Also, the partitioning of concentrations of indicator bacteria into runoff and infiltration was observed and discussed.

Chapter 4 builds on Chapter 3 as it describes and discusses the second experiment which focused on the effects of rainfall intensity on microbial release from manure that was applied over grass in soil boxes. In this chapter, total microbial release was taken as the sum of the total numbers of cells of of *Escherichia coli*, enterococci, and total coliforms that are recovered in surface runoff, soil leachate (having passed through the base of the soil profile), and contained within different soil depths following a rainfall event. The chapter elucidates the effect of rainfall intensity on the kinetics of bacteria and chloride ion removal with runoff as well as the effect of rainfall intensity on the concentrations of manure-bacteria remaining at different soil depths following rainfall.

Chapter 5 describes the study that was performed to address the effects of scale on making field scale or "real-world" predictions based on laboratory assessments. The objective of this particular study was to test the hypothesis that the relative numbers of manure-borne *Escherichia coli*, enterococci, total coliforms, and chloride ion that are transported out of a manure application area is affected by the size of the study area. The kinetics for runoff-removal of manure-bacteria and chloride during rainfall/irrigation are compared for three different-sized study areas: soil box (0.35 x 1 m), standard field plot (0.8 x 1 m), and corn field (36 x 77 m).

Chapter 6 summarizes the conclusions, analysis, and discussion of the three research studies in this thesis.

Chapter 2 – Release of Microorganisms from Land-Deposited Animal Waste and Animal Manures: Meta-analysis and Literature Review

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Abstract

Microbial pathogens present the leading cause of impairment to rivers, bays, and estuaries in the USA and agriculture is often viewed as the major contributor to such contamination. Microbial indicators and pathogens are released from land-applied animal manure during precipitation and irrigation events and carried by overland and subsurface flow that can reach and contaminate surface waters and ground water used for human recreation and food production. Microbial release is situation-specific and depends on a combination of physical, chemical, and biological factors, such as, manure source and consistency, manure application method and rate, manure age, cell properties important for microbial attachment/detachment, vegetation, and rainfall. Simulating release of manure-borne pathogens and indicator microorganisms is an essential component of microbial fate and transport modeling. While microbial release governs the quantities of available pathogens and indicators that move toward human exposure, a literature review on this topic is lacking. This critical review on microbial release from manure and animal waste is intended to provide a discussion on the published release studies to date, factors that impact the release process, and models used for simulating release. Current knowledge gaps are described and avenues for future research are suggested.

Introduction

Water contamination by microorganisms that are released from the fecal excrement of mammals and birds is of universal concern. It has been estimated that 10 to 100 billion tons of agricultural animal manure is generated on a global scale annually (Fayer and Trout, 2005) and in the United States alone, concentrated animal feeding operations (CAFOs) produce about 500 million tons of liquid and solid animal waste per year (Konewaran and Nierenberg, 2008). Worldwide livestock production and the volume of manure applied to agricultural lands for nutrient input and waste disposal is increasing in order to feed Earth's growing population (Forslund et al., 2011). Every load of fecal excrement contains high concentrations of microorganisms, some of which may be pathogens. During precipitation and irrigation events on lands where animal waste has been applied, enteric bacteria and protozoa are released from the feces into the suspension that enters surface runoff and/or infiltrates the underlying soil. Released microbes are carried with overland and subsurface flow and can contaminate surface waters and ground water that are used for recreational swimming/bathing, agricultural irrigation, water used as a carrier agricultural chemicals, prewashing fruits and vegetables, direct human consumption, production of shellfish in aquaculture, and other human activities. Some enteric pathogens are capable of causing acute gastrointestinal disease, long-term sequelae, and even death. Roughly 1 in 6 Americans experience foodborne illnesses each year, which cause an estimated annual financial loss of approximately $14 billion (Batz et al., 2012; Hoffmann et al., 2012), and nearly half of all reported foodborne illnesses can be attributed to produce contamination (Painter et al., 2013). Understanding the fate and transport of manure-borne pathogens is critical for sustaining human health and quality of life, which has a strong impact on the economy.

Manure and other animal waste in the environment

Animal waste can enter the environment in a variety of ways. Livestock excreta is collected and applied to crop and range lands in the form of fresh, stored, or composted manure to enhance soil fertility or to serve as a means for waste disposal (Sheldrick et al., 2003). Manure may be stored, mixed (e.g., feces, urine, and animal bedding), or processed to separate liquid and solid components, and these materials may be surface or subsurface applied to land or incorporated into soil by tillage. Animal waste inputs to land may also be directly deposited by livestock, wildlife, domestic pets, and humans. Compared with animal manure, cowpats and other direct deposits are not typically meant to serve as fertilizer, although they still may improve the fertility and physical quality of the soil where they are applied. Indirect fecal inputs, which also contribute to environmental contamination, can come from leakages in septic tanks and agricultural lagoons.

Pathogens

Common bacterial pathogens that enter the environment through livestock and animal waste are *Salmonella* spp., *Campylobacter jejuni*, Shiga toxin-producing *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Yersinia enterocolitica* (Guan and Holley, 2003; Mawdsley et al., 1995; Pell, 1997; Pond, 2004; Savichtcheva et al., 2006; Sobsey et al., 2006). Unlike bacteria, protozoa are eukaryotic and require a specific host to multiply, so they do not experience reproduction in manure, soil, water, or stream sediment. Protozoa may also act both as grazers and as parasites and they are generally less sensitive to chlorine or other microbial disinfectants that are used. Certain manure-borne protozoan parasites are infectious to humans and have the ability to multiply in the gut of a human host and cause disease. *Giardia* and *Cryptosporidium* (oo)cysts are two major manure-borne parasites with the former being

associated with both wildlife and livestock and the latter more often associated with livestock (Baldursson and Karanis, 2011; Barick et al., 2003; Pell, 1997). *Toxoplasma gondii* is another protozoan parasite of concern with regard to livestock-human transmission (Singh et al., 2013). Several viruses may cause disease in animals and a few that infect humans may be present in animal manure as well (Sobsey et al., 2006; Tauxe, 2002). For a more comprehensive review of pathogens in animal and livestock waste, their survival, and their transport please see Sobsey et al. (2006) and Mawdsley et al. (1995).

Microbial pathogens present the leading cause of impairment in rivers, reservoirs, streams, and estuaries in the USA and agriculture-related activities (e.g., crop production and grazing) has been designated as major sources of impairment to these water supplies (USEPA, 2009). Pathogens are capable of colonizing the digestive tracts of an entire herd of livestock in an agricultural feeding operation if they are consumed and may spread within the animal care system through the water, feed, and/or bedding. Primary sources of water-borne pathogens include cattle, swine, and poultry manures (Cardoso et al., 2012). All of these three livestock groups contribute to environmental inputs of *Salmonella*. Cattle are most commonly responsible for the inputs of *Cryptosporidium*, *Giardia*, *Campylobacter*, and pathogenic *E. coli.* Swine are primarily responsible for most inputs of *Yersinia enterocolitica* (Gerba and Smith, 2005). Sheep and goats, while grown in smaller quantities in the US, may make significant contributions to pathogen inputs as well (Freschet et al., 2008; Kudva et al., 1998; Moriarty et al., 2011).

Fecal indicator bacteria

Due to the relatively high cost of pathogen monitoring and research and the bio-safety concerns associated with pathogens, non-pathogenic indicator microorganisms are often enumerated, simulated, and regulated as being representative of fecal contamination to the

environment (Meays et al., 2004; Panhorst, 2002; Savichtcheva et al., 2006). Fecal indicator bacteria (FIB) are used in making these assessments. Ideally, FIB should be members of intestinal microflora present in the environment where other enteric microbes are present, they should survive longer than the hardiest enteric pathogens, their density should correlate well with fecal contamination in the environment, they should not multiply in environmental media, and they should be relatively easy to culture (Thurston-Enriquez et al., 2005; U.S. EPA, 1986).

Total coliforms

Coliforms are rod-shaped, non-spore forming, gram-negative bacteria that ferment lactose and produce acid and gas when incubated at 37^0 C (Edberg et al., 2000). Coliforms have been used as traditional indicators of fecal contamination due to their abundance in the intestines and fecal excrements of mammals. This group of bacteria, generally referred to as "total coliforms", is not often used for making microbial environmental risk assessments due to their wide distribution in nature and broad categorization (Rosen, 2000; Sinclair et al., 2012). While total coliforms include gut-associated bacteria, there are some genera and species in this group that are indigenous to soil, water, and vegetation. Although they are not optimal indicators of fecal contamination in the environment, total coliforms are monitored as an important indicator at drinking water treatment facilities because their absence indicates lack of any coliform subgroup.

Fecal coliforms

Fecal coliforms, also referred to as thermotolerant coliforms, are a subgroup of total coliforms that are distinguished by their ability to grow at 44-45^0 C (Alonso et al., 1999; Edberg et al., 2000). This group of coliform bacteria is not ubiquitous in nature and is more specific to the facultative anaerobes that populate the mammalian gut. Fecal coliforms have been a

traditional FIB in the past and are still used around the world today because of their relatively simple, non-hazardous, and cost-efficient enumeration methods. However, their usage as optimal FIB has been questioned due to background environmental concentrations from inputs of native wildlife that carry similar strains to livestock (Brooks et al., 2007; Coston-longares et al., 2008; Patni et al., 1985). The potential enumeration of soil-native fecal coliform strains, such as *Klebsiella*, may provide a false-positive indication of livestock fecal contamination in the environment (Drapcho, 2003), as well as lack of correlations with enteric pathogens. For example, Parajuli et al. (2007) stated that fecal coliform inputs by wildlife actually accounted for 15-30 % of the total land-deposited bacteria in a Kansas watershed.

Escherichia coli and enterococci

Escherichia coli, the most commonly recognized FIB, is a species within the fecal/thermotolerant coliform group. Bordalo et al. (2002) reported *E. coli* to account for up to 89 % of fecal coliforms in effluent entering into brackish and fresh estuarine waters. Another study reported *E. coli* may account for up to 35.2 % of the fecal coliform population in rivers, but their presence may be as low as 8.8 % in some areas (Alonso et al., 1999). *E. coli* generally is harmless to humans and it plays a vital role in the human digestive system. There are, however, several, less common, pathotypes of virulent *E. coli*, such as, Shiga toxin-producing *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, and enteroinvasive *E. coli* (U.S. CDC, 2012). Regardless, a strong correlation has been observed between elevated levels of *E. coli* in recreational water and occurrences of gastrointestinal disease (U.S. EPA, 1986), and the usage of "generic *E. coli*" as FIB is supported by the US Environmental Protection Agency and the World Health Organization.

Enterococci, a member of the bacterial group of streptococci, are another intestinal bacteria often used as FIB. Unlike coliforms, enterococci are coccoidal, gram-positive, and smaller in size. *E. coli* and enterococci are the primary FIB's that are used for environmental water quality regulation (Dufour and Ballantine, 1986; Guber et al., 2007). Compared with fecal coliforms, usage of *E. coli* and enterococci as FIB's in state regulations is supported by their significantly higher correlations with swimming-associated gastrointestinal symptoms (U.S. EPA, 1984). Enterococci is generally more human-specific than *E. coli* and both indicators are recommended for monitoring fresh waters while enterococci is the preferred indicator in marine water due to its ability to survive in the presence of salinity (U.S. EPA, 2012).

Other FIB's and surrogates

Other FIB's include: fecal streptococci (Borst et al., 2003; Stoddard et al., 1998) (although these are now grouped with enterococci (Byappanahalli et al., 2012)), staphylococci (Brooks et al., 2007) and fecal anaerobes – *Bacteroides* spp. and *Bifidobacterium* spp. (Savichtcheva et al., 2006). FIB's are present in livestock and wildlife feces, usually more often and in higher concentrations than are pathogens. Efficient usage of an indicator organism relies on the ability to accurately predict its release, transport, and survival, which differ among microorganisms based on their unique qualities for attachment/detachment and growth/inactivation among other physical and biochemical processes.

Surrogates, in the context of environmental microbiology, are defined as organisms, particles, or substances used to study the fate and transport of a pathogen in a specific environment (Sinclair et al., 2012). Viral phages – *Coliphage* and *Bacteriophage* – fall into this category and may be used in microbial contamination and fate and transport assessments (Savichtcheva et al., 2006). In addition, tracers, such as genetic markers (Oladeinde et al., 2014)

or fluorescent latex spheres and chemical solutes may be used as well (Sinclair et al., 2012). Stout et al. (2005) observed a high correlation (r=0.93) between the concentrations of fecal coliforms and total phosphorous in runoff that was transported across vegetated plots under simulated rainfall that suggested phosphorous may be used as a reasonable surrogate tracer of fecal coliforms that are released into runoff. Guber et al. (2006) reported similarity in the release kinetics of fecal coliforms and chloride ions, organic carbon, and water-soluble phosphorus from bovine slurry. Compared with FIB's, the inability of abiotic surrogates to multiply or die-off in solution, such as the aforementioned dissolved ions, must be considered when making fecal contamination assessments. This is especially true for recurrent rainfall events; for example, McDowell et al. (2006) noted release of NH_4-N and P from cowpats during a primary rainfall event to be similar to *E. coli*, but during recurrent rainfall events the release of these nutrients was significantly less than that of *E. coli* since the bacteria experienced regrowth in favorable environmental conditions between rainfall events.

Bacterial content in manure

The amount of indicator and pathogenic bacteria that colonize livestock herds, and shed by animals with their excreta, vary both spatially and temporally. Pachepsky et al. (2006) and Patni et al. (1985) have described asymmetrical statistical distributions for *E. coli* concentrations in manure with up to six orders of magnitude of variation. According to Meals and Braun (1999), livestock manure generally contains 10^6 to 10^7 fecal organisms per gram (g^{-1}). Other researchers have reported cow manure to normally contain $>10^9$ indigenous bacteria g^{-1} (Jiang et al., 2002).

Feces from a cattle herd that has been colonized by *Salmonella* may contain the pathogen at rates of 10^2 to 10^7 CFU g^{-1} feces (Himathongkham et al., 1999). *E. coli* in fresh sheep manure has been reported at values that exceed 10^9 CFU g^{-1} manure (Moriarty et al., 2011). Muirhead et

al. (2006a) reported *E. coli* concentrations in fresh cowpats from a herd of dairy cattle to range anywhere from 9.7×10^1 to 1.9×10^7 MPN per gram dry weight (gdw^{-1}) with a geometric mean of 2.1×10^5 MPN gdw^{-1}, yet in another study, Muirhead et al. (2005) noted *E. coli* concentrations in fresh cowpats to vary from 10^5 to 10^7 MPN gdw^{-1}. Likewise, Guber et al. (2007) stated that concentrations of *E. coli* and enterococci in fresh dairy cattle slurry produced at a concentrated animal feeding operation (CAFO) were $>10^6$ CFU ml^{-1} and between 10^5 and 10^6 CFU ml^{-1}, respectively. In a release study by Brooks et al. (2009), the concentrations of total coliforms, fecal coliforms, enterococci, and *C. perfringens* in poultry litter were approximately 10^3, 10^3, 10^6, and 10^6 CFU or MPN g^{-1} manure, respectively. Thurston-Enriquez et al. (2005) documented concentrations of *E. coli*, enterococci, and *Clostridium* to be 3.3×10^6, 5.9×10^5, and 1.3×10^4 CFU g^{-1} for fresh cattle manure,respectively, and 6.0×10^5, 2.2×10^5, and 1.2×10^5 CFU g^{-1} for fresh swine manure, respectively.

Temporal variability and spatial distributions of FIB's in manure are important to consider when making environmental assessments (Molina et al., 2005). The scale of microbial concentrations in manure and the wide variability that exists strongly expresses the need for using lognormal distributions to describe manure-borne microbes in research assessments (Guber et al., 2011).

Microbial fate and transport

The fate and transport of manure-borne microorganisms is dynamic and there are many processes involved, such as, manure application on land, microbial survival and redistribution in manure, release from manure, transport in overland or subsurface flow, remobilization from soil, transport in surface water, and so on (Fig. 2.1). Each process within the scope of fate and transport is unique and depends on a variety of chemical, biological, and physical factors. For

example, genetic strain variation has been shown to affect bacteria survival in manure compost (Kim et al., 2009), in stream sediment (Kiefer et al., 2012), and in several soils (Ibekwe et al., 2014). Temperature dependence of the survival of indicator bacteria and/or pathogens has been noted for cowpats (Martinez et al., 2013; Wang et al., 2004), cow and sheep slurry (Kudva et al., 1998), in agricultural soils (Howell et al., 1996; Jenkins et al., 2002), in surface waters (Pachepsky et al., 2014; Blaustein et al., 2013), in sewage water (Easton et al., 2005), and on farmyard surfaces (Williams et al., 2005). Survival has also been documented to be affected by sunlight (Sinton et al, 2007), soil water content (Berry and Miller, 2005), soil type (Franz et al, 2008; Jenkins et al., 2002), manure surface area (Muirhead and Littlejohn, 2009), manure application method (Sharples et al, 2004), and experiment type: lab or field (Van Kessel et al., 2007).

Surface transport of fecal microorganisms has been shown to be affected by manure type (Soupir et al., 2006), soil type (Kouznetsov et al., 2007; Kramers et al., 2012), and land use (Oliver et al., 2005), while subsurface transport is affected by soil texture and structure (Mosaddeghi et al., 2009) as well as the ionic strength of the soil solution (Wang et al., 2011). Transport is also affected by implementing conservation practices, such as vegetative filter strips (VFS) (Edwards et al., 1996; Fox et al., 2011; Kouznetsov et al., 2007; Lim et al., 1998; Srivastava et al., 1996; Zhang et al., 2001).

Developing tools that may be used to make predictions and risk assessments of microbial fate and transport requires an understanding of all of the situation-specific processes and how they may be affected by abiotic and biotic variables. Pachepsky et al. (2006) states that successful manure management evaluations require an integration of the interactions of overland flow, infiltration, partitioning, adsorption/detachment, straining, and biological growth and die-

off with projected flow regimes and pathogen loads. Muirhead and Monaghan (2012) suggested that an optimal fate and transport model would require a collective approach that accounts for microbial survival and transport in each respective microbial reservoir within a field (i.e., manure reservoir and soil reservoir) due to situation-specific characteristics that are associated with each reservoir. Theoretically, a collective approach that incorporates all fate and transport processes, from microbial release to survival and transport through soil, is needed in order to make the most accurate predictions and assessments for contamination in the environment.

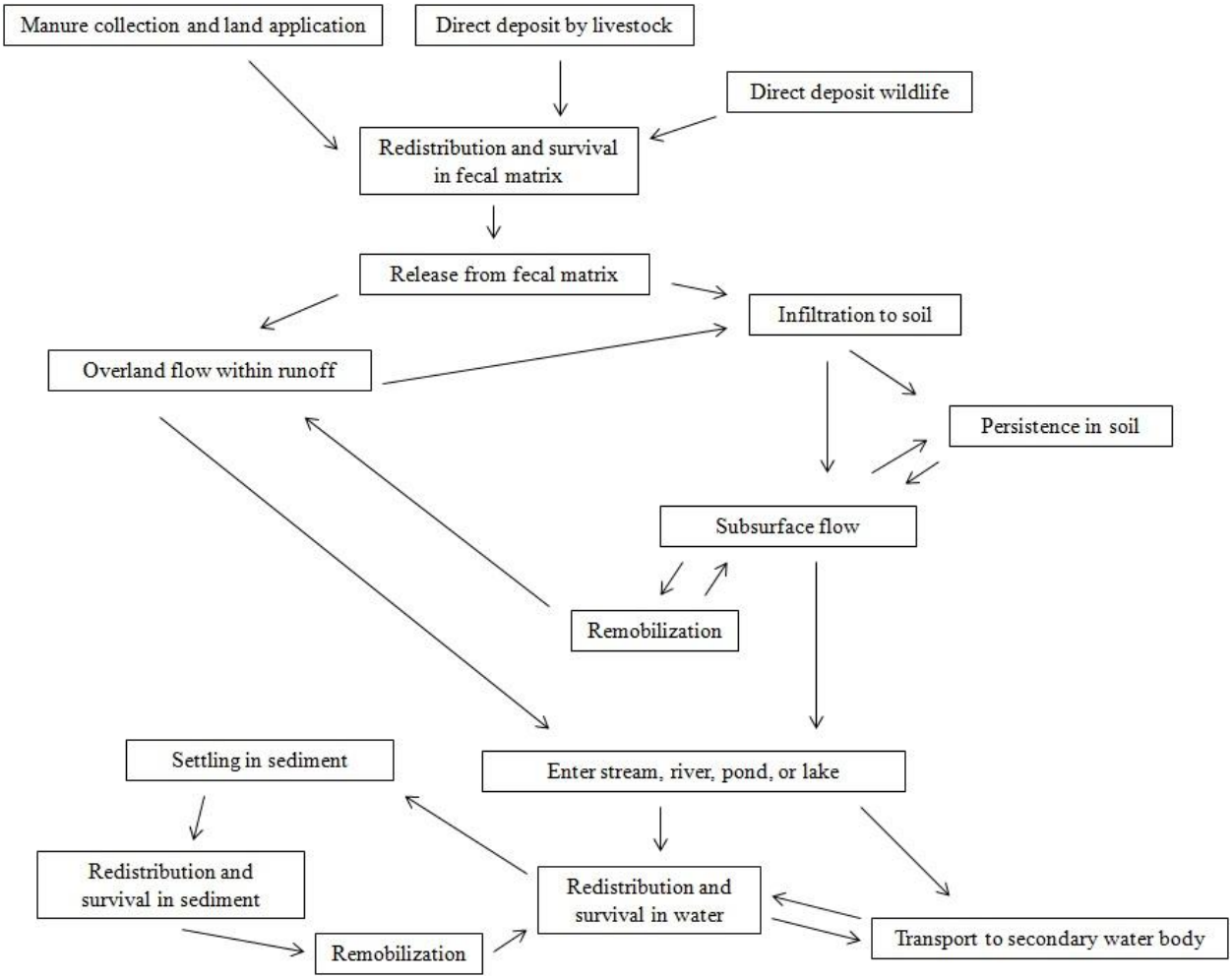

Figure 2.1 Schematic of fate and transport processes of manure-borne bacteria in the environment.

The microbial fate and transport processes that have been summarized in literature reviews include surface transport of pathogens (Tyrrel et al., 2003), subsurface transport of bacteria in drainage water (Jamieson et al., 2002), transport of coliforms and survival in aquifers (Foppen and Schijven, 2006), transport of bacteria from manure to contaminate water resources (Unc and Goss, 2004), modeling the fate and transport of manure-borne pathogens (Pachepsky et al., 2006), microbial fate in soil (van Veen et al., 1997), pathogen survival in swine manure (Guan et al., 2003), source tracking of bacteria in waters (Meays et al., 2004), bacteria in stream sediments (Pachepsky and Shelton, 2011), bacterial contamination of ground water (Crane and Moore, 1984), and pollutant transport in runoff from land with agricultural waste (Khaleel et al., 1980). While an extensive amount of literature about the processes of microbial fate and transport has been published, a literature review on the process of microbial release from manure is missing. The reviews by Unc and Goss (2004) and Pachepsky et al. (2006) discussed microbial release, but not as a focal point, and newer studies have provided additional, imperative information since the time of their publication. (Brooks et al., 2009; Cardoso et al., 2012; Dao et al., 2008; Guber et al., 2007; Hodgson et al., 2009)

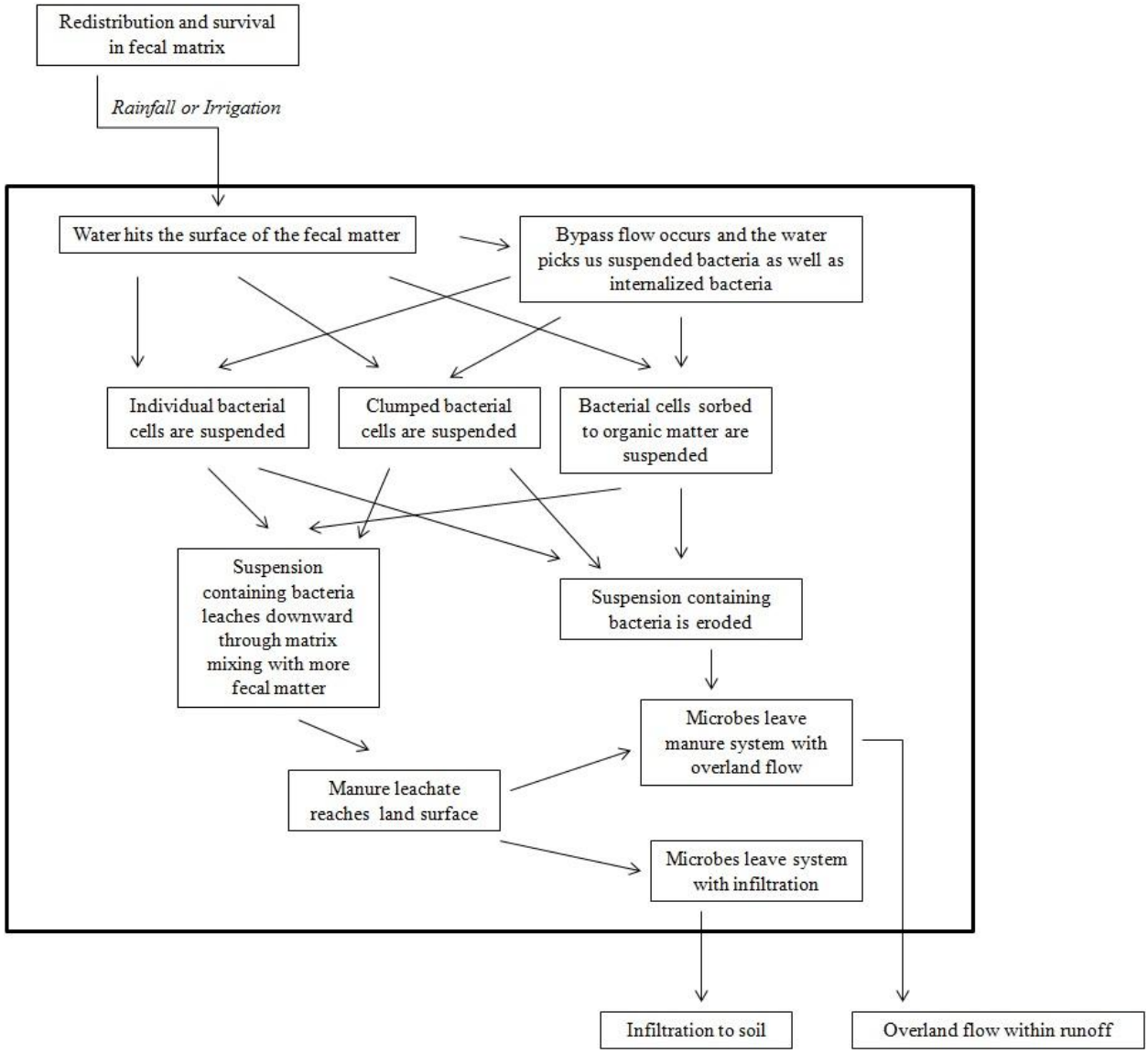

Figure 2.2 Possible processes affecting the release of microorganisms from animal waste are described in the center box. Release is shown to be preceded by redistribution within the fecal matrix and followed by overland flow and/or infiltration.

Microbial release

Release of microorganisms from animal waste is a critical component of microbial fate and transport. Release occurs as fecal material becomes suspended and eluted during precipitation. It is not really known how the content of the manure suspension is formed. Conceptually, one or more of the following occurs during release: (a) incoming rain suspends

manure constituents and then releases them by sloughing off erodible layers from the manure surface, (b) an internal mixing process occurs during the manure water absorbtion-event followed by release of a diluted manure solution upon saturation, or (c) the initial manure solution is pressed out of the manure-matrix and then the release of diluted, mixed components occurs. Released material may exit the manure matrix via water flow that runs off the manure surface and/or flow that leaches out of the base of the matrix, possibly accumulating microorganisms from aggregates within the manure matrix along the way. The actual release process probably occurs via some sort of a combination of these processes.

Microbial release from manure simply refers to the amount of microbiota removed from manure and displaced elsewhere. Release may be measured by the amount of microbes transferred away from solely a manure matrix (Bradford and Scijven, 2002; Shijven et al., 2004), or by the amount that is removed from a land area fully covered by manure with runoff and leachate (Guber et al., 2007). Thus, measuring release/removal can involve transport across a manure-covered portion of land. Along this coupled pathway of release-transport, the suspended material may interact with other parts of manure as well as with soil and vegetation.

Microbial release from animal waste and its subsequent removal with runoff/infiltration is affected by manure application rates (Brooks et al., 2007; Drapcho, 2003), the living-state of vegetation where manure is applied (Dao et al., 2008; Guber et al., 2007), whether manure is surface-applied or incorporated into the soil (Forslund et al., 2011), manure source (Soupir et al., 2003; Thurston-Enriquez, 2005), and manure age (Kress and Gifford, 1984). Precipitation variability impacts microbial release as well (Shijven et al., 2004; Thelin and Gifford, 1983). Further research on these factors as well as the effects of rainfall intensity, longer duration rainfalls, and different vegetative covers have been recommended (Ling et al., 2009).

Release establishes the content of microbes that can be removed from the manure application area and become a potential risk for human health. The amount of microbes released into manure suspension, the way that microbes are distributed in suspension (i.e., as cells clumped with each other, cells attached to soil particulates, or free-living "planktonic" cells), and the flow pathway of the released material (i.e., runoff or infiltration) impact the transport and, therefore, removal potential. Recognizing how release rates vary for different microbes, from different manure sources, and under different environmental conditions is important for predicting the consequences of situation-specific release events. From a technical standpoint, modeling the concentrations and total numbers of microbes released from animal waste during precipitation/ irrigation events is essential to assessing pathogen contamination risks. Understanding the release process, both qualitatively and quantitatively, is imperative to food safety control, environmental regulation, and agricultural waste management. This literature review will focus on microbial release from surface-applied animal manures from livestock in agricultural settings and will provide a discussion on the content of release work to-date, factors that impact release, models used for simulating release, and current knowledge gaps that suggest avenues for future research objectives on the topic of microbial release.

Scope of Microbial Release Studies

A thorough literature search on microbial release-related work (i.e. release and removal with runoff) yielded 29 published studies and a brief summary of the findings from each work is presented in Table 2.1. Some researchers have observed microbial release as eluting directly from a manure matrix, while others focused on release from manure that was applied over soil, and some described experiments that have on a combination of release and transport (where removal with runoff was reported, but manure did not cover the entire study area). The existing

literature on microbial release mainly comes from laboratory experiments using soil boxes or field experiments using small-scale plots, in which microbial release was induced via rainfall simulation. Rainfall intensities used in the studies involving simulated rainfall were within the range of 2.5 to 11 cm hr^{-1} and have mimicked normal, frequent rainfall events as well as rare, extreme events (Brooks et al., 2009; Cardoso et al., 2012; Dao et al., 2008; Edwards et al., 2000; Guber et al., 2007; Larsen et al., 1994; Lim et al., 2008; Mishra et al., 2007; Roodsari et al., 2005; Sistani et al., 2011; Soupir et al., 2003; Stout et al., 2005; Thurston-Enriquez et al., 2005). Several laboratory studies have been conducted with unique, non-rainfall methods to induce release, such as rolling vials containing manure and rainwater on a lab bench (Hodgson et al., 2009) or using mist and drip irrigation to induce release from a manure disc (Bradford and Schijven, 2002; Schijven et al., 2004). Release studies at the field-scale are relatively limited, probably due to challenges associated with collecting runoff at the edge-of-field, controlling precipitation or irrigation events at larger scales, and accumulating and applying several tons of manure to a large study area with limited farm equipment.

Table 2.1 Annotated bibliography of documented literature on microbial release from manure.

Reference	Brief Summary of Significant Findings
Bradford and Schijven, 2002	Increasing solution salinity caused a decrease in *Cryptosporidium* and *Giardia* (oo)cycsts released from cattle manure. A conceptual model used to predict manure and (oo)cyst release rates was developed and calibrated with experimental data.
Brooks et al., 2009	Enterococci, staphylococci, and *C. perfringens* are better indicators of microbial release from broiler litter than fecal coliforms and total coliforms. Application rate of broiler litter did not yield statistically significant differences in masses of released bacteria groups/species, but it did cause an increase in antibiotic resistance of microbes within litter.
Cardoso et al., 2012	*E. coli* and *Salmonella* are released at similar rates from swine slurry. While vegetation has a high capacity to lessen release and transport of microbes, its effectiveness depends on soil water content prior to rainfall and is most evident for drier pre-rain soil conditions that enhance infiltration.
Dao et al., 2008	More *E. coli* and enterococci are released from manure that is applied over dead grass than live grass since the leaf blades may intercept attachable bacteria and also attenuate raindrop impact energy that causes erosion. There is a high correlation with turbidity, concentration of bacteria, and concentration a phosphorus (P) fraction associated with manure particulates, in release. Assymetry in release patterns of bioavailable P and enteric microbes was evident.
Drapcho, 2003	Fecal coliform release is greater from lands where manure is surface-applied than from lands containing grazing cattle due to the crusting of cowpats that lessened the amount of microbial release from the latter.
Edwards et al., 2000	On land where cowpat deposition was simulated, the concentrations of released fecal coliforms, ammonia, optho-P in runoff all decreased exponentially with time.
Forslund et al., 2011	Compared with traditional methods of manure surface application, subsurface application of swine slurry limited the release of *Salmonella enterica* serovar Typhimurium bacteriophage 28B, *Cryptosporidium* oocysts, and *E. coli* into surface runoff, but it increased release and transport of the two former with leachate towards groundwater.
Garcia et al., 2012	In a beef cattle feedlot pen, increased soil compaction and soil bulk density favors partitioning of released manure constituents into surface runoff rather than infiltration.
Guber et al., 2006	The Bradford-Schijven (2002) model, which had uncorrelated parameters for irrigation rate and vegetation, is recommended for simulation of manure consituent release. Release kinetics, as quantified by release model parameters, of fecal coliforms were more similar to those of P and organic carbon than those of chloride ion.
Guber et al., 2007	The release rate of *E. coli* from dairy cattle slurry was about twice as fast as that of enterococci. Of the two indicators, *E. coli* release was more similar to that of bromide ion tracer. Thus, *E. coli* was suggested to be more associated with the liquid phase of manure.
Guber et al., 2011	At the field-scale, there was a high spatial variation in fecal coliform concentration in land-applied cattle manure. The coupled KINERSOS2-STWIR release and treansport model was calibrated.
Guber et al., 2013	A parameter for release efficiency was added to the reommended model from Guber et al. (2006).
Hodgson et al., 2009	A laboratory assay showed the relative percentage of *E. coli* and enterococci released during simulated rainfall to increase from sheep feces to beef cattle manure to dairy cattle slurry. The concentrations of *E. coli* and enterococci released from all fecal matrices decreased as the manures aged and dried out.

Kim et al., 2014	Data from a series of 144 rainfall simulation events on small-scale field plots was up-scaled to calibrate the field-scale model, KINEROS2/STWIR, for simulation of release and transport.
Kress and Gifford, 1984	Concentrations of fecal coliforms released from cowpats decreased as manure aged, although one hundred-day-old cowpats still released fecal coliform counts that exceeded water quality standards. Rainfall intensity had a greater effect on microbial release on aged manure than fresh manure.
Lim et al., 1998	Concentrations of fecal coliforms released from cattle manure and recovered in runoff were as high as 2×10^7 CFU 100 ml^{-1}.
Ling et al., 2009	*E. coli* release from soil was greatest when rainfall commenced soon after spray application.
McDowell et al., 2006	The release of NH_4-N and P from cowpats were similar to *E. coli* during a primary rainfall event, but, during recurrent rainfall events, the release of the nutrients was significantly lower than that of *E. coli*, since the bacteria experienced growth in favorable environmental conditions between rainfall events.
Muirhead et al., 2006a	The content of *E. coli* released from cowpats was highly variable and it strongly correlated with the starting content of *E. coli* in the source cowpat. Fractionation of suspended *E. coli* in the runoff samples showed only only about 8% of cells were transported as attached to dense particles (> 1.3 g ml^{-1}), while flocculation appeared not to have occurred, so the predominant transport of *E. coli* from cowpats occurred as single, planktonic cells.
Muirhead et al., 2006b	In soil inoculated with *E. coli* cells that were pre-attached to soils particles greater than 0.45um, the overland transport of *E. coli* was significantly lower than in overland flow transport during rainfall then in soil inoculated with unattached *E. coli* cells. Bacterial attachment to soil particles attenuates overland transport.
Muirhead et al., 2005	The majority of *E. coli* released from cowpats occured as single cells that were not attached to particles in suspension and not clumped with other cells. No differences in the "release state" of *E. coli* were seen for fresh and aged cowpats.
Pachepsky et al., 2009	Manure particles that are released from manure during rainfall as manure dissolves can serve as carriers, habitat, or nutrient source to released indicator bacteria and pathogens. The size distributions of particles that are released from dairy manure into runoff changed over time, and became relatively stable after 15 minutes of release.
Schijven et al., 2004	Rain droplet size and also the increased ratio of cow to calf manure had a positive effect on the release rate of *Cyrptosporidium* and *Giardia* (oo)cycts from manure. Temperature had little to no effect on microbial release.
Shelton et al., 2003	The concentration of fecal coliforms in leachate released from cattle manure was strongly corrleated with the turbitidy of leachate (R^2 = 0.807).
Sistani et al., 2009	Concentrations of *E. coli* released from surface-applied poultry litter into surface runoff decreased during successive rainfall events. Compared with traditional surface broadcasting, subsurface banding of poultry litter substantially reduced the content of *E. coli*, as well as that of nutrient contaminants, that were released into surface runoff.
Soupir et al., 2010	The percentage of cowpat *E. coli* and enterococci that were released and attached to soil particles in runoff ranged from 28-49% and at least 60% of soil-attached *E. coli* and enterococci had been attached to particles in the size range of 8-62 um. The majority of released bacteria are attached to manure colloids rather than oil particles and soil texture has little effect on release from cowpats.
Soupir et al., 2003	The percent of released fecal coliforms, *E. coli*, and enterococci that were removed with runoff increased from liquid dairy manure, to cowpats, to turkey litter. For all manure sources, the percent release was greatest for fecal coliforms, followed by *E. coli*, and then enterococci.

Thelin and Gifford, 1983	The concentration of fecal coliforms released from cowpats increased until an equilibrium concentration was reached within 10 minutes of release. Aged cowpats (30 days) released lower concentrations of fecal coliforms than fresh cowpats, although the contaminant concentration in runoff removed from aged manure still exceeded water quality standards.
Thurston-Enriquez et al., 2005	*E. coli*, enterococci, *C. perfringens*, *Giardia*, and *Cryptosporidium* were released from fresh cattle manure, aged cattle manure, and swine slurry at slightly different rates, and they were all released from each common manure source at a different rate as well. Only 0.01-6.99% of ecal indicator organisms (*E. coli*, enterococci, *C. perfringens*, and coliphage) were removed with runoff during 1.75 cm of rainfall. Runoff concentrations of *C. perfringens* and coliphage correlated well with that of protazoan parasites for swine slurry.

Concentrations of a variety of FIB's, pathogens, and a few viruses have been documented in published reports on microbial release from manures. The three most often used FIB's in these studies have been *E. coli,* enterococci, and fecal coliforms, in that order. Although the amount of release work on pathogens has been well outnumbered by those on indicators, the three pathogens that have been most often studied in release experiments are *Cryptosporidium, Giardia,* and *Salmonella.* Other FIB's and pathogens that have been documented by more than one source in release work are total coliforms and *C. perfringens* (Table 2.2).

Manure sources most often used in release work are cowpats, cattle manure and slurry, swine slurry, and poultry litter (Table 2.2). The initial concentrations of FIB in manure in these release studies typically range from 10^4 to 10^7 CFU g^{-1}, with the most often encountered concentrations around 10^6 CFU g^{-1} (Brooks et al., 2009; Guber et al., 2007; Thurston-Enriquez et al., 2005). Concentrations of pathogenic bacteria and protozoa in manure release experiments are usually less than FIB concentrations, unless the manure was inoculated for the study.

Table 2.2 Microorganisms and manure sources that have been used in microbial release studies.

Organism	Microbe Type	Manure Sources Used	References
Escherichia coli	FIB	Cowpat, Dairy Cattle Manure, Dairy Cattle Slurry, Beef Cattle Manure, Swine Slurry, Turkey Litter, Sheep Pat, Broiler Litter	Brooks et al., 2009; Cardoso et al., 2012; Dao et al., 2008; Forslund et al., 2012; Guber et al., 2007; Hodgson et al., 2009; Larsen et al., 1994; McDaniel et al, 2013; McDowell et al., 2006; Mishra et al., 2008; Muirhead et al., 2005; Muirhead et al., 2006a; Sistani et al., 2009; Soupir et al., 2003; Soupir et al., 2010; Thurston-Enriquez et al., 2005
Enterococci	FIB	Cowpat, Dairy Cattle Slurry, Beef Cattle Manure, Swine Slurry, Turkey Litter, Sheep Pat, Broiler Litter	Brooks et al., 2009; Guber et al., 2007; Hodgson et al., 2009; Mishra et al., 2008; Soupir et al., 2003; Soupir et al., 2010; Thurston-Enriquez et al., 2005
Fecal Coliforms	FIB	Cowpat, Dairy Cattle Manure, Dairy Cattle Slurry, Beef Cattle Manure, Broiler Litter	Brooks et al., 2009; Drapcho, 2003; Edwards et al., 2000; Guber et al., 2006; Guber et al., 2011; Kress and gifford, 1984; Lim et al., 1998; Mishra et al., 2008; Roodsari et al., 2005; Shelton et al., 2003; Springer et al., 1984; Stout et al., 2005; Thelin and Gifford, 1983
Total Coliforms	FIB	Cowpat, Dairy Cattle Slurry, Turkey litter, Broiler Litter	Brooks et al., 2009; Soupir et al., 2003
Staphylococci	FIB	Broiler Litter	Brooks et al., 2009
Clostridium perfringens	FIB; Pathogenic bacteria	Beef Cattle Manure, Swine Slurry, Broiler Litter	Brooks et al., 2009; Thurston-Enriquez et al., 2005
Salmonella	Pathogenic bacteria	Swine Slurry, Broiler Litter	Brooks et al., 2007; Cardoso et al., 2012
Campylobacter	Pathogenic bacteria	Broiler Litter	Brooks et al., 2009
Cryptosporidium	Pathogenic protozoa	Cowpat, Dairy Cattle Manure, Beef Cattle Manure, Swine Slurry	Bradford and Schijven, 2002; Schijven et al., 2004; Thurston-Enriquez et al., 2005
Giardia	Pathogenic protozoa	Cowpat, Dairy Cattle Manure, Beef Cattle Manure, Swine Slurry	Bradford and Schijven, 2002; Schijven et al., 2004; Thurston-Enriquez et al., 2005
Coliphage	Virus, Indicator	Beef Cattle Manure, Swine Slurry	Thurston-Enriquez et al., 2005
S. Typhimurium Phage 28B	Virus, Indicator	Broiler Litter	Forslund et al., 2013
Total Heterotrophic Bacteria	Other	Broiler Litter	Brooks et al., 2009

Microbe-Specific Release

During a continuous rainfall or irrigation event on land where manure is applied, manure constituents, including microbes, plant nutrients, and other ions and compounds, are released simultaneously, but at different rates and concentrations. In theory, dissolved ions and chemical compounds, and even free-living, planktonic microbes that are suspended in manure solution can naturally flow with the released material that is eluted from the manure matrix; whereas, solid manure components of manure components that happen to be sorbed to solid materials in manure may resist release. Microbial release efficiency, which depends on a microbe's potential to enter and flow with the formed suspension, is affected by the microbe's size, location, and distribution within the manure matrix, and whether or not the microbe happens to be associated with manure solids.

The release process for all manure-borne microorganisms is microbe-specific based upon the unique physiological properties of each microbe. Bacteria have specific physical and chemical properties that affect their efficiency of becoming dislodged from their micro-habitats (Lombard et al., 2011). The negatively charged cell surfaces on bacteria allow them to adsorb cations and attach to positively charged biopolymers in fecal organic material. Also, bacteria may sorb to the negatively charged clay surfaces in soil via cation bridging or by interaction with electrical charges in the diffuse layer surrounding a soil surface (Sobeck and Higgins, 2002). Bacteria surface charge, hydrophobicity, size, and surface structures such as flagella, fimbriae, and lipopolysaccharides (LPS) affects their ability to attach/detach from surfaces (Critzer and Doyle, 2010; Foppen and Schijven, 2006; Pachepsky et al., 2008). LPS is a major component in the outer membrane of gram-negative bacteria, yet it is non-existent in gram-positive bacteria. On the other hand, teichoic and mycolic acids that are located in the cell walls of gram-positive

bacteria, yet not gram-negative bacteria, are involved in attachment and dislodgement processes as well (Lombard et al., 2011). Thus, the release of gram-negative bacteria (i.e., *Salmonella*, *E. coli*, and other coliforms) and gram-positive bacteria (i.e., enterococci) may be fundamentally different based on their physiological structure and physiochemical properties for cellular attachment. The spatial distributions of gram-negative and gram-positive bacteria within the micro-habitats of the manure matrix may vary as well due to their unique structures, which would also have an effect on release.

Differences in microbial cell wall surface components (e.g., LPS or teichoic acids in cell wall of *E. coli* and enterococci, respectively) as well as microbial surface charges affect flocculation potential within manure suspension. Particle-associated bacteria in the environment are typically less mobile, sink faster, and experience different survival dynamics than planktonic bacteria (Fries et al., 2003). The manner in which bacteria are distributed in a released suspension – as individual cells, flocculated cells, cells attached to organic colloids and particulates, or cells attached to soil particles – has gained recent interest since mitigation strategies ought to be designed to counter the specific release- and transport-state of bacteria (McDaniel et al., 2013; Muirhead et al., 2005; Muirhead et al., 2006a; Muirhead et al., 2006b; Soupir et al., 2010). For example, the interception of single or flocculated cells flowing with runoff may rely on vegetation in VFS, while the catchment of cells that are attached to eroding soil particles may require other means for prevention of soil and sediment erosion. In addition, it may be important to consider that facilitated transport of the fractions of released bacteria that are attached to manure or soil particles can increase due to (1) raindrop erosion that breaks down the manure and, in effect, increases the transport of released fecal material and (2) as manure is

diluted and loosened, the contact between soil and fecal matter increases, causing more transport of bacteria attached to either manure or soil particles (Soupir et al, 2010).

Size distributions of particles and colloids in a suspension that is eluted from manure affects microbial release since the particles may contain adsorbed bacteria. Shelton et al. (2003) and Dao et al. (2008) stated that decreasing turbidity of released material corresponds with decreasing microbial concentrations in release and is indicative of decreasing sizes of particles suspended in runoff. In a study on the rainfall-induced release of *E. coli* and enterococci from cowpats, 60% of released FIB's were associated with particles in the size range of 8-62 microns (Soupir et al., 2010). Pachepsky et al. (2009) noted that the size of manure particles affect transport and retention of microbial pathogens in soil. The size distributions of particles released from dairy cattle slurry initially had an average size of approximately 7.96 μm and decreased before becoming stabilized at an average size of approximately 4.1 μm following 15 minutes of rainfall at a 32.4 mm hr^{-1} intensity (i.e., after rainfall depth reached 8.1 mm). This 2-fold size decrease in only 15 minutes could substantially affect the release of *E. coli* and enterococci, which are approximately 1-2 and 0.5 μm in size, respectively.

Studies have shown that release differs among microbial species even when the microbes have a common manure source (Brooks et al., 2009; Guber et al., 2007; Thurston-Enriquez et al., 2005). In a study on the rainfall-induced release of bacteria from bovine slurry that had been applied over grass in soil, Guber et al. (2007) reported the release-rate for *E. coli* to be almost twice as fast as that of enterococci. The release of bromide tracer in the same study was more similar to the release of the former than the latter, suggesting *E. coli* to be more associated with the manure liquid phase than enterococci (Guber et al., 2007). The location and release efficiency of these species within the surface-applied slurry likely contributed to the observed

differences in release. Compared with *E. coli*, the smaller eneterococci cells were likely more capable of remaining associated with manure solids, maybe held within manure aggregates, or attached to surface-soil and/or vegetation within the plots rather than being washed off (Guber et al., 2007). In another study, release was simulated in scintillation vials containing a mixture of manure and rainwater that were rolled on a lab bench. A significant difference in release-rate of *E. coli* and enterococci was noted for all manure types – dairy slurry, beef farmyard manure, beef feces, and sheep feces – that were used in the study (Hodgson et al., 2009). Evidence for microbe-specific release processes support the idea that an optimal release assessment should be based on release characteristics of multiple indicator microorganisms.

Factors Impacting Microbial Release

The release process is situation-specific and depends on a combination of physical, chemical, and biological factors such as manure source, manure consistency, manure application method and rate, manure age, vegetation, and rainfall.

Manure

Manure is animal dung that is used for fertilizing land and the term "manure" may refer to animal feces, the mixture of fecal and urine excrement, or the mixture of excrement combined with some sort of animal bedding (e.g., wood shavings, straw, sawdust). Manure may be applied to land in a fresh, aged, or composted state and it may also be applied as a liquid slurry, as a semi-solid, or as a solid material depending on the manure management. In CAFOs, the liquid and solid contents of manure may be separated, treated, and used differently in the field. Manure is a heterogeneous matrix that is made up of macro- and microscopic dietary fiber, microbial biopolymers, microbial colloids, and soluble components (Guber et al., 2006). Manure consists

of proportional liquid and solid contents that are affected by the physiology, diet, and lifestyle of the animal source. Physical and biochemical differences of the manure composition affect the release of microorganisms.

Animal source

The rate and extent of manure component release varies among animal waste types (Mawdsley et al., 1995). The microbiome of fecal matter depends on the chemical compounds and microflora that are processed in the gut of the animal that is producing it. Several studies have shown differences in FIB release based on animal source. Mossadeghi et al (2009) reported higher bacterial release-rates in poultry manure than in cow manure, suggesting poultry manure as the greater potential source of microbial contamination in runoff. Schijven et al. (2004) observed more efficient release of *Cryptosporidium* (oo)cysts in cow manure than calf manure and also more efficient release from a cow/calf manure mixture that contained an increased fraction of cow manure. This difference was probably due to the larger particle composition and more readily available space for water flow through cow manure as opposed to calf manure (Schijven et al., 2004). Soupir et al. (2003) reported the relative percentage of fecal coliforms, *E. coli*, and enterococci released from turkey litter to be ten-fold greater than the amount released from liquid dairy manure or cowpies during a 30 minute simulated rainfall event. Hodgson et al. (2009) reported the relative release of both *E. coli* and enterococci to sequentially increase when considering sheep feces, beef cattle manure, and dairy cattle slurry. In a rainfall-induced release study using cattle manure and swine slurry, Thurston-Enriquez et al. (2005) determined a difference not only in the amounts of *E. coli*, enterococci, *C. perfringens*, and *Cryptosporidium* released from the two manure sources, but also noted differences in the variability in total cells

released within treatment replications. Moreover, animal source affects microbial release from manure.

Manure consistency

The physical properties of manure, especially the proportion of solids and liquids within the matrix, seem directly related to release. The four states of manure consistency are liquid, slurry, semi-solid, and solid, which differ in ratio of liquid and solid contents depending on the animal source (Hamilton, 2011). Manure consistency can be affected by animal management (e.g., usage of straw or sawdust bedding in animal pen) and manure management (e.g., separation of solids and liquids at the storage facility, storage time, composting, etc.). Regardless of manure source, experimental conditions, and scale, the broad scope of release and removal with runoff studies show the dependency of percent of bacteria released based on rainfall depth to be drastically different for manures of different consistency (i.e., slurry, solid manure, and cowpats) (Fig. 2.3). The relationship between microbial release and rainfall appears to be strongest for release from slurry, then cowpats, and very weak for solid manure (Fig. 2.3). The liquid-state of slurry probably allows microbes to be released more easily, while the release from solid manure appears to be more complex and situation-specific. Microbial release from cowpats appears to be better explained by rainfall than release from solid manure (Fig. 2.3) and may be attributed to less variability in the microbial and physicochemical properties of the cowpats that were used in these experiments. It is noted that cowpats from research used in the dataset for the currect analysis had less variability than that of the solid manures – broiler litter, poultry litter, dairy manure, and beef manure.

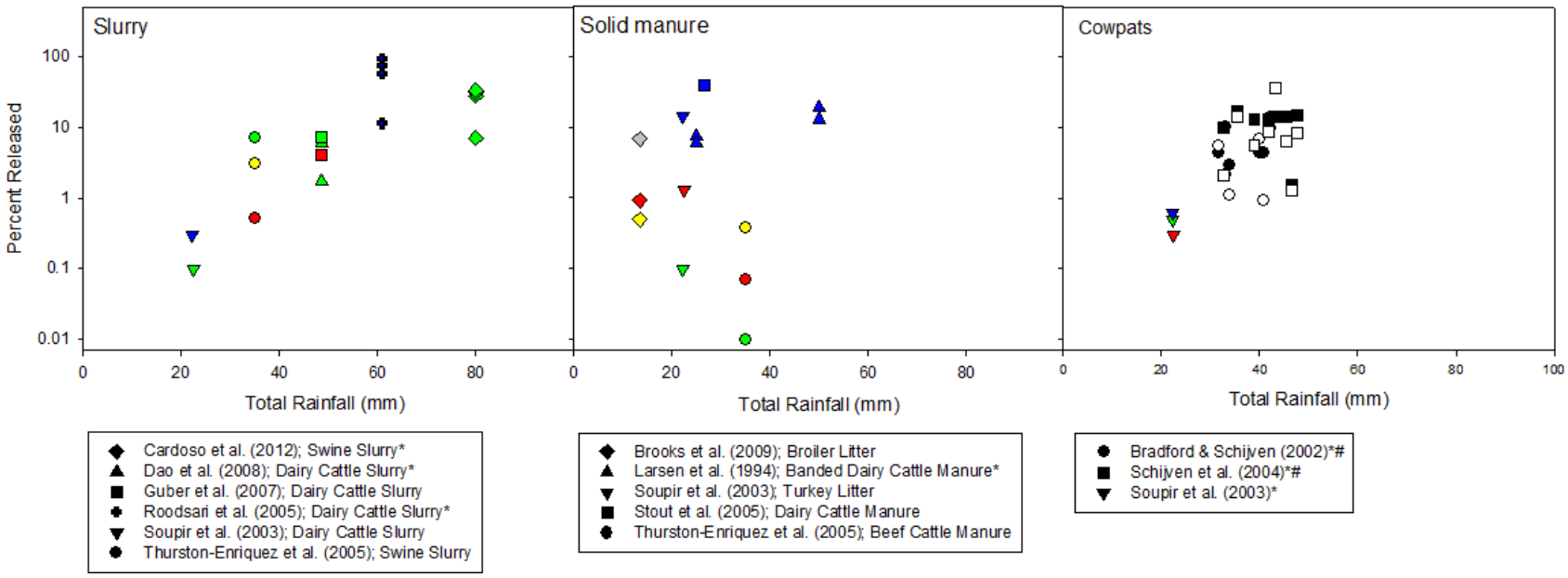

Figure 2.3 An illustration of the dependency of the percent mass of bacteria released from fresh manure and animal waste on total rainfall for slurry (left), solid manure (middle), and cowpats (right) based on data collected from published release studies. Colors correspond to microbe species/groups: green- *E. coli*, blue-fecal coliforms, red-enterococci, yellow-*C. perfringens*, gray-staphylococci, black-*Giardia*, and white-*Cryptosporidium*. Each datapoint represents a dataset corresponding to a publication listed in the key below each graph; the * indicates that manure in that particular release or removal with runoff study did not cover 100% of the plot area that was rained, and the # indicates that the manure was artificially packed into a "disc" and not a natural cowpat.

Perhaps the differences noted for microbial release from different animal sources (Thurston-Enriquez et al., 2005; Hodgson et al., 2009; Soupir et al., 2003) may be attributed to, at least to a degree, the liquid and solid contents of the manure. As manure solid content increased, sites for bacteria to live within the manure matrix also increased (Guber 2005). In a transport study, fecal coliforms association with aggregates in the manure-matrix was affected by water content as opposed to aggregate size (Guber et al., 2009). In another study, differences in the release of FIB's from liquid and solid manure were reported when both manure types were prepared from cattle at the same dairy facility (Soupir et al., 2003).

Manure aging

Manure ages between the time of fecal deposition and the onset of rainfall-induced release. During this time period, facultative anaerobes in manure may grow in the nutrient-rich organic material or they may become inactivated due to sunlight, desiccation, or other environmental stresses. Desiccation has been shown to occur at quicker rates for manure with greater surface areas (Muirhead et al., 2009). Manure aging alters the structure of aggregates and microhabitats within the manure matrix. For example, the degradation of manure causes the production of ammonia, volatile fatty acids, and other degradation products that have a negative effect on microbial survival (Thurston-Enriquez et al., 2005). Major changes to the manure matrix can occur in short periods of time – Bittman et al. (2005) stated that 85% of ammonia volatilization from dairy slurry applied on grass-covered land had occurred within 24 hours of surface and subsurface application.

Microbial die-off during the aging process may drastically lower the potential amounts of releasable bacteria. Evaporation during the aging process causes an increase in the dry manure contents, which surround viable cells in manure solids and make them less mobile and less

releasable (Hodgson et al., 2009). Hodgson et al. (2009) demonstrated that the bacterial concentrations released from dairy slurry, beef farmyard manure, and sheep feces all decreased as the manure aged and dried out. Kress and Gifford (1984) found concentrations of fecal coliforms released from cowpats decreased as manure aged. Manure application methods for satisfying nutrient requirements on cropland can be a significant source of bacterial loading and water contamination if rain occurs soon after the manure application without allowing time for aging to occur (Mishra et al., 2008). Ling et al. (2009) reported that *E. coli* was best released from swine slurry when rainfall occurred directly after spray application rather than aging prior to rainfall.

While manure age may have considerable impacts in reduced microbial release, the concentrations and total loads of microbes that are released from aged manure may still be relatively high. Springer et al. (1983) and Kress and Gifford (1984) reported one hundred-day-old cowpats to yield fecal coliform counts that exceeded water quality standards.

Thurston-Enriquez et al. (2005) stated that 3.5 cm of rainfall induced the release of up to 10^8 fecal indicator bacteria and 10^5 protozoan parasites from aged dairy cattle manure that was applied on 1.5 m^2 plots. Aging cowpats have been documented as a significant source of *E. coli* in overland flow over a one month period (Muirhead et al., 2005). During simulated rainfall at an intensity of 6.1 cm hr^{-1}, Thelin and Gifford (1983) measured concentrations of fecal coliforms in effluent from 30 day old cowpats to be 4 x 10^5 MPN 100 ml^{-1}. Compared with the fecal coliform concentrations that came from fresh manure, these values were only one order of magnitude lower. The major differences between fresh and aged manure in that study was that the rainfall time that had initiated release was longer for the aged manure treatment and that the aged fecal

material, which was drier due to desiccation, had required more water for saturation (Thelin and Gifford, 1983).

Manure application method

Some manure application methods include surface broadcasting, soil tillage/incorporation, soil injection, and subsurface banding. Compared with methods for subsurface application, surface application typically leads to greater rates of fecal contaminants that are released into overland flow and also greater rates of direct contamination of crops (Lamba, 2010). Sistani et al. (2012) reported concentrations of *E. coli* released into surface runoff to be approximately 6.5 times greater coming from land where broiler litter was surface applied than from land where broiler litter was subsurface banded. Microbial contamination to surface waters is less attributed to manure that is subsurface applied than that which is surface applied because, in theory, microbes in subsurface applied manure can only be transported to a surface water body after being returned to the soil surface. Returning to the soil surface may only be accomplished by means of upward flow with capillary rise or by subsurface flow toward a soil surface that exists downslope from the original underground location of applied manure. Underground bacteria may also resurface in soil following wind or water erosion that removes the surface layers of the porous media that are covering the bacteria or motile microbes (e.g., *E. coli* and *Salmonella*). These microbes are known to move towards a food source by means of chemotaxis and may resurface if an adequate gradient for upward transport is available. As soil water moves upward in soil to become evaporated by atmospheric gasses, concentrations of dissolved ions and compounds, including nutrients, in the soil solution re-locate near the soil surface. If conditions are favorable, microbial chemotaxis may move along these gradients to aquire nutrients. Another avenue of potential pollution may occur as motile subsurface bacteria

and pathogens follow nutrients downwards towards groundwater rather then trying to resurface. Gagliardi and Karns (2000) suggested that *E. coli* O157:H7 follows nitrogen sources (i.e., ammonia and nitrate) through soil. Furthermore, while subsurface manure application may seem to limit surface runoff contamination, its benefits come at the cost of higher expenses and greater risks for groundwater contamination by leaching of the manure microorganisms (Forslund et al., 2011). In an *in situ* field study, compared with surface application of pig slurry, the injection of pig slurry into a sandy clay loam soil was noted to release more *Cryptosporidium* into soil leachate (Forslund et al., 2011). Soil structure strongly affects water and bacterial movement through soil by not only controlling the velocity of water flow, but also by providing surface-sites for bacterial cellular attachment, both of which may limit microbial release from subsurface manure (Smith et al., 1985). Soil with a well developed macropore system enhances bacterial transport by increasing water flow velocity through its available pathways for water movement (Guber, 2005). Following subsurface manure release, preferential flow through macropores in well-structured soils accelerates the transport of leaching solution, enhancing the ability for bacteria in the solution to reach groundwater (Kramers et al., 2012). The maximum contamination depth of released pollutants is governed by soil structure and macroporous pathways (Mosaddeghi et al., 2009). In addition, soil pore size distributions ought to have a considerable impact on bacterial retention via straining. It is difficult to monitor release from subsurface applied manure without considering transport as well, since released bacteria are susceptible to physical straining as they move through porous media (Foppen and Schijven, 2006; Stevik et al., 2004).

Grazing livestock that provide excreta inputs to pasture land is another source of animal waste input to the environment. While this type of livestock waste is not applied to land as

manure fertilizer, it still may improve soil fertility and needs to be considered as another means of animal waste application to land that causes inputs of FIB's and pathogen contaminants. Drapcho (2003) stated that significantly higher loads of fecal coliforms were released from land where cattle manure was surface-spread than from land that contained fecal deposits from grazing cattle, even though the concentrations of bacteria in the feces from both lands were not significantly different. Manure crusting, which seemed to shelter bacteria from environmental stresses and impact energy of water droplets during precipitation, was attributed to lessening the amount of microbes released from the cowpats (Drapcho, 2003).

Manure application rate

Brooks et al. (2009) performed a release study using soil cylinders covered with different rates of poultry litter– 0 (control), 5, and 18 Mg ha^{-1}. The treatments with the lower rate of poultry litter were amended with enough inorganic NH_4NO_3 fertilizer to provide the plots with enough N to mimic the N inputs from the treatment with the higher litter application rate (Brooks et al., 2009). Except for the control, high contents of staphylococci, *C. perfringens*, and enterococci were measured in runoff for both litter treatments and the counts for each microbe released were not significantly different (Brooks et al., 2009). Perhaps the approximate 3.5-fold difference in manure application rate may not have been great enough to demonstrate statistically significant differences in observed microbial release.

Rainfall and irrigation water

Rainfall causes the compaction, slackening, detachment, and deposition of soil, and these actions contribute to the formation of a soil seal and potential crusting that significantly reduce infiltration and increase surface runoff (Zejun et al., 2002). Sharpley et al. (1985) reported the effective depth of interaction between surface soil and runoff to increase with rainfall intensities

from 5 to 16 cm hr^{-1} and soil slopes from 2 to 20% for 5 different soils. These differences were attributed to increased rainfall intensity creating a thicker surface seal. Theoretically, rainfall may also re-orient amorphous materials within manures to form a seal in a similar manner and it may also induce the formation of a surface seal at the manure-soil interface. Both processes would impact microbial release because a manure seal (which may even crust after drying) would protect microbes located beneath the manure surface from release.

Rainfall has been assumed as a key determinant in the fate and transport of pathogenic organisms. The majority of waterborne disease outbreaks in the United States from 1948-1994 were preceded by extreme precipitation events above the 90^{th} percentile ($p=0.002$) (Curriero et al., 2001). The impact energy of raindrops drives the dislodgement and erosion of solid aggregates and particles, and rainfall serves as a major source for surface runoff that transports suspended, eroded material and deposits it elsewhere in a landscape. Several studies have shown temporal changes in FIB's and (oo)cyst concentrations in surface water following precipitation events or simulated rainfall (Thelin and Gifford, 1983; Kress and Gifford, 1984; Mawdsley et al., 1996; Tate et al., 2000).

Rainfall intensity and duration

Rainfall intensity (Schijven et al., 2004; Kress and Gifford, 1984) and duration (Thelin and Gifford, 1983; Ling et al., 2009) affect the release of microbes from manure and soil. In a laboratory study by Schijven et al. (2004), *Cryptosporidium* release efficiencies from cattle manure were greater in treatments that received a dripping water application compared to mist irrigation. The impact energy from larger water droplets, especially when they had occurred at a more frequent application rate suggested that rainfall events with heightened rainfall intensities will increase levels of microbial release from manure on a farm (Schijven et al., 2004). Several

studies have shown that a higher volume of precipitation (i.e., higher intensity rainfall per unit time or longer duration of rainfall at the same rainfall intensity) caused an increase in microbial release from manure (Schijven et al., 2004; Kress and Gifford, 1984; Thelin and Gifford, 1983; Ling et al., 2009). In a study that had simulated heavy rainfall on soil where *E. coli* had been spray-applied, Ling et al. (2009) stated that when rainfall duration increased from 5 to 15 min, the concentration of *E. coli* in surface runoff increased by 2 orders of magnitude. While rainfall duration affected microbial release, the degree of the effect depended on the solid content of manure (Thelin et al., 1983). For example, in a study by Kress and Gifford (1984), rainfall intensity only had a greater impact on release of fecal coliforms from bovine manure as the manure became more air-dry. The effect of rainfall intensity and duration on release may be dependent on interactions with other factors, such as manure solid content.

Rainfall recurrence

With regard to rainfall recurrence, Mishra et al. (2008) reported that concentrations of fecal coliforms, *E. coli*, and enterococci in surface runoff were higher during a secondary rather than a primary rainfall event. The increased water content from rainfall between events 1 and 2 may have promoted bacterial survival and growth within the land-applied manure (Mishra et al., 2008). In agreement, McDowell et al (2006) saw manure-*E. coli* populations to grow between two rainfall events. On the contrary, Sistani et al. (2009) reported that concentrations of *E. coli* released from surface-applied poultry litter into surface runoff decreased during successive rainfall events. Thus, without growth of bacteria between rainfall events, lower concentrations of bacteria in released material are caused by the previous wash out events (Kress and Gifford, 1984).

Dissolved salts and temperature

The concentration of dissolved salts in irrigation water effect microbial release from manure as well. Irrigation waters induce microbial release similar to rainfall, and they may vary in salinity based on the surface water source. According to Bradford and Schijven (2002), the cumulative release of *Giardia* and *Cryptosporidium* (oo)cysts from manure was greatest when the electrical conductivity of the solution that induced the release was 0.3 dS m^{-1} and then the total percent release of these microbes decreased as the electroconductivity of solution sequentially increased from 5.0 to 9.5 and then to 14.8 dS m^{-1}. The increase in ionic strength in the solution may have caused a decrease in microbial release by stabilizing the manure after collapsing the double layer thickness that surrounded charged manure particles (Bradford and Schijven, 2002).

Furthermore, in theory, as precipitation temperature decreased, the viscosity of flowing water increased, which may have caused the release rate of bacteria from manures to have decreased (Farhangi et al., 2012). However, in the work of Schijven et al. (2004), solution temperature had little to no effects on the release of *Cryptosporidium* and *Giardia* (oo)cycts from calf and cow manure for the 5^0 C and 23^0 C solution temperatures that were studied.

Vegetation and soil

Microbial release from manure refers to the amount of microbiota removed from manure and displaced elsewhere. Release may be measured by the amount of microbes transferred away from a manure matrix or the content of microbes removed from a land area that is fully covered by manure via runoff and/or infiltration. As bacteria are removed from manure with runoff, they may be transported across a manure-covered portion of land to the area where the manure

coverage ends. Along this coupled pathway of release-transport, the suspended material may interact with other parts of manure as well as with soil and vegetation near the land surface.

Several release studies have looked at the effects of soil and vegetation on bacteria release from manure and removal with runoff. Dao et al. (2008) and Guber et al. (2007) noted more bacteria collected in runoff coming from soil boxes that had manure applied over dead grass than live grass in the box, suggesting that the status of vegetation had an effect on release. In a study on the release of fecal coliforms from bovine slurry applied on bare or vegetated sandy loam and clay loam plots, Guber et al. (2006) stated that the recommended microbial release model had a parameter that was significantly affected by vegetation presence or absence, but not by soil texture. Another study looked at the association of *E. coli* and enterococci to particle size distributions in release from cowpats that were applied on silty loam, loamy fine sand, and silty clay loam and the researchers reported no significant effects of soil texture on manure-bacteria released to runoff (Soupir et al., 2010). Compared with the presence of vegetation, the lack of soil texture effects on microbial release may be explained by above-ground factors (e.g., protruding vegetation stalks and leaves) having more influential interactions with the suspended material in overland flow. Perhaps, some microorganisms that are released from manure tend not to attach to soil particles during transport in overland flow. Soil texture likely has a more substantial affect on microbial transport than it does on microbial release and/or removal from study areas with runoff.

As microbes move across manure-covered land, they interact with above-ground vegetation. The release of microbes from a manure-covered area depends on the microbe's ability to bypass leaf blades and stalks and to avoid interception by surface vegetation to avoid being removed from the surface runoff (Dao et al., 2008). Surface filtration of colloidal

contaminants (e.g., bacterial indicators and pathogens) by vegetation is controlled by the physical contact processes and chemical attachment processes that are constantly interacting during laminar overland flow (Wu et al., 2012; Wu et al., 2014). Colloidal attachment efficiency is affected by Van der Waals attractive forces, electrostatic double layer forces, and hydrodynamic forces (Wu et al., 2012; Wu et al., 2014). Natural organic matter that coats vegetation (e.g., humic acids and fulvic acids) has been shown to play an important role in colloidal attachment efficiency as well (Wu et al., 2014). While colloidal filtration is enhanced by dense vegetation, such vegetation may also reduce microbial release by protecting the manure microhabitats from the impact energy of rain drops during a precipitation event.

Vegetation filter strips (VFS) have been recommended as a best management practice (BMP) to prevent manure-borne pathogens from reaching surface waters. VFS studies, which are not true microbial release studies, since the plot area is not fully covered by manure, have reported that vegetation reduced the transport of microbes from the respective plot area (Fox et al., 2011; Kouznetsov et al., 2007; Zhang et al., 2001; Edwards et al., 1996; Lim et al., 1998; Srivastava et al., 1996). The efficiency of VFS to remove released contaminants from surface runoff partially depends on the soil's infiltration capacity and the soil's antecedent moisture prior to the initiation of a rainfall event (Cardoso et al., 2012). Optimal design of VFS must consider total area for coverage, soil bulk density (and compaction), macroporosity, soil moisture content, and land slope (Fox et al., 2011). All of these factors affect infiltration fluxes, and, consequently, the mass of contaminant reduction from overland flow (Fox et al, 2011). Reducing microbial transport is not equivalent to reducing release since released microbes may flow with infiltrated material, but it does lessen the content of microbes in surface runoff, which is the manure effluent type that is most commonly used to measure microbial release from manure. In essence,

one should be careful when stating that vegetation may reduce release (when it really is only reducing transport), unless it is reducing release from an area that is fully covered by land-applied manure and a mass-balance assessment shows reduced release in combined runoff over soil and infiltration into soil.

Scale effects on runoff transport of manure-borne bacteria from application areas

Runoff and erosion have been shown to depend on the spatial scale. The runoff coefficient, defined as the percentage rainfall partitioned to runoff, and the sediment yield usually decrease with an increase in area where overland flow is observed. Cerdan et al. (2004) reported runoff coeffficients of 20%, 4.5%, and 1% for 4.50 ha plots, a 90 ha catchment, and an 1100 ha catchment, respectively. Delmas et al. (2012) compiled data from Nothwestern Europe and found values for the runoff coefficients for lands with a 2 to 5 % surface slope to be 30-50 %, 10-20 %, 5-10 %, and 0-5% for areal units of 10^{-4} ha (plot), 0.1-1 ha (field), 10 ha (hillslope), and 100 ha (catchment), respectively. Gomi et al (2008) observed runoff coefficients in a Japanese forested landscape of 20-40 % and 0.1-3 % for 10^{-4} ha plots and 0.02 ha hillslope areas, respectively. In an Israeli study of land under semiarid conditions, Kidron (2011) reported runoff coefficients for 12 ha plots in the range of 30 to70 % for 10^{-4} ha plots, 0 to 20 % for 2 ha plots, and <1 %.

The sediment concentration in runoff has been shown to follow a trend similar to that of runoff coefficients. Average sediment concentrations decreased by about 4 orders of magnitude with an increase of drainage area from 1 ha to 10^6 ha in Nothwesern Europe (Delmas et al., 2012). Also, in comparisons for Mediterranean conditions, Raclot et al. (2009) observed a 20-fold difference in specific yields between individual fields and catchments.

The presence of micro- and meso-topographic features is commonly invoked as a cause of the scale effect for erosion and runoff. When overland flow occurs, local (micro) depressions fill such that the depressions areovertopped and water will begin to flow downhill. The flow pattern looks like "a shallow sheet of water with threads of deeper, faster flow, diverging and converging around surface protuberances" with mixed occurrence of turbulent and laminar flow. This type of flow includes a braiding pattern of water threads, without the complete slope being covered by water (Van de Giesen, 2011), and it creates conditions for re-deposition of eroded sediment within the observation area. Another reason for the decreased runoff coefficients with increased plot sizes is due to increased relative infiltration downslope and to temporal changes in rainfall intensity such that runoff generated at a specific point may not necessarily reach the lower boundary of the land slope to be collected (Sheridan et al., 2014).

Following rainfall-induced release, manure constituents (e.g., microbial indicators and pathogens) that are removed from the manure application area are either suspended in runoff or attached to eroding sediment. Since both the runoff coefficient and sediment yield decrease with an increase in manure application area, one can expect that there may be a scale effect on manure-borne bacterial transport outside of a manure-covered area. Indirect evidence in favor of this assumption is presented in the work of Harmel et al. (2010). In rural Texas, the authors demonstrated that *E. coli* concentrations consistently decreased as watershed scale increased from field- to small watershed- to river basin-scale (Harmel et al., 2010). They noted that while it was not appropriate to conclude increasing scale as the sole or major cause of decreased *E. coli* concentrations, the inverse relationship was certainly present.

Modeling

Release models

Predictive release models for manure-borne bacteria often simulate the ratio of the concentration of bacteria released, *C* (often measured in runoff, not infiltration), to the initial concentration of bacteria in manure, C_0, over time. Roodsari et al. (2005) reported relative concentrations of fecal coliforms released from cattle manure into runoff (C/C_0) to generally decrease with time. Likewise, in a release experiment where rainfall was simulated over a field plot containing cowpats that were spatially distributed to mimic natural deposition, Edwards et al. (2000) reported the highest relative concentrations of released fecal coliforms in runoff to occur at the initiation of runoff and, over time, the subsequent concentrations decreased in an exponential fashion. According to Schijven et al. (2004), released concentrations of *Cryptosporidium* and *Giardia* (oo)cysts from cattle manure were always several orders of magnitude lower than the initial concentrations in manure, decreased gradually, and then tailed off. In agreement, Guber et al. (2006) reported microbial release kinetics to change from first order to zero order after approximately one hour of simulated rainfall on plots. In general, microbial release curves have been described as having a fast initial release stage followed by a slower log-linear release stage, which suggests the release process to be dynamic and variable (Guber et al., 2006; Guber et al., 2007; Bradford and Schijven, 2002; Schijven et al., 2004). This research suggests the temporal differences in release rates may be partially attributable to the depletion of manure components and exposed surface area of manure in time.

The C_0 is a value that depends on how it is defined and used. For instance, C_0 may be represented as bacteria content g^{-1} dry weight of manure, g^{-1} wet weight of manure, ml^{-1} total volume of manure, ml^{-1} liquid content of manure, or even ml^{-1} of runoff water. The Simulator of

Transport with Runoff and Infiltration model (STWIR), which has a microbial release subroutine, defines C_0 as the bacteria mass in cm^{-3} of manure (Guber et al., 2012) or as the bacteria mass in L of slurry (Guber et al., 2011; Martinez et al., 2014). Three kinetic-based microbial release models have been previously used where C_0 was defined by the bacteria content in manure divided by the manure water content which is based on the assumption that all bacteria are located within the liquid phase of manure (Guber et al., 2006; Guber et al., 2013). Results of these three models are typically reported as the ratio of concentrations of bacteria released over time to the initial concentration in the initial release from the manure (i.e., eluate) (i.e., C/C_0). These three models, along with the manure erosion equation employed in APEX (Williams et al., 2012), can be used to simulate the dependency of microbial release on time or on rainfall depth during a rainfall event.

Like C/C_0, the cumulative number of microorganisms that are released or the number divided by the total number at time zero, M/M_0, can be explained by the same four equations, using the appropriate derivations. Here are several microbial release models, converted from simulating C/C_0 to now simulate M/M_0:

1. The exponential release dependence model that is used by Guber et al. (2006) then becomes:

$$\frac{M}{M_0} = 1 - e^{(-k_e W)} \quad (1)$$

where M is the total number of bacteria released from manure per unit area, [M]=CFU bacteria m^{-2}; M_0 is the initial number of bacteria in manure per that area, [M_0]=CFU bacteria m^{-2}; W is the total amount of rainfall per unit area, [W]=L; and k_e is the rate constant for the exponential model, [k_e] = L^{-1}.

2. The power-rational release dependence model that is used by Guber et al. (2006) then becomes:

$$\frac{M}{M_0} = 1 - \frac{1}{(1+k_p \beta W)^{\frac{1}{\beta}}} \quad (2)$$

where M is the total number of bacteria released from manure per unit area, [M]=CFU bacteria m^{-2}; M_0 is the initial number of bacteria in manure per that area, [M_0]=CFU bacteria m^{-2}; W is the total amount of rainfall per unit area, [W]=L; k_p is the rate constant for the exponential model, [k_p] = L^{-1}; and β is a dimensionless shape parameter.

Eq. (1) and (2) are illustrated in Fig. 2.4. The following transformations of these equations help to understand how they work and also to see the difference between them.

According to (1)

$$\frac{1}{M_0}\frac{dM}{dW} = k_e \frac{M_0 - M}{M_0} \quad (3)$$

and according to (2)

$$\frac{1}{M_0}\frac{dM}{dW} = k_p \left(\frac{M_0 - M}{M_0}\right)^{1+\beta} \quad (4)$$

That is, the release rate in the exponential model is proportional to the relative remaining number (M_0-M)/M_0; whereas; the release rate in the power rational case is proportional to the relative remaining number to the 1+β degree. Because the relative remaining bacteria number is always less than 1 and β>1, one can expect that for the same values of the remaining relative bacteria numbers and for k_e=k_p, the release rate based on the power rational model will be less than the release rate based on the exponential model.

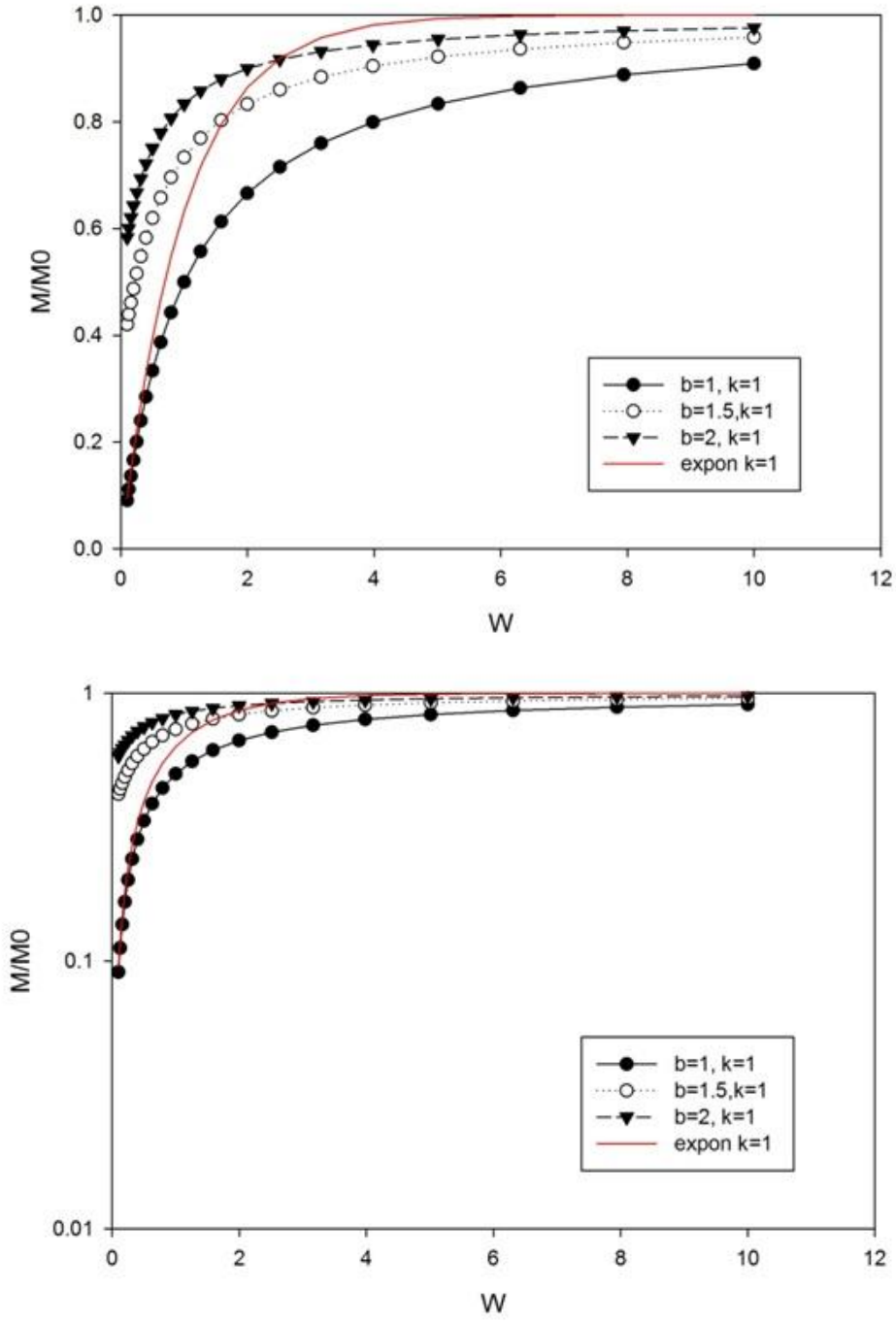

Figure 2.4 Simulations of the exponential dependence release model (Eq. 1) and power rational dependence release model (Eq. 2) on a linear scale (top) and log scale (below). The parameter values corresponding to each simulation are listed.

The next two microbial release models do not provide the asymptotic behavior that is shown in Fig. 2.4.

3. The Vadas et al. (2004) model was developed to describe inorganic phosphorus loss in runoff from surface-applied dairy, poultry, and swine manure. When adopted to microbial release, it becomes:

$$\frac{M}{M_0} = AW^n \tag{5}$$

where M is the total number of bacteria released from manure per unit area, [M]=CFU bacteria m^{-2}; M_0 is the initial number of bacteria in manure per that area, [M_0]=CFU bacteria m^{-2}; W is the total amount of rainfall per unit area, [W]=L; A is the rate constant for this model, [A] = L^{-n}; and n is a dimensionless parameter.

4. The manure erosion model in the Agricultural policy Extender model (APEX) is derived from the soil erosion model MUST (Williams et al., 2012). When adopted to microbial release, it becomes:

$$\frac{M}{M_0} = AW^{0.5} \tag{6}$$

where M is the total number of bacteria released from manure per unit area, [M]=CFU bacteria m^{-2}; M_0 is the initial total number of bacteria in manure per that area, [M_0]=CFU bacteria m^{-2}; W is the total amount of rainfall per unit area, [W]=L; and A is the rate constant for this model, [A] = $L^{-0.5}$

Recently, Guber et al. (2013) proposed the modification of his model (1) in the form:

$$\frac{M}{M_0} = E_r\left(1 - \frac{1}{(1+k_p\beta W)^{\frac{1}{\beta}}}\right) \tag{7}$$

where the multiplier, E_r, is called the parameter for "release efficiency"

In Eq. 7, as the amount of applied water grows, the total number of released bacteria approaches $E_r M_0$ and not M_0 as it would if all organisms were allowed to leave the manure. Perhaps, the *Er* parameter was developed because the value for C_0 was not properly represented.

Using release equations in field- and watershed-scale microbial fate and transport models

Equations 1, 2, 5, and 6 have been applied in field- and watershed-scale microbial fate and transport models (Benham et al., 2006; Guber et al., 2009; Kim et al., 2014; Moyer and Hyer, 2003; Williams et al., 2012) as well as in a pathogen risk assessment framework model (Whelan et al., 2014). Modeling the fate and transport of manure-borne indicator microorganisms and pathogens is critical for estimating the risk of microbial contamination throughout the environment and making appropriate management evaluations (Whelan at al., 2014; Chin et al., 2009; Benham et al, 2006). The Soil Water Assessment Tool (SWAT), which is recommended by U.S. EPA and NRCS, is a continuous-time model that was developed to help water resource managers assess hydrology, impacts of climate and management on water use, and non-point source loadings of pollution at the watershed scale (Arnold et al., 1998; Neitsch et al., 2005). Total Maximum Daily Loads (TMDLs) have been created to set regulatory pollutant limits for fecal contamination of receiving water bodies (Benham et al., 2006). Along with SWAT, the Hydrologic Simulation Program - Fortran (HSPF) is commonly used to support TMDL values for *E. coli*, enterococci, and fecal coliforms (Benham et al., 2006; Chin et al., 2009; Lawson et al., 2003; Moyer and Hyer, 2003,).

Watershed-scale models that are used to simulate the impairment of surface waters by fecal bacteria incorporate specific components of fate and transport, including microbial release. Thus, there is a necessity to correctly parameterize release in controlled experiments and be able

to estimate constants for parameters that can be used in models to simulate real-world events. In HSPF, the exponential-dependence release model (Eq. 1) is used to simulate release and a user-defined parameter sets the rate of runoff that is needed to transport 90% of the bacteria load across the land surface (Benham et al., 2006; Moyer and Hyer, 2003). In SWAT, pollutant (i.e., indicator and pathogen) loadings are estimated separately for each hydrologic response unit by using an analogue to Eq. 5 (Benham et al., 2006).

At the farm-scale, the APEX model (Williams et al., 2012) and the coupled Kinematic Runoff and Erosion Simulator/Simulator of Transport with Infiltration and Runoff model (KINEROS2/STWIR) (Kim et al., 2014; Guber et al., 2009) may be used to simulate manure-bacteria fate and transport. APEX, which is used in BMP development and evaluation, employs Eq. 6 (Williams et al., 2012), while Kim et al. (2014) developed field-scale parameters for the KINEROS2/STWIR model based on release values that generated from rainfall simulations over 144 field plots. Release is described in STWIR by the Bradford and Schijven (2002) release equation (Eq. 2) (Guber et al., 2009).

Knowledge Gaps

Factors that affect microbial release – manure application rate (Brooks et al., 2007; Drapcho, 2003), state of vegetation over which manure is applied (Dao et al., 2008; Guber et al., 2007), whether manure is surface-applied or incorporated into the soil (Forslund et al., 2011), manure type (Soupir et al., 2003; Thurston-Enriquez, 2005), and manure age (Kress and Gifford, 1984) – have been studied, but the data remains scarce, especially for pathogens, and given the variations in site-specific release conditions, multiplicity of the current data as well as new data collected on information described in the next several paragraphs would serve to advance this field. For example, aside from the longer rainfall time needed for manure to produce an effluent

as manure age increases, the implications of aging on total release and release kinetics are not well documented. It would be beneficial to study microbial release of manure with specific age times (e.g., 0, 2, 4, 8 weeks). In addition, since manure source and structure affects microbial release (Schijven et al., 2004; Hodgson et al., 2009), and this release may be affected by animal diet (e.g., grass vs. grain, or, more specifically, the contents of a mixed ratio diet), the effects of animal diet on microbial release from manure is a worthy avenue for future study.

Reports on release from surface applied manure far outnumber reports on subsurface applied manure. With continued innovation in agricultural technologies methods for effective and cost-efficient subsurface manure application (e.g., soil injection, subsurface banding, or soil incorporation via tillage), further research on the release or subsurface applied manure would be beneficial.

Compared with solid manure, the percent of FIB released from slurry is expressed by a much stronger dependency on total rainfall (Fig. 3). While the amount of published data on FIB released from liquid and solid manure is almost equal, future research should focus on solid manure because the microbial release process from this type of manure appears to be far more complex and situation-specific. Studies focusing on composted manure, aged manure, or specifically on the effect of the solid/liquid contents of manure on release would be beneficial as well.

Future studies of microbial release should be complemented by studies of release of pharmaceuticals, notably antibiotics and hormones, and microbial source tracking (MST) markers, which have become emergent contaminants. Antibiotics are administered to livestock to control animal disease and make the food supply safer by lowering the risk of bacterial transmission from animals to humans. Antibiotics deposited with animal waste have become a

growing public health concern because their presence in the environment selects for bacteria suited with corresponding antibiotic resistance genes. Antibiotic resistance genes can be acquired by mutation or horizontal gene transfer and then forwarded through intra- or inter-species horizontal gene transfer, potentially to pathogens. In addition, steroidal estrogenic hormones such as estradiol, estrone, and estriol, which are introduced to the open environment with animal waste, have been documented to adversely affect the reproductive biology of fish and other macro-vertebrates. In order to minimize the adverse effects of these emergent compounds, a better understanding of their release and transport, sorption, and degradation is needed (Hanselman et al., 2003). Furthermore, while the assessing and regulating microbial contamination in the environment with FIB and other surrogates is important, it alone does not point to the source of fecal contamination and provide an explanation of where attenuation ought to occur. MST, which aims to identify the original animal host group(s) of fecal microbes found in contaminated water, has been advanced with novel molecular biology methods, such as ribotyping, DNA fingerprinting, qPCR, length heterogeneity PCR (LH-PCR), and terminal restriction length polymorphism analysis (T-RFLP), as well as combined methods, such as, host-specific 16S rDNA that combines LH-PCR and T-RFLP (Meays et al., 2004). Specific host-associated genetic markers of microbial contaminants are used in making source attributions (Oladeinde et al., 2014). Comparative release studies using different MST markers alongside the standard FIB would improve current molecular and biochemical methods for environmental regulations. By obtaining data on the release of antibiotics, hormones, and/or MST markers from manure while extending research on the release of indicator microorganisms and pathogens, the work would serve as a double purpose that can answer complementary research questions.

Although the majority of release experiments and modeling efforts take place in laboratory or small-scale field plot settings, some researchers have attempted to model release at a relatively large scale where manure is land-applied to cover a field (Martinez et al., 2014; Guber et al., 2011). The scale effect of manure-borne bacteria release and/or transport outside of the manure application area has not been defined. However, if such a relationship exists, microbial release kinetics would be difficult to infer from the parameters developed from edge-of-field or edge-of-plot measurements, which would be dependent not only on local release kinetics per-se, but also on the conditions for released bacteria to be retained within the manure application area. An understanding of the effects of scale on release and transport interplay patterns is necessary to make field-scale inferences based upon lab-scale observations and also to improve parametization in field-scale and watershed-scale models.

Microorganisms released from animal manure may be transported with runoff or infiltration and the partitioning of release into the runoff and infiltration depends on the soil physical properties that govern these processes. Since topography controls physics of flow, it would be beneficial to determine the effects of slope on concentrations and loads of microbes released into both effluent types.

Release kinetics appear to be a two-stage process, with a fast initial release followed by a slower log-linear release (Guber et al., 2006; Guber et al., 2007; Schijven et al., 2004). However, data on the early mass release from the solid manure are lacking (Fig. 2.3). A better description of the early release of manure constituents and their initial release concentrations is needed to advance kinetic-based release models. Also, application of equations (1-2, and 5-7) to new data sets can be useful to determine the most efficient model for microbial release simulations.

Furthermore, all of the release models described in this text (Eq. 1-7) work under the assumption that microbial release is impacted by total rainfall, while there is no parameter for rainfall intensity. While rainfall intensity affects soil surface sealing (Zejun et al., 2002; Sharpley, 1985), it may similarly affect the seal formation on the surface of manure. In addition, more frequently occurring raindrops (i.e., as rainfall intensity increases) could cause sequential erosion of more manure components. If rainfall intensity does have significant impacts on microbial release and since the majority of microbes are thought to be released during the early time periods of rainfall, release that is induced during short, heavy rainfall events has probably been underestimated. Therefore, the effects of rainfall intensity on microbial release must be further investigated.

Conclusions

This review is an attempt to construct a critical analysis of microbial release from manure and animal waste. The release process is situation-specific and depends on a combination of physical, chemical, and biological factors, such as, manure source, manure consistency, manure application method and rate, manure age, vegetation, and rainfall. Kinetics for the dependency of cumulative release of manure-bacteria on total rainfall are non-linear with an initial fast stage of release that is followed by a slow stage of release. The release dependencies are situation-specific and more so for solid manure types than slurries. The effects of rainfall intensity, topography, and scale on microbial release and runoff-removal kinetics need additional investigation. Research directions that address these knowledge gaps as well as others discussed in this text will provide a better understanding of microbial release, both qualitatively and quantitatively, which will be used to ensure improvement of food safety and environmental risk assessment.

References

Alonso, J. L., Soriano, A., Carbajo, O., Alonso, J. L., Soriano, A., Carbajo, O., and Amoros, I., 1999. Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 C. Applied and Environmental Microbiology, 65(8): 3746-3749.

American Society of Agricultural Engineers, 2003. Manure production and characteristics. ASAE Standards. D384.1.

Arnold, J. G., Srinivasan, R., Muttiah, R. S., and Williams, J. R. 1998. Large area hydrologic modeling and assessment part I: Model development. Journal of American Water Resources Association, 34(1): 73-89.

Baldursson, S., and Karanis, P., 2011. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. Water Research, 45(20): 6603-6614.

Barick, R. S., Mohammed, H. O., White, M. E., and Bryant, R. B., 2003. Factors associated with the likelihood of *Giardia* spp. and C*ryptosporidium* spp. in soil from dairy farms. Journal of Dairy Science, 86(3): 784-791.

Batz, M. B., Hoffmann, S., and Morris, J. G., 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection, 75(7): 1278-1291.

Benham, B. L., Baffaut, C., Zeckorski, R. W., Mankin, K. R., Pachepsky, Y. A., Sadeghi, A. M., Brannan, K. M., Soupir, M. L., and Habersack, M. J., 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. Transactions of ASABE, 49(4): 987-1002.

Berry, E. D., and Miller, D. N., 2005. Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. Journal of Environmental Quality, 34(2): 656-663.

Bittman, S., van Vliet, L. J. P., Kowalenko, C. G., McGinn, S., Hunt, D. E., and Bounaix, F., 2005. Surface-banding liquid manure over aeration slots. Agronomy Journal, 97(5): 1304.

Blaustein, R. A., Pachepsky, Y., Hill, R. L., Shelton, D. R., and Whelan, G., 2013. *Escherichia coli* survival in waters: temperature dependence. Water Research, 47: 569-578.

Borst, M., and Selvakumar, A., 2003. Particle-associated microorganisms in stormwater runoff. Water Research, 37(1): 215-223.

Bradford, S. A., and Schijven, J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environmental Science & Technology, 36(18): 3916-3923.

Brooks, J. P., Adeli, A., Read, J. J., and McLaughlin, M. R., 2009. Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. Journal of Environmental Quality, 38(1): 218-229.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., and Harwood, V. J., 2012. Enterococci in the environment. Microbiology and Molecular Biology Reviews : MMBR, 76(4): 685-706.

Cardoso, F., Shelton, D., Sadeghi, A., Shirmohammadi, A., Pachepsky, Y., and Dulaney, W., 2012. Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. Journal of Environmental Management, 110: 1-7.

Cerdan, O., Le Bissonnais, Y., Govers, G., Lecomte, V., van Oost, K., Couturier, King, C., and Dubreuil, N. , 2004. Scale effect on runoff from experimental plots to catchments in agricultural areas in Normandy. Journal of Hydrology, 299: 4-14.

Chin, D. A., Sakura-Lemessy, D., Bosch, D. D., and Gay, P. A., 2009. Watershed-scale fate and transport of bacteria, Transactions of ASABE, 52(1): 145-154.

Costán-Longares, A., Montemayor, M., Payán, A., Méndez, J., Jofre, J., Mujeriego, R., and Lucena, F., 2008. Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. Water Research, 42(17): 4439-4448.

Crane, S. R., and Moore, J. A., 1984. Bacterial pollution of groundwater: A review. Water, Air, and Soil Pollution, 22: 67-83.

Critzer, F. J., and Doyle, M. P., 2010. Microbial ecology of foodborne pathogens associated with produce. Current Opinion in Biotechnology, 21: 125-130.

Curriero, F. C., Patz, J. A., Rose, J. B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the united states, 1948-1994. American Journal of Public Health, 91(8): 1194-1199.

Dao, T. H., Guber, A. K., Sadeghi, A. M., Karns, J. S., Shelton, D. P., and Pachepsky, Y. A., 2008. Loss of bioactive phosphorus and enteric bacteria. Soil Science, 2 (5): 511-521.

Delmas, M., Pak, L. T., Cerdan, O., Souchère, V., Le Bissonnais, Y., Couturier, A., and Sorel, L., 2012. Erosion and sediment budget across scale: a case study in a catchment of the European loess belt. Journal of Hydrology, 420-421: 255-263.

Dufour A. P., and Ballantine P., 1986. Bacteriological ambient water quality criteria for marine and freshwater recreational waters. US Environmental Protection Agency: EPA 440/445-484-002.

Drapcho, C. M., A. K. B., 2003. Fecal coliform concentration in runoff from fields with applied dairy manure. Master in Science Thesis, Louisiana State University, Baton Rouge, LA.

Easton, J. H., Gauthier, J. J., Lalor, M. M., and Pitt, R. E., 2005. Die-off of pathogenic *E. coli* O157:H7 in sewage contaminated waters. Journal of the American Water Resources Association, 41(5): 1187-1193.

Edberg S.C., Rice E.W., Karlin R.J., Allen M.J., 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology Symposium Supplement. 88:106S-116S.

Edwards, D. R., Daniel, T. C., and Moore Jr., P. A., 1996. Vegetative filter strip design for grassed areas treated with animal manures. Applied Engineering in Agriculture, 12(1): 31-38.

Edwards, D. R., Larson, B. T., and Lim, T. T., 2000. Runoff nutrient and fecal coliform content from cattle manure application to fescue plots, 36(4): 711-721.

Fayer, R., and Trout, J. M., 2005. Zoonotic protists in the marine environment. In: Oceans and Health: Pathogens in Marine Environment. Belkin, S. S., and Cowell, R. (Eds), Kluwer Academic Publishers, Dordrecht, pp. 143-163.

Foppen, J. W., van Herwerden, M., and Schijven, J., 2007. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology, 93(1-4): 236-54.

Forslund, A., Markussen, B., Toenner-Klank, L., Bech, T. B., Jacobsen, O. S., and Dalsgaard, A., 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a *Salmonella enterica* Serovar Typhimurium Bacteriophage through intact soil cores following surface application and injection of slurry. Applied and Environmental Microbiology, 77(22): 8129-8138.

Fox, G. A., Matlock, E. M., Guzman, J. A., Sahoo, D., and Stunkel, K. B., 2009. *Escherichia coli* load reduction from runoff by vegetative filter strips: a laboratory-scale study. Journal of Environmental Quality, 40(3): 980-988.

Franz, E., Semenov, A. V, Termorshuizen, A. J., de Vos, O. J., Bokhorst, J. G., and van Bruggen, A. H. C., 2008. Manure-amended soil characteristics affecting the survival of *E. coli* O157:H7 in 36 Dutch soils. Environmental Microbiology, 10(2): 313-327.

Freschet, G., Masse, D., Hien, E., Sall, S., and Chotte, J., 2008. Long-term changes in organic matter and microbial properties resulting from manuring practices in an arid cultivated soil in Burkina Faso. Agriculture, Ecosystems & Environment, 123(1-3): 175-184.

Fries, J. S., Characklis, G. W., and Noble, R. T. Attachment of fecal indicator bacteria to particle suspensions in the Neuse River Estuary, NC. Journal of Environmental Engineering-ASCE, 132(10): 1338-1345.

Gagliardi, J. V., and Karns, J. S., 2000. Leaching of *escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Applied Environmental Microbiology, 66(3): 887-883.

Gerba, C. P., and Smith, J. E., 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. Journal of Environmental Quality, 34(1): 42-48.

Gomi, T., Sidle, R. C., Miyata, S., Kosugi, K., and Onda, Y., 2008. Dynamic runoff connectivity of overland flow on steep forested hillslopes: scale effects and runoff transfer. Water Resources Research, 44(8): 1-16.

Guan, T. Y., Holley, R. A., H. O., and H. E. O., 2003. Pathogen survival in swine manure environments and transmission of human enteric illness — a review. Journal of Environmental Quality, 32: 383-392.

Guber, A. K., Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., and Sikora, L. J., 2006. Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and Environmental Microbiology, 72(12): 7531-7539.

Guber, A. K., Karns, J. S., Pachepsky, Y. A., Sadeghi, A. M., Van Kessel, J. S., and Dao, T. H., 2007. Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. Letters in Applied Microbiology, 44(2): 161-167.

Guber, A. K., Pachepsky, Y. A., Shelton, D. R., and Yu, O., 2009. Association of fecal coliforms with soil aggregates. Soil Science, 174(10): 543-548.

Guber, A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L., 2011. Uncertainty in modelling of faecal coliform overland transport associated with manure application in Maryland. Hydrological Processes, 25(15): 2393-2404.

Guber., A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L. 2012. STWIR, a microorganism transport with infiltration and runoff add-on module for the KINEROS2 runoff and erosion model documentation and user manual. User Manual. USDA-ARS. Beltsville, MD, USA.

Guber, A. K., Pachepsky, Y. A., Dao, T. H., Shelton, D. R., and Sadeghi, A. M., 2013. Evaluating manure release parameters for nonpoint contaminant transport model KINEROS2/STWIR. Ecological Modelling, 263: 126-138.

Hamilton, D. W., 2011. Consistency of manure/water mixtures. Stillwater, OK: Oklahoma Cooperative Extension Service. OSU Factsheet BAE 1751.

Himathongkham, S., Bahari, S., Riemann, H., and Cliver, D., 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiology Letters, 178(2): 251-257.

Hodgson, C. J., Bulmer, N., Chadwick, D. R., Oliver, D. M., Heathwaite, A. L., Fish, R. D., and Winter, M., 2009. Establishing relative release kinetics of faecal indicator organisms from different faecal matrices. Letters in Applied Microbiology, 49(1): 124-130.

Hoffmann, S., Batz, M. B., and Morris, J. G., 2012. Annual cost of illness and quality-adjusted life year losses in the united states due to 14 foodborne pathogens. Journal of Food Protection, 75(7): 1292-1302.

Howell, J. M., Coyne, M. S., and Cornelius, P. L., 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. Journal of Environment Quality, 25(6): 1216.

Ibekwe, A. M., Ma, J., Crowley, D. E., Yang, C., Johnson, A. M., Petrossian, T. C., and Lum, P. Y., 2014. Topological data analysis of *Escherichia coli* O157:H7 and non-O157 survival in soils. Frontiers in Cellular and Infection Microbiology, doi: 10.3389/fcimb.2014.00122

Jamieson, R. C., Gordon, R. J., Sharples, K. E., Stratton, G. W., and Madani, A., 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering, 44: 1-9.

Jenkins, M. B., Bowman, D. D., Fogarty, E. A., and Ghiorse, W. C, 2002. *Cryptosporidium parvum* oocyst inactivation in three soil types at various temperatures and water potentials. Soil Biology and Biochemistry, 34(8): 1101-1109.

Jiang, X., Morgan, J., Doyle, M. P., Jiang, X., Morgan, J., and Doyle, M. P., 2002. Fate of *Escherichia coli* O157 : H7 in manure-amended soil. Applied and Environmental Microbiology, 68(5): 2605-2609.

Khaleel, R., Reddy, K. R., and Overcash, M. R., 1980. Transport of potential pollutants in runoff water from land areas receiving animal wastes: a review. Water Research, 14(5): 421-436.

Kiefer, L. A., Shelton, D. R., Pachepsky, Y., Blaustein, R., & Santin-Duran, M., 2012. Persistence of *Escherichia coli* introduced into streambed sediments with goose, deer and bovine animal waste. Letters in Applied Microbiology: 345-353.

Kim, J., Luo, F., and Jiang, X., 2009. Factors impacting the regrowth of E*scherichia coli* O157:H7 in dairy manure compost. Journal of Food Protection, 72(7): 1576-1584.

Kidron, G. J., 2011. Runoff generation and sediment yield on homogeneous dune slopes: scale effect and implications for analysis. Earth Surface Processes and Landforms, 36(13): 1809-1824.

Kim, K., Whelan, G., Purucker, S. T., Bohrmann, T. F., Cyterski, M. J., Molina, M., Gu, Y., Pachepsky, Y., Guber, A., and Franklin, D. H., 2014. Rainfall-runoff model parameter estimation and uncertainty evaluation on small plots. Hydrological Processes, 28(20): 5220-5235.

Koneswaran, G., and Nierenberg, D., 2008. Global farm animal production and global warming: impacting and mitigating climate change. Environmental Health Perspectives, 116(5): 578-582.

Kouznetsov, M. Y., Roodsari, R., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Shirmohammadi, A., and Starr, J. L., 2007. Modeling manure-borne bromide and fecal coliform transport with runoff and infiltration at a hillslope. Journal of Environmental Management, 84(3): 336-346.

Kramers, G., Holden, N. M., Brennan, F., Green, S., and Richards, K. G., 2012. Water content and soil type effects on accelerated leaching after slurry application. Vadose Zone Journal, 11(1). doi:10.2136/vzj2011.0059.

Kress, M., and Cifford, G. F., 1984. Fecal coliform release from cattle fecal deposits. Water Resources Bulletin, 20(1): 61-66.

Kudva, I. T., Blanch, K., and Hovde, C. J., 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Applied and Environmental Microbiology, 64(9): 3166-3174.

Lawson, L., 2003. HSPF Model Calibration and Verification for Bacteria TMDLs. Guidance Memo No. 03-2012, Commonwealth of Virginia, Department of Environmental Quality.

Lim, T. T., Edwards, D. R., Workman, S. R., Larson, B. T., and Dunn, L., 1998. Vegetated filter strip removal of cattle manure constituents in runoff. Transactions of ASAE, 41(5): 1375-1381.

Ling, T. Y., Jong, H. J., Apun, K., and Sulaiman, W. H. W., 2009. Quantifying *Escherichia coli* release from soil under high-intensity rainfall. Transactions of ASABE, 52(3): 785-792.

Lombard, N., Prestat, E., Dirk van Elsas, J., and Simonet, P., 2011. Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78: 31-49.

Martinez, G., Pachepsky, Y. A., Shelton, D. R., Whelan, G., Zepp, R., Molina, M., and Panhorst, K., 2013. Using the Q_{10} model to simulate *E. coli* survival in cowpats on grazing lands. Environment International, 54: 1-10.

Martinez, G., Pachepsky, Y. A., Whelan, G., Yakirevich, A. M., Guber, A., and Gish, T. J., 2014. Rainfall-induced fecal indicator organisms transport from manured fields: Model sensitivity analysis. Environment International, 63: 121-129.

Mawdsley, J. L., Bardgett, R. D., Merry, R. J., and Pain, B. F., 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology, 2: 1-15.

McDaniel, R. L., Soupir, M. L., Tuttle, R. B., and Cervantes, A. E., 2013. Release, dispersion, and resuspension of *Escherichia coli* from direct fecal deposits under controlled flows. Journal of the American Water Resources Association, 49(2): 319-327.

McDowell, R. W., Muirhead, R. W., and Monaghan, R. M., 2006. Nutrient, sediment, and bacterial losses in overland flow from pasture and cropping soils following cattle dung deposition. Communications in Soil Science and Plant Analysis, 37(1-2): 93-108.

Meays, C. L., Broersma, K., Nordin, R., and Mazumder, A., 2004. Source tracking fecal bacteria in water: A critical review of current methods. Journal of Environmental Management, 73(1): 71-9.

Mishra, A., Benham, B. L., and Mostaghimi, S., 2008. Bacterial transport from agricultural lands fertilized with animal manure. Water, Air, and Soil Pollution, 189(1-4): 127-134.

Molina, M., 2005. Temporal and spatial variability of fecal indicator bacteria: Implications for the application of MST methodologies to differentiate sources of fecal contamination. EPA/600/R-05/107.

Moriarty, E. M., Mackenzie, M. L., Karki, N., and Sinton, L. W., 2011. Survival of *Escherichia coli*, enterococci, and *Campylobacter* spp. in sheep feces on pastures. Applied and Environmental Microbiology, 77(5): 1797-1803.

Mosaddeghi, M. R., Mahboubi, A. A., Zandsalimi, S., and Unc, A., 2009. Influence of organic waste type and soil structure on the bacterial filtration rates in unsaturated intact soil columns. Journal of Environmental Management, 90(2): 730-739.

Moyer, D. L., and Hyer, K. E., 2003. Use of the hydrological simulation program-FORTRAN and bacterial source tracking for development of fecal coliform total maximum daily load (TMDL) for Blacks Run, Rockingham County, Virginia. U.S. Department of the Interior U.S. Geological Survey, Water-resources Investigations Report 03-4161.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2005. Erosion and subsequent transport state of *Escherichia coli* from cowpats. Applied and Environmental Microbiology, 71(6): 2875-2879.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2006a. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Letters in Applied Microbiology, 42(2): 83-87.

Muirhead, R. W., Collins, R. P., Bremer, P. J., 2006b. Interaction of *Escherichia coli* and Soil Particles in Runoff. Applied and Environmental Microbiology, 72(5): 3406-3411.

Muirhead, R. W., and Littlejohn, R. P., 2009. Die-off of *Escherichia coli* in intact and disrupted cowpats. Soil Use and Management, 25(4): 389-394.

Muirhead, R. W., and Monaghan, R. M., 2012. A two reservoir model to predict *Escherichia coli losses* to water from pastures grazed by dairy cows. Environment International, 40: 8-14.

Oladeinde, A., Bohrmann, T., Wong, K., Purucker, S. T., Bradshaw, K., Brown, R., Snyder, B., and Molina, M, 2014. Decay of fecal indicator bacterial populations and bovine-associated source-tracking markers in freshly deposited cow pats. Applied and Environmental Microbiology, 80(1): 110-118.

Oliver, D. M., Heathwaite, L., Haygarth, P. M., and Clegg, C. D., 2005. Transfer of *Escherichia coli* to water from drained and undrained grassland after grazing. Journal of Environmental Quality, 34(3): 918-925.

Pachepsky, Y. A., Sadeghi, A. M., Bradford, S. A., Shelton, D. R., Guber, A. K., and Dao, T., 2006. Transport and fate of manure-borne pathogens: Modeling perspective. Agricultural Water Management, 86(1-2): 81-92.

Pachepsky, Y. A., Yu, O., Karns, J. S., Shelton, D. R., Guber, A. K., & Kessel, J. S. Van., 2008. Strain-dependent variations in attachment of *E. coli* to soil particles of different sizes. International Agrophysics, 22: 61–66.

Pachepsky, Y. A., Guber, A. K., Shelton, D. R., and McCarty, G. W., 2009. Size distributions of manure particles released under simulated rainfall. Journal of Environmental Management, 90(3): 1365-1369.

Pachepsky, Y. A., and Shelton, D. R., 2011. *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. Critical Reviews in Environmental Science and Technology, 41(12): 1067-1110.

Pachepsky, Y. A., Blaustein, R. A., Whelan, G., and Shelton, D. R., 2014. Comparing temperature effects on *Escherichia coli*, *Salmonella*, and *Enterococcus* survival in surface waters. Letters in Applied Microbiology, 59(3): 278-283.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V, Braden, C. R., Angulo, F. J., and Griffin, P. M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerging Infectious Diseases, 19(3): 407-415.

Panhorst, K. A., 2002. Estimating bacterial loadings to surface waters from agricultural watersheds. Master in Science Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Parajuli, P., 2007. SWAT bacteria sub-model evaluation and application. PhD Dissertation, Kansas State University, Manhattan, KS.

Patni, N. K., Toxopeus, H. R., and Jui, P. Y., 1985. Bacterial quality of runoff from manured and non-manured cropland. Transactions of ASAE, 28(6): 1871-1878.

Pell, A. N., 1997. Manure and microbes: public and animal health problem? Journal of Dairy Science, 80(10), 2673-2681.

Raclot, D., Le Bissonnais, Y., Louchart, X., Andrieux, P., Moussa, R., and Voltz, M., 2009. Soil tillage and scale effects on erosion from fields to catchment in a Mediterranean vineyard area. Agriculture, Ecosystems & Environment, 134(3-4), 201-210.

Roodsari, R. M., Shelton, D. R., Shirmohammadi, A., Pachepsky, Y. A., Sadeghi, A. M., and Starr, J. L., 2005. Fecal coliform transport as affected by surface condition. Transactions of ASAE, 48(3), 1055-1061.

Rosen, B., 2000. Waterborne pathogens in agricultural watersheds. USDA Watershed Science Institute-Technical Note 2. University of Vermont, Burlington.

Savichtcheva, O., and Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research, 40(13), 2463-2476.

Schijven, J. F., Bradford, S. A., and Yang, S., 2004. Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: Physical factors. Journal of Environmental Quality, 33(4), 1499-1508.

Sharples, K. E., Stratton, G. W., Madani, S. A., Gordon, R. J., and Patterson, G., 2004. The survival of *E. coli* in agricultural soil treated with dairy manure. 2004 ASAE/CSAE Annual International Meeting, Ottowa, Canada.

Sharpley, A. N., 1985. Depth of surface soil-runoff Interaction as affected by rainfall, soil slope, and management. Soil Science Society of America Journal, 49: 1010-1015.

Sheldrick, W., Syers, J. K., and Lingard, J., 2003. Contribution of livestock excreta to nutrient balances. Nutrient Cycling in Agroecosystems, 119-131.

Sheridan, G. J., Noske, P. J., Lane, P. N. J., Jones, O. D., and Sherwin, C. B., 2014. A simple two-parameter model for scaling hillslope surface runoff. Earth Surface Processes and Landforms, 39(8): 1049-1061.

Sinclair, R. G., Rose, J. B., Hashsham, S. A., Gerba, C. P., and Haas, C. N., 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Applied and Environmental Microbiology, 78(6): 1969-1977.

Sinton, L. W., Braithwaite, R. R., Hall, C. H., and Mackenzie, M. L., 2007. Survival of indicator and pathogenic bacteria in bovine feces on pasture. Applied and Environmental Microbiology, 73(24): 7917-7925.

Sistani, K. R., Bolster, C. H., Way, T. R., Tobert, H. A., Pote, D. H., and Watts, D. B., 2009. Influence of poultry litter application methods on the longevity of nutrient and *E. coli* in runoff from tall fescue pasture. Water, Air, and Soil Pollution, 206: 3-12.

Smith, M. S., Thomas, G. W., White, R. E., and Ritonga, D., 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. Journal of environmental quality, 14(1): 87-91.

Sobeck, D. C., and Higgins, M. J., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Research, 36(3): 527-538.

Sobsey, M. D., Khatib, L. A., Hill, V. R., Alocilja, E., and Pillai, S., 2006. Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. Animal Agriculture and the Environment: National Center for Manure and Animal Waste White Papers, Rice, J. M, Caldwell, D. F., Humenik, F., J. (eds.) ASABE Pub. St. Joseph, Michigan. pp. 609-665.

Soupir, M., Tech, V., Hall, S., Mostaghimi, S., and Alphin, E. F., 2003. Bacteria release and transport from livestock manure applied to pastureland. 2003 ASAE Annual International Meeting. Las Vegas, NV.

Soupir, M. L., Mostaghimi, S., Yagow, E. R., Hagedorn, C., and Vaughan, D. H., 2006. Transport Of fecal bacteria from poultry litter and cattle manures applied to pastureland. Water, Air, and Soil Pollution, 169(1-4): 125-136.

Soupir, M. L., Mostaghimi, S., and Dillaha, T., 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. Journal of Environmental Quality, 39(3), 1019-1027.

Springer, E. P., Gifford, G. F., and Windham, M. P., 1983. Fecal coliform release studies and development of a preliminary nonpoint source transport model for indicator bacteria. Utah State University Reports, Paper 69.

Srivastava, P., Edwards, D. R., Daniel, T. C., and Costello, T. A., 1996. Performance of vegetative filter strips with varying pollutant source and filter strip lengths. Transactions of ASAE, 39(6): 2231-2239.

Stevik, T. K., Aa, K., Ausland, G., Hanssen, J. F., 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research, 38: 1355-1367.

Stoddard, C. S., Coyne, M. S., and Grove, J. H., 1998. Fecal bacteria survival and infiltration through a shallow agricultural soil: timing and tillage effects. Journal of Environmental Quality, 27(6): 1516.

Tauxe, R. V., 2002. Emerging foodborne pathogens. International Journal of Food Microbiology, 78: 31-41.

Tate, K. W., Atwill, E. R., George, M. R., McDougald, M. K. and Larsen, R. E., 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. Journal of Range Management, 53(3), 295-299.

Thelin, R., and Gifford, G. F., 1983. Fecal coliform release patterns from fecal material of cattle. Journal of Environment Quality, 12(1), 57-63.

Thurston-Enriquez, J. A., Gilley, J. E., and Eghball, B., 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. Journal of Water and Health, 3(2), 157-171.

Tyrrel, S. F., and Quinton, J. N., 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. Journal of Applied Microbiology, 94 Suppl: 87S-93S.

Unc, A., and Goss, M. J., 2004. Transport of bacteria from manure and protection of water resources. Applied Soil Ecology, 25(1): 1-18.

U.S. CDC, 2012. General Information: *E. coli* (*Escherichia coli*). Atlanta, GA, USA. < http://www.cdc.gov/ecoli/general/index.html>.

U.S. EPA, 1984. Health effects criteria for fresh recreational waters. EPA 600/1-84-004, Washington, D.C.

U.S. EPA, 1986. Ambient water quality criteria for bacteria. EPA 440/5-84-002, Washington, D.C.

U.S. EPA, 2009. National water quality inventory: report to Congress, 2004 reporting cycle. EPA/841/R-08/001, Washington, D.C.

U.S. EPA., 2012. Water: Monitoring assessment. 5.11 Fecal bacteria. <http://water.epa.gov/type/rsl/monitoring/vms511.cfm>.

Vadas, P. A., Kleinman, P. J. A., Sharpley, A. N., 2004. A simple method to predict dissolved phosphorus in runoff from surface-applied manures. Journal of Environmental Quality, 33: 749-756.

Vadas, P. A., Good, L. W., Moore, P. A., and Widman, N., 2009. Estimating phosphorus loss in runoff from manure and fertilizer for a phosphorus loss quantification tool. Journal of Environmental Quality, 38(4): 1645-1653.

Van de Giesen, N., Stomph, T., Ajayi, E. A., and Bagayoko, F., 2011. Scale effects in hortonian surface runoff on agricultural slopes in west africa: field data and models. Agriculture, Ecosystems & Environment, 142(1-2): 95-101.

Van Kessel, J. S., Pachepsky, Y. A., Shelton, D. R., and Karns, J. S., 2007. Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. Journal of Applied Microbiology, 103(4), 1122-1127.

van Veen, J. A., van Overbeek, L. S., and van Elsas, J. D., 1997. Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews, 61(2): 121-35.

Wang, L., Mankin, K. R., and Marchin, G. L., 2004. Survival of fecal bacteria in dairy cow manure. Transactions of ASAE, 47(4): 1239-1246.

Wang, L., Xu, S., and Li, J., 2011. Effects of phosphate on the transport of *Escherichia coli* O157:H7 in saturated quartz sand. Environmental Science & Technology, 45(22): 9566-9573.

Whelan, G., Kim, K., Pelton, M. A., Soller, J. A., Castleton, K. J., Molina, M., Pachepsky, Y., Zepp, R., 2014. An integrated environmental modeling framework for performing quantitative microbial risk assessments. Environmental Modelling & Software, 55: 77-91.

Williams, A. P., Avery, L. M., Killham, K., and Jones, D. L., 2005. Persistence of *Escherichia coli* O157 on farm surfaces under different environmental conditions. Journal of Applied Microbiology, 98(5): 1075-1083.

Williams, J. R., Izzaurralde, R. C., and Steglich, E. M., 2012. Agricultural Policy/Environmental Extender Model Theoretical Documentation. Version 0806. AgriLife Research Texas A&M System.

Wu, L., Gao, B., Muñoz-Carpena, R., and Pachepsky, Y. A., 2012. Single collector attachment efficiency of colloid capture by a cylindrical collector in laminar overland flow. Environmental Science & Technology, 46(16): 8878-8886.

Wu, L., Muñoz-Carpena, R., Gao, B., Yang, W., and Pachepsky, Y. A., 2014. Colloid filtration in surface dense vegetation: experimental results and theoretical predictions. Environmental Science & Technology, 48(7): 3883-3890.

Zejun, T., Tingwu, L., Qingwen, Z., and Jun, Z., 2002. The sealing process and crust formation at soil surface under the impacts of raindrops and polyacrylamide. 12th ISCO Conference, Beijing, China. pp 456-462.

Zhang, Q., Mankin, K. R., and Erickson, L. E., 2001. Modeling transport of *Cryptosporidium parvum* oocysts in overland flow. Proceedings of the 2001 Conference on Environmental Research: 32-38.

Chapter 3 – Impacts of Rainfall Intensity and Land Slope on the Release of Fecal Indicator Bacteria from Dairy Manure to Runoff and Infiltration

Abstract

Simulating the rainfall-induced release of manure-borne indicator microorganisms and other surrogates of fecal contamination is an essential component to microbial fate and transport modeling. The objectives of this work were to determine the effects of rainfall intensity and land slope on the release of *Escherichia coli*, enterococci, total coliforms, and chloride ion from dairy cattle manure in partitioning boxes. The partitioning boxes were designed to have manure applied on a mesh-covered frame (70 x 70 cm), and to have both runoff and infiltration collected from troughs above and below the mesh, respectively. Three levels of rainfall intensity (3, 6, and 9 cm hr^{-1}) and two levels of land surface slope (5% and 20%) were used and runoff and infiltration samples were taken at gradually increasing time intervals for one hour. The 'time-release' and 'rainfall depth-release' data were fit with the one-parametric exponential release model and the two-parametric power-rational release model. Concentrations of bacteria in the initial effluent were approximately one order of magnitude lower than their concentrations in the manure. For most release events, the regression of concentrations in synchronous runoff and infiltrate did not significantly differ from a one-to-one relationship. The microbial release kinetics began with a precipitous log-linear increase in cumulative numer of bacteria released during, approximately, the first 1 cm of rainfall, which was followed by a much slower release for the remainder of rainfall. Release kinetics for the three bacteria groups/species did not significantly differ. The rate-constant parameters in both models were substantially affected by

rainfall intensity when cumulative release was a function of time, but not when it was a function of rainfall depth. While the land slope did not significantly impact microbial release from manure, it did positively affect the partitioning of release into runoff. Based on the root-mean-squared-error and the Akaike information criterion for each model-fit, the power-rational dependence model is recommended for simulating the total number of bacteria released from manure as a function of rainfall depth. These results may be used to advance parameterization of microbial release models that are critical for risk assessment of microbial contamination in the environment.

Introduction

During rainfall and irrigation events on lands with surface-applied manure, the enteric bacteria and protozoa are released from their fecal source into a suspension that enters surface runoff and/or infiltrates the underlying soil. Released microorganisms are carried with overland and subsurface flow and can contaminate water sources that are used for recreational swimming/bathing, irrigation of produce, dissolution of agricultural chemicals for spreading, prewashing fruits and vegetables, direct human consumption, production of shellfish in aquaculture, and other human activities. Release of manure constituents occurs as fecal material becomes suspended and eluted during precipitation, yet it is not really known how the content of the suspension is formed. Conceptually, one or more of the following occurs during release: (a) incoming rain suspends manure constituents and then releases microbes by sloughing off and eroding of layers from the manure surface, (b) an internal mixing process occurs during manure absorbtion of rainwater, followed by release of microbes in a diluted manure solution, or (c) the initial manure solution is removed for the manure-matrix and the release of diluted, mixed components including microbes occurs. Released material may exit the manure matrix via water

flowing over the manure surface or infiltrating through the base of the matrix, accumulating microorganisms along the way. The actual release process probably occurs via some sort of a combination of the proposed processes.

Microbial release from animal waste is affected by manure application rate (Brooks et al., 2007; Drapcho, 2003), the living-state of vegetation where manure is applied (Dao et al., 2008; Guber et al., 2007), whether manure is surface-applied or incorporated into the soil (Forslund et al., 2011), manure type (Soupir et al., 2003; Thurston-Enriquez, 2005), and manure age (Kress and Gifford, 1984). Precipitation variability impacts microbial release as well (Shijven et al., 2004; Thelin and Gifford, 1983). Further research on these factors as well as the effects of others, including rainfall intensity and longer duration rainfalls have been recommended (Ling et al., 2009).

Microbial release kinetics have been described as having a fast initial release followed by a slower log-linear release (Bradford and Schijven, 2002; Guber et al., 2006; Guber et al., 2007; Schijven et al., 2004). Temporal differences in release-rate may be partially attributable to the depletion of manure components and exposed surface area in time. Predictive release models for manure-borne bacteria often simulate the ratio of the concentration of bacteria, C, released (often measured in runoff, not infiltration) to the initial concentration of bacteria in manure, C_0, as a function of time or rainfall depth. Like C/C_0, the cumulative number of microbes released as a fraction of the initial number of microorganisms, M/M_0, may be simulated by the same release models, after implementing the appropriate transformations. While M_0 can be measured C_0 is a subjective variable that depends on how it is defined and used. For instance, C_0 may be represented as bacteria content g^{-1} dry weight of manure, g^{-1} wet weight of manure, ml^{-1} total volume of manure, ml^{-1} liquid content of manure, or even ml^{-1} of runoff water. Two kinetic-

based microbial release models used by Guber et al. (2006) to simulate fecal coliform release from bovine slurry – the one-parametric exponential model and the two-parametric power rational model – had been operated under the assumption that manure-bacteria were concentrated into the liquid portion of manure, so C_0 was defined by the bacteria content in manure divided by the manure water content. Results of these models were reported as the concentration of bacteria released over time to the concentration in the initial release from the manure (i.e., eluate) (i.e., C/C_0) (Guber et al., 2006). The assumption that the content of bacteria in manure divided by the manure water content (i.e., the effective concentration in manure) corresponds to the concentration of bacteria in the initial portion of released solution coming from solid manure types has not yet been verified for solid manure types. Furthermore, compared with microbial release from liquid-based manures and slurry, that from solid manures (e.g., farmyard manure, litter) has a much weaker correlation with total rainfall and it appears to be a more complex, situation-specific process (Chapter 2). Data on C_0 and microbial release kinetics for release from solid manure type (e.g., dairy cattle manure from a CAFO) is lacking and would be beneficial to collect.

Rainfall has been assumed to be a key determinant in the fate and transport of pathogenic organisms. The majority of waterborne disease outbreaks in the United States from 1948-1994 were preceded by extreme precipitation events above the 90^{th} percentile ($p=0.002$) (Curriero et al., 2001). Rainfall intensity affects the total release of microorganisms from manure by providing more rainfall per unit time (Kress and Gifford, 1984; Schijven et al., 2004) as does rainfall duration (Ling et al., 2009; Thelin and Gifford, 1983). However, the effect of rainfall intensity on microbial release kinetics is unknown. Rainfall causes compaction, slackening, detachment, and deposition of soil, all of which are actions that contribute to the formation of a

seal and potential crust that significantly reduces infiltration and increases surface runoff (Zejun et al., 2002). In theory, similar to a soil surface seal that is induced by rainfall, rainfall may also re-orient amorphous manure material to form a surface seal, which would impact microbial release by protecting microbes that are located under the manure surface from release. For example, in a laboratory study by Schijven et al. (2004), *Cryptosporidium* release efficiencies from cattle manure were greater in treatments that received drip irrigation compared to mist irrigation, and the greater the application rate of water had a positive impact on microbial release. The impact energy from larger, more frequently occuring droplets was attributed to their results, suggesting that increases in rainfall intensity on a farm should increase microbial release from manure (Schijven et al., 2004). If rainfall intensity were to have a significant, positive effect on microbial release from manure, then the microbes that are released during short, intensive surges of rainfall are probably underestimated by current fate and transport models.

A better description of C_0 and the early release of microorganisms from solid manure as well as a clarified understanding of the effects of rainfall intensity on manure constituent release are needed to advance kinetic-based microbial release models so that their formulations do not oversimplify bacteria release which could lead to erroneous results. The objectives of this work were: 1) to compare the contents of *E. coli*, enterococci, total coliforms, and chloride ion within dairy cattle farmyard manure collected from a Concentrated Animal Feeding Operation (CAFO) to the concentrations of these components that are released into initial runoff and infiltration, 2) to model the dependency of the relative release numbers of microorganisms M/M_0 of these manure-constituents in response to time and rainfall depth.

Methods

Partitioning boxes

A controlled-intensity rainfall simulator was used to induce the release of manure-borne microorganisms, as well as other manure constituents, from dairy cattle manure in partitioning boxes. The partitioning boxes (70 x 70 cm) were made of wood and consisted of three components – a top support frame, a middle frame covered with nylon mesh with 185-μm openings (Component Supply Co./SKU Solutions, Fort Meade, FL), and a lower frame with an impervious, plastic-covered base. The three layers were secured together with C-clamps to create an upper manure application section and a lower leachate collection section. The boxes were elevated with 5-cm wooden feet at the corners so that a trough could be placed below the opening at the front of the lower section to collect leachate. A second trough was installed on each box below an opening at the front of the manure application section to collect surface runoff. In summary, the partitioning boxes were designed to have manure applied on a mesh-covered frame and to have both runoff and leachate collected from PVC troughs at the end of the mesh frame and below the base, respectively, during a simulated rainfall event (Fig. 3.1).

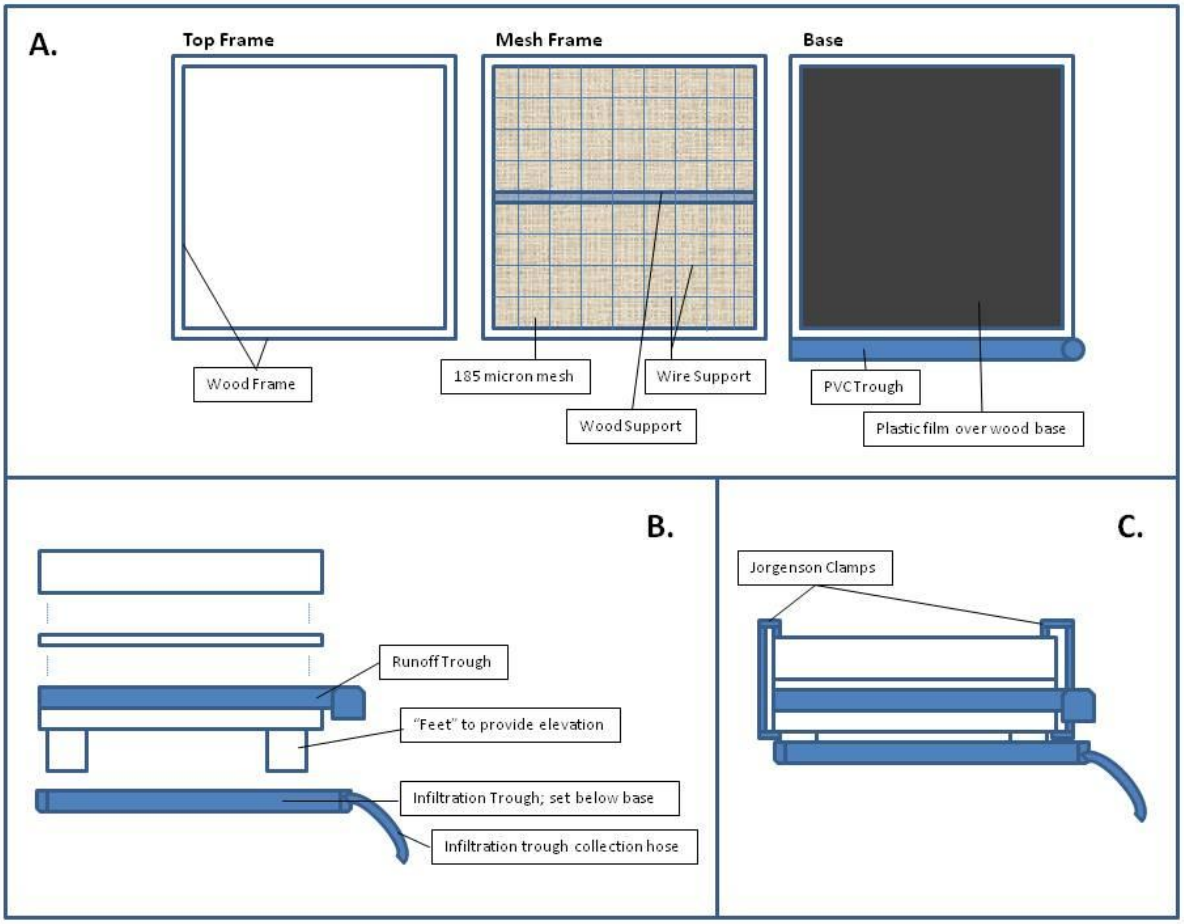

Figure 3.1 Partitioning box design. The inside dimensions are 70 x 70 cm. A – Overhead view of disassembled layers; B – Frontal view showing the assembly process; C – Frontal view of assembled box.

Manure and rainwater composition

The manure used in this study was obtained from dairy cattle in a CAFO at the USDA-ARS Dairy Research Facility, in Beltsville, MD. At this cattle feeding operation, the 2 to 5 year old dairy cattle are provided a corn silage-based TMR (total mixed ratio) diet in a free-stall barn. Similar to the work of Van Horn et al. (1994), a synthetic manure mix, consisting of fresh cattle excreta combined with saw dust bedding, was used to represent farmyard manure that is produced at dairy CAFOs and applied on cropland. Cattle feces and urine were sampled from 5

different cows in disinfected 5 gallon buckets, stirred in their respective buckets, and then mixed together at a 6/1 ratio (volumetric) of feces/urine to prepare the synthetic manure. This manure mixture was stored at 4° C until usage. On the morning of each experimental run, the synthetic manure was mixed with sawdust bedding to bring the dry solid content of manure up to approximately 30%. New manure was collected and prepared each week to ensure high concentrations of indigenous bacteria, and this standard method was used to insure the consistency of manure from week to week. Composite manure samples were collected on the day of each rainfall simulation event to obtain the average physical, chemical, and microbial contents of the manure throughout the study (Table 3.1). The composite manure samples were also analyzed for plant macro- and micro-nutrients in order to demonstrate the quality of the CAFO manure as a useful fertilizer (Appendix A).

Table 3.1 Chemical properties and microbial contents of the dairy cattle manure measured from composite manure samples that were collected on each morning of experimentation. The "±" separates average and standard deviation.

Manure Properties and Microbial Contents	
Solid Mass	29.23 ± 0.55 %
Wet Mass	70.77 ± 0.55 %
[1]pH	7.91 ± 0.12
[1]Carbon (C)	17.17 ± 1.85 %
[1]C:N ratio	45.60 ± 3.26
Total Coliforms	$4.31 \pm 1.43 \times 10^6$ CFU g^{-1}
Escherichia coli	$2.68 \pm 0.98 \times 10^6$ CFU g^{-1}
Enterococci	$2.29 \pm 0.90 \times 10^6$ CFU g^{-1}

[1]Analyses that were performed by the Penn State Agricultural Analytical Services Laboratory.

Rainwater was prepared to mimic the ion content and pH that is standard for rainfall in the Maryland, Pennsylvania, and Delaware region. The synthetic rainwater was made by adding reagent-grade chemicals to reverse-osmosis water to set concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+},

NH_4^+, NO_3^-, Cl^-, and SO_4^{2-} at 0.08, 0.03, 0.02, 0.12, 0.34, 1.36, 0.26, and 1.9 mg L^{-1}, respectively (Green et al., 2007; Dao et al., 2008). The rainwater solution was mixed in 500 gal holding tanks and pumped into a 100 gal tank that was connected to the rainfall simulator. Just before rainfall, the pH of the rainwater solution in the 100 gal tank was adjusted to 4.5 using necessary additions of HCl and/or NaOH.

Experimentation

Treatments followed a 2 x 3 factor design with two land slopes (5% and 20%) and three rainfall intensities (3, 6, and 9 cm hr^{-1}). The partitioning boxes were set to mimic 5% and 20% land slopes to generate differences in runoff/infiltration flux ratios. Rainfall intensities of 3, 6, and 9 cm hr^{-1} were chosen because of their linearity and correspondence to precipitation in the mid-Atlantic region (Table 3.2). Rainfall intensity was calibrated on the rain simulator by setting time intervals for pauses between nozzle oscillation sweeps (Fig. 3.2). The sprinkler nozzles (Veejet 80150; Spraying Systems Co., Wheaton, IL) were positioned to rain from a height of 3 m above the soil boxes so the raindrops could approach terminal velocity upon landing. The pressure of water flowing into the rainfall simulator was maintained at 41 N m^{-2} (i.e., 6 psi) to control rainfall intensity and rainfall distribution during each simulation event. This rain simulator design allowed for raindrop impact energy to be approximately 275 kJ/ha-mm, which is about the same for a natural rainfall event with rainfall intensity greater than 2.5 cm hr^{-1} (Meyer and Harmon, 1979). A full description of the rain simulator is provided in Meyer and Harmon (1979). All six treatments were performed in triplicate in randomized order. All rainfall simulations took place indoors to avoid confounding effects of wind or sunlight.

Table 3.2 Frequency-intensity-duration estimates for 10 minute and 60 minute durations of rainfall in Washington, D.C. (NOAA, 2013).

Average Recurrence Interval (years)	Rainfall Intensity (cm hr^{-1}) for 10-min duration	Rainfall Intensity (cm hr^{-1}) for 60-min duration
1	8.66	3.07
2	10.38	3.76
5	12.36	4.75
10	13.80	5.49
25	15.54	6.48
50	16.92	7.29
100	18.29	8.10

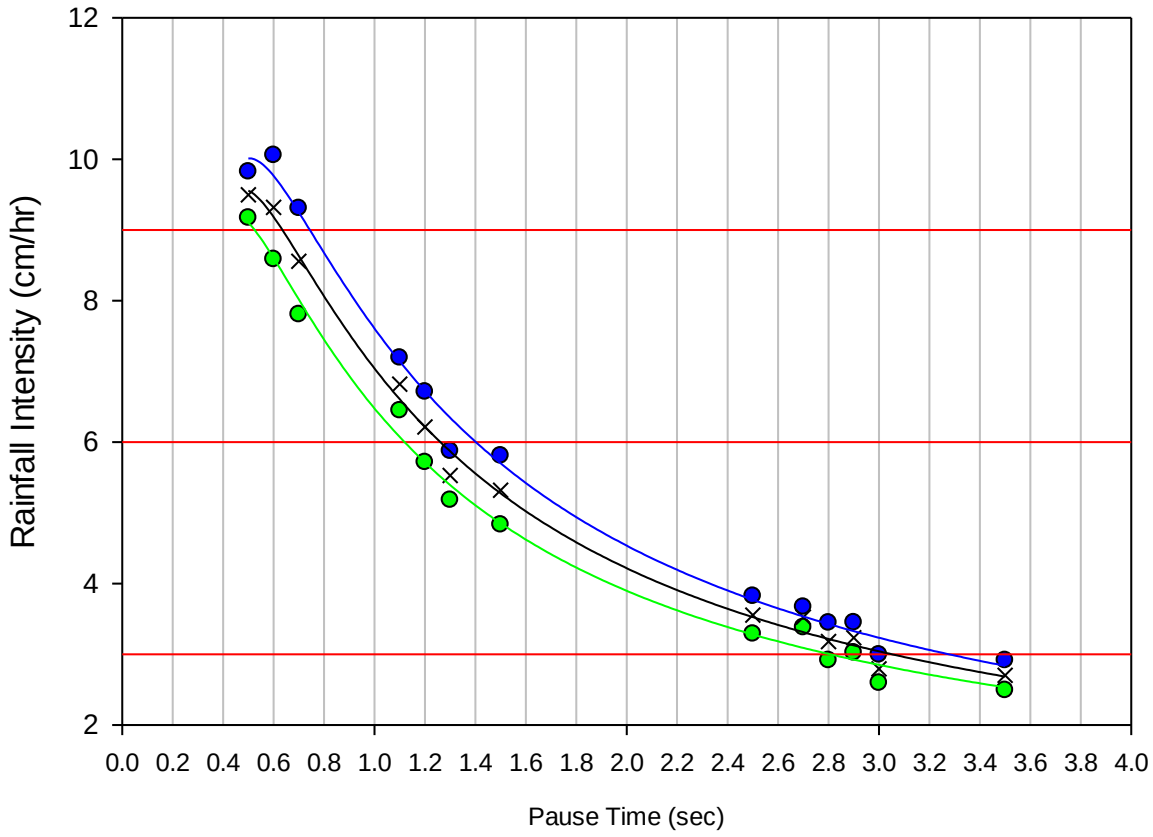

Figure 3.2 Rainfall simulator application intensities for a height of 3 m and pressure of 41 N m^{-2} (i.e., 6 psi), averaged across 60 x 60 cm (blue) and 80 x 80 cm (green) around the center of spray. Points marked with "X" represent the estimated values for 70 x 70 cm around the center of spray. Pause times of 2.8, 1.2, and 0.6 seconds represent approximate rainfall intensities of 3, 6, and 9 cm hr^{-1}, respectively.

Manure was applied to the mesh layer of each partitioning box at the rate of 60 ton ha^{-1} wet weight (i.e., 2.94 kg box^{-1}). Three samples of manure were collected from each box before rainfall to analyze for initial manure contents. During rainfall, runoff and leachate were collected from their respective troughs in sterile 100-ml bottles upon their initial release (time 0) and then subsequently at 1, 2, 4, 7, 10, 15, 20, 30, 40, 50, and 60 minutes. A photograph of the rainfall simulation and sample collection are shown in Fig. 3.3. In order to collect both effluent types simultaneously, the sampling schedule of 0-60 min release would always follow the relative release time of the first effluent type released (e.g., if leachate was released before runoff, then after the initial runoff sample was collected, the remaining runoff samples were collected on the relative time schedule of the leachate collection). All collection reference times and the duration time for each collection were recorded.

Microbiological and Chemical analyses

The water content of manure samples was measured by calculating water loss after samples were dried in an oven at 100^0 C for 24 h to a constant dry weight. Wet manure samples were each blended with sterile deionized water (2 g manure 200 ml^{-1} water) on high speed for 2 minutes (model 34BL97; Waring Laboratory, Torrington, CT) to produce a homogenous slurry mixture. Slurry was allotted 1 hr of settling time before processing. The manure slurries and the runoff and leachate samples were spread-plated on CHROMagar™ ECC (Chromagar, Paris, France) to enumerate *E. coli* and total coliforms and on m-Enterococcus agar (Neogen Corporation, Lansing, MI) to enumerate enterococci. The CHROMagar™ ECC plates were incubated at 37° C for 24 hours and blue colony forming units (CFUs) were reported as *E. coli* and mauve CFUs were reported as coliforms that were not *E.coli*. Thus, the total coliform CFUs were the sum of the blue and mauve colonies on this agar. The m-Enterococcus agar plates were

incubated at 37° C for 48 hours and red CFUs were reported as enterococci. Chloride ion content of each sample was measured with the QuantiChrom™ Chloride Assay Kit (Abnova, Taipei, Taiwan).

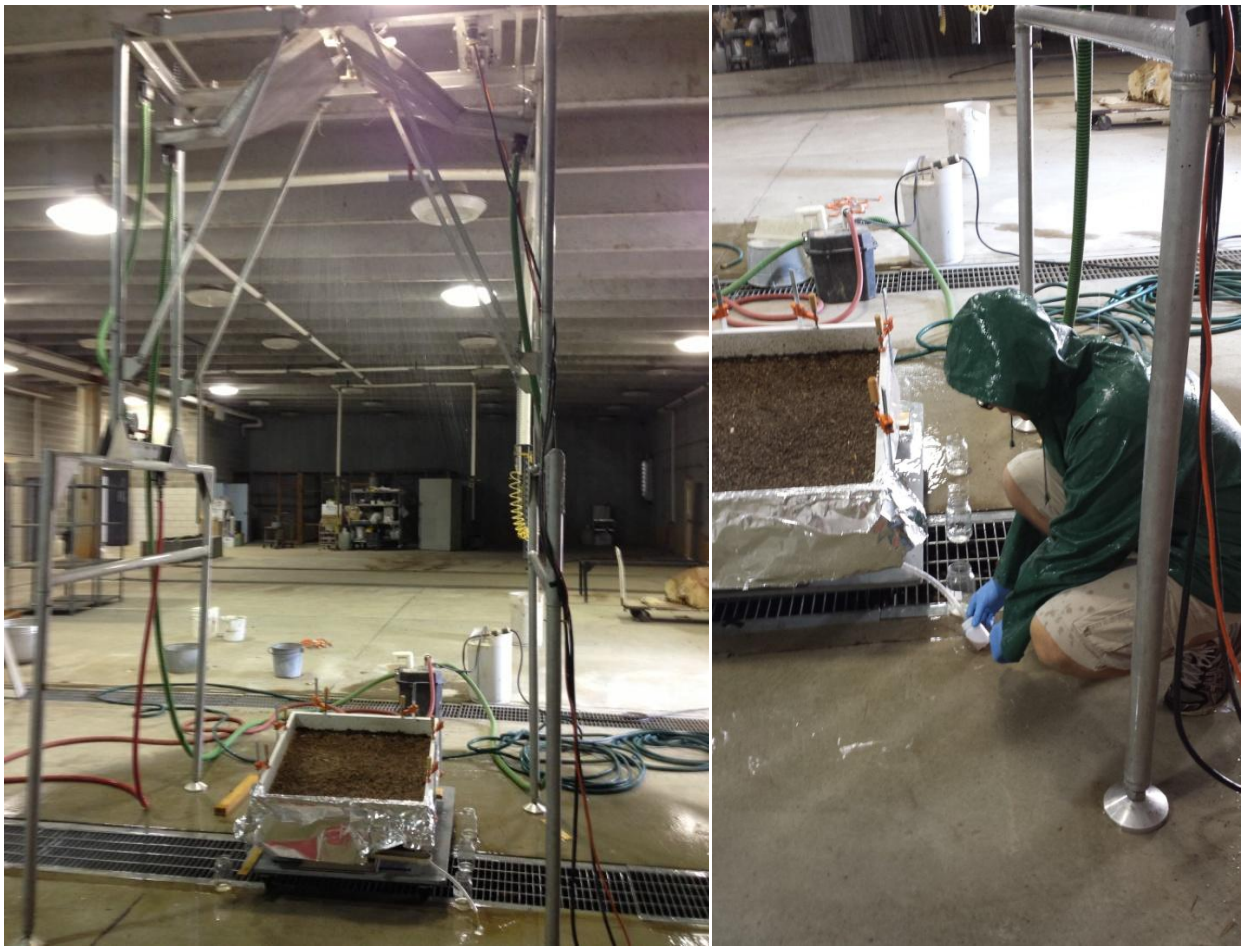

Figure 3.3 Photograph of rainfall simulation event (left) and infiltration sample collection from trough located under the partitioning box (right) (runoff collection on opposite side not shown). Aluminum foil was used to cover the PVC troughs in order to conceal any openings to prevent sample contamination/dilution by rainfall.

Release Modeling

For each release simulation event, the concentrations of *E. coli*, enterococci, total coliforms, and Cl^- in time continuous runoff and infiltration samples were integrated, based on

the respective effluent flow rate, to quantify the cumulative number of bacteria and cumulative mass of chloride released into each effluent type, and the cumulative numbers or masses of the individual manure-constituents released into runoff and infiltration were interpolated to quantify cumulative release from manure (i.e., into both runoff and infiltration).

The total relative number of bacteria and mass of Cl^- released from manure during rainfall was modeled as a function of rainfall-depth and as a function of time with the one-parametric exponential dependence release model (Equation 1) and the two-parametric power-rational dependence release model (Equation 2) that were previously described in Guber et al. (2006) (Fig. 3.4).

Eq. (1) $$\frac{M}{M_0} = 1 - e^{(-k_e W)}$$

Eq. (2) $$\frac{M}{M_0} = 1 - \frac{1}{(1+k_p \beta W)^{\frac{1}{\beta}}}$$

Here, M is total number of bacteria or Cl^- mass released per unit area of manure, [M] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is initial total number of bacteria or Cl^- mass per unit area of applied manure, [M_0] = CFU (for bacteria) or mg (for Cl^-); k_e and k_p are rate constant parameters, [k_e] and [k_p] = cm^{-1} (for release dependency on rainfall depth) or min^{-1} (for release dependency on time); W is rainfall depth or minutes of rainfall, [W] = cm rainfall (for release dependency on rainfall depth) or min (for release dependency on time); and β is a dimensionless shape parameter.

Transformations of these equations (Equations 3 and 4) help to understand how they work and also to see the difference between them:

Eq. (3) $$\frac{1}{M_0}\frac{dM}{dW} = k_e \frac{M_0 - M}{M_0}$$

Eq. (4) $$\frac{1}{M_0}\frac{dM}{dW} = k_p \left(\frac{M_0 - M}{M_0}\right)^{1+\beta}$$

Here, the total number of bacteria or Cl^- mass release rate in the exponential model is proportional to the relative remaining number of bacteria or Cl^- mass $(M_0 - M)/M_0$; whereas, the

release rate in the power rational case is proportional to the relative remaining number or mass to the $1+\beta$ degree. Since the relative remaining number or mass is always less than 1 and $\beta>1$, one can expect that for the same values of the remaining relative number or mass and for $k_e=k_p$ the release rate with the power rational model will be less than that in the exponential model.

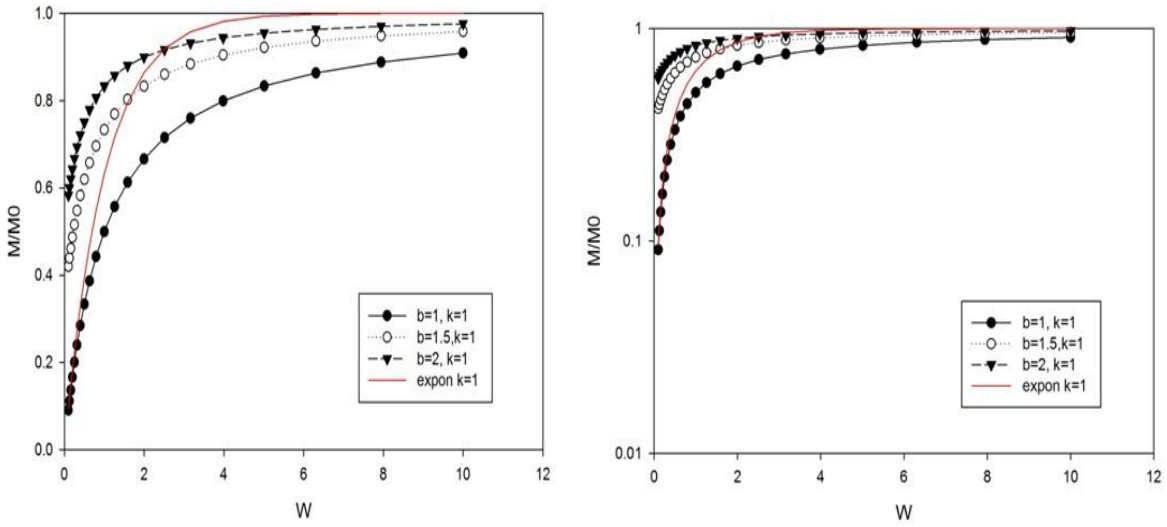

Figure 3.4 An example of simulations of the exponential dependence release model and power rational dependence release model with M/M_0 (i.e., number released/numer applied) on a linear scale (left) and log scale (right). Parameter values corresponding to each simulation are listed.

Eq. 1 and 2 were fitted to 'time- release' and 'rainfall depth-release' data with a FORTRAN code REL_BACT, which was based on the Marquardt-Levenberg optimization algorithm as implemented in van Genuchten (1981) (Appendix C).

Data Analysis

Based on flow rate into runoff and infiltration, the volume of water released that was partitioned into either effluent was calculated. The concentration of bacteria in manure was compared to the concentration of bacteria in the initial release that was collected in surface runoff. The concentrations of total coliforms, *E. coli*, and enterococci in synchronous runoff and infiltration were analyzed for one-to-one regression. A two-factor ANOVA was applied to

determine the effects of rainfall intensity and land slope on release model parameters for each bacteria group/species and for Cl^-.

Release model performance was assessed by root-mean-squared-error (RMSE) and Akaike information criterion (AIC) values that were generated from each model-fit. RMSE were computed as:

$$\mathrm{RMSE} = \sqrt{\frac{\mathrm{RSS}}{\mathrm{n}}}$$

where RSS is the residual sum of squares and *n* is the number of measurements. The RMSE units are dimensionless. The preferred model has smaller RMSE.

The Akaike information criterion (AIC) provides a means for model selection and accounts for the interplay between the model goodness of fit and the complexity of the model (Burnham and Anderson, 2002). In this work, the AIC test takes into account the fact that Eq. 1 and Eq. 2 have a different number of parameters (i.e., one and two, respectively). The corrected Akaike statistic is:

$$AIC = nln\left(\frac{RSS}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1}$$

where RSS is the residual sum of squares, *n* is the number of measurements, and *k* is the number of model parameters.

The AIC units are dimensionless. Of the two models, the one that performs best has the smaller corrected Akaike statistic.

The correlation between bacteria concentrations and Cl^- concentrations in release was determined. The Steiger's Z-test (Steiger, 1980), which is used to test whether one predictor (e.g., Cl^-) correlates equally with two criterion variables (e.g., *E. coli* and enterococci), was applied to compare the correlations that the different bacteria concentrations had with Cl^- concentration in release.

Results

Partitioning of water and bacteria between runoff and infiltrate

Land slope affected the amount of rainwater solution that was partitioned into runoff and infiltration. At the 5 % land slope, runoff and infiltration accounted for approximately 1-3 % and 97-99 % of the total volume of released material, respectively; whereas, at the 20 % land slope, runoff and infiltration accounted for approximately 20-22 % and 78-80 % of the total volume of released material, respectively (Table 3.3).

Table 3.3 Total amount of water released from manure and partitioned into runoff and infiltration during each treatment (i.e., combined rainfall intensity and land slope). The "±" separates average and standard deviation.

Rainfall intensity (cm hr^{-1})	Land slope (%)	$\frac{\text{Volume of water released}}{\text{Area of partitioning box}}$ (cm)	Volume of release partitioned to infiltration (%)	Volume of release partitioned to runoff (%)
3	5	2.97 ± 0.45	99.1 ± 0.9	0.9 ± 0.9
3	20	3.04 ± 0.09	78.8 ± 3.2	21.2 ± 3.2
6	5	6.05 ± 0.52	98.5 ± 0.5	1.5 ± 0.5
6	20	6.29 ± 0.10	80.0 ± 2.9	20.0 ± 2.9
9	5	9.66 ± 0.44	97.8 ± 1.5	2.2 ± 1.5
9	20	9.29 ± 0.26	78.8 ± 1.4	21.2 ± 1.4

Manure components were released and carried from the manure within the rainwater solution that flowed with surface runoff and infiltration. Although the partitioning of flow into runoff and infiltration was uneven (Table 3.3), the concentrations of bacteria within their respective solutions were similar. In this study, for each of the 6 treatments, the release of 3 different bacterial groups were analyzed, which provided a total of 18 "release events". Of these 18 "release events", 10 of them yielded concentrations of bacteria in synchronous runoff and infiltration that did not significantly differ from the one-to-one ordinary least squares regression line (i.e., slope=1 and y-intercept=0) when α was set to 0.05 (Fig. 3.5). There were 7 out of 9 release events for treatments at the 5 % land slope that had 1:1 release concentrations in

runoff:infiltration, while only 3 of the 9 release events for treatments at the 20 % land slope had 1:1 release concentrations in runoff:infiltration. There were no observed trends for differences in deviation from 1:1 release for the different bacteria group/species, and there were no observed differences in deviation from 1:1 release for different rainfall intensities (Fig. 3.5). Thus, while the partitioning of bacteria concentrations into runoff:infiltration was 1:1 most of the time, only an increase in land slope caused deviation from 1:1 release (Fig. 3.5).

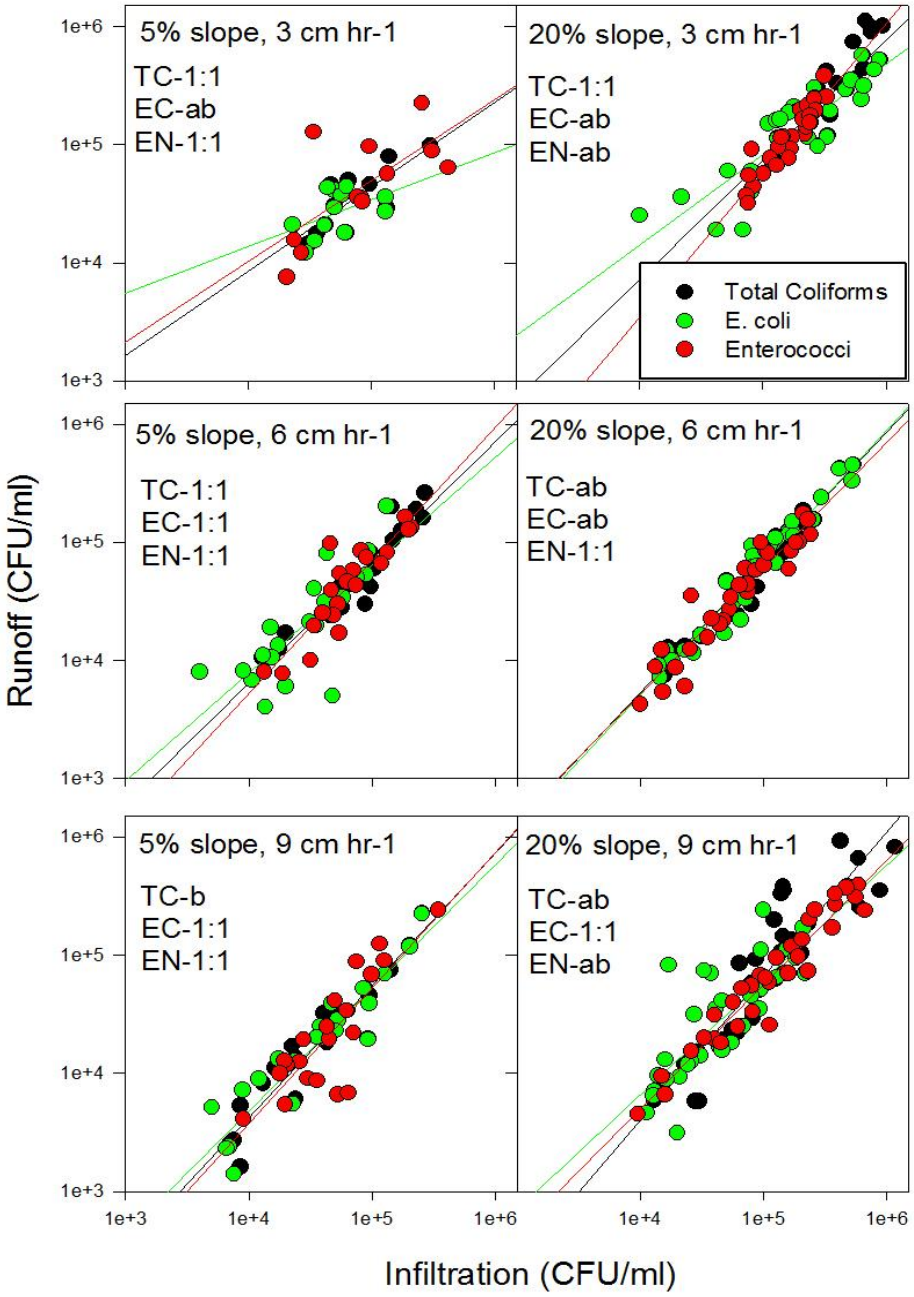

Figure 3.5 Concentrations of bacteria released into synchronous runoff and infiltration. Significant differences from the one-to-one ordinary least squares regression line (slope=1 and y-intercept=0) are indicated for slope as "a" and y-intercept as "b" next to bacteria type (TC – total colifroms, EC – *E. coli*; EN – Enterococci) in each graph legend, while "1:1" indicates no significant difference (α=0.05).

Initial release concentrations versus initial concentrations in manure

The concentrations of *E. coli*, enterococci, and total coliforms in initial release from manure were on average 1.8×10^5, 1.6×10^5, and 2.6×10^5 CFU ml^{-1} of release (runoff or infiltration, whichever was eluted first), respectively, and these concentrations were greater than one order of magnitude below the starting concentration of each bacteria in the manure (Table 3.4). $C_{Initial\ Release}/C_{Manure}$ was much greater for chloride than for bacteria (Table 4). For all bacteria in the study, the $C_{Initial\ Release}/C_{Manure}$ was not significantly affected by rainfall intensity or land slope (Table 3.5).

Table 3.4 Concentration of bacteria and chloride ion in initial release compared with their concentrations in manure. The "±" separates average and standard deviation.

Manure Constituent	$\frac{\text{Content in manure}}{\text{Manure water content}}$	Concentration in initial release	$\frac{C_{\text{Initial Release}}}{C_{\text{Manure}}}$
Total Coliforms	$3.49 \pm 1.22 \times 10^6$ CFU ml^{-1}	$0.26 \pm 0.18 \times 10^6$ CFU ml^{-1}	7.37%
E. coli	$2.62 \pm 0.76 \times 10^6$ CFU ml^{-1}	$0.18 \pm 0.06 \times 10^6$ CFU ml^{-1}	6.81%
Enterococci	$2.16 \pm 0.71 \times 10^6$ CFU ml^{-1}	$0.16 \pm 0.06 \times 10^6$ CFU ml^{-1}	7.24%
Chloride ion	0.701 ± 0.196 mg ml^{-1}	0.239 ± 0.115 mg ml^{-1}	37.67%

Table 3.5 Results from two-factor ANOVA (p-values are displayed) on the effect of rainfall intensity and land slope on $C_{Initial\ Release}/C_{Manure}$ for total coliforms, *E. coli*, and enterococci.

Bacteria group/species	Rainfall Intensity	Land Slope	Interactions
Total Coliforms	0.433	0.416	0.714
E. coli	0.684	0.160	0.433
Enterococci	0.674	0.309	0.976

Cumulative manure constituent release as effected by slope and rainfall intensity

For all treatments, the bacteria release kinetics began with a precipitous log-linear increase in cumulative mass released during the first 1 cm of rainfall, which was followed by a

much slower, steady release for the remainder of rainfall (Fig. 3.6). The bacteria release model parameters were substantially affected by rainfall intensity when release was a function of time, but not when it was a function of rainfall depth (Fig. 3.6; Table 3.6). Compared with the release model parameters generated when simulating the release of bacteria, the parameters generated for the release of Cl^- appeared to be much more affected by rainfall intensity and land slope when release was a function of either time or rainfall depth (Table 3.6). Although, the latter may have been due to Cl^- release reaching 100 % after a given rainfall depth, while bacteria release always approached an asymptote at a lower percentage value.

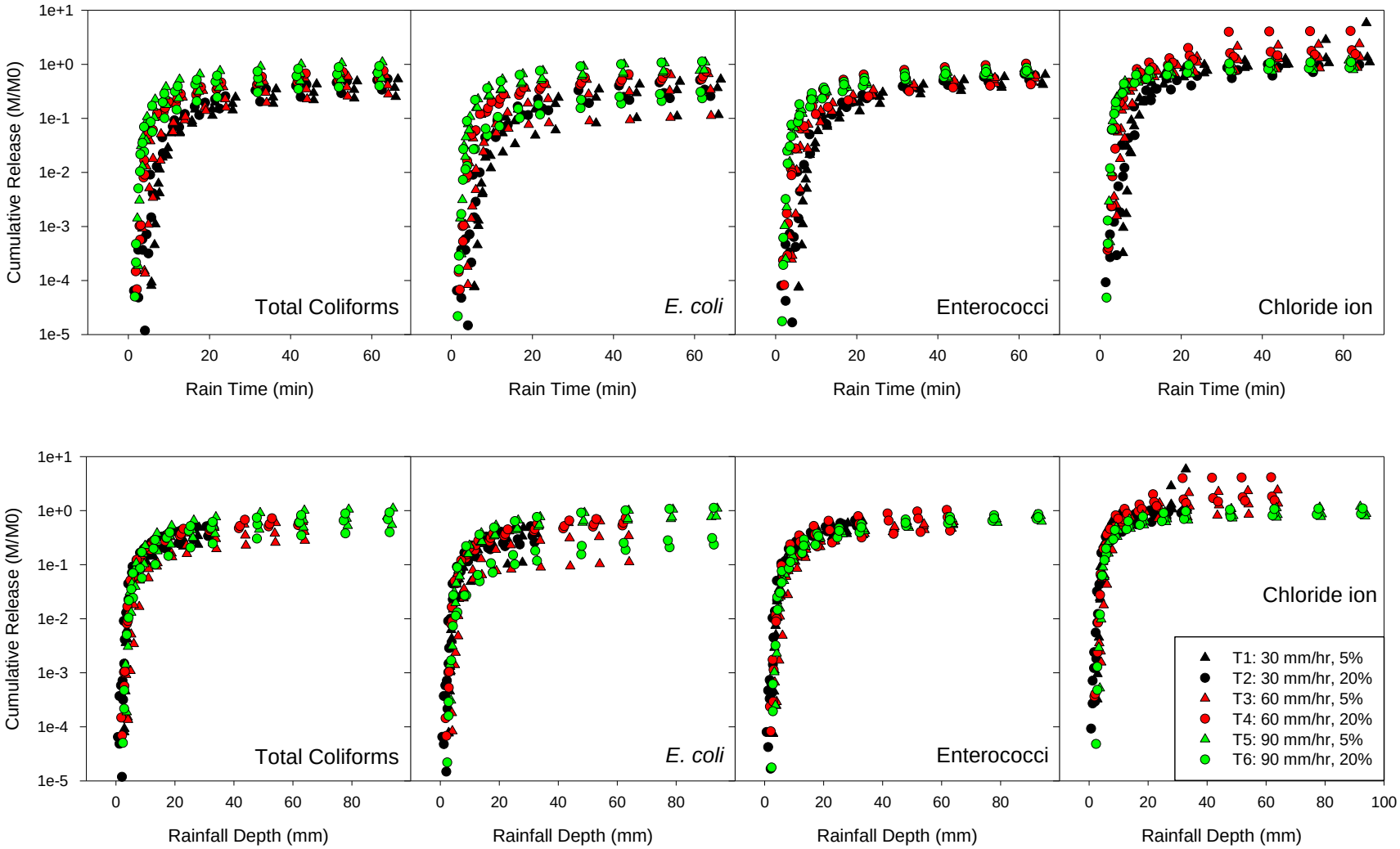

Figure 3.6 Relative cumulative masses of bacteria and chloride ion released as a function of time (top) and rainfall depth (bottom). Datasets for the the Treatments 1-6 are differentiated by color and shape in the legend.

Table 3.6 Results from two-factor ANOVA (p-values are listed) on the effect of rainfall intensity and land slope on model parameters for the dependency of release of each manure constituent on time of rainfall depth.

Model; Parameter; Units	Manure Constituent	*Release kinetics as funtion of time*			*Release kinetics as funtion of rainfall depth*		
		Rainfall Intensity	Land Slope	Interactions	Rainfall Intensity	Land Slope	Interactions
Eq.1; K_e; min^{-1} (for time) or cm^{-1} (for rainfall depth)	Total Coliforms	0.039	0.860	0.426	0.630	0.875	0.384
	E. coli	0.054	0.699	0.438	0.499	0.853	0.425
	Enterococci	0.040	0.156	0.482	0.262	0.146	0.513
	Chloride ion	0.046	0.211	0.622	0.002	0.386	0.004
Eq. 2; K_p; min^{-1} (for time) or cm^{-1} (for rainfall depth)	Total Coliforms	0.001	0.643	0.212	0.490	0.814	0.224
	E. coli	0.012	0.372	0.211	0.443	0.491	0.257
	Enterococci	0.000	0.340	0.190	0.680	0.284	0.193
	Chloride ion	0.000	0.140	0.002	0.002	0.031	0.000
Eq. 2; β; none	Total Coliforms	0.681	0.184	0.415	0.456	0.117	0.318
	E. coli	0.509	0.305	0.316	0.507	0.246	0.458
	Enterococci	0.386	0.340	0.736	0.680	0.216	0.413
	Chloride ion	0.004	0.962	0.044	0.099	0.197	0.638

Performance of release models

For the release of bacteria, the average root-mean-squared-error (RMSE) was 0.033 and 0.018 for the power-rational model and exponential model-fits, respectively. RMSE values were lower for the power-rational model fits than the exponential model fits for 94.4% of the model-fits (n=54 → 3 bacteria x 6 treatments x 3 replications=54). Similarly, Cl^- release curve fits yielded a lower average RMSE when fit to the power rational model (0.329) than the exponential model (0.346). Also, application of the Akaike test, which assesses model quality based on goodness of fit while penalizing for model complexity, showed the power rational model was favorable for simulating bacteria release in the majority of cases (Fig. 3.7). With regard to the generated Akaike Infromation Criterion, Cl^- release was better simulated by the exponential dependence model

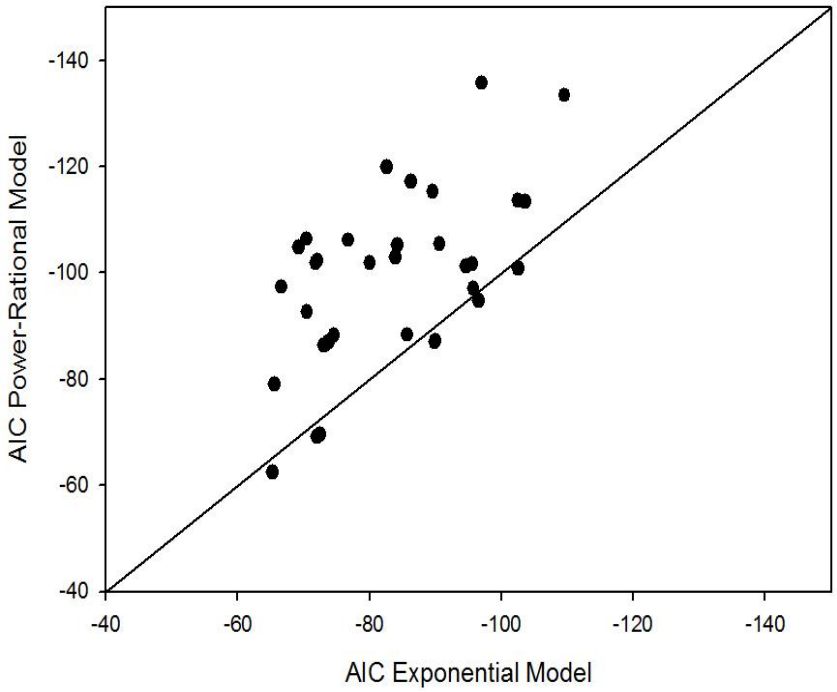

Figure 3.7 Akaike information criterion (AIC) yielded from the power-rational model fits for bacteria release vs. the exponential model fits for bacteria release. The line shown demonstrates

1:1 relationship. Lower the Akaike Information Criterion (AIC) values indicate the "stronger" model.

Concentrations of bacteria vs. chloride ion

Each gram of wet manure contained approximately 10^6 CFUs of total coliforms, *E coli*, and enterococci (Table 3.1) as well as 0.701 ± 0.196 mg of chloride ion. There was a moderate, positive relationship between the concentrations of bacteria and Cl^- measured in infiltrate. Linear regression showed that the variability of concentration of total coliforms, *E. coli*, and enterococci in infiltration could only be explained by the concentration of Cl^- in infiltration 47.6 %, 54.6 %, and 43.6 % of the time, respectively (Figure 3.8). Indicator bacteria in runoff were not correlated with the Cl^- concentrations since Cl^- concentrations were highly variable for the small volumes of runoff samples that remained after microbial processing took place. In addition, the correlation coefficients for the relationship between concentration of bacteria (CFU ml^{-1}) and chloride ion (ppm) in the collected infiltration samples was 0.690, 0.739, 0.661 for total coliforms, *E. coli*, and enterococci, respectively (n=213). Application of the Steiger's *Z*-test (Steiger, 1980) was used to determine whether the Cl^- in infiltration correlates equally with the two different bacteria in infiltration. The test showed that the correlation of *E. coli* and enterococci concentrations with concentrations of Cl^- concentration in infiltration was significantly different (p=0.023) and that the correlation of *E. coli* and total coliform concentrations with concentrations of Cl^- in infiltration was substantially different (p=0.087). The correlations of total coliform and enterococci concentrations with concentrations of Cl^- in infiltration were not different (0.845). Thus, of the three indicator bacteria in the study, the concentrations of released *E. coli* were slightly more similar to that of Cl^- than the other two indicator bacteria.

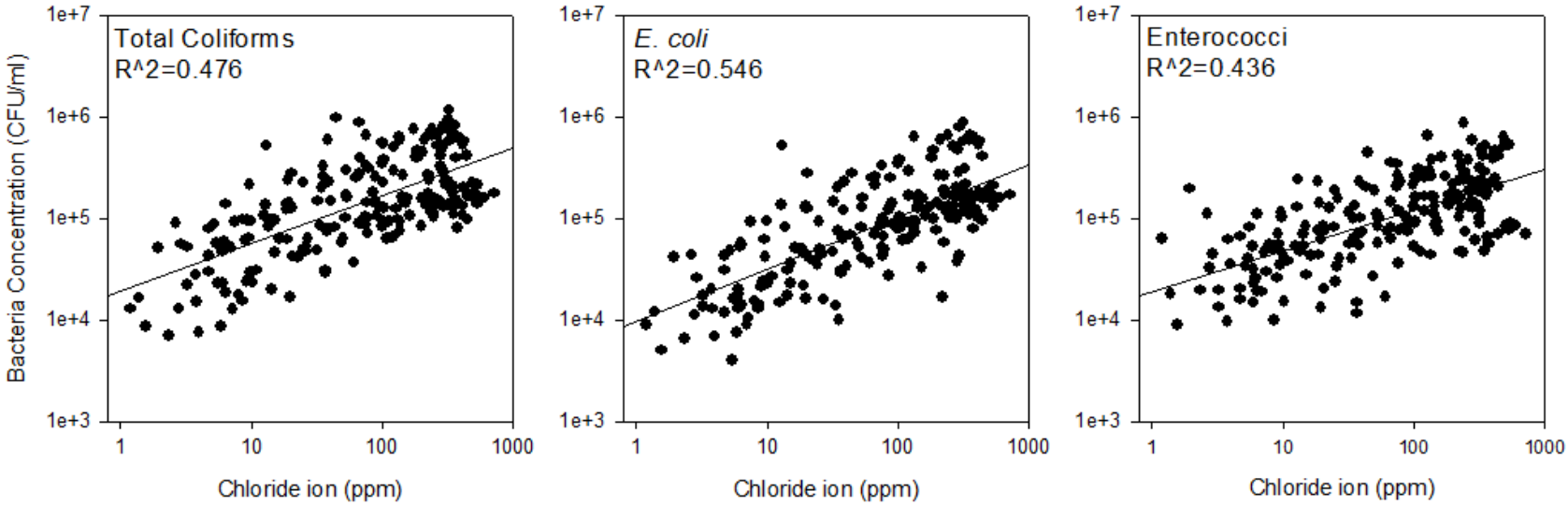

Figure 3.8 Linear regression of the concentration of each bacteria group/species (total coliforms-left, *E. coli*-middle, and enterococci-right) vs. the concentration of Cl^- in the collected infiltration samples.

Discussion

Predictive release models for manure-bacteria simulate the ratio of the concentration of bacteria released, C, to the initial concentration of bacteria in the manure, C_0, over time. C_0 for manure is a subjective term that depends on how it is defined and used. Three models described in Guber et al. (2006) and Guber et al. (2013) defined C_0 as an "effective concentration" in manure that represents the initial concentration of bacteria in release. Guber et al. (2006) and Guber et al. (2013) assumed C_0 in their work equal to the content of bacteria in manure divided by the manure water content. Essentially all bacteria of interest in that work were assumed to be concentrated only in the liquid phase of manure since the manure used was a slurry (Guber et al., 2006). In this study, the concentrations of *E. coli*, enterococci, and total coliforms in the initial release were all more than one order of magnitude below their starting contents in manure divided by the manure water content (Table 3.4). While the work of Guber et al. (2006) used bovine slurry, solid bovine manure was used in this study. Compared with the work of Guber et al. (2006), the initial release efficiency value for $C_{Initial\ Release}/C_{Manure}$ is lower than the value observed by Guber et al. (2006). This difference can be explained by 1) the solid manure providing more surfaces for bacteria, 2) the catchment of bacteria in manure aggregates as they are leaching through the manure prior to becoming released, and 3) bacteria or manure particulates containing adsorbed bacteria clinging to surfaces within the partitioning box such as in the pores of the mesh frame. Bacteria have specific physical and chemical properties that affect their capability of becoming dislodged from their micro-habitats (Lombard et al., 2011). Bacteria surface charge, hydrophobicity, and size as well as surface structures such as flagella, fimbriae, and lipopolysaccharides (LPS) affect their ability to attach/detach from other solid structures (Critzer and Doyle, 2010; Foppen and Schijven, 2006; Pachepsky et al., 2008). Unlike

bacteria, ions and chemical compounds dissolved within the manure solution may be leached upon initial release. In this study, the ratio of Cl^- in the initial release to Cl^- in the manure solution was not 1, which was likely due to dilution within the manure solution prior to the initial release or the flow of a fraction of the rainfall water bypassing the manure particles. It should be noted that the ratio was still much greater than that of any of the indicator bacteria (Table 3.2). This result may mean that rainwater may be diluting the manure solution while the matrix absorbs water prior to release, then this diluted solution containing some bacteria is leached with the initial release. Indications are that most bacteria remain associated with the solids in the manure matrix or are present within the matrix in an area that is not accessible to flow.

The change of M/M_0 with rainfall depth appeared to be a two-stage process with a precipitous early release stage and a much slower stage (Fig. 3.6). These findings are consistent with the work of Guber et al. (2006), where microbial release experienced a distinct rate-change and the kinetics shifted from first-order to zero-order kinetics after approximately one hour of simulated rainfall over bovine slurry on a hill-slope. In addition, Schijven et al. (2004) reported that concentrations of *Cryptosporidium* and *Giardia* (oo)cysts released from artificially constructed cowpats decreased gradually before experiencing a rate-change where the concentrations tailed-off. Relative concentrations and/or masses of fecal indicator bacteria in the release (C/C_0 and/or M/M_0) generally decreased with time (Roodsari et al., 2005; Cardoso et al, 2012) and the temporal differences in release-rate may be partially attributed to the depletion of the manure components and the exposed surface area in time.

The two-stage runoff-removal process described in this study may have resulted from a period of time where an initial washout of planktonic bacteria and suspended manure particulates containing adsorbed bacteria occurred and after the easily accessible pathways were leached out,

the manure-liquid mixture became almost void of microbes. The second stage began where there was an almost-constant rate of shaving and sloughing of manure into the moving water with the number of bacteria in solution being proportional to the shaved and sloughed amounts of manure. A partial manure-seal may have also formed and sheltered many remaining bacteria from release, protected the larger manure particulates from surface erosion, and stabilized the manure aggregates inside the matrix. Compared with the release of bacteria during the "initial washout" of the manure liquid phase, the process of loosening and removing the manure matrix with its constituents was probably much slower.

Another important finding was that the ratio of mass removed to the initial mass for Cl^- approaches 1 (i.e., 100 %) while the ratio of the total number of bacteria removed to the total initial number of bacteria remains stable at a value less than 1 (Fig. 3.6). The dissolved ions and chemical compounds within the manure may be completely washed out of the manure during rainfall while the bacteria may remain in the manure at high concentrations following rainfall.

Pachepsky et al. (2009) noted that manure particles can affect transport and retention of microbial pathogens and indicators in the soil. In their work, the size distributions of particles released from dairy cattle slurry began around an average of 7.96 μm and decreased before becoming stabilized at an average size around 4.1 μm following 15 minutes with a rainfall depth of 8.1 mm. This 2-fold decrease in particle size distribution prior to stabilization occurred at just under 1 cm of rainfall that corresponded to a rainfall depth similar to the rainfall depth at the "break point" described in this study. The rainfall may have substantially affected the release and subsequent transport of FIB, especially since a large quantity of *E. coli* and enterococci that are released from the manure are transported with the manure colloids (Soupir et al., 2010). The

change in size of eluted particulates observed by Pachepsky et al. (2009) might be interpreted as the onset of the predominantly sloughing stage in runoff-removal.

Rainfall intensity had a strong effect on the release of manure-bacteria when M/M_0 was a function of time because increasing the volume of rainwater per unit-time (i.e., rainfall intensity) caused substantially more bacteria to be released from the surface-applied manure. This finding is in agreement with other studies that have shown precipitation volume to positively affect microbial release (Schijven et al., 2004; Kress and Gifford, 1984; Thelin and Gifford, 1983; Ling et al., 2009). More importantly, though, the dependency of M/M_0 on rainfall depth was not significantly affected by rainfall intensity (Table 3.6), which supports the assumption that microbial release kinetics may be simulated by a dependency on rainfall depth without considering intensity.

Even though land slope did not appear to impact microbial release kinetics (as supported by the release model parameters in Table 3.6), it did have a significant effects on the partitioning of released matter. As land slope increased, water flow partitioned into surface runoff increased. While the concentrations of bacteria (CFU ml^{-1}) in synchronous runoff and infiltration did not differ most of the time (Fig. 3.5), they did differ slightly more when land slope was at 20 %. However, the differences were not one-directional in favor of higher concentrations consistently in runoff or infiltration, so this observation can probably just be attributed to increased land slope having caused more variability in the runoff. Thus, while the concentrations of bacteria (CFU ml^{-1}) in synchronous runoff and infiltration did not differ most of the time, increased land slope did have a positive effect on the volume of water moving with runoff and an increased microbial mass (CFUs) released into the surface runoff. In other words, the volume and intensity of runoff governed the partitioning of bacteria masses that were released from the manure.

Dissimilarity between the release of total coliforms, *E. coli*, and enterococci from the manure suggested organism-specific release processes that may be attributed to each bacteria's unique physiological properties. For example, bacteria have specific physical and chemical properties that affect their efficiency of becoming dislodged from their micro-habitats (Lombard et al., 2011). Bacteria surface charge, hydrophobicity, size, and surface structures such as flagella and fimbriae affect its ability to attach/detach from surfaces (Foppen and Schijven, 2006). Lipopolysaccharides and teichoic acids are unique components to the outer membrane of gram-negative and gram-positive bacteria, respectively, and are involved with their attachment and dislodgement processes (Lombard et al., 2011). Evidence for microbe-specific release supports the idea that an optimal release assessment should be based on release simulations of multiple indicator microorganisms. For example, in a lab assay simulating the release of *E. coli* and enterococci from dairy slurry, beef farmyard manure, beef feces, and sheep feces, differences in release rates between the two bacteria was noted for all manure types (Hodgson et al., 2009). Another study investigated the rainfall-induced dissolution of bovine slurry applied over vegetated soil and found that the release rate for *E. coli* was almost twice the release rate enterococci (Guber et al., 2007). In that study, the release of dissolved bromide ion tracer was closer in similarity to the release of the former than the latter, suggesting that *E. coli* was more associated with the liquid phase of that manure than enterococci (Guber et al., 2007). In this study, the release of an inorganic surrogate tracer (i.e., dissolved Cl^- in the manure) was more similar to the release of *E. coli* than enterococci or total coliforms (Fig. 3.8) and the correlation between *E. coli* with Cl^- in infiltration was substantially different from either that of enterococci or total coliforms with Cl^- in infiltration. The differences in location and release efficiency of bacterial species within surface-applied manure may be attributed to the differences seen in

microbial release studies. Compared with *E. coli*, smaller enterococci cells were more likely to remain held within manure aggregates within the manure matrix rather than being washed off. Thus, the release of gram-positive bacteria (e.g., enterococci) and gram-negative bacteria (e.g., *Salmonella* and *E. coli*) may be fundamentally different based on their distributions in micro-niches within the manure matrix and their unique physiochemical properties for cellular attachment and microbial flocculation in suspension.

Utilizing an optimal release model is essential for simulating and regulating fecal contamination to the environment. Guber et al. (2006) recommended the power-rational release model (Bradford and Schijven, 2002) for simulating release of indicator bacteria from dairy cattle slurry on field plots. Based on our assessment of model performance by root-mean-squared-error and the Akaike information criterion, the same model is recommended here for simulation of indicator bacteria release from solid manure.

Conclusions

Release of microorganisms from animal manure is an important component of microbial fate and transport as it establishes the content of microbes that enter the transport phase and become a subsequent risk for human health. In this study, the concentration of *E. coli*, total coliforms, and enterococci in the initial release from manure was about one order of magnitude lower than their concentrations in manure, while the release for Cl^- was much greater. Thus, rainwater may be diluting the manure liquid phase during the period where manure absorbs water prior to release, and then the diluted solution (containing some bacteria) is leached with the initial release, but most bacteria remain associated with solids in the manure matrix. Manure constituent release kinetics were expressed by a two-stage release process, having a precipitous early release, for about the first 1 cm of rainfall, and a slower, almost constant-rate of release for

the remainder of rainfall. The relative release (i.e., M/M_0) of Cl^- approached 1, while that for bacteria approached an asymptote at a value lower than 1. In essence, dissolved substances in manure solution may be completely leached during rainfall, while bacteria remain a part of the solid manure matrix throughout rainfall. Following the "initial washout" of the manure liquid phase, the release depends on the bacteria's efficiency of being sloughed off from the manure matrix surfaces. Microorganisms in manure were partitioned into runoff and infiltration at similar solution concentrations, but as land slope increased, more water, thus more bacteria, was partitioned into surface runoff. Compared with the exponential release model, the power-rational dependence model is recommended for simulating the total relative mass of manure-bacteria released as a function of rainfall depth. This study should be extended to evaluate microbial release from manure that is applied over soil in laboratory and field studies to gain a better understanding of the dynamic release process in a natural system where hydrological properties governing release partitioning depend on manure interactions with soil and vegetation.

References

Batz, M. B., Hoffmann, S., and Morris, J. G., 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection, 75(7): 1278-1291.

Benham, B. L., Baffaut, C., Zeckorski, R. W., Mankin, K. R., Pachepsky, Y. A., Sadeghi, A. M., Brannan, K. M., Soupir, M. L., and Habersack, M. J., 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. Transactions of ASABE, 49(4): 987-1002.

Bradford, S. A., and Schijven, J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environmental Science & Technology, 36(18): 3916-3923.

Brooks, J. P., Adeli, A., Read, J. J., and McLaughlin, M. R., 2009. Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. Journal of Environmental Quality, 38(1): 218-229.

Burnham, K. P. and Anderson, D. R., 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag. 2nd ed.

Cardoso, F., Shelton, D., Sadeghi, A., Shirmohammadi, A., Pachepsky, Y., and Dulaney, W., 2012. Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. Journal of Environmental Management, 110: 1-7.

Chin, D. A., Sakura-Lemessy, D., Bosch, D. D., and Gay, P. A., 2009. Watershed-scale fate and transport of bacteria, Transactions of ASABE, 52(1): 145-154.

Curriero, F. C., Patz, J. A., Rose, J. B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the united states, 1948-1994. American Journal of Public Health, 91(8): 1194-1199.

Dao, T. H., Guber, A. K., Sadeghi, A. M., Karns, J. S., Shelton, D. P., and Pachepsky, Y. A., 2008. Loss of bioactive phosphorus and enteric bacteria. Soil Science, 2 (5): 511-521.

Drapcho, C. M., A. K. B., 2003. Fecal coliform concentration in runoff from fields with applied dairy manure. Master in Science Thesis, Louisiana State University, Baton Rouge, LA.

Dufour A. P., and Ballantine P., 1986. Bacteriological ambient water quality criteria for marine and freshwater recreational waters. US Environmental Protection Agency: EPA 440/445-484-002.

Foppen, J. W., van Herwerden, M., and Schijven, J., 2007. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology, 93(1-4): 236-54.

Forslund, A., Markussen, B., Toenner-Klank, L., Bech, T. B., Jacobsen, O. S., and Dalsgaard, A., 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a *Salmonella enterica* Serovar Typhimurium Bacteriophage through intact soil cores following surface application and injection of slurry. Applied and Environmental Microbiology, 77(22): 8129-8138.

Gerba, C. P., and Smith, J. E., 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. Journal of Environmental Quality, 34(1): 42-48.

Guber, A. K., Shelton, D. R., and Pachepsky, Y. A., 2005. Transport and retention of manure-corne coliforms in soil. Vadose Zone Journal, 4(3) doi:10.2136/vzj2004.0097.

Guber, A. K., Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., and Sikora, L. J., 2006. Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and Environmental Microbiology, 72(12): 7531-7539.

Guber, A. K., Karns, J. S., Pachepsky, Y. A., Sadeghi, A. M., Van Kessel, J. S., and Dao, T. H., 2007. Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. Letters in Applied Microbiology, 44(2): 161-167.

Guber, A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L., 2011. Uncertainty in modelling of faecal coliform overland transport associated with manure application in Maryland. Hydrological Processes, 25(15): 2393-2404.

Guber., A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L. 2012. STWIR, a microorganism transport with infiltration and runoff add-on module for the KINEROS2 runoff and erosion model documentation and user manual. User Manual. USDA-ARS. Beltsville, MD, USA.

Guber, A. K., Pachepsky, Y. A., Dao, T. H., Shelton, D. R., and Sadeghi, A. M., 2013. Evaluating manure release parameters for nonpoint contaminant transport model KINEROS2/STWIR. Ecological Modelling, 263: 126-138.

Hamilton, D. W., 2011. Consistency of manure/water mixtures. Stillwater, OK: Oklahoma Cooperative Extension Service. OSU Factsheet BAE 1751.

Himathongkham, S., Bahari, S., Riemann, H., and Cliver, D., 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiology Letters, 178(2): 251-257.

Hodgson, C. J., Bulmer, N., Chadwick, D. R., Oliver, D. M., Heathwaite, A. L., Fish, R. D., and Winter, M., 2009. Establishing relative release kinetics of faecal indicator organisms from different faecal matrices. Letters in Applied Microbiology, 49(1): 124-130.

Hoffmann, S., Batz, M. B., and Morris, J. G., 2012. Annual cost of illness and quality-adjusted life year losses in the united states due to 14 foodborne pathogens. Journal of Food Protection, 75(7): 1292-1302.

Jiang, X., Morgan, J., Doyle, M. P., Jiang, X., Morgan, J., and Doyle, M. P., 2002. Fate of *Escherichia coli* O157 : H7 in manure-amended soil. Applied and Environmental Microbiology, 68(5): 2605-2609.

Kim, K., Whelan, G., Purucker, S. T., Bohrmann, T. F., Cyterski, M. J., Molina, M., Gu, Y., Pachepsky, Y., Guber, A., and Franklin, D. H., 2014. Rainfall-runoff model parameter estimation and uncertainty evaluation on small plots. Hydrological Processes, 28(20): 5220-5235.

Kress, M., and Cifford, G. F., 1984. Fecal coliform release from cattle fecal deposits. Water Resources Bulletin, 20(1): 61-66.

Lawson, L., 2003. HSPF Model Calibration and Verification for Bacteria TMDLs. Guidance Memo No. 03-2012, Commonwealth of Virginia, Department of Environmental Quality.

Ling, T. Y., Jong, H. J., Apun, K., and Sulaiman, W. H. W., 2009. Quantifying *Escherichia coli* release from soil under high-intensity rainfall. Transactions of ASABE, 52(3): 785-792.

Lombard, N., Prestat, E., Dirk van Elsas, J., and Simonet, P., 2011. Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78: 31-49.

Martinez, G., Pachepsky, Y. A., Whelan, G., Yakirevich, A. M., Guber, A., and Gish, T. J., 2014. Rainfall-induced fecal indicator organisms transport from manured fields: Model sensitivity analysis. Environment International, 63: 121-129.

McDaniel, R. L., Soupir, M. L., Tuttle, R. B., and Cervantes, A. E., 2013. Release, dispersion, and resuspension of *Escherichia coli* from direct fecal deposits under controlled flows. Journal of the American Water Resources Association, 49(2): 319-327.

Meyer, L. D., and Harmon, W. C., 1979. Multiple-intensity rainfall simulator for erosion research on row sideplots. Transactions of the ASAE, 22: 100-103.

Moyer, D. L., and Hyer, K. E., 2003. Use of the hydrological simulation program-FORTRAN and bacterial source tracking for development of fecal coliform total maximum daily load (TMDL) for Blacks Run, Rockingham County, Virginia. U.S. Department of the Interior U.S. Geological Survey, Water-resources Investigations Report 03-4161.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2005. Erosion and subsequent transport state of *Escherichia coli* from cowpats. Applied and Environmental Microbiology, 71(6): 2875-2879.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2006. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Letters in Applied Microbiology, 42(2): 83-87.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V, Braden, C. R., Angulo, F. J., and Griffin, P. M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerging Infectious Diseases, 19(3): 407-415.

Panhorst, K. A., 2002. Estimating bacterial loadings to surface waters from agricultural watersheds. Master in Science Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Pachepsky, Y. A., Yu, O., Karns, J. S., Shelton, D. R., Guber, A. K., & Kessel, J. S. Van., 2008. Strain-dependent variations in attachment of *E. coli* to soil particles of different sizes. International Agrophysics, 22: 61–66.

Pachepsky, Y. A., Guber, A. K., Shelton, D. R., and McCarty, G. W., 2009. Size distributions of manure particles released under simulated rainfall. Journal of Environmental Management, 90(3): 1365-1369.

Roodsari, R. M., Shelton, D. R., Shirmohammadi, A., Pachepsky, Y. A., Sadeghi, A. M., and Starr, J. L., 2005. Fecal coliform transport as affected by surface condition. Transactions of ASAE, 48(3), 1055-1061.

Savichtcheva, O., and Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research, 40(13), 2463-2476.

Schijven, J. F., Bradford, S. A., and Yang, S., 2004. Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: Physical factors. Journal of Environmental Quality, 33(4), 1499-1508.

Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., Stout, W. L., Karns, J. S., and Gburek, W. J., 2003. Release rates of manure-borne coliform bacteria from data on leaching through stony soil. Vadose Zone Journal, 2, 34–39.

Sobeck, D. C., and Higgins, M. J., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Research, 36(3): 527-538.

Soupir, M., Tech, V., Hall, S., Mostaghimi, S., and Alphin, E. F., 2003. Bacteria release and transport from livestock manure applied to pastureland. 2003 ASAE Annual International Meeting. Las Vegas, NV.

Soupir, M. L., Mostaghimi, S., and Dillaha, T., 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. Journal of Environmental Quality, 39(3), 1019-1027.

Steiger, J. H., 1980. Tests for comparing elements of a correlation matrix. Psychological Bulletin, 87, 245-251.

Stout, W. L., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Saporito, L. S., and Sharpley, A. N., 2005. Runoff transport of faecal coliforms and phosphorus released from manure in grass buffer conditions. Letters in Applied Microbiology, 41(3): 230-234.

Thelin, R., and Gifford, G. F., 1983. Fecal coliform release patterns from fecal material of cattle. Journal of Environment Quality, 12(1), 57-63.

Thurston-Enriquez, J. A., Gilley, J. E., and Eghball, B., 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. Journal of Water and Health, 3(2), 157-171.

U.S. EPA, 1984. Health effects criteria for fresh recreational waters. EPA 600/1-84-004, Washington, D.C.

U.S. EPA, 1986. Ambient water quality criteria for bacteria. EPA 440/5-84-002, Washington, D.C.

U.S. EPA., 2012. Water: Monitoring assessment. 5.11 Fecal bacteria. <http://water.epa.gov/type/rsl/monitoring/vms511.cfm>.

Van Horn, H. H., Wilkie, A. C., Powers, W. J., and Nordstedt, R. A., 1994. Components of dairy manure management systems. Journal of Dairy Science, 77(7): 2008–2030.

van Genuchten, M. Th. 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research Report No. 119, U. S. Salinity Laboratory, Riverside, CA, p. 88.

Whelan, G., Kim, K., Pelton, M. A., Soller, J. A., Castleton, K. J., Molina, M., Pachepsky, Y., Zepp, R., 2014. An integrated environmental modeling framework for performing quantitative microbial risk assessments. Environmental Modelling & Software, 55: 77-91.

Zejun, T., Tingwu, L., Qingwen, Z., and Jun, Z., 2002. The sealing process and crust formation at soil surface under the impacts of raindrops and polyacrylamide. 12th ISCO Conference, Beijing, China. pp 456-462.

Chapter 4 – Rainfall Intensity-dependent Release of Indicator Bacteria From Dairy Cattle Manure Applied on Vegetated Soil

Abstract

Simulating the rainfall-induced release of fecal indicator bacteria from manure is an essential component to microbial fate and transport modeling. The objectives of this work were to determine the effect of rainfall intensity on the release of three indicator bacteria – *Escherichia coli*, enterococci, and total coliforms – and a surrogate tracer, chloride ion, from manure and to test the performance of three kinetic-based models in describing their removal with runoff. Dairy cattle manure from a CAFO was applied over Kentucky 31 tall fescue in soil boxes (sandy loam; 100 x 35 x 15 cm). Three levels of rainfall intensity (3, 6, and 9 cm hr^{-1}) were applied and runoff and soil-leachate samples were collected at gradually increasing time intervals for one hour of release. Following rainfall, soil was sampled at 0, 2, 5, and 10 cm depths. The one-parametric exponential dependence model, the two-parametric Bradford-Schijven model, and the two-parametric Vadas-Kleinman-Sharpley model were fitted to the manure constituent runoff-removal curves. The ratio of the bacteria concentrations in initial runoff to their starting concentrations in manure was substantially lower than that of dissolved chloride. The dependency of cumulative bacteria runoff-removal on rainfall depth included a several orders of magnitude increase (approximately 10^4 to10^9 CFU, which was 0.001 to 30 % of the total mass) of the removed mass of bacteria during the initial 1 to 2 cm of rainfall, afterwards the mass removal rate drastically decreased and became closer to zero. Cumulative runoff-removal of chloride followed a similar two-stage process, although, unlike for the bacteria, the asymptote in

the "second stage" for chloride approached 1. Although rainfall intensity did not substantially affect most parameters for model fits, it significantly affected the content bacteria found at different depths of the soil profile after rainfall. Model performance, assessed with the root-mean-squared-error and the Akaike information criterion, was organism-dependent. The Vadas-Kleinman-Sharpley model performed better for total coliform runoff-removal, while the Bradford-Schijven model yielded the best simulation results for *E. coli* and enterococci runoff-removal.

Introduction

Animal manure is collected as a byproduct of livestock production and is applied to crop and range lands as a fertilizer to enhance soil quality (Sheldrick et al., 2003). Microbial release from land-applied manure is a critical component of microbial fate and transport as it governs the quantity of microbes available for transport that may cause environmental and food safety contamination. The quantities of land-applied microbes that are transported from manure-covered land into surface runoff or the quantities of microbes that move downward into the soil with infiltration depends on the quantities of bacteria released from the manure matrix. As released manure-bacteria are removed with surface runoff, the suspended colloids in runoff may interact with other parts of the manure matrix as well as with soil and vegetation. Thus, the presence and living condition of vegetation has an impact on microbial release via runoff-removal from land-applied manure (Dao et al., 2008; Guber et al., 2007). Microbial release from animal waste is also impacted by the animal source of manure (Soupir et al., 2003; Thurston-Enriquez, 2005), manure application rate (Brooks et al., 2007; Drapcho, 2003), manure age (Kress and Gifford, 1984), manure consistency (Soupir et al., 2003), and whether manure is surface-applied or incorporated into the soil (Forslund et al., 2011). Precipitation variability

impacts microbial release as well (Shijven et al., 2004; Thelin and Gifford, 1983). Further research investigating the impacts of these factors as well as the effects of rainfall intensity, longer duration rainfalls, and different vegetative covers have also been recommended (Ling et al., 2009).

Due to the relatively high cost of manure-borne pathogen research and the associated bio-safety concerns, non-pathogenic indicator microorganisms are often enumerated, simulated, and regulated as representive of potential fecal contamination to the environment (Meays et al. 2004; Panhorst, 2002; Savichtcheva et al., 2006). *Escherichia coli* and enterococci are the primary FIB used for environmental water quality regulation (Dufour and Ballantine, 1986; Guber et al., 2007). Use of these FIB's has been supported by the strong correlations that have been observed between elevated levels of these bacteria in recreational waters and occurrences of gastrointestinal disease (U.S. EPA, 1984).

Runoff-removal models for these manure-borne bacteria simulate the ratio of the concentration of bacteria in runoff, C_{Runoff}, to the initial concentration of bacteria in the manure, C_0, over time or rainfall depth. Like C_{Runoff}/C_0, the cumulative mass of microbes removed from manure with runoff, M_{Runoff}/M_0, may be simulated with the same models after accounting for bacterial masses rather than concentrations. While M_0 has an intrinsic value, C_0 is a subjective term that depends on how the concentration of bacteria in manure is defined. For instance, C_0 may be represented as bacteria content g^{-1} dry weight of manure, g^{-1} wet weight of manure, ml^{-1} total volume of manure, ml^{-1} liquid content of manure, or even ml^{-1} of runoff water. Three kinetic-based microbial release models that were used by Guber et al. (2006) to simulate the rainfall-induced release of fecal coliforms from bovine slurry to runoff are the one-parametric exponential model, the two-parametric Bradford-Schijven model (Bradford and Schijven, 2002),

and the two-parametric Vadas-Kleinman-Sharpley model (Vadas et al., 2004). These models have been used under the assumption that manure-bacteria were concentrated into the liquid portion of manure such that C_0 was defined by the bacteria content in manure divided by the manure water content. Results of these models have been reported as the concentration of bacteria removed in runoff over time to the concentration in the initial runoff from the manured area (i.e., C_{Runoff}/C_0) (Guber et al., 2006). The assumptions, that the content of bacteria in manure divided by the manure water content is the effective concentration in manure and that it corresponds to the concentration of bacteria in the initial portion of runoff from solid manure types, have not yet been verified for solid manure types. Furthermore, compared with microbial release from liquid-based manures and slurry, the release from solid manures (e.g., farmyard manure, litter) has a much weaker correlation with total rainfall and it appears to be a more complex, situation-specific process (Chapter 2). Data on C_0 and microbial runoff-removal kinetics for removal from solid manure types is lacking and would be beneficial to collect.

Rainfall intensity affects the total release of microorganisms from manure by providing more rainfall per unit time (Kress and Gifford, 1984; Schijven et al., 2004) as does an increase in rainfall duration at a constant intensity per increase in unit time (Ling et al., 2009; Thelin and Gifford, 1983). The effects of rainfall intensity on microbial runoff-removal kinetics and the parameters for models that simulate C_{Runoff}/C_0 or M_{Runoff}/M_0 as a function of rainfall depth are unknown. Rainfall causes compaction, slackening, detachment, and deposition of soil, all of which are actions that contribute to the formation of a seal and potential crust that may significantly reduce infiltration and increase surface runoff (Zejun et al., 2002). Based on results from rainfall simulations at varying intensities from 5-16 cm hr^{-1} over various soils, Sharpley et al. (1985) reported rainfall to positively impact the effective depth of interactions between

surface soil and runoff. If a surface seal were to form at the soil, at the manure, or even at the soil-manure interface during rainfall, microbial release from manure and removal with runoff may be impacted. A soil-seal formation would enhance released material to flow with surface runoff rather than infiltrate soil, while a manure-seal could protect microbes from release. Microbial fate and transport models consider microbial release to be dependent on rainfall depth and not on rainfall intensity. If rainfall intensity were to have positive effects on microbial release from manure and runoff-removal, then parameters used in current models may be underestimating the potential loss of bacteria especially for high-intensity rainfall events.

Furthermore, the refinement of current kinetic-based models that simulate microbial runoff-removal from solid manure requires better descriptions of the ratio of $C_{Initial\ Runoff}/C_{Manure}$, the clarification of the effects of rainfall intensity on manure constituent total release and runoff-removal, and more information concerning runoff-removal model parameters for different manure constituents during rainfall events. The objectives of this work were to determine the effects of rainfall intensity on the release of *E. coli*, enterococci, total coliforms, and chloride ion from dairy cattle manure and to test the performance of three kinetic-based models in describing the observed runoff-removal of the indicator bacteria.

Methods

A variable-intensity rainfall simulator (Meyer and Harmon, 1979) was used to induce the release of manure-constituents from dairy cattle manure that was collected from a concentrated animal feeding operation (CAFO) and applied over Kentucky 31 tall fescue in soil boxes.

Soil box preparation

The soil boxes (100 x 35 x 15 cm) were designed, based on the work of Isensee and Sadeghi (1999) and Sadeghi and Isensee (2001), to be packed with soil and seeded with fescue. Eighteen soil boxes were built, and each box was equipped with one height-adjustable runoff drain (10 mm diam.) positioned at the front of the box and three infiltration drains (6 mm diam.) positioned at the center of the base at 1 cm, 34 cm, and 67 cm up from the front of the box. A mesh screen (1 mm^2 openings) was set over each infiltration drain hole in order to help prevent drain clogging during the transmission of leachate. Two aluminum angle partitions (14 mm height) were attached to the base of each box directly in front of the 34- and 67-cm base drains to aid in the collection of leachate from each section of a box. The soil box design is illustrated in Fig. 4.1.

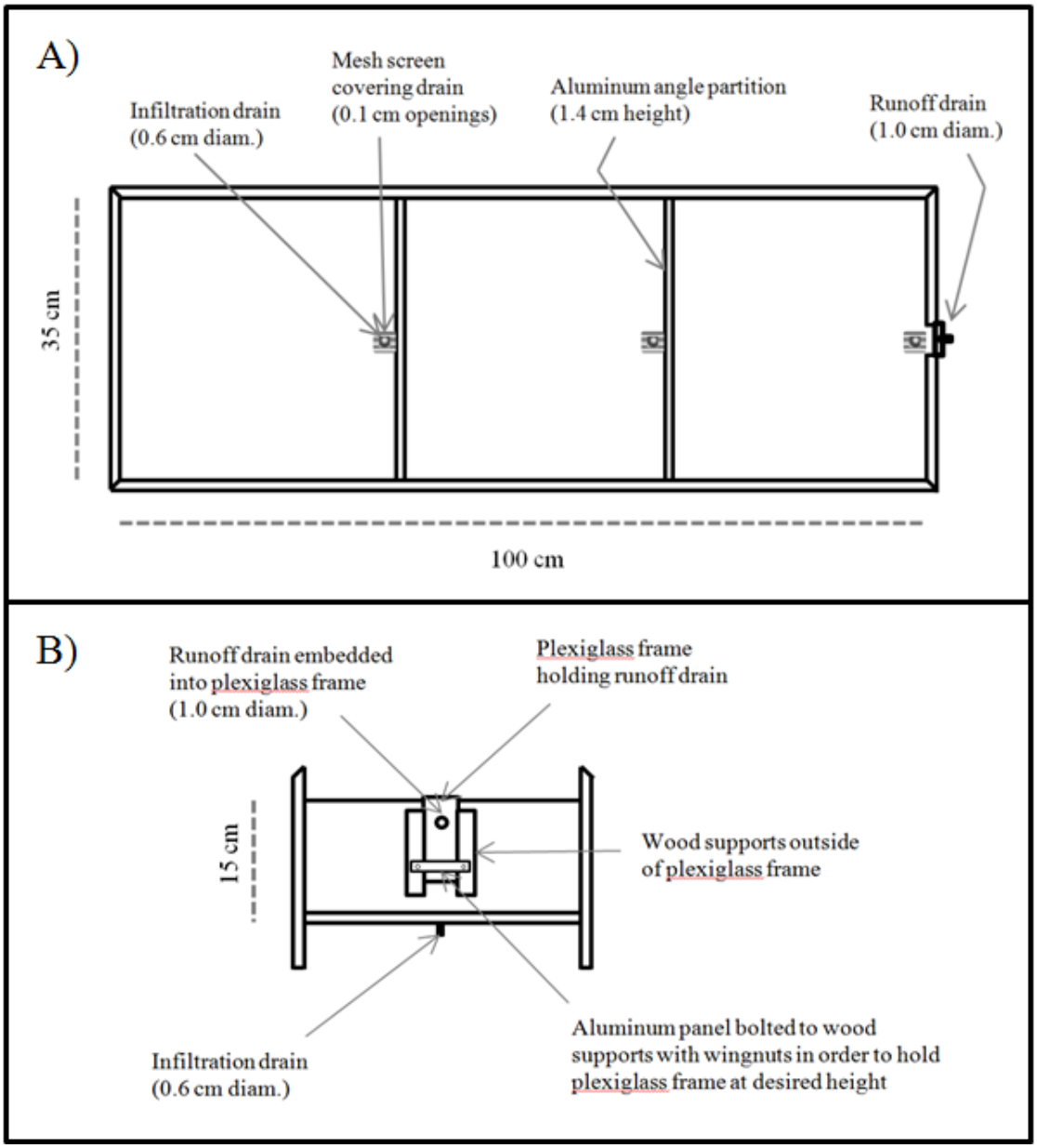

Figure 4.1 Top (A) and frontal (B) view of soil box with inside dimensions of 100 x 35 x 15 cm.

Packing the soil boxes was performed in layers in order to create uniform bulk density and soil conditions throughout the depth of soil within each box (Isensee and Sadeghi, 1999; Sadeghi and Isensee, 2001) (Fig. 4.2). Each box received an initial layer of 7 kg of air-dried sand (≤ 2mm) that was spread across the base of the box, packed down with a 20 x 20 cm plywood board, and then lightly grooved at the surface with a hand cultivator tool. Over the sand layer, a 7 kg layer of air-dried sandy loam soil (a mixture of various USDA-ARS Beltsville A horizons of no single soil series), that had been screened for rocks and gravel, was poured into the box, spread evenly, packed flat with the plywood board, and then scored at the surface. Six more layers of topsoil were added with this method. Each fully-packed soil box contained 1 layer of sand at the base and 7 layers of topsoil; thus, the total mass of soil packed into each box was 56 kg. A description of the physicochemical properties of the topsoil used in this work is provided in Table 4.1.

Figure 4.2 Soil box packing process – left, empty box; middle, pouring layer of topsoil over the base layer of sand; right, grooving the soil surface (after having packed it flat with a board) to prep it before pouring on the next topsoil layer.

Table 4.1 Properties of topsoil used in packing the soil boxes. All analyses were performed by the Penn State Agricultural Analytical Services Laboratory.

Soil Component	*Value*
Sand	63.8 %
Silt	24.8 %
Clay	11.4 %
[1]pH	7.0
[2]Soluble Salts	0.36 (mmhos cm^{-1})
[3]Total Carbon	2.23 %
[3]Total Nitrogen (N)	0.20 %
[4]Ammonium-N	1.25 ppm
[4]Nitrate-N	63.6 ppm

[1]1:1 (soil:water) method; [2]1:2 (soil:water) method; [3]combustion method; [4]electrode method

Packed soil boxes were placed in a temperature-controlled hoop house set to operate at 18^0 C located at the USDA-ARS North Farm in Beltsville, MD. The soil in each box was watered and cross-scored at the surface; then, Kentucky 31 tall fescue grass seed was applied to each box at the rate of 49 g m^{-2} (i.e., 10 lbs 1000 ft^{-2}). Kentucky 31 had been chosen as the fescue for this research since it has been previously used in microbial release research studies (Edwards et al., 2000; Sistani et al., 2009). Each seeded box was watered twice a day until grass germination and once a day following germination. After 20 days of grass growth, the soil boxes were over-seeded at the rate of 49 g m^{-2}, and then 2 kg of topsoil were added as cover to the newly added seed. Daily watering continued up until germination of the over-seeded grass, after which time the watering frequency was reduced to once every 2-3 days. In total, there was 58 kg of soil in each box that resulted in an estimated bulk density of 1.34 ± 0.07 g cm^{-3}. After settling, the height-adjustable runoff drains were fastened into place (Fig. 4.1) and sealed with silicone. The grass blades were trimmed to a height of 7.5 cm on a biweekly basis and on the day before each rainfall simulation event with a hand-held turf trimmer (model gs500; Back and Decker,

Towson, Maryland), and excess trimmings were removed. The soil boxes were kept in the hoop house up until experimentation (Fig. 4.3).

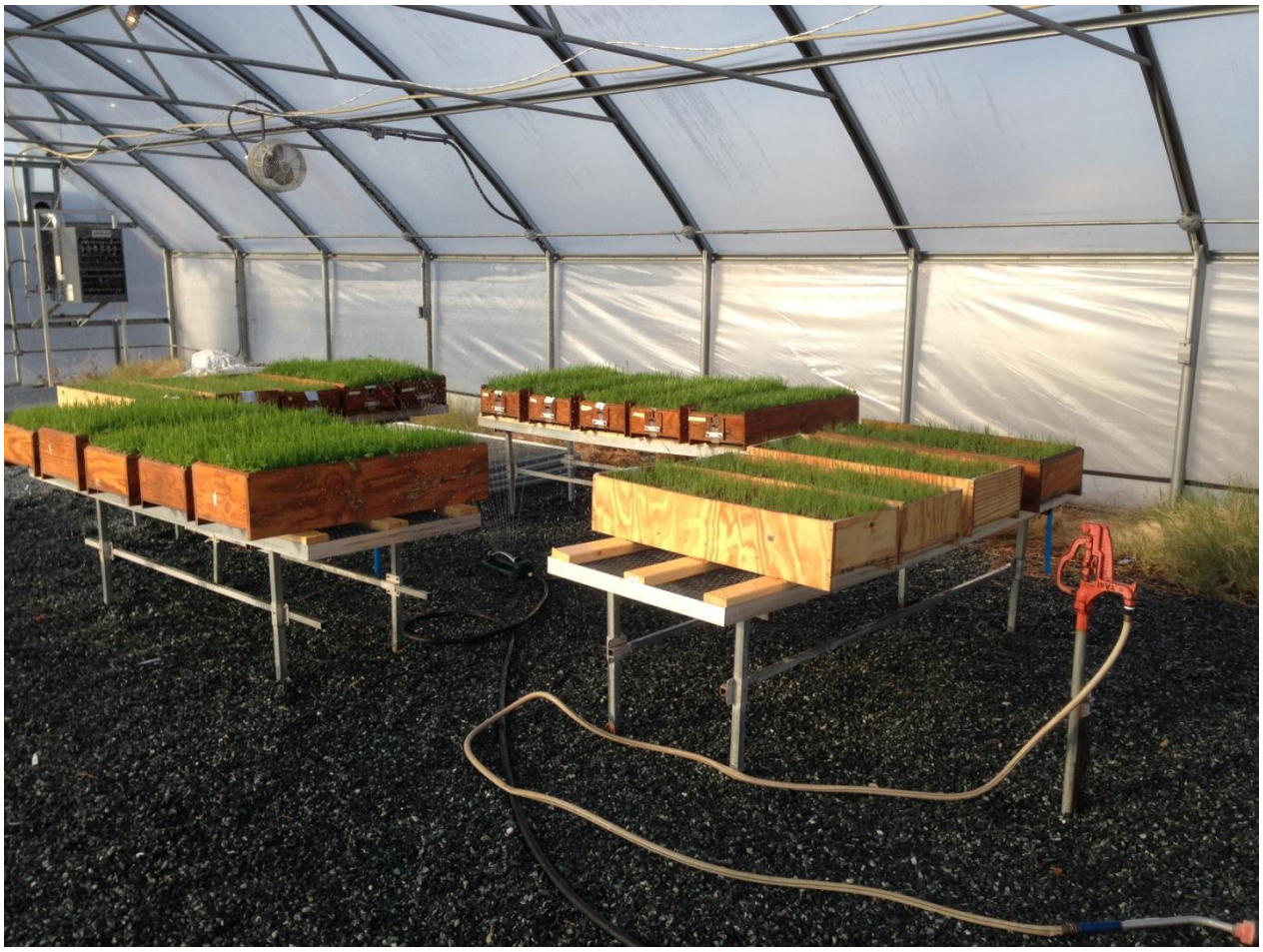

Figure 4.3 Kentucky 31 tall fescue growing in soil boxes (sandy loam soil) in a temperature-controlled hoop house (set at 18^0 C) at the USDA-ARS North Farm in Beltsville, MD. In the photo, the boxes can be seen to be watered by a sprinkler positioned in the center of the hoop house tables.

Manure and rainwater composition

The manure used in this study was obtained from dairy cattle at a CAFO at the USDA-ARS Dairy Research Facility, in Beltsville, MD. At this cattle feeding operation, the 2-5 year old dairy cattle are provided a corn silage-based TMR (total mixed ratio) diet in a free-stall barn. Based on the characterization of manure components by Van Horn et al. (1994), synthetic

manure was prepared to represent the farmyard manure that is produced at dairy CAFOs and applied to cropland as fertilizer. Cattle feces and urine were sampled from 5 different cows in disinfected 5 gallon buckets, stirred in their respective buckets, and then mixed together at a 6/1 ratio of feces/urine (volumetric) to prepare the synthetic manure. This manure mixture was stored at 4^0 C until usage. On the morning of each rainfall simulation event, the synthetic manure was mixed with sawdust bedding to bring the dry solid content of manure up to approximately 30 %. New manure was collected and prepared each week to ensure high concentrations of indigenous FIB, and the constant mixing ratio was used to control for manure structure. Composite manure samples were collected just before each rainfall simulation event to obtain the average physical, chemical, and microbial contents of the manure that was used throughout the study (Table 4.2). The composite manure samples were also analyzed for plant macro- and micro-nutrients in order to demonstrate the quality of the CAFO manure as a useful fertilizer (Appendix A).

Table 4.2 The physical properties, chemical properties, and microbial contents of the dairy cattle manure measured from composite manure samples that were collected on each morning of experimentation. The "±" separates average and standard deviation.

Manure Properties and Microbial Contents	
Solid Mass	29.6 ± 2.7 %
Wet Mass	70.4 ± 2.7 %
[1]pH	8.25 ± 0.16
[1]Carbon (C)	14.9 ± 1.0 %
[1]C:N ratio	40.9 ± 5.6
Total Coliforms	$6.78 \pm 4.66 \times 10^5$ CFU g^{-1}
Escherichia coli	$5.30 \pm 4.24 \times 10^5$ CFU g^{-1}
Enterococci	$3.81 \pm 1.64 \times 10^6$ CFU g^{-1}

[1]Analyses that were performed by the Penn State Agricultural Analytical Services Laboratory.

Rainwater was prepared to mimic the ion content and pH standard for rainfall in the Maryland, Pennsylvania, and Delaware region. The synthetic rainwater was made by adding reagent-grade chemicals to reverse-osmosis water to set concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, NH_4^{+}, NO_3^{-}, Cl^{-}, and SO_4^{2-} at 0.08, 0.03, 0.02, 0.12, 0.34, 1.36, 0.26, and 1.9 mg L^{-1}, respectively (Green et al., 2007; Dao et al. 2008). The rainwater solution was mixed in 500 gal holding tanks and pumped into a 100 gal tank that was connected to the rainfall simulator. Just before rainfall, the pH of the rainwater solution in the 100 gal tank was adjusted to 4.5 using necessary additions of dilute HCl and/or NaOH.

Rainfall simulations

Treatments followed a 3 x 2 factor design with three rainfall intensities (3, 6, and 9 cm hr^{-1}) and either manure application or no manure application (control). Rainfall intensity was calibrated on the rainfall simulator by setting time intervals for pauses between nozzle oscillation sweeps (see: Chapter 3, Fig. 3.2). The sprinkler nozzles (Veejet 80150; Spraying Systems Co., Wheaton, IL) were positioned to rain from a height of 3 m above the soil boxes so the raindrops could approach terminal velocity upon landing. The pressure of water flowing into the rainfall simulator was maintained at 41 N m^{-2} (i.e., 6 psi) to control rainfall intensity and distribution during each rainfall simulation event. This rain simulator design allowed for raindrop impact energy to be approximately 275 kJ/ha-mm, which is about the same for a natural rainfall event with rainfall intensity greater than 2.5 cm hr^{-1} (Meyer and Harmon, 1979). A full description of the rain simulator is provided in Meyer and Harmon (1979). All six treatments were performed in triplicate in a randomized order and all rainfall simulations took place indoors to avoid confounding effects of wind or sunlight.

Antecedent water contents of soil in each box were established with a pre-wetting rainfall simulation event for 30 minutes at an intensity of 3 cm hr^{-1} that occurred 24 hours before the scheduled rainfall events within the study. For the manure application treatments, manure was applied over the grass in each box at the rate of 60 ton ha^{-1} wet weight (i.e., 2.1 kg box^{-1}). Three samples of manure were collected from each box prior to rainfall to analyze for the initial contents of the manure constituents in the land-applied manure. One disinfected hose of PVC vinyl tubing was connected to the runoff drain and another was connected to the three infiltration drains. The fully prepped box was positioned on a 5 % slope underneath the rainfall simulator and rainfall was initiated. For the control treatments, no manure was applied, and the boxes were set up and rained on the same way as previously described.

During rainfall, surface runoff and soil leachate were collected from the respective drain hoses in sterile 100 ml bottles upon initial runoff (time 0) and then subsequently at 1, 2, 4, 7, 10, 15, 20, 30, 40, 50, and 60 minutes. A photograph of a rainfall simulation event is shown in Fig. 4.4. All collection reference times and the duration time for each collection were recorded. Following rainfall, soil cores (25 mm diam.) were taken at 10, 30, 50, 70, and 90 cm up from the front of the box and the soil core samples were divided at 0-2cm, 2-5 cm, and 5-10 cm depths. The cores taken at 10 and 90 cm were used for water content analysis, while those collected at 30, 50, and 70 cm were used for microbial and chemical analyses. All samples were stored on ice in a cooler between the time of collection and laboratory analysis.

Figure 4.4 Photograph of a rainfall simulation event (left) and soil sample collection (right).

Microbiological and chemical analyses

The water contents of manure and soil samples were measured by calculating water loss after samples were dried in an oven at 100^0 C for 24 h to a constant dry weight. Wet manure and soil samples were each blended with sterile deionized water (2 g sample 200 ml^{-1} water) using a high speed blender for 2 minutes (model 34BL97; Waring Laboratory, Torrington, CT) to produce a homogenous slurry mixture. Slurry was allotted 1 hr of settling time before processing. The manure and soil slurries and the runoff and leachate samples were spread-plated on CHROMagarTM ECC (Chromagar, Paris, France) to enumerate *E. coli* and total coliforms and on m-Enterococcus agar (Neogen Corporation, Lansing, MI) to enumerate enterococci. The

CHROMagarTM ECC plates were incubated at 37^0 C for 24 hours and blue colony forming units (CFUs) were reported as *E. coli* and mauve CFUs were reported as coliforms that were not *E.coli*. Thus, the total coliform CFUs were the sum of the blue and mauve colonies on this agar. The m-Enterococcus agar plates were incubated at 37^0 C for 48 hours and red CFUs were reported as enterococci. Chloride ion content of each sample was measured with the QuantiChromTM Chloride Assay Kit (Abnova, Taipei, Taiwan).

Runoff-removal modeling

For each rainfall simulation event, the concentrations of *E. coli*, enterococci, total coliforms, and Cl^- in chronologically collected runoff and infiltration samples were multiplied by the water flux , based on the resepective effluent flow rate, and the products were integrated to quantify the cumulative numbers of bacteria and cumulative cloride ion masses released into each effluent type. These cumulative numbers and masses were converted into relative ratio values M_{Runoff}/M_0 by dividing the quantity in runoff by the initial total quantity of bacteria for each bacteria group. Dependency of M_{Runoff}/M_0 of each of the bacteria groups/species and Cl^- on time and on rainfall depth were simulated using the following three microbial runoff-removal models:

1. The exponential release dependence equation that is used in the watershed-scale HSPF model for microbial fate and transport (Benham et al., 2006; Moyer and Hyer, 2003):

   Eq. 1. $$\frac{M_{Runoff}}{M_0} = 1 - e^{(-k_e W)}$$

   where M_{Runoff} is total number of bacteria or Cl mass removed per unit area of manure with runoff, $[M_{Runoff}]$ = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is initial total number or mass per unit area of applied manure, $[M_0]$ = CFU (for bacteria) or mg (for Cl^-); k_e is the rate constant parameter, $[k_e] = cm^{-1}$ (for removal dependency on rainfall depth) or min^{-1} (for removal dependency on time); W is rainfall depth or minutes of after rainfall started,

and [W] = cm rainfall (for removal dependency on rainfall depth) or min (for removal dependency on time)

2. The Bradford and Schijven (2002) equation that is used in the farm-scale STWIR microbial fate and transport model (Guber et al., 2009; Kim et al., 2014):

Eq. 2. $$\frac{M_{Runoff}}{M_0} = 1 - \frac{1}{(1+k_p \beta W)^{\frac{1}{\beta}}}$$

where M_{Runoff} is total number of bacteria or Cl mass removed per unit area of manure with runoff, [M_{Runoff}] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is initial total number or mass per unit area of applied manure, [M_0] = CFU (for bacteria) or mg (for Cl^-); k_p is the rate constant parameter, [k_p] = cm^{-1}, W is rainfall depth or minutes of rainfall, [W] = cm rainfall (for removal dependency on rainfall depth) or min rainfall (for removal dependency on time); and β is a dimensionless shape parameter.

3. The Vadas et al. (2004) equation, which was originally developed to describe inorganic phosphorus loss in runoff from surface-applied dairy, poultry, and swine manure, and now may be used to predict contaminant release (e.g., microbial indicators and pathogens) in the watershed-scale SWAT model (Benham et al., 2006):

Eq. 3. $$\frac{M_{Runoff}}{M_0} = AW^n$$

where M_{Runoff} is total number of bacteria or Cl mass removed per unit area of manure with runoff, [M_{Runoff}] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is initial total number or mass per unit area of applied manure, [M_0] = CFU (for bacteria) or mg (for Cl^-); A is the rate constant parameter, [A] = cm^{-n} (for removal dependency on rainfall depth) or min^{-n} (for removal dependency on time); W is rainfall depth or minutes of rainfall, [W] = cm rainfall (for removal dependency on rainfall depth) or min (for removal dependency on time); and n is a dimensionless parameter.

Eq. 1, 2, and 3 were fitted to the 'rainfall depth-removal with runoff' data and the 'rainfall time-removal with runoff' data using a FORTRAN code REL_BACT, which was based

on the Marquardt-Levenberg optimization algorithm as implemented in van Genuchten (1981) (Appendix C).

Data Analysis

A two-factor ANOVA was used to assess the effects of rainfall intensity and manure application on runoff partitioning.

Concentrations of manure constituents released into initial surface runoff were compared with their starting concentrations in manure. An ANOVA was used to evaluate the effects of rainfall intensity on microbial and Cl^- runoff removal model parameters. An ANOVA was also used to test for differences in removal model parameters for the different manure constituents in the study. The probability of difference in runoff-removal kinetics of *E. coli* and enterococci was determined with a two-tailed Student's t-test with α set at 0.05.

Runoff-removal model performance for the three kinetic models was assessed by the root-mean-squared-error (RMSE) and the Akaike information criterion (AIC) values that were computed from each model fit. RMSE were computed as:

$$\mathrm{RMSE} = \sqrt{\frac{\mathrm{RSS}}{\mathrm{n}}}$$

where RSS is the residual sum of squares and *n* is the number of measurements. The RMSE units are dimensionless. The expectation was that the preferred model would have smaller RMSE.

The Akaike information criterion (AIC) provides a means for model selection and accounts for the interplay between the model goodness of fit and the complexity of the model (Burnham and Anderson, 2002). In this study, the AIC test considers that Eq. 1, 2, and 3 have a

different number of parameters (one, two, and two, respectively). The corrected Akaike statistic is:

$$AIC = nln\left(\frac{RSS}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1}$$

where RSS is the residual sum of squares, *n* is the number of measurements, and *k* is the number of model parameters.

The AIC units are dimensionless. Of the three models, the one that performed the best would be expected to have the smaller corrected Akaike statistic.

To determine the efficiency of Cl^- as a surrogate for bacteria removed with runoff, the correlations between bacteria concentrations in surface runoff and Cl^- concentrations in surface runoff were determined. The Steiger's Z-test (Steiger, 1980), which is used to test whether one predictor (e.g., Cl^-) correlates equally with two criterion variables (e.g., *E. coli* and enterococci), was used to compare the correlations that the different bacteria concentrations had with Cl^- concentration in the surface runoff.

Physical straining of bacteria from soil leachate was assessed by comparing concentrations of bacteria measured in synchronous runoff and leachate and also by measuring the content of bacteria at different soil depths following rainfall. A two-factor ANOVA was used to evaluate the effects of rainfall intensity and soil depth on the concentrations of bacteria remaining within the soil at specific profile depths following a specific simulated rainfall event.

The total number of bacteria released from manure was estimated by adding the total quantities collected in the runoff (M_{Runoff}), the total quantities collected in the leachate ($M_{Leachate}$), and the total quantities remaining in the soil after rainfall (M_{Soil}). The quantities of bacteria remaining at the 0-2, 2-5, and 5-10 cm soil depths after rainfall was estimated by taking the

average concentration of bacteria at the respective depth (CFU gdw^{-1}) and multiplying it by the mass of soil in the box at the respective depth (i.e., soil depth (2, 3, or 5 cm) x box length (100 cm) x box width (35 cm) x average bulk density for soil (g cm^{-3})). The numbers of bacteria remaining in the soil at 0-2, 2-5, and 5-10 cm depths was added together to estimate M_{Soil}. The M_{Soil}, M_{Runoff}, and $M_{Leachate}$ quantities were added together to compute the total quantities of bacteria released from manure ($M_{Release}$). The relative percent release of each manure constituent during rainfall was equal to $M_{Release}/M_{Manure}$.

Results

Runoff partitioning as influenced by surface seal

In all treatments the majority of rainwater was recovered as runoff as opposed to leachate. Application of manure to the soil boxes had a positive effect on the amount of rainwater that was recovered as runoff (Fig. 4.5). For the control and the manure application treatments, as rainfall intensity increased, the amount of water recovered as runoff increased (Fig. 4.5). A two-factor ANOVA showed that both rainfall intensity ($p=0.050$) and manure application ($p=0.013$) both had significant effects on runoff partitioning and that the interactions of these factors on runoff partitioning were substantial (0.239).

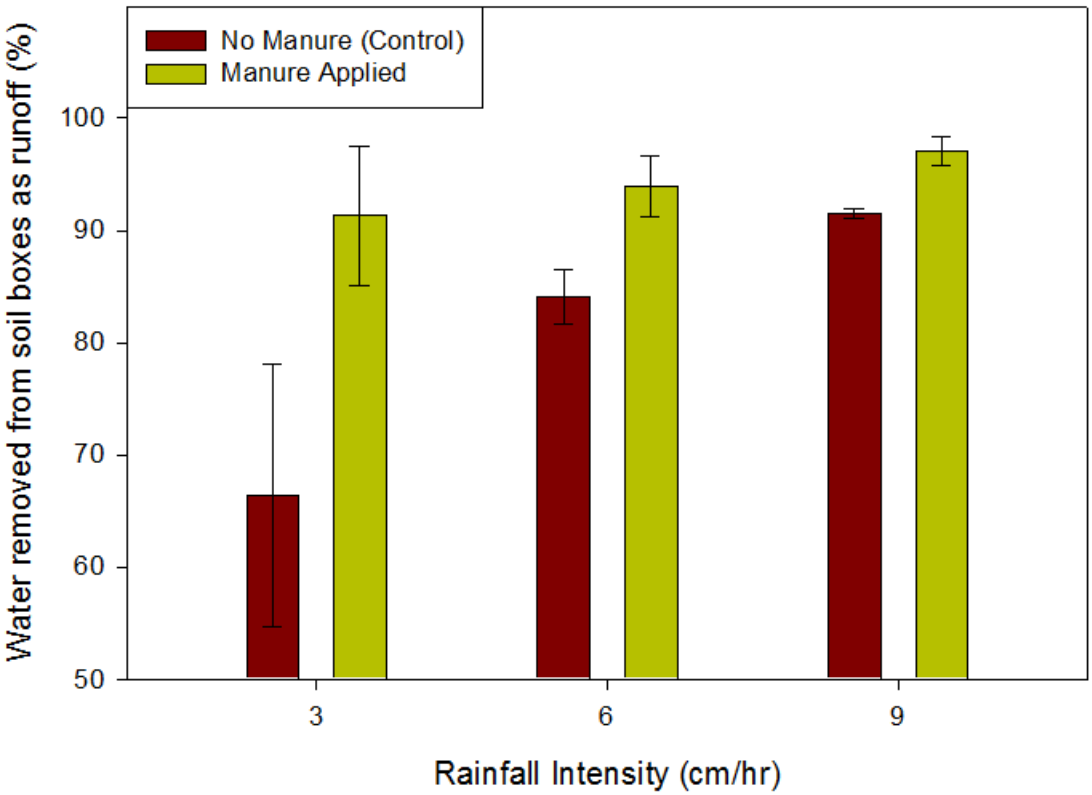

Figure 4.5 Percent of applied rainwater that was recovered as runoff (as opposed to infiltration) during the 3, 6, and 9 cm hr^{-1} rainfall events on the control and manure application soil boxes.

Background content of bacteria in soil

While control treatment samples analyzed did not contain *E. coli* and enterococci, total coliforms were measured in the control soil at concentrations from 10^4 to10^5 CFU gdw^{-1} and in the control runoff and leachate at concentrations that exceeded 10^5 CFU ml^{-1}.

Initial surface runoff concentrations versus initial contents in manure

The average concentrations of *E. coli*, enterococci, and total coliforms that were released from manure into the initial surface runoff were 1.9 x 10^5, 7.0 x 10^4, and 3.2 x 10^5 CFU ml^{-1}, respectively (Table 4.2). The concentrations of *E. coli* and enterococci in initial runoff were more than one order of magnitude below their starting concentrations in the manure (i.e., $C_{Initial}$

$_{Runoff}/C_{Manure}$) (Table 4.3). The $C_{Initial\ Runoff}/C_{Manure}$ for total coliforms was more than twice that of *E. coli* and enterococci, but this difference may be attributed to the background content of total coliforms in soil that had contributed to the measured value in runoff samples. The average concentrations of Cl^- released into the initial surface runoff was 125 ppm and the $C_{Initial\ Runoff}/C_{Manure}$ for this dissolved anion was substantially greater than *E. coli* and enterococci (Table 4.3).

Table 4.3 Concentration of bacteria and chloride ion in initial runoff compared with their concentrations in manure. The "±" separates average and standard deviation.

Manure Constituent	$\frac{\text{Content in manure}}{\text{Manure water content}}$ (i.e., C_{Manure})	Concentration in initial runoff (10^5 CFU/ml) (i.e., $C_{Initial\ Runoff}$)	$\frac{C_{Initial\ Runoff}}{C_{Manure}}$
Total Coliforms	$9.95 \pm 1.93 \times 10^5$ CFU ml^{-1}	$1.91 \pm 0.29 \times 10^5$ CFU ml^{-1}	19.2 %
Escherichia coli	$7.87 \pm 1.65 \times 10^5$ CFU ml^{-1}	$0.70 \pm 0.22 \times 10^5$ CFU ml^{-1}	8.9 %
Enterococci	$52.5 \pm 26.0 \times 10^5$ CFU ml^{-1}	$3.19 \pm 1.07 \times 10^5$ CFU ml^{-1}	6.1 %
Chloride ion	0.589 ± 0.193 mg ml^{-1}	0.125 ± 0.072 mg ml^{-1}	21.3 %

Runoff-removal kinetics model performance

The kinetics for the runoff-removal of *E. coli* and enterococci was initially a precipitous log-linear increase in cumulative mass removed during the first 1 to 2 cm of rainfall with approximately 10^4 to10^9 CFU which comprised 0.001 % to 30 % of the total mass. After the initial increase, the mass removal rate drastically decreased and became closer to zero (Fig. 4.6). The Cl^- followed a similar runoff-removal dependency on rainfall depth although its cumulative runoff-removal asymptote approached 100 % while the cumulative runoff-removal of the indicator bacteria only approached about 60 % (Fig. 4.6).

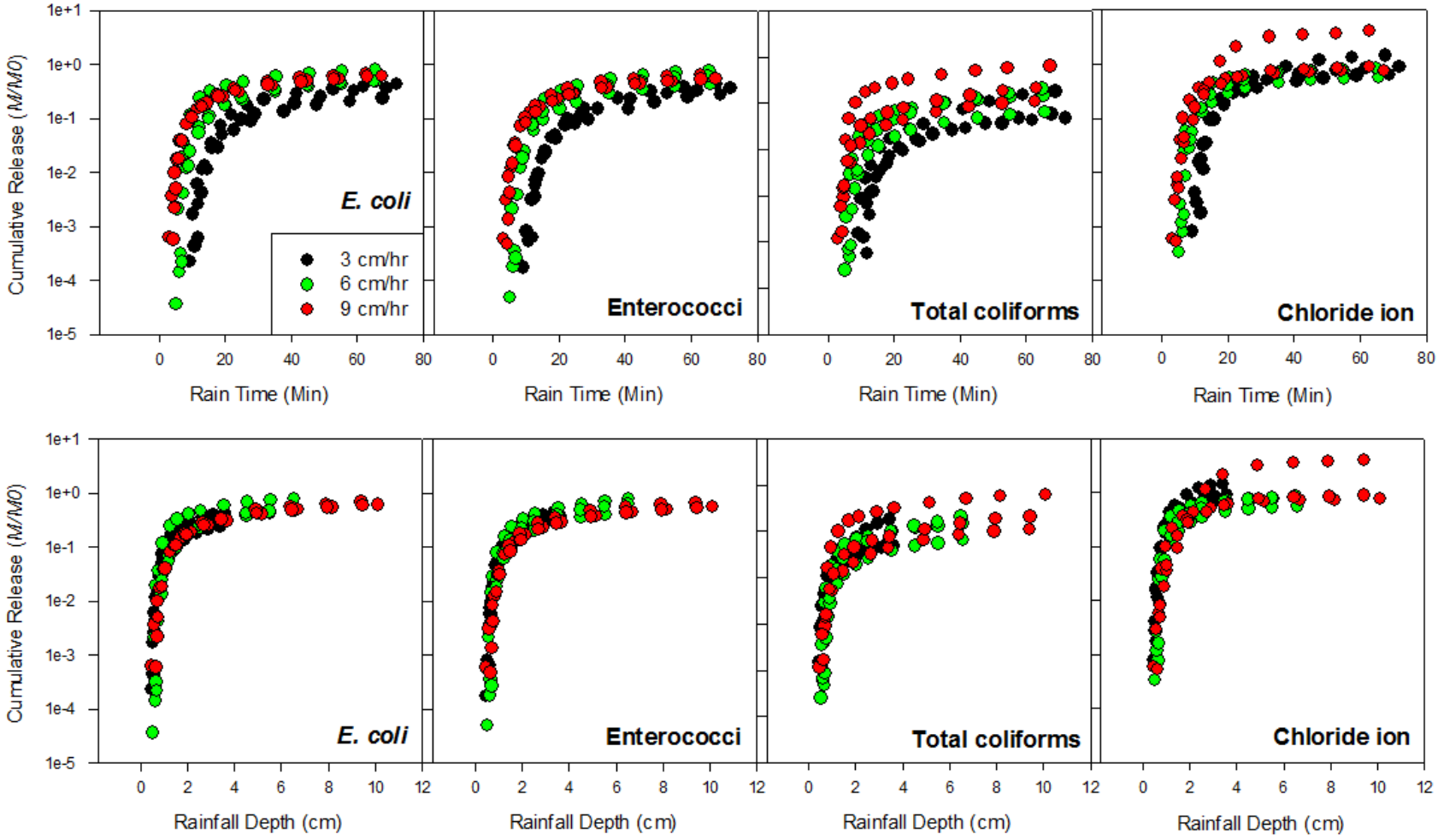

Figure 4.6 Relative cumulative mass of *E. coli*, enterococci, total coliforms, and chloride ion removed from manure with surface runoff as a function of time (top) and rainfall depth (bottom) for rainfall intensities of 3 (black circles), 6 (green circles), and 9 (red circles) cm hr^{-1}.

The total number of bacteria and Cl^- mass removed from manure into manure related to the initial values in M_{Runoff}/M_0 increased with the total volume of rainwater. The dependency of M_{Runoff}/M_0 on time differed for the 3, 6, and 9 cm hr^{-1} rainfall simulation events and was likely because the soil boxes received more water per unit time. However, the kinetics for the dependency of M_{Runoff}/M_0 on rainfall depth did not appear to be affected by rainfall intensity. In fact, according to an ANOVA (α=0.05), rainfall intensity did not have significant effects on any of the manure-constituent runoff-removal model parameters except for the parameter *n* in Eq. 3 and the significant effect was only for enterococci (Table 4.4).

Table 4.4 Results from an ANOVA (p-values are listed) for the effect of rainfall intensity on the parameters Eq. 1, 2, and 3 that simulated the runoff-removal of manure constituents dependent on rainfall depth.

Model	Parameter; Units	*E. coli*	Enterococci	Total coliforms	Chloride ion
Eq. 1	k_e; cm^{-1}	0.567	0.737	0.504	0.287
Eq. 2	k_p; cm^{-1}	0.561	0.402	0.674	0.311
Eq. 2	β; none	0.943	0.439	0.430	0.548
Eq. 3	A; cm^{-1}	0.692	0.588	0.498	0.391
Eq. 3	n; none	0.155	0.011	0.288	0.516

Since there were four manure constituents in this study (i.e., *E. coli*, enterococci, total coliforms, and Cl^-), three manure-application treatments (i.e., 3, 6, and 9 cm hr^{-1}), and five model parameters generated for runoff-removal (i.e., parameters from Eq. 1, Eq. 2, and Eq. 3), there were a total of 60 different parameters that were generated to describe the manure-constituent removal. Runoff-removal model parameters substantially differed amongst the *E. coli*, enterococci, total coliforms, and Cl^- (Table 4.5).

Table 4.5 Results from an ANOVA (p-values are listed) for differences in *E. coli*, enterococci, total coliforms, and chloride ion runoff-removal model parameters at each rainfall intensity. Lower p-values indicate substantial differences in model parameters among the manure-constituents at the given rainfall intensity.

Model	Parameter; Units	3 cm hr^{-1}	6 cm hr^{-1}	9 cm hr^{-1}
Eq. 1	k_e; cm^{-1}	0.099	0.126	0.295
Eq. 2	k_p; cm^{-1}	0.338	0.381	0.107
Eq. 2	β; none	0.230	0.053	0.004
Eq. 3	A; cm^{-1}	0.017	0.239	0.346
Eq. 3	n; none	0.153	0.178	0.664

Since *E. coli* and enterococci are the two leading FIB's (Dufour and Ballantine, 1986; Guber et al., 2007), their runoff-removal curve-fits were specifically compared at each of the applied rainfall intensities using the two-tailed Student's t-test. There was a moderate probability of similarity between the parameters generated for the *E. coli* and enterococci runoff-removal dependencies and, on average, the probability of similarity decreased as rainfall intensity increased (Table 4.6). Thus, kinetics for the removal of *E. coli* were similar, but as rainfall intensity increased, the differences in their removal kinetics become more apparent.

Table 4.6 Probability of similarity in runoff-removal model parameters generated for *E coli* and enterococci, during rainfall at 3, 6, and 9 cm hr^{-1}.

Model	Parameter; Units	3 cm hr^{-1}	6 cm hr^{-1}	9 cm hr^{-1}
Eq. 1	k_e; cm^{-1}	0.931	0.538	0.370
Eq. 2	k_p; cm^{-1}	0.877	0.820	0.156
Eq. 2	β; none	0.503	0.400	0.907
Eq. 3	A; cm^{-1}	0.959	0.414	0.097
Eq. 3	n; none	0.680	0.534	0.635
Average		0.790	0.541	0.433

Runoff-removal model performance basedd on the RMSE and AIC associated with each curve-fit was organism-dependent. The Vadas-Kleinman-Sharpley model (Eq. 3) performed

better for simulating total coliform removal, while the Bradford-Schijven model (Eq. 2) yielded the best simulation results for *E. coli* and enterococci removal (Table 4.7). The Bradford-Schiijven model also performed best for the Cl^- removal simulations (Table 4.7).

Table 4.7 Average and standard deviation for the root-mean-squared-error (RMSE) and Akaike information criterion (AIC) from fitting Eq. 1, 2, and 3 to the manure constituent runoff-removal curves. The lowest RMSE and most negative AIC values, which represent the best model, are displayed in bold font for each manure-constituent.

Manure Constituent	Model	RMSE	AIC
E. coli	Eq. 1	0.023 ± 0.009	-90.4 ± 8.8
	Eq. 2	**0.011 ± 0.005**	**-104.3 ± 9.9**
	Eq. 3	0.025 ± 0.014	-86.4 ± 13.1
Enterococci	Eq. 1	0.017 ± 0.007	-97.1 ± 10.2
	Eq. 2	**0.009 ± 0.004**	**-109.4 ± 9.7**
	Eq. 3	0.022 ± 0.010	-89.4 ± 12.1
Total coliforms	Eq. 1	0.486 ± 0.880	-45.6 ± 44.0
	Eq. 2	0.177 ± 0.182	-62.3 ± 46.8
	Eq. 3	**0.048 ± 0.056**	**-78.3 ± 21.7**
Chloride ion	Eq. 1	0.249 ± 0.530	-58.6 ± 32.2
	Eq. 2	**0.046 ± 0.066**	**-80.6 ± 21.4**
	Eq. 3	0.103 ± 0.124	-57.4 ± 17.1

Concentrations of bacteria and chloride ion in surface runoff

Each gram of wet manure contained approximately 10^6 CFU of each bacteria group/species (Table 4.1) as well as 0.589 ± 0.193 mg of Cl^-. The correlation coefficients for the relationship between concentration of bacteria (CFU ml^{-1}) and Cl^- (ppm) in the runoff samples were 0.500, 0.615, 0.595 for total coliforms, *E. coli*, and enterococci, respectively (n=107). All the bacteria indicators had concentrations in runoff that were positively correlated with the Cl^- concentrations ($p<0.001$). Although the positive correlation was significant, the positive releationship was very weak. Linear regression showed that the variability of concentrations of

total coliforms, *E. coli*, and enterococci in runoff could only be explained by the concentrations of Cl^- in the runoff 25.0 %, 37.8 %, and 35.4 % of the time, respectively (Fig. 4.7). Since the correlations of Cl^- with bacteria in runoff was significant, Cl^- may still be useful as a surrogate tracer for bacteria released from the manure into runoff, but when used in this manner care should be taken because this relationship is weak.

Application of the Steiger's *Z*-test (Steiger, 1980) showed that the correlations of *E. coli* and total coliform concentrations with the concentrations of Cl^- in surface runoff were substantially different from one another ($p=0.064$). The correlations of *E. coli* and enterococci concentrations with the concentrations of Cl^- in surface runoff were moderately different ($p=0.387$). The correlations of total coliform and enterococci concentrations with the concentrations of Cl^- in surface runoff were not different (0.876). Thus, of the three indicator bacteria used in this study, the concentrations of *E. coli* in runoff were most similar to that of Cl^-.

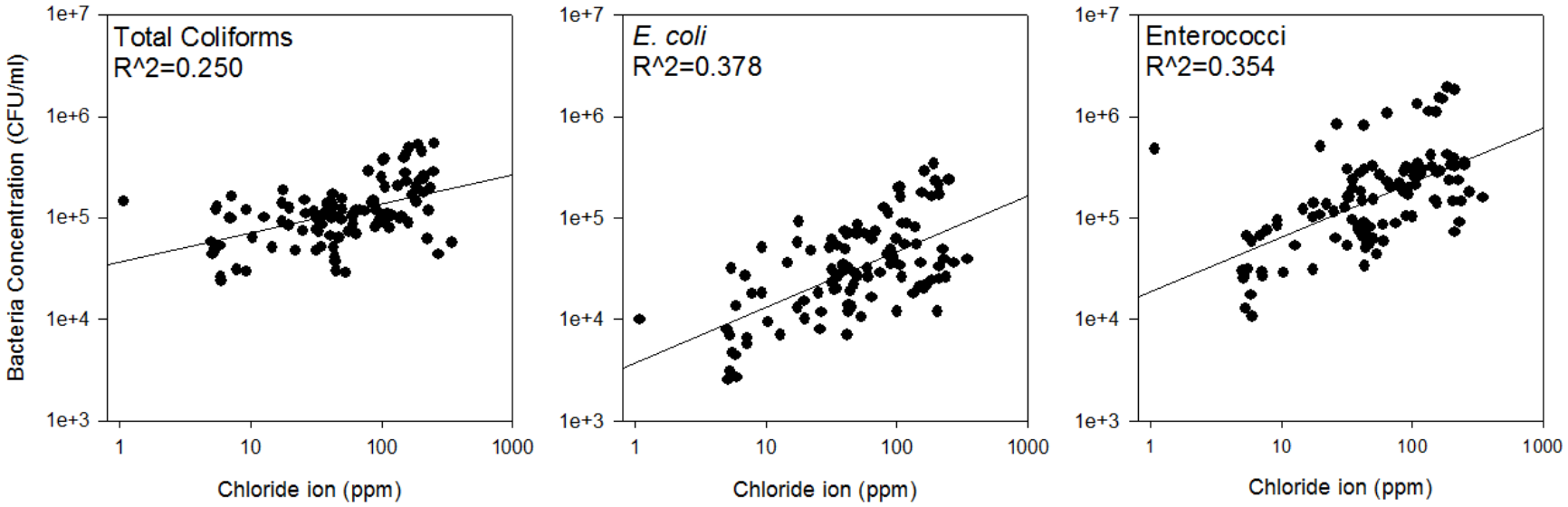

Figure 4.7 Linear regression of the concentration of total coliforms (left), *E. coli* (middle), and enterococci (right) vs. the concentration of chloride ion within surface runoff.

Retention of bacteria in soil

The concentrations of *E. coli* and enterococci found in surface runoff were always greater than their simultaneously occurring concentrations in soil leachate when leached through approximately a 10-cm soil profile (Fig. 4.8). These differences indicated that physical straining of indicator bacteria from leachate may have occurred as the leachate moved downward through the soil profile. It should be noted that this particular comparison is questionable because it takes some time for water to move through soil and comparing the simultaneous values in runoff and infiltration may not strictly be a valid comparison. In addition, the concentrations of *E. coli* and enterococci in soil following rainfall generally decreased with soil depth (Fig. 4.9).

Since total coliforms were indigenous in the soil, the straining effect was not noticeable for them and their concentrations in soil leachate were actually greater for the control treatments than for the manure application treatments (Fig. 4.8). The runoff and infiltration concentrations of total coliforms from the controls mapped differently from the concentrations of total coliforms coming from the manure-amended soils (Fig. 4.8).

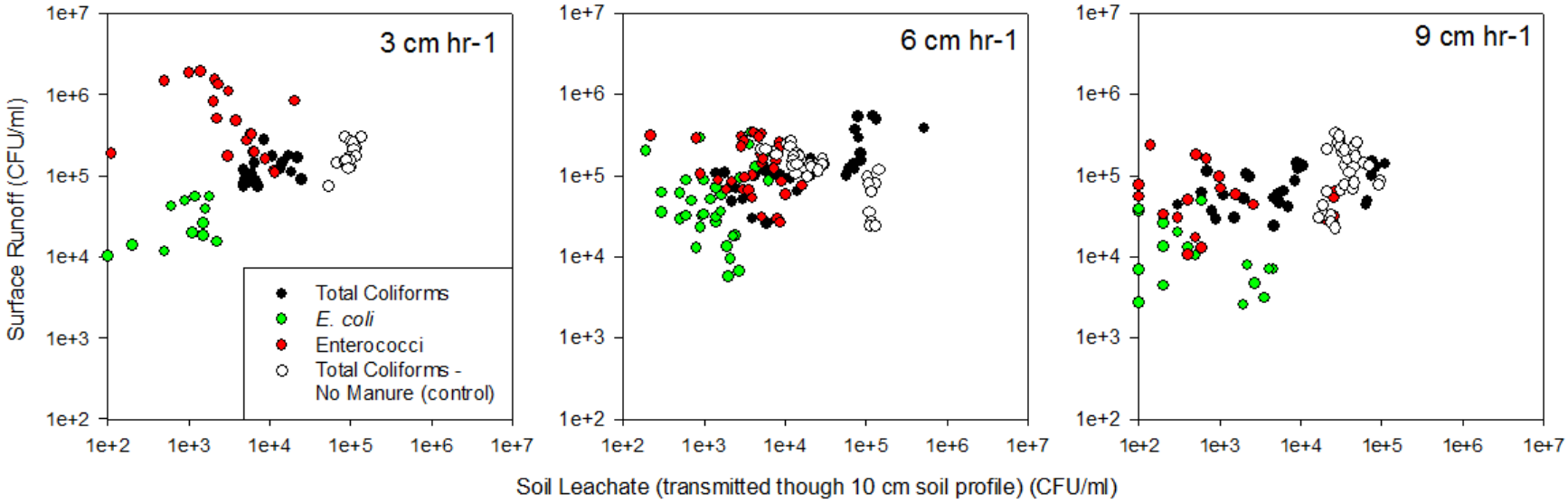

Figure 4.8 Concentrations of total coliforms, *E. coli*, and enterococci in synchronously collected runoff and soil leachate. Leachate was transmitted through, approximately, a 10 cm soil profile (sandy loam). Concentrations of total coliforms in runoff and leachate for the control treatment (no manure application) are also shown, while concentrations of *E. coli* and enterococci in the control treatment were trivial.

A two-factor ANOVA showed rainfall intensity and soil depth to both have had significant effects ($p<0.05$) on the concentrations of *E. coli* and enterococci that remained in the soil after rainfall and that the two variable factors experienced interactions for both bacterial species (*E. coli*, $p=0.026$; enterococci, $p<0.001$). As rainfall intensity and soil depth both increased, concentrations of indicator bacteria in the soil decreased (Fig. 4.9).

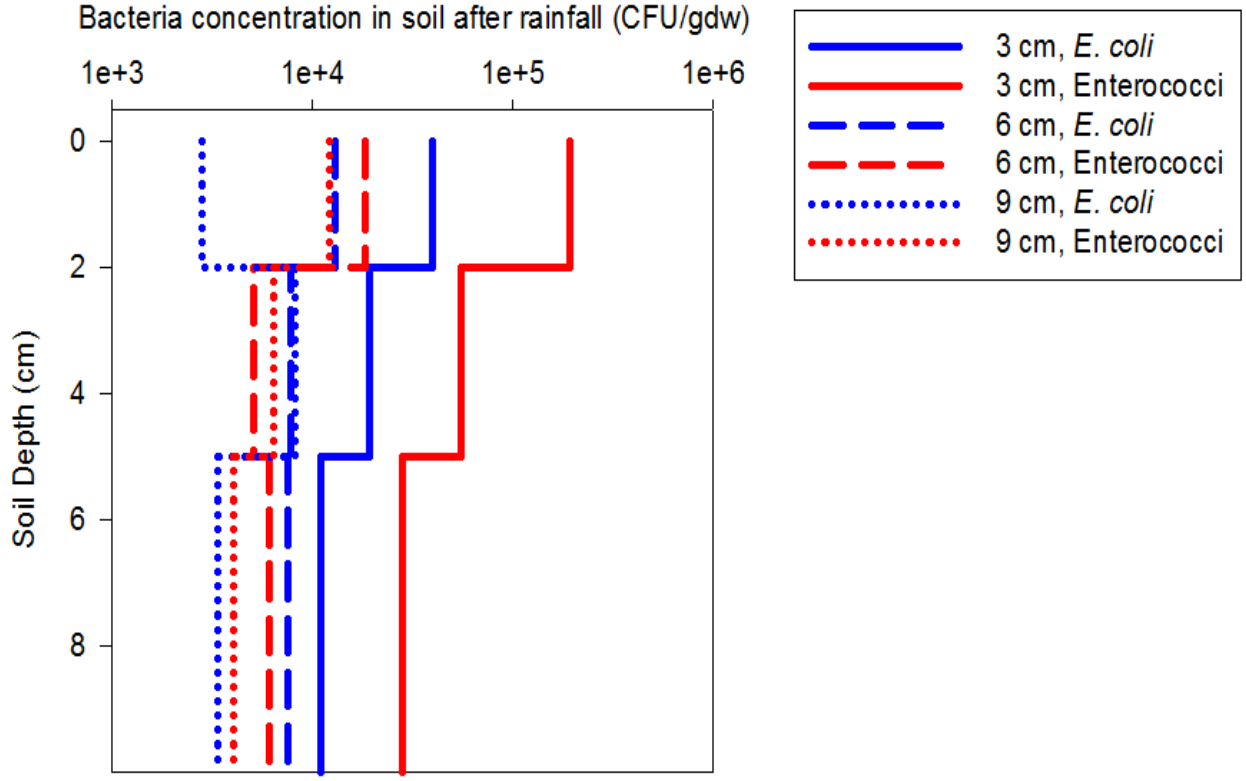

Figure 4.9 Concentrations of *E. coli* and enterococci found in the soil profile (measured at 0, 2, 5 10 cm depths) of the manure application treatments following rainfall.

Total bacteria released from manure during rainfall

A total number-release calculation was used to describe the partitioning of released loads of *E. coli* and enterococci into runoff, soil, and soil leachate during rainfall. During the 3 cm hr^{-1} rainfall events, the majority of released *E. coli* had been partitioned to flow with infiltration into the soil, while the majority of enterococci had been partitioned to flow with surface runoff (Table

4.8). At the 6 and 9 cm hr^{-1} rainfall events, more than twice as much of each bacterial species was partitioned into the surface runoff than into the soil (Table 4.8). For all treatments, the released bacteria that had been partitioned into the soil constituted less than 1.5 % of the bacteria that were transported out of the base of the soil profile with leachate.

The total released mass of *E. coli* was greater than the mass of enterococci when considering summation of the mass that was removed with the runoff and the leachate, and the mass which remained in the soil (Table 4.8).

Table 4.8 The starting total number of *E. coli* and enterococci in manure, and the total number of bacteria released from manure that had been released in runoff, soil leachate, and soil (post-rainfall). Total release of bacteria from manure was calculated as the sum of all total numbers removed from manure (i.e., total bacteria released from manure = removal with runoff + removal with leachate + release that had remained in soil).

Rainfall Intensity	Bacteria	Total number of bacteria applied to plot within the 2.1 kg of manure (CFU)	Removal of bacteria with surface runoff during rainfall (CFU (%))	Removal of bacteria with leachate during rainfall (CFU (%))	Bacteria released from manure that remained in soil after rainfall (CFU (%))	Total release of bacteria from manure during rainfall (CFU (%))
3 cm hr^{-1}	*E. coli*	1.31E+09	4.53E+08 (34.7%)	1.96E+06 (0.15%)	6.77E+08 (51.8%)	1.13E+09 (86.7%)
	Enterococci	1.58E+10	5.74E+09 (36.4%)	3.71E+07 (0.24%)	2.17E+09 (13.8%)	7.95E+09 (50.4%)
6 cm hr^{-1}	*E. coli*	1.41E+09	8.33E+08 (58.9%)	2.71E+06 (0.19%)	3.79E+08 (26.8%)	1.21E+09 (85.9%)
	Enterococci	3.54E+09	2.20E+09 (62.1%)	9.98E+06 (0.28%)	2.67E+08 (7.5%)	2.48E+09 (69.9%)
9 cm hr-1	*E. coli*	7.82E+08	4.89E+08 (62.5%)	4.62E+05 (0.06%)	2.14E+08 (27.4%)	7.03E+08 (90.0%)
	Enterococci	3.89E+09	2.27E+09 (58.2%)	2.66E+06 (0.07%)	2.28E+08 (5.9%)	2.50E+09 (64.2%)

Discussion

E. coli and enterococci worked well as FIB's since these bacteria were not detected in runoff, leachate, and soil of the control treatments. When considered for the manure application treatments, these bacteria were present in the land-applied manure, they were detected to have been removed from with runoff and leachate during rainfall, and they remained within the soil profile after rainfall. The use of total coliforms as an indicator of fecal contamination in runoff and leachate was more complicated because of the background concentrations of total coliforms in the soil. For the manure application treatments, the concentrations of total coliforms that were detected in runoff, leachate, and soil could have been released from the manure source as fecal contaminants or they could have been released from the soil as indigenous strains. This result was somewhat expected since some genera and species within the group of total coliforms are known to be indigenous to soil, water, and vegetation (Drapcho, 2003). The differences seen in the partitioning of concentrations of total coliforms into runoff/infiltration during the control treatments and manure-amended soil treatments is attributable to different bacterial species of coliforms that comprised the total coliform populations that were enumerated from samples from the respective treatments. Because of their wide distribution in nature and their broad categorization, total coliforms are not often used in microbial environmental risk assessments (Rosen, 2000; Sinclair et al., 2012).

Manure constituents, including bacteria, plant nutrients, and other dissolved ions and compounds, are released from manure simultaneously, but at different rates and concentrations. In theory, dissolved ions and chemical compounds, and even free-living, planktonic bacteria in manure solution can naturally flow with the suspended matter as it is eluted from the manure matrix; whereas, solid manure components that are part of the manure matrix resist release. In

this study, the ratio of $C_{Initial\ Runoff}$ to C_{Manure} for Cl^- was about 0.21 and that for *E. coli* and enterococci was substantially lower at about 0.06-0.08 (Table 4.3). Since chloride is a dissolved anion within the manure solution and since bacteria may be associated with either the manure solid or liquid phases, these results suggest that rainwater absorbed by manure prior to release is diluting the manure liquid phase such that the Cl^- and the suspended bacteria contents of the diluted manure liquid phase are leached with the initial release. Another explanation may be that there was a bypass flow of water that is characterized by the lower Cl^- concentrations in runoff than in the manure liquid phase but for this hypothesis to be true approximately 80% of leachate water did not participate in Cl^- displacement. Unlike dissolved ions, most bacteria remained intact within the manure matrix and were associated with manure solids even while the liquid phase components were released. Microbial initital release efficiencies, which may contribute to the bacteria's ability to dislodge from solid surfaces in a matrix, depends on the potential of bacteria to enter and flow with the formed manure-suspension. This is affected by bacterial surface charge, hydrophobicity, and size as well as cellular surface structures such as flagella, fimbriae, and lipopolysaccharides (LPS) (Critzer and Doyle, 2010; Foppen and Schijven, 2006; Pachepsky et al., 2008). The negatively charged surfaces of bacteria may also connect to negatively-charged surface sites within a manure or soil matrix via cation bridging or by interaction with charges in the diffuse layer surrounding a charged surface (Sobeck and Higgins, 2002). Bacterial association with the manure solid phase may explain why the $C_{Initial\ Runoff}/C_{Manure}$ for bacteria was lower than that for dissolved chloride. More specifically, the lower relative concentrations of bacteria that were seen in the initial runoff may be attributed to one or more of the following: 1) the solid manure providing surfaces for bacteria to remain within the manure matrix, 2) the Kentucky 31 tall fescue and the soil surface particulates providing surfaces for

bacterial attachment, 3) the interception of bacteria by grass blades during overland flow, 4) the catchment of bacteria by manure aggregates during overland, and/or 5) the presence of bacteria in parts of manure matrix that is initially not accessible to overland flow. As microbes and other colloids are transported across manure-covered land, the bacteria interact with the above-ground component of vegetation and with soil at the soil-manure interface. The removal of microbes from a manure-covered area with runoff depends on the microbe's ability to bypass leaf blades and stalks and to avoid interception and segregation from surface runoff (Dao et al., 2008). Surface filtration of colloidal contaminants such as bacterial indicators and pathogens by vegetation is controlled by the physical contact processes and the chemical attachment processes that are constantly interacting during laminar overland flow (Wu et al., 2012; Wu et al., 2014). Colloidal attachment efficiency is affected by Van der Waals attractive forces, electrostatic double layer forces, and hydrodynamic forces (Wu et al., 2012; Wu et al., 2014). Natural organic matter containing humic acids and fulvic acids coats vegetation and plays an important role in colloidal attachment efficiency as well (Wu et al., 2014). While colloidal filtration is enhanced by dense vegetation, such vegetation may also reduce microbial release by protecting the manure-bacteria microhabitats from rainfall-induced erosion. In a study on the release of fecal coliforms from bovine slurry applied on bare or vegetated plots that consisted of sandy loam and clay loam soils, Guber et al. (2006) stated the microbial release kinetics were significantly affected by vegetation presence. Dao et al. (2008) and Guber et al. (2007) both found that more manure-borne bacteria were removed from manure with runoff in soil boxes that had manure applied over dead grass than when the manure was applied over live grass and suggested that the living-state of vegetation has a negative impact on microbial collection in runoff. In this study, the Kentucky 31 tall fescue was healthy and well-developed, and its blades may have filtered

bacteria out of the runoff, which would have influenced the initial relative concentrations of bacteria in surface runoff to be lower than the concentrations of a dissolved substance in runoff. A more important factor is that the association of manure-bacteria with the manure matrix probably had the greatest influence of $C_{Initial\ Runoff}/C_{Manure}$ for relative bacteria concentrations being lower than relative concentrations of Cl^-.

Similar to the $C_{Initial\ Runoff}/C_{Manure}$ for the microorganisms that is described in this study, Schijven et al. (2004) reported concentrations of *Cryptosporidium* and *Giardia* (oo)cysts to have been released from artificially constructed cowpats at several orders of magnitude lower than their initial concentration in the manure. Guber et al. (2006) assumed the initial concentration of bacteria released from land-applied bovine slurry to be equivalent to the starting concentration in manure. The manure used in Guber et al. (2006) was bovine slurry which would have had a dominating liquid phase that would have collapsed the solid matrices of manure so that the manure contained less sites for bacteria association with manure solids or inaccessible flowing water. Thus, $C_{Initial\ Runoff}/C_{Manure}$ and the ensuing microbial runoff-removal kinetics are likely to be manure-specific. Soupir et al. (2003) reported differences in the total mass of FIB's removed from solid and liquid manures that had been prepared from cattle at the same dairy facility. Compared with microbial removal from liquid-based manures and slurry, the removal from solid manures would have a much weaker relationship with total rainfall as removal seems to be a more complex, situation-specific process (Chapter 2).

Simulating the rainfall-induced runoff-removal of fecal indicator bacteria from manure is an essential component to microbial fate and transport modeling. In this study, the dependency of microbial removal with surface runoff (M_{Runoff}/M_0) on rainfall depth appeared to be a two-stage process with a several order of magnitude increase of the removed mass during the initial 1 to 2

cm of rainfall from approximately 10^4 to10^9 CFU that constituted 0.0001 to 30 % of the total mass. After the initial increased removal rate, the mass removal-rate drastically decreased and became closer to zero (Fig. 4.5). These findings are consistent with the work of Guber et al. (2006) on slurry where microbial removal experienced a distinct rate-change and the removal kinetics shifted from first-order to zero-order kinetics after approximately one hour of simulated rainfall over bovine slurry on a hill-slope. In support of those findings, Schijven et al. (2004) had previously reported that concentrations of *Cryptosporidium* and *Giardia* (oo)cysts released from artificially constructed cowpats decreased gradually before experiencing a rate-change where the concentrations "tailed-off". Since microbial runoff-removal kinetics in this study and in other similar studies have approached an asymptote after a "break point" at a given rainfall depth, then, presumably, any rainfall intensity that occurs ont manured land may induce a similar load of total removal of bacteria from manure with runoff as long as the rainfall event has provided enough total rainfall to initiate the "break point" and onset of the secondary removal stage. In support of this statement, of the five total parameters within the three runoff-removal models (i.e., k_e for Eq. 1, k_p and β for Eq. 2, and A and n for Eq. 3) that were fitted to all manure-constituent M_{Runoff}/M_0 dependencies on rainfall depth, only n from Eq. 3 of the enterococci release simulations was significantly affected by rainfall intensity (Table 4.4). The assumption that microbial runoff-removal kinetics may be simulated as a factor of total rainfall rather than as a factor of rainfall intensity is seemingly validated by this study.

The two-stage runoff-removal process described in this study and in previous research may have resulted from a period of time where an initial washout of planktonic bacteria and suspended manure particulates containing adsorbed bacteria occurred. After the easily accessible pathways were removed in leachatet and the manure-liquid became almost void of microbes,

then a second stage occurred that included an almost-constant rate of shaving manure to moving water with the number of bacteria proportional to the shaved amount of manure. Pachepsky et al. (2009) noted that manure particles effected transport and retention of microbial pathogens in soil and that the size distributions of particles released from dairy cattle slurry started the removal process with an average approximate size of 7.96 μm that decreased before becoming stabilized at an average approximate size of 4.1 μm after 15 minutes of rainfall at 32.4 mm hr^{-1} that corresponded to a rainfall depth of 8.1 mm. This 2-fold decrease in particle size distribution prior to stabilization occurred at slightly less than 1 cm of rainfall and corresponded to a rainfall depth similar to the rainfall depth at the "break point" described in this study. This quantity of rainfall could have substantially affected the release and subsequent transport of FIB's especially since a large quantity of *E. coli* and enterococci that are released and transported are attached to manure colloids (Soupir et al., 2010). The change in the average size of eluted particulates observed by Pachepsky et al. (2009) may have indicated the onset of the predominantly sloughing stage in runoff-removal.

The occurrence of two-stage kinetics for the runoff-removal of Cl^- was evident as well; although, the asymptote in the second stage for Cl^- approached 100 % while the removal for *E. coli* and enterococci only reached about 60 %. It must be surmised that during runoff-removal, all manure constituents may experience a two-stage dynamic, but only dissolved substances and not those the associated with the manure solid phase may become fully released during a rainfall event. For bacteria that are associated with manure solids in the matrix to become released, the manure aggregates containing bacteria must become suspended within runoff and this suspension may only occur if these manure components are sloughed off of the matrix or scoured into suspension. Since there will always be bacteria associated with manure solids that are not

removed during rainfall, 100 % of the land-applied bacteria within manure cannot be removed from the manure during rainfall. While dissolved ions (e.g., Cl-) are removed completely during a heavy rainfall, a plethora of contaminant bacteria can remain in manure after rainfall and may be released during a subsequent rainfall event. This potential avenue of bacterial loss should be considered with regard to risk-assessment and food safety.

As rainfall intensity increased for both the manure application treatments as well as the control treatments, the amount of rainwater recovered as runoff, as opposed to infiltration, significantly increased ($p=0.050$). Thus, surface-seal formation is enhanced at increasing rainfall intensities with and without manure applied on soil. Manure application to the soil had an even more significant effect on enhancing runoff partitioning ($p=0.013$). Therefore, a seal at the soil, the manure, and/or at the soil/manure interface may have been forming to positively influence runoff partitioning. The seal may have been formed by both the disintegration of soil surface aggregates caused by the transmitted kinetic energy of raindrop impacts and by the pieces of colloidal manure blocking soil pores.

In this study, the bacteria that had been released from manure were partitioned to flow with surface runoff or infiltrate the soil. The bacteria that infiltrated the soil moved downward to exit the soil profile or remained within the soil profile during the rainfall event. Of the 3, 6, and 9 cm hr^{-1} rainfall intensities applied in this study, the 3 cm hr^{-1} rainfall caused the greatest relative amount of bacteria to be released into the soil infiltrate and the lowest relative amount of bacteria to be released into the surface runoff (Table 4.8). Increased rainfall intensities caused higher contents of *E. coli* and enterococci to be removed with surface runoff rather than to infiltrate the soil. Rainfall intensity also had a substantial effect on the contents of bacteria remaining within the soil profile at the 0-, 2-, 5-, and 10-cm depths following rainfall. Since the soil caused

apparent physical straining of bacteria from leachate (Fig. 4.9), soil depth also had significant effects on the contents of bacteria remaining in soil post-rainfall. As rainfall intensity and soil depth increased, the concentrations of FIB's remaining in the soil decreased. The effects of rainfall intensity on the amount of bacteria remaining in soil post-rainfall may have been caused by an enhanced formation of a soil surface seal upon increased rainfall intensity that would have caused the partitioning of more bacteria into the surface runoff rather than the infiltration.

While the runoff-removal kinetics of *E. coli* and enterococci were not significantly different from one another, the probability of differences between the two FIB's increased along with rainfall intensity (Table 4.6). The release of *E. coli* was substantially greater than that of enterococci (Table 4.8). Release creates microbial transport with runoff and infiltration, and while the two species of bacteria may have flowed into surface runoff at similar rates, soil contained relatively larger quantities of *E. coli* than enterococci. Compared with *E. coli*, enterococci likely remained in manure aggregates and/or became attached to soil and/or vegetation surfaces within the plots rather than being washed off (Guber et al., 2007). The same occurrence was likely observed in this study, and, like in Guber et al. (2007), the release of the inorganic surrogate Cl^- tracer was more similar to the release of *E. coli* than enterococci.

Dissimilarity between the release of *E. coli* and enterococci from the manure suggests a species-specific release process that may be attributed to location of habitat within manure and soil as well as unique physiological properties since both of these factors may effect bacterial release efficiency. Different species will compartmentalize to different microhabitats within porous media structure. Bacteria have specific physical and chemical properties that affect their efficiency of becoming dislodged from their micro-habitats (Lombard et al., 2011). Bacterial surface charge, hydrophobicity, size and surface structures such as flagella, fimbriae, and

lipopolysaccharides (LPS) influence their ability to attach/detach from solid surfaces (Critzer and Doyle, 2010; Foppen and Schijven, 2006; Pachepsky et al., 2008). LPS are unique to the cell wall of gram-negative bacteria and absent in gram-positive bacteria (Lombard et al., 2011); thus, the release of gram-positive enterococci and gram-negative *E. coli* may be fundamentally different based on unique physiochemical properties for cellular attachment. Evidence for microbe-specific release supports the idea that an optimal release assessment should based on release simulations of multiple indicator microorganisms.

Utilizing a recommended microbial runoff-removal model is essential for measuring, simulating, and regulating fecal contamination to the environment. In this study, runoff-removal model performance was assessed using the RMSE and the AIC and appeared to be organism-dependent. The Vadas-Kleinman-Sharpley model performed better for simulating total coliform runoff-removal, while the Bradford-Schijven model yielded the best simulation results for *E. coli* and enterococci runoff-removal. Since *E. coli* and enterococci are the premier FIB's, the Bradford-Scijven model is recommended as for simulating the runoff-removal of FIB's from dairy cattle manure.

Conclusions

Microbial release from manure heavily influences the potential pathogens and indicator microorganisms that enter a state of environmental transport and can move with overland and subsurface flow to contaminate surface waters and groundwater resources. Due to background concentrations of indigenous total coliforms in soil, *E. coli* and enterococci are recommended as FIB's. The initial concentrations of *E. coli* and enterococci released from manure and collected in surface runoff were greater than one order of magnitude less than their initial concentrations in manure. Kinetics for the FIB's removal from manure in runoff began with a precipitous removal

during the early onset of rainfall with reductions of approximately 10^4 to10^9 CFU (i.e., about 0.0001 to 30 % of the total amount). The initial reductions observed during the initial 1 to 2 cm of rainfall were followed by a slower, almost constant-rate of removal for the remainder of additional rainfall. The two-stage runoff-removal process may be explained by the rapid leaching of the manure liquid phase that contains dissolved ions and some bacteria during the first stage of runoff-removal that was followed by a second stage during which the bacteria remaining in manure were slowly sloughed off of the matrix. Microbes were partitioned into surface runoff and infiltration and those that flowed with the latter typically remained in the soil possibly because of physical straining rather than exiting the base of the soil profile with leachate. While rainfall intensity did not generally affect microbial runoff-removal kinetics, it had significant effects on the amount of bacteria remaining in soil following a rainfall event. As rainfall intensity and soil depth both increased, the concentration of bacteria in the soil decreased. The positive effect of rainfall intensity on bacteria runoff-removal and the inverse effects of rainfall intensity on bacteria remaining in soil after rainfall are attributable to soil, manure, and/or soil/manure interface seal formation. Total microbial release from manure varied for *E. coli* and enterococci and the former was released from manure at a faster rate especially with infiltration flow into the soil. The microbe-specific runoff-removal was best simulated by the Bradford-Schijven model. This study could be extended to investigate microbial release at the field scale and assess the effects of scale on runoff-removal model parameterization.

References

Arnold, J. G., Srinivasan, R., Muttiah, R. S., and Williams, J. R. 1998. Large area hydrologic modeling and assessment part I: Model development. Journal of American Water Resources Association, 34(1): 73-89.

Batz, M. B., Hoffmann, S., and Morris, J. G., 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection, 75(7): 1278-1291.

Benham, B. L., Baffaut, C., Zeckorski, R. W., Mankin, K. R., Pachepsky, Y. A., Sadeghi, A. M., Brannan, K. M., Soupir, M. L., and Habersack, M. J., 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. Transactions of ASABE, 49(4): 987-1002.

Blaustein, R. A., Pachepsky, Y., Hill, R. L., Shelton, D. R., and Whelan, G., 2013. *Escherichia coli* survival in waters: temperature dependence. Water Research, 47: 569-578.

Bradford, S. A., and Schijven, J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environmental Science & Technology, 36(18): 3916-3923.

Brooks, J. P., Adeli, A., Read, J. J., and McLaughlin, M. R., 2009. Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. Journal of Environmental Quality, 38(1): 218-229.

Burnham, K. P. and Anderson, D. R., 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag. 2nd ed.

Cardoso, F., Shelton, D., Sadeghi, A., Shirmohammadi, A., Pachepsky, Y., and Dulaney, W., 2012. Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. Journal of Environmental Management, 110: 1-7.

Chin, D. A., Sakura-Lemessy, D., Bosch, D. D., and Gay, P. A., 2009. Watershed-scale fate and transport of bacteria, Transactions of ASABE, 52(1): 145-154.

Critzer, F. J., and Doyle, M. P., 2010. Microbial ecology of foodborne pathogens associated with produce. Current Opinion in Biotechnology, 21: 125-130.

Curriero, F. C., Patz, J. A., Rose, J. B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the united states, 1948-1994. American Journal of Public Health, 91(8): 1194-1199.

Dao, T. H., Guber, A. K., Sadeghi, A. M., Karns, J. S., Shelton, D. P., and Pachepsky, Y. A., 2008. Loss of bioactive phosphorus and enteric bacteria. Soil Science, 2 (5): 511-521.

Drapcho, C. M., A. K. B., 2003. Fecal coliform concentration in runoff from fields with applied dairy manure. Master in Science Thesis, Louisiana State University, Baton Rouge, LA.

Dufour A. P., and Ballantine P., 1986. Bacteriological ambient water quality criteria for marine and freshwater recreational waters. US Environmental Protection Agency: EPA 440/445-484-002.

Foppen, J. W., van Herwerden, M., and Schijven, J., 2007. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology, 93(1-4): 236-54.

Forslund, A., Markussen, B., Toenner-Klank, L., Bech, T. B., Jacobsen, O. S., and Dalsgaard, A., 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a *Salmonella enterica* Serovar Typhimurium Bacteriophage through intact soil cores following surface application and injection of slurry. Applied and Environmental Microbiology, 77(22): 8129-8138.

Guber, A. K., Shelton, D. R., and Pachepsky, Y. A., 2005. Transport and retention of manure-corne coliforms in soil. Vadose Zone Journal, 4(3) doi:10.2136/vzj2004.0097.

Guber, A. K., Pachepsky, Y. A., Shelton, D. R., and Yu, O., 2009. Association of fecal coliforms with soil aggregates. Soil Science, 174(10): 543-548.

Guber, A. K., Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., and Sikora, L. J., 2006. Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and Environmental Microbiology, 72(12): 7531-7539.

Guber, A. K., Karns, J. S., Pachepsky, Y. A., Sadeghi, A. M., Van Kessel, J. S., and Dao, T. H., 2007. Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. Letters in Applied Microbiology, 44(2): 161-167.

Guber, A. K., Pachepsky, Y. A., Dao, T. H., Shelton, D. R., and Sadeghi, A. M., 2013. Evaluating manure release parameters for nonpoint contaminant transport model KINEROS2/STWIR. Ecological Modelling, 263: 126-138.

Himathongkham, S., Bahari, S., Riemann, H., and Cliver, D., 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiology Letters, 178(2): 251-257.

Hodgson, C. J., Bulmer, N., Chadwick, D. R., Oliver, D. M., Heathwaite, A. L., Fish, R. D., and Winter, M., 2009. Establishing relative release kinetics of faecal indicator organisms from different faecal matrices. Letters in Applied Microbiology, 49(1): 124-130.

Hoffmann, S., Batz, M. B., and Morris, J. G., 2012. Annual cost of illness and quality-adjusted life year losses in the united states due to 14 foodborne pathogens. Journal of Food Protection, 75(7): 1292-1302.

Ibekwe, A. M., Ma, J., Crowley, D. E., Yang, C., Johnson, A. M., Petrossian, T. C., and Lum, P. Y., 2014. Topological data analysis of *Escherichia coli* O157:H7 and non-O157 survival in soils. Frontiers in Cellular and Infection Microbiology, doi: 10.3389/fcimb.2014.00122

Isensee, A. R., and Sadeghi, A. M., 1999. Quantification of runoff in laboratory-scale chambers. Chemosphere, 38(8): 1733-1744.

Kim, K., Whelan, G., Purucker, S. T., Bohrmann, T. F., Cyterski, M. J., Molina, M., Gu, Y., Pachepsky, Y., Guber, A., and Franklin, D. H., 2014. Rainfall-runoff model parameter estimation and uncertainty evaluation on small plots. Hydrological Processes, 28(20): 5220-5235.

Kiefer, L. A., Shelton, D. R., Pachepsky, Y., Blaustein, R., & Santin-Duran, M., 2012. Persistence of *Escherichia coli* introduced into streambed sediments with goose, deer and bovine animal waste. Letters in Applied Microbiology: 345-353.

Kress, M., and Cifford, G. F., 1984. Fecal coliform release from cattle fecal deposits. Water Resources Bulletin, 20(1): 61-66.

Lawson, L., 2003. HSPF Model Calibration and Verification for Bacteria TMDLs. Guidance Memo No. 03-2012, Commonwealth of Virginia, Department of Environmental Quality.

Ling, T. Y., Jong, H. J., Apun, K., and Sulaiman, W. H. W., 2009. Quantifying *Escherichia coli* release from soil under high-intensity rainfall. Transactions of ASABE, 52(3): 785-792.

Lombard, N., Prestat, E., Dirk van Elsas, J., and Simonet, P., 2011. Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78: 31-49.

Mawdsley, J. L., Bardgett, R. D., Merry, R. J., and Pain, B. F., 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology, 2: 1-15.

Meays, C. L., Broersma, K., Nordin, R., and Mazumder, A., 2004. Source tracking fecal bacteria in water: A critical review of current methods. Journal of Environmental Management, 73(1): 71-9.

Meyer, L. D., and Harmon, W. C., 1979. Multiple-intensity rainfall simulator for erosion research on row sideplots. Transactions of the ASAE, 22: 100-103.

Moyer, D. L., and Hyer, K. E., 2003. Use of the hydrological simulation program-FORTRAN and bacterial source tracking for development of fecal coliform total maximum daily load (TMDL) for Blacks Run, Rockingham County, Virginia. U.S. Department of the Interior U.S. Geological Survey, Water-resources Investigations Report 03-4161.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2006. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Letters in Applied Microbiology, 42(2): 83-87.

Panhorst, K. A., 2002. Estimating bacterial loadings to surface waters from agricultural watersheds. Master in Science Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Pachepsky, Y. A., Yu, O., Karns, J. S., Shelton, D. R., Guber, A. K., & Kessel, J. S. Van., 2008. Strain-dependent variations in attachment of *E. coli* to soil particles of different sizes. International Agrophysics, 22: 61–66.

Pachepsky, Y. A., Guber, A. K., Shelton, D. R., and McCarty, G. W., 2009. Size distributions of manure particles released under simulated rainfall. Journal of Environmental Management, 90(3): 1365-1369.

Roodsari, R. M., Shelton, D. R., Shirmohammadi, A., Pachepsky, Y. A., Sadeghi, A. M., and Starr, J. L., 2005. Fecal coliform transport as affected by surface condition. Transactions of ASAE, 48(3), 1055-1061.

Rosen, B., 2000. Waterborne pathogens in agricultural watersheds. USDA Watershed Science Institute-Technical Note 2. University of Vermont, Burlington.

Sadeghi, A. M., and Isensee, A. R., 2001. Impact of hairy vetch cover crop on herbicide transport under field and laboratory conditions. Chemosphere, 44(2): 109–118.

Savichtcheva, O., and Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research, 40(13), 2463-2476.

Schijven, J. F., Bradford, S. A., and Yang, S., 2004. Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: Physical factors. Journal of Environmental Quality, 33(4), 1499-1508.

Sharpley, A. N., 1985. Depth of surface soil-runoff Interaction as affected by rainfall, soil slope, and management. Soil Science Society of America Journal, 49: 1010-1015.

Sheldrick, W., Syers, J. K., and Lingard, J., 2003. Contribution of livestock excreta to nutrient balances. Nutrient Cycling in Agroecosystems, 119-131.

Sinclair, R. G., Rose, J. B., Hashsham, S. A., Gerba, C. P., and Haas, C. N., 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Applied and Environmental Microbiology, 78(6): 1969-1977.

Sobeck, D. C., and Higgins, M. J., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Research, 36(3): 527-538.

Soupir, M., Tech, V., Hall, S., Mostaghimi, S., and Alphin, E. F., 2003. Bacteria release and transport from livestock manure applied to pastureland. 2003 ASAE Annual International Meeting. Las Vegas, NV.

Soupir, M. L., Mostaghimi, S., and Dillaha, T., 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. Journal of Environmental Quality, 39(3), 1019-1027.

Steiger, J.H., 1980. Tests for comparing elements of a correlation matrix. Psychological Bulletin, 87, 245-251.

Stout, W. L., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Saporito, L. S., and Sharpley, A. N., 2005. Runoff transport of faecal coliforms and phosphorus released from manure in grass buffer conditions. Letters in Applied Microbiology, 41(3): 230-234.

Tate, K. W., Atwill, E. R., George, M. R., McDougald, M. K. and Larsen, R. E., 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. Journal of Range Management, 53(3), 295-299.

Thelin, R., and Gifford, G. F., 1983. Fecal coliform release patterns from fecal material of cattle. Journal of Environment Quality, 12(1), 57-63.

Thurston-Enriquez, J. A., Gilley, J. E., and Eghball, B., 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. Journal of Water and Health, 3(2), 157-171.

U.S. EPA, 1984. Health effects criteria for fresh recreational waters. EPA 600/1-84-004, Washington, D.C.

U.S. EPA, 1986. Ambient water quality criteria for bacteria. EPA 440/5-84-002, Washington, D.C.

U.S. EPA., 2012. Water: Monitoring assessment. 5.11 Fecal bacteria. <http://water.epa.gov/type/rsl/monitoring/vms511.cfm>.

Vadas, P. A., Kleinman, P. J. A., Sharpley, A. N., 2004. A simple method to predict dissolved phosphorus in runoff from surface-applied manures. Journal of Environmental Quality, 33: 749-756.

Van Horn, H. H., Wilkie, A. C., Powers, W. J., and Nordstedt, R. A., 1994. Components of dairy manure management systems. Journal of Dairy Science, 77(7): 2008–2030.

van Genuchten, M. Th. 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research Report No. 119, U. S. Salinity Laboratory, Riverside, CA, p. 88.

Whelan, G., Kim, K., Pelton, M. A., Soller, J. A., Castleton, K. J., Molina, M., Pachepsky, Y., Zepp, R., 2014. An integrated environmental modeling framework for performing quantitative microbial risk assessments. Environmental Modelling & Software, 55: 77-91.

Wu, L., Gao, B., Muñoz-Carpena, R., and Pachepsky, Y. A., 2012. Single collector attachment efficiency of colloid capture by a cylindrical collector in laminar overland flow. Environmental Science & Technology, 46(16): 8878-8886.

Wu, L., Muñoz-Carpena, R., Gao, B., Yang, W., and Pachepsky, Y. A., 2014. Colloid filtration in surface dense vegetation: experimental results and theoretical predictions. Environmental Science & Technology, 48(7): 3883-3890.

Zejun, T., Tingwu, L., Qingwen, Z., and Jun, Z., 2002. The sealing process and crust formation at soil surface under the impacts of raindrops and polyacrylamide. 12th ISCO Conference, Beijing, China. pp 456-462.

Chapter 5 – Scale Effects on Fecal Indicator Bacteria Transport from Land-applied Manure

Abstract

Release and transport of pathogenic organisms from land-applied livestock manure affects the microbial quality of surface waters which is of the utmost importance for irrigation, recreation, household water use, aquaculture, industrial processes, and other surface water uses. The information on microorganism release rates and controls is traditionally collected at experimental plots or indoor soil boxes. The objective of this work was to test the hypothesis that the relative mass of manure-constituents – total coliforms, *Escherichia coli*, enterococci, and chloride ion – transported out of a manure application area is affected by the size of the study area. Experiments were conducted at three study scales using soil boxes (0.35 x 1 m), standard field plots (0.8 x 1 m), and a corn field (36 x 77 m). Stockpiled dairy cattle manure was accumulated at the dairy herd production center in Beltsville, MD, in the months of January to April 2014, and then applied to the study areas at the rate of 60 ton ha^{-1} at the OPE3 field site of the USDA-ARS Beltsville Agricultural Research Center. The corn field received sprinkler irrigation at the rate of 1.4 cm per hr^{-1} for 2 h from the time of runoff initiation and the field plots and soil boxes received a similar rate of precipitation via a controlled-intensity rainfall simulator. Each study area had the same soil and manure application rate and all experiments were performed during the same week. A strong dependence on scale was observed for the percent of land-applied bacteria and Cl^- that was removed from the study area with runoff. The microorganism retention processes that emerge at the field-scale appear not to be manifested at smaller scales. Scale effects need to be factored in when existing data on fecal indicator bacteria released from

manures that are applied to simulate field-scale fate and transport of manure-borne microorganisms and their effects on microbiological water quality.

Introduction

In order for surface runoff to occur on a field during a precipitation event, the rainfall intensity must exceed the infiltration capacity of the soil (Van de Geisen, 2011). A network of overland flow is established as the water that fills and overflows depressions on a field forms channels based on the connectivity of the heterogeneous properties of soil that govern the infiltration of water (Gomi et al., 2008; Van de Geisen, 2011). Due to an increase in spatial heterogeneity in soil-surface conditions with an increase in scale, the network of overland flow at the field-scale is considerably different than when examined in the laboratory or in small-scale field plots (Kidron et al., 2011; Mounirou et al., 2012; Penuela et al., 2013).

The effects of scale has been documented for the runoff coefficients (i.e., the percent of rainfall becoming runoff) and sediment concentrations in runoff (Cerdan et al., 2004; Delmas et al., 2012; Gomi et al., 2008; Kidron et al., 2011; Raclot et al., 2009). Both of these processes have an effect on microbial transport since (a) microbial indicators and pathogens that are released from manure during rainfall or irrigation are transported out of the manure application area in surface runoff and/or in subsurface flow, and (b) in situations where manure-bacteria have sorbed to soil particles, soil erosion may greatly contribute to microbial transport from of the manured application area (Soupir et al., 2010). Since the effects of scale have been observed for sediment yields in runoff (i.e., the relative amounts of eroding soil that is transported out of the study area) (Delmas et al., 2012; Raclot et al., 2009) and some manure-bacteria are known to be transported in runoff attached to soil particles especially during prolonged rainfall events when the contact between manure and soil is increased as the manure is dissipated (Soupir et al.,

2010), the effects of scale may also be evident for bacteria yields (i.e., the relative amount bacteria eroding from manure that are transported out of the study area). Indirect evidence in favor of this assumption is presented in the work of Harmel et al. (2010). In a study conducted in a rural area in Texas, the authors demonstrated that *Escherichia coli* concentrations consistently decreased as scale increased from field- to small watershed- to river basin-scale (Harmel et al., 2010). They noted that while it was not appropriate to conclude increasing scale as the sole or major cause of decreasing *E. coli* concentrations, the inverse relationship was certainly present. In addition, to develop a transport coefficient for *E. coli* from soils on a dairy pasture in New Zealand, Muirhead and Monaghan (2012) conducted experiments with soil boxes (20 x 80 cm) and field plots (100 x 200 cm), and they assessed the relationship between concentrations of *E. coli* in soil and the concentrations of *E. coli* collected in surface runoff during rainfall at both experimental scales. They reported a similar, positive regression line slope representing the concentrations of *E. coli* measured in soil and that measured in runoff at both scales, yet a significantly lower regression line y-intercept for the plots than the boxes (Muirhead and Monaghan, 2012). Thus, while there was a general trend of increasing concentrations of *E. coli* in runoff with an increase in *E. coli* concentrations in soil, there was greater average concentrations of *E. coli* in runoff removed from the soil boxes rather than from the field plots when the concentrations of *E. coli* in the soil within the boxes and within the plots was equivalent (Muirhead and Monaghan, 2012). While increasing the experimental scale may not have been the only cause of the noted effects, the inverse relationship between scale and the *E. coli* concentrations in surface runoff was evident (Muirhead and Monaghan, 2012).

Although the majority of microbial release experiments and modeling efforts have occurred in the laboratory or at small-scale field settings, some researchers have attempted to

model release at a relatively large scale where manure was surface broadcasted to cover a field (Martinez et al., 2014; Guber et al., 2011). Unfortunately, there is currently no published research that has demonstrated the effects of scale on manure-borne bacteria release and transport from the manure application area. Where such a relationship exists, microbial release kinetics and release model parameters have been difficult to infer from parameters developed from edge-of-box or edge-of-plot measurements. The difficulties have arisen because the results would be dependent not only on local release kinetics per-se, but also on the conditions for released bacteria to be retained within the manure application area. An understanding of the scale effects on release and transport interplay patterns is necessary to make field-scale inferences based upon lab-scale observations and also to improve parameterization in field-scale and watershed-scale models, such as STWIR (Guber et al., 2009; Kim et al., 2014), SWAT (Benham et al., 2006), and HSPF (Benham et al., 2006; Moyer and Hyer, 2003).

The objective of this work was to conduct microbial release experiments using laboratory soil boxes, field plots, and corn field scales to evaluate if the percent recovery of land-applied total coliforms, *E. coli*, enterococci, and chloride ion from manure is affected by the size of the study area. We hypothesize that the scale effects on runoff have important implications on the rainfall-induced release and transport of pathogens and indicator microorganisms from land-applied manure.

Methods

Microbial release and transport experiments were conducted using 0.35 m^2 laboratory soil boxes, 0.8 m^2 standard field plots, and an approx. 2772 m^2 cornfield and are hereafter referred to as lab-scale, plot-scale, and field-scale, respectively. Evaluations were made in April 2014 and all study areas had the same soil type and manure type.

Soil and Manure

The field studies occurred at the OPE3 field site at the USDA-ARS Beltsville Agricultural Research Center in Belsville, MD. The plot-scale experiment was conducted on land directly next to the field-scale experiment, and the lab-scale experiment used soil from the same field. The OPE3 Field site is primarily used for corn production research and dairy manure is annually applied to this field. The soil in the study area has been classified as a course loamy, siliceous, mesic Typic Hapludults (Guber et al., 2011; Gish and Kung, 2007) with an average bulk density of 1.27 g cm^{-3} (Gish et al., 2009). The soil profile has been described to consist of coarse loamy sand in the top soil at 0-30 cm, sandy loam at 30-80 cm, coarse sand at 80-150 cm, and gravelly sand in the lowest horizon at 150-250 cm (Guber et al., 2011). Due to heterogeneous properties of soil that have observed in this field, a composite of soil samples was collected from the surface horizon during all three experiments and the general soil properties representative of the surface soil used in each experiment are shown in Table 5.1.

Table 5.1 Properties of a composite sample of surface soil used in each of the three experiments (lab-, plot-, and field-scale). The composite sample from the lab-, plot-, and field-scale studies consisted of 10, 10, and 30 individual soil samples taken just before manure application in each experiment, respectively. The Penn State Agricultural Analytical Services Laboratory performed the soil analyses for the results displayed in this table.

Soil Component	Lab-scale	Plot-scale	Field-scale
Sand (%)	64.8	[6]50.7	
Silt (%)	21.2	[6]34.5	
Clay (%)	13.9	[6]14.7	
Textural Class	Sandy Loam	[6]Loam	
[1]pH	6.7	6.7	6.8
Organic Matter (%)	1	3.2	2.7
Total Carbon (%)	0.68	2.37	1.93
Total Nitrogen (N) (%)	0.06	0.21	0.17
[4]CEC (meq 100 g^{-1})	5.8	8.8	6.6
% Saturation of CEC (K, Mg, Ca)	6.0, 8.7, 47.7	11.6, 18.1, 47.7	14.2, 22.4, 63.4
[3]Acidity (meq 100 g^{-1})	2.2	2	0.0
Soluble Salts (mmhos cm^{-1})	0.06	0.06	0.03
Ammonium-N (ppm)	2.7	23.8	6.3
Nitrate-N (ppm)	2.5	85.6	28.8
[2]Phosphorous (P) (ppm)	194	176	181
[2]Potassium (K) (ppm)	136	398	363
[2]Magnesium (Mg) (ppm)	57	192	176
[2]Calcium (Ca) (ppm)	551	842	832
[2]Zinc (Zn) (ppm)	3.2	7	6.4
[2]Copper (Cu) (ppm)	0.9	1.6	1.5
[2]Sulfur (S) (ppm)	8.9	12	10.1

[1]1:1 soil:water pH, [2]Mehlich 3 (ICP), [3]Mehlich Buffer pH, [4]Summation of Cations, [5]1:2 soil:water, [6]Based on composite of 30 samples taken at the OPE3 Field.

Stockpiled dairy cattle manure was accumulated at the dairy herd production center at the USDA-ARS in Beltsville, MD, during the months of January to April of 2014. Dairy manure was from heifers and dry cows and consisted of the hay bedding material that was scraped from their outdoor feeding pads. During the final month of the manure accumulation period, manure samples were taken on a weekly basis to moniter that their counts of *E. coli* and enterococci would be in the range of 10^5-10^6 CFU g^{-1} at the time of experimentation. In late April 2014, the accumulated manure was used in the lab-, field-, and plot-scale experiments and a series of experiments were performed in the stated order during the same week to control for manure freshness. In all three experiments, manure was surface-applied over the soil at the rate of 60 tons ha^{-1}. The physical and chemical properties of the manure as well as its microbial contents from the morning of each experiment are displayed in Table 5.2. Manure samples from each study were also analyzed for their content of plant macro- and micro-nutrients (Appendix A).

Table 5.2 The physical properties, chemical properties, and microbial contents of the dairy manure measured from composite manure samples collected on each morning of each experiment. The composites from the lab-, plot-, and field-scale studies consisted of 9, 10, and 33 individual samples taken and mixed together, respectively. The "±" separates average and standard deviation.

Manure Content	Lab-scale	Plot-scale	Field-scale
Solid Mass (%)	22.6	48.5	26.8
Wet Mass (%)	77.4	51.5	73.2
[1]pH	8.59	8.86	8.75
[1]Carbon (%)	10.9	10.9	12.2
[1]Carbon:Nitrogen ratio	19.5	8.1	16.4
Enterococci (CFU g^{-1})	2.70×10^6	3.32×10^5	3.57×10^6
E. coli (CFU g^{-1})	9.31×10^6	4.02×10^5	7.91×10^5
Total coliforms (CFU g^{-1})	1.40×10^7	5.56×10^5	1.63×10^6

[1]Analyses performed by the Penn State Agricultural Analytical Services Laboratory.

Field-scale Study Design

The field-scale study took place at the OPE3 Field A site at the USDA-ARS Beltsville Agricultural Research Center in Belsville, MD. The elevation of land within the experimental area decreased along an average 2 % grade towards the lowest elevation point at the edge of the field where an H-flume was installed for the collection of edge-of-field surface runoff during an irrigation event.

Five truck-loads of manure were surface broadcasted on the OPE3 Field A at the rate of 60 ton ha^{-1} to establish total manure coverage of the land (Fig. 5.1). Two parallel irrigation pipelines (40 m long) were set on the field to provide irrigation that would induce the release and transport of microorganisms from the manure that had been applied on the field. Each irrigation line contained 5 sprinkler heads that were positioned 10 m apart. Prior to irrigation, manure was sampled along 33 grid points on the field. The 33 points consisted of 3 rows of 11 sample points,

and each row was located on either side of the irrigation pipeline (Fig. 5.2). Water collection jars were placed at each of the 33 grid points to measure the irrigation water volume during the event. Two transects were designated for tracer application to the land between the two irrigation lines. The layout of the experimental set up and topography of the field is illustrated in Fig. 5.2. The exact latitude, longitude, and elevation for each point were obtained with the GPS Total Station® 4700 (Trimble, Navigation Limited, Sunnyvale, CA).

Figure 5.1 Surface broadcasting of manure at the OPE3 Field.

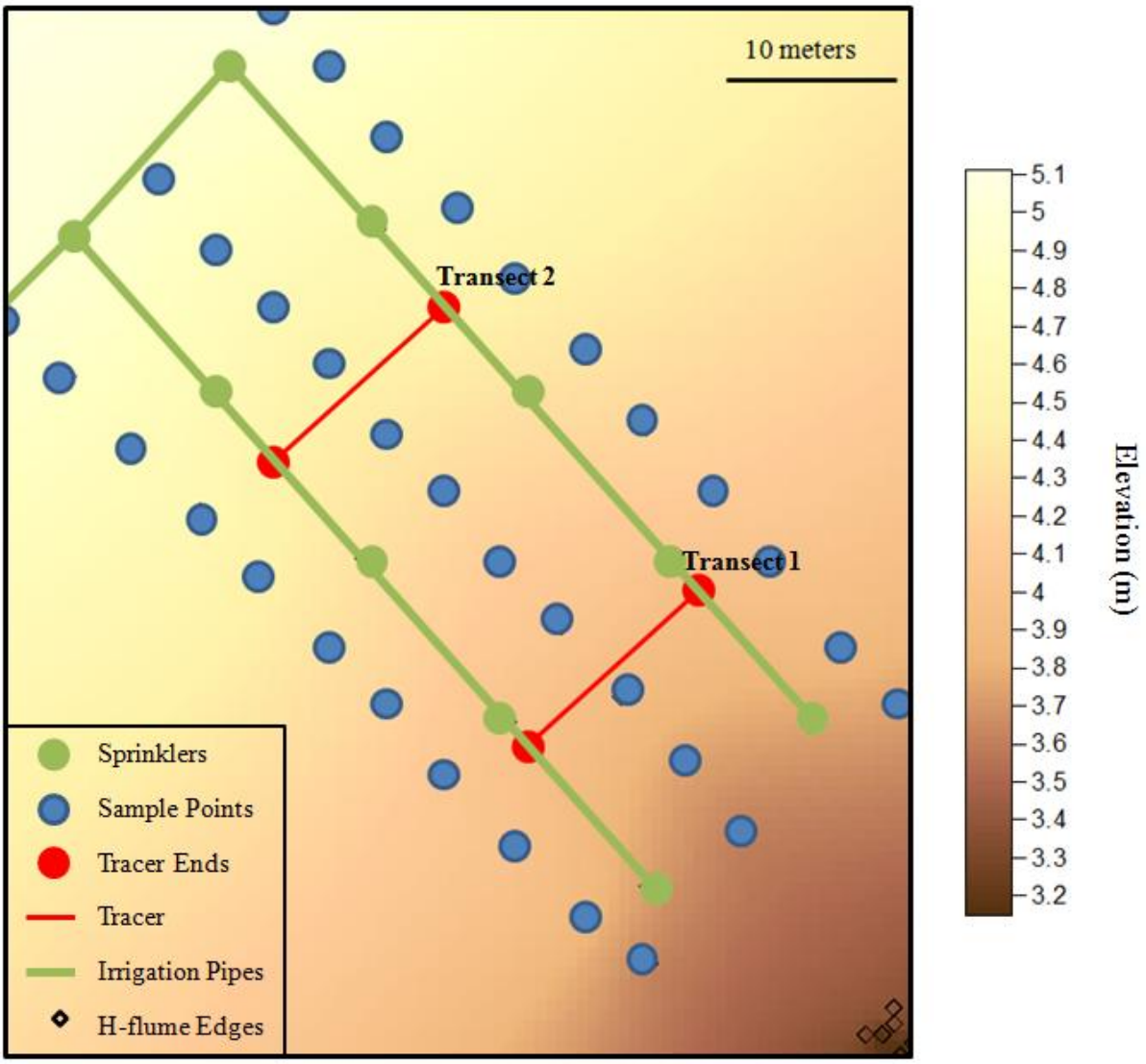

Figure 5.2 Layout for the experimental set up and topography at the OPE3 Field site.

Irrigation water was applied to the manured field at the delivery rate of 1.4 cm hr^{-1} (Fig. 5.3). During the irrigation event, surface runoff was collected in acid-washed, sterile glass bottles at 5 minute intervals for two hours following runoff initiation. At the collection time of each runoff sample, the stage of water in the flume was also recorded in order to measure the flow-rate and cumulative flux over time. At 7.5 and 17.5 minutes of runoff, 25 liters of KBr tracer (5 g Br^{-} l^{-1}) was applied onto the field through a manifold hose that was extended across Transect 1

and Transect 2, respectively (Fig. 5.2). The manifold was 15 m long and contained holes at every 0.2 m. The Br^- tracer was applied at the transects to assess the average transport velocity and percent recovery of runoff water moving across the field from each transect to the runoff collection flume. After two hours of runoff, the irrigation system was turned off and , runoff samples were collected for one additional hour as the field drained. Following irrigation, soil cores were taken from the top 5 cm of soil at each of the 33 sample grid-points (Fig. 5.2). All manure, runoff, and soil samples were stored on ice in a cooler until laboratory analysis.

Figure 5.3 Irrigation of the OPE3 Field after the manure was applied. The H-flume, irrigation pipelines and sprinklers, and some of the manure/soil sampling points (i.e., the orange flags on the field) can be seen. Up-field from the irrigation area, a blue tarp can be seen on the field. That tarp is covering one of the research plots that was used, two days after this photo was taken, in the plot-scale study.

Plot-scale Study Design

Just outside of the irrigation area from the field-scale experiment, two field research plots (80 x 100 cm) were installed where manure had been applied for the plot-scale experiment. On the day of the original manure application at OPE3 Field, two tarps had been used to cover the manure that was to be used for this study. The tarps covered the plots to prevent the irrigation water from the field-scale study from contacting this section of manure on the field and to prevent the manure-bacteria from becoming inactivated by sunlight (Fig. 5.3). Each tarp was propped up to an average height of 50 cm to prevent direct contact with the manure.

The perimeter of each research plot was delineated by a stainless-steel weir that was inserted 20 cm into the ground by force with a dead-blow hammer. A stainless-steel runoff collection pan was disinfected with 70 % ethanol and then attached to the front end of each plot (Fig. 5.4). A rainfall simulator was programmed to deliver rainfall onto each plot at the rate of 1.8 cm hr^{-1} (Fig. 5.5). Water delivered by the rain simulator was pumped from two 500 gal tanks connected to the simulator via hose. A wind screen was hung up around the rainfall simulator to block wind from interfering with the precipitation event (Fig. 5.4). The sprinkler nozzles (Veejet 80100; Spraying Systems Co., Wheaton, IL) were positioned to rain from a height of 3 m above the plots so the raindrops could approach terminal velocity upon landing. By using these specific nozzles and maintaining the pressure of water flowing into the rainfall simulator at 41 N m^{-2} (i.e., 6 psi), a constant rainfall intensity at the desired rate and a homogenous rainfall distribution on each plot was established (Fig. 5.5). This rain simulator design allowed for raindrop impact energy to be approximately 200 kJ/ha-mm which is approximately equivalent to a natural rainfall

event with rainfall intensity less than 2.5 cm hr^{-1} (Meyer and Harmon, 1979). A full description of the rain simulator is provided in Meyer and Harmon (1979).

Five manure samples were collected from each plot prior to rainfall. During rainfall, runoff samples were taken from the runoff collection pan in sterile plastic bottles at five minute intervals for two hours following initiation of runoff (Fig. 5.4). All collection reference times and the duration time for each collection were recorded. Following rainfall, five soil samples were collected from the top 5 cm of soil within each plot. All manure, runoff, and soil samples were stored on ice in a cooler until laboratory analysis.

Figure 5.4 Experimental set up at the plot-scale experiments at OPE3 Field. The top photo shows the field site, including the rainfall simulator and the water tanks. On the bottom-left, a sample runoff sample is being collected from a plot inside the wind-tent. On the bottom-right, the plot is shown and the runoff collection pan may be observed.

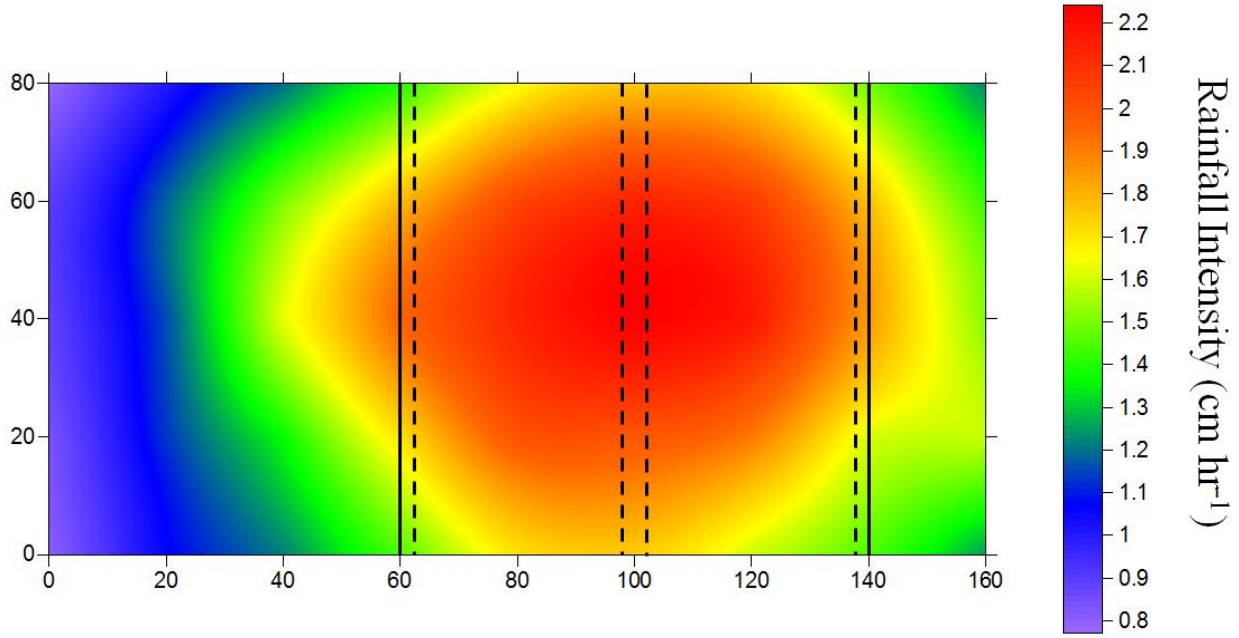

Figure 5.5 Map of rainfall delivery from the calibrated rainfall simulator that was used for the lab- and plot-scale experiments. The units on the x- and y-axes are centimeters. Solid lines show the position of the outer boundaries of the research plots under the simulator during the plot-scale study. Dashed lines show the position of the outer boundaries of the soil boxes under the simulator during the lab-scale study. Note that there is only 80 cm of height shown in on this map, so the research plots and soil boxes both extended 10 cm above and 10 cm below the area that is seen here (since they were both 1 m long).

Lab-scale Study Design

Three soil boxes (100 x 35 x 15cm) constructed to the specifications of Isensee and Sadeghi (1999) and Sadeghi and Isensee (2001) were used in the lab-scale study. Each box was equipped with one 10-mm diam. height-adjustable runoff drain positioned at the front end of the box and three 6-mm diam. infiltration drains positioned at the center of the base at 1 cm, 34 cm, and 67 cm from the front of the box. A mesh screen with 1 mm^2 openings was positioned over each infiltration drain in order to help prevent drain clogging during transmission of leachate. Two 14-mm height aluminum angle partitions were attached to the base of each box directly in front of the 34- and 67-cm base drains approximately 33 and 66 cm from the front end of the box

to aid in collection of sectioned leachate. For an illustration of the soil box design, see: Chapter 4, Fig. 4.1.

The base of each soil box was filled with an initial layer of 7 kg of ≤ 2mm air-dried sand that was spread across the base of the box, packed down with a 20- x 20-cm plywood board, and then grooved at the surface with a hand cultivator tool. A backhoe was used to remove soil from OPE3 Field and place it directly in the soil boxes over the sand layer (Fig. 5.6). Even the organic detritus from the surface of the soil remained in place during the loading process in order to maintain the same soil that was used in the plot- and field-scale experiments. The loaded soil boxes were brought to an indoor research facility at the USDA-ARS Beltsville, MD. The boxes were watered and the soil was allotted time to settle as it drained for two days to bring the water potential to field capacity.

Figure 5.6 Using a backhoe bucket to load soil from the OPE3 Field into three soil boxes for the lab-scale study.

On the morning of experimentation, manure was applied to the soil in each box at the rate of 60 t ha^{-1} and three manure samples were collected from each box. One disinfected hose of PVC vinyl tubing was connected to the runoff drain and another was connected to the three infiltration drains to aid in collection of surface runoff and leachate, respectively. The fully prepped box was positioned on a 2 % slope to mimic the average land slope at the OPE3 Field and placed underneath the rainfall simulator set to operate on the same rainfall intensity settings as the intensity used in the plot-scale experiment (Fig. 5.5). After rainfall was initiated, surface runoff and leachate were collected in sterile 100-ml bottles upon initial release (time 0) and then at five minute intervals for two hours. All collection reference times and the duration time for each collection were recorded. Following rainfall, five soil samples were collected from the top 5 cm of soil. All manure, runoff, and soil samples were stored on ice in a cooler until laboratory analysis.

Chemical and Microbiological Analyses

The surface runoff, manure, and soil samples that were collected during each of the three experiments were analyzed for their contents of total coliforms, *E. coli*, enterococci, and chloride ion (Cl^-). The water content of manure and soil samples was measured by calculating water loss after samples were oven-dried at 100^0 C for 24 h to a constant dry weight. The wet manure and soil samples were mixed with sterile deionized water at the rate of 2-g sample $200ml^{-1}$ water and blended at high speed for 2 minutes (model 34BL97; Waring Laboratory, Torrington, CT) to produce a homogenous slurry mixture. Slurry was allotted 1 hr of settling time before processing. The manure and soil slurries and the runoff and leahate samples were spread-plated on CHROMagarTM ECC (Chromagar, Paris, France) to enumerate *E. coli* and total coliforms and on m-Enterococcus agar (Neogen Corporation, Lansing, MI) to enumerate enterococci. The

CHROMagarTM ECC plates were incubated at 37^0 C for 24 hours and blue colony forming units (CFUs) were reported as *E. coli* and mauve CFUs were reported as coliforms that were not *E.coli*. Thus, the total coliform CFUs were the sum of the blue and mauve colonies on this agar. The m-Enterococcus agar plates were incubated at 37^0 C for 48 hours and red CFUs were reported as enterococci. The Cl^- content of each sample was measured with the QuantiChromTM Chloride Assay Kit (Abnova, Taipei, Taiwan).

Since the KBr tracer was applied to the field during the irrigation event of the field-scale study, bromide ion (Br^-) content of each runoff sample collected during the field-scale experiment was determined. The Br^- content was measured with the Bromide Combination Electrode (model no. 9635BNWP, Thermo Fisher Scientific, Beverly, MA, USA).

Removal with runoff modeling

The cumulative numbers of *E. coli*, enterococci, total coliforms, and the cumulative mass of Cl^- released from manure (M_0) into surface runoff (M_{Runoff}) were calculated by multiplying the values of the respective bacterial concentrations and Cl^- released into runoff by flow rates and integrating results over time. The dependency of M_{Runoff}/M_0 of each of the bacteria groups/species and Cl^- on rainfall/irrigation depth were simulated using three manure-constituent runoff-removal models (Eq. 1, 2, 3). Curve-fits were made using a FORTRAN code REL_BACT, which was based on the Marquardt-Levenberg optimization algorithm as implemented in van Genuchten (1981). The three models used were:

1. The exponential release dependence equation used in the watershed-scale Hydrologic Simpulation Program-FORTRAN model (HSPF) for microbial fate and transport (Benham et al., 2006; Moyer and Hyer, 2003):

Eq. 1. $$\frac{M_{Runoff}}{M_0} = 1 - e^{(-k_e W)}$$

where M_{Runoff} is the tota number of bacteria or Cl^- mass removed per unit area of manure within runoff, [M_{Runoff}] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is the initial number of bacteria or mass of Cl^- per unit area of applied manure, [M_0] = CFU (for bacteria) or mg (for Cl^-); k_e is the rate constant parameter, [k_e] = cm^{-1}; and W is rainfall depth, [W] = cm rainfall.

2. The Bradford and Schijven (2002) equation used in the farm-scale STWIR model for microbial fate and transport (Guber et al., 2009; Kim et al., 2014):

Eq. 2. $$\frac{M_{Runoff}}{M_0} = 1 - \frac{1}{(1+k_p \beta W)^{\frac{1}{\beta}}}$$

where M_{Runoff} is the tota number of bacteria or Cl^- mass removed per unit area of manure within runoff, [M_{Runoff}] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is the initial number of bacteria or mass of Cl^- per unit area of applied manure; k_p is the rate constant parameter, [k_p] = cm^{-1}; W is rainfall depth, [W] = cm rainfall; and β is a dimensionless shape parameter.

3. The Vadas et al. (2004) equation, which was originally developed to describe inorganic phosphorus loss in runoff from surface-applied dairy, poultry, and swine manure, and now may be used to predict contaminants, microbial indicators, and pathogen release from manure in the watershed-scale SWAT model (Benham et al., 2006):

Eq. 3. $$\frac{M_{Runoff}}{M_0} = AW^n$$

where M_{Runoff} is the tota number of bacteria or Cl- massved per unit area of manure within runoff, [M_{Runoff}] = CFU (for bacteria) or mg (for Cl^-) m^{-2}; M_0 is the initial number of bacteria or mass of Cl^- per unit area of applied manure; A is the rate constant parameter, [A] = cm^{-n}; W is rainfall depth, [W] = cm rainfall; and n is a dimensionless shape parameter.

Data Analysis

The runoff flow rate at the time of collection of each runoff sample and the time between each sample collection were used to compute the cumulative flux of runoff during each rainfall/irrigation event. Runoff coefficients calculated as the percent of applied water that was recovered in runoff were determined for each scale.

For the field-study, the transport time of Br^- from Transect 1 and Transect 2 to the runoff collection flume was measured as the lapse of time between applications of tracer solution and peak 1 and peak 2 of Br^- concentrations in surface runoff. The average velocity of water moving from each transect to the flume was calculated as distance travelled over transport time. The cumulative flux of Br^- in runoff was calculated to determine the Br^- recovery rate.

The concentrations of *E. coli*, enterococci, total coliforms, and Cl^- in initial surface runoff were compared with their starting concentrations in manure. Parameters for the dependencies of M_{Runoff}/M_0 for these manure constituents based on rainfall depth following the time of initial runoff were computed for each release event. The averages and standard deviations of parameter values for the plot- and lab-scale experiments were calculated since these experiments had two and three replications, respectively. The runoff-removal kinetics of manure constituents at the lab-, plot-, and field scales were compared.

Runoff-removal model performance was assessed by the root-mean-squared-error (RMSE) and the Akaike information criterion (AIC) values that were computed from each model fit. RMSE were computed as:

$$\mathrm{RMSE} = \sqrt{\frac{\mathrm{RSS}}{\mathrm{n}}}$$

where RSS is the residual sum of squares and *n* is the number of measurements. The RMSE units are dimensionless. The preferred model should have smaller RMSE values.

The Akaike information criterion (AIC) provides a means for model selection and accounts for the interplay between the model goodness of fit and the complexity of the model (Burnham and Anderson, 2002). In this study, the AIC test takes into account the fact that Eq. 1, 2, and 3 have one, two, and two parameters, respectively. The corrected Akaike statistic is:

$$AIC = nln\left(\frac{RSS}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1}$$

where RSS is the residual sum of squares, *n* is the number of measurements, and *k* is the number of model parameters.

The AIC units are dimensionless. Of the three models, the one that performs best should have the smaller corrected Akaike statistic.

To determine the efficiency of Cl^- as a surrogate tracer for microbial release and transport from the manured area, the correlation between Cl^- concentrations in surface runoff and *E. coli*, enterococci, and total coliforms were established. In addition, the Steiger's *Z*-test (Steiger, 1980) was used to test whether the Cl^- values correlate equally with the *E. coli* and enterococci values. The Z-test was applied to compare the correlations that the different bacteria had with Cl^- in surface runoff at each experimental scale.

The total relative number of *E. coli*, enterococci, total coliforms, and mass of Cl^- released from manure and recovered in surface runoff (M_{Runoff}/M_0) were determined for each scale. The total relative numbers of each bacteria group/species that were released from manure and remained in the top 5 cm of soil after rainfall or irrigation ($M_{Soil\ 5cm}/M_0$) was determined for each scale as well. This calculation was performed by taking the average concentration of bacteria in the top 5 cm of soil (CFU gdw^{-1}) after rainfall/irrigation and multiplying that value by the mass of dry soil in the top 5 cm of the soil profile in each respective experiment such that 5 cm soil depth x study area in cm^2 x 1.27 g cm^{-3} average soil bulk density for soil (Gish et al., 2009). The

total relative number of each bacteria released from manure and recovered in leachate ($M_{Leachate}/M_0$) was only determined for the lab-scale study since it was the only study in which leachate was collected. The values for M_{Runoff}/M_0 and $M_{Soil\ 5cm}/M_0$ for *E. coli*, enterococci, and total coliforms at each scale were compared to illustrate the effects of scale on cumulative microbial release and transport.

Results

Rain time and runoff coefficients at different scales

For the field-scale experiment, surface runoff arrived at the H-flume at the edge of the manure-covered field at 87 minutes of irrigation time. The irrigation system ran for two hours following runoff initiation. After rainfall was discontinuted and the field drained, surface runoff samples continued to be collected as the runoff flow rate decreased (Fig. 5.7). The flow rate of runoff peaked around a rate of 4 L min^{-1} and the cumulative flux of runoff water reached approximately 450 liters over the course of the sample collection time (Fig. 5.7).

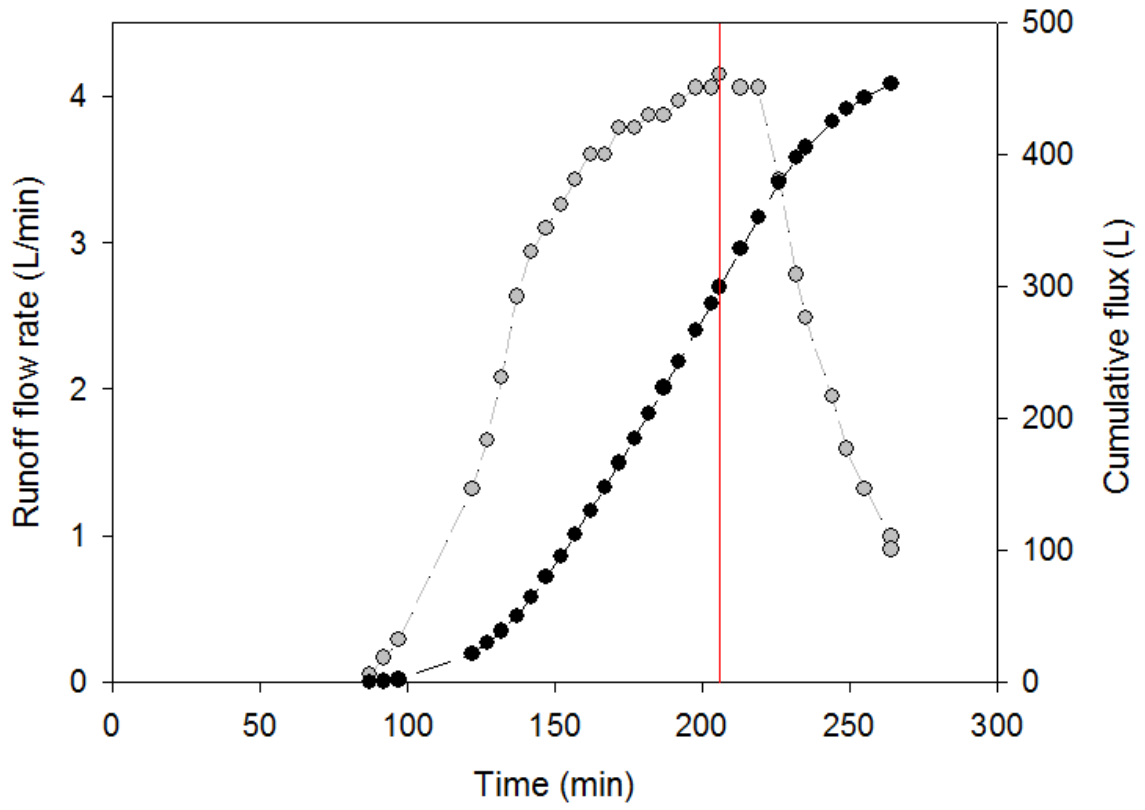

Figure 5.7 Hydrograph of runoff collected at H-flume during the field-scale study. Flow rate is shown in gray, cumulative flux is shown in black, and the red lines denotes time that irrigation system was turned off.

The total irrigation time at the field was 207 minutes (120 minutes of runoff) at the irrigation rate of 1.4 cm hr^{-1}, based on the average volume collected in the rain jars at the 33 grid points. The irrigation coverage area on the field was estimated to be 2772 m^2 such that the irrigation water volume was about 130 m^3 , based on 2772 m^2 area x 0.047-m depth of irrigation water. Conversion to a volume basis equates to 130,000 liters of water applied to the field and the recovery of 450 liters in runoff indicated a runoff coefficient of 0.346 %. Most of the land-applied water had infiltrated the soil during the time period between the initiation of irrigation start and the initiation of runoff.

The time of runoff initiation at the lab- and plot-scales was also delayed and the delay time varied among reps (Fig. 5.8) that resulted in variations in the cumulative flux of runoff

between reps (Fig. 5.8). Runoff was initiated during rep 1 and 2 of the plot-scale study at 31 and 89 minutes of rainfall, respectively, while it began during reps 1, 2, and 3 of the lab-scale study at 21, 31, and 34 minutes of rainfall, respectively (Fig. 5.8). The differences in runoff initiation time among treatments at the field-, plot-, and lab-scale as well as differences in rain duration within replications at the same scale may be attributed to heterogeneity in manure and soil. The runoff coefficients at the plot-scale and lab-scale were 32.9 ± 21.6 % and 45.0 ± 12.1 %, respectively. With a runoff coefficient of only 0.346 % for the field-scale, there was a very strong effect of scale on the amount of water applied to each study area that was recovered at runoff collection points (Fig. 5.9).

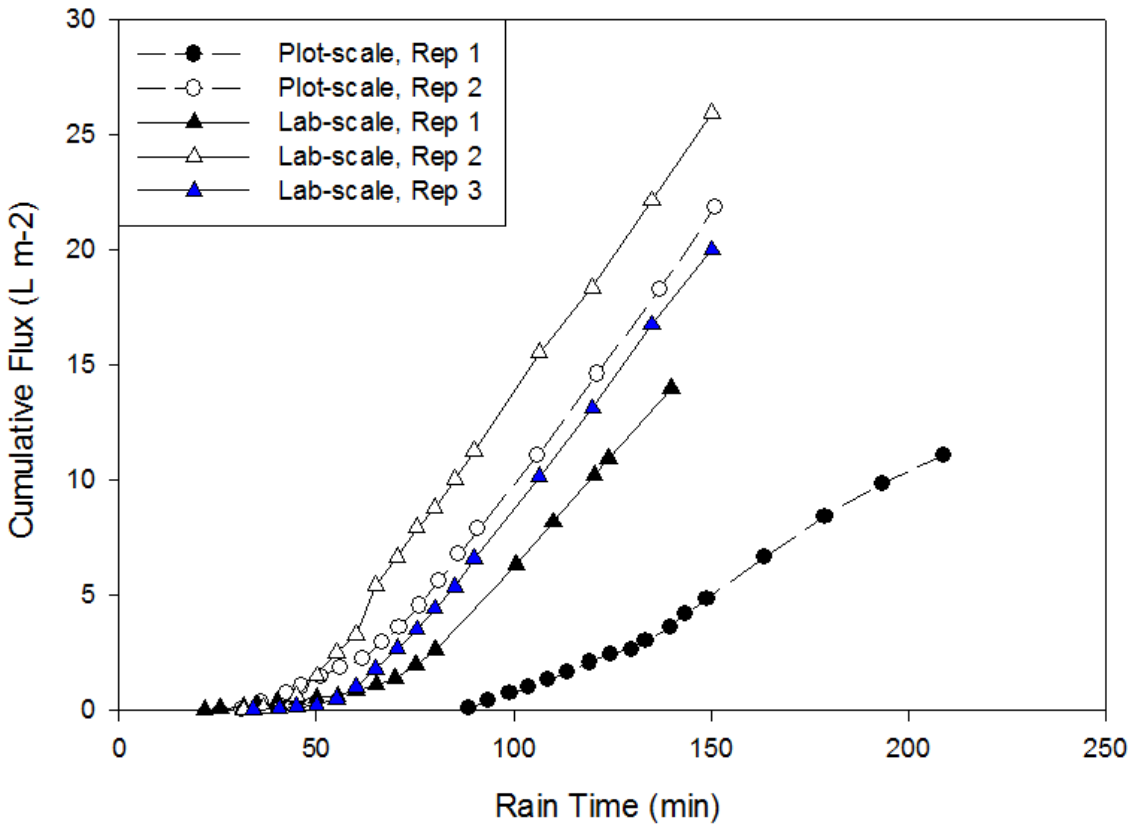

Figure 5.8 Cumulative flux of runoff for the plot- and lab-scale experiments.

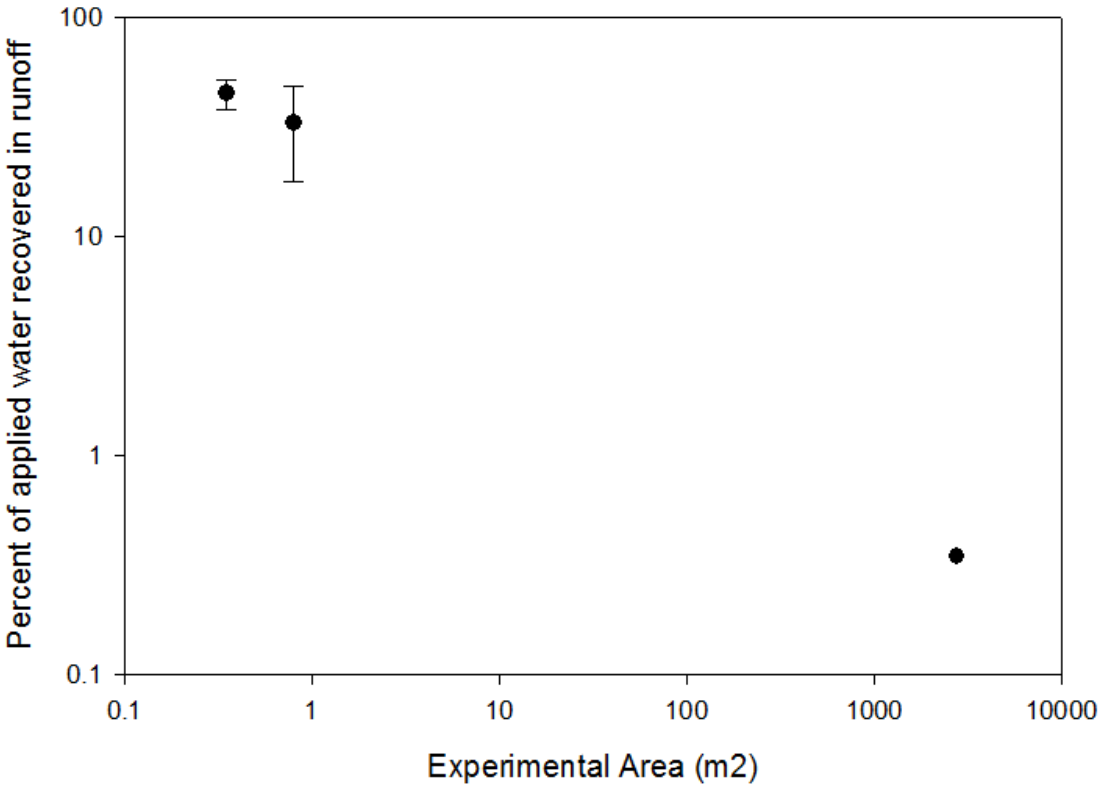

Figure 5.9 Effect of scale on the percent of applied water that was recovered in runoff. Error bars indicate standard error for the treatments that were replicated.

Transport of Br^- tracer at the field-scale

The KBr tracer that was applied during the field-scale study at Transect 1 and Transect 2 induced two peaks of Br^- concentration in the collected surface runoff samples. The tracer solution was first applied at Transect 1 at 7.5 minutes after runoff initiation and the application time lasted for two minutes, thus the reference time for the Transect 1 application was 8.5 minutes of runoff duration that corresponded to 95.5 minutes of rainfall. The tracer solution was then applied acrossTransect 2 at an irrigation time of 17.5 minutes for 1.5 minutes, thus the reference time for Transect 2 application was 18.25 minutes of runoff that corresponded to 105.25 minutes of rainfall. The first and second peaks of Br^- concentrations in collected runoff occurred at approximately 130.5 and 154.0 minutes of rainfall, respectively (Fig. 5.10). Thus, the

travel times of Br^- tracer from Transect 1 and Transect 2 to the runoff collection flume were approximately 35 minutes (i.e., 130.5-95.5) and 49 minutes (i.e., 154 -105.25), respectively. Since Transect 1 and 2 were located at 28 and 46 meters up-field from the runoff collection flume, the average transport velocity of Br^- from Transect 1 and 2 across the field to the flume was 0.80 m min^{-1} (i.e., 28 m in 35 min) and 0.94 m min^{-1} (i.e., 46 m in 49 min), respectively.

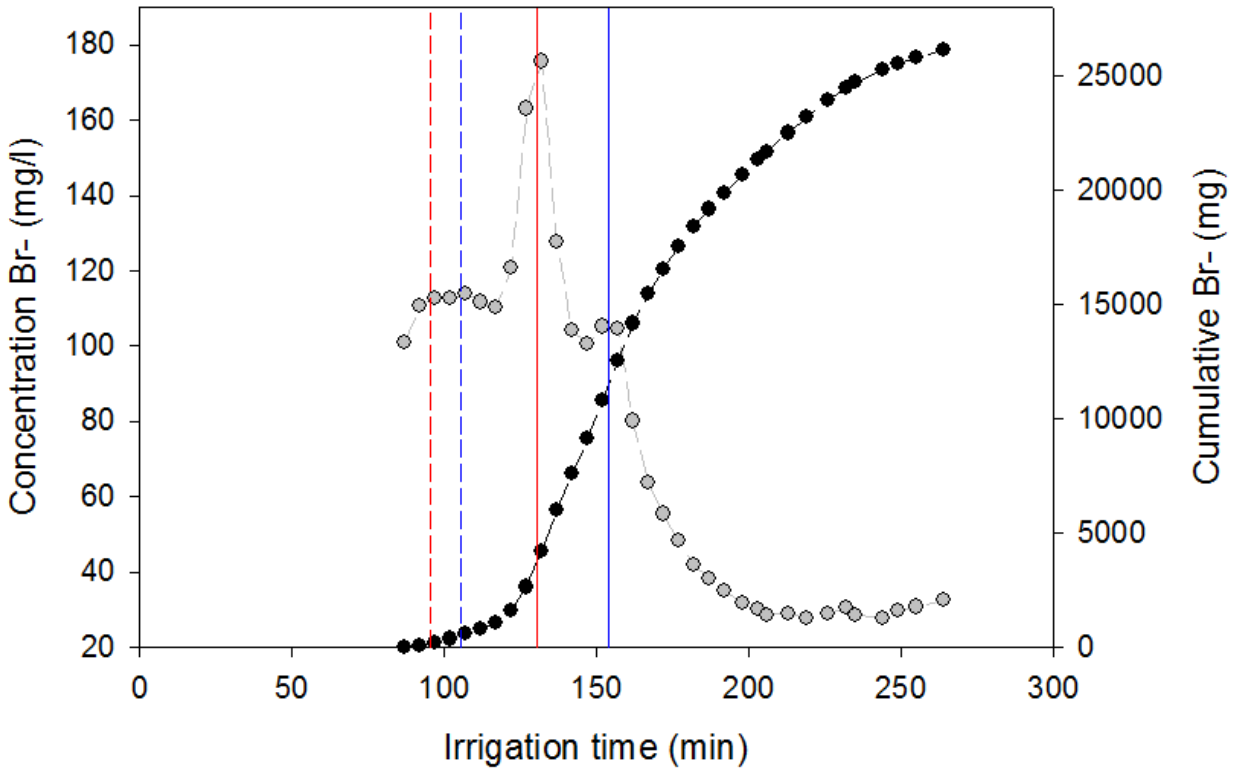

Figure 5.10 Br^- concentration (gray points) and cumulative flux (black points) in surface runoff during the field-scale study. The time of tracer application at Transect 1 and 2 are indicated by the dashed red and blue lines, respectively. The time of Br^- concentration peak 1 and 2 in runoff are indicated by the solid red and blue lines, respectively.

Within the 50 liters of tracer solution that was applied on the field, there was a total of 250 g of dissolved bromide ion. The total mass of Br^- recovered in runoff was approximately 27 g, which was a 10.8 % recovery rate.

Initial surface runoff concentrations versus initial contents in manure

The average concentrations of total coliforms, *E. coli*, and enterococci in initial surface runoff were typically in the range of 10^5-10^6 CFU ml^{-1}, regardless of scale, which was greater than one order of magnitude below the starting concentration of bacteria in manure. The trend seen for slightly greater $C_{Initial\ Runoff}/C_{Manure}$ for total coliforms than the other two bacteria may have been due to the background content of indigenous total coliforms that were present in the soil. Although total coliforms were present in the soil at lower concentrations than in the manure, the other bacteria were not detected in the soil prior to manure application.

The average concentrations of Cl^- in initial surface runoff were 459, 184, and 191 ppm for the lab-scale, plot-scale, and field scale studies, respectively. Although the $C_{Initial\ Runoff}$ for the dissolved anion was more than one order of magnitude less than its C_{Manure}, the ratio of $C_{Initial\ Runoff}/C_{Manure}$ of the Cl^- was greater most of the time than the ratios for the indicator bacteria and suggested that rainfall/irrigation induced a more rapid leaching of the dissolved anion than the manure-associated bacteria (Table 5.3). There was no noticeable effect of scale on $C_{Initial\ Runoff}/C_{Manure}$ (Table 3).

Table 5.3 The average concentration of total coliforms, *E. coli*, enterococci, and Cl^- in initial runoff ($C_{Initial\ Runoff}$) divided by the average concentration in manure (i.e., the content of the respective manure constituent in manure divided by the manure water content) (i.e., C_{Manure}) for each study displayed in terms of percent (i.e., $100*C_{Initial\ Runoff}/C_{Manure}$).

Manure Constituent	Field-scale	Plot-scale	Lab-scale
Total Coliforms	8.91 %	2.27 %	4.59 %
Escherichia coli	5.23 %	2.90 %	4.25 %
Enterococci	6.57 %	0.79 %	4.03 %
Chloride ion	9.39 %	1.03 %	7.97 %

Runoff-removal kinetics and model performance

The rainfall simulator used for the lab- and plot-scale experiments had been calibrated to consistently deliver rainfall at the rate of 1.8 ± 0.2 cm hr^{-1} (Fig. 5.5). The rate of irrigation water delivery during the field-scale experiment was slightly different and based on the volume of water collected in the rain jars at the 33 sample points, the irrigation rate at the field was 1.4 ± 0.5 cm hr^{-1}. Since the rainfall/irrigation intensities varied somewhat among treatments, which was due to complications of controlling irrigation intensity at the field-scale, and since the runoff initiation time varied not only across scale, but also within replications at plot- and lab-scales (Fig. 5.8), the M_{Runoff}/M_0 of the manure constituents was modeled based on their dependencies on rainfall depth after runoff initiation rather than total rainfall depth. The manure constituent release began with a several orders of magnitude increase in cumulative mass released during several mm of rainfall following runoff initiation after which time the mass release-rate appeared to decrease as M_{Runoff}/M_0 approached an asymptote (Fig. 5.11). The dependency for M_{Runoff}/M_0 on rainfall depth appeared to be strongly dependent on scale and as scale was reduced, the M_{Runoff}/M_0 asymptote appeared to approach a higher value (Fig. 5.11). Thus, the runoff-removal rate-constants for manure constituents decreased with increased scale. The parameter values

generated by fitting Eq. 1, 2, and 3 to the data shown in Fig. 5.11 are displayed in the Appendix B.

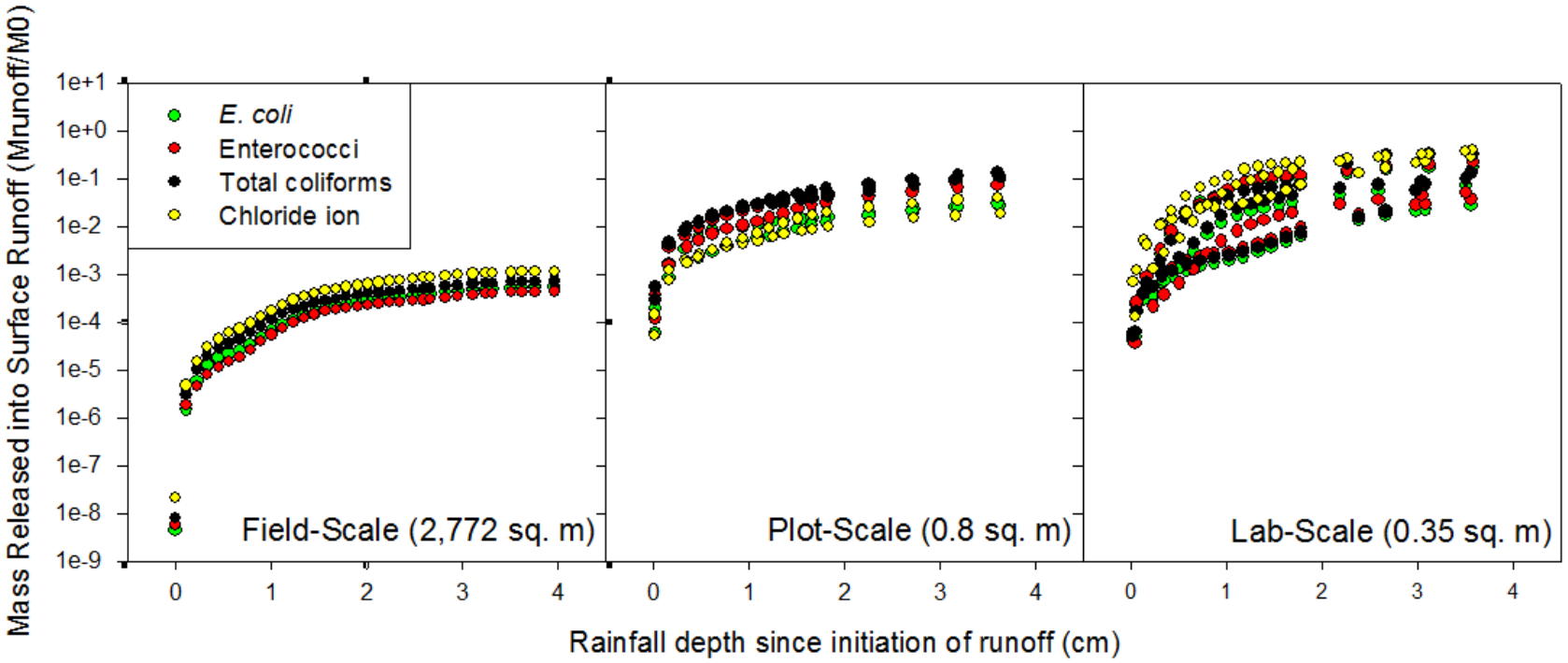

Figure 5.11 Relative cumulative mass of *E. coli*, enterococci, total coliforms, and Cl^- that was released from manure and recovered in edge-of-field surface runoff as a function of rainfall depth beyond the time of runoff initiation at the field-, plot-, and lab-scales. Since the plot- and lab-scale studies were performed with replicates, a release dependency plot is displayed for each individual replicate.

Runoff-removal model performance, assessed with the RMSE and AIC for each curve-fit, was somewhat situation-specific. Since the lowest RMSE and most negative AIC indicate the "best" model, Eq. 2 appeared to perform better than Eq. 1 and Eq. 3 most of the time, but there were still some cases when Eq. 3 performed better than Eq. 2 and Eq. 1 (Table 5.4).

Table 5.4 The root-mean-squared-error (RMSE) and Akaike information criterion (AIC) from fitting Eq. 1, 2, and 3 to the manure constituent runoff-removal curves at each scale. The values at the plot- and lab-scales are the averages among the replications.

		Field-Scale		Plot-Scale		Lab-Scale	
Manure Constituent	Model	RMSE	AIC	RMSE	AIC	RMSE	AIC
E. coli	Eq. 1	2.02E-05	-709.96	1.79E-03	-213.92	6.51E-03	-172.73
	Eq. 2	3.30E-05	-698.34	4.44E-04	-259.07	4.39E-03	-195.04
	Eq. 3	6.70E-05	-690.01	4.48E-04	-258.78	4.83E-03	-192.84
Enterococci	Eq. 1	2.83E-05	-690.04	4.18E-03	-185.70	7.46E-03	-168.81
	Eq. 2	3.88E-05	-689.56	1.57E-03	-215.68	3.92E-03	-202.11
	Eq. 3	3.89E-05	-669.07	1.72E-03	-212.69	4.53E-03	-199.28
Total coliforms	Eq. 1	2.02E-05	-709.98	5.91E-03	-173.22	2.88E-02	-127.98
	Eq. 2	3.30E-05	-698.37	2.54E-03	-205.32	1.18E-02	-160.01
	Eq. 3	2.74E-05	-690.02	2.39E-03	-206.33	1.31E-02	-157.44
Chloride ion	Eq. 1	7.53E-05	-644.48	1.52E-03	-231.94	3.17E-02	-117.69
	Eq. 2	6.65E-05	-650.67	8.94E-04	-240.47	1.12E-02	-150.89
	Eq. 3	6.66E-05	-650.62	9.19E-04	-239.75	1.46E-02	-143.75

Manure aging and correlation of manure-bacteria and Cl^- in surface runoff

The plot-scale study was performed two days after the field-scale study. During that short lag-time, the manure slightly aged as it dried, which is indicated by the manure that was used in plot-scale study having a lower percentage of liquid content than the manure used in the other studies (Table 5.2). The starting concentrations of bacteria in manure slightly decreased as the

manure dried, hence the observation of the range in bacteria concentrations in runoff being lower for the plot-scale study than the lab- and field-scale studies (Fig. 5.12). During the short period of manure aging, the Cl^- content in manure did not appear to change much, hence the Cl^- concentrations in runoff was in the range of 80-800 ppm for release events at all scales (Fig. 5.12). Manure bacteria can be associated with manure liquid contents and/or manure solids. Because some of the liquid evaporated, there was a decrease in the amount of bacteria that were associated with manure liquids during the time between the field-scale and plot-scale experiments. These deceases in the amounts of liquid and bacteria did not allow conclusive comparisons of the correlations of bacteria concentrations to Cl^- concentrations in runoff at different scales to be made.

There was a noticeable increase in bacteria correlations to Cl^- in runoff when going from the plot- to the field-scale (Fig. 5.12). At the field-scale, the variability in concentrations of *E. coli*, enterococci, and total coliforms in surface runoff could be explained by Cl^- concentrations in surface runoff 74.5, 70.4, and 85.1 % of the time, respectively (Fig. 5.12). According to the Steiger's Z-test, there were differences in the correlations between three bacteria and Cl^- in the runoff and the similarity seen between correlations of Cl^- with each bacteria group/species was greatest at the field-scale (Table 5.5). Overall, the correlations of Cl^- with each bacteria in runoff were significant at all scales ($p<0.05$), but one must be careful when using Cl^- as a surrogate tracer for bacteria in surface runoff because the significant relationship is not one-to-one.

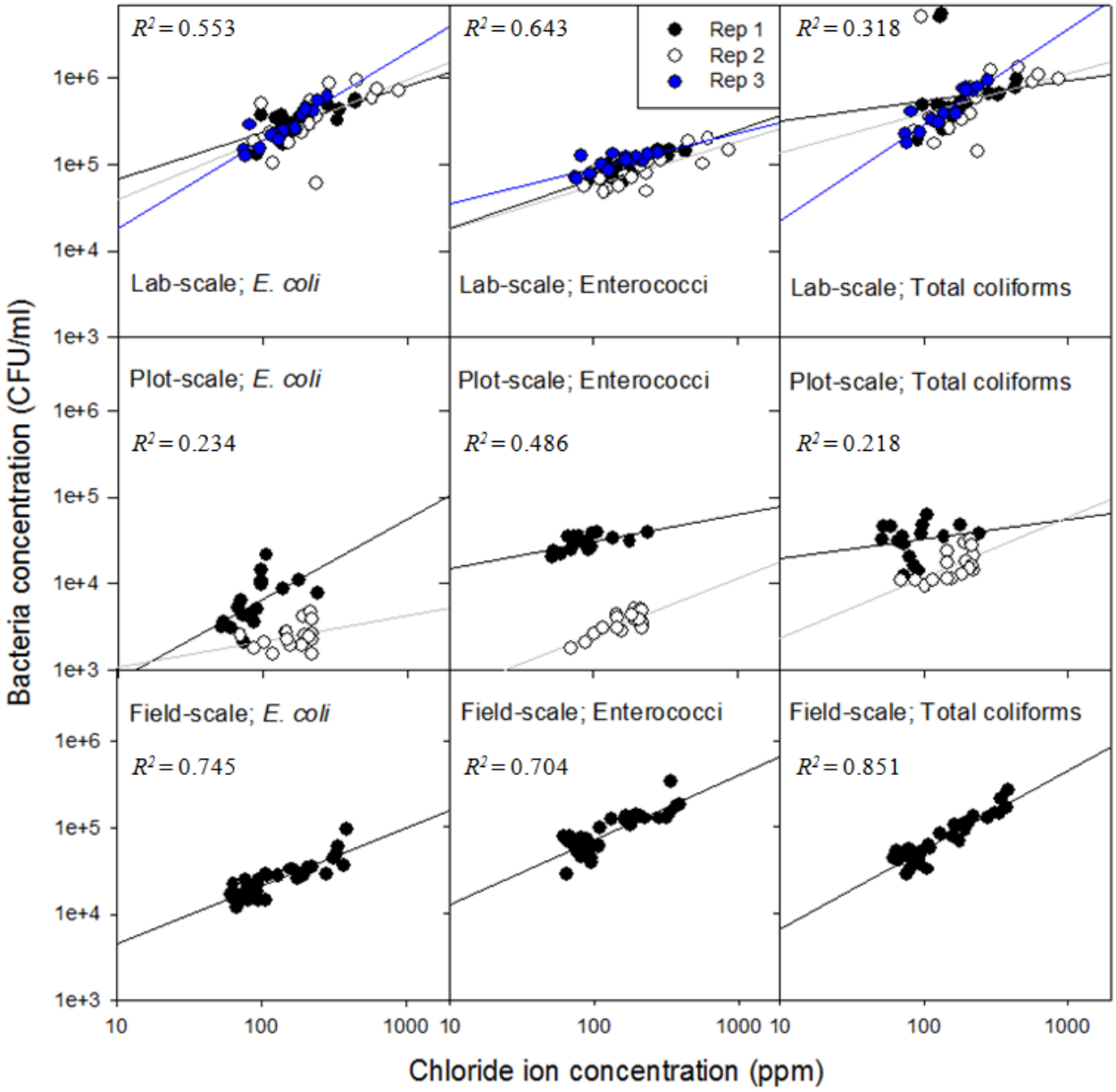

Figure 5.12 Linear regression of the concentrations of *E. coli* (left column), enterococci (middle column), and total coliforms (right column) vs. the concentrations of Cl^- in surface runoff from the lab- (top row), plot- (middle row), and field-scale (bottom row) studies. The lab-, plot-, and field-scale studies had 3, 2, and 1 replications, respectively, and the data for each replication are distinguished by circle color. The determination coefficient, R^2, is presented with each graph, and for the lab- and plot-scale, the reported R^2 values are the average R^2 value among replications.

Table 5.5 Results from Steiger's *Z*-test (p-values shown), which tests for probability of similarity between two criterion variables (i.e., concentrations of different bacteria) with a single predictor (i.e., concentration of Cl^-).

	Lab-scale	*Plot-scale*	*Field-scale*
E. coli/Cl^- vs. Enterococci/Cl^-	0.346	0.256	0.679
E. coli/Cl^- vs. Total coliforms/Cl^-	0.239	0.504	0.989
Total coliforms/Cl^- vs. Enterococci/Cl^-	0.325	0.067	0.990
Average	0.303	0.276	0.886

Total bacteria released from manure during rainfall

The total numbers of the indicator bacteria in the land-applied manure at the lab-, plot, and field-scale studies were around 10^{10}, 10^9, and 10^{13} CFU, respectively, on the day of experimentation (Table 5.6). During each rainfall/irrigation event, bacteria that were released from manure were transported away from the manure with surface runoff and infiltration. The ratios of mass of bacteria recovered in runoff to the starting mass of bacteria in manure substantially decreased as scale increased. The average percentages of land-applied total coliforms, *E. coli*, and enterococci recovered in surface runoff were 12.8 %, 7.9 %, and 0.06 % at the lab-, plot-, and field-scales, respectively (Table 5.6) (Fig. 5.13). The average percentages of land-applied Cl^- that was recovered in surface runoff were 35.0 %, 2.9 %, and 0.12 % at the lab-, plot-, and field-scales, respectively (Fig. 5.13). Thus, there was a general trend of a higher Cl^- recovery rate than that of the indicator bacteria.

With regard to the bacteria that were transported from surface-applied manure into the soil, there was much less of an effect of scale. The average percentages of land-applied total coliforms, *E. coli*, and enterococci that were measured in the top 5 cm of soil after rainfall/irrigation were 10.5 %, 10.2 %, and 5.3 % at the lab-, plot-, and field-scales, respectively (Table 5.6) (Fig. 5.13). The concentration of Cl^- in the soil at the OPE3 field before and after

irrigation was very low and most of the collected samples were out of the measurable range of the Cl^- test kit. The samples that were in the range of the Cl^- analysis showed an average content of 34 mg Cl^- kg^{-1} soil after rainfall. Since this was very similar to the Cl^- content in soil prior to rainfall ($p=0.829$), the amount of Cl^- released from manure and recovered in the top 5 cm of soil after rainfall was considered to be zero.

Table 5.6 The average starting mass of total coliforms, *E. coli*, and enterococci in the land-applied manure (M_0), the average mass of each bacteria group/species removed from the study area in surface runoff (M_{Runoff}), and the average mass of each bacteria group/species remaining in the top 5 cm of soil after rainfall ($M_{Soil\ 5cm}$) for the lab-, plot-, and field-scale experiments. Percentages for M_{Runoff}/M_0 and $M_{Soil\ 5cm}/M_0$ are listed as well.

Indicator Bacteria	Lab-scale study			Plot-scale study			Field-scale study		
	M_0 (CFU)	M_{Runoff} (CFU); M_{Runoff}/M_0 (%)	$M_{Soil\ 5cm}$ (CFU); $M_{Soil\ 5cm}/M_0$ (%)	M_0 (CFU)	M_{Runoff} (CFU); M_{Runoff}/M_0 (%)	$M_{Soil\ 5cm}$ (CFU); $M_{Soil\ 5cm}/M_0$ (%)	M_0 (CFU)	M_{Runoff} (CFU); M_{Runoff}/M_0 (%)	$M_{Soil\ 5cm}$ (CFU); $M_{Soil\ 5cm}/M_0$ (%)
Total coliforms	3.59×10^{10}	6.75×10^{9}; 18.8 %	1.28×10^{9}; 3.56 %	2.15E+09	2.66×10^{8}; 12.4 %	1.49×10^{8}; 6.92 %	3.39E+13	2.51×10^{10}; 0.074 %	2.14×10^{12}; 6.30 %
E. coli	2.72×10^{10}	2.51×10^{9}; 9.20 %	1.03×10^{9}; 3.77 %	1.45E+09	4.25×10^{7}; 2.92 %	2.92×10^{8}; 20.05 %	1.74E+13	9.66×10^{9}; 0.056%	9.47×10^{11}; 5.45%
Enterococci	8.07×10^{9}	8.41×10^{8}; 10.4 %	7.78×10^{8}; 9.64 %	1.63E+09	1.37×10^{8}; 8.42 %	6.08×10^{8}; 3.74 %	7.54E+13	3.36×10^{10}; 0.044 %	3.14×10^{12}; 4.16 %

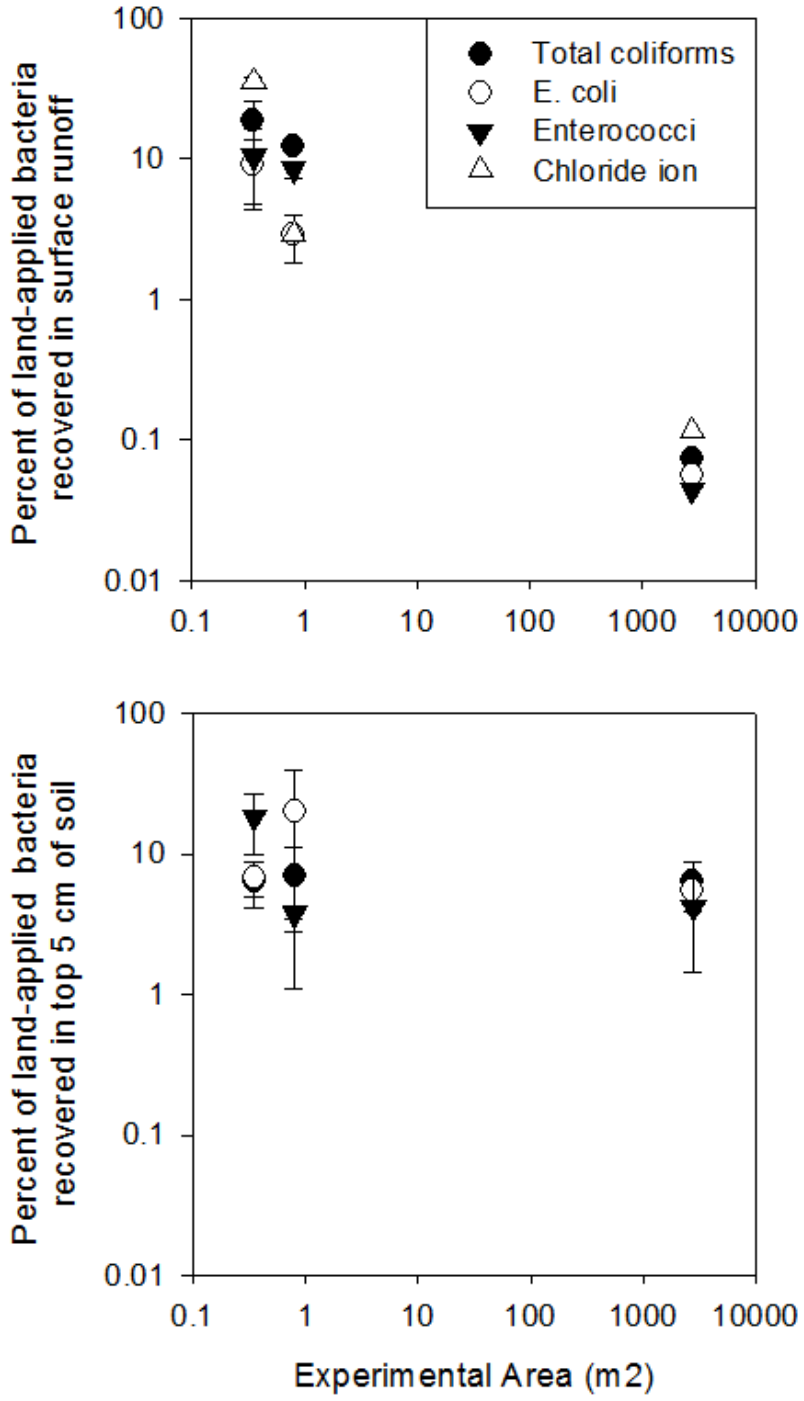

Figure 5.13 The percent of bacteria that were released from land-applied manure and recovered in surface runoff (top), and the percent of bacteria that were released from land-applied manure measured in the top 5 cm of soil after rainfall (bottom). Error bars indicate standard error.

The average cumulative numbers of bacteria released from manure that were recovered either in surface runoff and in the top 5 cm of soil after rainfall/irrigation were 23.3 %, 18.1 %, and 5.4% at the lab-, plot-, and field-scales, respectively. Thus, while the effects of scale were most substantial for bacteria that were recovered in surface runoff, it is also seemingly evident for the numbers of bacteria that are released from manure during rainfall/irrigation determined by summing the number recovered in runoff plus the mass recovered in the top 5 cm of surface soil.

At the lab-scale, which was the only scale where leachate water was collected (with a soil profile of approx. 10 cm), the average percent mass of land-applied *E. coli*, enterococci, and total coliforms recovered in leachate were 4.3 %, 2.9 %, and 7.0 %, respectively. The average percentage of land-applied Cl^- that was recovered in leachate during the lab-scale study was 25.0 %. Therefore, assuming the amount of Cl^- remaining in soil was trivial, at the lab-scale, the average percentages of indicator bacteria and Cl^- released from manure into the soil determined by the sum of $M_{Soil\ 5cm}$ and $M_{Leachate}$ were 15.6 % and 25.0 %, respectively.

Discussion

Generation of runoff on a field requires the depressions at the soil surface to become filled with water until their maximum storage capacity is reached, the water overflows, and then travels along a pathway towards a point of lower elevation (Van de Geisen et al., 2011). During a rainfall-runoff event, the filling and overflowing of topographic micro-depressions, meso-depressions, and/or macro-depressions is a progressive, continuous process (Penuela et al., 2013). A network for overland flow is formed as channels unite the overflowing depressions based on the connectivity of the heterogeneous properties of soil that govern the infiltration of water (Gomi et al., 2008). Runoff does not necessarily flow across the entire landscape to the

point of lowest elevation because it may be more energetically favorable for moving water to infiltrate soil prior to reaching that point (Van de Geisen, 2011). In the network of connected surface runoff channels, the smaller channels may feed into larger pathways for preferential surface flow, similar to how a headwater stream leads into a first order stream, which then meets with another first order stream to form a second order stream, and so on. The connectivity of an overland flow network is a function of land use, slope, and surface runoff potential, all of which become more heterogeneous as scale increases (Delmas et al., 2011).

In this study, scale influenced the time of rainfall/irrigation that initiated surface runoff. At the lab-, plot-, and field-scales the first runoff samples were collected on average at 29, 60, and 87 minutes of rainfall/irrigation, respectively. The same positive relationship between scale and runoff delay time was seen in the work of Penuela et al. (2013). Conceptually, as scale at any given site increases, the amount of topographic depressions in the study area must increase; therefore, with an increase in scale and potentially an increased volume to be stored in depressions, a greater volume of water is needed to form a complete overland flow network by filling and connecting all soil surface depressions (Penuela et al., 2013). Also, at different scales, the soil macrobiota and networks of live/dead roots are likely going to be different, which would impact soil compaction rates for runoff generation.

This study showed that the total percentage of land-applied water collected in runoff or the runoff coefficient decreased as experimental area increased. The average runoff coefficients were 45.0 ± 12.1 %, 32.9 ± 21.6 %, and a mere 0.346 % at the lab- (0.35 m^2), plot- (0.8 m^2), and field-scales (2772 m^2), respectively. Other authors have also seen an inverse relationship between scale and runoff coefficients. Cerdan et al. (2004) reported runoff coefficients of 20%, 4.5%, and 1% for 4.5 ha plots, a 90 ha catchment, and an 1100 ha catchment, respectively.

Likewise, Delmas et al. (2012) compiled data from Nothwestern Europe and found values for the runoff coefficients to be 30-50%, 10-20%, 5-10%, and 0-5% for areal units of 10^{-4} ha (plot), 0.1-1 ha (field), 10 ha (hillslope), and 100 ha (catchment), respectively, at lands with a 2 to 5% surface slope. In addition, Gomi et al (2008) observed runoff coefficients of 20-40% and 0.1-3% for 10^{-4} ha plots and 0.02 ha hillslope areas, respectively, in a Japanese forested landscape. In an Israeli study for land in semiarid conditions, Kidron (2011) reported runoff coefficients in the range of 30 to70 % for 10^{-4} ha plots, 0 to 20% for 2 ha plots, and <1% for 12 ha plots.

The presence of micro- and meso-topographic features are commonly invoked as a cause of the scale effect for runoff (Langhans et al., 2014). As scale increases, new processes that contribute to connectivity and patchiness of overland flow emerge (Dos Santos et al., 2012), and the interconnectedness of the different sized channels within the overland flow network becomes more complex (Van de Geisen, 2011). When overland flow occurs, local (micro) depressions fill up and after such depressions are filled, they will be overtopped and water and will start to flow downhill. The flow pattern looks like "a shallow sheet of water with threads of deeper, faster flow, diverging and converging around surface protuberances" with mixed occurrence of turbulent and laminar flow (Van de Giesen, 2011). This type of flow includes a braiding pattern of water threads, without the complete slope being covered by water, and it creates conditions for re-deposition of water and material suspended in water (e.g., eroded sediment and manure) within the observation area (Van de Giesen, 2011). In effect, runoff that begins uphill from a given collection location (e.g., the runoff drain, runoff pan, and H-flume used in the lab-, plot-, and field-scale studies in this work) should have a lower probability of arriving at said point than runoff that begins from a shorter distance uphill from said point. Due to a relative increase of the infiltration downslope and to temporal changes in rainfall intensity (e.g., the spinning irrigation

sprinkler heads in the field-scale study), runoff generated at a specific point will not necessarily reach the lower boundary of the land slope (Sheridan et al., 2014). Evidence for this phenomenon was seen in this study at the field-scale when the second Br^- concentration peak in runoff (i.e., Br^- tracer moving from Transect 2, which was 46 m uphill from the flume) was more dilute than the first Br^-concentration peak in runoff (i.e., Br^- tracer moving from Transect 1, which was 28 m uphill from the flume).

As the length of the study area increased, the land-applied total coliforms, *E. coli*, enterococci that were released from manure and removed with surface runoff had to have been suspended in the runoff for a longer time and transported across further distances to arrive at the runoff collection point. The manner in which the bacteria were distributed in suspension is important for considering the settling of bacteria from the runoff suspension on the field, according to Stokes' Law. Particle-associated bacteria are less mobile as the larger particles will sink faster (Fries et al., 2003). The effects of scale have been observed for sediment yield in runoff (i.e., the relative amount eroding soil that is transported out of the study area) (Delmas et al., 2012; Raclot et al., 2009) and some manure-bacteria are known to be transported in runoff attached to soil particles, especially during prolonged rainfall when the contact between manure and soil increases as manure is dissipated (Soupir et al., 2010). It would logically follow that the effects of scale should also be evident for bacteria yield (i.e., the relative number of bacteria eroding from manure that are transported out of the study area) and is one potential explanation for the negative effects of scale on bacteria removal with runoff. Another possible explanation is that the preferential overland flow pathways within catchments control most of the water that impacts the runoff coefficient (Cerdan et al., 2004). Therefore, the noted inverse relationship for percent of bacteria recovered and scale could have been caused by the preferential overland flow

pathways with bacteria suspensions becoming diluted over time, while the pathways of lower-order channel flow in the network (where manure had been less diluted) contributed less to the total bacteria removed within collected runoff. In other words, much of the starting content of bacteria in manure remained in the manure in the spots on the field that were not directly connected to preferential overland flow. In addition, the effects of scale on bacteria and Cl^- removal with runoff can simply be explained by the same principles that govern the scale effects on runoff coefficients. Since the movement of manure constituents depends on the amount of runoff that moves out of the study area. In theory, the effects of scale would be expected to actually be greater on the bacteria yield than the runoff coefficient because of mechanisms for bacteria "settling out" of the moving runoff. In support of this hypothesis, there was less than a 2 orders of magnitude difference in the runoff coefficients at the smallest and largest scales in this study (Fig. 5.9), while there was greater than a 2 orders of magnitude difference for all land-applied bacteria and Cl^- recovered in surface runoff at the smallest and largest scales in this study (Fig. 5.13).

The connectivity of 'infiltrating' and runoff producing' areas become more variable with an increase in scale (Cerdan et al., 2004). Since the complexity of the connectivity of an overland flow network increases with scale, it is reasonable to assume that surface runoff becomes less 1-dimenional as scale increases. During simulated rainfall at the lab-scale (0.35 x 1 m) and plot-scale (0.8 x 1 m), the runoff flow pathway must have been close to 1-dimensional due to a homogenous surface slope for these short, 1 meter long study areas that had artificial outer boundary limits (i.e., wooden edges of the soil box, and sheet metal edges of the field plots) that would re-direct any outward 2-D flow to move forward, downhill towards the runoff collection location. Flow velocity at the finer scale (e.g., the lab- and plot-scale studies in this work) is

more affected by soil roughness that contributes to the friction factor for resistance to moving water, while as scale increases, flow velocity becomes more and more dependent on the spatial distributions of morphologies that influence connectivity of the overland flow network on the field such as the spatially distributed topographic depressions and surface seals (Penuela et al., 2013).

In order to accept these conclusions as true effects of scale and determine that all of the field-runoff leads to the flume at the edge-of-field (rather than losing runoff collection at another edge-of-field location), the flow at the field-scale needed to be generally proven to be 1-dimensional like that at the lab- and plot-scales even though the flow network was more complex. The lack of differences in flow velocity from the two tracer applications demonstrates that flow on the field was generally 1-dimensional towards the flume. Thus, the noted effects of scale on the transport of bacteria out of manured areas in this work are acceptable.

The models that simulated the dependency of M_{Runoff}/M_0 for bacteria and Cl^- on rainfall depth must be considered as models for manure bacteria removal with runoff rather than models for microbial release from manure (as they had been used in Chapter 3) since transport was involved and infiltration water was not collected on the field. Based on the lowest RMSE and most-negative AIC associated with fitting the M_{Runoff}/M_0 dependencies to Eq. 1, 2, and 3, the Bradfod-Schijven model (Eq. 2) performed the best. This runoff-removal model has been recommended by others in research comparing these same three models for the release of manure-bacteria from bovine slurry (Guber et al., 2006; Guber et al, 2013).

The dependency of M_{Runoff}/M_0 for bacteria and Cl^- on rainfall depth followed a two-stage kinetic process with a precipitous early removal after which time the mass release-rate drastically decreased and became closer to zero (Fig. 5.11). The two-stage runoff-removal process may have

resulted from a period of time of an initial washout of planktonic bacteria and loose manure particulates containing adsorbed bacteria, after which time a seal or semi-seal of manure formed, and the small manure aggregates containing bacteria had to be sloughed off from the manure matrix to enter runoff. Compared with the precipitous release of bacteria during the first stage, the release of organic matter that was sloughed was probably much slower, hence the observation of a well-defined, slower second stage. The dependencies of M_{Runoff}/M_0 on time were similar for each bacteria species/group and increasingly similar as the study area expanded (Fig. 5.11). This observation reinforces the assumption that the physics of microbial transport with water are most responsible for the scale effects.

The relative concentrations of Cl^- in initial surface runoff (i.e., C_{Runoff}/C_{Manure}) were slightly larger than that for bacteria (Table 5.3), and Cl^- seemed to have a slightly greater recovery rate than the bacteria (Fig. 5.11). These trends may be explained by a slightly more rapid leaching of the dissolved anion from the manure than the leaching observed for the bacteria. Bacteria in manure can be associated with the liquid and solid manure phases as the bacteria may be planktonic or be a part of the manure matrix, while dissolved Cl^- is generally only associated with the liquid phase of manure. The liquid phase of manure is mostly leached during a rainfall event, while the solid phase must be sloughed off. In theory, the bacteria content removed with runoff should be less than the Cl^- content because of its partial association with manure solids that are not removed from the field during rainfall.

Surrogates, in the context of environmental microbiology, are defined as organisms, particles, or substances used to study the fate of a pathogen in a specific environment (Sinclair et al., 2012). Surrogates for fecal indicator bacteria have been investigated in the works of Stout et al. (2005) and Guber et al. (2006). Stout et al. (2005) observed a high correlation (r=0.93)

between the concentrations of fecal coliforms and total phosphorous in runoff that was transported across vegetated plots under simulated rainfall, suggesting phosphorous as a reasonable surrogate of fecal coliforms release, and Guber et al. (2006) reported similarity in the release kinetics of fecal coliforms and Cl^-, organic carbon, and water soluble phosphorus from bovine slurry. Likewise, this study showed that the concentration of Cl^- in runoff had a significant correlation to that of bacteria. Also, interestingly, as the size of the study area increased, the correlations of Cl^- and bacteria became stronger (Fig. 5.12) and the correlations of Cl^- with the different bacteria became more similar (Table 5.5). The latter may have been due to less of a difference among the bacteria-bacteria correlations as scale increased. Perhaps, at the smaller scales, the microbe-specific release processes that are indicated by dissimilarity in release kinetics (i.e., the M_{Runoff}/M_0 dependencies) are more relevant, while at a larger-scale, the removal of bacteria with runoff becomes more dependent on overland flow network properties such as channel flow branching, dispersion, ponding, and spatially distributed flow velocities due to the connectivity of soil-surface heterogeneities. Regardless, compared with manure-bacteria, the inability of abiotic surrogates to multiply or die-off in solution must be considered if and when making assessments of fecal contamination with them.

While there were effects of scale on the content of manure constituents recovered in surface runoff (i.e., M_{Runoff}), there were no effects of scale on the load of bacteria released from manure that was recovered in the top 5 cm of soil after rainfall (i.e., $M_{Soil\ 5cm}$) (Table 5.6) (Fig. 5.13). Conceptually, bacteria that were partitioned to flow with infiltration water (as opposed to runoff) only needed to travel a very short distance in pore space (0-0.05 m) to become recoverable in the top 5 cm of soil after rainfall, while the content of bacteria recovered in runoff was effected by transport rates of bacteria from any distance on the 77 x 36 m field to the

collection flume. That said, many of the bacteria residing in the top 5 cm of soil after rainfall probably moved across a distance greater than only a few cm, either during a period of overland transport before infiltrating the soil and/or, during a period of underground movement with subsurface flow through the soil until immobilization via straining or by cellular attachment to soil particles.

In this study, at the field-scale approximately 10 billion CFU of each indicator bacteria group/species – *E. coli* (9.7×10^9 CFU), enterococci (3.4×10^{10} CFU), and total coliforms (2.5×10^{10} CFU) – were transported beyond the edge of the field through the collection flume (Table 5.6). This massive quantity was outnumbered by the amount of indicator bacteria that was estimated to have been released from manure and remained in the top 5 cm of soil post-rainfall – *E. coli* was 9.5×10^{11} CFU, enterococci was 3.1×10^{12} CFU, and total coliforms were 2.1×10^{12} CFU (Table 5.5). Based on the bacteria recovery in soil leachate from the lab-scale study, the majority of the bacteria that were released from manure were thought to have either 1) been transported out of the study area in runoff and/or 2) remained in the top 5 cm of soil. In effect, for the field-scale study, since the amount bacteria recovered in runoff and in post-rainfall soil at the OPE3 Field was less than half of the original amount applied to the field (Table 5.6), the total land-applied *E. coli*, enterococci, and total coliforms that remained in the manure on the OPE3 field following irrigation must have exceeded trillions of CFUs if low bacteria losses to leachate beyond 5 cm of soil may be assumed. A conceptual framework for the risk associated with manure application on fields must account for 1) the high content of bacteria transported out of the application area with surface runoff that may be deposited on land distant from the field or in a nearby surface water body, 2) the even more massive load of manure-bacteria remaining in the top few cm of soil after rainfall that may grow and become remobilized at a later time, and 3) the

even more immense load of bacteria that actually remain in the manure on the field and may grow and become mobilized as well.

Conclusions

Three separate experiments on the release and transport of indicator bacteria from land-applied manure at variable scales – soil box (0.35 x 1 m), standard field plot (0.8 x 1 m), and corn field (36 x 77 m) – were conducted, in parallel, using the same soil and manure. Scale was seen to have strong, inverse effects on the recovery rates of applied irrigation/rain water in runoff and on the content of *Escherichia coli*, enterococci, total coliforms, and chloride ion that were released from land-applied manure and recovered in surface runoff. Rainfall depth-dependent runoff-removal kinetics for bacteria appeared to follow a two-stage process comprised of a precipitous initial washout of planktonic bacteria and loose manure particulates followed by a stage dominated by the sloughing off of bacteria from manure. The Bradford-Schijven model performed best for simulating the edge-of-field transport of manure-constituents with runoff.

After a two hour irrigation event at the field scale, many bacteria, much more than the amount removed with runoff, remained in the top 5 cm of soil and even more remained in the manure. Because post-rainfall manure- and soil-bacteria may potentially become remobilized at a subsequent precipitation event, a conceptual model for the release and transport of land-applied bacteria in manure on a field must consider the content removed with runoff and that which remains in near-surface soil and in manure. In order to use data from laboratory and/or plot studies to predict the amounts of bacteria removed from land applied manure at the field scale, appropriate up-scaling of release and transport model parameters is required. It would be beneficial to repeat this study in future years to build a dataset that could be used to advance the parameterization of field scale microbial release and transport models.

References

Benham, B. L., Baffaut, C., Zeckorski, R. W., Mankin, K. R., Pachepsky, Y. A., Sadeghi, A. M., Brannan, K. M., Soupir, M. L., and Habersack, M. J., 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. Transactions of ASABE, 49(4): 987-1002.

Bradford, S. A., and Schijven, J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environmental Science & Technology, 36(18): 3916-3923.

Burnham, K. P. and Anderson, D. R., 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag. 2nd ed.

Cerdan, O., Le Bissonnais, Y., Govers, G., Lecomte, V., van Oost, K., Couturier, King, C., and Dubreuil, N. , 2004. Scale effect on runoff from experimental plots to catchments in agricultural areas in Normandy. Journal of Hydrology, 299: 4-14.

Curriero, F. C., Patz, J. A., Rose, J. B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the united states, 1948-1994. American Journal of Public Health, 91(8): 1194-1199.

Dao, T. H., Guber, A. K., Sadeghi, A. M., Karns, J. S., Shelton, D. P., and Pachepsky, Y. A., 2008. Loss of bioactive phosphorus and enteric bacteria. Soil Science, 2 (5): 511-521.

Delmas, M., Pak, L. T., Cerdan, O., Souchère, V., Le Bissonnais, Y., Couturier, A., and Sorel, L., 2012. Erosion and sediment budget across scale: a case study in a catchment of the European loess belt. Journal of Hydrology, 420-421: 255-263.

dos Santos, J. C. N., de Andrade, E. M., de Queiroz Palacio, H. A., de Araujo Meto, R. R., and da Silva Filho, J. A., 2012. Measurement of soil erosion on different spatial scales with vegetation cover of caatinga in the northeast of Brazil. 21st Century Watershed Technology Conference and Workshop, ASABE. Bari, Italy.

Fries, J. S., Characklis, G. W., and Noble, R. T. Attachment of fecal indicator bacteria to particle suspensions in the Neuse River Estuary, NC. Journal of Environmental Engineering-ASCE, 132(10): 1338-1345.

Gish, T. J., and Kung, K, 2007. Procedure for quantifying a solute flux to a shallow perched water table. Geoderma, 138(1-2): 57-64.

Gish, T. J., Pruger, J. H., Kustas, W. P., Hatfield, J.L., McKee, L. G., Russ, A. L., 2009. Soil moisture and metalochar volatilization observations over three years. Journal of Environmental Quality, 38 (5): 1785-1795.

Gomi, T., Sidle, R. C., Miyata, S., Kosugi, K., and Onda, Y., 2008. Dynamic runoff connectivity of overland flow on steep forested hillslopes: scale effects and runoff transfer. Water Resources Research, 44(8): 1-16.

Guber, A. K., Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., and Sikora, L. J., 2006. Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and Environmental Microbiology, 72(12): 7531-7539.

Guber, A. K., Karns, J. S., Pachepsky, Y. A., Sadeghi, A. M., Van Kessel, J. S., and Dao, T. H., 2007. Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. Letters in Applied Microbiology, 44(2): 161-167.

Guber, A. K., Pachepsky, Y. A., Shelton, D. R., and Yu, O., 2009. Association of fecal coliforms with soil aggregates. Soil Science, 174(10): 543-548.

Guber, A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L., 2011. Uncertainty in modelling of faecal coliform overland transport associated with manure application in Maryland. Hydrological Processes, 25(15): 2393-2404.

Harmel, R. D., Karthikeyan, R., Gentrym T., and Srinivasan, R., 2010. Effects of agricultural management, land use, and watershed scale on *E. coli* concentrations in runoff and streamflow. Transactions of ASABE, 53(6): 1833-1841.

Isensee, A. R., and Sadeghi, A. M., 1999. Quantification of runoff in laboratory-scale chambers. Chemosphere, 38(8): 1733-1744.

Kidron, G. J., 2011. Runoff generation and sediment yield on homogeneous dune slopes: scale effect and implications for analysis. Earth Surface Processes and Landforms, 36(13): 1809-1824.

Kim, K., Whelan, G., Purucker, S. T., Bohrmann, T. F., Cyterski, M. J., Molina, M., Gu, Y., Pachepsky, Y., Guber, A., and Franklin, D. H., 2014. Rainfall-runoff model parameter estimation and uncertainty evaluation on small plots. Hydrological Processes, 28(20): 5220-5235.

Langhans, C., Govers, G., Diels, J., Stone, J. J., and Nearing, M. A., 2014. Modeling scale-dependent runoff generation in a small semi-arid watershed accounting for rainfall intensity and water depth. Advances in Water Resources, 69: 65–78.

Lombard, N., Prestat, E., Dirk van Elsas, J., and Simonet, P., 2011. Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78: 31-49.

Martinez, G., Pachepsky, Y. A., Whelan, G., Yakirevich, A. M., Guber, A., and Gish, T. J., 2014. Rainfall-induced fecal indicator organisms transport from manured fields: Model sensitivity analysis. Environment International, 63: 121-129.

McDaniel, R. L., Soupir, M. L., Tuttle, R. B., and Cervantes, A. E., 2013. Release, dispersion, and resuspension of *Escherichia coli* from direct fecal deposits under controlled flows. Journal of the American Water Resources Association, 49(2): 319-327.

Meyer, L. D., and Harmon, W. C., 1979. Multiple-intensity rainfall simulator for erosion research on row sideplots. Transactions of the ASAE, 22: 100-103.

Mishra, A., Benham, B. L., and Mostaghimi, S., 2008. Bacterial transport from agricultural lands fertilized with animal manure. Water, Air, and Soil Pollution, 189(1-4): 127-134.

Moyer, D. L., and Hyer, K. E., 2003. Use of the hydrological simulation program-FORTRAN and bacterial source tracking for development of fecal coliform total maximum daily load (TMDL) for Blacks Run, Rockingham County, Virginia. U.S. Department of the Interior U.S. Geological Survey, Water-resources Investigations Report 03-4161.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2006. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Letters in Applied Microbiology, 42(2): 83-87.

Muirhead, R. W., and Monaghan, R. M., 2012. A two reservoir model to predict *Escherichia coli* losses to water from pastures grazed by dairy cows. Environment International, 40: 8-14.

Peñuela, A., Javaux, M., and Bielders, C. L. 2013. Scale effect on overland flow connectivity at the plot scale. Hydrology and Earth System Sciences, 17(1): 87–101.

Raclot, D., Le Bissonnais, Y., Louchart, X., Andrieux, P., Moussa, R., and Voltz, M., 2009. Soil tillage and scale effects on erosion from fields to catchment in a Mediterranean vineyard area. Agriculture, Ecosystems & Environment, 134(3-4), 201-210.

Sadeghi, A. M., and Isensee, A. R., 2001. Impact of hairy vetch cover crop on herbicide transport under field and laboratory conditions. Chemosphere, 44(2): 109–118.

Schijven, J. F., Bradford, S. A., and Yang, S., 2004. Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: Physical factors. Journal of Environmental Quality, 33(4), 1499-1508.

Sheridan, G. J., Noske, P. J., Lane, P. N. J., Jones, O. D., and Sherwin, C. B., 2014. A simple two-parameter model for scaling hillslope surface runoff. Earth Surface Processes and Landforms, 39(8): 1049-1061.

Sistani, K. R., Bolster, C. H., Way, T. R., Tobert, H. A., Pote, D. H., and Watts, D. B., 2009. Influence of poultry litter application methods on the longevity of nutrient and *E. coli* in runoff from tall fescue pasture. Water, Air, and Soil Pollution, 206: 3-12.

Soupir, M. L., Mostaghimi, S., and Dillaha, T., 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. Journal of Environmental Quality, 39(3), 1019-1027.

Steiger, J.H. (1980). Tests for comparing elements of a correlation matrix. Psychological Bulletin, 87, 245-251.

Stout, W. L., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Saporito, L. S., and Sharpley, A. N., 2005. Runoff transport of faecal coliforms and phosphorus released from manure in grass buffer conditions. Letters in Applied Microbiology, 41(3): 230-234.

U.S. EPA, 2009. National water quality inventory: report to Congress, 2004 reporting cycle. EPA/841/R-08/001, Washington, D.C.

Vadas, P. A., Kleinman, P. J. A., Sharpley, A. N., 2004. A simple method to predict dissolved phosphorus in runoff from surface-applied manures. Journal of Environmental Quality, 33: 749-756.

Van de Giesen, N., Stomph, T., Ajayi, E. A., and Bagayoko, F., 2011. Scale effects in hortonian surface runoff on agricultural slopes in west africa: field data and models. Agriculture, Ecosystems & Environment, 142(1-2): 95-101.

Van Horn, H. H., Wilkie, A. C., Powers, W. J., and Nordstedt, R. A., 1994. Components of dairy manure management systems. Journal of Dairy Science, 77(7): 2008–2030.

van Genuchten, M. Th. 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research Report No. 119, U. S. Salinity Laboratory, Riverside, CA, p. 88.

Chapter 6 – General Conclusions

The release of microorganisms from animal manure is an important component of microbial fate and transport as it establishes the content of microbes that enter a state of transport and move toward human exposure. Microbial release from manure is situation-specific and it depends on a combination of physical, chemical, and biological factors, such as, manure source, manure consistency, manure application method and rate, manure age, vegetation, and rainfall. Experiments were conducted to determine the effects of rainfall intensity, topography, and scale on the release of *E. coli*, enterococci, total coliforms, and chloride ion from land-applied dairy cattle manure, and to test the performance of three knetic-based models on describing the observed release and runoff-removal. Results and observations obtained from this work are detailed in the following bullets.

- The initial elution released from manure contained a diluted concentration of the manure-constituents in the manure liquid phase and some bypass water, and while some indicator bacteria were leached with the initial release, most bacteria remained part of the solid manure matrix.
    - The concentrations of *E. coli*, total coliforms, and enterococci that were released with the initial portion of runoff (or infiltration) were about one order of magnitude below their corresponding starting concentrations in manure.
    - Compared with the relative concentration of bacteria in initial release, that of dissolved Cl^-, while still less than 1, was much greater.
- The kinetics for the dependency of cumulative release and runoff-removal of manure-bacteria and Cl^- on rainfall depth were expressed by a two-stage process, having a

precipitous early stage, for about 1 to 2 cm of rainfall, and a slower, almost-constant rate for the remainder of rainfall.

    - The two-stage microbial removal process may be explained by a precipitous initial washout of free-living bacteria and suspended manure particulates containing adsorbed bacteria, and after the easily accessible pathways were leached and the manure-liquid became almost void of microbes, the second stage began where an almost-constant rate of shaving and sloughing manure to moving water with the number of bacteria proportional to the shaved and sloughed amount of manure.

- Since most all dissolved ions, but only some bacteria, were leached with the manure liquid phase, only the cumulative release of the former approached 100 %, while the cumulative release of the latter reached an asymptote at a lower relative value.
- During rainfall-induced release, microorganisms were partitioned into runoff and infiltration at similar concentrations.
    - The physics of the hydrology in a given situation, as influenced by land slope, governed the directional flow of released bacterial loads.
- Of the fraction of released bacteria that flowed with infiltration water into soil (as opposed to runoff), most were retained in the top few cm of the soil profile due to straining.
- Rainfall intensity had no substantial effect of on the kinetics for microbial release or runoff-removal from manure, which verified the assumption that bacterial release from manure, or removal with runoff, can be modeled as a factor of rainfall depth.

- Compared with the traditionally used exponential-dependence model and also the Vadas-Kleinman-Sharpley model, the Bradford-Schijven model consistently performed best for simulation of the rainfall-induced bacterial release and runoff-removal from manure.
- Microbial fate and transport models would benefit by implementing the Bradford-Schijven model as a subroutine for microbial release from land-applied manure (if not having done so already).
- Rainfall intensity and soil profile depth both had an inverse effect (with interactions) on the concentration of land-applied bacteria remaining in the soil after rainfall.
- The release-rate of *E. coli* from manure appeared to exceed that of enterococci.
- Manure-borne *E. coli* was transported into and through soil at a faster rate than enterococci.
- The correlation between Cl^- and *E. coli* concentrations in release was stronger than the correlations of Cl^- with the other bacteria.
- Of the three indicator bacteria in study, *E. coli* was most associated with the manure liquid phase.
- Because of microbe-specific release, using more than one group/species of indicator bacteria would be beneficial for risk assessment of fecal contamination in the environment.
- Scale was seen to have a strong, inverse effect on the runoff recovery of land-applied *E. coli*, enterococci, total coliforms, and chloride ion.
    - In order to use data from laboratory or field plot studies to predict the amount of bacteria removed from land-applied manure at the field scale, appropriate up-scaling of runoff-removal model parameters is required.

- Because post-rainfall soil-bacteria may potentially become remobilized at a subsequent precipitation event, a conceptual model for the release and transport of land-applied indicator bacteria and pathogens in manure must consider the content of bacteria removed with runoff and that which remains in surface soil.

The results from this series of experiments can be used for improvement of microbial fate and transport models that are critical for risk assessment of microbial contamination in the environment.

Appendices

Appendix A. Plant nutrient content of the dairy cattle manure used in the experiments described in Chapter 3-"Partitioning Boxes Release Study" (A1), Chapter 4-"Soil Boxes Release Study" (A2), and Chapter 5-"Scale Effect Transport Study" (A3).

Table A-A1 Nutrient content of the synthetic dairy cattle manure used in the "Partitioning Boxes Release Study". The "±" separates average and standard deviation. All analyses were performed by the Penn State Agricultural Analytical Services Laboratory.

Plant Nutrient	**mg kg^{-1} manure (wet weight)**
Total Nitrogen (N)	3289 ± 104
Organic N	2948 ± 77.1
Ammonium N (NH_4-N)	340 ± 63.5
Nitrate Nitrogen (NO_3-N)	8.2 ± 1.8
Phosphate (P_2O_5)	1606 ± 86.2
Potash (K_2O)	1356 ± 204
Calcium (Ca)	2459 ± 90.7
Magnesium (Mg)	744 ± 36.2
Sulfur (S)	381 ± 40.8
Copper (Cu)	9.1 ± 0.0
Manganese (Mn)	51.3 ± 15.6
Zinc (Zn)	45.4 ± 0.0
Iron (Fe)	70.8 ± 3.6
Sodium (Na)	263 ± 63.5
Aluminum (Al)	17.2 ± 1.8

Table A-A2 Nutrient content of the synthetic dairy cattle manure used in the "Soil Boxes Release Study". The "±" separates average and standard deviation. All analyses were performed by the Penn State Agricultural Analytical Services Laboratory.

Plant Nutrient	**mg kg^{-1} manure (wet weight)**
Total Nitrogen (N)	3800 ± 390
Ammonium N (NH_4-N)	660 ± 220
Nitrate Nitrogen (NO_3-N)	34.8 ± 17.7
Phosphate (P_2O_5)	1900 ± 370
Potash (K_2O)	2120 ± 520
Calcium (Ca)	2340 ± 340
Magnesium (Mg)	920 ± 45
Sulfur (S)	440 ± 55
Copper (Cu)	42.4 ± 5.6
Manganese (Mn)	51.3 ± 15.6
Zinc (Zn)	7.6 ± 1.5
Iron (Fe)	65.8 ± 9.1
Sodium (Na)	412 ± 170
Aluminum (Al)	17.7 ± 4.0

Table A-A3 Average nutrient content of the dairy cattle manure used in the lab-, plot-, and field scale experiments in the "Scale Effect Study". Results are representative of manure composite samples that were taken on the morning of each respective experiment. The content of each macro- and micro-nutrient is reported in mg kg^{-1} manure (wet weight). All analyses were performed by the Penn State Agricultural Analytical Services Laboratory.

Plant Nutrient	**Lab-Scale**	**Plot-Scale**	**Field-Scale**
Total Nitrogen (N)	4808	12129	6817
Ammonium N (NH_4-N)	338	422	689
Nitrate Nitrogen (NO_3-N)	317	2563	532
Phosphate (P_2O_5)	2790	8736	2632
Potash (K_2O)	6300	24657	11719
Calcium (Ca)	2336	7861	3155
Magnesium (Mg)	991	4146	1625
Sulfur (S)	651	1842	850
Copper (Cu)	47.6	132	62.0
Manganese (Mn)	36.29	122	48.38
Zinc (Zn)	9.1	27.2	12.1
Iron (Fe)	381	875	1538
Sodium (Na)	447	1805	696
Aluminum (Al)	97.5	345	745

Appendix B. Parameter values for model fits.

Table A-B1 Parameter values for fitting Eq. 1, 2, and 3 to the dependencies of M_{Runoff}/M_0 on rainfall depth upon initiation of runoff for *E. coli*, enterococci, total coliforms, and chloride ion from the "Scale Effect Transport Study" (Chapter 5). The units are listed next to each parameter. The "±" separates the value and standard error associated with each curve-fit. The values presented for the plot- and lab-scale are the average value and average standard error of the replicates in that study.

Scale	Manure Constituent	k_e (Eq. 1); $[k_e]$ = cm^{-1}	k_p (Eq. 2); $[k_p]$ = cm^{-1}	β (Eq. 2); β is dimensionless	A (Eq. 3); $[A]$ = cm^{-n}	n (Eq. 3); n is dimensionless
Field-Scale	Enterococci	1.18E-04 ± 2.27E-06	1.15E-04 ± 2.00E-06	8.19E-01 ± 3.10E-02	9.42E-05 ± 4.72E-06	1.22E-00 ± 4.56E-02
	E. coli	1.47E-04 ± 3.11E-06	1.43E-04 ± 2.79E-06	8.01E-01 ± 3.27E-02	1.14E-04 ±6.32E-06	1.25E-00 ± 5.02E-02
	Total coliforms	1.97E-04 ± 2.96E-06	1.95E-04 ± 2.87E-06	8.85E-01 ± 3.20E-02	1.72E-04 ± 7.75E-06	1.13E-00 ± 7.35E-02
	Chloride ion	3.12E-04 ± 5.62E-06	3.07E-04 ± 5.85E-06	8.79E-01 ± 3.96E-02	2.70E-04 ± 1.54E-05	1.14E-00 ± 5.04E-02
Plot-Scale	Enterococci	2.30E-02 ± 6.22E-04	2.37E-02 ± 2.81E-04	7.83E-01 ± 1.73E-02	1.87E-02 ± 5.41E-04	1.22E-00 ± 2.94E-02
	E. coli	8.12E-03 ± 2.57E-04	7.88E-03 ± 8.48E-05	9.97E-01 ± 1.96E-02	7.92E-03 ± 1.42E-04	1.06E-00 ± 1.87E-02
	Total coliforms	3.27E-02 ± 9.02E-04	3.43E-02 ± 4.56E-04	7.84E-01 ± 2.12E-02	2.65E-02 ± 7.58E-04	1.19E-00 ± 2.97E-02
	Chloride ion	8.01E-03 ± 2.19E-04	8.08E-03 ± 1.48E-04	8.80E-01 ± 2.84E-02	6.79E-03± 2.90E-04	1.13E-00 ± 3.87E-02
Lab-Scale	Enterococci	3.05E-02 ± 1.19E-03	3.24E-02 ± 8.72E-04	6.45E-01 ± 2.72E-02	2.38E-02 ± 1.44E-03	1.53E-00 ± 6.27E-02
	E. coli	2.84E-02 ± 1.02E-03	2.98E-02 ± 9.16E-04	7.05E-01 ± 3.66E-02	2.42E-02 ± 1.55E-03	1.45E-00 ± 7.35E-02
	Total coliforms	4.59E-02 ± 4.78E-03	3.78E-02 ± 3.76E-03	4.59E-01 ± 3.09E-02	2.18E-02 ± 3.30E-03	2.60E-00 ± 1.78E-01
	Chloride ion	1.04E-01 ± 5.73E-03	1.13E-01 ± 3.67E-03	6.02E-01 ± 2.46E-02	7.10E-02 ± 4.55E-03	1.43E-00 ± 6.42E-02

Appendix C. The code used to run the three models on the bacteria and Cl- release (into runoff or leachate as in Chapters 3 and 4) or removal with runoff (as in Chapter 5).

```
!  BAC_REL.f90
!
!  FUNCTIONS:
!  threemodels - Entry point of console application.
!

!****************************************************************
**************
!
!  PROGRAM: BAC_REL
!
!  PURPOSE: Entry point for the console application.
!
!****************************************************************
**************

    program BAC_REL

!  BAC_REL.f90
!
!  FUNCTIONS:
!  Interpolat - Entry point of console application.
!

!****************************************************************
**************
!
!  PROGRAM: Interpolat
!
!  PURPOSE: Entry point for the console application.
!
!****************************************************************
**************

    Integer*2 Reason

    Character*5 NameSR(20),NameSI(20)
    Character*20,fnamer,fnamei,fnamem,fnamefit
    Dimension raintimeI
(100),ColltimeI(100),volumeI(100),TCI(100),ECI(100),ENI(100)
    Dimension raintimeR
(100),ColltimeR(100),volumeR(100),TCR(100),ECR(100),ENR(100)
    Real*4 MRRR(100),MRRI(100)
    Dimension
tr(0:100),cumR(0:100),tI(0:100),cumI(0:100),ycI(0:100),ycR(0:10
0)
    Dimension totalR(0:100),totalI(0:100)
    Dimension AR(100),AI(100)
    real*4 massTC,massEC,massEN

    DIMENSION X(400), Y(400), F(400), R(400), x0(0:400),
y0(0:400)
    DIMENSION B(10), E(10), ST(10)
    DIMENSION D(10,10)
    Common /IMODEL/imd,c0
    open(10,file='release_curve.txt')
    read(10,*) t0
    ic=0
    DO WHILE(1.EQ.1)
    ic=ic+1
    read(10,*,IOSTAT=Reason) x0(ic),TCR(ic),ECR(ic),ENR(ic)
    IF (Reason < 0) EXIT
    Enddo

    NR=ic-1
    print *,NR
    close(10)

Do i=1,NR
    x0(i)=x0(i)-t0
Enddo


    Do ibac=1,3

    cumR(0)=0.
! mass release rates
    if(ibac.EQ.1) then
        Do i=1,NR
            AR(i)=TCR(i)
        Enddo
            fnamer='runmassT.txt'
            fnamem='massreleaseT.txt'
            fnamefit='fitT.txt'
            totM=massTC
    endif
    if(ibac.EQ.2) then
        Do i=1,NR
            AR(i)=ECR(i)
        Enddo
            fnamer='runmassE.txt'
             fnamem='massreleaseE.txt'
            fnamefit='fitE.txt'
            totM=massEC
```

```
    endif
    if(ibac.eq.3) then
        Do i=1,NR
            AR(i)=ENR(i)
        Enddo
            fnamer='runmassN.txt'
            fnamem='massreleaseN.txt'
            fnamefit='fitN.txt'
            totM=massEN
    endif


    Do i=1,NR
       Y0(i)=AR(i)
    enddo
    close(31)
    nob=NR
    open(51,file=fnamefit)

   Do imd=1,4
       ! i=1 exponential
       ! i=2 power fractional Bradford Shijven
       ! i=3 Vadas
    !==============
    ! Start fitting
    !==============
    !get y as log log(M/Mo)
    Do i=1,nob
        x(i)=x0(i)
        y(i)=y0(i)
!        y(i)=alog10(y0(i))
   enddo
    if(imd.EQ.1.OR.imd.EQ.4) then
        np=1
        b(1)=1

    else
        np=2
        b(1)=1.E-03
        b(2)=1.

    endif
    mit=20
    call marqu(np,nob,b,x,y,f,r,sumb,sdev,e,d,st,mit)
      print *,sumb
      do i=1,np
         print *, i,b(i),st(i)
      enddo
      AICCL = nob*ALOG(sumb/nob)+2*NP+2*NP*(NP+1.0)/(NOB-NP-
1.0)
      write(51,*) 'np=',np,'; RMSE=', sqrt(sumb/(nob-1)),
'AKAIKEc=', AICCL
      write(51,100)
      do i=1,np
         write(51,101) i,b(i),st(i)
      enddo
      write(51,102)
      do i=1,nob
        write(51,103) i,x(i),y(i),f(i),r(i)
     enddo
   enddo  !imd


   close(51)
    enddo !ibac
    stop
100   format(45('-'),/'Parameter # | Estimated Mean | Stand.
Error|'/,45('-'))
101 format(5x,i3,7x,E12.4,3x,e12.4)
102   format(54('-'),/,'Point # | Argument | Measured |
Estimated | Residual |',/,'       | value    | function |
function  |  value   |',/,54('-'))
103   format(i3,3x,4e12.4)
    end program fourmodels


      subroutine marqu(np,nob,b,x,y,f,r,sumb,sdev,e,d,st,mit)

      dimension y(nob),x(nob),f(nob),r(nob),st(np),b(np),e(np),
and
               c(10), p(10), q(10), a(10,10), d(10,10),
and
               delz(200,10),dz(200)

      data eps /0.0005/

      print *, "***********************************"

      ga = 0.02
      sumb = 0.0
      call model(b,np,f,nob,x)
      do 10 k = 1,nob
      z = y(k) - f(k)
      r(k) = z
      if(abs(z) .gt. 1.0e-37) sumb = sumb + z * z
 10   continue
print *,mit
      do 200 nit = 1,mit
      print *,b
      ssq = sumb
      ga = 0.1 * ga
```

```
      do 30 j = 1,np
      temp = b(j)
      b(j) = 1.01 * b(j)
      call model(b,np,dz,nob,x)
      do 15 i = 1,nob
      delz(i,j) = dz(i)
15    continue
      sum = 0.0
      do 20 k = 1,nob
      delz(k,j) = 100.0 * (delz(k,j) - f(k))
      tmp = delz(k,j) * r(k)
      sum = sum + tmp
20    continue
      q(j) = sum / b(j)
      b(j) = temp
      c(j) = temp
30    continue
      sum3 = 0.0
      do 60 i = 1,np
      do 50 j = 1,i
      sum = 0.0
      do 40 k = 1,nob
      temp = delz(k,i) * delz(k,j)
      sum = sum + temp
40    continue
      d(j,i) = sum / (b(j) * b(i))
      d(i,j) = d(j,i)
50    continue
      e(i) = sqrt(d(i,i))
      q(i) = q(i) / e(i)
      if(abs(q(i)) .gt. 1.0e-37) sum3 = sum3 + q(i) * q(i)
60    continue
70    do 90 i = 1,np
      do 80 j = 1,i
      a(j,i) = d(j,i) / e(j) / e(i)
      a(i,j) = a(j,i)
80    continue
90    continue
      do 100 i = 1,np
      p(i) = q(i)
100   a(i,i) = a(i,i) + ga
      call matinv(a,np,p)
      sum1 = 0.0
      sum2 = 0.0
      do 110 i = 1,np
      temp = p(i) * q(i)
      sum1 = sum1 + temp
      temp = p(i) * p(i)
      sum2 = sum2 + temp
110   continue
      an = sqrt((sum1/sum2)*(sum1/sum3))
      angle = 57.2958 * atan((sqrt(1-an**2))/an)
      step = 1.0
120   do 130 i = 1,np
130   b(i) = p(i) * step / e(i) + c(i)
      do 140 i = 1,np
      if(c(i)*b(i) .le. 0.0) go to 160
140   continue
      sumb = 0.0
      call model(b,np,f,nob,x)
      do 150 k = 1,nob
      z = y(k) - f(k)
      r(k) = z
      if(abs(z) .gt. 1.0e-37) sumb = sumb + z*z
150   continue
      if(sumb-ssq .lt. 1.0e-8) go to 180
160   if(angle .gt. 30.0) go to 170
      step = 0.5 * step
      go to 120
170   ga = 10.0 * ga
      go to 70
180   do 190 i = 1,np
      if(abs(c(i)-b(i)) .gt. eps*abs(b(i))) go to 200
190   continue
      go to 210
200   continue
210   call matinv(d,np,p)
      sdev = sqrt(sumb/float(nob-np))
      do 220 i = 1,np
      e(i) = sqrt(amax1(d(i,i),1.0e-20))
      st(i) = e(i) * sdev
220   continue
      return
      end
      subroutine matinv(a,np,b)

      dimension a(10,10),b(np),indx1(10),indx2(10)

      do 10 j = 1,np
10    indx1(j) = 0
      i = 0
20    amax = -1.0
      do 40 j = 1,np
      if(indx1(j) .ne. 0) go to 40
      do 30 k = 1,np
      if(indx1(k) .ne. 0) go to 30
      p = abs(a(j,k))
      if(p .le. amax) go to 30
      ir = j
      ic = k
      amax = p
30    continue
```

```
 40   continue
      if(amax .le. 0.0) go to 120
      indx1(ic) = ir
      if(ir .eq. ic) go to 60
      do 50 l = 1,np
      p = a(ir,l)
      a(ir,l) = a(ic,l)
      a(ic,l) = p
 50   continue
      p = b(ir)
      b(ir) = b(ic)
      b(ic) = p
      i = i + 1
      indx2(i) = ic
 60   p = 1.0 / a(ic,ic)
      a(ic,ic) = 1.0
      do 70 l = 1,np
      a(ic,l) = a(ic,l) * p
 70   continue
      b(ic) = b(ic) * p
      do 90 k = 1,np
      if(k .eq. ic) go to 90
      p = a(k,ic)
      a(k,ic) = 0.0
      do 80 l = 1,np
      a(k,l) = a(k,l) - a(ic,l) * p
 80   continue
      b(k) = b(k) - b(ic) * p
 90   continue
      go to 20
 100  ic = indx2(i)
      ir = indx1(ic)
      do 110 k = 1,np
      p = a(k,ir)
      a(k,ir) = a(k,ic)
      a(k,ic) = p
 110  continue
      i = i - 1
 120  if(i .gt. 0) go to 100

      return
      end
      subroutine model(b,np,y,nob,x)

      dimension b(np),y(nob),x(nob)
      Common /IMODEL/imd,c0
       if (iMD.EQ.1) then
       do 10 i = 1,nob
       y(i)=(1-exp(-b(1)*abs(x(i))))
!         y(i)=alog10(1-exp(-b(1)*x(i)))
 10    continue
       Endif
       if (imd.EQ.2) then
       do 20 i = 1,nob
         y(i)=1-1./(1+b(1)*b(2)*x(i)**(1./b(2)))
!          y(i)=alog10(1-1./(1+b(1)*b(2)*x(i))**(1./b(2)))
 20    continue
       Endif
       if (iMD.EQ.3) then
       do 30 i = 1,nob
       y(i)=b(1)*abs(x(i))**b(2)
!          y(i)=alog10(1-exp(-b(1)*x(i)))

 30    continue
       Endif

      return
      end
```

Glossary

The following acronyms were used at various points in the text of this thesis:

AIC: Akaike information criterion

CAFO: concentrated animal feeding operation

CFU: colony forming unit

EC: *Escherichia coli*

EN: enterococci

FIB: fecal indicator bacteria

FORTRAN: Formula Translating System

HSPF: Hydrological Simulation Program - FORTRAN

KINEROS2/STWIR: Kinematic Runoff and Erosion model coupled with Simulator of Transport with Infiltration and Runoff

MPN: most probable number

MST: microbial source tracking

RMSE: root-mean-squared-error

STWIR:

SWAT: Soil and Water Assessment Tool

TC: total coliforms

Bibliography

Alonso, J. L., Soriano, A., Carbajo, O., Alonso, J. L., Soriano, A., Carbajo, O., and Amoros, I., 1999. Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 C. Applied and Environmental Microbiology, 65(8): 3746-3749.

American Society of Agricultural Engineers, 2003. Manure production and characteristics. ASAE Standards. D384.1.

Arnold, J. G., Srinivasan, R., Muttiah, R. S., and Williams, J. R. 1998. Large area hydrologic modeling and assessment part I: Model development. Journal of American Water Resources Association, 34(1): 73-89.

Baldursson, S., and Karanis, P., 2011. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. Water Research, 45(20): 6603-6614.

Barick, R. S., Mohammed, H. O., White, M. E., and Bryant, R. B., 2003. Factors associated with the likelihood of *Giardia* spp. and C*ryptosporidium* spp. in soil from dairy farms. Journal of Dairy Science, 86(3): 784-791.

Batz, M. B., Hoffmann, S., and Morris, J. G., 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection, 75(7): 1278-1291.

Benham, B. L., Baffaut, C., Zeckorski, R. W., Mankin, K. R., Pachepsky, Y. A., Sadeghi, A. M., Brannan, K. M., Soupir, M. L., and Habersack, M. J., 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. Transactions of ASABE, 49(4): 987-1002.

Berry, E. D., and Miller, D. N., 2005. Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. Journal of Environmental Quality, 34(2): 656-663.

Bittman, S., van Vliet, L. J. P., Kowalenko, C. G., McGinn, S., Hunt, D. E., and Bounaix, F., 2005. Surface-banding liquid manure over aeration slots. Agronomy Journal, 97(5): 1304.

Blaustein, R. A., Pachepsky, Y., Hill, R. L., Shelton, D. R., and Whelan, G., 2013. *Escherichia coli* survival in waters: temperature dependence. Water Research, 47: 569-578.

Borst, M., and Selvakumar, A., 2003. Particle-associated microorganisms in stormwater runoff. Water Research, 37(1): 215-223.

Bradford, S. A., and Schijven, J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environmental Science & Technology, 36(18): 3916-3923.

Brooks, J. P., Adeli, A., Read, J. J., and McLaughlin, M. R., 2009. Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. Journal of Environmental Quality, 38(1): 218-229.

Burnham, K. P. and Anderson, D. R., 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag. 2nd ed.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., and Harwood, V. J., 2012. Enterococci in the environment. Microbiology and Molecular Biology Reviews : MMBR, 76(4): 685-706.

Cardoso, F., Shelton, D., Sadeghi, A., Shirmohammadi, A., Pachepsky, Y., and Dulaney, W., 2012. Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. Journal of Environmental Management, 110: 1-7.

Cerdan, O., Le Bissonnais, Y., Govers, G., Lecomte, V., van Oost, K., Couturier, King, C., and Dubreuil, N. , 2004. Scale effect on runoff from experimental plots to catchments in agricultural areas in Normandy. Journal of Hydrology, 299: 4-14.

Chin, D. A., Sakura-Lemessy, D., Bosch, D. D., and Gay, P. A., 2009. Watershed-scale fate and transport of bacteria, Transactions of ASABE, 52(1): 145-154.

Costán-Longares, A., Montemayor, M., Payán, A., Méndez, J., Jofre, J., Mujeriego, R., and Lucena, F., 2008. Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. Water Research, 42(17): 4439-4448.

Crane, S. R., and Moore, J. A., 1984. Bacterial pollution of groundwater: A review. Water, Air, and Soil Pollution, 22: 67-83.

Critzer, F. J., and Doyle, M. P., 2010. Microbial ecology of foodborne pathogens associated with produce. Current Opinion in Biotechnology, 21: 125-130.

Curriero, F. C., Patz, J. A., Rose, J. B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the united states, 1948-1994. American Journal of Public Health, 91(8): 1194-1199.

Dao, T. H., Guber, A. K., Sadeghi, A. M., Karns, J. S., Shelton, D. P., and Pachepsky, Y. A., 2008. Loss of bioactive phosphorus and enteric bacteria. Soil Science, 2 (5): 511-521.

Delmas, M., Pak, L. T., Cerdan, O., Souchère, V., Le Bissonnais, Y., Couturier, A., and Sorel, L., 2012. Erosion and sediment budget across scale: a case study in a catchment of the European loess belt. Journal of Hydrology, 420-421: 255-263.

dos Santos, J. C. N., de Andrade, E. M., de Queiroz Palacio, H. A., de Araujo Meto, R. R., and da Silva Filho, J. A., 2012. Measurement of soil erosion on different spatial scales with vegetation cover of caatinga in the northeast of Brazil. 21st Century Watershed Technology Conference and Workshop, ASABE. Bari, Italy.

Dufour A. P., and Ballantine P., 1986. Bacteriological ambient water quality criteria for marine and freshwater recreational waters. US Environmental Protection Agency: EPA 440/445-484-002.

Drapcho, C. M., A. K. B., 2003. Fecal coliform concentration in runoff from fields with applied dairy manure. Master in Science Thesis, Louisiana State University, Baton Rouge, LA.

Easton, J. H., Gauthier, J. J., Lalor, M. M., and Pitt, R. E., 2005. Die-off of pathogenic *E. coli* O157:H7 in sewage contaminated waters. Journal of the American Water Resources Association, 41(5): 1187-1193.

Edberg S.C., Rice E.W., Karlin R.J., Allen M.J., 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology Symposium Supplement. 88:106S-116S.

Edwards, D. R., Daniel, T. C., and Moore Jr., P. A., 1996. Vegetative filter strip design for grassed areas treated with animal manures. Applied Engineering in Agriculture, 12(1): 31-38.

Edwards, D. R., Larson, B. T., and Lim, T. T., 2000. Runoff nutrient and fecal coliform content from cattle manure application to fescue plots, 36(4): 711-721.

Fayer, R., and Trout, J. M., 2005. Zoonotic protists in the marine environment. In: Oceans and Health: Pathogens in Marine Environment. Belkin, S. S., and Cowell, R. (Eds), Kluwer Academic Publishers, Dordrecht, pp. 143-163.

Foppen, J. W., van Herwerden, M., and Schijven, J., 2007. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology, 93(1-4): 236-54.

Forslund, A., Markussen, B., Toenner-Klank, L., Bech, T. B., Jacobsen, O. S., and Dalsgaard, A., 2011. Leaching of *Cryptosporidium parvum* oocysts, *Escherichia coli*, and a *Salmonella enterica* Serovar Typhimurium Bacteriophage through intact soil cores following surface application and injection of slurry. Applied and Environmental Microbiology, 77(22): 8129-8138.

Fox, G. A., Matlock, E. M., Guzman, J. A., Sahoo, D., and Stunkel, K. B., 2009. *Escherichia coli* load reduction from runoff by vegetative filter strips: a laboratory-scale study. Journal of Environmental Quality, 40(3): 980-988.

Franz, E., Semenov, A. V, Termorshuizen, A. J., de Vos, O. J., Bokhorst, J. G., and van Bruggen, A. H. C., 2008. Manure-amended soil characteristics affecting the survival of *E. coli* O157:H7 in 36 Dutch soils. Environmental Microbiology, 10(2): 313-327.

Freschet, G., Masse, D., Hien, E., Sall, S., and Chotte, J., 2008. Long-term changes in organic matter and microbial properties resulting from manuring practices in an arid cultivated soil in Burkina Faso. Agriculture, Ecosystems & Environment, 123(1-3): 175-184.

Fries, J. S., Characklis, G. W., and Noble, R. T. Attachment of fecal indicator bacteria to particle suspensions in the Neuse River Estuary, NC. Journal of Environmental Engineering-ASCE, 132(10): 1338-1345.

Gagliardi, J. V., and Karns, J. S., 2000. Leaching of *escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Applied Environmental Microbiology, 66(3): 887-883.

Gerba, C. P., and Smith, J. E., 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. Journal of Environmental Quality, 34(1): 42-48.

Gish, T. J., and Kung, K, 2007. Procedure for quantifying a solute flux to a shallow perched water table. Geoderma, 138(1-2): 57-64.

Gish, T. J., Pruger, J. H., Kustas, W. P., Hatfield, J.L., McKee, L. G., Russ, A. L., 2009. Soil moisture and metalochar volatilization observations over three years. Journal of Environmental Quality, 38 (5): 1785-1795.

Gomi, T., Sidle, R. C., Miyata, S., Kosugi, K., and Onda, Y., 2008. Dynamic runoff connectivity of overland flow on steep forested hillslopes: scale effects and runoff transfer. Water Resources Research, 44(8): 1-16.

Guan, T. Y., Holley, R. A., H. O., and H. E. O., 2003. Pathogen survival in swine manure environments and transmission of human enteric illness — a review. Journal of Environmental Quality, 32: 383-392.

Guber, A. K., Shelton, D. R., and Pachepsky, Y. A., 2005. Transport and retention of manure-corne coliforms in soil. Vadose Zone Journal, 4(3) doi:10.2136/vzj2004.0097

Guber, A. K., Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., and Sikora, L. J., 2006. Rainfall-induced release of fecal coliforms and other manure constituents: comparison and modeling. Applied and Environmental Microbiology, 72(12): 7531-7539.

Guber, A. K., Karns, J. S., Pachepsky, Y. A., Sadeghi, A. M., Van Kessel, J. S., and Dao, T. H., 2007. Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions. Letters in Applied Microbiology, 44(2): 161-167.

Guber, A. K., Pachepsky, Y. A., Shelton, D. R., and Yu, O., 2009. Association of fecal coliforms with soil aggregates. Soil Science, 174(10): 543-548.

Guber, A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L., 2011. Uncertainty in modelling of faecal coliform overland transport associated with manure application in Maryland. Hydrological Processes, 25(15): 2393-2404.

Guber., A. K., Pachepsky, Y. A., Yakirevich, A. M., Shelton, D. R., Sadeghi, A. M., Goodrich, D. C., and Unkrich, C. L. 2012. STWIR, a microorganism transport with infiltration and runoff add-on module for the KINEROS2 runoff and erosion model documentation and user manual. User Manual. USDA-ARS. Beltsville, MD, USA.

Guber, A. K., Pachepsky, Y. A., Dao, T. H., Shelton, D. R., and Sadeghi, A. M., 2013. Evaluating manure release parameters for nonpoint contaminant transport model KINEROS2/STWIR. Ecological Modelling, 263: 126-138.

Hamilton, D. W., 2011. Consistency of manure/water mixtures. Stillwater, OK: Oklahoma Cooperative Extension Service. OSU Factsheet BAE 1751.

Harmel, R. D., Karthikeyan, R., Gentrym T., and Srinivasan, R., 2010. Effects of agricultural management, land use, and watershed scale on *E. coli* concentrations in runoff and streamflow. Transactions of ASABE, 53(6): 1833-1841.

Himathongkham, S., Bahari, S., Riemann, H., and Cliver, D., 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiology Letters, 178(2): 251-257.

Hodgson, C. J., Bulmer, N., Chadwick, D. R., Oliver, D. M., Heathwaite, A. L., Fish, R. D., and Winter, M., 2009. Establishing relative release kinetics of faecal indicator organisms from different faecal matrices. Letters in Applied Microbiology, 49(1): 124-130.

Hoffmann, S., Batz, M. B., and Morris, J. G., 2012. Annual cost of illness and quality-adjusted life year losses in the united states due to 14 foodborne pathogens. Journal of Food Protection, 75(7): 1292-1302.

Howell, J. M., Coyne, M. S., and Cornelius, P. L., 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. Journal of Environment Quality, 25(6): 1216.

Ibekwe, A. M., Ma, J., Crowley, D. E., Yang, C., Johnson, A. M., Petrossian, T. C., and Lum, P. Y., 2014. Topological data analysis of *Escherichia coli* O157:H7 and non-O157 survival in soils. Frontiers in Cellular and Infection Microbiology, doi: 10.3389/fcimb.2014.00122

Isensee, A. R., and Sadeghi, A. M., 1999. Quantification of runoff in laboratory-scale chambers. Chemosphere, 38(8): 1733-1744.

Jamieson, R. C., Gordon, R. J., Sharples, K. E., Stratton, G. W., and Madani, A., 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering, 44: 1-9.

Jenkins, M. B., Bowman, D. D., Fogarty, E. A., and Ghiorse, W. C, 2002. *Cryptosporidium parvum* oocyst inactivation in three soil types at various temperatures and water potentials. Soil Biology and Biochemistry, 34(8): 1101-1109.

Jiang, X., Morgan, J., Doyle, M. P., Jiang, X., Morgan, J., and Doyle, M. P., 2002. Fate of *Escherichia coli* O157 : H7 in manure-amended soil. Applied and Environmental Microbiology, 68(5): 2605-2609.

Khaleel, R., Reddy, K. R., and Overcash, M. R., 1980. Transport of potential pollutants in runoff water from land areas receiving animal wastes: a review. Water Research, 14(5): 421-436.

Kiefer, L. A., Shelton, D. R., Pachepsky, Y., Blaustein, R., & Santin-Duran, M., 2012. Persistence of *Escherichia coli* introduced into streambed sediments with goose, deer and bovine animal waste. Letters in Applied Microbiology: 345-353.

Kim, J., Luo, F., and Jiang, X., 2009. Factors impacting the regrowth of E*scherichia coli* O157:H7 in dairy manure compost. Journal of Food Protection, 72(7): 1576-1584.

Kidron, G. J., 2011. Runoff generation and sediment yield on homogeneous dune slopes: scale effect and implications for analysis. Earth Surface Processes and Landforms, 36(13): 1809-1824.

Kim, K., Whelan, G., Purucker, S. T., Bohrmann, T. F., Cyterski, M. J., Molina, M., Gu, Y., Pachepsky, Y., Guber, A., and Franklin, D. H., 2014. Rainfall-runoff model parameter estimation and uncertainty evaluation on small plots. Hydrological Processes, 28(20): 5220-5235.

Koneswaran, G., and Nierenberg, D., 2008. Global farm animal production and global warming: impacting and mitigating climate change. Environmental Health Perspectives, 116(5): 578-582.

Kouznetsov, M. Y., Roodsari, R., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Shirmohammadi, A., and Starr, J. L., 2007. Modeling manure-borne bromide

and fecal coliform transport with runoff and infiltration at a hillslope. Journal of Environmental Management, 84(3): 336-346.
Kramers, G., Holden, N. M., Brennan, F., Green, S., and Richards, K. G., 2012. Water content and soil type effects on accelerated leaching after slurry application. Vadose Zone Journal, 11(1). doi:10.2136/vzj2011.0059.
Kress, M., and Cifford, G. F., 1984. Fecal coliform release from cattle fecal deposits. Water Resources Bulletin, 20(1): 61-66.
Kudva, I. T., Blanch, K., and Hovde, C. J., 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Applied and Environmental Microbiology, 64(9): 3166-3174.
Lawson, L., 2003. HSPF Model Calibration and Verification for Bacteria TMDLs. Guidance Memo No. 03-2012, Commonwealth of Virginia, Department of Environmental Quality.
Lim, T. T., Edwards, D. R., Workman, S. R., Larson, B. T., and Dunn, L., 1998. Vegetated filter strip removal of cattle manure constituents in runoff. Transactions of ASAE, 41(5): 1375-1381.
Ling, T. Y., Jong, H. J., Apun, K., and Sulaiman, W. H. W., 2009. Quantifying *Escherichia coli* release from soil under high-intensity rainfall. Transactions of ASABE, 52(3): 785-792.
Lombard, N., Prestat, E., Dirk van Elsas, J., and Simonet, P., 2011. Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78: 31-49.
Martinez, G., Pachepsky, Y. A., Shelton, D. R., Whelan, G., Zepp, R., Molina, M., and Panhorst, K., 2013. Using the Q_{10} model to simulate *E. coli* survival in cowpats on grazing lands. Environment International, 54: 1-10.
Martinez, G., Pachepsky, Y. A., Whelan, G., Yakirevich, A. M., Guber, A., and Gish, T. J., 2014. Rainfall-induced fecal indicator organisms transport from manured fields: Model sensitivity analysis. Environment International, 63: 121-129.
Mawdsley, J. L., Bardgett, R. D., Merry, R. J., and Pain, B. F., 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology, 2: 1-15.
McDaniel, R. L., Soupir, M. L., Tuttle, R. B., and Cervantes, A. E., 2013. Release, dispersion, and resuspension of *Escherichia coli* from direct fecal deposits under controlled flows. Journal of the American Water Resources Association, 49(2): 319-327.
McDowell, R. W., Muirhead, R. W., and Monaghan, R. M., 2006. Nutrient, sediment, and bacterial losses in overland flow from pasture and cropping soils following cattle dung deposition. Communications in Soil Science and Plant Analysis, 37(1-2): 93-108.
Meays, C. L., Broersma, K., Nordin, R., and Mazumder, A., 2004. Source tracking fecal bacteria in water: A critical review of current methods. Journal of Environmental Management, 73(1): 71-9.
Meyer, L. D., and Harmon, W. C., 1979. Multiple-intensity rainfall simulator for erosion research on row sideplots. Transactions of the ASAE, 22: 100-103.

Mishra, A., Benham, B. L., and Mostaghimi, S., 2008. Bacterial transport from agricultural lands fertilized with animal manure. Water, Air, and Soil Pollution, 189(1-4): 127-134.

Molina, M., 2005. Temporal and spatial variability of fecal indicator bacteria: Implications for the application of MST methodologies to differentiate sources of fecal contamination. EPA/600/R-05/107.

Moriarty, E. M., Mackenzie, M. L., Karki, N., and Sinton, L. W., 2011. Survival of *Escherichia coli*, enterococci, and *Campylobacter* spp. in sheep feces on pastures. Applied and Environmental Microbiology, 77(5): 1797-1803.

Mosaddeghi, M. R., Mahboubi, A. A., Zandsalimi, S., and Unc, A., 2009. Influence of organic waste type and soil structure on the bacterial filtration rates in unsaturated intact soil columns. Journal of Environmental Management, 90(2): 730-739.

Moyer, D. L., and Hyer, K. E., 2003. Use of the hydrological simulation program-FORTRAN and bacterial source tracking for development of fecal coliform total maximum daily load (TMDL) for Blacks Run, Rockingham County, Virginia. U.S. Department of the Interior U.S. Geological Survey, Water-resources Investigations Report 03-4161.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2005. Erosion and subsequent transport state of *Escherichia coli* from cowpats. Applied and Environmental Microbiology, 71(6): 2875-2879.

Muirhead, R. W., Collins, R. P., and Bremer, P. J., 2006a. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Letters in Applied Microbiology, 42(2): 83-87.

Muirhead, R. W., Collins, R. P., Bremer, P. J., 2006b. Interaction of *Escherichia coli* and Soil Particles in Runoff. Applied and Environmental Microbiology, 72(5): 3406-3411.

Muirhead, R. W., and Littlejohn, R. P., 2009. Die-off of *Escherichia coli* in intact and disrupted cowpats. Soil Use and Management, 25(4): 389-394.

Muirhead, R. W., and Monaghan, R. M., 2012. A two reservoir model to predict *Escherichia coli losses* to water from pastures grazed by dairy cows. Environment International, 40: 8-14.

Oladeinde, A., Bohrmann, T., Wong, K., Purucker, S. T., Bradshaw, K., Brown, R., Snyder, B., and Molina, M, 2014. Decay of fecal indicator bacterial populations and bovine-associated source-tracking markers in freshly deposited cow pats. Applied and Environmental Microbiology, 80(1): 110-118.

Oliver, D. M., Heathwaite, L., Haygarth, P. M., and Clegg, C. D., 2005. Transfer of *Escherichia coli* to water from drained and undrained grassland after grazing. Journal of Environmental Quality, 34(3): 918-925.

Pachepsky, Y. A., Sadeghi, A. M., Bradford, S. A., Shelton, D. R., Guber, A. K., and Dao, T., 2006. Transport and fate of manure-borne pathogens: Modeling perspective. Agricultural Water Management, 86(1-2): 81-92.

Pachepsky, Y. A., Yu, O., Karns, J. S., Shelton, D. R., Guber, A. K., & Kessel, J. S. Van., 2008. Strain-dependent variations in attachment of *E. coli* to soil particles of different sizes. International Agrophysics, 22: 61–66.

Pachepsky, Y. A., Guber, A. K., Shelton, D. R., and McCarty, G. W., 2009. Size distributions of manure particles released under simulated rainfall. Journal of Environmental Management, 90(3): 1365-1369.

Pachepsky, Y. A., and Shelton, D. R., 2011. *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. Critical Reviews in Environmental Science and Technology, 41(12): 1067-1110.

Pachepsky, Y. A., Blaustein, R. A., Whelan, G., and Shelton, D. R., 2014. Comparing temperature effects on *Escherichia coli*, *Salmonella*, and *Enterococcus* survival in surface waters. Letters in Applied Microbiology, 59(3): 278-283.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V, Braden, C. R., Angulo, F. J., and Griffin, P. M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerging Infectious Diseases, 19(3): 407-415.

Panhorst, K. A., 2002. Estimating bacterial loadings to surface waters from agricultural watersheds. Master in Science Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Parajuli, P., 2007. SWAT bacteria sub-model evaluation and application. PhD Dissertation, Kansas State University, Manhattan, KS.

Patni, N. K., Toxopeus, H. R., and Jui, P. Y., 1985. Bacterial quality of runoff from manured and non-manured cropland. Transactions of ASAE, 28(6): 1871-1878.

Pell, A. N., 1997. Manure and microbes: public and animal health problem? Journal of Dairy Science, 80(10), 2673-2681.

Peñuela, A., Javaux, M., and Bielders, C. L. 2013. Scale effect on overland flow connectivity at the plot scale. Hydrology and Earth System Sciences, 17(1): 87–101.

Raclot, D., Le Bissonnais, Y., Louchart, X., Andrieux, P., Moussa, R., and Voltz, M., 2009. Soil tillage and scale effects on erosion from fields to catchment in a Mediterranean vineyard area. Agriculture, Ecosystems & Environment, 134(3-4), 201-210.

Roodsari, R. M., Shelton, D. R., Shirmohammadi, A., Pachepsky, Y. A., Sadeghi, A. M., and Starr, J. L., 2005. Fecal coliform transport as affected by surface condition. Transactions of ASAE, 48(3), 1055-1061.

Rosen, B., 2000. Waterborne pathogens in agricultural watersheds. USDA Watershed Science Institute-Technical Note 2. University of Vermont, Burlington.

Sadeghi, A. M., and Isensee, A. R., 2001. Impact of hairy vetch cover crop on herbicide transport under field and laboratory conditions. Chemosphere, 44(2): 109–118.

Savichtcheva, O., and Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research, 40(13), 2463-2476.

Schijven, J. F., Bradford, S. A., and Yang, S., 2004. Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: Physical factors. Journal of Environmental Quality, 33(4), 1499-1508.

Sharples, K. E., Stratton, G. W., Madani, S. A., Gordon, R. J., and Patterson, G., 2004. The survival of *E. coli* in agricultural soil treated with dairy manure. 2004 ASAE/CSAE Annual International Meeting, Ottowa, Canada.

Sharpley, A. N., 1985. Depth of surface soil-runoff Interaction as affected by rainfall, soil slope, and management. Soil Science Society of America Journal, 49: 1010-1015.

Sheldrick, W., Syers, J. K., and Lingard, J., 2003. Contribution of livestock excreta to nutrient balances. Nutrient Cycling in Agroecosystems, 119-131.

Shelton, D. R., Pachepsky, Y. A., Sadeghi, A. M., Stout, W. L., Karns, J. S., and Gburek, W. J., 2003. Release rates of manure-borne coliform bacteria from data on leaching through stony soil. Vadose Zone Journal, 2, 34–39.

Sheridan, G. J., Noske, P. J., Lane, P. N. J., Jones, O. D., and Sherwin, C. B., 2014. A simple two-parameter model for scaling hillslope surface runoff. Earth Surface Processes and Landforms, 39(8): 1049-1061.

Sinclair, R. G., Rose, J. B., Hashsham, S. A., Gerba, C. P., and Haas, C. N., 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Applied and Environmental Microbiology, 78(6): 1969-1977.

Sinton, L. W., Braithwaite, R. R., Hall, C. H., and Mackenzie, M. L., 2007. Survival of indicator and pathogenic bacteria in bovine feces on pasture. Applied and Environmental Microbiology, 73(24): 7917-7925.

Sistani, K. R., Bolster, C. H., Way, T. R., Tobert, H. A., Pote, D. H., and Watts, D. B., 2009. Influence of poultry litter application methods on the longevity of nutrient and *E. coli* in runoff from tall fescue pasture. Water, Air, and Soil Pollution, 206: 3-12.

Smith, M. S., Thomas, G. W., White, R. E., and Ritonga, D., 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. Journal of environmental quality, 14(1): 87-91.

Sobeck, D. C., and Higgins, M. J., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Research, 36(3): 527-538.

Sobsey, M. D., Khatib, L. A., Hill, V. R., Alocilja, E., and Pillai, S., 2006. Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. Animal Agriculture and the Environment: National Center for Manure and Animal Waste White Papers, Rice, J. M, Caldwell, D. F., Humenik, F., J. (eds.) ASABE Pub. St. Joseph, Michigan. pp. 609-665.

Soupir, M., Tech, V., Hall, S., Mostaghimi, S., and Alphin, E. F., 2003. Bacteria release and transport from livestock manure applied to pastureland. 2003 ASAE Annual International Meeting. Las Vegas, NV.

Soupir, M. L., Mostaghimi, S., Yagow, E. R., Hagedorn, C., and Vaughan, D. H., 2006. Transport Of fecal bacteria from poultry litter and cattle manures applied to pastureland. Water, Air, and Soil Pollution, 169(1-4): 125-136.

Soupir, M. L., Mostaghimi, S., and Dillaha, T., 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. Journal of Environmental Quality, 39(3), 1019-1027.

Springer, E. P., Gifford, G. F., and Windham, M. P., 1983. Fecal coliform release studies and development of a preliminary nonpoint source transport model for indicator bacteria. Utah State University Reports, Paper 69.

Srivastava, P., Edwards, D. R., Daniel, T. C., and Costello, T. A., 1996. Performance of vegetative filter strips with varying pollutant source and filter strip lengths. Transactions of ASAE, 39(6): 2231-2239.

Steiger, J. H., 1980. Tests for comparing elements of a correlation matrix. Psychological Bulletin, 87, 245-251.

Stevik, T. K., Aa, K., Ausland, G., Hanssen, J. F., 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research, 38: 1355-1367.

Stoddard, C. S., Coyne, M. S., and Grove, J. H., 1998. Fecal bacteria survival and infiltration through a shallow agricultural soil: timing and tillage effects. Journal of Environmental Quality, 27(6): 1516.

Stout, W. L., Pachepsky, Y. A., Shelton, D. R., Sadeghi, A. M., Saporito, L. S., and Sharpley, A. N., 2005. Runoff transport of faecal coliforms and phosphorus released from manure in grass buffer conditions. Letters in Applied Microbiology, 41(3): 230-234.

Tauxe, R. V., 2002. Emerging foodborne pathogens. International Journal of Food Microbiology, 78: 31-41.

Tate, K. W., Atwill, E. R., George, M. R., McDougald, M. K. and Larsen, R. E., 2000. *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. Journal of Range Management, 53(3), 295-299.

Thelin, R., and Gifford, G. F., 1983. Fecal coliform release patterns from fecal material of cattle. Journal of Environment Quality, 12(1), 57-63.

Thurston-Enriquez, J. A., Gilley, J. E., and Eghball, B., 2005. Microbial quality of runoff following land application of cattle manure and swine slurry. Journal of Water and Health, 3(2), 157-171.

Tyrrel, S. F., and Quinton, J. N., 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. Journal of Applied Microbiology, 94 Suppl: 87S-93S.

Unc, A., and Goss, M. J., 2004. Transport of bacteria from manure and protection of water resources. Applied Soil Ecology, 25(1): 1-18.

U.S. CDC, 2012. General Information: *E. coli* (*Escherichia coli*). Atlanta, GA, USA. < http://www.cdc.gov/ecoli/general/index.html>.

U.S. EPA, 1984. Health effects criteria for fresh recreational waters. EPA 600/1-84-004, Washington, D.C.

U.S. EPA, 1986. Ambient water quality criteria for bacteria. EPA 440/5-84-002, Washington, D.C.

U.S. EPA, 2009. National water quality inventory: report to Congress, 2004 reporting cycle. EPA/841/R-08/001, Washington, D.C.

U.S. EPA., 2012. Water: Monitoring assessment. 5.11 Fecal bacteria. <http://water.epa.gov/type/rsl/monitoring/vms511.cfm>.

Vadas, P. A., Kleinman, P. J. A., Sharpley, A. N., 2004. A simple method to predict dissolved phosphorus in runoff from surface-applied manures. Journal of Environmental Quality, 33: 749-756.

Vadas, P. A., Good, L. W., Moore, P. A., and Widman, N., 2009. Estimating phosphorus loss in runoff from manure and fertilizer for a phosphorus loss quantification tool. Journal of Environmental Quality, 38(4): 1645-1653.

Van de Giesen, N., Stomph, T., Ajayi, E. A., and Bagayoko, F., 2011. Scale effects in hortonian surface runoff on agricultural slopes in west africa: field data and models. Agriculture, Ecosystems & Environment, 142(1-2): 95-101.

Van Horn, H. H., Wilkie, A. C., Powers, W. J., and Nordstedt, R. A., 1994. Components of dairy manure management systems. Journal of Dairy Science, 77(7): 2008–2030.

van Genuchten, M. Th. 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research Report No. 119, U. S. Salinity Laboratory, Riverside, CA, p. 88.

Van Kessel, J. S., Pachepsky, Y. A., Shelton, D. R., and Karns, J. S., 2007. Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. Journal of Applied Microbiology, 103(4), 1122-1127.

van Veen, J. A., van Overbeek, L. S., and van Elsas, J. D., 1997. Fate and activity of microorganisms introduced into soil. Microbiology and Molecular Biology Reviews, 61(2): 121-35.

Wang, L., Mankin, K. R., and Marchin, G. L., 2004. Survival of fecal bacteria in dairy cow manure. Transactions of ASAE, 47(4): 1239-1246.

Wang, L., Xu, S., and Li, J., 2011. Effects of phosphate on the transport of *Escherichia coli* O157:H7 in saturated quartz sand. Environmental Science & Technology, 45(22): 9566-9573.

Whelan, G., Kim, K., Pelton, M. A., Soller, J. A., Castleton, K. J., Molina, M., Pachepsky, Y., Zepp, R., 2014. An integrated environmental modeling framework for performing quantitative microbial risk assessments. Environmental Modelling & Software, 55: 77-91.

Williams, A. P., Avery, L. M., Killham, K., and Jones, D. L., 2005. Persistence of *Escherichia coli* O157 on farm surfaces under different environmental conditions. Journal of Applied Microbiology, 98(5): 1075-1083.

Williams, J. R., Izzaurralde, R. C., and Steglich, E. M., 2012. Agricultural Policy/Environmental Extender Model Theoretical Documentation. Version 0806. AgriLife Research Texas A&M System.

Wu, L., Gao, B., Muñoz-Carpena, R., and Pachepsky, Y. A., 2012. Single collector attachment efficiency of colloid capture by a cylindrical collector in laminar overland flow. Environmental Science & Technology, 46(16): 8878-8886.

Wu, L., Muñoz-Carpena, R., Gao, B., Yang, W., and Pachepsky, Y. A., 2014. Colloid filtration in surface dense vegetation: experimental results and theoretical predictions. Environmental Science & Technology, 48(7): 3883-3890.

Zejun, T., Tingwu, L., Qingwen, Z., and Jun, Z., 2002. The sealing process and crust formation at soil surface under the impacts of raindrops and polyacrylamide. 12th ISCO Conference, Beijing, China. pp 456-462.

Zhang, Q., Mankin, K. R., and Erickson, L. E., 2001. Modeling transport of *Cryptosporidium parvum* oocysts in overland flow. Proceedings of the 2001 Conference on Environmental Research: 32-38.